The effect of the supplementation with a primary carbon source on the resistance to oxygen exposure of methanogenic sludge

C. Estrada-Vázquez*, H. Macarie, M.T. Kato***, R. Rodríguez-Vázquez*, F. Esparza-García* and H.M. Poggi-Varaldo***

* CINVESTAV-IPN, Dept. Biotechnology and Bioengineering, Environmental Biotechnology R&D Group, P.O. Box 14-740, México D.F., 07000, México. (E-mail: *hectorpoggi2001@yahoo.com*)

** IRD, Marseille, France

*** Federal University of Pernambuco, Recife, Brazil

Abstract Anaerobic methanogenic consortia have a considerable resistance to oxygen exposure. Yet, most research has been focused on the study of the tolerance to oxygen of anaerobic immobilized biomass. Less is known on the potential of the anaerobic suspended biomass for withstanding exposure to oxygen and the effect of a primary degradable substrate on such resistance. Thus, the objective of this work was to determine the effect of the amount of a primary degradable substrate (sucrose) on the resistance of a methanogenic suspended biomass to oxygen exposure. It was found that the inhibition of disperse anaerobic sludge by oxygen exposure decreases when the concentration of the supplemented carbon source increases. This is in agreement with the fact that aerobic respiration of the added substrate by the facultative heterotrophic bacteria, always present in this type of sludge, has been found in previous studies as one of the main mechanisms protecting methanogens against O_2. From a practical point of view, this suggests that aeration of anaerobic systems should be possible without inhibiting the activity of methanogenic bacteria if an adequate ratio between oxygen and COD feeding is maintained. Such a ratio will depend however on the wastewater initial COD concentration.

Keywords Anaerobic; carbon source; methanogenic activity; oxygen exposure; resistance; sludge

Water Science and Technology **Vol 48 No 6 pp 119–124** © IWA Publishing 2003

Introduction

Biological treatment of wastes in reactors possessing several electron acceptors has been considered with increased interest in the recent past (Estrada-Vázquez *et al.*, 2000, 2001a). To some extent, it is a consequence of the success and wide acceptance of anaerobic digestion as a wastewater treatment option (Poggi-Varaldo and Rinderknecht-Seijas, 1996; Macarie, 2000; Monroy *et al.*, 2000), and it is one of its logical further developmental steps (Estrada-Vázquez *et al.*, 2000). The combined environment methanogenesis-aerobiosis shows promise for the treatment and quality-polishing of dilute non-complex wastewaters, toxic effluents and groundwater remediation.

It has been shown that anaerobic methanogenic consortia have a considerable resistance to oxygen exposure. However, most research has been focused on the study of the tolerance to oxygen of anaerobic immobilized biomass such as anaerobic granules from UASB digesters (Guiot *et al.*, 1993; Kato *et al.*, 1993a and b; Macarie and Guiot, 1996) and bioparticles from fluidized bed reactors (Zitomer and Shrout, 2000). Little is known on the potential of the anaerobic suspended biomass for withstanding exposure to oxygen and the effect of a primary degradable substrate on such resistance. The aim of this work was to determine the influence of the amount of a primary degradable substrate (sucrose) on the resistance of a methanogenic suspended biomass to oxygen exposure.

Materials and methods

The anaerobic sludge used as inoculum for all the study was drawn from lab scale continuous, completely mixed digesters fed with a synthetic wastewater containing 25 g COD/L (sucrose, sodium acetate, mineral salts) and operated at 35°C and 25 days hydraulic retention time (Estrada-Vázquez *et al.*, 2001b). The sludge was characterized by the following particle size distribution (on total suspended solids basis) determined accordingly to Laguna *et al.* (1999): 15.10% passed the 250 μm mesh and was retained in the 97 μm mesh; 5.57% was retained in mesh size 58 μm; 79.33% was captured in the dish (actual 0 μm). The mean particle diameter was 65.0 μm (arithmetic) and 60.2 μm (geometric), assuming a minimum diameter of 29 μm for the dish. Because of its small size, and also because flocks were not apparent, the inoculum is called *disperse anaerobic sludge* (DAS) throughout the article.

