Optimization of Anaerobic Digestion of Sewage Sludge Using Thermophilic Anaerobic Pre-Treatment

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Effect of hyper-thermophilic pre-treatment on thermophilic anaerobic digestion of primary sludge

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Abstract

The present study focuses on a two-step process for the treatment and stabilisation of primary sludge. The process consisted of an hyper-thermophilic hydrolysis step operated at 70°C and a hydraulic retention time (HRT) of 2 days followed by a thermophilic (55°C) anaerobic digestion step at a HRT of 13 days. A one-step anaerobic digester operated at 55°C and 15 days HRT was used as a reference process. The two-step process was characterized by a 13% higher organic matter removal efficiency and better pathogen reduction effect than the conventional one-step digestion. The microbial community of the digester fed with pre-treated sludge was characterised by a higher activity compared to that of the digester treating raw sludge. Moreover, the pre-treatment of the primary sludge resulted at a 48% increase of the methane potential (20.09 and 13.56 mmole CH₄/gVS with and without pre-treatment respectively) and at a 115% increase of the methane production rate. Finally it was shown that the extra energy requirements for the operation of a pre-treatment step would be covered by the energy produced from the extra methane production and in addition there would be a significant energy surplus of 2.17 kJ / d.

**Key words:** Energy balance; Hyper-thermophilic pre-treatment; Microbial activity; Primary sludge; Thermophilic anaerobic digestion; Two-step process
1. Introduction

During the anaerobic digestion the organic compounds such as carbohydrates, proteins, and fats are initially hydrolyzed and fermented by fermentative bacteria to volatile fatty acids (VFA) (e.g. acetate, propionate, butyrate, iso-butyrate etc.), alcohols, H$_2$ and CO$_2$. The VFA other than acetate as well as some alcohols are subsequently oxidized by syntrophic bacteria to acetate, H$_2$ and CO$_2$ and these are finally converted into biogas (methane and carbon dioxide) by methanogens (Stams, 1994). Anaerobic digestion is a widely used method for the treatment of sewage sludge (Mata-Alvarez et al. 2000). In comparison with other methods of waste treatment, such as land filling, incineration and composting, anaerobic digestion has the advantages of: a) reducing the amount of waste, b) generating energy in the form of methane that can be used for production of heat and/or electricity and c) resulting to a nutrient-containing final product that is suitable for application on the farmland as fertilizer or soil improving agent. Thus, the anaerobic digestion process can be considered as a sustainable method for sewage sludge treatment and it is worthy of further study.

Anaerobic digesters are usually operated under mesophilic or thermophilic conditions at temperatures of 30-40°C or 50-60°C, respectively (Buhr and Andrews, 1977; Ahring 1995). Most of the anaerobic digesters in Europe were started and operated in mesophilic manner (de Baere, 2000). However, recognizing the advantage of the thermophilic process that derives from higher metabolic activities of the thermophiles makes the shift to the thermophilic operational temperature attractive. Higher metabolic activities and substrate conversion rates of the thermophilic microorganisms is reflected by a higher methane production rate. The latter allows the shortening of the retention time of the waste/wastewater in the digester, treating larger volumes of sludge in existing capacities or building of new reactors with smaller volume (Buhr
and Andrews, 1977). Several cases of successful shift of sewage sludge treatment in full-scale digesters documented that the thermophilic operation could be stable and reliable (Ahring et al. 2002; Nielsen and Petersen, 2000; Zabranska et al. 2000). According to the new regulations made by EPA (USA) and European Commission for disposal of anaerobically digested sewage sludge, the final product of the mesophilic digestion is classified as Class B biosolids with restricted use on agricultural land ( ). Even though the thermophilic digestion is yet not declared as a method for obtaining Class A biosolids, the number of reports documenting the pathogen reduction effect of this method to levels that satisfy the demand for Class A classification is increasing (Watanabe 1997; Krugel et al. 1998; Huyard 2000; Nielsen and Petersen, 2000). This indicates that most probably the role of the thermophilic anaerobic digestion should be re-evaluated (Aitken and Mullennix, 1992; Ahring et al. 2002).

