Acetoclastic methanogens in an anaerobic digester could be susceptible to trace metal supplementation
C. Park, A. Bega, C. Unlu, R. A. Chadderton, W. R. McKean, P. M. Kohl
J. A. Hunt, J. Keaney, J. L. Willis and M. Duran

ABSTRACT
The objective of this study was to investigate the effects of nutrient supplementation on anaerobic biomass. While many studies emphasized the importance of supplementing trace metals such as iron, cobalt, and nickel for maximum methanogenic activity, there is no evidence whether such supplements, even at relatively low concentration, could perturb anaerobic biomass. Effects of supplementing nutrients, including yeast extract, on anaerobic biomass from two full-scale mesophilic digesters, operating under different conditions, at the North East Water Pollution Control Plant in Philadelphia, Pennsylvania, USA, were assessed using biochemical methane potential tests. The results show that acetoclastic methanogens from a recently cleaned digester was not stimulated by nutrient supplementation at relatively low concentrations and a slight perturbation was observed when supplementation was at a relatively high concentration. Furthermore, greater degree of susceptibility to the trace metal supplementation was observed for biomass from another digester that had not been cleaned for over 10 years, thus it had reduced active volume due to grit accumulation. For instance, supplementation of 200 mg/L of iron as FeCl₂·4H₂O to the biomass from the reduced-active-volume digester caused 17% reduction in CH₄ production, as compared to a control which did not receive any supplements, while the same concentration had no effect on the biomass from full-active-volume digester. Results strongly suggest that acetoclastic methanogens stressed due to reduced hydraulic/solids retention time may be susceptible to trace metal addition. Therefore, trace metal supplementation for anaerobic digesters should be considered on a case by case basis.

Key words | anaerobic digestion, biochemical methane potential, trace metals, yeast extract

INTRODUCTION
Anaerobic digestion of wastewater treatment biosolids is a vital process for stabilization of excess biosolids and beneficial recovery of methane gas. Biogas production, an alternative fuel source, is one of the major advantages of anaerobic biotechnology and the reason for the promising future of the anaerobic digestion. Many studies have revealed the importance of operational parameters including temperature, mixing, hydraulic retention time, solids loading, and co-digestion for successful operation of anaerobic digesters. Along with these operation parameters, literature emphasized the importance of supplementing trace metals such as, iron (Fe), cobalt (Co), and nickel (Ni), for maximum methanogenic activity (Owen et al. 1978; Speece et al. 1983; Shen et al. 1995; Gonzalez-Gil et al. 1999; Kida et al. 2001; Paulo et al. 2004).

Anaerobic microbial groups require various nutrients and trace metals to optimize their growth (Parkin & Owen 1986). Lack of sufficient nutrients and trace metals can severely limit the growth of microorganisms, consequently the process efficiency; causing process failure.
Rittmann & McCarty (2001) have listed the typical form of addition and the required amount of some of the most significant nutrients for methanogens, including Fe, Ni and Co.

According to Speece et al. (1983), Fe, Ni, Co, and sulfide are required for methanogens to cleave acetate. Most of the natural methane production and approximately seventy percent of the total biogas from anaerobic digestion comes from the cleavage of acetate; a pathway of acetoclastic methanogens (McCarty & Smith 1986). Methane formation involves enzymes and coenzymes, unique only to methanogens that carry Co, Ni, Fe, Mo, and Zn based non-heme active part (Deppenmeier et al. 1996).

Ni requirement is unique only to methanogens (as part of methyl M reductase F430) and has shown to stimulate methane production as high as 10 g acetate/g VSS d (Speece et al. 1983). It is believed Co is involved in the methyl transferase and co-dehydrogenase (Takashima & Speece 1990). Zitomer et al. (2008a,b) concluded that propionate utilization rates were more frequently stimulated by nutrient addition. Co addition on UASB increased methane production three times stimulating both acetogens and methanogens. In addition, supplementation of both Ni and Co resulted in an increase of methane production from methanol conversion by methanogens. Methane generation rate, however, decreased as the concentrations of the Co increased.

Another study demonstrated that nutrient limitations could be avoided when trace metals are added continuously (at reported experimental values in literature) so that they are bioavailable for the cells at any moment (Gonzalez-Gil et al. 1999). Micronutrients might be present in the wastewater but not at the needed concentrations nor bioavailable thus addition of trace metal cocktail might stimulate the process.

The effects of iron and other trace nutrients on full-scale temperature-phased anaerobic digestion (TPAD) were reported by Kemp et al. (2008). The study demonstrated the beneficial effects of trace metal addition in controlling VFA levels and struvite deposition which causes scaling and subsequent maintenance problems in closed pipes and heat exchangers. The addition of iron as ferric chloride (FeCl₃) resulted in decrease of VFA concentration by half after only two week.

