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An Experimental Study on the Effects of Oxygen in Bio-gasification - Part 1

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Abstract. An experimental study was carried out to understand the possible impacts of limited aeration in an anaerobic bio-gasification process. A number of anaerobic bioreactors were operated at 35 ^oC, both under semi-continuous and batch feed modes, with different oxygenation loads. Two series of batch experiments clearly indicated an increasing methane yield in the range of oxygenation loads of 0 – 16 % (% O_2 of COD input). In the semi continuous feed mode, four completely mixed bioreactors operated under oxygenation levels of 0, 1.3, 2.6 and 3.9 % produced biogas at approximately equal level and constant rates. The methane generation rate at the low oxygenation level of 1.3 % was higher than the strict anaerobic condition, while higher oxygenation levels induced increasingly negative impact on methane production. Accumulation of volatile fatty acids at the start up of the continuous feed reactors was lower for the aerated than the strict anaerobic reactor. The positive effect of oxygen on methane production has a much larger range in the batch feed mode compared to the semi-continuous feed mode. This suggests that methane production can be optimized by some limited aeration in the first of two or more stages of anaerobic digestion.

Key words

Aeration, Bio-gasification, Methane, Oxygen effects

1. Introduction

Even a minor aeration effect is conventionally regarded as a negative factor leading to process inhibition in anaerobic digestion, AD, for biogas production; bio-gasification. This perception even suggests de-aeration of anaerobic digesters at the start-up. Only a limited number of studies have been carried out on investigating the true nature of oxygen involvement in anaerobic bio-gasification. Most of the operating anaerobic digesters are, however, subjected to different levels of limited and unintended aeration effects caused by the various interactions between the digesters and the open environment; such as feeding, effluent and gas removal, mixing, sludge recycle and containment failure. The true outcome of aeration effects are neither very properly quantified in many experimental studies nor handled in standard models intended for describing AD. Botheju et al. [1] attempted to extend the Anaerobic Digestion Model no. 1 (ADM1, [2]) to include the aeration effects in bio-gasification.

Inhibitory nature of oxygen towards strict anaerobic organisms has been explored before [3; 4]. It is also possible that the fermentation process is distracted by the direct conversion of soluble C into CO_2 in the presence of O_2 by the aerobic respiration of facultative microorganisms present in an anaerobic reactor. These facultative organisms are responsible for carrying out the initial stages of anaerobic degradation known as hydrolysis (liquefaction) and fermentation (acidogenesis). It is also known that the hydrolysis rates are faster under aerobic conditions compared to an anaerobic environment [5]. Enhanced hydrolysis by limited aeration in anaerobic digestion has been observed [6; 7]. Hydrolysis can often be the rate limiting step of anaerobic biodegradation, when the substrate is primarily composed of particulate organic matter [8]. The overall impact of oxygenation on methane generation therefore depends on a number of different biochemical processes occurring simultaneously or sequentially.

This article describes an experimental attempt to explore impacts of limited aeration on methane potential in anaerobic digestion systems. Performances of four different semi-continuously fed bio-reactors, and two series of miniaturised batch mode bio-reactors, operating under different aeration conditions, are observed and compared based on product formation versus aeration levels.

2. Methodology

A. Experimental set-up – continuous reactors

Four semi continuously (daily) fed, completely mixed bioreactors each having a liquid volume of 300 ml and a total working volume of 0.5 L were operated under strict anaerobic and oxygen loaded conditions to evaluate the effects of oxygenation on the reactor performance. Initial inoculum was taken from an active mesophilic (at 35 ^oC) bio-gasification plant fed with chemically precipitated primary sludge from municipal wastewater (*Porsgrunn* municipality, Norway). Inoculum was initially filtered through a 0.5 mm sieve to remove larger particulates and then purged with N_2 gas until the dissolved oxygen reading of 0.3 mg/L is indicated. A sterile synthetic substrate (Table 1) was fed once a day with an organic loading rate of 0.36 kg COD/m^3.d and with a hydraulic retention time (HRT) of 30 days until a pseudo steady state was achieved. A thermostatic heating cabinet (*Termaks*®) was used for incubation at 35 °C. The feed substrate was so chosen that it consists mainly of particulate chemical oxygen demand (COD) which has to be hydrolysed before consumed by microorganisms. The reactors were exposed to four different oxygenation levels of 0, 1.3, 2.6 and 3.9 % O_2 (as % of feed COD load) by daily air injection after daily feeding.

