Advantages of anaerobic digestion of sludge in microaerobic conditions

P. Jenicek, J. Koubova, J. Bindzar and J. Zabranska

ABSTRACT

The paper reviews results and experience of microaerobic experiments at both high and low sulphide concentrations and evaluates advantages and drawbacks of the anaerobic digestion of sludge in microaerobic conditions as regards biogas quality, digested sludge quality, organic pollutants biodegradability and methanogenic activity of biomass. The innovative microaerobic modification of the anaerobic sludge digestion technology was studied in both laboratory and full scale. Microaerobic conditions are obtained by dosing of a limited amount of the air into the liquid phase of the anaerobic digester. It was shown that anaerobic bacteria including methanogens can be active also in such system. In a mixed culture, even strict anaerobes can survive without inhibition, if the facultative microorganisms are able to consume the present oxygen quickly and fully. Until now, the microaerobic conditions were predominantly used for hydrogen sulphide removal from biogas. In the paper the role of the surplus oxygen was studied also at low sulphide concentration, when the oxygen is consumed in high extent for other processes beside sulphide oxidation.

Key words | anaerobic digestion, biodegradability, methanogenic activity, microaerobic conditions, sulphide oxidation

P. Jenicek (corresponding author)
J. Koubova
J. Bindzar
J. Zabranska
Department of Water Technology and Environmental Engineering,
Institute of Chemical Technology Prague,
Technicka 5,
166 28 Prague,
Czech Republic
E-mail: *jenicekp@vscht.cz*

INTRODUCTION

Among sludge treatment technologies anaerobic digestion is established and well-proven technology. The innovative microaerobic modification of anaerobic sludge digestion technology combines the anaerobic and in micro-scale the aerobic condition. In the field of biological wastewater treatment the combination of anaerobic and aerobic processes is well known and often used. It brings many benefits, mostly related to excellent treatment efficiency and low energy consumption. In the field of sludge treatment the combination of different operational conditions is much scarcer.

The successive combination of anaerobic sludge treatment followed by post-aeration was successfully tested to improve nitrogen removal (Parravicini *et al.* 2008), however in this case both processes are separated in time and space. Untill lately, there was a lack of knowledge about the possibility of combining both anaerobic and aerobic (or anoxic) processes (Jenicek *et al.* 2002) at the same time simultaneously in one biological reactor.

The coupling of oxidative and reductive environments is common in natural environments since the homogeneous distribution of oxygen is rare (Stephenson *et al.* 1999). Conjunction and symbiosis of anaerobic and aerobic processes into the microaerobic operation of anaerobic digestion presents a special case (Jenicek *et al.* 2005). It was found out that limited and controlled presence of oxygen inside a digester is not dangerous, it can even have a positive effect in some aspects (Stephenson *et al.* 1999; Zitomer & Shrout 2000; Khanal & Huang 2003*a*; Tang *et al.* 2004; van der Zee *et al.* 2007).

The microaerobic condition is obtained by dosing of a limited amount of air (oxygen) into an anaerobic reactor.

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It was shown that anaerobic bacteria, including methanogens, can be active in such systems (Kato *et al.* 1993). As has been reported, the methanogenic activity can sometimes be even higher under microaerobic conditions in comparison with a purely anaerobic system (Zitomer & Shrout 1998). On the other hand it was proved that also strictly aerobic bacteria can be autochthonous inhabitants of anaerobic environment (Thierry *et al.* 2004). Even strict anaerobes can survive in mixed culture without any inhibition in the presence of oxygen, when facultative microorganisms consume the oxygen quickly and fully.

Microaerobic conditions–definition

The term microaerobic (or micro-aerobic) may be found in the literature referring to very different systems. In one case it represents an aerobic system with low oxygen supply, while in another it describes an anaerobic system into which a trace amount of oxygen is supplied. In fact, an imponderable amount of oxygen is dosed with fresh wastewater or sludge into most anaerobic systems. The term "microaerophilic condition" can be found also, despite the fact that this adjective should be reserved for organisms mainly–"microaerophilic microorganisms".

Characterization of microaerobic conditions often differs being applied for any special variation of basic anaerobic, anoxic or aerobic conditions.

Chu & Mavinic (1998) designated conditions as microaerobic when oxygen demand exceeds oxygen supply, such as in thermophilic aerobic digestion. Kalyuzhnyi (1998) used the term microaerobic preacidification for a case, where the reaction vessel was kept open and oxygen transfer by liquid surface was not prevented.