Speece et al. (1986), showed the stimulatory effects of an array of trace metals and macro nutrients in conventional mesophilic digesters on a survey of 30 digesters. Zitomer et al. (2008a,b) reported that the majority (77%) of biomass samples collected from thermophilic and mesophilic digesters benefited from Fe, Ni, and Co supplementation, each at 25 mg/L as cation, with an increase in acetate and propionate utilization rates of 35 and 50%, respectively. The effects of yeast extract on methanogens were also investigated as literature shows notable synergism between yeast and municipal wastewater sludge (Zitomer et al. 2008a,b). The importance and economical feasibility of trace metal assay (containing Fe, Ni, Co, Se, Zn, Mo, Bo and Tu) was also suggested as weekly routine supplement, especially during start-up or change in loading rate (Speece 1996).

Emphasis is mostly on the beneficial effects of trace metal supplementation on anaerobic digesters. However, there is no published record, at least to the best of the authors' knowledge, on whether such supplements, even at relatively low concentration, could perturb anaerobic biomass, particularly acetoclastic methanogens.

The aim of this investigation was to assess effects of nutrient supplementation on anaerobic biomass form two full-scale digesters at North East Water Pollution Control Plant (NEWPCP) in Philadelphia, Pennsylvania, USA, using Biochemical Methane Potential (BMP) test developed by Owen et al. (1978). The BMP tests were conducted in bench-scale 165 mL capacity serum bottles. The objective was to determine whether anaerobic biomass in NEWPCP digesters would be stimulated by supplementation of Fe, Ni, Co, a macro nutrient cocktail, a trace metal cocktail, and combination of macro and trace metal cocktails (Vanderbilt Media). Evaluation of supplementation effects were compared based on ultimate, or 30-day, methane production.

METHODS

Philadelphia Water Department (PWD) operates three wastewater treatment plants treating approximately 500/1000 (average/peak) MGD domestic and pretreated industrial wastewater. The biosolids generated are digested
mesophically under design HRT of 18 days, mixing ensured by a sludge circulation, heating provided by sludge heating pumps. The anaerobic biomass used in this study was taken from the circulation line of two pancake digesters at NEWPCP: (1) Digester A, recently cleaned and assumed to have full design HRT of 18 days; (2) Digester B, not cleaned for the past 10 years and expected to have shorter HRT, thus biomass is likely to be under stress.

**Biochemical methane potential testing and nutrient assay**

The effects of nutrient supplementation on NEWPCP digester biomass were evaluated by BMP tests conducted according to a slightly modified protocol developed by Owen et al. (1978). BMP test is a measure of biochemically produced methane from a given substrate usually expressed in units of methane volume per mass of chemical oxygen demand (COD) equivalent of the substrate. For BMP testing, 45 mL biomass samples from Digester A and B was measured into 165 mL serum bottles and each serum bottle received the corresponding concentration of either Fe, Ni, Co, a macro nutrient cocktail, a trace metal cocktail, or the combination of macro nutrient and trace metal cocktails (Vanderbilt Media). The effects of Fe were assessed at concentrations between 50 and 400 mg/L as FeCl₂·4H₂O; Co between 1 and 10 mg/L as CoCl₂·6H₂O; and Ni between at 0.1 and 0.5 mg/L as NiCl₂·6H₂O. The constituents and concentrations of micro and macro nutrient cocktails are listed in Table 1. In addition, the effect of 20 mg/L concentration of yeast extract was investigated. Calcium acetate (Ca(C₂H₃O₂)₂) at 5,000 mg/L (approximately 3,750 mg/L as acetate,) was added to all serum bottles in order to avoid substrate limiting of acetoclastic methanogens, therefore any change in methane production will be a result of nutrients investigated. Then volume in each serum bottle was completed to 60 mL using distilled water. Afterwards, each serum bottle was deoxygenated by purging it with 30% CO₂ and 70% N₂ mixture for approximately 30 seconds. Once the head space of the serum bottles was set to atmospheric pressure by releasing the gas with a needle all serum bottles were placed in the environmental room at 35 ± 2°C, on a rotating shaker, shaking continuously at 135 rpm.

