Nitrogen removal from aquaculture pond water by heterotrophic nitrogen assimilation in lab-scale sequencing batch reactors

Peter De Schryver, Willy Verstraete *

Laboratory of Microbial Ecology and Technology (LabMET), Ghent University, Coupure Links 653, B-9000 Ghent, Belgium

**Abstract**

The potential use of sequencing batch reactors (SBRs) as an alternative bio-flocs technology (BFT) approach in aquaculture was explored. One SBR was dosed with glycerol and one with acetate for the decrease of the nitrogen concentration in simulated aquaculture water by microbial assimilation. At an optimal C/N ratio between 10 and 15, the nitrogen removal efficiency reached up to 98% (=110 mg N L\(^{-1}\) reactor day\(^{-1}\)) for both SBRs. The estimated biomass productivity reached 0.62–0.94 g C L\(^{-1}\) reactor day\(^{-1}\) for the glycerol SBR and 0.54–0.82 g C L\(^{-1}\) reactor day\(^{-1}\) for the acetate SBR. The floc protein content, indicating biomass quality, reached up to 57% if grown on glycerol. With acetate, it attained a value of 61%. The highest average poly-β-hydroxybutyrate (PHB) content was 16% on a dry weight basis for the acetate biomass.

© 2008 Elsevier Ltd. All rights reserved.

1. Introduction

In intensive land-based aquaculture systems, densities of animal biomass can easily reach high values. For example, fish is cultured at densities up to 50 kg of living biomass m\(^{-3}\) (Conte, 2004). In these feed-driven pond systems, only 20–30% of the nitrogen input is converted into harvestable products (Azim et al., 2003; van Dam et al., 2002). The remainder is excreted and typically accumulates as inorganic nitrogen within closed systems, reaching concentrations toxic to aquatic culture species (Avnimelech, 2007). In contrast to recirculating aquaculture systems (RAS) which have a limited water exchange of about 10% of the pond volume each day (Twarowska et al., 1997), bio-flocs technology (BFT) with zero or minimal water exchange was developed to cope with this accumulation of toxic nitrogen species.

BFT is based on the assimilation of inorganic nitrogen species (ammonia, nitrite and nitrate) by the microbial community present within the pond water. This can be accomplished by aiming at a high C/N ratio in the water (Azim et al., 2007). Either the use of food formulated with a lower protein content (Avnimelech, 1999; Hargreaves, 2006) or supplying additional carbon sources like glucose or starch to the pond water (Crab et al., 2007) can be used as control methods. The bacterial biomass grown on the metabolized organic substrate can be taken up by the fish or shrimp as an additional food source. As such, the nutrients excreted after primary consumption by the aquaculture species and the nutrients originating from non-eaten food are recycled into harvestable microbial biomass. It was shown that a doubling of the nitrogen use by tilapia could be obtained through application of this technology (Avnimelech, 2006). Overall, BFT offers the advantages of lower impact on the environment due to lower external water requirements, the removal of toxic inorganic nitrogen species and the in situ production of additional feed for the culture species. It is estimated that the decrease in feed costs per kg annually produced live weight using BFT can be in the order of 15%. Based on investment and operation costs, nitrogen control can be performed at about half the price of normal pond aquaculture with an external trickling filter (De Schryver et al., 2008).

The microbial metabolism for the decomposition of organic matter necessitates the continuous presence of oxygen (Azim et al., 2007). The addition of carbonaceous substrate to the water may result in sudden and temporary lower dissolved oxygen (DO) concentrations. Anecdotal data suggest that such DO drops can result in fish mortality (Colt, 2006). Secondly, excessive turbidity may have negative effects on sensitive fish species and not all are adaptable to growing in turbid water (Avnimelech, 2006). Although preference should be given to basic BFT, i.e., co-culture of aquaculture species and heterotrophic bacterial biomass within the same solution, the considerations mentioned above sometimes require the application of BFT in a compartmental design as was described by Avnimelech (2006). In such applications, the culturing of fish and the microbial production are performed in separate compartments. This allows for a better control of turbidity, oxygenation and return of microbial protein back into the fish compartment.

---

* Corresponding author. Tel.: +32 9 264 59 76; fax: +32 9 264 62 48.
E-mail address: Willy.Verstraete@Ugent.be (W. Verstraete).
In this lab-scale research study, the treatment of simulated fish pond water was explored by means of sequencing batch reactors (SBRs) as an external compartment of the bio-flocs technology. This approach was set up with the goals (1) to provide a simple reactor design for application in intensive aquaculture, (2) to determine the effect of the C/N ratio on the assimilation of the ammonia nitrogen in microbial biomass rather than nitrifying it into nitrate and (3) to assess floc quality (protein and poly-β-hydroxybutyrate) and floc morphology.

