Comparison of microbial activity in anaerobic and microaerobic digesters

P. Jenicek, C. A. Celis, J. Koubova and D. Pokorna

ABSTRACT

Microaerobic alternative of anaerobic digestion offers many advantages especially when sulfide concentration in the digester is high. For better understanding of the microaerobic technology more detailed characterization of biomass activity is needed. Two equal digesters were operated under the same condition except of microaeration in one of them. During long term operation of anaerobic and microaerobic digesters the sludge quality and the biomass activity was monitored. The activity of sulfide oxidizing bacteria of microaerobic biomass was significantly higher in comparison with anaerobic biomass. The activity of sulfate reducing bacteria was comparable. The activity of methanogenic bacteria activity depended on sulfide concentration more than on microaeration. The extent of foaming problems was lower in the microaerobic than in the anaerobic digester.

Key words | anaerobic digestion, foaming, methanogenic bacteria, microaerobic conditions, sulfate reducing bacteria, sulfide oxidizing bacteria

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INTRODUCTION

Recently, it has been shown in many studies that the use of microaerobic conditions in anaerobic digestion can be beneficial. The application of this technology is an efficient method of hydrogen sulfide removal from biogas (Buisman *et al.* 1990; Stephenson *et al.* 1999; Krishnakumar *et al.* 2005; van der Zee *et al.* 2007) and sulfide toxicity suppression (Cirne *et al.* 2008). The implementation of microaerobic condition has in many cases improved the efficiency of anaerobic digestion and enhanced hydrolysis and biodegradability of some organic compounds (Zacharias *et al.* 1995; Johansen & Bakke 2006). The experiences with microaerobic process show that benefits usually outweigh potential drawbacks such as oxidation of part of the organic substrate or methane or lower methane concentration (Jenicek *et al.* 2008).

The general definition of microaerobic condition was not adopted yet. In this paper, microaerobic biomass means the biomass cultivated in anaerobic system with limited (trace) oxygen consumption. With respect to the oxidation-reduction potential (ORP), the microaerobic system can be marked generally as a system in which micro-consumption of oxygen causes a limited ORP increase (Khanal & Huang 2003).

In a mixed culture, even strict anaerobes can survive without any inhibition, if facultative microorganisms are able to consume the present oxygen quickly and fully. It was proved that the presence of limited amount of oxygen in digester does not destroy the digestion process even in the system where the oxygen is not consumed by sulfide oxidation (Kato *et al.* 1993; Jenicek *et al.* 2008).

Microaerobic conditions can be obtained by dosing of a limited amount of air (or oxygen) into anaerobic reactor. There are two basic configurations of microaerobic technology. The air can be pumped into the gas space of the digester or into the mixed liquor suspension. In the first alternative, the risk of biogas pipe clogging by sulfur is higher; at the second alternative a bigger surplus of oxygen is needed. Moreover because of closer contact of sludge with oxygen the likelihood of the anaerobic bacterial consortia affection is more significant in the second case.

Less attention has been paid till now to the comparison of the specific bacterial activity and digested sludge quality after a shift from strictly anaerobic to microaerobic sludge digestion. Tang *et al.* (2004) reported that microaeration has no obvious effect on the phylogenetic diversity of microorganisms. However the results indicated that ratio between hydrogenotrophic and acetoclastic methanogens changed due to microaeration. Zitomer & Shrout (1998) reported that the methanogenic activity can sometimes be even

doi: 10.2166/wst.2011.579

higher under microaerobic conditions in comparison with strictly anaerobic system.

The presented paper aims to evaluate the changes in microbial activity after switch from fully anaerobic to microaerobic conditions. The comparison was focused on two fields: chemical composition of the sludge and specific activity of different bacterial groups of the sludge especially methanogenic bacteria (MB), sulfate reducing bacteria (SRB) and sulfide oxidizing bacteria (SOB). Measurement of specific activity of different trophic groups was used for monitoring of the biomass quality changes, because the biomass activity is, from the technological point of view, the most important characteristic of sludge.

MATERIAL AND METHODS

The experiments were carried out in two lab-scale reactors with 10 L of working volume (Figure 1). The start-up of both reactors was conducted in anaerobic condition. After start-up, the operation of the first reactor was changed to microaerobic. Second reactor remained anaerobic and served as referential. Waste activated sludge of municipal wastewater treatment plant was used as the substrate for reactors; the sulfur content was increased by natrium sulfate addition. The average sludge composition was following: total suspended solids (TSS) 32.8 g/L, volatile suspended solids (VSS) 20.1 g/L, pH 7.84.

