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Overcoming sodium toxicity by utilizing grass leaves as co-substrate during the start-up of batch thermophilic anaerobic digestion

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HIGHLIGHTS

▸ Sodium toxicity in anaerobic digestion can be overcome by adding grass clippings as co-substrate.
▸ Different grass turf species can be used as co-substrate to decrease sodium toxicity.
▸ Betaine could be a significant compound in grass causing reduction in sodium inhibition.

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ABSTRACT

Sodium toxicity is a common problem causing inhibition of anaerobic digestion, and digesters treating highly concentrated wastes, such as food and municipal solid waste, and concentrated animal manure, are likely to suffer from partial or complete inhibition of methane-producing consortia, including methanogens. When grass clippings were added at the onset of anaerobic digestion of acetate containing a sodium concentration of 7.8 g Na^+/L, a total methane production about 8 L/L was obtained, whereas no methane was produced in the absence of grass leaves. In an attempt to narrow down which components of grass leaves caused decrease of sodium toxicity, different hypotheses were tested. Results revealed that betaine could be a significant compound in grass leaves causing reduction to sodium inhibition.

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1. Introduction

Anaerobic digestion of wastes, in particular solid wastes, results in the solubilization of organic and inorganic salts. The inhibitory effect of accumulating salts on the methanogenic microbiota in the anaerobic digester is a problem that is not well understood. One of the ions that always accumulates and that has been shown to be toxic to methanogenic Archaea is sodium.

Although sodium is essential for bacterial growth (Dimroth and Thomer, 1989), high sodium concentrations increase osmotic stress that can result in decreased cell activity and cell plasmolysis (Uygur, 2006). The occurrence of high sodium concentrations in an anaerobic reactor can generally be attributed to a high sodium concentration in the influent waste stream or sodium addition during operation of the digestion process. Industries, such as the seafood processing industries, utilize raw materials containing high sodium salts resulting in the generation of a salty wastewater. High sodium concentrations in an anaerobic digester can also arise from the addition of alkaline solution in the form of sodium hydroxide (NaOH), sodium carbonate (Na_2CO_3) or sodium bi-carbonate ($NaHCO_3$) to neutralize acidity during start-up and operation.

Although anaerobic digestion of saline wastewaters such as effluents from tannery industries (Lefebvre et al., 2006), seafood-processing (Omil et al., 1995) and oil and gas production (Ji et al., 2009) have been studied, solutions to the problem of inhibitory high sodium salts are still limited. One way of tackling the sodium salts problem, is by allowing the anaerobic sludge to acclimate to high sodium concentrations (Vyrides and Stuckey, 2009), but this technique requires time for the methanogens to adapt to the saline conditions which in turn results in a prolonged period before the anaerobic reactor can achieve its full-loading capacity. Mendez et al. (1995) stated that a start-up period of 9 months was required for the adaptation of anaerobic sludge to effectively treat saline seafood-processing wastewater. The use of halophilic methanogens as an inoculum has also been reported as an approach to deal with high sodium salts problems (Riffat and Krongthamchat, 2007). However, in a practical sense, it may be difficult to obtain halophilic methanogens for anaerobic reactors located far from the sea. One possible organic compound, which can cause

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antagonism against sodium toxicity, is glycine-betaine (GB) (Yerkes et al., 1997; Vyrides et al., 2010). GB $(CH_3)_3N^+CH_2COO^-$), also known as betaine, is a trimethylated derivative of glycine (Rudulier and Bouillard, 1983). GB is one of the "compatible solutes" involved in osmoregulation at high osmotic pressure in many plants (Oishi and Ebina, 2005) and halophilic methanogens (Robertson et al., 1990; Lai and Gunsalus, 1992). Compatible solutes are soluble organic compounds, not involved in normal cell metabolism (Yerkes et al., 1997), although high concentrations of these solutes are accumulated within bacterial cells that are under salt stress. However, using GB to decrease sodium toxicity in commercial-scale anaerobic digesters would be too costly.

This study originated from observations that thermophilic anaerobic digestion can be started up successfully in the presence of high sodium bicarbonate concentrations (330 mM) added to avoid early build-up of volatile fatty acids (Suwannoppadol et al., 2011). It therefore appears that factors present in this type of digestions mitigate the usually observed inhibition by salt. To test if plant material (grass leaves) contributes to overcoming salt inhibition, thermophilic digestion of acetate was carried out with turf soil as source of methanogens and grass leaves as co-substrate. Moreover, compounds present in grass leaves such as betaine and potassium were investigated as a possible cause of overcoming sodium toxicity.

2. Methods

2.1. Inoculum sources and grass leaves

Turf soil samples were used as source of methanogens (Suwannoppadol et al., 2012). Leaves and soil were collected from a grassy area at Murdoch University, Perth, Australia. After removing grass leaves and the main grass roots the soils were used immediately as thermophilic anaerobic inoculum. All experiments were carried out in 100 mL serum vials with 10 or 15 g of turf soil and 40 or 50 mL of culture medium described in Section 2.2. One-hundred grams per liters of fresh grass leaves was added to test for reduction of sodium toxicity where applicable. For mesophilic tests, 40 mL of mesophilic anaerobic sludge (soluble chemical oxygen demand = 1541 mg/L, total solids = 32.3 g/L, total suspended solids = 29.0 g/L, volatile solids = 24.3 g/L, and volatile suspended solids = 24.2 g/L) was used as inoculum and the final working volume was adjusted to 60 mL with culture medium. Mesophilic anaerobic sludge was collected from the Woodman Point Wastewater Treatment Plant treating municipal wastewater located in Perth, Western Australia.

