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Enhancing anaerobic digestion of agricultural-residues by microaerobic conditions

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Abstract

Biogas plants treating agricultural-residues are associated with limited bioenergy output due to the high content of hardly degradable lignocellulosic fibers in the feedstock. Hence, effective treatment techniques are needed to enhance holocellulose deconstruction, and thus, increase the energy budget of anaerobic digestion (AD) process. In the present research, microaerobic conditions were applied in reactors that were previously operated under strict anaerobic conditions as a tool to increase the biodegradability of the lignocellulosic material and thereby improve the methane production in lignocellulose-based AD. Initially, two levels of oxygen loads (i.e. 5 and 15 mL O_2 /gVS) were examined during the AD of lignocellulosic biomass at batch mode. Low and high oxygen loads were connected with positive (+10%) and negative (-4%) impact in methanation process, respectively. Subsequently, the experimental results were validated by the amended BioModel. Furthermore, continuous mode experiments were conducted to more closely mimic real-life applications. Monitoring of a continuous digester fed with agricultural residues showed that the injection of 7.3 mL O_2 /gVS/d –which value was defined from mathematical optimization –was capable of improving the methane yield by ~7%. In addition, oxygen injection did not create any risk of inhibition incidents. Concerning microbial community structure, the bacterial population was relatively robust and was not markedly affected by oxygen addition. In contrast, some archaeal representatives were found to have increased relative abundance on oxygen exposure. More specifically, the aero-tolerant *Methanosarcina* and *Methanobacterium* spp. were the most dominant methanogens at microaerobic conditions.

Keywords

Agricultural-residues; anaerobic co-digestion; microaerobic conditions; 16S rRNA gene sequences

1 Introduction

The recalcitrant phenolic structure of lignin is the major barrier that should be broken down in order to increase the access of fermentable organic matter to the microbes, and thus, increase the degradability of lignocellulosic residues [1]. For this reason, various pretreatment methods (i.e. physical, chemical, biological or combination of them) are available focusing on disrupting the naturally created protection shield [2]. Despite the vast diversity of alternative methods, most of the pretreatments are not very flexible

and commonly need an extra process step before the anaerobic digestion (AD). In addition, the demand for this separate step is associated with extra costs for energy, equipment, facilities, construction and operation [3]. However, investments to address the mentioned needs can finally eliminate or overshadow the increased methane production. In contrast, the perspective of reducing the supply chain steps by performing an effective treatment in parallel with the biologically mediated AD process can improve the overall process balance [4].

In this context, the establishment of microaerobic conditions into an AD reactor appears as a promising solution to boost the methane yield of lignocellulosic biomass [5]. Specifically, oxygen can increase lignin oxidation due to the fact that the bacterial excretion of enzymes (e.g. laccases, peroxidases), which are able to break down the irregular polyphenolic polymer, is favored by the presence of oxygen [6]. These oxidative enzymes use oxygen as final electron acceptor to oxidize the phenolic hydroxyl groups of lignin [7, 8]. However, the oxygen should be carefully injected under a controlled environment to avoid substrate's overoxidation and loss of organic matter that can be conducted at a high oxidation-reduction potential [6].

Despite the fact that limited amounts of oxygen can increase the delignification of recalcitrant lignocellulosic residues, microaerobic conditions are not widely established in real-life applications. The reason behind is that a non-controlled oxygen addition can provoke strong inhibition to the sensitive methanogenic archaea leading to a markedly hindered methanation process [9]. On this topic, our preliminary study monitoring oxygen addition in AD culture showed that controlled operation can boost lignocellulose deconstruction and thus, increase the biogas production by ~7% injecting mLO_2/gVS [6]. However, the methane yield was deteriorated by ~13%, even by a slight increment on microaeration levels to $15 \text{ mLO}_2/\text{gVS}$. Thus, the positive impact directly shifted to negative at higher oxygen loads that were inhibitory to methanogenic archaea. Nevertheless, the mentioned research did not assess the effect of oxygen on methane production in correlation with changes in microbial communities. Hence, a robust conclusion about the impact of oxygen on microbial and archaeal microbes cannot be drawn.

