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Short Communication

Improving methane production and phosphorus release in anaerobic digestion of particulate saline sludge from a brackish aquaculture recirculation system

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HIGHLIGHTS

- Both specific methanogenic activity – SMA and phosphatase activity – PA are considered.
- First study on effect of compatible solutes on SMA and PA of anaerobic biomass.
- Salinity >35 g/L severely deteriorates SMA of biomass adapted to high salinity.
- 1 mM Trehalose (T) and glycine betaine (GB) conducive to enhancing SMA and acid PA.
- T and GB likely improve CH_4 yield and P release from saline waste in AD.

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ABSTRACT

In this study, batch tests were conducted to examine the effects of trehalose and glycine betaine as well as potassium on the specific methanogenic activity (SMA), acid and alkaline phosphatase activity of anaerobic biomass and phosphorus release in anaerobic digestion of saline sludge from a brackish recirculation aquaculture system. The results of ANOVA and Tukey's HSD (honestly significant difference) tests showed that glycine betaine and trehalose enhanced SMA of anaerobic biomass and reactive phosphorus release from the particulate waste. Moreover, SMA tests revealed that methanogenic sludge, which was long-term acclimatized to a salinity level of 17 g/L was severely affected by the increase in salinity to values exceeding 35 g/L. Addition of compatible solutes, such as glycine betaine and trehalose, could be used to enhance the specific methane production rate and phosphorus release in anaerobic digestion from particulate organic waste produced in marine or brackish aquaculture recirculation systems.

1. Introduction

With the increasing demand of seafood and the subsequent exploitation and over-exploitation of marine fish occurring in some regions in the world a more efficient, environmentally friendly, and economical approach to produce seafood is required. Nowadays, marine/brackish recirculation aquaculture systems (RAS) seem to be a promising approach to meet the requirements for a high production efficiency and low discharge of waste streams (Zhang et al., 2013a).

However, the relatively concentrated stream from recirculation systems needs to be treated due to the high solids COD and nutrient concentrations. Anaerobic digestion is successfully applied to treat domestic waste sludges as well as organic streams from industries to achieve sludge reduction and energy recovery. However, the full-scale application of anaerobic digestion in marine/brackish fish farms has not been reported. Thus far, the high salinity of wastewater and/or sludge is a challenge to biological treatment processes such as anaerobic digestion (Dereli et al., 2012), particularly for sludges with high solids contents (Zhang et al., 2013b). Methanogenesis generally is considered as the rate-limiting step in anaerobic digestion of wastewaters (Khanal, 2008), however, in anaerobic digestion of organic particulate waste, enzymatic hydrolysis can also be rate-limiting process (Cavaleiro et al.,

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2013; Xu et al., 2012). Moreover, phosphorus solubilization by phosphatase in anaerobic digestion of organic solids waste is a prerequisite prior to recovering P from the aqueous phase, for instance by struvite precipitation (Zhang et al., 2013a). Thus, there is a need to enhance the anaerobic digestion process of salty solid wastes.

Compatible solutes and potassium are reported to enhance the methane production from waste streams containing high salt concentrations (Oh et al., 2008; Vyrides et al., 2010). This may be attributed to microbes that are able to accumulate the solutes and balance osmotic stress caused by high salinity, and in this way maintain and even further enhance their activity (Ocon et al., 2007; Oh et al., 2008; Vyrides et al., 2010; Vyrides and Stuckey, 2009). Therefore, it was hypothesized that the presence of solutes may be indirectly conducive to phosphorus release from the particulate matters in anaerobic digestion and improves the specific methanogenic activity (SMA). Moreover, to our knowledge no investigation on the effect of compatible solutes on phosphatase activity of microorganisms and phosphorus release from salty particulate waste is reported.

In this work, the compatible solutes, trehalose and glycine betaine, and the salt potassium chloride were utilized in batch assays to examine their effects on SMA and phosphatase activity (PA) of anaerobic biomass collected from a completely stirred tank reactor (CSTR) fed with concentrated sludge from a brackish RAS. In Addition, their effects on phosphorus release (represented as reactive phosphorus) from the concentrated sludges were evaluated. In this current study the effect of salinity on SMA of the anaerobic biomass adapted long term to saline conditions has also been investigated by using *NaCl* addition.

2. Methods

2.1. Seeding sludge and substrate

Seed sludge for all batch tests was obtained from a semi-continuous CSTR (4.0 L). The CSTR was fed with sludge from a brackish RAS with a salinity level of 13–17 g/L and was operated for 5 months under mesophilic conditions (35 °C) with stable performance in terms of biogas production and SMA (0.08–0.14 g COD–CH_4/(d gVSS)). The sludge from the brackish RAS was also used as the substrate for all the PA batch assays. Initial pH and salinity of seed sludges for SMA and PA batches were 7.4, 17 g/L and 7.4, 21 g/L, measured at 25 °C, respectively. The differences in salinity of the seed sludges from the CSTR were due to the different operational periods.