The economy of a biogas plant is directly linked to the amount of biogas produced per unit of raw material treated. In practice, only about half of the organic material is converted and therefore there is a large potential for increasing the biogas yield of sewage sludge. The optimization of the anaerobic digestion process strongly depends on the increase of the hydrolysis efficiency since the organic matter of sewage sludge mainly exists in particulate form and hydrolysis is the rate-limiting step of the whole process (Vandevoorde et. al, 1988; Eliosov and Aegaman, 1995; Eastman and Ferguson, 1981). Novel pre-treatment methods have been developed in order to disintegrate the flock structure of the sludge, improve the solubility of solid organic matter and consequently increase the methane production rate. Muller (2001) reviewed the physical, chemical and biological pre-treatment methods in terms of energy consumption, operation reliability, dewaterability of the effluent and pathogens reduction and found the thermal and the biological pre-treatment to be the most efficient and feasible.
Understanding the role of different microbial groups in the process of anaerobic digestion led to the development of a two-phase concept already in early 1970’s (Pohland and Ghosh, 1971). The concept is based on the combination of two digesters in series, where the first reactor is operated as a hydrolysis/acidification reactor at a short retention time delivering the pre-hydrolysed sludge into a second methanization reactor operated at a longer retention time. So far, the two-stage digestion has been investigated in combinations of: two mesophilic reactors ( ), a moderate thermophilic (55-60°C) reactor coupled to a mesophilic reactor (Oles et al. 1997; Dichtl 1997), two thermophilic (55°C) reactors ( ) and finally an hyper-thermophilic reactor (75°C) combined with a mesophilic reactor (Zhao and Kugel, 1996) and all studies obtained very promising results. The increase of the performance of the two-stage thermophilic digestion will significantly contribute to the future application of this method for treatment of sewage sludge.

The fact that the thermophilic hydrolytic bacteria exhibit a broad temperature optimum in a range of 55-75°C (Wiegel, 1992) makes of great interest the development of a two-phase process combining a 70°C hydrolysis/acidification with a 55°C methanogenesis since such a process will have the additional advantages of the increased hydrolysis performance and the fulfilment of the sanitation requirements due to the elevated temperature of the hydrolysis step. The effect of a pre-treatment step at 70°C on mesophilic and thermophilic anaerobic digestion of primary and secondary sludge was investigated and different pre-treatment durations were tested in the preliminary study of Gavala et al. (2003).

The scope of the present study was the thorough investigation of the effect of a pre-treatment step at 70°C on thermophilic (55°C) anaerobic digestion of primary sludge using continuously
operated digesters (CSTR-type). Process stability, microbial activity, organic matter destruction efficiency, pathogen reduction and economic feasibility of the two-step process versus the one-step thermophilic (55°C) process were assessed.
2. Materials and methods

2.1. Analytical methods

Determinations of the total (TS) and volatile (VS) solids and total suspended (TSS) and volatile (VSS) solids were carried out according to standard methods (APHA, 1989). For the quantification of volatile fatty acids (VFA), acidified samples with 17% H₃PO₄ were analysed on a gas chromatograph (Hewlett Packard 5890 series II) with a flame ionisation detector and a capillary column (Hewlett Packard FFAP 30 m, inner diameter 0.53 mm, film 1 μm). Biogas composition in methane was quantified with a gas chromatograph (Shimadzu GC-8A) equipped with a flame ionisation detector and a packed column (Porapak Q, 80/100-mesh). The medium (BA medium) used in batch tests was prepared from the following stock solutions (chemicals in g l⁻¹ of distilled water): (A) NH₄Cl, 100; NaCl, 10; MgCl₂·6H₂O, 10; CaCl₂·2H₂O, 5; (B) K₂HPO₄·3H₂O, 200; (C) resazurin, 0.5; (D) trace metals and selenite solution: FeCl₂·4H₂O, 2; H₃BO₃, 0.05; ZnCl₂, 0.05; CuCl₂·2H₂O, 0.038; MnCl₂·4H₂O, 0.05; (NH₄)₆Mo₇O₂₄·4H₂O, 0.05; AlCl₃, 0.05; CoCl₂·6H₂O, 0.05; NiCl₂·6H₂O, 0.092; ethylene-di-amine-tetra-acetate, 0.5; Na₂SeO₃·5H₂O, 0.1; HCl 37%, 1ml; (E) vitamin solution according to Wolin et al. (1963). The following volumes of stock solutions were added to 916 ml of distilled water: A, 10 ml; B, 2 ml; C, 1 ml; D, 1 ml; E, 10 ml. 50 ml of a 52 g l⁻¹ NaHCO₃ solution were added as well. The medium was gassed with 80% N₂ – 20% CO₂, dispensed and autoclaved. Before inoculation the medium was reduced with a 25 g l⁻¹ Na₂S·9H₂O solution to a ratio of 0.1 ml / 10 ml of medium.

