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Treatment of produced water: targeting dissolved compounds to meet a zero harmful discharge in oil and gas production

Thesis for the degree of Philosophiae Doctor

Trondheim, June 2009

Norwegian University of Science and Technology
Faculty of Engineering Science and Technology
Department of Hydraulic and Environmental Engineering

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PREFACE

This thesis is submitted for the fulfilment of the Ph.D. degree at the Norwegian University of Science and Technology (NTNU). The work has been performed at the Department of Hydraulic and Environmental Engineering and supervised by Professor TorOve Leiknes.

I received my first M.Sc degree in 2001 at the Department of Environmental Engineering, Faculty of Industrial Chemistry of Gheorghe Asachi Technical University in Iasi (Romania) in the field of environmental management. Then I continued my studies in Germany where I obtained my second M.Sc. in 2004 at the Department of Environmental Engineering and Energy Management of Technical University Hamburg-Harburg (TUHH) in the field of environmental engineering.

The present study has been done within the scope of the Joint Industrial Program (JIP) on Treatment of Produced Water: Characterization and new treatment strategies (2004-2009), financed by the Norwegian Research Council, through the Petromaks program and several industrial partners.

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In particular, I am deeply grateful to my supervisor Professor TorOve Leiknes for intelligently guiding my work towards the completion of this PhD thesis. I also thank Professor Hallvard Ødegaard for giving me the opportunity to be involved in this research project at NTNU.

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I am also grateful to Cetco Oilfield Services Company and Catalina Biosolutions, which provided the organoclay (Crudesorb) and the blend of naturally occurring microbes, respectively, used in my experiments.

Finally, I am forever indebted to my parents and my younger brother Marian for their continuous support and encouragement during the strenuous periods of my work on the way to complete this thesis.

Ciprian

ABSTRACT

High amounts of dissolved compounds are discharged into the sea with the produced water generated from the offshore oil and gas platforms. Some of these compounds are toxic to the environment, having important contributions to the environmental impact factors (EIF) calculated for produced water discharges. No performance standards currently exist for the removal of dissolved compounds from produced water. However, the overall goals for oil, natural components and chemicals in produced water require reducing the input into the sea of oil and other substances resulting from produced water from offshore installations. The ultimate aim is to eliminate pollution from those sources and ensure that effort is made to give priority to actions related to the most harmful components of produced water.

The goal of this study is to acquire further knowledge and technology to attain the "Zero Harmful Discharge" policy initiated by the Norwegian authorities. The ambition is to study a treatment method to meet the requirements of the future performance standards for the removal of harmful dissolved organic compounds.

The dissolved compounds can be removed by physical/chemical methods such as stripping, oxidation, membrane technology, extraction, sorption as well as biological treatment. Required process size is a key factor limiting the application of several of the alternatives described above, especially if very low effluent concentrations must be achieved. Handling of the "waste" stream that could be spent sorption media, off-gas that requires further treatment or concentrate from a membrane process, is another common limitation.

The scope of this study was to investigate the feasibility of a treatment concept that combines selective in-line sorption of selected dissolved compounds (BTX) coupled with biological regeneration of the spent media in a fluidized bed reactor (FBR). The research work was focused on the following issues: selective sorption of dissolved aromatic compounds but not of organic acids from produced water, assessment of the efficiency of the bioregeneration process and feasibility of the treatment concept based on sorption and biological regeneration in a continuous process.
BTEX were selected to be used in the tests because they are found by far in the highest concentration in produced water among the dissolved aromatic compounds, while acetic acid was chosen for the same reason as the representative of the organic acids group.
In the first instance, the sorption properties of several different types of sorption media were investigated. Focus was made on sorbents, which seemed to be promising for this application based on reports found during the literature review. This included also an assessment of sorption kinetics of the selected media. After identifying the most appropriate sorbent, laboratory experiments were conducted to study the biodegradation of the sorbed compounds by the microorganisms grown on the sorbent granules. Finally, a laboratory-scale experimental setup was built and operated in order to study and determine the feasibility and potentials of the treatment concept.
Results indicated that selective sorption of BTX compounds from wastewater was achieved by using an organoclay. This sorbent showed good affinity for BTX

compounds and at the same time retained only to a low extent acetic acid from wastewater. Off-line biological regeneration of the organoclay was shown to be feasible under the studied conditions. Operating conditions depended on wastewater quality, therefore FBR operation had to be tuned first to sorb the entire amount of BTX compounds from produced water and then completely biodegrade the previously sorbed compounds in order for the organoclay bed to recover its entire sorption capacity. Long-time off-line bioregeneration experiments indicated that the organoclay bed lost a part of its sorption capacity over time. Possible causes for this phenomenon could be: accumulation of organic compounds and minerals, particle attrition, desorption and biodegradation of the tailoring agent (dimethyl dihydrogenated ammonium chloride).

SUMMARY

High amounts of dissolved compounds are discharged into the sea with the produced water generated from the offshore oil and gas platforms. Some of these compounds are toxic to the environment, having important contributions to the environmental impact factors (EIF) calculated for produced water discharges. No performance standards currently exist for the removal of dissolved compounds from produced water. However, the overall goals for oil, natural components and chemicals in produced water require reducing the input into the sea of oil and other substances resulting from produced water from offshore installations. The ultimate aim is to eliminate pollution from those sources and ensure that effort is made to give priority to actions related to the most harmful components of produced water.

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The dissolved compounds can be removed by physical/chemical methods such as stripping, oxidation, membrane technology, extraction, sorption as well as biological treatment. Required process size is a key factor limiting the application of several of the alternatives described above, especially if very low effluent concentrations must be achieved. Handling of the "waste" stream, which could be spent sorption media, off-gas that requires further treatment or concentrate from a membrane process, is another common limitation.

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BTEX were selected to be used in the tests because they are found by far in the highest concentration in produced water among the dissolved aromatic compounds, while acetic acid was chosen as the representative of the organic acids group for the same reason.

Studies have been conducted to find a sorbent with a significant affinity for the dissolved aromatics and very low affinity for organic acids. Several different types of sorption media (activated carbons, natural zeolites, surfactant-modified zeolites, and organically modified clays) were studied during the sorption tests. An organoclay called Crudesorb showed the best performance, since it sorbed only to a low extent acetic acid and at the same time had good sorption abilities for BTX compounds.

The sorption properties of the organoclay were further studied at different values of pH (3, 5 and 8), acetic acid concentrations (0, 160 and 320 mg/l), salinity (0, 35 and 70 g/l) and temperature (20, 40 and 60 °C). Results showed that there was a sorption competition between BTX and acetic acid. Sorption of BTX compounds decreased with increasing initial acetic acid concentration and temperature, and increased proportionally with salinity. Sorption of acetic acid, however, decreased with increasing pH, temperature and salinity.

The biological regeneration of the organoclay used to sorb the BTX (benzene, toluene and p-xylene) compounds from saline water was studied in a laboratory-scale fluidized bed reactor. The system was operated with and without oxygen and nutrients supply in order to study the importance of biological regeneration process for BTX removal. Sorption was the only removal mechanism of BTX when no inoculum, nutrients and oxygen were supplied into the system. The hydrocarbons broke through the organoclay bed in the following order: benzene, toluene and p-xylene. This suggested that the hydrophobic surface of organoclay preferentially sorbed the BTEX components based on their solubilities. A commercial inoculum was used to develop a microbial culture, which formed a biofilm on the surface of the fluidized organoclay particles after oxygen and nutrients (at a stoichiometric ratio of 100/5/1: COD/N/P) were supplied into the system. A membrane contactor was used for aeration in order to prevent stripping of the volatile BTX compounds. Results demonstrated that the use of an organoclay as biomass carrier in a fluidized bed reactor produced a system in which both sorption and biodegradation removal mechanisms for BTX reduction were present. The BTX removal was therefore enhanced when microorganisms were present in the FBR as compared to the case when sorption was the only removal mechanism.

Off-line bioregeneration of the organoclay loaded with BTX compounds in the fluidized bed reactor system was further investigated by alternating sorption and biodegradation steps. Different operation modes of the fluidized bed system were studied in order to obtain an efficient bioregeneration of the organoclay bed in a short period. The most important parameters that were varied were the duration of the sorption and biodegradation steps as well as the duration of the aeration operation. One important issue was to deliver the nutrients necessary for the microbial growth in the beginning of the biodegradation steps and not before the sorption steps. Nutrients were supplied into the system in the form of Bushnell-Haas medium (at a stoichiometric ratio of 100/5/1: COD/N/P). Results demonstrated that the biological regeneration of an organoclay, loaded with BTX compounds, could be achieved in a fluidized bed reactor system by alternating sorption and biodegradation treatment steps and tuning the operation of the fluidized bed reactor system.

Long time off-line bioregeneration experiments were carried out to investigate the efficiency of bioregeneration process over a longer period of operation. The best performance was observed when sorption steps of 2 h were alternated with bioregeneration steps of 6 h (5 h aeration). Results indicated that the organoclay bed lost a part of its sorption capacity over time and therefore, in a continuous system, the spent media should be replaced with fresh media at a calculated rate in order to compensate for the loss of sorption capacity.

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NOTATIONS

Abbreviations

AA	acetic acid
AC	activated carbon
ALS	almond shells
API	American Petroleum Institute
BAC	biological activated carbon
BDTDA	benzyldimethyltetradecyl ammonium
BET	Brunauer-Emmet-Teller (isotherm)
BOD	biochemical oxygen demand
BTEX	benzene, toluene, ethylbenzene, and o-, m-, and p-xylene
BTMA	benzyltrimethyl ammonium
BV	bed volume
CDW	cell dry weight
CEC	cation exchange capacity (meq/g)
CFU	colony forming units
COD	chemical oxygen demand
CP	(mono) chlorophenol
CS	cotton stalks
CSLM	confocal scanning laser microscopy
DAPI	4,6-diamidino-2-phenylindole
DCM	dichloromethane
1,2-DCB	1,2-dichlorobenzene
DCP	dichlorophenol
DGGE	denaturing gradient gel electrophoresis
DNA	deoxyribonucleic acid
DO	dissolved oxygen
DP	date pits
DREAM	dose related risk and effects assessment model (software)
DS	dry solids
E. coli	Escherichia coli
ED	electrodialysis
EDC	3-Ethyl-1-[3-(dimethilamino) propyl] carbodiimide
EDTA	ethylenediaminetetraacetic acid
EIF	environmental impact factor
EPA	Environmental Protection Agency
FAME	fatty acid methyl ester
FAS	fixed film activated sludge
FBR	fluidized bed reactor
FID	flame ionization detector
FISH	fluorescent in situ hybridization
F/M	food to microorganisms ratio
GAC	granular activated carbon
GC	gas chromatograph
GC/MS	gas chromatograph/mass spectrometer system

HDTMA	hexadecyltrimethyl ammonium
HOC	hydrophobic organic chemicals
HPLC	high performance liquid chromatography
HRT	hydraulic retention time
IR	infra red
LMH	litres per square metre and hour
MAR	micro autoradiography
MBBR	moving bed biofilm reactor
MD	membrane distillation
MGP	manufactured gas plant
MPN	most probable number
MPPE	macroporous polymer extraction
MS	mass spectrometer
MSD	mass selective detector
MTBE	methyl tert-butyl ether
MW	molecular weight
m-xylene	meta-xylene
NCS	Norwegian Continental Shelf
NF	nanofiltration
NOC	non-ionic organic compounds
NOM	natural organic matter
NPD	Norwegian Petroleum Directorate
NPD	naphthalene, phenanthrene, dibenzothiophene
NZ	natural zeolite
OECD	Organization for Economic Co-operation and Development
OGP	International Association of Oil and Gas Producers
OiW	oil in water
OLF	Norwegian Oil Industry Association
OLR	organic loading rate
OS	olive stones
OSPAR	Convention for the Protection of the Marine Environment of the North-East Atlantic
OTU	operational taxonomy units
o-xylene	ortho-xylene
PAA	polyacrylamide
PAC	powder activated carbon
PAH	polycyclic aromatic hydrocarbons
PCP	pentachlorophenol
PCR	polymerase chain reaction
PEC	predicted environmental concentration
PID	photo ionization detector
pK_a	acid dissociation constant
PLFA	phospholipid fatty acid
PNEC	predicted no effect concentration
PS	peach stones
PSIG	pounds per square inch gauge
P&T	purge and trap

PW	produced water
PWRI	produced water re-injection
p-xylene	para-xylene
rDNA	ribosomal deoxyribonucleic acid
RCF	relative centrifugal force
RE	refinery effluent
RFLP	restricted fragment length polymorphism
RNA	ribonucleic acid
RO	reverse osmosis
rRNA	ribosomal ribonucleic acid
SBR	sequencing batch reactor
SCOD	soluble chemical oxygen demand
SEM	scanning electron microscope
SFFR	submerged fixed film reactor
SMO	standard mineral oil
SMP	soluble microbial products
SMZ	surfactant-modified zeolite
SPME	solid phase microextraction
SRT	solids retention time
SS	suspended solids
TAE	tris-acetate-ethylenediaminetetraacetic acid (EDTA)
TBE	tris-borate-ethylenediaminetetraacetic acid (EDTA)
TCE	trichloroethylene
2,4,6-TCP	2,4,6-trichlorophenol
TEMED	tetramethylethylenediamine
TFEA	2,2,2 trifluoroethylamine
ThOD	theoretical oxygen demand
TOC	total organic carbon
TOP Water	treatment of produced water
T-RFLP	terminal restriction fragment length polymorphism
TSS	total suspended solids
UASB	upflow anaerobic sludge blanket
USEPA	United States Environmental Protection Agency
UV	ultra violet
VOC	volatile organic carbon
VS	volatile solids
XRD	X-ray diffraction

Fundamental quantities

L	length
M_C	mass of contaminant
M_{O2}	mass of oxygen
M_S	mass of substrate
M_{Sor}	mass of sorbent
M_X	mass of bacteria
T	time

Symbols

Latin letters

A	biofilm surface area (L^2)
A_0	Arrhenius constant (dimension depends on the model)
C_0	initial concentration of sorbate in solution ($M_C\,L^{-3}$)
C_e	equilibrium concentration of sorbate in solution ($M_C\,L^{-3}$)
C_f	substrate concentration within biofilm ($M_S\,L^{-3}$)
C_{OC}	sorbate concentration in the organic phase ($M_C\,M_{Sor}^{-1}$)
C_S	saturation concentration in liquid phase ($M_C\,L^{-3}$)
C_W	equilibrium solute concentration in water phase ($M_C\,L^{-3}$)
D	molecular diffusitivity in bulk liquid ($L^2\,T^{-1}$)
d_c	characteristic cell diameter (L)
dC	gradient in substrate concentration between the bulk liquid and the biofilm surface ($M_S\,L^{-3}$)
D_f	molecular diffusitivity in biofilm ($L^2\,T^{-1}$)
E_a	activation energy or temperature characteristics ($J\,mol^{-1}$)
foc	fractional organic carbon content of the sorbent (-)
h_S	overall mass transfer coefficient for the substrate (LT^{-1})
J	substrate flux into the biofilm ($M_S\,L^{-2}T^{-1}$)
k_d	decay coefficient which accounts for cell death (T^{-1})
k_{e1}	rate constant of pseudo-first-order sorption (T^{-1})
k_{e2}	rate constant for second-order sorption ($M_{Sor}\,M_C^{-1}T^{-1}$)
K_d	linear sorption coefficient ($L^3\,M_{Sor}^{-1}$)
K_F	Freundlich adsorption constant ($L^3\,M_{Sor}^{-1}$)
K_L	adsorption intensity or Langmuir coefficient ($L^3\,M_{Sor}^{-1}$)
K_i	substrate inhibition constant ($M_S\,L^{-3}$)
K_I	substrate interaction coefficient (dimension depends on model)
K_{OC}	organic carbon-based partition coefficient of the sorbed compound ($L^3\,M_{Sor}^{-1}$)
K_{OW}	octanol/water partition coefficient (-)
K_S	half-saturation constant ($M_S\,L^{-3}$)
L	length of diffusion layer (L)
m_{O2}	specific rate of O_2 consumption for maintenance ($M_{O2}\,M_X^{-1}T^{-1}$)
q	specific substrate utilization rate (T^{-1})
q_e	equilibrium solid phase concentration of the contaminant on the sorbent ($M_C\,M_{Sor}^{-1}$)
q_m	theoretical saturation capacity ($M_C\,L^{-3}$)
q_{max}	maximum specific substrate utilization rate ($M_S\,M_X^{-1}T^{-1}$)
q_t	amount of solute sorbed at time t ($M_C\,M_{Sor}^{-1}$)
Q	flow ($L^3\,T^{-1}$)
Q_{10}	temperature coefficient (Arrhenius equation)
Q_R/Q	recycle ratio (-)
r_T	temperature dependent rate constant (dimension depends on model)
R	universal gas constant ($8.31\,J\,mol^{-1}\,K^{-1}$)

S	substrate concentration ($M_S L^{-3}$)
S_0	initial substrate concentration ($M_S L^{-3}$)
S^*	concentration of inhibitory substrate at which the highest growth rate is achieved ($M_S L^{-3}$)
S_e	amount adsorbed on solid at equilibrium in Freundlich equation ($M_C M_{Sor}^{-1}$)
S_f	substrate concentration within the biofilm ($M_S L^{-3}$)
S_m	adsorption capacity of the adsorption maximum ($M_C M_{Sor}^{-1}$)
t	time (T)
T	temperature (ºC or K)
V	volume (L^3)
X	biomass concentration ($M_X L^{-3}$)
X_0	initial biomass concentration ($M_X L^{-3}$)
X_f	average biomass concentration in the biofilm ($M_X L^{-3}$)
Y	biomass growth yield coefficient ($M_X M_S^{-1}$)
$Y_{X/O2}$	biomass yield on O_2 ($M_X M_S^{-1}$)
z	distance normal to biofilm surface (L)

Greek letters

β	constant related to the adsorption energy (Dubinin-Radushkevich equation)
ε	Polanyi adsorption potential (J)
μ	specific microbial growth rate (T^{-1})
μ_{max}	maximum specific growth rate ($M_S M_X^{-1} T^{-1}$)
ρ_c	density of cells ($M_X L^{-3}$)
θ	temperature coefficient (dimension depends on model)

1. INTRODUCTION

1.1 Produced Water - facts and figures

1.1.1 General overview

Produced water is the largest volume waste stream in the oil and gas exploration and production processes. It is a by-product of the production of oil and gas hydrocarbons from underground reservoirs which consists of formation water, which is water naturally present in the reservoir, and/or in the case of gas production, condensed water. In addition, the effluent stream from oil production can also contain: (a) seawater that has been injected to maintain reservoir pressure and that has broken through to production wells and (b) occasionally some smaller oily streams like displacement water from oil storage facilities, process and drainage water (Ray and Engelhardt, 1992).

Around 17 million cubic metres of water are produced daily in offshore operations worldwide together with the 120 million barrels of oil equivalent. About 40% of the daily water production (7 million cubic metres) is discharged offshore.
In 2007 the amount of produced water generated on the Norwegian Continental Shelf was 183 million cubic metres. This means an increase by 5% compared with 2006. The amount of produced water discharged into the sea was 162 million cubic metres, an increase by 12%, the main cause for this being the problems with water injection in several fields (Figure 1).

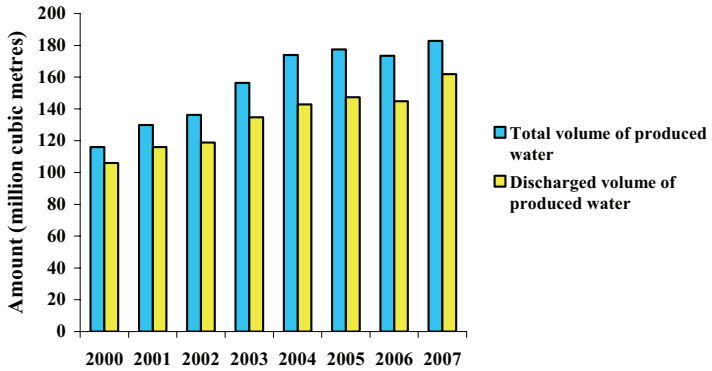


Figure 1. Amounts of produced water on the Norwegian Continental Shelf (OLF, 2007)

The composition of produced water varies from one field to another, within the field, and during its life span. Fields that produce gas or gas/condensate usually produce only condensed water during their early life, a fluid that contains very few salts and inorganic

compounds, but may contain high concentrations of dissolved light hydrocarbons. Productivity of gas wells decreases very rapidly – and even stops – when significant quantities of reservoir water are being produced. Therefore, the quantity of water associated with the gas production typically remains low, but its composition evolves markedly.
On the other hand, oil fields usually start producing reservoir water at a rather early stage of production at low water to oil ratios. Later, as fields mature, the ratio between water and oil can reach high values (up to 10:1), and the composition of the produced reservoir water changes, but significantly less than in gas fields. Also, oil field production is often enhanced by injection of water, to maintain the reservoir pressure. When the injected water breaks through into the production stream, it dilutes the formation water and the discharged produced water progressively approaches the injected water in composition and character (OGP, 2005).

The components of produced water can be grouped into the following categories:

- Inorganic components;
- Organic constituents;
- Production and processing chemicals;
- Other substances and properties.

1.1.2 Inorganic constituents

Formation water has similar properties to seawater, but normally has higher salinity and lower pH. Produced water composition will eventually change if seawater is injected to maintain reservoir pressure.

1.1.2.1 Major constituents

Depending on the geology of the field and production process, salinity of produced water can vary from almost fresh to saturated. The concentration of total dissolved salts in North Sea produced water can have values between 3 g/l and far above the average concentration in seawater.

Table 1. Produced water characteristics (Ray and Engelhardt, 1992)

Produced water parameters	pH	Chloride (g/l)	Temperature (°C)
Oil fields			
Brent	6-7.7	12.4-14.8	3-80
Other northern	6-7.7	14.7-16.9	
Central North Sea	6-7.7	81.0-100	
Gas fields			
UK sector	3.5-5.5	0.1-277	10-50
Dutch sector	3.8-5.5	0.1-189	13-45
North Sea seawater	8.1	18.8-19.5	3-17

The data in Table 1 on pH and chloride content of the effluents and of seawater illustrate that the effluents from different fields have very variable pH values and salinities. Normally the produced water coming from gas fields has lower pH values (3.5-5.5) while that resulting from oil fields is characterized by higher pH values (6-7.7).
Water injection is not applied in gas fields; therefore, produced waters are primarily a mixture of formation waters and condensed waters. The effluents could also contain drainage water. The chloride content of the discharges varies from almost fresh – the condensed water, to salty formation water with a chloride content of about 14 times that of seawater. These produced waters also are more acidic than those discharged from the oil platforms.
On the other hand the temperature of produced water can be very different depending on the field and can take values between 3-80 ºC.
Table 2 lists the average concentration values of some of the major anionic constituents in produced water and in seawater.

Table 2. Major inorganic constituents in produced water (mg/l) (OGP, 1994)

Component	World wide discharge average	North Sea discharge average	World-wide seawater average
Bicarbonate	771	615	28
Chloride	60874	44630	19000
Sulphate	325	814	900
Sulphide	140	-	-
Nitrate	1	1	0.67
Phosphate	0	0	0.09

1.1.2.2 Trace constituents

Metals are the main inorganic constituents thought to be of environmental concern.
Both industry and government carried out studies to determine the concentrations of trace metals in formation and produced waters. Over the last 10 years, sampling and analysis methodologies of trace metals improved considerably. The most commonly studied metals are iron, cadmium, chromium, copper, lead, mercury, nickel, arsenic and zinc. Due to different geological characteristics of the reservoirs, the results are characterized by considerable variability; for instance, gas fields usually provide higher values of heavy metals than oil fields. Furthermore, produced water generated from mature fields has significantly less trace metal content than that from early production fields. Corrosion of galvanised equipment is also believed to be a source of zinc and lead in some produced waters.

Table 3. Tons of heavy metals discharged into the North Sea (OLF, 2007)

Metal	2000	2001	2002	2003	2004	2005	2006	2007
Arsenic	0.080	0.052	0.104	0.013	0.144	0.057	0.073	0.063
Cadmium	0.103	0.035	0.055	0.012	0.010	0.006	0.010	0.008
Copper	4.790	4.290	3.230	3.090	1.760	1.080	1.780	1.930
Lead	23.800	2.450	4.180	1.940	1.100	1.630	2.290	2.230
Chromium	1.180	1.030	0.694	0.809	0.580	0.458	0.482	0.538
Mercury	0.116	0.016	0.020	0.007	0.005	0.004	0.005	0.003
Nickel	0.248	0.204	0.335	-	-	-	-	-
Zinc	3.570	1.840	4.510	-	-	-	-	-

1.1.3 Organic constituents

The organic components of produced water can be subdivided in the following two general categories:

- Dispersed oil;
- Dissolved organic compounds.

1.1.3.1 Partitioning of organic constituents in produced water

Oil is the common term that refers to a wide spectrum of compounds, mostly hydrocarbons, which can have very different properties. Oil is present in produced water both as dispersed droplets and in the dissolved phase. Some compounds such as the aliphatic hydrocarbons are found predominantly in the dispersed phase due to their solubility properties, while others, such as carboxylic acids are normally found in the water phase. Depending on their molecular weight and structural complexity, aromatic compounds (organic compounds which contain one or several benzene rings) are found either in one phase or another and some even in both phases. Low molecular weight aromatics, such as benzene, toluene, ethylbenzene and xylene (referred as BTEX) and naphthalene are fairly soluble in the water phase. On the other hand, heavier PAHs (PAHs - polycyclic aromatic hydrocarbons are chemical compounds that consist of fused aromatic rings and do not contain hetero-atoms or carry substituents) are significantly less soluble and mostly remain in the dispersed oil phase.
Faksnes *et al*. (2004) studied partitioning of organic compounds between dispersed oil and water phase. Experiments performed with produced water from Statfjord B and Gullfaks C indicated that significant amounts of some of the organic components are in dispersed oil. PAHs, especially 4-6 ring PAHs and C6-C9 phenols (a class of chemical compounds consisting of a hydroxyl group (-OH) attached to an aromatic hydrocarbon group) are mostly associated with dispersed oil. Partition of naphthalenes (two-ring aromatic compounds) in oil phase is enhanced by the increase in dispersed oil concentrations. C0-C3 and C4-C5 phenols are mainly in the water phase. However, partitioning can vary significantly within each group of compounds.

1.1.3.2 Dispersed oil

Dispersed oil contains small discrete oil droplets suspended in water, while soluble oil is present in a dissolved form. The amount of dispersed oil in a produced water stream is influenced by several different factors:

- Density of oil;
- Shear history of the droplet;
- Amount of oil coalescence;
- Interfacial tension between the water and the oil.

Oil content is defined by the method used to measure it. The most frequently used methods are Infra Red Spectrometry (IR), Ultra Violet (UV) Spectrometry, Gas Chromatography (GC) and Gravimetry. Each of these techniques has advantages and drawbacks. The methods used for sampling, preservation and extraction can also influence the determination of oil concentration. Usually, the analytical parameters are defined in such a way that dispersed hydrocarbons only are estimated. Although the oil content in produced water discharged to the North Sea varies from installation to installation, the overall average concentration is relatively stable. The target performance standard for dispersed oil, expressed as a monthly average is 30 mg/l from 01.01.2007 (OSPAR, 2001).

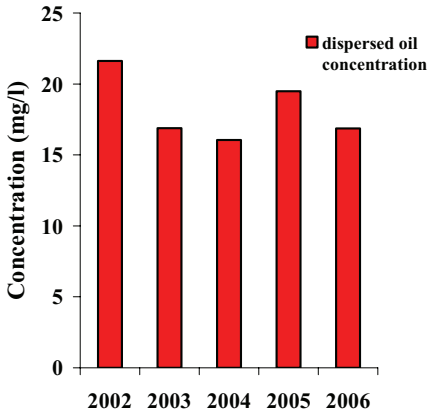


Figure 2. Average concentrations of dispersed oil discharged with PW in the Norwegian sector (compiled from OLF, 2007)

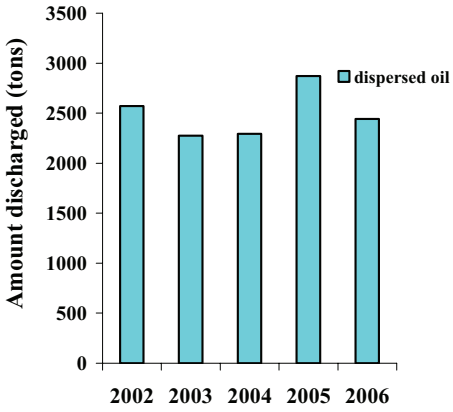


Figure 3. Amounts of dispersed oil discharged with PW in the Norwegian sector (OLF, 2007)

1.1.3.3 Dissolved organic compounds

Generally, the bulk of dissolved compounds in produced water is provided by the non-hydrocarbon organic compounds. In 2007, the contribution from specific organic compound groups was the following (OLF, 2007):

- Carboxylic acids 93.6%;
- BTEX 4.8%;
- Phenols 0.5%
- EPA PAHs 0.13%
- Alkylphenols (C1-C3) 0.89%
- Alkylphenols (C4-C9) 0.03%

The amount and nature of soluble organic compounds that may be in the produced water stream also will vary depending on several different factors:

- Type of oil;
- Volume of water production;
- Artificial lift technique;
- Age of production.

With respect to its chemical characteristics, the dissolved organic compounds can be divided in the following classes:

- Aliphatic hydrocarbons;
- Phenols;
- Carboxylic acids;
- Low molecular weight aromatic compounds.

Carboxylic acids

Carboxylic acids (or organic acids) are found in the highest concentrations among all organic components in produced water. Their concentrations can range between 40 and 349 mg/l (data provided by OLF and individual OGP member companies). Carboxylic acids account for most of the total organic carbon content (TOC) of produced water, and are not considered to be environmentally harmful. The amount of carboxylic acids discharged on the Norwegian Continental Shelf in 2007 increased by 3% compared with the amount discharged in 2006.

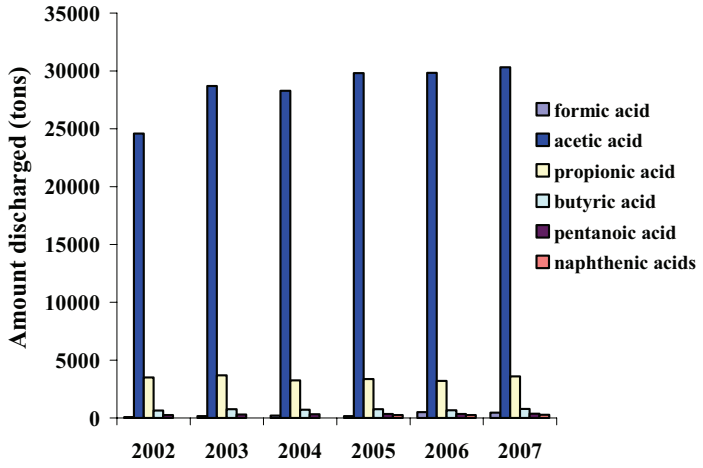


Figure 4. Amounts of carboxylic acids discharged on the Norwegian Continental Shelf (OLF, 2007)

Low molecular weight carboxylic acids, such as acetic acid and propionic acid, are most abundant. Acetic acid is present in the highest concentrations in the produced water discharged from the North Sea platforms (Table 4).

Table 4. Average concentrations of carboxylic acids (mg/l) in PW discharged in the Norwegian sector (OLF, 2007)

Type of carboxylic acid	2002	2003	2004	2005	2006	2007
Formic acid	0.5	1.1	1.4	1.0	3.4	2.7
Acetic acid	206.7	212.9	197.9	202.4	206.1	187.4
Propionic acid	29.4	27.3	22.7	22.9	22.2	22.2
Butyric acid	5.4	5.6	4.9	5.1	4.6	4.8
Pentanoic acid	2.1	2.2	2.1	2.2	2.3	2.3
Naphthenic acids	-	-	-	1.7	1.8	1.7

Phenols

The second largest group of dissolved organic compounds in produced water is the phenols. Phenol is the most abundant compound in this group (Figure 5, Table 5). Phenol partitions almost entirely into solution. Solubility of alkylphenols decreases with increasing molecular weight. Studies on C4-C9 phenols have indicated effects on hormone balance and reduced reproduction abilities in cod exposed to alkylated phenols (endocrine disruptors). These compounds are also believed to bioaccumulate. A study that was carried out in 2002 using DREAM software claimed that there is no significant risk of reproductive effects on the population levels of cod, saithe and haddock in the North Sea as a result of alkyl phenol discharges in produced water (Myhre *et al*., 2004).

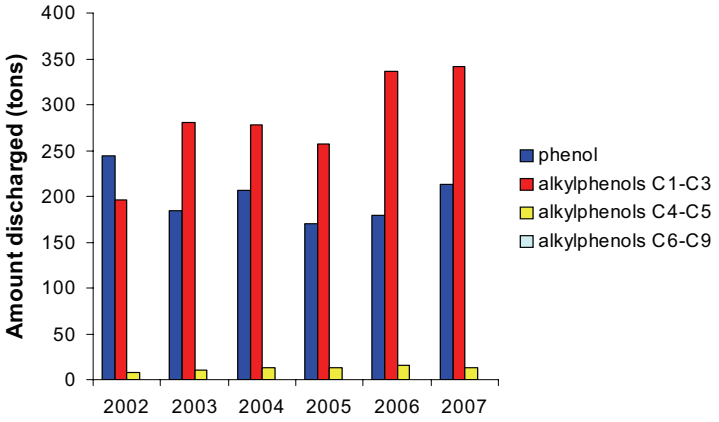


Figure 5. Amounts of phenols discharged on the Norwegian Continental Shelf

Table 5. Average concentrations of phenols (μg/l) in PW discharged in the Norwegian sector. Compiled from OLF data (2007)

Type of compound	2002	2003	2004	2005	2006	2007
Phenol	2047.8	1366.9	1449.2	1155.1	1239.5	1315.1
Alkylphenols C1-C3	1651.9	2086.5	1947.9	1749.6	2320.9	2108.7
Alkylphenols C4-C5	66.7	74.9	89.7	90.1	107.5	77.3
Alkylphenols C6-C9	2.2	2.9	1.5	2.0	0.9	1.0

Aromatic compounds

Other organic components are denoted "total aromatics". Aromatics are termed as mono- and polycyclic aromatic compounds containing only hydrogen and carbon atoms. Dibenzothiophene, a sulphur-containing compound, is the only exception in this group. Due to their wide range of concentrations in produced water, and differences in potential for causing environmental effects, aromatic compounds are divided into the following groups:

- BTEX: monocyclic aromatic compounds - benzene, toluene, ethylbenzene, and xylenes (ortho, meta and para isomers);
- NPD: naphthalene, phenanthrene and dibenzothiophene, including their C1-C3 alkyl homologues. These are 2-3 ring aromatic compounds;
- PAH: polycyclic aromatic hydrocarbons. These are represented by the 16 EPA PAHs (except naphthalene and phenanthrene that are included in the NPD-group).

Table 6. Amounts of aromatic compounds (tons) discharged in the Norwegian sector (OLF, 2007)

Group of compounds	2002	2003	2004	2005	2006	2007
BTEX	1089	861	1485	1479	1644	1826
NPD	146	142	170	163	154	124
EPA PAHs (excluding NPD)	1.2	1.8	1.6	1.9	1.7	1.1

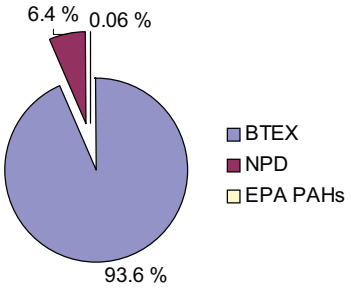


Figure 6. Aromatics (by class) in PW (compiled from OLF, 2007)

BTEX

Benzene, toluene, ethylbenzene and xylenes (BTEX) are found in the highest concentrations among aromatic compounds in produced water. They are moderately soluble in seawater, highly volatile and are biodegraded rapidly in the water environment. BTEX compounds have a low affinity for partitioning into lipid tissues of aquatic organisms. They are not persistent in sea water and are not accumulated to any degree by marine organisms. BTEX are removed from seawater by: 1) vaporization; 2) adsorption on particles and sedimentation; 3) biodegradation; and 4) photolysis. The most important mechanism of BTEX loss from seawater is vaporization. Generally, toxicity increases with increasing molecular weight, but exposure of marine animals to these compounds is extremely low due to their rapid loss from seawater.

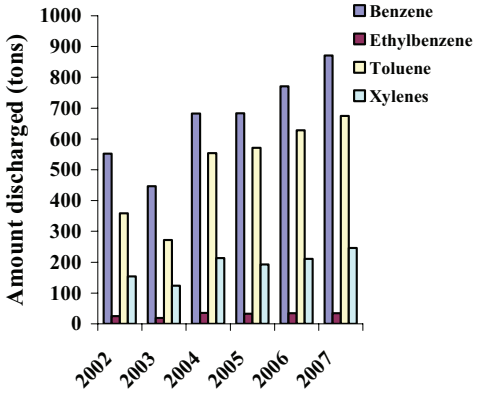


Figure 7. Amounts of BTEX discharged on the Norwegian sector (compiled from OLF, 2007)

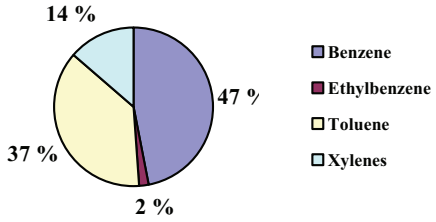


Figure 8. Percentage of each component of BTEX group in PW discharges (compiled from OLF, 2007)

Table 7. Average concentrations of BTX (mg/l) in PW discharges in the Norwegian sector (compiled from OLF, 2007)

Compound	2002	2003	2004	2005	2006	2007
Benzene	4.6	3.3	4.7	4.6	5.3	5.3
Ethylbenzene	0.2	0.1	0.2	0.2	0.2	0.2
Toluene	3.0	2.0	3.8	3.8	4.3	4.1
Xylenes	1.3	0.9	1.4	1.3	1.4	1.5
SUM BTEX	9.1	6.3	10.4	10.0	11.3	11.2

NPDs

The most abundant compounds in NPD group are naphthalene and its alkyl homologues. Compared with the high molecular weight PAHs, naphthalenes have lower bioaccumulation potential and are rapidly biodegraded in the aquatic environment. Therefore, naphthalenes pose a relatively low environmental risk. The other compounds

in this group such as dibenzothiophenes are moderately toxic, but not mutagenic or carcinogenic.

Table 8. Amounts of NPDs discharged in the Norwegian sector and average discharge concentrations – (compiled from OLF, 2007)

	tons			µg/l		
Compound	2005	2006	2007	2005	2006	2007
naphthalene	39.13	63.07	49.45	265.72	435.76	305.58
C1-naphthalene	59.93	50.25	43.94	406.94	347.17	271.52
C2-naphthalene	27.25	21.14	16.09	185.04	146.07	99.40
C3-naphthalene	21.96	11.23	7.81	149.09	77.56	48.28
phenanthrene	2.55	1.72	1.52	17.34	11.90	9.38
C1-phenanthrene	3.24	1.35	1.89	21.99	9.29	11.65
C2-phenanthrene	3.34	1.98	1.82	22.71	13.69	11.27
C3-phenanthrene	0.47	0.19	0.38	3.16	1.29	2.32
Dibenzothiophene	0.75	0.45	0.43	5.08	3.10	2.65
C1-dibenzothiophene	1.95	1.52	0.69	13.26	10.51	4.26
C2-dibenzothiophene	2.10	1.45	0.66	14.23	10.04	4.10
C3-dibenzothiophene	0.47	0.34	0.07	3.22	2.36	0.44
SUM NPD	163.14	154.69	124.74	1107.78	1068.76	770.85

PAHs

PAHs (polycyclic aromatic hydrocarbons) have a wide range of structures and properties. The higher the molecular weight of a compound in this group the lower its solubility and the higher its potential for bioaccumulation in marine organisms. PAHs partition mostly in the oil droplets or particulate matter. Their toxicity is variable and depends on the particular compound, exposure (acute or chronic) and the nature of the organism exposed to PAHs. They are of environmental concern, although they represent only a small fraction of the aromatic compounds in produced water, due to possible mutagenic, carcinogenic or teratogenic effects. In addition, some PAHs may be endocrine disruptors. Higher molecular weight PAHs are thought to be more toxic to marine life than lower molecular weight aromatics. PAHs can be biodegraded, but at relatively low rates. During the microbial degradation of PAHs, compounds more toxic than the parent compounds are often produced. These intermediate metabolic products may also be mutagenic or carcinogenic even if their parent compounds are not.

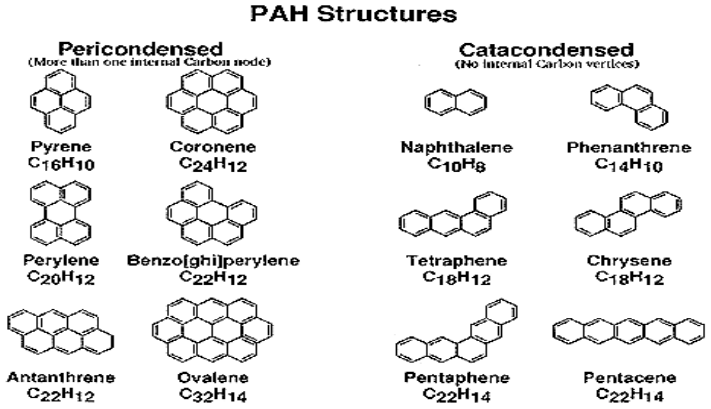


Figure 9. Chemical structures of several polycyclic aromatic hydrocarbons (PAHs)

Table 9. Amounts of PAHs in PW discharges in the Norwegian sector and average discharge concentrations (compiled from OLF, 2007)

	kg			µg/l		
Compound	2005	2006	2007	2005	2006	2007
Acenaphthylene	155	185	45	1.05	1.26	0.31
Acenaphthene	276	238	200	1.87	1.62	1.36
Fluorene	1769	1308	1132	12.01	8.88	7.69
Anthracene	118	36	36	0.80	0.24	0.24
Fluoranthene	88	53	38	0.60	0.36	0.26
Pyrene	117	64	64	0.79	0.43	0.43
Chrysene	74	61	40	0.50	0.41	0.27
Benzo(a)anthracene	32	29	13	0.22	0.20	0.09
Benzo(a)pyrene	11	14	6	0.07	0.10	0.04
Benzo (g,h,i)perylene	21	17	5	0.14	0.12	0.03
Benzo(b)fluoranthene	25	132	13	0.17	0.90	0.09
Benzo(k)fluoranthene	5	13	2	0.03	0.09	0.01
Indeno(1,2,3-c,d)pyrene	5	12	2	0.03	0.08	0.01
Dibenzo(a,h)anthracene	9	12	3	0.06	0.08	0.02

1.1.4 Production and process chemicals

Process chemicals are used in offshore operations for specific purposes to enhance treatment and reduce or mitigate different types of operating problems. Large numbers of special additives are available for use in the production system of a well to enhance the recovery and pumping efficiency of hydrocarbons, protect the production system from corrosion, and increase the separation of oil, gas and water. Many of these chemicals are more soluble in oil than in produced water and therefore remain mostly in oil phase. Some of the chemicals are water-soluble, concentrate in produced water phase, and are disposed with it. The point in the production stream where the chemical

is added influences the amount that may be discharged in the produced water stream. The number of additives used in a particular production system is usually low and depends on the specific production problems encountered in the well.
Biocides are usually added to the water treatment system for produced water intended for re-injection. Biocides may also be used to control sulphide production by anaerobic archaea and bacteria in the production stream. Biocides used for this purpose include hypochlorite and gluteraldehyde.
Corrosion inhibitors may be water-soluble or oil-soluble. Most of corrosion inhibitors used on the North Sea platforms are oil-soluble. Only small amounts remain in the treated produced water. Corrosion inhibitors are complex mixtures of medium to high molecular weight nitrogen-containing fatty acids, based on rosin acids (from plants).
Scale inhibitors are water-soluble and tend to remain in the produced water fraction of the production stream. They inhibit formation of barium or calcium scale on the inside of production pipes and usually are nitrogen-containing phosphates esters, such as phosphatidal ethanolamine or organic phosphonates.
Large amounts of gas-treating chemicals are used to treat gas production streams and about one-third of the amount used is discharged in produced water. These chemicals include methanol, ethylene glycol, and triethylene glycol. Methanol and ethylene glycol are used to prevent gas hydrate formation and they remain associated with the water phase. Triethylene glycol is used in a closed loop system to dehydrate the gas. It is recycled and therefore only small amounts enter the produced water stream. If produced water is sour (contains high concentrations of H_2S and CO_2), it may be sweetened in a closed loop system containing polyethylene glycol dimethyl ether, which acts as an absorbent for the sour gas. Only a small amount of polyethylene glycol enters the produced water stream and is discharged with it.

1.1.5 Other substances and properties

1.1.5.1 Total Suspended Solids (TSS)

Since total suspended solids have never raised significant concern, little information is available about these materials. Concentrations of TSS between 3 and 85 mg/l were found by a study of the discharges in the North Sea (OGP, 1994), while a study of 10 Louisiana platforms found concentrations ranging from 12 to 840 mg/l.

1.1.5.2 COD and BOD

There are relatively few measurements of either chemical oxygen demand (COD) or biochemical oxygen demand (BOD) because oxygen demand of produced water is normally not a major issue in offshore discharges in the North Sea (except in special situations such as near shore discharges in shallow waters).

Table 10. Concentrations of COD and BOD at different platforms (OGP, 2005)

Produced Water (origin)	COD (mg O_2/l)	BOD_5 (mg O_2/l)
Northern North Sea platform	130-2070	-
Central North Sea platform	4160 (av.)	465
Central North Sea condensate platform	4508 (av.)	1010
Southern North Sea: 8 gas/condensate platforms	400-15800	28-6700
Southern North Sea: 7 gas/condensate and 4 oil platforms	96-11500	-
Oil platform in USA waters	100-3000	300-2000
Offshore California oil production	-	750-1220

1.2 TOP Water project

1.2.1 Introduction to TOP Water project

Chapter 1.2 consists of a discussion based on several reports prepared by SINTEF (Helness, 2005, 2006; Melin, 2005a).

The present study was done as a part of the TOP Water project (acronym for Treatment Of Produced Water), which focuses on new treatment strategies/technologies and characterization of produced water. The goal of this study was to acquire further knowledge and technology to attain the "Zero Harmful Discharge" policy initiated by the Norwegian authorities. The ambition was to study a treatment method to meet the requirements of assumed very strict future performance standards for the removal of harmful dissolved organic compounds.

One of the challenges of offshore treatment of produced water is the achievement of a compact process (small footprint) due to special constraints regarding space and weight on the top of offshore platforms.

There is a significant difference in quality and flow between the produced waters generated on different sites or over the lifetime of the same field. A screening for the most representative cases of produced water qualities and flows had to be done because of the huge variations of these two characteristics.

Data for produced water quality after the initial separation of the well stream and after existing produced water treatment were necessary, since the treatment technologies to be investigated in TOP Water project could be alternatives to existing produced water treatment methods, or technologies for additional treatment after existing produced water treatment steps.

In order to evaluate the efficiency of the experimental results obtained in the project, it was required to set well-defined performance targets.

Parameters that could be measured directly in the laboratory were necessary to estimate experimental results in this research project. Targets for removal efficiency or effluent concentration were also defined for assessment in TOP Water project (Helness, 2006).

1.2.2 Quality and flow of produced water

Several sources were used to compile data on quality and flow of produced water (Helness, 2006; Melin, 2005a):

- Environmental reports from different installations on the Norwegian Continental Shelf (NCS) for 2006 and 2007 (OLF 2006, 2007);
- Reports from the International Organisation of Oil & Gas Producers (OGP 2002, 2005);
- Annual report on discharges from OSPAR (OSPAR, 2008);
- Other reports, papers and presentations on the subject of produced water.

The range of values found in the above-mentioned sources and the average values for the NCS for the interval 2005-2007 reported by OLF are indicated in Table 11.

Table 11. Concentrations of compounds in produced water used for EIF calculation

Compounds	Minimum	Maximum	OLF, average (05-07)
Aliphatics (mg/l)	10	64	17.5
BTEX (mg/l)	0.04	38	10.9
Naphthalenes (mg/l)	0.08	9	0.98
PAH 2-3 ring (mg/l)	0.02	1.5	0.13
PAH 4-6 ring (mg/l)	0.001	0.04	0.002
Phenols C0-C3 (mg/l)	0.3	17	3.3
Phenols C4-C5 (mg/l)	0.004	11	0.09
Phenols C6+ (mg/l)	0.00004	0.32	0.001
Copper (Cu) (mg/l)	0.02	9.5	0.0026
Zinc (Zn) (mg/l)	0.01	26	0.04
Nickel (Ni) (mg/l)	0.002	3.1	0.005
Lead (Pb) (mg/l)	0.0004	2.1	0.005
Cadmium (Cd) (mg/l)	0.0003	25	0.00015
Mercury (Hg) (mg/l)	-	0.3	0.00005

The environmental risk of a discharge could not be assessed by employing only these data. In order to evaluate treatment concepts, several supplementary parameters had to be utilized. These were suspended solids (SS), pH as well as BOD and organic acids concentrations for biological treatment.

