COASTAL BIOGAS

Cluster On Anaerobic digestion, environmental Services and NuTrient

Interreg South Baltic

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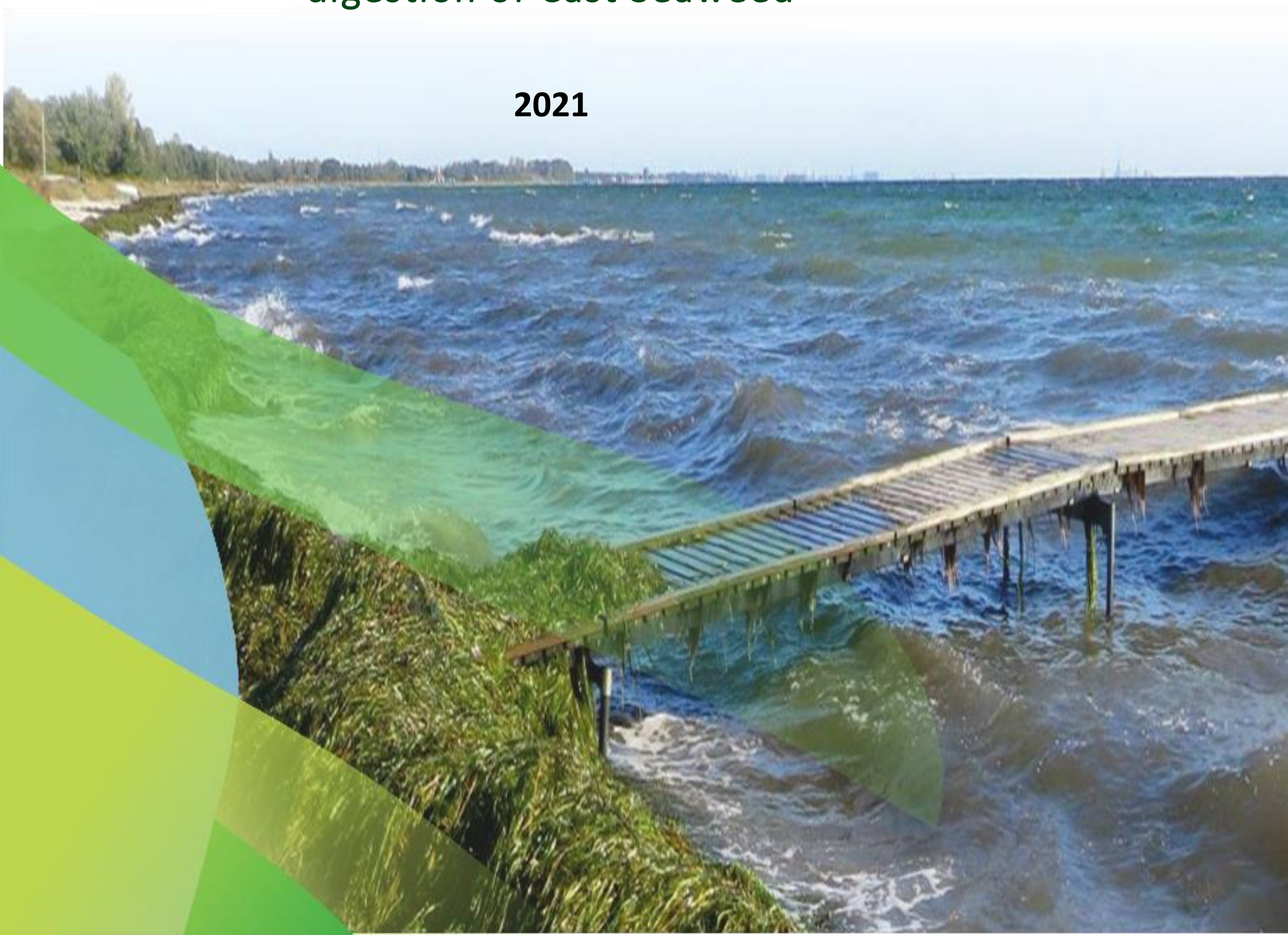
Report 4.3.1. Pretreatment and Quasi-continuous Co-digestion of Cast Seaweed

2021



https://www.coastal-biogas.eu/

Preface

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Cover photo

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List of Abbreviations/Glossary

A		Coefficient of the disintegration kinetic equation	%
A_{COD}	-	Degree of algae biomass disintegration measured COD method	%
AD	-	Anaerobic digestion	-
B		Coefficient of the disintegration kinetic equation	-
BPM	-	Bio-methane potential measurement	-
C_{CH4}	-	Methane concertation (concentration?) at i-th cycle	V/V
COD_0	-	Chemical oxygen demand of untreated sample	$mgO_2\ dm^{-3}$
COD_{NaOH}	-	Chemical oxygen demand of an alkaline hydrolysed sample	$mgO_2\ dm^{-3}$
COD_X	-	Chemical oxygen demand disintegrated sample	$mgO_2\ dm^{-3}$
FID		Flame Ionization Detector	-
m_0	-	Mass of the aluminium evaporating dish	g
m_1	-	Mass of the aluminium dish and the sample before drying	g
m_2	-	Mass of the aluminium dish and the sample after drying	g
m_b	-	Mass of wet biomass	t
m_c	-	Mass of the crucible	g
m_F	-	Mass of feedstock	kg
m_s	-	Mass of the crucible and sample before calcination	g
OLR	-	Organic loading rates	kg VS/m^3 day
P	-	Pressure in the OxiTop bioreactor	Pa
R	-	Gas constant	J mol K^{-1}
SC	-	Sand content	%
STP	-	Standard temperature and pressure	-
t	-	Time	s
T	-	Temperature	K
TCD		Thermal conductivity detector (katharometer)	-
TS	-	Total solids	%
TS_1	-	Total solids before washing	%

TS_2	-	Total solids after washing	%
V	-	Volume of the headspace in the OxiTop bioreactor	m^3
V_{bg}	-	Biogas production	m^3
VFA	-	Volatile fatty acids	mg CH_3COOH/dm^3
VS	-	Volatile solids	%

Introduction

The key parameter for the evaluation of substrates to be used in anaerobic digestion plants and pretreatment methods is the biogas potential. It states the maximum amount of biogas that can be obtained from a given amount of substrate and therefore represents the benchmark for any technical application for biogas production.

Discontinuous batch tests are biological test systems, which allow a direct evaluation of factors influencing the yield. Several standards and guidelines are available (e.g. VDI 4630, 2016, DIN EN ISO 11734, 1998 or Angelidaki et al., 2009) for performing anaerobic digestion by means of batch experiments. The experiments show a biogas yield, which can be used for estimation of the biogas potential and provide additional information on degradation kinetics.

The batch test is a widely used investigation system for the determination of the biogas potential of different organic materials. As was reported in literature, interlaboratory tests and investigations analysing the impact of inoculum have revealed a significant variability in the results of the test. Chemical methods of biogas potential determining were also investigated due to the much lower variability of the results. However, chemical methods have limited correlation to the measurement results obtained in the batch tests.

It is still not possible to clearly determine which of the methods is more accurate.

Nevertheless, in order to assess the technical feasibility of using cast seaweed in anaerobic digestion, it is necessary to carry out experiments as similar as possible to the conditions of the technical process. For this purpose, measurements of digestion of marine biomass were carried out in quasi-continuous measurements in reactors with a total capacity of 10 dm^3 and 1 000 dm^3. The results of the experiments performed in comparison to the batch tests are presented in the further part of the report.

1 Characteristics of the Biomass Used in the Digestion Experiments

1.1 Characteristics of Cattle Slurry

In the co-digestion experiments cattle slurry obtained from a commercial dairy farm located in Barkoczyn (Poland) was used as an inoculum, with a level of total solids of about 12.2% at the firsts test runs. This level dropped after a few months, due to the storage conditions to 11.39%. The collected fresh cattle slurry was stored at -18°C before it was used in the experiments. The fermentation of a pure cattle slurry was run as a reference case to the other tests performed, in which the feedstock biomass mixture was prepared with a use of the specific cattle slurry and macroalgae, untreated and treated in hydrothermal, mechanical, chemical and combined methods. The characteristics of the cattle slurry used in the semi-continues digestion tests are shown in Table 1.

Table 1. Basic parameters of the inoculum (cattle slurry)

No.	Parameter	Unit	Value
1.	Total Solids	%	12.2
2.	Volatile Solids	%	84.44
3.	VFA	$mgCH_3COOH/dm^3$	9462
4.	Alkalinity	$mgCaCO_3/dm^3$	16337
5.	Phosphorus in total	$mgPO_4/dm^3$	41.1
6.	Ammonia*⁾	$mgNH_4/dm^3$	11.2

*⁾ measured by Hach LCK 304 test

The high content of total solids and volatile solids affects the high value of volatile fatty acids contents, which is close to 9 500 mg CH_3COOH/dm^3. Cattle slurry also contains significant amounts of ammonia nitrogen and phosphorus 41.1 mg PO_4/dm^3 and 11.2 mg NH_4/dm^3 respectively.

In Table 2, the elemental analysis of cattle slurry used as inoculum is presented. The nitrogen content in the biomass was about 3.2% and carbon 33.4%. The C/N ratio was a little higher than 10. The C/N ratio and the pH play an important role in the anaerobic digestion process [1]. The pH influences the chemical equilibria of ammonia hydrogen sulphide and volatile fatty acids, which could inhibit the activity of the microorganisms. The ideal pH range for anaerobic digestion has been reported to be 6.8–7.4 [2]. Biomass with low C/N ratio and high pH need to be balanced in anaerobic reactors. It is also reported in the literature that substantial increase in biogas generation was observed whereas the ratio of inoculum to substrates increase from 0.5 to 1.0 [3]. Based on this information and results abstained (obtained?) in BMP tests, it was decided, that the inoculum in all conducted experiments should account for 75% of the feedstock.

Table 2. Elemental analysis of inoculum (cattle slurry)

No.	Element	Unit	Value	Standard Deviation
1.	Nitrogen	%	3.24	0.24
2.	Carbon	%	33.38	1.64
3.	Hydrogen	%	0.20	0.01
4.	Sulphur	%	40.00	2.31

An example of the results of anaerobic digestion of cattle slurry carried out in the OxiTop system are presented in Figure 1 and Figure 2. As it is shown in Figure 1, the highest rate of biogas production was observed at the 10th day of digestion.

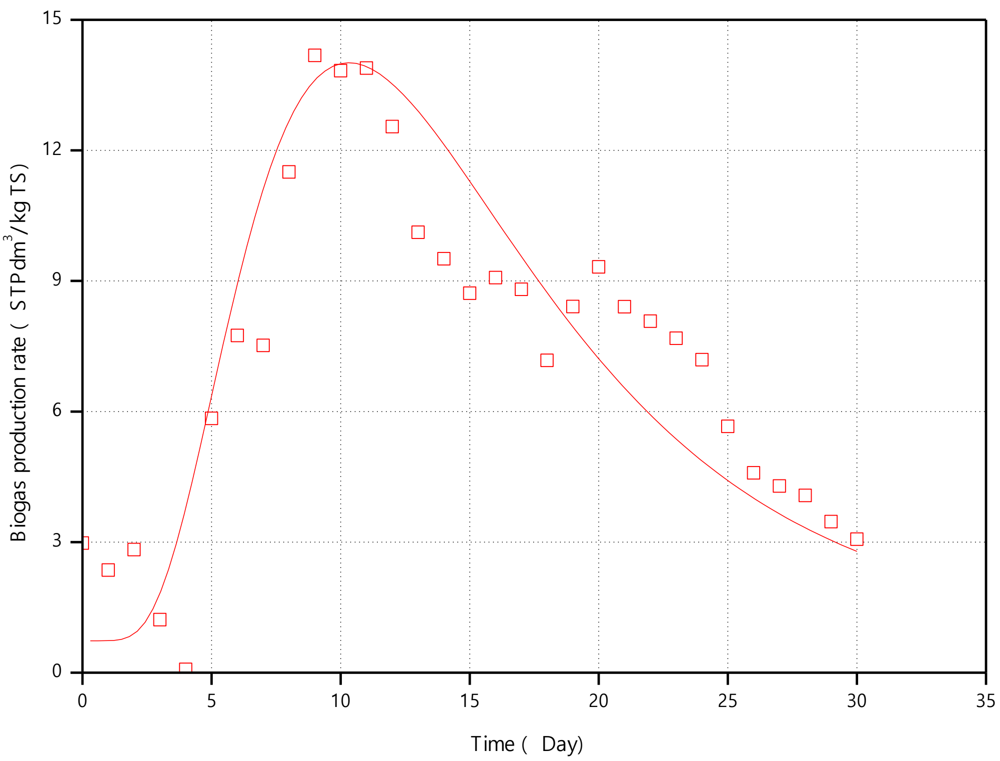

Figure 1. Kinetic of biogas production from cattle slurry in BPM test

The total methane concentration in the produced biogas varied in the ranged 45-48% by volume and the average biogas production was 222.2±11.0 STP dm^3/kg VS.

1.2 Marine Biomass

In order to carry out the digestion measurements in a semi-continuous process, it was necessary to obtain significant amounts of the cast seaweeds. For the experiments conducted in 10 dm^3 bioreactor approximately 9 kg of marine biomass were needed. For the experiments performed in 1 000 dm^3 bioreactors about 250 kg of wet seaweeds for were used per test.

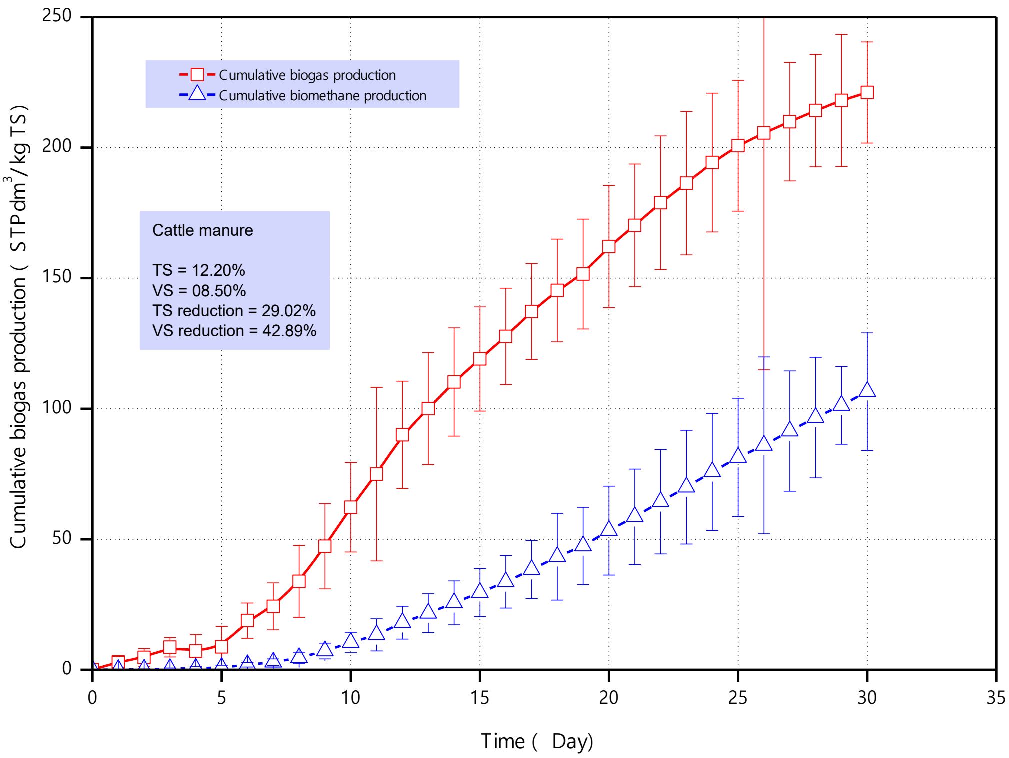

Figure 2. Cumulative biogas and bio-methane production during BMP test of cattle slurry

It was not possible to obtain such significant amounts of one type of algae from the Polish coast. Therefore, a mixture of seaweed collected from the beach in Gdańsk-Brzeźno was used for the digestion experiments (**Fehler! Verweisquelle konnte nicht gefunden werden.**).

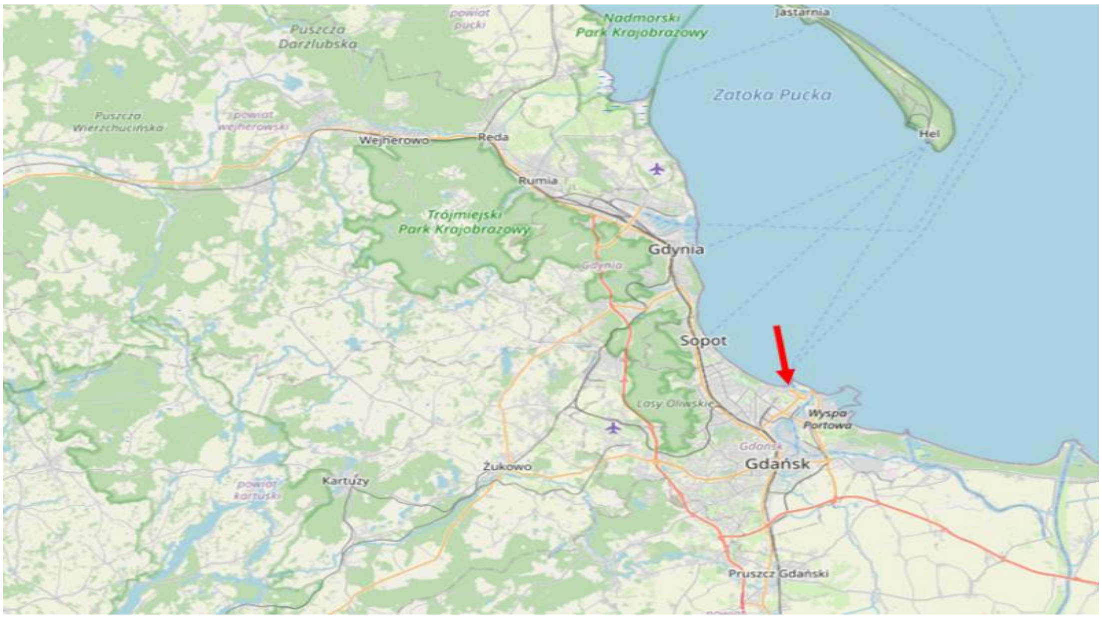

Figure 3. Map with sea biomass sampling points. [Source: https://www.openstreetmap.org]

The obtained biomass was contaminated with leaves, bird feathers, branches, and contained significant amounts of sand. The collected mixture of biomass was stored at -18°C before it was used in experiments. Each portion of biomass, before mixing with cattle slurry, was washed to remove sand, which is described in more detail in chapter 3.2.

The characteristics of the marine biomass used in the semi-continuous digestion experiments are presented in Table 3. The main species of algae present in the collected beach marine biomass from Gdańsk-Brzeźno were red and brown algae, which have established their supremacy in the South-Baltic region. It is estimated that the algae appeared on the beach for about two days and were thrown onto the coast after a storm.

The collected algae had a small amount of the total solid, approximately 16.9%, and about 73% of volatile solids.