The reaction mixture was kept homogeneous by mechanical mixing and operational temperature was kept at $40 \pm 1\,^{\circ}C$. The volumetric loading rates of digesters were 2.0 g/L.d and 0.15 g /L.d for COD and SO_4^{2-} respectively. The retention time in both reactors was on average 30 days. To reach the microaerobic conditions and oxidize the sulfide produced by SRB, one reactor was connected to an air supply by peristaltic pump with flow rate fixed to 350 mL/d.

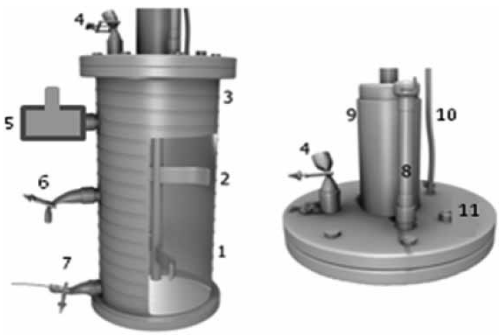

Figure 1 | The scheme of the reactor: 1. body of reactor, 2. paddle-wheel stirrer, 3. resistance heater, 4. input pipe for substrate, 5. vessel for ORP analysis, 6. sampling point, 7. air supply, 8. thermometer, 9. water-cap, 10. biogas output to gas flow meter, 11. septum for biogas sampling.

Analytical procedures were carried out according to standard methods for the examination of Water and Wastewater (American Public Health Association/American Water Works Association/Water Environment Federation 1998), the biogas composition and volatile fatty acids were determined by gas chromatograph GC 8000Top equipped with a heat conductivity detector HWD 800 (Dohanyos *et al.* 1997). The elemental composition of sludge was assessed by X-ray fluorescence analysis, using the ARL 9400 XP sequential WD-XRF spectrometer. It is equipped with the Rh anode end-window X-ray tube type 4GN fitted with 75 µm Be window. All peak intensity data were collected by software WinXRF in vacuum.

Activity of methanogenic bacteria (MB activity)

The assessment of the specific MB activity was carried out in serum bottles under mesophilic conditions without mixing using initial F/M ratio of 1 g COD/g VSS. All tests were carried out in three replicates. The volume of produced methane was monitored and maximum methane production rate calculated according to guidelines proposed by Angelidaki *et al.* (2009). The production of biogas was measured volumetrically using water displacement method. The biogas composition was determined by gas chromatography. The tests were performed with acetate as organic substrate and therefore the activity of acetoclastic methanogens was assessed.

Activity of sulfate reducing bacteria (SRB activity)

For the determination of SRB activity, a modified methanogenic activity test was used. The SRB activity test proceeded under mesophilic conditions, in serum bottles with volume of 120 mL. The volume of liquid phase was 80 mL, volume of tested biomass was 30 mL. Acetate was used as the organic substrate needed for the sulfate reduction. The initial ratio $S\text{-}SO_4^{2-}/COD$ was 0.03. Solution of natrium sulfate was added into the batch bottles to achieve the initial sulfate concentration of 500 mg/L in the liquid phase. Each experiment was carried out in triplicate. At the start, the bottles were closed and the headspace was flushed with nitrogen. The samples for analysis of liquid phase were taken daily and the concentration of sulfate was determined in the centrifuged sample.

Activity of sulfide oxidizing bacteria (SOB activity)

Testing of SOB activity was performed in Erlenmeyer flasks (total volume 1.3 liter). The sludge was tested without any pretreatment at original concentration (about 15 g/L VSS) at operational temperature of digesters. The sulfide was dosed as Na_2S to achieve an initial sulfide concentration of 200 mg/L. The flask was tightly closed and mixed by gas recirculation (flow 1L/min). The activity of SOB was characterized by value of sulfide removal rate in mg/(g.h). The volumetric ratio between gaseous and liquid part in the testing flask was 8:5, that means the initial mass ratio of oxygen to sulfide sulfur about 2. After sulfide addition, a chemical change of sulfur compounds (sulfide precipitation for example) can take place and therefore the test was performed also in nitrogen atmosphere to separate other processes from biochemical and chemical oxidation. The decrease of sulfide concentration in liquid phase and oxygen in gas phase was then monitored. The SOB activity was expressed as difference between sulfide removal rate in N_2 atmosphere and sulfide removal rate in air atmosphere.

