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ORIGINAL PAPER

Iron flocculation stimulates biogas production in *Microthrix parvicella*-spiked wastewater sludge

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Abstract Municipal wastewater sludge has been used for fertiliser and biogas production for several decades. Chemical compounds such as iron and aluminium are common coagulants used in wastewater treatment plants to remove suspended solids, phosphorus and micro-organisms. This laboratory study explores whether ferric chloride ($FeCl_3$ as PIX-111) or aluminium chloride ($AlCl_3$ as PAX-18) flocculation could stimulate biogas production in wastewater sludge contaminated with *Microthrix parvicella*. In a fermentation process run in three replicates, cumulative methane production was in average about 25 % higher using the iron flocculated sludge than using the aluminium flocculated sludge; this difference was statistically significant ($P < 0.05$) in the subsequent runs of the semi-continuous process. In all runs, the iron flocculated sludge produced less ($P < 0.05$) hydrogen sulphide in the biogas than the aluminium flocculated sludge. The numbers of *M. parvicella* stayed at the similar levels throughout the process. It is concluded that biogas production is higher and more stable with iron coagulant in comparison with aluminium coagulant, presumably due to the reduced formation of hydrogen sulphide. Thus, iron coagulants seem to be better than aluminium coagulants to stimulate the methane production process. Both coagulants significantly suppressed multiplication of *M. parvicella* in the biogas reactor, i.e. they did not evoke foaming in this experiment.

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Introduction

Municipal sludge has often been treated by anaerobic mesophilic digestion prior to being spread on the land. The reasons are that this treatment procedure produces energy, reduces the volume of sludge and makes more convenient its further treatment. Iron (Fe^{3+}) and aluminium (Al^{3+}) salts are widely used as electro-coagulants to remove phosphorus, suspended solids (SS), enteric micro-organisms etc. in wastewater treatment (Morse et al. 1998; DeWolfe et al. 2003). These coagulants are cheap and have been demonstrated to be effective under a variety of conditions. Aluminium-based coagulants have been reported to lead to the presence of relatively high residual aluminium concentrations in the treated water (Yang et al. 2010). Since aluminium is a toxic element for some aquatic organisms, the use of aluminium-based coagulants has raised concerns about its safety in water treatment (Rosseland et al. 1990).

The micro-organism, *Microthrix parvicella*, can cause problems in wastewater treatment, especially in temperate climates and in treatment plants during low loads. In fact, problems with *M. parvicella* have been reported in many countries, e.g. Denmark, The Netherlands, Italy, Greece, Czech Republic, Germany, France and UK. Westlund et al. (1998) reported that the biogas production of all three large wastewater treatment plants (WWTP) in the greater Stockholm area has experienced serious foaming problems caused by *M. parvicella*. It is regarded to be one of the most serious disturbing filamentous micro-organisms in activated sludge of WWTP since it can cause massive foaming (Eikelboom et al. 1998; Noutsopoulos et al. 2007).

The destruction (or disinfection) of this micro-organism in a WWTP is difficult since it can adapt to different environmental conditions (Martins et al. 2004; Jenkins et al. 2005). Furthermore, it has been hypothesised that *M. parvicella* might inhibit methanogenesis and reduce biogas production from wastewater sludge. It is also assumed that *M. parvicella* or related filamentous species can be eliminated during the biogas production process due to heat and anaerobic condition (Eikelboom et al. 1998; Noutsopoulos et al. 2007). It is difficult to detect this organism by cultivation due to its slow growth rate in laboratory media (Tandoi et al. 1998), and staining is difficult since the presence of other bacteria can obscure the results (Eikelboom and van Buijsen 1983). Recently, a polymerase chain reaction (PCR) method based on *M. parvicella* 16S rRNA gene has been published to aid identification of this organism (Kaetzke et al. 2005).