Table 12. Concentrations of SS, organic acids and BOD in produced water

Compounds	Minimum	Maximum	Average (reference)
Suspended solids (mg/l)	3	840	- (OGP, 2005)
BOD (mg/l)	300	2000	465 (OGP, 2005)
Organic acids (mg/l)	40	1135	232 (OLF, 2007)

Data available in the literature mostly refers to treated produced water. Influent concentrations of dispersed oil to produced water treatment were determined taking into account data collected from several fields operated by StatoilHydro and removal efficiencies reported for hydrocyclone treatment (Figure 10) (Thorsen-Haugen, 2004).

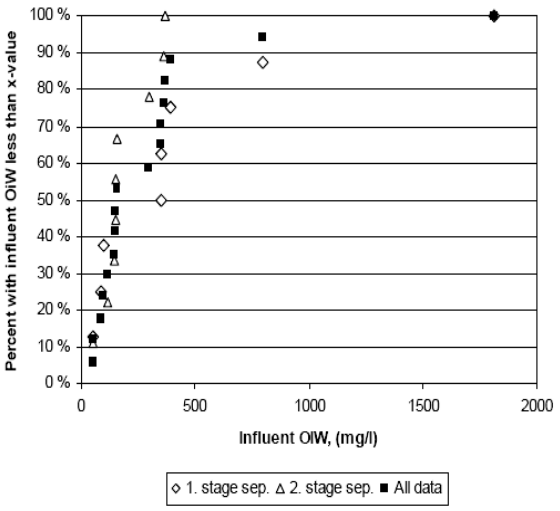


Figure 10. Influent concentrations of dispersed oil (OiW) to produced water treatment process

Two cases were selected for produced water quality:

- Case 1 - produced water quality after production separators;
- Case 2 - produced water quality after treatment with hydrocyclones.

Concentrations of semi soluble compounds, which partition between oil and water phase in produced water, were estimated for case 1 taking into account the increase in dispersed oil concentration from case 2 to case 1 and the partition ratios reported in literature (Faksnes *et al.,* 2004).

Table 13. Produced water quality after production separators (Case 1) and after hydrocyclones (Case 2) based on literature data

Compounds	Case 1	Case 2
Dispersed oil, (mg/l)	350	25
BTEX, (mg/l)	8	8
Naphthalenes, (µg/l)	6000	1000
PAH 2-3 ring, (µg/l)	1800	150
PAH 4+ ring, (µg/l)	30	2
Phenols, C0-C3, (µg/l)	4000	4000
Phenols, C4-C5, (µg/l)	400	150
Phenols C6+, (µg/l)	15	2
Metals (Zn), (µg/l)	100	100
Metals (Cu, Ni), (µg/l)	20	20
Metals (Pb, Cd, Hg), (µg/l)	5	5
Organic acids, (mg/l)	250	250
BOD	700	300
Suspended solids, (mg/l)	50	50
pH	~7	~7

In order to acquire relevant information on flows of produced water discharges, data have been compiled for 27 sites.
Figure 11 illustrates the percent of sites with a produced water discharge less than the x-axis value. Produced water flows of 3000, 8000 and 30000 m^3/day (marked with dotted lines on Figure 11) have been selected to cover a broad range of flows. These were also used in the EIF calculations presented later in this study.

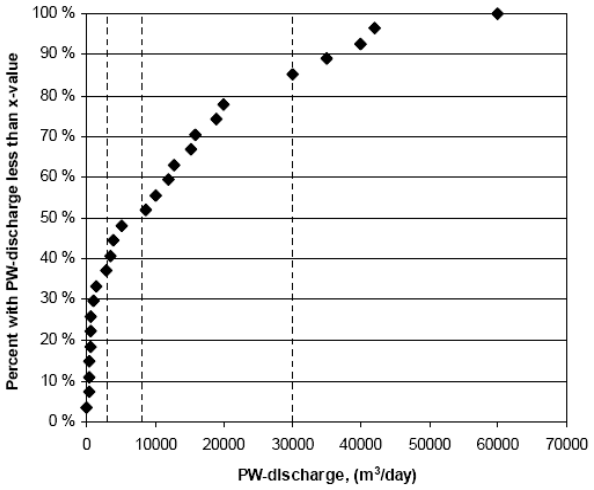


Figure 11. Produced water discharge flows based on data for 2004.
Dotted lines are for 3000, 8000 and 30000 m^3/day

Figure 11 indicates that 60% of platforms had produced water discharges higher than 3000 m^3/day, while only 15% of the rigs had discharges over 30000 m^3/day. About 50% of the rigs had produced water discharges higher than 8000 m^3/day.
Evaluation of different treatment methods was carried out based on the quality cases correlated with data on produced water discharge flows.

1.2.3 Treatment targets

The ambition of TOP Water project was to study treatment concepts able to meet future performance standards. Treatment targets were defined based on EIF calculations since no information was available on the performance standards that would be agreed in the future.
The standards for dispersed oil calculated as a weighted monthly average were reduced to 30 mg/l from the beginning of 2007. Currently, there are no performance standards for other components in produced water, but the Norwegian authorities set operational goals for oil, natural components and chemicals in produced water.
The targets for discharges of oil and other naturally occurring substances are:

- No discharges, or minimisation of discharges of chemicals on the authorities list of chemicals for priority actions;

- No discharges of other substances if the discharges can lead to adverse effects in the environment.

The environmental impact factor (EIF) is normally used to assess the environmental risk of a discharge in the North Sea and for ranking measures (i.e. treatment technologies, substitution of chemicals, produced water re-injection (PWRI)) for reaching the goal of "zero harmful discharge".
Data on the composition and flow of produced water discharges are necessary for EIF model calculations. This model simulates the spreading of a discharge and calculates the risk of a harmful effect in the recipient. Comparison of concentration of compounds (PEC) with the concentration where no effect is expected (PNEC) is used to perform risk calculation. The EIF for a discharge is related to a recipient water volume of 100000 m^3 (a grid with cells of 100 x 100 x 10 m) and is the volume of water with a risk > 5 % divided by 100000. The reported EIF is the maximum value calculated for the 30-day period (Melin, 2005a).
In order to obtain a basis for defining treatment targets and assessment of technologies, EIF calculations were employed to estimate the concentration levels of naturally occurring components in produced water that would give a discharge with no harmful effect. An EIF = 1 or lower was defined as "zero harmful discharge" for the purpose of this evaluation.
Calculations were performed for discharges of 3000, 8000 and 30000 m^3/day containing only one component or mixtures of all components.

Table 14. Concentrations in discharges, resulting in an EIF = 1 for each component discharged separately and for discharges containing all components

Compound	3000 m^3/day individual	3000 m^3/day Sum of comp.	8000 m^3/day individual	8000 m^3/day Sum of comp.	30000 m^3/day individual	30000 m^3/day Sum of comp.
Dispersed oil	**28.4820**	**4.615**	**10.3020**	**1.6232**	**2.7876**	**0.46150**
BTEX	**16.2010**	**2.838**	**6.5280**	**1.03**	**1.7**	**0.28380**
Naphthalenes	**2.0013**	**0.3506**	**0.8064**	**0.128**	**0.21**	**0.03506**
PAH 2-3 ring	**0.1430**	**0.025**	**0.0576**	**0.0091**	**0.015**	**0.0025**
PAH 4-6 ring	**0.0353**	**0.0055**	**0.0128**	**0.002**	**0.00345**	**0.00055**
Phenols C0-C3	**9.5300**	**1.6694**	**3.8400**	**0.6088**	**1**	**0.16694**
Phenols C4-C5	**0.3431**	**0.0601**	**0.1382**	**0.0219**	**0.036**	**0.00601**
Phenols C6+	**0.0282**	**0.0046**	**0.0102**	**0.0016**	**0.00276**	**0.00046**
Zinc (Zn)	**0.4384**	**0.0768**	**0.1766**	**0.028**	**0.046**	**0.00768**
Copper (Cu)	**0.0191**	**0.0033**	**0.0077**	**0.0012**	**0.002**	**0.00033**
Nickel (Ni)	**1.1627**	**0.2037**	**0.4685**	**0.0743**	**0.122**	**0.02037**
Cadmium (Cd)	**0.0267**	**0.0047**	**0.0108**	**0.0017**	**0.0028**	**0.00047**
Lead (Pb)	**0.1734**	**0.0304**	**0.0699**	**0.011**	**0.0182**	**0.00304**
Mercury (Hg)	**0.0076**	**0.0013**	**0.0031**	**0.00049**	**0.0008**	**0.00013**

The aim of these calculations was to obtain a basis for defining treatment targets and for assessing technologies in this project. A comparison of concentrations found for the discharges containing all components with the average values reported for the Norwegian Continental Shelf (NCS) is presented in Table 15.
Bold font was used to put in evidence values found by EIF calculation to be lower than the NCS averages. Yellow was utilized to highlight the cells in the table where the NCS average is higher than all EIF results.
Results showed that in order to ameliorate produced water treatment with respect to naturally occurring compounds, focus should be put on dispersed oil, BTEX, naphthalenes and the most water-soluble PAHs and phenols.
It was assumed that the effluent standards corresponding to the concentrations found in the EIF calculations for a discharge of 8000 m^3/day containing all components have to be achieved as basis for assessment of treatment concepts and technologies.

Table 15. Comparison of the average values reported for the NCS with the concentrations found for the discharges containing all components

Compounds	OLF, average (05-07)	3000 m^3/day Sum of comp.	8000 m^3/day Sum of comp.	30000 m^3/day Sum of comp.
Aliphatics (mg/l)	17.5	**4.615**	**1.6232**	**0.46150**
BTEX (mg/l)	10.9	**2.838**	**1.03**	**0.28380**
Naphthalenes (mg/l)	0.98	**0.3506**	**0.128**	**0.03506**
PAH 2-3 ring (mg/l)	0.13	**0.025**	**0.0091**	**0.0025**
PAH 4-6 ring (mg/l)	0.002	0.0055	0.002	**0.00055**
Phenols C0-C3 (mg/l)	3.3	**1.6694**	**0.6088**	**0.16694**
Phenols C4-C5 (mg/l)	0.09	**0.0601**	**0.0219**	**0.00601**
Phenols C6+ (mg/l)	0.001	0.0046	0.0016	**0.00046**
Copper (Cu) (mg/l)	0.02	0.0768	0.028	**0.00768**
Zinc (Zn) (mg/l)	0.04	**0.0033**	**0.0012**	**0.00033**
Nickel (Ni) (mg/l)	0.005	0.2037	0.0743	0.02037
Lead (Pb) (mg/l)	0.005	**0.0047**	**0.0017**	**0.00047**
Cadmium (Cd) (mg/l)	0.00015	0.0304	0.011	0.00304
Mercury (Hg) (mg/l)	0.00005	0.0013	0.00049	0.00013

1.2.4 TOP Water treatment concept

TOP Water project has addressed an end of pipe treatment solution, which is one of several options in produced water management. The proposed produced water treatment concept consists of three steps (Helness, 2005 and 2006):

- Pre-treatment;
- Removal of dispersed compounds;
- Removal of remaining dissolved compounds.

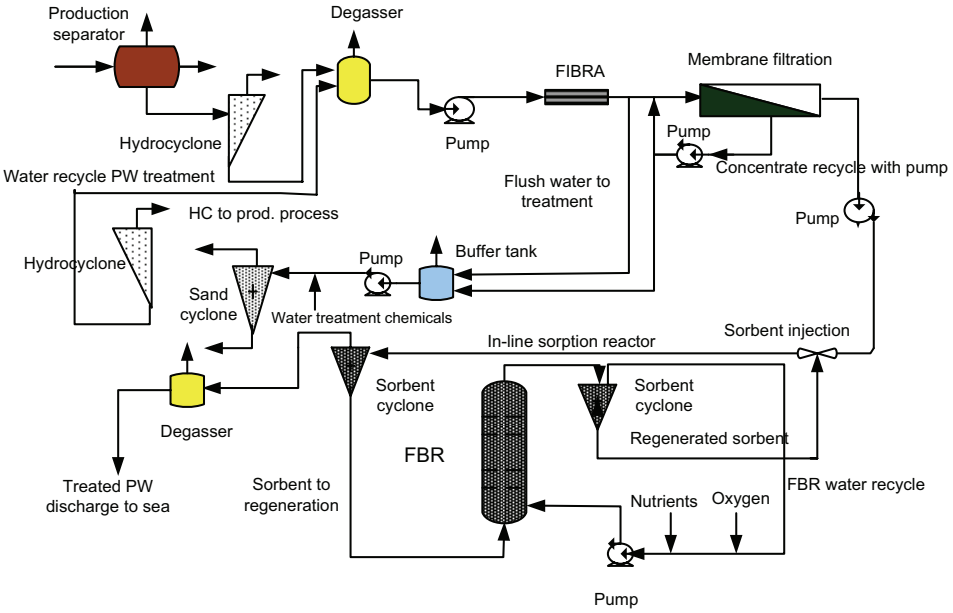


Figure 12. Simplified process flow diagram of the TOP Water treatment concept

1.3 Problem statement

In 2007, the volume of produced water discharged on the Norwegian Continental Shelf totalled about 162 million m^3. This is expected to increase in the coming years primarily due to an increased water production from the major fields (OLF, 2007). The current performance standard on the Norwegian Continental Shelf (NCS) is 30 mg/l for dispersed oil calculated as a weighted monthly average. No performance standards currently exist for the other components in produced water, including the dissolved compounds. Semi-soluble hydrocarbons such as benzene, toluene, ethylbenzene and xylenes (BTEX), polycyclic aromatic hydrocarbons (PAHs), naphthalenes, phenanthrenes and dibenzothiophenes (NPD), phenol and alkylated phenols have a significant contribution to the EIF of a produced water discharge and therefore, the following overall goals were set for the oil and gas industry at OSPAR conventions (OSPAR, 2001 - amended in 2006):

- Reduce the input of oil and other substances into the sea resulting from produced water from offshore installations, with the ultimate aim of eliminating pollution from those sources;
- Ensure that effort is made to give priority to actions related to the most harmful components of produced water.

1.4 Scope of the study

The aim of TOP Water project was to acquire further knowledge and technology to attain the "Zero Harmful Discharge" policy initiated by the Norwegian authorities. Produced water characterization was done by two other PhD students at Ugelstad laboratory, pre-treatment of produced water was studied by SINTEF, while another PhD candidate investigated the removal of dispersed oil.
The ambition of the present study was to investigate treatment methods to meet the requirements of assumed very strict future discharge standards for the removal of dissolved organic compounds that have an important contribution to the EIF of a produced water discharge.
TOP Water is a knowledge building project and developing and providing a treatment technology, which can be applied offshore, is beyond the scope of the PhD study. However, results of the research work could contribute to further development of a viable offshore treatment technology for dissolved constituents.
Since targeted dissolved aromatic compounds are very numerous, it was decided to focus the experiments only on a small group of components. BTEX were selected to be used in the tests because they are found by far in the highest concentration in produced water among the dissolved aromatic compounds (see chapter 1.1.3.3).
On the other hand, acetic acid was selected for the tests because it is the most representative component of the organic acids group because it is found in produced water in concentrations much higher than the concentrations of the other organic acids (Table 4).

The present study investigated the following issues:

- Selective sorption of dissolved aromatic compounds, but not of organic acids, from produced water;
- Influence of pH, acetic acid concentration, salinity and temperature on sorption process in batch experiments;
- Sorption kinetics and sorption capacity for BTX and acetic acid in batch experiments;
- Designing and building a lab-scale experimental setup (FBR system) to evaluate the feasibility of the proposed treatment concept;
- Sorption kinetics and sorption capacity for BTX in FBR tests;
- Development of a microbial culture able to feed on BTX as sole carbon source;
- Simultaneous sorption and biological regeneration in the FBR system;
- Off-line bioregeneration of the spent sorbent in the FBR system;
- Loss of sorption capacity during long time FBR treatment processes alternating sorption and bioregeneration steps;
- Assessment of the feasibility of the proposed treatment concept.

2. LITERATURE REVIEW AND PROPOSED TREATMENT CONCEPT

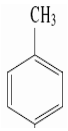
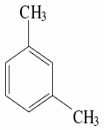
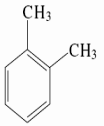
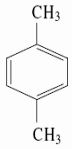
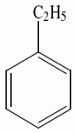
2.1 Properties of monoaromatic hydrocarbons

Monoaromatic compounds such as BTEX have large migration abilities and toxicities and therefore are classified into the group of most dangerous compounds to the environment (Coates *et al*., 2002; An, 2004). High contributions to EIF values were calculated for these compounds based on a combined environmental risk and hazard assessment of produced water discharges, accounting for both composition and amount of the discharge. The EIF concept is described in detail by Johnsen *et al*. (2000). It can be used to identify which of the naturally occurring compounds or man-added chemicals contribute most to the environmental risk. The EIF is not only a valuable tool for quantifying the total environmental impact from a single or a group of platforms but also for evaluating the contribution of different compounds in produced water.

The fate of these compounds in the environment can be predicted based on their physical and chemical properties, which are summarized in Table 16 (Plyasunov and Shock, 2000; Kermanshahipour *et al*., 2005; Farhadian *et al*., 2006).

Monoaromatic hydrocarbons have relatively high water solubilities therefore, they spread without difficulty in contaminated waters. BTEX have high vapour pressure and low molecular weight, which means that the compound is more likely to volatilize out of water solution (Lee *et al*., 2004). The behaviour of pollutants in remediation procedures such as air stripping processes can be predicted by using Henry's law constant. The order in which the BTEX diffuse and volatilize through air stripping from water is ethylbenzene > xylene > toluene > benzene (Langwaldt and Puhakka, 2000). Also, the decimal logarithm of octanol-water partition coefficients is consistent with the already mentioned fact that monoaromatic pollutants are hydrophilic, soluble in water and can be easily removed from water by evaporation and stripping (Zytner, 1994; Lipson and Siegel, 2000; Lin and Chou, 2006). Theoretical oxygen demand (ThOD), which corresponds to the stoichiometric amount of oxygen required to fully oxidize a given molecule, and total organic carbon (TOC) in an aqueous sample are two additional parameters often used to characterize aqueous solutions of these compounds.

Table 16. Properties of BTX compounds

Properties (unit)	Benzene	Toluene	*m*-Xylene	o-Xylene	*p*-Xylene	Ethylbenzene
Chemical structure						
Chemical formula	C_6H_6	C_7H_8	C_8H_{10}	C_8H_{10}	C_8H_{10}	C_8H_{10}
Molecular weight (g/mol)	78.11	92.14	106.17	106.17	106.17	106.17
Some trade names and synonyms	Benzol90 Pyrobenzol Coalnaphtha Phene	Phenylbenzene Methylbenzene Methacide Toluol	*m*-Xylol metaxylene 1,3 dimethyl-benzene	o-Xylol orthoxylene 1,2 dimethyl-benzene	*p*-Xylol paraxylene 1,4 dimethyl-benzene	Ethylbenzol Phenyl-ethane EB
Some applications	Solvent in chemical industry, LAB, fuel	Solvent, TNT, urethane, benzene, fuel	Solvent, *p*- and *o*-xylene, fuel	Solvent, PA, fuel, plasticizers	Solvent, DMT, polyester fiber, fuel	Styrene, solvent, fuel
Water solubility (mg/L) at 25 °C	1785.5	532.6	161.5	171.5	181.6	161.5
Boiling point temp. (°C)	80.0	110.6	139.1	144.5	138.3	136.1
Vapor pressure (mmHg) at 20 °C	95.19	28.4	8.3	6.6	3.15	4.53
Melting point temp. (°C)	5.50	−94.9	−47.8	−25.2	13.2	−94.9
Specific density (°C)	0.8765^{20}	0.8669^{20}	0.8642^{20}	0.8802^{10}	0.8611^{20}	0.8670^{20}
Octanol–water partition coeff. 25 °C (log *P*)	2.13	2.73	3.20	3.12	3.15	3.15
Henry's law constant at 25 °C (kPa m^3/mol)	0.557	0.660	0.730	0.551	0.690	0.843
Content in water equilibrated with (as ppm)	18	25	20	20	20	3
Gasoline diesel fuel	7.89	22.8	13.95	13.95	13.95	–
			For 1 mg/L of substance:			
ThOD	3.076	3.13	3.17	3.17	3.17	3.17
TOC (as ppm)	0.923	0.913	0.906	0.906	0.906	0.906

2.2 Methods for BTX detection

Determination of monoaromatic compounds in water samples at the mg/l level requires pre-concentration before analysis, if a GC technique is employed (Demeestere *et al.*, 2007). The pre-concentration methods can be classified into three families: solute concentration in a gas, liquid or on a solid phase. The first approach makes use of techniques such as headspace sampling or the purge and trap process. Both are based on vaporization of solutes from aqueous samples. Headspace sampling involves direct analysis of the resulting gas phase, while purge and trap method utilizes analyte adsorption onto a porous support, followed by desorption prior to GC injection. These methods are recommended for VOC analysis in contaminated water, since they are rapid, cost efficient and can be automated (Kuran and Sojak, 1996; Ohlen *et al*., 2005; Gusmão *et al*., 2006, 2007). Other methods such as GC/MS, GC/FID, and GC/PID using purge and trap are recommended by different standard methods for the determination of all forms of monoaromatic compounds in contaminated water (USEPA standard method 5030C, 2003; USEPA standard method 8260B, 1996; Environment Canada, 2005; Rosell *et al*., 2003; Zein *et al*., 2006). Solvent extraction is the second possibility to pre-concentrate volatile compounds from water samples. This method has as potential disadvantages solvent peak overlapping and, at low concentration levels, the need for highly purified material. Many studies on detection and determination of benzene, toluene and xylenes (BTX) compounds in water by GC/MS indicate that sample preparation by extraction with solvents such as n-$C_{12}H_{26}$ and n-$C_{16}H_{34}$ leads to accurate results. On the other hand, detection after extraction with dichloromethane (DCM), pentane, hexane, octane, n-C_9H_{20}, n-$C_{10}H_{22}$ and n-$C_{11}H_{24}$ give poor results (Farhadian *et al.*, 2006). Recently, solid-phase microextraction (SPME) has been developed as the third approach. This technique combines sampling, isolation and enrichment in one-step and does not use any solvent for sample preparation. It involves the utilization of a small-diameter optical fibre coated with a polymeric phase introduced in an aqueous sample. Analytes are retained by partition into a stationary phase and then are thermally desorbed in the injector of a gas chromatograph (Theodoridis *et al.*, 2000). The difficulty is to gain an accurate quantitative knowledge of the partition phenomena between adsorbent and liquid phase (Djozan *et al*., 2004; Ji *et al*., 2006; Ouyang and Pawliszyn, 2006). General methods used in this area are based on treatment of standard solutions. Adsorption characteristics depend on the composition of the liquid medium and this can lead to potentially high errors during solute quantitation. High-performance liquid chromatography (HPLC) fitted with an ultraviolet (UV) detector can also be employed for detection of aromatic compounds (Kelly *et al*., 1996; Zepeda *et al*., 2006; Kim *et al*., 2006). Depending on the method and equipment, these methods can measure concentrations down to parts per billion (ppb). The present columns available for HPLC are not able to separate very accurately all forms of monoaromatic hydrocarbons (for example ethylbenzene and xylene isomers that have similar retention times). Decontaminated containers such as disposable bottles are usually employed to collect samples from water sources. Sterile disposable gloves and other clean sterilized sampling equipment should be used. Standard guidelines for sampling, preservation, handling and analysis of monoaromatics in contaminated water have been defined (Kuran and Sojak, 1996; USEPA standard method 8260B, 1996; USEPA, 1997; Environment Canada, 2005).

Several different methods such as chemical extraction (benzylsuccinate, trimethylbenzene, catechol 2, 3 dioxygenase), physical methods (depletion of dissolved oxygen, nitrate and sulphate or production of dissolved ferrous iron, sulphide and carbon dioxide), biological (bioassay tools) or numerical, physical and kinetic models can be used for on-line monitoring of monoaromatics degradation during bioremediation processes (Lin *et al*., 2002; Johnson *et al*., 2003; Schulze and Tiehm, 2004; Maurer and Rittmann, 2004; Bekins *et al*., 2005; Gödeke *et al.*, 2006; Hendrickx *et al*., 2006; Hu *et al*., 2006; Kao *et al*., 2006; Atteia and Guillot, 2007; Biggerstaff *et al*., 2007; Morasch *et al*., 2007).

2.3 Technologies for the removal of dissolved compounds

Dissolved compounds are the only hydrocarbons remaining in produced water after the removal of oil droplets. The most important groups of dissolved aromatic compounds that need to be removed are BTEX and light phenols (C0 – C3). Heavy phenols, NPDs and PAHs with many fused aromatic rings are mostly removed with dispersed oil. Treatment methods able to remove heavy phenols, NPDs, PAHs, and had potential for removal of heavy metals, were given priority over the others (Helness, 2005).

2.3.1 Stripping

This treatment method would work well especially for volatile compounds such as BTEX and light phenols. Its efficiency for heavier compounds such as PAHs can be enhanced by increasing the temperature (API, 1995).
Although stripping is not employed for the treatment of the main produced water flows on oil and gas platforms, it has the reputation of being proven technology in the oil/gas industry (OSPAR, 2002).
OGP (2002) classified steam stripping as a technique efficient for BTEX treatment, but not one that can be utilized to remove NPDs and PAHs in oil/gas industry.
The main disadvantage of stripping is that it creates new waste streams that need further treatment:

- Separation of hydrocarbons from condensed vapours is required for steam stripping;
- Off-gas treatment is required for air stripping.

Costs of implementing stripping for the treatment of dissolved compounds can be very high, especially for steam stripping, which is more energy intensive. A large stripping column would probably be required in order to comply with the stringent effluent standards set in the TOP Water project.
Organic fouling is not expected to be a critical factor, since significant upstream treatment is performed. On the other hand, inorganic scaling caused by iron and calcium and inefficiency for heavy metals removal could be two major drawbacks of this treatment technology.

2.3.2 Oxidation

Another option for the treatment of dissolved organic compounds is chemical oxidation, which makes use of ozone and/or hydrogen peroxide. The main advantage of this technique is the relatively simple operation, while the high-energy consumption for ozone generation and the toxic waste generated by the process are the main drawbacks (API, 1995). Chemical oxidation is believed to require long contact times for an efficient degradation of the target soluble compounds (Klasson *et. al.*, 2002). Another interesting alternative is advanced oxidation employing UV light and titanium dioxide as catalyst. This method has a potential advantage over chemical oxidation, since it does not generate waste streams. However, fouling of the UV lamps and/or catalyst may be a major disadvantage of this technique. All oxidation methods are inefficient for removal of heavy metals.

2.3.3 Extraction

An extraction liquid immobilized in polymer beads is used by macroporous polymer extraction (MPPE) to extract dissolved compounds from the produced water stream.
Two parallel units are usually employed by this technology. While one line is effectively used to treat produced water, the other one is regenerated with low-pressure steam. Removal efficiencies of 98% for PAHs and NPD, and 99.4% for BTEX where reported during MPPE testing on Åsgaard A.
Since size of the process is thought to have a major influence on the removal efficiency of MPPE technology, it is expected that the achievable performance with this technique would be significantly limited on offshore platforms (Grini *et. al.* 2003).
Meijer (2003) estimated a footprint of 42 m^2 and a height of 10 m for an MPPE unit designed to treat a produced water flow of 300 m^3/h. A major drawback of MPPE technology is the loss of activity over time. Therefore, the MPPE media need to be replaced at intervals depending on produced water quality and pre-treatment. A replacement interval of one year has been previously reported (OSPAR, 2002). MPPE technology is not suitable for the removal of heavy metals.
Another treatment technology utilizing extraction process is CTour. This uses gas condensate, provided by the scrubbers in the gas compression train, for an in-line extraction process. The condensate, which is injected upstream of the de-oiling hydrocyclones, behaves as a solvent, extracting dissolved hydrocarbons and small oil droplets from produced water.
Downstream hydrocyclones are normally employed to remove the large, low-density droplets created by condensate and hydrocarbons. Therefore, CTour removes the dissolved aromatics, enhancing at the same time the removal of dispersed oil in hydrocyclones. Condensate composition has a significant influence on the efficiency of extraction process. Grini *et al.* (2003) reported increased concentrations of BTEX in produced water down stream of hydrocyclones and no removal of C0 – C3 phenols in a CTour process. A disadvantage of this technology may be the shortage of condensate with required quality.

2.3.4 Membranes

Dissolved organic compounds and heavy metals can be removed from produce water by reverse osmosis (RO) membranes. This technology is more energy intensive than nanofiltration (NF) membranes, since it requires higher pressure for operation.
NF-process utilizes membranes with larger pores and therefore, would be less effective than a RO-process for removal of compounds with low molecular weight (API, 1995). However, it is believed that sufficient removal of BTEX and light phenols can be achieved with NF membranes. This type of membranes is efficient for rejecting divalent metal ions. A major drawback reported for both processes (NF and RO) is membrane fouling (API, 1995; OGP, 2002; Hayes and Arthur, 2004).
Other disadvantages of membrane treatment are the short lifetime of membrane material and the relatively low flux rates.
The concentrate generated in the process must undergo further treatment to separate hydrocarbons from water. The resulting water is recycled upstream of the pre-treatment step. A significant challenge of NF or RO operation would be to achieve very high recovery in order to avoid a broad treatment scheme for the concentrate stream.
An alternative to NF and RO is electro dialysis (ED) that utilizes an electric field as driving force for separation. Compared with NF and RO, ED has three major advantages:

- High water recovery;
- Low pressure requirement;
- Resistance to fouling.

The major drawbacks of ED are the inefficiency to remove BTEX and naphthalenes as well as the high-energy costs (Hayes and Arthur, 2004).
Membrane distillation (MD) can separate two liquid phases using an evaporation/condensation process. Proper operation of this technique requires that the temperature on the feed/concentrate side of the membrane is higher than on the effluent/permeate side. The transfer of water across the membrane is facilitated by the difference in vapour pressure between the two sides of the membrane. The difference in boiling points can be used to achieve good separation of water and aromatic compounds such as PAHs, phenols and most BTEX, except benzene, which has a low boiling point (80 °C). Failure to remove benzene is a major disadvantage of MD because benzene is the most abundant and harmful member of the BTEX group.
Gryta *et al.* (2001) reported a flux of 10 LMH (litres per square metre and hour) for the treatment of oily water (bilge water) with MD after pre-treatment with UF. Although MD is less sensitive to fouling than pressure driven membrane processes, scaling could be a major concern and reduce the achievable recovery or result in the need for scaling inhibitors.

2.3.5 Adsorption

Adsorption can be carried out with adsorbents that can be regenerated or have to be disposed after their adsorption capacity is exhausted. It is more cost-efficient to use

adsorption media with regenerative properties, especially if a low-cost on-site regeneration method is available.
Activated carbon is an established adsorbent usually employed in municipal and industrial wastewater treatment. Although the spent carbon was reported to be regenerated on-site by wet air oxidation, it is mainly regenerated off-site (Hayes and Arthur, 2004). Rank *et al.* (2002) investigated the utilization of a surface modified zeolite (SMZ) to remove BTEX compounds from produced water. The spent surface modified zeolite was successfully regenerated by air sparging. However, this regeneration method is problematic, since it transfers the pollutant from the water phase (produced water) to gaseous phase (off-gas from air sparging). The off-gas stream requires further treatment before being released into the atmosphere. Surface modified zeolites are not suitable for the removal of heavy metals, since their surface is modified with a tailoring agent (hydrophobic surfactant) designed to increase their affinity for hydrophobic aromatic compounds.

2.3.6 Biological treatment

Removal of dissolved aromatic compounds can be also achieved in aerobic or anaerobic bioreactors. Depending on the nature of biomass growth, biological treatment can be divided in suspended growth (activated sludge) and attached growth (biofilm) processes.
Attached growth processes utilize carriers, on which microorganisms establish a biofilm, feeding on the organic compounds and nutrients available in water. Biofilm systems are usually more compact processes and therefore, the footprint and volume requirements would be lower than in the case of activated sludge processes. This is a significant advantage, since stringent footprint and weight requirements are in place on offshore platforms.
Different reactor configurations have been used for the treatment of aromatic compounds. The most common technologies are fluidized bed reactors (FBR), moving bed biological reactors (MBBR), submerged fixed film reactors (SFFR) and fixed film activated sludge (FAS) (Voice *et al*, 1992, 1995; Guerin, 2002; Pruden *et al*., 2003).
Fluidized bed reactors employing granular activated carbon as carrier were reported to establish more rapidly biofilm structures due to sorption of pollutants on the carbon particles (Sutton and Mishra, 1994). Fluidized bed systems have been also used in laboratory scale for anaerobic treatment (Ballapragada *et al*., 1997; Magar *et al*., 1999).
All these types of systems are based on fixed film approach, which can retain larger concentrations of biomass, therefore increasing microbial degradation when operated as continuous processes. Attached biomass is believed to be more resistant to toxicity than suspended biomass.
In bioreactors based on suspended growth, microorganisms form flocks or granules in suspension that can be removed from water by sedimentation. Suspended growth treatment processes are commonly used in activated sludge or upflow anaerobic sludge blanket reactors. Activated sludge processes consist of an aeration basin and a mechanical clarifier utilized to separate the sludge from the effluent for recycle. Different types of aeration nozzles and air blowers can be used for aeration of water.

Previous laboratory- and pilot-scale studies (Petrasek *et al*., 1983; Melcer and Bedford, 1988) reported that the degradation of toxic organics in activated sludge reactors was low and could not be optimized.
In upflow anaerobic sludge blanket (UASB) reactors, the organic pollutants are biodegraded by suspended granular biomass. This process generates methane and carbon dioxide. The main drawbacks of this technology are the requirements for temperatures higher than 28 °C (Mohn and Kennedy, 1992) and high organic carbon concentrations (Woods *et al*., 1989).

Since effluent requirements set in the TOP Water project are very stringent, large biological reactors would be required, even if compact biofilm processes are employed.
In addition to dissolved aromatic compounds, produced water contains high amounts of organic acids that are more easily biodegradable than the aromatics. If treated together with the aromatics, organic acids would pose a major burden on the biological process due to requirements for increased reactor volume and high oxygen and nutrients consumption.
In the case assumed in the TOP Water project, oxygen consumption and nutrient requirements would be approximately 270-630 mg O_2/l, 3-8 mg P/l and 15-35 mg N/l.
A major drawback of biological treatment is sludge production, which would amount ~1600 - 3200 kg DS/day for the produced water flow of 8000 m^3/d considered in the TOP Water project. Offshore sludge handling is difficult to achieve and if this operation is to be done onshore, then the costs would increase considerably.
Most of sludge would result from the degradation of organic acids, whose removal is not required because they have a low contribution to the EIF of a produced water discharge.
Biodegradation of heavy metals is not possible, but these pollutants may be retained by sludge. If this is the case, sludge could become harmful and it needs to be treated before being discharged.

2.3.7 Discussion and proposal of a treatment concept

The proposal of the treatment concept was done based on the previous work done in the TOP Water project by SINTEF (Helness, 2005 and 2006; Melin, 2005b).
High footprint and volume requirements of most of the investigated technologies, except CTour, are major drawbacks that would limit their utilization on offshore platforms. In some cases, the contaminants are transferred from liquid phase to solid or gaseous phase or concentrated, resulting in waste streams that need further treatment (off-gas from air sparging, concentrate from membrane treatment and spent adsorbents).
An interesting alternative to these processes, which showed many disadvantaged, would be an in-line treatment process making use of the existing pipes on the oil/gas rigs.

Utilization of pipes has several advantages over pressure vessels, since pipes have lower footprint requirements and it is easier to find the necessary space (e.g. run the pipe along the edge of the platform deck). It is also believed that a pipe reactor would provide a process closer to plug flow conditions.

If an in-line sorption process is employed, the sorption media could be separated by cyclones from the produced water stream. Removal of dissolved compounds in the pipe reactor could be also achieved with MPPE beads. The main drawbacks of such a process would be the erosion of beads, the energy requirements for the low-pressure regeneration of beads and the necessity of a separation unit for hydrocarbons and condensed steam.
One possibility could be to use MPPE beads and low-pressure steam for regeneration.
Granular activated carbon (GAC) or surface modified zeolites (SMZ) could be used in the pipe reactor instead of MPPE beads to sorb the soluble aromatics. It is expected that erosion of these two types of sorbents would be less significant than in the case of MPPE beads, since GAC and zeolites have been successfully employed in processes with moving media such as fluidized bed reactors.
There are two main options for the regeneration of spent GAC or SMZ:

- Air sparging, which uses air to remove the sorbed pollutants from the spent sorption media;
- Biological regeneration of spent sorbents in a fluidized bed reactor.

As previously discussed, utilization of air sparging (Rank *et. al.*, 2002) would generate an off gas, which needs further treatment before being discharged into the atmosphere.
On the other hand, biological regeneration of spent media in a fluidized bed reactor seems to be a more attractive option.
Gisvold *et al.* (2000) investigated the biological regeneration of a natural zeolite used to adsorb ammonia from municipal wastewater. Xing and Hickey (1994) bioregenerated successfully a GAC in a fluidized bed reactor treating BTX from groundwater (FBR). The reported BTX concentration in the influent to the GAC-FBR was 4.26 mg/l and this was reduced to an average of 0.015 mg/l (> 99 % removal). The treatment system was operated at an organic loading rate of 6.7 kg COD/m^3-day and a hydraulic retention time (HRT) of 3 minutes. The biological part of the process was the rate-limiting step, since the adsorption of BTX was about 3 times faster than the biological regeneration.
In order to decrease the organic loading on the biological treatment process, it is necessary that selective sorption of the target dissolved compounds and little or no sorption of organic acids is achieved. Organic loading provided by BTEX, phenols, NPDs and PAHs is expected to be low, therefore, the size of the reactor as well as the requirements for oxygen and nutrients would be significantly reduced.
Based on the literature review correlated with the challenges of TOP Water project, an in-line sorption process coupled with biological regeneration of the sorption media was proposed for the treatment of dissolved compounds. The concept assumes that sorption media can be injected in the pipe transporting produced water, sorb the soluble harmful compounds, is extracted at the end of the pipe reactor and subsequently bioregenerated continuously in a fluidized bed reactor (FBR), before being re-injected in the pipe for a new sorption cycle (Figure 13).

In order to implement into practice the proposed concept, solutions must be found to several important challenges referring to sorption, desorption and biodegradation of the target dissolved compounds, as well as to the construction of the process on an offshore platform.

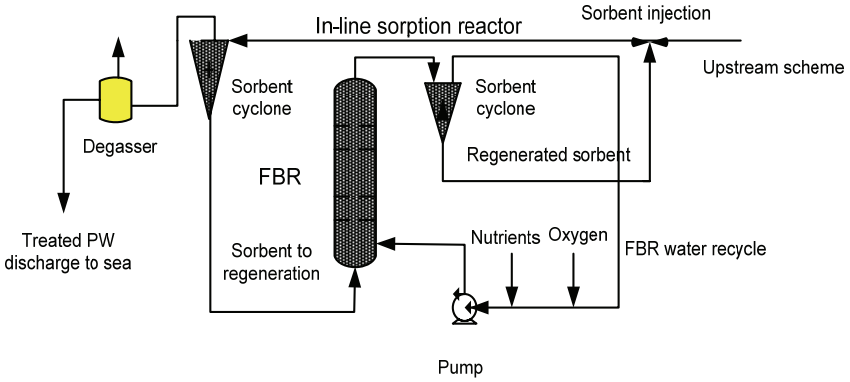


Figure 13. Proposed treatment scheme for the removal of dissolved compounds

2.4 Sorption coupled with FBR bioregeneration for BTX removal

2.4.1 BTX sorption

2.4.1.1 Characteristics of sorption process

Sorption refers to the action of either absorption or adsorption. Absorption is the incorporation of a substance in one state into another of a different state (e.g. liquids being absorbed by a solid or gases being absorbed by a liquid). Adsorption is the physical adherence or bonding of ions and molecules onto the surface of another molecule. It is a process that occurs when a gas or liquid solute accumulates on the surface of a solid or a liquid (adsorbent), forming a film of molecules or atoms (the adsorbate). Adsorption is different from absorption, in which a substance diffuses into a liquid or solid to form a solution. Physical adsorption is caused mainly by van der Waals and electrostatic forces between adsorbate molecules and the atoms that compose the adsorbent surface. Therefore, adsorbents are characterized first by surface properties such as surface area and polarity. Large adsorption capacities are usually provided by large specific surface areas, but the creation of a large internal surface area in a limited volume inevitably gives rise to large numbers of small sized pores between adsorption surfaces. The accessibility of adsorbate molecules to the adsorption surface is determined by the size of micropores, so the pore size distribution of micropores is another important property for characterizing adsorptivity of adsorbents.
In addition to micropores, some adsorbents have also larger pores that result from granulation of fine powders or fine crystals into pellets or originate in the texture of raw materials. These pores called macropores are several micrometers in size. They function

as diffusion paths for adsorbate molecules from outside the granule to the micropores. Adsorbents containing macropores and micropores are often said to have “bi-dispersed” pore structures.
Surface polarity corresponds to affinity with polar substances such as water. Polar adsorbents are thus called “hydrophilic” and aluminosilicates such as zeolites, porous alumina, silica gel or silica-alumina are examples of adsorbents of this type. On the other hand, nonpolar adsorbents are considered “hydrophobic”. Examples of nonpolar adsorbents are carbonaceous adsorbents, polymer adsorbents and silicate. These adsorbents have more affinity for oil than water (Suzuki, 1990).

2.4.1.2 Types of sorbents

Activated carbons

Activated carbon, also called activated charcoal or activated coal, is prepared from carbon-containing source materials (anthracite, brown coal, lignite, wood, nut shell and petroleum) processed to make it extremely porous and thus to have a very large surface area available for adsorption or chemical reactions. The source materials are first pyrolyzed and carbonized at several hundred degrees centigrade. The volatile fraction and low molecular products of pyrolisis are removed during this process, while the residual carbonaceous materials undergo the subsequent activation process. This employs different oxidizing gases, such as steam, at above 800 °C or carbon dioxide, at higher temperatures. The activation process favours the formation of micropores. Activated carbon yield from raw materials is in most cases less than 50% and sometimes below 10%.
Most adsorption takes place in micropores that are in the form of two-dimensional spaces between two graphite-like walls, two-dimensional crystallite planes composed of carbon atoms. Normally, the distance between the two-neighbouring planes of graphite is 3.76 Å (0.376 nm). This figure is larger in the case of activated carbons that have a rather disordered crystallite structure (turbostratic structure), since adsorbate molecules are not accessible otherwise.

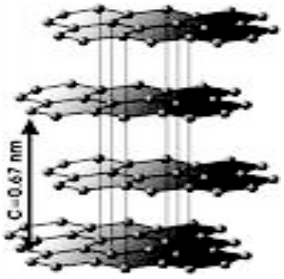


Figure 14. Chemical structure of activated carbon

Typically, activated carbons have a trimodal pore size distribution (micropores, mesopores and macropores). The conditions of the initial pyrolisis and activation

procedures have a significant influence on the actual distribution and total pore volume associated with each pore size range. Table 17 provides information about pore ranges, but it is possible to prepare activated carbons with even higher porosity, surface area, and adsorptive capacity by utilizing special techniques.

Table 17. Pore sizes in typical activated carbons

	Micropores	Mesopores or transitional pores	Macropores
Diameter (Å)	<20	20-500	>500
Pore volume (cm^3/g)	0.15-0.5	0.02-0.1	0.2-0.5
Surface area (m^2/g)	100-1000	10-100	0.5-2

Although a slight polarity may arise from surface oxidation, the surface of activated carbon is commonly nonpolar. Thus, carbon adsorbents are considered to be hydrophobic and organophilic. They are mainly employed for the adsorption of organics in water purification and solvent recovery systems and as a general-purpose adsorbent in different air purification systems. In order to decrease the mass transfer resistance, activated carbons used for adsorption from liquid phase, generally have larger pore diameters than those used for adsorption from the gas phase.
Generally, this type of media show very little selectivity in the adsorption of molecules with variable sizes. However, it is possible to prepare activated carbons with a very narrow distribution of micropore size that behave as molecular sieves, by using special activation procedures.

Zytner (1994) investigated the sorption and desorption characteristics of BTEX compounds for five media: granular activated carbon, sandy loam soil, organic top soil, clay soil, and peat moss. Results indicated that the Freundlich isotherm described well the sorption and desorption of dissolved BTEX on the sorbents tested. An important factor in both sorption and desorption was the organic carbon content of the media. The observed sorption abilities for BTEX decreased in the following order: GAC > peat moss > organic top soil > clay soil > sandy loam soil.

Daifullah and Girgis (2003) investigated the potential of five residues of botanical origin to be employed as activated carbon precursors. These were: date pits (DP), cotton stalks (CS), peach stones (PS), almond shells (ALS), and olive stones (OS). In order to be able to compare the results, a standard activation procedure based on impregnation with 50% H_3PO_4 and heat treatment at 773 K was used. The adsorption properties of the manufactured carbons for BTEX compounds decreased in the order: PS, ALS, CS, OS and DP, respectively. Uptake of benzene, toluene, ethylbenzene and p-xylene (BTEX) was evaluated for each component in the mixture and in terms of total. In the case of PS, the adsorption capacities were 3 mg benzene/g, 6.5 mg toluene/g, and 8.7 mg p-xylene/g. The determined adsorption capacities for the five activated carbons were lower than those of a commercial powder activated carbon (PAC) produced by Prolabo, which adsorbed 7.95 mg benzene/g, 9.2 mg toluene/g and 9.92 mg p-xylene/g.
Uptake of the hydrophobic organic molecules is believed to be determined by the complexity of the carbon surface with developed porosity and high content of hydrophilic oxygen functionalities. The order of uptake was observed to be consistent

with previous investigations and increased in the order: benzene < toluene < ethylbenzene < xylenes. This means that the sorption of BTEX compounds increased with the increase of molecular weight and decreased with the increase of water solubility of sorbate.

Zeolites

Zeolites are porous crystalline aluminosilicates whose framework consists of an assemblage of SiO_4 and AlO_4 tetrahedra. Shared oxygen atoms join these structures to form an open crystal lattice containing pores of molecular dimensions into which guest molecules can penetrate. The crystal lattice determines the micropore structure, which is therefore precisely uniform with no distribution of pore size. This feature distinguishes the zeolites from the traditional microporous adsorbents. The structures of zeolite frameworks can be regarded as built up from assemblages of secondary building units, which are themselves polyhedra made up of several SiO_4 and AlO_4 tetrahedra (Figure 15).

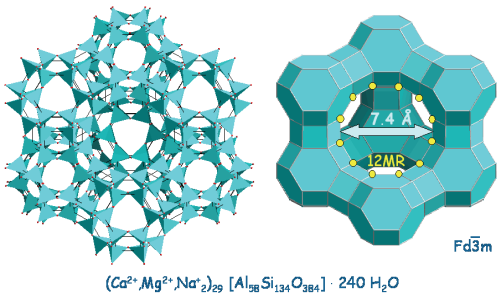


Figure 15. Zeolite framework structure (courtesy of W. Schmidt)

Each vertex represented in Figure 15 indicates the location of a Si or Al atom. The yellow circles mark the positions of the neighbouring tetrahedral centres which form a window (12MR = 12-membered ring, which means that the window is formed by 12 tetrahedra that are connected via shared oxygen atoms).
One negative charge is introduced in the framework by each aluminium atom. These charges must be balanced by exchangeable cations. They play a very important role in determining the adsorptive properties of zeolites and are located at preferred sites within the framework. A useful and widely exploited method of modifying the adsorptive properties of zeolites is provided by changing the exchangeable cations by ion exchange.
The Si/Al ratio in a zeolite is never less than 1.0 but there is no upper limit and pure silica analogues of some of the zeolite structures have been prepared. While the aluminium rich sieves have very high affinities for water and other polar molecules, the microporous silicas, such as silicalite, are essentially hydrophobic and adsorb n-paraffins in preference to water. The transition from hydrophilic to hydrophobic was observed to take place at a Si/Al ratio between 8 and 10 Adsorbents with very broad adsorptive properties can be prepared by appropriate choice of framework structure,

Si/Al ratio and cationic form. The selectivity required for a particular separation may be possible by tailoring the adsorptive properties of the zeolite.
The intracrystalline diffusivity and hence the kinetic selectivity, and in extreme cases, the molecular sieve properties are determined mainly by the free diameters of the windows in the intracrystalline channel structure. In some zeolites, these channels are constricted by six-membered oxygen rings with free diameter of about 2.8 Å. Only small polar molecules such as H_2O and NH_3 can penetrate since these pores are very small. The limiting constrictions are eight-membered oxygen rings with a free diameter of 4.2 Å in the "small-port" zeolites such as type A, chabazite, and erionite, while in the "large-port" zeolites, such as X and Y and mordenite, access is through twelve membered oxygen rings which have free diameters of 7-7.4 Å.
By blocking the cations, the free diameter of the windows is reduced and this causes a dramatic decrease in the diffusivity of guest molecules. The number and nature of cations affect the extent to which the windows are obstructed, since different cations show different affinities for the window sites. Sometimes it is possible to design kinetic selectivity and even to attain a molecular sieve separation between species that can both diffuse easily in an unobstructed sieve by appropriate choice of cationic form (Ruthven, 1984).

