university of groningen

MSc. Industrial Engineering and Management
Research Project

Evaluation of the Impact of Physical and Chemical Pretreatment on the Anaerobic Digestibility of Sewage Sludge

Research Group:
Products and Processes for Biotechnology
in the Biobased Economy

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Project Abstract

Project Prelude: The master research project is focused on investigating pretreatment methods to enhance the anaerobic digestibility of municipal sewage sludge. The research aims to perform a screening of various available methods. In this regard, three key chemical and physical pretreatments, namely heat, alkaline hydrolysis, and sonication, are investigated via the application of standard protocols of biomethane and substrates quantification.

Research Objective: This project aims to investigate to what extent the application of thermal degradation, alkaline hydrolysis, and sonication pretreatment enhances the anaerobic digestibility of sewage sludge by looking at a controlled inoculated representative bacteria culture over 15 days.

Design and Scope: The research project treats the anaerobic digestion process as a grey box; reaction mechanisms are only studied at an abstract level. Where necessary, macroeconomics values are assumed within the Netherlands and the European Union (EU), and operational parameters are based on the wastewater plant of Garmerwolde. The core goal is addressed by defining the variable boundaries via an in-depth literature review and an experimental study. The project strives mainly towards generating a solid knowledge foundation rather than absolute optimization.

Principal Variables and Methods: The driving independent variable is the pretreatment method and conditions. Triplicates of each method are prepared and evaluated using an inoculated control biomass. The digestibility is evaluated by quantifying biomethane production over the defined time; quantification is evaluated semi-daily for 15 days. The biogas composition is evaluated via gas chromatography on the final day. Substrates and solids are further quantified to provide support to the primary data; the supporting variables are quantified before and after pretreatment and after the 15-day inoculation.

Results: Heat and alkaline hydrolysis are observed to enhance digestibility in both methane production and startup significantly. Heat treatment resulted in up to 16.27% of the theoretical production to be reached compared to just 9.6% of the standard (control) setup, whereas Alkaline hydrolysis resulted in the more substantial value of 30.8% potential reached. Solubilization of substrates is also enhanced. Sonication indicated a less visible outcome; the startup is highly inhibited; however, the biogas composition indicates that the process was diverging from the untreated sample on day 15. A literature review provides support for the hypothesis that a lengthy low dosage sonication has potentially resulted in poor solubilization and flocculation; however, further research is necessary to confirm this.

Conclusion: Low-temperature heat treatment and alkaline hydrolysis are identified to enhance the digestion process substantially. However, the introduction of chemicals and alteration in the effluent may lead to operational limitations, as well as regulations to consider regarding the effluent. For this, further analysis of critical temperature and dosage boundaries in addition to an in-depth evaluation of externalities together with a representative stakeholder analysis is recommended. Further analysis of sonication is discouraged due to the solid content of the samples; literature has noted that this factor is likely going to lead towards an unfavorable energy balance.

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1 Introduction to the Research Project

1.1 Project Background

Sewage Sludge (SS) is a critical byproduct of wastewater treatment processes. Historically, SS was classified as a waste stream disposed of mainly via combustion, landfilling, and discharge to surface waters. The latter practices remained the industrial norm until the 1980s when the threat of heavy metals and sediments accumulating in fauna was clarified. In most European states, exercises such as landfilling are since restricted [1]. As a result of the global population growth trends, especially those in urban areas, the volume of sludge produced from wastewater treatment plants has increased, posing an increasing demand for improved treatment processes and methods of waste valorization [1].

SS is a complex mixture of partially settled organic and inorganic solids and phosphorous, precipitates, and biomass generated via digestion [2]. This wide array present in a single mixture results in a challenging treatment process, which can accumulate up to half of the overall wastewater treatment's process cost in addition to the potential for environmental hazards [3]. Consequently, a wide array of research and development is since striving towards increasing the harm mitigation towards population and environment while assuring an extended valorization of the waste stream through means such as the component recovery [3].

A conventional wastewater treatment method utilizes a waste-activated sludge plant (AB-Process). This system operates an anaerobic digestion (AD) stage to treat the thickened sludge. Via employment of a favorable environment, the growth of specific bacterial species is promoted, which provides contaminant removal and biogas generation [4]. The resulting biogas is used in the system as a means of energy recovery in the form of heat and electricity [5].

To briefly discuss, AD is defined as "a microbiologically mediated process during which organic carbon present in biopolymers and other degradable compounds is converted to its most reduced form, methane (CH_4) and its most oxidized form, carbon dioxide (CO_2) in the absence of oxygen" [6]. A schematic illustration of the process with variables of interest for this research is represented in Figure 1. The AD process involves a wide array of bacteria engaging in an orderly procedure to treat and digest the organic material consisting of carbohydrates, proteins, and lipids, through the four stages, namely [7]:

- **Hydrolysis:** Cleavage of complex organics into soluble monomers and polymers. The process stage is catalyzed mainly by extracellular enzymes such as cellulase, protease, amylase, and lipase [8].

- **Acidogenesis:** Mediated by the fermentative organism, this stage converts the relatively soluble products from the previous step into volatile fatty acids (VFAs), hydrogen gas (H_2), carbon dioxide (CO_2), and ammonia (NH_3) [8].

- **Acetogenesis:** moderated and driven by the acetogenic bacteria, fatty acids from the previous stage, excluding acetate, are converted into acetic acid (CH_3COOH), hydrogen gas (H_2), and carbon dioxide (CO_2) [8].

- **Methanogenesis:** Lastly, the final organic material is degraded into biogas. In this process, the two archaea bacteria groups of acetolactic methanogens and hydrogenotrophic methanogens contribute to 2/3 and 1/3 of the output methane (CH_4), respectively [8].

This research project, per the suggestion of the problem owner of the project, the University of Groningen's (RuG) Products and Processes for Biotechnology in the Biobased Economy (PPBBE), investigates the proposal that a potential route for energy recovery and waste valorization is to introduce a pretreatment stage to enrich the substrates fed to the anaerobic digester. AD of SS and hence the biomethane production is influenced primarily by temperature, available nutrients (feedstock), the composition of the medium, and the loading rate [9]. Due to its complex microstructure and components, AD of SS is bottlenecked by the hydrolysis step. This stage directly relates to free accessible surface area availability and substrate structure [8]. Pretreatment methods, both physical and chemical, strive to disrupt the extracellular polymeric substances (EPS) matrices and the cell walls resulting in increased availability of nutrients for digestion [10].

The overarching project requests investigating the impact of three pathways of thermal degradation, sonication, and low-temperature alkaline hydrolysis on mesophilic AD (digestion temperature of 37 °C).

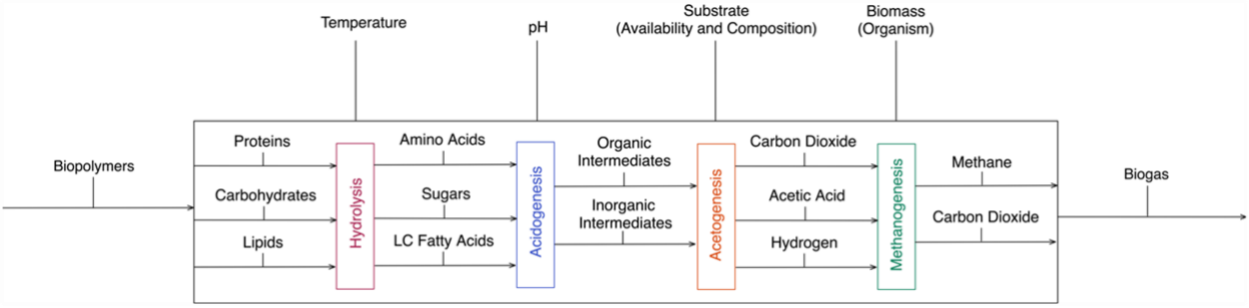


Figure 1: Schematic overview of the anaerobic digestion of biopolymers into biogas

1.2 Aim and Success Criteria

The research goal of this project is to investigate the effect of thermal degradation, sonication, and alkaline hydrolysis on the digestibility of sewage sludge by a controlled complex representative culture of bacteria over a defined period. The aim of this investigation is thus to obtain more biomethane generation via the application of the pretreatments prior to digestion.

The research goal is addressed by investigating a critical point defined per preliminary literature search for each pretreatment which would exemplify the potential of each approach. The effects are then evaluated against the status quo represented by the untreated sample. Additionally, the solubilization of carbohydrates and proteins as key substrates are quantified to support the analysis further. Moreover, a general preliminary evaluation of externalities in the form of emissions based on the necessary investment and energy recovery is provided.

1.3 Research Question

The central research question (RQ) of this investigation is defined as:

"What is the effect of applying thermal degradation, alkaline-hydrolysis, and sonication pretreatment on the anaerobic digestibility of sewage sludge, and what are the recommendations towards optimizing the efficiency in biomethane generation by a controlled complex representative culture of bacteria over a defined period of time?"

It should be stated that given that this investigation is still within the screening phase of the overarching research, the project strives towards the generation of knowledge basis in favor of the business relevance under the defined time constraint. Hence, variable boundaries are selected mainly based on their contribution to the research rigor rather than a defined efficiency goal for a given treatment.

1.4 Guiding Questions

The following guide questions are defined to aid the research project's progress.

- **GQ1 - Principles of the Methods:** What are the corresponding method's fundamental chemical and physical mechanisms involved in the pretreatment of SS?
- **GQ2 -Potential Enhancements:** How does the pretreatment affect the digestibility and biogas generation? Is the added cost leading to a positive net value generation?
- **GQ3- Drawbacks:** What are the critical drawbacks of the process regarding the system's efficacy in terms of effluent quality?
- **GQ4- Externalities:** Is there welfare loss or gain associated with applying the pretreatment stage?