Table 3. Basic parameters of marine biomass used in the digestion experiments

No.	Parameter	Unit	Value
1.	Total Solids	%	16.86%
2.	Volatile Solids	%	73.04%
3.	VFA	$mgCH_3COOH/dm^3$	13.74
4.	Alkalinity	$mg\ CaCO_3/dm^3$	1.37
5.	Phosphorus in total	$mgPO_4/dm^3$	881
6.	Ammonia*)	$mgNH_4/dm^3$	?
7.	Sand contents	%	?

*) measured by Hach LCK 304 test

2 Analytical Procedures

2.1 Total Solids

The total solids were determined by the gravimetric method in accordance to the PN-75/C-04616.01 standard. The test sample was placed in a previously weighed aluminium evaporating dish and reweighed with an accuracy of d = 0.001 g. Next, the sample dish was placed in an oven, preheated to 105°C and kept for 5 hours. After obtaining a constant weight, the sample was cooled in a desiccator and reweighed.

2.2 Volatile Solids

The volatile solids (VS) were determined by the gravimetric method in accordance to the PN-75/C-04616.01 standard. The sample, previously dried at the temperature of 105°C, was grounded and poured into the calcined porcelain crucible. The crucible with the sample was weighed and placed in the chamber furnace for 5 hours at 500°C. After calcining, the sample was cooled in a desiccator and weighed.

2.3 Biogas Composition Measurements

The gas phase composition was determined using two methods. One with a SRI 310c gas chromatograph with a thermal conductivity detector (TCD), flame ionization detector (FID) and a packed ShinCarbon ST Micropacked Column. The gas phase samples were collected in 0.5 dm^3 tedlar bags, from which 0.3 cm^3 of the sample was withdrawn using a gas-tight syringe and dispensed directly into the gas chromatograph injector. The tests were carried out using argon as the carrier gas. The second method included using a GFM 416 Gas Data certified analyser with infrared detector for methane and carbon dioxide analysis and electrochemical cells to measure any combination of oxygen, carbon monoxide, hydrogen sulphide, and hydrogen.

2.4 Volatile Fatty Acids and Ammonium Nitrogen

The Volatile fatty acids (VFA) and ammonium nitrogen levels were determined with a use of Hach cuvette tests LCK365 and LCK502 respectively according to the producer's guideline. Collected samples were centrifuged and the decanted liquid was filtrated with 0.2 µm filter before photometric analysis.

2.5 Alkalinity

The acid capacity was determined with a use of Hach cuvette tests LCK362 according to the producer's guideline. Collected samples were centrifuged and the decanted liquid was filtrated with a 0.2 µm filter before photometric analysis.

2.6 Determination of Sand Contents

A sample of the collected algae biomass was divided into two parts, one of which was mixed with tap water in a beaker with a volume of about 1 dm^3. The beaker was then set aside until the particles of density higher than the water had become completely sediment. Next, biomass was decanted and filtered on a paper filter. The process was repeated twice. Subsequently, the total solids were determined for bought (both?) samples (washed and unwashed).

The sand content was determined as the loss of volatile solids after washing the sample with fresh water using equation (1):

$$RS = \frac{(1 - \mathrm{VS}_1) - (1 - \mathrm{VS}_2)}{1 - \mathrm{VS}_1} \cdot 100 \quad (1)$$

Where:
VS_1 – volatile solids before washing and pretreatments
VS_2 – volatile solids after washing and pretreatments

As it can be seen from the results presented in Table 4, the location of biomass collecting is crucial in terms of the sand content in seaweed. When the algae are collected from the beach, the sand content is much higher than when the algae are collected from shallow water. It has also been found that sand removing is easier to conduct if the algae are fresh. It is much more difficult to wash off the sand from the biomass if it was lying on the beach 1-3 days.

Table 4. Sand content in marine biomass

Type of algae	Sand content, [%]	Place of sampling
Enteromorpha compressa	11.65	Shallow water
Enteromorpha plumosa	4.96	Shallow water
Potamogeton pectinatus	4.00	Shallow water
Zostera marina	20.88	Beach
Pheaophyta	7.80	Shallow water

3 Pretreatment of Biomass

3.1 Introduction

The core function of different pretreatments is to make organic matter more accessible to the microorganisms by breaking down the complex biopolymers, enhancing the bio-digestibility of the algal biomass through accessibility of microbial enzymes, and disrupting cell walls by bringing out the chemical substances from polymers into more available compounds, to ultimately improve fermentation and biofuel yield. There are many methods to increase biodegradability of seaweed (Figure 4). With varying effectiveness, mechanical (cutting, drying), thermal (heating), alkaline (NaOH), acid (HCl) or enzymatic hydrolysis (cellulose or hemicellulose) could be applied. Also, the combination of the pretreatment methods could significantly increase the methane production [4].

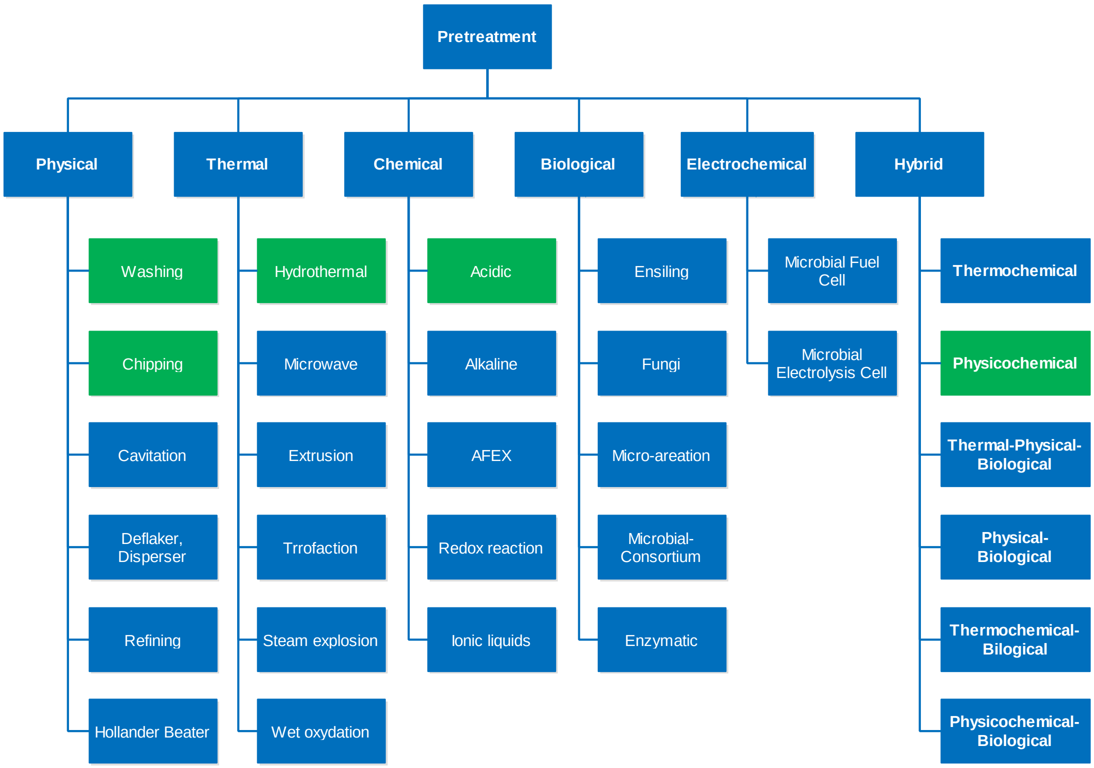

Figure 4. Types of pretreatment methods. The methods marked in green were applied in this work

On an industrial scale, the use of pretreatment of biomass, and in particular seaweed, in digestion process can bring many benefits, ranging from reducing the abrasive effect of hard mechanical impurities (such as sand) and ending with obtaining a greater production of biogas from a unit amount of biomass. The current study focuses on washing, thermal and chemical pretreatment as well as combined chemical and mechanical methods, and effects of pretreatment in semi-continuous digestion of marine biomass in the 10 dm^3 and 1 000 dm^3 bioreactors volume.

3.2 Washing and Sand Removal

Collected seaweed biomass very often contains sand, which can negatively affect the durability of bioreactors and other biogas plant equipment.

Most of the biomass is lighter than water and floats, whereas most sand and rocks are heavier and sink, which makes the water bath effective for rock removal. However, the water bath has serious disadvantages, including:

- Moisture pick-up in the biomass, which if used as fuel in a biomass boiler causes problems in the combustor and decreases output.
- If you operate in a very cold climate, the wet biomass can freeze into lumps.
- The cleaning water quickly becomes contaminated and must be continually refreshed. Treating the wastewater can be costly, unless the facility already has a plant with a large wastewater treatment system.

In the case of seaweed (as opposed to other types of biomass), due to the significant water content, the most frequently used method of their management is methane fermentation, which takes place in the aquatic environment. Therefore, apart from the classic methods used in the separation of contaminants present in biomass, such as stones, sand, plastics, it is possible to separate them in a water bath. Washing of the seaweed initially takes place in order to clean the seaweed biomass from sand and other impurities.

In order to reduce the sand content in seaweed collected from the beach, there were performed several experiments. There were chosen four methods of pretreatment: mixing untreated seaweed in fresh water, mixing untreated seaweed in sulphuric acidic solution with a pH level of 2, mixing mechanically disintegrated seaweed in fresh water and mixing of mechanically disintegrated seaweed in sulphuric acidic solution with a pH level of 2. Mechanical disintegration was performed with a laboratory grinder (total power 1 200 W, rotary speed 24 000 min^{-1}, screen mash number 60 – 200 mesh) for 120 s. Seaweed washing was performed in a 600 dm^3 reactor equipped with paddle mixer to provide adequate mixing (rotary speed 60 min^{-1}). Averaged washing time for each method in pilot scale was 2 hours.

The samples of washed seaweed were taken out of the reactor and gently dried out with towel paper. To calculate the sand removal degree, the volatile solids parameter was compered between fresh and untreated seaweed and the pretreated ones.

As it can be seen from the results presented in Table 5, mechanical disintegration didn't have any significant influence on the sand removal rate and the process is more effective performed in an acidic solution.

Table 5. Degree of sand removal in different pretreatment methods

Type of sample	Sand contents [%]	Degree of sand removal [%]
Fresh seaweed	39.11%	-
Mechanical disintegrated seaweed	9.88%	74.75%
Mechanical disintegrated seaweed in pH 2	6.11%	84.39%
Untreated seaweed	9.06%	76.83%
Seaweed in pH 2	4.46%	88.61%

3.3 Mechanical Pretreatment

Mechanical treatment can significantly enhance accessibility for the microorganisms to the surface of biomass and is a popular technique, which involves the use of blades, knives and hammers, to chip, grind, mill and shred biomass into small particles prior to anaerobic digestion. To increase the biomethane potential, the marine biomass was pretreated which increases the surface to volume ratio and helps to improve the hydrolysis of complex carbohydrates to sugar, by making it more available for the microorganisms. Ball milling the biomass is the most commonly used process [5], however due to the difference in the cell wall ultrastructure, the benefits from size reduction might have different effects. For instance, mechanical wet milling of *L. digitata* using cutting discs did not enhance glucose release [6]. Similarly it was found that neither vibro-ball milling nor centrifugal milling of *Ulva lactuca* affected its sugar release [7]. Another study where *Pelvetia canaliculate* was first dried for 24 hours at 80^{o}C and then milled in a conventional ball milling (ceramic cylinder with alumina balls) to a particle sizes of 1 mm and 2 mm, resulted in lowered methane yield with respect to the raw seaweed [8]. On the other hand, centrifugal milling of *Gelidium sesquipedale* ended up with 129% increase in sugar released [7]. Another method involves beating, carried out by pounding the seaweed against a plate, enabling the production of seaweed pulp at different consistencies depending on the machine setting. It was found that beating was the most effective pretreatment method of *Laminaria spp.* Compered to milling and microwaving [8]. Beating might also be carried out through the utilisation of a modified Hollander beater, made of an elliptic channel with a bladed drum, which is normally used in the paper industry. A 50 minutes pretreatment time of *Pelvetia canaliculata* resulted in a 45% increase in methane yield compered to non-treated algae [9]. Other methods such as a mechanical maceration which involves homogenisation (by hand blender) of the algae to a paste resulted in methane yield *of U. lactuca, G. vermiculophylla and C. linum* increase by 68%, 11% and 17% respectively [10].

3.4 Chemical Pretreatment

For chemical pretreatment the most common acids and base are: NaOH, sulphuric, nitric, hydrochloric, phosphoric, citric, lactic, acetic and oxalic acid. The biomass is treated with different dilutions where the lower ones need less substance for neutralization and higher concentrations might increase the production of inhibitor compounds.

3.4.1 Alkali or Acidic Treatment

It is suspected that addition of alkalis (the most often NaOH) causes swelling of fibres and increases pore size which enables release of sugars [11].Also, the saponification reaction which may take place, results in cleavage of ester linkages between hemicelluloses and lignin. Therefore, their fragments are accessible to the enzymes [12]. Acid pretreatment is based on the susceptibility of the glucosidic bonds between hemicellulose and cellulose to acid. Hydronium ions, which originate from the acid catalyst, cause breakdown of the long cellulose and hemicellulose chains into sugar monomers [13]. The addition of acids (such as H_2SO_4, HCl) was proposed to hydrolyse cellulose, hemicellulose or laminarin [14,15]. Treating *F. vesiculosus* with 0.2 M HCl (80°C, 12 hours) resulted in 2.5 times higher methane yield compared to untreated seaweed. However, another study concluded that similar treatment of Ulva spp. with 0.1 M – 0.2 M HCl and NaOH at 60°C, 75°C and 90°C showed no different results compared to just thermal pretreatment [16]. Another study investigated the influence on releasing of macromolecules of different chemical compounds. Among the set of pretreatments studied (sulphuric, nitric, hydrochloric, phosphoric acids and sodium hydroxide), nitric acid was to be the most effective in enhancing the realise of macromolecules [14]. It is noteworthy that the use of acids has disadvantages such as: hazard risks when concentrated acids are used, high operating cost of acid recycling and necessity to use acid-resistant equipment, which is related to high investments costs [15].

3.4.2 Peroxide Treatment

Combing thermal treatment and hydroxyl radical reaction step (0.018% H_2O_2 and 11.9 mM $FeSO_4$) shows a higher cellulose to glucose conversion rate (88.1%) when compared to alkali and acid pretreatment of seaweed [17]. The biomass does not need the neutralisation process with a lower utility cost compared with acid or alkaline pretreatment. It is also possible that the method decreases the risk of inhibitory furfurals production [18].

3.5 Hydrothermal Pretreatment

Thermal and hydrothermal pretreatment (THP) are seen as simple processes with an implementation cost that varies according to the reaction temperature and the exposure time [5]. Some technologies of hydrothermal pretreatment have been implemented on a technical scale. It is reported by many researchers, that THP improves the conversion of the organic waste into bio-energy improving biodegradability of biomass especially containing high contents of cellulose and lignocellulose. Hydrothermal pretreatment consumes more energy than conventional thermal pretreatment due to the reaction configuration of elevated temperature and pressure, coupled with the steady state supply of water into the reactor device [19]. The technology also sanitizes, dewaters and reduces the viscosity of biomass, thereby improving the nutritional quality of the digestate generated during anaerobic digestion.

Hydrothermal process occurs in a reactor at 165 - 170°C and 5 - 6 bar for 20 - 30 minutes. The total solid of the treated biomass is about 16 - 18% to reduce the heat consumption. The use of waste heat from power plant in hydrothermal pretreatment can be an attractive method that balances investment and operating costs with the benefits of biomass pretreatment.

3.6 Hybrid Pretreatment Methods

Hybrid methods use the advantages of several pretreatment procedures to increase the bioavailability of biomass, reduce the time and heat and energy required to carry out the process. Among the many combinations of hybrid methods, the following can be distinguished: thermo-chemical, physico-chemical, thermo-physico-chemical, physico-biological, thermos-chemical-biological, physico-chemical, etc. In the presented a hybrid method of pretreatment based on mechanical grinding and thermal processing was used. In semi-continuous digestion experiments in the 10 dm^3 bioreactor, the grinding of the marine biomass was down using the laboratory knife mill presented on Figure 5. The total power of the mill was 1 200 W, the rotary speed: 24 000 min^{-1}, screen size 200 mesh. The milling was performed in duration time from 15 to 180 s.

Figure 5. Knife mill used for marine biomass grinding experiments conducted in 10 dm^3 bioreactors

Figure 6. Hammer mill used for marine biomass grinding experiments conducted in 1 000 dm^3 bioreactors

In experiments on a semi-technical scale (1 000 dm^3 bioreactor), due to the significant amount of marine biomass needed for conducting digestion, grinding was performed in an industrial hammer mill shown in Figure 6. The electric power of the mill was 4 kW and the rotational speed about 7 400 min^{-1}. A screen with 10 mm circular hols was used for grinding mix of algae.

Figure 7. Autoclave used for thermal pretreatment in lab-scale digestion. [Source: https://www.mt.com]

Figure 8. Heated tank used for thermal pretreatment in semi-technical scale of digestion

After grinding the mix of algae, the biomass was hydrolysed in a sulfuric acid solution at pH 2 at 95°C for 6 hours. In the case of fermentation experiments carried out in bioreactors with a volume of 10 dm^3, the hydrolysis was conducted in a laboratory autoclave with a capacity of about 2 dm^3 (Figure 7). For the process on a semi-technical scale, acid hydrolysis was carried out in a heated tank (total volume 800 dm^3) equipped with a stirrer, which is shown in Figure 8.

4 Algae Digestion in a Quasi-continuous Process in 10 dm^3 Bioreactors

4.1 Methodology of Semi-continuous Experiments

Anaerobic digestion experiments in quasi-continuous process of marine biomass were carried out in the system presented in Figure 9. The laboratory installation is designed to conduct research on methane fermentation in the mesophilic and thermophilic digestion process on a quasi-continuous laboratory scale. The system consists of twin bioreactors and the necessary equipment for their operation. Stainless steel (AISI 316L) was used in the construction of the reactors as well as fittings and piping.

Figure 9. The quasi-continuous digestion system equipped in two bioreactors with a capacity of 10 dm^3 of each

The system includes bioreactors with a total capacity of 10 dm^3, equipped with a stirrer and a heating jacket. It is possible to carry out the anaerobic digestion process with a dry matter content up to 12%. Bioreactors have connectors for the pH and redox electrodes, and temperature sensors located on the circumference of the reactor at an angle of 15° at a height of 80 mm from the bottom of the reactors. In addition, the bioreactors are equipped with a sight glass, a mechanical stirrer with gas-tight sealing, bearing in the top cover of the bioreactor. The permissible operating pressure of bioreactors is 150 kPa. The rotational speed of the agitator can be adjusted in the range of 2-70 min^{-1}. The paddle mixer was equipped with three levels of position adjustable blades. Bioreactors are heated by a water jacket with temperature control by an external thermostatic system. The amount of produced biogas was determined on-line with a volumetric flow meter placed on the biogas discharge pipe. The accuracy of the flow meter indications was 0.1 STP cm^3/minute. The analysis of the biogas composition was performed using a gas analyser and additionally (in order to check the correct operation of the analyser), a gas chromatography with an FID and TCD detector was used.

The installation was equipped with a supervision and data acquisition software developed in the LabView ™ environment. The software allows to control the fermentation process such as: temperature, stirrer speed, mass loading and unloading to bioreactors, as well as records all measured parameters. The view of the control panel is shown in Figure 10.