In addition, to better understand the biogas process at microaerobic conditions a deeper understanding of the basic kinetics is also needed. Each AD microbial group do not respond to the same extend on oxygen exposure, and hence, inhibition or stimulation of microbial growths and activities result in bioenergy output changes. Previously, attempts to model microaerobic conditions in AD process have focused almost exclusively on the extension of the ADM1 model to consider the oxygen effects on the different microbial

groups as well as in the hydrolytic steps of AD [9]. The ADM1-Ox model proposed by Botheju et al. [10] includes seven aerobic uptake processes as well as oxygen effects on process kinetics. In this work the bioconversion “BioModel” developed by Angelidaki et al. [11] and later extended by Kovalovszki et al. [12] is used to model and simulate the effect of microaerobic conditions on the biodegradability of lignocellulosic feedstock.

The present study focuses on further paving the way for a successful implementation of microaerobic conditions in a strictly anaerobic environment. For this purpose, microaerobic conditions were initially established during the AD of straw and then, the amended BioModel was used to examine the effect of oxygen on biomass degradability. Subsequently, the co-digestion of straw with cattle slurry was performed under continuous mode operation injecting pure oxygen. The microbial community shifts were monitored by applying high throughput 16S rRNA amplicon sequencing.

2 Materials and methods

2.1 Inoculum and substrates characteristics

Digestate from Snertinge biogas plant, which co-digests animal slurries and wastes from ethanol industry under thermophilic conditions, was used as inoculum. Before usage, the inoculum was sieved to remove large particles and the remaining undigested organic matter. Then, it was stored in a thermophilic incubator for ten days to decrease the background methanation. Wheat straw and cattle slurry were collected from a livestock farm in Denmark. The lignocellulosic substrate was treated with a cutting mill (SM 200, Retsch GmbH, Germany) to reach size distribution less than 0.5 cm. In addition, cattle slurry was sieved to remove large lignocellulosic fibers that could create operational problems (i.e. clogging). The main physicochemical characteristics of the inoculum after storage and substrates after sieving and size decrease are presented in Table 1.

2.2 Batch mode operation

The effect of oxygen on the methane yield of lignocellulosic residues was initially examined in batch assays based on the guidelines of Angelidaki et al. [13]. Each reactor had a total and working volume of 547 and 200 mL, respectively. The reactors were filled with 1 g wheat straw, 160 mL inoculum and 40 mL distilled water. The background methane productivity of the digestate was defined by blank reactors

containing only water and inoculum. To evaluate the accuracy of the experiment, control reactors containing pure cellulose at the same organic load (i.e. 4.33 gVS/L) were set up. Three different sets of straw-filled reactors were prepared based on oxygen levels: 0, 5 and 15 mL O_2 /gVS, according to our previous research [6]. Oxygen was injected to the working volume of the reactors and the bottles were shaken daily to improve the oxygen mass transfer. Methane generation was measured every three days by sampling the headspace of the digesters until no significant increase ($p < 0.05$) was detected. All batch reactors were conducted in triplicates.

2.3 Continuous mode operation

The effect of microaerobic conditions was examined by monitoring a continuously fed digester. The continuously stirred tank reactor (CSTR) had a total and working volume of 5.0 and 3.0 L, respectively. The CSTR was operated under thermophilic conditions (54 ± 1 °C), at hydraulic retention time (HRT) of 15 days and organic loading rate (OLR) of 1.7 gVS/L/d. The co-digestion of agricultural-residues was examined using cattle slurry and wheat straw. The feedstock composed of 85:15 slurry: straw on organic matter basis. The experimental period was separated in two periods. At the initial period (P-I, 0-15 days), the CSTR was operated under strictly anaerobic conditions. At the second period (P-II), microaerobic conditions were established (P-II, 16-45 days) by injecting 7.3 mL O_2 /gVS/d following the outcome of a thoroughly examined optimization study [6]. Specifically, response surface methodology was selected to describe the interactions of dissimilar variables (i.e. microaerobic period, oxygen load, pulse frequency). This methodology allowed the formulation of a second-order polynomial model and then, the optimal conditions that can maximize the methane yield were estimated. During the experiment, the biogas in the headspace was continuously recirculated to improve oxygen transfer to the liquid [14]. Biogas production was daily recorded using the water displacement method. Methane content in the biogas, pH and volatile Fatty acids (VFA) accumulation were measured twice a week.