1.5 Project Relevance

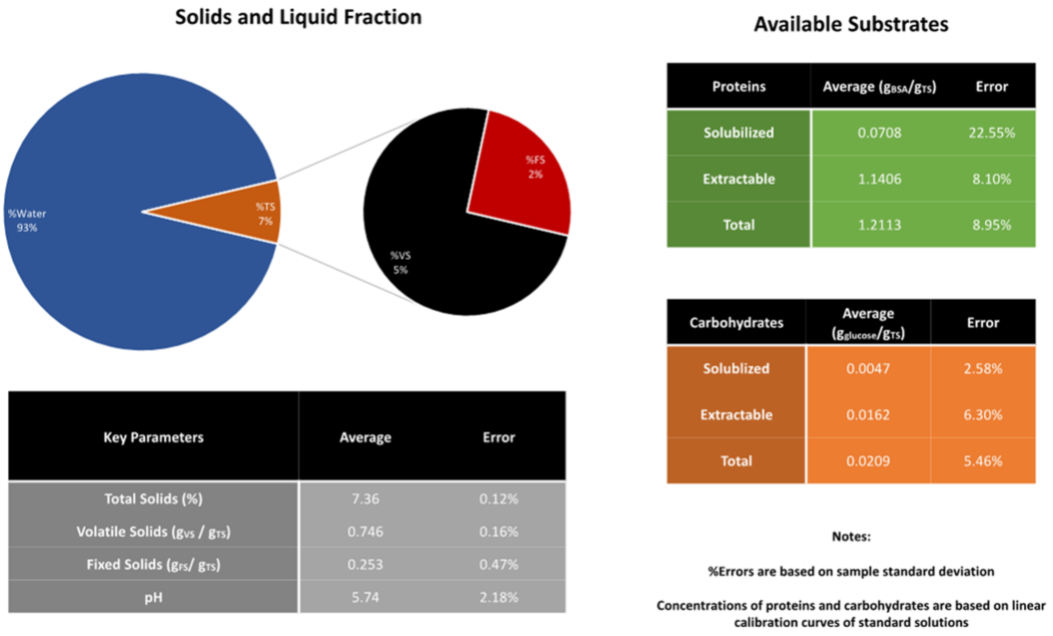
The overarching system of this research is a two-stage activated sludge plant (AB-Process) operated by Noorderzijlvest in Garmerwolde, the Netherlands. The AB process is responsible for approximately 40,000 m^3 of daily sewage treatment, from which 4 million m^3 of biogas is generated [4]. Despite the energy recovery in the form of electricity and heat from gas generators at the plant, demand for approximately 1.5 million kWh of electricity persists, which is currently outsourced. The current energy consumption rate of the plant is specified at 0.33 kWh/m^3 influent [11] [5].

Enhancement pathways for digestibility of SS can promote cost reduction, both private and in terms of externalities, via increased biomethane generation in addition to improved sludge stabilization and pathogen reduction [12].

2 Literature Review on Treatment Methods

2.1 Choice of Variables for Given Pretreatment Methods

The effect of the pretreatment method is directly dependent on the properties of the initial SS sample. Information about the untreated sludge is thus initially evaluated prior to investigating the pretreatments on the 3rd of January 2022 using the definitive versions of the protocols, as will be discussed in 3.2. This information is presented in Data Set 1. A literature review is utilized to select the definitive variables. A summary of the expected effect induced by the pretreatments and definitive experimental variables are represented in Figure 2, Figure 3 and Figure 4.



Key Parameters	Average	Error
Total Solids (%)	7.36	0.12%
Volatile Solids (g_{VS} / g_{TS})	0.746	0.16%
Fixed Solids (g_{FS}/ g_{TS})	0.253	0.47%
pH	5.74	2.18%

Proteins	Average (g_{BSA}/g_{TS})	Error
Solubilized	0.0708	22.55%
Extractable	1.1406	8.10%
Total	1.2113	8.95%

Carbohydrates	Average ($g_{glucose}/g_{TS}$)	Error
Solublized	0.0047	2.58%
Extractable	0.0162	6.30%
Total	0.0209	5.46%

Data Set 1: Information about the Solids (%TS, VS & FS) and available substrates (Proteins and Carbohydrates) in standard untreated sludge (Aerobic SS from Garmerwolde)

2.1.1 Heat Treatment (Thermal)

2.1.1.1 Principle of Thermal Degradation

The critical principle of utilizing heat as a means for pretreatment is the disintegration of SS and, therefore, an increase in the solubilization of the solids [13]. Application of heat is sub-grouped into High-Temperature Thermal (HTT) and Low-Temperature Thermal (LTT) based on whether the desired temperature falls above or below 100 °C [13]. In both cases, however, the mechanism strives to enhance the bottleneck stage of AD, hydrolysis, via disruption of gel structure and lysis [14]. The Garmerwolde plant currently operates at an atmospheric pressure condition [5]. This research focuses on LTT to avoid introducing pressure as a variable to provide comparative analysis.

The pretreatment provides a pathway for an improved digestibility of sludge as well as its dewaterability [13]; LTT in the range of 65 °C to 95 °C can provide potential pathways for the increase in the solubilization of proteins and sugars; direct linear correlation is identified between the change in the temperature and solubilization [15]. In terms of chemical oxygen demand (COD), gains of up to 20% are reported [16]. To exemplify, proteins and sugar solubilization of thermal pretreatment at 95 °C were observed to lead to an increase of up to 18.6% ± 1.8%, and 7.4% ± 1.9%, respectively, relative to untreated samples [15]. The effect of LTT is more dominant on carbohydrate solubilization than proteins. This is due to carbohydrates occupying the exopolymer of sludge, whereas proteins are located within the cells [17]. The subsequent effect of heat treatment on biogas production can accumulate to an increase of 50% relative to untreated sample as investigated in an Up-flow Anaerobic Sludge Blanket Reactor (UASB) treated at 75 °C for 7 hours [13]. For the SS investigated in this project, the problem owner has noted that they have identified mild enhancements (~4%) in biomethane generation relative to the untreated sample from a 2 hours pretreatment at 45 °C [18].

The choice of both treatment time and temperature is significant for evaluating LTT [16]. The first significant factor is the effect on proteins. The impact of treatment on the digestibility of proteins is dependent on the initial culture of proteins; this correlates with the protein's structure, size, charge, and amino acid sequence [19]. Protein denaturation occurs at temperatures of ~75°C and higher [20]; this effect can hence lead to protein precipitation. In addition to proteins, the secondary factor of interest is the complex biological structure of flocs; combined with electrostatic, ionic, and hydrogen bond effects, present the potential for an increase in non-covalent energy that can potentially lead to the occurrence of coagulation and precipitation at temperatures above 90 °C [15].

In addition, the enhancements are amplified due to the pretreatment's sanitary impact upon the effluent acquired via the destruction of pathogens [14]. Additionally, thermal treatments provide an odor removal improvement [10]. In terms of process costs, the combined effect of dewaterability and the reduction in viscosity can offer implications to practical aspects such as the filterability of the processed stream [13]. The primary drawbacks of thermal treatment methods are the need for high capital investment, the release of ammonia, and the introduction of heavy metals to the soluble phase [16][10].

2.1.1.2 Choice of Variables for Heat Treatment

Based on the information discussed earlier, the most promising boundary for evaluating the effect induced by LTT falls between 75 °C and 90 °C. Heat treatment of 2 hours at 80 °C is chosen as an indicator of LTT for this research. The choice of treatment time is primarily driven by the problem owner's plans for their next sample series whereby a similar 2-hour treatment at 80 °C is to be applied; similar conditions are utilized as one of the data points in the problem owner's future trial. Thus, a consistent treatment period allows a cross-trial reliability screening of the methods.

2.1.1.3 Specific Guiding Question: Heat Treatment

"What is the extent of thermal disintegration and substrate solubilization of sewage sludge from a 2-hour treatment at 80 °C, and how does this affect the biomethane potential?"

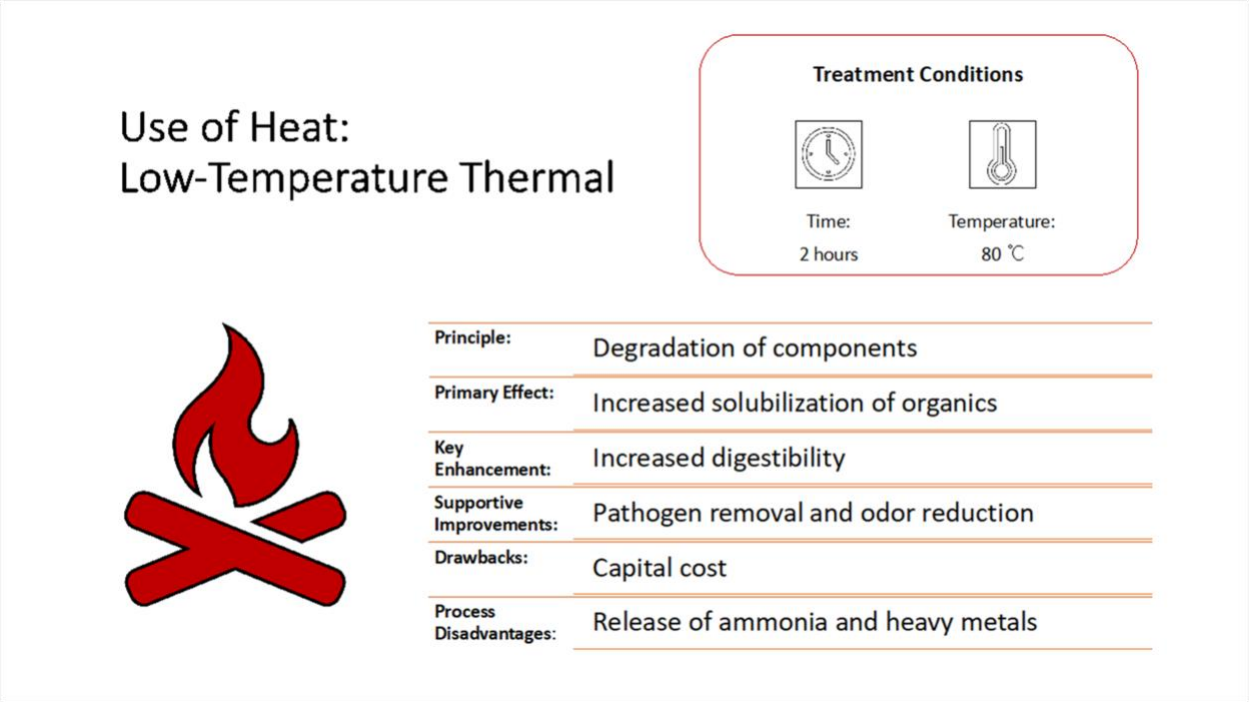


Figure 2: Summary of essential information regarding thermal pretreatment and the definitive experimental conditions

2.1.2 Sonication

2.1.2.1 Principle of Soundwaves and Sonication

Sound waves provide a mechanical route towards the destruction or deactivation of biological cells. Ultrasound offers a cyclical sound pressure in the form of compression and expansion within cavitation bubbles with frequencies of 20 kHz and higher [21].