2. Methods

2.1. Reactor design and operation

The experiments were performed in two 4.5 L sequencing batch reactors. Both had an internal diameter of 8 cm and a liquid filled height of 60 cm (=3 L working volume) and were maintained at a temperature of 24–26 °C (optimal temperature for tilapia culture). The microbial biomass was intensively mixed by means of two fine bubbling diffusive airstones. The first was placed at the bottom of the reactor and the second was placed at half of the water height. Each reactor was supplied with air by means of an 8 W air pump (Rena Air 400, Rena, France). The reactors were initially subjected to consecutive cycles of 3 h comprising an anaerobic feeding phase (Rena Air 400, Rena, France). The reactors were initially subjected to consecutive cycles of 3 h comprising an anaerobic feeding phase of 1 h 52 min, a settling phase of 3 min and 5 min withdrawing the effluent (de Kreuk and van Loosdrecht, 2004). From day 15, the feeding phase was aerated as well. After settling, 2.5 L of the supernatant was removed. Thus, the reactor selected for microbial biomass settling could be calculated based on its containing 16% organic nitrogen. Protein content was extracted to obtain organic nitrogen concentrations. Protein content could be calculated based on its containing 16% organic nitrogen.

2.2. Nitrification batch tests

With the biomass from the SBRs, batch nitrification tests were performed. Erlenmeyers flasks of 1 L (n = 3) were filled with 0.5 L microbial suspension from either the glycerol SBR or the acetate SBR. These were spiked from a NH4Cl solution to obtain a total ammonia nitrogen (TAN) concentration of ca. 14 mg L−1 and placed on a shaker at 150 rpm and 25 °C. Initial volatile suspended solids (VSS), TAN, nitrite nitrogen (NO2–N) and nitrate nitrogen (NO3–N) concentrations were determined. Every half hour during a total of 3 h, samples for TAN, NO2–N and NO3–N analysis were taken. Sampling was performed by setting the flasks for 2 min before the actual sample time and filtering 10 mL of the supernatant over a 0.45 μm filter (Chromafil® AO-45/25, Machery-nagel, Germany). The samples were stored at 4 °C until further analysis.

2.3. Analyses

2.3.1. Physicochemical parameters

For the determination of the total suspended solids (TSS), VSS, ash and total chemical oxygen demand (CODt), a 20 mL grab sample was taken from the reactor and frozen immediately at −20 °C until further analysis. Analyses were performed according to standard methods (Greenberg et al., 1992).

For the determination of the soluble chemical oxygen demand (CODs), TAN, NO2–N and NO3–N, a 20 mL grab sample was taken from the reactor, filtered over a 0.45 μm filter (Chromafil® AO-45/25, Machery-nagel, Germany) and stored at 4 °C until further analysis. The CODs of the influent was determined on 5 mL samples from the influent vessels and stored at 4 °C prior to the analysis that was performed according to standard methods (Greenberg et al., 1992). For TAN, a 5 mL sample was measured spectrophotometrically via the Nessler method (Greenberg et al., 1992) NO2–N and NO3–N were analyzed on a 10 mL sample with ion chromatography using an ion chromatograph (IC 761 Compact, Methrom) with a Metrosep A sup 5 column and a Metrosep A 4/5 guardcolumn. The eluent consisted of 3.2 mM Na2CO3 and 1.0 mM NaHCO3 with a flow of 0.7 mL min−1. Kjeldahl nitrogen was determined on a 10 mL grab sample stored at −20 °C. Samples were analyzed using a Gerhardt Vadopost 20 distillation apparatus (C. Gerhardt Fabrik und Lager chemischer Apparate GmbH and Co. KG, Köningswinter, Germany) according to standard methods (Greenberg et al., 1992). From the obtained Kjeldahl nitrogen values, TAN was subtracted to obtain organic nitrogen concentrations. Protein content could be calculated based on its containing 16% organic nitrogen.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Overview of the increase in C/N ratio during SBR operation together with the resulting chemical oxygen demand and carbon loading rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period (days)</td>
<td></td>
</tr>
<tr>
<td>0–11</td>
<td>2.5</td>
</tr>
<tr>
<td>11–32</td>
<td>5</td>
</tr>
<tr>
<td>32–50</td>
<td>10</td>
</tr>
<tr>
<td>50–60</td>
<td>15</td>
</tr>
</tbody>
</table>

* 1 g glycerol (C3H8O3) = 1.2 g COD (experimentally determined); 0.4 g C/g glycerol.