Surfactant modified zeolites

Surfactants have been used to modify the surface characteristics of soils and create sorbents able to immobilize other compounds (Lee *et al.*, 1989; Burris and Antworth, 1992; Danzer and Grathwohl, 1998). Cationic-surfactant-modified zeolites were also prepared, tested and proposed for the removal of a broad range of compounds such as the hydrophobic organic chemicals (HOCs), inorganic cations, anions, and inorganic oxyanions (Haggerty and Bowman, 1994; Bowman *et al*., 1995, 2000; Li and Bowman, 1998). The cationic surfactant-modified zeolites are usually prepared by mixing the natural zeolites with a solution containing the surfactant (for example hexadecyltrimethyl ammonium chloride – HDTMA), which is sorbed quantitatively up to about twice the zeolite external cationic capacity of 100 meq/kg. The surfactant molecule is too large to penetrate the Angstrom-sized internal pore spaces of the zeolite and forms a coating on zeolite surface that is very resistant to washoff by aqueous and organic solutions (Figure 16).

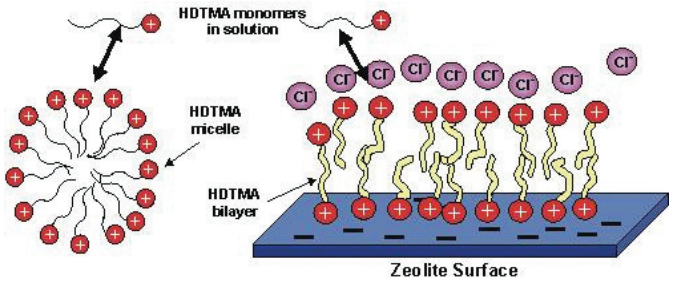


Figure 16. Zeolite modified with HDTMA (courtesy of Professor Robert Bowman)

The sorbed surfactant creates an organic-rich layer and the charge on the surface is reversed from negative to positive. The positive charge on the outward-pointing surfactant head groups is balanced by anions from solution, forming an electrical double layer.

Li e*t al***.** (2000) studied the sorption of benzene, phenol and aniline by a surfactant-modified zeolite (SMZ), prepared at different HDTMA surface coverages. Results demonstrated that benzene was strongly retained by SMZ and its sorption was described by a linear sorption isotherm, suggesting a partition-type mechanism.

Karapanagioti *et al*. (2005) studied the removal of benzene, toluene, ethylbenzene, 1,2-dichlorobenzene, naphthalene, and phenanthrene by several surfactant-modified sorbents. They investigated one cationic (HDTMA) and three anionic (DOWFAX-8390, STEOL-CS330, and Aerosol-OT) surfactants for their sorptive abilities after being immobilized onto different sorbents (zeolite, alumina and Canadian River Alluvium). The most stable surfactant-modified sorbent was the HDTMA–zeolite system, since it showed the lowest surfactant desorption. Modified sorbents manufactured with both anionic and cationic surfactants demonstrated higher sorption capacity and affinity than the unmodified Canadian River Alluvium containing only natural organic matter. The affinities of the surfactant-modified sorbents (Koc) for most HOCs are lower than octanol/water partition coefficient (Kow) normalized to the organic carbon content (foc) and the density of octanol (Koc octanol); naphthalene and phenanthrene are the exceptions to this rule.

Clays

Clay minerals consist of small crystalline particles that are made of silica tetrahedral sheets (with a silicon ion tetrahedrally coordinated with four oxygens) and the aluminium or magnesium octahedral sheet (where an aluminium or magnesium ion is octahedrally coordinated with six oxygens of hydroxyls) (Grim, 1968). Aluminium can replace silica in the tetrahedral layers, while iron, magnesium, manganese, and other cations of similar size can replace aluminium in the octahedral layer. In some cases, during the formation process, the replacement of ions in the structure of clay minerals is called isomorphous substitution. The net negative charge created on the clay particle surface has to be neutralized, since the valence of the replaced cations is often lower than that of the original ions. Therefore, the clay surface attracts cations such as sodium, potassium, and calcium that neutralize the layer charge. These ions, called exchangeable cations, may be further replaced by other cations. Clays consist mainly of bentonite, a chemically altered volcanic ash whose major constituent is the mineral montmorillonite. It has a cation exchange capacity of 70-95 meq/g.
These characteristics of clays enable them to readily adsorb inorganic and metallic cations from wastes. Studies carried out by Theng (1974) and Raussell-Colom and Serratosa (1987) demonstrated that many polar organics such as alcohols, amines and ketones are adsorbed onto the external clay surface, interlayer space and probably on clay particle edges by electrostatic attraction and ion exchange reactions.

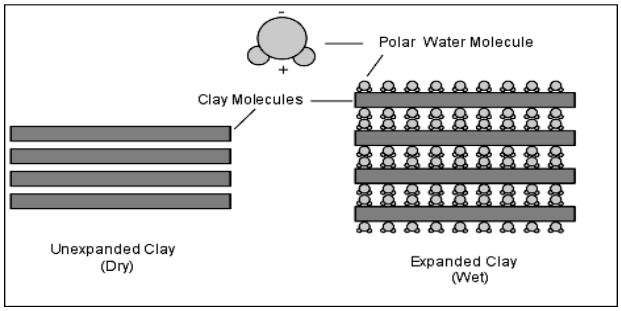


Figure 17. Clay structure and its expansion in water

Organoclays

Natural clays have the ability to attract the inorganic rather than the organic constituents of wastes, which have larger molecular sizes than the inorganic cations. Besides that, clays are of hydrophilic nature and this allows polar water molecules to cover the clay surface and reduce the attraction of hydrophobic organic species (Chiou *et al*., 1983).
Many researchers have studied the possibility of changing the two basic characteristics of these minerals, namely, interlammelar distance and hydrophobicity of clays in order to find a solution for the complexities involved in clay-organic compounds interactions and further enhance the adsorption of organics by clays. Experiments carried out by Boyd *et al*. (1988a,b), McBride e*t al*. (1977) and Evans and Pancoski (1989) demonstrated that a stationary phase is formed in the clay particles when the quaternary ammonium cations such as HDTMA replace the exchangeable inorganic cations on their surfaces. Moreover, due to larger dimension of the HDTMA cations compared with that of the replaced cations, the interlamellar distance (basal spacing) of the mineral increases and additional space is created in the particles. This process facilitates the retention of other organic compounds. The presence of quaternary ammonium cations changes the properties of clay surface from hydrophilic to hydrophobic (organophilic). Therefore, these sorbents can retain fewer water molecules, while their sorption capacity for hydrophobic organic compounds is significantly increased. The organophilic surface of modified clays enables them to remove oil and other organic compounds of low polarity. The hydrophobic chains of organoclay particles extend into the water and since “like dissolves like”, the chains dissolve into the organic compounds, removing them from water by a partition mechanism. In contrast to adsorption of oil by carbon that takes place inside the pores, the partition reaction takes place “outside” of the clay particles. The simplest definition of partitioning is that liquid compounds dissolve into other liquid compounds of a similar nature when both are present in a third liquid of a different nature. An example would be two nonpolar liquids dissolving into each other when present in a polar liquid.

Gitipour *et al*. (1997a,b) investigated the utilization of an organoclay (bentonite modified with dimethyl dihydrogenated ammonium chloride) for binding benzene, toluene, ethylbenzene, and o-xylene (BTEX compounds) in contaminated liquids. The efficiency of the modified clay in removing aromatic compounds from liquids was assessed by carrying out sorption isotherm and column leach experiments. On the other hand, changes in the basal spacing of bentonite caused by its interactions with the organic compounds were evaluated by X-ray diffraction analyses. Results showed increases in the basal spacing of the modified bentonite due to the interaction between the clay and aromatics. The removal efficiencies of the organoclay for benzene, toluene, ethylbenzene and o-xylene compounds were 75%, 87%, 89%, and 89%, respectively, proving that the modified bentonite can be used as a practical organic sorbent for waste water treatment systems.

Koh and Dixon (2001) compared the abilities of three organo-minerals prepared from Na-montmorillonite, sericite, and zeolite to sorb benzene, phenol, and toluene from contaminated wastewater. Quaternary ammonium cations with various molecular weights such as benzyldimethyltetradecyl ammonium (BDTDA), Hyamine 1622, and benzyltrimethyl ammonium (BTMA) were exchanged on the surface of the natural minerals in order to manufacture the organo-minerals. The amounts of quaternary ammonium cations exchanged onto these minerals decreased in the order: montmorillonite > zeolite > sericite. This demonstrated that the exchanged amounts depended on the Ca/Mg cation exchange capacity (CEC) of each mineral.
A brief comparison of the performances obtained with the three organo-minerals with the performances achieved with activated carbon, indicated that all modified mineral complexes sorbed less organic pollutants than the activated carbon. BDTDA-montmorillonite was the best performing organo-mineral because its sorption capacity increased with each addition of surfactant up to about 70% and 66% of the activated carbon adsorption for benzene and toluene, respectively. BTMA-zeolite sorption was 65% of the activated carbon adsorption for benzene and approximately 50% of the activated carbon adsorption for toluene. Only little sorption of phenol was observed on the untreated zeolite or sericite surfaces. Organo-minerals showed a poor efficiency in removing phenol. The only exceptions were BDTDA- and BTMA-montmorillonite, which sorbed about 35% as much phenol as adsorbed by activated carbon.

2.4.2 BTX biodegradation

2.4.2.1 Pathways for BTX degradation

Biodegradation of BTX compounds can be achieved under both aerobic and anaerobic conditions. During the degradation of light aromatic hydrocarbons under aerobic conditions, microorganisms produce carbon dioxide, water and sludge. On the other hand, compounds such as methane, CO_2 and mineral salts are generated during the microbial degradation under anaerobic conditions. The stoichiometries under aerobic and anaerobic conditions for the overall reactions describing the biodegradation of benzene, toluene, ethylbenzene and xylenes isomers are given in table 18. The energy

released during electron transfer processes occurring during biochemical reactions is further utilized for growth and cell maintenance.

Available literature on BTX removal under aerobic conditions indicates that each of these compounds could be degraded through at least one pathway leading to a substituted catechol (Andreoni and Gianfreda, 2007). Benzene is degraded to catechol (Tsao *et al*., 1998; Johnson *et al*., 2003), while toluene and ethylbenzene are degraded via several separate pathways leading to the production of 3-methylcatechol and 3-ethylcatechol, respectively. Xylenes are metabolized to mono-methylated catechols (Jindrova *et al*., 2002; Stephens, 2006).

Table 18. Properties of BTEX and degradation stoichiometry (Langwaldt and Puhakka, 2000; Cunningham *et al*., 2001; Villatoro-Monzon *et al*., 2003; Roychoudhury and Merrett, 2006)

Compound	Some properties	Overall reaction
Benzene (B)	C_6H_6 MW = 78.11 g/mol Water solubility = 1791 ppm at 25 °C Density = 0.8787 g/cm^3 Theoretical oxygen demand (ThOD) for 1 ppm benzene in water = 3.076 ppm	$C_6H_6 + 7.5O_2 \rightarrow 6CO_2 + 3H_2O$ $C_6H_6 + 6NO_3^- + 6H^+ \rightarrow 6CO_2 + 6H_2O + 3N_2$ $C_6H_6 + 15Mn^{4+} + 12H_2O\ 6CO_2 + 30H^+ + 15Mn^{2+}$ $CC_6H_6 + 30Fe3^+ + 12H_2O \rightarrow 6CO_2 + 30H^+ + 30Fe^{2+}$ $C_6H_6 + 3.75$ $SO_4^{2-} + 7.5H^+ \rightarrow 6CO_2 + 3.75H_2S + 3H_2O$ $C_6H_6 + 4.5H_2O \rightarrow 2.25CO_2 + 3.75CH_4$
Toluene (T)	C_7H_8 MW = 92.14 g/mol Water solubility = 535 ppm at 25 °C Density = 0.8669 g/cm^3 ThOD for 1 ppm toluene in water = 3.13 ppm	$C_7H_8 + 9O_2 \rightarrow 7CO_2 + 4H_2O$ $C_7H_8 + 7.2NO_3^- + 7.2H^+ \rightarrow 7CO_2 + 7.6H_2O + 3.6N_2$ $C_7H_8 + 18Mn^{4+} + 14H_2O \rightarrow 7CO_2 + 36H^+ + 18Mn^{2+}$ $C_7H_8 + 36Fe3^+ + 14H_2O \rightarrow 7CO_2 + 36H^+ + 36Fe^{2+}$ $C_7H_8 + 4.5SO_4^{2-} + 9H^+ \rightarrow 7CO_2 + 4.5H_2S + 4H_2O$ $C_7H_8 + 5H_2O \rightarrow 2.5CO_2 + 4.5CH_4$
Ethylbenzene (E) and isomer xylenes (X) (*meta* xylene, *ortho* xylene, and *para* xylene)	C_8H_{10} MW = 106.16 g/mol Water solubility (E) = 161 ppm at 25 °C Water solubility (X) = 146–175 ppm at 25 °C Density (E) = 0.867 g/cm^3 Density (X) = 0.861–0.88 g/cm^3 ThOD for 1 ppm E or X in water = 3.17 ppm	$C_8H_{10} + 10.5O_2 \rightarrow 8CO_2 + 5H_2O$ $C_8H_{10} + 8.4NO_3^- + 8.4H^+ \rightarrow 8CO_2 + 9.2H_2O + 4.2N_2$ $C_8H_{10} + 21Mn^{4+} + 16H_2O \rightarrow 8CO_2 + 42H^+ + 21Mn^{2+}$ $C_8H_{10} + 42Fe3^+ + 16H_2O \rightarrow 8CO_2 + 42H^+ + 42Fe^{2+}$ $C_8H_{10}85.25SO_4^{2-} + 10.5H^+ \rightarrow 8CO_2 + 5.25H_2S + 5H_2O$ $C_8H_{10} + 5.5H_2O \rightarrow 2.75CO_2 + 5.25CH_4$

Bacteria are usually the microorganisms that have the physiological and metabolic capabilities for BTX removal, but fungi can also play a significant role (Van Hamme *et al*., 2003; Schulze and Tiehm, 2004; Nikolova and Nenov, 2005). The presence of an electron acceptor, nutrients and suitable environmental conditions are the most important requirements for BTX biodegradation. Typical electron acceptors in microbial metabolism are O_2, nitrate, Fe (III), sulphate and CO_2. Aerobic processes use O_2 as

electron acceptor, while the other acceptors are involved in anaerobic processes. During bioremediation operations, microorganisms can utilize BTX as carbon source for their growth. Nutrients such as nitrogen, phosphorus, sulphur and trace elements must be supplied to the medium.

Microbial activity does not depend only on medium composition, but also on environmental conditions such as temperature, pH, salinity and pressure (Granger *et al.*, 1999; Lin *et al*., 2002; Villatoro-Monzon *et al*., 2003; Chakraborty and Coates, 2004; Jahn *et al*., 2005).

BTX compounds have been observed to be utilized as carbon and energy source by several pure and mixed cultures of microorganisms (Table 19). These cultures are normally bacterial consortia from domestic or industrial sludge, soil contaminated with oil products and polluted groundwater (Solano-Serena *et al.*, 1999, 2000; Guerin, 2002; Pruden *et al*., 2003; Cattony *et al*., 2005; Ohlen *et al.*, 2005; Kermanshahipour *et al*., 2005, 2006; Zein *et al.*, 2006).

Degradation of combinations of BTEX components by pure bacterial strains such as *Rhodococcus rhodochrous* (Deeb and Alvarez-Cohen, 1999), several strains of *Pseudomonas putida* (Alagappan and Cowan, 2003) and *Alcaligenes xylosoxidans* (Yeom and Yoo, 2002) has been studied. It was reported that, in order to efficiently degrade all BTEX components simultaneously, especially when o-xylene was present, a bacterial consortium was required. O-xylene has been observed to be significantly more difficult to biodegrade than the other BTEX compounds (Deeb and Alvarez-Cohen, 1999; Attaway and Schmidt, 2002).

Fungi have been also reported, besides bacteria, to degrade BTEX and MTBE. White-rot fungi have been mainly employed to evaluate fungally mediated biodegradation of soil pollutants (Pointing, 2001). These organisms oxidize aromatic hydrocarbons by co-metabolism, and significant mineralization can only be achieved through the synergic interaction of fungi and bacteria (Kotterman *et al.*, 1998; Boonchan *et al.,* 2000).

Recently, fungi capable of growing on volatile aromatic hydrocarbons as their sole source of carbon and energy have been isolated from soil (Cox *et al.,* 1993; Prenafeta Boldú *et al.,* 2001; Woertz *et al*., 2001). These fungal isolates are believed to be suitable as biocatalysts in air biofilters treating BTEX vapours, since they were noticed to be tolerant to acidic and dry conditions (van Groenestijn *et al*., 2001).

Deeb and Alvarez (1999) isolated a pure culture from a consortium enriched at 35 °C. It was identified as *Rhodococcus rhodochrous*. Each component of the BTEX group was degraded individually and in mixtures by this culture, following the same degradation patterns as the mixed cultures. Moreover, it was shown that *Rhodococcus rhodochrous* used benzene, toluene and ethylbenzene as primary carbon and energy sources. Studies were conducted with the consortium as well as the pure culture in order to evaluate potential substrate interaction patterns. Ethylbenzene was found to be the most potent inhibitor of benzene, toluene, and xylene degradation in pure as well as in mixed cultures. This inhibition was more pronounced in the case of *Rhodococcus rhodochrous* than in the case of the parent consortium. It was also reported that the degradation of BTX in binary mixtures did not proceed until all the ethylbenzene was degraded.

Table 19. Microorganisms involved in degradation of monoaromatic pollutants (Farhadian *et al*., 2007)

Organism	Source of pollutant	Reference(s)
Rhodococcus rhodochrous	BTEX	Deeb and Alvarez-Cohen (1999)
Pseudomonas sp. ATCC 55595	BT (p-)X	Collins and Daugulis (1999)
Pseudomonas putida and *Pseudomonas fluorescens*	BTE (o-)X	Shim and Yang (1999)
Rhodococcus sp. RR1 and RR2	BTE (m-/p-)X	Deeb and Alvarez-Cohen (2000)
Pseudomonas putida F1	BTE, TCE	Parales *et al.* (2000)
Pseudomonas putida	BTEX	Attaway and Schmidt (2002)
Cladophialophora sp. strain T1	BTEX	Prenafeta-Boldú *et al.* (2002)
Rhodococcus sp. strain DK17	BTE (o-) X	Kim *et al.* (2002)
Pseudomonas putida strain mt-2	T (m-/p-) X	Morasch *et al.* (2002)
Blastochloris sulfoviridis ToP1	T	Van Hamme *et al.* (2003)
Pseudomonas putida	BTEX	Shim *et al*. (2002, 2005)
Pseudomonas putida PaW1	Aromatic compounds and chloroaliphatics	Leahy *et al*. (2003)
Pseudomonas aeruginosa	B	Kim *et al*. (2003)
Rhodococcus pyridinovorans PYJ-1	BT (m-)X	Jung and Park (2004)
Achromobacter xylosoxidans	BTEX	Nielsen *et al*. (2006)
Geobacteraceae	BTX	Botton *et al.* (2007)
Ralstonia picketti and *Alcaligenes piechaudii*	BTEX	Plaza *et al*. (2007)

Alfreider and Vogt (2007) investigated bacterial diversity and aerobic biodegradation potential in a BTEX-contaminated aquifer. Their study was based on 16S ribosomal DNA sequence analysis. Sampling stations in the centre of the pollution plume were found to be dominated by a bacterial consortium affiliated with various members of the class of *Proteobacteria* and *Firmicutes*, including different sporulating and non-sporulating sulphate reducing bacteria and members of the genus *Geobacter*. Several phylotypes, which were also observed in the centre of the pollution plume, were also revealed in the non-polluted samples taken from outside the plume. A few genera of beta *Proteobacteria* subclass, which was not associated with other phylotypes obtained in this study, dominated bacterial sequences taken from the fringe of the plume. Presence of microorganisms with genetic potential to degrade aromatic compounds via the meta-cleavage pathway was demonstrated at all sampling stations, since all samples tested positive for catechol 2,3-dioxygenase genes.

Hendrickx *et al*. (2005) studied the dynamics of a bacterial aquifer community during contact with a groundwater plume polluted with BTEX. Denaturing gradient gel electrophoresis analysis of PCR amplified 16S rRNA genes and PCR detection of degradation genes were employed to investigate the effect of benzene, toluene, ethylbenzene, and xylenes (BTEX) contamination on the response of the aquifer

bacterial community. Only *tmoA*-like genotypes were detected at the uncontaminated location, while bacteria with *tmoA*- and *xylM/xylE1*-like BTEX catabolic genotypes colonized the aquifer at the contaminated location. *Proteobacteria* were dominant in the communities growing in the contaminated part of the aquifer, while *Actinobacteria* and *Proteobacteria* were detected at the uncontaminated location.

Plaza *et al.* (2007) employed two different bacteria, *Ralstonia picketti* (BP-20) and *Alcaligenes piechaudii* (CZOR L-1B), isolated from extremely polluted soils contaminated with petroleum hydrocarbons, to investigate the biodegradation of benzene, ethylbenzene, toluene, and xylenes (BTEX) compounds. Identification of isolates was done by PCR and Fatty Acid Methyl Ester (FAME). Biodegradation of BTEX used individually or as a mixture was evaluated. *Alcaligenes piechaudii* was a better degrader of BTEX both in mixture and individually. Differences between BTEX biodegradation in mixture and individually were observed, especially in the case of benzene. For both bacteria tested, biodegradation rates of all BTEX in the mixture were lower than those determined when individual compounds were used as substrate. Removal of toluene, m- and p-xylenes was higher than that of the other BTEX in all experiments. Biodegradation process did not generate any metabolic intermediates. Culture techniques were utilized to investigate biosurfactant production. Presence of 3-hydroxy fatty acids, important in biosurfactant production, was observed by FAME analysis. Results indicated that the microbial culture had a significant contribution to the removal of aromatic hydrocarbons.

Kim *et al*. (2003) determined the model parameters describing biodegradation of benzene by *Pseudomonas aeruginosa* for various initial benzene (100 -700 mg/l) and microbial concentrations (10^7 – 10^9 colony forming units [CFU]/ml). The estimated values of maximum substrate utilization rate (q_{max}) and half-saturation constant (K_S) were in the range of 61 to 105 mg/l/d and about 270 mg/l.

Shim and Yang (1999) used a co-culture of *Pseudomonas putida* and P*seudomonas fluorescens* immobilized in a fibrous matrix to degrade benzene, toluene, ethylbenzene, and o-xylene in synthetic waste streams. Under hypoxic conditions, complete and simultaneous degradation of BTEX mixture was attained in the bioreactor. Cells in the bioreactor adapted very well to the available substrate and therefore, they were not sensitive to benzene toxicity. A long, slim morphology was observed for cells in the fibrous bed, which is different from the normal short-rod shape found for suspended cells in solution.
Substrate inhibition kinetics was observed when BTEX were utilized as a sole carbon source for cell growth. Cells immobilized in the fibrous bed were shown to be more resistant against toxic substrate (especially benzene) than the original seeding cultures present as free cells under similar conditions. It was therefore concluded that the co-culture immobilized in the fibrous bed could be used to treat industrial waste streams containing high concentrations of BTEX.

Nielsen *et al*. (2006) estimated the oxygen requirements of *Achromobacter xylosoxidans* for aerobic degradation of BTEX in a bioscrubber. Biomass yield on O_2, $Y_{X/O2,}$ for benzene and ethylbenzene, was 1.96 ± 0.25 mg CDW mg O_2 $^{-1}$ and 0.98 ± 0.17 mg

CDW mg O_2 $^{-1}$, respectively. On the other hand, the specific rate of O_2 consumption for maintenance, m_{O2}, was approximated as 0.041 ± 0.008 mg O_2 mg CDW^{-1} h^{-1} and 0.053 ± 0.022 mg O_2 mg CDW^{-1} h^{-1}, respectively.

Botton *et al.* (2007) studied the biodegradation of BTX compounds in an iron-reducing aquifer. Iron-reducing enrichments derived from an iron-reducing aquifer polluted with landfill leachate were employed to develop a microbial community able to biodegrade BTX compounds. The structure of this community was linked with the degradation potential for benzene, toluene or xylene (BTX). Characterization of the culture was done by 16S rRNA gene-based analysis, targeting the benzylsuccinate synthase-encoding bssA gene and phospholipid fatty acid (PLFA) profiling in combination with tracking of labelled substrate. The dominance of Geobacteraceae in all enrichments inoculated with polluted aquifer material was indicated by 16S rRNA gene analysis. A decrease in species richness combined with an increase in biodegradation rates was noticed upon cultivation in all primary incubations and successive enrichments. The same Geobacteraceae phylotype remained common and dominant, indicating its involvement in BTX degradation. However, it is believed that another member of the enrichments was responsible for the oxidation of toluene, benzene and xylene, since the bssA gene sequenced in BTX degrading enrichments differed considerably from those of Geobacter isolates. It was therefore concluded that BTX compounds were degraded by a bacterial consortium, in which Geobacteraceae used intermediate metabolites. Utilization of ^{13}C-toluene combined with PLFA analysis proved that the enriched Geobacteraceae were assimilating carbon originally present in toluene. This study suggested that Geobacteraceae play a key role in the natural in situ bioremediation of each member of the BTX group.

Prenafeta-Boldú *et al*. (2002) investigated the pattern of degradation of BTEX mixtures by a fungus capable of growing on aromatic hydrocarbons. The model fungus *Cladophialophora* sp. strain T1 (=ATCC MYA-2335) that grows on toluene, was selected for the biodegradation experiments. This strain, which was isolated from a BTEX-polluted soil, was observed to have the best degradative capacity in terms of substrate specificity among all the fungal isolates examined (Prenafeta-Boldú *et al.*, 2001). Focus was put on two key issues: biodegradation kinetics of multiple substrates and the extent of degradation of each BTEX component. Based on this information, techniques utilizing fungi to biodegrade BTEX could be designed. These can be applied especially under solid state like environmental conditions, such as those in air biofilters or acidic soils where fungal growth is higher than bacterial growth (Bossert and Bartha, 1984; Prenafeta-Boldú *et al.*, 2004).

2.4.2.3 Characterization techniques

Overview of available techniques

Total cell counts and fluorescence in situ hybridization (FISH) can be used to obtain quantitative information about the distribution and activity of the microbial communities based on a single cell approach. The molecular data are usually compared

with the results obtained by cultivation-dependent techniques, including most probable number (MPN) counts of total cultivable bacteria. In recent years, the most practiced approach to study microbial diversity and processes in the natural environment was the analysis of genes. Several genetic tools exist nowadays to characterize microbial communities from various habitats without prior cultivation (Kowalchuk *et al.,* 2004). In fact, specific physiological properties and the ecological role of particular populations cannot be assessed by molecular approaches alone. The ideal investigation should ascertain the identity and function of naturally occurring bacteria and consequently a combination of techniques and concepts from different disciplines is necessary to obtain a better knowledge of the interactions between microorganisms and their environments (Brinkhoff *et al.*, 1998).

Chemical staining

Metabolically active bacteria can be counted by staining with 4,6-diamidino-2-phenylindole (DAPI). Fluorescence microscopy is usually used to count the cells stained with DAPI and excited with UV light. Staining can be applied to sludge samples (mixed bacteria culture) or after isolation of pure cultures.

Culture based determination

The biomass sample is diluted and spread on a plate containing an artificial laboratory growth media. Standardized methods and tests are then carried out to determine the physiology and morphology of isolated cultures. Based on the physiological and morphological characteristics of the cultures, a relatively accurate identification of bacteria can be achieved.
Development of molecular methods in microbiology caused a significant amelioration of biomass characterization compared to the traditional culture based methods.

rRNA libraries

These methods are based on the extraction of DNA from the bacteria and isolation of DNA-sequences that code for 23S rRNA and 16S rRNA molecules the bacteria use in protein synthesis. These sequences are 3000 and 1500 base pair long respectively and contain highly variable regions (distinct for different bacteria) as well as highly conserved regions (common for all bacteria). The relation between different bacteria can be determined by comparing the rDNA sequences. This is achieved by amplifying the rDNA sequences using polymerase chain reaction (PCR). Specific oligonucleotide primers that bind to conserved regions of the rDNA in two places are utilized in PCR. The enzyme called DNA-polymerase is then used to copy the rDNA sequences between the primers. Before the sequences can be determined, DNA strands with different sequences must be isolated and amplified. Cloning is usually used to fulfil these operations by inserting one single rDNA strand in a plasmid, which is then incorporated into a bacterial cell (*E. Coli*). When the amplification in the *E. Coli*-clones is completed, the isolated rDNA can be extracted and sequenced.

Known sequences of other bacteria are compared with the obtained sequences and in this way, a phylogenetic tree illustrating the relationship between the clones and known bacteria can be established.

DNA or RNA fingerprinting

This method also starts with extraction of DNA from bacteria, but instead of rDNA sequencing from the total biomass, separation of the different rDNA strands is done by different methods in order to obtain a fingerprint of the rDNA in the biomass.

Detection of catechol dioxygenase genes

A central step in the catabolic pathways of aromatics under aerobic conditions is the cleavage of the aromatic ring, which is typically catalyzed by ortho- or meta-cleavage dioxygenases (Cerniglia, 1984). Therefore, catechol dioxygenase genes are suitable targets for monitoring bacteria involved in the degradation of aromatic compounds. To assess the presence of catechol 1,2-dioxygenase- and catechol 2,3-dioxygenase genes, two different primer pairs are usually applied (Alfreider and Vogt, 2007).

Denaturing gradient gel electrophoresis (DGGE)

DGGE can be used for analysing diversity in a microbial culture. Extraction of rDNA from a biomass sample followed by amplification of rDNA by PCR is the first step of the analysis. A PCR product with a number of different DNA fragments separated in a gel is obtained. Separation of DNA fragments of the same length but with different base pair sequences can be attained by using DGGE because different base pair sequences give different melting temperatures and the partially melted DNA fragment has lower mobility in the DGGE gel compared to the helical form (Muyzer *et. al.*, 1993). Increased resolution is obtained by employing DGGE as compared to neutral gel electrophoresis for separation of PCR product. DGGE techniques do not provide quantitative results.

Restricted fragment length polymorphism (RFLP)

This approach is used in fingerprinting to classify rDNA strands that have been separated by cloning or DGGE. RFLP utilizes enzymes that cut the rDNA strands at locations with specific sequences. The fragments are separated in a gel according to their lengths, which determine how far the fragment will migrate in the gel. After separation of the fragments, different RFLP-patterns will be obtained with strands of rDNA with different sequences. RFLP method is commonly carried out on rDNA from clones and the results used to group clones with equal RFLP-patterns into RFLP-types or operational taxonomy units (OTU). This technique can also be employed after rDNA is separated from the total biomass in a DGGE-gel. Although RFLP gives lower resolution compared to sequencing, it is faster and can be used for screening and selection of clones or bands in a DGGE gel for sequencing. RFLP is not used directly on samples with rDNA strands from the total biomass and does not provide quantitative results.

Terminal restriction fragment length polymorphism (T-RFLP) is a variation of RFLP, where one end of the rDNA strand is marked. The interpretation of results after enzymatic cutting of the rDNA is based on comparing the length of the terminal marked fragment. T-RFLP can be used on samples with rDNA strands from the total biomass, which is a major advantage of this technique over RFLP. This method is not quantitative.

Fluorescent in situ hybridization (FISH)

FISH is a cytogenetic technique that can be employed to detect and localize the presence or absence of specific DNA sequences on chromosomes. It uses oligonucleotide probes that bond to rRNA inside whole bacterial cells. A fluorophore, which gives a certain colour when exposed to laser light of a certain wavelength is connected to the gene. Tailor made probes with different resolutions can be designed to target bacteria species, genus, group or all bacteria. Comparison of microscope images of samples hybridized with different probes is utilized to study the composition of a biomass sample. In contrast to the gene methods described above, quantitative results can be obtained with FISH. If combined with other methods, FISH can be used as a tool to link bacteria to a certain metabolism. One possible combination of FISH is with micro autoradiography (MAR), where radioactive labelled substrates (i.e. ^{13}C) are used to monitor the metabolism. FISH can detect only bacteria that are targeted by the probes. Methods such as DGGE are better suited as initial step to characterize the diversity of a bacterial community. An optimisation of the procedure is usually required, since FISH relies on the penetration of the cell wall by the gene probe.

2.4.3 FBR treatment technology

2.4.3.1 Characteristics of FBRs

A fluidized bed reactor is a treatment system in which the wastewater is fed upward to a bed of particles (sand, activated carbon, anthracite, glass etc) at a velocity sufficient to expand or fluidize the carrier bed. The carrier bed provides a large surface area for microbial growth when the influent velocity is beyond the point of minimum fluidization. If particulate fluidization conditions are maintained, the bed expands smoothly without the violent, turbulent characteristics of gas/solid fluidization.
The specific surface area in a FBR is about 1000 m^2/m^3 of reactor volume, which is greater than any of the other fixed film packings. The fluid velocity within the necessary treatment retention times is provided by effluent recirculation. Hydraulic retention times in FBRs range from 3 to 20 min. Microorganisms grow as a biofilm on granular carrier material. This mode of operation allows the density of the biomass to be maintained at high concentrations without having to recycle solids as in the activated sludge process.

2.4.3.2 Biofilms in FBRs

Biofilm formation

Scientists in many different fields (biofouling, biocorrosion, bioconversion, medicine, limnology, etc) are studying biofilm growth because biofilms occur in virtually any system where microorganisms are present.
Biofilm development on solid surfaces is a series of complex but discrete and well-regulated steps:

1) Surface conditioning. Trace organics and not bacteria are the first to be associated with the surface of the carriers. After the solid surface comes in contact with water, an organic layer deposits on the water/solid interface (Mittelman, 1985a,b). A"conditioning layer" is formed by the organic substances and this neutralizes excessive surface charge and surface free energy that may prevent bacteria cells from attaching to the surface. The adsorbed organics are often used as substrate by bacteria.

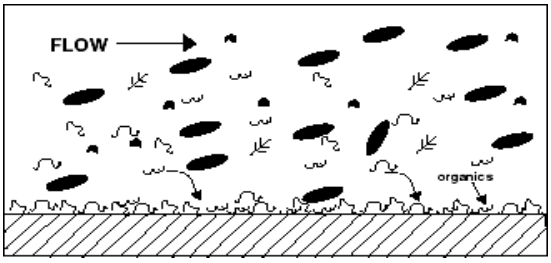


Figure 18. Adsorption of organic molecules on clean surfaces
(Charaklis and Marshall, 1990)

2) Adhesion of pioneer bacteria. Some of the bacteria from the water phase will approach the surface of carriers and become entrained within the boundary layer, the quiescent zone near the surface where flow velocity is close to zero. A fraction of these cells will adsorb to the surface for some finite time, and then desorb. This is called reversible adsorption. This initial attachment is based only on electrostatic attraction and physical forces and not on chemical interactions. Some of the adsorbed cells begin to form structures that enable them to adhere permanently to the surface. These cells become irreversibly adsorbed (Figure 19).

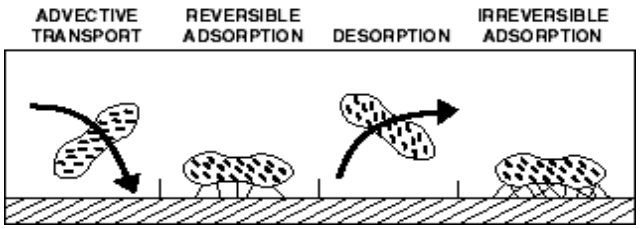


Figure 19. Transport of bacteria cells to the conditioned surface, adsorption, desorption, and irreversible adsorption (Characklis and Marshall, 1990)

3) Slime formation (Glycocalyx). Bacteria succeed in holding the biofilm together and cement it to a solid surface by excreting extracellular polymeric substances or sticky polymers. In addition, these polymers strands trap scarce nutrients and protect bacteria from biocides. According to Mittelman (1985a,b), "attachment is mediated by extracellular polymers that extend outward from the bacterial cell wall, much like the structure of a spider web. The pioneer cells proceed to reproduce and their daughter cells produce their own glycocalyx, greatly increasing the volume of ion exchange surface. A thriving colony is established in this way.

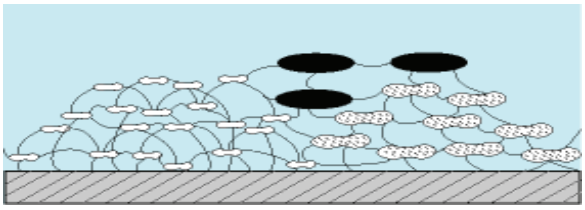


Figure 20. Biofilm made up of microbes and a "spider web" of extracellular polymers (Mayette, 1992)

The loosely organized glycocalyx matrix occupies 75-95% of a mature biofilm compared to bacterial cells which count only for 5-25% of the total volume (Geesey *et al*., 1994).

4) Secondary colonization. In addition to trapping nutrient molecules, the glycocalyx net also snares other types of microbial cells through physical restraint and electrostatic interaction. These secondary colonizers metabolize wastes from primary colonizers as well as produce their own waste that other cells use in turn. According to Borenstein (1994), these bacteria and fungi become associated with the surface following colonization by the pioneering species over a matter of days.

5) Fully functioning biofilm. The mature fully functioning biofilm is a complex metabolically cooperative made up of different species each living in a customized microniche. Different species live together in the biofilm helping each other to exploit food supplies and to resist antibiotics through neighbouring interactions. The different bacteria produce different enzymes that can break down compounds that no single species could digest alone. Biofilms are permeated at all levels by a network of channels through which water, bacterial waste, nutrients, enzymes, metabolites and oxygen travel in different directions. Gradients of chemicals and ions between micro zones provide the power to shunt the substances around the biofilm (Coghlan, 1996).

Biofilm modelling

Biofilm models are usually based on the concept of idealized biofilm consisting of a homogeneous matrix of bacteria and extracellular polymers. It is also assumed that the biofilm has a uniform cell density (X_f) and a locally uniform thickness.

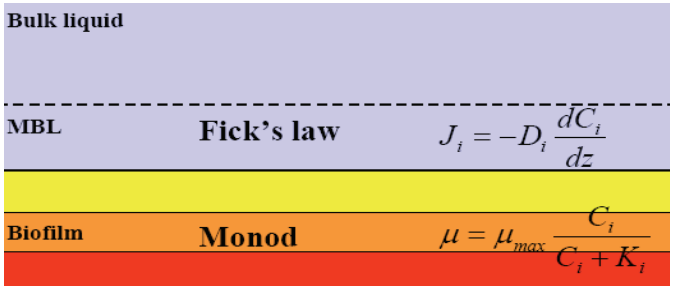


Figure 21. Mass transport and kinetics in a biofilm

Four processes are normally combined in biofilm kinetics:

1) External mass transport from the bulk liquid to the biofilm surface through diffusion layer;
2) Mass transport in the biofilm by molecular diffusion;
3) Biodegradation in biofilm;
4) Microbial growth in the biofilm.

An effective diffusion layer is used to model mass transport resistance from bulk liquid to the biofilm surface. Parameter *dz* is defined as the equivalent depth of liquid through which the actual mass transport can be described by molecular diffusion alone. Substrate flux (*J*) is determined by applying Fick's first law across the diffusion layer:

$$J = -D \frac{dC}{dz} \qquad (1)$$

where *D* is molecular diffusitivity in liquid and *dC* is the gradient of substrate concentration between the bulk liquid and the biofilm surface. The effect of external mass transfer (liquid film diffusion) is usually not significant and can be neglected (Christiansen *et al.* 1995)

It is assumed that substrate concentration within the biofilm changes only in the direction that is normal to the surface of the biofilm (noted with y in order to distinguish it from the depth of the effective diffusion layer described above). The following equation can be used to describe the steady-state substrate concentration within a differential section of biofilm when substrate utilization is according to the Monod relationship and mass transport is by molecular diffusion (Williamson and McCarty, 1976):

$$D_f \frac{\partial^2 S_f}{\partial y^2} = \frac{q_{\max} X_f C_f}{K_S + C_f} \qquad (2)$$

where D_f is molecular diffusivity in biofilm, C_f is substrate concentration within biofilm and X_f is average cell density in the biofilm. Molecular diffusion in biofilm matrix is about 50 to 80% of the value in pure water (Siegrist and Gujer, 1985).

Rittmann and McCarty (1980) described the net growth rate of bacterial mass for a differential section of ideal biofilm:

$$\frac{\partial AX_f dy}{\partial t} = Y \frac{q_{\max} C_f}{K_S + C_f} AX_f dy - k_d AX_f dy \qquad (3)$$

where A is biofilm surface area. The use of biofilm models is complicated due to nonlinear character of the equations and requires graphical or numerical methods. Diffusion can be neglected for a thin biofilm and this simplifies kinetic modelling (Wang *et al*., 1986; La Motta and Cascante, 1996).

Biofilm structure is not ideal as normally assumed in models. Thin biofilms and partially covered carriers are the result of low surface substrate loading rate and high detachment forces. Rough and weak biofilms develop with protruding elements with high surface substrate loading and low detachment forces. Diffusion in the biofilm matrix is increased by irregularities on biofilm surface because turbulent zone reaches the biofilm surface and produces eddy diffusion.
Figure 22 indicates how the biofilm morphology is influenced by detachment forces and substrate loading in FBR (Tijhuis *et al.*, 1996).

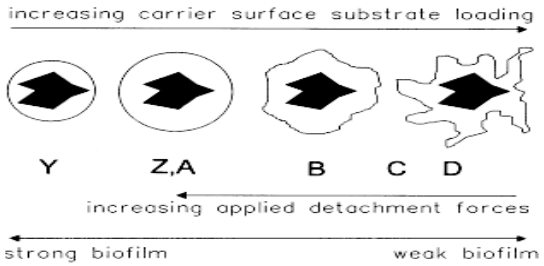


Figure 22. Influence of substrate loading and shear forces on the appearance of biofilms on fluidized carriers (Tijhuis et al. 1996)

Voice *et al*. (1992) studied the development of a biofilm on non-adsorbing and adsorbing media used in FBR systems for the treatment of water contaminated with BTX. The investigation of the media surfaces was done by SEM (scanning electron microscopy). The biofilm growth was faster on the particles with adsorptive properties (GAC) and this was proven by visual observation and data collected on oxygen consumption and BTX concentrations in the effluent. This observation is consistent with previous findings showing that media with adsorptive properties support richer microbial communities than material with non-adsorptive properties (Pirbazari *et al.*, 1990). It is believed that this results from the ability of carbon to concentrate chemicals necessary for microbial growth while providing an environment that is well protected from fluid shear forces for cell attachment. Since the activated and non-activated

carbons were observed to have similar surface textures, it is unlikely that the differences can be explained by shear-force protection alone.

The understanding of biofilm structure was enhanced after the utilization of new methods such as confocal scanning laser microscopy (CSLM) was introduced in biofilm studies. These investigations showed that the structure of biofilm is more complicated than usually assumed from scanning electron microscopy.

Massol-Deyá *et al*. (1995) used CSLM to study petroleum-degrading biofilms from laboratory and field-scale FBRs and demonstrated that the classical model of random growth patterns leading to a uniform biofilm structure with active cells at the surface was not supported. It was found that there was a base film of 10 to 75 μm on which the structured, more porous film was built. Interaggregate channels in a mosaic pattern that extended into biofilms having thicknesses of 20 to 300 μm were as well observed. This biofilm structure was believed to facilitate transport of nutrients, substrates, and gases deeper into the matrix than is possible by diffusion alone.

Several researches (Costerton *et al*., 1994) updated the concept of biofilm based on the results obtained by CSLM and other new methods (Figure 23). The new model shows that biofilms consist of slimy extrusions penetrating upwards from the carrier surface. Oxygen and nutrients can diffuse in the interstitial water between the extrusions. New challenges are raised by this new concept for biofilm modelling, especially taking into account the tolerance of biofilms towards toxic compounds.

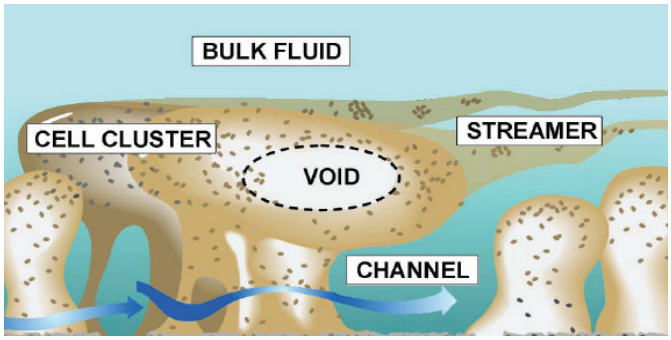


Figure 23. Current biofilm model (Costerton *et al.*, 1994)

2.4.3.3 Mechanisms of BTX removal in a FBR

Bioregeneration is the recovery of sorption capacity of a sorbent by microbial activities (Aktaş and Çeçen, 2007). The achievement of bioregeneration process has the following microbiological and technological preconditions (Klimenko *et al*., 2003):

- Reversibility of sorption process;
- Presence of a microbial culture capable of utilizing the sorbed pollutants as carbon source;

- Presence of sufficient amounts of mineral compounds (nitrogen, phosphorus, sulphur and other microelements) necessary for a normal metabolism of the sorbate;
- Optimum conditions for microbial activity (oxygen concentration, temperature, pH, etc);
- Optimum proportion between the concentrations of microorganisms and the sorbed compounds.

Two mechanisms are usually proposed in the literature to explain the bioregeneration process (Aktaş and Çeçen, 2007). The first one involves desorption of the sorbed pollutant molecules due to a concentration gradient between the sorbent surface and bulk liquid. This concentration gradient is caused by the activity of microorganisms present as a biofilm onto the surface of the sorbent or in the bulk liquid. The desorbed molecules pass through the biofilm where they undergo partial or complete mineralization. A new portion of sorbate molecules (substrate) passes through the biofilm towards the sorbent from aqueous solution. A part of these molecules is degraded as well and then an additional amount of molecules can sorb onto the sorbent surface. This amount is equivalent to the bioregenerated sorbent surface. This mechanism also involves the difference in the Gibbs free energy between the molecules in solutions and the sorbed molecules that was indicated to be a driving force for bioregeneration (Klimenko *et al*., 2002).

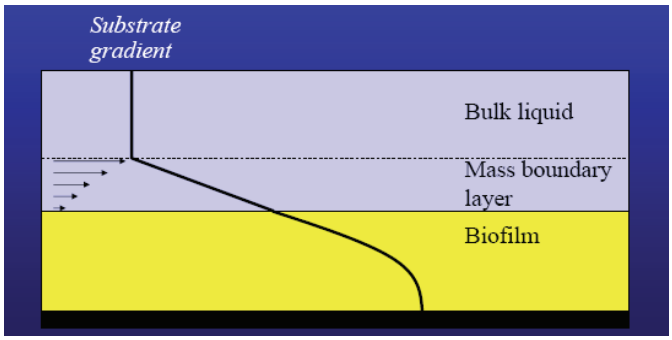


Figure 24. Classical biofilm concept

The second mechanism involves extracellular enzymes. According to this theory the microorganisms excrete exoenzymes which diffuse into the pores of the sorbent (interlammelar spaces in the case of the organoclay) and react with the sorbed substrates. The hydrolitic decay of the substrate may occur or desorption of the resulting enzyme metabolite may take place due to weaker sorbability of metabolite. Andrews and Tien (1980) suggested that the low diffusivity of large hydrolitic enzymes into the pores would lead to a slow bioregeneration process.

Available data do not allow determining if the bioregeneration phenomenon depends solely on a mechanism involving desorption or if it also involves exoenzymes activities. Further work is necessary to support either one of these hypotheses.

2.4.3.4 FBRs in treatment of organics

Long solids retention times or cell residence times in the bioreactor, corresponding to a slow specific growth rate of the microbial population is required for the microbial degradation of complex organics. Efficient removal of complex organic compounds at a short liquid contact time or hydraulic retention time relative to more conventional biological systems is enhanced by the accumulation of high biomass concentrations. The chances of inhibition due to toxic or inhibitory feed inputs are minimized by the long SRT attainable in FBRs. The use of a sorbent as packing material provides additional benefits including more rapid initial removal upon start-up and a greater removal of slowly degradable or recalcitrant compounds. The pollutants are removed by sorption during the start-up period and concentrated on the surface of the sorbent. With microbial growth, biodegradation becomes an important removal mechanism in addition to extending the life of the sorption media through bioregeneration.

