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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 CH₄/gVS_{added}/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.

Keywords: Micronutrients; Thermophilic AD; Response surface methodology; Biogas

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.

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).

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., 2003; 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.

Table 1 Characteristics of food waste original feedstock and acidogenic AD effluent.		
Parameter	FW (3 g VS/L)	Acidogenic effluent
pH	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.

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 two-phase 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 (CaCl₂·4H₂O, MnCl₂·4H₂O, CoCl₂·6H₂O, NiCl₂·6H₂O) 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 number of gas flow meters was connected to the acquisition system. The reactors were sealed with rubber stoppers and then connected to mechanical agitator which stirred the sludge for 1 min on and off at 80 rpm. The reactors were sparged with nitrogen gas before sealed to create anaerobic conditions and incubated at 55 °C. The gas produced was monitored using automated data logging system. Three separate runs were carried out – Run 1: Ca-Mg mixture (high concentration metals); Run 2: Co-Ni mixture (low concentration metals); and Run 3: optimized micronutrient supplement. Run 1 and 2 were conducted in duplicate, while Run 3 in triplicate. All experiments lasted for 20 days.

The effect of the formulated micronutrient supplement on methane production was further studied in continuous two-phase thermophilic AD system consisting of a 10 L reactor for hydrolysis and acidogenesis, and a 20 L reactor for methanogenesis (Winpack, USA). The reactors were monitored for pH, ORP and biogas production. Both reactors were maintained at 55 °C using heating jackets, while the HRT was 5 and 15 days for acidogenic and methanogenic phase, respectively. The feedstock in the acidogenic phase was FW with OLR 3 g VS/L/day and pH 4.5 ± 0.3. The effluent from the acidogenic phase was the feedstock in the methanogenic phase. Before the micronutrient supplementation, the two-phase AD system was at stable operation for 6 months, but at later stage the methanogenic phase experienced some VFA accumulation and the methane production dropped.

2.4 Response Surface Methodology (RSM)

A central composite design with two factors was used to design the experiments in the batch studies. Methane production was chosen as the response variable and the concentrations of Ca, Mg, Co and Ni were chosen as the independent variables. This design required the use of three levels of the individual variables - a lower limit 1), an upper limit (1) and the centre point (0). The lower limit was chosen 0 mg/L for every element and the upper limit was chosen based on literature review and preliminary experiments as explained in Section [2.2](#). Hence, the three concentrations used for each micronutrient were: 0, 250 and 500 mg/L for Ca²⁺; 0, 500 and 1000 mg/L for Mg²⁺; 0, 5 and 10 mg/L for Co²⁺; and 0, 2.5 and 5 mg/L for Ni²⁺. The micronutrients were divided into two groups for evaluation on the basis of their similarity and synergistic effect: Ca-Mg (similar chemical characteristics, required in larger concentrations), and Ni-Co (both involved in methanogenesis and substrate utilization, required in lower concentrations). The statistical analysis software MINITAB 17 (SBTI, San Marcos, Texas) was used for this purpose.

2.5 Analytical techniques

The pH-readings in the batch experiments were conducted with a compact titrator (Mettler Toledo) equipped with a pH probe (Mettler Toledo DGi 115-SC). Total (CODt) and soluble (CODs) chemical oxygen demand measurements were made using COD digestion vials (Hach) and a spectrophotometer DR2800 (Hach, USA) as per standard methods (APHA, 1995). Ammonia was measured colorimetrically using a DR2800 spectrophotometer (Hach, USA). The micronutrient content in the AD effluent was analysed using Inductively Coupled Plasma-Mass Spectrometer (ICP-MS NexION 300D, Perkin Elmer). Volatile fatty acids (VFAs) content was measured using a gas chromatograph (Agilent Technologies 7890A, USA), equipped with a flame ionization detector (FID) (Agilent Technologies, USA). A selective capillary column (DB-FFAP) was applied for good separation of nine VFA compounds including acetic, propionic, *iso*-butyric, *n*-butyric, *iso*-valeric, *n*-valeric, *iso*-caproic, *n*-caproic and heptanoic acids. A gas chromatograph (Agilent Technologies 7890 A, USA) equipped with a thermal conductivity detector (TCD) was used to measure the methane, hydrogen and carbon dioxide content in biogas. The C/N ratio of FW was determined by measuring the total carbon and nitrogen using an elemental analyzer (Vario EL cube CHNOS, Germany).