<table>
<thead>
<tr>
<th>Trace metal and macro nutrient cocktails*</th>
<th>Trace metal cocktail (or micro nutrient)</th>
<th>Macro nutrient cocktail</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constituent</td>
<td>Conc. (mg/L)</td>
<td>Constituent</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>100</td>
<td>NH₄Cl</td>
</tr>
<tr>
<td>CoCl₂·6H₂O</td>
<td>10</td>
<td>MgSO₄·7H₂O</td>
</tr>
<tr>
<td>MnCl₂·4H₂O</td>
<td>0.5</td>
<td>KCl</td>
</tr>
<tr>
<td>NH₄VO₃</td>
<td>0.5</td>
<td>Na₂S·9H₂O</td>
</tr>
<tr>
<td>CuCl₂·2H₂O</td>
<td>0.5</td>
<td>(NH₄)₂HPO₄</td>
</tr>
<tr>
<td>ZnCl₂</td>
<td>0.5</td>
<td>FeCl₂·4H₂O</td>
</tr>
<tr>
<td>AlCl₃·6H₂O</td>
<td>0.5</td>
<td>(NaPO₃)₁₃Na₂O</td>
</tr>
<tr>
<td>NaMoO₄·2H₂O</td>
<td>0.5</td>
<td>KI</td>
</tr>
<tr>
<td>H₂BO₃</td>
<td>0.5</td>
<td>NaHCO₃</td>
</tr>
<tr>
<td>NiCl₂·6H₂O</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>NaWO₄·2H₂O</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Na₂SeO₃</td>
<td>0.3</td>
<td></td>
</tr>
</tbody>
</table>

*Concentrations are for entire chemical including salt and water.

Three control reactors used were: (1) Seed Control that received only 45 mL sludge from Digester A or B; (2) Feed Control (or Acetate Control) in which calcium acetate was added compared to seed control and (3) Yeast Control in which yeast was added instead of acetate. Cumulative methane generated in a nutrient supplemented serum bottle was compared to that in control reactors that received no nutrient. The maximum theoretical methane production from calcium acetate alone at 35°C was 108 mL. Each concentration investigated was tested in triplicate (with a total of 63 reactors for each digester) to ensure data quality.

The behavior of triplicate was similar and values reported are the average of three measurements with few exceptions where average of duplicates was used when one reactor was an obvious outlier or when one reactor was lost. All reactors that had an associated CV of cumulative methane greater than 8% were considered outliers. Data presented is sufficiently accurate in terms of repeatability with average CV of cumulative methane generation in triplicate for the digester with longer (less stressed biomass) and shorter HRT (more stressed biomass) of 2.6 and 3.6%, respectively. The CV of acetate control reactors were 3.6 (for Digester A) and 6.4% or less (for Digester B).
Analytical protocols

Daily biogas production was monitored by a liquid displacement method for at least 30 days. The method consists of a 21 G 1 precision glide needle connected to a 100 mL burette which is connected to a 100 mL graduated cylinder filled with diluted acid solution. The amount of the gas produced in each bottle was measured by determining the volume of the liquid displaced by the gas vented into the system. Total gas production was used to calculate methane production after determining the methane content of the biogas.

Biogas composition of the reactors was analyzed for CH₄ and CO₂ by a Hewlett Packard™, Model 6890 gas chromatograph equipped with a thermal conductivity detector. A short packed column was used to separate CH₄ and CO₂ (Alltech Associates Inc, Deerfield, IL). Helium was the carrier gas at a flow rate of 21.8 mL/min. The inlet and detector temperature were 150 and 165°C, respectively, with nitrogen as a makeup gas. Initial oven temperature was 75°C and increased to a maximum of 200°C for 2 minutes. Pure gases (Micromat™–14 Cylinder from Matheson Tri-Gas®, Alltech Associates, Inc. Deerfield, IL) were used to develop calibration curves for CH₄ and CO₂. In addition, standard gas (9.99% methane) was run periodically in triplicates as a quality check of the calibration. Methane percentages were calculated by assuming other components were negligible.

RESULT AND DISCUSSION

The average cumulative methane production in acetate control bottles at Day 30 for Digester A (recently cleaned) was approximately 80% of the theoretical value while for Digester B was 70%. This clearly indicates biomass from Digester B are under stress due to operation conditions (short HRT) since the expected methane is approximately 13% less than that of the biomass from Digester A (longer HRT). The cumulative methane production from the Seed Control reactors for both, Digester A and B at 30 days of incubation was 42 and 71 mL, respectively. The comparison also suggests that there were more undigested volatile solids in the digester with shorter HRT.

Figures 1–3 show cumulative methane generation when trace metals were added. For both cultures, methane yield generally decreased with increasing dose of the trace metal and did not reach methane yield of the Acetate Control reactor. The results clearly indicate that there was no benefit in terms of increased methane production from supplementation of these three trace metals on acetate utilizing methanogens for both digesters. On the contrary, the results show that certain concentrations of Fe and Ni resulted in slight decrease in CH₄ production. It is important to note that Digester B biomass (digester with short HRT) showed greater susceptibility to nutrient supplementation. This is evidenced by the fact that supplementation of FeCl₂·4H₂O to Digester B biomass, at a concentration of 200 mg/L, resulted in approximately 17% decrease in cumulative biochemical methane generation as compared to cumulative CH₄ generated in the control reactor (see Figure 1(b)). Although the next higher dose of Fe supplement, 400 mg/L, showed lesser sensitivity to the
supplementation generating only 11% less methane than the control, the difference in response, 6%, is within the experimental variation range. However, the same dose of FeCl₂·4H₂O supplemented to Digester A biomass, 200 mg/L, did not affect methane production at all (only 1% difference in cumulative methane generation), giving a drastically different response to Fe supplementation from that of Digester B biomass.

