# THE START-UP OF SBR USING BIOFLOC TECHNOLOGY TREATING SOLID AQUACULTURE WASTE

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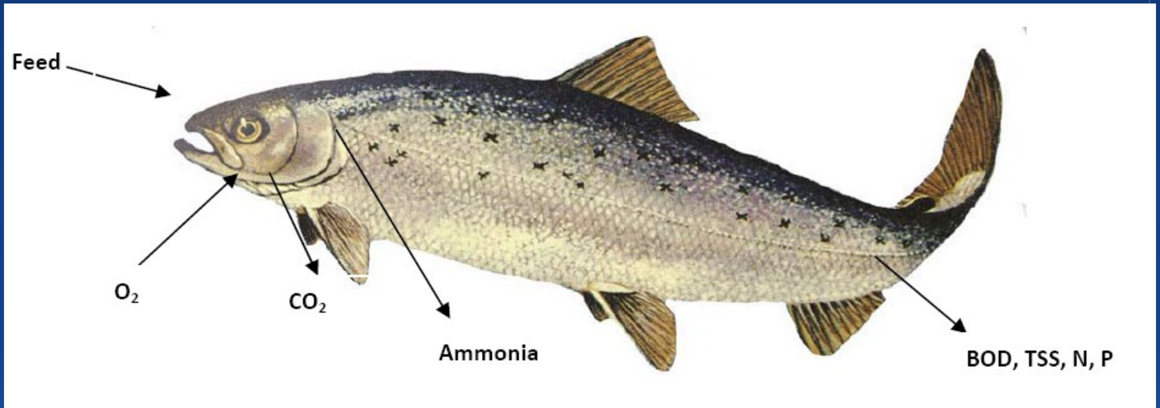
**The scope of digestion in fish is limited caused a relatively large fraction of feed remains undigested and excreted (Amirkolaie, 2005) .**




**General Mass Balance on a feeding fish (from Chen et al. 1998)**

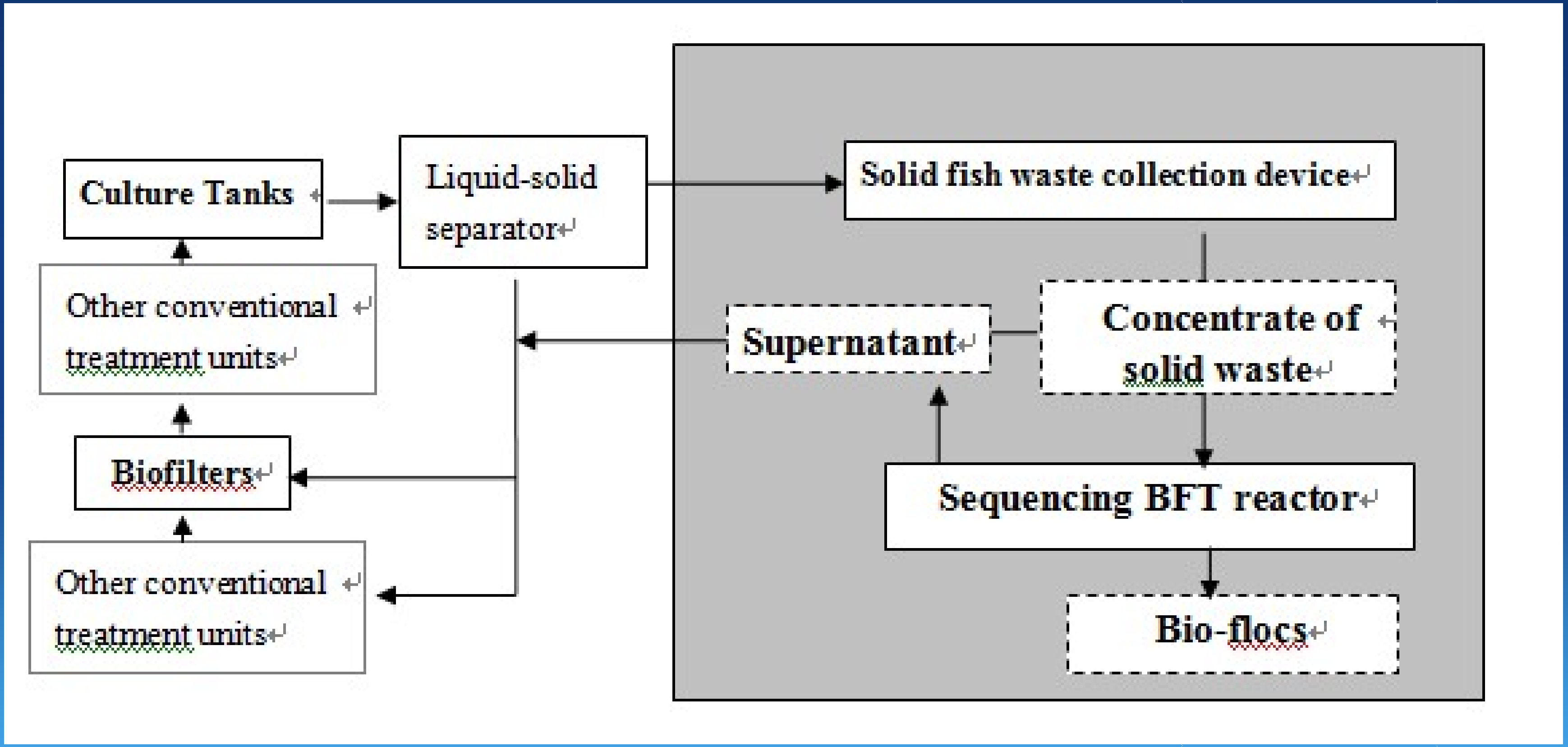
**In a properly managed recirculating aquaculture system (RAS) , 11 and 40% of applied feed has been estimated to accumulate as sludge discharged to the environment (Hopkins et al., 1994).**