* 1 g acetate (CH3COOH) = 0.9 g COD (experimentally determined); 0.4 g C/g acetate.
and was expressed as a percentage of the bio-floc dry matter weight (DMW) (AOAC, 1999). Dissolved oxygen was measured by means of a Hach Lange HQ40D equipped with a IntelliChlor LDO oxygen sensor and built-in thermometer (Hach Lange, Belgium).

2.3.2. Poly-β-hydroxybutyrate (PHB)

The PHB content of the biomass in frozen 10 mL grab samples from the reactors was determined by acid hydrolysis of the PHB with the formation of crotonic acid (Karr et al., 1983). Samples were thawed in warm water and centrifuged for 10 min at 7000g. The pellets were resuspended in 1 mL of distilled water and transferred to pre-weighed Eppendorf tubes. Samples were centrifuged for 5 min at 13,000g, dried overnight at 100°C and weighed again for determination of the biomass DMW. Dried pellets were digested with 1 mL of 96% H2SO4 at 100°C for 1 h to form crotonic acid. The reaction mixture was then cooled to room temperature on ice, diluted 50-fold with 0.014 M H2SO4 and crotonic acid concentrations were determined by HPLC using a Dionex ASI-100 autosampler injector (Dionex Corporation, Sunnyvale, CA, USA) equipped with an Aminex HPX-87 H ion-exchange organic acids column (300 x 7.8 mm) (Bio-Rad Laboratories, Richmond, CA, USA). The solvent used was 0.014 M H2SO4 at a flow rate of 0.7 mL min⁻¹. The elution peaks were monitored at 210 nm with a Dionex UV detector. PHB content was calculated from a calibration curve for standards of commercial PHB (Goodfellow Cambridge Ltd., Huntingdon, England) treated in the same manner as the samples and expressed as a percentage on biomass DMW.

2.3.3. Bio-floc morphology

Photographs of the microbial biomass morphology within the reactors were taken using a stereomicroscope (WILD Heerbrugg, Belgium) on a 10 mL grab sample from the reactors. Images were processed by means of the image processing software ImageJ.

2.3. Statistical analysis

For the glycerol SBR as well as the acetate SBR, the differences in the mean nitrogen removal efficiency, the mean COD removal efficiency and the mean protein content were statistically analyzed by One-way ANOVA with the C/N ratio being the main factor. A Tukey’s post-hoc test (p < 0.05) was used if equal variances could be assumed or a Tamhane’s T² post-hoc test (< 0.05) was used if equal variances could not be assumed.

Comparison between the glycerol SBR and the acetate SBR concerning the protein content in the reactor flocs or the effluent flocs was performed with a t-test (p < 0.01).

3. Results

3.1. Reactor performance for nitrogen and carbon removal

An increase in the C/N ratio resulted in a significantly higher nitrogen removal efficiency for both SBRs (Table 2). The maximum concentrations in nitrite and nitrate nitrogen detectable at the end of the sampled cycles were 0.4 ± 0.9 mg L⁻¹ and 0.4 ± 0.2 mg L⁻¹ for the glycerol SBR whereas these were 1.3 ± 0.8 mg L⁻¹ and 0.5 ± 0.5 mg L⁻¹ for the acetate SBR. In additional batch tests, no autotrophic nitrification was observed (data not shown). Without the presence of carbon, the biomass of both the glycerol SBR and the acetate SBR did not significantly decrease TAN while no production of nitrite or nitrate could be observed.

The average COD removal efficiency per experimental period defined by the C/N ratios 2.5–10 ranged from 88 ± 3.2% to 91 ± 6.6% for the glycerol SBR and from 84 ± 2.6% to 87 ± 11% for the acetate SBR (Fig. 1). When the C/N ratio was increased to a final value of 15, a significant decrease (p < 0.05) in COD removal efficiency to 65 ± 10% could be observed in case of the glycerol SBR. For the acetate SBR, this was a non-significant decrease (p > 0.05) to 74 ± 17% (average calculated excluding the outlier value of 11.4% on day 57). The maximum amount of feed carbon that could be removed on a daily basis occurred at the C/N ratio of 10 and attained 1.56 g C L⁻¹ day⁻¹ in the glycerol SBR and 1.36 g C L⁻¹ day⁻¹ in the acetate SBR.