2.2. Culture medium composition and carbon source

To adjust the working volume of serum vial, culture medium, sterilized grass juice (chemical oxygen demand 3331 mg/L or filtered grass juice were used. When necessary, the pH of culture medium was adjusted to 7.5 ± 0.2 with 1 M HCl.

The culture medium contained (per liter): 0.3 g KH_2PO_4, 0.6 g NaCl, 0.1 g $MgCl_2{\cdot}2H_2O$, 0.08 g $CaCl_2{\cdot}2H_2O$, 1.0 g NH_4Cl, 3.5 g $KHCO_3$, 10 mL of vitamin solution, and 5 mL of trace element solution. Vitamin solution contained (per liter): 2.0 mg biotin, 2.0 mg folic acid, 10.0 mg pyridoxine hydrochloride, 5.0 mg thiamin hydrochloride, 5.0 mg riboflavin, 5.0 mg nicotinic acid, 5.0 mg DL-calcium pantothenate, 0.1 mg vitamin B_{12}, 5.0 mg *p*-aminobenzoate, and 5.0 mg lipoic acid. Trace element solution contained (per liter): 12.8 g nitrilotriacetic acid, 1.35 g $FeCl_3{\cdot}6H_2O$, 0.1 g $MnCl_4{\cdot}H_2O$, 0.024 g $CoCl_2{\cdot}6H_2O$, 0.1 g $CaCl_2{\cdot}2H_2O$, 0.1 g $ZnCl_2$, 0.025 g $CuCl_2{\cdot}2H_2O$, 0.01 g H_3BO_3, 0.024 g $Na_2MoO_4{\cdot}4H_2O$, 1.0 g NaCl, 0.12 g $NiCl_2{\cdot}6H_2O$, 4.0 mg $Na_2SeO_3{\cdot}5H_2O$, 4.0 mg $Na_2WO_4{\cdot}2H_2O$.

2.3. Preparation of sterilized grass leaves, sterilized grass juice, filtered grass juice, and ash from grass leaves

Mix-species of grass leaves were collected at least 2 cm above the soil profile to minimize contamination by the soil. To prepare sterilized grass leaves, 5 g of grass leaves were autoclaved at 120 °C for 40 min.

To prepare sterilized grass juice (chemical oxygen demand = 3331 mg/L), 5 g of fresh grass leaves and 50 mL of culture medium were blended with a mechanical blender (DeLonghi, model DBL740) for 15 min. The grass residue was removed from the grass juice by filtering through cloth with pore size of 1 mm and the filtrate was sterilized by autoclaving at 120 °C for 40 min or by filtration through a 0.2 μm filter paper (Whatman).

To prepare a solution of ash from grass leaves, 5 g of grass leaves was combusted in a furnace at 550 °C for 24 h. The ash was dissolved in 50 mL of culture medium and neutralized by addition of 1 M HCl.

2.4. Experimental design

Experiments were conducted in duplicate 100 mL serum vials (Wheaton) sealed with butyl rubber stoppers and aluminum crimps at 55 °C or the mesophilic experiment at 37 °C. To establish anaerobic conditions, the headspaces of all serum vials were flushed with N_2/CO_2 (80/20%) for 30 s. All samples were incubated in a water bath (Paton, model RW 1812) with shaking (30 oscillations/min). All serum vials were depressurized to atmospheric pressure after the first hour of incubation. The volume of biogas produced was measured using a 50 mL glass syringe (Popper & Sons, Inc.).

2.5. Analysis

For VFA analysis, 0.25 mL of liquid sample was removed via syringe through the rubber seal of the serum test vials. The supernatant was centrifuged at 12,000 rpm for 5 min (Hermle Z233M2) to obtain a clear solution. The supernatant was used for VFA analysis or stored at −20 °C. The VFA concentrations of samples were analyzed by gas chromatography (GC) using a Varian Star 3400 equipped with a Varian 8100 auto sampler and a flame ionization detector (FID) as described by Walker et al. (2009).

The methane concentration in biogas was analyzed with a Varian Star 3400 gas chromatograph equipped with a thermal conductivity detector as described by Charles et al. (2009).

Betaine in grass leaves was analyzed by ChemCentre, Perth, Western Australia, a nationally accredited analytical laboratory. Betaine present in grass leaves was analyzed by using a combination of liquid chromatography and mass spectrometry (LC–MS). For grass extraction, 0.25 g of grass leaves sample was extracted with 10 mL of 50% methanol. After filtration, the extracted grass solution was transferred to HPLC for LC–MS analysis. HPLC was done with a Zorbax Eclipse Plus C column (18 4.6 × 50 mm, 1.8 μm) and a pump system (Agilent 1260 Infinity Binary Pump System). The mobile phase consisted of 10 mM ammonium formate pH 3 (A) and acetonitrile (B) at an isocratic with 10% acetonitrile for 10 min. MS analyses were carried out on Triple Quad LCMS operating in electro-spray ionization (ESI) mode. Analytical data was acquired in Multiple Reaction Monitoring (MRM) mode (precursor ion 118, product ion 59, positive ion mode). After the LC and MRM protocols were selected, standard curves were determined. Five different concentrations of betaine were prepared and

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