3 Results and discussion

3.1 Micronutrient availability

The bioavailability of micronutrient elements was determined using the Visual Minteq software. Table S1 shows the concentration of the species used to model the metal speciation analysis. Normal methanogenic conditions of pH = 7, ORP = -500 mV and ammonia, carbonate and phosphate species were used. For the determination of bioavailability the maximum concentration of trace metals was used (Ca: 500 mg/L, Mg: 1000 mg/L, Co: 10 mg/L, Ni: 5 mg/L). Table S2 shows the results of the speciation analysis for trace metal ions. 94% and 91.6% of added Ca and Mg were predicted to remain in solution as Ca^{2+} and Mg^{2+} ions, respectively, while 92% of Co was predicted to remain in the form of bioavailable Co^{2+} ions. 83.3% of Ni (added in the smallest amount) was predicted to be as Ni^{2+} ions, while approximately 15% of Ni was predicted to remain in soluble ammonium and phosphate complexes. Therefore, all trace metals would be mostly in bioavailable forms, and the AD system would not suffer with accumulation of precipitants (insoluble crystals) that inhibit biogas production and cause toxic effects.

3.2 Optimization of micronutrient mixtures

3.2.1 Run 1: Ca-Mg mixture

Fig. 1 presents the effect of Ca-Mg mixture on methane production. It seems that the addition of micronutrients had a positive effect in methane production. More specifically, the addition of micronutrients not only increases the production, but it accelerated the production rates as well. This suggests that the trace elements Ca-Mg were at suboptimal concentrations in the AD system and their addition was necessary for better process performance. The production of methane was increased by 30-60% at various dosages of the micronutrients, with the combination of 250 mg/L Ca^{2+} and 500 mg/L Mg^{2+} showing the highest yield.

Fig. 1 Effect of Ca-Mg mixtures on methane production.

The RSM analysis of Ca-Mg concentrations for optimized methane production is shown in Fig. 2 in the form of three dimensional response surface of the predicted methane production. The regression equation used for the model was:

$$\text{CH}_4 = 1170 + 4.178\text{Ca} + 1.381\text{Mg} - 0.00583\text{Ca} * \text{Ca} - 0.000720\text{Mg} * \text{Mg} - 0.000861\text{Ca} * \text{Mg}$$

(1)

Fig. 2 Three-dimensional response surface of the effects of Ca-Mg supplements on methane production.

Fig. S1 shows the 2D surface graphs for optimizing Ca-Mg concentrations for maximum methane production. The predicted optimal concentrations for Ca and Mg were 303 and 777 mg/L, respectively, which could theoretically produce 2.3 L of methane in a 20 days batch experiment, leading to a 75% increase in production.

Table 2 shows the ANOVA results for the response surface analysis of the addition of Ca-Mg mixture for optimizing methane production. Based on the significant strong relationship between the model and the response variable (p-value <0.005), the high R-squared and R-squared (predicted) values of 93.31% and 88.53%, respectively, and the signifying acceptable goodness-of-fit statistics, the model could reliably be used to predict the effect of addition of Ca-Mg mixture on methane production. In the regression model, the p-values of the both Ca-Mg linear coefficients were highly significant (<0.005), signifying that both had a large effect on methane production. The squared value of Ca*Ca was highly significant (p-value <0.005) and the squared value of Mg*Mg was significant (<0.05), which suggests that both elements were important for methane production, but calcium was more important among the two. On the other hand, the Ca-Mg had a high p-value (>0.05), which suggests that the synergistic effect of Ca-Mg on methane production was not that prominent. This was expected as both micronutrients affect different metabolic pathways. Ca and Mg are similarly important as activators of various enzymes which are important for the growth and functioning of bacterial communities, but the primary role of Ca is to inhibit the toxicity of long chain fatty acids (Kleybocker et al., 2012). Long chain fatty acids (LCFAs) are the major inhibitors for biogas production (Kuang et al., 2006; Luostarinen et al., 2009), when over acidification happens. The role of Mg is to stimulate the production of single cells (Harris, 1987; ~~Xun et al., 1988~~; Schmidt and Ahring, 1993). The high sensitivity of single cells (which are necessary for catabolizing acetic acid) to lysis is an important factor in the loss of aceticlastic activity in anaerobic reactors. This problem is heightened in thermophilic reactors as cell lysis is accelerated.

