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Algae and rotifer turbidostats: studies on stability of live feed cultures

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Abstract

A two stage turbidostat was developed according to Boraas & Bennet (1988), but with highly improved turbidity sensors. The first stage was an algal turbidostat where algal density was regulated by turbidity measurements. Algal density was also held constant in the second stage (rotifer production) according to turbidity measurements. Additionally, the growth rates were monitored. The regulation system allowed an effective on-line process control. Initially, the production of rotifers in long-time studies was variable. However, after further improvements of the turbidity measurement, fluctuations in the rotifer turbidostat decreased significantly.

Introduction

Batch cultures are commonly used in live food aquaculture to produce rotifers as food for fish larvae. This technique creates highly variable conditions both in abundance as well as in biochemical content of the rotifers. In addition, there is a very high risk that the cultures will become unstable and the growth yield may not be very high. The same is also true for semi-continuous cultures (see review by Snell, 1991).

There is a great demand for more accurate monitoring of the growth process and for receiving early warning of impending crashes. Swimming speed (Snell et al., 1987; Korstad et al., 1995) and egg ratio (Korstad et al., 1995) are good indicators for the status of the culture. However, a disadvantage of such indicators is that they are labour intensive. Furthermore, even with daily measurements, major changes or crashes may occur in the interval between measurements.

Improvements have been made by using the inherent regulation and stabilization characteristics of chemostats. In this type of continuous culture growth rate is regulated by food limitation. James & Abu-Rezeq (1989) maintained two stage cultures for aquaculture, algae in the first stage and rotifers in the second stage, with the volume of 1 m^3 for the rotifer stage for up to 6 month (James, 1993). In steady-state growth, yield may be optimized and biochemical composition does not vary. However, steady states in chemostats are also subject of fluctuations, especially when running at low dilution rates (see review by Rothhaupt, 1993). In most examples, rotifer chemostats showed oscillations.

For stable production of rotifers, the turbidostat is a very attractive system. It combines both early warning monitoring and effective regulation of algae and rotifer densities in the two stages of the system. In the turbidostat of Boraas & Bennet (1988), algal densities in the rotifer stage are held constant, as regulated by turbidity measurements. The system presented here is a further development of this system, with improved turbidity sensors to regulate algal concentrations in both stages. This system was used to test whether the turbidostat produces a more stable culture system than the chemostat.

Methods

Principles of construction

Rotifers and algae were cultivated in a two stage system (Walz, 1993). In the first stage, algae (*Monoraphidium minutum*) were grown in a 948 ml vessel which was operated as a turbidostat (Figure 1). This vessel was exposed to 6 cool-white and 6 warm-white fluorescent lamps. Mineral medium (Chu 12) as described by Walz (1993) was pumped in by a peristaltic pump. Before the medium pipe reached the vessel, it was combined with an air-supply sterilized by 0.2 μm PTFE-Filter (Sartorius Midisart 2000). The volume of the culture vessel was constant as the overflow was blown out into an output vessel.

The algae suspension was carried by a peristaltic pump to the second stage, the 248 ml rotifer vessel (*Brachionus calyciflorus*). The construction was identical to the algal vessel, but maintained in darkness. Both vessels were held at a constant temperature of 20 °C by water jackets connected to external thermostats.

Principles of operation

Improved turbidity sensors according to the principle of Boraas & Bennet (1988) were connected to both culture vessels to measure algal concentrations. The light transmittance was measured through the wall of a syringe which pulled out and pushed back 10 ml of algal suspension. Wall growth, which would interfere with the light beam, commonly a serious problem in such systems, was prevented by the activity of the syringe plunger. About 150 000 measurements could be done without a change of the sensor. There was a linear relationship between light attenuation of the sensors and algal volume measured by Coulter counter ($r^2 = 0.993$, $P = 0.001$).

The output vessels were located on electric balances and the output was determined by weight increase in the vessels. The calculated output volume was divided by the culture volume to give the dilution rate (D). Light attenuations in the two stages were measured every two minutes and compared to predetermined threshold value by a graphic Labview program (National Instruments Inc., Austin, Texas). In the algal turbidostat the pump proceeded to introduce fresh medium when the light attenuation was higher than the threshold value. In contrast, in the rotifer vessels the pump started when the attenuation value was lower than the set-value (as a result of grazing) to carry in new algal suspension.

Data on attenuation values, thresholds, output volumes, pump operation times and dilution rates were saved on files together with date, clock times and running times. The program was able to show the actual situation on diagrams on the monitor. Variability was calculated by the relative variation coefficient (V_r = standard error $*$ 100/mean value).