The pathogen reducing effect (PRE) was determined by using faecal streptococci (FS) as indicator organism as suggested by the Nordic Committee on Food Analysis (1992). The determination of the FS was carried out as described in Bendixen (1999). By comparing the FS values in sludge before and after treatment, a numerical value of the PRE is obtained. The results are expressed in log₁₀-reduction units according to the equation 1.
\[ \text{PRE} = \log_{10} N_i - \log_{10} N_e \]  

(1)

where \( N_i \) and \( N_e \) are the average number of faecal streptococci in 1 ml of the influent (before treatment) and effluent (after treatment) respectively.

5 2.2. Continuous experiments

The primary sludge used was taken from Lundofte Wastewater Treatment Plant, Lyngby, Denmark. It was collected just after the gravity sedimentation tank, distributed in 5 l containers and subsequently stored at \(-20^\circ\text{C}\). Two batches of primary sludge were collected and used throughout the experimental period.

Two experimental set-ups were used in this study: Set-up (a) was used as test process, and Set-up (b) was used as reference process for comparison purposes. Figure 1a shows the schematic diagram of Set-up (a), which was a two-step thermophilic anaerobic digestion process. It consisted of two continuously stirred tank reactors (CSTR): Reactor P and Reactor A with active volumes of 0.5 l and 3.0 l respectively. Two water baths were used to keep the temperature of Reactor P and Reactor A at 70\(^\circ\text{C}\) and 55\(^\circ\text{C}\) respectively by circulating warm water to the double-layered wall of the reactors. Two 1.0 l bottles were served as influent and effluent bottle. The influent bottle was connected with a plastic bag containing \( \text{N}_2/\text{CO}_2 \) gas for securing anaerobic conditions. The produced biogas in Reactor A was sequentially flowing through the effluent tube, the effluent bottle and finally through the biogas flow meter. Two peristaltic pumps were used to drive the primary sludge from the influent bottle into Reactor P and the effluent of Reactor P into Reactor A, respectively. Programmable timers controlled the stirrer for the influent bottle, the mixers of the reactors and the pumps. Biogas samples for methane analysis were taken through a
A septum located on the tube between the effluent bottle and the gas meter. Manual valves were used for sludge sampling or for closing the tubes when necessary. Set-up (b) consisted of one CSTR, Reactor B, which was identical to Reactor A (Fig. 1b).

Reactors A and B were simultaneously started-up on day 1 by inoculation with 0.5 l anaerobic mixed liquor from a laboratory-scale thermophilic anaerobic reactor treating primary sludge. Initially, the reactors were operated at 55ºC and at a hydraulic retention time (HRT) of 15 days. They were fed with primary sludge from the first batch until the day 35 and with sludge from the second batch for the rest of the experimental period, which lasted until day 103. After both reactors reached a steady state (day 53), reactor P was started up at HRT of 2 days and connected to reactor A. At the same, the HRT of reactor A was reduced to 13 days and started to be fed with the effluent of the reactor P. The performance of the two set-ups was monitored by measuring the pH, the concentration of VFA, solids and biogas production and composition throughout the experimental period. Once the steady state was reached measurement of the reactors took place and the performances of Set-up (a) and (b) were compared regarding organic matter removal and pathogen reducing effect. Also mixed anaerobic liquor from digesters A, B and P was used as inoculum for two series of batch experiments that were carried out in order to compare the microbial activities of digesters A and B and the methane potential of the pre-treated and raw primary sludge respectively.