Table 2 ANOVA results for response surface analysis of addition of Ca and Mg mixture.

Source	DF	Adj SS	Adj MS	F-value	P-value
Model	5	1,299,958	259,992	19.53	0.001
Linear	2	558,910	279,455	20.99	0.001
Ca	1	260,625	260,625	19.57	0.003
Mg	1	298,285	298,285	22.4	0.002
Square	2	694,758	347,379	26.09	0.001
Ca*Ca	1	366,375	366,375	27.52	0.001
Mg*Mg	1	89,551	89,551	6.73	0.036
2-Way interaction	1	46,290	46,290	3.48	0.105
Ca*Mg	1	46,290	46,290	3.48	0.105
Error	7	93,201	13,314		
Lack-of-fit	3	87,812	29,271	21.73	0.006
Pure error	4	5389	1347		
Total	12	1,393,159			
R-sq	R-sq (adj)		R-sq (pred)		
93.31%	88.53%		37.66%		

It is difficult to evaluate the derived data with other studies as very few were conducted to determine the optimal concentrations of these micronutrients for thermophilic AD of food waste. The results agreed in some extent with similar studies as the optimal concentrations determined here fell squarely within the suggested concentrations for Ca and Mg (Takashima and Speece, 1990).

3.2.2 Run 2: Co-Ni mixture

Fig. 3 shows the methane production after adding mixtures of Co (0, 5 and 10 mg/L) and Ni (0, 2.5 and 5 mg/L). Similar to Run 1, the addition of micronutrients greatly increased methane production of thermophilic AD process. The enhancement was between 35% and 40% over control. Fig. 4 shows the RSM based on optimization of Co-Ni concentrations for methane production. The quadratic equation used for this model was:

CH₄ = 1131.0 + 52.2Co + 158.2Ni – 2.13Co * Co – 17.23Ni * Ni – 6.59Co * Ni

(2)

Fig. 3 Effect of Co-Ni mixtures on methane production.

Fig. 4 Three-dimensional response surface of the effects of Co-Ni supplements on methane production.

Fig. S2 shows the 2D plots of Co-Ni concentrations optimized for maximizing methane production. The optimized combination was 7 mg/L Co^{2+} and 3 mg/L Ni^{2+} , which could theoretically increase biogas yield by 45%.

Table 3 shows the detailed ANOVA data of the addition of Co-Ni for optimizing methane production. The relationship between the model and the response variable (methane generation) was highly significant (p -value < 0.005),

while the high R-squared value (>93%) suggests that the model had considerable goodness-of-fit statistics. Hence, this model could be safely used to predict the relationship between the concentrations of Co-Ni and methane production. The effect of addition of both micronutrients was highly significant for methane generation (p-value <0.005). The square term of Ni was highly significant (p-value <0.005), while Co was only significant at low p-value (p < 0.1). This implies that Ni addition was extremely important for increasing biogas production, while Co was still important but not that critical. Moreover, the addition of both micronutrients was significant (p < 0.05), suggesting that Co-Ni had an important synergistic effect. This is because both elements are directly involved in metabolic processes relevant to methanogenesis. Co is a key element of vitamin B12, which is involved in the activity of methyl transferase required for the transfer of methyl groups from compounds including methanol in methanogenesis (Madigan et al., 2003). Co is also found in carbon monoxide dehydrogenase (CODH), which plays an essential role in methanogenesis from acetate (Madigan et al., 2003, Zandvoort et al., 2006). CODH also requires Ni, which is found in cofactor F430 in methyl-coenzyme M reductase (MCR) (Takashima and Speece, 1990). F430 is a porphinoid (Shima et al., 2002) that plays a role in autotrophic methanogenesis, making Ni especially important when H₂ and CO₂ are the sole sources of energy. Ni is at the catalytic centre of most hydrogenases (Shima et al., 2002) and may be involved in membrane stability (Jarrell and Sprott, 1982).

Table 3 ANOVA results for response surface analysis of addition of Co and Ni mixture.