The effect of Fe^{3+} and Al^{3+} on the anaerobic digestion and biogas production is not clearly identifiable. Thus, Johnson et al. (2003) presented that an iron rich primary sludge reduced gas production by 32 % as compared to an iron-poor sludge but in contrast, Lee (2008) found that addition of iron compounds to sludge increased gas production. Westlund et al. (1998) have demonstrated that aluminium compounds can control growth of *M. parvicella* in wastewater sludge. However, the enzymatic pathways producing methane are catalysed by many metalloenzymes, i.e., there is a clear requirement for many trace metals (Glass and Orphan 2012), but the effects of different coagulant strategies on biogas production are not known. Therefore, the aim of this study was to examine the effect of $FeCl_3$-based and $AlCl_3$-based (PIX-111 and PAX-18, both commercial coagulants from Kemira, Finland) flocculated sludges on biogas production and also their effect on numbers of *M. parvicella*. The number of *M. parvicella* spiked was estimated with the PCR method of Kaetzke et al. (2005) during the biogas production processes. The study material for biogas production tests was obtained from Kuopio and Siilinjärvi WWTP, and each biogas production experimental run was conducted for 30–32 days. This research work was done between September 2010 and January 2012 at the University of Eastern Finland.

Materials and methods

Flocculation of sludge using PIX and PAX

Untreated wastewater from Siilinjärvi (Finland) municipal WWTP was flocculated using PIX-111 ($FeCl_3$) at a dose of 80 mg Fe^{3+} L^{-1} or PAX-18 ($AlCl_3$) at a dose of 38 mg Al^{3+} L^{-1}. The flocculated sludge was collected and stored at +4 °C for 5–10 days. These sludge portions were spiked with pure culture of *M. parvicella* and used in these experiments. Three subsequent experimental runs using inocula from the previous experimental runs were performed as three parallel determinations.

Experimental protocol

In the first experimental run, 2 L of PIX or PAX flocculated sludge was poured into the 5-L glass biogas reactors, and 1 L of inoculum sludge (from Kuopio Lehtoniemi WWTP biogas reactor) was added in the same biogas reactors. These biogas reactors were capped and mixed carefully by shaking. The reactor's cap nozzles were connected by a plastic pipe to a plastic gas bag (TECOBAG by TESSERAUX, Germany), and the reactors were placed into the incubator at 35 ± 1 °C for 30 days corresponding to the conditions in Lehtoniemi full-scale biogas process. When the gas bags were filled or looked swollen, the biogas produced (CH_4, CO_2, O_2, H_2S, NH_4 and the rest, which is mainly N_2 and nitrogen oxides) was measured in a gas analyser (GA 2000 plus, Geotechnical Instrument, UK). The gas volume was measured using suction measuring cylinder under ambient air pressure.

In the second run, 2 L of freshly collected/prepared PIX or PAX flocculated sludge was poured into the new 5 L glass reactors in a similar manner to the first experimental run. Then, 1 L of digested slurry from the first experimental run (after completion of the first experimental run) was added in the same reactors as inocula. The inocula from PIX-treated reactors were added into the PIX treatment reactors, and the inocula from PAX-treated reactors were added into the PAX treatment reactors. The mixtures were mixed, capped, connected to a gas bag and incubated at 35 ± 1 °C for 30 days. The produced biogas was analysed as in the first experimental run.

The third experimental run was repeated as in the second experimental run, but here, the inocula were taken from the second experimental runs. In this experimental run, the incubation time was 32 days. The gas generation was analysed as described previously.

Physico-chemical analyses

The collected sludge was analysed for *M. parvicella,* pH, chemical oxygen demand (COD_{Cr}), organic matter (OM), dry matter (DM), Fe^{3+}, Al^{3+}, total Kjeldahl nitrogen (N) and total phosphorus (P). OM was analysed by burning at 550 °C and DM by heating at 105 °C before and after all experimental runs. Total P was analysed by the colorimetric ascorbic acid method using sulphuric acid + nitric acid digestion (SFS-EN 13346). The total N was analysed by the Kjeldahl method (ISO 1871), and Fe^{3+} and Al^{3+} were analysed by the flame atomic absorption spectrophotometer

(FAAS) method. All the above-mentioned analyses were conducted according to the methods recommended by the American Public Health Association (APHA 2005). COD_{Cr} was analysed by Hach DR 2010 spectrophotometer (Hach Company, Loveland, Colorado) with method 8000 (analysed according to the manufacturer's instructions). The pH value was measured with a WTW pH 340 metre (Weilheim, Germany). The data are presented in Table 1.