2.3. Batch experiments

A series of batch tests for the determination of the substrate consumption activities were performed in order to investigate the effect of the pre-treatment step on the activities of the different microbial groups (acetogens and methanogens) of the thermophilic anaerobic digestion
of primary sludge. Batch tests were carried out in triplicates at 55°C in 58 ml serum vials sealed with butyl rubber stoppers and aluminum crimps. The serum bottles contained 15 ml of BA medium and acetate or butyrate or propionate as sodium salts was added at a final concentration of 20, 10 and 5 mM, respectively. The bottles were inoculated with 5 ml of anaerobic mixed liquor from the thermophilic digester A or B. When hydrogen was used as the substrate, the serum bottles were pressurized to 2 atm with a gas mixture of 20%CO₂ and 80% H₂. Triplicate controls were also prepared containing only 15 ml of BA medium and 5 ml of anaerobic mixed liquor (no substrate added). VFA concentrations or methane production were followed throughout the experiments and the activities of acetogenic bacteria to degrade intermediate VFA (e.g. butyrate, propionate) to acetate and the activities of methanogenic archaea to convert acetate to methane (by aceticlastic methanogens) and H₂/CO₂ to methane (by hydrogen-utilizing methanogens) were determined. In case of experiments with hydrogen as substrate, the calculations for the determination of the activities were based on the methane production. Specific methanogenic activity (SMA) and maximum specific substrate consumption rate (MSSCR) were used to describe the above-mentioned microbial activities. SMA can be defined as the substrate-dependent methane production rate per unit mass of biomass, i.e. the rate with a saturating concentration of substrate present when background methane production rate has been diluted to an insignificant level (Sørensen & Ahring, 1993). In the plot of methane produced versus time, the initial slope divided by the VSS mass gives the specific methanogenic activity (SMA). In the plot of VFA concentration versus time, the initial slope divided by the VSS concentration gives the maximum specific substrate consumption rate (MSSCR).

A second series of batch experiments were performed in order to determine the methane potential and the maximum methane production rate of the pre-treated and raw primary sludge. Batch tests
were carried out in triplicates in serum vials sealed with butyl rubber stoppers and aluminium crimps. The serum bottles contained inoculum from digester A supplemented with pre-treated primary sludge (effluent from digester P, P-A) and inoculum from digester B supplemented with raw primary sludge (PS-B) and were incubated at 55°C. Also inoculum from digester P supplemented with raw primary sludge (PS-P) was incubated at 70°C. Inoculum from digesters A and B were incubated separately at 55°C (no substrate added, A and B respectively) and served as controls. Also raw primary sludge was incubated at 55°C and 70°C (PS and PSE respectively) and pre-treated primary sludge (effluent of reactor P, P) was incubated at 55°C without inoculum added to test if there were any indigenous methanogenic archaea. Methane production was followed throughout the experiments.

2.4. Mathematical expressions and calculations

2.4.1. Organic matter destruction efficiency

In this study, organic matter content was expressed as VS. The organic matter destruction efficiency was calculated by the following equation 2:

\[ \alpha = \left( \frac{\text{VS}_i - \text{VS}_e}{\text{VS}_i} \right) \times 100 \% \] (2)

where \( \alpha \) ---- organic matter destruction efficiency

\( \text{VS}_i \) ---- VS content in the influent of the process, g/l;

\( \text{VS}_e \) ---- VS content in the effluent of the process g/l.

2.4.2. Energy balance
The energy consumption during the operation of an anaerobic digestion system covers the heating of the influent sludge, the pumping of the sludge, the stirring of the digesters and the heat losses through the piping and through the digester boundaries. It has been shown that the major part of the heat requirement of a full-scale plant for thermophilic sludge digestion concerns the heating of the influent sludge. On the other hand and depending on the outside temperature, the heat losses account only to 2-8% of the heat needed for the influent sludge heating (Zupancic and Ros 2003). The energy requirements for pumping and mixing are estimated to $1.8 \times 10^3 \text{ kJ/m}^3$ and $3.0 \times 10^2 \text{ kJ/(m}^3\text{.d)}$ respectively (information from Lundofte Wastewater Treatment Plant, Lyngby, Denmark). In this study, the construction materials, the configuration and the operation of the reactors of the process (a) were identical to those of process (b). Therefore, the extra energy needed for the two-step process (a) is the energy used for running the pretreatment reactor.

In practice, the outflow from the pretreatment reactor in process (a) has to be cooled down from 70°C to 55°C in order to be fed to the second reactor. By using a heat exchanger, the heat released from cooling the effluent of the pretreatment reactor from 70°C to 55°C can be utilized to heat the influent sludge of the pretreatment reactor, and thus can be efficiently recovered. In this study we assumed that 85% of the heat can be recovered by the heat exchanger. Consequently, the energy input in the form of heat and electricity for the pretreatment step is calculated according to the following equations:

\[ E_{\text{input,heat}} = \rho \times Q_a \times \gamma \times (t_1 - t_2) \times (1 - \phi) \times (1 + \kappa) \quad (3) \]

\[ E_{\text{input,electricity}} = Q_a \times 10^{-6} \times \theta + V_p \times \omega \quad (4) \]

