



Microbial floc meal as a replacement ingredient for fish meal and soybean protein in shrimp feed

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ABSTRACT

Microbial flocs produced in suspended growth bioreactors could offer the shrimp industry a novel alternative feed. In this study, microbial flocs were produced in sequencing batch reactors (SBRs) using tilapia effluent and sugar as a growth media. It was determined that 1 kg of microbial floc could be produced per 1.49 kg of sucrose. These microbial flocs were tested as an ingredient for shrimp feed over a 35 day feeding trial. Two control diets (absent of microbial flocs) were compared against three dietary treatments (microbial floc inclusion). Control 1 and microbial floc diets (diets 1–3) were formulated to be equivalent for levels of crude protein, total fat, crude fiber, calcium, magnesium, phosphorus, potassium, and sodium. Controls 1 and 2 did not contain microbial flocs and differed slightly from each other in soybean oil, krill meal, and mineral/salt levels. For diet 1 (microbial floc 7.8%) and diet 2 (microbial floc 15.6%), soybean protein isolate on a protein basis was replaced with microbial flocs at a 7.8 and 15.6% inclusion level on a dry matter basis. For diet 3, fishmeal was replaced with microbial flocs at 7.8% and fish oil at 0.50% (microbial floc 7.8% + fish oil). Four juvenile *Litopenaeus vannamei* were stocked per tank and each dietary treatment was tested in 12 replicates over a 35 day feeding trial. No differences were observed between final survival rates (93 to 100%) between any of the dietary treatments. Growth (weight gain per week) for control 1, control 2, diet 1, diet 2, and diet 3 were respectively 1.09 ± 0.14 , 0.88 ± 0.14 , 1.64 ± 0.03 , 1.61 ± 0.03 , 1.63 ± 0.04 g/week. The total gain in weight for the three diets containing microbial floc of 8.07 to 8.18 g in five weeks with an initial weight of 0.44 ± 0.005 g is truly exceptional. Tukey's HSD (Honestly Significant Differences) test revealed that each of the three microbial floc diets significantly ($P < 0.01$) outperformed each control in terms of weight gain per week with no differences in survival.

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1. Introduction

Traditional Penaeid shrimp culture has a long proven history using pond culture in tropical climates. The global shrimp market has expanded from less than \$1 billion to \$5.8 billion (US) from 2000 to 2005 (FAO, 2008). To meet this growing demand, the shrimp industry is shifting from extensive rearing systems to more intensive rearing systems. Presently, shrimp production in earthen ponds using cutting edge technology in tropical zones can produce two to four crops per year while only one crop per year can be produced per year in a temperate zone. To combat limitations in temperate areas, indoor recirculating aquaculture systems could be implemented to simulate a tropical environment. Therefore, more than one crop could be produced per year. Moreover, there are numerous drawbacks and concerns regarding outdoor intensive systems. Drawbacks include stressed animals, increased disease, increased oxygen demands, and decreased water quality. Generally, these risks can be reduced, while maintaining a

high density of animals, when a controlled indoor environment is used (e.g. recirculating aquaculture systems). Recirculating aquaculture systems use numerous technologies to clean water for reuse within the culture system (Timmons et al., 2002) or even from one animal to another (Kuhn et al., 2007). Recirculating systems often include the following technologies; nitrification (e.g. fluidized sand filters), oxygenation (e.g. sparge cone), disinfection (e.g. ultraviolet sterilization), and solids removal (e.g. drum filters) (Skjølstrup et al., 2000; Menasveta, 2002; Timmons et al., 2002).

Clear water recirculating systems have numerous benefits over outdoor intensive pond systems, especially in temperate climates. However, implementation of recirculating aquaculture systems has not yet translated into a solution for intensive shrimp culture. This is because numerous studies have demonstrated that shrimp reared in clear water using initial stocking densities above 300/m³ have a significantly lower growth rate than those reared in dirty water using much lower initial stocking densities. This becomes increasingly evident when shrimp tested in clear water are compared directly to shrimp in systems with a high productivity of natural organisms (e.g. algae, bacteria, and other natural biota) (Tacon et al., 2002; Izquierdo

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et al., 2006; Moss et al., 2006). McLean et al. (2006) reported that shrimp can grow equally as well when fed a yeast based diet compared to a fishmeal diet. These naturally occurring organisms may contribute nutritionally and serve as a pre-/probiotic and/or unknown growth promoter. For these reasons, it was hypothesized that production of microbial flocs in sequencing batch reactors (SBRs) using effluent from a tilapia production facility and a carbon source (e.g. sugar) could produce a viable alternative ingredient for shrimp feed.