*Table 2 Reported efficiencies of solids removal units in RAS*

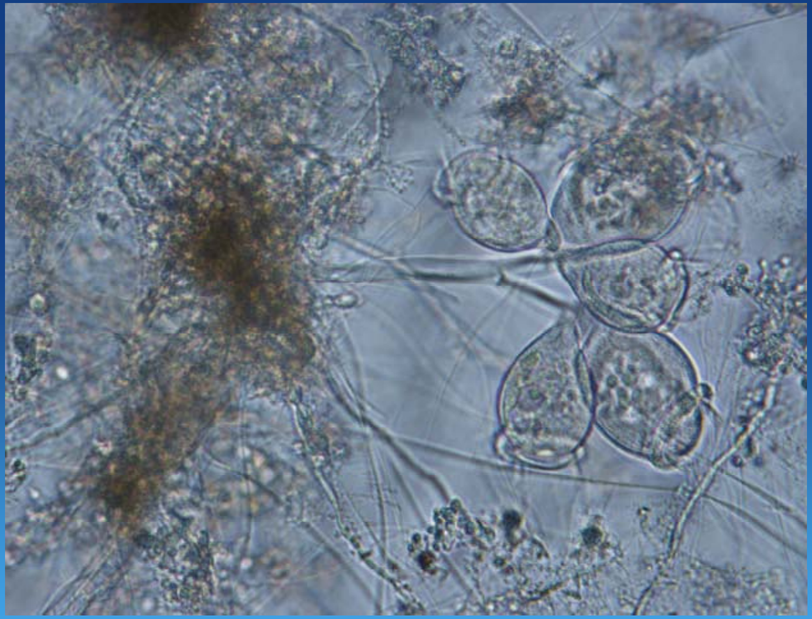
| Kind of filter | Removal efficiency | Source |
|---|---|---|
| Particle separators (Cornell dual-drain) | 92% of TSS | Timmons & Ebeling, 2007 |
| Particle separators (Eco-tramp®) | 98% of feed waste and 92% of excrements | www.aquaoptima.com |
| Swirl separator + floating plastic bead bioclarifier + fluidized sand bead | 85% of TSS (Overall) | Pfeiffer et al., 2008 |
| Swirl separators + drum filter | 88 % of TSS (63 and 22%, respectively) | Couturier et al, 2009 |
| Microscreens (25-100 μm) | 71-77% | (Cripps & Bergheim, 2000; Kelly et al., 1997; Cripps, 1995) |