In the line between rotifer vessel and syringe sensor an additional light barrier was introduced. On passing this barrier, rotifers were counted twice, once leaving the vessel and again returning to it. With the help of setting thresholds, larger or smaller particles could be excluded from counting. The data could be stored on the computer file.

Results

In the second part of the 4 month period of the experiment an improved light transmittance sensor was installed. The operation in the algal vessel, as in the rotifer reactor, was in the chemostat mode during the first two weeks until algal concentration had declined and rotifer density had increased. Then it was switched to the turbidostat mode in both vessels. The light attenuation coefficient in the algal turbidostat was constant (Figure 2, panel a). In contrast to the stability of this signal, the total algal volume as determined by Coulter Counter fluctuated (panel b). A similar difference was observed for the rotifer vessel (panel c and d). Some failures occurred in the transmittance sensor. For that reason also the rotifer density and the dilution rate in this vessels fluctuated in the first part of the experiment (Figure 2 panel e, f).

After installing improved sensors in both vessels on 10.01.1996, the dilution rates of the *Brachionus* vessel (Figure 2, panel f) soon reached constant values which resulted in a steady state with fairly constant rotifer densities 10 days later (panel e). The relative variation coefficient of the *Monoraphidium* biovolume in the first stage (Figure 2, panel b) lowered from 2.8% in the first period to 2.0% in the second period. Similarily, in the *Brachionus* turbidostat the variation of the residual algal volume decreased from 4.1% to 3.1% (panel e). The first decline of algae in the chemostat mode was not included in the calculations. This period was likewise not considered in calculating rotifer density variation coefficient, which decreased from 7.1% in the first period to 4.6% in the second period.

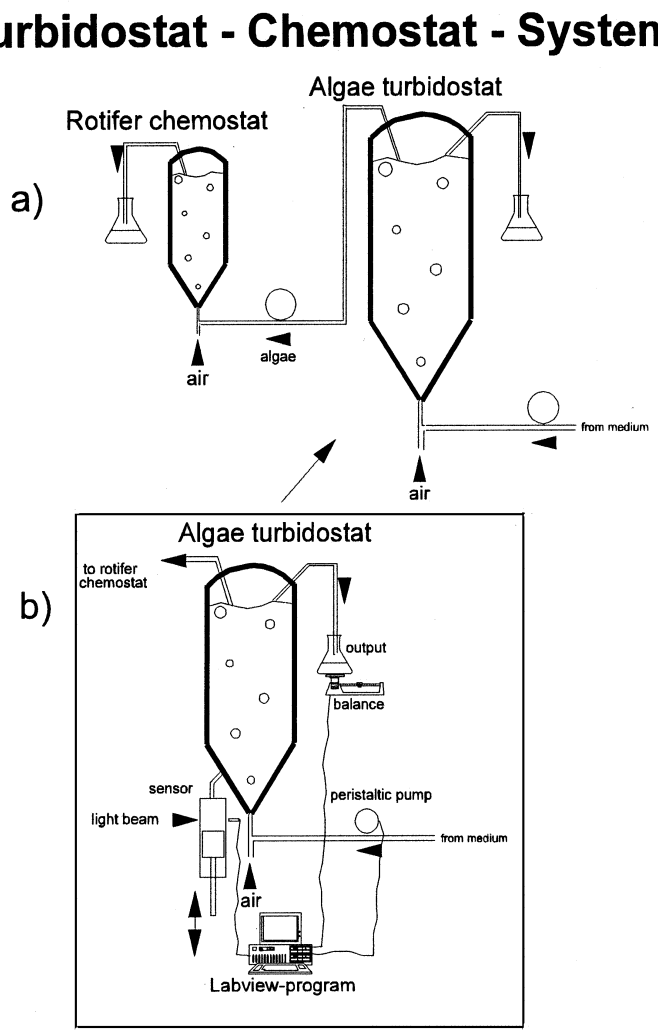

Figure 1. (a) Two stage turbidostat – chemostat system with the production of algae in the first stage and of rotifers in the second stage. The rotifer stage is presented here in the chemostat mode. (b) Detail of the algal turbidostat. When the rotifer stage is operated as turbidostat, this construction corresponds also to the rotifer reactor. Further explanations are given in the text.