Biological treatment of aquaculture effluent using suspended growth processes has been demonstrated with (Schneider et al., 2006; Schneider et al., 2007) and without (Sharrer et al., 2007) carbon supplementation. Carbon supplementation is necessary if nitrogen removal is not efficient. Microbial flocs produced in biological reactors would be different from naturally occurring organisms found in pond systems because they would be produced externally from shrimp while treating a fish effluent in dark reactors. Microbial flocs produced in SBRs could be dried and incorporated into a pelleted feed for shrimp. If this alternative feed proved to be successful, it could offer the shrimp industry a new culture option in clear water recirculating aquaculture systems. A very significant further justification is the need to have alternative lower cost ingredients replacing marine animal meals and traditional plant meals. For these reasons, this study investigated if it would be possible to produce microbial floc in SBRs as a potential ingredient for replacing fish meal and soybean meal in shrimp feed.

2. Materials and methods

2.1. Experimental design

Microbial flocs generated in SBRs by Virginia Tech researchers at a shrimp pilot facility (Virginia Shrimp Farms, Martinsville, Virginia, US) were used as a test ingredient in shrimp feed, over a 35 day feeding trial. Two control diets (absence of microbial flocs) were compared against three dietary treatments (microbial floc inclusion). Each dietary control/treatment consisted of 12 replicates (each tank was a replicate) over six systems in a randomized block design. Each 20 L tank was stocked with four juvenile shrimp. This feeding trial was conducted indoors at the Texas A&M AgriLife Research Mariculture Laboratory (Port Aransas, Texas, US) using indoor recirculating systems with seawater renewal. This is essentially a clean or clear water system with no natural productivity present.

2.2. Sequencing batch reactors used to produce microbial flocs

Wastewater was diverted from a tilapia farm where recirculating aquaculture systems are used. This effluent was pumped to an aerated well-mixed equalization tank (11,300 L). Wastewater in the equalization tank was monitored for five different days over a one week period to determine water quality in terms of nitrate (95.7 ± 10.9 mg/L), nitrite (0.56 ± 0.11 mg/L), pH (7.09 ± 0.02), total ammonia nitrogen (TAN, 10.8 ± 2.7 mg/L), and soluble total organic carbon (TOC, 20.8 ± 3.1 mg/L). Effluent was manually drained into two commercially available, 5100 L SBRs (Model CA-15d, Cromaglass Corp., Williamsport, Pennsylvania, US). Atmospheric air and water pumps were used to aerate and agitate the tilapia effluent with microorganisms (microbial flocs) which reduce dissolved organic matter and assimilate and/or oxidize ammonia and nitrite. Carbon (Granulated white sugar, i.e. sucrose, Kroger Co., Cincinnati, Ohio, US) was supplemented at a target rate of 80 mg carbon/L every 24 h. The SBRs were operated inside a building and had manhole covers to prevent light penetration. Well-mixed aerobic batch tests were performed with and without carbon supplementation at 19 °C over a six hour period. These batch tests were repeated to determine consistency.

Total ammonia nitrogen was determined spectrophotometrically using methods approved by the US Environmental Protection Agency

for the analysis of wastewater (HACH Co., Loveland, Colorado, US). Soluble chemical oxygen demand (COD), soluble TOC, and volatile suspended solids (VSS) were measured in accordance with APHA (2005). Microbial floc levels were measured as VSS (Metcalf and Eddy, 2003).

2.3. Microbial flocs as an ingredient for shrimp feed

Nutritional composition reported for microbial flocs (Table 1) was analyzed by A&L Eastern Laboratories, Inc. (Richmond, Virginia, US). Two independent sampling events (14 days between events) were performed to determine microbial floc nutritional consistency. Microbial flocs were harvested as a dietary ingredient for the shrimp feeding trial during the first sampling event. Settled microbial flocs were harvested from SBRs by siphoning and were air dried in 5 cm layers to solids levels greater than 86%. Microbial flocs were subsequently ground into fine material using a stand mixer with grain mill attachment (KitchenAid® Professional 600 Series, Saint Joseph, Michigan, US).