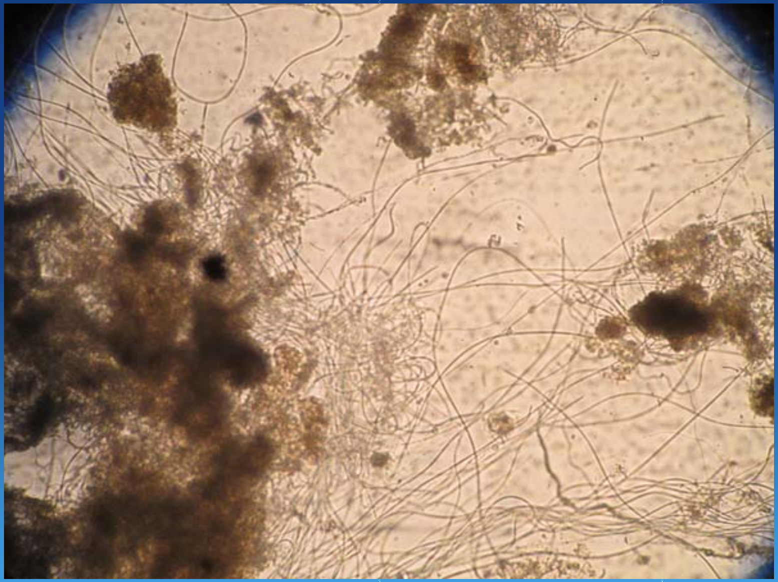
**(del Campo, Ibarra et al. 2010)**

## A hybrid technology of BFT and RAS, uses the production of heterotrophic bacteria to convert the nutrients in sludge from RAS into bacterial biomass.



**For SBR using BFT, the start-up progress is essential to set up an appropriate microbial community and the operation efficiency.**





# Experimental RAS and SBRs

**Pre-treatment of waste**

Lab-scale external biofloc reactors

**The average stocking density of *S. barcoo* in each RAS was 40 ± 2.2 kg/m$^3$. The fish were fed a commercial pellet diet (moisture 3%; crude protein 45.0%; crude lipid 8.0%; Ca 1.8%; P 1.5%; lysine $\geqslant$ 2.9%; and methionine 1.4%)**

# Experiment 1: Inorganic nitrogen dynamics in SBR using BFT to treat aquaculture sludge: start-up and assimilation of TAN and nitrate

## (1) Start-up phase:

Two treatments (three replications each) of the following:
a. Glucose (carbon content 40%) added to the biofloc reactors (SBR-glu) and b. Control biofloc reactors without an added carbon source (SBR-con).

DOC:TAN in the glucose treatment was maintained at >10.

Each reactor was supplied with 8 L of effluent from the RAS.

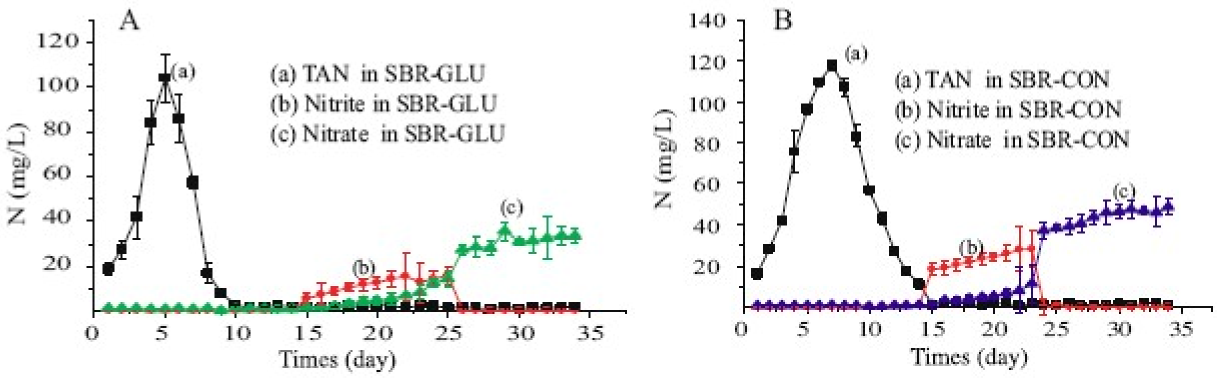



Fig. 1. (A) Changes in TAN (a), $NO_2^-$-N (b), and $NO_3^-$-N (c) concentrations during the start-up period of sequencing batch reactors with glucose added; (B) changes in TAN (a), $NO_2^-$-N (b), and $NO_3^-$-N (c) concentrations during the start-up period of sequencing batch reactors for the control group.

