



Short Communication

Effect of oxygen on the microbial activities of thermophilic anaerobic biomass



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HIGHLIGHTS

- The effect of oxygen on thermophilic anaerobic biomass was studied.
- Hydrolysis and acidogenesis of easily degradable substrates were not affected.
- Methanogenic activity lowered 40% with a lag phase of one day.
- A fraction of the methanogenic community was irreversibly inhibited.

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ABSTRACT

Low oxygen levels ($\mu\text{g O}_2 \text{ L}^{-1}$) in anaerobic reactors are quite common and no relevant consequences are expected. On the contrary, higher concentrations could affect the process. This work aimed to study the influence of oxygen (4.3 and 8.8 $\text{mg O}_2 \text{ L}^{-1}$, respectively) on the different microbial activities (hydrolytic, acidogenic and methanogenic) of thermophilic anaerobic biomass and on the methanogenic community structure. Batch tests in presence of oxygen were conducted using specific substrates for each biological activity and a blank (with minimum oxygen) was included. No effect of oxygen was observed on the hydrolytic and acidogenic activities. In contrast, the methane production rate decreased by 40% in all oxygenated batches and the development of active archaeal community was slower in presence of 8.8 $\text{mg O}_2 \text{ L}^{-1}$. However, despite this sensitivity of methanogens to oxygen at saturation levels, the inhibition was reversible.

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1. Introduction

Anaerobic digestion (AD), as the name itself conveys, is a process that occurs in environments devoid of oxygen. Nevertheless, some oxygen reaches the digester either unintentionally (Botheju and Bakke, 2011) or as a specific treatment (eg. removal of sulphides). Oxygen is commonly perceived as a toxic agent in AD, especially to the acetogenic and methanogenic microorganisms, which are obligate anaerobes, and therefore, it can impair their functioning and even cause cell lysis (Botheju and Bakke, 2011). However, some anaerobic bacteria are tolerant to oxygen and sometimes even capable of consuming it (Zhou et al., 2007).

Several studies have investigated the effect of oxygen on AD performance, mainly in mesophilic range (35–37 °C). Most of them agreed on detecting a positive impact on hydrolysis (Johansen and

Bakke, 2006; Zhu et al., 2009; Jagadabhi et al., 2010), while the influence on acidogenesis and methanogenesis is not clear. Some authors found that organic acid production was impaired by oxygen (Zhu et al., 2009), whilst others observed an increased production when different air flows were applied (Jagadabhi et al., 2010). Similarly, Celis-Garcia et al. (2004) observed a decrease in the methanogenic activity in presence of oxygen, while Lim and Wang (2013) achieved better methane yields after aeration (0.0375 $\text{L O}_2 \text{ L}^{-1} \text{ reactor d}^{-1}$).

Little information is available on the effect of oxygen on thermophilic AD, a promising technology due to the higher metabolic rates and destruction of pathogens (El-Mashad et al., 2004), but with limited applications because of the higher sensitivity of thermophilic microorganisms to toxicity and/or changes in operational and environmental conditions. The aim of the present work was to investigate the impact of oxygen at saturation level on the different microbial activities of thermophilic biomass (hydrolytic, acidogenic and methanogenic) and on the methanogenic community structure.

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2. Materials and methods

2.1. Thermophilic biomass

The inoculum was taken from a lab-scale (14 L) thermophilic (55 °C) digester treating a mixture of pig manure, maize silage and food waste (88%, 4% and 8% w/w, respectively), with a hydraulic retention time (HRT) of 20 d and an organic loading rate (OLR) of around 2 g COD L⁻¹ d⁻¹.

2.2. Microbial activity tests

All activity tests were performed in triplicate in bottles of 122 mL (100 mL liquid volume) or 550 mL (500 mL liquid volume) following the protocol described by Regueiro et al. (2012) and the substrate to inoculum ratio was set to 1 g COD g⁻¹ VSS. The biomass concentration varied between 1 and 3 g VSS L⁻¹, which was obtained by diluting the raw inoculum with an aqueous medium containing the main micro- and macronutrients (Angelidaki et al., 2009). Prior to substrate and inoculum addition, the aqueous medium was aerated for 3 h at two different temperatures (20 °C and 65 °C) in order to reach dissolved oxygen concentrations of 8.8 and 4.3 mg O₂ L⁻¹, respectively. In each test, a blank with non-aerated medium was included as control. After substrate and inoculum addition, the bottles were promptly closed with a rubber septum and placed in a shaker (50 °C, 120 rpm).