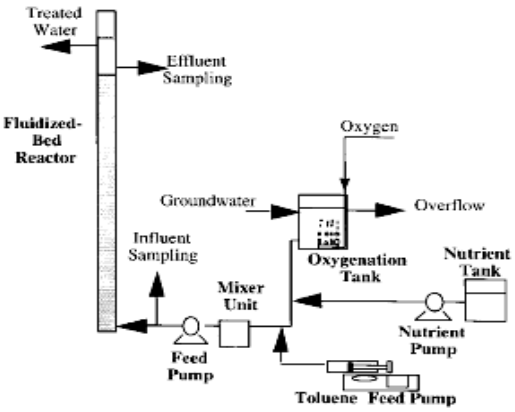


Figure 25. Schematic of experimental GAC-FBR system (Zhao *et al*. 1999)

For aerobic processes, oxygenation is done by passing the influent through an oxygenation tank to pre-dissolve oxygen. Adding air to the fluidized bed reactor would discharge packing to the effluent. For municipal wastewater treatment, FBRs have been mainly used for post-dinitrification. Aerobic FBRs are frequently used to treat groundwater contaminated with hazardous substances. Both adsorption and biological degradation are usually achieved by employing activated carbon as packing in these kinds of applications (Sutton and Mishra, 1994). FBR technology has the following main advantages for the treatment of hazardous substances:

- Reduced reactor size due to the increased volumetric treatment efficiency associated with the development of high biomass concentrations;
- Long sludge retention time (SRT) for microorganisms necessary to degrade the xenobiotic and toxic compounds;
- Sorption of shock loads or non-biodegradable toxic compounds onto the carrier material;

- High quality effluent with low concentrations of total suspended solids (TSS) and chemical oxygen demand (COD);
- Oxygenation method prevents stripping and emission of toxic organic compounds to the atmosphere;
- Simple and reliable operation.

Verheul *et al.* (1994) stated that a biological process for elimination of organic contaminants has to meet the following three criteria:

1) Achieve a low concentration of pollutant in effluent;
2) Removal must be mainly through degradation and not through volatilization or adsorption;
3) High mean transformation rates must be attained in the reactor.

FBRs are known for providing low effluent concentrations for the treatment of organic pollutants. Utilization of internal oxygenation systems decreases the amount of air emissions from FBRs treating volatile organics. Grey *et al*. (1995) did not detect any off-gas flows of contaminants during treatment of groundwater polluted with PAHs and BTEX from a manufactured gas plant (MGP) site by a pilot plant FBR. Reported concentration of volatile organic compounds in gas samples was less than 1 mg/l. Biodegradation is the major removal mechanism after the biofilm has been established, even when GAC is used as carrier in the FBR.

The conditions that must be fulfilled to get a high transformation rate in a biofilm reactor are (Verheul *et al*., 1994):

1) High biomass concentration in the reactor;
2) Development of a biofilm with large specific surface area to decrease biofilm thickness and therefore diffusion limitation in biofilm;
3) High substrate concentration at the surface of the biofilm.

FBRs are normally characterized by high biomass concentrations and large surface areas. A high substrate concentration at the biofilm surface can be attained by operating the reactor in a plug-flow mode. Voice *et al* (1992) degraded BTX compounds in a GAC- FBR system obtaining high removal rates at only 3.3 min hydraulic retention time (HRT). This is achievable in aerobic FBRs when influent concentrations of organics are low and enough oxygen can be dissolved without oxygenation in recycling system. Recycle ratios can be adjusted in order to get the desired amount of mixing. FBRs have been extensively used in bioremediation. Table 20 lists several applications of FBRs in treatment of organics.

Table 20. BTEX removal from polluted water through FBRs

Source of pollutant(s)	Type of reactor	Analytical method	Results	Remark(s)	References
BTEX, phenols, PAH (coal tar)	FBR Media: activated carbon Inoculum: from water polluted with coal tar	pH, phenols by HPLC, BTEX by GC/MS (purge and trap), COD, TSS, PAHs, NH_3	Pollutants removal: > 90% HRT: 3-26 h	No data about stripping of aromatic compounds by aeration and adsorption of pollutants on activated carbon	Guerin (2002)
BTEX and MTBE	Aerobic FBR; V = 7.88 l, T = 20 °C, pH = 7.4-7.9 Media: GAC Inoculum: from a mixed culture grown on MTBE as sole carbon source	pH, DO, COD, TOC, BTEX and MTBE by GC/FID (purge and trap)	Influent: 2 mg/l each of the BTEX; Effluent: 1.4-2.2 mg/l; Addition of BTEX compounds did not inhibit MTBE degradation	No data about stripping of aromatic compounds by aeration and adsorption of pollutants on activated carbon	Pruden *et al.* (2003)
TEX and chlorinated ethylene (paint contaminated site)	Two stage FBR (5 l each reactor); T = 30 °C; DO = 5-6 mg/l; Media: polyurethane foam cubes (surface area 1000 m^2/m^3); Inoculum: sludge from a pilot plant treating groundwater.	GC/FID (head space)	One stage FBR pilot was not enough to treat the contaminated groundwater; HRT = 12 h Influent: 1.2 mg/l toluene, 0.65 mg/l ethylbenzene; 1.75 mg/l p-xylene and m-xylene and 0.17 mg/l o-xylene; Removal efficiency > 98%.	Pure oxygen supplied to bioreactors to prevent contaminants loss by stripping; Adsorption of pollutants onto solid supports is not studied.	Ohlen *et al.* (2005)
BTEX (contaminated groundwater)	Aerobic FBR (4320 gallons/day); Media: activated carbon This system contained secondary suspended solids filtration and activated carbon polishing.	Not mentioned	Over 3 months operation with BTEX contaminated groundwater; Removal efficiency > 98% of BTEX in the main bioreactor.	No data about stripping of aromatic compounds by aeration and adsorption of pollutants on activated carbon	Spectrum-environmental services, Inc (1996)

Source of pollutant(s)	Type of reactor	Analytical method	Results	Remark(s)	References
Toluene (synthetic)	Aerobic FBR; V = 14.7 l; pH = 8 T = 20 °C Media: GAC (0.9 mm) Inoculum: from a bioreactor supplied with toluene for 1 year	Toluene by GC/FID	Adsorption capacity > 70% of initial levels during the first 2 months; Adsorption capacity after 6 months operation was 40%, 52% and 57% of the initial value for equilibrium toluene concentrations of 0.1, 3 and 3 mg/l respectively; No correlation between the thickness of the biofilm and the residual adsorptive capacity.	Pure oxygen was used for oxygenation and toluene stripping was minimized; Adsorption of toluene by GAC was studied.	Zhao et al. (1999)

The above table (Table 20) shows that GAC is used as biomass carrier in most of FBRs. The advantages of GAC as biomass carrier were demonstrated by Voice *et al*. (1992, 1995) who studied the treatment of groundwater contaminated with BTX in three FBRs with (1) adsorptive removal capacity, only using GAC without microorganisms, (2) combined biological and adsorptive removal mechanisms, using GAC covered by a microbial biofilm and (3) biological removal mechanism, using non-activated carbon (without adsorptive properties) covered by a microbial biofilm. The influent BTX concentrations to all three systems were initially 1 mg/l each and the HRT was between 3.3 and 3.6 min. Removal efficiencies of BTX compounds in the FBR combining both mechanisms were 92 to 99.7% at a HRT of 3.3 min and at a loading rate of 3.8 kg COD/m^3-day. Results showed that the system with combined removal mechanism released much less BTX during the start-up of the biological systems and the growth of the biofilm was more rapid as compared to biological non-adsorptive system. Immediately after the start-up period, the effluent quality of the adsorption only unit started to deteriorate showing that biological activity was necessary to maintain good effluent quality. Under steady-state conditions, BTX removal efficiencies were similar for both biological systems. An enhanced BTX removal and more stable operation during 3 and 6 fold step-load increases of influent BTX concentrations was observed in the system utilizing both adsorption and biodegradation as removal mechanisms. Thus, it was concluded that the FBR using GAC as carrier is less likely to be affected by process disturbances. Bioregeneration of GAC was observed in the system combining adsorption and biodegradation processes, since microorganisms seemed to be able to utilize some of the adsorbed BTX after a period of increased organic loading rate in the FBR treating BTX polluted water.

Zhao *et al*. (1999) claimed that the adsorption capacity of biocoated carbon remained at greater than 70% of initial levels during the first two months. After six months of operation, the remaining capacity was still approximately 40, 52 and 57% of the initial value for equilibrium toluene concentrations of 0.1, 3 and 10 mg/l, respectively.

Hickey *et al.* (1995) attained 99.7% PAHs removal in a pilot scale FBR at an influent concentration of 6.2 mg/l and HRT of 6 to 7 min; the organic loading rate was 4.6 kg COD/m^3-day. It was estimated that after treating for 7 months the PAH containing wastewater, about 1.2% of removed PAHs were retained on the GAC and the rest was biodegraded.

Jackson and Hayes (1994) used a HRT of only 3.3 min to obtain 50% reduction of TCE (trichloroethylene) at influent concentration of 430 µg/l in a methanotrophic FBR.

Concentrations of non-biodegradable toxic organic compounds can be kept at low levels by GAC in FBRs to prevent toxic effects on microorganisms (Pfeffer and Suidan, 1989). This is normally achieved by performing frequent replacements of small portions of the GAC in order not to lose too much biomass and to maintain long enough solids retention time. Anthracite was used instead of GAC as biomass carrier in anaerobic FBR to decrease toxicity of 2,4,6-TCP (trichlorophenol) by Petrozzi *et al*. (1993). 2,4,6-TCP desorbed easier from anthracite after toxic loading and therefore it was regenerated faster. The main drawbacks of GAC are: it is expensive and susceptible to attrition, especially by biomass removal systems, and therefore requires replenishing (Folsom *et al.*, 1995).

In the last years, FBRs were employed in increasing numbers for the treatment of organic pollutants. Since FBRs can be used to obtain high effluent qualities with high

loading rates, they are likely to become more common in bioremediation in the near future. Relatively small reactors can be used because FBRs attain high volumetric removal rates.

2.4.3.5 Operation parameters of FBRs

Sutton and Tracy (1989) set up a strategy for start-up and subsequent equilibrium operation of GAC-FBR systems. This is as follows:

1. An enriched microbial seed is added to the reactor which is operated on total recycle (batch mode) or at low feed rate in order to attain a high concentration of biomass and to achieve a significant degradation of the biodegradable material;
2. When adsorbable material starts to pass through the system due to increase in feed rate, the replacement of the packing material should start. The rate of biomass buildup in the reactor may limit the GAC replacement rate. The reactor SRT must not be reduced below the critical level and therefore the replacement rate of GAC has to be carefully controlled. The rate of GAC replacement can be increased if necessary, when biomass concentration in the reactor increases.
3. When the biomass concentration reaches a "steady state", the rate of GAC replacement will be reduced as biodegradation has reached its maximum level and the GAC life is increased by bioregeneration.

If the concentration of adsorbable material is low and/or the capacity of GAC is high, the biomass in the reactor may become fully established before GAC replacement becomes necessary. Step 2 mentioned above is not required in such situations. If all the adsorbable material is biodegradable, GAC replacement may not be necessary at all. Adsorption capacity of GAC tends to moderate changes in feed substrate concentrations to the FBR, once "steady state" conditions have been reached. The adsorption process would preserve effluent quality during shock loads until the microbial population increases to accommodate the load increase.

The most important parameters in fluidized bed operation are (Tchobanoglous, 1998; Langwaldt and Puhakka, 2000; Farhadian *et al*., 2006):

- Temperature: rate of biological reaction in mesophilic conditions increases by a factor of 2 for each 10 °C rise in temperature. On the other hand, volatilization of BTX compounds is favoured by an increase in temperature;
- Hydraulic retention time (HRT = V/Q): for an effective process control, a flexible handling of influent flow rates is required;
- Influent quality: efficiency of bioreactors is strongly affected by variation in influent quality. Volatilization of VOC compounds is normally inhibited by the presence of dissolved chemicals such as organic compounds and salts and increased by mixing and thermal energy;
- Flow regime: can impact on efficiency of bioreactors that can be set up to operate as plug flow or completely mixed reactors;
- Biomass: potential problems may be created by the attachment and detachment of biomass. Uncontrolled growth of biomass results in large oxygen demands. Start-up and efficiency of FBRs are influenced by the quantities and qualities of biomass applied as inoculum;

- Nutrient requirements: depend on the type of contaminants treated; macronutrients (such as N, P) and micronutrients (such as trace elements) must be supplied proportional to the COD in contaminated water and elemental biomass composition;
- pH: control of pH is of significant importance since contaminated water may not have adequate buffer capacity;
- Electron acceptor: aerobic condition – O_2; anaerobic condition – compounds such as NO_3^- and SO_4^{2-};
- Packing materials: efficiency of FBRs is strongly influenced by the properties of the carrier material (surface area, porosity and nature); estimation of sorption and desorption capacity of media as well as bioregenerability of packing must be taken into account in biofilm reactors;
- OLR = QS/V: organic loading rate can determine the capacity of bioreactor for organic pollutant removal;
- F/M = QS/VX: food to microorganisms ratio is important, since it has a significant impact on the efficiency of biological systems;
- Recycle ratio (Q_R/Q): recycle flow may be necessary for the fluidization of the packing material;
- Gas control: gas produced in bioreactors must be controlled. Diffusion and stripping of volatile organic compounds by aeration must be addressed in aerobic reactors. In anaerobic conditions, the biogas produced can evaporate and strip BTX from the liquid phase.

2.4.3.6 Bioregeneration methods

Bioregeneration has been reported in two different treatment system configurations:

- Simultaneous sorption and biodegradation, which occur during the continuous operation of BAC-FBR treatment systems (Ha *et al*., 2000; Vinitnantharat *et al*., 2001);
- Off-line bioregeneration which involves removal of sorbed organic matter from loaded sorbents through desorption and biodegradation occurring inside a closed batch system (Goeddertz *et al*., 1988; Roy *et al*., 1999; Silva *et al*., 2004);

Bioregeneration can be optimized by varying the nature of microorganisms, the environmental conditions and the loading on sorbent (Vinitnantharat *et al*., 2001).

Simultaneous sorption and biodegradation

Some researchers have claimed that bioregeneration of activated carbon occurs simultaneously to wastewater treatment during powder activated carbon (PAC) processes (Kim *et al*., 1997; Seo *et al*., 1997). Other reports contradicted this hypothesis and pretended that the process is a simple combination of adsorption and biodegradation (Xiaojian *et al*., 1991; Çeçen, 1994; Bornhardt *et al*., 1997; Garner *et al*., 2001) with no bioregeneration of activated carbon occurring.
Several other authors (Ha *et al*., 2000; Vinitnantharat *et al*., 2001; Badriyha *et al*., 2003) reported the regeneration of activated carbon occurring simultaneously to wastewater

treatment in biological activated carbon (BAC) processes. BAC systems involve GAC particles covered with a biofilm and are usually employed in fluidized bed reactors. Sirotkin *et al*. (2001) suggested three phases to describe the BAC treatment processes:

1) The initial phase consists of the preferential adsorption of contaminants from wastewater to GAC, when the adsorption rate surpasses considerably the biodegradation rate;
2) The second phase corresponds to the primary adsorption equilibrium, when the rate of adsorption and biodegradation are comparable;
3) The third phase consists of dynamic conditions where the biodegradation rate surpasses the adsorption rate and desorption from pores might occur resulting in regeneration of activated carbon.

In BAC systems, biofouling caused by excessive microbial growth can considerably hamper the bioregeneration process (Vuoriranta and Remo, 1994; Scholz and Martin, 1997).

Off-line bioregeneration

This type of bioregeneration involves removal of sorbed organic matter from loaded sorbents by desorption and biodegradation taking place in a closed batch system. Off-line bioregeneration of spent sorption media is carried out in reactors, in which a mixture of acclimated bacteria, nutrients and dissolved oxygen are recirculated to biodegrade sorbed organic matter (Goeddertz *et al*., 1988).
Investigations involving off-line bioregeneration were used to draw most of the conclusions on bioregeneration processes, since it is difficult to differentiate between sorption/desorption and biodegradation, when they occur simultaneously. Therefore, off-line bioregeneration processes are usually preferred to simultaneous sorption and biodegradation processes occurring in biological activated carbon (BAC) treatment for availability of nutrients and dissolved oxygen, and for avoiding problems such as persistence of many organic compounds and operational difficulties including hydraulic short-circuiting and excessive head loss. Kolb and Wilderer (1997) showed that an off-line BAC bioregeneration process was more efficient than a simultaneous BAC operation.

2.5 Hypotheses

2.5.1 Hypotheses referring to sorption of target compounds

2.5.1.1 Selective sorption of target compounds can be achieved

One of the key issues of the project is to identify sorbents with high selectivity for the target soluble compounds and little or no sorption for organic acids. Failure of the treatment process would occur if such sorbents are not available on the market or are not identified during screening tests since this would impact on the biological treatment that would probably be unfeasible if selective sorption was not achieved.

2.5.1.2 Sorption media are not contaminated with other compounds

Contamination with other compounds present in produced water (oil residues, soluble microbial products, production chemicals, and salts) may affect the selectivity and sorption capacity of sorbents. This would cause a reduction of the total removal capacity of the sorption media leading to no compliance with respect to project effluent targets.

2.5.1.3 Good sorption kinetics can be attained

The proposed treatment concept assumes that the target dissolved compounds are removed during a short contact time (10-15 minutes) between the sorbent and the produced water stream. Sorption kinetics (and its changes due to operating conditions like temperature for example) is a key issue of the treatment process, since any rates decrease would result in a poor performance of the treatment system.

2.5.1.4 Sorbent properties do not change over time (ageing)

Surface chemistry and available surface area are the main factors influencing sorption capacity of sorbents. Friction or wear that might occur at different stages of the treatment (for example during separation in hydrocyclones) can alter surface area of sorption media. Even if sorbents with modified surface are used, surface chemistry and surface activity remain critical parameters. It is expected that any degradation caused by ageing or surface wear would lead to a loss of sorption capacity.

2.5.2 Hypotheses referring to sorbent regeneration

2.5.2.1 Efficient bioregeneration can be achieved

Another key issue of the project is that biomass does not feed on other compounds or on the sorbents themselves (especially if they have a modified surface), leaving the BTX compounds undegraded. Treatment performance would be strongly affected if efficient biodegradation cannot be achieved and the process must be re-designed to compensate for that.

2.5.2.2 Good biodegradation kinetics can be attained

Degradation kinetics is a fundamental design parameter of the treatment process. Produced water properties such as temperature, pH, salinity or heavy metals content have a significant impact on degradation kinetics. Therefore, investigations should be carried out in order to define the process window where the bioregeneration reactor shows an adequate performance.

2.5.2.3 Microbial culture is resistant against toxic shocks

The growth and development of the microbial culture employed to degrade BTX compounds depends on specific conditions. It is believed that if the incoming produced water contains very high loads of BTX compounds, the biomass could be killed or severely weakened. Therefore, the impact of high concentrations of these dissolved compounds on the efficiency of biological treatment should be evaluated.

2.6 Expected performance

Attaining selective sorption of the target dissolved compounds and little or no sorption of organic acids is a key issue due to the high concentrations of organic acids in produced water. If this is achieved, the organic loading on the subsequent bioregeneration process would be significantly decreased, since the organic loading provided by the dissolved aromatic compounds is relatively low.

Produced water quality after hydrocyclone treatment and a discharge flow of 8000 m^3/day, which is close to the average flow of produced water discharges from Norwegian offshore installations (data reported in 2004), were taken into account for the calculation of the expected performance (Helness, 2005 and 2006). In this case, the soluble compounds correspond to a soluble COD concentration of ~35 mg SCOD/l. For a produced water flow of 8000 m^3/d, the SCOD loading on an in-line sorption process with biological regeneration would be 280 kg SCOD/d. The required reactor volume for biological regeneration will be 47 m^3 if a removal rate of 6 kg SCOD/m^3-day is considered. A HRT of 8.5 minutes is obtained, if the same volume is considered in the sorption part of the process.

Based on literature review, the following assumptions are made for the in-line sorption and biological regeneration treatment processes (Helness, 2005 and 2006):

- Biological degradation rate in FBR: 0.3 g COD/g VS-d;
- Biomass concentration in FBR: 20 kg VS/m^3 reactor;
- Nutrient requirement: COD/N/P: 100/5/1;
- Waste sludge can be safely discharged into the sea without further treatment;
- Filling of sorption media in FBR: 50%;
- Sorption capacity of sorbent: 0.4 g SCOD/g sorbent;
- Required HRT in the sorption step if 50% filling of sorbent is employed: 1/3 of HRT in FBR.

It is expected that the sludge amounts generated by the biological regeneration process are significantly lower than for a biological process treating the main produced water flow. Membrane contactors could be employed to provide bubble free aeration in order to avoid stripping of volatile compounds.

3. THEORY OF SORPTION AND BIODEGRADATION OF DISSOLVED COMPOUNDS IN A FBR

3.1 Sorption process

3.1.1 Sorption isotherms

An adsorption isotherm describes the relation between the amount or concentration of adsorbate that accumulates on the adsorbent and the equilibrium concentration of dissolved adsorbate. Although adsorption can be irreversible, an adsorption isotherm is an expression of the principle of microscopic reversibility. Many isotherm models have been described in the literature and some of them will be discussed below.

3.1.1.1 Langmuir isotherm

It has been successfully applied to fit the sorption of many pollutants such as benzene, toluene etc. A basic assumption of Langmuir isotherm is that sorption takes place at specific homogeneous sites in the sorbent. It is considered that no further sorption can take place at a site after it is occupied by a solute. The Langmuir adsorption isotherm (Langmuir, 1916) is expressed as follows:

$$S_e = \frac{K_L S_m C_e}{1 + K_L C_e} \qquad (4)$$

where S_e is the amount adsorbed on solid at equilibrium (mg/kg), C_e is the equilibrium liquid concentration (mg/l), S_m is the adsorption capacity of the adsorption maximum (mg/kg), and K_L is the adsorption intensity or Langmuir coefficient (l/mg). Equation (4) can be rearranged to the following form:

$$\frac{C_e}{S_e} = \frac{1}{K_L S_m} + \frac{C_e}{S_m} \qquad (5)$$

3.1.1.2 Brunauer, Emmett, Teller (BET) isotherm

This isotherm has been developed from theory as an extension to the Langmuir isotherm for the case where multilayer adsorption was occurring. It is formulated as follows:

$$q_e = \frac{BCQ_0}{(C_S - C)\left[1 + \frac{(B-1)C}{C_S}\right]} \qquad (6)$$

where q_e is the final (equilibrium) solid phase concentration of the contaminant on the sorbent, B and Q_0 are constants and C_S is the saturation concentration of solute in water (Droste, 1994).

3.1.1.3 Freundlich isotherm

In 1906, Freundlich reported that if the concentration in solution at equilibrium, C_e, was increased to the power of $1/n$, the amount of solute sorbed being S_e, then the $C_e^{1/n}/S_e$ was constant at a fixed temperature (Freundlich, 1906). The main applications of this experimental model are especially on non-ideal sorption on heterogeneous surfaces as well as multi-layer sorption. It is expressed by the following equation:

$$S_e = K_F C_e^{1/n} \qquad (7)$$

The Freundlich isotherm has been derived by assuming an exponentially decaying sorption site energy distribution. By taking the logarithm of both sides, this equation can be rearranged in the linear form as follows:

$$\log S_e = \log K_F + \frac{1}{n} \log C_e \qquad (8)$$

3.1.1.4 Redlich-Peterson isotherm

This isotherm (Redlich, 1959) incorporates the features of both the Langmuir and Freundlich equations and is described by three parameters. It has a linear dependence on concentration in the numerator and an exponential function in the denominator and can be expressed as follows:

$$S_e = \frac{AC_e}{1 + BC_e^g} \qquad (9)$$

where A, B and g ($0 < g < 1$) are the isotherm constants. At high concentrations, the Redlich-Peterson isotherm approaches Freundlich isotherm, while at low concentrations it is similar to Henry's law. It has two limiting cases that can be summarized as follows:

$$S_e = \frac{AC_e}{1 + BC_e} \qquad (10)$$

where $g = 1$, i.e. the Langmuir form results.

$$S_e = \frac{AC_e}{1 + B} \qquad (11)$$

where $g = 0$, i.e. the Henry's law form results. In order to obtain values for the isotherm constants, a trial and error procedure has previously been applied to a pseudo-linear form of Redlich–Peterson isotherm, although a linear analysis is not possible for a three-parameter isotherm. By calculating the logarithms, equation *(9)* can be converted to a pseudo-linear form:

$$\ln\left(A\frac{C_e}{S_e}-1\right)=g\ln(C_e)+\ln(B) \qquad (12)$$

Isotherm parameter A is varied in this method in order to obtain the maximum value of correlation coefficient for the linear regression of $\ln(C_e)$ against $\ln(A(C_e/S_e)-1)$.

3.1.1.5 Linear sorption isotherm

The sorption of non-ionic organic contaminants (NOCs) to an organic rich substrate can follow a linear sorption isotherm or Henry's law in the following form:

$$C_S = K_d C_W \qquad (13)$$

where C_S is the amount of solute sorbed (mg/kg), C_W the equilibrium aqueous solute concentration (mg/l), and K_d is the linear sorption coefficient. K_d is related to K_{OC}, the organic carbon-based partition coefficient of the sorbed compound, and $f$oc the fractional organic carbon content of the sorbent, by the following equation:

$$K_d = K_{OC} f_{OC} \qquad (14)$$

K_{OC} is a reasonable constant for a certain sorbed compound, and thus, K_d increases linearly with respect to f_{OC}. In a partitioning process, the sorbed compound distributes itself between two immiscible phases. The partition coefficient K_{OC} reflects the ratio of sorbate concentrations in the organic phase (C_{OC}) and in the aqueous phase (C_W):

$$K_{OC} = \frac{C_{OC}}{C_W} \qquad (15)$$

3.1.1.6 Dubinin-Radushkevich equation

This equation is defined as:

$$q_e = q_m \exp(\beta\varepsilon^2) \qquad (16)$$

where q_e is the amount of solute sorbed at equilibrium, q_m is the theoretical saturation capacity, β is a constant related to the adsorption energy, and ε is the Polanyi adsorption potential.

3.1.1.7 Sorption isotherms of BTX

Li *et al.* (2000) studied the sorption isotherms of benzene, phenol and aniline by a surfactant-modified zeolite (SMZ) at different hexadecyltrimethyl ammonium concentrations (HDTMA) surface coverages. It was noticed that all sorption isotherms were linear and could be described by a distribution coefficient (K_d). The K_d values of benzene, phenol, and aniline on SMZ increased with HDTMA loading, up to monolayer coverage at neutral pH, where all species exist primarily in their neutral forms. Further increases in HDTMA loading did not increase the K_d values of the solutes, beyond monolayer coverage. Since sorption of neutral species was primarily by partitioning into the bound HDTMA organic pseudo phase, the K_d values were consistent with the relative octanol/water partition coefficients of the three compounds. Results indicated that sorption of target species can be maximized by tailoring the HDTMA surface coverage.

Fuierer and Bowman (2001) studied the sorption and microbial degradation of toluene on a surfactant-modified zeolite support. The toluene sorption isotherm was linear, reflecting a partitioning type mechanism. Linear sorption isotherms were also observed for other nonpolar organics (Bowman *et al.*, 1995).

Alther (2002) investigated the sorption of BTX and naphthalene on an organoclay. The surfactant used to modify the surface of the clay was dimethyl dihydrogenated ammonium chloride. The sorption of BTX was fitted well to a linear sorption isotherm. It was observed that the changes in pH did not affect the sorption of BTEX onto organoclay, an important characteristic of partition removal mechanisms. Column sorption tests were also performed and the results indicated that benzene was the first to elute out of the column, followed by toluene and naphthalene.
Gitipour *et al*. (1997a) obtained as well linear isotherms for the sorption of BTEX onto a similar organoclay (using dimethyl dihydrogenated ammonium chloride for surface modification).

Fuller *et al*. (2007) studied the influence of quaternary alkylammonium amendment length on sorption mechanisms of modified bentonites for four non-ionic organic compounds: benzene, carbon tetrachloride, TCE, and 1, 2-DCB. A linear rather than a curvilinear isotherm was determined for three- and four-carbon-chain functional groups on the ammonium cation.
Tetrapropyl and tetrabutyl ammonium clays showed a non-competitive uptake in binary system, which was not significantly influenced by temperature fluctuations. Sorption was characterized by linear isotherms that indicated a partitioning uptake mechanism for these organoclays.
The Dubinin–Radushkevich equation has been used to fit the adsorptive organoclays (tetramethyl and tetraethyl ammonium clays) in order to study the application of the Polanyi–Manes potential theory to organoclay adsorption. Results demonstrated that carbon tetrachloride and TCE, which have similar physical and chemical characteristics, behaved according to the Polanyi–Manes theory.

3.1.2 Sorption kinetics

The global kinetic expressions such as Lagergren pseudo-first-order, pseudo-second-order, and Elovich rate equations are usually employed to describe the adsorption kinetics.

3.1.2.1 Pseudo-first-order process

The first-order equation for the sorption kinetics of a solute from a liquid solution was given by Lagergren (1989):

$$\frac{dq_t}{dt} = k_{e1}(q_e - q_t) \qquad (17)$$

where q_e is the amount of solute sorbed at equilibrium (mg/g); q_t is the amount of solute sorbed at time t (mg/g); and k_{e1} is the equilibrium rate constant of pseudo-first-order sorption (l/h). Integrating equation *(17)* for the boundary conditions $t = 0$ to $t = t$ and $q_t = q_t$ gives (Ho and McKay, 1999):

$$q_t = q_e - q_e \exp(-k_{e1}t) \qquad (18)$$

Equation *(18)* expresses the integrated rate law for pseudo first-order reaction.

3.1.2.2 Pseudo-second-order process

Ho *et al*. (1996) described the kinetics of heavy metals sorption on peat by a pseudo second-order reaction rate equation:

$$\frac{t}{q_t} = \frac{1}{k_{e2}q_e^{\,2}} + \frac{t}{q_e} \qquad (19)$$

where k_{e2} is the second-order reaction rate constant for sorption. These two models are widely used in the analysis of sorption kinetics of various substances by different sorbents.

3.1.2.3 Elovich rate equation

The Elovich equation is as follows:

$$\frac{dq_t}{dt} = a\exp(-bq_t) \qquad (20)$$

Integrating equation *(20)* with the conditions (q_t = 0 at t = 0; q_t = q_t at t = t) and subsequently linearizing the integrated equation will lead to the following mathematical expression:

$$q_t = \frac{1}{b}\ln(ab) + \frac{1}{b}\ln(t + t_0) \qquad (21)$$

where a and b are the parameters of the Elovich rate equation; t_0 is equal to $1/(ab)$.
If $abt >> 1$, equation *(21)* can further be simplified as:

$$q_t = \frac{1}{b}\ln(ab) + \frac{1}{b}\ln(t) \qquad (22)$$

Moazed and Viraraghavan (2005) tested a bentonite organoclay to remove oil from water. Standard mineral oil (SMO), Kutwell 45 (KUT45), and Valcool (VAL) (two cutting oils), refinery effluent (RE) and produced water (PW) from production wells at Estevan, Saskatchewan were used to prepare the oil-in-water emulsions. The concentrations of oil in oily waters varied from 26 to 381 mg/l. The equilibrium time for the sorption of oil by organoclay, determined in batch experiments, was less than 1 h for all emulsions.

Results showed that the kinetics of oil sorption by the organoclay was modelled well by Lagergren's pseudo first-order and Ho's pseudo second-order equations. Freundlich model was indicated by the batch sorption experiments as the most appropriate isotherm for SMO, KUT45, and produced water sorption by the organoclay. The Langmuir and BET isotherms described well the sorption data for VAL and RE, respectively. Results demonstrated also that the organoclay was an excellent medium for treating oily waters.

3.2 Biodegradation process

3.2.1 Biodegradation kinetics

For designing and modelling bioremediation processes, it is necessary to have knowledge of microbial growth and degradation kinetics of environmental organic pollutants. Specific microbial growth rate (μ) is described by the equation proposed by Monod. It assumes that a single essential substrate is the growth-limiting factor:

$$\mu = \frac{\mu_{\max} S}{K_S + S} \qquad (23)$$

where K_S is half-saturation constant, μ_{max} is maximum specific growth rate, and S is growth limiting substrate concentration. Specific growth rate asymptotically approaches maximum specific growth rate when substrate concentration is increased. Substrate concentration at which specific growth rate (μ) is half of its maximum value (μ_{max}) is called half saturation constant. Although Monod equation looks very similar to

Michaelis-Menten model for enzyme kinetics, it is empirical and has no mechanistic basis. Merchuk and Asenjo (1995) gave a particular interpretation to the growth rate-substrate concentration dependence, taking into consideration the mass transfer from the bulk liquid to the surface of the cell. The derived equation for a spherical cell assuming that the substrate concentration at the cell boundary would be much smaller than in the bulk liquid is:

$$K_S = \frac{\mu_{\max}}{\left[\frac{6Yh_S}{\rho_C d_C}\right]} \qquad (24)$$

where Y is biomass yield, h_S is overall mass transfer coefficient for the substrate, ρ_c is density of cell, and d_c is characteristic cell diameter. The above equation predicted accurately some experimentally obtained K_S values.

The role of K_S in equation *(23)* becomes unimportant when it has low values and the specific growth rate follows zero-order kinetics being equal to the maximum specific growth rate: $\mu = \mu_{max}$. If substrate concentration is lower than K_S, its role in denominator is insignificant and first-order kinetics can be used: $\mu = (\mu_{max}/K_S)S$.

Increase in biomass (X) in time (t) is obtained from the following equation:

$$\frac{dX}{dt} = \mu X - k_d X \qquad (25)$$

where k_d is decay coefficient which accounts for cell death. The following equation can be used to calculate the decrease in substrate concentration:

$$-\frac{dS}{dt} = \frac{\mu X}{Y} \qquad (26)$$

Usually μ/Y is replaced by specific substrate utilization rate (q) and μ_{max}/Y by maximum specific substrate utilization rate (q_{max}). Equation *(26)* can be written in case of Monod kinetics as follows:

$$-\frac{dS}{dt} = \frac{q_{\max} SX}{K_S + S} \qquad (27)$$

The affinity of microorganisms for a certain substrate is measured by the half saturation constant. Those microorganisms that show low K_S (high affinity) for a substrate have an advantage over other microorganisms at low substrate concentration. It is suggested that ratio μ_{max}/K_S (or q_{max}/K_S) describes better the affinity for substrate and allows better

comparison between different microorganisms in case of competitive situations because both μ and K_S affect growth and substrate removal rates.

Most organic pollutants, which act as substrate for microorganisms, start to inhibit growth at high concentrations. Haldane equation is used to model specific growth rate:

$$\mu = \frac{\mu_{\max} S}{K_S + S + \frac{S^2}{K_i}} \qquad (28)$$

where K_i is substrate inhibition constant. It is the substrate concentration where the growth rate is half of maximum due to substrate inhibition. This equation approaches Monod equation when K_i approaches infinity.

The description of inhibitory substrate removal is obtained by substituting equation *(28)* in equation *(26)*:

$$-\frac{dS}{dt} = \frac{q_{\max} SX}{K_S + S + \frac{S^2}{K_i}} \qquad (29)$$

Haldane growth curve goes through certain maximum specific growth rate after which it starts to decline when substrate concentration further increases. The following equation can be used to calculate substrate concentration at this point (S^*):

$$S^* = \sqrt{K_S K_i} \qquad (30)$$

Wayman and Tseng (1976) proposed a model where specific growth rate is modelled with monod kinetics *(23)* up to S^* and above with equation *(31)*:

$$\mu = \frac{\mu_{\max} S}{K_S + S - K(S - S^*)} \qquad (31)$$

where K is a constant.

Cultivation in batch or continuous conditions is employed to determine the biodegradation kinetics parameters for BTX removal. The data is fitted with the well-known Monod equation (Kelly *et al.*, 1996; Bekins *et al*., 1998; Bielefeldt and Stensel, 1999; Lovanh *et al*., 2002; Okpokwasili and Nweke, 2005; Zepeda *et al*., 2006). Kelly *et al.* (1996) reported that utilization rates of benzene, toluene and xylene in discontinuous operations were 1.32, 1.42 and 0.83 mmol/l-h, respectively. Maximum growth specific rate (μ_{max}) for biomass degrading monoaromatic compounds has been reported to be in the range of 0.046-0.383 h^{-1}.

The experiments conducted by Bielefeldt and Stensel (1999) indicated that the values of kinetic coefficients for the individual BTX components are influenced by the operating solids retention time (SRT) in the reactor and the nature of the substrate mixture.
Suarez and Rifai (1999) showed that the biodegradation rates of fuel hydrocarbons followed first order kinetics with rate constants up to 0.445 day^{-1} under aerobic conditions and up to 0.522 day^{-1} under anaerobic conditions. The average reaction rates found in the literature for monoaromatic hydrocarbons were 0.3% day^{-1} for benzene, 4% day^{-1} for toluene, 0.3% day^{-1} ethylbenzene and 0.4% day^{-1} for xylenes, respectively.

3.2.2 Factors influencing biodegradation kinetics

3.2.2.1 Effects of retention time

Shim *et al.* (2002) increased the retention time in a fibrous bed reactor from 0.3 to 0.4 h and this enhanced the removal efficiency of the organic pollutants (BTEX) but decreased their degradation rates. At retention times of 1.5 h or longer, nearly 100% degradation efficiency for all BTEX compounds was observed. M-xylene appeared to be the easiest to degrade among all the BTEX compounds. It was followed by toluene and ethylbenzene; p- and o-xylenes were the most difficult to degrade due to their higher concentrations in the treated groundwater.

3.2.2.2 Effects of temperature

Temperature has an important effect on microbial kinetics and it is difficult or expensive to control during bioremediation. The effects of temperature on microbial kinetics are commonly described by Arrhenius equation:

$$r_T = A_0 e^{(-E_a / RT)} \qquad (32)$$

where r_T is the temperature dependent rate constant, E_a is activation energy, R is the universal gas constant (8.31 J $mol^{-1}K^{-1}$), T is temperature (Kelvin), and A_0 is a constant. Although originally proposed to describe the effects of temperature on the rates of chemical reactions, Arrhenius extended his formula to the influence of temperature on the rates of biological processes and reactions. A more general term called the temperature characteristic is normally used to replace activation energy in microbial kinetics.
The equation can be written as follows, when comparing rate constants at two different temperatures:

$$\frac{r_{T1}}{r_{T_2}} = \exp\left[\left(\frac{E_a}{RT_1T_2}\right)(T_1 - T_2)\right] \qquad (33)$$

where r_{T1} and r_{T2} are rate constants at temperatures T_1 and T_2, respectively. The so-called temperature coefficient (Q_{10}) is often used to describe substrate removal rates for a temperature difference of 10 °C and is obtained from equation *(33)* by substituting $T_1 = T_2 + 10$ °C:

$$Q_{10} = \frac{r_{t+10}}{r_t} = \exp\left(\frac{10E_a}{RT_1T_2}\right) \qquad (34)$$

Equation *(33)* can be written as follows, when it is assumed that E_a/RT_1T_2 is approximately constant:

$$r_{T1} = r_{T_2}\theta^{(T_1-T_2)} \qquad (35)$$

where θ is temperature coefficient. This equation is commonly used in wastewater engineering and temperature is expressed as ºC. Temperature T_2 is usually set at 20 °C.

Deeb and Cohen (1999) developed a microbial consortium derived from gasoline-contaminated aquifer and enriched on toluene in a chemostat at 20 °C. This was used to biodegrade benzene, ethylbenzene and xylenes. In batch studies conducted to quantify the effect of temperature on the microbial activity of the 20 °C enriched consortium, cell growth on toluene increased approximately four times due to an increase in the incubation temperature from 20º to 35 °C. A temperature optimum of 35 °C was determined from a range of temperatures tested (7 to 65 °C). When a consortium was enriched in a continuous growth reactor at 35 °C, BTEX degradation rates increased and lag times decreased relative to the 20 °C-enriched consortium. It was observed that although the degradation rates for individual BTEX compounds were higher for the 35 °C-grown cells relative to 20 °C-grown cells, the rate of ethylbenzene degradation in a BTEX mixture was consistently lower than that for the 20 °C-enriched consortium. These findings indicated that the enrichment at a higher temperature promoted fundamental changes in the composition of the consortium.

Interactions between different pairs of BTX compounds were studied by Chang *et al.* (1993) who used *Pseudomonas* sp. which were able to biodegrade these compounds as the sole carbon source. The following equation for competitive inhibition was used to model substrate interactions:

$$\mu = \frac{\mu_{\max} S_1}{K_S\left(1 + \frac{S_2}{K_i}\right) + S_1} \qquad (36)$$

where S_1 is substrate concentration and S_2 is concentration of interacting substrate. The results obtained from single substrate experiments indicated that K_S could be used to quantify substrate interactions (K_i).

Oh *et al.* (1994) used a similar model for interactions between benzene and toluene degradation by mixed culture and a *Pseudomonas* sp. The following equation was used by Oh *et al*. (1994) to describe benzene degradation:

$$\mu = \frac{\mu_{\max} S_1}{K_S + S_1 + K_I S_2} \qquad (37)$$

Another equation was employed to model toluene degradation since it followed inhibitory kinetics:

$$\mu = \frac{\mu_{\max} S_1}{K_S + S_1 + \frac{S_1^{\,2}}{K_i} + K_I S_2} \qquad (38)$$

For benzene degradation in the presence of toluene, K_i was over 20 times higher than for toluene degradation in the presence of benzene. This demonstrates that utilization of benzene was more severely inhibited by the presence of toluene although both compounds served as growth substrates.

Deeb and Cohen (2000) observed that substrate concentration patterns over a range of BTEX concentrations (0 to 80 mg/l) for individual aromatics were found to differ significantly from patterns for aromatics in mixtures. Individually, toluene was degraded fastest, followed by benzene, and the xylenes. Biodegradation rates decreased in the following order in BTEX mixtures: ethylbenzene, toluene, benzene and xylenes.
A range of substrate interaction patterns including no interaction, stimulation, competitive inhibition, non-competitive inhibition, and cometabolism was noticed due to the concurrent presence of multiple BTEX compounds. Presence of o-xylene slightly increased benzene and toluene degradation rates, while the presence of toluene, benzene or ethylbenzene had a negative effect on xylene degradation rates. Ethylbenzene was shown to be the strongest inhibitor of BTEX mixture. A mixture of competitive and non-competitive inhibition kinetics was proposed during the attempted quantification of the inhibition effects caused by ethylbenzene. On the other hand, benzene, toluene, and the xylenes had a negligible effect on the biodegradation of ethylbenzene. Cometabolism of o-, m-, and p-xylene was demonstrated to be a positive substrate interaction.

3.2.2.3 Effects of loading rate

BTEX degradation rates generally increase with increasing total loading rate (Shim *et al.,* 2002). At low loading rates (< 2 mg/l-h), all BTEX compounds were completely degraded with 100% removal efficiency, and therefore, the degradation rates were equal to the loading rates.
On the other hand, when the loading rate was further increased, the degradation rates and thus the removal efficiencies decreased. Overloading or washout effects were believed to be the main causes of the unexpected drop in reactor performance. The

biodegradation rates calculated for p- and o-xylenes were lower than for the other BTEX compounds, at the same loading rates. This demonstrated that they were not degraded as efficiently as the other members of BTEX group by the co-culture used in the study (Shim *et al*., 2002).

3.2.2.4 Effects of initial substrate to biomass ratio (S_0/X_0)

In a batch experiment, this ratio influences the values of kinetic constants in mixed culture (Chudoba *et al*., 1992). Biomass growth is favoured, if S_0/X_0 is high. This can change the proportion between the slow-growing and fast growing microorganisms and therefore, the culture composition and the kinetics are not representative for the original culture. In the presence of abundant substrate, the physiological state of microorganisms can also change influencing the kinetic constants. Kinetics measured with high S_0/X_0 ratios are termed intrinsic in the terminology proposed by Grady *et al*. (1996), since they indicate the maximum activity of the microbial culture. On the other hand, little growth was reported during tests done at low S_0/X_0 ratio. Therefore, it is strongly believed that kinetic parameters are representative for the growth conditions where the culture was growing prior to the test. The term of extant (currently existing) was proposed by Grady *et al*. (1996) for kinetic constants obtained under low S_0/X_0 ratio. The difference is important when predicting effluent concentrations for continuous-flow treatment systems with kinetic constants obtained from batch tests.

3.2.2.5 Effects of nutrient composition

Nutrient composition can have a significant effect on biodegradation kinetics. Degradation of chlorobenzoic acids by *Pseudomonas* sp. and *Alcaligenes* sp. was often changed from Haldane to Monod type kinetics. On the other hand, the presence of yeast extract in the growth media, resulted in an increased affinity for the substrates (Armenante *et al.*, 1995). Moreover, the pentachlorophenol (PCP) sensitivity of *Flavobacterium* sp. cells grown under carbon- or phosphate-limited conditions was higher than in the case of cells grown under ammonium- or sulphate-limitations (Topp and Hanson, 1990).

3.2.2.6 Effects of growth history

The growth kinetics of microorganisms is strongly affected by their growth history. Substrate concentration or growth rate in the culture prior to the experiments seems to be an important factor. An unspecified pure culture grown at higher specific growth rates before testing, exhibited higher μ_{max} values and lower K_S values for 2-chlorophenol (2-CP) degradation as compared to those grown at lower growth rates (Templeton and Grady, 1988). In steady-state chemostat where the bacteria were grown prior to batch experiments (Sokol, 1987), the degradation kinetics of phenol by *P. Putida* showed increased maximum specific growth rates as well as increased K_S and K_i for increased phenol concentrations. Even higher variations among kinetic constants were observed

for phenol degradation kinetics in unsteady-state conditions, before a new steady state was established (Sokol, 1988). Changes in microbial kinetics have been noticed even in pure culture studies during long-term steady-state operation of chemostats (Höfle, 1983; Rutgers *et al*., 1987).

3.2.3 Degradation kinetics in mixtures of organic environmental contaminants

Usually the presence of other compounds in mixtures can inhibit, stimulate or have no effect on the biodegradation rates of each individual compound (Burback and Perry, 1993; Chang *et al.*, 1993; Bouchez *et al.*, 1995; Tiehm and Fritzsche, 1995). The stimulating effect may come from an increase in biomass quantity since some compounds are better growth substrates, improved enzyme induction, other substrates acting as primary substrates, or cometabolism. When a mixture has many components, it becomes very difficult to distinguish between the different substrate interactions. Arvin *et al*. (1989) observed that presence of toluene or xylene stimulated benzene degradation by a mixed culture but when the two compounds were found together in the mixture, the stimulating effect was less than the sum of the effects from toluene and xylene alone.

4. MATERIALS AND METHODS

4.1 Sorption experiments

4.1.1 Sorption screening experiments

4.1.1.1 Materials

Three different types of media were used during the screening tests:

- Activated carbons: Cecarbon (Ceca) and Filtrasorb 400 (Chemviron Carbon);
- Natural zeolites: ZS 500 RW (GSA Resources Inc.), WID (Zeoponix Inc.), and Cabsorb SOS 820 (GSA Resources Inc.);
- Surfactant modified zeolites: Bowman SMZ (Prof. Robert Bowman, New Mexico Tech), Zeoloc SMZ (Zeotech corporate), and ZeoSand SMZ (Zeo Inc.) (Table 21).

Table 21. Chemical composition of the zeolites used in the screening tests

Type of medium	Composition (weight %)									
	SiO_2	Al_2O_3	Na_2O	Fe_2O_3	CaO	MgO	K_2O	P_2O_5	MnO	TiO_2
WID*	67.4	10.6	0.59	1.7	2.23	0.45	4.19	0.10	<0.01	0.27
ZS 500 RW	69.50	16.60	2.40	4.33	4.49	0.89	1.32	-	-	0.47
ZeoSand**	68.1	10.7	0.6	1.7	2.2	0.5	4.3	-	-	0.3

*WID and the natural zeolite used to prepare Bowman SMZ are extracted from the same mine (the same properties)

** The surface of this natural zeolite was modified by Zeo Inc. to prepare the ZeoSand SMZ

The commercial names of these media were replaced with acronyms (Table 22), when discussing the results of the experimental work:

Table 22. Acronyms of the tested sorption media

No	Commercial name	Acronym
1	Cecarbon	AC 1
2	Filtrasorb 400	AC 2
3	ZS 500 RW	NZ 1
4	WID	NZ 2
5	Cabsorb SOS 820	NZ 3
6	Bowman SMZ	SMZ 1
7	Zeoloc SMZ	SMZ 2
8	ZeoSand SMZ	SMZ 3

Solutions used for the tests

The chemicals were purchased from Sigma-Aldrich. All solutions were prepared with water purified with a Milli-Q system. Initially, the screening tests were performed with fresh water, but saline water with 35 g/l NaCl was used in later experiments since this imitates better the characteristics of produced water. The screening tests were carried out with acetic acid and BTEX solutions. The BTEX solutions contained the average concentrations of each component in produced water (Table 23).

Table 23: Concentrations of BTEX in PW – (OLF, 2005)

No	Chemical name	Concentration (mg/l)
1	Benzene	4
2	Toluene	3.2
3	Ethylbenzene	0.2
4	Xylenes	1.2
5	Formic acid	1.2
6	Acetic acid	163
7	Propionic acid	18
8	Buthanoic acid	4
9	Valeric (pentanoic) acid	1.8

4.1.1.2 Methods

A method developed by Fuierer and Bowman (2001) was modified and employed to prepare the media for the sorption tests. First the media were washed with 50 pore volumes purified water to remove fines and then dried in oven at 150 °C for 24 h. The surfactant-modified zeolites (SMZ) were washed again with 15 pore volumes purified water in order to remove the excess of surfactant from zeolite's surface. The media were then left to dry at room temperature. The pH of solutions was adjusted to a value of 7, which is the average for produced water. For this purpose, NaOH and HCl solutions with a concentration of 0.1 M were used. The prepared solutions were transferred to 60 ml crimp cap vials containing 5 g of one of the sorbents. For each sample, 2 duplicates were prepared in order to check the reproducibility of the results. The vials were then placed on a reciprocating shaker and shaken for 2 h at 25 °C and 100 strokes/min. Based on literature review the adsorption equilibria of acetic acid (Husson and King, 1999) and BTEX (Fuierer and Bowman, 2001) are reached after less than 2 h. A syringe was used to collect approximately 25 ml sample from each vial, and transfer it to the TOC analyzer vials.
The samples were filtered through filter paper in order to remove fine particles, which might affect sample analysis. A total organic carbon (TOC) analyzer (Tekmar Dohrmann Apollo 9000) was used to measure the concentrations of compounds in the samples.