**Changes in DIN in biofloc systems are remarkably similar to those in conventional RAS. It can be characterized by time lags in peak concentrations of ammonia and then nitrite as the different populations of bacteria development.**

## (2) Assimilation of ammonium and nitrate phase:

The goal of this phase was to focus on the capacity of the reactors to assimilate TAN and $NO_3^-$-N .

After the start-up period when the DIN in reactors were stable. Glucose (15 g) and 0.5 g of $NH_4Cl$ were added to SBR-glu; only 0.5 g of $NH_4Cl$ was added to SBR-con.

Two days later, the capacity of the reactors to assimilate nitrate was tested. An additional 10 g of glucose and 0.5 g of $KNO_3$ were added to the SBR-glu, and only 0.5 g of $KNO_3$ was added to SBR-con.

# Assimilation of ammonium

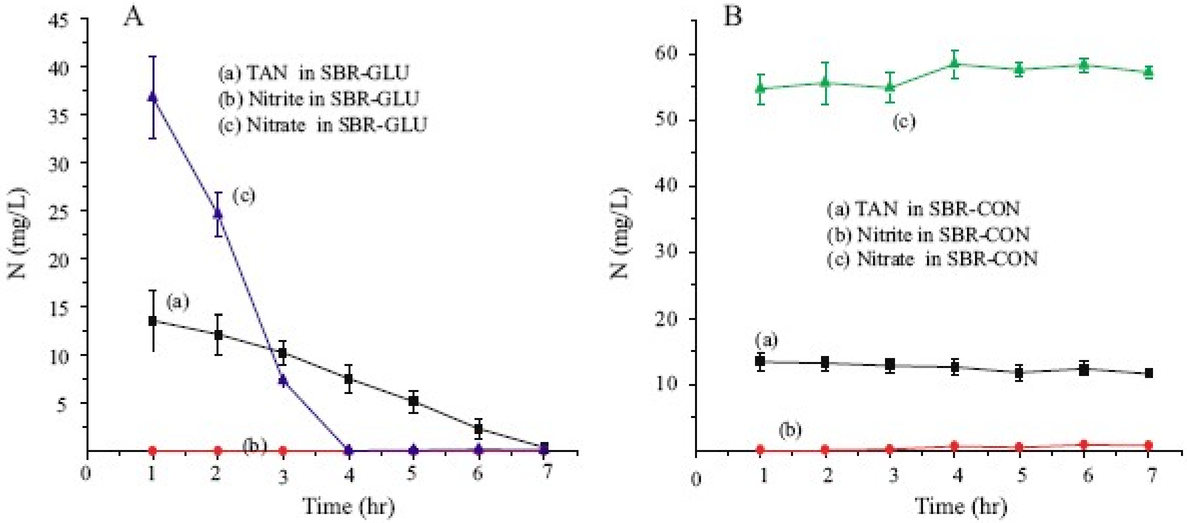



Fig. 3. (A) Changes in TAN (a), $NO_2^-$-N (b), and $NO_3^-$-N (c) concentrations during the start-up period of sequencing batch reactors with glucose added in the assimilation of ammonium experiment. (B) Changes in total ammonia nitrogen (TAN), nitrite ($NO_2^-$-N), and nitrate ($NO_3^-$-N) concentrations of sequencing batch reactors for the control group in the assimilation of ammonium experiment.

**The additions of 15 mg N/L of $NH_4Cl$ and 10 g of glucose were observably eliminated within 6 h, while no production of $NO_2^-$-N or $NO_3^-$-N was observed during these 6 h in SBR-glu. Interestingly, $NO_3^-$-N in SBR-glu decreased along with TAN and DOC, and the reduction in $NO_3^-$-N in this trial was 36.72 mg N/L. The reduction in DIN was 50 mg N/L in 6 h.**