Source	DF	Adj SS	Adj MS	F-value	P-value
Model	5	176,639	35327.8	19.16	0.001
Linear	2	88,849	44424.7	24.1	0.001
Co	1	31,284	31284.3	16.97	0.004
Ni	1	57,565	57565.2	31.23	0.001
Square	2	60,688	30,344	16.46	0.002
Co*Co	1	7814	7814.1	4.24	0.079
Ni*Ni	1	32,016	32015.6	17.37	0.004
2-Way interaction	1	27,101	27101.4	14.7	0.006
Co*Ni	1	27,101	27101.4	14.7	0.006
Error	7	12,904	1843.5		
Lack-of-fit	3	12,644	4214.5	64.66	0.001
Pure error	4	261	65.2		
Total	12	189,543			
R-sq	R-sq (adj)		R-sq (pred)		
93.19%	88.33%		32.60%		

It should be mentioned that there are no similar studies, but the findings here are comparable with those reported by Pobeheim et al. (2010). The suggested concentration of Co agrees with the range mentioned in the current study, while Ni is higher by an order of magnitude. This is due to the mesophilic conditions and different substrates that were applied.

3.3 Formulation of micronutrient supplement

A micronutrient supplement was formulated with Ca, Mg, Co and Ni containing 303, 777, 7, and 3 mg/L, respectively, based on the previous findings. Research data on the effect of formulated micronutrient supplement, optimized Ca-Mg mixture, and optimized Co-Ni mixture in methane production are presented in Fig. 5. It seems that the synergistic effect of micronutrient supplement greatly enhanced the thermophilic AD process compared to individual mixtures. The addition of micronutrient supplement was highly significant (p < 0.005) in improving methane production. The total methane production was 1722 mL, an increase of 2.7 times over control. There was also

significant improvement ($p < 0.05$) in the production in comparison to the addition of individual mixtures (Ca-Mg and Co-Ni, respectively) with at least 20% increase in total methane production. The batches with the individual mixtures (Ca-Mg, Co-Ni) had similar production implying that the benefits of adding both group of elements were equivalent. The gas production profiles, however, presented some differences. The batch fed with the Ca-Mg mixture appeared a lag phase likely resulted from the improvements in cellular stability and inhibition as described earlier in Section 3.2. The batch fed with Co-Ni had a shorter lag phase, which could be attributed to the metabolic enhancements in methane generation pathways. The combined benefits of the two groups as micronutrient supplement was not only the higher production, but also a major reduction in processing time. It took less than 5 days for 85% of the total methane production.

Fig. 5 Methane production using the formulated micronutrient supplement, and mixtures of Ca-Mg, and Co-Ni.

Table 4 presents the effluent characteristics (VFAs, TS removal, total and soluble COD, and pH) at the end of the batch experiments. The experiment with the formulated micronutrient supplement showed a significant reduction in effluent VFAs and COD and showed a minor increase in TS destruction compared to experiments with individual mixtures and control. More specifically, the residual VFAs in the batch with micronutrient supplement showed 28-30% and 45% reduction compared to Ca-Mg and Co-Ni mixtures and control, respectively. The addition of formulated micronutrient supplement greatly enhanced VFA utilization, which contributed to improved methane production and decreased acidification. The latter was apparent in the noticeably higher pH of the batch with formulated micronutrient supplement (7.8 compared to 7.1 in control). There was an almost 3-times reduction in CODs on addition of the micronutrients, again pointing to improved substrate utilization. There was a minor increase in TS destruction (76.8% in micronutrient supplement vs. 72.5 in control), while similar solid destruction presented the other batches. Regarding trace metals residual concentration, it was found that more than 95% of elements in micronutrient supplement were utilized by the microbial activity (data not shown). This suggests that minor accumulation of precipitates was possible, which could be toxic in long run, and it could also affect the economic viability and sustainability of micronutrients addition.

Table 4 Effluent characteristics after micronutrients addition.