4.1.2 Sorption properties of the organoclay

4.1.2.1 Materials

Two types of sorbents were tested in this study:

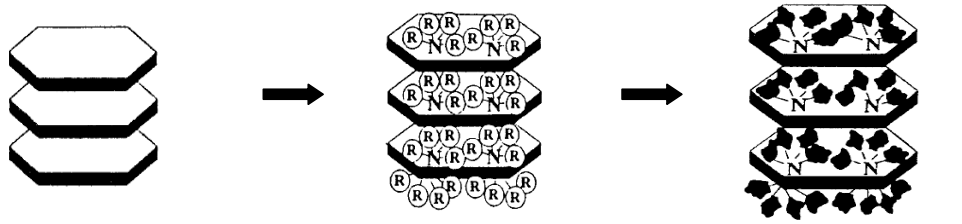
- A natural zeolite: ZS 500 RW (GSA Resources Inc.);
- An organoclay (organically modified clay): Crudesorb (Cetco Oilfield Services Company).

Description of the ZS 500 RW zeolite

Zeolites are naturally occurring hydrated aluminosilicates characterized by cage-like structures, high surface areas and high cation exchange capacities. ZS 500 RW is a chabazite, this type of rock being the most important member of the 48 minerals in the zeolite group (Table 21).
The aromatics are too large to enter the framework of the chabazite. This mineral has a platy morphology, a high physical exterior surface area (around 100 m^2/g), very strongly polarizing properties and since the aromatics have numerous potential points of contact, they can bind fairly strongly to the exteriors of chabazite's crystals.

Description of the Crudesorb clay

Crudesorb is a proprietary sorbent of Cetco Oilfield Services Company. It is an organoclay produced from bentonite modified with a quaternary amine. The clay mineral montmorillonite, a chemically altered volcanic ash, is the major constituent of bentonite. Montmorillonite has an ion-exchange capacity of 70-90 meq/g. Figure 26 illustrates how the nitrogen end of a quaternary amine is exchanged onto the surface of the clay for sodium and calcium, thereby becoming organophilic. In this way, swelling in water is minimized while swelling in organic fluids is enhanced (Vinka *et al*., 2007). In the case of Crudesorb, the quaternary amine is the dimethyl (dihydrogenated) ammonium chloride (12-18 carbons). According to manufacturers, the modified bentonite is thermally stable up to 220 °C. Beyond this temperature bentonite will start losing weight and a moisture content of 2% (Gitipour *et al*., 1997a). If the entire cation exchange capacity (CEC) of the clay is reached, Crudesorb medium becomes a non-ionic sorbent. The carbon chains will stand up perpendicularly to the clay platelet in the presence of water, removing oils and other non-polar or slightly polar organics by a partition phenomenon (Faschan *et al*., 1993)



Individual stack of expandable clay

Clay surface modified with quaternary amine

Saturation of organophilic clay with contaminant

Figure 26. Structure of modified clay platelets; N-nitrogen; R-radicals (Gitipour *et al.*, 1997b)

Some organically modified clays are able to remove anions from the liquid phase. Two mechanisms have been indicated as explanation for this behaviour:

- The organoclays have a certain anion exchange capacity caused by the exchange of the chlorine end of the amine with an anion present in the liquid phase. The long-chain quaternary amines (C18) have a much lower anion exchange capacity than the short-chains (C12). Since commercial quaternary amine surfactants contain a mixture of C12 - C22 amines, it is believed that this anion exchange ability is due to the short-chain impurities (Alther, 1995);
- Usually not all the quaternary amine chains are attracted to the clay platelets by ion exchange. Some of them tie to the attached chains by a form of tail-tail interaction. The result of this tail-tail interaction is that a positive charge extends into the water, causing negatively charged anions to be removed by ionic attraction (Alther, 2002).

Solutions used for the tests

The chemicals used were supplied by Sigma-Aldrich and VWR Norway. The procedure used in this study followed that utilized in the initial screening tests (Scurtu *et al.*, 2006). All solutions were prepared with water purified with a Millipore system (Millipore CO., USA). Saline water containing 35 g/l NaCl (average salinity of seawater) was used in all tests except when the influence of salinity on the sorption process was studied. The laboratory investigations were carried out with acetic acid because the average concentration (160 mg/l) is far greater than the concentrations of the other carboxylic acids present in produced water. The BTEX compounds were selected as the representatives of the dissolved aromatic compounds since they are found in higher concentrations and are more water soluble than the PAHs and phenols. Compared to the previous screening tests only benzene, toluene and p-xylene were included in the experiments. Ethylbenzene and ortho- and meta-xylene were excluded in order to get results that are more reliable with the GC/MS analysis. Therefore, the abbreviation BTEX was replaced with BTX. This is not expected to have an important influence on the results because the compounds that were not considered for the experiments are only found in very small concentrations in produced water.

4.1.2.2 Methods

The natural zeolite and the organoclay were prepared for the experiments using the modified method based on the procedure developed by Fuierer and Bowman (2001) as described above. The organically modified clay was also flushed with purified water and thereafter left to dry at room temperature, in accordance with the recommendations given by the manufacturer. The pH of solutions was adjusted with NaOH and HCl solutions (0.1 M). The prepared solutions were transferred to 60 ml crimp cap vials containing 5 g sorbent. The volume of solution added to each vial was as follows:

- 55 ml for the vials containing no sorbent;
- 50 ml for the vials containing the mentioned amount of sorbent.

These volumes were chosen in order to have a small headspace in the vials that improved the mass transfer during the shaking period. Two duplicates were prepared for each vial in order to check the accuracy of the obtained results. The vials were then shaken for 2 h on a reciprocating shaker at 150 strokes/min and 25 °C, except when the influence of temperature on the sorption process was studied.
BTX concentrations were determined by GC/MS analysis (Agilent GC-6890N, MS-5975) coupled with a headspace auto sampler (Teledyne Tekmar HT3™) for sample preparation. A Tekmar Dohrmann Apollo 9000 instrument was utilized to measure the amount of total organic carbon present in the samples. Since the samples contained only two types of organic compounds (BTX and acetic acid), the concentration of acetic acid was determined by subtracting the concentrations of BTX compounds from the total concentration of organic compounds.

4.2 FBR operation

4.2.1 Materials

4.2.1.1 Description of the Crudesorb clay

A detailed description of the Crudesorb properties is given above in chapter 4.1.2.1.
Crudesorb is an established sorbent already in use for the treatment of produced water on many offshore platforms in the form of a media filter immobilized in canisters. The produced water flow is forced through the canisters and the organic compounds are sorbed by the Crudesorb bed resulting in a clean effluent. In the FBR system the organoclay is utilized in the form of a fluidized bed of particles (not used in a fixed bed as in the case of canisters), which sorb the BTX compounds and provide the support for the growth of a microbial culture responsible for the biodegradation of sorbed compounds.

4.2.1.2 Solutions used for the tests

The chemicals used were supplied by Sigma-Aldrich and VWR Norway. All solutions were prepared with water purified with a Millipore system (Millipore CO., USA). The saline solutions were prepared with a commercial product, Tropic Marin, a pharmaceutically pure sea salt containing all 70 trace elements in the natural concentrations of the ocean.
The matrix used for culturing the BTX degraders consisted of 3.27 g/l Bushnell-Haas powder (commercial nutrient medium) and 30 g/l Tropic Marin sea salts dissolved in purified water. Each litre of Bushnell-Haas broth contained 0.2 g $MgSO_4$, 0.02 g $CaCl_2$, 1.0 g KNO_3, 0.05g $FeCl_3$, 1.0 g $(NH_4)_2HPO_4$, and 1.0 g KH_2PO_4.

4.2.1.3 Fluidized bed reactor system

The experimental configuration (Figure 27) consisted of a glass reactor (2.8 cm diameter and 92 cm height), two computer controlled peristaltic pumps (Masterflex L/S, model 7550-30) and two glass tanks (for BTX solution and overflow waste). The glass reactor as well as the two glass tanks was connected at the top to Tedlar gas bags (impermeable for BTX vapours) with single Teflon fittings in order to prevent the loss of BTX vapours from the system. The Tedlar bag connected to the top of the glass reactor also prevented pressure build-up in the system. The Tedlar bag connected to the BTX tank was filled with air before the experiments were started and the air filled the headspace created in the tank by pumping the BTX solution into the glass reactor. Viton tubing (resistant against BTX) was used to transfer the liquids into the system. Temperature and pressure were measured using transducers connected to a National Instruments FieldPoint data acquisition system. A bed of glass beads at the bottom of the reactor was used in order to obtain a uniform inflow.
The glass reactor was filled with 165 g organoclay, which provided a non-fluidized bed volume of 195 cm^3 (32 cm in height). Peristaltic pump 1 was used to pump BTX solution into the glass reactor while pump 2 was employed to recirculate the fluid from the top into the feed of the reactor in a closed loop. A membrane contactor (model microza UMP-1147 M) was used for oxygenation of the liquid stream. The membrane contactor was preferred to other more common aeration methods as it provides bubble free aeration, thereby preventing BTX stripping from the liquid phase. Compressed air was supplied to the membrane contactor and both the air inflow and outflow were controlled by flow regulators.
The total volume of the system including the glass reactor, the membrane contactor and the tubing was around 0.7 l. This experimental setup was used to carry out the tests in which sorption was the only removal mechanism of BTX as well as the experiments in which both sorption and biodegradation were present. The difference was made by the supply of a microbial inoculum together with nutrients and oxygen in the case when biodegradation of the sorbed BTX compounds was investigated.

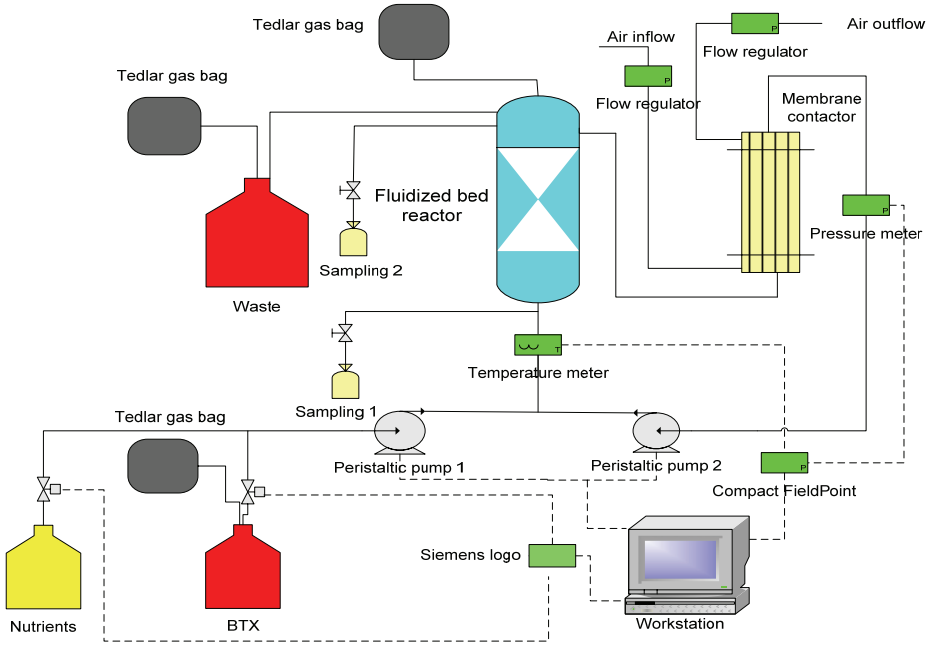


Figure 27. Experimental setup of the fluidized bed system

4.2.2 Methods

4.2.2.1 Method for the screening test for BTX degraders

The matrix described in chapter 4.2.1.2 was utilized for culturing BTX degraders. The final pH of the matrix was adjusted to 7.5 with NaOH solution (0.1 M). The solution was then saturated with oxygen (compressed air was bubbled into the liquid) to provide optimum conditions for microbial growth. The samples were prepared in 120 ml glass serum vials closed with crimp caps and teflon septa. Each vial contained 85 ml of the matrix (sea salts and Bushnell-Haas powder dissolved in distilled water) and either 3 ml of sludge (from an ozonation-biofiltration system or a municipal wastewater treatment pilot plant) or 2 g carbon pellets containing a commercial blend of microorganisms.
When natural seawater was used to provide the microorganisms for culturing BTX degraders, the samples contained only 85 ml natural sea water in which 3.27 g/l Bushnell-Haas powder was dissolved. No sea salts and no additional inoculum were used in this case (seawater contains salts and microorganisms). Blank samples (no microbes added initially) containing only sea salts and Bushnell-Haas nutrients were prepared in addition to the samples containing a microbial source.

Pure BTX compounds were added in equal proportions to each vial to ensure a total concentration of 100 mg/l. The culture was incubated in a shaker at 150 rpm and 25 °C for 9 days. 15 ml samples were then collected from each vial and sent to an external laboratory for DAPI analyses (see appendix A).

4.2.2.2 Method for carrying out the BOD test

The BOD test was performed following the OECD guidelines for testing the biodegradability of chemicals in seawater (OECD, 1992). The synthetic seawater matrix used during the biochemical oxygen demand (BOD) test was the same as the one used for the screening test. The test substance was a BTX mixture (2.5 mg/l) while the reference substance was sodium acetate (2 mg/l). The reference compounds are used to check the microbial activity of the samples. Sodium benzoate, sodium acetate and aniline are examples of chemicals that may be used for this purpose. The reference compounds must be degraded within a reasonably short time span, otherwise it is recommended that the test be repeated. The tests were conducted with a target cell concentration of 1.0 E+06 cells/ml (Scurtu *et al*., 2008a).
The BOD test was run in 270 ml BOD glass bottles closed with glass stoppers. 27 bottles were used in total since the samples had to be sacrificed for measuring the DO concentrations. Samples were sacrificed after 7, 14 and 28 days and 3 duplicates were prepared for each type of sample. The bottles were filled up completely (no headspace) to prevent the partition of BTX compounds between the liquid and gaseous phases. All bottles were stored in a dark place at constant temperature (~ 25 °C).

4.2.2.3 Method for BTX biodegradation test

The BOD test carried out previously provided information about the degradation of the total BTX compounds over a period of 28 days by measuring the amount of dissolved oxygen consumed after certain time intervals. The BOD analysis did not provide information on the biodegradation of each individual component of the BTX group. A distinction of the individual BTX concentrations was achieved by GC/MS analysis. Preparation of the liquid matrix for the biodegradation test followed the same procedure as previously reported (distilled water with Tropic Marin salts and Bushnell-Haas nutrients). The tests were conducted with a target cell concentration of 1.0 E+06 cells/ml (Scurtu *et al*., 2008b).

4.2.2.4 Method for establishing a microbial culture in the FBR

The commercial inoculum selected after the above-described screening test was further enriched following a method described by Fuierer and Bowman (2001) until a high concentration of BTX degraders was obtained. The enrichment was made by transferring a 3 ml aliquot from the initial microbial culture (obtained during the screening tests) to vials containing the same matrix used in the screening and BOD tests and 100 mg/l BTX (total concentration). The enrichment operation was repeated twice

and the final inoculum obtained was transferred into the fluidized bed reactor. BTX were provided as the sole carbon source in the reactor. The fluidized bed system was operated for around 7 days under batch conditions by supplying small amounts of BTX and nutrients every 30 min and recirculating the solution in a closed loop in the FBR. Continuous aeration was provided into the system and the dissolved oxygen (DO), BTX and bacteria concentrations were monitored. When the sorption capacity of the organoclay bed in the FBR was completely exhausted or a different type of test had to be carried out, the existent bed was replaced with a bed of fresh media containing the same amount of organoclay. A microbial culture was then established into the reactor following the steps mentioned above.

4.2.3 Operation of the fluidized bed reactor

4.2.3.1 Operation of FBR during sorption and simultaneous sorption and biodegradation experiments

Peristaltic pump 1 was used to pump BTX solution into the glass reactor at a rate of 50 ml/min while pump 2 was employed to recirculate the fluid from the top into the feed of the reactor in a closed loop at a rate of 100 ml/min (Figure 27). This resulted in an empty bed contact time (the time the fluid spends in an empty bed the size of the sorbent bed) of 3.9 minutes. The experimental setup used to carry out the tests combining the effects of sorption and biodegradation of BTX is the same as the one used for the sorption experiments. The difference was made by the supply of a microbial inoculum together with nutrients and oxygen in the case when biodegradation of the sorbed BTX compounds was investigated. The nutrients were supplied in the form of Bushnell-Haas medium at a stoichiometric ratio of 100/5/1: COD/N/P together with the BTX compounds using peristaltic pump 1.

4.2.3.2 Tuning of off-line bioregeneration experiments

In this experimental design, sorption and bioregeneration were conducted as cyclic and separate batch processes. The FBR was operated in three different modes in order to achieve a rapid sorption of the BTX compounds, followed by an efficient bioregeneration of the spent organoclay bed. All modes started with a 5 min dosage of a BTX containing solution followed by a 2 h sorption step, a bioregeneration step of variable durations and a period of recirculation of the liquid in the system under oxygen deprivation. A new treatment cycle was performed by pumping again BTX containing solution into the system and alternating the above-enumerated steps in consecutive cycles. The BTX containing solution was pumped in the FBR for 5 min at a rate of 100 ml/min and then the fluid was recirculated in the reactor at a rate of 250 ml/min during the sorption, bioregeneration and recirculation without aeration steps.
The FBR was operated in the following three operation modes:

- 6 h cycles (2 h sorption, 2 h bioregeneration, 2 h recirculation without aeration). The solution pumped for 5 min into the system at the beginning of each treatment cycle contained also nutrients in the form of Bushnell-Haas medium at

a stoichiometric ratio of 100/5/1: COD/N/P. The BTX concentrations were 142 mg/l benzene, 145 mg/l toluene and 198 mg/l p-xylene that provided a BTX amount equal to the sorption capacity of the organoclay bed (Scurtu *et al*., 2008b). This step was followed by a period of 1 h and 55 min during which the solution was recirculated without aeration for the completion of sorption process, a bioregeneration step in which the liquid was recirculated in the FBR for 2 h under continuous aeration and a 2 h recirculation step without aeration designed to decrease the DO concentration in the system.

- 8 h cycles (2 h sorption, 5 h bioregeneration, 1 h recirculation without aeration). The concentrations of BTX pumped into the system at the beginning of each treatment cycle were 106 mg/l benzene, 109 mg/l toluene and 148 mg/l p-xylene that provided a BTX amount equal to 75% of the sorption capacity of the organoclay bed. The solution did not contain nutrients. This was followed by a 1 h and 55 min period of recirculation of the fluid in the FBR, in which sorption took place, a 5 min nutrients dosage step (50 ml/min) at a stoichiometric ratio of 100/5/1: COD/N/P, a 5 h bioregeneration step in which the solution was recirculated under oxygen provision and a 1 h recirculation period without aeration.
- 6 h cycles (2 h sorption, 4 h bioregeneration). The solution pumped in the FBR for 5 min at the beginning of each treatment cycle had exactly the same composition as in the previously described 8 h cycle operation mode. A similar sorption step of 1 h and 55 min followed, after which the nutrients were supplied into the system for 5 min at a rate of 50 ml/min and a stoichiometric ratio of 100/5/1: COD/N/P. The subsequent bioregeneration step lasted 4 h, but aeration was provided only in the first 3 h and 30 min.

4.2.3.3 Long time off-line bioregeneration experiments

The goal of these experiments was to study the efficiency of bioregeneration process over a longer period. During the previous tuning experiments, it was observed that the most efficient operation mode of the FBR was in 6 h cycles (2 h sorption, 4 h bioregeneration).

The FBR system was operated initially in the 6 h cycle mode described above for a period of 36 days only with BTX followed by a second period of 36 days with BTX and a low concentration of acetic acid (2 mg/l in the feed solution).

After these two tests, it was concluded that the bioregeneration of the organoclay was not complete and therefore an additional set of experiments with a bioregeneration period increased from 4 to 6 h was carried out. Aeration was provided only during the first 5 h.

The FBR was operated during these long time off-line bioregeneration experiments as follows:

- 5 min dosage of BTX solution (amount equivalent to 75% of the total sorption capacity of the organoclay bed);
- 2 h sorption – including the previous 5 min, achieved by recirculating the fluid into the FBR (250 ml/min) without oxygen supply;
- 5 min dosage of nutrient solution into the FBR;

- 4 h (increased to 6 h in the second set of experiments) bioregeneration steps – including the previous 5 min (oxygen supplied during the first 3.5 h or 5 h for the experiments with 4 and 6 h bioregeneration steps, respectively).

4.3 Analyses

4.3.1 Detection of BTX (headspace auto sampler, GC/MS system)

Concentrations of BTX compounds were measured with a GC/MS system (Agilent GC-6890N, MS-5975) coupled with a headspace auto sampler (Teledyne Tekmar HT3^{TM}) for sample preparation.
The method used to analyze the BTX samples was a modified version of EPA method 5021. This method is characterized by simplicity and minimal carryover.
Although purge and trap (P&T) is widely used for the analysis of low-level volatiles in water, recent advances in system inertness and performance are enabling new approaches using static headspace sampling. The detector 5975 MSD is designed with a highly inert source. The performance electronics in the 5975 allow for fast scan rates without loss of signal. The performance advantages of the Teledyne Tekmar HT3^{TM} with respect to sensitivity and repeatability offer analysts an attractive alternative that can match or exceed that of P&T for many analytes.
Instrumental settings used in the volatile BTX analysis are listed in Tables 24 and 25.

Table 24. HT3 Static Headspace Parameters (loop Method)

Variable	Value
GC Cycle time	20.00 min
Valve Oven Temp	100 °C
Transfer line Temp	100 °C
Standby Flow Rate	50 ml/min
Platen/Sample Temp	85 °C
Sample Equil. Time	30.00 min
Mixer	on
Mixing time	20.00 min

Variable	Value
Mixing Level	Level 5
Mixer Stabilization Time	0.50 min
Pressurize	10 PSIG
Pressurize Time	1.50 min
Pressurize Equil. Time	0.50 min
Loop Fill Pressure	5 PSIG
Loop fill Time	0.50 min
Loop Fill. Equil. Time	0.50 min
Inject Time	1.00 min

Table 25. GC/MS parameters for BTX analysis

Column	HP-5 MS 30 m length, 0.25 mm diameter, 0.25 μm film
Carrier	He at 0.8 ml/min
Injector	Split ratio: 25:1, total flow 22.5 ml/min, temp. 220 °C
Oven	Initial temperature 35 °C, hold for 2.00 min
Step 1	Temperature increase to 85 °C, rate of 14 °C/min, hold for 0.00 min
Step 2	Temperature increase to 210 °C, rate of 40 °C/min and hold for 5.00 min (total 13.70 min)
MS	Interface at 280 °C, MS quad 150 °C, MS source at 230 °C

4.3.2 Detection of acetic acid (derivatization and GC/MS analysis)

A technique for rapid, room temperature derivatization of aqueous carboxylic acids to the corresponding 2,2,2-trifluoroethylamide derivative developed by Ford *et al.* (2007) was modified and used for the detection of acetic acid in aqueous samples. 3-Ethyl-1-[3-(dimethilamino) propyl] carbodiimide hydrochloride (EDC) and 2,2,2 trifluoroethylamine hydrochloride (TFEA) were added to the water samples containing acetic acid. Amidization was essentially complete within ten minutes, and subsequent liquid-liquid extraction of the amides with methyl tert-butyl ether (MTBE) demonstrated recoveries of 85%. The fluorinated amides produced had good chromatographic characteristics for gas chromatography and were easily detected by quadrupole mass spectroscopy (Agilent 5975 MSD).

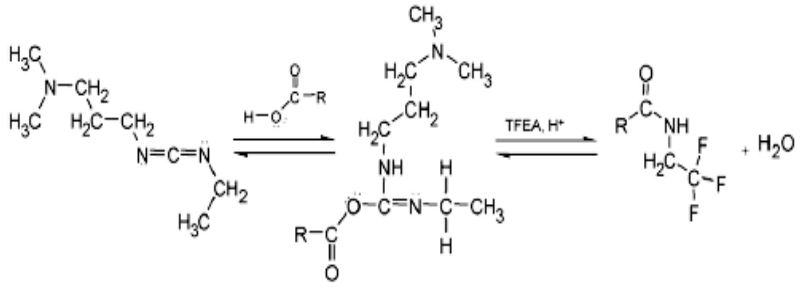


Figure 28. Derivatization of carboxylic acids by carbodiimides and amines

The derivatization of carboxylic acids by carbodiimides and amines is a multistep process based on the reaction of an acylisourea (generated by the reaction of the carbodiimide and carboxylic acid) with the amine (shown in Figure 28 for EDC and TFEA).

Derivatization

A 1.0 ml volume of aqueous pH 5.0 dibasic phosphate buffer (0.5 M), 1.0 ml of aqueous EDC (0.4 M), and 1.0 ml of aqueous TFEA (0.4 M) were added to 10.0 ml of a given

aqueous sample containing acetic acid. The resulting solution was mixed for approximately 1 min and then allowed to react for 10 min at room temperature. The mixture was extracted with 2 ml pure MTBE containing 42.5 μM 1,4-dibromobenzene (50 times the amount used by Ford *et al.,* 2007) as external standard and immediately analyzed.

Standards

Pure standards (98%) of the above amides were prepared under the following conditions: equal parts of aqueous pH 5.0 phosphate buffer, 0.4 M aqueous EDC, 0.4 M aqueous TFEA, and water samples containing variable concentrations of acetic acid. These were combined (at 25 °C) and extracted with MTBE after 10 min. 1 ml samples were taken from the MTBE phase and analyzed by GC/MS.

Derivative analysis

After derivatization, 1 ml samples were taken from the MTBE phases and transferred to 1.5 ml vials that were subsequently placed on the automatic liquid sampler. The settings of the GC/MS system used to analyze the samples are described in the table below:

Table 26. GC/MS parameters for the analysis of acetic acid derivative

Column	HP-5 MS 30 m length, 0.25 mm diameter, 0.25 μm film
Carrier	He at 1 ml/min
Injector	Splitless, total flow 73.6 ml/min, temp. 250 °C
Oven	Initial temperature 40 °C, hold for 2.00 min
Step 1	Temperature increase to 100 °C, rate of 5 °C/min, hold for 1.00 min
Step 2	Temperature increase to 200 °C, rate of 10 °C/min and hold for 0.00 min (Total 25.00 min)
MS	Interface at 280 °C, MS quad 150 °C, MS source at 230 °C

4.3.3 Analysis of biomass samples

Water and sorbent (organoclay particles) samples were collected from the FBR during the experiments and sent to an external laboratory for analysis. The counts of microbial cells were determined in both water and particle phases by epifluorescence microscopy (DAPI method).
Analysis of biomass diversity was done by DGGE on DNA extracted from the biomass samples. A detailed description of the techniques (Brakstad, 2008) used for biomass characterization is provided in Appendix A.

4.3.4 Other analytical methods

The pH and DO concentrations were determined using a multiparameter meter (VWR – Symphony SP 70 D).

5. RESULTS AND DISCUSSION

5.1 Sorption experiments

5.1.1 Sorption screening tests

5.1.1.1 Experimental work

The first sorption-screening test was carried out with 160 mg/l acetic acid dissolved in purified water. Results showed that the surfactant was washed off from the surface of the surfactant modified zeolites although these media had been washed twice with purified water. This is proven by the high total organic carbon (TOC) levels measured when only the media without acetic acid was present in the vials. Figure 29 shows the TOC values measured in each vial containing media without acetic acid, media and acetic acid or only acetic acid solution. A solution containing 160 mg/l acetic acid should have a TOC concentration of 64 mg/l but the value measured during the test was 55 mg/l, probably due to stripping of acetic acid during sample handling.
The results presented in Figure 29 were used to calculate the amount of acetic acid (mg) sorbed by each gram of different media used in the experiments. When these amounts were calculated, it was taken into consideration the measured TOC values, the volume of sample in the vial, the amount of media introduced in each vial and the percent of carbon contained by acetic acid. The results of these calculations are presented in Figure 30, which indicates the amount of acetic acid sorbed by each gram of sorbent utilized. The same calculation method was used to obtain the data for Figures 31, 32, 33 and 34. The only difference is that for Figures 31 and 34 the percent of carbon contained by BTEX was taken into account.

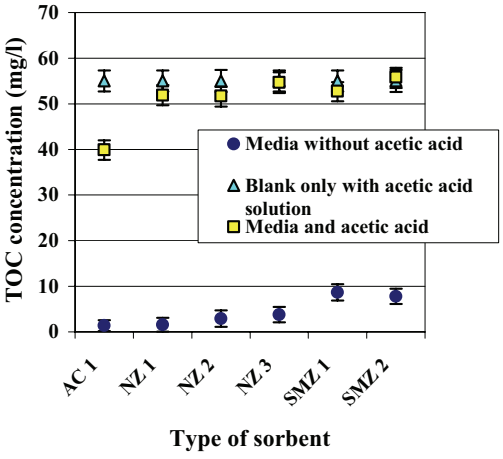


Figure 29. Screening test with 160 mg/l acetic acid and different media

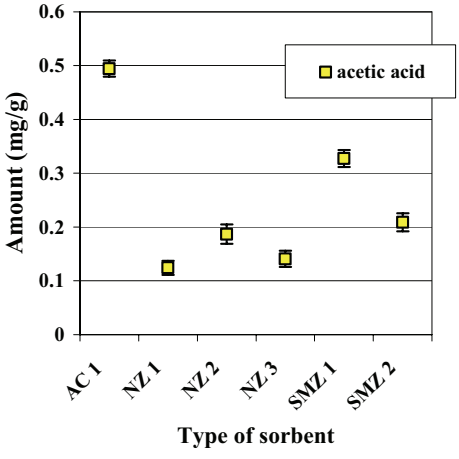


Figure 30. Amount of acetic acid sorbed by each sorption medium utilized

The experiment indicated that the surfactant (HDTMA) was washed off from the surface of the SMZ and in this way, it might inhibit the biological regeneration of the spent sorbent since it is believed to be toxic for the microorganisms. AC 1 showed the highest adsorption of acetic acid and did not meet the requirements of the treatment process.
The natural zeolites (NZ 1, NZ 2 and NZ 3) had the lowest affinities for acetic acid and therefore were the most suitable for the proposed treatment scheme.

The second screening experiment tested the sorption of BTEX by different media. Solutions contained the average concentrations of each component of the BTEX group as indicated in Table 23. Saline water with a NaCl concentration of 35 g/l was used.

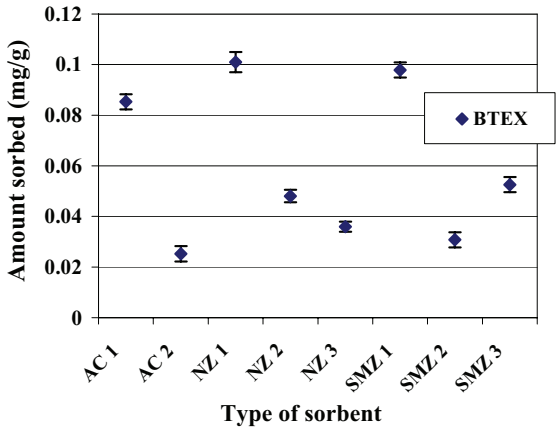


Figure 31. Amount of BTEX sorbed by each sorption medium utilized (saline water solution)

Again high TOC concentrations were measured in the vials containing only surface modified zeolites showing release of surfactant into solution (data not shown here). The natural unmodified zeolite NZ 1 proved to be the most efficient in adsorbing BTEX compounds (Figure 31).

The third sorption experiment was similar to the first one since it focused on the adsorption of acetic acid by different media. The only difference was the utilization of saline water in this test (35 g/l NaCl).

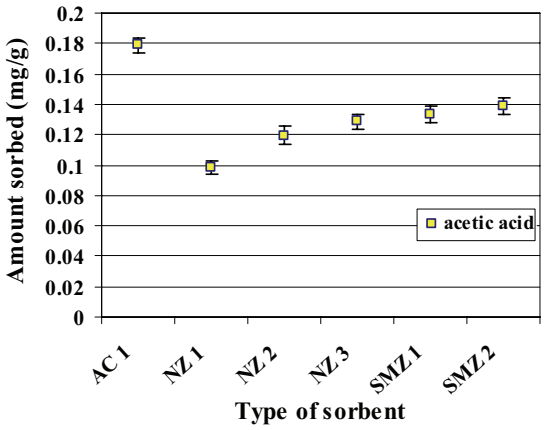


Figure 32. Amount of acetic acid sorbed by each sorption medium utilized (saline water solution)

A comparison between figures number 30 and 32 indicates that the amounts of acetic acid adsorbed by the different media in saline water were lower than the amounts adsorbed in fresh water. Again, the natural zeolites (NZ 1, NZ 2 and NZ 3) showed lower adsorption, while AC 1 adsorbed the highest amount of acid. NZ 1 had again the lowest adsorption, which is consistent with the previous results displayed in Figure 30.

The results of these experiments led to the conclusion that the natural zeolite NZ 1 was the most suitable sorbent for the requirements of the proposed treatment concept and the following tests were carried out using this medium.
Previous calculations indicated that the retention time in the pipe reactor, where the adsorption process takes place, must be less than 10 minutes due to the special conditions existing on offshore platforms. Therefore the adsorption kinetics for the first 10 minutes was studied by measuring the amounts of acetic acid and BTEX sorbed by NZ 1 after 1, 3, 5, 7 and 10 minutes.

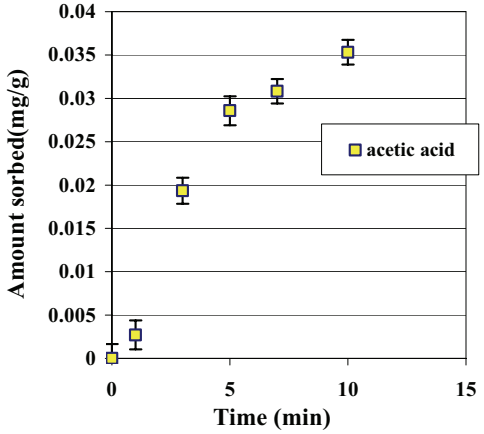


Figure 33. Sorption kinetics of acetic acid onto NZ 1 (saline water solution)

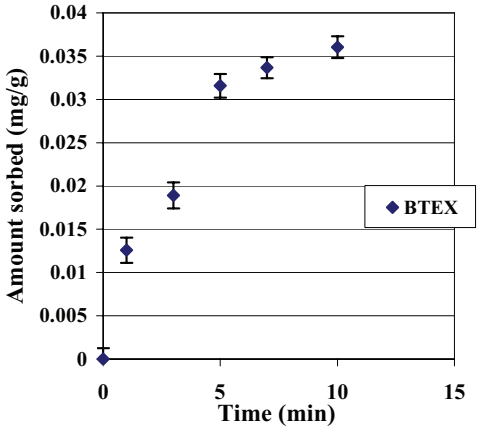


Figure 34. Sorption kinetics of BTEX onto NZ 1 (saline water solution)

The two curves (Figures 33 and 34) showing the adsorption kinetics for acetic acid and BTEX were quite similar. The amounts of BTEX and acetic acid sorbed after 10 minutes were around 0.035 mg/g NZ 1. Figures 31 and 32 indicate that after 2 h the sorbed amounts of acetic acid and BTEX were around 0.1 mg/g NZ 1. These results are comparable with the data obtained by Fuierer and Bowman for toluene sorption onto SMZ 1 (Fuierer and Bowman, 2001). Their experimental work demonstrated that the sorption equilibrium was reached shortly after 1 h contact time between the toluene solution and the SMZ 1 on a rotary shaker at 100 rpm. The sorbed amounts found by these researchers at equilibrium were in the range between 0.15-0.3 mg toluene/g SMZ 1 (initial toluene concentration = 120 mg/l).

5.1.1.2 Theoretical considerations

The results of experiments with fresh and saline water with a NaCl concentration of 35 g/l showed that ionic strength influenced the sorption process: the higher the ionic strength, the lower the sorption of the other anions present in produced water. Chloride anions compete with the other anions for the same sorption sites, therefore decreasing their sorption. This phenomenon has a particular importance for the sorption of organic acids that are highly dissociated at the average pH of produced water (6-7). In high ionic strength

waters, the anions of organic acids will be less sorbed, the chloride ions occupying a part of the sorption sites of the media.
Therefore, pH value has a very important influence on the sorption of organic acids since their dissociation degrees depend on its value (Figure 35). pH of produced water can vary between 3.5 and 8. The lower pH values are measured at the gas fields while the higher values are characteristic to oil fields. In the central North Sea, the pH varies between 6 and 7.7 and the chloride concentration is up to 5 to 6 times that of sea water (81-100 g/l).

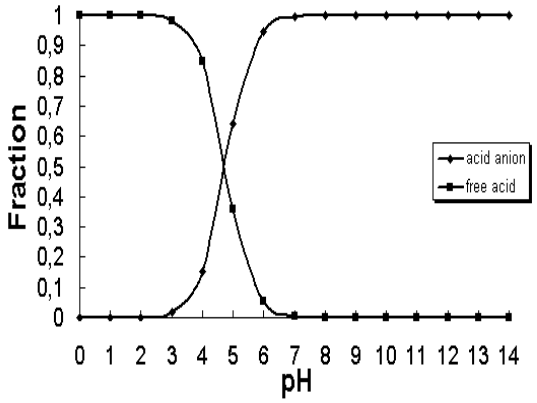


Figure 35. Distribution diagram of acetic acid (pKa = 4.75) species as a function of pH

The most representative organic acids present in produced water have pK_a values between 3.75 (formic acid) and 4.86 (propionic acid). At the normal pH of produced water in the North Sea, organic acids are highly dissociated, the anionic species being predominant. The natural zeolites have a negatively charged surface and therefore they are not able to retain to high extents the anions of organic acids, while the surfactant-modified zeolites have a positively charged surface and this favours the retention of anions. This fact is shown also by the experimental results: all natural zeolites tested showed lower sorption of acetic acid than the surfactant-modified zeolites. Therefore, natural zeolites are more suitable for the requirements of the proposed treatment concept since the sorption of organic acids is not desired.
Three natural zeolites were tested (NZ 1, NZ 2 and NZ 3) and the best sorption of BTEX was provided by NZ 1 (containing chabazite). This finding confirms the results obtained by Janks and Cadena (1991). Their experiments proved that chabazite containing zeolites sorbed BTEX better than clinoptilolite-based zeolites (NZ 2 in our case).

5.1.1.3 Conclusions on sorption screening tests

The following conclusions were drawn on the results of the sorption screening tests:

- A small fraction of the surfactant (HDTMA) was released from the surface of surfactant-modified zeolites. This was a major impediment since this compound

might be toxic to biological activity during regeneration process and can constitute a burden on the aquatic environment if discharged into the sea;

- Natural unmodified zeolites showed the lowest sorption of acetic acid in both fresh and saline water solutions, while activated carbons proved to be the most unselective by sorbing the highest amounts of acid;
- The sorption of acetic acids was lower in saline water due to the presence of chloride ions which compete with the dissociation products of acetic acid for the same sorption sites;
- At the normal pH values of produced water in the North Sea (6 -7.7), the organic acids are highly dissociated, the dominant species being the anions that cannot be sorbed normally to a high extent by natural unmodified zeolites, which have a negatively charged surface. However, they can be retained by the surfactant modified zeolites since these types of sorbents have a positively charged surface;
- Sorption kinetics of acetic acid and BTEX onto NZ 1 were similar. Results showed that the sorption process was very strong during the first 10 minutes, the amount of acetic acid and BTEX sorbed being around one third of the sorption capacity of NZ 1 for these compounds;
- Experimental work indicated that NZ 1 had the lowest affinity for acetic acid and the highest affinity for BTEX, among the tested media. Therefore, NZ 1 was considered the most suitable sorbent for the requirements of the treatment concept.

5.1.2 Sorption properties of the organoclay

The organoclay marketed under the name Crudesorb is a commercial sorbent extensively used in the treatment of produced water in the form of a media filter immobilized in canisters. Unfortunately it was not available during the previous screening tests and therefore it was decided to compare its sorption properties with those of NZ 1 through a brief sorption test.
Four parameters were considered when designing the set of experiments to be carried out: pH, concentration of acetic acid, salinity and temperature. The influence on the sorption process of each of the four variables was studied by keeping three parameters constant and varying the value of the fourth one (Scurtu *et al*., 2007).

5.1.2.1 Effect of pH

The first experiments were designed to provide a comparison between the natural zeolite ZS 500 RW (NZ 1) and the organically modified clay Crudesorb. The solutions used contained 35 g/l NaCl, 5 mg/l benzene, 5 mg/l toluene and 5 mg/l p-xylene. Sorption of the two different classes of compounds was studied at pH 3, 5 and 8 because acetic acid (pk_a = 4.75) is found in molecular form at low pH and dissociated at high pH.

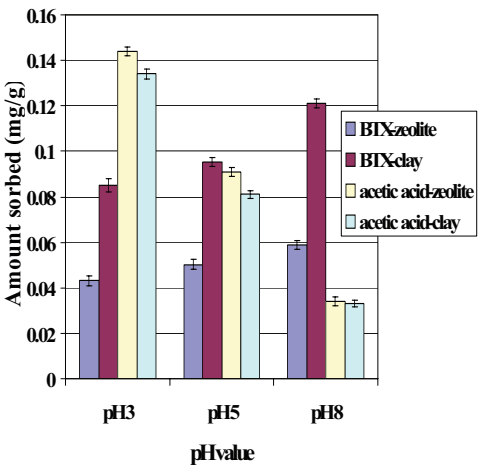


Figure 36. Total BTX and acetic acid sorption at different pH values

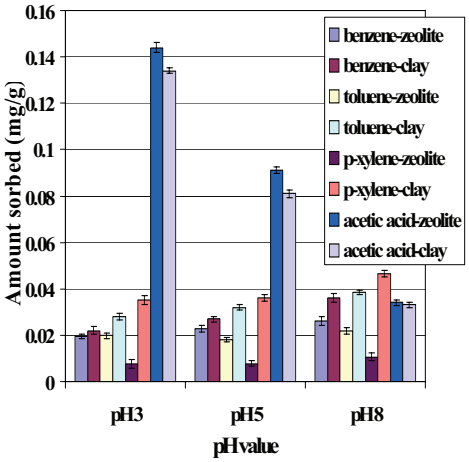


Figure 37. BTX and acetic acid sorption at different pH values

Results clearly showed the influence of pH on the sorption of benzene, toluene and p-xylene in mixture with acetic acid. Figures 36 and 37 indicated that the organically modified clay sorbed around twice as much BTX compounds as the natural zeolite. On the other hand, the organoclay retained a lower amount of acetic acid than the natural zeolite at all pH values. The amount of acetic acid sorbed by each of the two media decreased with the increase of pH because the acid dissociated and the predominant species, the acetate anion, was not retained to a high extent by any of the sorbents. The undissociated acetic acid molecules dominating at low pH are more hydrophobic than the ionized form and hydrophobic bonding becomes the driving force for sorption. This finding is consistent with the results obtained by other researchers (Anirudhan and Ramachandran, 2007) who observed that the uptake of humic acids by an organoclay decreased dramatically as the pH of the solution was increased from 3 to 10.

It can also be observed that in the case of the natural zeolite, which has a hydrophilic surface, the retention increased in the order p-xylene < toluene < benzene while in the case of the modified clay, which has a hydrophobic surface, the uptake increased in the inverse order: benzene < toluene < p-xylene. Results suggested that the removal of BTX components followed the general solubility principles, corresponding to an increasing octanol/water partition coefficient in the order benzene (1.64), toluene (2.25.) and xylene (2.76). The octanol/water partition coefficient is expressed as the ratio of concentrations of a compound in the two phases of a mixture of octanol and water that are immiscible at equilibrium. Hence, these coefficients are a measure of differential solubility of the compound between these two solvents.

Since the modified clay showed a better performance than the natural zeolite with regard to the BTX and acetic acid sorption during the first tests, subsequent experiments were only conducted with the modified clay to assess the effects of acetic acid concentration, salinity and temperature.

5.1.2.2 Effect of acetic acid concentration

It was observed during the first set of experiments that the amount of BTX sorbed by the modified clay decreased proportionally with the increase of acetic acid amount retained. Since the concentration of organic acids varies in produced water, its influence on the retention of the BTX compounds was studied by running experiments with 3 different initial acetic acid concentrations (0, 160 and 320 mg/l). The tests were done at pH 5, where approximately half of the acetic acid is in the form of acetate anion (dissociated), while the other fraction is in the molecular form (undissociated).

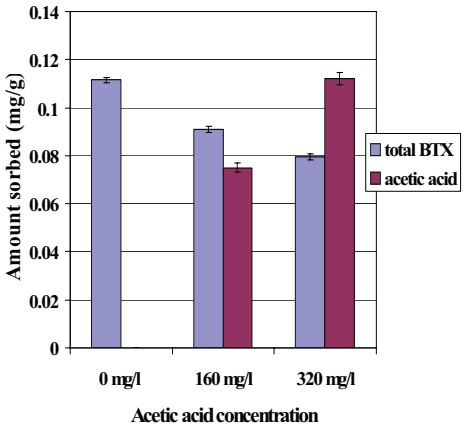


Figure 38. Total BTX and acetic acid sorption at different acetic acid concentrations

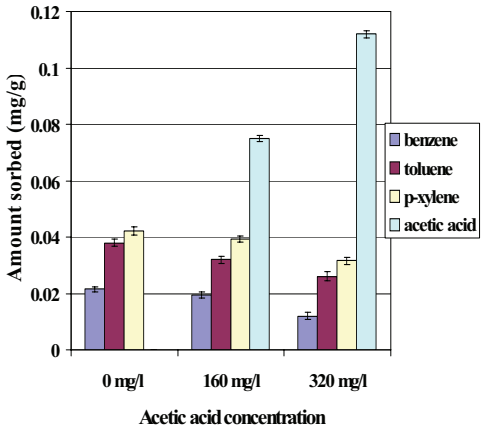


Figure 39. BTX and acetic acid sorption at different acetic acid concentrations

Figures 38 and 39 indicated that the amount of acetic acid sorbed increased with increasing initial concentration of acid. On the other hand, the amount of BTX sorbed decreased proportionally with the increasing concentration of acetic acid, showing a sorption competition between the two different groups of substances.

The BTX compounds, as other organic non-polar substances, are sorbed onto organoclay by partition. The simplest definition of this mechanism is that liquid compounds dissolve into other liquid compounds of a similar nature, when they are present in a third liquid of a different nature. The partition mechanism is characterized by linear sorption isotherms and is specific only to organoclays modified with long chain quaternary amines.

The organoclay is mainly able to retain the molecular form of acetic acid and not so much the acetate anion. Solubility of a carboxylic acid decreases as the length of its hydrocarbon chain increases, and thus its molecules have a stronger tendency to move from the solution to the surface of the sorbent. The carboxylic group gives the polar character in carboxylic acids while the aliphatic group contributes to their non-polar character. Thus, the polar character in low molecular weight carboxylic acids is increased, in contrast to what occurs in high molecular weight homologues. The alkyl

group of carboxylic acid is perpendicularly oriented to the non-polar sorbent and the carboxyl group towards the solution (Freitas *et al*., in press). According to the Duclaux-Traube rule the sorption capacity is proportional to the increment of the – CH_2 group in the sorbate chain. Thus, the uptake of carboxylic acids on the organoclay increases in the following order: formic acid < acetic acid < propionic acid < butyric acid < pentanoic (valeric) acid.

The observed competition between BTX and acetic acid sorption can be explained by the fact that both classes of compounds bind to the aliphatic chain of the organic surfactant. This phenomenon occurs when the concentrations of these compounds in the solution exceed a certain level.

5.1.2.3 Effect of salinity

The salinity of produced water is variable ranging from zero in condensed water up to several times the salt concentration of seawater. Its influence was assessed by carrying out experiments at three different salinities (NaCl concentrations of 0, 35 and 70 g/l) and keeping the other parameters constant (pH = 5, temperature = 25 °C and acetic acid concentration = 160 mg/l).