Starch (Panreac) and glucose (Panreac) were used as substrates in the hydrolytic and acidogenic activity test, respectively. During the 24-h experiments, samples of the supernatant (1.5 mL) were taken every 3 h to determine the remaining substrate concentrations. In the methanogenic activity test, a mixture of volatile fatty acids (50% acetic acid, 25% propionic acid and 25% butyric acid; COD basis) was used and methane production over the 10-day experiment was checked daily. Two methanogenic experiments with two different biomasses (with high and low methanogenic activity, respectively) were conducted in order to evaluate whether the initial biomass status affects the response to oxygen presence.

The specific activity (g COD g⁻¹ VSS d⁻¹) was calculated as the ratio between the maximum slope of substrate consumption/production of methane (g COD L⁻¹ d⁻¹) and the biomass concentration (g VSS L⁻¹). Statistical analyses were performed with IBM SPSS Statistics 20.0.0 software. The Brown–Forsythe test was applied for non-homogenous variances (acidogenic activity), while in case of homogeneity (hydrolytic and methanogenic activities), the one-factor analysis of variance (ANOVA), followed by the Dunnett's T3 test (*P* values ≤ 0.05) was used.

2.3. Analytical methods

The concentration of starch was calculated as the difference between the total sugar (Dubois et al., 1956) and the reducing sugar (DNS method) concentrations. Glucose concentration was determined using a commercial enzyme kit (GOD-PAP/Trinder; Spinreact). Biogas production was monitored using a pressure transducer (Centrepoints electronics), and biogas composition

was analysed by gas chromatography (HP, 5890 Series II, thermal conductivity detector, stainless steel column and helium as carrier gas).

2.4. Fluorescent *in situ* hybridization (FISH)

FISH technique was used to identify active microbial populations. Fresh biomass samples were taken on days 0, 2, 7 and 9 of the second methanogenic activity test and prepared according to the procedure described by Amann et al. (1995). Hybridization, probe selection and data analysis were conducted following the protocol explained by Regueiro et al. (2012).

3. Results and discussion

3.1. Specific hydrolytic activity

No differences were observed between the control and the two aerated batches (Fig. S1): almost 70% of the starch was consumed rapidly during the first 6 h, followed by a slower degradation till the end of the experiment. Consequently, the specific hydrolytic activities were similar, ca. 1.6 g COD g⁻¹ VSS d⁻¹ (Table 1), suggesting that neither 4.3 mg O₂ L⁻¹ nor 8.8 mg O₂ L⁻¹ affected starch hydrolysis. These results agreed with Nguyen et al. (2007), who did not observe a positive effect on the hydrolysis of the organic fraction of municipal solid waste under mesophilic conditions when micro-aeration with an air flow of 1 L min⁻¹ for 2 h every 6 h during 3 and 7 days was applied (daily oxygen dose ca. 4.5 L O₂ kg⁻¹ VS d⁻¹). In contrast, Johansen and Bakke (2006) reported a 50% higher hydrolysis of carbohydrates and proteins, but not of lipids, in mesophilic reactors treating primary sludge when an air flow of 500 mL d⁻¹ was applied intermittently for 15 s every minute during a 4-day experiment (daily oxygen dose ~3.7 L O₂ kg⁻¹ VS d⁻¹). Similarly, Zhu et al. (2009) achieved a better hydrolysis of easily biodegradable carbohydrates and proteins in mesophilic reactors treating vegetable and flower waste when the oxygen dose was higher than 35 L O₂ kg⁻¹ VS, while lower doses impaired it. On the contrary, Fu et al. (2015) noticed a faster transformation of the complex structure of corn straw into simple organic compounds in thermophilic batch reactors regardless the initial oxygen load (between 5 and 40 L O₂ kg⁻¹ VS). In brief, the effect of oxygen on the hydrolytic step seems to be strongly dependent on the oxygen dose, the aeration strategy and the type of substrate treated. Hence, the low initial oxygen loads of 1 and 2 L O₂ kg⁻¹ VS and the use of an easily hydrolysable substrate, such as starch, probably explain the negligible effect attained in this study.