The primary principle of ultrasonication is applying a series of compression and rarefactions; the cycles lead to positive pressure exerted upon the liquid (compression), bringing about the cells together followed by the rarefaction, with this, the negative pressure is applied, pulling molecules from one another. The immense pressure leads to the formation of cavitation bubbles which proceed to pursue growth in successive cycles until reaching an unstable, varying diameter resulting in violent collapse over a short span of a few microseconds [21]. The bubble formation process can be regarded as a competition between the liquid strength and the acoustic pressure. Under the assumption that the velocity of the sound in the sample and the overall density remain controlled, the acoustic pressure will be directly correlated with the square root of intensity. To create the cavitation bubbles, the acoustic pressure must surpass the cavitation threshold of the medium [22]. The threshold sonication intensity at the sonicator probe horn necessary to form cavitation bubbles in SS is reported similar to that of highly impure water at ~20 $\frac{W}{cm^2}$ [23].

The effect of this treatment is levied on the AD principally as a result of disintegration proceeding to spiflicates of the cell walls, leading to the facilitation of the intercellular availability of substances [21]. The effect of sonication on particle disruption is indicated to be dependent on the floc size, whereby macro-particles (>4.4 µm) were more prone to disruption than the micro-particles (<4.4 µm). Binding forces

between the flocs as well as the surface area may be the cause of this effect [22]. Aside from the hydromechanical shear force, sonication results in an increase in temperature and oxidizing effects as a consequence of the ultrasound radiations [21].

Although under a well-optimized process, sonication can initially reduce the particle size, should the process continue too long, it will introduce a re-flocculation phenomenon; released biopolymers will proceed to form hydroxyl and carboxy bounds and, in essence, will induce a glue-like effect that regathers the flocs [21]. Effective use of ultrasound can provide up to a 50% rise in the biogas generation [21]. Literature indicates that specific energy (SE) values of 50 $\frac{kJ}{kg_{TS}}$ provide an optimal balance between the disintegration and the energy investment [21]. The problem owner has already identified that a noticeable increase in biomethane generation occurs relative to untreated sludge with applied specific energy of ~31000 $\frac{kJ}{kg_{TS}}$ is and a sonication density of 3000 $\frac{W}{l}$ [18].

Regarding the substrates, the literature indicates a complex behavior for SS with high solid content whereby, solubilization increases fractionally despite the significant disintegration in the matrix [24]. Regarding proteins, high Total Solid (TS) content is identified to inhibit the effect of sonication due to a reduction in the formation of cavitation bubbles [21].

The principal drawback of sonication is its operational energy cost; the treatment process follows a complex efficiency pattern involving two critical stages of electricity to vibration and vibration to cavitation. Given the current estimations of 30% - 40% electrical production efficiencies of generators in low-frequency ultrasound, the pretreatment proposes an increased burden due to a reduced rate of applied efficiency. It hence results in a net negative operational energy balance in SS with high solid concentrations (>4%) [20]. Lastly, in terms of costs, although beyond the scope of this research project, it is recommended that the process shall evaluate the maintenance costs due to the erosions of the sonotrode over time [24].

2.1.2.2 Choice of Variables for Sonication

Given that the SS investigated in this research contains a 7.3% TS fraction, the research deems a standard single-stage sonication applied uniformly potentially unlikely to provide a net gain regarding the invested energy. Considering this factor, the independent variable conditions are altered towards identifying the effect of sonication on the degradation and solubilization of nutrients present. In other words, sonication is treated as a potential initial stage for another follow-up treatment.

The available Sonicator in the laboratory facilities of Nijenborgh 4 is that of Sonics and Material's inc.'s VC 300, which, ideally, generates a total power input of 300 Watts at the frequency of 20 kHz. The sonotrode can generate intensities of ~2800 $\frac{W}{cm^2}$ at the probe horn, surpassing the cavitation threshold. It must be noted that sonication can potentially lead to the solubilization of heavy metals. Literature notes that sonication density of 1200 $\frac{W}{l}$ for a duration of 30 minutes can lead to the increased solubilized heavy metal content of up to 7%, with a minimum density of 800 $\frac{W}{l}$ is identified in the literature for heavy metal solubilization [25]. To maximize the total available substrates, sonication of 40 minutes (with 50% pulses) at a reduced overall density of ~300 $\frac{W}{L}$ is applied; This simulates a lengthy sonication duration at a low intensity which remains below the threshold for the interference of heavy metal solubilization.

The investigation expects that a lengthy high, intensity sonication will indicate the upper value of the maximum available substrates. However, simultaneously, biomethane production may be inhibited due to the re-flocculation, as mentioned earlier.

2.1.2.3 Specific Guiding Question: Sonication

"What is the maximum substrate availability of the available sewage sludge that can be provided from a sonication treatment, and to what extent does the re-flocculation inhibit the access to these substrates?"

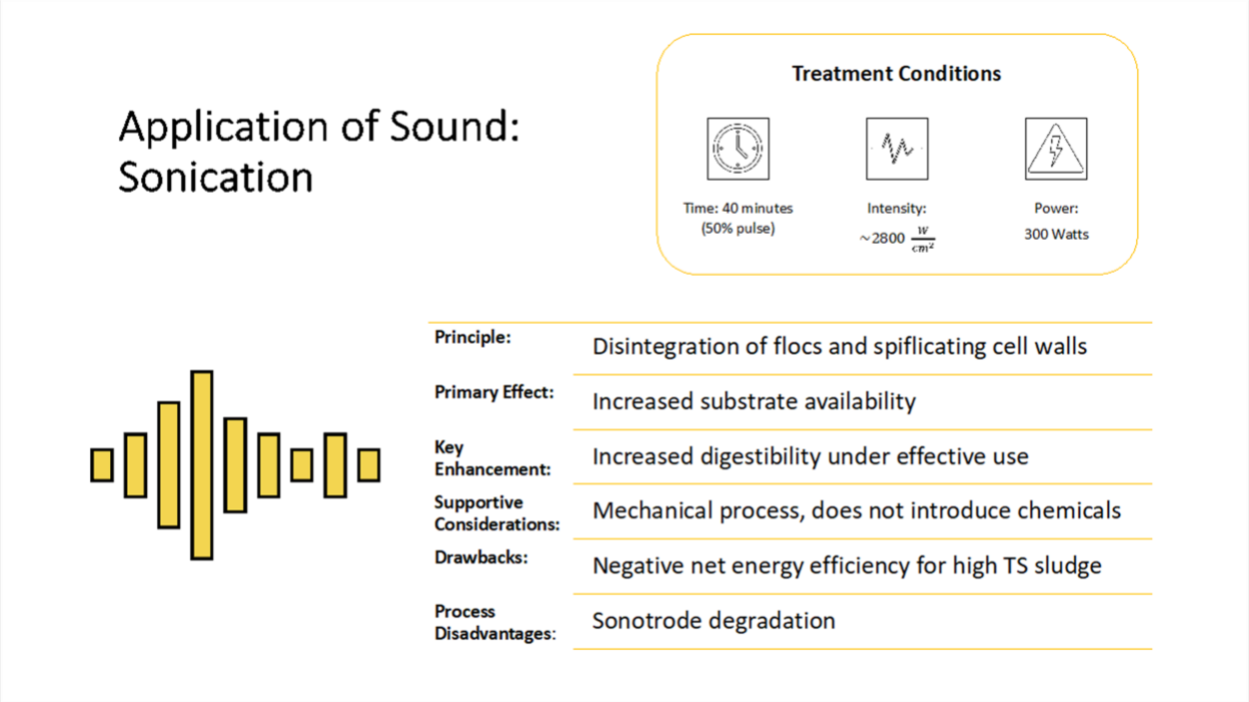


Figure 3: Summary of essential information regarding sonication pretreatment and the definitive experimental conditions

2.1.3 Alkaline-Hydrolysis

2.1.3.1 Principle of Physio-Chemical Treatment

Application of chemical pretreatment using acidic or basic reagent results in increased substrate availability and improved digestibility. Acidic compounds are more effective for lignocellulosic material, whereas alkali treatment favors the lignin breakdown [10]. For the system at hand, alkaline-hydrolysis is chosen as the primary route of investigation due to the increased buffer capacity that the base treatment induces on the digested sludge being favored in the industry [10].

Application of Alkali treatment is a relatively effective approach towards increased sludge solubilization. Common treatments include, in order of highest efficacy first, the use of sodium hydroxide (NaOH), potassium hydroxide (KOH), and magnesium hydroxide ($Mg(OH)_2$). The overall sludge solubility

proceeds to increase relative to the given dose of alkaline and treatment temperature conditions; The conditions, however, are bound with an upper limit such as too high concentrations of sodium (Na^+) and potassium (K^+), inhibiting the process [26].

The principal mechanisms involved in the Alkalinization of sludge are that of Alkali-Hydrolysis. The processes provide for a breakdown route via solvation and saponification, which impose depolymerization and cleavage of linkages; This results in the previously uneasy to digest substrates to be available to the digestive enzymes [10].

Alkali treatment methods are often combined with thermal treatment due to the reduced overall requirement for chemicals and energy. Solid disintegration values of up to 75.6% relative to an untreated sample of sludge are observed in literature for the combined alkalinization and low temperature (<100 °C) heat treatment [27].

Besides the direct effect from the alkaline hydrolysis, a supportive effect is further induced by introducing a negative charge on the cell surface because of increased pH. The resulting electrostatic repulsion can further lead to the desorption of extracellular polymers. However, it must be noted that too high of alkalinity after pretreatment can result in reduced cellular viability due to the harsh impact of the environment leading to the inability of organisms to retain their appropriate turgor pressure [28].

Alkalization by sodium hydroxide (NaOH) can potentially result in up to 40 times increase in proteins and 41 times increase in the carbohydrate solubilization under treatment conditions of up to 720 hours and sample pH of 12; it must, however, be noted that the most considerable impact is achieved after 0.25 hours [29]. The increased presence of nitrogen ammonia (NH_3-N) by factors of 45.9% - 62.4% is a further indication of protein decomposition due to the alkali treatment [30].

During the alkali treatment stage, the biomass present may consume some of the added bases. Furthermore, degradation of the substances can contribute to the formation of acidic compounds. This decreases the sample's pH after applying the treatment process relative to the initial alkalinity induced [31]. Alkalization of sludge can also lead to potential enhancement in the quality of effluent, since pathogens such as *Escherichia coli* and viable helminth eggs are destroyed [32].

Furthermore, studies on waste-activated sludge have indicated that the settling and dewaterability are affected in high alkaline hydrolysis treatment. The primary cause suggested is the induced ion-exchange whereby cations are displaced from the flocs by the charge gradient created by sodium ions (Na^+). Moreover, when combined with heat, the effect is amplified due to the hydration of proteins, carbohydrates, and other intracellular macromolecules leading to an increased overall free water content of the sample [28].