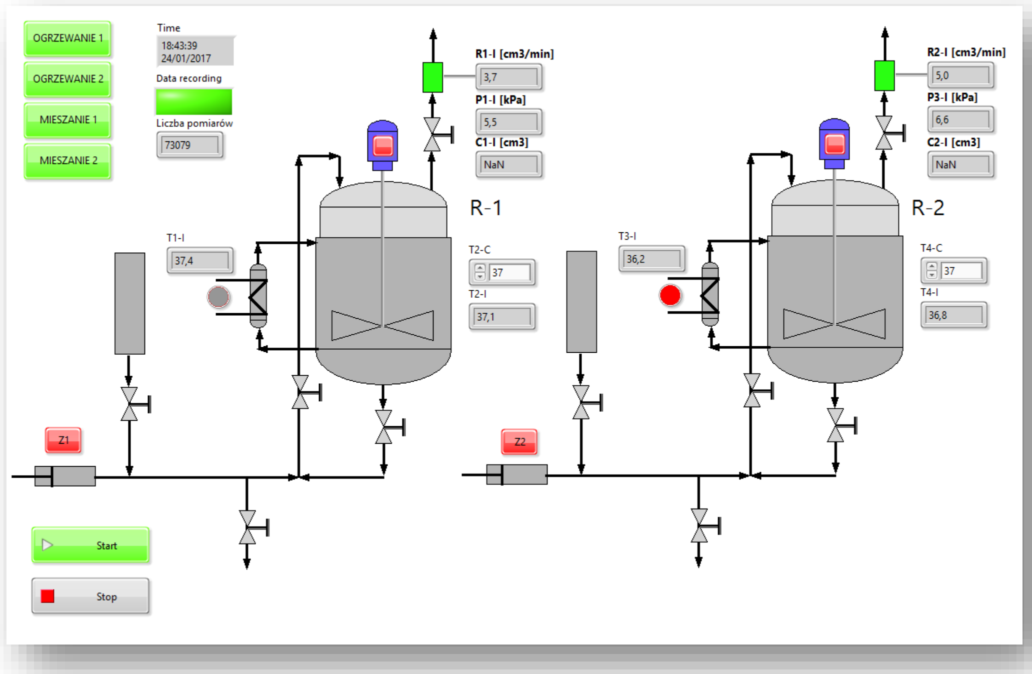

Figure 10. Control system of 10 dm^3 digestion bioreactors

4.2 Results and Discussion

4.2.1 Cattle Slurry

The results of biogas production potential measurements in laboratory tests were verified in the quasi-continuous fermentation process, which reflects much better the industrial biogas processes than BPM tests. Figure 14 shows the biogas and biomethane production and also the temperature of the bioreactor during the stable phase of the cattle slurry quasi-continuous digestion process. The changes in the intensity of the biogas production and the temperature of the bioreactor, caused by cyclic feeding of the biomass to the bioreactor, are evident. The average values of the biogas and biomethane production rates were also marked. The process was carried out for a constant OLR of 4 kg VS/m^3 day. The parameters of the experiment are presented in Table 6.

The daily average biogas and biomethane productions were determined from linear correlation of cumulative data (Figure 15) and were about 7.46 and 3.74 STP dm^3/day respectively, which seems to be a good result for medium loaded digesters. It is assumed that the amount of produced biogas should be about 1 STP dm^3 per 1 dm^3 of the biomass volume. In the cattle slurry digestion experiment, this value was about 1.07 STP dm^3/dm^3.

Table 6. Parameters of quasi-continuous cattle slurry digestion in 10 dm^3 reactors

No	Parameter	Value	Units
1	The active volume of the bioreactor	7.0	dm^3
2	Hydraulic retention time	30	Days
3	Daily feedstock amount	0.238	dm^3/day
4	Biogas production	266.6	STP dm^3/kg VS
5	Biomethane production	137.2	STP dm^3/kg VS
6	Daily biogas production	7.47	STP dm^3/day
7	Daily biomethane production	3.74	STP dm^3/day
8	Methane concentration	51.5	%
9	TS of feedstock	12.20	%
10	TS of digestate	8.58	%
11	VS of feedstock	88.44	%
12	VS of digestate	75.01	%
13	OLR	4.00	kg VS/m^3 day

Figure 16 shows changes in the concentration of biomethane, carbon dioxide and hydrogen sulphide. The concentration of methane in the produced biogas was stable during the experiment and amounted to about 52%, and carbon dioxide at the level of 40%. Hydrogen sulphide concentration varied much more, in a range from 150 to over 1 200 ppm.

The VFA (Figure 17) of the cattle slurry, are high and was about 9 000 mgCH_3COOH/dm^3 and was decreasing during fermentation below 8 000 mgCH_3COOH/dm^3. The alkalinity was also high and was decreasing during digestion, so the ratio of VFA to alkalinity was almost constant at the level of about 0.6.

4.2.2 Mix of Algae and Cattle Slurry in Ration 1:3

Figure 19 shows the biogas and biomethane production and also the temperature of the bioreactor during the stable phase of a quasi-continuous digestion process of a mix of algae and cattle slurry (1:3 TS ratio). The average values of the biogas and biomethane production rates were 272.1 and 90.7 STP dm^3/kg VS respectively. The process was carried out at constant OLR value (4 kg VS/m^3 day) what needed about 0.4 dm^3 of the feedstock per day. The parameters of the conducted experiment are presented in Table 7.

The daily average biogas and biomethane production were determined from linear correlation of cumulative data (Figure 20) and ware about 7.62 and 2.54 STP dm^3/day respectively. The daily average biogas production is about 2% higher than during pure cattle slurry digestion but the biomethane production was 34% less, which means that the concentration of methane in biogas obtained from the algae and cattle slurry mixture is much lower, (about 33%). The amount of produced biogas per 1 dm^3 of the algae and cattle slurry biomass volume was about 1.09 STP dm^3/dm^3.

Table 7. Parameters of quasi-continuous mix of algae and cattle slurry (1:3 ratio) digestion

No	Parameter	Value	Units
1	The active volume of the bioreactor	7.0	dm^3
2	Hydraulic retention time	30	Days
3	Daily feedstock amount	0.399	STP dm^3/day
4	Biogas production	272.1	STP dm^3/kg VS
5	Biomethane production	90.7	STP dm^3/kg VS
6	Daily biogas production	7.62	STP dm^3/day
7	Daily biomethane production	2.54	STP dm^3/day
8	Methane concentration	33.32	%
9	TS of feedstock	7.87	%
10	TS of digestate	5.55	%
11	VS of feedstock	78.91	%
12	VS of digestate	71.90	%
13	OLR	4.00	kg VS/m^3 day

Figure 21 shows changes in the concentration of biomethane, carbon dioxide and hydrogen sulphide. The concentration of methane in the produced biogas was stable during the experiment at amounted to 40%, and carbon dioxide at the level of 55%. Hydrogen sulphide concentration varied much more, in a range from 10 to over 750 ppm.

The VFA content (Figure 22) in the mixture of algae and cattle slurry is high and was about 4 000 $mgCH_3COOH/dm^3$ and was decreasing during fermentation below 2 000 $mgCH_3COOH/dm^3$. The alkalinity was also high and was decreasing during digestion and the ratio of VFA to alkalinity was slightly decreasing through the process from 0.35 to 0.15. The low ratio of VFA to alkalinity may indicate the low bioavailability of the organic components in the mixture of algae and cattle slurry.

4.2.3 Hydrothermal Pretreatment at 95°C

4.2.3.1 Hydrothermal Pretreatment at 95°C during 60 minutes

As was shown in chapter 4.2.2, the addition of the algae mix to the cattle slurry almost does not influence the biogas production but decreases the concentration of methane. For the increase of the bioavailability of marine biomass, a number of experiments of pretreatment of algae were carried out. One of the promising methods is thermal hydrolysis which was described in paragraph 3.5. Presented in the current paragraph, results concern low temperature hydrolysis, performed at 95°C for 60 minutes.

In Figure 29 are presented results of the biogas and biomethane production and also the temperature of the bioreactor during stable phase in a quasi-continuous digestion process of a mixture of hydrothermally pretreated algae and cattle manure in ratio 1:3. The average values of the biogas and biomethane production rates are marked by the dashed lines. The process was carried out for a constant OLR of 4 kg VS/m^3 day. The parameters of the digestion experiment are presented in Table 8.

The daily average biogas and biomethane productions were determined from linear correlation of cumulative data (Figure 30) and ware about 8.37 and 4.90 STP dm^3/day respectively. The biogas production was 10% higher in comparison to pure cattle slurry and 12% in comparison to untreated mixture of algae and cattle slurry. When comparing the biomethane yield, an increase of about 28% higher in comparison to pure cattle slurry and 93% in comparison to untreated mixture of algae and cattle slurry can be noticed. The amount of produced biogas per 1 dm^3 of the biomass volume of hydrothermally pretreated algae at 95°C for 60 minutes and cattle manure was about 1.2 STP dm^3/dm^3.

Table 8. Parameters of quasi-continuous mixture of hydrothermally pretreated at 95°C for 60 minutes algae and cattle manure (ratio 1:3) digestion

No	Parameter	Value	Units
1	The active volume of the bioreactor	7.0	dm3
2	Hydraulic retention time	30	days
3	Daily feedstock amount	0.477	STP dm^3/day
4	Biogas production	298.8	STP dm^3/kg VS
5	Biomethane production	174.9	STP dm^3/kg VS
6	Daily biogas production	8.37	STP dm^3/day
7	Daily biomethane production	4.90	STP dm^3/day
8	Methane concentration	58.54	%
9	TS of feedstock	6.32	%
10	TS of digestate	3.19	%
11	VS of feedstock	82.93	%

No	Parameter	Value	Units
12	VS of digestate	75.60	%
13	OLR	4.00	kg VS/m^3 day

Figure 31 shows changes in the concentration of biomethane, carbon dioxide and hydrogen sulphide. The concentration of methane in the produced biogas was stable during the experiment and amounted to about 58%, and carbon dioxide to a level of 38%. Hydrogen sulphide concentration varied in a range from 75 to over 750 ppm.

The VFA (Figure 32) of the mix of hydrothermally pretreated at 95°C for 60 minutes algae and cattle slurry is high and was about 6 500 mg CH_3COOH/dm^3 and was decreasing during fermentation below 4 000 mg CH_3COOH/dm^3. The alkalinity was also high and was decreasing during digestion. The ratio of VFA to alkalinity was high and slightly decreasing through the process from 0.9 to 0.7.

4.2.3.2 Hydrothermal Pretreatment at 95°C during 24 hours

Hydrothermal pretreatment at 95°C was also carried out during 24 hours to determine the effect of the process time on the production of biogas and biomethane. Figure 34 shows the biogas and biomethane production as also temperature of the bioreactor during stable phase of mixture of hydrothermally pretreated at 95°C for 24 hours algae and cattle manure in ratio 1:3 in quasi-continuous digestion process. The process was carried out for a constant OLR of 3.72 kg VS/m^3 day which was a little lower than for hydrothermal pretreatment during 1 hour. The parameters of the experiment are presented in Table 9.

The daily average biogas and biomethane production were about 7.2 and 4.3 STP dm^3/dm^3 respectively (Figure 35), which in the range of standard deviation are almost the same lake for biomass pretreated during 1 hour.

Table 9. Parameters of quasi-continuous mixture of hydrothermally pretreated at 95°C for 24 hours algae and cattle manure (ratio 1:3) digestion

No	Parameter	Value	Units
1	The active volume of the bioreactor	7.0	dm^3
2	Hydraulic retention time	30	Days
3	Daily feedstock amount	0.477	STP dm^3/day
4	Biogas production	278.1	STP dm^3/kg VS
5	Biomethane production	164.9	STP dm^3/kg VS
6	Daily biogas production	7.25	STP dm^3/day
7	Daily biomethane production	4.30	STP dm^3/day
8	Methane concentration	59.30	%
9	TS of feedstock	6.07	%
10	TS of digestate	2.92	%
11	VS of feedstock	81.06	%

No	Parameter	Value	Units
12	VS of digestate	75.24	%
13	OLR	3.72	kg VS/m^3 day

Figure 36 shows changes in the concentration of biomethane, carbon dioxide and hydrogen sulphide. The concentration of methane in the produced biogas was stable during the experiment at amounted of about 60%, and carbon dioxide at the level of 39%. Hydrogen sulphide concentration varied in a range from 500 to over 700 ppm.

The VFA (Figure 37) of the mix of hydrothermally pretreated at 95°C for 24 hours algae and cattle slurry are high and was about 6 200 mgCH_3COOH/dm^3 and was decreasing during fermentation below 5 500 mgCH_3COOH/dm^3. The alkalinity was also high and was decreasing during digestion and the ratio of VFA to alkalinity was slightly decreasing through the process from 0.3 to 0.27.

4.2.4 Hydrothermal Pretreatment at 160°C

The second experiment of hydrothermal pretreatment of marine biomass was performed at 160°C for 30 minutes. Figure 39 shows the biogas, biomethane production and temperature of the bioreactor during stable phase in a quasi-continuous digestion process of a mixture of hydrothermally pretreated algae and cattle manure in ratio 1:3. The average values of the biogas and biomethane production rates are marked by dashed lines. The process was carried out for a constant OLR of 4 kg VS/m^3 day. The parameters of the experiment are presented in Table 10.

The daily average biogas and biomethane production are presented in Figure 40 as a linear correlation of cumulative data. The daily biogas yield was about 12.38 STP dm^3/day and shows an increase of about 66% and 62% in comparison to pure cattle slurry and untreated mixture of algae and cattle slurry respectively. The biomethane production (6.11 STP dm^3/day) was about 59% higher in comparison to cattle slurry and 141% in comparison to a mixture of algae and cattle slurry respectively. The amount of produced biogas per 1 dm^3 of the biomass volume for a mix of hydrothermally pretreated at 160°C for 30 minutes algae and cattle manure was about 1.77 STP dm^3/dm^3.

Table 10. Parameters of quasi-continuous mixture of hydrothermally pretreated at 160°C for 30 minutes algae and cattle manure (ratio 1:3) digestion

No	Parameter	Value	Units
1	The active volume of the bioreactor	7.0	dm^3
2	Hydraulic retention time	30	Days
3	Daily feedstock amount	0.427	STP dm^3/day
4	Biogas production	441.9	STP dm^3/kg VS
5	Biomethane production	218.2	STP dm^3/kg VS
6	Daily biogas production	12.38	STP dm^3/day
7	Daily biomethane production	6.11	STP dm^3/day

No	Parameter	Value	Units
8	Methane concentration	49.38	%
9	TS of feedstock	7.80	%
10	TS of digestate	5.66	%
11	VS of feedstock	75.80	%
12	VS of digestate	73.36	%
13	OLR	4.00	kg VS/m^3 day

Figure 41 shows changes in the concentration of biomethane, carbon dioxide and hydrogen sulphide. The concentration of methane in the produced biogas was stable during the experiment at amounted of about 50%, and carbon dioxide at the level of 46%. Hydrogen sulphide concentration varied much more, in a range from 100 to over 750 ppm.

The VFA (Figure 42) of the mix of hydrothermally pretreated at 160°C for 30 minutes algae and cattle slurry are high and were about 7 000 mg CH_3COOH/dm^3 and decreased slightly during fermentation below 6 000 mg CH_3COOH/dm^3. The alkalinity was also high and was decreasing during digestion and the ratio of VFA to alkalinity was almost stable at 0.38 to 0.33 levels and in the optimal range.

4.2.5 Combined Pretreatment - Mechanical and Acid Hydrolysis

During the investigation of influence pretreatment method on the biogas yield combined methods were also carried out by coupling mechanical disintegration with acid hydrolysis. In the first step, the marine biomass (a mixture of algae) was mechanically disintegrated in a laboratory knife mill presented in Figure 5 for 120 s and in the next step the grinded biomass was hydrolysed at ambient temperature by sulphuric acid during 6 hours (pH 2). Then, the hydrolysed biomass was neutralized to pH 7 with sodium bicarbonate. The results of biogas and biomethane production and also the temperature for a mixture of combined mechanical and acid pretreatment of algae and cattle manure in ratio 1:3 in a quasi-continuous digestion process is presented in Figure 24. The process was carried out for a constant OLR of 4 kg VS/m^3 day. The parameters of the experiment are presented in Table 11.

The average values of the biogas and biomethane production rates were 292.4 and 179.6 STP dm^3/kg VS respectively. The biogas production was 10% higher in comparison to pure cattle slurry and 7% in comparison to untreated mixture of algae and cattle slurry. When comparing the biomethane yield, an increase of about 10% in comparison to pure cattle slurry and 98% in comparison to untreated mixture of algae and cattle slurry can be noticed. The daily average biogas and biomethane productions were determined from linear correlation of cumulative data (Figure 25). In the mix of combined mechanical and acid pretreatment algae and cattle manure digestion experiment, the daily biogas production per volume of digested biomass reached about 1.17 STP dm^3/dm^3.

Table 11. Parameters of quasi-continuous mixture of combined mechanical and acid pretreatment of algae and cattle manure (ratio 1:3) digestion

No	Parameter	Value	Units
1	The active volume of the bioreactor	7.0	dm^3
2	Hydraulic retention time	30	Days
3	Daily feedstock amount	0.518	STP dm^3/day
4	Biogas production	292.4	STP dm^3/kg VS
5	Biomethane production	179.6	STP dm^3/kg VS
6	Daily biogas production	8.19	STP dm^3/day
7	Daily biomethane production	5.03	STP dm^3/day
8	Methane concentration	61.41	%
9	TS of feedstock	6.01	%
10	TS of digestate	2.89	%
11	VS of feedstock	81.80	%
12	VS of digestate	75.25	%
13	OLR	4.00	kg VS/m^3 day

Figure 26 shows changes in the concentration of biomethane, carbon dioxide and hydrogen sulphide. The concentration of methane in the produced biogas was stable during the experiment and amounted to about 61%, and carbon dioxide at the level of 38%. Hydrogen sulphide concentration varied less than typically, in a range from 500 to over 600 ppm.

The VFA (Figure 27) of the mix of combined mechanical and acid pretreatment algae and cattle slurry are high and was about 7 000 $mgCH_3COOH/dm^3$ and was decreasing slightly during fermentation to a level below 6 000 $mgCH_3COOH/dm^3$. The alkalinity was also at elevated level and was decreasing during digestion and the ratio of VFA to alkalinity was almost stable at around 0.3 level.

5 Algae Digestion in a Quasi-continuous Process in 1 000 dm³ Bioreactors

5.1 Technical Description of Digestion Plant 1 000 dm³ Bioreactors

The installation is designed for digestion of organic fraction of biodegradable solid wastes in thermophilic process in semi-technical scale. The size of the digester (1 000 dm³) avoids the sampling errors resulted from inhomogeneity of raw material. The system consists mainly of three modules: bio-thermal treatment system of feed, twin digesters and dewatering and digested treatment system. The flow diagram and photo of the installation is presented at Figure 11 and **Fehler! Verweisquelle konnte nicht gefunden werden.**.