3.2. Floc composition

The protein content of the biomass increased in both reactors concomitantly with higher C/N ratios (Table 3). However, this increase was only significant for the shift in C/N ratio from 5 to 10 in case of the acetate SBR. Although average protein content values for the biomass in the glycerol SBR were higher than those for the acetate SBR, no significant differences between the two could be
observed (t-test, p > 0.01). For both reactors at a C/N ratio of 2.5, the protein content of the microbial biomass present in the effluent was significantly lower than the protein content of the reactor biomass (t-test, p < 0.01). However, no significant differences between the effluent and the reactor values could be observed at higher carbon loading rates (t-test, p > 0.01).

The VSS content of the flocs increased and the ash content decreased as a function of time (Fig. 2). Starting at a C/N ratio of 5, the VSS content steadily increased until it remained constantly as a function of time (Fig. 2). Starting at a C/N ratio of 5, the VSS content steadily increased until it remained constantly.

### 3.3. Floc morphology

During the first 15 days of the experimental period (the period at a C/N ratio of 2.5 and the beginning of the period at a C/N ratio of 5), feeding was performed under oxygen limited conditions with DO values of 0.1–0.6 mg O₂ L⁻¹ during feeding. This was followed by an aeration phase with DO values of 7.5–8.0 mg O₂ L⁻¹. The relatively smooth surface of the inocula granules in both reactors became more filamentous after the C/N ratio was increased from 2.5 to 5 on day 12. From day 15 on, the feeding phase was performed under fully aerobic conditions. Starting on day 20, the walls of the reactors were cleaned regularly (ca. every 2 days) to remove biofilm growth. The biomass detached from the interior was left in the reactor for wash out. In the glycerol SBR, this resulted in granules morphologically similar to these on day 2 but larger in size (up to 10 mm). At the same time in the acetate SBR, mainly smaller granules (<2 mm), irregularly in shape but with a smoother exterior appeared. On day 32, the C/N ratio was increased from 5 to 10 what resulted in slightly larger granules but with the presence of more filaments on the granule surface. From day 50 on, the C/N ratio was 15 and the granules in both the glycerol SBR and the acetate SBR became floccular with long filamentous extrusions and irregular shapes.

### 4. Discussion

#### 4.1. Reactor performance for nitrogen and carbon removal

In BFT, intensive heterotrophic growth in the culture water results in turbidity and may induce variations in DO levels due to increased microbial metabolism resulting from carbon dosing. Such varying DO levels may have a negative influence on the aquaculture organisms. The implementation of external heterotrophic growth reactors in which high turbidity and large variations in DO do not influence the species in the aquaculture pond, may offer a solution. In such a concept, the water treated in the external reactor is redirected to the pond while the release of microbial protein is controlled depending on the protein requirements by the culture species. The excess in microbial protein in the reactor can be harvested and used in other aquaculture units.

In this study, TAN assimilating microbial biomass with the quality to be reused as additional food source was grown in such SBRs. An influent TAN concentration of 13–14 mg L⁻¹ represents the daily nitrogen waste produced in an aquaculture system at a fish/shrimp culture density of 22–23 kg m⁻³ (De Schryver et al., 2008). In RAS, this nitrogen is converted into nitrate that tends to accumulate in the water (Gutierrez-Wing and Malone, 2006). In the past, complete removal could only be obtained by further denitrification into nitrogen gas or discharge into surrounding water bodies (van Rijn et al., 2006). In BFT, the goal is to assimilate the nitrogen into microbial biomass rather than nitrifying it. In the alternative SBR approach, the development of nitrifiers in the reactors could be avoided. This was demonstrated when the SBR biomass was spiked in batch nitrification tests with ammonia (data not shown). Neither removal of TAN nor production of nitrite and nitrate could be observed in the absence of an additional carbon source.
source. This suggests that the main driving force for the TAN removal within the reactors indeed was heterotrophic microbial growth. This can be related to the short SBR cycles, since heterotrophs have a growth rate and microbial biomass yield per unit substrate that is a factor 10 higher than that of nitrifying bacteria (Hargreaves, 2006). Due to the dependence of carbon, nitrogen removal efficiencies were limited at low doses of glycerol and acetate. Under these conditions, carbon was the limiting nutrient in heterotrophic nitrogen assimilation resulting in a steady and high efficiency for carbon removal. Resulting from an increase in carbon loading rate, the nitrogen removal increased along until at a C/N ratio of 15 the highest nitrogen removal efficiencies were observed (up to 98%). These values are slightly higher than the nitrogen removal efficiencies of 85–90% as observed in other researches (Schneider et al., 2006a,b, 2007). However, the number of studies focusing on the treatment of aquaculture effluents via heterotrophic nitrogen assimilation is limited. The use of established techniques like nitrification/denitrification can result in similar removal efficiencies up to 96% as was shown for example by Sharrrer et al. (2007) and Visvanathan et al. (2008). At the C/N ratio of 15, the carbon removal efficiency decreased suggesting an over-supply of carbon. The optimal C/N ratio for nitrogen removal is thus situated between 10 and 15, which lies close to the value of 10 suggested by Avnimelech (1999) for microbial nitrogen assimilation in BFT. Hargreaves (2006) also suggested providing a substrate with a C/N ratio of 10 to promote nitrogen limitation for bacterial growth to increase nitrogen removal efficiency. In case all ammonium is converted into nitrate, the nitrogen can also be removed by denitrification. Stoichiometrically, a C/N ratio of 1:1:1 is necessary for denitrification based on acetate (Reyes-Avila et al., 2004). The costs for carbon supplementation to obtain denitrification can thus be about 10 times less than that for microbial assimilation. However, the capital and operational costs for the nitrification/denitrification systems together with the lower feeding costs due to microbial protein production make the nitrogen assimilation a competitive technique.