2.4. Shrimp

Postlarvae of less than 1 mg/shrimp were obtained from Oceanic Institute (OI) and were free of pathogens listed by the US Marine Shrimp Farming Program (USMSFP, 2006), including taura syndrome virus (TSV), white spot syndrome virus (WSSV), yellow head virus (YHV), infectious hypodermal and hematopoietic necrosis virus (IHHNV), and infectious myonecrosis virus (IMNV). These shrimp were from OI's "Kona" line which is a reference strain of shrimp that originated from Sinaloa, Mexico. This shrimp line has been domesticated for over 15 years and is used as a positive control in disease-challenge studies because of its consistent performance over time (Hennig et al., 2004). These 20 day old postlarvae were reared in a recirculating aquaculture system with a high sea water exchange rate of 17.5%/day. Water quality parameters were ideal. Dissolved oxygen, salinity, and temperature averaged 6.01 mg/L, 31.7 ppt, and 28.1 °C, respectively. For the first week, postlarvae shrimp were fed artemia and crumbled commercial feed (Rangen 45/10, Buhl, Idaho, US). For the remaining 16 days, prior to experiment initiation, shrimp were fed the Rangen 45/10 diet only until the average shrimp weighed 435 mg.

2.5. Experimental systems for shrimp

Six systems using recirculating technology were used to culture shrimp. Each system comprised of two rows (front and back) with 12

Table 1

Composition of microbial flocs on dry matter basis, mean values with standard errors, as determined by laboratory analysis ($n = 2$).

Parameter	Microbial flocs
	[g/100 g]
Crude protein	49.0 ± 1.5
Carbohydrate ^a	36.4 ± 0.9
Total ash	13.4 ± 0.6
Crude fat	1.13 ± 0.09
Crude fiber	12.6 ± 0.1
Calcium	1.28 ± 0.07
Phosphorus	1.29 ± 0.08
Sodium	1.27 ± 0.03
Potassium	0.75 ± 0.13
Magnesium	0.41 ± 0.05
	[mg/kg]
Zinc	181 ± 1
Copper	92.5 ± 3.0
Manganese	35.0 ± 0.5

^a Calculated value (Merrill and Watt, 1973): carbohydrate = 100 – (ash + crude protein + moisture + total fat).

tanks per row (24 tanks per system). Five out of the 12 tanks in a row were dedicated to this study. Each opaque tank had a cover and an air diffuser and was 20 L with a bottom area of 0.09 m². Each system included 100 µm filtration, aerated nitrification sump, and ultraviolet sterilization. Each tank had a recirculation rate of 5400%/day, and seawater renewal rate of 85.7%/day. Seawater was pumped from the Corpus Christi Ship Channel about 1 km from the Gulf of Mexico. This seawater was pumped through a series of pressurized sand filters (50, 25, and 1 µm respectively) and was stored in three 53,000 L opaque fiberglass reservoirs prior to use.

2.6. Water quality in experimental systems used for shrimp

Each experimental system for shrimp was monitored daily for dissolved oxygen (DO), salinity, and temperature using a YSI 85 meter (Yellow Springs, Ohio, US). Nitrate, nitrite, pH, and (TAN) were measured weekly using methods designed for seawater samples (Spotte, 1979). More detail for nitrate, nitrite, and TAN procedures can be found in Strickland and Parsons (1972), Mullin and Riley (1955), and Solorzano (1969), respectively.

2.7. Diets and feeding regime for shrimp

Control 1 and microbial floc diets (diets 1–3) were formulated (Table 2) to be equivalent for levels of crude protein, total fat, crude fiber, calcium, magnesium, phosphorus, potassium, and sodium. Controls 1

and 2 did not contain microbial flocs and differed slightly from each other in soybean oil, krill meal, and mineral/salt levels. Inclusion of microbial flocs was used in diets 1, 2, and 3. For diet 1 (microbial floc 7.8%) and diet 2 (microbial floc 15.6%), soybean protein isolate was replaced on a protein basis with microbial flocs at a 7.8 and 15.6% inclusion level. The 7.8% and 15.6% microbial floc inclusion levels, respectively, replaced approximately 50 and 100% of the soybean protein isolate as fed. For diet 3 (microbial floc 7.8% + FO), fishmeal was replaced on a protein basis with microbial flocs at 7.8% and fish oil at 0.50%. In other words, 37% of the fishmeal was replaced with microbial floc, as fed. The two control diets were semi-purified feeds which allowed not only the crude protein but also crude fat, marine fat, total ash, crude fiber, Ca, P, Mg, Na and K to be very similar for both the control and experimental diets. The following adjustments were made for the experimental diets; (1) crude fat, polyunsaturated fatty acids (PUFA), and highly unsaturated fatty acids (HUFA) were kept constant by adjusting levels of refined menhaden fishmeal and pure soybean oils, (2) total ash by adjusting the levels of acid washed diatomaceous earth, (3) crude fiber by adjusting reagent grade cellulose, (4) Ca, P, Mg, Na, and K by adjusting reagent grade CaCO₃, CaHPO₄, MgO, NaCl, and KCl, and (5) vitamins, Zn, Cu and Mn were essentially constant and not limiting since the same level of a vitamin–mineral premix was added to all diets. Diets were extruded at 1.98 mm diameter and were crumbled into irregular shaped spheres using a food processor and were graded using sieves to be between 1 and 2 mm in diameter.