Where \( E_{\text{input,heat}} \) = heat requirement for pretreatment, kJ/d;
\( E_{\text{input,electricity}} \) = electricity requirement for pretreatment, \( kJ/d \);

\( \rho \) = Specific density of primary sludge, \( 1 \, g/ml \);

\( Q_a \) = Flow of influent sludge fed to Process (a), \( 230 \, ml/d \);

\( \gamma \) = Specific heat of primary sludge, \( 4.18 \times 10^{-3} \, kJ/(g \cdot ^\circ C) \);

\( t_1 \) = Temperature of pretreatment, \( 70 \, ^\circ C \);

\( t_2 \) = Temperature of Digester A, \( 55 \, ^\circ C \);

\( \phi \) = Percentage of heat recovered, \( 85\% \);

\( \kappa \) = Percentage of heat loss through the piping and through the digester boundaries, \( 8\% \);

\( \theta \) = Electrical energy consumption for pumping, \( 1.8 \times 10^3 \, kJ/m^3 \);

\( V_p \) = Working volume for pre-treating influent of reactor, \( 4.62 \times 10^{-6} \, m^3 \);

\( \omega \) = Electrical energy consumption rate for stirring, \( 3.0 \times 10^2 \, kJ/(m^3 \cdot d) \).

The biogas is the only exploitable energy source which is produced during the anaerobic digestion processes because the chemical energy of the methane can easily be converted to heat and electricity by a CHP (combined heat and power) unit. If the 70/90 \(^\circ C\) standard system is used in the CHP unit, about 35\% of the chemical energy of methane can be converted to electrical energy, 55\% to heat and the rest 10\% is lost (Jenbacher AG, 1999). Thus, the extra energy obtained from process (a) can be attributed to the increase of methane production due to the pretreatment step and can be calculated by the following equations:

\[
E_{\text{output,heat}} = \xi \times (Q_a \times C_a \times \eta_a - Q_b \times C_b \times \eta_b) \times \psi \tag{5}
\]

\[
E_{\text{output,electricity}} = \xi \times (Q_a \times C_a \times \eta_a - Q_b \times C_b \times \eta_b) \times \pi \tag{6}
\]

Where \( E_{\text{output,heat}} \) = Heat production from extra methane produced by process (a), \( kJ/d \);
$E_{\text{output,electricity}} = $ Electricity production from extra methane produced by of process (a), kJ/d;

$\xi = $ Calorific value of methane, $8.02 \times 10^2$ kJ/mol; 

$\eta_a = $ Methane potential of digester A (see section 3.2, table 4), $20.09 \times 10^{-3}$ mol/g-VS; 

$\eta_b = $ Methane potential of digester B (see section 3.2, table 4), $13.56 \times 10^{-3}$ mol/g-VS; 

$C_a = $ VS concentration of the pre-treated primary sludge (see section 3.1, table 2), 7.10 g-VS/l; 

$C_b = $ VS concentration of the raw primary sludge (see section 3.1, table 1), 9.58 g-VS/l; 

$Q_a = $ Flow of influent sludge fed to digester A, 0.230 l/d; 

$Q_b = $ Flow of influent sludge fed to digester B, 0.200 l/d; 

$\psi = $ Efficiency of the CHP unit for heat generation, 55%; 

$\pi = $ Efficiency of the CHP unit for electricity generation, 35%.

3. Results and discussion

3.1. Continuous experiments

The characteristics of the two batches of primary sludge used for the experiments and the reactors A, B and P after steady state was reached are shown in tables 1 and 2 respectively. The pH of reactor P was lower than that of A and B and VFA concentration was considerably higher in reactor P, due to hydrolysis and acidogenesis of the organic matter, with acetic and propionic acid being the main acids generated. The organic matter destruction efficiency of processes (a) and (b) was 52.4 and 39.1 % respectively. The 36% of the total destruction of solids (measured as VSS) happened in reactor P during process (a) while the rest 19% took place in reactor A. Comparing the organic matter destruction efficiency of the two processes, we observe that
process (a) resulted in a 13% higher efficiency than process (b). This supports the initial hypothesis that a pre-treatment at 70°C enhances the solubilisation of solids thus resulting in increased digestibility of the sludge. Regarding pathogen reducing effect (PRE) it was calculated that process (b) resulted in a PRE of 2.6. On the other hand process (a) resulted in a complete destruction of faecal streptococci indicating that pre-treatment at 70°C had a decisive effect on pathogens destruction.