Foaming potential and foam stability

These parameters were assessed as additional characteristic of digested sludge, because presence of filamentous foam forming bacteria was elevated. It has been reported, that anaerobically treated excess activated sludge exhibits the tendency to cause foaming in digester. For the description and comparison of foam quantity and quality the 'bubble test' has been developed (Zaplatilkova 2004). The testing was based on the test described by Pagilla *et al.* (1996) and modified with respect to the character of anaerobic sludge. The final test was carried out by bubbling of 1 liter of sludge by nitrogen with the flow rate of 1 L/min in 2 L volumetric cylinders. The level of foamy sludge is recorded after five minutes of bubbling and the foaming potential (FP) is calculated from this value. Five minutes after the stopping of the gas flow the level of foamy sludge is recorded again to calculate index of stability (IS). FP describes the capability of the sludge to create foam. IS gives the information about stability of the foam created. The mathematical definition of FP and IS are following:

$$FP = \frac{V_5}{V_0}$$

$$IS = \frac{(V_{ST} - V_0)}{(V_5 - V_0)} * 100$$

where V_0 is the volume of sludge at the beginning of measuring (usually 1 liter), V_5 is the volume of foamy sludge after 5-minute bubbling and V_{ST} is the volume of foamy sludge 5 minutes after the gas flow stop.

RESULTS AND DISCUSSION

The basic results of anaerobic digestion process for both reactors are shown in Table 1. It is evident that despite air dosing, the specific methane production in the microaerobic digester was higher. Significantly better quality of sludge liquor as regards soluble COD and N_{ammon} was proved. Rest of nitrogen from dosed air caused lower methane concentration in biogas of microaerobic digester.

In the evaluated period the hydrogensulfide concentration in biogas produced in the anaerobic reactor was not very high (high content of Fe caused the removal of sulfide by precipitation of FeS), therefore H_2S removal efficiency by microaerobic digester is not as high as usually reported (Jenicek *et al.* 2008; Fdz-Polanco *et al.* 2009), however biogas quality is sufficient for most of biogas exploitation ways. Figure 2 shows the H_2S concentration in days of MB activity testing to show the potential relation mentioned below.

Sludge composition

Small differences were found in elemental composition of digested sludge from microaerobic and anaerobic reactor (Table 2). Expected increase of sulfur content due to accumulation of elemental S in sludge was confirmed, however this increase was not very high because of continuous removal of sludge (and sulfur) from digester. The increase was consistent with sludge retention time in digester and with actual

Table 1 | Comparison of sludge digestion results (average value for all period of operation)

Parameter	Microaerobic digester	Anaerobic digester
pH	8.04	8.06
Soluble COD (g/L)	4.47	6.71
VFA (mg/L)	65	63
N_{ammon} (g/L)	2.78	3.04
Specific methane production ($L/kg\ VSS_{added}$)	280	239
Methane in biogas (vol.%)	60.7	64.8
Hydrogensulfide in biogas (mg/m^3)	107	283

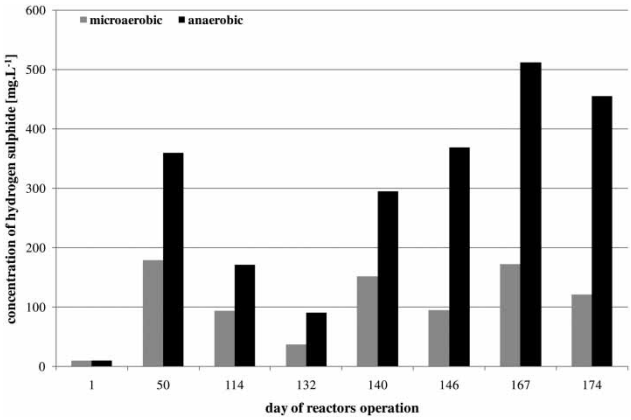

Figure 2 | Hydrogensulfide concentration in biogas in days of methanogenic activity testing.