	VFA (mM)	TS reduction (%)	CODs (g/L)	CODt (g/L)	pH
Micronutrient supplement	1.8	76.8 ± 0.03	3.03 ± 0.04	18.14 ± 0.14	7.8
Ca-Mg mixture	2.3	74.7 ± 0.12	7.80 ± 0.06	22.58 ± 0.16	7.4
Co-Ni mixture	2.5	76.8 ± 0.04	6.97 ± 0.02	24.36 ± 0.04	7.5
Control	3.1	72.5 ± 0.14	8.36 ± 0.09	26.56 ± 0.15	7.1

3.4 Effect of micronutrient supplement on continuous two-phase thermophilic AD

The formulated micronutrient supplement was tested in the methanogenic phase of two-phase thermophilic AD system. Fig. 6a illustrates the methane yield in the methanogenic reactor. The two-phase thermophilic AD process was carried out without any micronutrient supplementation for 150 days. The methane yield averaged 0.3–0.35 L CH₄/gVS_{added}/day till day 80, when there was some sludge wash-out. This caused temporary reduction in methane productivity and afterwards it fluctuated due to unstable conditions. At day 150, the methane yield reached the lowest value 0.2 L CH₄/gVS_{added}/day. At this point, the micronutrient supplement was added to the reactor. This was done by preparing a 20× stock solution in distilled water and adding it to the feed (effluent from the acidogenic phase). The concentration of the various trace metals in the methanogenic phase was adjusted to the formulated micronutrient supplement.

Fig. 6 Monitoring the methanogenic phase of continuous thermophilic AD process, (a) methane yield; (b) pH and ORP variations.

The methane productivity was enhanced continuously for several weeks until the trace elements depleted. The micronutrient supplement was added again on day 170 and 190 to maintain the optimal concentration of the micronutrients in the methanogenic reactor. The methane production reached the ultimate yield 0.46 L CH₄/gVS_{added}/day, which was nearly twice the production before the addition of the supplement. The methane production stabilized at around 0.43 L CH₄/gVS_{added}/day, which was 40% increase over the stable methane production in the early days of the AD system. This indicates that the micronutrient supplement not only increased the methane productivity, but also stabilized the failing thermophilic AD process.

The methanogenic reactor was also monitored for pH and ORP variations (Fig. 6b). Similar to methane yield, pH and ORP showed fairly stable profiles till day 80, after which some destabilization appeared. The pH dropped below 6 after day 110, which was at suboptimal level for methanogenesis. The pH decrease signified possible VFA accumulation. ORP presented similar trend (at suboptimal level), with a lot of destabilization after day 110. However, upon the addition of the micronutrient supplement on day 150, both pH and ORP quickly recovered. They reached optimal and stable levels again within two weeks and remained at those levels for the duration of the experiment.

As mentioned previously, the effect of formulated micronutrient supplement can be compared to other studies with similar substrates. Climenhaga and Banks (2008) used a micronutrient recipe described by Gonzales-Gil et al. (2000), which consisted of 2000 mg/L of CoCl₂·6H₂O and 142 mg/L of NiCl₂·6H₂O amongst other trace metals such as Fe, Al, Zn, Cu, Mn, etc. to stabilize methane production from single stage mesophilic AD of catering wastes in semi-CSTR. In that study, the AD process presented partly failure and stopped producing biogas after 60 days of operation at 25 day HRT, while the micronutrient supplementation allowed for stable methane yield at 0.4–0.5 L/gVS_{added}/day. In the current study, the methane yield was stabilized at similar levels, but less micronutrients (4 opposed to 10) was used and at much lower concentrations. The optimal concentrations could also help to minimize the additional cost and reduce potential toxicity. Furthermore, Zhang and Jahng (2012) used trace metal supplementation to stabilize methane production from food waste in CSTR reactor. A mixture of Co, Fe, Mo and Ni (2, 100, 10 and 5 mg/L,

respectively) was shown to stabilize methane yield at 0.3–4.3 L/gVS_{added}/day, consistent with the results found in this study. Although all studies had different substrates and reactor setups, an improvement in substrate utilization and methane production was noticed on the addition of trace metals.