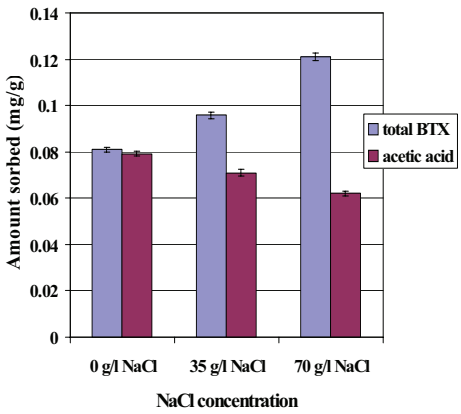


Figure 40. Total BTX and acetic acid sorption at different NaCl concentrations

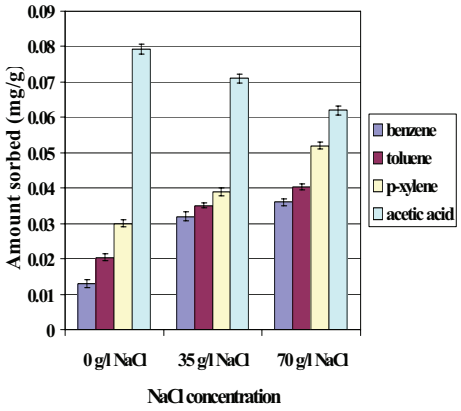


Figure 41. BTX and acetic acid sorption at different NaCl concentrations

The results illustrated in Figures 40 and 41 showed that the sorption of BTX compounds increased proportionally with the concentration of NaCl. This is the so-called salt-out effect on the sorption process that can qualitatively be pictured as follows: the ionization of NaCl in the equilibrium solution causes the binding of water molecules into hydration shells, which reduces the volume of aqueous solution by a process called electrostriction. This may reduce the aqueous solubility of BTX compounds and thus, increase their diffusion in the organic phase. The salt-out effect has been shown to enhance sorption of hydrophobic compounds by increase of ion strength (Tremblay *et*

al., 2005). Results presented here are consistent with those obtained by Karickhoff *et al*. (1979) who found that pyrene sorption coefficients increased by 15% when NaCl concentration was raised to 0.34 M and El-Nahhal and Safi (2004) who observed that phenanthrene sorption increased going from pure water to a NaCl concentration of 150 g/l.

On the other hand, uptake of acetic acid showed a different trend when the ionic strength of the solution was increased. The following explanations were suggested for this phenomenon:

- Sorption competition between BTX and acetic acid. Since more BTX are sorbed by the modified clay, less space is left for acetic acid;
- Cl^- anions compete with acetate anions for the possible anion exchange sites or for the positively charged cations (electrostatic attraction) present on the surface of clay;
- Acetate anions form an outer-sphere surface type complex on the surface of the modified clay.

The decrease of acetic acid sorption onto the organoclay when the ionic strength was increased is consistent with the results published by other research groups (Abate *et al*., 2006; Anirudhan and Ramachandran, 2007).

5.1.2.4 Effect of temperature

The fourth parameter considered in this study was temperature, which was expected to have an important effect on the sorption process since it influences solubilities of solutes. Temperature of produced water varies between 3 and 80 °C, depending on the field.

Due to technical limitations, the experiments were run at 20, 40 and 60 °C, the other 3 parameters being kept constant (pH = 5, acetic acid concentration = 160 mg/l, NaCl concentration = 35 g/l).

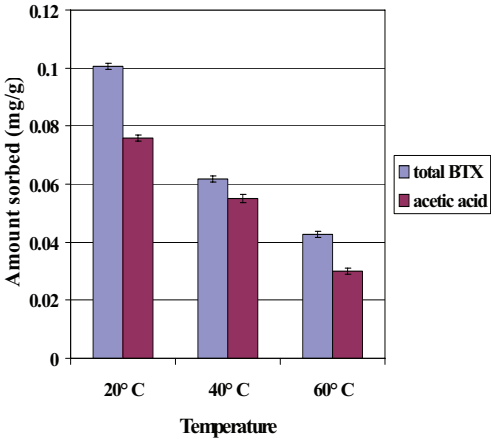


Figure 42. Total BTX and acetic acid sorption at different temperatures

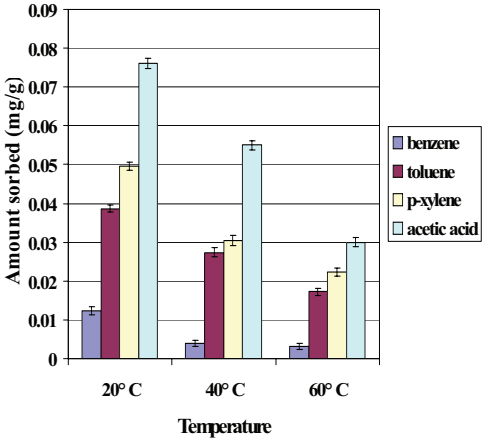


Figure 43. BTX and acetic acid sorption at different temperatures

The amounts of both BTX compounds and acetic acid sorbed on the organoclay decreased when the temperature was increased from 20 to 60 °C (Figures 42 and 43). The increase of temperature negatively influenced the uptake capacity of sorbent due to desorption of molecules at the interface, *i.e.* increase of BTX and acetic acid solubilities in aqueous solutions. Similar temperature effects on organic acids sorption were reported in other studies (Tahir and Rauf, 2004; Freitas *et al*., in press).
The partition coefficients of BTX between the aqueous and the solid phases (modified clay) were larger at higher temperatures (data not shown here). Therefore, the effects of temperature on the partition coefficients can be attributed primarily to changes in BTX water solubility. This finding is similar to the conclusions drawn by Tremblay *et al*. (2005) who discovered that the sorption of phenanthrene and fluoranthene on suspended particulate matter decreased when the temperature was increased from 2 to 20 °C.
In batch sorption experiments, Piatt *et al*. (1996) found for phenanthrene an average increase of the partition coefficient between the aqueous phase and sorbent of about 11% with a decrease in temperature of 22 °C.

5.1.2.5 Study of sorption kinetics

After studying the influence of pH, initial acetic acid concentration, salinity and temperature on BTX sorption onto Crudesorb after 2 h, more insight was obtained into the sorption kinetics by measuring the concentrations of all compounds in the liquid phase after 1, 5, 10, 30, 60, 120, 240 and 1440 minutes. Two types of experiments were run:

- Without acetic acid;
- With 160 mg/l acetic acid. In this case, the pH was adjusted to a value of 5, at which the acetic acid is half dissociated.

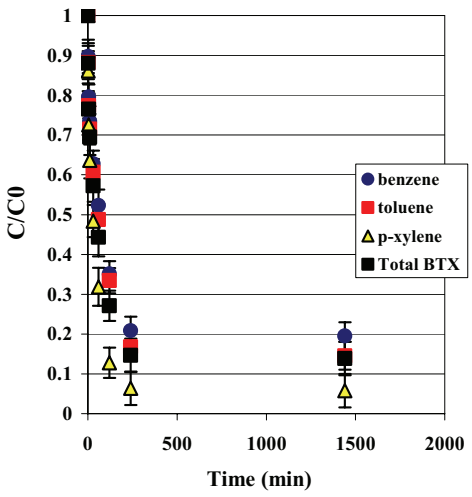


Figure 44. Sorption kinetics of BTX onto organoclay

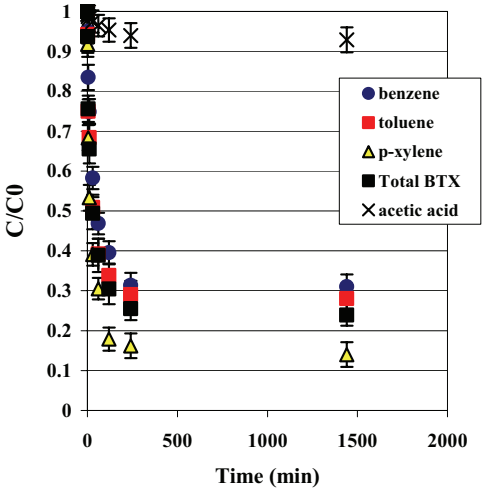


Figure 45. Sorption kinetics of BTX onto organoclay (acetic acid present in solution)

The results presented in graphs 44 and 45 show the ratio between the concentration of a compound in solution at a given time (C) and its initial concentration in the liquid phase (C_0). The initial concentrations of dissolved compounds used in the sorption tests were 5 mg/l for each BTX and 160 mg/l for acetic acid. Like in the previous tests, p-xylene was the most strongly retained member of the BTX group, while benzene was the least sorbed.

Table 27. Amounts of BTX and acetic acid sorbed at equilibrium

	q_e (mg/g)	
Compound	without acetic acid	with acetic acid
benzene	0.046	0.025
toluene	0.048	0.039
p-xylene	0.063	0.053
Total BTX	0.157	0.117
Acetic acid	-	0.125

The values determined for q_e indicated the sorption capacity of the organoclay for each of the BTX compounds and acetic acid in a batch test. Results showed that there was a sorption competition between BTX compounds and acetic acid since the sorption capacities for BTX were lower when acetic acid was present in solution.

Batch kinetics data obtained for the experiment using both BTX compounds and acetic acid were fitted to the Lagergren (equation *17*) and Ho (equation *19*) models by linear regression analysis.

Integrating equation *(17)* with the conditions ($q_t = 0$ at $t = 0$; $q_t = q_t$ at $t = t$) gives:

$$\ln(q_e - q_t) = \ln(q_e) - k_{e1}t \qquad (39)$$

where q_t = amount of solute sorbed at time t (mg/g); q_e = amount of solute sorbed at equilibrium (mg/g); k_{e1} = pseudo-first-order rate constant of sorption (1/min). The calculated values of k_{e1} for acetic acid, total BTX, benzene, toluene and p-xylene were 0.0303, 0.0321, 0.0248, 0.0389 and 0.03138, respectively.
The following figures were drawn by plotting $-\ln(q_e-q_t)/\ln(q_e)$ versus $k_{el}t$.

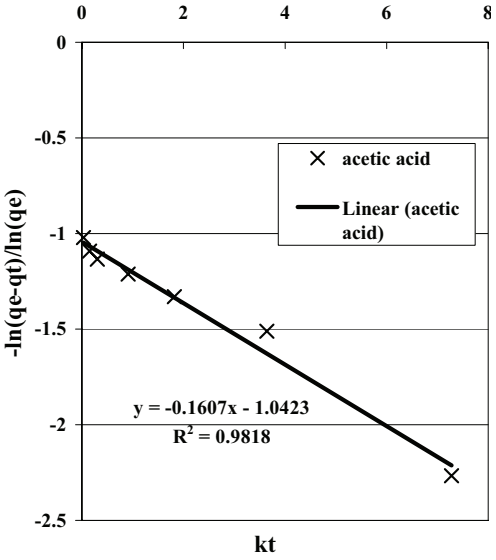


Figure 46. Pseudo-first-order linear equation for the sorption of acetic acid

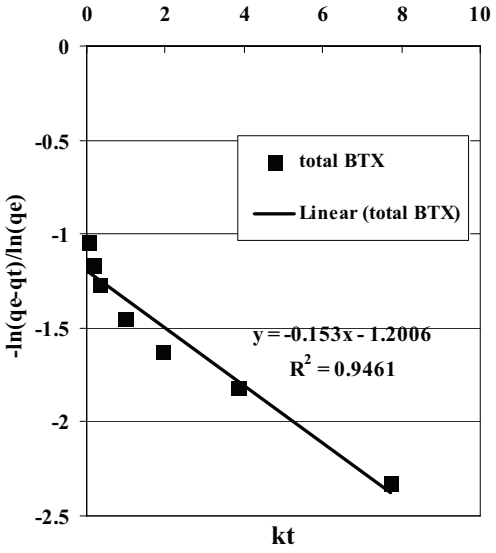


Figure 47. Pseudo-first-order linear equation for the sorption of total BTX

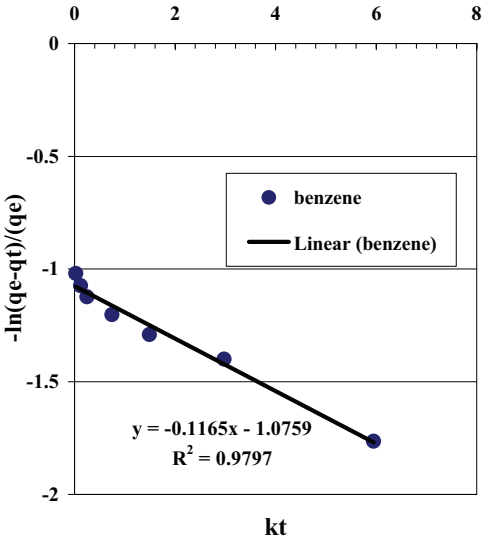


Figure 48. Pseudo-first-order linear equation for the sorption of benzene

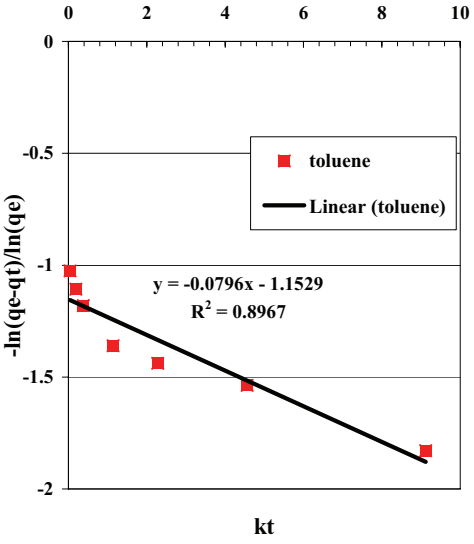


Figure 49. Pseudo-first-order linear equation for the sorption of toluene

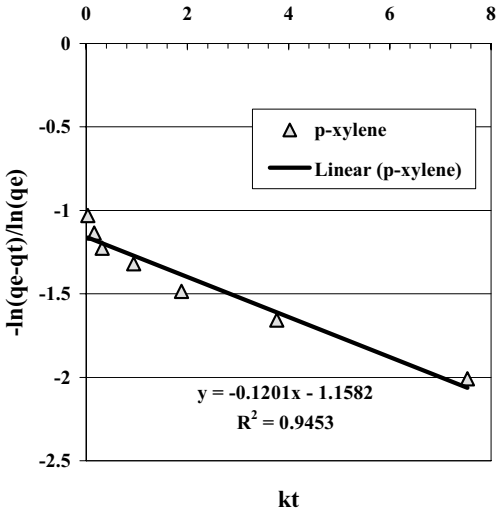


Figure 50. Pseudo-first-order linear equation for the sorption of p-xylene

The coefficients of determination (R^2) calculated for pseudo-first-order linear equations describing the sorption of acetic acid, total BTX, benzene, toluene and p-xylene were: 0.9818, 0.9461, 0.9797, 0.8967 and 0.9453, respectively.

Ho's model (equation *19*) was used to fit the data to the pseudo-second-order linear equation. The following diagrams were drawn by plotting t/qt versus t.

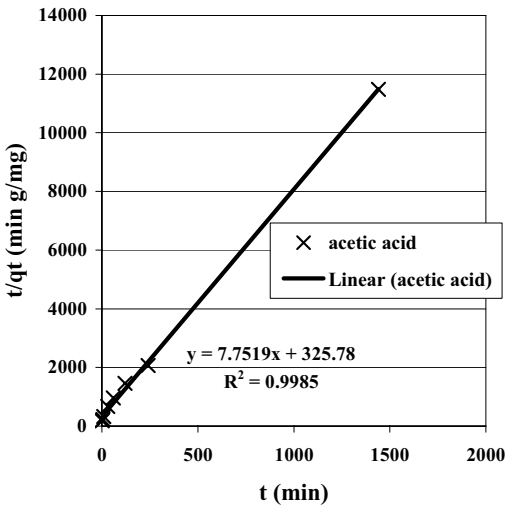


Figure 51. Pseudo-second-order linear equation for the sorption of acetic acid

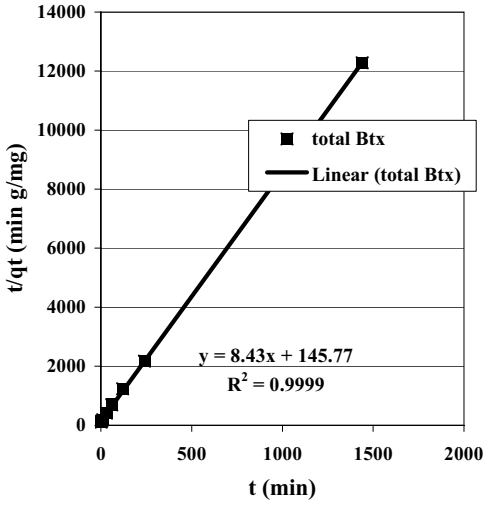


Figure 52. Pseudo-second-order linear equation for the sorption of total BTX

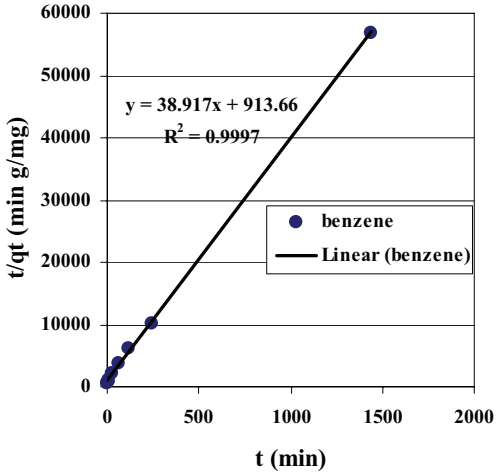


Figure 53. Pseudo-second-order linear equation for the sorption of benzene

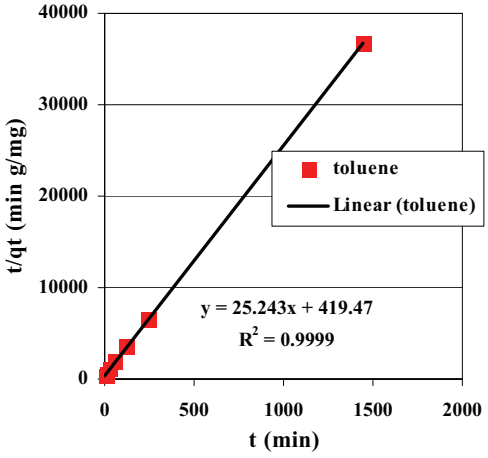


Figure 54. Pseudo-second-order linear equation for the sorption of toluene

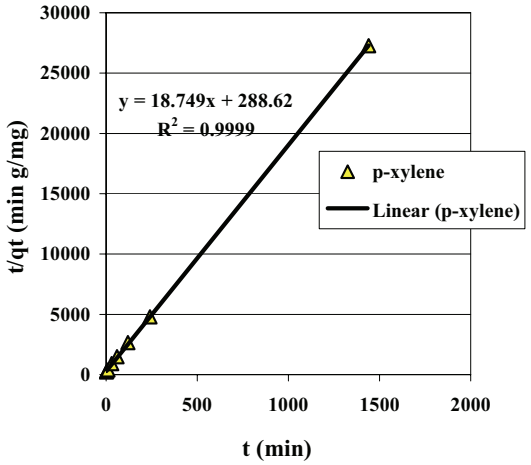


Figure 55. Pseudo-second-order linear equation for the sorption of p-xylene

The calculated values of k_{e2} (pseudo-second-order rate constant of sorption (g mg^{-1} min^{-1})) for acetic acid, total BTX, benzene, toluene and p-xylene were 0.1844, 0.4875, 1.6576, 1.5190 and 1.2179, respectively.
The coefficients of determination (R^2) calculated for pseudo-second-order linear equations describing the sorption of acetic acid, total BTX, benzene, toluene and p-xylene were 0.9985, 0.9999, 0.9997, 0.9999 and 0.9999, respectively.

Comparing the values of R^2 for pseudo-first-order and second-order equations, the latter fitted better to the experimental data and was selected to predict the sorption kinetics of BTX and acetic acid on the organoclay.
The pseudo-second-order model was used to calculate the initial sorption rates in the batch experiment for acetic acid, total BTX, benzene, toluene and p-xylene. These were 0.00307, 0.006860, 0.00109, 0.002386 and 0.003464 mg g^{-1} min^{-1}, respectively.

5.1.2.6 Conclusions on sorption tests with the organoclay

This study compared, in the first instance, the uptakes of BTX and acetic acid by a natural zeolite (NZ 1) and an organoclay. The organoclay showed better sorption properties for BTX and lower affinity for acetic acid than the zeolite and was therefore chosen for subsequent tests to assess effects of varying operating conditions. Influence of pH, initial acetic acid concentration, salinity and temperature on the sorption properties of the organoclay was studied by varying the value of one of the four parameters and keeping constant the values of the other three. The following conclusions were drawn:

- The uptake of BTX increased when less acetic acid was sorbed and vice-versa. This indicated that there was a sorption competition between BTX compounds and acetic acid on the hydrophobic chains of the quaternary amine immobilized on the surface of the clay.
- Sorption of acetic acid decreased proportionally with the increase of pH since the molecular form of the acid was better retained than the ionic form by the modified clay.
- The sorption onto the organoclay increased in the following order: benzene < toluene < p-xylene. This observation showed that the retention of BTX compounds by organoclay took place by a partition mechanism and increased with the decrease of solubility in aqueous solutions.
- Retention of BTX was enhanced when the concentration of NaCl was increased from 0 to 70 g/l. This can be explained by the salt-out effect. On the other hand, uptake of acetic acid decreased when salinity was increased.
- Sorption of both BTX and acetic acid decreased with the increase of temperature since this influenced the solubilities of the solutes.

5.2 FBR operation

As previously mentioned, the adopted treatment concept is based on selective sorption of BTX compounds onto an organoclay (Crudesorb) and biological regeneration of the spent media in a fluidized bed reactor. Similar systems using activated carbon instead of organoclay as sorbent were described by Xing and Hickey (1994*)* and Zhao *et al.* (1999)**.** Two sets of experiments were planned in order to investigate whether bioregeneration is a viable process for the recovery of the sorption capacity of Crudesorb:

- Without providing a microbial inoculum, nutrients and oxygen supply;

- With nutrients and oxygen supply after the addition of an inoculum containing a special blend of naturally occurring microorganisms cultured on a substrate containing BTX as the sole carbon source.

The aim of this study was to demonstrate that the use of an organoclay as biomass carrier in fluidized bed reactors produces a system in which both sorption and biodegradation removal mechanisms for BTX reduction are present. Sorption is the only removal mechanism for organic compounds when no nutrients and oxygen are supplied into the system. In this case, breakthrough of organic pollutants occurs in the system after the sorption capacity of the organoclay is exhausted. On the other hand, the breakthroughs of the pollutants are expected to be delayed or even prevented if the sorption process is coupled with biological degradation of sorbed compounds.

5.2.1 Sorption experiments with the FBR

Initial experiments were carried out without adding a microbial inoculum, oxygen and nutrient supply. Results are shown in Figure 56.

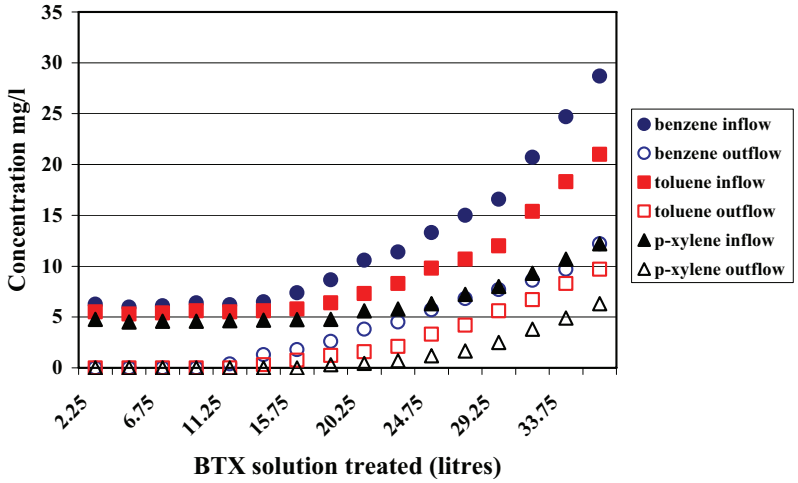


Figure 56. Removal of BTX compounds by sorption in a fluidized bed of organoclay

The order of breakthrough based on the volume of water treated at which the component was first detected was as follows: benzene (11.25 l water or 58 bed volumes), toluene (13.5 l water or 70 bed volumes) and p-xylene (22.5 l water or 115 bed volumes). These results suggested that the removal of BTX components followed the general solubility principles, corresponding to an increasing octanol/water partition coefficient in the order benzene (1.64), toluene (2.25.) and p-xylene (2.76) (Silalahi *et al.*, 2008).
The average benzene, toluene and p-xylene concentrations in the influent to the fluidized bed system were 6.3, 5.4 and 4.4 mg/l, respectively (total COD = 50.2 mg/l).

The observed sorption rates of the compounds were 0.34 mg benzene/g organoclay-h, 0.29 mg toluene/g organoclay-h and 0.24 mg p-xylene/g organoclay-h, which means 2.71 mg COD/g organoclay-h or 0.0650 kg COD/kg organoclay-day. Benzene was sorbed at a higher rate than toluene and p-xylene due to a higher concentration in the influent. The sorption capacities calculated based on the obtained results were: 0.43 mg benzene/g organoclay, 0.44 mg toluene/g organoclay and 0.60 mg p-xylene/g organoclay. These values are about 10 times higher than the sorption capacities previously measured in batch experiments. A possible explanation is the better contact between the solution and the organoclay in the fluidized bed reactor compared to the batch test configuration.
Sorption capacities of the organoclay for BTX compounds reported by Cetco Oilfield Services Company (the producer of this sorbent) were between 4 and 6 times higher. This was determined by pumping a solution containing 10 mg/l of each BTX compound through a column containing a fixed bed of 100 g organoclay. The order of breakthrough, based on the bed volumes (BV) at which the component was first detected was: benzene (150 BV), toluene (175 BV) and xylene (300 BV). Since the organoclay has a density of 0.85 g /cm^3, it means that the sorption capacities were: 1.75 mg benzene/g organoclay, 2.04 mg toluene/g organoclay and 3.51 mg p-xylene/g organoclay.
Daifullah and Girgis (2002) determined the adsorption capacities for BTX of five different types of activated carbons produced from different precursors: date pits (DP), cotton stalks (CS), peach stones (PS), almond shells (ALS), and olive stones. The adsorption capacities of these activated carbons decreased in the order: PS, ALS, CS, OS, and DP. In the case of PS the adsorption capacities were: 3.0 mg benzene/g, 6.5 mg toluene/g, and 8.7 mg p-xylene/g. The adsorption capacities determined for the five activated carbons were lower than those of a commercial powder activated carbon (PAC) produced by Prolabo (7.9 mg benzene/g, 9.2 mg toluene/g and 9.9 mg p-xylene/g).
Sorption capacities of activated carbons are higher than those of organoclay, but compared to organoclay, activated carbons have lower selectivity for the BTX compounds.

5.2.2 Biodegradation experiments

5.2.2.1 Screening test for BTX degraders

The study of BTX biodegradation was started by selecting a good microbial inoculum able to achieve high degradation rates of the target compounds. Four different types of microbial sources were employed to grow microbial cultures, which fed on BTX as sole carbon source. The origins of microorganisms were as follows:

1. Natural sea water from Trondheim fjord, Norway;
2. Sludge from an ozonation – biofiltration system for biodegradation of natural organic matter (NOM) and selected organic micropollutants;
3. Sludge from a conventional municipal wastewater treatment system;
4. A commercial blend of naturally occurring microorganisms provided by Catalina Biosolutions (USA based company).

Results illustrated in Figure 57 demonstrated that the microbial culture prepared with the commercial blend of microorganisms provided by Catalina Biosolutions contained the highest concentration of bacteria (8.36 E+07), while the lowest concentrations were determined in the blank samples and in those in which sludge from the ozonation-biofiltration plant was added as microbial source. Based on the results of the screening test, it was decided to continue this study using only the inoculum produced with the commercial microbial blend.

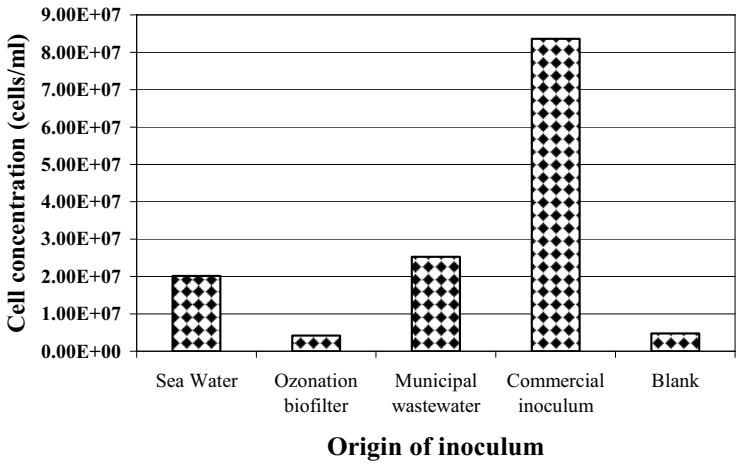


Figure 57. Concentrations of microbial cells in different cultures of microorganisms

5.2.2.2 BOD test

The aim of the test was to obtain more information on the degradation rates of BTX compounds by the selected culture of microorganisms.
Three different types of samples were prepared for the BOD test:

1. Microbial inoculum + synthetic sea water matrix (blanks);
2. Microbial inoculum + sodium acetate (2 mg/l) + synthetic sea water matrix.
3. Microbial inoculum + BTX (2.5 mg/l) + synthetic sea water matrix;

Table 28 shows the values of DO concentrations measured in the BOD bottles in the beginning of the test and after the indicated number of days.

Table 28. Values of DO concentrations (mg/l) measured in the samples

No.	Days	0	7	14	28
1.	Inoculum + synthetic matrix (blanks)	7.3	7.3	7.2	7.1
2.	Inoculum + acetate + synthetic matrix	7.3	6.3	5.9	5.6
3.	Inoculum + BTX + synthetic matrix	7.2	3	1.4	0.2

The percentage of BTX compounds and sodium acetate biodegraded during the test was calculated according to:

$$\%\, biodegradation = \frac{mg\ O_2/mg\ tested\ (or\ reference)\ substance}{mg\ ThOD/mg\ tested\ substance} \times 100 \qquad (40)$$

The theoretical oxygen demand (ThOD) of a substance $C_cH_hCl_{cl}N_nNa_{na}O_oP_pS_s$ of the molecular weight MW is calculated with the following formula:

$$ThOD = \frac{16[2c + \frac{1}{2}(h - cl - 3n) + 3s + \frac{5}{2}p + \frac{1}{2}na - o]}{MW} \qquad (41)$$

This calculation implies that C is mineralized to CO_2, H to H_2O, P to P_2O_5 and Na to Na_2O. Halogen is eliminated as hydrogen halide and nitrogen as ammonia.
The theoretical amounts of oxygen needed to biodegrade 1 mg of benzene, toluene or p-xylene are 3.077, 3.13 and 3.17 mg respectively, while 0.78 mg O_2 is necessary to biodegrade 1 mg sodium acetate.

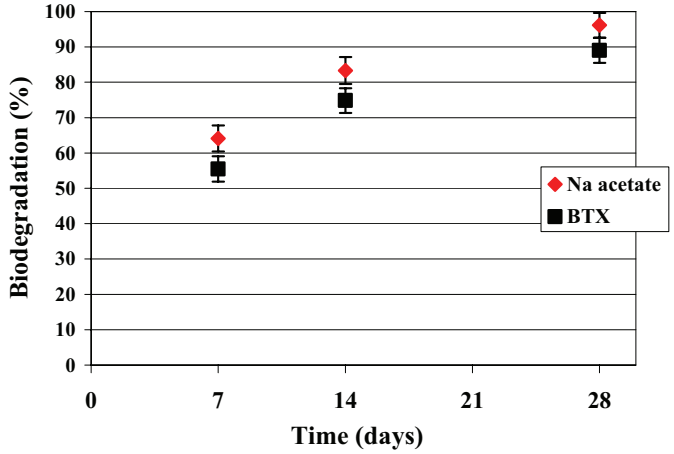


Figure 58. Biodegradation (%) of BTX compounds and sodium acetate

The amounts of reference compound (sodium acetate) biodegraded after 7, 14 and 28 days were 64%, 84% and 96% of the initial amount, respectively (Figure 58). Since sodium acetate was reasonably degraded within a short time span, it was concluded that the BOD test was successful.
The calculated biodegradation of the BTX compounds was 55%, 75% and 89% of the initial amount after 7, 14 and 28 days respectively (Figure 58). This demonstrated that the inoculum prepared from the commercial microbial blend was a viable source of BTX degraders that could be further used to inoculate the lab-scale fluidized bed reactor.

5.2.2.3 BTX biodegradation test

The BOD test carried out previously provided information about the degradation of the total BTX compounds over a period of 28 days by measuring the amount of dissolved oxygen consumed after certain time intervals (Scurtu *et al*., 2008a). Since that experiment did not offer detailed data about the biodegradation of each individual member of the BTX group, a second test, in which the BTX concentrations were determined by GC/MS at the same time intervals as in the BOD test was performed. The initial concentrations of each of the BTX compounds were 1 mg/l (compared to 2.5 mg/l total BTX in the previous BOD test). The same procedure as in the BOD test was used to prepare the liquid matrix for this second biodegradation test (purified water with Tropic Marin salts and Bushnell-Haas nutrients).

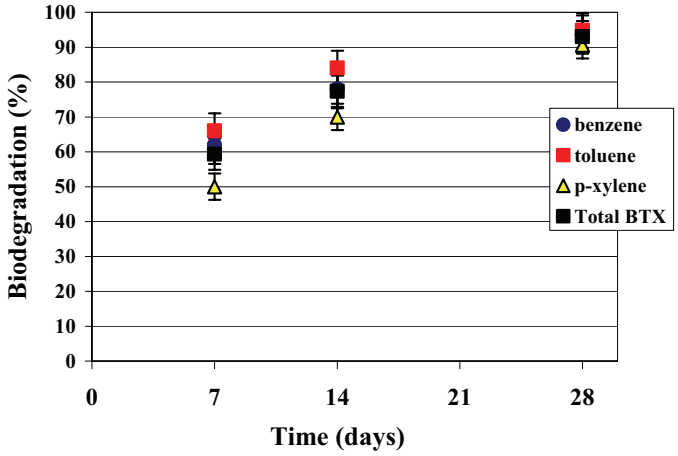


Figure 59. Biodegradation (%) of BTX compounds

The biodegradation calculated for each of the BTX components was as indicated in Table 29:

Table 29. BTX biodegradation (%) at different time intervals

Compound	Number of days		
	7	14	28
benzene	62%	78%	93%
toluene	66%	84%	94%
p-xylene	50%	70%	90%
Total BTX	59%	77%	92%

Results indicated that the biodegradation increased in the following order: p-xylene < benzene < toluene. Consequently, toluene seemed to be the most biodegradable member of the BTX family while p-xylene is the least biodegradable. This finding is supported by the results obtained by Voice *et al*. (1992)**.** The biodegradation calculated for total BTX was 59%, 77% and 93% of the initial amount after 7, 14 and 28 days respectively (Figure 59, Table 29). This fitted well with the results of the previously done BOD test.

5.2.3 Simultaneous sorption and biodegradation of BTX in the FBR

Tests were conducted with the fluidized bed system designed to combine the effects of sorption and biodegradation. The aim of these experiments was to determine if this process configuration enhances the performance of the sorbent with respect to BTX removal. A culture of BTX degraders was established in the fluidized bed reactor by enriching the culture obtained from the commercial inoculum during the screening test and transferring it into the experimental system as described above.

A continuous feed of synthetic seawater containing BTX and nutrients (at a stoichiometric ratio of 100/5/1:COD/N/P) was started when the bacteria cell concentration was around 1.0 E+8 cells/ml and the BTX concentration less than 1 mg/l. Other groups of researchers confirmed the growth of biofilms on different types of materials such as activated carbon, a synthetic carbonaceous sorbent, an anion exchange resin, sand and glass beads (Pirbazari *et al.*, 1990)
The data presented in Figure 60 provide the same type of information as Figure 56, but indicate the breakthroughs of BTX compounds in a system in which the removal of these contaminants was caused by the combined effects of sorption and biodegradation.

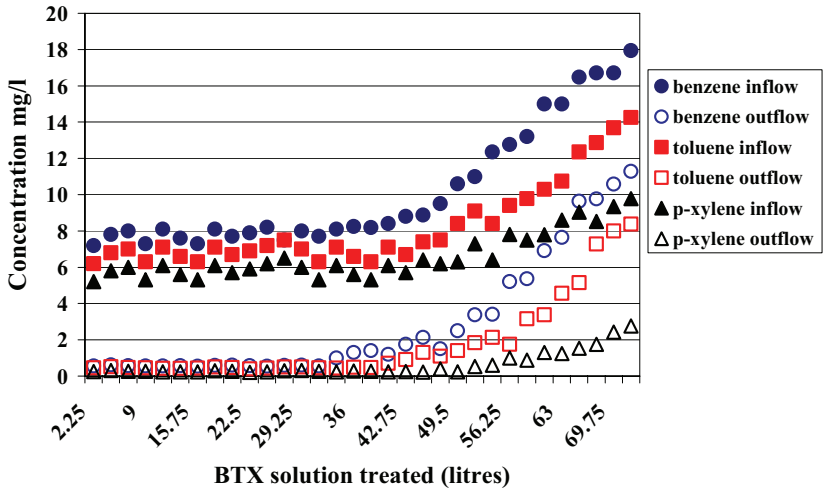


Figure 60. Removal of BTX compounds by sorption and biodegradation in a fluidized bed of organoclay

The breakthrough order based on the volume of water treated at which the compound was first detected was similar to that observed when sorption was the only removal mechanism (Figure 56). The difference was that all three BTX compounds were first detected in the effluent of the fluidized bed reactor after a larger volume of BTX solution was treated: benzene (33.75 l or 173 bed volumes), toluene (42.75 l or 219 bed volumes) and p-xylene (56.25 l or 288 bed volumes). The average benzene, toluene and p-xylene concentrations in the influent to the fluidized bed system were calculated to be

7.7, 6.7 and 5.7 mg/l respectively (total COD = 62.73 mg/l). The observed biodegradation rates of BTX compounds were: 1.72 kg benzene/m^3-day, 1.54 kg toluene/m^3-day and 1.21 kg p-xylene/m^3-day (13.94 kg COD/m^3-day or 0.0591 kg COD/kg organoclay-day). Results showed the superior performance of sorption-biodegradation process configuration compared with the system based only on sorption. The biological activity in the fluidized bed reactor delayed but did not prevent the breakthrough of BTX compounds, probably due to an overloaded system with respect to its biological regeneration capacity. One hypothesis is that the biofilm of microorganisms detected on the organoclay particles biodegraded the sorbed BTX compounds and in this way, the sorbent recovered to some extent its sorption properties. However, microorganisms not attached to the organoclay may have contributed to the biodegradation of the BTX compounds in the water phase, thereby reducing the concentrations the sorbent was exposed to.

5.2.4 Tuning of FBR operation for off-line bioregeneration of the spent organoclay

5.2.4.1 Aim of tuning experiments

The lab-scale FBR system was used to conduct sorption and bioregeneration experiments as cyclic and separate batch processes. This experimental design was closer to the proposed treatment concept based on in-line sorption coupled with biological regeneration in a FBR.
Sorption capacity of the organoclay was reported to be as follows: 0.43 mg benzene/g organoclay, 0.44 mg toluene/g organoclay and 0.60 mg p-xylene/g organoclay (Scurtu *et al*., 2008a). Since the fluidized bed contained 165 g organoclay, it means that the FBR system removed a total of 70.95 mg benzene, 72.60 mg toluene and 99.00 mg p-xylene respectively, before the breakthrough of all compounds occurred.

5.2.4.2 Operation in 6 h cycles (2 h sorption, 2 h bioregeneration, 2 h recirculation without aeration)

The experiment consisted of pumping into the FBR an amount of BTX equal to the determined sorption capacity in order to exhaust the sorption capacity of the organoclay bed and then regenerate the media by biodegrading the sorbed pollutants.
Experiments started after the DO concentration in the solution was decreased below 1 mg/l by stopping aeration in the system. Influent flows at the beginning of each treatment cycle contained both BTX compounds and nutrients. Initially, the nutrients necessary for the bioregeneration were supplied at the beginning of the test and not after the sorption process to avoid dilution of the BTX solution in the FBR. Subsequently the liquid was recirculated in the FBR for 1 h and 55 min without aeration at a rate of 250 ml/min in order to complete the sorption process and to exhaust the sorption capacity of the organoclay bed.
BTX concentrations were first measured 5 min after the start of each treatment cycle (at the end of BTX dosage steps) and every 30 minutes during sorption and bioregeneration

steps. Breakthrough of all BTX compounds was observed due to the high concentrations of pollutants pumped into the FBR. Although p-xylene was supplied in higher concentration than benzene and toluene, its concentration in the effluent was lower than those of the other two compounds (Figure 61). This showed once more the affinity of organoclay for the more hydrophobic compounds with higher octanol/water partition coefficients.

The concentrations of all BTX components dropped steeply due to sorption onto organoclay, until equilibrium was reached. The following concentrations were measured at sorption equilibrium: 0.74 mg/l benzene, 0.72 mg/l toluene and 0.62 mg/l p-xylene.

A membrane contactor was used to supply oxygen into the system for 2 h immediately after the completion of the sorption step. The DO consumption increased sharply after the oxygen supply was started, where influent concentration to the FBR was around 8.2 mg/l (saturation concentration for the given salinity and temperature) and less than 1 mg/l in the effluent.

The BTX concentrations in the liquid phase dropped below 0.1 mg/l, however, DO consumption remained high until the end of the period indicating that the microorganisms fed on the BTX compounds sorbed onto the organoclay.

The aeration was stopped after 2 h and the liquid phase was recirculated in the system for two more hours. DO concentration dropped below 0.5 mg/l and the experiment continued with a new treatment cycle (BTX dosage for 5 min). The concentrations of BTX compounds measured after pumping BTX into the FBR in the second cycle were higher than those measured in the previous cycle. A possible explanation for this phenomenon could be the incomplete recovery of sorption capacity during the previous bioregeneration step. BTX compounds were sorbed at slightly slower rates than in the first sorption cycle and the equilibrium concentrations in the liquid phase were higher than at the end of the previous sorption cycle (3.4 mg/l for benzene, 2.9 mg/l for toluene and 2.7 mg/l for p-xylene). During the subsequent bioregeneration step the BTX concentrations in the liquid phase dropped again under 0.1 mg/l (Figure 61).

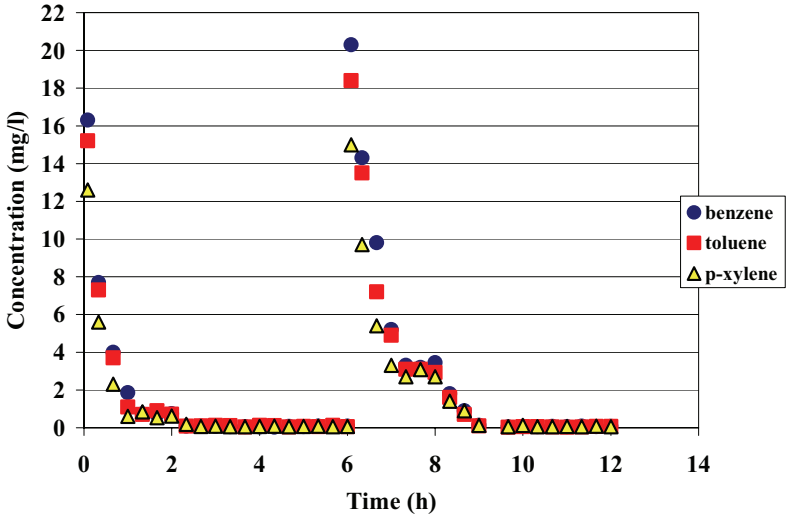


Figure 61. Profile of BTX concentrations (2 h sorption, 2 h bioregeneration, 2 h recirculation without aeration)

5.2.4.3 Operation in 8 h cycles (2 h sorption, 6 h bioregeneration)

Since the concentrations of BTX measured in the FBR at the end of the sorption step of the second cycle were higher than in the first cycle and the calculated DO consumptions were high at the end of both bioregeneration steps (when oxygen was supplied only for 2 h into the system), it was believed that the sorbed BTX were not completely biodegraded. Therefore, it was decided to lower the amount of BTX pumped into the system from 100% to 75% of the sorption capacity of the organoclay bed, increase the aeration period from 2 to 5 h and monitor the profile of DO consumption.
One important issue for the treatment process was to have a low microbial activity during the BTX sorption steps. This could be insured by low DO concentrations and absence of nutrients in the system. Therefore it was decided to supply the nutrients at the beginning of the bioregeneration steps when oxygen supply was also started.

The concentrations of BTX measured into the reactor at the end of BTX dosage steps were lower than in the previous experiment since the amounts of BTX pumped were only 75% of the total sorption capacity of the organoclay bed. On the other hand, the concentrations of BTX measured in the FBR after the sorption steps were lower (< 0.1 mg/l) than those measured in the previous experiment at the end of the sorption step of the second treatment cycle (about 3 mg/l for each of the BTX compounds) - when more BTX were pumped into the system. The concentrations of all BTX compounds into the liquid phase were lower than 0.05 mg/l at the end of the subsequent bioregeneration and recirculation without aeration treatment steps (Figure 62).

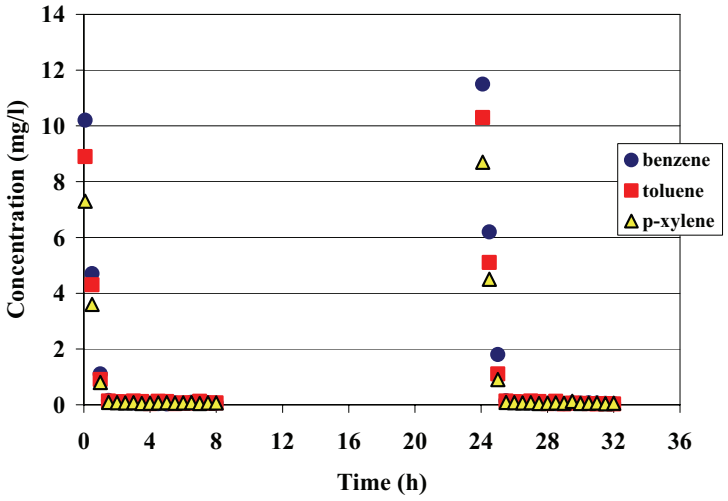


Figure 62. Profile of BTX concentrations (2 h sorption, 6 h bioregeneration)

The sorption rates of the BTX compounds measured for both treatment cycles were 0.16 mg benzene/g organoclay-h, 0.17 mg toluene/g organoclay-h and 0.23 mg p-xylene/g organoclay-h, which means a total of 1.75 mg COD/g organoclay-h (0.0420 kg COD/kg organoclay-day). On the other hand, the biodegradation rates of BTX were 0.30 kg benzene/m^3-day, 0.31 kg toluene/m^3-day and 0.42 kg p-xylene/m^3-day, which means a total of 3.22 kg COD/m^3-day (0.0136 kg COD/kg organoclay-day).

DO consumption was calculated by subtracting DO concentration measured in the effluent from DO concentration in the influent to the FBR. DO consumption quickly increased after the aeration was started reaching around 210 mg DO/l/h and decreasing to 6 mg DO/l/h when the carbon source (BTX) was being depleted.

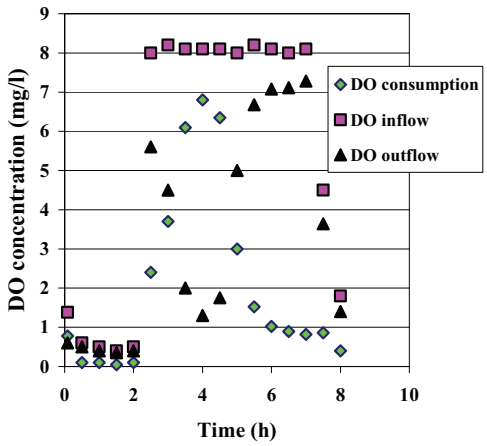


Figure 63. Profile of DO concentrations during the first cycle

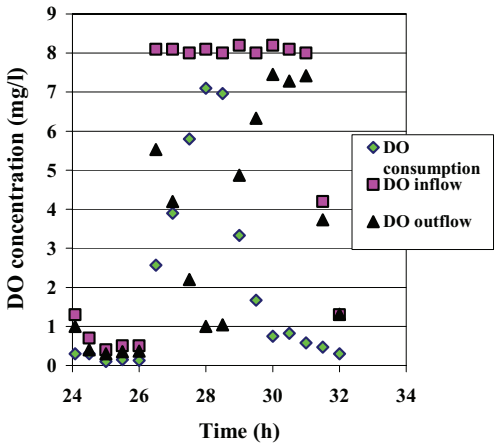


Figure 64. Profile of DO concentrations during the fourth cycle

Based on data shown in Figures 63 and 64 it was concluded that the bioregeneration process lasted for approximately 4 h. In this period, a significant microbial activity took place into the system proven by the high DO consumption measured. Microorganisms used up the sorbed BTX compounds as carbon source and when concentrations became insufficient to support microbial growth, oxygen consumption decreased steeply.

5.2.4.4 Operation in 6 h cycles (2 h sorption, 4 h bioregeneration)

The oxygen consumption in the last 1.5 h of the 5 h aeration step of the previous treatment configuration was low and therefore, it was decided to shorten the aeration period from 5 to 3.5 h. In this way a low DO concentration level could be attained in the system at the end of each bioregeneration step with shorter recirculation steps with no oxygen supply. Based on these observations it was concluded that the treatment cycle should last 6 h and consist of the following treatment steps:

- 5 min dosage of BTX solution (amount equivalent to 75% of the total sorption capacity of the organoclay bed);

- 2 h sorption – including the previous 5 min, achieved by recirculating the fluid through the FBR (250 ml/min) without oxygen supply;
- 5 min dosage of nutrient solution to the FBR;
- Oxygen supplied only for 3.5 h after each sorption step.