4.2. Floc composition

The nutritional composition of the bio-flocs is of high importance to represent a valuable additional food source. One of the main parameters that determines the suitability as feed is the protein content. In normally regulated feeds, the protein content averages 18–50% based on DMW (Craig and Helfrich, 2002). When these values are compared with the protein content of the biomasses in this assay, it can be noted that these lay within the upper part of this range (Table 3). The large standard deviations for the effluent values at a C/N ratio of 5 and 10 can be ascribed to the relatively small biomass amounts in the grab samples of the effluents. This resulted in larger diurnal and weekly variations of the calculated protein content within the periods 11–32 days and 32–50 days. At the highest C/N ratio, a protein content of 50% on DMW was surpassed in 80% and 75% of the measurements for the glycerol SBR and the acetate SBR, respectively. The ash content was higher compared to the optimal value of less than 8% in fish feed (Craig and Helfrich, 2002) (Fig. 2). For acetate as carbon source (i.e., ash content of 40%), results were in accordance with previous experiments performed at our lab (Crab et al., unpublished data). The ash content of the glycerol flocs averaged a value of 10%. Microbial productivity could be estimated based on the amount of carbon that is metabolized during one operation cycle, of which 40–60% accounts for new bacterial cell production (Avnimelech, 1999). This yielded maximum production values of 0.62–0.94 g C L⁻¹ day⁻¹ for the glycerol SBR and 0.54–0.82 g C L⁻¹ day⁻¹ for the acetate SBR. Based on these values, the potential of the bio-flocs as additional feed in a fish culture unit can be calculated. For the glycerol SBR, the average maximum microbial production of 0.78 g C L⁻¹ reactor day⁻¹ corresponds to a production of 2.3 g C day⁻¹. Since half of the bacterial cell dry weight is carbon, this corresponds to a production of about 4.6 g CDW day⁻¹ (Verstraete and van Vaerenbergh, 1986). The maximum microbial production occurred at a C/N ratio of 15 at which the protein content of the flocs was on average 61%. This results in a production of ca. 2.8 g microbial protein day⁻¹. Under the operation conditions of this research, a major part (2.5/3) of the flocs can theoretically be returned to the fish pond. Assuming that similar to conventional feed only 25% of the protein from the flocs ends up in fish biomass, the microbial protein contribution related to the biomass from the acetate SBR was calculated to be 0.4 g protein day⁻¹. This corresponds to a contribution of up to 7% on the daily protein requirement for the culture of 1 kg fish live weight.