3.2. Batch experiments

Acetic, butyric and propionic acids consumption during the batch experiments with inoculum from digesters A and B are shown in figures 2a, 2b and 2c respectively. Methane production from hydrogen during the batch experiments with inoculum from digesters A and B and hydrogen is presented in figure 3. The maximum specific substrate consumption rate and specific methanogenic activity have been calculated for both digesters A and B and are presented in table 3. Digester A was characterized by a higher activity compared to digester B regarding VFA consumption and methane production from hydrogen (44, 30 and 31 % increase of acetic, propionic and butyric acids consumption rate respectively and 46% increase in methane production rate from hydrogen). This was due to the higher VFA concentration in the influent of digester A, which was thermally pre-treated and thus partly hydrolyzed/acidified primary sludge from digester P. The enhanced microbial activities in digester A suggest that the same efficiencies in organic matter removal and methane recovery could be obtained at a lower HRT thus resulting at reduced digester volume for a full-scale plant.

The methane produced during the batch experiment with inoculum from digester B supplemented with primary sludge (PS-B) is presented in figure 4a. The control values, namely the methane
produced during the incubation of inoculum from digester B have been subtracted. Also the profile of the methane produced during incubation of the primary sludge (PS) at 55°C is presented in the same figure. The methane produced during the batch experiment with inoculum from digester A supplemented with pre-treated primary sludge, namely the effluent of digester P (P-A) is shown in figure 4b. The control values, namely the methane produced during the incubation of inoculum from digester A have been subtracted. Finally, the methane produced during the batch experiment with inoculum from digester P supplemented with primary sludge (PS-P) is presented in figure 4c. The control values, namely the methane produced during the incubation of inoculum from digester P have been subtracted. Also the profile of the methane produced during incubation of the primary sludge at 70°C (PSE) is shown in the same figure.

The methane production is expressed in mmole CH₄ per g of VS of the primary sludge (raw or pre-treated) added at the beginning of each batch set. The average value from the triplicates is used and the standard deviation is shown in all figures. It is noticeable that thermophilic and hyperthermophilic methanogenic archaea are present in primary sludge. Primary sludge produced 4.5 and 0.1 mmole methane per g VS when incubated at 55 and 70°C respectively.

The methane potential and the methane production rate during the experiments with inoculum from digesters B and P and raw primary sludge added and inoculum from digester A and pre-treated primary sludge added have been calculated and presented in table 4. Pre-treatment of the primary sludge resulted at a 48% increase of the methane potential. This most probably means that a part of the primary sludge solids that were non-hydrolysable at 55°C and therefore did not contribute to the methane produced during the thermophilic anaerobic digestion process were actually hydrolysed at 70°C during the pre-treatment step and thus become susceptible to the anaerobic fermentation. Moreover the methane production rate during the anaerobic digestion of
the pre-treated primary sludge was 115% higher compared with that during the anaerobic
digestion of the raw primary sludge.

3.2.1. Net energy production

The calculated values of the heat and electricity requirements for the hyper-thermophilic pre-
treatment digester ($E_{\text{input,heat}}$ and $E_{\text{input,electricity}}$) and the heat and electricity production from the
extra methane produced by process (a) ($E_{\text{output,electricity}}$ and $E_{\text{output,heat}}$) are presented in Table 5. These values indicate that the extra energy requirements for the operation of a pre-treatment step
(like in process a) would be covered by the energy produced from the extra methane production
and in addition there would be a significant energy surplus. Also, the higher rate of the methane
production in the two-step process (table 4) indicates that a smaller bioreactor could be equally
sufficient and therefore the capital cost of the methanogenic digester would be lower. Moreover,
the cost of operating and maintaining a smaller digester would be lower as well. Concluding, an
hyper-thermophilic / thermophilic two step process for the stabilisation of primary sludge results
not only in increased organic matter destruction efficiency and complete pathogens destruction
but moreover in enhanced methane production that surpasses the extra energy requirements.
4. Conclusions

The present study examines the effect of a hyper-thermophilic (70°C) pre-treatment step on the thermophilic (55°C) anaerobic digestion of sludge. It has been shown that the process with the pre-treatment step (process a) resulted in 13% higher organic matter removal efficiency than the one step thermophilic process (process b). This suggests that the pre-treatment at 70°C enhances the solubilisation of sludge solids thus increasing the digestibility of sludge. Also process (a) completely destructed the faecal streptococci, which has been chosen as an indicator pathogen in the primary sludge, compared to the process (b) that resulted in a lower pathogen reducing effect (PRE) of 2.6.