Preparation of *M. parvicella*

R2A agar plates (Reasoner and Geldreich 1985) with colonies of *M. parvicella* strain RN1 (Rossetti et al. 1997) were kindly provided by Drs. V. Tandoi and S. Rossetti (CNR—Water Research Institute, Rome, Italy). The cells were transferred to new R2A agar plates and incubated at 25 °C for 2–3 months to produce more *M. parvicella* biomass and to ensure that this bacterium was a pure culture.

The *M. parvicella* colonies from 10–20 plates were harvested by washing with 2–5 mL of sterile water. Since this solution was gelatinous, it was mixed using a sterile UltraTurrax-homogenizer (IKA, Germany). Homogenised *M. parvicella* solutions of 40 mL (i.e. $\log_{10}$ of copy numbers mL^{-1} was 5.2) were spiked in 6 L of PIX flocculated sludge and 6 L of PAX flocculated sludge. After spiking and careful mixing, about 50 mL of the sludge sample was taken into a sterile test tube for further analysis, and the rest of the sludge was divided into six parallel reactors: three for flocculated with PIX and three for flocculated with PAX as described above in the first, second and third experimental runs.

The quantitative analysis of *M. parvicella*

The amount of *M. parvicella* was analysed from the initial inoculum, from the PIX and PAX flocculated sludges spiked with *M. parvicella* and from the slurries after biogas production. From each reactor, a 10 mL sample of sludge was taken and centrifuged, and then, the pellets were lyophilised. About 20 mg (exact weight was recorded for

Table 1 Characteristics of PIX or PAX flocculated sludge spiked with *M. parvicella* in the first, second and third biogas production experimental runs ($N = 3$)

Concentrations in sludge before and after biogas production	The first experimental run		The second experimental run		The third experimental run	
	PIX	PAX	PIX	PAX	PIX	PAX
COD_{Cr} g L^{-1}						
Before	39.1	34.2	51	33	36.4	41.5
After	25.0 ± 1.9	23.1 ± 8.0	37.0 ± 5.15	24.1 ± 0.81	28.8 ± 4.3	17.0 ± 1.3
OM % in FW						
Before	2.2	1.8	3.3	3.2	2.4	2.0
After	1.6 ± 0.07	1.4 ± 0.14	2.2 ± 0.08	2.0 ± 0.07	1.8	1.6 ± 0.04
DM %						
Before	3.6	3.3	6.2	6.3	5.3	6.0
After	2.7 ± 0.1	2.6 ± 0.04	5.18	5.0	4.5	4.5 ± 0.05
N g kg^{-1} DW						
Before	23.7	22.5	17.5	16.8	19.3	12.5
After	19.3 ± 0.2	17.0 ± 0.55	17.4 ± 0.3	13.8 ± 0.3	15.6 ± 0.4	13.6 ± 0.1
P g kg^{-1} DW						
Before	58.9	54.8	73.4	80.5	56.7	41.0
After	84.5 ± 1.2	94.9 ± 3.6	68.5 ± 1.5	51.9 ± 1.2	66.3 ± 0.7	59.2 ± 2.4
Fe^{3+} g kg^{-1} DW						
Before	45.8	27.7	56.7	10.4	97.1	27.5
After	63.6 ± 3.4	22.6 ± 0.5	71.7 ± 2.2	27 ± 0.4	127.7 ± 0.7	30.1 ± 0.6
Al^{3+} g kg^{-1} DW						
Before	9.5	32.9	5.2	51.8	5.8	28
After	4.5 ± 0.1	25.4 ± 1.4	8.9 ± 0.2	28.8 ± 0.3	6.8 ± 0.1	38.2 ± 0.3
pH						
Before	6.7	6.7	6.3	6.1	7.1	6.9
After	7.1	7.2	7.5	7.4	7.5	7.3

FW fresh weight, *DW* dry weight

calculation) of lyophilised samples was taken for DNA isolation using QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the instructions of the manufacturer. The extracted DNA was stored at −20 °C until further analysis.