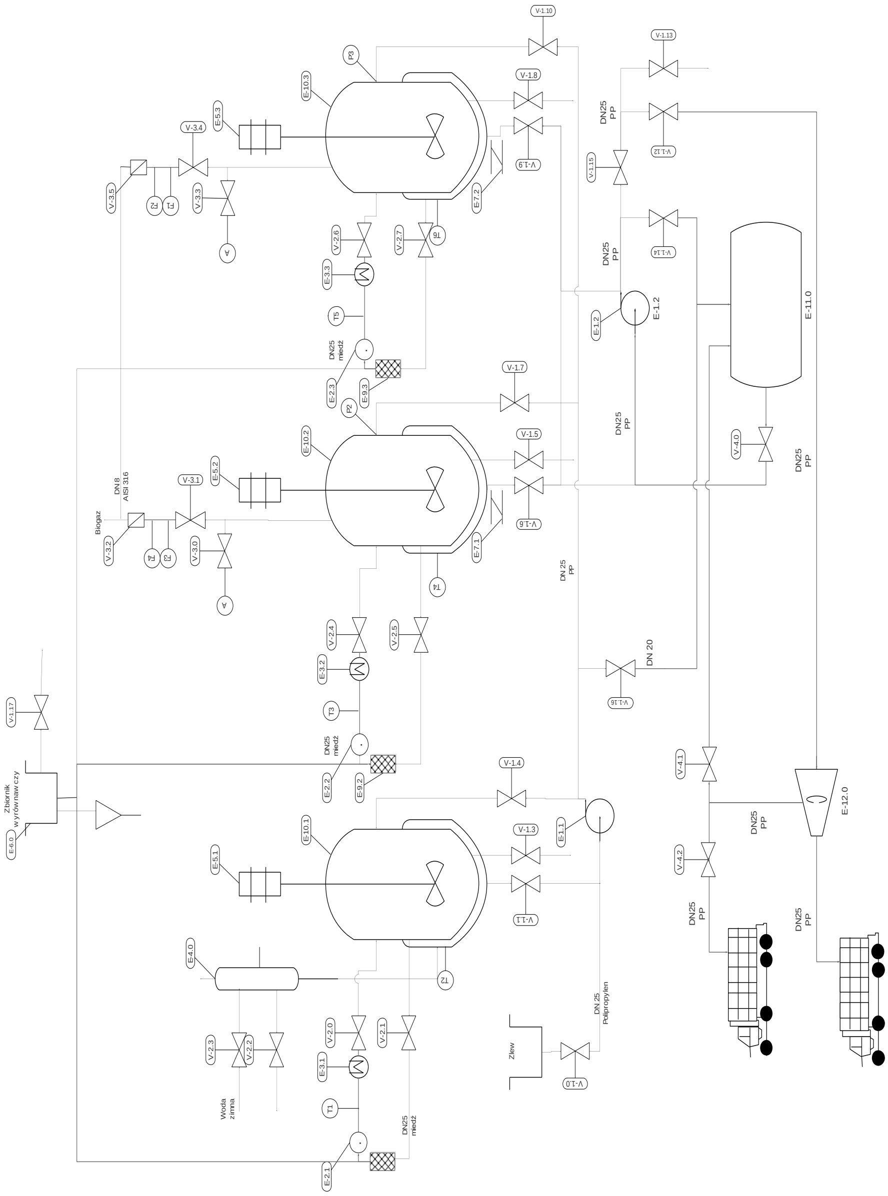

Figure 11. Flowsheet diagram of quasi-continuous digestion system

Figure 12. Picture of the digestion (1 000 dm^3 digester) and hydrolysis system

The bioreactor is made from stainless steel AISI 316L and the fitting from PP-aluminium composite. The system includes thermalizes bioreactor (E-10.1) with total volume 800 dm^3, completed with agitator (E-5.1) and heat jacket. The duplex of bioreactor with total volume of 1 000 dm^3 completed with mixers (E-5.2 and E-5.3), heat jacket and capacity strain gauges (E-7.1 and E-7.2), enable to measure of bioreactors weight (E-10.2 and E-10.3). The heating of bioreactors is conducted by jacket filled isopropyl glycol and utilizing the electric heater (E-3.1, E-3.2, E-3.3) controlled by solid state relay and PID regulator. The above solution guarantee high precision of temperature control in bioreactors with accuracy ±0.2°C. The weighting system provide precision of mass measurement with accuracy ±1 kg. The installation is completed with biogas flowmeter (F1, F3) and biogas flow canter (F2, F4) summing the quantity of produced biogas. The screw pump E-1.1 is used to feed the bio-thermal treatment system and digesters. For unloading the bioreactors and transporting digested to centrifuge (E-12) the pump E-1.2 is used.

The system was equipped with control and logging system programmed in LabView™ environment. The software enables controlling of digestion (temperature, speed of agitator rotation, loading and unloading of bioreactors sets), and also logging all measured parameters and sets. The front panel view was presented at Figure 13.

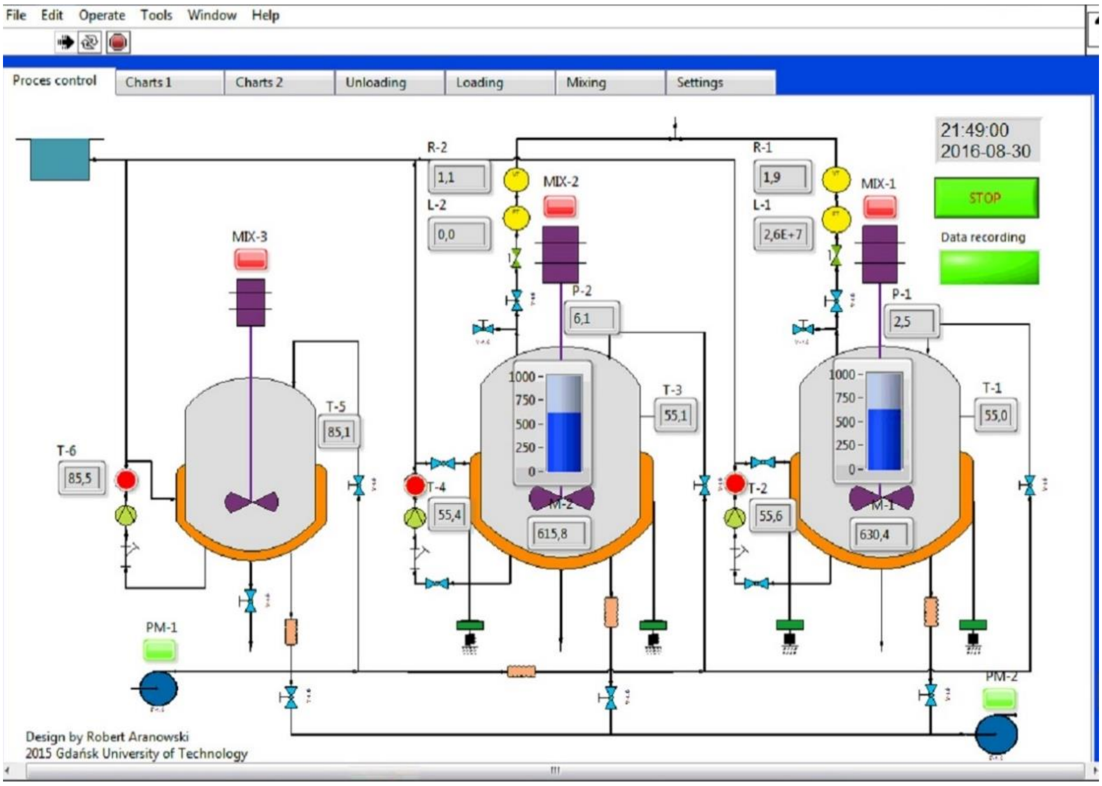

Figure 13. Front panel of the anaerobic fermentation control system

5.2 Results and Discussion

To determine the effect of the bioreactor size on biogas production, three experiments carried out in the 10 dm^3 reactors were selected to be conducted also in 1 000 dm^3 reactors.

5.2.1 Digestion of Cattle Slurry on a Pilot Scale

Figure 44 shows the biogas and biomethane production as also temperature of the bioreactor during the stable phase of a cattle slurry quasi-continuous digestion process in pilot scale (1 000 dm^3). The results of this experiment were used as reference values for the study of co-fermentation of marine biomass. The changes in the intensity of biogas production and temperature of bioreactor caused by cyclic feeding of the biomass to the bioreactor are, in intensity, very similar to experiments conducted in the 10 dm^3 digesters. The process was carried out for a constant OLR (4 kg VS/m^3 day) the same as in the 10 dm^3 bioreactors experiments. The detailed process parameters are presented in Table 12.

The daily average biogas and biomethane production were determined from linear correlation of cumulative data (Figure 45) and were about 667.24 and 343.34 STP dm^3/day respectively, and were a little higher than the analogous measurements conducted in laboratory scale and 10 dm^3 digesters. The amount of produced biogas was about 1.11 STP dm^3 per 1 dm^3 of the biomass volume per day.

Table 12. Parameters of quasi-continuous cattle slurry digestion in 1 000 dm^3 reactors

No	Parameter	Value	Units
1	The active volume of the bioreactor	600.0	dm^3
2	Hydraulic retention time	30	days
3	Daily feedstock amount	20.430	STP dm^3/day
4	Biogas production	277.7	STP dm^3/kg VS
5	Biomethane production	142.9	STP dm^3/kg VS
6	Daily biogas production	667.24	STP dm^3/day
7	Daily biomethane production	343.34	STP dm^3/day
8	Methane concentration	50.78	%
9	TS of feedstock	12.20	%
10	TS of digestate	8.33	%
11	VS of feedstock	88.44	%
12	VS of digestate	74.31	%
13	OLR	4.00	kg VS/m^3 day

Figure 46 shows changes in the concentration of biomethane, carbon dioxide and hydrogen sulphide. The concentration of methane in the produced biogas was changing during the experiment from about 38% to 61%, same as carbon dioxide from 35% reaching around 60%. Hydrogen sulphide concentration varied in a range from 200 to over 1 100 ppm.

The VFA (Figure 47) of the cattle slurry is high and was about 5 500 mg CH_3COOH/dm^3 and was decreasing during fermentation below 4 500 mg CH_3COOH/dm^3. The alkalinity was also high and was decreasing during digestion, so the ratio of VFA to alkalinity was almost constant on the level about 0.3.

5.2.2 Mix of Algae and Cattle Slurry in Ration 1:3

Figure 49 shows the biogas and biomethane production and also the temperature of the bioreactor during the stable phase in a quasi-continuous digestion process of a mix of algae and cattle slurry (1:3 TS ratio). The changes in the intensity of the biogas production and temperature of the bioreactor, caused by cyclic feeding of the biomass to the bioreactor, are evident. The average values of the biogas and biomethane production rates are marked. The process was carried out for a constant OLR of 4 kg VS/m^3 day. The parameters of the experiment are presented in Table 13.

The daily average biogas and biomethane production were determined from linear correlation of cumulative data (Figure 50) and were about 725.64 and 290.12 STP dm^3/day respectively. The daily average biogas production is about 9% higher than during pure cattle slurry digestion, but the biomethane production was 15% less, which means that the concentration of methane in the biogas obtained from the algae and cattle slurry mixture is lower, (about 40%). The amount of produced biogas per 1 dm^3 of the biomass volume mixture of algae and cattle slurry was 1.21 STP dm^3/dm^3.

Table 13. Parameters of quasi-continuous algae and cattle slurry digestion in 1 000 dm^3 reactors

No	Parameter	Value	Units
1	The active volume of the bioreactor	600.0	dm3
2	Hydraulic retention time	30	days
3	Daily feedstock amount	34.200	STP dm^3/day
4	Biogas production	302.4	STP dm^3/kg VS
5	Biomethane production	120.9	STP dm^3/kg VS
6	Daily biogas production	725.64	STP dm^3/day
7	Daily biomethane production	290.12	STP dm^3/day
8	Methane concentration	42.33	%
9	TS of feedstock	7.87	%
10	TS of digestate	5.81	%
11	VS of feedstock	78.91	%
12	VS of digestate	70.63	%
13	OLR	4.00	kg VS/m^3 day

Figure 51 shows changes in the concentration of biomethane, carbon dioxide and hydrogen sulphide. The concentration of methane in the produced biogas was rising for the most of the time from 40% to 60% with a short drop during the experiment. Carbon dioxide levels varied much, starting at 60%, falling to around 35% and then stabilizing to some point at 45%. Hydrogen sulphide concentration varied in a range from below 400 to over 700 ppm.

The VFA (Figure 52) of the mix of algae and cattle slurry is high and was about 4 000 mg CH_3COOH/dm^3 and was decreasing during fermentation below 2 000 mg CH_3COOH/dm^3. The alkalinity was also high and was decreasing during digestion and the ratio of VFA to alkalinity was slightly decreasing through the process from 0.35 to 0.18.

5.2.3 Hydrothermal Pretreatment at 95°C

Figure 59 shows the biogas and biomethane production and also temperature of the bioreactor during the stable phase in a quasi-continuous digestion process of a mixture of hydrothermally pretreated at 95°C for 60 minutes algae and cattle manure in ratio 1:3. The changes in the intensity of biogas production and the temperature of bioreactor, caused by cyclic feeding of the biomass to the bioreactor, are evident. The average values of the biogas and biomethane production rates are also marked. The process was carried out for a constant OLR of 4 kg VS/m^3 day. The parameters of the experiment are presented in Table 14.

The daily average biogas and biomethane production were determined from linear correlation of cumulative data (Figure 60) and ware about 779.89 and 456.54 STP dm^3/day respectively, which seems to be a good result for medium loaded digesters. It is assumed that the amount of produced biogas should be about 1 STP dm^3 per 1 dm^3 of the biomass volume. In the mix of hydrothermally pretreated at 95°C for 60 minutes algae and cattle manure digestion experiment, this value was about 1.3 STP dm^3/dm^3.

Table 14. Parameters of quasi-continuous mixture of hydrothermally pretreated at 95°C for 60 minute algae and cattle manure (ratio 1:3) digestion

No	Parameter	Value	Units
1	The active volume of the bioreactor	600.0	dm^3
2	Hydraulic retention time	30	days
3	Daily feedstock amount	40.900	STP dm^3/day
4	Biogas production	324.8	STP dm^3/kg VS
5	Biomethane production	190.2	STP dm^3/kg VS
6	Daily biogas production	779.89	STP dm^3/day
7	Daily biomethane production	456.54	STP dm^3/day
8	Methane concentration	57.91	%
9	TS of feedstock	6.32	%
10	TS of digestate	3.47	%
11	VS of feedstock	82.93	%
12	VS of digestate	74.21	%
13	OLR	4.00	kg VS/m^3 day

Figure 61 shows changes in the concentration of biomethane, carbon dioxide and hydrogen sulphide. The concentration of methane in the produced biogas was slightly rising during the experiment from around 40-45% to 60% level, while carbon dioxide dropped at the beginning from 55% to 40% and became stable at this level. The hydrogen sulphide concentration varied much more, in a range from 400 to over 800 ppm.

The VFA (Figure 62) of the mix of hydrothermally pretreated at 95°C for 60 minutes algae and cattle slurry is high and was about 2 700 mg CH_3COOH/dm^3 and was decreasing during fermentation below 1 800 mg CH_3COOH/dm^3. The alkalinity was also high and was decreasing during digestion and the ratio of VFA to alkalinity was stable through the process at around 0.3 level.

5.2.4 Combined Pretreatment - Mechanical and Acid Hydrolysis

Figure 54 shows the biogas and biomethane production and also the temperature of the bioreactor during the stable phase in a quasi-continuous digestion process of a mixture of combined mechanical and acid pretreatment of algae and cattle manure in ratio 1:3. The changes in the intensity of the biogas production and temperature of bioreactor, caused by cyclic feeding of the biomass to the bioreactor, are evident. The average values of the biogas and biomethane production rates were also marked. The process was carried out for a constant OLR of 4 kg VS/m^3 day. The parameters of the experiment are presented in Table 15.

The daily average biogas and biomethane production were determined from linear correlation of cumulative data (Figure 55) and ware about 842.62 and 517.47 STP dm^3/day respectively, which seems to be a good result for medium loaded digesters. It is assumed that the amount of produced biogas should be about 1 STP dm^3 per 1 dm^3 of the biomass volume. In the mix of combined mechanical and acid pretreatment algae and cattle manure digestion experiment, this value was above the assumed value and reached about 1.4 STP dm^3/dm^3.

Table 15. Parameters of quasi-continuous mixture of combined mechanical and acid pretreatment of algae and cattle manure (ratio 1:3) digestion

No	Parameter	Value	Units
1	The active volume of the bioreactor	600.0	dm^3
2	Hydraulic retention time	30	days
3	Daily feedstock amount	44.400	STP dm^3/day
4	Biogas production	350.9	STP dm^3/kg VS
5	Biomethane production	215.5	STP dm^3/kg VS
6	Daily biogas production	842.62	STP dm^3/day
7	Daily biomethane production	517.47	STP dm^3/day
8	Methane concentration	60.36	%
9	TS of feedstock	6.01	%
10	TS of digestate	3.11	%
11	VS of feedstock	81.80	%
12	VS of digestate	73.79	%
13	OLR	4.00	kg VS/m^3 day

Figure 56 shows changes in the concentration of biomethane, carbon dioxide and hydrogen sulphide. The concentration of methane in the produced biogas was rising during the experiment from 50% to 61%, while carbon dioxide was decreasing from 42% to 35%. The hydrogen sulphide concentration varied less than typically, in a range from 500 to over 700 ppm.

The VFA (Figure 57) of the mix of combined mechanical and acid pretreatment algae and cattle slurry were stable through the experiment at 2 000 mg CH_3COOH/dm^3 level. The alkalinity was relatively high and was stable during digestion so the ratio of VFA to alkalinity did not vary and maintained at 0.3 level.

Summary

The biomethane potential measurements test is an established system for the determination of the biogas potential of wide organic materials. Many publications show that the small BPM scale affects the measurement error especially because of the heterogeneity of marine biomass and the different type of inoculum used in the measurements.

Many researchers made an attempt to estimate the BPM based on measurements of physical and chemical properties of biomass, such as total solids and volatile solids, chemical biomass composition, total organic carbon and chemical oxygen demand, but the obtained results, depending on the type of biomass and the method used, differed by up to 70% [20,21].

As shown in Table 16, the differences in the values of biogas production during digestion of cattle slurry experiments carried out on various size of bioreactors reach 31% in the case of biogas production and 25% in the case of biomethane. The average concentration of methane and carbon dioxide in biogas increased by 17% in the experiment conducted in 10 dm^3 bioreactors and by 16% for 1 000 dm^3.

Table 16. Results of cattle slurry digestion in various size of bioreactors

No.	Parameter	Units	1 dm^3	10 dm^3	1 000 dm^3
1	The active volume of the bioreactor	dm^3	0.1	7.00	600.00
2	Hydraulic retention time	Days	30.00	30.00	30.00
3	Daily feedstock amount	STP dm^3/day	-	0.24	20.43
4	Biogas production	STP dm^3/kg VS	211.18	266.60	277.70
5	Biomethane production	STP dm^3/kg VS	114.32	137.20	142.90
6	Daily biogas production	STP dm^3/day	-	7.47	667.24
7	Daily biomethane production	STP dm^3/day	-	3.74	343.34
8	Methane concentration	%	43.91	51.50	50.78

In the case of mixture of algae and cattle slurry co-digestion (Table 17), the production of biogas in 1 000 dm^3 bioreactors was almost the same as in the batch tests, and in the case of 10 dm^3 reactors it decreased by 10%. Biomethane yield in the bioreactors capacity of 10 dm^3 decreased by 10% and in the case of 1 000 dm^3 it increased by 20%. In 10 dm^3 bioreactors, the average methane concentration decreased by 5% and in the case of 1 000 dm^3 it increased by 21%.