2.4 Microbial community analysis

Samples (50 mL) were collected at steady-state of strictly anaerobic conditions (AD, 15th day) and steady-state of microaerobic period (MA, 45th day) in order to define the microbial changes due to oxygen injection. The sampling points are highlighted in red rhombus in Fig. 2a. PowerSoil® DNA Isolation Kit

(QIAGEN Bioinformatics, Germany) was used to isolate and purify the genomic DNA from samples. The guidelines of the manufacturer's protocol was followed with slight adjustments towards improved quality of the recovered DNA [15]. Sequencing of the 16S rRNA gene (V4 hypervariable region) was performed with universal primers 515F/806R by Illumina MiSeq desktop sequencer at the Ramaciotti Centre for Genomics (Sydney, Australia). NanoDrop (Thermo Scientific) and Qubit fluorimeter (Life Technologies) were to assess purity and quantification, respectively. The raw reads can be found in Sequence Read Archive (SRA) database (<http://www.ncbi.nlm.nih.gov/sra>) at an accession number of PRJNA354694. The detailed sample IDs along with statistics of sequencing are given in Supplementary material. The raw sequences were processed using CLC Workbench software (V.8.0.2) with Microbial genomics module plug in (QIAGEN) and analyzed as previously described [15]. The most abundant **operational taxonomic units (OTUs)** are presented in the Supplementary Dataset and the consensus sequences were manually verified using BLAST (16S ribosomal RNA database) using previously defined threshold for taxonomic assignment [16]. The Multi experiment viewer software was used to depict the relative abundance of microbial members as heat map (MeV 4.9.0) with Pearson correlation with complete linkage method for OTUs clustering [17].

2.5 Analytical methods

The values of pH, TS, VS, TKN and NH_4^+ were defined according to Standard methods for the examination of water and wastewater [18]. The methane concentration in all AD reactors and the VFA content in all samples were determined using gas-chromatographers (GC-TRACE, Thermo Scientific), as previously described [19]. All analyses were performed in triplicate samples.

2.6 Modelling approach and computational method

Due to the nature of the substrate, oxygen effect is considered solely to affect the carbohydrate fraction of the substrate. Similar to the approach suggested by de Gracia et al. [20], a first order aerobic hydrolysis kinetic equation for insoluble carbohydrates ($\text{C}_6\text{H}_{10}\text{O}_5$)_{is} was incorporated into the BioModel to model the oxygen effects on this hydrolytic step (1.1)

$$r_{ae,hyd_carb} = k_{0,ae} \left(\frac{K_i}{\Sigma VFA + K_i} \right) \left(\frac{1}{1 + \frac{K_{s,O_2}}{[O_2]}} \right) [(C_6H_{10}O_5)_{is}] \quad (1.1)$$

Where $k_{0,ae}$ is the maximum specific hydrolysis rate constant for carbohydrates under aerobic conditions. Inhibition due to oxygen concentration –not represented in the original BioModel –was incorporated by means of a non-competitive inhibition term and it was considered to affect the anaerobic hydrolysis, acidogenic, lipolytic, acetogenic and aceticlastic methanogenic steps, respectively (1.2-1.8)

$$r_{hyd_carb} = k_0 \left(\frac{K_i}{\Sigma VFA + K_i} \right) \left(\frac{1}{1 + \frac{K_{i,O_2}}{[O_2]}} \right) [(C_6H_{10}O_5)_{is}] \quad (1.2)$$

$$r_{Carb} = \mu_{max,Carb}(T) \left(\frac{1}{1 + \frac{K_{s,Carb}}{[(C_6H_{10}O_5)_s]}} \right) \left(\frac{1}{1 + \frac{K_{s,NH_3}}{[T - NH_3]}} \right) \left(\frac{1}{1 + \frac{[LCFA]}{K_{i,LCFA}}} \right) \left(\frac{1}{1 + \frac{[O_2]}{K_{i,O_2}}} \right) X_{Carb} F(pH) \quad (1.3)$$

$$r_{AA} = \mu_{max,AA}(T) \left(\frac{1}{1 + \frac{K_{s,AA}}{[AA]}} \right) \left(\frac{1}{1 + \frac{[LCFA]}{K_{i,LCFA}}} \right) \left(\frac{1}{1 + \frac{[O_2]}{K_{i,O_2}}} \right) X_{AA} F(pH) \quad (1.4)$$

$$r_{GTO} = \mu_{max,GTO}(T) \left(\frac{1}{1 + \frac{K_{s,GTO}}{[GTO]}} \right) \left(\frac{1}{1 + \frac{K_{s,NH_3}}{[T - NH_3]}} \right) \left(\frac{1}{1 + \frac{[LCFA]}{K_{i,LCFA}}} \right) \left(\frac{1}{1 + \frac{[O_2]}{K_{i,O_2}}} \right) X_{GTO} F(pH) \quad (1.5)$$