The amplification of *M. parvicella* DNA was carried out in a 25-μL reaction mixture containing 2.5 μL (10 μmol) of two primers (S-S-M.par-0828-S-21 and S-S-M.par-1018-A-17 for *M. parvicella* 16S rRNA gene) described by Kaetzke et al. (2005), 12.5 μL of SYBR Green master mix (Maxima SYBR green/Rox qPCR master mix (2x), Fermentas), 1 μL of template DNA (*M. parvicella* plasmid DNA or 1:10 diluted sample) and 6.5 μL of nuclease-free water. Monitoring the fluorescence was made by the MyiQ single colour real-time PCR system (Bio-Rad) with following the cycling conditions: 95 °C for 10 min 45 cycles (94 °C for 10 s, 60 °C for 20 s, 72 °C for 20 s and 86 °C for 15 s) for the measurement of the SYBR Green I signal and a final extension step (72 °C for 5 min). Melting points of the PCR products were determined by the melting curve analyses at 55 °C for 1 min, 80 cycles at 55 °C for 10 s and 72 °C for 10 min. The suitable dilution of the sample DNA was chosen by testing the inhibitory effect of sludge samples at different dilutions. There were negative and positive controls in every qPCR run. The negative controls contained only nuclease-free water and the positive controls the cloned 16S rRNA gene plasmid DNA of *M. parvicella*, instead of sample DNA.

For the quantitative analysis, the standard curve and amplification efficiency were determined with the cloned *M. parvicella* 16S rRNA plasmid DNA. For the cloning, DNA from pure culture of *M. parvicella* RN1 was amplified with the protocol using a 50-μL volume reagent solution, i.e. 22.3 μL of nuclease-free water, 25 μL of 2x premix F, 1.25 μL of primers [S-S Microthrix par 0828-S21 (10 μM) and S-S Microthrix par 1018 A-17 (10 μM) and 0.2 μL of Taq DNA polymerase (Invitrogen)]. Template DNA from 1 μL broth or one colony from an agar plate was added to the reagent mixture. Then, the reaction mixture was amplified in a PCR block (MJ research PTC-200) according to the protocol used by Kaetzke et al. (2005). The PCR protocol used was 94 °C for 5 min, 35 cycles of 94 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s and then 72 °C for 10 min. The reaction was stopped and kept at 16 °C until being removed from the block. The *M. parvicella* 16S rRNA PCR product (190 bp) was purified by High Pure PCR Product Purification Kit (Roche). It was ligated to pDrive vector and transformed to *E. coli* according to the instructions of the manufacturer (Qiagen PCR Cloning Kit, Qiagen). Plasmid DNAs were sequenced by primers T7 and P13 to ensure that the selected clone contained the qPCR primer positions. Sequencing was performed in a commercial laboratory using the Applied Biosystems 3730XL automated sequencing system (Macrogen Ltd, Seoul, South Korea Macrogen). Sequences from this study are under accession numbers HG530471-HG530490 in the EMBL-bank.

For the standard curves, tenfold dilution series down to 10^{-7} were made with the positive control plasmid DNA containing *M. parvicella* 16S rRNA gene. Triplicate qPCRs were pipetted from each dilution, and the qPCRs were performed as described earlier. Copy numbers of *M. parvicella* were calculated from the dilution series of positive control plasmid DNA. The detection limits were 2.3×10^{1}–2.3×10^{8} gene copies. The slope of the regression curve of dilution series was 3.51, and correlation of determination (r^2) was 0.97.

Statistical analysis

The data from biogas components, different parameters of sludge at the start and during processes, and DNA copy numbers of *M. parvicella* were collected in Microsoft Excel file. The data were transformed to SPSS 19 and analysed using normality test before statistical analysis. The statistical significances between the differences of the coagulants on gas production were analysed in each batch and pooled data from all three batches by one-way ANOVA. Correlations between the different variables were analysed using Pearson bivariate analysis. The Mann–Whitney test was used in cases where the data were not normally distributed. Levels of significances (P), rations of variances between groups and variances within groups (F) and the correlation coefficients (r) were calculated.