Table 1: Substrate composition

Compound	*Composition (g/L)*
Starch	3.90
Peptone	3.01
Yeast extract	3.58
KH_2PO_4	0.29
K_2HPO_4	0.29

B. Experimental set-up – Batch test series 1 and 2

Two batch test series were also conducted to study the effects of different oxygen loads on bio-gasification under batch feed conditions. These test series were comprised of miniaturised bioreactors in the form of polypropylene syringes (*BD Plastipak*®, supplied by *VWR international*) of 100 ml total volume each filled with 50 ml of inoculum added with 1 ml of feed substrate (starch). The generation of biogas is directly indicated by the piston displacement along the volumetrically labelled syringe body. 1 ml of starch solution, having a COD of 54.5 g/L, was fed to the each bioreactor except for the control reactors containing only the inoculum. Syringes were fitted with hypodermic needles to intake intended oxygen headspace (in the form of air) at the beginning (0, 4, 8, 16 and 32 ml of air) and also to sample the produced biogas for headspace analysis. Needles were kept air tight during the experiment, using rubber stoppers. The incubation was done at 35 °C, mesophilic condition, inside a heating cabinet (*Termaks*®). The reactors were operated until the gas generation stopped (in about 17 days in both series).

Series 1 and 2 batch experiments were operationally identical except for the different types of inoculum used. Inoculum for the series 1 was taken from the same municipal digester as mentioned in part *2. A.* Inoculum for the series 2 was taken from a lab scale mesophilic (at 35 °C) semi-continuous anaerobic bioreactor (3.3 L liquid volume, 33 days HRT) fed with the substrate indicated in Table 1. All batch reactors were run in parallels, in series 1 in quadruplets and series 2 in duplicates. Initial preparation steps of the inoculums (filtration and N_2 purging) were as in part *2. A.*

C. Analytical procedures

Volumetric biogas generation, gas composition, total and soluble CODs, pH and the volatile fatty acids of acetic, propionic, iso-butyric, butyric, iso-valeric, valeric, iso-caproic, caproic and heptanoic were analysed on the samples collected on daily basis from the semi-continuous reactors.

For the batch test series, the biogas generation was recorded on daily basis and the other parameters were measured at the end of the experimental duration, avoiding the disturbance of batch condition.

Biogas composition (CH_4, CO_2, N_2 and O_2) was determined using a Gas Chromatograph (Hewlett-Packard -P series, micro GC), with helium as the carrier gas. 12 ml of gas sample was injected during a 35 s time period. The instrument was calibrated by a synthetic biogas mixture of 50 % CH_4 and 50 % CO_2 (provided by *Yara Industrial AS*).

Liquid samples were centrifuged at 9000 rpm at 15 min. duration and then the liquid phase was filtered through 0.45 μm syringe filters prior to the soluble component analyses. Volatile fatty acids (VFAs) were analysed by a stationary Hewlett Packard GC with flame ionization detector and a capillary column (FFAP 30 m, inner diameter 0.250 mm, film 0.25 μm). The oven was programmed to ascend from 80 ºC to 180 ºC at a rate of 30 ºC/min., and after that to 230 ºC at a rate of 100 ºC/min. The carrier gas used was helium (99.9 %) at 24 ml/min. flow rate, and the injector and detector temperatures were set to 200 and 250 ºC, respectively.

Closed reflux colorimetric method [9] was used to determine the total and soluble chemical oxygen demands, using *Hach*® *DR 2000* spectrophotometer. Total/volatile suspended solids (TSS/VSS), total/volatile solids (TS/VS) and alkalinity were also determined using accredited standard test methods [9].

3. Results

A. Semi-continuous reactors

The four reactors maintained under different oxygenation levels show a similar average biogas generation rate of about 60 ml/d, quite independent from their oxygenation condition (Fig. 1).