The microbial activities of two thermophilic methanogenic digesters with and without a pre-treatment step were also measured and compared. When a pre-treatment step preceded, the methanogenic digester was characterised by a higher activity regarding acetate, propionate and butyrate consumption and methane production from hydrogen (44, 30 and 31 % increase of acetic, propionic and butyric acids consumption rate respectively and 46% increase in methane production rate from hydrogen). The enhanced microbial activities suggest that the same efficiencies in organic matter removal and methane recovery could be obtained at lower HRT thus resulting at reduced digester volume for a full-scale plant. Moreover, pre-treatment of the primary sludge resulted at a 48% increase of the methane potential (20.09 and 13.56 mmole CH₄/gVS with and without pre-treatment respectively) and at a 115% increase of the methane production rate. Taking into account the increased methane production and calculating the energy balance of the two-step process it was showed that the extra energy requirements for the operation of a pre-treatment step (like in process a) would be covered by the energy produced
from the extra methane production and in addition there would be a significant energy surplus of 2.17 kJ / d. Last but not least, the higher rate of the methane production in the two-step process indicates that a smaller bioreactor could be equally sufficient and therefore the capital, operating and maintaining cost of the methanogenic digester would be lower.
**Acknowledgments**

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**References**


Table 1. Characteristics of the two batches of the primary sludge
(Values are given as average of triplicates with standard deviation.)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Primary sludge</th>
<th>1&lt;sup&gt;st&lt;/sup&gt; batch</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; batch</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS, g/l</td>
<td></td>
<td>17.90 ± 0.11</td>
<td>15.02 ± 0.20</td>
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<td>VS, g/l</td>
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<td>11.2 ± 0.13</td>
<td>9.58 ± 0.21</td>
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<td>TSS, g/l</td>
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<td>14.48 ± 0.03</td>
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<td>VSS, g/l</td>
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<td>9.05 ± 0.16</td>
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<td>Propionic acid, mM</td>
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<td>Butyric acid, mM</td>
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<td>2.47 ± 0.42</td>
<td>1.17 ± 0.03</td>
</tr>
<tr>
<td>FS (CFU/ ml)</td>
<td></td>
<td>Not measured</td>
<td>4.7 x 10&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Table 2. Characteristics of the digesters A, B and P at steady state
(Values are given as average of triplicates with standard deviation.)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Digester P</th>
<th>Digester A</th>
<th>Digester B</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>pH</strong></td>
<td>6.6 ± 0.1</td>
<td>7.1 ± 0.1</td>
<td>7.1 ± 0.1</td>
</tr>
<tr>
<td><strong>TS, g/l</strong></td>
<td>11.92 ± 0.92</td>
<td>10.75 ± 0.50</td>
<td>14.06 ± 0.34</td>
</tr>
<tr>
<td><strong>VS, g/l</strong></td>
<td>7.10 ± 0.54</td>
<td>4.56 ± 0.22</td>
<td>5.83 ± 0.29</td>
</tr>
<tr>
<td><strong>TSS, g/l</strong></td>
<td>10.23 ± 0.98</td>
<td>9.21 ± 0.56</td>
<td>12.41 ± 0.64</td>
</tr>
<tr>
<td><strong>VSS, g/l</strong></td>
<td>5.77 ± 0.54</td>
<td>4.05 ± 0.22</td>
<td>5.11 ± 0.30</td>
</tr>
<tr>
<td><strong>% CH₄</strong></td>
<td>Not measured</td>
<td>69 ± 2</td>
<td>63 ± 3</td>
</tr>
<tr>
<td><strong>Biogas production, ml/d</strong></td>
<td>Not measured</td>
<td>706 ± 71</td>
<td>696 ± 76</td>
</tr>
<tr>
<td><strong>Acetic acid, mg/l</strong></td>
<td>722 ± 79</td>
<td>32 ± 26</td>
<td>25 ± 13</td>
</tr>
<tr>
<td><strong>Propionic acid, mg/l</strong></td>
<td>526 ± 61</td>
<td>16 ± 18</td>
<td>17 ± 18</td>
</tr>
<tr>
<td><strong>Isobutyric acid, mg/l</strong></td>
<td>114 ± 97</td>
<td>18 ± 17</td>
<td>18 ± 17</td>
</tr>
<tr>
<td><strong>Butyric acid, mg/l</strong></td>
<td>187 ± 26</td>
<td>10 ± 17</td>
<td>9 ± 12</td>
</tr>
<tr>
<td><strong>FS (CFU/ ml)</strong></td>
<td>Not measured</td>
<td>No colonies</td>
<td>1.1 x 10³ grown</td>
</tr>
</tbody>
</table>
Table 3. Maximum specific substrate consumption rates and specific methanogenic activities of digesters A and B