Microaerobic condition is sometimes described by a limited concentration of dissolved oxygen below 1 mg/L (Ergüder & Demirer 2005), below 0.2 mg/L (Ma & Love 2001) or below 0.05 mg/L (Deniz *et al.* 2004). Zitomer & Shrout (1998) used the oxygen loading rate for comparison of alternatives of microaerobic conditions (0.1 – 1 g O_2/L.day).

The relation of oxygen dose to oxidized substances such as O_2:S or O_2:COD (Cirne *et al.* 2008) or the relation to biogas production such as oxygen (air) flow rate: biogas (or methane) rate seems to be suitable for the efficient control of microaerobic process.

Table 1 | Examples of oxidation-reduction potential values referred to as microaerobic conditions (in mV)

Aerobic	Microaer.	Anaerobic	Literature
$>+110$	0 to -200	≤ -150	Noparatnaraporn *et al.* (1986)
$\geq -100^{*}$	≤ -150	-460	Ohta *et al.* (1996)
Not presented	-230 to -180	-280	Khanal & Huang (2003*b*)

*Signed as aerated.

The oxidation-reduction potential (ORP) is theoretically an excellent tool for the characterization of a microaerobic system; however the data reported in the literature vary again to a great extent (see Table 1). The probable reason for the variation is the specificity of each system. Moreover, there is a lack of information in some reported cases on whether the results are expressed in relation to the hydrogen electrode in accordance with the standard procedure–ORP_H (Eh), (Pitter *et al.* 2005).

Such an inexplicit situation is caused by the absence of a precise and clear definition of the microaerobic conditions. The microaerobic system referred to in this paper, can be characterized as a system with zero concentration of oxygen and limited (trace) oxygen consumption. With respect to the ORP, the microaerobic system, regardless of whether it is microaerobic in relation to anaerobic or anoxic condition, can be defined generally as a system in which micro-consumption of oxygen causes a limited ORP increase.

METHODS

The basic analytical procedures were carried out according to the Standard Methods (APHA (2005)), the biogas composition and volatile fatty acids were determined by gas chromatography. AOX was determined using the AOX analyser LTX-2000 (Labtech, Czech Republic) according to the standard method ISO 9562 (shaking procedure).

The assessment of biodegradability and specific methanogenic activity of biomass was carried out at mesophilic conditions without mixing in three replicates. The volume of methane produced is obtained by multiplying the headspace volume by the (%) of CH_4 in the headspace as determined by GC analysis. according to guidelines proposed by Angelidaki *et al.* (2009).

Table 2 | The technological parameters of digesters and average results before implementation of micro-aeration

Digester	A	B
Volume (m^3)	2 × 1,500	2,100
Operational temperature	40	38
Biogas production (m^3/d)	1,220	830
Hydr. retention time (d)	25	23
H_2S concentration (mg/m^3)	4,380	7,580

The full scale experiments were carried out in the anaerobic mesophilic digesters of two municipal wastewater treatment plants, their basic technological parameters are illustrated in Table 2. The air was injected in both cases into the sludge recirculation stream of digesters (Jenicek *et al.* 2008).

The additional laboratory experiments were performed in two identical laboratory digesters (C and D) (volume 11 L). The design and equipment of the reactors is shown in Figure 1.

The first one was operated as the microaerobic digester and the second one as the fully anaerobic reference digester, both with hydraulic retention time of 18 days. The digested material was the mixture of thickened surplus activated sludge with the addition of bone flour to increase the biogas production, both characterized in Table 3.

The mass ratio activated sludge: bone flour was about 50:1. Despite the low concentration of hydrogen sulphide the effect of the air dosing was tested to find out the influence of the oxygen if the sulphide oxidation is negligible.