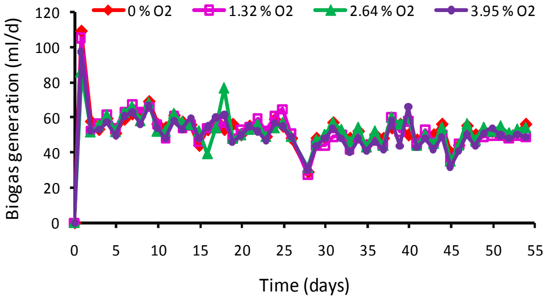

Fig. 1: Biogas generation rates of differently O_2 loaded semi-continuous reactors.

Most of the biogas in all cases was methane but the methane content was influenced slightly by the aeration level. Figure 2 illustrates the moving averaged methane (CH_4) generation rates of the four reactors during the experiment. Accordingly, 1.3 % O_2 fed reactor indicated a higher CH_4 generation rate compared to the strict anaerobic reactor (0 % O_2). Further, 3.9 % O_2 load showed an increasingly negative impact on methane generation compared to the strict anaerobic reactor. The low methane production measured during the first weeks in the 2.6 % O_2 reactor was due to a malfunctioning gas sampling bag. After it was replaced the gas generation was almost similar to the complete anaerobic case.

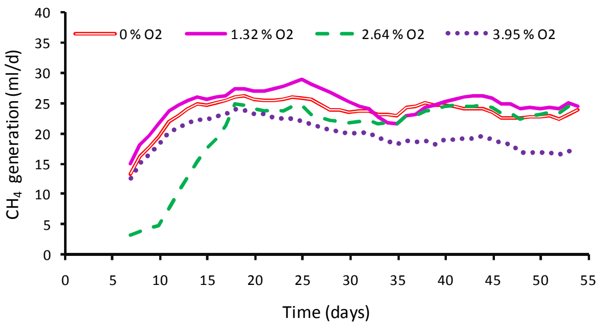

Fig. 2: CH_4 generation (moving averages) of differently O_2 loaded semi-continuous reactors.

Acetic acid dominated the VFAs content of all reactors (data not shown). Oxygenation considerably reduced the initial accumulation of VFAs at the start up period of the reactors (Fig. 3). This observation is consistent with the measured pH variations. A prominent pH drop was observed for the strict anaerobic reactor at the start up, compared to the oxygen fed reactors. Another study [10] reported that pH in complete mixed methanogenic reactors recovered more quickly under micro-aeration condition. Further, an alkalinity test carried out at the end of the experimental operation indicated no major variation among the different reactors. The average alkalinity of the four reactors was 2864 (±32) mg $CaCO_3$ /L. Over 2000 mg $CaCO_3$ /L is considered to be the appropriate level of alkalinity for safe digester operation [11] while values below 1000 mg $CaCO_3$ /L can put the digester stability at risk [12].

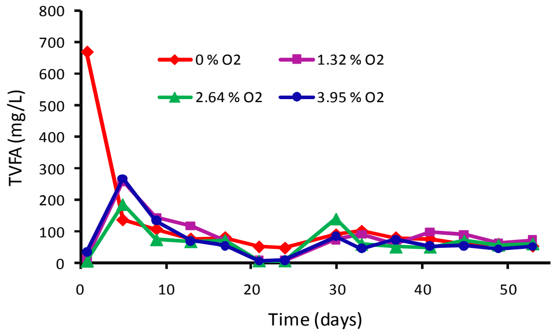

Fig. 3: TVFAs profiles for semi-continuous reactors.

B. Batch Reactor series

Figure 4 demonstrates the observed biogas and methane yields (ml CH_4 per gram COD starch feed) under different oxygen loading conditions of 0, 2, 4 , 8 and 16 % (% of COD input) tested in the batch experiment series 1 and 2. Both test series indicated increasing biogas and methane yields under increasing oxygen loading. When the condition changes from complete anaerobic to 16 % aerobic, the CH_4 yield increases by 55 %, i.e from 476 to 738.5 ml/g COD, in series 1. For the series 2, it is a 30 % increase from 372.5 to 484 ml/g COD. The nature of the inoculum seems to play a significant role on the level of oxygen induced enhancement of the methane yield. The methane yield figures used here are calculated based on the initial starch COD feed and do not account for the additional soluble COD (sCOD) generated by the hydrolysis of particulate matter present in the inoculum.