Table 2 | Relative elemental composition of digested sludge from microaerobic and anaerobic reactor (major elements of mineral fraction) (132nd day)

Element	Microaerobic sludge (%)	Anaerobic sludge (%)	Std. dev.	Ratio anaerobic/ aerobic
Fe	28.7	29.2	0.10	0.983
P	17.3	17.1	0.10	1.012
Ca	11.9	11.9	0.09	1.000
S	9.92	9.68	0.09	1.025
Na	9.58	9.66	0.09	0.992
Al	8.48	8.30	0.09	1.022
Si	5.60	5.43	0.07	1.031
K	3.67	3.80	0.06	0.966
Mg	1.43	1.44	0.04	0.993

efficiency of biogas desulfurization. High Fe content is caused by Fe^{3+} addition in wastewater treatment technology where the treated activated sludge originates from. Lower amount of Fe in microaerobic sludge can be explained by lower total sulfide concentration (Table 3). The analysis will be continued to confirm the mentioned sludge uniformity and suggested trends as regards S content.

Table 3 shows that also variation in basic parameters of the sludges is small. Bigger difference can be found in sludge liquor composition (soluble COD). Higher concentration of total sulfide in anaerobic sludge is not in contradiction with data about sulfur in Table 2 and indicates the oxidation of sulfide.

Activity of methanogenic bacteria – MB

The activity of methanogenic bacteria was periodically monitored during the digesters operation. The results of activity tests are shown in Figure 3. Immediately after the start of microaeration, decrease (16%) of MB activity was observed.

Table 3 | Comparison of basic parameters of digested sludge from microaerobic and anaerobic reactor (average value and standard deviation for all period of operation)

	Microaerobic digester		Anaerobic digester	
Parameter	Average	Std. dev.	Average	Std. dev.
TSS (g/L)	29.8	1.6	27.6	0.8
VSS (g/L)	17.6	0.9	15.9	0.4
VSS/TSS (%)	54.3	0.9	54.4	0.9
Soluble COD (g/L)	4.14	0.74	5.28	1.41
Total sulfide (mg/L)	858	64	928	68
ORP_H (mV)	−270	13	−305	4

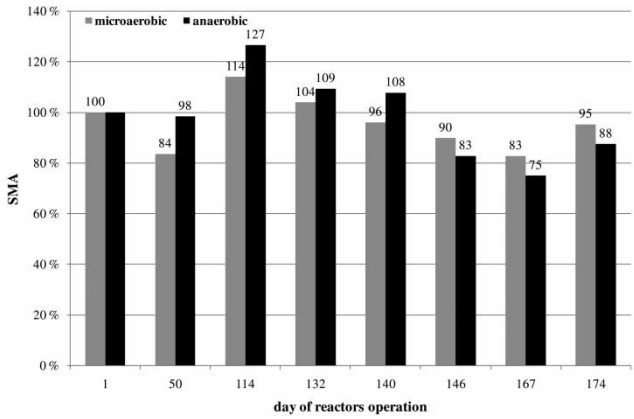

Figure 3 | The course of methanogenic bacteria activity.

When sulfate dosing was initiated, methanogenic activity of both anaerobic and microaerobic biomass increased, possibly because of previous deficit of sulfur as nutrient. During further operation, the MB activity dropped again in both reactors, however, decrease of activity of microaerobic biomass was smaller and therefore its activity started to be higher in comparison with the anaerobic biomass. This activity development was affected by many factors and dissolved sulfide concentration was probably the most important of them. Sulfide concentration was gradually rising in both reactors; however, the concentration in microaerobic reactor was always significantly lower. The relations between dissolved sulfide concentration and specific MB activity are shown in Figure 4. While the activity of microaerobic biomass is independent on sulfide concentration, the activity of microaerobic biomass seems to be decreasing with increasing dissolved sulfide concentration. IC_{50} (median inhibition values) reported for methanogenic activity range between 30 and 250 mg/L (Lens *et al.* 1998), however free H_2S is most toxic form and therefore also pH value is important. At actual pH of digester the maximum concentration of free H_2S was 14 and 24 mg/L for the microaerobic and anaerobic digester, respectively.