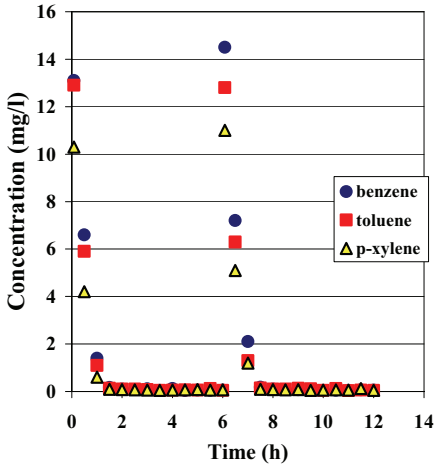


Figure 65. Profile of BTX concentrations (2 h sorption, 4 h bioregeneration)

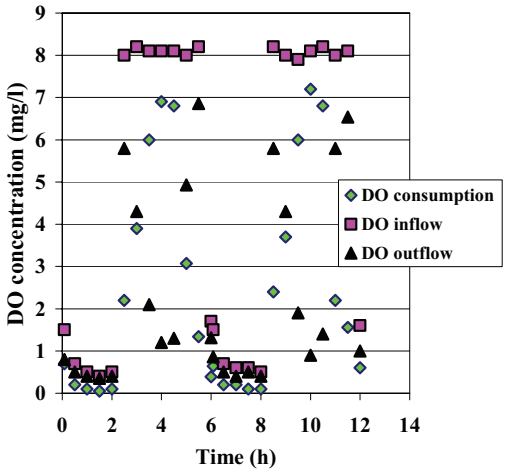


Figure 66. Profile of DO concentrations (2 h sorption, 4 h bioregeneration)

The concentrations of BTX compounds were less than 0.1 mg/l after the sorption step and less than 0.05 mg/l after the bioregeneration steps (Figure 65). The observed sorption rates of the BTX compounds were similar to those calculated for the previous operation mode, while the biodegradation rates of BTX were 0.46 kg benzene/m^3-day, 0.47 kg toluene/m^3-day and 0.64 kg p-xylene/m^3-day, which means a total of 4.91 kg COD/m^3-day (0.0208 kg COD/kg organoclay-day).
The data presented in Figure 66 show that the residual DO concentration at the end of the bioregeneration cycles was around 1.5 mg/l and the DO consumption was low.

5.2.5 Long time off-line bioregeneration experiments

5.2.5.1 Operation in 6 h cycles (2 h sorption, 4 h bioregeneration)

Experiment with BTX compounds

This experiment was carried out over a period of 36 days in order to study the efficiency of off-line bioregeneration of the organoclay, loaded with BTX compounds, in the fluidized bed reactor system. The previous tests showed that the off-line bioregeneration of the organoclay was feasible but this had to be verified through additional experiments over a longer period.

The FBR system was operated following the 6 h cycle operation mode found to provide the most efficient bioregeneration of the organoclay during the previous tuning experiments as described in the section dedicated to the FBR operation.
Water samples were taken from the FBR every fourth day, exactly at the same time, as follows:

- Before pumping a new load of BTX solution;
- At the end of the BTX dosage steps;
- At the end of the sorption steps;
- At the end of the bioregeneration steps.

The concentrations of BTX compounds were measured by GC/MS and the profile of BTX concentrations over time was determined:

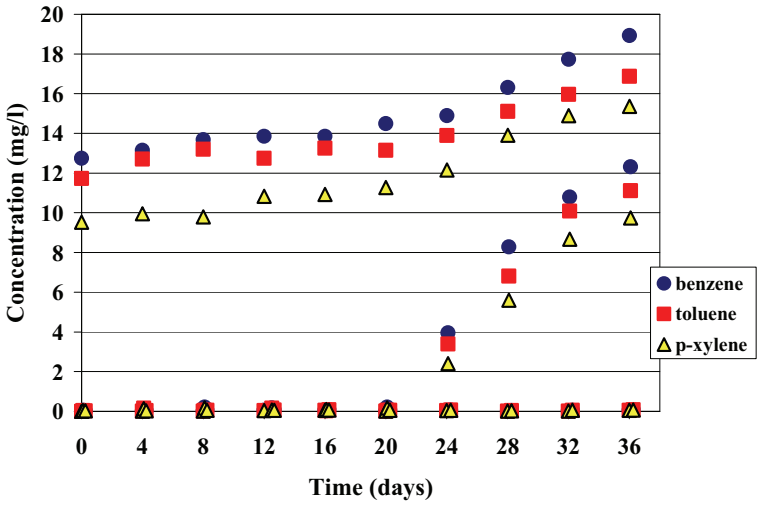


Figure 67. Profile of BTX concentrations in the FBR (2 h sorption, 4 h bioregeneration)

Diagram 67 shows that the organoclay partially lost its sorption capacity over time. This loss was demonstrated by the increasing amounts of BTX measured into the reactor at the end of the BTX dosage steps and the amounts of BTX detected in the water phase after the sorption steps, starting with day 24 counted from the beginning of the test. Since only a BTX amount equal to 75% of the sorption capacity of the organoclay bed was pumped into the system in the beginning of each treatment cycle, it means that a reserve of 25% of the sorption capacity remained available for further sorption. Even though the organoclay lost a small part of its sorption capacity, this did not result in a residual amount of BTX after the sorption steps until day 24 thanks to the spare sorption reserve of 25% of the total sorption capacity of the organoclay bed. This spare capacity covered the loss of sorption capacity and helped sorb the increasing residual amounts of BTX in the system after the BTX dosage steps until day 24 (578 h after the start). In this day the losses exceeded the spare sorption capacity and the organoclay was not able anymore to sorb the entire amount of BTX pumped in the beginning of each treatment cycle and residual amounts of BTX were further on detected at the end of each sorption

step. Microorganisms biodegraded the residual amounts of BTX and most of the sorbed compounds in such a way that the concentrations of BTX compounds at the end of all bioregeneration steps were close to 0.
The occurrence of off-line bioregeneration was proven by the small differences between the residual concentrations of BTX in the FBR after two consecutive BTX dosage steps. If there was no bioregeneration, the organoclay bed would become completely loaded with BTX after only two BTX dosage steps (which summed up pump an amount of BTX equal to 150% of the sorption capacity of the organoclay bed). A brief calculation demonstrates that if no bioregeneration occurred into the system, the residual concentrations of BTX after the second sorption step (second treatment cycle) would be very high. The obtained results indicated that the concentrations of BTX compounds after the BTX sorption steps were low at all times before day 24, showing that the organoclay bed was able to sorb a significant part of the pumped BTX compounds during the entire duration of the experiment.
Based on these results it was estimated that the organoclay bed lost around 1% of its sorption capacity per day (25% of its sorption capacity in 24 days). The decrease in sorption capacity continued also between day 24 and day 36 (the end of the test). When the experiment was stopped, the loss of sorption capacity of the organoclay bed was around 35% of its total sorption capacity. Theoretically, the residual amounts of BTX were expected to increase in the order benzene < toluene < p-xylene since more p-xylene was pumped in the FBR in the beginning of each cycle, but in practice they decreased in this order, the concentration of p-xylene in solution being lower than those of benzene and toluene. One explanation could be the higher hydrophobicity of p-xylene (higher octanol/water partition coefficient), which resulted in stronger bonds between the organoclay and this compound.
Sorption and biodegradation rates for this test were similar to those calculated for the tuning experiment with 2 h sorption and 4 h bioregeneration (3.5 h aeration).

Experiment with BTX compounds and acetic acid

The organoclay bed whose sorption capacity was exhausted during the previous test when it was repeatedly loaded with BTX compounds and bioregenerated over a long period was replaced with a fresh bed of the same volume and a microbial culture was established in the FBR as described above in the methods section.
The test lasted as well 36 days and studied the variation in sorption capacity of the organoclay bed loaded with BTX compounds and a small amount of acetic acid. The reason for adding acetic acid is that produced water contains significant concentrations of carboxylic acids and acetic acid is found by far in the highest concentration.
The operation mode of the FBR was the same as the one employed in the previous experiments, the only difference being provided by the composition of the feed solution pumped into the reactor during the first step of a treatment cycle. The solution contained 2 mg/l acetic acid in addition to the amount equal to 75% of the sorption capacity of the organoclay bed (106 mg/l benzene, 109 mg/l toluene and 148 mg/l p-xylene).
The sampling protocol was the same as the one used in the previous test: four samples were collected every fourth day as described above.

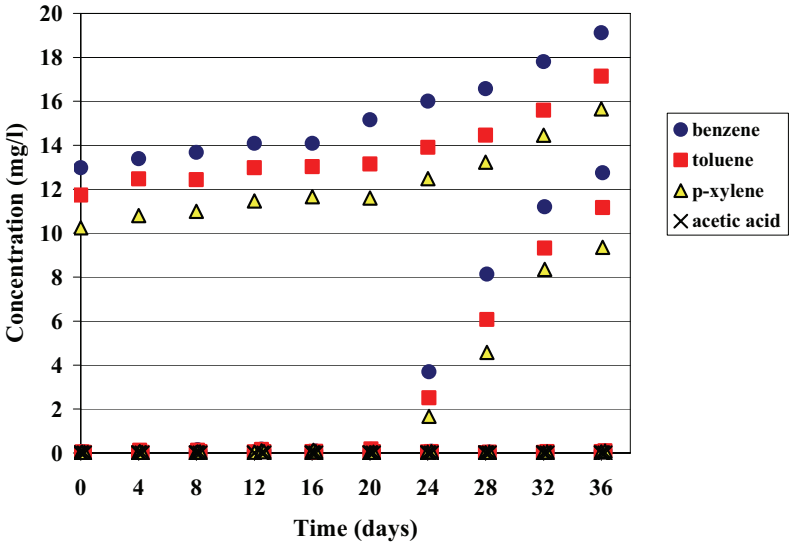


Figure 68. Profile of BTX and acetic acid concentrations in the FBR (2 h sorption, 4 h bioregeneration)

Results (Figure 68) were similar to those obtained when no acetic acid was added to the feed solution. Acetic acid was not detected at any time into the FBR, which means that it was completely sorbed during the first 5 minutes of each treatment cycle (BTX and acetic acid dosage steps).

Since the loss of sorption capacity of the organoclay bed was in the same range as in the previous experiment it means that the acetic acid was desorbed from the organoclay particles and subsequently biodegraded. One possible explanation for this phenomenon could be the weaker bonds formed between the acetic acid (a fairly polar compound) and the non-polar tailoring agent (dimethyl dihydrogenated ammonium) used to modify the surface of the clay. On the other hand, acetic acid is a readily biodegradable compound and therefore, it was easily metabolized by microorganisms. Several researchers claimed that the presence of small concentrations of acetic acid could lower the metabolic flux of BTX compounds. However, this negative effect might be counteracted by an acetic acid supported increase in biomass, which leads to faster biodegradation rates (measured as the rate of BTX degradation per cell) (Lovanh *et al*., 2002).

The sorption rates of the BTX compounds were 0.16 mg benzene/g organoclay-h, 0.17 mg toluene/g organoclay-h, 0.23 mg p-xylene/g organoclay-h and 0.0030 mg acetic acid/g organoclay-h, which means a total of 1.756 mg COD/g organoclay-h (0.0421 kg COD/kg organoclay-day), while the biodegradation rates of BTX were 0.46 kg benzene/m^3-day, 0.47 kg toluene/m^3-day, 0.64 kg p-xylene/m^3-day and 0.009 kg acetic acid/m^3-day, which means a total of 4.92 kg COD/m^3-day (0.0209 kg COD/kg organoclay-day).

5.2.5.2 Operation in 8 h cycles (2 h sorption, 6 h bioregeneration) with low concentration of acetic acid

One of the operation modes tested during the tuning of FBR operation had an aeration step of 5 h. Results indicated a high DO consumption only in the first 3.5 h and therefore, it was decided, at that time, to shorten the bioregeneration period to 4 h. However, DO consumption profile showed that the microbial activity continued at lower intensity after the 3.5 h period. This is believed to occur as a consequence of desorption of remaining sorbed BTX compounds due to the concentration gradient between the loaded organoclay bed and the liquid phase in the FBR. Microorganisms can feed on the low amounts of desorbed BTX, consuming small amounts of DO.
In order to verify this theory, it was decided to increase the bioregeneration period from 4 to 6 h and provide continuous aeration during the first 5 h of each bioregeneration step. Profiles of BTX, acetic acid and DO concentrations were monitored.

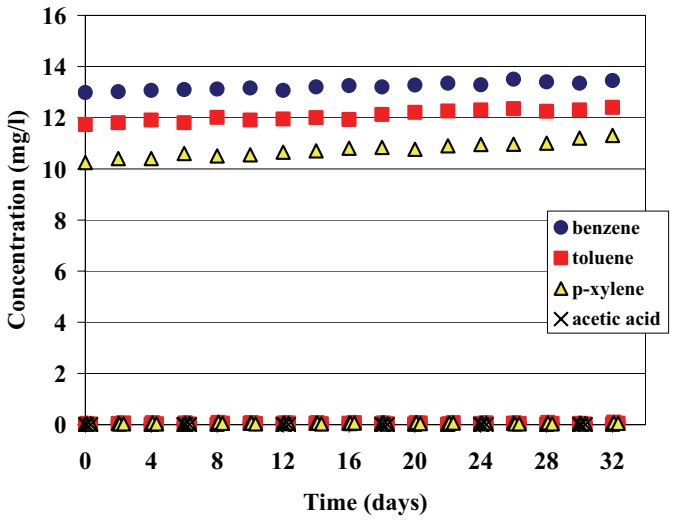


Figure 69. Profile of BTX and acetic acid concentrations in the FBR (2 h sorption, 6 h bioregeneration)

The FBR was run in this operation mode for 32 days (Figure 69), but the exhaustion of the entire 25% spare sorption capacity was not observed within this period, since BTX compounds were not detected at any time into the solution after the sorption steps. However, it is believed that the organoclay bed lost a small fraction of its sorption capacity over time, since the concentrations of all BTX in the liquid phase constantly increased after the 5 min dosage steps. This increase demonstrated that sorption kinetics decreased towards the end of the test.

Acetic acid was supplied in a concentration of 2 mg/l (as in the previous test) together with the BTX solution. Again, acetic acid was not detected at any time in the system

because it was sorbed by the organoclay bed during the 5 min dosage steps, in the beginning of each treatment cycle, and then biodegraded by the microbial culture.

The sorption rates calculated for this experiment were similar to those measured during the previous test with 2 mg/l acetic acid in the feed solution (2 h sorption and 4 h bioregeneration): 0.16 mg benzene/g organoclay-h, 0.17 mg toluene/g organoclay-h and 0.23 mg p-xylene/g organoclay-h and 0.0030 mg acetic acid/g organoclay-h, which means a total of 1.756 mg COD/g organoclay-h (0.0421 kg COD/kg organoclay-day). On the other side, biodegradation rates of BTX were 0.30 kg benzene/m^3-day, 0.31 kg toluene/m^3-day, 0.42 kg p-xylene/m^3-day and 0.006 kg acetic acid/m^3-day, which means a total of 3.23 kg COD/m^3-day (0.0137 kg COD/kg organoclay-day).

DO concentration profiles were monitored in the beginning and towards the end of the experiment (26 days after the start). Each time the DO concentration was monitored for 8 h in two consecutive days (with two not monitored cycles in between).

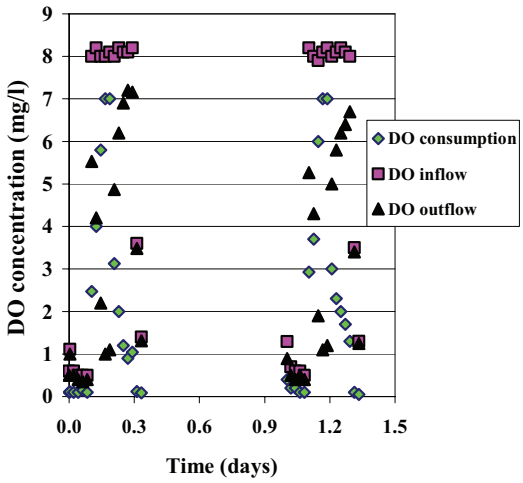


Figure 70. Profile of DO concentrations in the beginning of the experiment

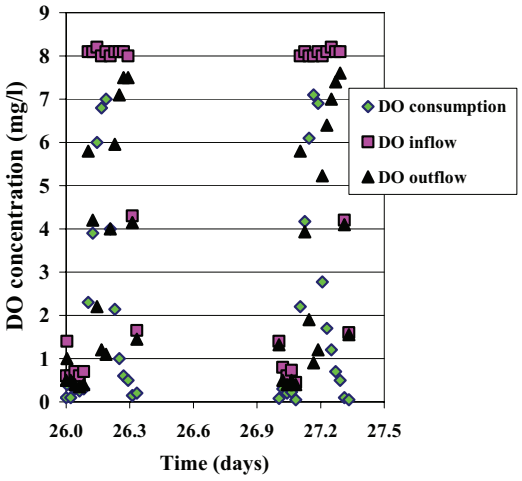


Figure 71. Profile of DO concentrations 26 days after the start

Results confirmed the presence of a low microbial activity in the system 3.5 h after the beginning of each bioregeneration step. This demonstrated that small amounts of substrate (BTX) were available for biodegradation. Two mechanisms are believed to make the substrate available:

- Desorption of the remaining amounts of sorbed BTX due to concentration gradient and long contact time between the organoclay particles and liquid phase in the FBR;
- Microorganisms produce exoenzymes that hydrolyze the sorbed BTX compounds and the resulting products desorb and are available for biodegradation.

Compared with the previous long time experiment with only 3.5 h aeration period, this operation mode extended microbial activity in the system with a period of low intensity biodegradation.
A brief comparison of the two DO concentrations profiles (Figures 70 and 71), measured at different intervals after the start of the experiment showed that the duration of microbial activity slightly decreased over time because of loss of sorption capacity. One hypothesis is that less BTX compounds have desorbed during the bioregeneration steps towards the end of the test.

5.2.5.3 Operation in 8 h cycles (2 h sorption, 6 h bioregeneration) with high concentration of acetic acid

This experiment was similar to the previous one, the only difference being the addition of 50 mg/l instead of 2 mg/l acetic acid to the feed solution containing BTX. The duration of each bioregeneration step was 6 h, aeration being provided only in the first 5 h.
The system was run in this operation mode for 28 days (Figure 72), but the exhaustion of the spare sorption capacity did not occur in the system since BTX concentrations at the end of all sorption steps were close to 0. On the other hand, the acetic acid was not significantly sorbed by the organoclay bed, being detected into the reactor after all 5 min dosage and 2 h sorption steps. Since the BTX and acetic acid concentrations measured in the liquid phase after the 5 min dosage steps slightly increased over time, it is thought that the organoclay bed lost a small fraction of its sorption capacity.

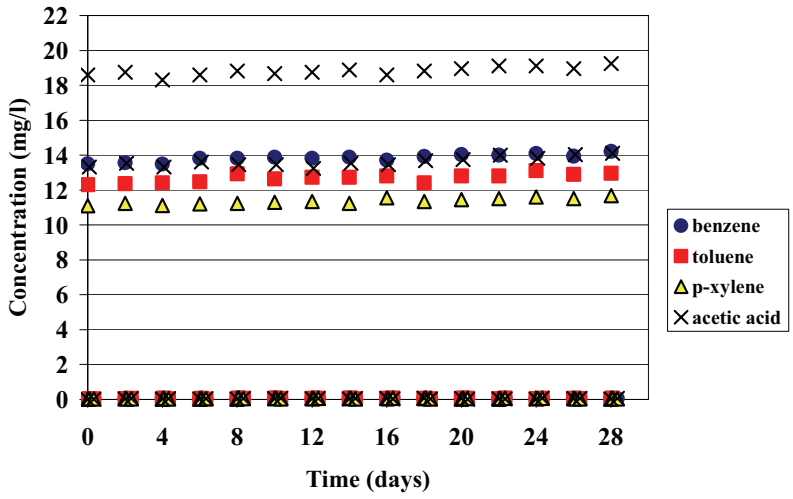


Figure 72. Profile of BTX and acetic acid concentrations in the FBR (2 h sorption, 6 h bioregeneration, high concentration of acetic acid)

The average amount of acetic acid removed between the end of the 5 min dosage steps and the end of the sorption steps was 3.63 mg acetic acid that means 0.022 mg acetic acid/g organoclay, which is lower than the amount of acetic acid sorbed during the same period in the batch tests (0.06 mg acetic acid/g organoclay). An explanation for this could be the higher concentration of acetic acid (160 mg/l) and lower concentration of BTX (5 mg/l each of the BTX) used in the batch tests.
The acetic acid was not detected at any time at the end of the bioregeneration steps, which demonstrates that it was degraded by microorganisms, since it is known as an easily biodegradable organic compound.
The observed biodegradation rate for acetic acid was 0.14 kg acetic acid/m^3-day. The biodegradation rates of BTX compounds were the same as in the previous experiment, which means that the biodegradation rate for all organic compounds was 3.37 kg COD/m^3-day (0.0143 kg COD/kg organoclay-day).

DO concentrations profiles were monitored during two treatment cycles in the beginning and 20 days after the start of the experiment (Figures 73 and 74). Each time the DO concentration was monitored for 8 h in two consecutive days (as in the previous test).

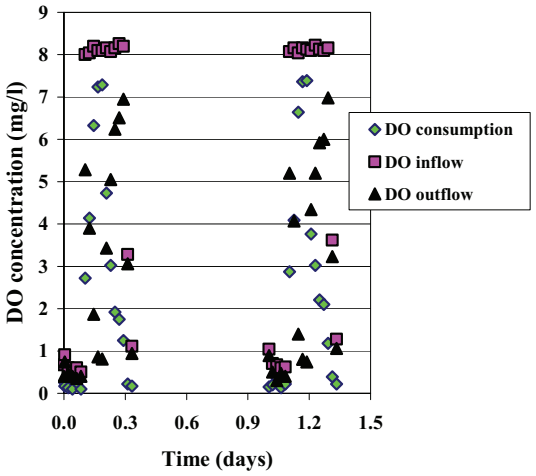


Figure 73. Profile of DO concentrations in the beginning of the experiment

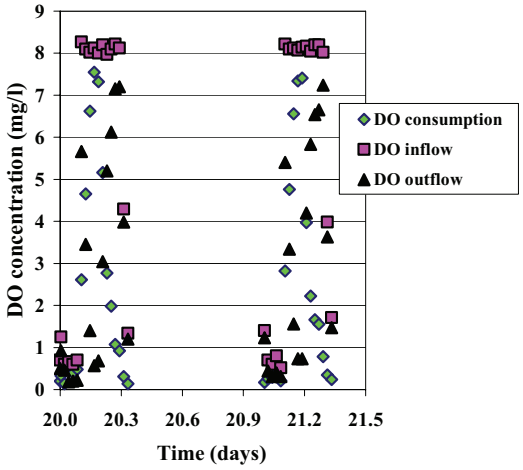


Figure 74. Profile of DO concentrations 20 days after the start

A comparison between the profiles of DO concentrations for the experiment with 2 mg/l acetic acid and the current experiment with 50 mg/l acetic acid indicated that the oxygen consumption slightly increased in the second case.

5.2.5.4 Theories for loss of sorption capacity

Accumulation of organic substances

It is reported in the literature that organic substances can load the pores of sorbents without any possibility for bioregeneration (Kameya *et al*., 1997). These substances affect mainly the small pores less than 20 nm. X-ray diffraction (XRD) analysis was extensively used to study the sorption of BTX compounds on organoclay. This method provided information regarding the orientation of the sorbed molecules or the number of sorbed layers into the basal spacing (or interlammelar distance), which is the distance between the organoclay platelets. Taking into consideration the molecular size of BTX compounds, it is strongly believed that each of these organics form single-layer interlammelar complexes in the modified clay, with the plan of the molecules longitudinally oriented perpendicular to the silicate surface (Gitipour *et al*., 1997a). The natural bentonite has before surface modification a basal spacing of 12.41 Å that increases to 27.17 Å after the interaction with dimethyl dihydrogenated ammonium chloride, the tailoring agent. In the modified bentonite samples, significant changes were observed in the basal spacing of the clay particles because of sorption of BTX compounds. The interlammelar distance increased from 27.17 Å to 33.53 Å, 34.74 Å, and 36.21 Å corresponding to 23%, 28% and 33% changes in the interlammelar spacing for the samples exposed to benzene, toluene and o-xylene, respectively (Gitipour *et al*., 1997b).
All these data show that BTX compounds have a high affinity for the modified bentonite binding to the tailoring agent exchanged onto organoclay platelets. Therefore, some of the sorbed BTX compounds might be sorbed irreversibly by the organoclay without any possibility for desorption.

Accumulation of minerals

Both the Bushnell-Haas nutrient medium and the Tropic Marin salts used to prepare the liquid matrix for experiments contained different mineral salts consisting of inorganic elements, which are believed to accumulate and block the pores of sorbents. Among these elements, Ca was found to accumulate in the highest amount in the form of sulphates and carbonates (Kameya *et al*., 1997). Accumulation of minerals in activated carbons is small and the pore volume decrease is believed to be mainly caused by the accumulation of organic substances.

Accumulation of soluble microbial products

Microorganisms can produce soluble microbial products (SMP) and it has been reported that sorption of SMP can reduce sorption capacity (Olmstead, 1989). It is believed that in order for the SMP to decrease the sorption capacity, the sorbent may have to contact the SMP for an extended period to produce an observable effect. Apparently, these materials do not desorb significantly, and thus the sorption capacity cannot be restored.

Particle attrition

Movement of sorbent particles in the FBR caused attrition and a change in the initial pore size distribution and pore structure of the organoclay bed. These phenomena can lead to a shrinking of the basal spacing and an increase of diffusion resistance. On one hand, this can result in a decrease of organoclay sorption capacity and on the other hand in lower sorption rates caused by the diffusion limitations.

Desorption of the tailoring agent

The tailoring agent used to modify the surface of the organoclay used in our tests is a quaternary amine called dimethyl dihydrogenated ammonium chloride. There are many studies in the literature which investigated the stability of non-ionic organic complexes formed between clays and different tailoring agents under various ionic strengths.
El-Nahhal and Safi (2003) measured the adsorption of benzyltrimethyl ammonium (BTMA), a tailoring agent with similar properties to dimethyl dihydrogenated ammonium, onto montmorillonite and its desorption from the organoclay complex under various NaCl concentrations. Results showed a decrease in amount of BTMA adsorbed as the NaCl concentration increased from 0 to 100 g/l. Release of BTMA from the organoclay complex (clay–BTMA) increased as NaCl concentration reached 100 g/l; however, reported results showed that the desorption of BTMA was always less than 10% of the adsorbed amount.
Sorption of phenanthrene by the clay–BTMA complex increased as NaCl concentration was increased. However, experimental results indicated the stability of organoclay complex (clay–BTMA) under various NaCl concentrations and that its sorption capacity towards phenanthrene was not significantly affected.
In our case, it is possible that a small amount of the adsorbed dimethyl dehydrogenated ammonium was desorbed due to salts present in the synthetic seawater prepared from Tropic Marin. This process might affect negatively the sorption capacity of the organoclay bed since the BTX compounds bind to the adsorbed tailoring agent.

Microorganisms biodegrade the tailoring agent

Although most of tailoring agents (quaternary ammines) are biocidal (Gilbert and Al-Taae, 1985), they were reported to have been biodegraded microbially.
Tests on biodegradation of decyltrimethyl ammonium bromide indicated that it was utilized as a sole carbon source by a mixed population of two bacteria isolated from soil and wastewater (Dean-Raymod and Alexander, 1977). Dimethyl ammonium chloride and distearydimethyl ammonium chloride were mineralized in aqueous solutions under aerobic conditions (Sullivan, 1983). At initial concentrations between 0.1 and 20 mg/l, extensive biodegradation of octadecyltrimethyl ammonium chloride in aqueous solution was observed by Games *et al*. (1982). In the studies cited above, biodegradation of quaternary ammonium chlorides (QACs) was observed only in solution. No extensive studies of biodegradation of sorbed dimethyl dihydrogenated ammonium chloride have been previously reported.

Li *et al.* (1998) reported that a quaternary amine called hexadecyltrimethyl ammonium HDTMA bound to zeolite surface was resistant to microbial degradation, with more than 98% of the original HDTMA remaining after 12-17 weeks of incubation under aerobic or anaerobic conditions. While aqueous HDTMA was biocidal, HDTMA bound on surfactant-modified zeolite (SMZ) did not inhibit microbial growth. Results of this study demonstrated that the SMZ appeared to be suitable as a sorbent for long-term in situ applications.

5.2.6 Conclusions on FBR operation experiments

The following conclusions can be drawn upon the results obtained during the experiments carried out with the FBR:

- FBR tests without a microbial culture demonstrated the ability of organoclay to remove BTX compounds from produced water by a sorption mechanism;
- This property of organoclay correlates with increasing octanol/water partition coefficient of each individual compound. The higher this partition coefficient the more strongly the compound is retained by the modified clay. Therefore, the amount sorbed increased in the following order: benzene < toluene < p-xylene;
- Utilization of organoclay as biomass carrier in a FBR configuration produced a system in which BTX removal capacity was enhanced compared to a process with only sorption and no biodegradation;
- Off-line bioregeneration of the organoclay loaded with BTX compounds in a FBR is a feasible process that can be achieved by alternating sorption and biodegradation treatment steps;
- Microbial activity in the FBR was decreased during sorption steps when nutrients were supplied at the beginning of the bioregeneration steps and not at the beginning of the sorption steps, since microorganisms can not grow properly without a source of nutrients, even if residual oxygen is present during the sorption steps;
- Long-time off-line bioregeneration experiments alternating sorption and biological regeneration showed that the organoclay bed partially lost its sorption capacity;
- Possible causes for the partial loss of organoclay's sorption capacity could be: accumulation of organic compounds and minerals, particle attrition, desorption and biodegradation of the tailoring agent (dimethyl dihydrogenated ammonium chloride).

5.3 Characterization of biomass

The aim of biomass characterization was to evaluate if changes in FBR operation and composition of feed solution could be correlated with variation in bacterial diversity.
The investigation of off-line bioregeneration of the organoclay bed was divided in 5 experimental periods:

1. Tuning experiments (variable duration of bioregeneration process);
2. Test only with BTX. FBR operated in 6 h cycles;
3. Test with BTX and 2 mg/l acetic acid. FBR operated in 6 h cycles;
4. Test with BTX and 2 mg/l acetic acid. FBR operated in 8 h cycles;
5. Test with BTX and 50 mg/l acetic acid. FBR operated in 8 h cycles.

Figure 75 indicates the relation between the organic loading rate on bioregeneration process and the increase of COD concentration in the liquid phase after the dosage steps, 24 days after the start of the different tests.

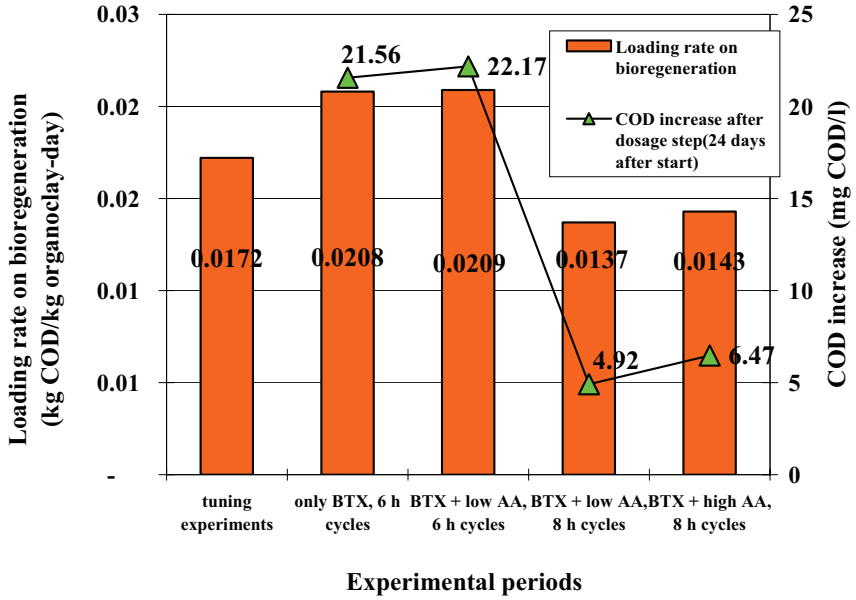


Figure 75. Relation between the loading rate on biological process and the increase of COD after dosage steps 24 days after the start of experiments

A period of 24 days was selected for the calculation of COD increase in the reactor after dosage of organic compounds in the FBR because in the 2nd and 3rd tests the exhaustion of the 25% spare sorption capacity of the organoclay bed occurred 24 days after the start of the experiments. This increase was calculated as the difference between the COD concentration in the reactor after the first dosage step (day 1 of the experiment) and the COD concentration determined after the dosage step, 24 days after the start of the experiment.

Increase of COD concentration was not plotted for the tuning experiments because the system was operated for short periods during these tests and this could not be estimated. Results showed that the higher the organic loading rate on the bioregeneration process, the higher the increase of COD concentration in the reactor after the dosage steps and probably the faster the exhaustion of the 25% spare sorption capacity. The latter

conclusion is supported by the experimental results which showed that the 25% spare sorption capacity was exhausted 24 days after the start of tests 2 and 3 and was not exhausted 32 and 28 days after the start of experiments 4 and 5, respectively.

The presence of biomass into the FBR system was verified by collecting and analyzing 3 different types of samples (Figure 76):

1. Water samples from the bulk liquid in the FBR;
2. Water phase samples – water filtrated from particle samples;
3. Particle samples collected from the organoclay bed.

The concentrations of bacteria in these samples were determined by epifluorescence staining (DAPI), while the bacteria diversity was investigated by DGGE (see Appendix A).

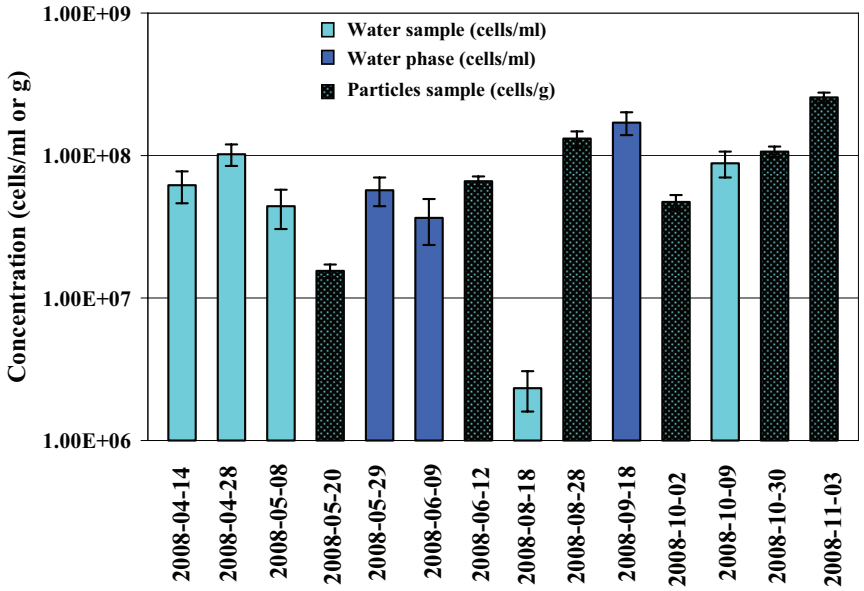


Figure 76. Concentrations of bacteria cells in 14 different samples collected from the FBR system

Figure 77 provides information about the nature of each of the 14 samples, number of days after the start of the first experiment when the samples were collected and the organic loading rate on bioregeneration process for the experiment carried out at the time of sampling. The different types of samples were analyzed for biomass concentration and diversity. Samples were collected at least twice during each experimental period.

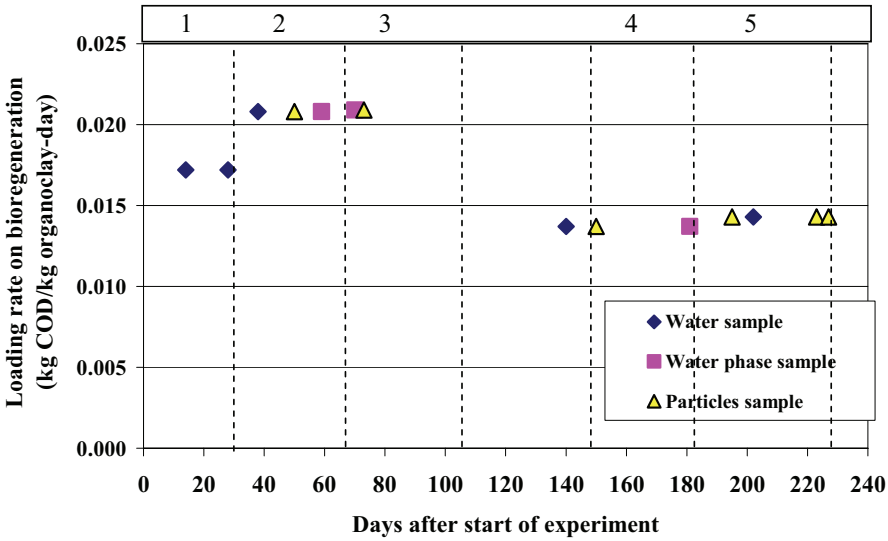


Figure 77. Sample types, collection days and loading rate on bioregeneration process at the time of sampling

The water sample labeled with the date 18.08.2008 (Figure 76) was collected before starting experiment 4 (Figure 77) and therefore the concentration of bacteria determined by epifluorescence staining (DAPI) was low.

The DGGE gel containing 15 biomass samples collected during the 5 different experimental periods is displayed in Figure 78. These 15 samples include the 14 samples characterized in Figures 76 and 77 and an additional water phase sample collected on the 04.09.2008 (sample 10 on the DGGE gel), which was not analyzed for bacteria concentration by DAPI method.
Numbering and a brief description of the different experimental periods are provided at the bottom of the figure, while the biomass samples are assigned numbers from 1 to 15. The most relevant bands are numbered on the gel for a better identification. Different numbers were assigned only to bands having a different position on the gel.

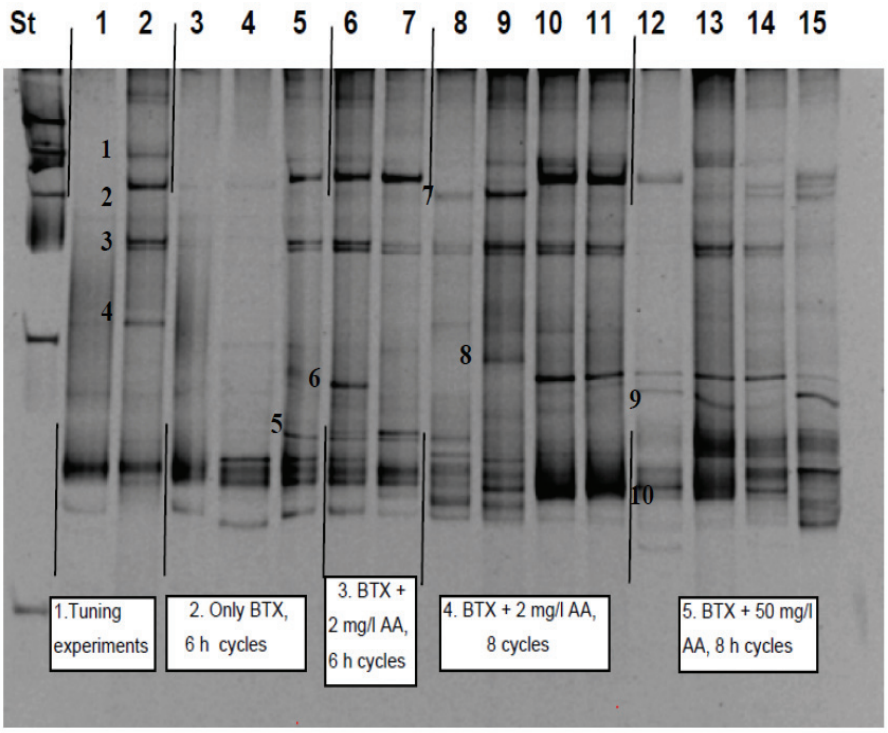


Figure 78. DGGE gel with biomass samples collected in experimental periods 1-5

Overall, the DGGE profile of the biomass samples did not indicate major differences in the composition of the microbial community responsible for the biodegradation of the organic compounds over the 5 different experimental periods.
Presence of several distinct bands during all these periods demonstrated that the biodegradation process has always been carried out by a diverse microbial community. The observed bands showed variable intensities both during a certain experimental period and from test to test. Some of the samples (1 and 3) showed relatively low DNA intensity that could be caused either by a low concentration of microorganisms in these samples or a low extraction efficiency.

The following observations were made based on the presence and intensity of the bands:

- Bands 2 and 3 are found at least in one sample of each experimental period and therefore they are believed to represent the BTX degraders in the community;
- Intensity of band 2 decreased during experimental period 5 (high acetic acid concentration), possibly because some of the BTX degraders could not compete efficiently under conditions with increased acetic acid concentration present in solution. DGGE is not a quantitative method but a high intensity of a band shows that the corresponding microorganisms are found in higher concentrations than in the bands with lower intensity.

- Band 6 is present only in the samples taken during periods when acetic acid was fed into the bioreactor. This could mean that it contains those microorganisms able to feed on acetic acid but not on BTX;
- Band 7 is found only in samples taken during the tests when the FBR was operated in 8 h cycles (long time bioregeneration steps). Probably the microorganisms present in band 7 grew better at lower concentrations of organic compounds sorbed on the surface of the organoclay bed. An alternative explanation could be that these bacteria needed a longer contact time with the contaminated media in order to be able to feed on the sorbed compounds.

5.4 Modelling of off-line bioregeneration processes in a FBR

5.4.1 Mathematical model

The scope of this mathematical modelling is to provide a tool that could be used for the following purposes:

1. Estimate the remaining spare sorption capacity of the organoclay bed;
2. Estimate when the spare sorption capacity will be exhausted;
3. Estimate the COD concentration in the water phase after the spare sorption capacity will be exhausted.

The COD amount accumulated in the reactor after a given period of operation is related to the amounts of COD dosed into the system in the beginning of each treatment step and the amounts of COD biodegraded during the bioregeneration steps.
The following equation was proposed for the modelling of off-line bioregeneration processes alternating sorption and bioregeneration treatment steps in a FBR:

$$\frac{d(COD)}{dt} = \frac{d(COD_D)}{dt} + \frac{d(COD_{BIO})}{dt} \qquad (42)$$

COD_D = amount of COD dosed in the reactor in the beginning of one treatment cycle (mg COD);
COD_{BIO} = amount of COD biodegraded during one treatment cycle (mg COD);

$$S_i = S_{init} - \sum_{0}^{i-1} \left(\frac{d(COD_{Di})}{dt} + \frac{d(COD_{BIOi})}{dt}\right) \qquad (43)$$

where i = number of treatment cycle;
S_i = remaining spare sorption capacity after i sorption steps (mg COD);
S_{init} = initial spare sorption capacity (mg COD); it represents the remaining sorption capacity after the first sorption step;

It is assumed that COD_D was the same for each treatment cycle since the amount of COD dosed into the reactor in the beginning of each cycle was constant over time.

COD_D was equal to the amount of COD sorbed (COD_S) by the organoclay bed since the analyses of water samples showed that at the end of each sorption step the entire amount of COD dosed into the system (COD_D) was sorbed by the organoclay bed. This was valid until the spare sorption capacity was exhausted. After that point, increasing amounts of COD were measured into the reactor after each sorption step.
It is also assumed that COD_{BIO} was constant over time since the parameters influencing biological activity (amounts of nutrients, substrate and biomass as well as temperature) were similar for each treatment cycle.
Taking into account these assumptions, equation *(43)* can be written as:

$$S_i = S_{init} - (i-1)\left(\frac{d(COD_D)}{dt} + \frac{d(COD_{BIO})}{dt}\right) \qquad (44)$$

$$COD_D = COD_S \qquad (45)$$

where COD_S is the amount of COD sorbed by the organoclay bed during each treatment cycle before the spare sorption capacity of the organoclay bed is exhausted.

$$COD_S = q_e m_{org} \qquad (46)$$

The following equation can be written by substituting equation *(46)* in equation *(45):*

$$\frac{d(COD_D)}{dt} = q_e m_{org} \qquad (47)$$

q_e = amount of COD sorbed by 1 g organoclay at sorption equilibrium for this specific case (mg COD/g organoclay); it was calculated by dividing the amount of COD dosed into the reactor in the beginning of each treatment step by the amount of organoclay in the fluidized bed (equal to 75% of the sorption capacity of 1 g organoclay for BTX compounds – see chapter 5.2.1);
m_{org} = mass of organoclay bed (g); it was constant during the experiments.

The rate of substrate removal is proportional to the rate of microorganism growth. Assuming that substrate is the limiting factor, the rate of microbial growth becomes dependent on the amount of substrate present as well as the number of microorganisms in the system.

$$\frac{d(COD_{BIO})}{dt} = -\frac{1}{Y}\frac{dN}{dt} = -\frac{1}{Y}k' q_e m_{org} N \qquad (48)$$

Y = yield factor (microbial cells/mg COD removed);
k' = rate constant (mg COD^{-1}*h^{-1});
N = number of microbial cells in the FBR;

$$k_t = \frac{1}{Y} k' \qquad (49)$$

k_t = biodegradation parameter (cell^{-1}*h^{-1})

Equation *(49)* is substituted in equation *(48)*:

$$\frac{d(COD_{BIO})}{dt} = -\frac{1}{Y}\frac{dN}{dt} = -k_t q_e m_{org} N \qquad (50)$$

Equation *(51)* is obtained by substituting equations *(47)* and *(50)* in equation *(44)*:

$$S_i = S_{init} - (i-1)(q_e m_{org} - q_e m_{org} k_t N t_{bio}) = S_{init} - (i-1) q_e m_{org} (1 - k_t N t_{bio}) \qquad (51)$$

t_{bio} = duration of each bioregeneration step (h);

5.4.2 Parameter estimation

Equation *(51)* was used to estimate parameter k_t. The estimation was made using the experimental data obtained after carrying out two off-line bioregeneration experiments with and without acetic acid (2 mg/l in the feed solution) and 4 h bioregeneration periods.

Experimental results showed that S_{init} (25% spare sorption capacity) was exhausted after 24 days of continuous operation of the FBR (experiments with and without 2 mg/l acetic acid in the feed solution and 4 h bioregeneration periods).

Based on the experimental setup and method used to run the test, the values of the known parameters in equation *(51)* were:

m_{org} = 165 g;

S_{init} = 189.75 mg COD (25% of the total sorption capacity of the organoclay bed amounting to 759.00 mg COD). The total sorption capacity of the organoclay bed was calculated by multiplying the amount of BTX compounds sorbed at equilibrium (when the entire sorption capacity is exhausted) by 1 g of organoclay (0.43 mg benzene/g organoclay, 0.44 mg toluene/g organoclay and 0.60 mg p-xylene/g organoclay - see chapter 5.2.1) by the amount of clay in the fluidized bed (m_{org} = 165 g) and converting the result to COD.

COD_D = 75% of the total sorption capacity of the organoclay bed;

q_e = 3.45 mg COD/g organoclay;

COD_D = 3.45*165 = 569.25 mg COD;

$N = 5.69*10^{10}$ cells in the FBR;

N was estimated by summing up the numbers of bacteria in the particle and water phases. The average concentrations of bacteria in the particle and water phases were determined based on the results presented in Figure 76 (the 14 samples considered for biomass characterization). The water sample taken on 18.08.2008 was not included in the calculation since it was collected before the start of the experiment. The average concentrations of bacteria in the particle and water phases were 1.0 E+08 cells/g organoclay and 8.0 E+07 cells/ml, respectively. The concentration of bacteria in the particle phase was estimated assuming that the concentration of particles in the samples

taken for DAPI analyses was the same as that in the fluidized organoclay bed (0.44 g particles/ml). The volume of the entire treatment system (including tubing and membrane) was around 0.7 l, while the volume occupied by the organoclay bed was 0.195 l.
t_{bio}= 4 h; the duration of the bioregeneration steps was 4 h for the first 2 long time off-line bioregeneration experiments;
S_i = 0 (when the 25% spare sorption capacity was exhausted);
i = 96 (since the spare sorption capacity was exhausted 24 days after the start of the experiments and there were 4 treatment cycles/day);

The values of the known parameters described above were substituted in equation *(51),* where k_t was the only unknown*:*

$k_t = 0.0437*10^{-10}$ (cell^{-1}*h^{-1})

5.4.3 Modelling of experiment with 2 h sorption and 6 h bioregeneration (2 mg/l acetic acid in the feed solution)

The increases of residual COD concentrations observed after the 5 min BTX dosage steps, 24 days after the start of the experiments with 4 h and 6 h bioregeneration periods and 2 mg/l acetic acid in the feed solution, were compared (Figure 75). These increases were calculated as the differences between the COD concentrations in the reactor after the first dosage steps (day 1 of the experiments) and the COD concentrations after the dosage steps, 24 days after the start of the experiments. In the second experiment, the longer bioregeneration process was more efficient in removing the sorbed BTX compounds, therefore the increase of the residual COD concentration measured after the BTX dosage step was 4.5 times lower than the increase measured for the experiment with 4 h bioregeneration periods. A correlation was assumed between the increase of COD concentration after the BTX dosage steps and the loss of sorption capacity. The steeper this increase, the sooner the spare sorption capacity would be exhausted.
Taking into account that the increase was 4.5 times lower for the experiment with 6 h bioregeneration period and assuming a linear correlation in the model, it was expected that the 25% spare sorption capacity would be exhausted 108 days after the start of the experiment (4.5*24 days).
The number of treatment cycles performed until the spare sorption capacity would be exhausted is calculated using the estimated value of 108 days and the 8 h duration of a treatment cycle (3 cycles/day).
i = 108*3 = 324 cycles;
t_{bio}= 6 h; the bioregeneration period was increased from 4 to 6 h;
S_{init}, m_{org}, q_e and N were the same as for the previous experiment. The value of q_e did not include the amount of acetic acid sorbed since this was very low and could be neglected.