Preliminary results on automatic rotifer counting

Recently a rotifer counter has been added to count by means of light barriers. The rotifers are counted every two minutes as they enter or leave the syringe of the turbidity sensor (see above). The counting technique is on-line and is an improvement of the rotifer Coulter measurements of Boraas (1983). First results (Figure 3) indicate large outliers, probably due to aggregates of algae or to overlap occurrencies. The numbers counted automatically are correlated with the numbers counted under the stereomicroscope ($r^2 = 0.90$, $P < 0.0001$). In the automatic counter numbers above 500 N ml^{-1} were lower than the manually counted ones due to co-occurrences.

Discussion

The turbidostat is a very new tool for the production of rotifers. Boraas & Bennet (1988) were the first to describe a rotifer turbidostat system and its use to select fast growing strains of rotifers (Bennet & Boraas, 1988). The turbidostat is a very good tool for such experiments because it functions optimally at maximum growth rates whereas the chemostat is bet-

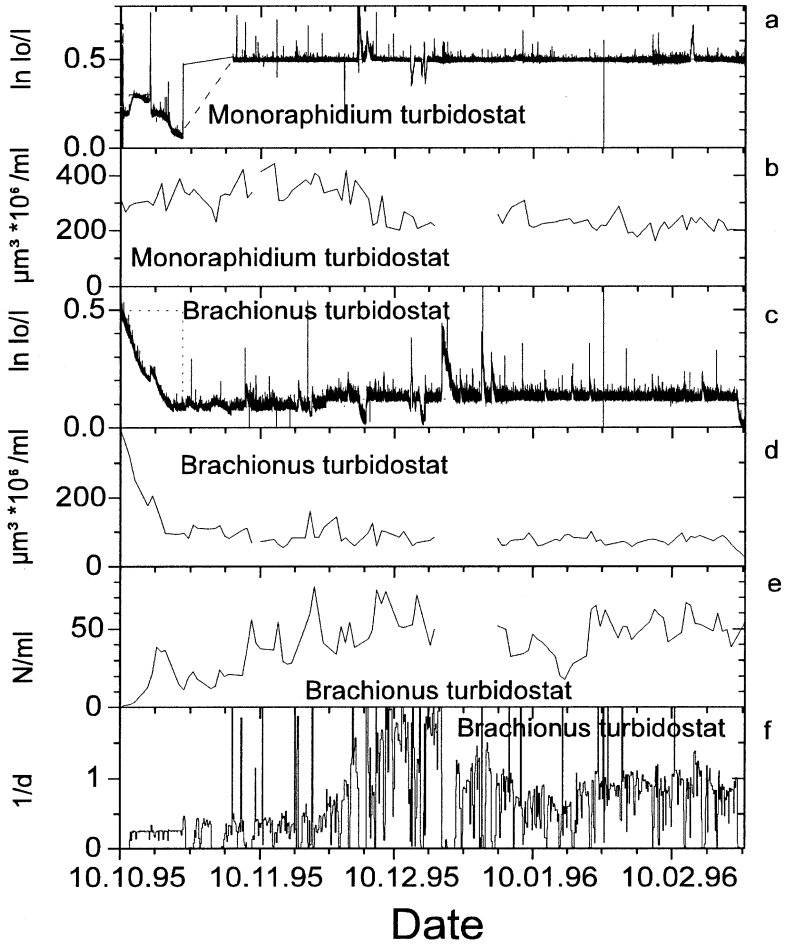

Figure 2. Results of the long-term experiment with *Monoraphidium minutum* in a turbidostat in the first stage and *Brachionus calyciflorus* in a turbidostat in the second stage. Panel (a) Light attenuation in the algal stage, (b) Algal volume in the algal stage, (c) Light attenuation in the rotifer stage, (d) Residual algal volume in the rotifer stage, (e) Rotifer density in the rotifer stage counted under the stereomicroscope, (f) Dilution rate of the rotifer stage.

ter for lower or middle range growth rates (Bennet & Boraas, 1993).

The differences between the attenuation measurements with low variability and the parallel measurements of algal volumes by the Coulter Counter with large variability in the first experimental period are striking (Figure 2, panels a, b and c, d before 10.01.1996). Although there was a strict relationship between light attenuation and Coulter volume calibrations at any time, there was no constant cell volume/cell concentration relationship over time, i.e. the cells differed in size. Therefore, same cell volumes could give different light attenuations at different cell numbers. The light attenuation signal is correlated to biomass (Günther & Bergter, 1971). Due to the regulation behaviour of the turbidostat, this error gave a positive feed back between algal growth and the size. This could be seen by the significant larger fluctuation of rotifer numbers ($V_r = 6.0\%$) in the turbidostat (first experimental period) when compared to an unpublished experiment in chemostat mode ($V_r = 4.6\%$, *F*-Test, $P = 0.001$).