3.2. Specific acidogenic activity

The consumption of glucose was similar in the control and in the two aerated bottles: it proceeded slowly during the first 9 h and accelerated afterwards till negligible glucose levels at the end of the experiment (Fig. S2). Therefore, no statistically significant differences were observed among the acidogenic activity

Table 1
Microbial activity results (in g COD g⁻¹ VSS d⁻¹). Values are presented as mean ± standard deviation.

	SHA	SAA	SMA 1st experiment	SMA 2nd experiment
Control	1.58 ^a ± 0.04	5.32 ^a ± 0.19	0.29 ^a ± 0.02	0.17 ^a ± 0.00
20 °C (8.8 mg O ₂ L ⁻¹)	1.60 ^a ± 0.06	4.88 ^a ± 0.16	0.16 ^b ± 0.02	0.11 ^b ± 0.01
65 °C (4.3 mg O ₂ L ⁻¹)	1.72 ^a ± 0.05	4.55 ^a ± 0.15	0.15 ^b ± 0.01	0.10 ^b ± 0.01

SHA: specific hydrolytic activity; SAA: specific acidogenic activity; SMA: specific methanogenic activity. Values with different superscript letters in columns are significantly different (*P* ≤ 0.05).

values, which varied between 4.55 and 5.32 g COD g⁻¹ VSS d⁻¹ (Table 1). Contradictory results are reported in literature regarding the effect of oxygen on acidogenesis. Jagadabhi et al. (2010) reported a 4-fold higher acidogenic activity in a mesophilic reactor treating grass silage after the addition of 2.5 L air for one day (daily oxygen dose of 10 L O₂ kg⁻¹ VS d⁻¹). Similarly, Xu et al. (2014) indicated a 3-fold increased production of acetic and butyric acid in a mesophilic reactor when an air flow rate of 1 L min⁻¹ for 12 min every 3 h was applied (daily oxygen dose of 63 L O₂ kg⁻¹ VS d⁻¹). In contrast, Zhu et al. (2009) observed a negative impact on acidogenesis in mesophilic reactors treating vegetable and flower waste when a daily oxygen dose of 18 L O₂ kg⁻¹ VS d⁻¹ was applied, while it was promoted at higher doses (≥35 L O₂ kg⁻¹ VS d⁻¹). Once again, the oxygen dose and the aeration strategy seems to determine the influence of oxygen on acidogenesis and the no effect observed in this study was probably due to the low levels applied (1 and 2 L O₂ kg⁻¹ VS).

3.3. Specific methanogenic activity

The two methanogenic activity experiments gave similar results: after a lag phase of one day in the aerated batches, a fast methane production occurred for five days followed by the typical plateau at the end of the experiment when the substrate was consumed (Fig. 1a, Fig. S3). The specific methanogenic activity of the control batches was around 2-fold higher (0.29 and 0.17 g COD g⁻¹ VSS d⁻¹ in the first and second experiment,

respectively, Table 1) than the aerated bottles, which displayed similar values (0.16 and 0.10 g COD g⁻¹ VSS d⁻¹ in the first and second experiment, respectively). The lower activity values of the second experiment were associated with the deteriorated performance of the thermophilic digester when the biomass was taken for the test. Botheju and Bakke (2011) also observed a 3-day lag phase in methane production in mesophilic reactors when the anaerobic inoculum was subjected to initial oxygen concentrations of 3, 5 and 7 mg O₂ L⁻¹. Celis-Garcia et al. (2004) tested different initial oxygen concentrations (1–10 mg O₂ L⁻¹) in batch mesophilic assays with anaerobic granular and biofilm sludge and they observed a decrease in the methanogenic activity of 75% and 65% for granular and biofilm sludge, respectively, at 8 mg O₂ L⁻¹. However, at around 4 mg O₂ L⁻¹, the inhibition was lower (50% and 30%, respectively). In this study performed with thermophilic suspended biomass, a 40%-decrease in the methanogenic activity was noticed regardless the oxygen level (4.3 or 8.8 mg O₂ L⁻¹), which suggests that the thermal shock could also be influencing negatively the methanogenic activity. This finding is in agreement with El-Mashad et al. (2004), who found that a temporary upward temperature change affected severely the thermophilic microflora.

