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Electron-acceptor utilization and methanogenesis in brackish aquaculture sludge

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ABSTRACT

Anaerobic digestion (AD) of sulfate-rich brackish aquaculture sludge (BAS) is an effective method to reduce negative environmental impact and produce biogas. However, the mechanisms of electron-acceptor utilization during AD of BAS, and the effects of elevated sulfate, nitrate and nitrite levels on biogas production, have never been investigated. The current study is the first to demonstrate the mechanisms of electron-acceptor utilization in BAS under methanogenic conditions. Nitrate, nitrite and iron were almost completely depleted during the first day of AD, and methane production began only when these electron acceptors were removed. In contrast, sulfate reduction also started immediately, but took 15–28 d, dependent on the treatment, and largely coincided with methane production. The volume of produced biogas, the lag phase for methane production and methane content in the biogas were affected by the inhibition of sulfate reduction and by the increase in nitrate and nitrite concentrations. However, the average methane-production rates in all treatments were similar. Our data suggest that methanogenesis in BAS under naturally high sulfate levels results in the highest possible methane-producing capability of the system; no additional manipulations are required to further increase methane production, suggesting the robustness of AD of BAS under the currently reported conditions.

1. Introduction

Saline wastewater with high sulfate content is produced by various commercial activities, such as those of the pulp and paper industries, fermentation processes and edible oil production (Cirne et al., 2008; Hulshoff Pol et al., 1998). Left untreated, the increased load of sulfate-rich salty waste in the environment leads to various potential hazards, such as salinization of receiving bodies and production of harmful gases methane and sulfide, as well as increased loads of carbon, nitrogen and sulfur in the environment. Anaerobic digestion (AD) of sulfate-rich wastes is commonly used for methane production and harvesting as an alternative energy source, and has the added benefit of lower sulfate discharge into the environment with subsequent reduction of the negative ecological impact of both sulfate and its reduction products.

Inland brackish and marine water aquaculture production is another continuously expanding source of salty waste (FAO, 2008). Even in controlled recirculating aquaculture systems (RASs), which produce a smaller environmental footprint than traditional aquaculture, between 25 and 50% of applied fish feed accumulates in the form of salty sludge (Mirzoyan et al., 2012; Timmons and Ebeling, 2007). The volume of salty solid waste produced in the growth of 100 tons of fish is comparable to that produced by a mid-sized town (Hardy, 2001), and appropriate waste-management strategies are required to deal with the high load of sulfate-rich waste.

The use of AD is a relatively new (Mirzoyan et al., 2010 and references therein) and highly efficient approach to aquaculture sludge management. AD of brackish aquaculture sludge (BAS) from RASs has been reported to result in high rates of methane production (Gebauer, 2004; Gebauer and Eikebrokk, 2006; Mirzoyan et al., 2008) and, more importantly, high efficiency of solid-waste reduction, leading to more environmentally friendly waste-management practices in RASs (Mirzoyan and Gross, 2013).

Degradation of organic matter during AD involves sequential reduction of electron acceptors, with the compounds with higher redox potentials being reduced first. It is therefore predicted that

Abbreviations: AD, anaerobic digestion; RAS, recirculating aquaculture system; BAS, brackish aquaculture sludge; ORP, redox potential; RM-ANOVA, repeated-measures analysis of variance; UASB, upflow anaerobic sludge blanket; SMP, specific methane production; SRP, sulfate-reducing prokaryote.

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in AD, nitrate (NO_3^-) and nitrite (NO_2^-) will be reduced first, followed by ferric iron (Fe^{3+}) and finally sulfate (SO_4^{2-}) (Chidthaisong and Conrad, 2000). However, in environments with high organic matter content, the reduction processes are not mutually exclusive and might occur simultaneously (Chidthaisong and Conrad, 2000). While the efficiency of AD for methanogenesis in BAS is well established, the mechanisms governing electron-acceptor utilization during this process have not been described. Moreover, BAS is a challenging waste for AD: its high sulfate content and relatively high potential levels of nitrate and nitrite suggest a change in electron flow during AD, from methane production to sulfate reduction and denitrification, and subsequent inhibition of methanogenesis (Isa et al., 1986; Stams et al., 2003; Tugtas and Pavlostathis, 2007a).

The aim of the current study was to determine: (a) pathways of electron-acceptor utilization; (b) the role of increased sulfate reduction, and (c) the effect of elevated nitrate and nitrite levels on methane production during AD of BAS.

2. Materials and methods

2.1. Sludge source and analytical methods

BAS from R.A.M. RAS, Negev, Israel (located 50 km south of Beer Sheva, Israel, 30°58′ N, 34°43′ E) was collected and transported to the laboratory in an icebox (Mirzoyan et al., 2008).

pH and redox potential (ORP) were checked by laboratory meters. Volatile solids (VS) were analyzed according to the standard method (APHA, 1998). Nitrite, nitrate and sulfate concentrations were estimated in supernatants after centrifugation. Nitrite concentration was determined by the diazo colorimetric method (APHA, 1998), nitrate by the second-derivative method (Ferree and Shannon, 2001) and sulfate by the turbidometric method (APHA, 1998). Fe^{2+} was analyzed by the phenanthroline method (APHA, 1998).