Significant drawbacks of applying alkaline pretreatment are the combined cost of chemical input and the addition of inorganics to the output stream [20]. The issue of salts introduced is of a complex nature; The alkali is often converted into irrecoverable salts that can be incorporated within the biomass. [33]. Furthermore, the risk of recalcitrant compounds formation is present in this method [10]. The introduction of the residual chemicals can induce lignin structure alteration and microbial inhibition of AD [32].

2.1.3.2 Choice of Variables for Alkaline-Hydrolysis

A sample of 95% SS is mixed with 5% 2N NaOH overnight (12 hours) in refrigerated conditions to provide complete mixing representation. This amount of base is expected to provide an initial pH alteration of >12, given the acidic pH conditions of SS being already identified at approximately 5.7. Thermal treatment is followed using similar parameters of 2 hours of heating post-startup at 80 °C. Literature indicates that despite the enhancements to the hydrolysis stage, concentrations of 3.5 – 5 $\frac{g}{l}$ Na^+ results in mild inhibition of mesophilic methanogens, whereas concentrations 8 $\frac{g}{l}$ and higher lead to potent inhibition [34].

The critical investigation point for this treatment is whether the net gain in digestibility surpasses the added cost of employing a chemical treatment or not. Given that the dosage of NaOH is about 5 $\frac{g}{l}$, the investigation expects that the influence of Na^+ will be at 2.6 $\frac{g}{l}$ placing it below the lower range of mild inhibition. A mildly delayed startup is hence potentially anticipated.

2.1.3.3 Guiding Question for Low-Temperature Alkaline-Hydrolysis Treatment

"Does the addition of alkaline (NaOH) sufficiently enhance the overall gains from the digestion process to justify the higher investment in operating costs as well as the levied externalities?"

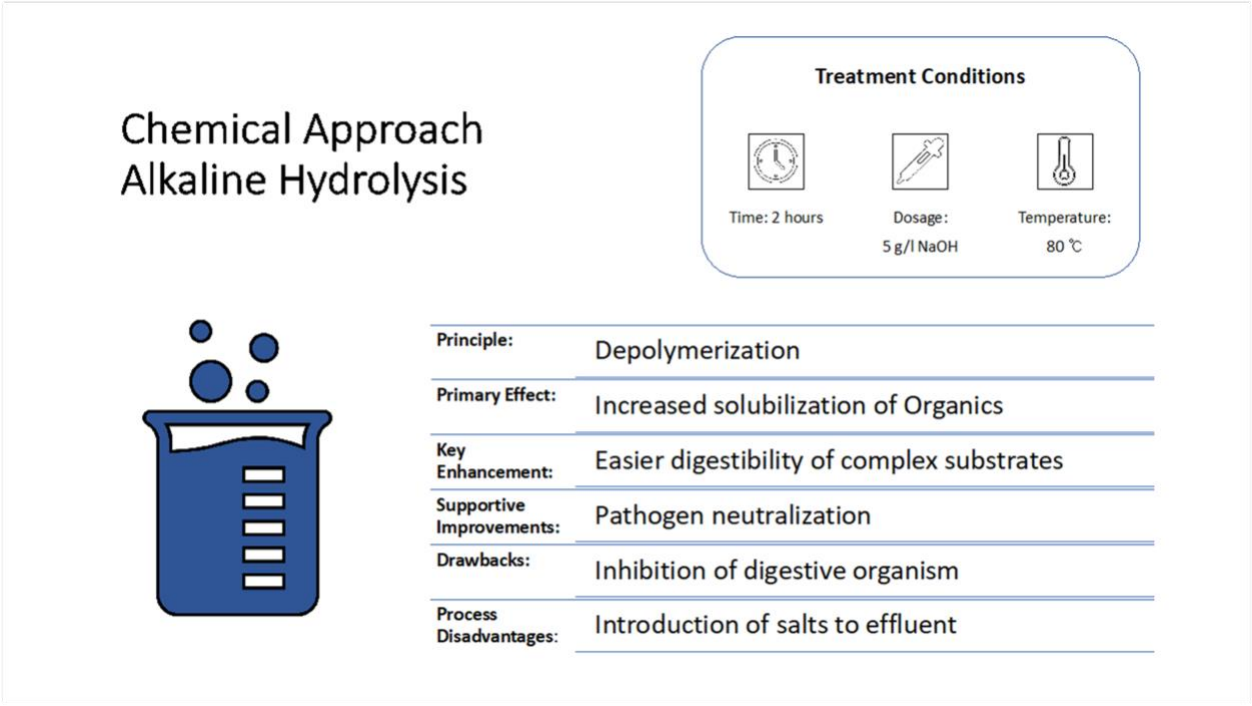


Figure 4: Summary of critical information regarding Alkaline-Hydrolysis pretreatment and the definitive experimental conditions

3 Experimental Methodology and Apparatus

3.1 Biomass Inoculation and Sample Preparation

The experimental setup follows a sequence of two stages: inoculation of the bacteria culture and pretreatment study.

3.1.1 Inoculation of Biomass

In the first phase, a standard biomass culture is anaerobically inoculated for a period of 9 days from 7 vessels containing ~300g of *"anaerobic sludge"* collected in April 2021 from the Garmerwolde treatment plant. Environmental temperature is controlled at 37 °C with mixing retained at 150 RPM incubation conditions. Quantifications for biomethane generation, solids, and solubilized substrates are performed per the standard protocol defined in section 3.2. A summary of the critical properties of the inoculations is represented in Data Set 2

Biomethane Generation of the Inoculations

Variable	Average	Error (%)
Total Biomethane Production (ml $gVSi^{-1}$)	374.75	1.83%
Biomethane Production Rate (ml $gVSi^{-1}$ h^{-1})	0.1742	15.99%
Initial Volatile Solids (g_{VS}/g_{TS})	0.6569	5.62%
Final Volatile Solids (g_{VS}/g_{TS})	0.6122	1.32%

Solid Contents of Biomass

Variable	Average	Error
Total Solids (%TS)	4.68%	0.48%
Volatile Solids (g_{VS}/g_{TS})	0.6122	1.32%
Fixed Solids (g_{FS}/g_{TS})	0.3878	2.08%

Available Substrates in Biomass

Proteins (g_{BSA}/g_{TS})		Error
Soluablized	0.0530	14.07%
Extractable	0.5697	7.01%
Total	0.6228	7.61%

Carbohydrates ($g_{glucose}/g_{TS}$)		Error (%)
Soluablized	0.0068	8.25%
Extractable	0.0112	6.38%
Total	0.0180	7.09%

Data Set 2: Key parameters of the inoculated biomass utilized to evaluate pretreatments.

3.1.2 Pretreatment Study

Pretreatments samples were prepared over two days, starting with the thermal pretreatment, with prepared samples being stored in refrigerated conditions. Approximately 900 ml of each treatment is prepared as per the variables defined. Untreated aerobic sludge is employed as the standard reference to compare the effect induced by the given treatment of concern. In the pretreatment study, for the evaluation of biomethane

potential, 50 g of biomass is introduced to 150 g of sample sludge and thereby inoculated in the incubator under the same conditions of 150 RPM at 37 °C for a total duration of 15 days. Substrates and solids are evaluated on day 0 and after 15 days of inoculation.

3.2 Standard Methods of Quantification

Quantification methods employed in this study are sludge digestibility, assessed by quantifying the biomethane potential; available substrates evaluated via solids and solubilized organics; and lastly, energy investment evaluated via a driven cost model. These methods are discussed in this subsection.

3.2.1 Biomethane Potential

To evaluate the biomethane potential of the flasks, the generated biogas from the batch flask in the incubator over the entire 15-day period is continuously purified via the application of a chemical absorption step into biomethane. The outgoing biomethane stream proceeds to a water displacement step where the resulting change in displacement is utilized to quantify biomethane generation and hence the digestion in the batch flask. The total generated biomethane value is evaluated against the total initially available volatile solids and specific energy applied per treatment. The general scheme of the setup is illustrated in Figure 5.

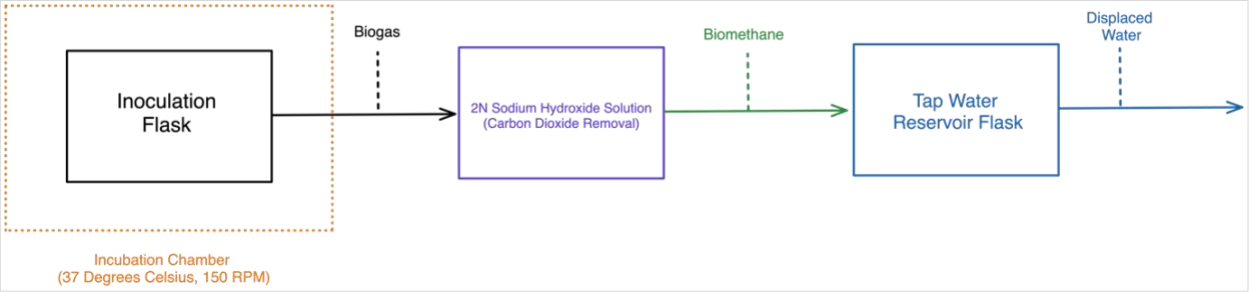


Figure 5: Key stages of the apparatus applied in the laboratory study of the biomethane production from the sample of concern. In the setup, the digestion takes place in the inoculation flask, and the subsequently produced biogas is enriched, providing a biomethane stream that is evaluated via water displacement in the tap water reservoir flask.

3.2.2 Solids (TS, VS, and FS)

The critical values regarding solids are defined as:

- **Total Solids (TS):** evaluated as the material remaining after evaporation; this is performed via oven drying at 105 °C overnight (12 hours) [35].
- **Volatile Solids (VS):** the mass of solids within the sample that is lost after the furnace at the temperature of 575 °C for >2 hours [35]. VS is used as an estimator of total available organic content.
- **Fixed Solids (FS):** Represent the remaining sample after the furnace [35]. Used as an indicator of total salt (inorganics).

3.2.3 Solubilized Substrates

For solubilized substrates, proteins and carbohydrates are considered. Proteins are quantified by the Lowry method at 550 nm in using Bovine Serum Albumin (BSA) as the reference protein. The method employs the Biuret combined with Folin-Ciocalteau reactions under a basic environment providing the resulting Heteropoly molybdenum Blue [32]. To achieve this quantification, 32 µl of hydrolyzed samples is mixed with 80 µl of 0.2 N Folin-Ciocalteau reagent and 96 µl of freshly prepared complex-forming reagent composing of 98.5 $\frac{g}{l}$ sodium carbonate (Na_2CO_3), 0.315 $\frac{g}{l}$ copper sulfate ($CuSO_4$) and 1.34 $\frac{g}{l}$ sodium potassium tartrate tetrahydrate ($KNaC_4H_4O_6 \cdot 4H_2O$) in an equal 1:1:1 volumetric ratio. Spectrophotometric quantification is carried out after a 30-minute reaction time [36].