Poly-β-hydroxybutyrate (PHB) is an intracellular biodegradable polymer produced by a wide variety of microorganisms and is involved in bacterial carbon and energy storage (Defoirdt et al., 2007). It may also be a valuable parameter to be considered in assessing bio-floc quality since it has shown its ability to act as a preventive or curative protector of Artemia franciscana against Vibrio infections (Defoirdt et al., 2007). In this study, the PHB accumulation in the biomass (w/w) in the glycerol SBR was 0.9% at a C/N ratio of 10 and 6.0% at a C/N ratio of 15. In case of acetate, the PHB accumulation in the biomass was higher with average values of 13% at a C/N ratio of 10 and 16% at a C/N ratio of 15. These higher values may be related to the fact that acetate is the prime precursor of PHB (Salehizadeh and Van Loosdrecht, 2004). The PHB content values in this report suggest that by this approach a substantial supply of PHB to the aquaculture organisms can be provided. Based on calculations by De Schryver et al. (2008), the bio-floc production can be estimated for fish culture applied with feed containing 30% protein and at a feed conversion ratio of 2.2 kg feed dosed kg⁻¹ fish live weight produced. In this case, about 0.19 kg bio-floc C can be grown on the waste products of each kg fish produced. This corresponds with a bio-floc DMW of about 0.4 kg (carbon on dry matter for bacterial biomass is ca. 50%) of which 25% is assumed to be eaten. At a PHB content of 15%, this corresponds to about 15 g of PHB, which is supplied to each kg of fish. By the application of BFT, the total feed supply is decreased to 1.7 kg kg⁻¹ fish produced (feed plus bio-flocs), which yields a PHB content of about 1% on the total fish feed supply. This is about six times higher than the amount of butyric acid which showed to induce a significant decrease of Salmonella enteritidis colonization in young chickens (Van Immerseel et al., 2004). More research is warranted on how the PHB content of the bio-flocs within the reactors can be increased, e.g., by inoculation with specific PHB producing strains, by variation in DO level or by changes in feast/famine regime.

4.3. Floc morphology

The increased occurrence of granule filaments was observed during shifts in the C/N ratio from 2.5 to 5, 5 to 10 and 10 to 15. Similar differences in the morphological structure of the two biomass types were observed by Tay et al. (2001) when they compared glucose and acetate as feeding carbon source. McSwain et al. (2005) summarized that in addition to carbon type, the DO
concentrations, shear force, settling time and even feast/famine regimes influence granule formation and morphology. During this study, more filamentous growth at higher substrate loading rates (a less pronounced feast/famine regime) was observed, which is consistent with the observations made by the latter authors. McSwaín et al. (2005) stated that floc-forming bacteria had a selective advantage (regarding to substrate-uptake kinetics and starvation sensitivity) over filamentous organisms at short shear relative to long famine periods (1:4). However, the degree of granulation may also be influenced by the lower DO concentrations at the beginning of each cycle. Filamentous organisms have increased oxygen affinity and are therefore able to outcompete their floc forming counterparts in periods of oxygen limitation. Martins et al. (2003) reported increased amounts of filamentous bacteria in flocs compared to floc formers at DO levels less than or equal to 1.1 mg O₂ L⁻¹. Increased aeration would not only increase DO levels but also the shear force applied to the flocs (Tay et al., 2001). At a C/N ratio of 5, the filamentous growth could be remedied by converting the oxygen limited conditions into fully aerobic conditions. However, since the power input in this lab-scale research is high (2.7 W L⁻¹), a more optimal reactor design is necessary for application in practice. By increasing the height/diameter ratio, DO levels or shear forces can be maintained by imposing lower power input values. The morphology effect was most striking when the highest carbon loading was imposed (5.42 g COD L⁻¹ day⁻¹ for the glycerol SBR and 3.77 g COD L⁻¹ day⁻¹ for the acetate SBR). The granules lost their compactness and loose floccular structures became abundant in the reactors. Li et al. (2006) made similar observations in their SBR at high carbon loading rate (4.2 g COD L⁻¹ day⁻¹) and long aerobic feeding phases (30 min). Overall, higher carbon loading rates and the resulting lower DO levels appear to support the flocs rather than the granular biomass.

5. Conclusions

In this study, the lab-scale SBRs envisaged as external growth reactors for bio-flocs technology aquaculture ponds were able to provide adequate ammonia removal. Significant nitrogen assimilation was exhibited by the heterotrophic microbial biomass present using both glycerol and acetate as carbon source. This could be accomplished without leaving excessive amounts of carbon in the effluent, provided that an optimal C/N ratio ranging between 10 and 15 was maintained. Moreover, the quality of the bio-flocs in terms of protein content was similar to fish feed while the accumulation of PHB can represent a major added value.

Acknowledgements

This work was funded by the “Fonds voor Wetenschappelijk Onderzoek” in Flanders on the project “Probiot-induced functional responses in aquatic organisms”. The authors would also like to thank ir. Bart De Gussem, Dr. ir. Tuba Hande Ergüder and ir. Siegfried Vlaeminck for the critical reading of the manuscript and helpful suggestions.

References