Table 17. Results of co- digestion of mixture of algae and cattle slurry in various size of bioreactors

No.	Parameter	Units	1 dm^3	10 dm^3	1000 dm^3
1	The active volume of the bioreactor	dm^3	0.1	7.00	600.00
2	Hydraulic retention time	Days	30.00	30.00	30.00
3	Daily feedstock amount	STP dm^3/day	-	0.40	34.20
4	Biogas production	STP dm^3/kg VS	303.28	272.10	302.40
5	Biomethane production	STP dm^3/kg VS	100.74	90.70	120.90
6	Daily biogas production	STP dm^3/day	-	7.62	725.64

No.	Parameter	Units	1 dm^3	10 dm^3	1000 dm^3
7	Daily biomethane production	STP dm^3/day	-	2.54	290.12
8	Methane concentration	%	35.10	33.32	42.33

Also, in the case of fermentation experiments of a mixture of algae and cattle slurry mechanically disintegrated and acidic hydrolysed (Table 18), differences in the average amount of biogas and biomethane produced are visible at the level of 20 and 24%, respectively, for 1 000 dm^3 reactors.

Table 18. Results of co-digestion of mechanically and acidic pretreated mixture of algae and cattle slurry in various size of bioreactors

No.	Parameter	Units	1 dm^3	10 dm^3	1 000 dm^3
1	The active volume of the bioreactor	dm^3	0.1	7.00	600.00
2	Hydraulic retention time	Days	30.00	30.00	30.00
3	Daily feedstock amount	STP dm^3/day	-	0.52	44.40
4	Biogas production	STP dm^3/kg VS	292.45	292.40	350.90
5	Biomethane production	STP dm^3/kg VS	174.06	179.60	215.50
6	Daily biogas production	STP dm^3/day	-	8.19	842.62
7	Daily biomethane production	STP dm^3/day	-	5.03	517.47
8	Methane concentration	%	59.52	61.41	60.36

Tables 19 - 21 present a comparison of the other results of the influence of pretreatment methods on the production and composition of biogas. Only in the case of thermalizes at 95°C during 24 hours, a decrease in biogas production was observed in bioreactors with a volume of 10 dm^3 compared to batch tests. In other cases, higher yields of biogas and biomethane were observed for quasi-continuous experiments carried out in bioreactors with a volume of 10 and 1 000 dm^3.

Table 19. Results of co-digestion thermally pretreated mixture of algae and cattle slurry (temperature 95°C, 60 minutes) of in various size of bioreactors

No.	Parameter	Units	1 dm^3	10 dm^3	1 000 dm^3
1	The active volume of the bioreactor	dm^3	0.1	7.00	600.00
2	Hydraulic retention time	Days	30.00	30.00	30.00
3	Daily feedstock amount	STP dm^3/day	-	0.48	40.90
4	Biogas production	STP dm^3/kg VS	250.55	298.80	324.80
5	Biomethane production	STP dm3/kg VS	152.12	174.90	190.20
6	Daily biogas production	STP dm^3/day	-	8.37	779.89
7	Daily biomethane production	STP dm^3/day	-	4.90	456.54
8	Methane concentration	%	61.23	58.54	57.91

Table 20. Results of co-digestion thermally pretreated mixture of algae and cattle slurry (temperature 95°C, 24 h) of in various size of bioreactors

No.	Parameter	Units	1 dm³	10 dm³	1 000 dm³
1	The active volume of the bioreactor	dm³	0.1	7.00	-
2	Hydraulic retention time	Days	30.00	30.00	-
3	Daily feedstock amount	STP dm³/day	-	0.48	-
4	Biogas production	STP dm³/kg VS	281.79	278.10	-
5	Biomethane production	STP dm³/kg VS	164.94	164.90	-
6	Daily biogas production	STP dm³/day	-	7.25	-
7	Daily biomethane production	STP dm³/day	-	4.30	-
8	Methane concentration	%	58.53	59.30	-

Table 21. Results of co-digestion thermally pretreated mixture of algae and cattle slurry (temperature 160°C, 30 minutes) of in various size of bioreactors

No.	Parameter	Units	1 dm³	10 dm³	1 000 dm³
1	The active volume of the bioreactor	dm³	0.1	7.00	-
2	Hydraulic retention time	Days	30.00	30.00	-
3	Daily feedstock amount	STP dm³/day	-	0.43	-
4	Biogas production	STP dm³/kg VS	322.06	441.90	-
5	Biomethane production	STP dm³/kg VS	174.57	218.20	-
6	Daily biogas production	STP dm³/day	-	12.38	-
7	Daily biomethane production	STP dm³/day	-	6.11	-
8	Methane concentration	%	54.95	49.38	-

A standard method for the transfer of batch test to depict digestion process behaviour of continuous tests or full-scale systems is still under development. The results of conducted batch tests and in quasi-continuous tests of marine biomass co-digestion in 10 and 1 000 dm³ bioreactor show significant deviation. These results are difficult to interpret even if the same inoculum and biomass composition were used. For this reason, biomethane potential measurements should be carried out under conditions closest to the full scale, not only because of the similarity of the process, but also because of the smallest influence of disturbing the digestion process with external factors.

Conclusion

The measurements of the biogas potential of mixtures of cattle slurry and marine biomass obtained from the beach in Gdańsk-Brzeźno show that seaweed is a difficult substrate for biogas production and requiring special pretreatment methods to increase the bioavailability of the ingredients.

It should be noticed that mono-substrate digestion of marine biomass is difficult to conduct while co-fermentation with other substrates (cattle slurry) is a stable process up to 25% by mass of marine biomass content in the mixture.

The removal of sand is the most important step in the pretreatment of marine biomass. High sand contents in the feedstock may adversely affect the durability of bioreactors and other biogas plant devices as a result of abrasion.

The conducted sand removal experiments confirmed positive effect of decreasing pH on sand removal. It was also found that the comminution of the biomass before the sand separation process reduces the removal efficiency.

The highest production of biomethane was obtained for the co-fermentation of a mixture of algae and cattle slurry thermally pretreated at a temperature of 160°C for 30 minutes. The increase of thermalizes time at 160°C results, in the batch tests, in a decrease of the amount of produced biomethane.

Mechanical pretreatment results in the lowest increasing biogas and biomethane yield in the range from 4% to 24%.

Biomethane yield in case of the acid pretreatment increases the biomethane yield in comparison to untreated seaweed from 25% to 33%.

The hybrid method increases the biomethane yield from 36% to 64% with an average value about 51%

The results obtained in the quasi-continuous measurements of marine biomass digestion are 20% to 37% higher than the values obtained in the batch tests.

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6 Appendix

6.1 Quasi-continuous digestion experiments in 10 dm³ bioreactors

6.1.1 Cattle Manure

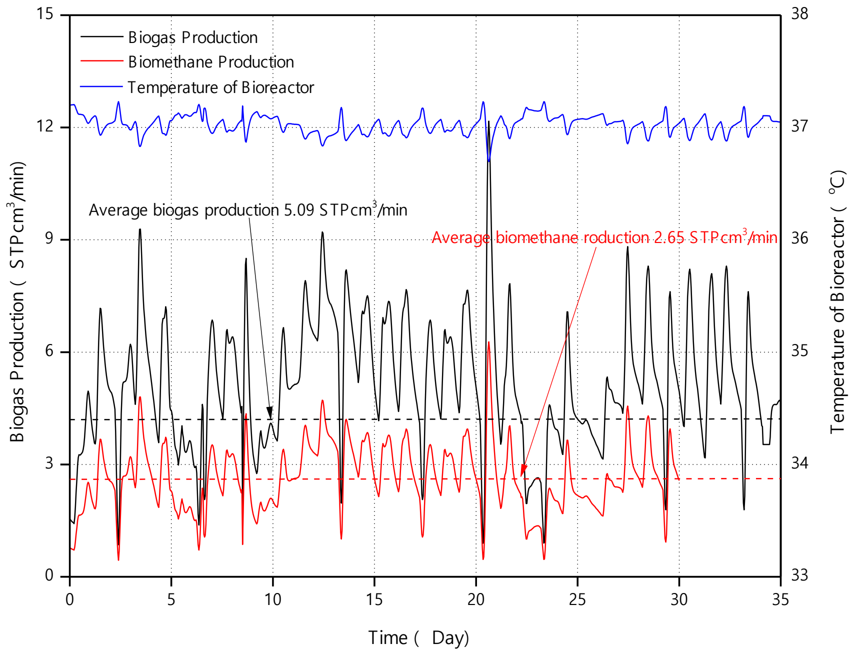

Figure 14. Production of biogas and biomethane and temperature changes in the 10 dm³ bioreactor during cattle manure mesophilic digestion

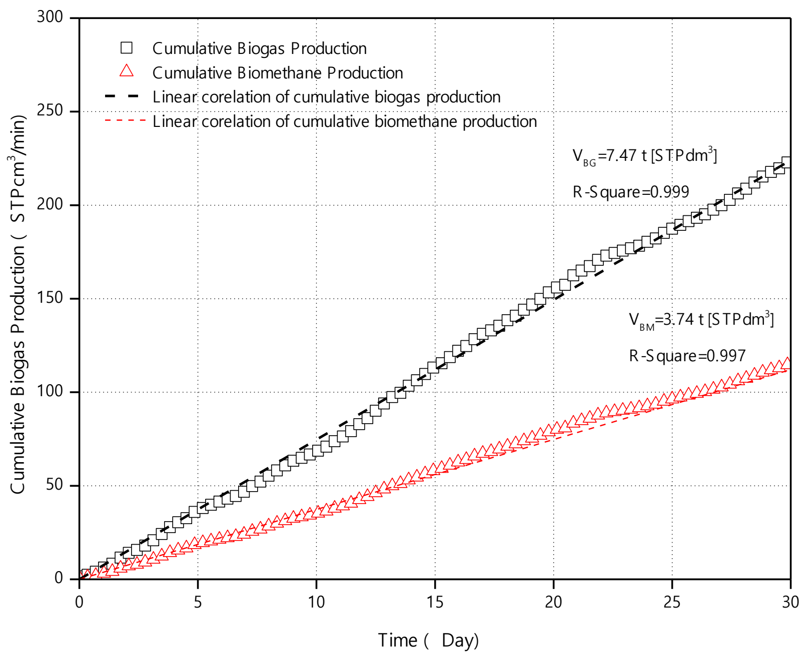

Figure 15. Cumulative biogas and biometan production in the 10 dm³ bioreactor during cattle manure mesophilic digestion

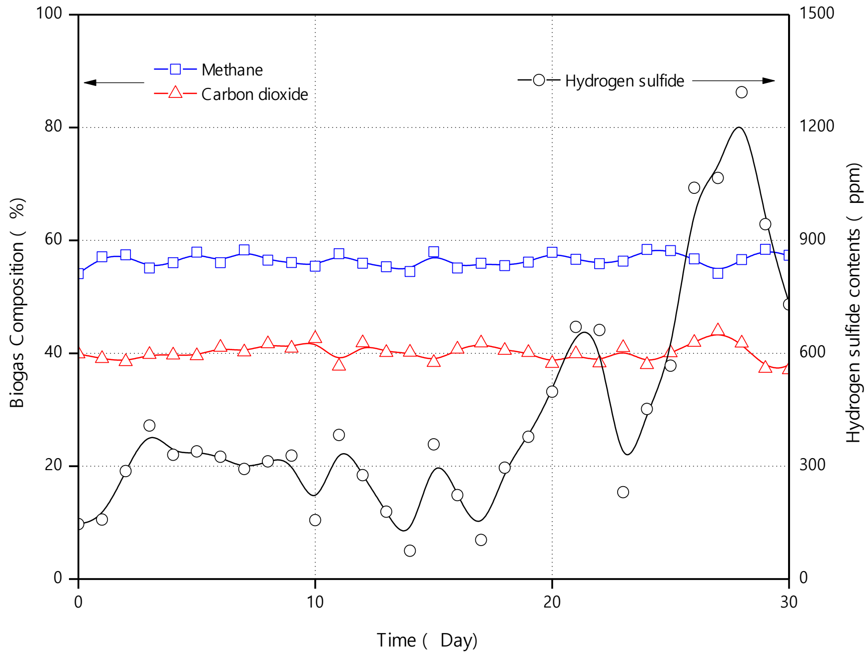

Figure 16. Concentration of methane. carbon dioxide and hydrogen sulphide during cattle manure mesophilic digestion in the 10 dm^3 bioreactor

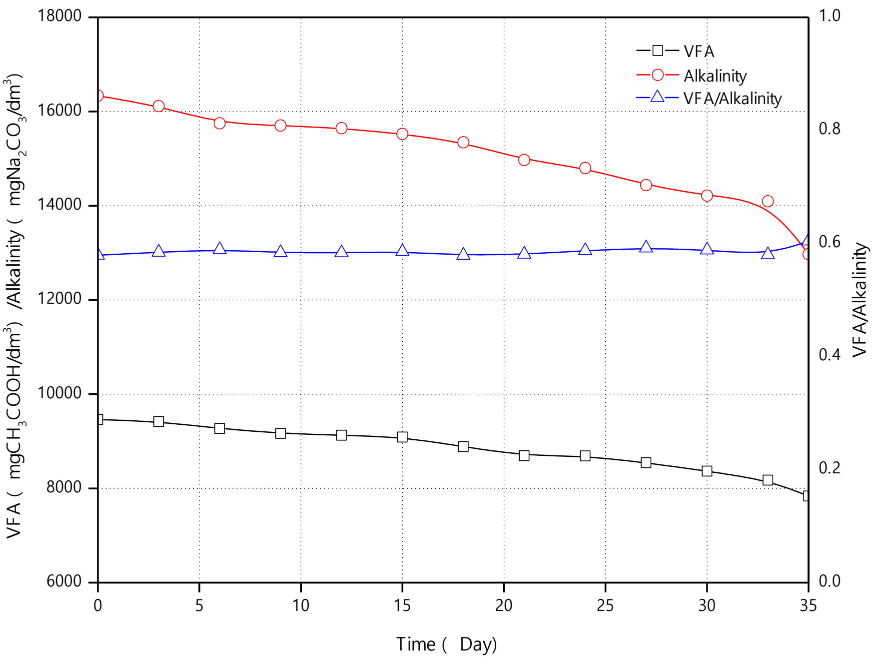

Figure 17. Changing of VFA. alkalinity VFA/alkalinity ratio during cattle manure mesophilic digestion in the 10 dm^3 bioreactor

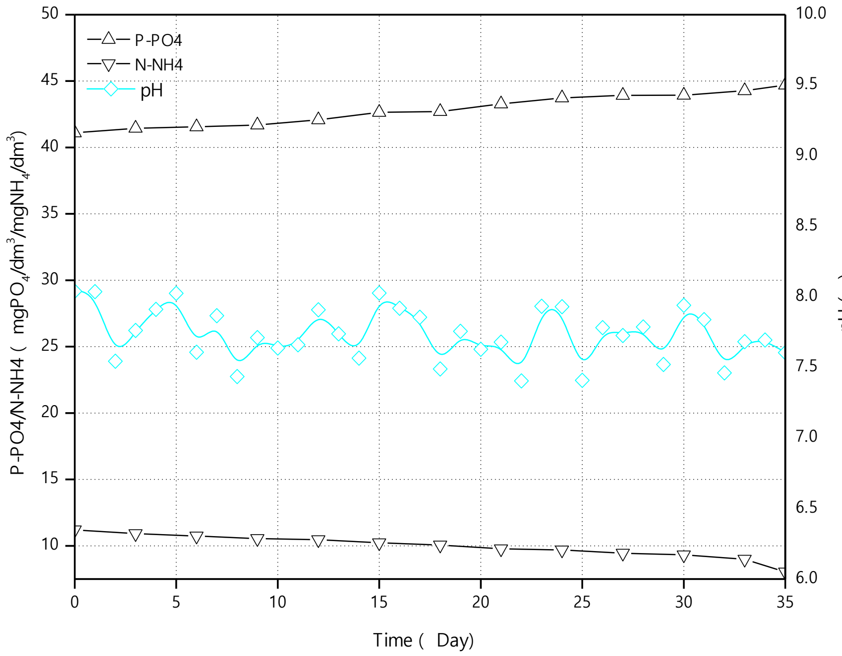

Figure 18. Change of phosphorus and ammonia concertation and pH during cattle manure mesophilic digestion in the 10 dm^3 bioreactor

6.1.2 Mixture of Algae and Cattle Manure in Ratio 1:3

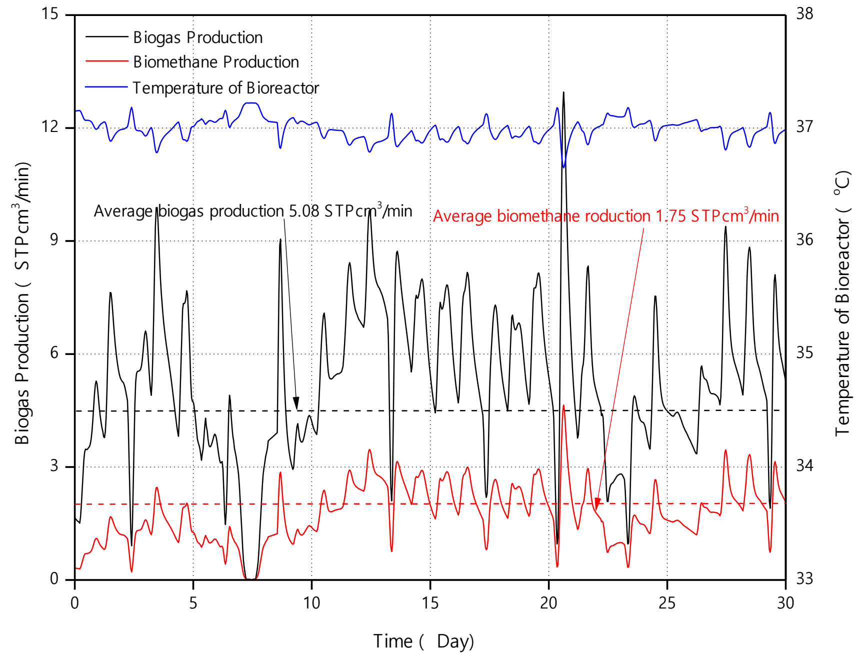

Figure 19. Production of biogas and biomethane and temperature changes in the 10 dm^3 bioreactor during mesophilic digestion of algae mix and cattle manure in ration 1:3