Results and discussion

Gas production from sludges

The characteristics of wastewater can continuously vary which means the characteristics of sludge also vary as it is shown in all three experimental runs (Table 1). Similarly, degradation rates varied as the content of the sludge was changed. The degradation of organic matter during gas production can be seen in Table 1. The gases (Table 2) are end products of an anaerobic degradation process made by a microbial consortium, and in addition, there can be many different intermediate products, such as sugars, fatty acids, alcohols, carbonic acids and amino acids (Zhang 2010). Therefore, variation in the substrate content in sewage sludge and thus the biogas production from sewage sludge is not easily predicted.

The COD_{Cr} describes chemical, oxygen-demanding degradation of mainly organic compounds, OM describes organic compounds regardless of their biodegradability,

Table 2 Volumes of different gases in semi-continuous biogas produced from sludge when either PIX or PAX was used as the coagulant ($N = 4$)

Gases (%)	First experimental run		Second experimental run		Third experimental run	
	PIX	PAX	PIX	PAX	PIX	PAX
CH_4	60 ± 4	59 ± 2	64 ± 2	63 ± 1	58 ± 4	58 ± 2
CO_2	30 ± 1	32 ± 1	33 ± 1	33 ± 1	29 ± 1	30 ± 1
Rest (mainly N and N oxides)	9 ± 3	8 ± 2	6 ± 0.4	8 ± 1	14 ± 2	11 ± 1

The gases produced from PIX or PAX were not significantly different in any experimental runs ($P > 0.05$)

and DM can contain both organic and inorganic compounds. Although PIX flocculated sludge produced high amount of biogas, there were no significant difference in COD and OM degradation in PIX and PAX flocculated sludge. When coagulant types (PIX and PAX) were not considered, it was found that COD and OM in residual sludge correlated positively to each other but r is only 0.69 ($P = 0.003$). On the other hand, there are still significant amounts of OM in sludge after biogas production, and this OM can be dewatered and used for compost.

During biogas production, the relation of CH_4 and CO_2 productions of PIX and PAX-treated sludge was similar (Table 2). The PIX flocculated sludge produced an average of 707 ± 106 L CH_4 kg^{-1} of OM, whereas the PAX flocculated sludge produced 549 ± 276 L CH_4 kg^{-1} of OM during one biogas production run of 30–32 days. This range of methane produced by the primary sewage sludge is similar to the values presented by Sato et al. (2001), i.e. 612 L CH_4 kg^{-1} of OM, but it is more than in the values described by Speece (2001).

The results showed that the CH_4 production from the PIX and PAX flocculated sludges were similar ($P > 0.05$) in the first experimental run, but in the second and third experimental runs of the semi-continuous biogas processes, the PIX flocculated sludge produced a significantly higher amount of CH_4 compared with the PAX flocculated sludge ($F = 24.73$, $P = 0.008$ in the second run and $F = 23.85$, $P = 0.016$ in the third run) (Fig. 1).

Both PIX (iron) and PAX (aluminium) have been commonly used to remove SS, P and microbes from wastewater (Hutnan et al. 2006). The removal of organic matter and nutrients during the biogas process from PIX and PAX flocculated sludge can be seen from the results of these physico-chemical parameters analysed before and after biogas production (Table 1). This experiment also found that iron content in sludge correlated with total biogas productions ($r = 0.76$, $P < 0.0001$) and CH_4 production ($r = 0.72$, $P < 0.001$). This may be because iron is an essential micronutrient for many of enzymes participated in the methane production pathway (Glass and Orphan 2012). Instead, Al^{3+} may be toxic or competing with iron and manganese of the adhesion sites on the microbial cell membrane or cell wall, which affects microbial growth (Cabirol et al. 2003). This research group also reported that the specific activity of methanogenic and acetogenic micro-organisms clearly declined if these micro-organisms were exposed to 1,000 mg L^{-1} $Al(OH)_3$. Similarly, the COD_{Cr} degradation was higher in sludges flocculated with PIX instead of PAX in our first and second experimental runs. Park and Novak (2007) also showed that the presence of iron increased the degradation of exocellular polymers during the anaerobic digestion of activated sludge.