As mentioned earlier, the digesters chosen for the study had a distinctive characteristic. Digester A was recently cleaned and assumed to have close to the full design HRT of 18 days as opposed to Digester B which had not been cleaned for the past 10 years. Digester B is expected to have much shorter HRT and presumably biomass is under stress compared to Digester A. A tracer study on these two full-scale digesters confirmed that Digester B had a shorter actual residence time (data not presented).

The results highlight that the response of the acetate utilizing methanogens to trace metal supplements is strongly related to the biomass condition. Furthermore, the results presented in Figures 1–3 show that the more stressed the biomass was, the more negatively significant effects trace metal addition had on the biomass activity, resulting in methane generation decrease. It is important to note that all three reactors supplemented with 100 mg/L of Fe in Digester B test did not produce any gas after the first week of incubation as shown in Figure 1(b). Any toxicity effect was excluded since higher concentration did not have the same effects. Possible explanations are either gas leak or an experimental error during the trace metal addition.

Similar to Fe addition, the reactors with Digester B biomass, supplemented with the highest concentration of 0.5 mg/L of NiCl₂·6H₂O, produced only 101 mL methane during the experimental period, approximately 31% less...
compared to the Acetate Control reactor (see Figure 2). The same concentration of Ni caused 17% decrease in CH$_4$ production for Digester A biomass tested.

The effect of Co addition on acetate utilizing methanogens is shown in Figure 3. Four concentrations of Co, 1, 2, 5, and 10 mg/L, were tested. Data shown are the average of triplicate with exception of the 2 mg/L Co for Digester B which is an average of duplicate.

All concentrations investigated, shown in Figure 3, resulted only in a slight decrease in cumulative CH$_4$ production for the biomass from both digesters, with the exception of the highest concentration of 10 mg/L of CoCl$_2$·6H$_2$O, at which additional methane production from supplemented acetate was not observed. Interestingly, the same dose of Co caused only a 2% reduction in methane generated by the biomass from Digester B (see Figure 3(b)).

This response is quite contrary to those from Fe and Ni supplementation. Assuming that there was no experimental error involved such as not adding acetate into the serum bottles, it is difficult to explain this unusual observation without further experiments.

Similarly, supplementation of micro and macronutrient cocktails, Vanderbilt media (combination of micro and macronutrient cocktails) and yeast extract did not seem to stimulate the acetoclastic methanogens (see Figures 4 and 5). The biomass form Digester B, with short HRT, seemed to be more easily perturbed from the cocktail supplementation. The decrease in cumulative methane was 14% (for macronutrient cocktail on Digester B) or less due to the supplements.

Yeast extract is considered as a source of organic micronutrients in the medium and a vitamin provider.
in particular vitamin B2 complex (Shen et al. 1993). Zitomer et al. (2008a,b) shows yeast waste codigestion with municipal biosolids resulted in notable synergism, increasing biogas production by over 50%. However, supplemental yeast extract did not seem to stimulate acetogens as shown in Figure 5. No synergism from yeast supplementation was observed in our study. In fact, slight decrease in methane production was observed for the biomass from both digesters, which was within acceptable variance range.

CONCLUSIONS

The following conclusions can be drawn from this study:

- Trace metal (Ni, Co, Fe), micro and macronutrient cocktails resulted in decrease in terms of CH4 production for both digesters.
- Similarly, supplemental yeast extract did not seem to stimulate the methanogens.
- Greater degree of susceptibility to the trace metal supplementation was observed for biomass from the digester that had not been clean for over 10 years.
- The data strongly suggest that acetoclastic methanogens stressed due to reduced hydraulic/solids retention time may be more susceptible to trace metal addition.
- Although some increase in rate of biodegradation (early parts of cumulative CH4 generation) was observed due to addition of some nutrients, the effect is minimal and it may not translate into any appreciable and practical benefit considering that the full-scale digesters at PWD NEWPCP are operated under SRT/HRT of 18 days.
- The results presented here provide further evidence that trace metal supplementation for anaerobic digesters should be considered on a case-by-case basis.

ACKNOWLEDGEMENTS

This research was funded by Philadelphia Water Department (PWD). The authors are grateful to the staff at NEWPCP for their assistance in collection of samples.

REFERENCES