Carbohydrates are quantified via the application of the Anthrone method at 630 nm in terms of glucose equivalent. Carbohydrates are available in various forms ranging from free sugars to more complex polysaccharides. Anthrone method proceeds towards breaking down the carbohydrates into simple sugars via acidic hydrolysis; Thereby, within a heated acidic environment, the monosaccharide glucose is dehydrated into hydroxymethylfurfural ($C_6H_6O_3$). The reaction of the product, as mentioned earlier with Anthrone ($C_{14}H_{10}O$), provides the green product with an absorption maxima of 630 nm (Orange) [37]. In this quantification approach, 300 µl of 0.1% freshly prepared Anthrone solution are mixed with 100 µl of sample; the mixture is thereby briefly vortexed and is hence heated at 100 °C for 5 minutes. After the heating, the sample is cooled in an ice bath for 5 minutes. Lastly, absorption of 200 µl of processed sample is evaluated 630 nm using spectrophotometer [36].

The concentrations are taken from 10 times diluted samples. The concentration for the liquid fraction is taken from the suspension remaining after 12 ml of the diluted sample is centrifuged at 15000 RPM for 20 minutes at refrigerated temperature. The supernatant is evaluated for the quantification of the solubilized fraction. The solubilized substrates represent the proteins and carbohydrates that are readily available in the supernatant without any extraction procedure.

Using the application of an intense ultrasonic treatment, remaining proteins and carbohydrates are solubilized; this fraction is an indicator for the proteins and carbohydrates that were not sufficiently solubilized by the pretreatment but are theoretically available. The remaining solid fraction is thereby evaluated using a modified protocol. Substrates are suspended in 1 N sodium hydroxide (NaOH) solution and ultrasonicated at 20 kHz for 16 minutes with a 50% pulse rate. The processed sample is then centrifuged at 15000 RPM for 20 minutes at refrigerated temperature (operating condition of the machine is set at 4 °C). The process is repeated once to detect the full spectrum of the available substrates. The concentrations of substrates are evaluated based on the resulting liquid fraction [38].

3.2.4 Complex Energy Input Model

Two types of energy are employed in this project: Thermal and Electrical. For comparative purposes, all costs are evaluated in terms of opportunity cost equivalent to electricity. Additionally, chemical costs are also evaluated in terms of electrical energy equivalent. Externalities are evaluated as CO_2 emission equivalent of gas generator reported at 0.41277 $\frac{kg_{CO2}}{kWh}$ [39].

To summarize and further clarify, the total equivalent kWh of electricity is considered as the private cost for the operation, while the equivalent value of emission of CO_2 to generate the given quantity of electricity is used as an indirect measure of externalities.

3.2.4.1 Thermal Energy Model

The thermal energy input for heat and alkaline hydrolysis is applied via exposure of the samples to a heating bath set at the desired temperature of 80 °C. During the startup phase, the total energy necessary to heat the sample is evaluated via a specific heat model using heat capacity (Cp) of water at 4184 $\frac{J}{kg*K}$. The maintenance energy is evaluated as heat loss through free convection to ambient air of 25 °C at a rate of 25 $\frac{W}{m^2*K}$; the surface temperature of the sample is assumed at a uniform 80 °C with a total cylindrical area evaluated using the base diameter of the flask. Specific Energy (SE) is thereby evaluated based on the initially available TS. The summary of equations is represented in Equation Set 1.

Thermal Energy Model

$$Heat\ Up\ Energy\ (Q_s) = Sample\ Mass\ (m) * Heat\ Capacity\ (Cp) * Temperature\ Change\ (\Delta T)$$

$$Maintainance\ Energy\ (Q_m) = Heat\ Loss\ Rate\ to\ Air\ (H_{air}) * Area\ (A) * Temperature\ Difference\ \ (\Delta T) * Time\ (t)$$

$$Specific\ Thermal\ Energy\ (SE_{th}) = \frac{Total\ Energy\ Input\ (Q_s + Q_m)}{Initially\ Available\ Total\ Solids\ (TS_0)}$$

Equation Set 1: Energy model of the heat input for Thermal and Alkaline-Hydrolysis pretreatments

The electrical and thermal efficiencies of the generators that use gas to produce electricity are reported as 40% and 50%, respectively [40]. Thus, each kWh of thermal energy is equivalent to the opportunity cost of 0.8 kWh of electricity.

3.2.4.2 Electrical Energy Input of Sonicator

The energy consumption of the Sonicator can be evaluated in terms of the specific supplied energy. The value generated is dependent on the equipment power (P_s), the volume of sample (V_s), the initial total solids (TS_0) content, and the total sonication time (t) [24]. The relationship is represented in Equation Set 2.

Sonicator Electrical Energy Input Model:

$$Specific\ Electrical\ Energy\ of\ Sonication\ (SE_{so}) = \frac{Power\ (P) * Sonication\ Time\ (t)}{Sample\ Volume\ (Vs) * Initially\ Available\ Total\ Solids\ (TS_0)}$$

Equation Set 2: Energy model of Sonicator

3.2.4.3 Chemical Costs in Terms of Energy Equivalent

European cost of 0.42 $\frac{€}{kg}$ is taken for NaOH [40]. As of October 2021, the European average industrial electricity price of 0.1283 $\frac{€}{kWh}$ is reported [41]. 1 kg of NaOH is thus defined as 3.2736 kWh of electricity equivalent in terms of opportunity cost.

3.2.4.4 Energy Recovery from Generated Methane

The theoretical higher heating value (HHV) of 37.78 $\frac{MJ}{m^3}$ for methane is considered as the total energy potential of biomethane. The same 40% and 50% for electrical and thermal generation efficiency are employed to calculate the total energy. 0.8 kWh electricity equivalent to 1 kWh of thermal heat under the opportunity cost model is taken to compare the net values. The energy recovery also mitigates the need for gas used for electricity generation, so emissions are also avoided. The amount of CO_2 emission avoided due to reuse of generated biomethane is evaluated using the similar value of 0.41277 $\frac{kg_{CO_2}}{kWh}$ based on the recovered electrical energy.

3.3 Theoretical Biomethane Potential

Under the assumption that VS value reflects the maximum digestible organic matter available for AD, a total theoretical yield of biomethane can be estimated based on the maximum yields driven from stoichiometric formulas of carbohydrates, proteins, and lipids which are reported as 0.415, 0.496, and 1.014 $\frac{L}{gVS}$ respectively.

SS organic matter distribution accounts for approximately 20% - 40% carbohydrates, 30% - 50% proteins, and less than 10% lipids which within the assumptions of this project constitutes up to 80% ± 7% of the %VS [15].

Maximum biomethane generation potential of SS is achieved applying the total initially available VS using the relationship represented in Equation Set 3.

$$Maximum\ Biomethane\ Potential = 87\% * VS_0 * \left(M_{carbs} + M_{proteins} + M_{lipid}\right)$$

$$With:$$

$$M_{carbs} = 40\% * 0.415 \frac{L}{gVS}$$

$$M_{Proteins} = 50\% * 0.496 \frac{L}{gVS}$$

$$M_{Lipids} = 10\% * 1.014 \frac{L}{gVS}$$

Equation Set 3: Calculation of maximum theoretical biomethane generation potential

4 Experimental Results

Triplicates of each pretreatment are prepared and transferred to the corresponding flasks. Additionally, an untreated sample is used as the reference standard. One of the thermal flasks was deactivated due to the reflux of NaOH solution. It is suspected that this has been caused by siphoning the solution due to the height difference. Accumulated biomethane production of the samples over 15 days is represented in Figure 6. The effect of each pretreatment on the carbohydrates and proteins is represented in Figure 7 and

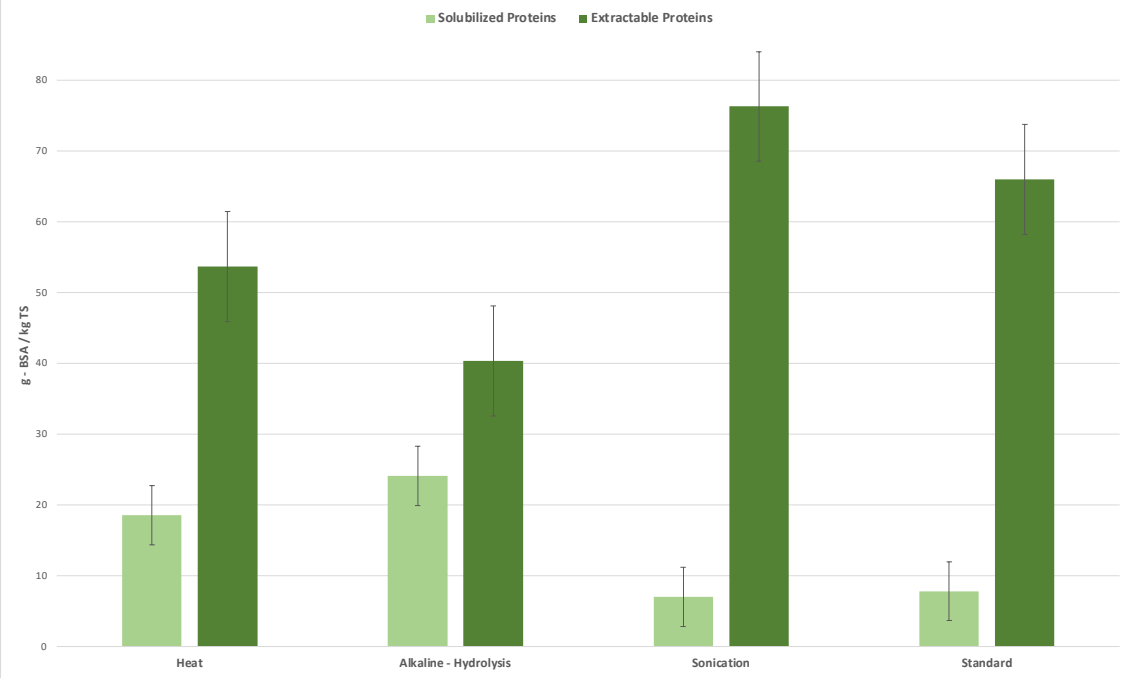


Figure 8 respectively. Changes in the solid contents are reflected in Table 1. Energy investment and emission costs are represented in Table 2.