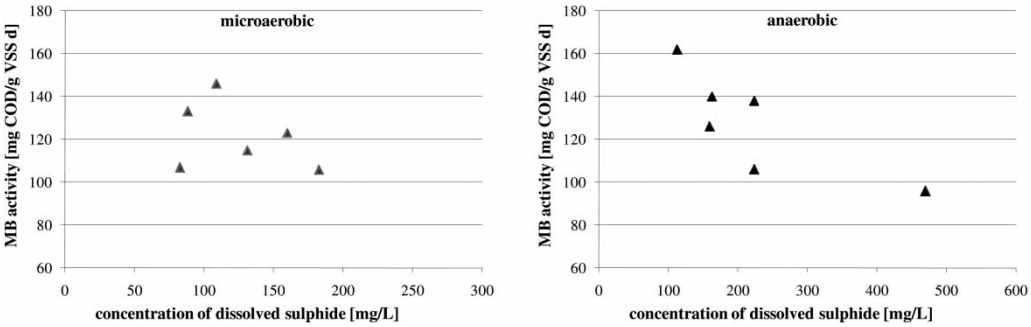

Figure 4 | Relation between specific methanogenic activity and concentration of dissolved sulfide.

Activity of sulfate reducing bacteria – SRB

The activity of SRB was measured with acetate as organic substrate. SRB activity was expressed as maximum sulfate removal rate. It was observed a twofold increase of the SRB activity during 110 days of reactors operation (Table 4). Because of competition of SRB with MB for the readily degradable organic substrate, the reduction of sulfate in both reactors was not complete. The results do not reflect the expectation of lower activity of SRB because of air presence in microaerobic digester. Microaerobic condition did not cause any inhibition of SRB; their activity was even 10% higher in comparison with strictly anaerobic biomass. The results are in agreement with findings of Tang *et al.* (2004) who reported that activity of SRB in the microaerobic digester was not repressed.

Activity of sulfide oxidizing bacteria – SOB

The activity of SOB was measured as maximum specific sulfide removal rate. The activity of original sludge used as inoculum for both reactors was low, because the content of available sulfur compounds in the treated sludge was small. This confirms previous findings of Jenicek *et al.* (2005) and Jenicek *et al.* (2010). During the period in which dosing of sulfate started, the activity of SOB increased step by step and the activity of the microaerobic biomass was always about 50–75% higher. Finally, the SOB activity was measured after long term biomass adaptation of both laboratory reactors to high sulfate content in the sludge treated. The results are shown in Table 5.

Table 4 | Change of SRB activity – expressed for sulfate and VSS in mg/(g.h)

Day of operation	Microaerobic digester	Anaerobic digester
1	0.98	0.98
110	2.0	1.8

Table 5 | Change of SOB activity – expressed for sulfide and VSS in mg/(g.h)

Day of operation	Microaerobic digester	Anaerobic digester
1	1.8	1.8
176	10.4	6.1

Foaming problems

The waste activated sludge treated contained in some periods increased amount of filamentous bacteria and therefore the risk of digester foaming was high. Especially during period with high foaming potential, the difference between microaerobic and anaerobic digester was high. The maximum foaming potential FP was 3.2 and 2.6 for the anaerobic and microaerobic reactor, respectively. In addition, the foam rising in anaerobic reactor was much more stable in comparison to the microaerobic reactor. The index of stability IS was 96% and 53% in the anaerobic and microaerobic reactor, respectively.

CONCLUSIONS

The data collected during long term monitoring of sludge quality and biomass activity of anaerobic and microaerobic digesters allow to draw following conclusions:

- The quality of sludge in both rectors is similar, only small differences were found in composition and activities, except of SOB activity.
- Slightly higher content of total S and lower content of S-S^{2-} in microaerobic sludge indicate the accumulation of elemental S.
- Methanogenic activity slightly decreased due to air dosing in the digester, however, the methanogenic activity of microaerobic biomass was higher than that of strictly anaerobic biomass at higher sulfide concentrations.

- Activity of sulfate reducing bacteria in the microaerobic reactor was not negatively affected by air dosing and was even slightly higher than the activity in the anaerobic reactor.
- Activity of sulfide oxidizing bacteria of the microaerobic biomass was significantly higher in comparison with the anaerobic biomass. The SOB activity was stimulated not only by microaerobic condition but also by sulfide concentration even in the anaerobic digester.
- The main differences in operational results of the microaerobic digester in comparison with anaerobic digester were: efficient H_2S removal, higher specific methane production, lower methane concentration in biogas due to dilution by nitrogen remained from the air dosed, better sludge liquor quality and lower foaming potential and foam stability.

ACKNOWLEDGEMENTS

This research was financially supported by The Czech Ministry of Education, Youth and Sports – project MSM6046137308.

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