# Assimilation of nitrate

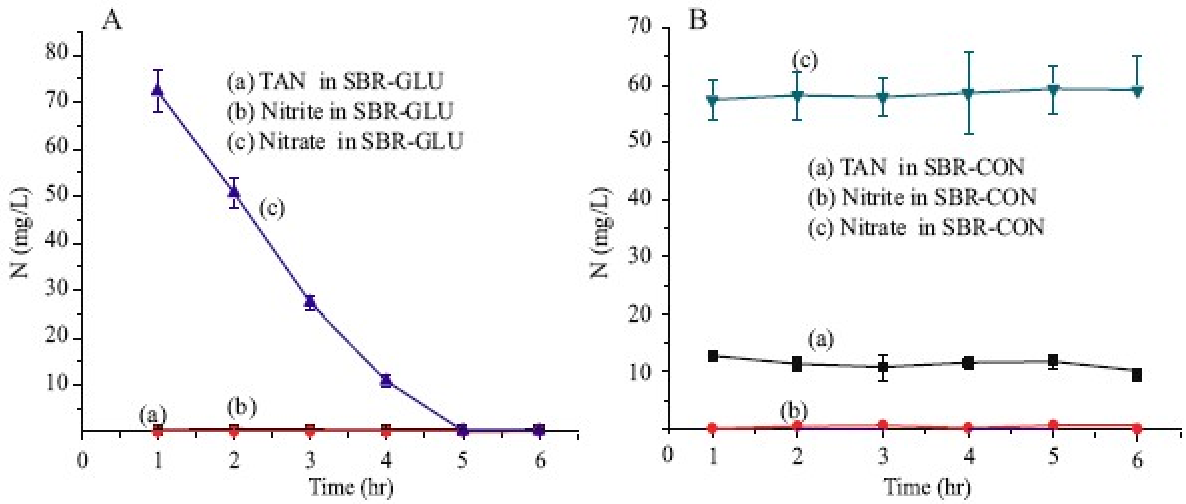



Fig. 5. (A) Changes in TAN (a), $NO_2^-$-N (b), and $NO_3^-$-N (c) concentrations during the start-up period of sequencing batch reactors with glucose added in the reducing nitrate experiment. (B) Changes in TAN (a), $NO_2^-$-N (b), and $NO_3^-$-N (c) concentrations during the start-up period of sequencing batch reactors for controls in the reduced nitrate experiment.

**A further 10 g of glucose and 0.5 g of $KNO_3$ were added to SBR-glu and 0.5 g of $KNO_3$ was added to SBR-con. The $NO_3^-$-N concentration in the SBR-glu decreased from 72.41±1.34 mg N/L to 0.10± 0.02 mg N/L within 5 h. The concentration of DOC decreased 489.8 mg/L within 5 h . The $NO_3^-$-N, TAN, $NO_2^-$-N and DOC levels remained nearly constant in SBR-con within 5 h.**

# Conclusion-1

1、The presence of nitrification during the start-up period of the reactors.

2、A good assimilation of TAN and $NO_3^-$-N was observed.

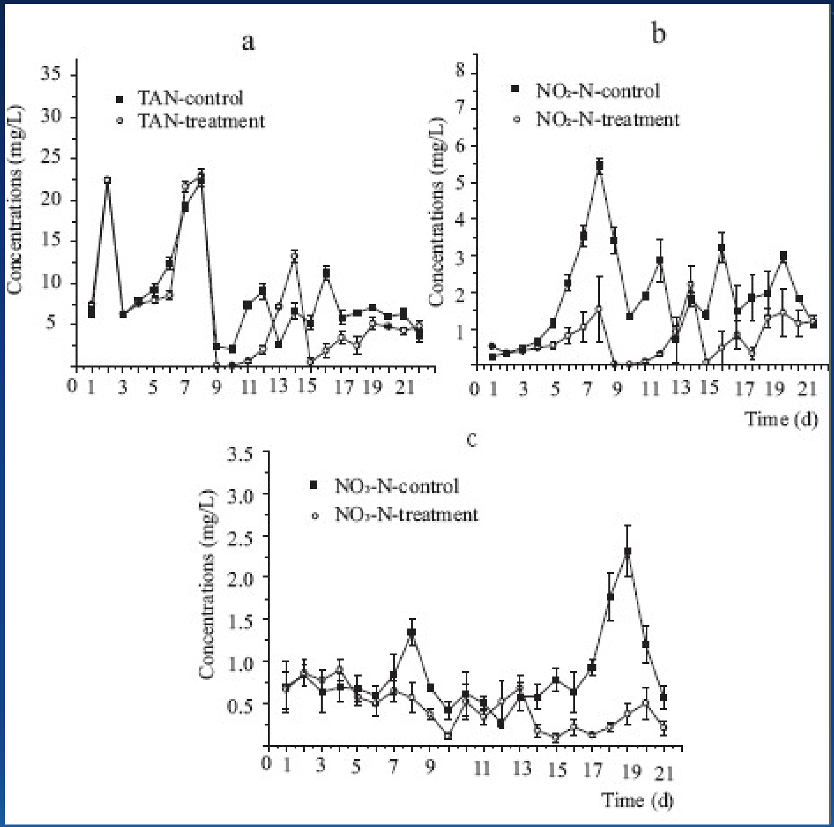
3、The bioflocs contained 30.42 ± 0.55% crude protein for SBR-glu and 26.32 ± 0.78% crude protein for SBR-con at the end of experiment.