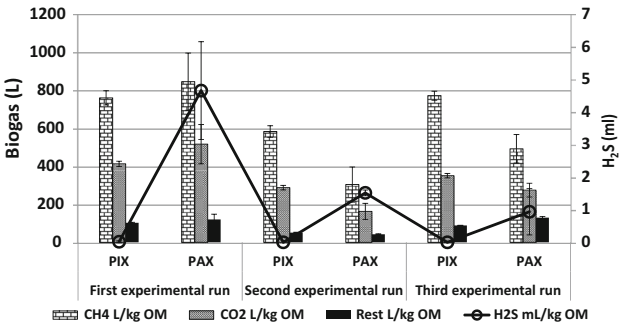

Fig. 1 Biogas production of sludge in three semi-continuous experimental stages. CH_4, CO_2, N_2 and nitrogen oxides (marked as *Rest*) in L kg^{-1} OM of sludge/slurry (*scale on left*). The H_2S produced by PIX (Fe^{3+}) or PAX (Al^{3+}) flocculated sludge is presented as mL kg^{-1} OM as point and line (*scale on right*). *Bars* show the standard deviations ($N = 3$)

The concentrations of Fe^{3+} and Al^{3+} before biogas production depended whether the coagulant used was iron-based or aluminium-based. Since a part of organic matter has been metabolised into biogas, the apparent concentration of Fe^{3+} and Al^{3+} in organic matter seems to have increased excluding the second experimental run where PAX was used as a coagulant (Table 1). In this case, it must be considered that there had been sulphide formation (Fig. 1) leading to formation of insoluble FeS, and this salt may have been attached to the walls of reaction vessels so that all of Fe may not be included to the determination of Fe concentration.

Role of H_2S

The production of H_2S from PIX (Fe^{3+}) flocculated sludge in gas phase was minimal, whereas it was noticeable in the

cases of the PAX (Al^{3+}) flocculated sludge. This difference between PIX and PAX was statistically significant in all experimental runs ($F = 28.638$, $P = 0.006$) as presented in Fig. 1 (presented as points and line). High concentrations of H_2S especially as dissolved H_2S at pH 7–8 have been described as a potential inhibitor of methanogenesis (Koster et al. 1986) and methanogenic micro-organisms (Chen et al. 2008). The higher standard deviation of CH_4 formation (Fig. 1) in the PAX flocculated sludge than in the PIX flocculated sludge could be a reflection of disturbance of methane formation possibly caused by H_2S.

There may have been some formation of H_2S in both PIX and PAX flocculated sludges. H_2S produced in the PAX reactors was in gas form as can be seen in Fig. 1. If PIX is used, its ferric ions (Fe^{3+}) are reduced to ferrous ions (Fe^{2+}) under anaerobic conditions as stated by Dewil et al. (2008). The Fe^{2+} ion will then form with H_2S a black insoluble ferrous sulphide (FeS) precipitated from the solution and also controls sulphide in anaerobic digestion (Ge et al. 2013). Thus, the sulphide concentration in the biogas process solution could well remain low if iron is used in the flocculation of the sludge. In contrast, the water solubility of aluminium sulphide is much higher (CRC Handbook 1979), and thus, sulphide will remain in the solution. The lack of soluble sulphide in biogas production may have stabilised the process so that the standard deviations of all gases (Fig. 1) are much lower when iron is used as the coagulant than if aluminium is used.

Furthermore, H_2S can cause anaerobic corrosion on metal surfaces (Postgate 1979). If there is a risk of metal corrosion, then iron coagulants are one way to reduce this risk. It should also be remembered that H_2S has the unpleasant smell of rotten eggs, and it is highly toxic to many living organisms, including humans even sometimes causing deaths (WHO 2003). This risk could be another reason for preferring iron coagulants since they reduce the presence of H_2S.

