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# Optimization of micronutrient supplement for enhancing biogas production from food waste in two-phase thermophilic anaerobic digestion

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#### ABSTRACT

The aim of this study was to enhance the biogas productivity of two-phase thermophilic anaerobic digestion (AD) using food waste (FW) as the primary substrate. The influence of adding four trace metals (Ca, Mg, Co, and Ni) as micronutrient supplement in the methanogenic phase of the thermophilic system was investigated. Initially, Response Surface Methodology (RSM) was applied to determine the optimal concentration of micronutrients in batch experiments. The results showed that optimal concentrations of 303, 777, 7 and 3 mg/L of Ca, Mg, Co and Ni, respectively, increased the biogas productivity as much as 50% and significantly reduced the processing time. The formulated supplement was tested in continuous two-phase thermophilic AD system with regard to process stability and productivity. It was found that a destabilized thermophilic AD process encountering high VFA accumulation recovered in less than two weeks, while the biogas production was improved by 40% yielding 0.46 L CH<sub>4</sub>/gVS<sub>added</sub>/day. There was also a major increase in soluble COD utilization upon the addition of micronutrient supplement. The results of this study indicate that a micronutrient supplement containing Ca, Mg, Co and Ni could probably remedy any type of thermophilic AD process.

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#### 1. Introduction

Food waste (FW) is one of the largest components of municipal solid waste (MSW) in most developed countries. Approximately 36.4 and 89 million tonnes of FW are generated annually in USA and EU, respectively (USEPA, 2012; EU, 2010). In developing countries such as China, FW forms up to 50% of the total MSW and it is expected to increase drastically in the future (Dai et al., 2013). Ineffective management of FW can cause many environmental issues such as hygiene and odour problems, greenhouse gas emissions, pollution, and loss of resources (Zhang et al., 2014). Food waste composition is highly variable depending on the source with characteristic high moisture content of 74-90%, VS/TS ratio of 80-97%, and C/N ratio of 14.7-36.4 (Miller and Clesceri, 2003). This makes FW easily biodegradable and good source for renewable energy production through bioconversion. Considering energy security, world hunger and environmental impacts, it would be more accurate to call FW a misplaced resource rather than waste.

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http://dx.doi.org/10.1016/j.wasman.2016.10.017 0956-053X/© 2016 Elsevier Ltd. All rights reserved. Anaerobic digestion (AD) is a promising approach for the bioconversion of FW into biogas with relatively low energy consumption, space requirement and cost (Speece, 1983). The two main groups of microorganisms (acidogens and methanogens) involved in AD differ widely in their nutritional needs, growth kinetics and environmental requirements. Any imbalance among them could cause process failure (Demirel and Yenigün, 2002). In particular, AD suffers with easily biodegradable substrate like FW due to enhancement of acidogenic stage (Ward et al., 2008). The scarcity of essential micronutrients, the presence of inhibitory agents such as long chain fatty acids (LCFA) and the excess of ammonia are some other factors that could further limit AD process (Chen et al., 2008).

Phase separation can improve the AD process through optimization of the acidogenic and/or the methanogenic stages (Jiang et al., 2012). The two-phase AD process mitigates imbalance and overloading, while higher organic loading rates (OLRs) or biogas productivity could be achieved (Shen et al., 2013). Thermophilic AD (carried out at 50–60 °C) is another operation mode to increase biogas productivity. The higher temperatures improve hydrolysis and physical degradation of the substrate, increase destruction of organic matter and enhance methane yields (Kim, 2004).

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Recently, there has been a spotlight on the role of certain trace metals or micronutrients in AD process. The micronutrients play significant role in several metabolic pathways in AD, however, their concentration is usually inadequate to affect them (Schattauer et al., 2011; Zhang et al., 2007). Micronutrients of particular interest are cobalt nickel, iron, magnesium and calcium. These micronutrients are essential for a variety of chemical, biochemical and microbiological reactions related to VFA utilization, methane generation and cell lysis. Cobalt and nickel form a part of carbon monoxide dehydrogenase (CODH), which plays an essential role in methanogenesis from acetate (Madigan et al., 2015; Zandvoort et al., 2006). Nickel is also found in the cofactor coenzyme F430 (Takashima and Speece, 1990), which plays an important role in autotrophic methanogenesis. Iron is part of cytochromes and ferredoxin in methylotrophic methanogens, and as iron-sulphur proteins in some enzymes (Takashima and Speece, 1990). Magnesium is known to stimulate the production of single cells (Schmidt and Ahring, 1993), which are required for avoiding the loss of aceticlastic activity in anaerobic reactors. Calcium can address the problem of overacidification in AD due to long chain fatty acid inhibition by forming insoluble salts with LCFAs (Kleybocker et al., 2012).

The micronutrients operate at the metabolic level, and their requirement is independent of source of the substrate. As such, micronutrient supplementation has been shown to be important for stabilizing and optimizing biogas production from food waste (Zhang and Jahng, 2012; Wei et al., 2014). While some research has been conducted to formulate a micronutrient supplement for use in AD, little has been done towards tailoring a micronutrient supplement for enhancing thermophilic AD of food waste.