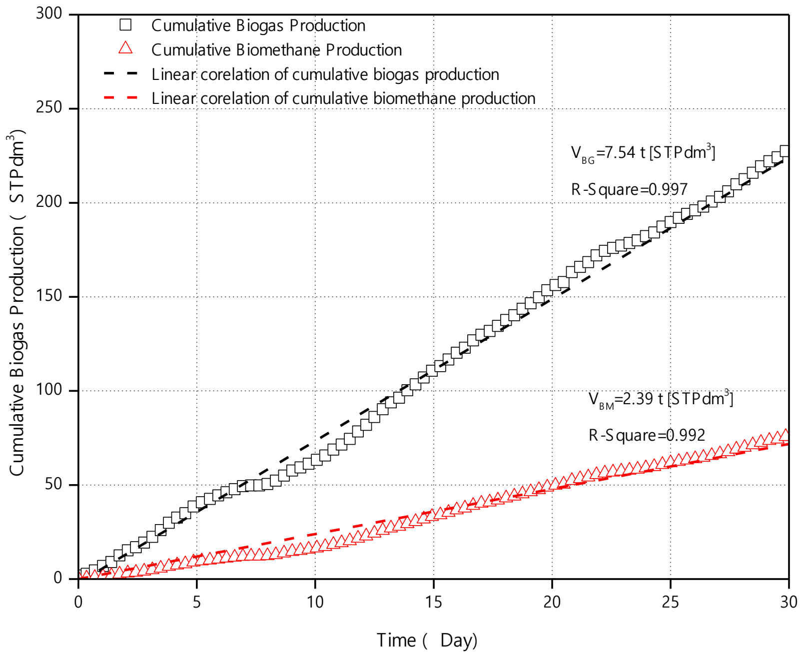

Figure 20. Cumulative biogas and biometan production in the 10 dm^3 bioreactor during mesophilic digestion of algae mix and cattle manure in ration 1:3

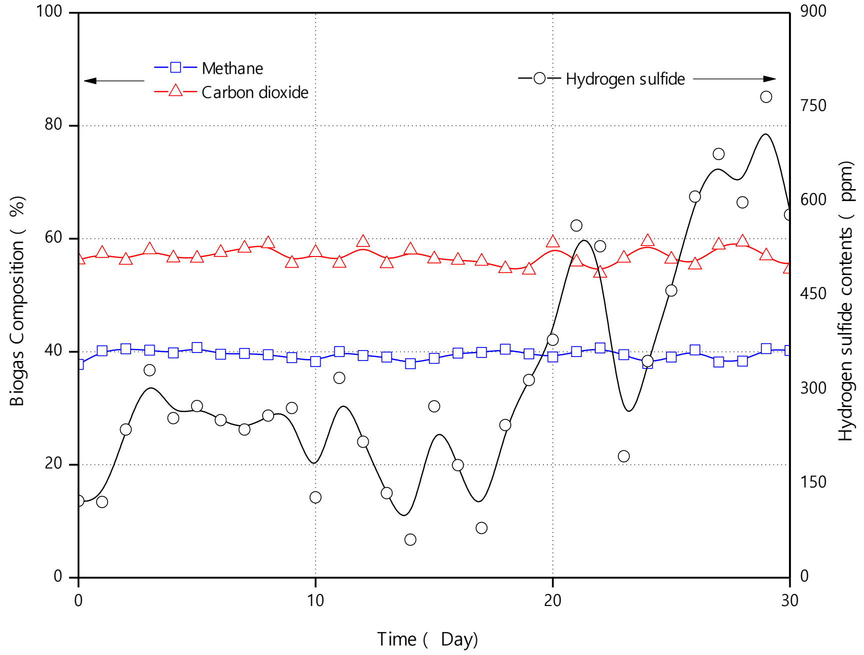

Figure 21. Concentration of bio-methane, carbon dioxide and hydrogen sulphide during mesophilic digestion of algae mix and cattle manure in ration 1:3

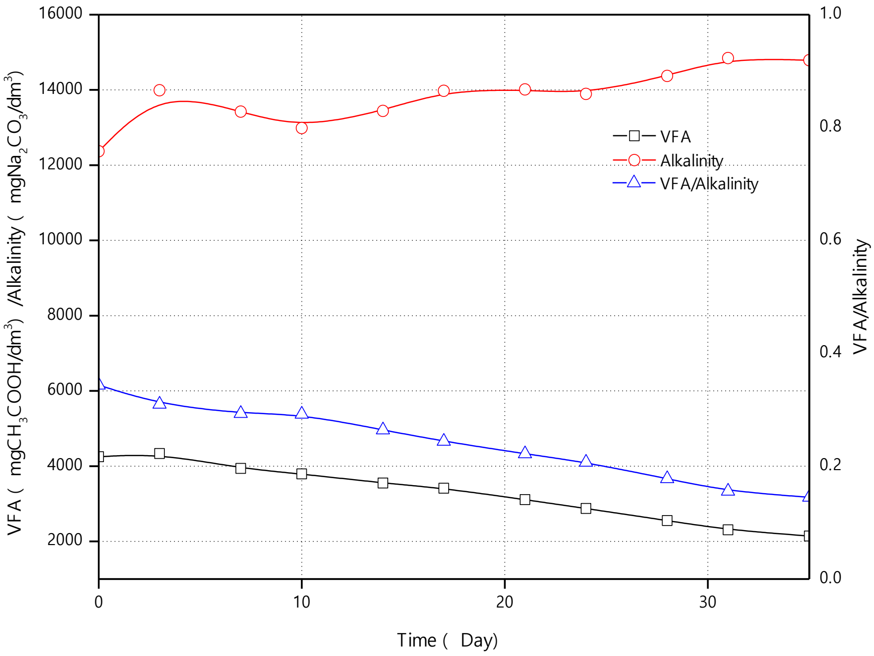

Figure 22. Changing of VFA. alkalinity VFA/alkalinity ratio during mesophilic digestion of algae mix and cattle manure in ration 1:3

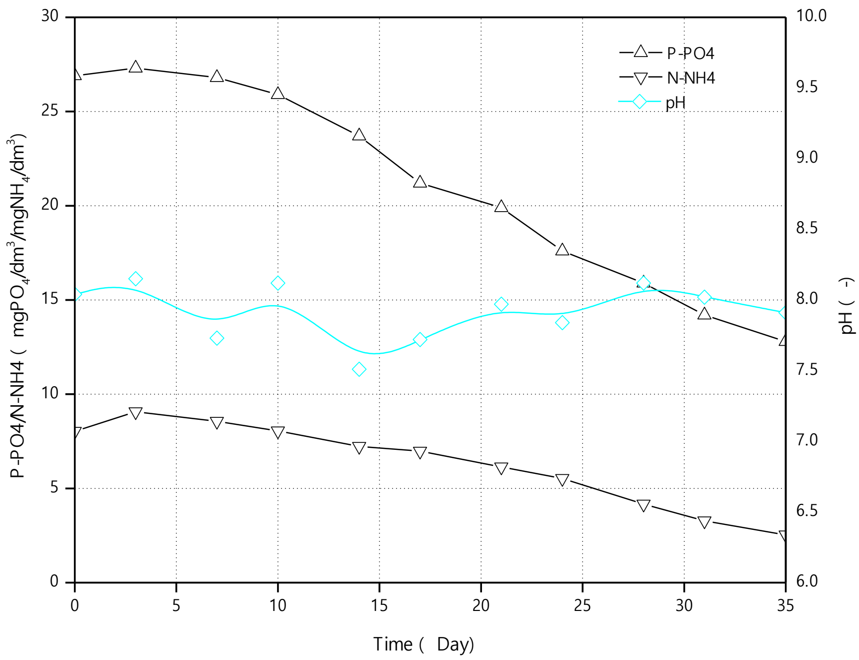

Figure 23. Change of phosphorus and ammonia concertation and pH during algae mix and cattle manure in ration 1:3 of mesophilic digestion in the 10 dm^3 bioreactor

6.1.3 Mixture of Combined Mechanical and Acid Pretreatment of Algae and Cattle Manure in Ratio 1:3

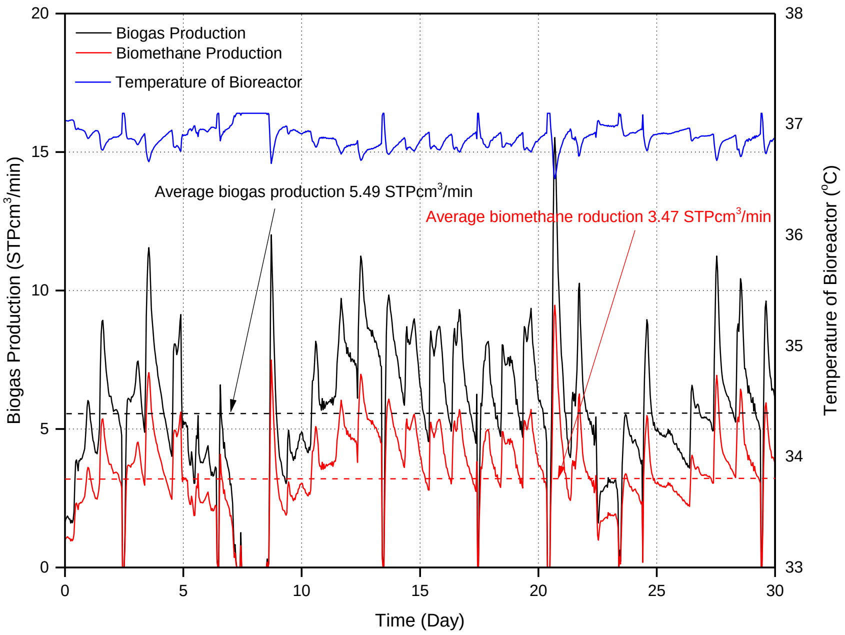

Figure 24. Production of biogas and biomethane and temperature changes in the 10 dm^3 bioreactor during mixture of combined mechanical and acid pretreatment of algae and cattle manure in ratio 1:3 mesophilic digestion

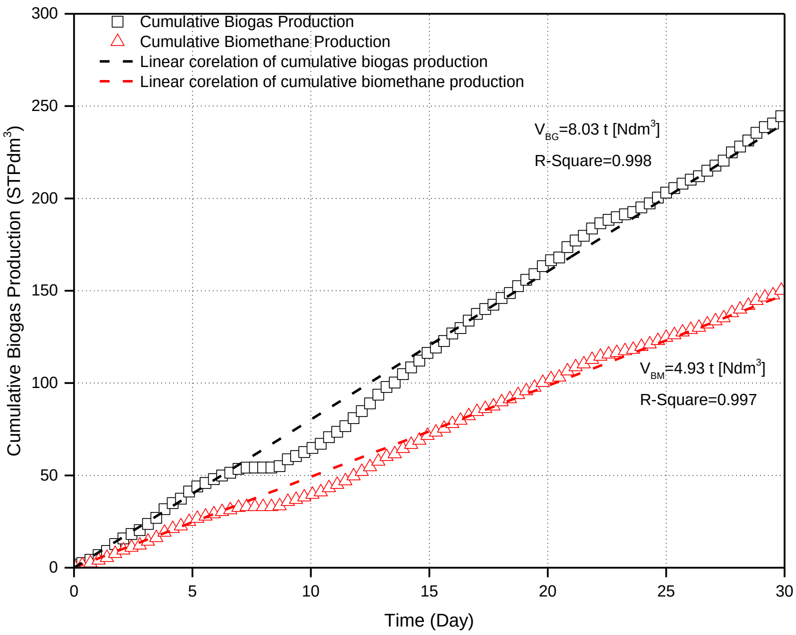

Figure 25. Cumulative biogas and biomethane production in the 10 dm^3 bioreactor during mesophilic digestion of mixture of combined mechanical and acid pretreatment of algae and cattle manure in ratio 1:3

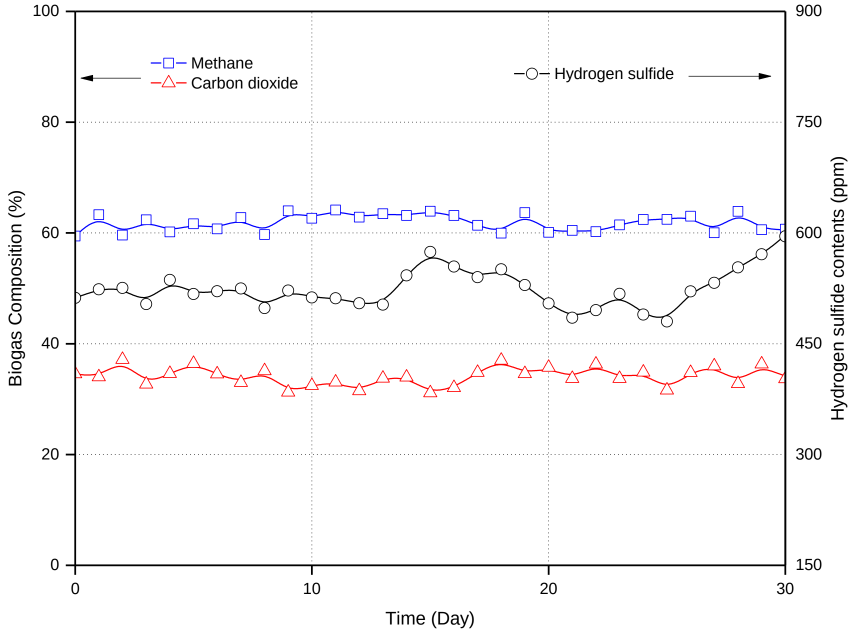

Figure 26. Concentration of methane. carbon dioxide and hydrogen sulphide during mesophilic digestion of mixture of combined mechanical and acid pretreatment of algae and cattle manure in ratio 1:3

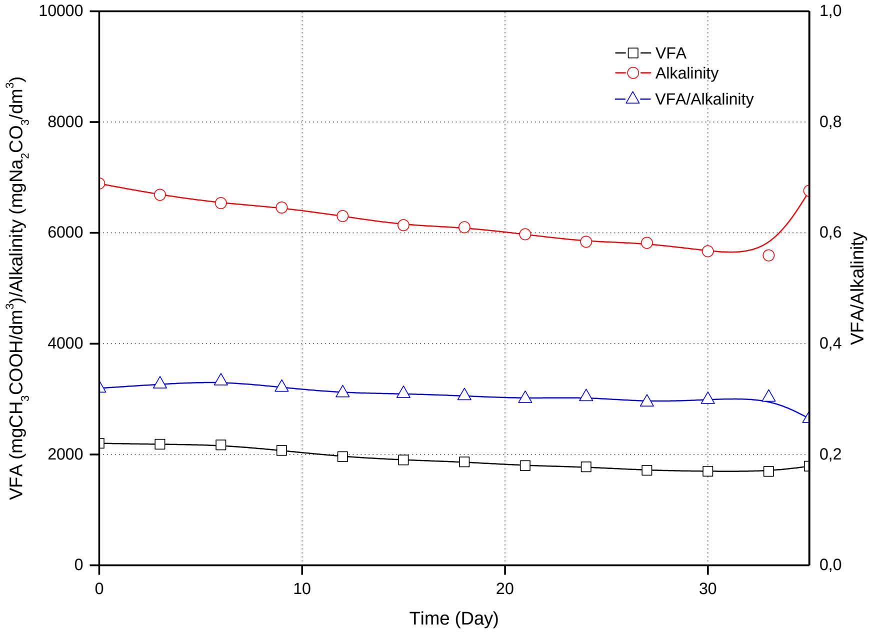

Figure 27. Changing of VFA. alkalinity VFA/alkalinity ratio during mesophilic digestion of combined mechanical and acid pretreatment of algae and cattle manure in ratio 1:3

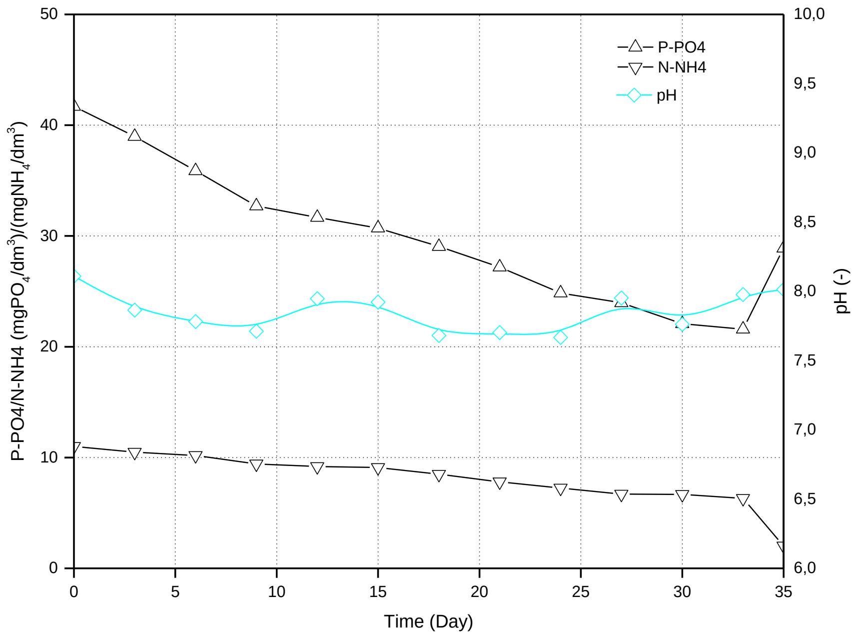

Figure 28. Change of phosphorus and ammonia concertation and pH during mixture of combined mechanical and acid pretreatment of algae and cattle manure in ratio 1:3 mesophilic digestion in the 10 dm^3 bioreactor

6.1.4 Mixture of Hydrothermally Pretreated at 95°C for 60 minutes Algae and Cattle Manure in Ratio 1:3

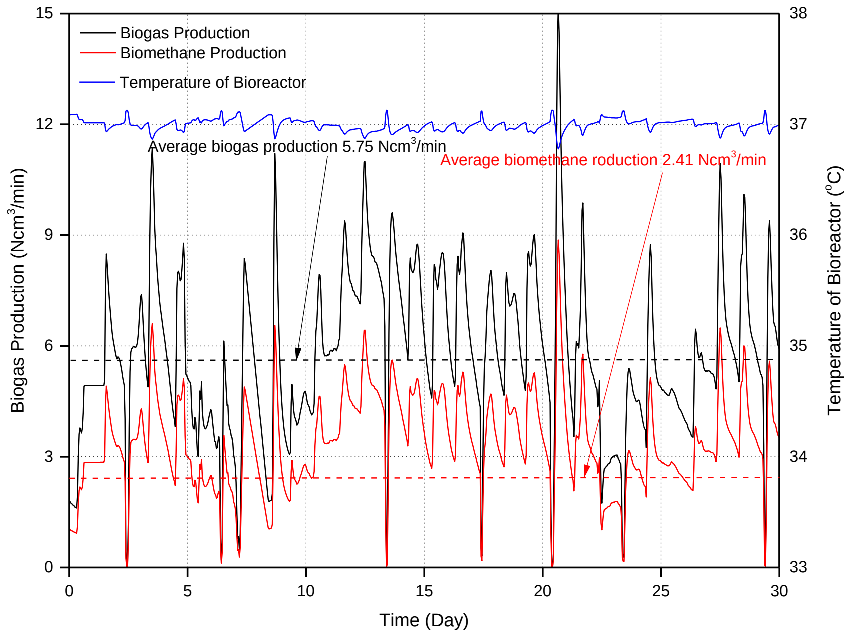

Figure 29. Production of biogas and biomethane and temperature changes in the 10 dm^3 bioreactor during mixture of hydrothermally pretreated at 95°C for 60 minutes algae and cattle manure in ratio 1:3 mesophilic digestion