The DAS was batch-incubated without and with sucrose (initial 0, 1, 2, and 4 g COD-sucrose/L), under a range of initial O_2 concentration in the headspace (IPOH) between 0 to 70% v/v (atmospheric pressure of 580 mm Hg in Mexico City). The assay was carried out in 160 mL serum bottles with 60 mL volume liquid and 100 mL in headspace. The final concentration of the sludge was 1,620 ± 140 mg VSS/L. Bottles were incubated at 35°C and 80 rpm (Kato *et al.*, 1993a). The sludge resistance was assessed in terms of the acetoclastic specific methanogenic activity (SMA) recovery (R) of the cultures after the incubation under oxygen, and an oxygen inhibitory concentration 50% IC_{50} (Kato *et al.*, 1993a; Estrada-Vázquez *et al.*, 2001b)

$$R = \frac{SMA_j}{SMA_c} \times 100 \qquad (1)$$

where SMA_j = specific acetoclastic methanogenic activity of the culture exposed to a given IPOH, and SMA_c = specific acetoclastic methanogenic activity of the control.

Incubations under oxygen exposure lasted 3 days. After the 3-day incubation, the spent media in the bottles were replaced by a medium containing sodium acetate 30 mmol l^{-1}, the headspace was flushed and replaced with N_2:CO_2 4:1, and the specific methanogenic activity was determined. Details on other analytical methods and procedures used in this work (serum bottle technique, SMA determination, pH, alkalinity, alpha parameter, COD, total suspended and volatile suspended solids, methane and carbon dioxide contents in biogas, etc.) can be found elsewhere (Campos-Velarde *et al.*, 1997; Poggi-Varaldo *et al.*, 1997; Estrada-Vázquez *et al.*, 2000 and 2001a).

Results and discussion

The increase of initial sucrose concentration significantly improved the resistance of DAS to oxygen, as can be seen from the recoveries of the SMA of the cultures (Figure 1). The positive effect was more important between 0 to 2 g/L of initial sucrose. The recoveries of DAS incubated with 2 and 4 g/L were very similar, where remarkable high R values of up to 90% were achieved. These results confirm and extend data reported by Kato *et al.* (1993a and b) and Estrada-Vázquez *et al.* (2001a and b) for disperse and granular methanogenic sludge, respectively, and reinforce the idea of a biochemical protection of the methanogenic bacteria in the inocula by consumption of the inhibitory oxygen via aerobic respiration. The latter is probably effected by the facultative microorganisms or even sometimes strict aerobes that are usually present in anaerobic consortia (Toerien and Hattingh, 1969; Assih *et al.*, 2002).

When the initial sucrose load is much higher than the oxygen load, the recoveries of the SMA are so high that there is no IC_{50} in the range of IPOHs tested (R values are between 75 to 95% in the range of 2.5 to 50% IPOH for bottles with DAS incubated with 2 and 4 g/L

sucrose). If the half oxygen inhibitory percentage or concentration IC_{50} is used as a measure of the toxic effect of O_2 exposure to the methanogenic bacteria in the DAS, the IC_{50} values increase with increasing sucrose concentrations (IC_{50} of 3.5, 16.9, < 50 and < 50 for the series of inocula incubated with initial concentrations of 0, 1, 2 and 4 g COD-sucrose/L, respectively, Table 1). There seems to exist an inverse relationship between the IC_{50} and the specific oxygen uptake rate (SOUR) of the anaerobic cultures, see Table 1. These results are in line with the argument of the possible role of the biochemical protection outlined above, and further confirm and generalize preliminary results reported by Estrada-Vázquez *et al.* (2001b).

If a load ratio γ in the experimental unit is defined as

$$\gamma = \left[\frac{Initial \cdot mass \cdot of \cdot oxygen \cdot in \cdot the \cdot bottle \cdot headspace}{Initial \cdot mass \cdot of \cdot COD \cdot available \cdot in \cdot the \cdot bottle \cdot liquid \cdot phase} \right] \times 100 \qquad (2)$$

It can be seen in Figure 2 that recoveries are apparently very high when $\gamma < 5\%$, either for DAS incubated with 0, 1, 2, or 4 g COD/L initial sucrose (i.e. independent of the initial sucrose concentration available in the liquid phase). Above 5%, the recovery pattern seems to depend on both the γ and the absolute value of initial sucrose concentration. Since the bottles with no supplementary sucrose had a basal concentration of degradable COD (200 mg/L average) coming from the digester liquor, their corresponding γ values, although great, could be calculated. Actually, for initial concentrations of added-sucrose of 0 and 1 g/L, the recoveries (10 to 55% and 35 to 40% , respectively) are much lower than those for DAS incubated at 2 and 4 g/L (range 75 to 90% for both series). This is somewhat counter-intuitive, since one might speculate that the load ratio can be the determining factor for the protection of the consortia, regardless of the absolute initial concentration of the supplemental carbon source. However, the analysis of the relationship between γ and R values of the SMA needs further investigation.