# Experiment 2: Effects of calcium and magnesium addition on the start-up of SBR reactor using BFT treating solid aquaculture waste.

# Experimental 2 - Design

**$MgCl_2.6H_2O$ and $CaCl_2$ were added as a $Ca^{2+}$ and $Mg^{2+}$ source: 800 mg/L and 400mg/L, respectively.**

**Sodium acetate was added into each reactor as dissolved organic carbon source to maintain DOC/TON >15.**




High degree of fluctuation in DIN levels were observed in all BFT reactors during the entire experimental period. The $NO_3^-$-N and $NO_2^-$-N concentrations in treatment group were significantly lower than those of control.

Fig. DIN throughout the experiment period. **(a) TAN, (b)** **$NO_2^-$**-N, **(c) $NO_3^-$**–N.

# Nutritional quality of bio-flocs

**Compositions of bio-flocs on 22th day**

| Composition | Control | $Ca^{2+}$ 400mg/L + $Mg^{2+}$ 800 mg/L |
|---|---|---|
| **Crude protein (%)** | **26.35±0.08[a]** | **15.32±0.50[b]** |
| Crude lipid (%) | 1.69±0.01[a] | 1.11±0.12[b] |
| Ash (%) | 8.38±0.39[a] | 4.20±0.14[b] |
| **Polysaccharide ($ug.g^{-1}$)** | **3.18±0.13[a]** | **12.78±1.26[b]** |
| Fatty acid composition (% crude lipid ) | | |
| Total saturated | 27.08±1.12[a] | 28.00±0.98[a] |
| Total monounsaturated | 42.79±2.23[a] | 45.78±3.12[a] |
| Total n-6PUFA | 6.21±0.12[a] | 3.14±0.44[b] |
| Total n-3PUFA | 6.74±0.94[a] | 10.57±1.02[b] |
| Total PUFA | 14.63+1.35[a] | 16.75+2.21[b] |
| unkonwn | 15.35+0.23[a] | 13.47+0.17[b] |
| $Ca^{2+}/Mg^{2+}$ | | |
| $Ca^{2+}$ (mg/g) | 101.36±0.09[a] | 188.76±0.15[a] |
| $Mg^{2+}$(mg/g) | 5.86±0.00[a] | 11.09±0.02[a] |

