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# The start-up and saline adaptation of mesophilic anaerobic sequencing batch reactor treating sludge from recirculating aquaculture systems

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ABSTRACT

Treatment of sludge from aquaculture is a matter of special importance and there is a need for salt-tolerant biological wastewater treatment to coincide with the development of brackish/marine aquaculture. The aims of the current study were to determine the ability of anaerobic sequencing batch reactor (ASBR) to anaerobically digest sludge from fresh-water recirculating aquaculture systems and the ability of adaptation to low saline conditions. The mesophilic ASBR were evaluated with loading rates between 0.12 and 0.41 g chemical oxygen demand (COD)/day at a 20-day hydraulic retention time (HRT) for start-up and with organic loading rates (OLR) of 0.39–0.41 g COD/L day at a 20-day HRT for saline adaptation. The average removal rate of total chemical oxygen demand (TCOD), total suspended solids (TSS) and volatile suspended solids (VSS) of the ASBR were above 97%, 96% and 91% during the stabilization period of the experimental reactors. The average daily gas production of ASBR was between 0.013 and 0.022 L/g TCOD from day 118. A sludge-mass reduction of up to 94 ± 2.3%, TCOD reduction of 44 ± 13% and VSS/SS of 39–70% were demonstrated for the reactor performance during the gas production period. However, the process of gas production was obviously inhibited, presumably by salt, and unstable due to the dissolved COD (DCOD), total ammonium nitrogen (TAN) and alkalinity of the effluents of the experimental reactors and TSS and sludge volume index (SVI) observed within the reactors. The daily gas production was observed to decrease during the saline adaptation period and stopped when the salinity of the effluents was higher than 8.7 ppt until the end of the experiment.

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## 1. Introduction

Aquaculture seems to be the fastest growing animal-food production method, fuelled by governmental support, the evolution of technological assistance and pessimistic forecasts for fisheries production, reflected by the fact that its contribution to world supplies of aquatic products has increased from 1 million tones per year in the early 1950s, to a production of 55.1 million tons in 2009 (FAO, 2010). With increased interest in environmentally friendly farming practices and the potential for regulatory action by the Environmental Protection Agency (EPA) and other agencies, the aquaculture industry has been focusing on ways to reduce waste (environmental impact) from aquaculture facilities. Because the scope of digestion in fish is limited, a relatively large fraction of feed remains undigested and is excreted (Amirkolaie, 2005). To produce 1 kg live weight of fish one needs 1–3 kg dry weight of feed (assuming a food conversion ratio of about 1–3) (Naylor et al., 2000). About 36% of the feed is excreted as a form of organic waste (Brune et al., 2003). In a properly managed farm, between 11 and 40% of applied feed has been estimated to accumulate (Hopkins et al., 1994). Recirculating aquaculture systems (RAS) provide many advantages over traditional aquaculture systems (Lin et al., 2005) because of their reuse of aquaculture water. The solids originating in a RAS are composed mainly of fish excretions with a small percentage of uneaten feed, and its volatile (organic) fraction ranges from 50% to 92% (Gebauer, 2004; Gebauer and Eikebrokk, 2006; Mirzoyan et al., 2008; Piedrahita, 2003).

Disposal of aquaculture sludge into wastewater-treatment systems is often prohibited, as it usually involves high volumes with high organic matter content, especially if the sludge includes salts that might interfere with the treatment of municipal sludge. Most commonly used sludge treatments employ flocculation/coagulation processes to reduce sludge volume prior to composting it for land dispersal (Mirzoyan et al., 2008). Anaerobic microbiological decomposition is a process in which microorganisms derive energy for growth by metabolizing organic material in an oxygen-free environment resulting in the production of methane ($CH_4$). Even though anaerobic digestion (AD) is commonly used for the stabilization of municipal, industrial and agricultural wastes, it is a novel approach for the treatment of sludge produced in RAS (Mirzoyan et al., 2010). Conventional anaerobic

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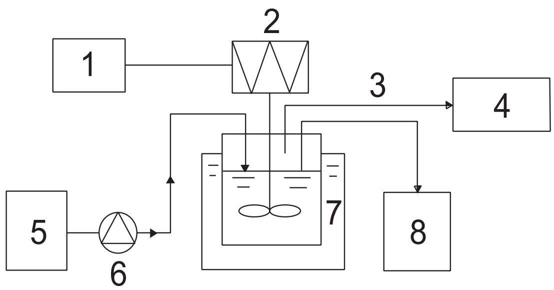


**Fig. 1.** Schematic diagram of the anaerobic sequencing batch reactor (ASBR). 1 timer; 2 stirrer; 3 biogas; 4 aluminum bags; 5 influent tank; 6 peristaltic pump; 7 water-bath; 8 effluent tank.

digesters employed to process thermally hydrolyzed sewage sludge used in previous studies on aquaculture sludge were continuous flow stirred tank reactors (CSTR) (Gebauer, 2004; Gebauer and Eikebrokk, 2006), the upflow anaerobic sludge blanket (UASB) reactor (Seghezzo et al., 1998) and a membrane biological reactor (MBR) (Mirzoyan et al., 2008; Tal et al., 2009). Recent developments such as the anaerobic sequencing batch reactor (ASBR) have made it possible to treat high-solids waste streams in a high-rate system (Wang et al., 2009).