The values of the known parameters described above were substituted in equation *(51),* where k_t was the only unknown*:*

$k_t = 0.0292*10^{-10}$ (cell^{-1}*h^{-1})

5.4.4 Modelling of experiment with 2 h sorption and 6 h bioregeneration (50 mg/l acetic acid in the feed solution)

In this case the increase of the residual amount of COD measured in the liquid phase after the dosage step, 24 days after the start of the experiment, was only 3.4 times lower than in the case of the experiments with 4 h bioregeneration (2 mg/l acetic acid in the feed solution).
Therefore, it was expected that the 25% spare sorption capacity would be exhausted 82 days after the start of the experiment (3.4*24).

The number of treatment cycles performed until the spare sorption capacity would be exhausted is calculated using the estimated value of 82 days and the 8 h duration of a treatment cycle (3 cycles/day).
i = 82*3 = 246 cycles;
t_{bio}= 6 h;
q_e = 3.54 mg COD/g organoclay. In this case q_e was higher than in the previous experiments due to the addition of 50 mg/l acetic acid in the feed solution. The amount of acetic acid sorbed at the end of each sorption step by 1 g organoclay was 0.09 mg acetic acid/g organoclay. Acetic acid was not sorbed completely by the organoclay bed during the 2 h sorption steps, but it was removed during the subsequent bioregeneration steps.
S_{init}, m_{org} and N were the same as in the previous experiment.

The values of the known parameters described above were substituted in equation *(51),* where k_t was the only unknown*:*

k_t = 0.0292*10^{-10} (cell^{-1}*h^{-1})

5.4.5 Verification of the model

In order to verify the accuracy of the model, 6 data points (values of i) were selected for each of the 3 experiments (4 h bioregeneration period, 2 mg/l acetic acid; 6 h bioregeneration period, 2 mg/l acetic acid; 6 h bioregeneration period, 50 mg/l acetic acid). Three of the data points were chosen to be before the predicted number of treatment cycles after which the spare sorption capacity would be exhausted, while the other two were selected to be after the exhaustion would take place into the system.

5.4.5.1 Experiment with 2 h sorption and 4 h bioregeneration

The data set in section 5.4.2 was used to verify the accuracy of the model for this experiment:
COD_D = 75% of the sorption capacity of the organoclay bed;
m_{org} = 165 g;
COD_D = 3.45*165 = 569.25 mg COD;
S_{init} = 189.75 mg COD (25% of the total sorption capacity of the organoclay bed);

q_e = 3.45 mg COD/g organoclay;
$N = 5.69*10^{10}$ cells in the FBR;
t_{bio}= 4 h;
k_t = 0.0437*10^{-10} (cell^{-1}*h^{-1})

The values of the known parameters were introduced in equation *(51)*. The values of S_i were then calculated for the six data points selected (values of *i*) – Table 30.

Table 30. Remaining spare sorption capacity after *i* treatment cycles (4 h bioregeneration, 2 mg/l acetic acid

Type of experiment	*i* (number of treatment cycle)	S_i (remaining sorption capacity)
4 h bioregeneration, 2 mg/l acetic acid	0	191.74
	46	99.86
	71	49.93
	96	0
	121	-49.93
	146	-99.86

The positive values of S_i ($S_i > 0$) in the table above indicate that the spare sorption capacity would not be exhausted after the corresponding number of cycles.
At S_i = 0, the spare sorption capacity of the organoclay bed would be completely exhausted.
The negative values of S_i ($S_i < 0$) in the table above show the concentration of COD in the liquid phase after the corresponding number of cycles.

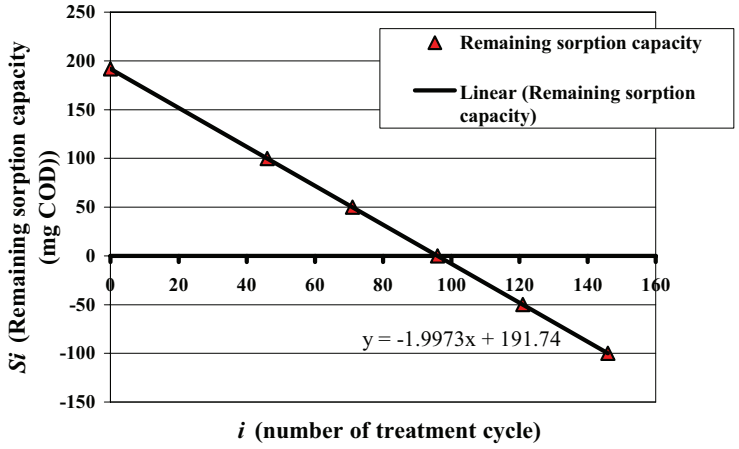


Figure 79. Predicted sorption capacity remaining after *i* treatment cycles (4 h bioregeneration, 2 mg/l acetic acid)

5.4.5.2 Experiment with 2 h sorption and 6 h bioregeneration (2 mg/l acetic acid in the feed solution)

The data set in section 5.4.3 was used to verify the accuracy of the model for this experiment:
COD_D = 75% of the sorption capacity of the organoclay bed;
m_{org} = 165 g;
COD_D = 3.45*165 = 569.25 mg COD;
S_{init} = 189.75 mg COD (25% of the total sorption capacity of the organoclay bed);
q_e = 3.45 mg COD/g organoclay;
$N = 5.69*10^{10}$ cells in the FBR;
t_{bio}= 6 h;
$k_t = 0.0292*10^{-10}$ (cell^{-1}*h^{-1})

The values of the known parameters were substituted in equation *(51)*. The values of S_i were then calculated for the six data points selected (values of i) - Table 31.

Table 31. Remaining spare sorption capacity after i treatment cycles (6 h bioregeneration, 2 mg/l acetic acid)

Type of experiment	i (number of treatment cycle)	S_i (remaining sorption capacity)
6 h bioregeneration, 2 mg/l acetic acid	0	190.33
	224	58.74
	274	29.37
	324	0
	374	-29.37
	424	-58.74

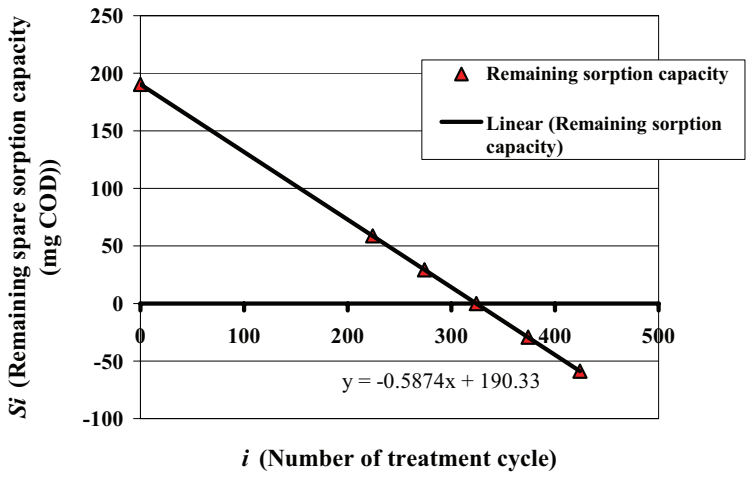


Figure 80. Predicted sorption capacity remaining after i treatment cycles (6 h bioregeneration, 2 mg/l acetic acid)

5.4.5.3 Experiment with 2 h sorption and 6 h bioregeneration (50 mg/l acetic acid in the feed solution)

The data set in section 5.4.4 was used to verify the accuracy of the model for this experiment:
COD_D = 75% of the sorption capacity of the organoclay bed;
m_{org} = 165 g;
COD_D = 3.54*165 = 584.1 mg COD;
S_{init} = 189.75 mg COD (25% of the total sorption capacity of the organoclay bed);
q_e = 3.54 mg COD/g organoclay;
$N = 5.69*10^{10}$ cells in the FBR;
t_{bio}= 6 h;
$k_t = 0.0292*10^{-10}$ ($cell^{-1}*h^{-1}$)

The values of the known parameters were introduced in equation *(51)*. The values of S_i were then calculated for the six data points selected (values of i) - Table 32.

Table 32. Remaining spare sorption capacity after i treatment cycles (6 h bioregeneration, 50 mg/l acetic acid)

Type of experiment	i (number of treatment cycle)	S_i (remaining sorption capacity)
6 h bioregeneration, 50 mg/l acetic acid	0	190.52
	146	77.44
	196	38.72
	246	0
	296	-38.72
	346	-77.44

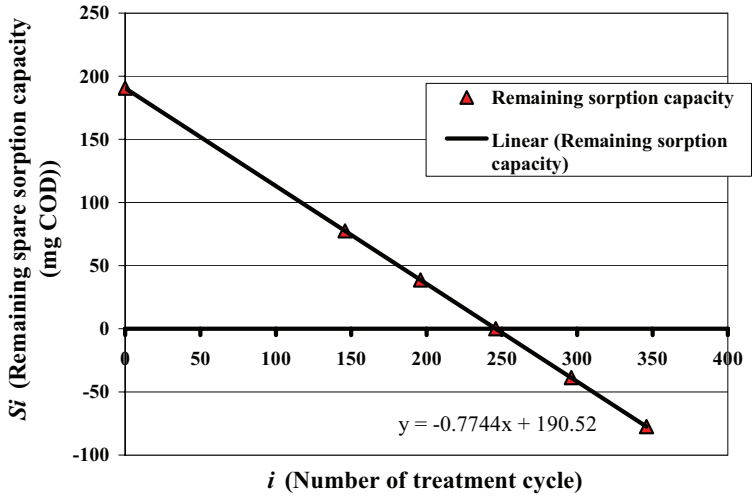


Figure 81. Predicted sorption capacity remaining after i treatment cycles (6 h bioregeneration, 50 mg/l acetic acid)

5.4.6 Conclusions on modelling of off-line bioregeneration processes in a FBR

The following observations were made on the results:

- Increase of residual COD concentration after the BTX and acetic acid dosage steps could be used to predict when the exhaustion of the spare sorption capacity would occur;
- Value of parameter k_t depends on the duration of the bioregeneration steps (t_{bio}). k_t decreases with the increase of t_{bio} and is constant for the same t_{bio};
- k_t could be used to predict the number of treatment cycles after which the spare sorption capacity would be exhausted if no information is available on the residual COD concentration after the BTX and acetic acid dosage steps. Based on the available data, an increase of 50% of t_{bio} resulted in a decrease of 49.6% of k_t. Therefore the variation of t_{bio} could be used to calculate first k_t and then i by substituting the known values in equation *(51)*.

Table 33. Variable parameters of the mathematical model for the 3 different experiments

Type of experiment	q_e (mg COD/g organoclay)	t_{bio} (h)	k_t (cell^{-1}*h^{-1})
4 h bioregeneration, 2 mg/l acetic acid	3.45	4	0.0437
6 h bioregeneration, 2 mg/l acetic acid	3.45	6	0.0292
6 h bioregeneration, 50 mg/l acetic acid	3.54	6	0.0292

The data in Table 33 indicate that the biodegradation parameter (k_t) depends on the duration of the bioregeneration steps (t_{bio}) since all the other factors influencing biodegradation were similar for all the three different experiments. The value of k_t was lower for the experiments with 6 h bioregeneration steps since the COD load/cell*h decreased**.**

6. DISCUSSION OF RESULTS IN RELATION TO HYPOTHESES

6.1 Hypotheses referring to sorption of target compounds

6.1.1 Selective sorption of target compounds can be achieved

Hypothesis:
One of the key issues of the project was to identify sorbents with high selectivity for the target soluble compounds and little or no sorption for organic acids. Failure in achieving the effluent targets set in the project would occur if such sorbents were not available on the market or were not identified during screening tests.

Results showed that the organoclay had a high affinity for BTX compounds and a low affinity for acetic acid and therefore was able to remove selectively the aromatic compounds from the water phase despite the high concentration of acetic acid present in solution. Batch sorption tests were run with 5 mg/l initial concentration of each BTX compound and 160 mg/l initial concentration of acetic acid. The amounts sorbed at equilibrium were 0.025 mg benzene/g organoclay, 0.039 mg toluene/g organoclay, 0.053 mg p-xylene/g organoclay and 0.125 mg acetic acid/g organoclay, respectively. The amounts of each BTX sorbed when acetic acid was not present in solution were higher: 0.046 mg benzene/g organoclay, 0.048 mg toluene/g organoclay and 0.063 mg p-xylene/g organoclay (section 5.1.2.5). This demonstrated that there was a sorption competition between acetic acid and BTX compounds onto the organoclay. Sorption of acetic acid was strongly dependent on pH, since acetic acid was completely dissociated at high pH values and the anions were much less sorbed than the molecular form present in solution at low pH values. On the other hand, BTX compounds showed high affinity for the hydrophobic chains of the surface tailoring agent (dimethyl dihydrogenated ammonium chloride), therefore being strongly retained onto the surface of the organoclay.
The sorption capacities of the organoclay for BTX compounds measured during FBR operation without biological regeneration were around 10 times higher than the values obtained in the batch tests at sorption equilibrium: 0.43 mg benzene/g organoclay, 0.44 mg toluene/g organoclay and 0.60 mg p-xylene/g organoclay (section 5.2.1). This higher sorption capacity measured in the FBR is believed to be a consequence of the better contact between the fluidized organoclay particles and the liquid phase containing the dissolved compounds.

6.1.2 Sorption media are not contaminated with other compounds

Hypothesis:
Contamination with other compounds present in produced water (oil residues, soluble microbial products, production chemicals, and salts) may affect the selectivity and sorption capacity of sorbents. This would cause a reduction of the total removal capacity of the sorption media leading to no compliance with respect to project effluent targets.

Experiments were carried out only with BTX and acetic acid, therefore the impact of other components of produced water such as oil residues and production chemicals was not investigated. However, it is believed that sorption capacity of the organoclay for BTX compounds during FBR operation decreased, among other reasons, due to sorption of soluble microbial products (SMP) produced by microorganisms and salts coming from the commercial Tropic Marin sea salts and Bushnell-Haas nutrients used to prepare the seawater matrix used for the tests. Apparently, soluble microbial products and salts can accumulate on sorbents, and thus sorption capacity cannot be restored.

6.1.3 Good sorption kinetics can be attained

Hypothesis:
The removal efficiency of dissolved compounds could also be affected by sorption kinetics in addition to sorbent concentration. Sorption kinetics (and its changes due to operation conditions like temperature for example) was a key issue of the treatment process, since any decrease in kinetics would result in no compliance with effluent targets.

Sorption kinetics of BTX onto the organoclay fitted a pseudo-second-order equation. This demonstrated that sorption of BTX was stronger in the beginning of sorption process and weaker towards the point where equilibrium was reached. This is a positive characteristic because in the proposed treatment concept the contact time between sorbent and produced water in the pipe reactor is expected to be very short. Unfortunately, the sorption kinetics and the sorption capacity of the organoclay used in these experiments were lower than those reported in the literature for other sorbents such as activated carbons. The organoclay was chosen for its higher selectivity for BTX compounds.

6.1.4 Sorbent properties do not change over time (ageing)

Hypothesis:
Surface chemistry and available surface area were thought to be the main factors influencing sorption capacity of sorbents. Friction or wear that might occur at different stages of the treatment (for example during separation in hydrocyclones) could alter surface area of sorption media. It was expected that any degradation caused by ageing or surface wear would lead to a loss of sorption capacity.

It is believed that sorbent properties did change over time due to different phenomena. Movement of sorbent particles in the FBR caused attrition and a change in pore size distribution and pore structure of the organoclay bed. A visual comparison carried out between the fresh organoclay granules and the granules collected from the FBR after a long time operation, indicated that particle size became smaller over time, probably due to friction phenomenon, which occurred between the fluidized particles or between particles and the wall of the glass reactor. This probably caused a decrease of basal spacing in organoclay structure and an increase of diffusion resistance. On one hand,

this could lead to a decrease of organoclay sorption capacity and on the other hand to lower sorption rates caused by diffusion limitations.
The partial loss of sorption capacity could be also caused by desorption of tailoring agent used to modify the surface of the clay (dimethyl dihydrogenated ammonium chloride). This phenomenon was studied in the literature and it was reported that desorption of the quaternary amine increased with increase of salinity. However, results reported in the literature showed that the degree of desorption was always less than 10% of the adsorbed amount (El-Nahhal and Safi, 2003).

6.2 Hypotheses referring to sorbent regeneration

6.2.1 Efficient bioregeneration can be achieved

Hypothesis:
Another key issue of the project was that the biomass would not feed on other compounds or on the sorbents themselves (especially if they had a modified surface) leaving the BTX compounds undegraded. Treatment performance would be strongly affected if efficient biodegradation could not be achieved and the process must be re-designed to compensate for that.

Biodegradation tests were carried out only with BTX as representatives of the target dissolved aromatic compounds and acetic acid as representative of organic acids group. Growth on BTX compounds was proven by the results of a BOD test and another brief biodegradation experiment in which a GC/MS system was utilized to quantify the degradation rates of each member of BTX family. The microbial culture used during the biodegradation experiments was enriched for BTX degraders and then transferred into the FBR system, where it was acclimatized before starting the tests.
During the tests done with the FBR system both low (2 mg/l) and high (50 mg/l) concentrations of acetic acid were added to the concentrated BTX solution dosed into the reactor. When dosed in high concentration, acetic acid was not sorbed completely by the organoclay bed, but it was biodegraded by microorganisms during bioregeneration steps. Although the bacteria culture was enriched for BTX degraders (BTX used as sole carbon source), microorganisms could induce enzymes able to break down acetic acid because it is more easily biodegradable than BTX. Microorganisms fed on both BTX and acetic acid but this did not seem to affect the efficiency of BTX degradation since the loss of sorption capacity of the organoclay bed was very low over time.

6.2.2 Good biodegradation kinetics can be attained

Hypothesis:
Degradation kinetics was considered to be a fundamental design parameter of the treatment process. Produced water properties such as temperature, pH, salinity or heavy metals content have a significant impact on degradation kinetics. Therefore,

investigations should be carried out in order to define the process window where the bioregeneration reactor shows an adequate performance.

Tests were carried out at constant water properties (pH ~ 7, t ~ 20 °C, 35 g/l sea salts and no heavy metals). Influences of variation of water temperature, pH, salinity and heavy metal content were not investigated.
Biodegradation rates calculated for the experiment in which simultaneous sorption and biodegradation were used as removal mechanisms of BTX compounds in the FBR were 1.72 kg benzene/m^3-day, 1.54 kg toluene/m^3-day and 1.21 kg p-xylene/m^3-day, which means 13.94 kg COD/m^3-day or 0.0591 kg COD/kg organoclay-day. However, the high BTX concentrations in the solution dosed into the reactor overloaded the system and breakthrough of all BTX components was observed in the FBR (section 5.2.3).

Biodegradation rates were also determined for BTX and acetic acid (50 mg/l in the feed solution) degradation during long time off-line bioregeneration experiments carried out with the FBR system (2 h sorption and 6 h bioregeneration). In this case, the observed rates were lower than during the simultaneous sorption and biodegradation experiment: 0.30 kg benzene/m^3-day, 0.31 kg toluene/m^3-day, 0.42 kg p-xylene/m^3-day and 0.14 kg acetic acid/m^3-day, which means a total of 3.37 kg COD/m^3-day (0.0143 kg COD/kg organoclay-day) (section 5.2.5.3).
These results are in the range of the results reported in the literature for BTX biodegradation (Voice *et al*, 1992).

6.2.3 Microbial culture is resistant against toxic shocks

Hypothesis:
The growth and development of the microbial culture employed to degrade BTX compounds was expected to depend on specific conditions. It was believed that if the incoming produced water contained very high loads of BTX compounds, the biomass could be killed or severely weakened. Therefore, the impact of high concentrations of these dissolved compounds on the efficiency of biological treatment had to be evaluated.

During the batch experiments carried out to enrich a microbial culture for BTX degraders, high concentrations of BTX were dosed in the vials (100 mg/l total BTX concentration). Despite the high concentration and the fact that at that time the culture was not specialized to grow on BTX as sole carbon source, a significant growth was observed by DAPI analysis.
The ability of microorganisms to cope with high BTX concentrations was also proven by the FBR experiments, when high concentrations of BTX (106 mg/l benzene, 109 mg/l toluene and 148 mg/l p-xylene), equal to 75% of the sorption capacity of the organoclay bed, were pumped into the system in the beginning of each treatment cycle. It is unlikely that the microorganisms would be killed by a toxic load of aromatic compounds, since the average concentrations in produced water reported by OLF (Norwegian Oil Industry Association) in 2007 for benzene, toluene and xylenes were 5.4 mg/l, 4.1 mg/l and 1.5 mg/l, respectively. The maximum total BTX concentration reported by OLF was 38 mg/l.

7. DISCUSSION IN RELATION TO EXPECTED PERFORMANCE

Produced water quality after hydrocyclone treatment and a discharge flow of 8000 m^3/day that is close to the average flow of produced water discharges from Norwegian offshore installations, were considered for the calculation of expected performance of a full-scale treatment system using in-line sorption coupled with biological regeneration. In this particular case, the soluble compounds correspond to a soluble COD of ~ 35 mg SCOD/l. SCOD loading on an in-line sorption process coupled with biological regeneration would be 280 kg SCOD/day. The removal rate measured during the off-line bioregeneration experiment operated with 2 h sorption and 6 h bioregeneration (50 mg/l acetic acid in the feed solution) was 3.37 kg COD/m^3-day. This gives a required reactor volume of 83 m^3. During the sorption tests done with the FBR, without a microbial culture, an influent concentration of 50.2 mg COD/l (6.3 mg benzene, 5.4 mg/l toluene and 4.4 mg/l p-xylene) was removed by the organoclay bed (28% filling of the reactor) at 14 min HRT. If the same HRT and organoclay filling are assumed for the pipe reactor, the required volume for the in-line sorption process will be 78 m^3.
The following observations can be made if the original assumptions stated in section 2.6 are compared with the performance of the treatment system measured during the laboratory tests:

- The sorption capacity determined for the organoclay was 4.6 mg COD/g, which is much smaller than the capacity assumed when the treatment concept was proposed (0.4 g COD/g sorbent). Therefore the volume of the in-line sorption reactor calculated based on the experimental data was 78 m^3 at a HRT of 14 min, while in section 2.6 it was expected to be 47 m^3.
- The biodegradation rates measured during the tests were lower than those considered for the initial calculations of the expected performance (3.37 kg COD/m^3-day compared to 6 kg COD/m^3-day). The volume calculated for the bioreactor (83 m^3 at a HRT of 15 min) was therefore higher than the volume assumed initially (47 m^3 at an HRT of 8.5 min). However the filling with sorption media was only 28% of the reactor volume during the experiments, which is lower than the 50% filling considered in the original assumptions. It is expected that a higher filling percentage would increase the amount of biomass and thus decrease the volume of the reactor.

8. CONCLUSIONS

- Selective sorption of BTX compounds from wastewater was demonstrated by using an organoclay. This sorbent showed good affinity for BTX compounds and at the same time retained only to a low extent acetic acid from wastewater. A sorption competition was observed between BTX compounds and acetic acid onto the organoclay. Sorption of acetic acid decreased proportionally with the increase of pH because the molecular form of acetic acid found in solution at low pH was better retained than the ionic form by the modified clay. Sorption of BTX was described as a partition mechanisms and it was observed to increase with the increasing octanol/water partition coefficient of a compound (benzene < toluene < p-xylene). BTX sorption increased with the increase of salinity from 0 to 70 g/l because of salt-out effect. Sorption of both BTX and acetic acid decreased with the increase of temperature since this influences solubilities of solutes.

- Sorption capacities of organoclay particles, fluidized in the reactor, were about 10 times higher than in the case of batch experiments probably due to better contact between sorbent particles and liquid phase containing the dissolved compounds. Sorption capacities of organoclay bed for BTX measured during FBR sorption tests were: 0.43 mg benzene/g organoclay, 0.44 mg toluene/g organoclay and 0.60 mg p-xylene/g organoclay. These capacities are lower than those reported in the literature for other sorbents such as activated carbons. However, the organoclay was preferred for its higher selectivity for BTX compounds. Sorption kinetics tests showed that sorption process is much stronger in the first minutes than towards the point where sorption equilibrium is reached.

- FBR experiments using only sorption and simultaneous sorption and biodegradation for BTX removal showed that the use of an organoclay as biomass carrier in a FBR produces a system in which BTX removal capacity is enhanced compared to a process with only sorption and no biodegradation.

- High biodegradation rates were calculated for the experiment in which simultaneous sorption and biodegradation of BTX were investigated: 1.72 kg benzene/m^3-day, 1.54 kg toluene/m^3-day and 1.21 kg p-xylene/m^3-day, which means 13.94 kg COD/m^3-day or 0.0591 kg COD/kg organoclay-day. However, breakthrough of all BTX components was observed in the system due to the high BTX concentrations in the solution pumped into the FBR, which overloaded the system.

- Off-line biological regeneration of the organoclay is feasible in a FBR by alternating sorption and biological regeneration steps. Operating conditions depend on wastewater quality, therefore FBR operation must be tuned first to sorb the entire amount of BTX compounds from produced water and then completely biodegrade the previously sorbed compounds in order for the organoclay bed to recover its entire sorption capacity.

- A FBR operation cycle of 8 h, with 2 h sorption without aeration after BTX and acetic acid dosage and 6 h biological regeneration with 5 h continuous aeration after

nutrients dosage, was found to provide the best results. The biodegradation rates of BTX and acetic acid (50 mg/l in the feed solution) were 0.30 kg benzene/m^3-day, 0.31 kg toluene/m^3-day, 0.42 kg p-xylene/m^3-day and 0.14 kg acetic acid/m^3-day, which means 3.37 kg COD/m^3-day (0.0143 kg COD/kg organoclay-day).

- Long-time off-line bioregeneration experiments indicated that the organoclay bed lost a part of its sorption capacity over time. Possible causes for this phenomenon could be: accumulation of organic compounds and minerals, particle attrition, desorption and biodegradation of the tailoring agent (dimethyl dihydrogenated ammonium chloride).

9. SUGGESTIONS FOR FURTHER RESEARCH

In order to understand better how the proposed in-line sorption coupled with biological regeneration concept will perform when employed for the treatment of real produced water, the following aspects must be further addressed:

- Possibilities to ensure optimum sorbent concentration into the pipe reactor. Sorbent concentration in the pipe reactor controls removal capacity of the target dissolved compounds. Any loss of media would lead to a decrease in removal rate of target compounds, even if the system is designed to provide the right concentration of sorption media. Loss of media will essentially originate from failure in extraction of sorbents from produced water stream.

- Influence of temperature, pH, salinity and heavy metals concentrations on the biological regeneration process. Properties of produced water can be significantly different depending on its origin. Temperature of produced water can vary between 3 and 80 °C, depending on the field. This parameter has a strong influence on microbial kinetics and it is difficult or expensive to control during bioremediation. The pH of produced water can have values as low as 3.5 in the case of produced water from gas fields and as high as 8 in the case of produced water from oil fields. Salinity of produced water is also variable ranging from 0 in condensed water up to several times the salt concentration of seawater. Salinity and pH can influence the composition of microbial culture growing in the FBR used for produced water treatment. Concentrations of heavy metals are also different from site to site. Heavy metals can be toxic to microorganisms if found in high concentrations and therefore could inhibit degradation of sorbed target compounds.

- Influence of concentrations and ratios of nutrients on the biodegradation process. Composition of nutrients solution and ratios between different nutrients were reported to influence biodegradation abilities of microorganisms.

- Causes for loss of sorption capacity during FBR treatment of produced water. Further studies should determine more accurately the causes for the observed loss of sorption capacity as well as the weight of their individual contributions to the total loss.

- Review process design and test in larger scale (pilot) before possible full-scale implementation.

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APPENDIX A. TECHNIQUES FOR BIOMASS CHARACTERIZATION

1. DAPI method for bacteria counting

1.1 Objective and application

Cell concentrations were determined by counting the total number of bacteria under the microscope after staining with 4,6-diamino-2-phenylindole (DAPI) (Porter and Feig, 1980). DAPI was used to stain all cells and a UV (A)-filter was employed with the microscope for counting cell numbers.
The bacteria counts were expressed as number of cells per ml (water samples) or g (particle samples).

1.2 Experimental part

1.2.1 Sample processing

Water samples
The water samples were diluted with distilled water (9 ml), filtered through a sterile filter in test tubes and then homogenized for 1 minute on a mixer. It was important that the samples were particle free and therefore they were filtered through a glass fibre filter before further dilution. 1 ml sample was further diluted in 9 ml distilled water in the next test tube (the so-called 10-folds-dilution). The samples were stirred very well between the dilution steps.

Sediment samples
The samples containing both organoclay and liquid phase were shaken in such a way that the particles were mostly in suspension. 1 ml sample was transferred from the suspension to an Eppendorf-tube and centrifuged for 1 minute at relatively low speed (2000-3000 rcf). The supernatant was removed and 1 ml sterile water was added and the centrifugation operation was repeated. The supernatant was again removed and 1 ml sterile water was added once more and the sample was suspended and treated in an ultrasound bath. The treated sample was centrifuged as described above and the supernatant was taken care of. Dilutions of supernatant were prepared and stained with DAPI.

1.2.2 Epifluorescence staining – DAPI

Bacterial cells were stained with DAPI solution (final concentration 0.6 µg/ml in particle-free deionised water) that was added to 10 ml samples and mixed well. The filtration of samples was carried out 5 minutes after this operation.

1.2.3 Filtration

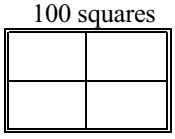
All samples were filtered through black 0.22 µm polycarbonate filters. The microscope slides were then prepared and sample number, dilution and volume filtered were marked directly on the glass. After filtration, the filters were left to dry for about 1 minute. The microscope slides were properly fixed on the filters to prevent them from being blown away. One drop of non-fluorescent immersion oil (Merck for DAPI) was placed on microscope slides before placing the filters. Another oil drop was placed on the filter before the cover glass slide was set on the filter. At 100x magnification a third drop of oil was placed on the cover glass slide.

1.2.4 Microscope analysis

The prepared samples were analysed by a Leitz Dialux 20 microscope (with Ploemopak fluorescence unit and UV-filter A) at a magnification of 100x (Ernst Leitz Wetzlar GmbH, Wetzlar, Germany).

1.2.5 Counting of bacteria numbers

Diameter filter holder: 15.2 mm
Area of the filtered sample: 182.5 mm^2



-> area = $6.4 \cdot 10^{-3}$ mm^2/100 squares

Area of 1-square = $(6.4 \cdot 10^{-3})/100 = 6.4 \cdot 10^{-5}$ mm^2
Number of "1-squares" per filter = (181.4 mm^2)/ ($6.4 \cdot 10^{-5}$ mm^2) = **$2.84 \cdot 10^6$**
Total bacteria / number of counted squares = X (bacteria/ square)
$X \cdot 2.84 \cdot 10^6$ = number of bacteria on the filter
Number of bacteria/ml filtered sample = number of bacteria/ml

Correction for dilution:

Dilution series

1	2	3	4	(number of bacteria/ml)/dilution = concentration in the sample
1	0.1	0.01	0.001	

2. PCR and DGGE

2.1 Nucleic acid extraction

2.1.1 Water samples

The samples were first filtered through 0.22 µm Sterivex GV filters (Millipore Corp., Bedford, Ma, U.S.A). After filtration each filter was filled with 2 ml lysis buffer (50 mM Tris-HCl, pH 8.0; 40 mM EDTA; 750 mM sucrose).
The microbial cells retained in the filter units were then lysed enzimatically. Lysis was carried out by incubating each filter with 2 µg lysozyme (from 20 mg/ml stock solution) at 37 °C for 30 minutes. The lysates were transferred to sterile tubes, the Sterivex filters were rinsed with lysis buffer (at 55 °C for 10 minutes) and the solutions from each filter were mixed. The lysates were extracted with warm phenol-chloroform-isoamylalcohol (25:24:1) according to a standard procedure (Sambrook and Russel, 2001). Each lysate (3 ml) was then mixed with 6 ml warm (60 °C) Tris-HCL buffer phenol-chloroform-isoamylalcohol (pH 8), strongly shaken, kept warm for 5 minutes and cooled on ice. The samples prepared in this way were then phase-separated by centrifugation (4000 x g; for 5 minutes at 4 °C). Sodium acetate (0.2 volume with 10 M solution) was added to each water phase and re-extracted with 5 ml Tris-HCl buffer phenol-chloroform-isoamylalcohol followed by centrifugation. Water phases were then extracted with 5 ml warm (60 °C) chloroform-isoamylalcohol (24:1) and centrifuged as described above. The extracted water phases were then precipitated with 2.5 volume 96% ethanol (for 3 hours at 20°C). The precipitates were pelletized by centrifugation (4000 x g) and rinsed with 75% ethanol. After re-centrifugation the pellets were dried (N_2) and dissolved in 100 µl sterile ultrapure water (Biochrom AG, Berlin, Germany). The nucleic acids were then preserved frozen (-20 °C).

2.1.2 Particle samples

DNA extractions were performed for the comparison of DNA contents in the different phases of particle samples. 3 replicates were prepared for each sample and their volumes were measured. The replicates were then filtered through a filter and the volumes of the particle-free filtrates were determined, while the particle phases were rinsed through the filter with 2 ml particle-free water (MilliQ). The water volumes were then mixed and the DNA extracted (”FastDNA Spin kit for soil”). The particles rinsed on the filter were weighed (wet weight), dried at 30 °C over night and the dry weight determined. The DNA was extracted from particles with the”FastDNA Spin kit for soil”. The DNA amounts in all samples were measured on the NanoDrop ND-1000 (NanoDrop Technologies, Wilmington, USA).

2.2 Polymerase chain reaction (PCR)

2.2.1 Oligonucleotides

Oligonucleotide primers were prepared specific for *Bacteria* (Teske *et al*., 1996):

Bacteria
- 341fBac: 5'-CCT-ACG-GGA-GGC-AGC-AG-3' (forward primer);
- 907rBac: 5'-CCC-CGT-CAA-TTC-CTT-TGA-GTT-3' (reverse primer);
- Expected PCR product: 567 bp.

The primers were synthesized by EuroGentec. The primers were diluted in sterile water at concentrations of 50 µM, distributed in 50 µl aliquots and stored at ÷20 °C.

2.2.2 Deoxynucleotides

Stock solutions of deoxynucleotides (d'NTP) were prepared by diluting 100 mM of the d'NTPs 2'-deoxyadenosine 5'-triphosphate (d'ATP), 2'-deoxysythymidine 5'-triphosphate (d'TTP), 2'-deoxyguanosine 5'-triphosphate (d'GTP) and 2'-deoxycytidine 5'-triphosphate (d'CTP) (Amersham Pharmacia Biotech). Each d'NTP (100 µl) was diluted in sterile water (600 µl), resulting in final concentrations of 10 mM of each d'NTP. Solutions were distributed in 50 µl aliquots and stored at ÷20 °C.

2.2.3 PCR amplification

A touchdown PCR was performed for the amplification of partial 16S rDNA sequences of *Bacteria*. A PCR mix of 100 µl mix consisted of 20 µl d'NTP (10 mM), 10 µl forward primer (50 µM), 10 µl reverse primer (50 µM), 55 µl sterile water and 5 µl AmpliTaq DNA polymerase (Perkin Elmer Roche Molecular Systems, Branchburg, NJ, USA). DNA template (1-10 µl) was diluted in 10 µl [10x] PCR buffer with 15 mM $MgCl_2$ (Perkin Elmer Roche) and with sterile water to a final volume of 90 µl. The mixture was heated (95 °C) for 5-10 minutes on a heating block. A PCR mix of 10 µl was applied to each sample when the samples were still in the heating block (95 °C) and then they were immediately transferred to an iCycler DNA Thermal Cycler (BioRad Labs. Inc., Hercules, CA, USA).

PCR was run as a touchdown method to reduce the generation of spurious by-products, and with the following sequence cycles:

- Denaturation: 95 °C for 1 minute;
- Primer annealing: 65-55 °C for 1 minute;
- DNA synthesis (primer extension): 72 °C for 3 minutes;
- Number of cycles: 35.

During the first 10 cycles, the annealing temperature was gradually reduced from 65 to 55 °C with 1 °C for each cycle during the first 10 cycles, followed by 25 cycles with annealing temperature of 55 °C.

2.3 Agarose gel electrophoresis

2.3.1 Analytical agarose gel electrophoresis

PCR products were analysed by horizontal agarose gel electrophoresis. Samples (27 µl) were mixed with [10x] gel-loading TBE buffer (3 µl) (0.9 M Tris, 0.9 M borate, 20 mM EDTA, pH 8.3, 50% (v/v) glycerol, 0.25% (w/v) bromophenol blue). A low DNA Mass Ladder (Gibco BRL, Paisley, UK) was used as standard, 12 µl standard in 3 µl [10x] gel-loading TBE buffer.

Gels were prepared by heating agarose (2.0 g; Sigma) in 160 ml [0.5x] TBE (0.045 M Tris, 0.045 M borat, 1 mM EDTA, pH 8.3) in a microwave oven (4 minutes), followed by cooling to 50 °C in water bath. Ethidium bromide (10 µl) from a stock solution (10 mg/l ethidium bromide in sterile water) was applied to the agarose, and the melted gel was cast horizontally in a plastic tray (open ends of the tray sealed) with a comb of 15-well or 20-wells in the electrophoresis apparatus (BioRad). The gel was set at room temperature for 20 minutes, submerged in [0.5x] TBE buffer, and the comb and seals carefully removed.

Prepared samples and standard (see above) were applied to the submerged gel wells (20 µl sample and 10 µl standard) and electrophoresis run with constant voltage (150 V) in 1.5-2 hours at room temperature. Gel documentation was performed over a UV-transilluminator table (BioRad). The gels were photographed by black-white Polaroid film (0.1 – 0.5 second exposure time), or by digital camera (GelDoc, 2000, BioRad).

2.4 Denaturing gradient gel electrophoresis (DGGE)

2.4.1 Analytical DGGE

By DGGE, PCR products were generated with general primers defining *Bacteria* (341fBAC and 907rBAC). To the primer 341f BAC a 40 mer GC-clamp was added to the 5'-end (5'-CGC-CCG-CCG-CGC-GCG-GCG-GGC-GGG-GCG-GGG-GCA-CGG-GGG-G-3').

DGGE was performed with 6% (w/v) polyacrylamide (PAA) gels in [0.5x] TAE buffer (20 mM Tris-acetat, pH 7.4; 10 mM acetate; 0.5 mM EDTA) with a 20-70% gradient of the denaturing agents urea and formamide (100% denaturing agents corresponded to 7 M urea and 40% (v/v) deionised formamide) in a DCode Universal Mutation Detection system (BioRad).

Stock solutions of PAA/Bis-acrylamide (Bis) (40%) consisted of 38.93 g acrylamide and 1.07 g Bis dissolved in deionised water to 100 ml, while stock solutions of [50x] TAE buffer were generated by mixing 242 g Tris, 57.1 g acetic acid, and 100 ml 0.5 M EDTA to a total volume of 1000 ml with deionised water. Linear gradient gels (thickness 1 mm) were prepared by mixing PAA and Bis with denaturating agents to generate a 20 to 70% linear gradient in a gradient delivery system (BioRad model 475). For the preparation of one gel, 18 ml of each solution was mixed with 200 µl ammonium persulphate (10% (w/v) in deionised water) and 20 µl TEMED (BioRad), and the mixtures immediately transferred to each of two 30-ml syringes, which were subsequently mounted in the gradient delivery system. The gel was cast as a parallel gradient gel (16 x 16 cm) with 1 mm thickness and allowed to polymerize for approximately 1 hour, and with a comb of 15 wells. The electrophoresis tank was filled with [1x] TAE buffer that was heated to 60 °C in the tank, and 1-2 polymerised gels were placed vertically in the electrophoresis tank.

Each PCR product sample (10 µl) was mixed with 10 µl sample buffer (0.05% bromophenol blue, 0.05% xylene cyanol, 70% glycerol, diluted in deionised water), and the complete volume (20 µl applied to each well).

Vertical electrophoresis was performed with continuous temperature (60 °C) and voltage (150 V) until both markers had migrated to the bottom of the gel (approximately 4.5 hours).

After electrophoresis, the gels were stained in SYBR Gold (Molecular Probes, Leiden, The Netherlands), diluted 1:10,000 in [1x] TAE for 20-30 minutes. The gels were then photographed with the GelDoc system (BioRad).

APPENDIX B. LIST OF PAPERS

1) Scurtu C., Leiknes T., Helness H. and Melin E. (2006). Research of a selective, bio-regenerable adsorbent for the dissolved aromatic compounds in the wastewaters from offshore platform operation. Proceedings - *4th International Conference on Marine Waste Water Disposal and Marine Environment*, Antalya Turkey, 6-10 November 2006.
2) Scurtu C., Leiknes T. and Helness H. (2007). Study of an organoclay for the sorption of dissolved organic compounds in the wastewaters from offshore platform operation. Proceedings - *2nd IWA ASPIRE Conference*, Perth, Australia, 28 October - 1 November 2007. *Water Science and Technology*, 58.9 (2009), 1495-1503.
3) Silalahi S., Scurtu C., Leiknes T. and Helness H. (2008). Research into new strategies in removing dispersed oil and dissolved compounds from produced water. Proceedings - *6th Produced Water Workshop,* Aberdeen, UK, 23-24 April 2008.
4) Scurtu C., Leiknes T. and Helness H. (2008). Biological regeneration of an organoclay in a fluidized bed reactor for the treatment of wastewaters from offshore platform operation. Proceedings – *4th IWA Young Water Professional Conference*, Berkeley, CA, USA, 16-18 July 2008.
5) Scurtu C., Leiknes T. and Helness H. (2008). Combination of organoclay sorption and biodegradation in a fluidized bed reactor for the treatment of wastewaters from oil and gas industry. Proceedings – *IWA Chemical Industries Conference*, Beijing, China, 9-11 November 2008. Submitted for possible publication to *Water Science and Technology* by conference organizers (December 2008).

Additional conference presentations (without papers)

1) Scurtu C., Leiknes T. and Helness H. (2008). Combination of organoclay sorption and biodegradation in a fluidized bed reactor for the treatment of wastewaters from oil and gas industry. Proceedings – 3rd Annual Produced Water Management Summit, Abu Dhabi, United Arab Emirates, 16-19 November 2008.
2) Scurtu C., Leiknes T. and Helness H. (2009). Evaluation of organoclay sorption coupled with biological regeneration for removal of BTX in produced water. Proceedings – Produced Water Management 2009, Stavanger, Norway, 21-22 January 2009.

APPENDIX C. PHOTOGRAPHS

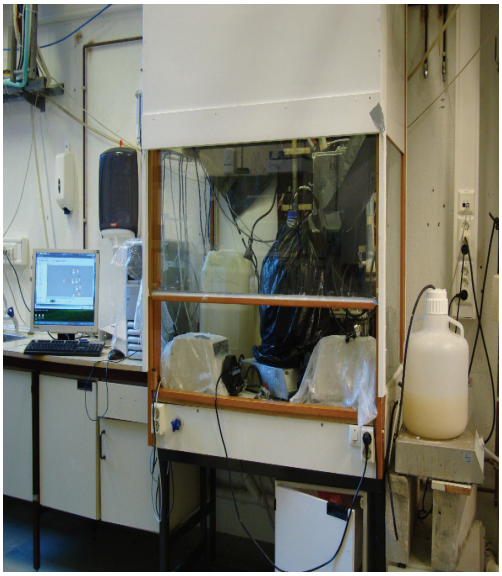


Figure 82. Experimental setup, control and data acquisition system

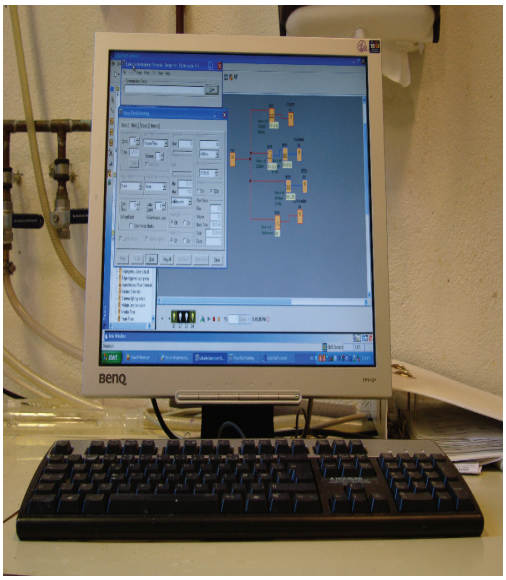


Figure 83. Softwares for controlling the pumps and the valves

Figure 84. Close view of the experimental setup

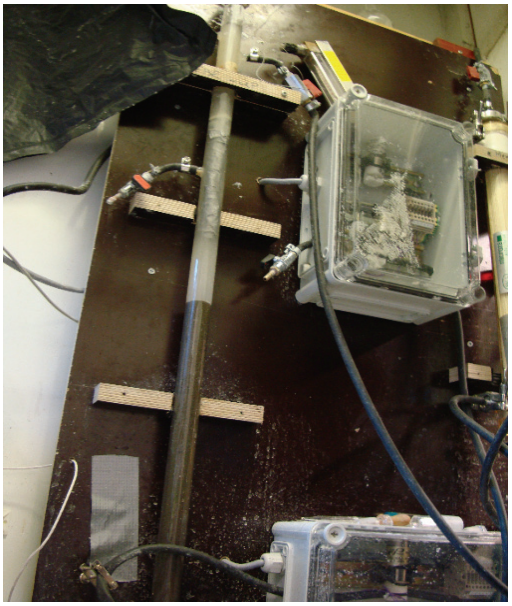


Figure 85. Fluidized bed reactor

Figure 86. Peristaltic pump used to supply BTX, acetic acid and nutrients solution in the FBR

Figure 87. Membrane contactor used to provide bubble free aeration

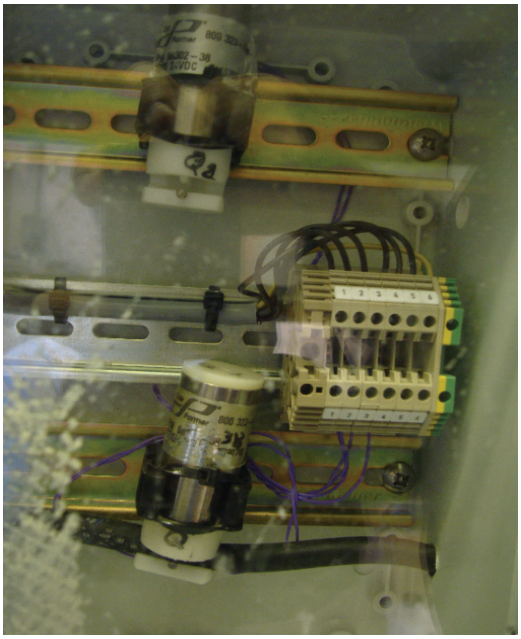


Figure 88. Valves used to control fluid flows in the treatment system

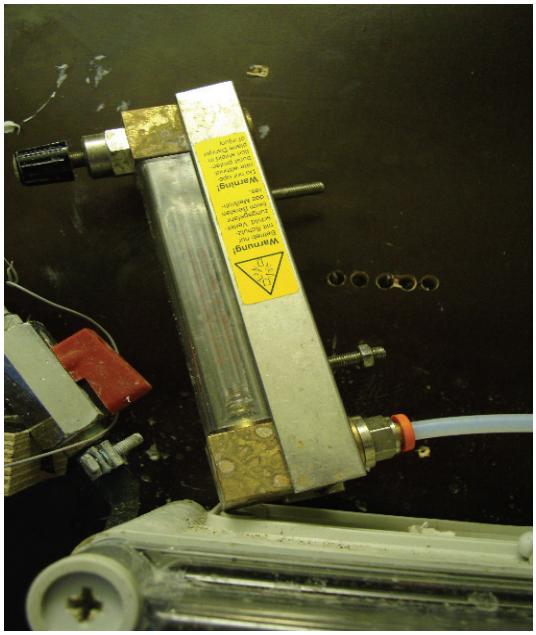


Figure 89. Flow meter used to control aeration