Biogas was analyzed for the presence of methane by gas chromatography (Varian GC 3800) using a thermal conductivity detector and a Hayesep Q packed column (Varian).

2.2. Experimental setup

Twenty-eight 250-mL serum bottles were flushed with nitrogen and filled with 200 mL sludge. The sludge was seeded with 1.5 mL BAS from upflow anaerobic sludge blanket (UASB) reactors operating for 12 months (Mirzoyan and Gross, 2013). Four treatments were tested, seven bottles per treatment, as follows: "Raw" – raw sludge; "Mo" – raw sludge supplemented with sodium molybdate (sulfate-reduction inhibitor) to a final concentration of 10 mg L^{-1} (2.5 mM) MoO_4^{2-}; "NO_3" – raw sludge supplemented with 50 mg L^{-1} NO_3^-–N as sodium nitrate, and "NO_2" – raw sludge supplemented with 20 mg L^{-1} NO_2^-–N as sodium nitrite.

All bottles were flushed with N_2 gas and capped with butyl rubber stoppers to ensure anaerobic conditions. Bottles were incubated for 28 d in a dark room at 25 °C and were manually shaken by hand for 30 s a day to avoid concentration gradients between the solid and liquid phase. Gas and sludge samples were collected daily during the first week of the experiment, every 2 d during the second week and every 3 d during the last 2 weeks. Each bottle was used only twice for sampling to minimize artifacts associated with changes in sample volume and solid-to-liquid ratio. Periodically, additional two random bottles from each treatment were sampled to check data reproducibility.

During each sampling, 14 mL of sludge sample was withdrawn. From this, 10 mL was immediately analyzed for pH and ORP, then centrifuged (10,000 RPM for 5 min), and the supernatant was frozen at −20 °C for further analyses of nitrite, nitrate and sulfate. Into the remaining 4 mL, immediately after sampling, 0.5 mL methol-reductant solution was introduced to prevent Fe^{2+} oxidation. Then samples were centrifuged at 10,000 RPM for 5 min and the supernatant was used for Fe^{2+} analysis.

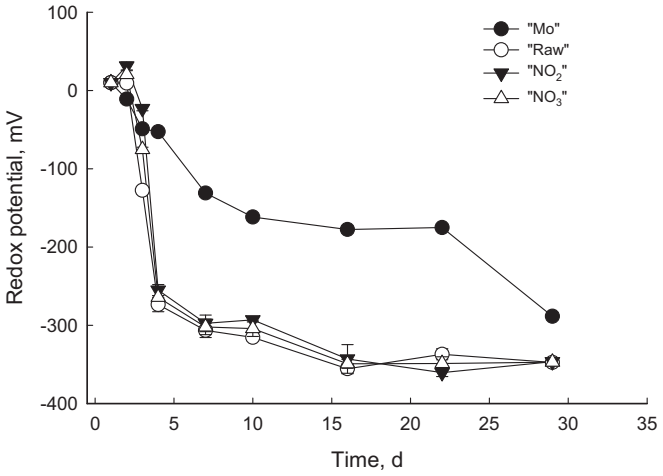

Fig. 1. Redox potential change over time in raw brackish aquaculture sludge that was anaerobically digested under different electron-acceptor regimes. "Raw" – raw sludge; "Mo" – raw sludge supplemented with MoO_4^{2-} to a final concentration of 10 mg L^{-1}; "NO_3" – raw sludge supplemented with 50 mg L^{-1} NO_3^-–N; "NO_2" – raw sludge supplemented with 20 mg L^{-1} NO_2^-–N.

The biogas was volumetrically collected by a syringe from the headspace of each bottle and analyzed for the presence of methane by gas chromatography.

The sulfate-reduction rates were calculated by fitting data to exponential function (SigmaPlot 3.1 package (SPSS, 1997)).

The quality differences between raw and treated sludge were compared by repeated-measures analysis of variance (RM-ANOVA) followed by a post-hoc test, when needed, using the SigmaStat 3.1 package (SPSS, 1997).

3. Results

3.1. Redox potential

The ORP of the sludge samples was measured at different time points as an indicator of the anoxic/anaerobic conditions developed in the sludge. The initial ORP ranged between 0 and 32 mV and decreased quickly in all treatments except for "Mo" (Fig. 1; Table 1), which developed anoxic conditions significantly more slowly than the other groups ($P < 0.05$). In treatments "Raw", "NO_3" and "NO_2", after 5 d of digestion, the ORP reached about −250 mV, indicating highly reduced conditions which were maintained for the rest of the study. In the molybdate-supplemented samples, however, the ORP reached only −132 mV on day 5 and approximately −300 mV toward the end of the experiment (Fig. 1), significantly higher than in the rest of the treatments.

3.2. Nitrate removal

Initial NO_3^-–N concentration in all treatments was 48 mg L^{-1}, except for 75 mg L^{-1} in treatment "NO_3" where an additional 50 mg L^{-1} NO_3^-–N was added (Fig. 2; Table 1). The NO_3^-–N concentration in nitrate-supplemented samples was lower than its expected concentration of 98 mg L^{-1}, probably due to high rates of nitrate reduction during the time elapsed between the introduction of nitrate and the first sampling (1–2 h).

Nitrate levels dropped rapidly in all treatments with no significant differences in nitrate-removal rates related to treatment