2.2. Reagents

NaCl (ACS reagent, ⩾99.0%, Sigma–Aldrich) was used to increase salinity. D-(+)-Trehalose dihydrate (99%) and anhydrous glycine betaine (>98%) were purchased from Alfa Aesar, Germany. Sodium acetate trihydrate (>99.0%, BDH, PROLABO) was purchased from VWR international.

2.3. Determination of enzymatic activity and analytic methods

SMA tests to examine the effect of salinity on SMA were conducted using the AMPTS II (Bioprocess Control, Sweden), using sodium acetate solution with COD of 2 g O_2/L as the substrate. The substrate was prepared in brackish water in order to use similar conditions as those present in the reactor where the anaerobic biomass was cultivated. The SMA assays were conducted in duplicates for each salinity level, which was achieved by addition of *NaCl*. The pH was maintained at 7.0 by a 10 mM-phosphate buffer. The control group for SMA was without addition of *NaCl*. Phosphate buffer stock solution contains 45.7 g/L of $K_2HPO_4{\cdot}3H_2O$ and 31.2 g/L of $NaH_2PO_4{\cdot}2H_2O$. Macronutrient stock solution consists of NH_4Cl (170 g/L), $CaCl_2{\cdot}2H_2O$ (8.0 g/L) and $MgSO_4{\cdot}7H_2O$ (9.0 g/L). Moreover, micronutrient stock solution is the mixture of $FeCl_2{\cdot}4H_2O$ (2.0 g/L), $CoCl_2{\cdot}6H_2O$ (2.0 g/L), $MnCl_2{\cdot}4H_2O$ (0.5 g/L), $CuCl_2{\cdot}2H_2O$ (30 mg/L), $ZnCl_2{\cdot}$ (50 mg/L), HBO_3 (50 mg/L), $(NH_4)_6Mo_7O_2{\cdot}4H_2O$ (90 mg/L), $Na_2SeO_3{\cdot}5H_2O$ (100 mg/L), $NiCl_2{\cdot}6H_2O$ (50 mg/L), EDTA (1.0 g/L), and HCl (36% 1.0 ml/L). In each 200 mL batch test were 10 mL phosphate buffer stock solution, 1.2 mL macronutrient stock solution and 0.12 mL micronutrient stock solution added individually. The increase in SMA is calculated using the equation (SMA increase (%) = $(SMA - SMA_{CG})/SMA_{CG} \times 100$), where CG represents control group. PA and reactive phosphorus assays (Zhang et al., 2013a) were conducted in the same triplicated bottles (100 mL) at each dosage of compatible solutes. The corresponding control group was also conducted in triplicates but without the addition of compatible solutes.

In the PA batch testing bottles, initial pH was 7.4 and the end pH was 7.7. PA assays were based on methods published in the literature (Calderon et al., 2012). Reactive phosphorus was analyzed using Merck kits. Salinity levels (g/L) were measured by means of a portable conductivity meter (Cond 340i, WTW, Germany) that was calibrated by a standard solution (HI6031, HANNA instruments, USA). Standard deviations of the measurements including SMA, PA and reactive phosphorus were assessed.

2.4. Statistical analysis methods

Analysis of variance (ANOVA) was used to examine effects of additions of trehalose, glycine betaine and *KCl* on SMA, PA and reactive phosphorus. In the analyses, the degrees of freedom of groups, error and total variances were 5, 6 and 11 for SMA, 9, 20 and 29 for PA, and 9, 20, and 29 for reactive phosphorus, respectively. Probability (p) of rejection of null hypothesis of no significant difference between the groups was used to evaluate the effects, and the significance level was set at 0.05. However, a one-way ANOVA does not indicate which means in the testing groups are significantly different from the others. Hence, in this study a multiple comparison test procedure based on Tukey–Kramer method, also called Tukey's HSD (honestly significant difference) test, was used to determine which groups were significantly different from the control group at the 95% confidence level.

3. Results and discussion

3.1. Effect of increased salinity on SMA of biomass using addition of NaCl

Averages of SMA of the batch assays at salinity levels of 17, 25, 35, 40, 44 and 49 g/L were 80, 63, 38, 23, 13 and 3 mg COD-CH_4/(d gVSS), respectively. SMA seems to be decreasing linearly with the increasing salinity. The results indicate that with an increase in salinity, SMA of the sludge adapted to the salinity of 17 g/L decreased from 0.08 g COD-CH_4/(d gVSS) to 0.038 g COD-CH_4/(d gVSS) when the salinity was increased from 17 g/L to 35 g/L. The results show that sodium toxicity to methanogens exists even though the inoculum was long-term adapted to a high salinity level. The severe toxicity to methanogens may be caused by the increase in osmotic pressure (Vyrides and Stuckey, 2009), as a result of high sodium chloride concentrations, which severely affected metabolically active intracellular enzymes in living cells (Ocon et al., 2007; Oh et al., 2008). However, even with the further increase in salinity up to 44 g/L in the assays, there was still some