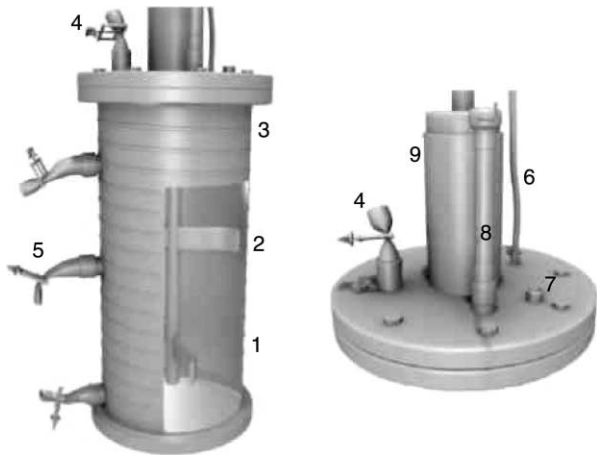

Figure 1 | Scheme of the reactor: 1—body of reactor; 2—paddle-wheel stirrer, 3—resistance heater; 4—input pipe for substrate; 5—sampling pipes; 6—output of biogas to gas-meter; 7—septum for biogas sampling; 8—regulation thermometer; 9—the water-cap.

Table 3 | The basic characteristic of treated material in laboratory digesters

	Activated sludge (g/L)	Bone flour (g/g)
COD	48.8	1.38
CODsoluble	1.83	–
TS	45.74	0.965
VS	29.57	0.671

RESULT AND DISCUSSION

Biogas desulphurization

The traditional use of microaerobic condition is connected with desulphurization of biogas, which may bring also detoxification of digesters - decrease of sulphide concentration under toxic level (Buisman *et al.* 1990; Janssen *et al.* 1999; Khanal & Huang 2003*a*; Krishnakumar *et al.* 2005; van der Zee *et al.* 2007). Intimate contact of aerobes with anaerobes may reduce the accumulation of toxic intermediates as the aerobes would achieve an in situ removal of these anaerobic metabolites. The microbial diversity and exchange of metabolites may promote system stability (Stephenson *et al.* 1999).

Long-term full-scale experience with microaerobic desulphurization of biogas shows that the efficiency of the hydrogen sulphide removal from biogas of about 99% is achievable at high initial concentration (4,000–8,000 mg/m^3), as shown in Table 4.

Biodegradability improvement

Improvement of biodegradability of organic compounds (or lower effluent COD) presents another proven advantage of

Table 4 | Comparison of average H_2S concentration in biogas during anaerobic and microaerobic operation of digesters

Digester	Anaerobic period (mg/m^3)	Microaerobic period (mg/m^3)	H_2S removal efficiency (%)
A	4380	41	99.06
B	7580	72	99.05
C	34	2.6	92.35

Table 5 | Comparison of the residual organic fraction in digested sludge from digesters operated at different conditions

VSS/TSS (%)		
Anaerobic period	**Microaer. Period**	**Digester characterization**
56.6	54.6	Full scale, high initial sulphide concentration
65.8	59.7	Full scale, high initial sulphide concentration
56.4	55.4	Lab scale, low initial sulphide concentration

microaerobic conditions. This refers to a higher degradation of chlorinated aromatic hydrocarbons (Zacharias *et al.* 1995), more efficient degradation of BTX compounds (Ma & Love 2001), higher efficiency of hydrolysis (Johansen & Bakke 2006) and higher hydrogen production (Eriksen *et al.* 2008), or lower COD of sludge liquor (Jenicek *et al.* 2008).

The improved biodegradability listed in Table 5 is probably caused by the complementing of reducing and oxidizing processes but also by the augmentation of microbial species diversity of microaerobic population in comparison with a strictly anaerobic one (Tang *et al.* 2004).

AOX removal efficiency

This parameter was monitored because of the strict concentration limits of AOX (adsorbable organically bound halogens) in stabilized sludge which is applied in agriculture in the Czech Republic and in the European Union as well. The preliminary result seems to be promising showing 30% lower average concentration of the AOX in the sludge from a microaerobic digester in comparison with an anaerobic digester over a test period of two months. The results are presented in Table 6.

Such results are interesting especially in the circumstances of the European Union legislation, where the AOX concentration limit for agricultural use of stabilized sludge is 500 mg/kg (Erhardt & Pruess 2001). The presented results proved the anticipated positive effect of the presence of oxygen or microaerobic conditions on biodegradability of halogenated organic compounds determined as the AOX. However, more detailed experiments are to be carried out in this field.