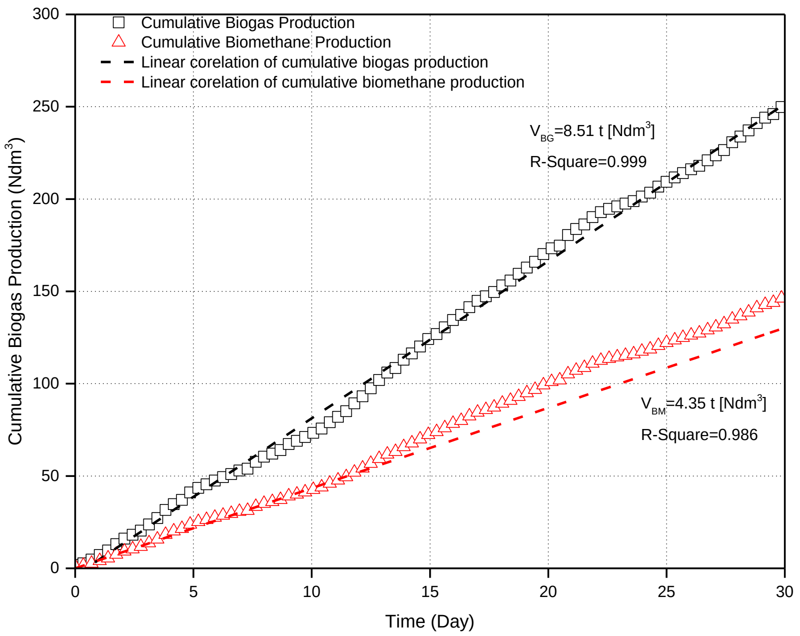

Figure 30. Cumulative biogas and biometan production in the 10 dm^3 bioreactor during mesophilic digestion of mixture of hydrothermally pretreated at 95°C for 60 minutes algae and cattle manure in ratio 1:3

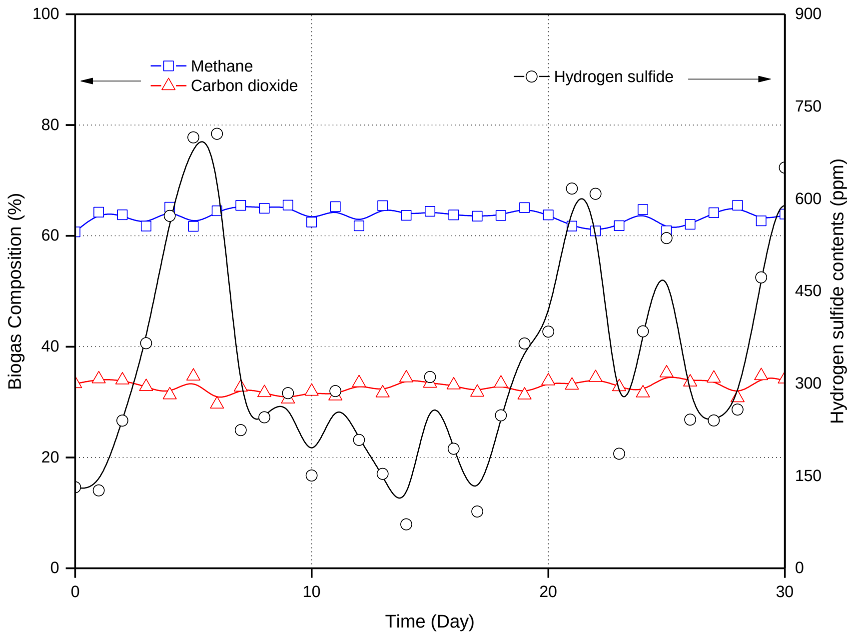

Figure 31. Concentration of methane. carbon dioxide and hydrogen sulphide during mesophilic digestion of mixture of hydrothermally pretreated at 95°C for 60 minutes algae and cattle manure in ratio 1:3

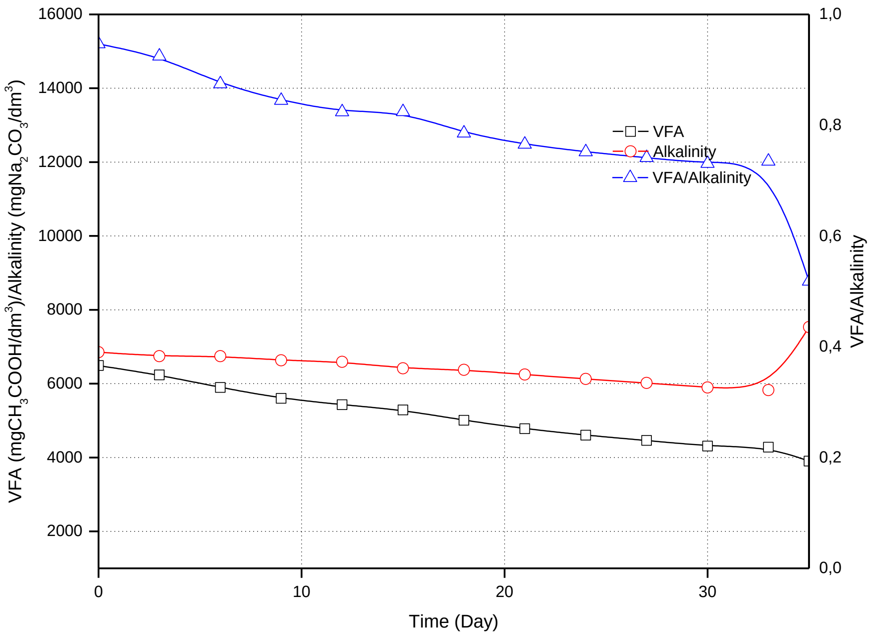

Figure 32. Changing of VFA. alkalinity VFA/alkalinity ratio during mesophilic digestion of mixture of hydrothermally pretreated at 95°C for 60 minutes algae and cattle manure in ratio 1:3

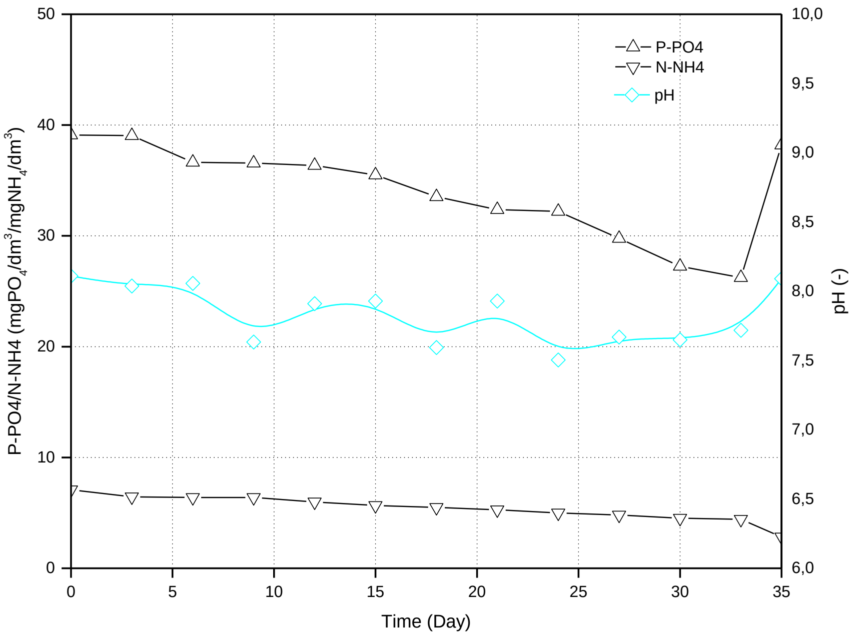

Figure 33. Change of phosphorus and ammonia concertation and pH during mixture of hydrothermally pretreated at 95°C for 60 minutes algae and cattle manure in ratio 1:3 of mesophilic digestion in the 10 dm^3 bioreactor

6.1.5 Mixture of Hydrothermally Pretreated at 95°C for 24 hours Algae and Cattle Manure in Ratio 1:3

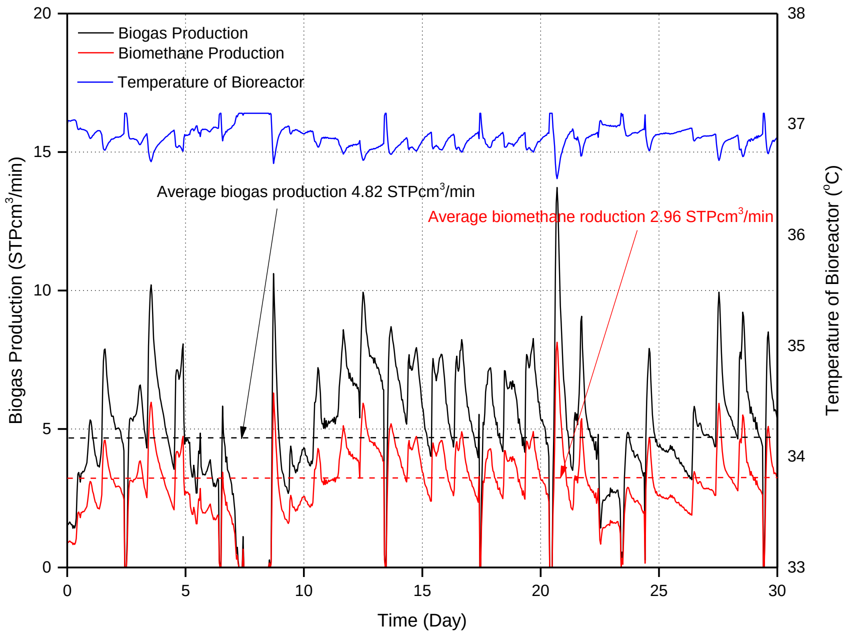

Figure 34. Production of biogas and biomethane and temperature changes in the 10 dm³ bioreactor during mixture of hydrothermally pretreated at 95°C for 24 hours algae and cattle manure in ratio 1:3 mesophilic digestion

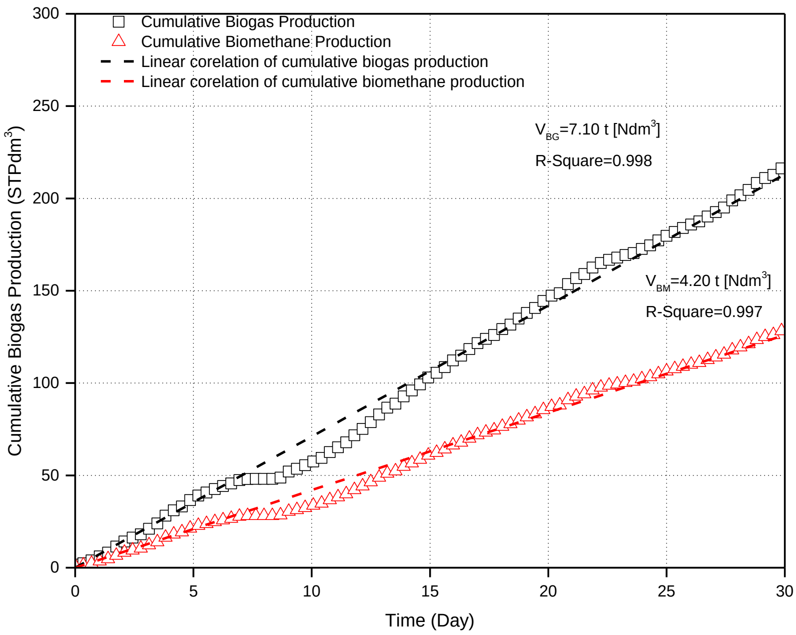

Figure 35. Cumulative biogas and biometan production in the 10 dm³ bioreactor during mesophilic digestion of mixture of hydrothermally pretreated at 95°C for 24 hours algae and cattle manure in ratio 1:3

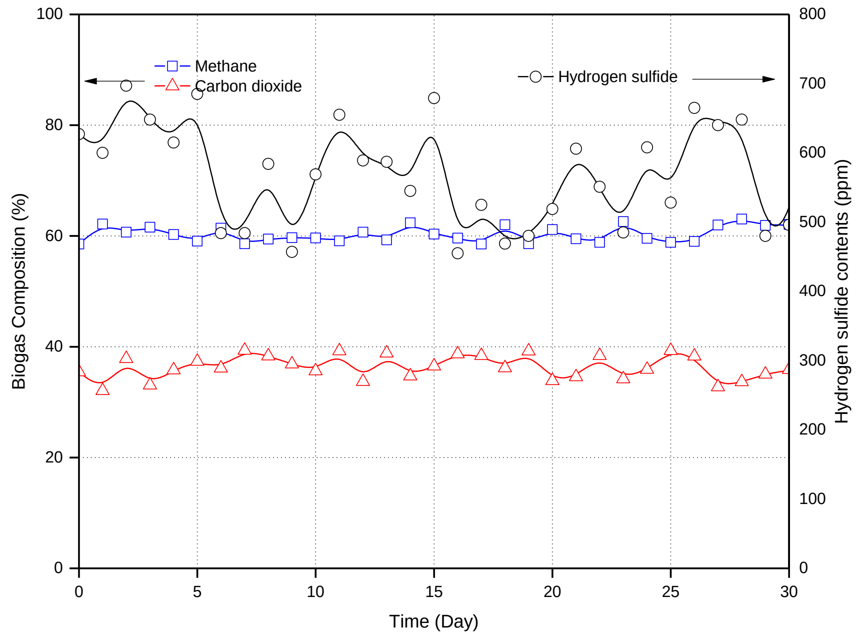

Figure 36. Concentration of methane. carbon dioxide and hydrogen sulphide during mesophilic digestion of mixture of hydrothermally pretreated at 95°C for 24 hours algae and cattle manure in ratio 1:3

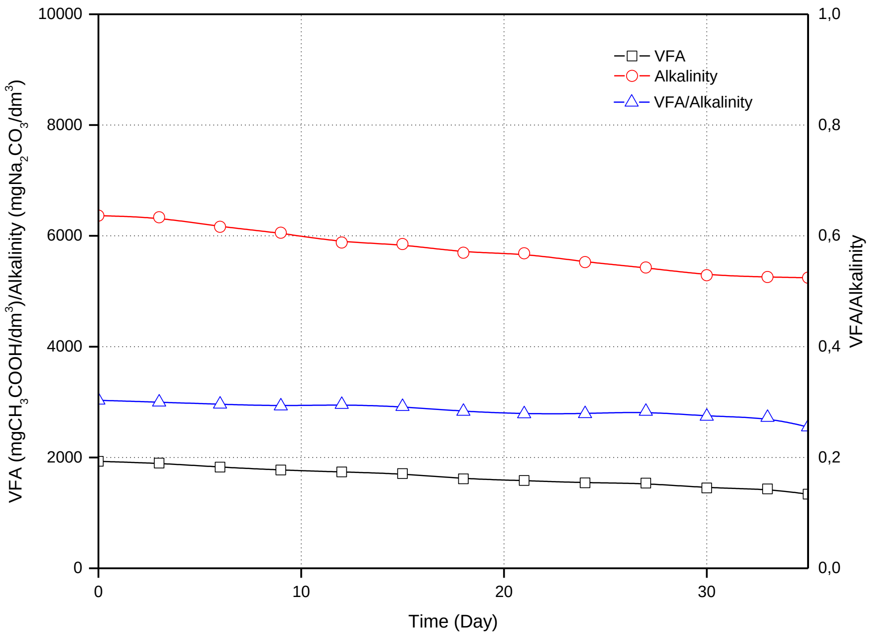

Figure 37. Changing of VFA. alkalinity VFA/alkalinity ratio during mesophilic digestion of mixture of hydrothermally pretreated at 95°C for 24 hours algae and cattle manure in ratio 1:3

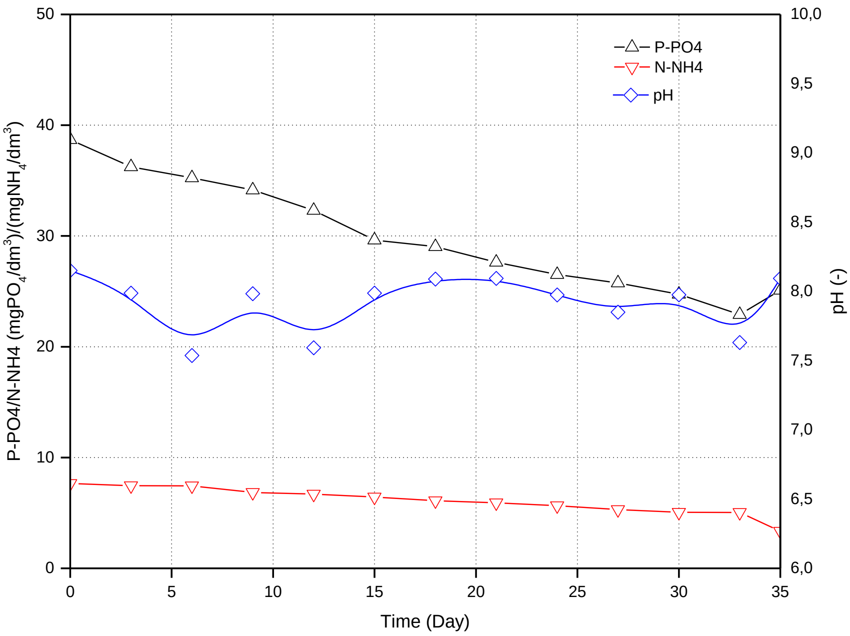

Figure 38. Change of phosphorus and ammonia concertation and pH during mixture of hydrothermally pretreated at 95°C for 24 hours algae and cattle manure in ratio 1:3 of mesophilic digestion in the 10 dm³ bioreactor

6.1.6 Mixture of Hydrothermally Pretreated at 160°C for 30 minutes Algae and Cattle Manure in Ratio 1:3

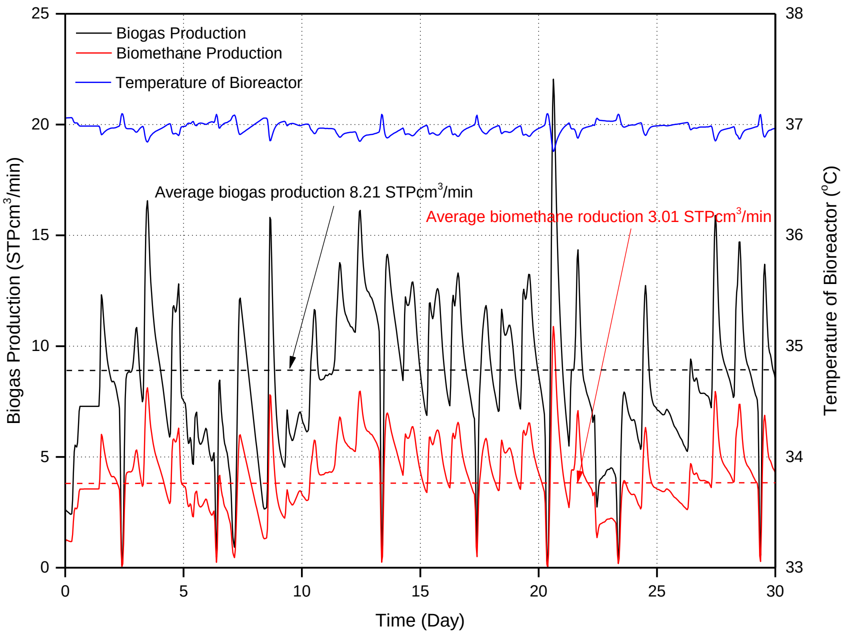

Figure 39. Production of biogas and biomethane and temperature changes in the 10 dm³ bioreactor during mixture of hydrothermally pretreated at 160°C for 30 minutes algae and cattle manure in ratio 1:3 mesophilic digestion