However, this situation changed to the opposite in the second period after improving the transmittance sensor on 10.01.1996. After that, the lowest variation coefficients in all vessels was recorded in the turbidostat. The variation of rotifer numbers was significantly lower compared to the first period (*F*-test, $P = 0.005$). Variation was also significantly lower for the algal biovolumes in both the first and the second stage ($P = 0.05$

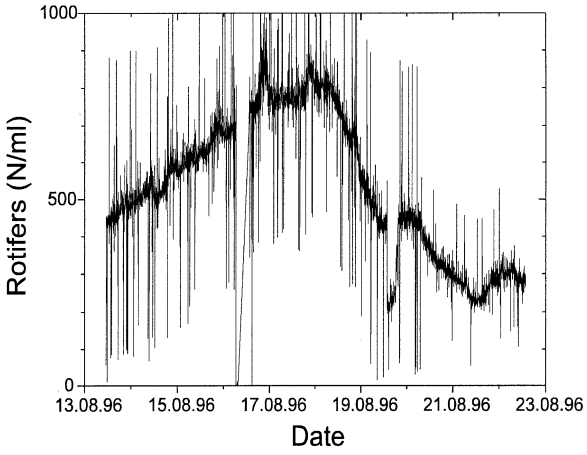

Figure 3. Example on automatic rotifer counting in a rotifer vessel.

and 0.001, respectively). Also the dilution rate, the dependent variable, was stabilized (Figure 2, panel f). In the second period the variation coefficient of the biovolume of residual algae in the rotifer vessel was significantly lower than in the unpublished chemostat experiment (3.1% against 9.4%, *F*-test, $P<0.001$). The fluctuation of the rotifer abundance decreased sigificantly from $V_r = 5.2\%$ in the chemostat to 4.1% in the turbidostat (*F*-test, $P = 0.001$).

The advantage of the turbidostat is not only established by the lower variability, but even more by its possibility to monitoring the growth process of algae and rotifers. This may be combined with warning signals passing threshold settings. The rotifer counting, however, by means of light barriers, is susceptible to aggregates of algae and detritus which, at the present situation, rendered these measurements impossible at higher concentrations of those particles. All these sensors have been also installed in vessels operated in the chemostat mode (as in the unpublished experiment cited above). For the chemostat no upscaling problems are to be expected, when sensors should be introduced into larger tanks (Rusch & Malone, 1991, for 225 l algal chemostats). The number of sensors, which can also immersed into the tanks, may be increased when applied to larger volumes. In contrast, upscaling problems of the turbidostat are unknown at present. But with the introduction of several sensors per tank upscaling problems should not be higher than with large chemostats (Abu-Rezq et al., 1997).

The costs to install sensors and electronic equipment for turbidostats are high and eventually may not give the equivalent value for the advantage of lower fluctuations. However, turbidostats are safely operated at high dilution rates. This will bring two further advantages:

– Rotifer growth is affected by algae nutrient limitation (Rothhaupt, 1995). In contrast to the chemostat, the algal growth in the turbidostat is not limited by nutrients. This provides a better quality of live feed for rotifers in turbidistats than in chemostats (operating at low to medium dilution rates). It is known that rotifer growth depends on growth rate of the algae in chemostats (Scott, 1980; Schmid-Araya, 1992). The same argument as that of algae quality for rotifer growth may be true for the food quality of rotifers for the nutrion of fish. Therefore, it may be beneficial to operate rotifer vessels in the turbidostat mode.

– No effort was made to increase the rotifer concentration during the experiments (51.7 rotifers ml^{-1} in the second period). Later, the number of rotifers could be increased by the factor of 10 by higher input algae concentrations (Figure 3). But even with about 50 rotifers ml^{-1} due to the high dilution rate of 0.9 d^{-1} in the second experimental period the production of 46.0 rotifers ml^{-1} d^{-1} (= 82.8 mg fresh weight, 8.3 mg dry weight) could be achieved. This value lies in the middle range of many rotifer aquaculture plants (Fulks & Main, 1991; Lubzens, 1987). The yield (rotifer biomass developed/ food consumed) on a wet weigth basis was very high, 0.6. The ingestion rate, calculated by: D^* (algal input concentration – algal output concentration)/rotifer concentration, was 1.4 g per g rotifer (d^{-1}). The loss by residual algae was 0.7 (d^{-1}). The total food necessary for the production of 1 g rotifer was, therefore, 2.1 g per day (wet weight basis).

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