Table 6 | Concentration of the AOX in the digested sludge in mg per kg of dry solids (lab–scale digesters C and D)

	AOX (mg/kg)	
Type of sludge (digester)	**Average**	**Standard deviation**
Microaerobic (C)	353	40
Anaerobic (D)	510	52

Table 7 | Specific methane production rate—1st test (lab–scale digesters C and D)

Substrate	Biomass	r_{CH_4}, max (ml/d g VSSinoculum)
Real substrate	Microaerobic C	15
	Anaerobic D	18
Acetate	Microaerobic C	18
	Anaerobic D	21

Methanogenic activity of biomass

The biomass activity was monitored during operation of lab-scale reactors by anaerobic batch tests (volume 80 ml). The methanogenic activity was expressed by maximum methane production rate (recalculated to standard temperature and pressure) related to concentration of biomass VSS (r_{CH_4}, max).

It was found that the specific methanogenic activity measured with real substrate and acetate was slightly lower in the microaerobic digester at stable operation of digesters (Table 7).

However, total biogas (and methane production as well) was surprisingly lower at the batch tests with microaerobic biomass (9 and 16% respectively) (Figures 2

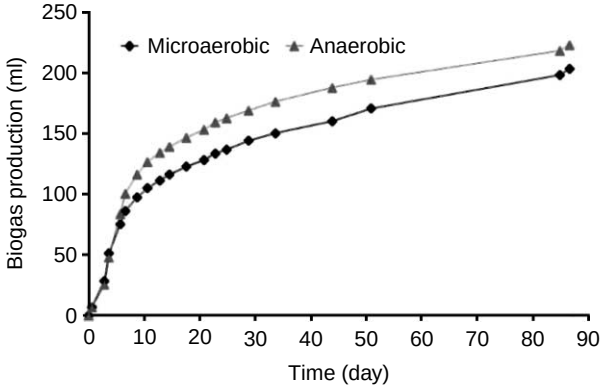

Figure 2 | Cumulative biogas production during the test with the substrate used to feed the reactors.

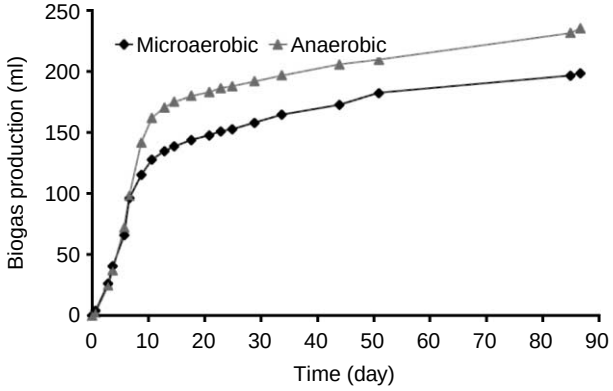

Figure 3 | Cumulative biogas production during the test with acetate.

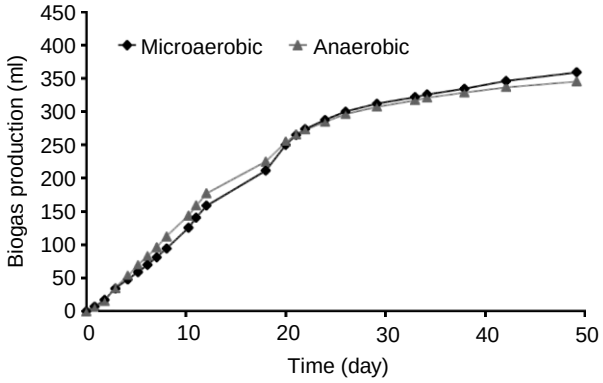

Figure 4 | Cumulative biogas production during the test with the substrate used to feed the reactors—biomass from overloaded digesters.

and 3). The mechanism of the substrate degradation was different, probably because the complete degradation of acetate was proved.

Whereas the first test was carried out during stable operation of digesters, the second set of the activity tests was performed in the period of overloading of both digesters. The significant decrease of specific biogas production and increase of soluble COD concentration (SCOD) was found. Even in such a situation the efficiency of hydrogen sulphide removal from biogas was not affected significantly as shown in Table 8.

The course of biogas production during the second testing shows that in this case the methanogenic activity measured with real substrate is comparable again but the difference in total biogas production ascertained previously had disappeared–Figure 4.

It is also interesting to note the course of the biogas production in the test with acetate as substrate–Figure 5. The microaerobic biomass was able to overcome the inhibition better and the recovery of methanogenic bacteria was much faster. The reason for this is likely the lower sulphide concentration in the microaerobic reactor.