Measured nutrient levels (A&L Eastern Laboratories, Inc., Richmond, Virginia, US) for each formulated diet are reported in Table 3. Shrimp were fed 15 times daily using modified automatic feeders (Lifeguard Automatic Fish Feeder, Pentair Aquatics®, El Monte, California, US) at a rate based on an excess food conversion ratio (FCR) of 2 assuming a weight gain of 1 g/week. Uneaten feed was removed daily by siphoning.

2.8. Shrimp performance indicators

Group weights of all shrimp were recorded on a per tank basis at study initiation. Shrimp were stocked at a density of 4 shrimp per tank (57 shrimp/m², or 0.20 shrimp/L). Survival rates were recorded daily and any moribund/dead shrimp were removed immediately from the study. At the termination of the experiment, final group weights of remaining shrimp were made on a per tank basis. Survival, weight gain, weight gain per week, and specific growth rates (SGRs) were used to assess dietary effects on shrimp performance. The following equation was used to determine SGRs values (Ricker, 1975).

$$SGR\left(\frac{1}{d}\right) = \frac{100 * [\ln \text{shrimp final mass (g)} - \ln \text{shrimp initial mass (g)}]}{\text{time (d)}}$$

2.9. Analysis of data

Statistical analysis was performed using SAS v9.1.3 for Windows (Cary, North Carolina, US). Differences in water quality were considered significant when $P < 0.05$. Analysis of variance (ANOVA) was used on shrimp performance data to look at effects of diet (5 levels). Additionally, weight gain data ($n = 12$, 12 tanks per treatment) was subjected to ANOVA with two blocking factors (system at 6 levels, row at 2 levels) to see if there were any effects of rows or systems. When appropriate, a Tukey's HSD (Honestly Significant Differences) post-hoc test was employed to check for differences between means. The 5% significance level was used for all tests. With the exception to initial weight and survival, the following transformation adapted from Manly (1971), was used to equalize the variance of all shrimp performance data.

Transformation = $\sqrt{e^x}$, where x is shrimp performance values

Table 2
Percent ingredient composition of diets used in feeding trial.

Ingredients	Treatment diets (g/100g dry matter)				
	Control 1	Control 2	Diet 1 Microbial floc 7.8%	Diet 2 Microbial floc 15.6%	Diet 3 Microbial FLOC 7.8% + FO
Microbial floc ^a	0	0	7.8	15.7	7.8
Wheat starch ^b	29.6	29.0	27.6	25.3	27.9
Fish meal ^c	15.0	15.0	15.0	15.0	9.4
Squid meal ^d	15.0	15.0	15.0	15.0	15.0
Krill meal ^d	10.0	10.5	10.0	10.0	10.0
Soybean protein isolate ^d	7.9	7.9	4.0	0.0	7.9
Lecithin ^e	4.0	4.2 ^f	4.0	4.0	4.0
Dicalcium phosphate ^b	3.0	6.6	2.8	2.8	3.6
Calcium carbonate ^g	2.0	1.5	2.0	1.8	2.1
Alginate ^h	2.0	2.0	2.0	2.0	2.0
Potassium chloride ^d	2.0	1.9	1.9	1.8	2.0
Cellulose ⁱ	2.5	2.0	1.6	0.80	1.6
Diatomaceous earth ⁱ	2.0	1.0	1.6	1.2	1.5
Magnesium oxide ^g	1.5	1.7	1.5	1.5	1.5
Chromic oxide ^j	1.0	0.0	1.0	1.0	1.0
Sodium hexametaphosphate ^j	1.0	1.0	1.0	1.0	1.0
Sodium chloride ^b	0.5	0	0.40	0.30	0.40
Mineral/vitamin premix ^d	0.46	0.50	0.36	0.36	0.36
Soybean oil ^k	0.30	0	0.20	0.20	0.20
Cholesterol ^b	0.20	0.20	0.20	0.20	0.20
Vitamin C (stay C) ^d	0.04	0.05	0.04	0.04	0.04
Fish oil ^c	0	0	0	0	0.50

^a Produced in SBRs as part of this study.

^b MP Biomedicals, Solon, Ohio, US.

^c Omega Protein, Houston, Texas, US.

^d Zeigler Bros., Inc., Gardners, Pennsylvania, US.