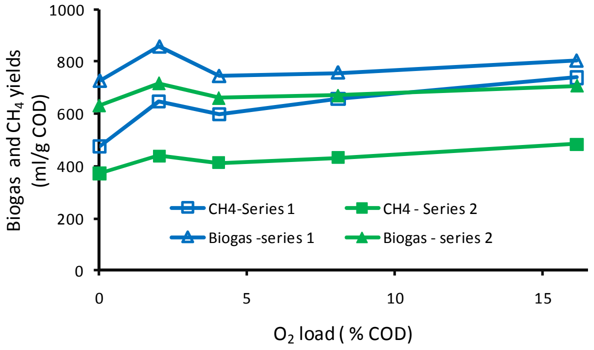

Fig. 4: CH_4 and biogas yields from batch experiments.

Analyses show almost a constant quantity of VSS and other solids (TS, TSS and VS) at varying O_2 loads (Fig. 5). According to Figure 5, series 1 maintains on average 14 % higher VSS than series 2. This difference in biomass quantity could have led to the higher methane yields observed in series 1.

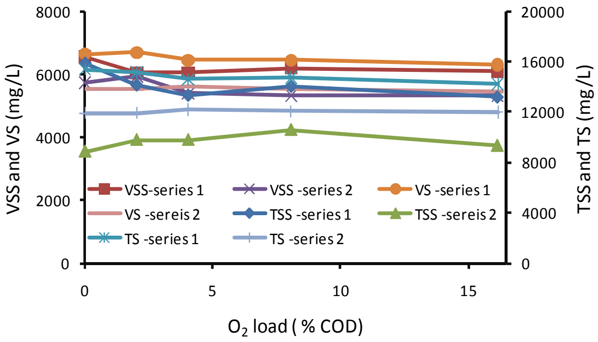

Fig. 5: Solids analyses for batch series 1 and 2.

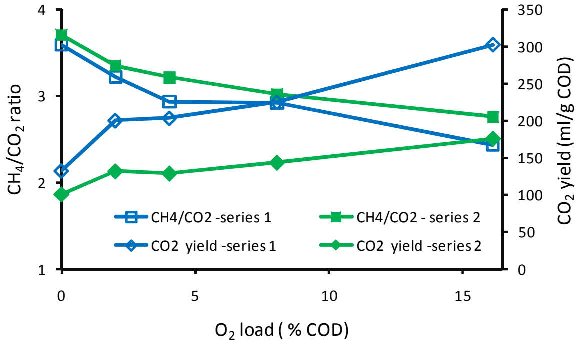

Fig. 6: CH_4/CO_2 ratios and CO_2 yields under different O_2 loading conditions, for batch series 1 and 2.

Despite of the increased CH_4 yield observed in oxygenated conditions, even higher increase of CO_2 production leads to reduced CH_4/CO_2 ratios at increasing oxygen loads, as depicted in Fig. 6. In series 1, CO_2 yield is increased by a factor of 2.3 (i.e. 128 %) when the aeration load increased from 0 to 16 %. The corresponding increase in series 2 is by a factor of 1.7 (or 74 %).

The VFA contents and the pH levels measured at the end of the experiment do not show any noteworthy variation with the applied oxygen load (Fig. 7). Measured VFA is low and almost completely represented by acetic acid. No other volatile acid was detected except a trace amount of caproic acid. pH stays close to neutral in both test series. Measured alkalinity at the end was also found to be similar for differently oxygenated reactors (data not shown). The VFA content is about twice higher in the series 2 compared to series 1(Fig. 7). The higher biomass content in the series 1 can be the reason for this higher reduction of VFA.