Table 8 | Performance of digester in the testing period (lab–scale digesters C and D)

		SCOD effluent (g/L)	CH_4 prod. (L/g TCOD)	H_2S (g/m^3)
1st test period	Microaer. C	8.1	0.12	15
	Anaerobic D	8.6	0.13	18
2nd test period	Microaer. C	14.5	0.07	18
	Anaerobic D	15.3	0.08	21

The combination of high VFA concentration and high sulphide concentration in the anaerobic digester can cause deeper inhibition of methanogenic bacteria.

Table 9 shows the value of specific methanogenic activity which can be evaluated for the states before and after recovery (at the test with acetate as substrate)–value in parenthesis. The faster recovery (400 hours versus 800 hours approximately) of microaerobic biomass is surprising, because untill now the microaeration was supposed to be unsuitable for unstable systems. This phenomenon should be studied in more detail. Anyway potential explanation of the better recovery of microaerobic biomass is their greater microbial species diversity (Tang *et al.* 2004).

Drawbacks of microaerobic technology

The distrust of operators to the introduction of oxygen into the digester presents the main drawback of microaerobic

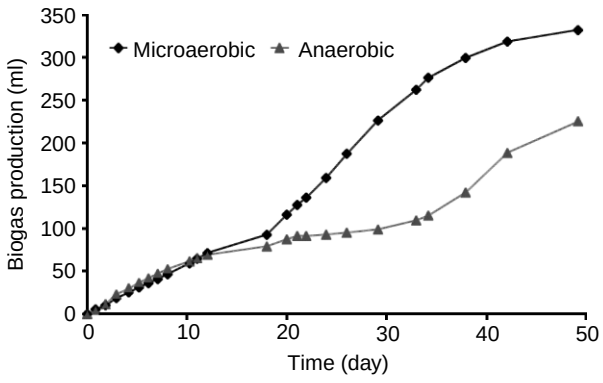

Figure 5 | Cumulative biogas production during the test with acetate—biomass from overloaded digesters.

Table 9 | Specific methane production rate—2nd test (lab–scale digesters C and D)

Substrate	Biomass	r_{CH_4}, max (ml/d g VSSinoculum)
Real substrate	Microaerobic C	15
	Anaerobic D	16
Acetate	Microaerobic C	6 (12)*
	Anaerobic D	7 (9)*

*Evaluated for period after recovery.

modification of anaerobic digestion, although the dose of oxygen is sufficiently low to keep the process absolutely safe.

If the oxygen is consumed for organic matter oxidation and not for sulphide oxidation only, the decrease of methane production can be expected as illustrated by the data in Table 10. However, the loss of methane is not significant and is often compensated by the improved efficiency of organic matter biodegradation and increased volume of produced biogas. The dose of oxygen is usually in the range of several percents of methane production (1–2% in our full scale experiments–Jenicek *et al.* 2008), accordingly the loss of methane of 0.5–1% can be calculated from stoichiometry. The same value represents the increase of CO_2 production in the case where all the oxygen is consumed for organic matter oxidation (Zitomer & Shrout 1998). Moreover, it is important to mention that in the case, where air is used for microaeration, the nitrogen from air will remain and dilute the biogas. On the other hand, Zitomer & Shrout (2000) reported that direct limited aeration of fluidized bed reactors (FBRs) could achieve increased methane production compared to strictly anaerobic FBRs treating high sulfate, high-COD wastewater.

Table 10 | Comparison of average CH_4 concentration in biogas during anaerobic and microaerobic operation of digesters

CH_4 (%)		
Anaerobic period	**Microaer. Period**	**Digester characterization**
65.8	64.5	Full scale, high initial sulphide concentration
65.9	65.4	Full scale, high initial sulphide concentration
70.8	66.7	Lab scale, low initial sulphide concentration

Another risk of microaerobic technology is associated with the lack of full scale experience. Long-term operation at higher extent of aeration can cause higher aerobic biomass production and deterioration of anaerobic granules, if this type of biomass is used. Stephenson *et al.* (1999) reported a decrease in the mean particle size and lower VSS levels retained in the reactors at stronger oxygenation.

Lastly, it is necessary to say that the paper does not intend to argue the application of the microaerobic process every time in each anaerobic process. Only well substantiated benefit and improvement of process such as desulphurization of biogas, suppression of sulphide toxicity, improved removal of specific toxicants, enhanced quality of digested sludge or another should be sufficient reason for the application of anaerobic digestion of sludge in microaerobic conditions.