4.1 Primary Variable: Biomethane Potential

Based on the theoretical maximum biomethane production potential as discussed in section 3.3, the available SS is in principle capable of up to 0.308 m^3 of biomethane generation based on the available VS. Experimental results of 15 days of incubation period indicate that only 9.6% of the potential theoretical biomethane generation for the standard untreated sample and the sonicated pretreatment series are achieved. Both samples are likely still in their startup period. On the other hand, heat and alkaline hydrolysis have reached 16.27% and 30.8% of the theoretical potential during the same time constraints.

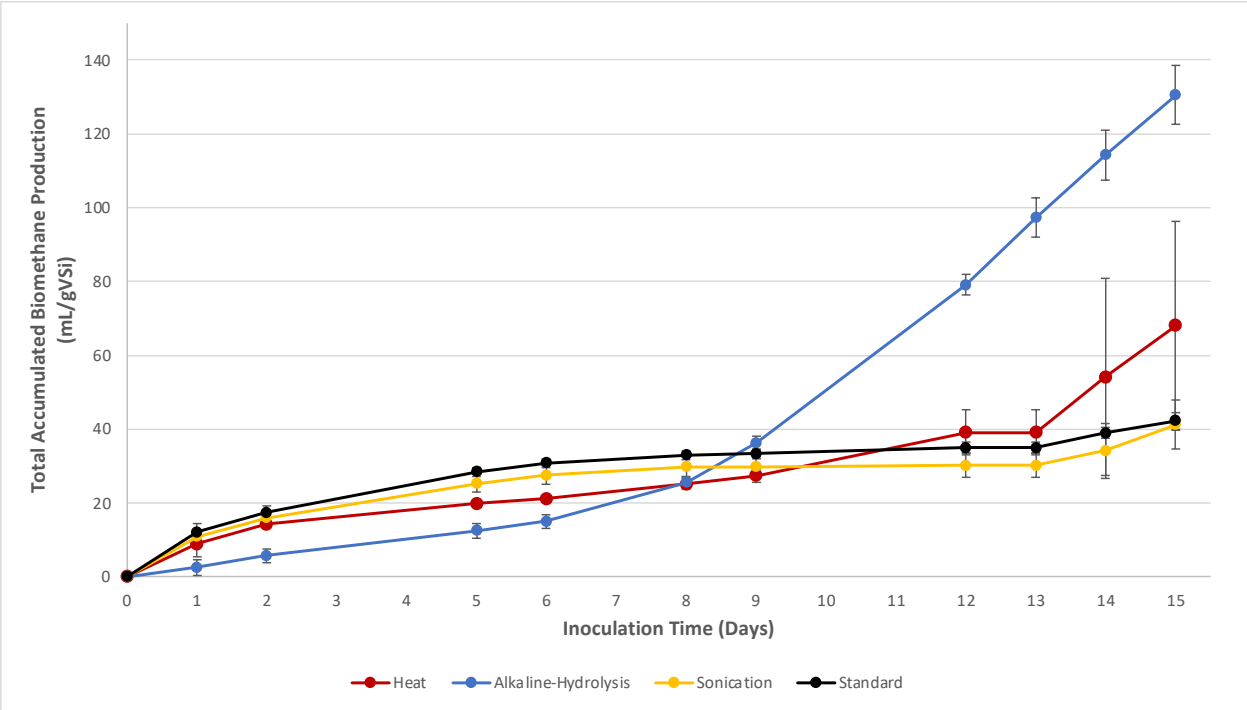


Figure 6: Accumulative biomethane production of pretreatment samples and standard over the 15-day inoculation period

4.2 Supportive Quantifications: Substrates and Solids

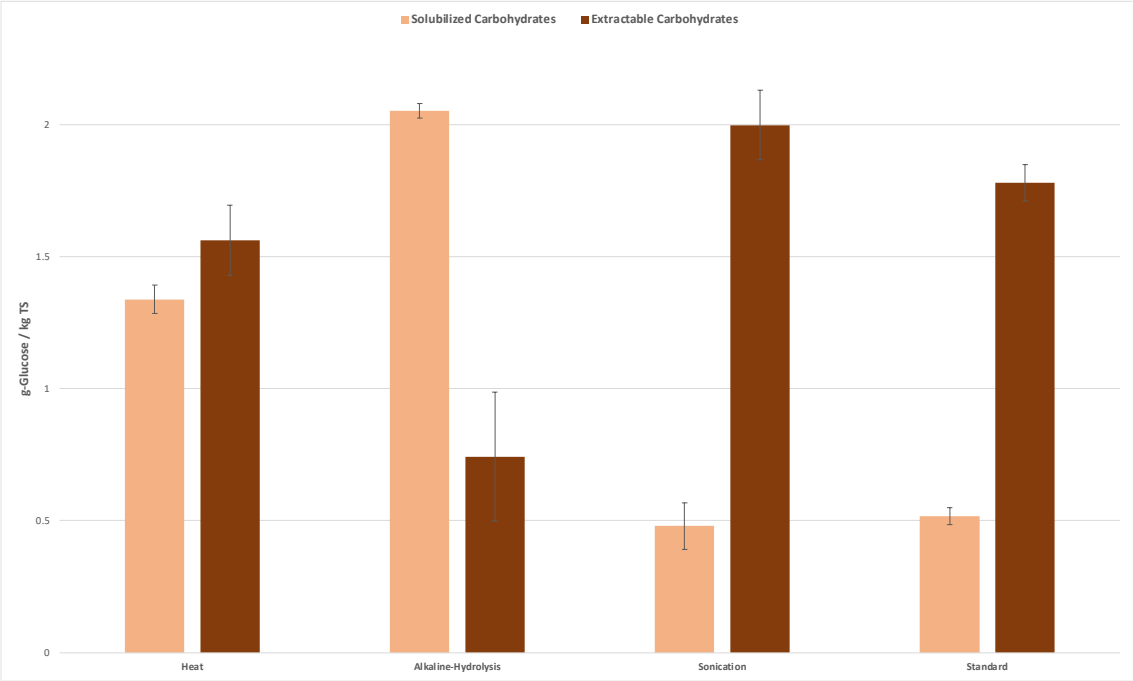


Figure 7: Effect of pretreatments on the availability of solubilized and extractable carbohydrates

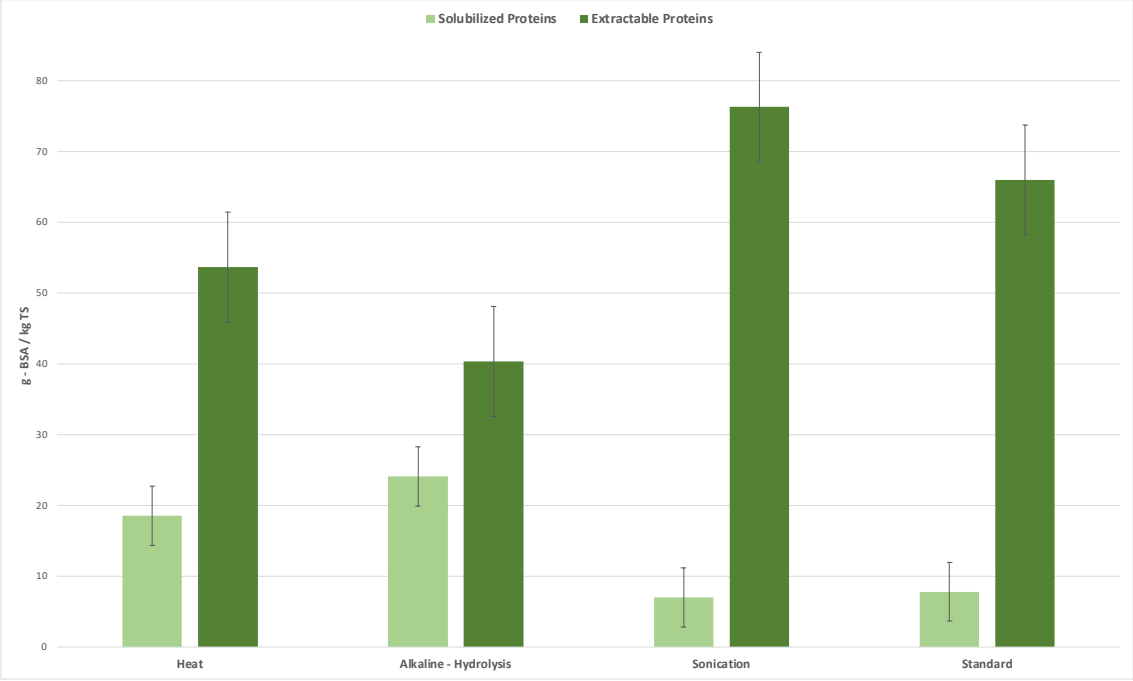


Figure 8: Impact induced by the pretreatment methods on the solubilized and extractable proteins

Table 1: Effect of pretreatments on the solid fraction of the samples

Pretreatment	Initial			Final			VS Reduction %
	TS	VS	FS	TS	VS	FS	
Heat	6.83%	72.32%	27.68%	6.15%	69.82%	30.18%	2.50%
Alkaline-Hydrolysis	6.67%	71.06%	28.94%	5.62%	68.24%	31.76%	2.81%
Sonication	6.67%	69.93%	30.07%	6.02%	62.55%	37.45%	7.39%
Standard	6.68%	68.53%	31.47%	6.60%	65.47%	34.53%	3.06%

Table 2: Energy balance and externalities evaluation of heat, and alkaline-hydrolysis pretreatments over the inoculation span of 15 days

Treatment	Invested SE (kWh/kg_{TS})	CO_2 Emission (kg_{CO_2}/kg_{TS})	Energy Recovery ($kWh_{Electricity}$ / kg_{TS})	CO_2 Avoided via Recovery (kg_{CO_2}/kg_{TS})	Net Energy Balance ($kWh_{Electricity}$ / kg_{TS})	Net CO_2 Balance (kg_{CO_2}/kg_{TS})
Heat	0.30	0.12	0.17	0.07	-0.13	-0.05
Alkaline - Hydrolysis	0.56	0.23	0.55	0.23	-0.02	-0.01

The substrates are observed to exhibit two sets of general behavior: General reduction in solubilized fraction for the heat and alkaline hydrolysis and solubilization of extractable fraction for the sonication pretreatment.

After 15 days of incubation, the solubilized proteins for heat and alkaline hydrolysis pretreatments are reduced by 48.17% ± 5.58% and 62.62% ± 6.48% respectively. Similarly, the solubilized carbohydrates are decreased by 40.42% ± 4.29 % and 54.13% ± 6.82% for the two mentioned pretreatments. Heat pretreatment indicated a significant reduction of 34.59% ± 5.26% in the available extractable proteins and 37.19 % ± 4.37% in extractable carbohydrates. Alkaline hydrolysis also suggested a similar trend of 20.56% ± 2.61% and 31.79% ± 6.53% reduction in extractable proteins and carbohydrates, respectively.