Typically, fish sludge is characterized by its low total solid (TS) content (1.5–3%) and C:N ratio compared to other animal production or industrial wastewater processes (Mirzoyan et al., 2008). The suitability of wastewater sludge for anaerobic digestion depends largely on its physical, chemical and biological characteristics, while there is relatively little information concerning the suitability of sludge from RAS for anaerobic treatment (Mirzoyan et al., 2008). Salt additives, particularly NaCl, are commonly used in freshwater aquaculture (Mazik et al., 1991). Treatment of saline organic wastewater from sources such as seafood processing plants and brackish aquaculture is a matter of special importance. In the present study, anaerobic sludge degradation was examined in a laboratory-scale ASBR to determine the anaerobic digestion ability of ASBR to treat sludge from fresh water RAS and the ability to adapt to low saline conditions.

## 2. Materials and methods

### 2.1. Experimental ASBR reactor

The experiment was carried out at a mesophilic temperature of 35 ± 1 °C, in three 4-L oroglas (poly-methyl acrylic acid methyl ester) ASBR reactors, and was placed in a water-bath and stirred continuously at 150 rpm. Reactors were 14 cm in diameter and 26 cm high with an operating volume of about 4 L (a work volume of 3 L and a headspace of 1 L) (Fig. 1). The ASBR operates in a cyclic batch mode with four distinct phases per cycle. The four phases are: feeding (15 min), reacting (46.5 h), thickening (1 h) and drawing (15 min). The thickening and drawing phases are the key steps in the ASBR operation. The thickening phase brings about an accumulation of sludge as the solids are kept within the reactor. Each reactor was loaded with 3 L of anaerobic sludge. The reactors were sampled and fed manually, through, respectively, a double siphon and a tube through the digester lid. Biogas was collected in 10-L aluminum bags. The experiment was conducted in the dark.

### 2.2. Inoculum

The inoculum was taken from the digested municipal sewage plant at Shanghai New-city. The main qualities of the inoculum were: TSS 24.35 g/L, VSS 7.13 g/L, VSS/SS 29.28%, Solids Volume Index (SVI) 32.85 mL/g.

### 2.3. Fish farming sludge (substrate)

Sludge was collected from a recirculating aquaculture system (RAS) used for the production of *Scortum bacoo* at the Center for Recirculating Aquaculture Systems of Shanghai Ocean University (Shanghai, China). The composition of the sludge is shown in Table 1. The sludge was collected once a day over a 3-month period in 2010. Fish were fed on a commercial pellet diet (moisture 3%; crude protein 45.0%; crude lipid 8.0%; Ca 1.8%; P 1.5%; lysine ≥2.9%; and methionine 1.4%) (Suzhou Tong Wei Special Feed Co., Ltd., Wujiang, China). The feed coefficient (kg feed used/kg fish produced) was 2.0. The sludge was frozen on the day of collection and was kept frozen at −18 °C until the experiment was started.

### 2.4. Start-up of the experimental ASBR

A 300 mL inoculum of sludge was introduced into each reactor and 300 mL of sludge was collected from each reactor every 2 days. The HRT was 20 days. The reactor was operated for a total of 165 days for start-up. The operation conditions are shown together with the performance in Tab. 2. During the first 77 days of operation (period I), the OLR was 0.12–0.14 g COD/L day, then increased 0.24–0.26 g COD/L day (days 78–113, period II) and finally 0.39–0.41 g COD/L day (days 114–165, period III). The start-up progress was achieved by stable gas production and the removal rate of COD was maintained above 80%.

Sludge samples of the influents and effluents were analyzed for TCOD, VSS and TSS every 2 days. The alkalinity (ALK), measured as $CaCO_3$ and pH of the effluent from the reactors, was measured every 2 days from day 77. The TSS, VSS, ALK, TAN, TCOD, DCOD, dissolved oxygen (DO) concentration, redox potential (ORP) of the effluent of the reactors and the gas production were measured every 2 days from day 114 to day 165. At the same time (period III), the VSS/SS and TCOD of the sludge within the reactors were measured irregularly to determine the performance of the reactor.

### 2.5. Saline adaptation of the ASBR

After establishment of the ASBR reactor, sea salt (Jintanbaojia Sea Salt Factory, Nantong, Jiangsu Province, China) was chosen to increase salinity of the sludge (already described in Table 1). The salinity of sludge was increased gradually (0.26 ± 0.15 ppt at a time) from 0 ppt to 10.46 ppt under experimental conditions, as described in the start-up experiment. The HRT and the OLR were 20 days and 0.39–0.41 g COD/L day, respectively. An aliquot of 300 mL of sludge was introduced into each reactor and 300 mL of sludge was collected from each reactor every 2 days. For practical wastewater treatment, the pH of the effluents should be maintained at >6.2 (Lettinga, 1995). When the pH-value decreased, 2 g $NaHCO_3$ was dissolved in the influent in the experiment. The DCOD, TAN, pH,

**Table 1**
Composition of the sludge collected from RAS for production *Scortum bacoo*.

| Component | SS (g/L) | VSS (g/L) | pH | TCOD (g/L) | DCOD (g/L) | TKN (g/L) | TAN (g/L) | $PO_4^{3-}$-P (g/L) | ORP (mV) |
|---|---|---|---|---|---|---|---|---|---|
| Content | 129.6–145.3 | 35.2–50.8 | 6.3–6.7 | 182.7–241.2 | 25.3–32.5 | 20.1–24.3 | 3.9–4.2 | 1.2–1.4 | −130 to 260 |

SS, suspended solids; VSS, volatile suspended solid; TCOD, total chemical oxygen demand; DCOD, soluble chemical oxygen demand; TKN, total kjeldahl nitrogen.

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