CONCLUSIONS

The results and experience gained during laboratory and full-scale operation of microaerobic systems can be summarized as following:

- The application of microaerobic conditions is an efficient method for the hydrogen sulphide removal from the biogas. The efficiency of the H_2S removal from biogas of about 99% is a realistic value at a high concentration in the range of grams per m^3.
- The application of microaerobic conditions is an efficient method for sulphide toxicity suppression.
- The dosing of the limited amount of oxygen in the digester does not destroy the digestion process even in systems where oxygen is not consumed by prompt sulphide oxidation.
- The dosing of the limited amount of oxygen in the digester does not destroy the digestion process even in overloaded systems.
- The microaeration does not cause the significant decrease of the specific methanogenic activity of the biomass.
- At the air dosing into digester the remaining nitrogen dilutes the methane in biogas. Part of methane or organic compounds from which the methane is arising can be oxidized by present oxygen.

- The expected decrease of methane production connected with oxidation processes must not happen in all cases, thanks to deeper degradation of organic material. The VSS/TSS ratio of the digested sludge decreased in all experiments with microaerobic conditions, due to better efficiency in VSS degradation.
- A slight decrease of the soluble COD concentration, ammonia nitrogen and phosphate concentration in the sludge liquor was observed in systems where the microaerobic conditions were applied.
- A decrease of some recalcitrant compounds concentration (AOX for example) was observed thanks to the combination of anaerobic with (micro)aerobic conditions.

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REFERENCES

Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J. L., Guwy, A. J., Kalyuzhnyi, S., Jenicek, P. & van Lier, J. B. 2009 Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. *Water Sci. Technol.* **59**(5), 927–934.

APHA 2005 *Standard Methods for the Examination of Water and Wastewater*, 21st edition. AWWA, Washington, DC.

Buisman, C. J. N., Geraats, S., Ijspeert, P. & Lettinga, G. 1990 Optimization of sulphur production in a biotechnological sulphide removing reactor. *Biotechnol. Bioeng.* **35**, 50–56.

Chu, A. & Mavinic, D. S. 1998 The effects of macromolecular substrates and a metabolic inhibitor on volatile fatty acid metabolism in thermophilic aerobic digestion. *Water Sci. Technol.* **38**(2), 55–61.

Cirne, D. G., van der Zee, F. P., Fdz-Polanco, M. & Fdz-Polanco, F. 2008 Control of sulphide during anaerobic treatment of S-containing wastewaters by adding limited amounts of oxygen or nitrate. *Rev. Environ. Sci. Biotechnol.* **7**, 93–105.

Deniz, T., Cinar, Ö. & Grady, C. P. L., Jr 2004 Effects of oxygen on biodegradation of benzoate and 3-chlorobenzoate in a denitrifying chemostat. *Water Res.* **38**, 4524–4534.

Ergüder, T. H. & Demirer, G. N. 2005 Investigation of granulation of a mixture of suspended anaerobic and aerobic cultures under alternating anaerobic/microaerobic/aerobic conditions. *Process Biochem.* **40**, 3732–3741.

Erhardt, W. & Pruess, A. 2001 Organic contaminants in sewage sludge for agricultural use. *Joint Research Centre Institute for Environment and Sustainability Soil and Waste Unit*.

Eriksen, N. T., Nielsen, T. M. & Iversen, N. 2008 Hydrogen production in anaerobic and microaerobic *Thermotoga neapolitana*. *Biotechnol. Lett.* **30**, 103–109.

Janssen, A. J. H., Lettinga, G. & de Keizer, A. 1999 Removal of hydrogen sulphide from wastewater and waste gases by biological conversion to elemental sulphur, Colloidal and interfacial aspects of biologically produced sulphur particles. *Colloids Surf. A Physicochem. Eng. Asp.* **151**, 389–397.

Jenicek, P., Maca, J., Keclik, F. & Bindzar, J. 2008 Use of microaerobic condition for the improvement of anaerobic digestion of solid wastes. *Water Sci. Technol.* **58**(7), 1491–1496.

Jenicek, P., Smejkalova, P. & Horecky, P. 2005 Biological microaerobic sulphide removal with respect to specific biomass activity. *Proc. of Int. Conf. Anaerobic Digestion of Solid Wastes*, Copenhagen, pp. 145–148.