<table>
<thead>
<tr>
<th></th>
<th>Maximum specific substrate consumption rate, mmol·g-VSS⁻¹·h⁻¹</th>
<th>Specific methanogenic activity, mmol CH₄·g-VSS⁻¹·h⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Digester A</td>
<td>Digester B</td>
</tr>
<tr>
<td>Acetate</td>
<td>52.47·10⁻²</td>
<td>29.40·10⁻²</td>
</tr>
<tr>
<td>Propionate</td>
<td>4.92·10⁻²</td>
<td>3.44·10⁻²</td>
</tr>
<tr>
<td>Butyrate</td>
<td>21.10·10⁻²</td>
<td>14.66·10⁻²</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>2.04·10⁻²</td>
<td>1.11·10⁻²</td>
</tr>
</tbody>
</table>
Table 4. Methane potential and methane production rates calculated for digesters A, B and P from the second series of batch experiments.

<table>
<thead>
<tr>
<th>Batch experimental sets</th>
<th>Methane potential</th>
<th>Methane production rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mmole CH$_4$ / g VS</td>
<td>mmole CH$_4$ / g VS / d</td>
</tr>
<tr>
<td>Inoculum from B and primary sludge added (PS-B)</td>
<td>13.56 ± 0.91</td>
<td>0.98</td>
</tr>
<tr>
<td>Inoculum from P and primary sludge added (PS-P)</td>
<td>0.23 ± 0.01</td>
<td>0.07</td>
</tr>
<tr>
<td>Inoculum from A and pre-treated primary sludge added (P-A)</td>
<td>20.09 ± 0.16</td>
<td>2.11</td>
</tr>
</tbody>
</table>

Table 5. Energy balance of hyper-thermophilic / thermophilic two-step process

<table>
<thead>
<tr>
<th></th>
<th>Extra requirement (kJ/d)</th>
<th>Extra production (kJ/d)</th>
<th>Net production (kJ/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat</td>
<td>$E_{\text{input,heat}} = 2.34$</td>
<td>$E_{\text{output,heat}} = 3.01$</td>
<td>+0.67</td>
</tr>
<tr>
<td>Electricity</td>
<td>$E_{\text{input,electricity}} = 0.42$</td>
<td>$E_{\text{output,electricity}} = 1.92$</td>
<td>+1.50</td>
</tr>
</tbody>
</table>
Figure 1. Schematic diagram of the two-step thermophilic anaerobic digestion process (a) and the one-step thermophilic anaerobic digestion process (b).

Figure 2. Consumption of acetic (a), butyric (b) and propionic (c) acids during the batch experiments with inoculum from digesters A and B.

Figure 3. Methane production from hydrogen during the batch experiments with inoculum from digester A and B.
1-N₂/CO₂ gas bag, 2-Influent bottle, 3-Biogas meter, 4-Valve, 5-Peristaltic pump, 6-Mixer, 7-Stirrer, 8-Gas sampling septum, 9-Timer, 10-Reactor P, 11-Effluent bottle, 12-Water bath (75.5°C), 13-Reactor A, 14-Water bath (57.5°C)
Figure 2.

\[ \text{digester A: } y = -0.4992x + 16.499, \quad R^2 = 0.9957 \]
\[ \text{digester B: } y = -0.3853x + 21.188, \quad R^2 = 0.8875 \]

\[ \text{digester A: } y = -0.0468x + 3.694, \quad R^2 = 0.9981 \]
\[ \text{digester B: } y = -0.0451x + 3.821, \quad R^2 = 0.9909 \]

\[ \text{digester A: } y = -0.2008x + 8.2771, \quad R^2 = 0.9926 \]
\[ \text{digester B: } y = -0.1921x + 7.8201, \quad R^2 = 1 \]
Figure 3

Digester A: \( y = 0.0004x - 0.0015, \ R^2 = 0.9677 \)

Digester B: \( y = 0.0003x - 0.0004, \ R^2 = 0.9826 \)