$$r_{LCFA} = \mu_{max,LCFA}(T) \left(\frac{1}{1 + \frac{K_{s,LCFA}}{[LCFA]} + \frac{[LCFA]}{K_{i,LCFA}}} \right) \left(\frac{1}{1 + \frac{K_{s,NH_3}}{[T - NH_3]}} \right) \left(\frac{1}{1 + \frac{[O_2]}{K_{i,O_2}}} \right) X_{LCFA} F(pH) \quad (1.6)$$

$$r_A = \mu_{max,A}(T) \left(\frac{1}{1 + \frac{K_{s,A}}{[A]}} \right) \left(\frac{1}{1 + \frac{K_{s,NH_3}}{[T - NH_3]}} \right) \left(\frac{1}{1 + \frac{[HAc]}{K_{i,HAc}}} \right) \left(\frac{1}{1 + \frac{[LCFA]}{K_{i,LCFA}}} \right) \left(\frac{1}{1 + \frac{[O_2]}{K_{i,O_2}}} \right) X_A F(pH) \quad (1.7)$$

$$r_{HAc} = \mu_{max,HAc}(T) \left(\frac{1}{1 + \frac{K_{s,HAc}}{[HAc]}} \right) \left(\frac{1}{1 + \frac{K_{s,NH_3}}{[T - NH_3]}} \right) \left(\frac{1}{1 + \frac{[NH_3]}{K_{i,NH_3}}} \right) \left(\frac{1}{1 + \frac{[LCFA]}{K_{i,LCFA}}} \right) \left(\frac{1}{1 + \frac{[O_2]}{K_{i,O_2}}} \right) X_{HAc} F(pH) \quad (1.8)$$

Aerobic uptake of soluble carbohydrates was represented by the following stoichiometry (1.9)

Monod type kinetic was used to model the aerobic process for soluble carbohydrates degradation according to equation (1.10)

$$r_{ae,Carb} = \mu_{max,Carb}(T) \left(\frac{1}{1 + \frac{K_{s,Carb,Ae}}{[(C_6H_{10}O_5)_s]}} \right) \left(\frac{1}{1 + \frac{K_{s,NH_3}}{[T - NH_3]}} \right) \left(\frac{1}{1 + \frac{[LCFA]}{K_{i,LCFA}}} \right) \left(\frac{1}{1 + \frac{K_{s,O_2}}{[O_2]}} \right) X_{Carb} F(pH) \quad (1.10)$$

Where X represents the concentration of the different biomass-microbial groups in the BioModel. Values for the new parameters, $k_{0,ae}$, K_{i,O_2} , and K_{s,O_2} were identified by minimization of the square-error criteria between the BioModel output and the experimental data. The Levenberg-Marquardt algorithm [21] was used to solve the non-linear least square minimization problem. Methane cumulative production under the different microaerobic experimental conditions was used to perform the parameter estimation. Gaseous component (CH_4 , CO_2 , NH_3 , H_2S , and O_2) in liquid and gas phases were modeled as described in Lovato et al. [22].

2.7 Statistics

ANOVA accompanied with a Post Hoc test (Tukey HSD) was conducted using OriginPro 9.0.0 SR2 software to define statistically significant differences ($p < 0.05$) among experimental measurements. In addition, descriptive statistics was performed for all variables; mean values and standard deviations were computed.

3 Results and discussion

3.1 Batch & model simulation results

Initially, batch assays were conducted to evaluate the effect of oxygen on lignocellulose degradability and the experimental measurements were validated by the amended BioModel. First, for the anaerobic conditions experiment (Fig. 1a) the maximum specific hydrolysis rate constant and biodegradability coefficient for carbohydrates, respectively were the only parameters considered for the model calibration (Table 2). Subsequently, the parameters were fixed and then, the experimental data-sets of both microaerobic conditions were used to estimate the new set of parameters, $k_{0,ae}$, K_{i,O_2} , and K_{s,O_2} . As observed in Fig. 1b, c model-fitting results exhibited a good agreement with experimental data.