Jenicek, P., Zabranska, J. & Dohanyos, M. 2002 Adaptation of the methanogenic granules to denitrification in anaerobic-anoxic USSB reactor. *Water Sci. Technol.* **45**(10), 335–340.

Johansen, J. E. & Bakke, R. 2006 Enhancing hydrolysis with microaeration. *Water Sci. Technol.* **53**(8), 43–50.

Kalyuzhyi, S., Fedorovich, V. & Nozhevnikova, A. 1998 Anaerobic treatment of liquid fraction of hen manure in UASB reactors. *Bioresour. Technol.* **65**, 221–225.

Kato, M. T., Field, J. A. & Lettinga, G. 1993 High tolerance of methanogens in granular sludge to oxygen. *Biotechnol. Bioeng.* **42**, 1360–1366.

Khanal, S. K. & Huang, J. C. 2003*a* Anaerobic treatment of high sulfate wastewater with oxygenation to control sulphide toxicity. *J. Environ. Eng.* **129**, 1104–1111.

Khanal, S. K. & Huang, J. C. 2003*b* ORP-based oxygenation for sulfide control in anaerobic treatment of high-sulfate wastewater. *Water Res.* **37**, 2053–2062.

Krishnakumar, B., Majumdar, S., Manilal, V. B. & Haridas, A. 2005 Treatment of sulphide containing wastewater with sulphur recovery in a novel reverse fluidized loop reactor (RFLR). *Water Res.* **39**, 639–647.

Ma, G. & Love, N. G. 2001 Creating anoxic and microaerobic conditions in sequencing batch reactors treating volatile BTX compounds. *Water Sci. Technol.* **43**(3), 275–282.

Noparatnaraporn, N., Sasaki, K., Nishizawa, Y. & Nagai, S. 1986 Simulation of vitamin B12 formation in aerobically-grown *Rhodopseudomonas gelatinosa* under microaerobic condition. *Biotechnol. Lett.* **8**(7), 491–496.

Ohta, H., Moriki, D., Miyagi, A., Watanabe, T., Kato, K. & Fukui, K. 1996 Microaerophilic property of *Actinobacillus actinomycetemcomitans* in fructose-limited chemostat cultures. *FEMS Microbiol. Lett.* **138**, 191–196.

Parravicini, V., Svardal, K. & Kroiss, H. 2008 Post-aeration of anaerobically digested sewage sludge for advanced COD and nitrogen removal: results and cost-benefit analysis at large-scale. *Water Sci. Technol.* **57**(7), 1087–1094.

Pitter, P., Sykora, V. & Kral, P. 2005 The problems with interpretation of the results of oxidation-reduction potential (ORP) measurement. *Vodni hosp.* **55**(5), 134–135 (in Czech).

Stephenson, R. J., Patoine, M. A. & Guiot, S. R. 1999 Effects of oxygenation and upflow liquid velocity on a coupled anaerobic/aerobic reactor system. *Water Res.* **33**(12), 2855–2863.

Tang, Y., Shigematsu, T., Ikbal, , Morimura, S. & Kida, K. 2004 The effects of micro-aeration on the phylogenetic diversity of microorganisms in a thermophilic anaerobic municipal solid-waste digester. *Water Res.* **38**, 2537–2550.

Thierry, S., Mariaca, P., Ramirez, F. & Macarie, H. 2004 Strict aerobes, autochthonous or passenger microorganisms in the anaerobic reactor? *Proc. of the 10th World Congress on Anaerobic Digestion., Montreal, Canada*, pp. 167–173.

van der Zee, F. P., Villaverde, S., Garcia, P. A. & Fdz-Polanco, F. 2007 Sulfide removal by moderate oxygenation of anaerobic sludge environments. *Bioresour. Technol.* **98**(3), 518–524.

Zacharias, B., Lang, E. & Hajery, H. H. 1995 Biodegradation of chlorinated aromatic hydrocarbons in slow sand filters simulating conditions in contaminated soil-pilot study for in situ clearing of an industrial site. *Water Res.* **29**(7), 1663–1671.

Zitomer, D. H. & Shrout, J. D. 1998 Feasibility and benefits of methanogenesis under oxygen-limited conditions. *Waste Management* **18**(2), 107–116.

Zitomer, D. H. & Shrout, J. D. 2000 High-sulfate, high-chemical oxygen demand wastewater treatment using aerated methanogenic fluidized beds. *Water Environ. Res.* **72**, 90–97.