Usually, the micronutrient supplement is externally added in AD system (Facchin et al., 2013). However, it is imperative to determine the optimal levels as overdose can lead to excess cost and eco-toxicity. Response surface methodology (RSM), a collection of mathematical and statistical techniques that can be used for analysing the effects of such independent variables and optimize their effect on a single response (Box and Draper, 1987). RSM can be applied in Design of Experiments to improve process design and optimize the response of two or more variable without knowing the relationship between the factors. The objective of using RSM is to determine the optimal operating conditions for the system to achieve a required output. Hence, RSM has been used to optimize various factors in anaerobic digestion such as substrate mixing ratio, feeding rate, C/N ratio and substrate pretreatment (Kim, 2004; Wang et al., 2012; Menon et al., 2015).

In this study, a micronutrient supplement containing Ca, Mg, Co, and Ni was formulated to improve methane generation in two-phase thermophilic AD system using food waste as substrate. RSM was initially applied to optimize the concentration of Ca, Mg, Co, and Ni in batch experiments. The efficiency of the formulated supplement was tested in continuous mode thermophilic AD system considering process stability and biogas yield.

#### 2. Materials and methods

#### 2.1. Substrate and inoculum

The primary substrate was FW collected from a canteen in Nanyang Technological University, Singapore. It mostly consisted of leftovers containing rice, noodles, fruit peels, boiled vegetables, cooked meat and oil. Non-biodegradable materials such as bones or plastics were manually separated. Afterwards, the FW was grinded, and filtered through a fine wire sieve, creating a paste with particles smaller than 1 mm in diameter which was then diluted with water to the concentration of 3 g VS/L. This was used as feedstock in the acidogenic phase of thermophilic AD process. The effluent from the acidogenic phase was used as substrate in the methanogenic phase of AD without any pre-treatment or dilution. Table 1 presents the main characteristics of FW and acidogenic effluent.

The original seed inoculum was mesophilic AD sludge that obtained from Ulu Pandan Water Reclamation Plant, Singapore. Its conversion to thermophilic sludge was done using a method described by de la Rubia et al. (2005) with some changes. Briefly, a 20 L CSTR bioreactor was operated with organic loading rate (OLR) of 0.5 g VS/L/day FW as substrate and hydraulic retention time (HRT) of 30 days for a period of 2 weeks at mesophilic conditions. The temperature was then directly raised to thermophilic conditions (55 °C) using heating jacket and after a period of starvation for 2 days, the reactor was fed with 0.5 g glucose/L/day for 2 weeks, while other conditions remained unchanged. The substrate was then changed to FW with an OLR of 0.5 g VS/L/day. Once biogas production and solid destruction were stabilized, a twophase thermophilic AD system was adopted with an OLR of 3 g VS/L/day (more details in Section 2.3). The effluent from the methanogenic phase was collected and used as inoculum in the batch experiments.

#### 2.2. Micronutrient supplement

The concentration of micronutrients (Ca, Mg, Co, and Ni) was measured by the quantity of individual component typically found in food waste and the actual requirement in AD process. The maximum concentrations were chosen based on extensive literature review and preliminary experiments. As such, the maximum concentrations used were 500 mg/L for Ca, 1000 mg/L for Mg (Lo et al., 2010), 10 mg/L for Co (Pobeheim et al., 2010) and 5 mg/L for Ni (Takashima and Speece, 1990). All trace metals were added as dissolved salts (CaCl<sub>2</sub>·4H<sub>2</sub>O, MnCl<sub>2</sub>·4H<sub>2</sub>O, CoCl<sub>2</sub>·6H<sub>2</sub>O, NiCl<sub>2</sub>·6H<sub>2</sub>-O) in the feed for the methanogenic phase. In addition, Visual Minteq, a chemical equilibrium model was applied to calculate the metal speciation and solubility equilibria.

#### 2.3. Experimental setups

The optimal concentration of micronutrient supplement was determined using biochemical methane potential (BMP) tests. The substrate to inoculum ratio (SIR) was maintained at 0.3 (based on VS) (Angelidaki et al., 2009). The BMP tests were conducted in the Automated Methane Potential Testing System (AMPTSII) (Bioprocess Control, Sweden). AMPTS consisted of 15 parallel reactors of 500 mL capacity (working volume of 400 mL), and the same

Fable 1								
Characteristics	of food	waste	original	feedstock	and	acidogenic	AD	effluent

Parameter	FW (3 g VS/L)	Acidogenic effluent
рН	5.1	4.2
ORP (mV)	-59	-388
TS (%)	3.2	2.1
VS/TS (%)	93.51	74.54
Acetate (mM)	1.22	40.17
Propionate (mM)	0.15	1.86
Butyrate (mM)	0.05	15.52
TVFAs (mM)	1.98	59.69
CODt (g/L)	33.55	28.26
CODs (g/L)	8.95	16.57
Ca (mg/L)	13.3	36.2
Co (mg/L)	2.27	0.84
Fe (mg/L)	51.79	16.25
Mg (mg/L)	15.8	5.18
Ni (mg/L)	14.18	n.d

n.d. - not detected.

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