The experimental results showed that both microaerobic conditions **affected** the cumulative methane production (Fig. 1b, c). The oxygen load of 5 mLO₂/gVS (31 mgO₂/L) had a positive impact (+10%), whilst the 15 mLO₂/VS (93 mg O₂/L) had a negative one (-4%), when compared to the control experiment (Fig. 1a), respectively. It has been documented that when using mono-saccharide as substrate, oxygen loads of 44 and 88 mg/L had a negligible but negative impact on the methane generation potential [23]. In this study, straw was used as substrate and therefore an effect on the hydrolysis due to aerobic activity of facultative microorganisms could be expected, but not necessarily in the ultimate methane potential as reported in other studies [24]. This can be observed in Fig. 2a which represents the concentration of the soluble carbohydrates –a hydrolytic product –over time for the anaerobic and microaerobic experimental conditions, respectively.

The maximum value of the soluble carbohydrates attained ultimately, corresponding to an enhancement of 15% in the hydrolytic pathway provoked by the microaerobic conditions of 15 mLO₂/gVS (Fig. 2b). This is expected as the increased hydrolysis rate is directly linked with the oxygen load as observed in Fig. 3c. Once the oxygen is depleted, there is a shift after five days to anaerobic conditions again.

3.2 Effect of microaerobic conditions in continuous mode operation

The co-digestion of straw with livestock slurry was conducted in a lab-scale CSTR and the outcome measures from reactor's monitoring are depicted in Fig. 3. At steady-state conditions of P-I, the methane

in the biogas was $70.2 \pm 0.3\%$ and the bioenergy yield was $259 \pm 5 \text{ mLCH}_4/\text{gVS}$ (Fig. 2a). In parallel, pH was stable at 7.93 ± 0.06 , while negligible accumulation of TVFA was observed ($0.11 \pm 0.01 \text{ g/L}$) (Fig. 3b). Subsequently, $7.3 \text{ mL O}_2/\text{gVS}$ were daily injected to reactor's working volume for the entire P-II.

During the first HRT of microaerobic conditions, the methane production was not affected. However, during the second HRT the impact of oxygen was revealed and at the newly established steady-state the methane yield was $276 \pm 7 \text{ mLCH}_4/\text{gVS}$ or 7% higher than that of the anaerobic operation (i.e. P-I). In parallel, the methane concentration in biogas ($69.9 \pm 1.1\%$), pH (7.92 ± 0.09) and TVFA ($0.08 \pm 0.01 \text{ g/L}$) stayed at the same range with the strictly AD. Hence, an acclimation period was mandatory for the microorganisms to be adapted into the new conditions. It should be noted that the results do not differ significantly ($p > 0.05$) with the outcome of our previous study [6], in which the optimization model predicted a 9% increase in methanation process using comparable oxygen injection load. Monitoring the biochemical parameters showed that the methane production of lignocellulose-based AD was favored by the applied microaerobic conditions. It is indicated that the injected oxygen concentrations were not able to partially or totally inhibit the AD of agricultural residues.

3.3 Effect of microaerobic conditions on microbial populations

Alpha diversity estimated the OTUs diversity and the rarefaction curves indicated that the sequencing data were satisfactory to deliver all the adequate information for the content of microbial communities (Supplementary Data). In addition, principal coordinate analysis was conducted to evaluate composition and variability of microorganisms (Supplementary Data).

Regarding bacterial population, the most abundant strains belonged to *Firmicutes* and *Bacteroidetes* phyla, which commonly dominate biogas reactors treating similar agricultural residues [25, 26]. In both samples, the most dominant OTU was *Clostridia* sp. 20, affiliated to *Firmicutes* (Fig. 4). Conducting a BLASTn search to the NCBI database of 16S ribosomal RNA sequences for both bacteria and archaea, the strain was found to be only 90% similar to *Hydrogenispora ethanolica* of class *Clostridia*.

The dominance of *Clostridia* into biogas reactors treating livestock slurry has been previously reported in the literature [27, 28]. At the end of the experiment (i.e. 2 HRTs of microaerobic conditions), *Clostridia* sp. 85 of *Ruminococcaceae* family increased its relative abundance by 12.5-fold. Species able to decompose structural sugars are known within *Ruminococcaceae* family, due to their characteristics to excrete enzymes

which can hydrolyse the glycosidic bonds in complex carbohydrates and other components of carbohydrate-active enzymes that help the colonization in lignocellulose-based biogas reactors [29, 30]. Nevertheless, the establishment of microaerobic environment did not create distinct shifts in the global bacterial consortium. A rationale to explain the lack of remarkable changes can be attributed to the fact that a large fraction of the detected bacteria are facultative anaerobes. Hence, they can use the exogenous oxygen and rapidly reestablish the anaerobic environment avoiding the colonization obligatory aerobic bacteria. Furthermore, the long-term operation of the AD reactor could also contribute in a relative establishment of a robust and adapted to the feedstock bacterial community.