Fig. 7a shows the influent and effluent VFAs concentration of the methanogenic reactor. Although the concentration of VFAs in the influent was nearly constant, there was a major build-up of VFAs in the reactor (during destabilization stage). By the day 150, the amount of VFAs was nearly doubled compared to initial stage of the AD process. This caused pH drop (as seen in Fig. 6) and hence reduced methane production. After micronutrient supplement addition on day 150, there was a drastic increase of VFAs utilization and methane production indicating the direct impact of micronutrient availability. The VFAs concentration was further reduced after the second addition of the supplement and stabilized by the time of the third addition. The VFAs concentration presented 50% reduction compared to destabilization stage. These results could be particularly attributed to the addition of Ca and Co. Calcium is well known to play an important role in cell permeability (Chen et al., 2008) as well as enhance the action of other metals (Oleszkiewicz and Sharma, 1990). Cobalt addition is particularly important in improving VFA utilization (Zandvoort et al., 2006; Gallert and Winter, 2008) and for acting synergistically with Ni for VFA uptake and enhancing methanogenic activity (Murray and van den Berg, 1981).

Fig. 7 Methanogenic reactor monitoring, (a) influent/effluent VFAs; (b) influent/effluent soluble COD; (c) solids destruction.

Fig. 7b shows the influent and effluent CODs variations of the methanogenic reactor. While the effluent CODs was fairly constant till day 150, varying between 6 and 8 g/L, a drastic reduction was seen after the addition of the micronutrient supplement. After the first addition, the CODs levels dropped rapidly to 3 g/L and remained around 2 g/L after the second and third addition. This is comparable with effluent CODs levels seen in other studies (Zhang et al., 2011; Zhang and Jahng, 2012). Such drastic reduction in CODs could explain the significant improvement in methane productivity. The influence of each micronutrient was explained at earlier parts.

The VS and TS destruction efficiency profiles are presented in Fig. 7c. VS destruction was around 70% before process destabilization. After day 70, the VS destruction was reduced to 35% suggesting low substrate utilization efficiency and increased wash-out of untreated waste. Although there was a slow recovery after day 120, micronutrient supplementation on 150 lead to an immediate improvement in VS destruction. By day 190, VS destruction efficiency was higher than 80%. Regarding TS destruction efficiency, it showed a minor improvement after micronutrient supplementation. This improvement could be attributed to a healthier biomass with enhanced substrates utilization rates as stated above. Studies have shown similar improvement in VS removal, but not to this level (Yirong et al., 2014). Co addition has been identified to improve the health of the biomass (Agler et al., 2008). The supplementation with Mg is vital, since it is known to spur the growth of cells directly involved in substrate utilization and prevent biomass washout. This is especially significant as the role of magnesium is mostly neglected in most studies related to micronutrient supplementation.

It should be noted that the beneficial effects of Ca, Mg, Co and Ni on substrate utilization and methanogenic pathways could only be inferred based on previous studies. More research is required to identify the direct effects of these micronutrients. This can be achieved through investigations on gene products and bacterial community dynamics which could pinpoint the changes caused by the addition of micronutrients to the composition of the methanogenic community. While community dynamics studies could shed some light on the effect of such additions (Karlsson et al., 2012), metagenomic studies would be of particular interest, considering most microbial communities present in AD process are uncultured (Streit and Schmitz, 2004).

4 Conclusions

It was found that a micronutrient supplement consisted of Ca, Mg, Co and Ni (303, 777, 7 and 3 mg/L, respectively) could enhance methane production and stabilize the methanogenic phase of anaerobic digestion process. The biogas yield was 0.46 L CH₄/gVS_{added}/day, while there was increased utilization of soluble COD. In most cases, these elements are not present in optimal concentrations, thus, the AD process usually operates at suboptimal rates or even fails. The supplement could be simply mixed with the substrate (or acidified effluent) and added to the reactor. Special emphasis and attention should be given to metal accumulation in case the sludge is utilized as soil conditioner.

5 Uncited references

Ahring (2003), Lin and Chen (1999), Nagao et al. (2012), Nges and Liu (2010), Peleg et al. (2012), United Nations Industrial Development Organization (2012) and Zhang et al. (2013).

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.wasman.2016.10.017>.

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Appendix A. Supplementary material

[Multimedia Component 1](#)

Supplementary data 1

Highlights

- CH₄ yield of two phase thermophilic AD was enhanced by micronutrient addition.
- The supplement consisted of Ca, Mg, Co and Ni.
- Optimal concentration of micronutrients identified using response surface analysis.

- VS destruction efficiency was more than 80%.
-

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