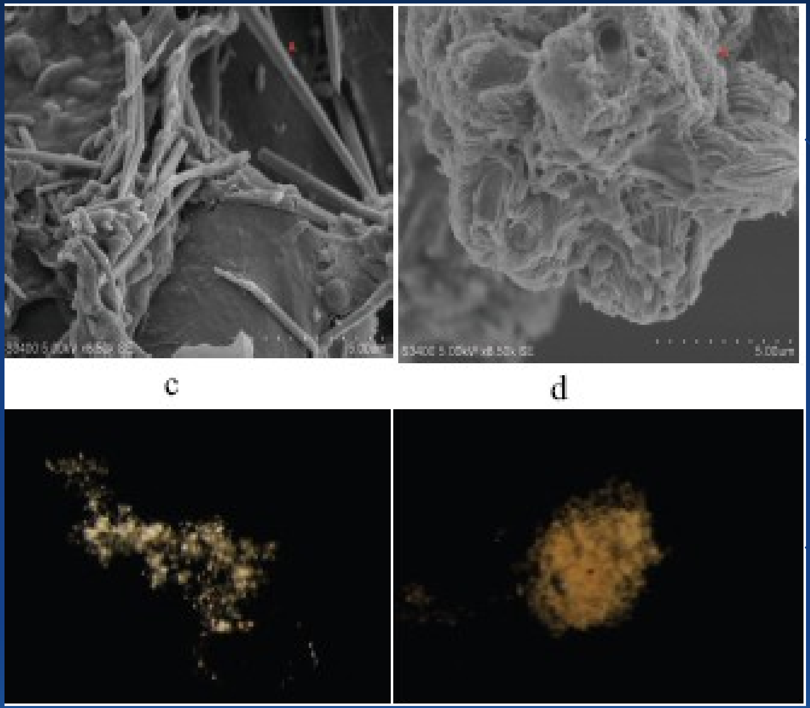
# Yields and nutrient conversion

| | Control | $Ca^{2+}$ 400mg.$L^{-1}$ + $Mg^{2+}$ 800 mg.$L^{-1}$ |
|---|---|---|
| Initial MLSS (mg.$L^{-1}$) | 2000 | 2000 |
| Initial N content in reactors (mg.$L^{-1}$) | 30.35±1.98 | 30.35±1.98 |
| Final TN contents in supernatant (mg.$L^{-1}$) | 7.12±0.24 | 1.27±0.08 |
| Final N content in crude protein in flocs (mg.$L^{-1}$) | 34.16± 1.23 | 34.00±1.09 |
| Final MLSS (mg.$L^{-1}$) | 854±23[a] | 1700±35[b] |
| Final MLVSS (mg.$L^{-1}$) | 571±11[a] | 825±20[b] |

**Yields and nitrogen conversion in the current experiment on 22$^{th}$ day**

# Observation of bio-flocs

Control




Treatments

**SEM of flocs on 22 d for treatment and control, (a) surface of flocs of control, (b) surface of flocs of treatments, (c) flocs size of control, (d) floc size of treatments**

# Conclusion-2

1、Alcium and magnesium addition could improve bio-flocs' settle-ability, increase polysaccharide， saturated fatty acid and mounsaturated content, while decrease the crude protein content of bio-flocs.

2、Bacteria biomass was enhanced by addition of $Ca^{2+}$ and $Mg^{2+}$ and about 415 g MLVSS/kg aquacultural solid waste was produced.

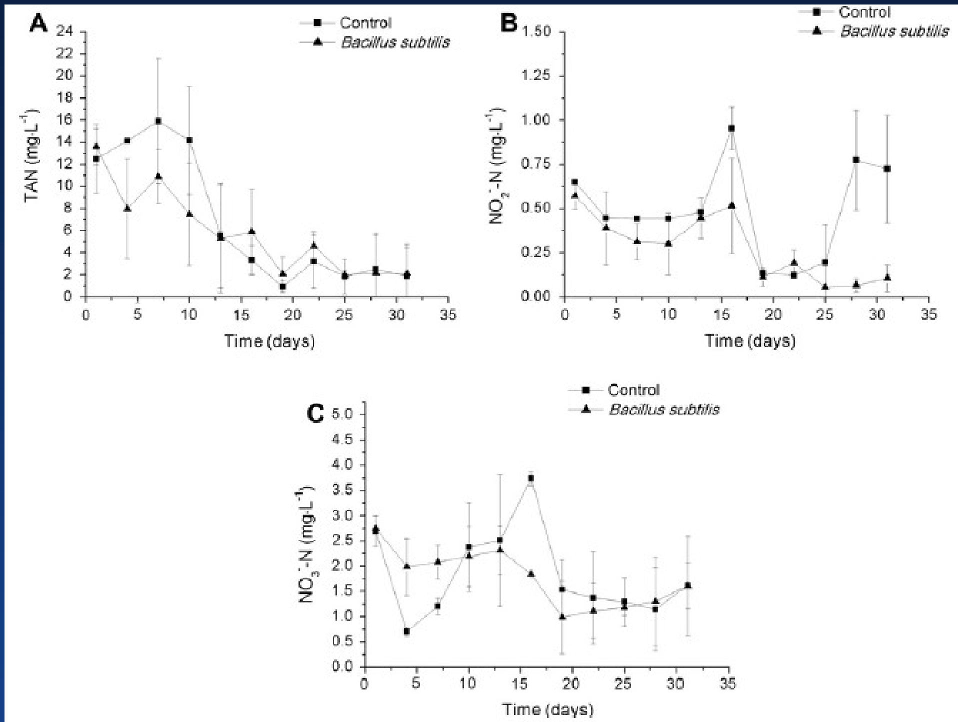
3、The average conversion rate of nitrogen in aquacultural solid waste sludge was more than 100% for control and treatment by BFT in the current experiment.