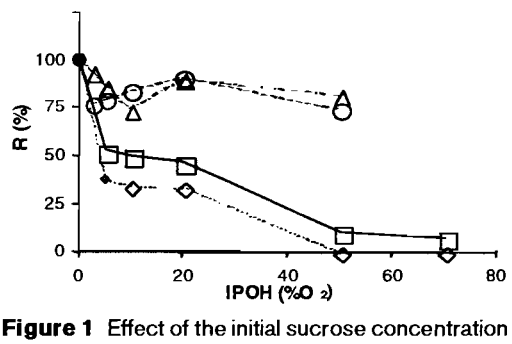

Figure 1 Effect of the initial sucrose concentration recovery of the methanogenic of activity (R) *versus* the initial recovery

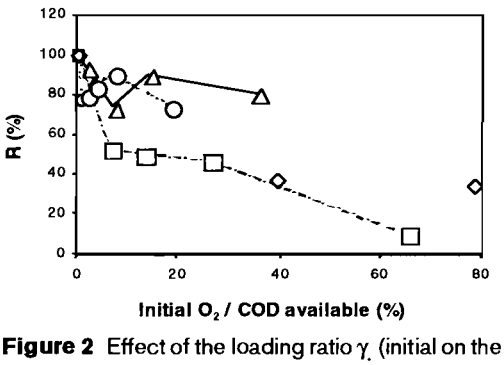

Figure 2 Effect of the loading ratio γ, (initial on the mass of O_2 in the headspace to initial mass aceto-clastic COD available) on the activity percentage of oxygen recovery

Symbols in Figure 1 and 2: 0 g/L sucrose: ◇; 1 g/L sucrose: □; 2 g/L sucrose: △; 4 g/L, ○

Table 1 Half oxygen inhibitory concentration IC_{50} of disperse methanogenic sludge exposed to oxygen

Incubation conditions	IC_{50}[a] (%)	SOUR (mg O_2/gVSS.day)[b]
0 g COD-sucrose/L	3.5	259
1 g COD-sucrose/L	16.9	505
2 g COD-sucrose/L	> 50	547
4 g COD-sucrose/L	> 50	839

Notes

[a]Inhibitory concentration 50% of oxygen; [b]specific oxygen uptake rate; experimental values at a total equivalent oxygen concentration of 980 mg O_2/L liquid phase of bottle (Kato *et al.*, 1993a)

The time courses of oxygen and methane in the bottle headspace are depicted in Figure 3 and 4, respectively.

They further support the idea that high recoveries R are related to a lower oxygen exposure during the incubation due to oxygen consumption. For instance, bottles with 50% IPOH have nearly 2, 0.6, 0.6 and 0.4 mmol O_2/bottle at the end of the 3-day incubation, for initial supplemented sucrose 0, 1, 2, and 4 g COD/L, respectively (Figure 3).

Figure 4A shows that the effect of oxygen exposure on the cultures not supplemented with sucrose was drastically negative: methane generation for the oxygen-exposed cultures decreased almost 8-fold as compared to the strict anaerobic control. In the series supplemented with 2 and 4 g COD- sucrose/L (Figure 4C, D) the negative effect of the IPOH on the methane generation was less drastic, and the bottles with low IPOH exhibited a methanogenesis nearly of the same order of that of the anaerobic control.