^e Cargill, Hamburg, Germany.

^f Solae Co., Saint Louis, Missouri, US.

^g VWR International, West Chester, Pennsylvania, US.

^h ChemPoint, Bellevue, Washington, US.

ⁱ Sigma-Aldrich, Saint Louis, Missouri, US.

^j Fisher Scientific, Pittsburg, Pennsylvania, US.

^k Federated Group Inc., Arlington Heights, Illinois.

Table 3
Composition of diets on a dry matter basis (g/100g).

Parameter	Treatment diets				
	Control 1	Control 2	Diet 1 Microbial floc 7.8%	Diet 2 Microbial floc 15.6%	Diet 3 Microbial floc 7.8% + FO
Crude protein	38.4 ± 1.6	41.9 ± 0.3	41.8 ± 0.1	41.2 ± 1.3	42.7 ± 0.1
Carbohydrate ^a	28.6 ± 1.6	21.9 ± 1.0	24.7 ± 0.6	22.2 ± 0.7	21.9 ± 2.0
Total ash	19.7 ± 0.1	19.7 ± 0.1	19.9 ± 0.1	21.6 ± 0.3	19.7 ± 0.1
Crude fat	5.70 ± 0.12	7.09 ± 1.42	5.88 ± 0.65	7.54 ± 0.38	8.19 ± 2.11
Crude fiber	1.87 ± 0.21	1.43 ± 0.16	1.31 ± 0.94	1.79 ± 0.29	1.60 ± 0.05
Calcium	2.82 ± 0.08	3.42 ± 0.08	2.94 ± 0.09	3.19 ± 0.01	3.16 ± 0.07
Phosphorus	1.98 ± 0.04	2.62 ± 0.06	2.04 ± 0.12	2.34 ± 0.02	2.22 ± 0.09
Potassium	1.34 ± 0.04	1.28 ± 0.02	1.38 ± 0.03	1.32 ± 0.01	1.33 ± 0.01
Magnesium	1.01 ± 0.02	1.16 ± 0.02	1.06 ± 0.01	1.23 ± 0.02	1.02 ± 0.00
Sodium	1.03 ± 0.03	0.88 ± 0.04	1.09 ± 0.02	1.06 ± 0.01	1.06 ± 0.01
Energy [kcal/100g dry matter]	420.7 ± 1	426 ± 2	426 ± 1	434 ± 2	430 ± 1

Values determined from laboratory analysis (mean ± standard errors) and calculated from ingredient inputs.

^a Calculated value (Merrill and Watt, 1973): carbohydrate = 100 – (ash + crude protein + moisture + total fat).

3. Results

3.1. Microbial floc generation in SBRs

Carbon supplementation enhanced removal rates of TAN (Fig. 1) and microbial floc generation (Fig. 2) in SBR batch tests used to treat tilapia effluent. Without carbon supplementation, TAN treatment averaged <1.0%/h. Carbon supplementation increased the observed mean rate to 26.0%/h. Generation of microbial flocs without carbon supplementation averaged <1.0 (mg VSS)/(L h) while carbon supplementation increased the observed mean rate to 95.1 (mg VSS)/(L h). This sugar source is 42% carbon based on soluble COD and TOC correlations. The following data represent calculated kinetic coefficients when sugar was used as a carbon supplement. Uptake of soluble COD as microbial flocs were generated can be observed in Fig. 3. The average uptake rate of soluble TOC was 0.14 (g carbon)/(g VSS h) or 0.32 (g sugar)/(g VSS h) for sugar. Meanwhile, the observed mean specific growth rate was 0.22 (g VSS)/(g VSS h) or 0.221/h. This unit is typically denoted as inverse time (Rittmann and McCarty, 2001; Metcalf and Eddy, 2003), for example, inverse hour (1/h). The observed yield coefficient was calculated to be 1.6 (g VSS)/(g carbon) or 0.67 (g VSS)/(g sucrose).

3.2. Diets and water quality

Composition of diets can be observed in Table 3. One-way ANOVA did not reveal any differences between means for proximate values

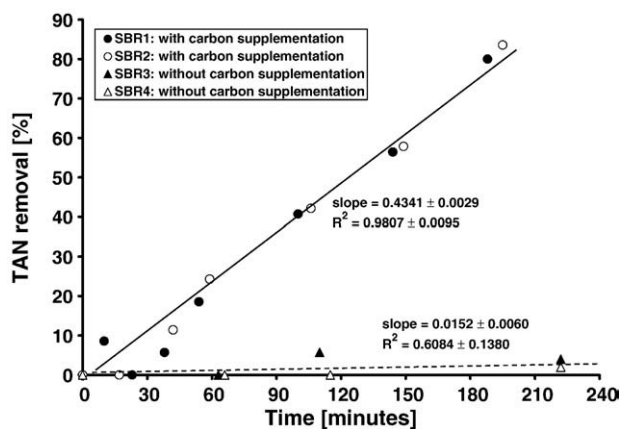


Fig. 1. Removal of TAN observed during batch treatment of aquaculture wastewater using microbial flocs in SBRs, with and without carbon supplementation.