**Experiment 3: Effects of *Bacillus subtilis* on nitrogen recycle from aquaculture solid waste by heterotrophic nitrogen assimilation in SBRs.**

# Experimental 3 - Design

**The *Bacillus subtilis* with a relative molecular weight of 8508.5Da. Before addition, the bacteria was inoculated to broth medium, and cultured in 37 ℃ for 24 hours with agitation. 10 mL of the bacteria with concentration of $2.044 \pm 0.091 \times 10^{11}$/ mL was added to three SBRs respectively as treatment group. The other three SBRs with no addition bacteria were control group.**

**Glucose was added into each reactor to maintain DOC:TON >15.**



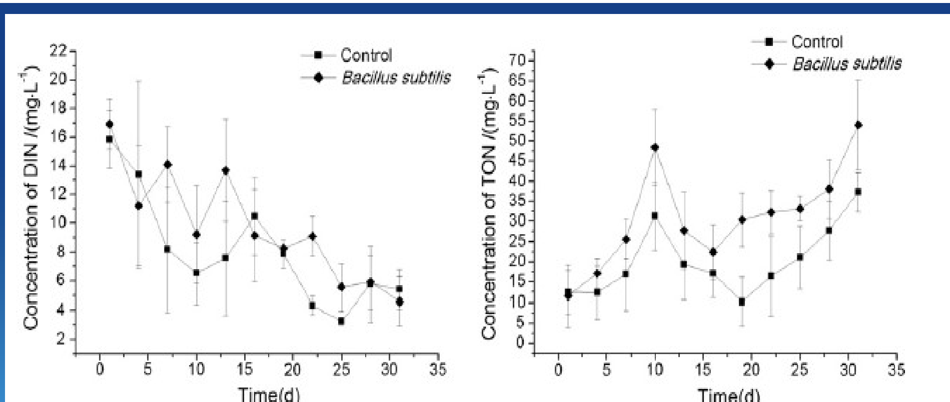




**Fig. 2. DIN and DON throughout the experiment period. (A) TAN. (B) $NO_2$–N. (C) $NO_3$–N.**

**The removal rate of dissolve inorganic nitrogen for treatments was 0.41±0.079 mg /L.d, which was 1.17 times of that in the control. The utility rate of total organic nitrogen for treatment reactors was 1.42±0.33mg/L.d， which was 1.71 times of that of the control.**

Table 1 EPS components of Bioflocs on 25 day (average ± stand deviation (minimum-maximum))

| Reactor | VSS ($mg \cdot g^{-1}$) | TOC-EPS ($mg \cdot g\ VSS^{-1}$) | Proteins-EPS ($mg \cdot g\ VSS^{-1}$) | Carbohydrates-EPS ($mg \cdot g\ VSS^{-1}$) | C-EPS/TOC (%) | P-EPS/TOC (%) | C-EPS/P-EPS |
|---|---|---|---|---|---|---|---|
| Control | 1.33±0.12 | 121.35 ±5.99 | 24.92±1.46 | 31.34±5.62 | 34.16% | 27.25% | 1.37±0.17 |
| *Bacillus subtilis* | 1.55±0.07 | 135.95 ±11.00 | 31.39±1.28 | 42.77±5.64 | 48.58% | 35.85% | 1.25±0.23 |

**On day 25, crude protein 29.65 ± 13.34% in the reactors with B. subtilis, 23.97 ± 11.62% in the control reactors.**

# Conclusion-3

1. ***B. subtilis*** **could improve the treatment effects of SBR for sludge from the RAS.**

2. **A significant amount of nitrogen assimilation was achieved in this system. After the addition of *B. subtilis*, $RR_{DIN}$, $UR_{TON}$ and $RR_{DOC}$ were greater than those in the control reactor.**

3. **The settling ability of the reactor containing Bacillus subtilits was superior to that of the control reactor. The SEM images revealed a more condensed surface of the bio-flocs particles in the reactor containing Bacillus subitilis.**

# Thanks for your attention!