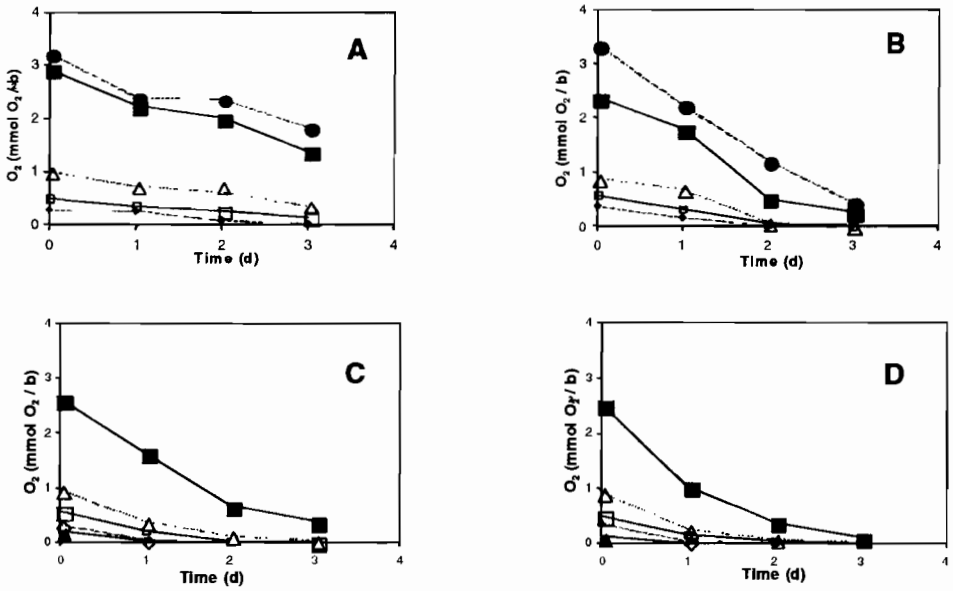

Figure 3 Time course of the oxygen content in the headspace of bottles during the 3-day incubation. A: 0; B: 1; C: 2; D: 4 g COD-sucrose/L supplemented. ▲ 0; □ 2.5; ◊ 5; ❐ 10; Δ 20; ■ 50; ● 70 initial oxygen percentage in the headspace

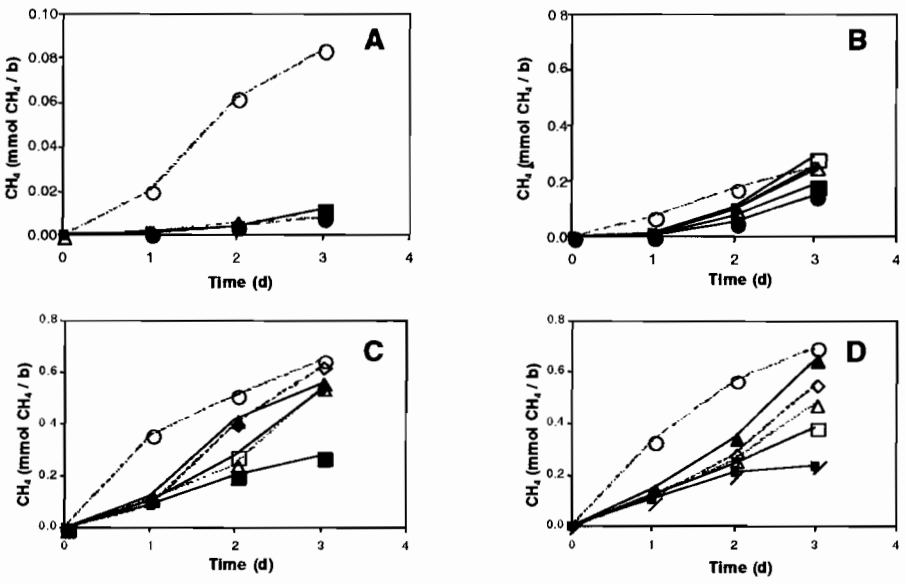

Figure 4 Time course of the methane content in the headspace of bottles during the 3-day incubation. Same keys and symbols as in Figure 3

Conclusions

The inhibition of disperse anaerobic sludge by oxygen exposure decreases when the concentration of the supplemented carbon source increases. This is in agreement with the fact that aerobic respiration of the added substrate by the facultative heterotrophic bacteria, always present in this type of sludge, has been found in previous studies as one of the main mechanisms protecting methanogens against O_2. From a practical point of view, this suggests that aeration of anaerobic systems should be possible without inhibiting the activity of methanogenic bacteria if an adequate ratio between oxygen and COD feeding is maintained. Such a ratio will depend however on the wastewater initial COD concentration.

Acknowledgements

The authors wish to thank Mr. Jorge Acevedo-Benítez, Ms. Claudia Hernández-Vivas, Ms. Aseneth Herrera-Martínez, for their excellent assistance with the operation of the inoculating reactors and some of the serum bottle tests, and Mr. Rafael Hernández-Vera for his help with the granulometric characteristics determination and lab management, all of them with the Dept. Biotechnology, Environmental Biotechnology R&D Group GBPANAT, CINVESTAV-IPN. The financial help of CONACYT (graduate scholarship to CE-V), CINVESTAV-IPN and COSNET is gratefully acknowledged.

References

Assih, E.A., Ouattara, A.S., Thierry, S., Cayol, J.-L., Labat, M. and Macarie, H. (2002). *Stenotrophomonas acidaminiphila* sp. nov., a strictly aerobic bacterium isolated from an upflow anaerobic sludge blanket (UASB) reactor. *Int. J. Syst. Evol. Microbiol.*, **52**, 559–568.

Campos-Velarde, M.D., Poggi-Varaldo, H.M., Ríos-Leal, E., Fernández-Villagómez, G. and López-Mercado, V. (1997). Chlorophenol and phenol-contaminated water treatment in biological fluidized beds. In: Leeson, A. and Alleman, B.C. (Eds.). *In situ and On-site Bioremediation. Vol 3*. Battelle Press, Columbus, OH, pp. 273–278.