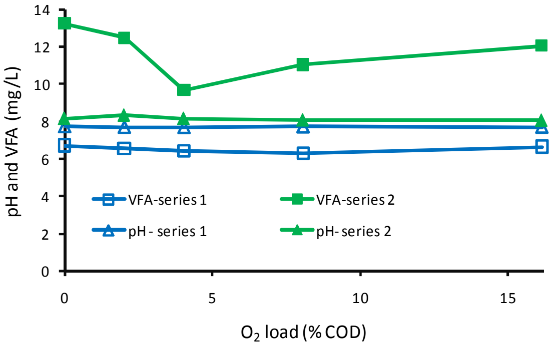

Fig. 7: pH and TVFA measured at the end of the batch experiments.

4. Discussion

A. Enhanced hydrolysis and overall effect on CH_4

Aeration can enhance the hydrolysis stage of anaerobic digestion [6; 7] giving rise to the improved digestion performances observed in the batch test series as well as in the 1.3 % O_2 loaded semi-continuous reactor (Fig. 4 and Fig. 2). Increased aeration in semi-continues reactors may, however, have partially inhibited the strict anaerobic organisms and also oxidised the substrate to CO_2, reducing the methane yield as observed clearly in the 3.9 % O_2 fed reactor (Fig. 2).

The batch feed condition evidently utilises oxygen in a more beneficial manner than in the case of semi-continuous reactors since much higher oxygen loads give increased methane yields. Batch experiments indicate a consistent increase in methane yield within the tested oxygen load range of 0 – 16 %. Meanwhile, the continuous reactor experiment demonstrates a clear negative effect at 3.9 % O_2 load. However, it must be noted that, semi-continuous reactor cultures were exposed to a much higher absolute oxygenation (irrespective of organic load) due to daily aeration, compared to the batch reactors which only had initial air headspaces.

The batch reactors can be interpreted as performing a sequential aerobic - anaerobic process assuming that all free oxygen is consumed quickly. The same effect can be obtained in full scale operation by staged bioreactors with oxygen enhanced hydrolysis first and subsequent fermentation and methanogenesis.

B. Oxygen Inhibition

Oxygen, being able to generate potentially toxic highly oxidising molecules such as peroxides and superoxides [13], not only inhibit the functioning of strict anaerobic acetogenic and methanogenic organisms but also could lead to rapid cell lysis. However, studies have found that these obligatory anaerobes are capable of surviving prolonged aeration by different means. Facultative organisms consuming oxygen rapidly can shield strict anaerobes from exposure to oxygen. Some strict anaerobes including certain strains of methanogens have a limited capability of tolerating oxygen exposure for a short time period [14; 15]. Diffusion barriers through sludge aggregates (e.g. flocs or granules) can also protect the strict anaerobic organisms residing inside [16].

C. Aerobic substrate consumption

Aerobic substrate consumption can straight forwardly generate CO_2, bypassing the anaerobic biochemical pathways leading to CH_4 generation. Any reduction of methane generation due to the decelerated anaerobic activity by oxygen inhibition and substrate loss can be compensated by the increased CO_2 production by aerobic respiration, hence keeping the volumetric biogas generation rate approximately constant as observed in the semi-continuous fed reactors (Fig. 1). In the case of batch feed condition, a considerably high CO_2 production is noted at increasing oxygen loads, compared to the increase of CH_4 yield. This gives rise to a reducing trend of CH_4/CO_2 ratio (Fig. 6). Increasing oxygenation therefore leads to reduced biogas quality.

D. VFAs accumulation

At the start up, metabolically more active and dominant fermentative organisms act faster than the slow growing less abundant secondary substrate consumers like acetogens and methanogens. This will create a temporary imbalance of the reaction system indicated by the accumulation of intermediate VFAs. This VFAs accumulation can in fact further prolong the start up time period by partially inhibiting the methanogenic organisms. Inhibitory effect of volatile fatty acids at high concentrations is well known [17; 18]. A limited aeration at this stage can be helpful by two means. First, at the availability of oxygen, mostly facultative acidogenic (fermentative) organisms tend to undergo aerobic respiration instead of fermentation. This process will greatly reduce the generation of VFAs but convert the substrate into CO_2. Secondly, it is also possible that a part of the generated VFAs can be oxidized to CO_2 through aerobic substrate consumption. This suggests that limited aeration can aid in establishing a stable digestion system faster, through minimized initial VFAs accumulation as observed in continuous feed experiment (Fig. 3). Rapid acquisition of an active methane phase (50 % CH_4 level) caused by micro-aerobic pre-treatment has been reported before [19].