Despite the different methanogenic activity values, the methane yields in the aerated batches were similar to the control in the first experiment (Fig. S3). This suggests that the inhibition of methanogens was reversible, as previously stated by Botheju and Bakke (2011). In contrast, in the second experiment, the methane yields in the aerated batches were lower than in the control (Fig. 1a), particularly in the bottles aerated at 65 °C, where the combination of high temperature and oxygen could have permanently damaged part of the strict anaerobes. This indicates that the status of the biomass prior to perturbation affects its ability to confront process disturbances, since oxygen impact was more pronounced when the biomass was already suffering from operational imbalance.

3.4. Influence of oxygen on the methanogenic community structure

FISH results showed that *Methanosarcina* was the dominant population (>80%) in all tests (data not shown). This finding is directly connected with the inoculum used, i.e. the operational conditions (thermophilic) and the substrate treated (mainly pig manure). The increase of active *Archaea* during the first days of the methanogenic experiment (day 2, Fig. 1b) was slower in the bottles aerated at 20 °C (8.8 mg O₂ L⁻¹), probably because facultative organisms out-competed strict anaerobes due to their higher specific activities and growth rates (Botheju and Bakke, 2011). In addition, the slightly lower number of active *Archaea* in the aerated batches (60%) than in the control (70%) at the end of the test suggests a possible permanent damage of part of these strict anaerobes. Yet, a long-term recovery of the methanogens cannot be excluded.

4. Conclusions

This study investigates the consequences of exposing thermophilic anaerobic biomass to two initial oxygen levels: 4.3 and 8.8 mg O₂ L⁻¹. Hydrolytic and acidogenic activities were not positively or negatively affected, while the methanogenic activity decreased by 40% regardless of the oxygen concentration and the background activity of the biomass prior to oxygen exposure. Moreover, a fraction of biomass might be irreversibly inhibited, especially when it was already partially disturbed and/or when several shocks are combined. Nevertheless, in a continuous operation, the growth of new biomass is likely to compensate the irreversible loss of activity.

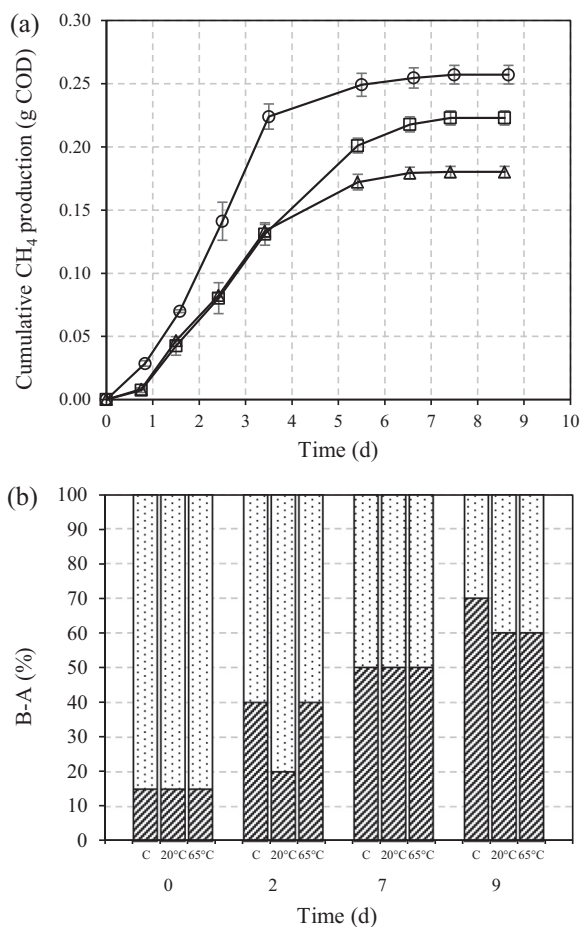


Fig. 1. Effect of oxygen on the specific methanogenic activity (a) and on the methanogenic community structure (b) during the second experiment. Control (○), aerated at 20 °C (□) and aerated at 65 °C (Δ). Bacteria (▨) and Archaea (▩).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.biortech.2016.03.085>.

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