Estrada-Vázquez, C., Poggi-Varaldo, H.M., Macarie, H. and Kato, M.T. (2000). Biological reactors of combined environments and their potential for bioremediation of groundwaters. *Proceedings 2nd. Congress of the Mexican Society for Remediation of Soils and Waters-SORESCA*. Puebla, Pue., México. (in CD-ROM). (In Spanish).

Estrada-Vázquez, C., Poggi-Varaldo, H., Macarie, H., Kato, M.T., Rodriguez-Vázquez, R., Esparza-García, F. and García-Mena, J. (2001a). Tolerance to oxygen exposure of anaerobic suspended biomass. In: *Proceedings, Part 2, 9th World Congress Anaerobic Digestion: Anaerobic Conversion for Sustainability*. 2–6 septembre 2001, Antwerpen, Belgium, pp. 575–577, ISBN 90-76019-16-9.

Estrada-Vázquez, C., Macarie, H., Kato, M.T., Rodriguez-Vázquez, R. and Poggi-Varaldo, H.M. (2001b). Resistencia a la exposición al oxigeno de lodos anaerobios suspendidos. *Interciencia*, **26**(11), 547–553.

Guiot, S.R., Frigon, J.C., Darrah, B., Landry, M.F. and Macarie, H. (1993). Coupled aerobic and anaerobic treatment of toxic wastewater, p. 1761–1769. In *Proceedings of the 7th forum for Applied Biotechnology*, Med. Fac. Landbouww. Univ. Gent, 58/4a, Gent, Belgium.

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

Kato, M., Field, J.A. and Lettinga, G. (1993b). Methanogenesis in granular sludge exposed to oxygen. *FEMS Microbiol. Letters*, **114**, 317–324.

Laguna, A., Ouattara, A., Gonzalez, O., Baron, O., Famá, G., El Mamouni, R., Guiot, S., Monroy, O. and Macarie, H. (1999). A simple and low cost technique for determining the granulometry of an upflow anaerobic sludge blanket reactor sludge. *Wat. Sci. Tech.*, **40**(8), 1–8.

Macarie, H. (2000). Overview of the application of anaerobic treatment to chemical and petrochemical wastewaters. *Wat. Sci. Tech.*, **42**(5–60), 201–213.

Macarie, H. and Guiot, S. (1996). Pentachlorophenol degradation using a simultaneous anaerobic-aerobic process: a new technology for wastewater treatment. In: *Frontiers of Biotechnology and Bioengineering*. E. Galindo (Editor). Mex. Soc. Biotechnol. Bioeng. – SMBB, México D.F., México. pp. 317–324. ISBN 968-7735-00-7. (In Spanish).

Monroy, O., Famá, G., Meraz, M., Montoya, L. and Macarie, H. (2000). Anaerobic digestion for wastewater treatment in Mexico: State of the technology. *Wat. Res.*, **34**, 1803–1816.

Poggi-Varaldo, H.M. and Rinderknecht-Seijas, N. (1996). Anaerobic Wastewater Treatment: Experiences in Mexico with Industrial Effluents. *In*: Niemczynowicz, J. (Ed.). *Integrated Water Management in Urban Areas: Searching for New, Realistic Approaches with respect to the Developing Countries*. Transtec Publications, Winterthur, Switzerland. Pp. 367–372. ISBN 0-87849-736-6.

Poggi-Varaldo, H.M., Valdés, L., Fernández-Villagómez, G. and Esparza-García, F. (1997). Solid substrate anaerobic co-digestion of paper mill sludge, biosolids, and municipal solid waste. *Wat. Sci. Tech.*, **35**(2–3), 197–204.

Toerien, D.F. and Hattingh, W.H.J. (1969). Anaerobic digestion. I. The microbiology of anaerobic digestion. *Wat. Res.*, **3**, 385–416.

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

Notation

COD	Chemical oxygen demand
DAS	Disperse anaerobic sludge
IC_{50}	Oxygen inhibitory concentration 50%, that is, the initial percentage of oxygen in the headspace that causes a 50% decrease in the specific methanogenic activity, with respect to the activity of the control
IPOH	Initial percentage of oxygen in the headspace of the bottle
R	Recovery of the specific acetoclastic methanogenic activity, given by Eq. (1)
SMA_c	Specific acetoclastic methanogenic activity of the control culture
SMA_i	Specific acetoclastic methanogenic activity of the culture exposed to a given IPOH
SOUR	Specific oxygen uptake rate
γ	Load ratio of the initial mass of oxygen in the bottle headspace to the initial mass of COD available in the bottle liquid phase, see Eq. (2)