On the other hand, interesting findings were associated with the archaeal consortium (Fig. 4b). For instance, *Methanosarcina thermophila* 1 (100% similarity), which dominated the methanogenic population in both samples, increased its relative abundance by 6.3-fold at microaerobic environment. Likewise, *Methanosarcina* sp. 8 was also found to be enhanced by 6.2-fold at the end of the experiment. The increased proliferation is attributed to the fact that *Methanosarcina* spp. are among the most aero-tolerant methanogens [9]. Furthermore, *Methanobacterium* sp. 17 was also favored during oxygenation conditions, which is in agreement with a previous research that detected increased abundance of *Methanobacterium* under comparable oxygen exposure levels [31]. In contrast, *Methanoculleus palmolei* 4 and *Methanothermobacter thermautotrophicus* 5 were inhibited and decreased their relative abundance by 7.8- and 37.0-fold, respectively. In accordance, Fu et al. [31] found that the relative abundance of *Methanoculleus* and *Methanothermobacter* was decreased under oxygenated conditions. In addition, the abundance of *M. palmolei* 2 remained unchanged during the entire experiment indicating oxygen tolerance. This is in accordance with previous report that found *M. palmolei* species to be tolerant to microaerobic [32] or aerobic conditions [33].

4 Conclusions

The present research demonstrated that controlled oxygen exposure can boost the methane production of agricultural residues. The amended BioModel was used to analyze the effect of oxygen on biomass degradability and to obtain a set of parameters for further experimental validation. Therefore, results from BioModel should be interpreted as qualitative indication of the effect of microaerobic conditions in the methanation process and not as absolute guidelines of oxygen loads that could enhance the hydrolytic

pathway. At continuous mode operation, the anaerobic digestion of agricultural residues was enhanced by 7% by the injection of 7.3 mL O₂/g VS/d. Moreover, the microbial analysis showed that the bacterial consortium was not markedly affected by the microaerobic conditions. Regarding methanogenic populations, the aero-tolerant *Methanosarcina thermophila* increased its relative abundance and efficiently carried out the methanogenesis step under the limited oxygen exposure. The findings of the present study indicate that controlled microaerobic condition can be considered as an effective tool to improve the bioenergy output of lignocellulose-based biogas production.

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Tables

Table 1 Characteristics of inoculum, wheat straw and cattle slurry

Characteristics	Unit	Inoculum	Wheat Straw	Cattle slurry
pH		8.11		7.69
Total Solids (TS)	g/kg	27.47 ± 0.24	928.40 ± 5.90	31.82 ± 0.52
Volatile solids (VS)	g/kg	17.11 ± 0.12	867.20 ± 3.90	22.13 ± 0.23
Total Kjeldahl Nitrogen (TKN)	g/kg	3.63 ± 0.13	4.82 ± 0.08	2.63 ± 0.11
Ammonium Nitrogen (NH_4^+)	g/kg	3.51 ± 0.12	0.76 ± 0.01	1.74 ± 0.54
Total Volatile Fatty Acids (TVFA)	g/L	0.90 ± 0.10	^a nd	3.61 ± 0.11

^and = Not determined

Table 2. Estimated parameters for anaerobic and microaerobic conditions

Parameter	Anaerobic	Microaerobic	R ²
k_0 (1/d)	0.1194		0.9998
$Y_{carb, sol}$ (g _{carb, sol} /g _{carb, is})	0.4337		
$k_{0,ae}$ (1/d)		1.0000	0.9898
K_{i,O_2} (g/L)		0.4969	
K_{s,O_2} (g/L)		0.7479	

Figures

Fig. 1. Cumulative methane production for anaerobic and microaerobic conditions, respectively.

Δ Experimental data; □ BioModel output.

Fig. 2. Simulation results for: a) Concentration profile for soluble carbohydrates at anaerobic and microaerobic conditions, b) carbohydrate hydrolysis rate profiles and c) carbohydrate hydrolysis profiles for microaerobic conditions at 15 mLO₂/gVS

Fig. 3 Monitoring of continuous mode operation (a) methane yield and sampling points (red rhombus) for 16S rRNA gene analysis and (b) pH and VFA accumulation.

Fig. 4 Heat maps of relative abundance of most interesting (a) bacteria and (b) archaea. Colour scale refers to the OTUs abundance and is reported on top of the two figures. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)