E. Implications

This study signifies the usefulness of providing a controlled amount of air in an anaerobic digestion process involving particulate organic matter. A common carbohydrate, starch, is used in this study. An early study by Johansen and Bakke [6] has pointed out that the enhancement of hydrolysis of both carbohydrates and proteins are possible through micro-aeration, but could not detect any positive improvement of lipid hydrolysis. However, in that particular study [6], an overall negative impact on the methane yield is observed, probably due to the much higher aeration levels used. Carbohydrates constitute a major fraction of municipal and agricultural organic wastes [11]. Lipids can however be present in high amounts in certain industrial wastes [20].

Enhanced CH_4 yields under the extended range of oxygen loading (0 – 16 %) observed in batch feed condition may imply that a similar result could be obtained in a continuous process where staged reactors are deployed instead of a single digester. Sequential continuous digesters have already been used in stage wise optimisation of hydrolysis/fermentation and methanogenesis [21; 22; 23]. Such already existing digester systems can easily be retrofitted to accommodate a limited aeration in the hydrolysis (1st stage) reactor. Aeration used in the first stage of the process will minimize the potential of oxygen inhibition on strict anaerobes and furthermore reduce the likelihood of direct consumption of soluble substrate (generated by hydrolysis) by aerobic respiration. This may also prevent the biogas quality deterioration observed in the experiments (Fig.6). Small amounts of lower quality biogas containing some air and high amount of CO_2 generated in the hydrolysis reactor can be collected separately in order to avoid contamination of high quality biogas generated in the successive digester(s).

Observation of the minimum initial accumulation of VFAs in the presence of oxygen can route a new strategy to recover failed digesters due to VFAs inhibition and consequent low pH. Process failures caused by sloppy increases in feed loading often found in WWTP operation. The common means to recover such digesters include costly and time consuming options like reduction of feed loading over time, use of chemicals to adjust pH, partial replenishment of the inoculum; and some occasions could still end up with messy replacement of the whole reactor content and a restart. Instead of such actions, a limited aeration can be a simple option. Conversely, this method has not yet been used in industrial scale digester recovery; thus a careful evaluation with a backup plan is advocated before practicing this suggestion.

F. Safety aspects

Importantly, special care must be taken to minimize the potential formation of explosive air-methane mixtures [24] in large or experimental scale digestion systems. Note that the explosivity range of methane air mixtures is about 5 – 15 % of CH_4 and hence there is a likelihood of creating such gas mixtures in an aerated hydrolysis reactor; the configuration suggested earlier. If such a scenario is observed, immediate actions must be taken to reset the aeration rate to evade the explosivity range. However, it is observed that the presence of various inert gases like CO_2, water vapour and N_2 can radically reduce the explosivity of methane air mixtures [25]. Consequently, when adequate and standard safety measures such as venting and spacing, flame traps at gas inlet/outlets, and inline CH_4 sensors with alarm systems are incorporated into the plant design, any significant threat from an explosion hazard would be trivial.

5. Conclusions

Under batch feed condition, oxygenation (0 – 16 %) in anaerobic digestion lead to enhanced methane yield apparently caused by the improved solubilisation of substrate matter. Increasing oxygenation however reduced the CH_4/CO_2 ratio due to the significant increase in CO_2 production.

The low oxygenation condition of 1.3 % O_2 lead to an enhanced digester performance while the higher oxygenation level of 3.9 % had a negative impact on methane generation, in the case of semi-continuously fed bioreactors. Level of oxygenation within the experimented range (0 ~ 4 % O_2) does not affect the volumetric biogas generation of these reactors.

Oxygenation can enhance the establishing of a stable digestion system through reduced initial VFA accumulation.

Batch operation conditions can utilise an extended range of oxygen loads more favourably for methane production, compared to the continuous feed operation tested. A staged continuously fed process can be designed to enhance the substrate conversion to methane by aeration in the first stage with down-stream methane production.

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