between diets. More specifically, no differences were observed between diets for determined crude protein ($P=0.1244$), total fat ($P=0.5646$), and fiber ($P=0.8822$), and calculated carbohydrate ($P=0.0528$).

Table 4 summarizes water quality measured during the 35 day feeding trial for each of the six experimental systems. No differences were observed between any water quality parameters, thereby confirming system uniformity. Water quality levels were within safe levels for normal shrimp health, growth, and survival.

3.3. Shrimp performance

As presented in Table 5, no differences were observed using ANOVA between initial weights of shrimp between 429 and 444 mg ($P=0.9032$) or final survival rates (93% to 100%) ($P=0.2079$). Tukey's HSD revealed that that all three microbial floc diets independently and significantly ($P<0.01$) outperformed either control in terms of weight gain, weight gain per week, and SGRs (Table 5). The over 1800% change in weight obtained with all three experimental diets as compared to the approximately 1100% change in rate for the control diets is very impressive. Also, microbial floc diets enhanced shrimp growth by an average of 65.1% over mean growth of control diets. More specifically, shrimp fed with microbial flocs on average grew, respectively, 49.2 and 84.8% faster than controls 1 and 2, respectively. No differences ($P=0.4904$) in weight gain were detected between

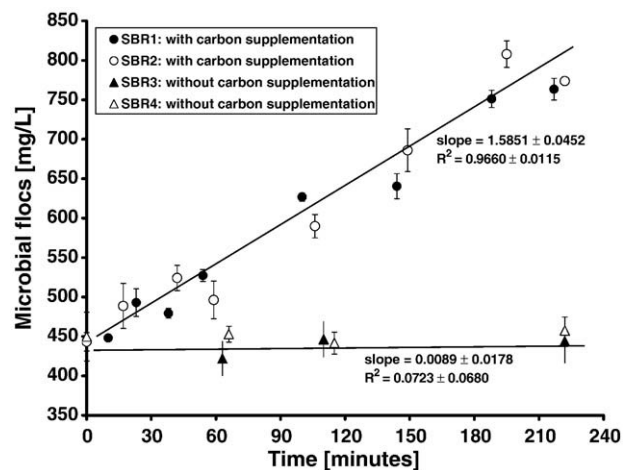


Fig. 2. Microbial floc generation observed during batch treatment of aquaculture wastewater in SBRs, with and without carbon supplementation.

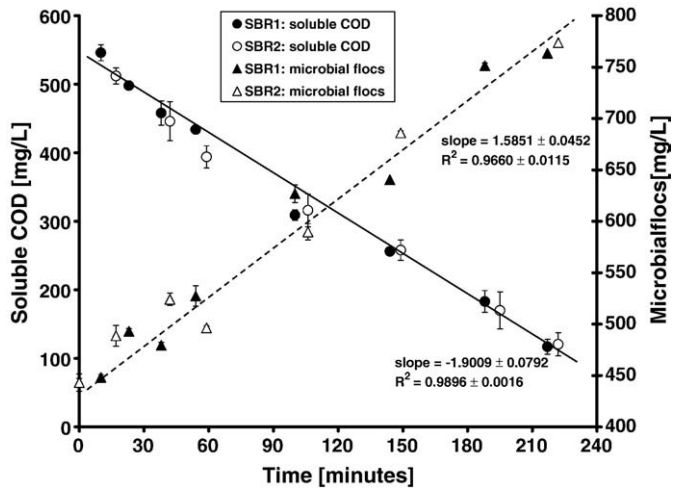


Fig. 3. Carbon supplementation effects on generation of microbial flocs with simultaneous removal of soluble COD (mean values with standard error bars).

front and back rows within the systems. Even though systems had an effect on weight gain ($P = 0.0365$), no pair wise differences were detected using Tukey's HSD.