Sonication and standard had similar behavior over the 15 days of the incubation period. Solubilized proteins increased by 54.18% ± 6.15% for sonication and 33.3% ± 10.05% for untreated standard reference samples. A similar effect is observed for carbohydrates, whereby a significant increase in the solubilized fraction of 77.38% ±12.97% is observed for sonication samples. The standard sample also indicated increased solubilization of 58.26% ± 5.3%.

The extractable fraction of the proteins for both standard and sonication samples followed similar reductions of 33.91% ± 4.68% and 33.72% ± 8.98% respectively. This trend is observed for the carbohydrates of the standards, whereby a reduction of 19.39% ± 3.48% is observed in the extractable fraction. The sonication pretreatment indicated a complex behavior regarding the extractable fraction of carbohydrates whereby only a minor reduction of 3.18% ± 0.85% is exhibited.

The pH of the samples is roughly evaluated using pH paper indicators after 15 days of the incubation period. A mildly acidic pH of ~6 for sonication and standard is observed; it is thus presumed that the pH is unaffected by the sonication treatment. Alkaline hydrolysis indicated a weakly basic environment of ~8; given the initial pH of 12, this points out that the flask has neutralized most of the alkali during the digestion. Lastly, heat pretreatment indicated a mildly alkaline pH environment of ~7 – 8, indicating an increase in alkalinity of the environment over the digestion process.

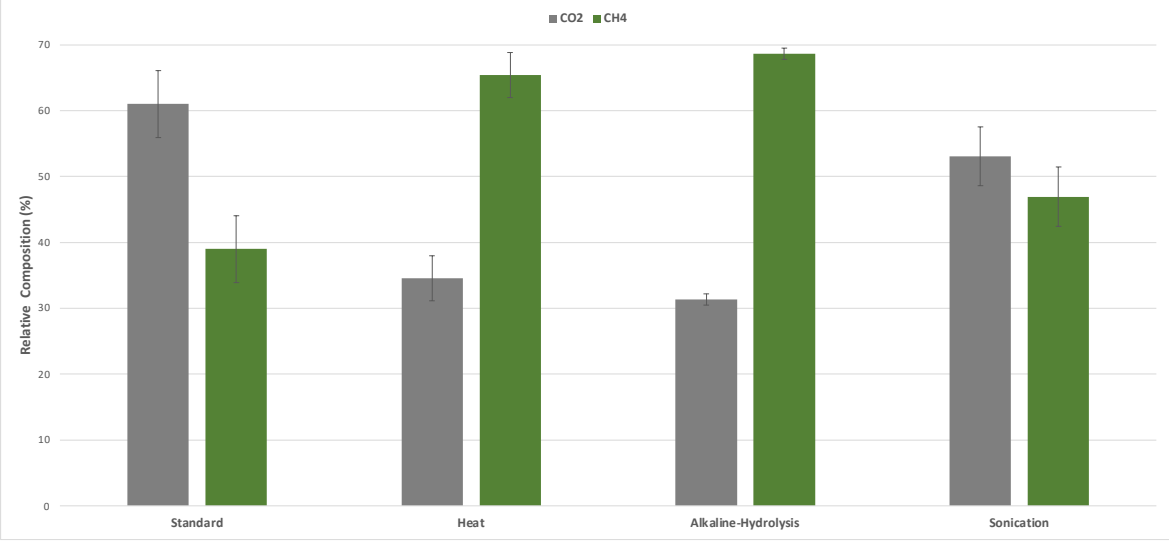


Figure 9: Relative biogas composition of CH_4 and CO_2 on day 15 of incubation in the output of the incubation flask

Lastly, the biogas composition of the flasks has been evaluated on the final day of inoculation by Gas Chromatography (GC) protocol developed and provided by Mr. G. Hofstede from the Hanze University of Applied Sciences. This information is illustrated in
Figure 9.

5 Discussion and Evaluation

This research project strived to address the research question by investigating and analyzing the three pretreatment methods and evaluating their effect on the AD of SS. The experimental analysis results, combined with the literature information, are hereby discussed for each method of interest. This is followed by evaluating the method and implications for the overarching research.

5.1 Primary Implications of the Pretreatments

Each pretreatment method that has been investigated provided valuable information and implications towards future research. In terms of the central goal of enhancing the AD process, heat and alkaline hydrolysis pretreatments indicated that within a short startup period, they can potentially provide a breakeven level of cost recovery while significantly enhancing digestibility. Sonication exhibited more complex behavior.

5.1.1 Heat Pretreatment: Potentially Promising Approach Towards Enhancing AD

Low-temperature thermal hydrolysis of the SS proved a potentially promising approach in enhancing the AD process. The results from the experimental study indicate a considerable improvement in the SS digestibility via low energy investment costs of ~0.3 $\frac{kWh}{kg_{TS}}$. Solubilization of substrates is also observed to increase. However, it should be noted that high variability in the biogas production does indicate a possibility for a lower reliability factor of this method necessitating further research on the consistency of the pretreatment.

The startup period of heat pretreatment is observed to be enhanced the most within the scope of this research. This may be as a result of minimized inhibition via sulfides [42]. In addition to the enhancement to the digestibility, it is reported in the literature and further qualitatively observed that the treatment reduces the viscosity of the treated stream [42]. The enhancement is due to reduced free water, denaturation and destruction of biopolymers, interactions between the compounds, and decreased particle size [42]. This may further enhance the energy efficiency of the process due to the direct relationship between pumping power and viscosity.

The most prominent issue that was presented by the heat treatment is that of sample expansion. SS utilized in the experiment are from the aerobic stream. To assure maximum dispersion of air bubbles, wastewater treatment processes employ aeration processes in the activated tank; In this process, oxygen is continuously fed to the aeration basin [43]. Although not quantified, the gas fraction imposed a significant potential for expansion. Various parameters affect the bubble formation process in SS; critically, the surface tension and buoyancy [43]. The sludge influent is thickened using mechanical and gravitational thickeners prior to entry to the digester. Thickening processes were employed to increase the TS content are increased in the aim of reducing the volume while retaining the liquid-like properties of the stream [44]. The research project proposes interaction and communication with the sludge provider on the parameters of the thickened SS to

evaluate the issue of expansion. It is proposed that reducing the trapped bubbles via potential alterations in the thickener may suffice in addressing this issue.

Lastly, it should be noted that considerations regarding ammonia are needed. Increased protein solubilization and breakdown after that leads to the release of ammonia and henceforth an increase in alkalinity of the stream [42]. The introduction of free ammonia (FA) may introduce additional inhibition routes to the process. FA is capable of diffusion into cells causing ionization forming ammonium. The resulting reaction leads to alteration and imbalance in the intracellular pH [42]. The effect of this inhibitory mechanism is dependent on the archaea and concentration. Methods such as pH neutralization can be employed as mitigation tactics [42]. Further studies on the total and free nitrogen are recommended to evaluate this possible inhibitory route.

5.1.2 Sonication: Complex Principle Requiring Further Research

Ultrasonic pretreatment proved to be a complex method. Results indicate that the initial solubilization of substrates is similar to the standard untreated sample. After the incubation period, the extractable fraction of substrates is barely reduced, while the solubilized portion is significantly higher than the standard. However, the extractable fraction of proteins and carbohydrates are both increased by >10%. Additionally, the biomethane production trend and composition being shifted to methane compared to the standard also supports the development of the trajectory.

Concrete analysis of the data is unfortunately complex as the information available is limited. Literature review on the matter suggests that this effect may be due to the re-flocculation effect of lengthy ultrasonication, which has inhibited the extraction and solubilization of substrates [21].

It should further be noted that, based on observations, two potential causes are suspected in this regard to be present in the methodology of this research. Firstly, the construct validity of the methods is potentially insufficient concerning quantified substrates; it is thus proposed that more than two rounds of extraction may be necessary to quantify the extractable portion fully, and hence the values reported may not be reflective of the actual total available substrates for extraction. Additionally, a more extended inoculation period is deemed necessary to evaluate the trajectory regarding the biomethane potential.

Another aspect for consideration is the low dosage of ultrasonication. Although the intensity has been above the cavitation threshold, it may be argued that the low dosage provided weak ultrasonication. Weak ultrasonic treatment has previously been identified as unable to dissolve insoluble substrates effectively. However, the treatment's enhancement of the hydrolyzed enzymes' ability to attack the substrates enhances the digestion phase [45]. Literature reports that such treatment can lead to a two-phase digestion process; In the first stage, the initial first 10-day period, processes involving organics and the surface charge are highly impacted. However, in the follow-up stage, the effects converge, which continues the methane potential trajectory in a 20 – 30 day span [45]. Based on the observations of results represented in the composition of biogas and biomethane production, a susception is deemed necessary on the possibility that this may indeed be a similar case. Further research is necessary to confirm this matter, especially concerning the potential for re-flocculation and construct validity of the method.

Additional extensions to the project can be induced subject to the availability of the equipment. This investigation focused mainly on the traditional approach of high intensity ultrasonication primarily because of the available equipment. This method focuses on the degradation of SS, which enhances the hydrolysis stage of AD. As noted in the literature research, however, the TS content of the available SS may be a

drawback of this approach [21]. Given this implication, for further extensions on the study of ultrasonication, the investigation proposes evaluating low power ultrasonication instead.
Low power ultrasonic treatment (LPUT) does not directly release substances into the suspension but rather enhances the availability of the organic to be attacked by the hydrolyzed enzyme. The effect of the LPUT approach is primarily towards promoting catalytic activity, cell growth, and membrane permeability. The approach, however, does require a high degree of optimization [46]. Application of a fully optimized low intensity and low dosage ultrasonic treatment can potentially improve digestibility as high as 67.6% [46]. Dependent on the availability of probes, low-intensity conditions can potentially be achieved to provide conditions for this proposed extension.

5.1.3 Alkaline Hydrolysis: Capable Pretreatment Subject to Stakeholder

From purely the perspective of enhancements to digestibility, the increased operational costs in the form of energy investments of ~0.56 $\frac{kWh_{electricity\ eqv.}}{kg_{TS}}$ can provide a significant enhancement to the biomethane potential and solubilization of substrates. The effect is sufficient that after a short initial inhibition, the inoculation sufficiently produces a consistent enhancement that is reflected in the almost breakeven of investments, as reflected in Table 2.