The presence of *M. parvicella* in Fe^{3+} and Al^{3+} flocculated sludges

The analytical method used to monitor the presence of *M. parvicella* was successful with relatively low standard deviations (Fig. 2). Biogas production was not significantly ($P = 0.71$) influenced by the numbers of *M. parvicella* irrespective of the coagulant type used. It has been reported that some biogas processing of WWTP sludge in the greater Stockholm area has experienced serious foaming problems caused by *M. parvicella* when ferrous sulphate was used as a coagulant (Westlund et al. 1998). However, PIX used in this study is ferrous chloride, and it did not show any foaming problems. Furthermore, after biogas production, *M. parvicella* numbers were slightly increased in PIX flocculated sludge in the first experimental run (Fig. 2). This might be because of low level of iron in the first experimental run compared with the second and third experimental runs with PIX (Table 1). Similarly, after biogas production, *M. parvicella* numbers were slightly increased in PAX flocculated sludge in the third experimental run (Fig. 2), and this might be because of the low level of aluminium in the third experimental run compared with levels of aluminium present in the first and second experimental runs (Table 1). This result indicates that further research is needed to find the doses of iron and aluminium needed to suppress the growth of *M. parvicella* in sludge.

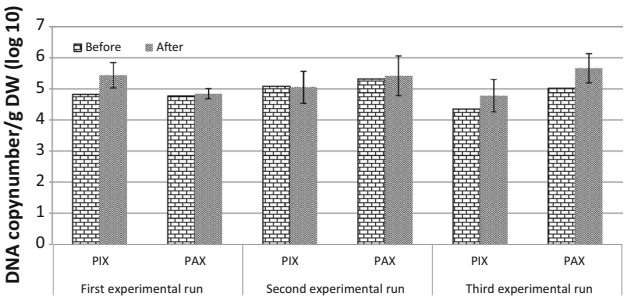

Fig. 2 *Microthrix parvicella* in Fe^{3+} (PIX) and Al^{3+} (PAX) flocculated sludge in three semi-continuous biogas production stages (before and after biogas production) ($N = 3$)

During the present experiment, there were no foam problems in biogas reactors, and the numbers of *M. parvicella* remained at about the same level before and after the anaerobic digestion (Fig. 2). Similar results are presented by Marneri et al (2009), who reported no foaming problem when the *M. parvicella*-infected sludge was used for anaerobic digestion in thermophilic and mesophilic conditions. Evidently, environmental conditions adjusted according to Kuopio Lehtoniemi WWTP biogas process, from which the inoculum was taken, did not stimulate excess growth of *M. parvicella*. In this respect, there were no differences with the two coagulants examined in our study. It has been claimed that polyaluminium chloride can control *M. parvicella* in wastewater treatment plants (Nielsen et al. 2005; Rossetti et al. 2005). Mamais et al. (2011) have reported that the Al^{3+} coagulant controlled *M. parvicella* by embedding the filaments inside the flocs, and this may be another reason why this bacterium did not cause any problems in our reactor. In contrast to our result, Marneri et al. (2009) stated that mesophilic anaerobic digestion achieved 76 % destruction of *M. parvicella* as analysed with fluorescence microscopy counting method.

Conclusion

Using PIX coagulant is better than PAX in wastewater treatment when the primary aims are to maximise biogas

production and to reduce H_2S concentration since PIX flocculated sludge produced a higher amount of CH_4 and a significantly lower amount of H_2S as compared to PAX flocculated sludge. The daily CH_4 production was 5.3 L kg^{-1} OM more with PIX flocculated sludge in comparison with PAX flocculated sludge. It is worth noting that H_2S can inhibit the methanogenesis, and H_2S is toxic to humans and it is also corrosive. The micro-organism, *M. parvicella*, in PIX and PAX flocculated sludge did not affect the biogas formation in anaerobic digestion, and the numbers of *M. parvicella* were not influenced by PIX or PAX flocculation. Furthermore, iron coagulated slurry might be better for composting and agriculture as compared to aluminium-treated slurry because the residual aluminium in slurry may be harmful when used on farms.

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