4. Discussion

Microbial flocs were produced in SBRs using tilapia effluent and sucrose as a carbon source. Carbon supplementation selects for heterotrophic bacteria (Avnimelech, 1999; Ebeling et al., 2006) and other heterotrophic microorganisms (Metcalf and Eddy, 2003). Batch tests in this study provided evidence of heterotrophic domination. Removal of TAN without carbon supplementation was not efficient (Fig. 1) and was 26 times slower than batches that were supplemented with carbon. Similar benefits of carbon supplementation were observed for microbial floc production, without carbon supplementation, no generation of microbial flocs was observed over a 240 minute period (Fig. 2).

The calculated yield coefficient $0.67 \text{ (g VSS)/(g sucrose)}$ represents the amount of microbial floc generated per unit sugar consumed by the heterotrophic microorganisms. Alternatively, this could be expressed as 1 kg of microbial flocs produced per 1.5 kg of sucrose on a dry matter basis. Even though the maximum specific growth rate was not determined for this study, the observed mean specific growth rate in this study, $0.221/\text{h}$, was twice as great as maximum values observed for treating aquaculture wastewater using molasses ($0.10\text{--}0.121/\text{h}$; Schneider et al., 2006). This is probably because refined sugar (sucrose) is readily biodegradable, and molasses is more complex because it consists of long chain polysaccharides. However, specific growth rates reported in this study are similar to growth rates of yeast (*Candida utilis*) grown on sugar cane stillage ($0.20\text{--}0.271/\text{h}$; Cabib et al., 1983; Bottaro Castilla et al., 1984) or for more generally described aerobically produced heterotrophic organisms ($0.2\text{--}0.51/\text{h}$; Rittmann and McCarty, 2001; Metcalf and Eddy, 2003).

Table 4

Water quality results in the systems used to test dietary effects on shrimp performance.

System	Dissolved oxygen [mg/l] <i>n</i> = 100	Nitrate-N [mg/L] <i>n</i> = 5	Nitrite-N [mg/L] <i>n</i> = 5	pH <i>n</i> = 4	Salinity [ppt] <i>n</i> = 100	Total ammonia-N [mg/L] <i>n</i> = 5	Temperature [°C] <i>n</i> = 100
1	5.76 (5.32–6.19)	0.78 (0–2.11)	0.08 (0–0.19)	8.39 (8.26–8.52)	30.6 (29.1–32.1)	0.11 (0.01–0.22)	30.0 (29.2–30.9)
2	5.72 (5.34–6.09)	0.91 (0–2.58)	0.09 (0–0.23)	8.31 (8.12–8.50)	30.3 (28.7–32.0)	0.12 (0–0.27)	30.1 (29.7–30.5)
3	5.74 (5.24–6.25)	0.74 (0–2.06)	0.10 (0–0.25)	8.33 (8.18–8.48)	30.5 (28.9–32.1)	0.12 (0–0.26)	29.9 (29.4–30.5)
4	5.85 (5.37–6.32)	0.76 (0–2.13)	0.09 (0–0.19)	8.34 (8.21–8.47)	30.5 (28.9–32.0)	0.12 (0.01–0.24)	30.1 (29.5–30.8)
5	5.62 (5.11–6.11)	0.73 (0–2.02)	0.08 (0–0.15)	8.31 (8.14–8.47)	30.3 (28.8–31.8)	0.13 (0–0.29)	30.0 (29.5–30.6)
6	5.77 (5.35–6.19)	0.80 (0–2.28)	0.07 (0–0.15)	8.31 (8.16–8.47)	30.2 (28.7–31.7)	0.09 (0–0.21)	30.0 (29.6–30.4)

Mean values with 95% confidence intervals (*n* denotes the number of sampling events).

Table 5

Mean values (mean transformation value) of initial weights and shrimp performance indicators ($n = 12$) at the end of the 35 day feeding trial.

Diet	Initial weight [g]	Survival [%]	Weight gain [g]	Weight gain per week [g/week]	SGR [1/day]
Control 1	0.44	93	5.46 ^a (26.1)	1.09 ^a (1.77)	7.17 ^a (41.7)
Control 2	0.43	93	4.42 ^a (19.0)	0.88 ^a (1.60)	6.63 ^a (35.5)
Diet 1					
Microbial floc 7.8%	0.43	100	8.18 ^b (61.5)	1.64 ^b (2.27)	8.54 ^b (71.8)
Diet 2					
Microbial floc 15.6%	0.44	98	8.07 ^b (58.7)	1.61 ^b (2.24)	8.45 ^b (69.4)
Diet 3					
Microbial floc 7.8% + FO	0.43	100	8.13 ^b (61.5)	1.63 ^b (2.26)	8.54 ^b (72.2)
Pooled error	0.09937	9.897	19.62 ¹	0.2648 ¹	16.51 ¹
<i>P</i> > <i>F</i>	0.9032	0.2079	<0.0001	<0.0001	<0.0001

Superscript letters denote significant differences ($P < 0.01$).