In line with the literature review, it is observed that the solubilized proteins and carbohydrates are increased by 3.09 and 3.96 times relative to the standard, respectively. This is further reaffirmed as the AD is effectively enhanced. It is likely, that the alkaline environment has facilitated this via inducing a charged surface on the bacteria resulting in polymeric substances to be desorbed in addition to potential cell lysis [47].

The application of alkaline hydrolysis can further be supported as within the same period of 15 days, the results indicate that approximately 30% of the theoretical maximum biomethane potential is achieved, which is a three times enhancement over the standard. The enhancement to the startup period and digestion rate may be a potential area of interest for further research; to support this suggestion, it should be noted that literature notes that alkaline-hydrolysis with an optimized dosage is capable of reducing the required hydraulic retention time of the process from 15 days down to 2 days [48]. Another significant potential advantageous enhancement of this pretreatment that unfortunately fell beyond this project's scope is the effect alkaline hydrolysis may induce on the selective release of phosphorous and nitrogen. This effect can positively enhance digestion. Combined thermal and alkaline treatment have been noted to release total phosphorous selectively and total nitrogen at enhanced relative rates of 94% and 76%, respectively [49].

A potential extension to the study of alkaline hydrolysis treatment is to employ higher temperatures. A significant constraint of high-temperature thermal pretreatment is the occurrence of Maillard reaction, which effectively hinders the usage of nutrients via the formation of melanoidins. However, alkaline hydrolysis provides for a substantial release and solubilization of proteins and their conversion to nitrogen-containing nutrient intermediates prior to solubilization of saccharides, which effectively avoids the Maillard reactions [47]. Alternatively, another monobasic alkaline such as potassium hydroxide (KOH) can also be investigated, inducing an alternative impact than sodium. However, the use of dibasic alkaline is not recommended due to the lower solubilization rate, which can lead to a lower level of enhancement to AD [16].

Although effective in improving digestibility and pathogen removal, the introduction of the chemical to the stream, however, does introduce a range of drawbacks both in terms of operational costs and externalities.

In terms of operational parameters, the addition of alkali can lead to corrosion and fouling of equipment [50]. This operational drawback necessitates the use of high-grade resilient materials and an increased maintenance cost [50].
Externally, introducing chemicals can potentially lead to problems in the effluent streams. This is a matter that is highly specific to the definitive stakeholder of concern and varies based on the receiving body of the effluent. In the case of NaOH, the addition of Na^+ ions can lead to an accumulation of sodicity in the effluent stream. To exemplify, introducing chemicals in the Eemskanaal, the effluent of Garmerwolde plant can introduce sodicity in surface soil exposed to the waterway leading to poor soil structure and lowered infiltration rate. Accumulation of sodicity can further affect the dispersion of the medium [51]. To resolve the issue, the use of chemical means such as gypsum is necessary for the long run to replace the ions with calcium and leach sodium from the soil [51]. Evaluation of the total externalities and regulatory limits based on the definitive stakeholder is necessary for this context.

5.2 Validity and Reliability of Research Methodology

The goal and corresponding research design strived towards promoting the knowledge basis for the research to decide upon the direction of choice regarding the available pretreatment methods. Quantified variables are those of biomethane potential, solids, and substrates. They are hereby evaluated, and recommendations are suggested.

5.2.1 Biomethane Potential Apparatus

The principal technique utilized for evaluating pretreatments is the use of experimental research. Via the manipulation of a singular independent variable, the effect on the prime variable of interest, the biomethane potential, is evaluated with a controlled device. Given the nature of the design, this approach provides a robust internal validity, but the external validity and hence applicability of methods in the industry do necessitate actual non-laboratory conditions. For this project, given that the experiments are preliminary and hence only used as a screening approach, the level of validity is deemed sufficient. However, further experimentations do require more extended inoculation periods to evaluate a better reflection of the total biomethane potential of the samples.

The construct validity of the apparatus was also evaluated via the use of GC on day 15 of the experimentation. Each sample's corresponding NaOH output flask was evaluated on their output composition. Regardless of the incubation flask's output composition, all outputs from NaOH flasks indicated an undetectable CO_2 fraction which supports that the apparatus has successfully removed the CO_2 from the stream, and hence the displacement of water is indeed a direct reflection of the methane production. Thus, for the 15 days of incubation, there has been sufficient NaOH available to remove the CO_2, and hence, replacing these flasks due to the conversion to Na_2CO_3 is not necessary under the experimental conditions of this project.

The reliability of the method is subject to severe drawbacks. It is observed that a suction effect is present in some of the samples that lead to the return of NaOH to the inoculation flask, hence deactivating the system. It is suspected that the cause is a siphoning effect due to the height difference between the chambers, however, this is not experimentally confirmed. Further research and safety measures, such as valves, are crucial for future trials.

5.2.2 Solids

Quantification of the solids followed a standardized method. A systematic error is present in the quantification caused by the miscalibration of the electronic balance between the measurement stages, especially for low masses; however, the degree of this factor is negligible compared to the standard deviation.

The primary area of extension suggested is an investigation regarding the dewaterability of the SS. The samples contain two critical water fractions naming immobilized water, which is bound chemically or physically to flocs and hence is non-evaporable, and free water, which behaves as bulk fraction [52].

The use of Differential Scanning Calorimetry (DSC) has been reported as a potential approach in evaluating the different fractions of water. DSC provides direct thermal analysis of bound water available in both the organic and inorganic phases. This approach provides a thorough evaluation of liquid-solid binding strength [52]. Evaluation of the mentioned parameters can be beneficial for potential upper-level customers, primarily since methods such as heat treatment affect the significant process parameter of dewaterability of the SS [13].

5.2.3 Quantifications of Substrates

Availability of the substrates is evaluated using modified Lowry (Proteins) and Anthrone (Carbohydrates) protocols. Throughout trials, both methods exhibited reproducible quantification with reasonable reliability both in terms of consistency and precision. The validity of the methods, however, is subject to caution.

For proteins, quantification methods are generally prone to indicate sensitivity to amino acid composition; The Lowry approach provides a route whereby the sensitivity is moderately constant among samples that permit estimations for situations, like this project, where protein mixtures are involved [32].

The major disadvantage of the approach employed for the quantification of proteins is that common substances such as K^+, NH_4^+, carbohydrates, and reducing agents proceed to interfere with the quantification. Additionally, the presence of amines is also considered a discouraging factor in utilizing this approach [53]. As mentioned in the results, potential routes for the formation of FA are present in the thermal treatment, and the addition of salts is essential in alkaline hydrolysis. Given the advantages the Lowry method provides, it is still deemed as suitable for a standardized method for future trials; however, evaluation of the interference via NH_4^+ and high carbohydrate concentration is recommended.

Given that the extractable fraction is evaluated under an alkali environment, mitigation measures were already applied whereby the concentration was evaluated via a modified calibration curve. The method is identified as consistent and similar in yield and accuracy for the SS sample [54]. However, it should still be noted that the protocol is prone to the underestimation of monomers from hemicellulose [54]. Given that the samples in this investigation are from municipal sewage, the methodology is deemed sufficiently consistent in providing the desired comparison; however, if future research extends to samples, especially those involving plant waste, caution is advised in employing this protocol.

Based on this information, although the method is deemed sufficient as a supportive indicator, extensions can be potentially valuable in the areas of organic quantifiers such as Chemical Oxygen Demand (COD). Alternatively, given that no specific information about the organic matter is desired, Total Organic Content (TOC) can be utilized as a faster approach to evaluate the organic matter, dependent on the availability of apparatus.

6 Conclusions and Recommendations

This master research project aimed to analyze and evaluate the effect of heat pretreatment, alkaline hydrolysis, and sonication on the anaerobic digestibility of municipal sewage sludge. The effect of the pretreatment was evaluated mainly via the application of a control representative inoculate biomass in combination with a standardized biomethane potential measurement apparatus. The results were thereby further supported via supporting quantifications of substrates and solids. Additionally, the project has provided a foundation for energy cost and externalities evaluation that can be further optimized as a standardized method for cross-comparison of varying pretreatment approaches.

A general literature review investigated the principal chemical and physical mechanisms involved in each pretreatment. Based on the information, pretreatment criteria were selected. Heat pretreatment followed a low-temperature thermal environment of 80 °C, indicating the region where protein denaturation occurs but falls below the upper range of 90 °C where the flocculation phenomenon appears. The 5% by mass alkali environment concentration using NaOH combined with a similar low-temperature thermal treatment of 80 °C was applied for alkaline hydrolysis.

Meanwhile, sonication indicated a complex result. Given the high solids content of available sludge, sonication aimed to evaluate a combination of low dosage with high intensity to evaluate whether sufficient extractable fraction can be created as a preliminary step to a follow-up treatment. Quantifications indicated that the total incubation time of 15 days was insufficient to evaluate the method; however, a noticeable alteration has been made relative to the standard. Further literature review on the observations provided a basis for suspecting that a lengthy low dosage sonication has potentially resulted in insufficient solubilization and flocculation; additionally, supportive variables may have been insufficiently quantified. Further research with a more extended incubation period, at least beyond the startup phase, and enhanced supportive variable quantification are deemed necessary to evaluate this result fully.

Heat and alkaline hydrolysis indicated promising enhancements in total biomethane production and shortened startup. Alkaline hydrolysis provided a significantly higher enhancement supported by increased solubilized proteins and carbohydrates with factors of 3.09 and 3.96 times relative to the untreated sludge. It must, however, be noted that the alkali pretreatment introduces ions to the effluent; considering this factor, although heat treatment provided 3.2 times less energy recovery potential, it may be more promising given the long-term operational and regulatory conditions. Evaluation of the implementation strategy with the involvement of a definitive stakeholder is necessary to provide a more detailed analysis.

In conclusion, based on the combined information of the literature review and experimental study, low-temperature thermal treatment, and low dosage alkaline hydrolysis are deemed as the most promising approaches for the sample at hand. The investigation recommends further overarching research to evaluate the critical boundary points of these pretreatments and to perform an in-depth evaluation of externalities together with a definitive stakeholder. Lastly, further analysis of sonication is discouraged due to the solid content of the samples leading to a likely unfavorable energy balance and the available equipment limitations of the facility; this project suggests that alternative approaches are more promising towards the provision of potentially valuable data. Lastly, a fundamental shortcoming of this research project is that the focus is on improving the digestibility of SS. This approach contradicts the value proposition of a wastewater treatment plant that aims to provide sewage treatment services. Reformulation of the central goal whereby digestibility is considered a secondary variable is recommended for future research to provide a more direct correlation with the demands of the industry.

7 Bibliography

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