¹ Reflects transformation values.

Proximate values (crude protein, crude fiber, crude fat, and total ash) were not different from each other between any of the diets (Table 3) and water quality in shrimp systems did not vary from each other (Table 4). Formulated diets and water quality values were considered optimal for shrimp culture (Davis et al., 1993; Lawrence and He, 1999; Van Wyk et al., 1999; Cuzon et al., 2004; Fox et al., 2006). This allowed direct comparisons between diets with and without microbial flocs to be made. Since the preceding nutrients are either essentially constant and/or not limiting in the control and experimental diets the differences in growth when the biofloc ingredient was included is probably not due to any of these nutrients. Survival rates did not vary between dietary treatments; however, shrimp growth was significantly improved ($P < 0.01$) for shrimp fed microbial flocs (Table 5). Even though numerous studies have reported enhanced survival, health, and growth rates of shrimp raised in ponds with high activity of algae, microbial flocs, and other natural biota (Avnimelech, 1999; Moss et al., 2000; Moss et al., 2001; Tacon et al., 2002; Burford et al., 2004; Cuzon et al., 2004; Izquierdo et al., 2006; Wasielesky et al., 2006), none of these studies used dark-produced microbial flocs. The results from this study are consistent with Kuhn et al. (2008) who reported enhanced growth of shrimp fed dark-produced microbial flocs produced from treating aquaculture wastewater (without carbon supplementation). This study differed in that shrimp were fed pelleted microbial flocs instead of being fed directly. Furthermore, the enhancement of growth was more consistent in this study compared to Kuhn et al. (2008) as was treatment of tilapia effluent with carbon supplementation.

It is not known exactly how microbial flocs enhance growth. Izquierdo et al. (2006) suggested lipid contributions of microbial flocs are important. Microbial flocs in this study were low in fats ($1.13 \pm 0.09\%$) and contributed very low lipid levels to the microbial floc diets (e.g. $<0.1\%$ of diet 1 or 3) and the crude fat and marine fat levels were very similar between control and experimental diets. Therefore, since

the contribution of lipids was limited and/or constant between the control and experimental a lipid was not likely the reason for enhanced growth. The enhanced growth was probably not due to microbial flocs having a more favorable amino acid profile and/or more digestible because all the essential amino acids were in excess. It is also speculated that microbial flocs are probiotics (Bairagi et al., 2002; Bairagi et al., 2004; Kesarcodi-Watson et al., 2008). Ultimately, more work needs to be done in order to fully understand the contributions of microbial flocs produced in SBRs and natural organisms found in ponds.

From January 2008 through May 2009, the global soymeal market varied approximately from a low mean of \$375 to a high mean of \$550/metric ton. During the same time period, fishmeal varied approximately from a low mean of \$1000 to a high mean of \$1225 (FAO, 2009). Lastly, initial estimates of cost for producing a metric ton of dry ingredient from the microbial floc is approximately \$400 to \$1000. With the significant increase in growth rate with the addition of the dry ingredient prepared from the microbial floc, the replacement fraction of either soybean and/or fish meal is feasible. Furthermore, the microbial flocs are generated from a process that cleans a waste stream, therefore, this is an added benefit for dealing with aquaculture effluents.

5. Conclusion

Numerous studies have demonstrated that shrimp are healthiest and grow best in ponds or systems that have high levels of algae, bacteria, and other natural biota. This study differed significantly because microbial flocs were generated externally from shrimp systems in dark reactors, using simple sugars and tilapia wastewater. Microbial floc inclusion significantly enhanced shrimp growth of over 1800% for the experimental diets versus about 1100% change in weight for the control diets ($P < 0.01$) over a 35 day feeding trial. Also, whatever the factor in the microbial flocs was the addition of 7.8% microbial floc was adequate to supply this unknown dietary nutrient/factor under the conditions of this experiment. Addition of dried microbial flocs into a feed is also a novel approach. Not only did this allow for a controlled study, but this technique can also be adapted by the shrimp industry. This could be especially important for inland industries that would like to implement clear water recirculating aquaculture systems. These clear water recirculating systems generally have benefits over traditional pond or flow-through systems in terms of climate control, biosecurity, conservation of water and salts, wastewater treatment, solids management, feed management, and oxygen demand. It is hoped that results from this study can be adapted by the shrimp industry as a means to increase economic and environmental sustainability. Lastly, these data indicate that replacing part of the soybean and/or fishmeal in shrimp feed will improve growth and potentially may reduce feed costs as well.

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