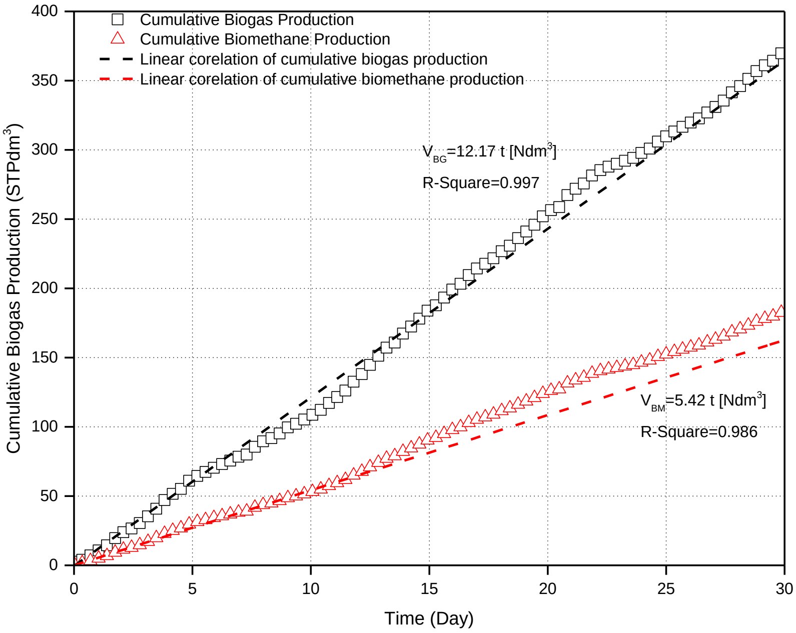

Figure 40. Cumulative biogas and biometan production in the 10 dm^3 bioreactor during mesophilic digestion of mixture of hydrothermally pretreated at 160°C for 30 minutes algae and cattle manure in ratio 1:3

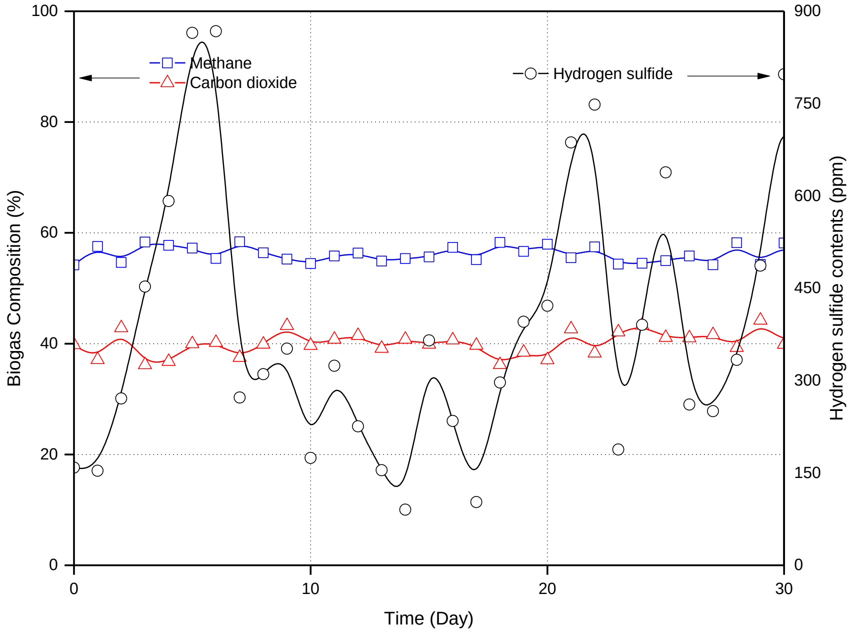

Figure 41. Concentration of methane. carbon dioxide and hydrogen sulphide during mesophilic digestion of mixture of hydrothermally pretreated at 160°C for 30 minutes algae and cattle manure in ratio 1:3

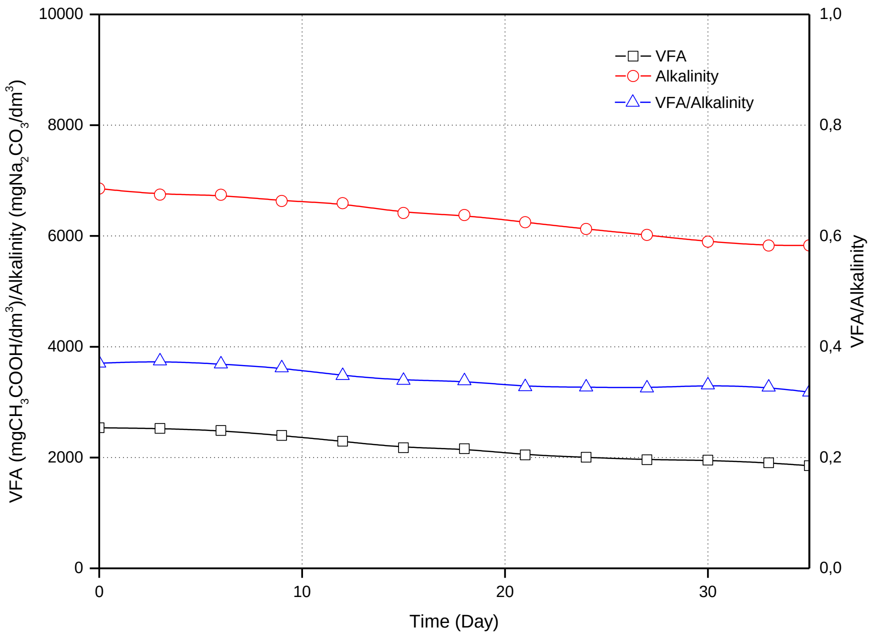

Figure 42. Changing of VFA. alkalinity VFA/alkalinity ratio during mesophilic digestion mixture of hydrothermally pretreated at 160°C for 30 minutes algae and cattle manure in ratio 1:3

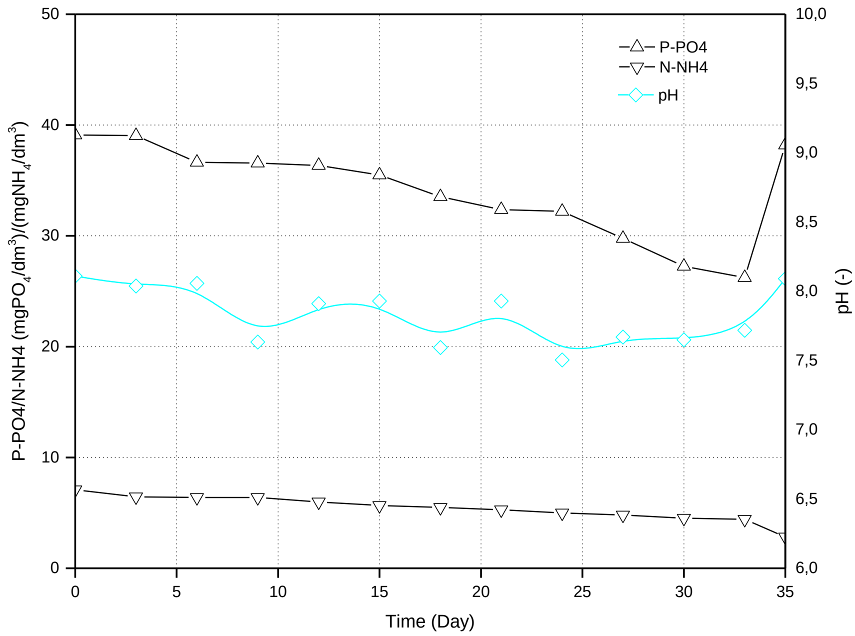

Figure 43. Change of phosphorus and ammonia concertation and pH during mixture of hydrothermally pretreated at 160°C for 30 minutes algae and cattle manure in ratio 1:3 of mesophilic digestion in the 10 dm^3 bioreactor

6.2 Quasi-continuous Digestion Experiments in 1 000 dm³ Bioreactors

6.2.1 Cattle Manure in the 1 000 dm³ Reactor

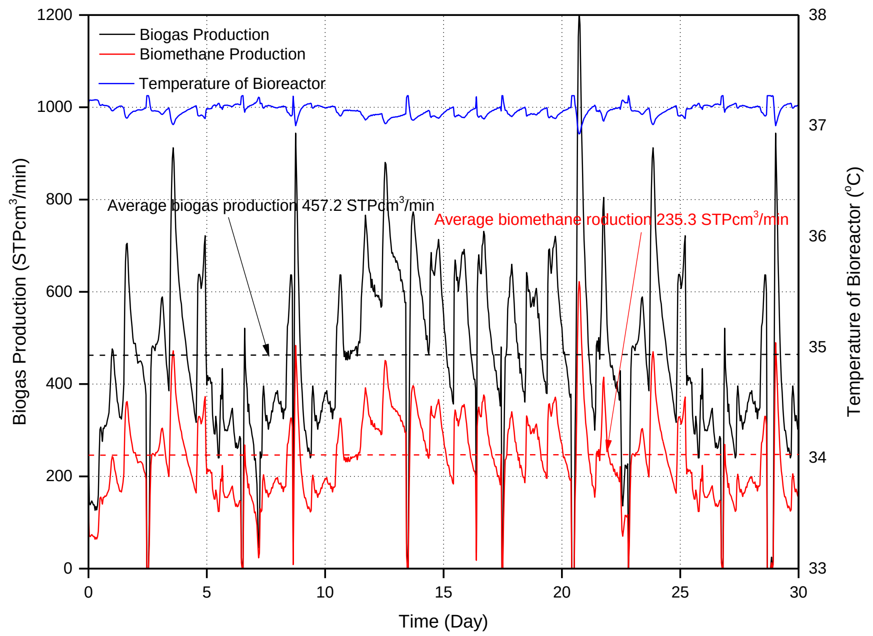

Figure 44. Production of biogas and biomethane and temperature changes in the 1 000 dm³ bioreactor during cattle manure mesophilic digestion

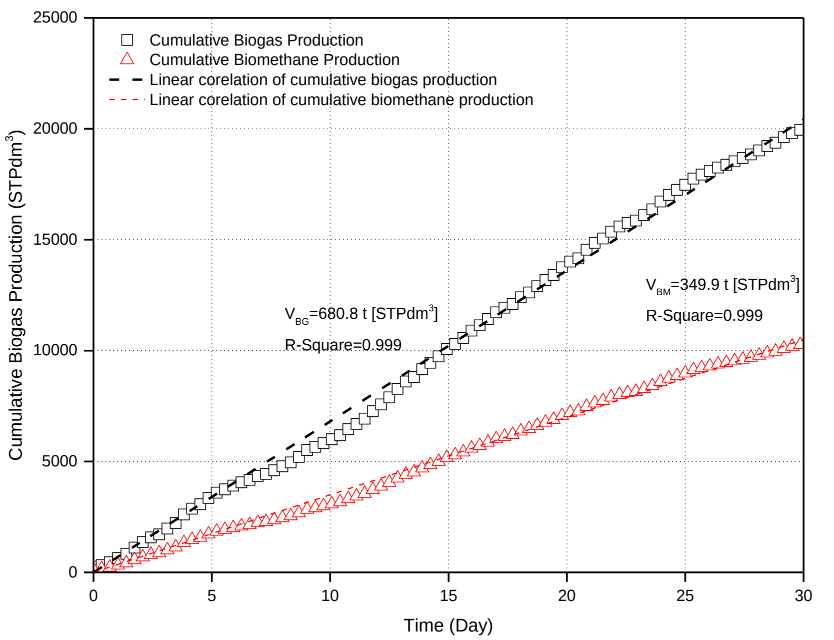

Figure 45. Cumulative biogas and biometan production in the 1 000 dm³ bioreactor during mesophilic digestion of cattle manure

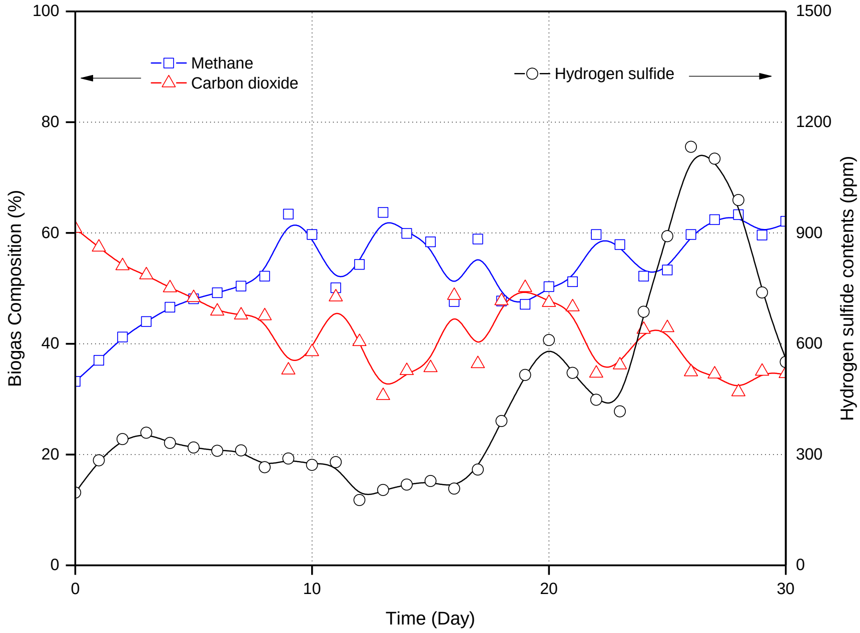

Figure 46. Concentration of methane, carbon dioxide and hydrogen sulphide during mesophilic digestion of cattle manure

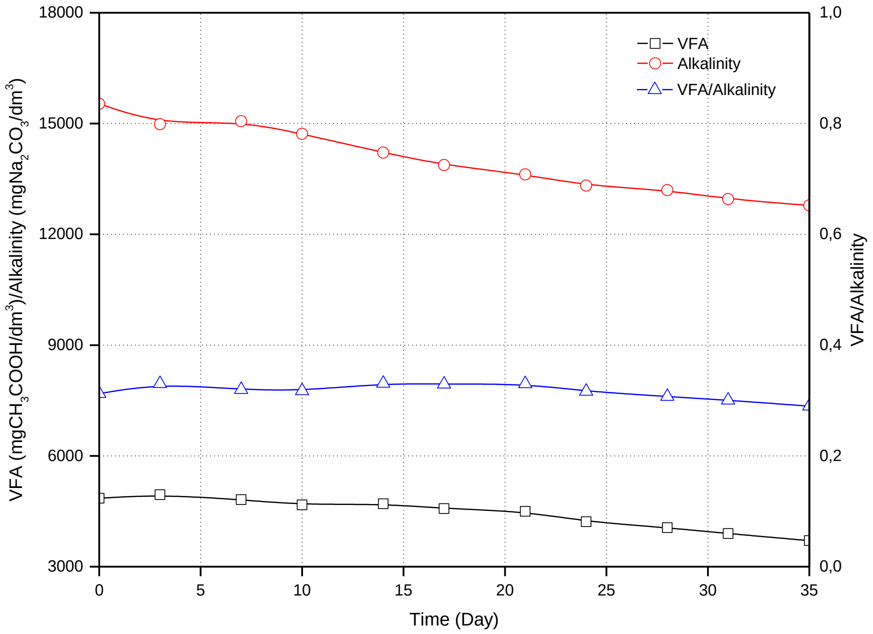

Figure 47. Changing of VFA, alkalinity VFA/alkalinity ratio during mesophilic digestion of cattle manure

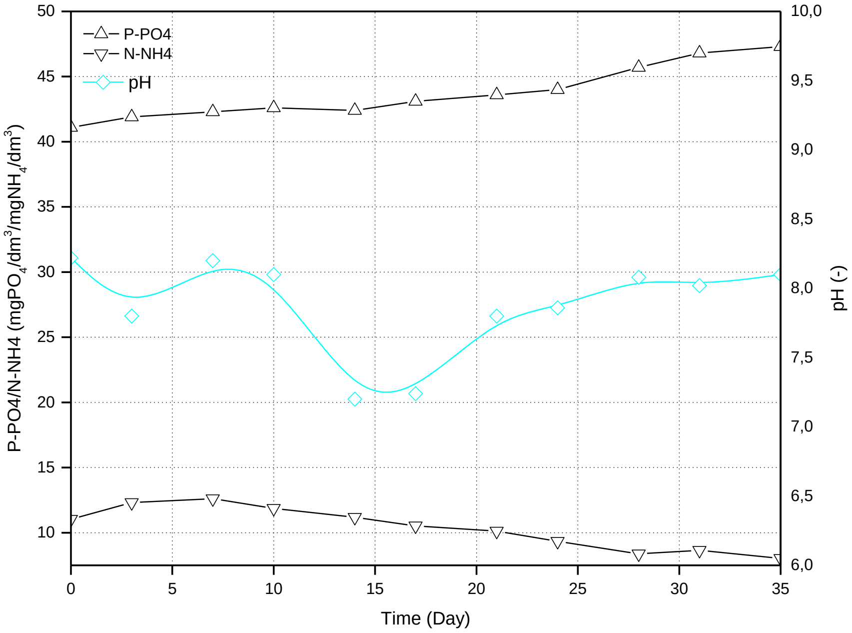

Figure 48. Change of phosphorus and ammonia concertation and pH during cattle manure of mesophilic digestion in the 1 000 dm^3 bioreactor

6.2.2 Mixture of Algae and Cattle Manure in ratio 1:3 in the 1 000 dm^3 Reactor

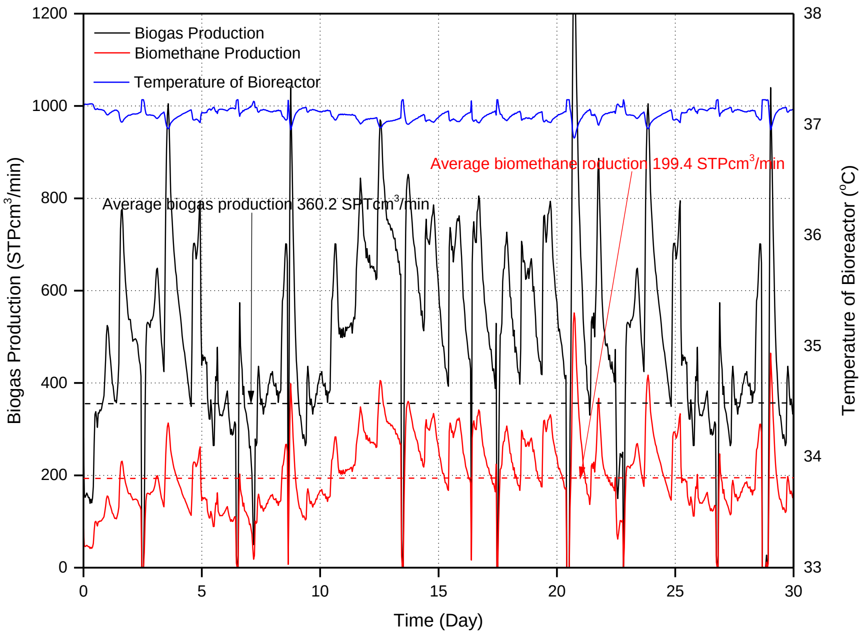

Figure 49. Production of biogas and biomethane and temperature changes in the 1 000 dm^3 bioreactor during algae mix and cattle manure in ration 1:3 mesophilic digestion

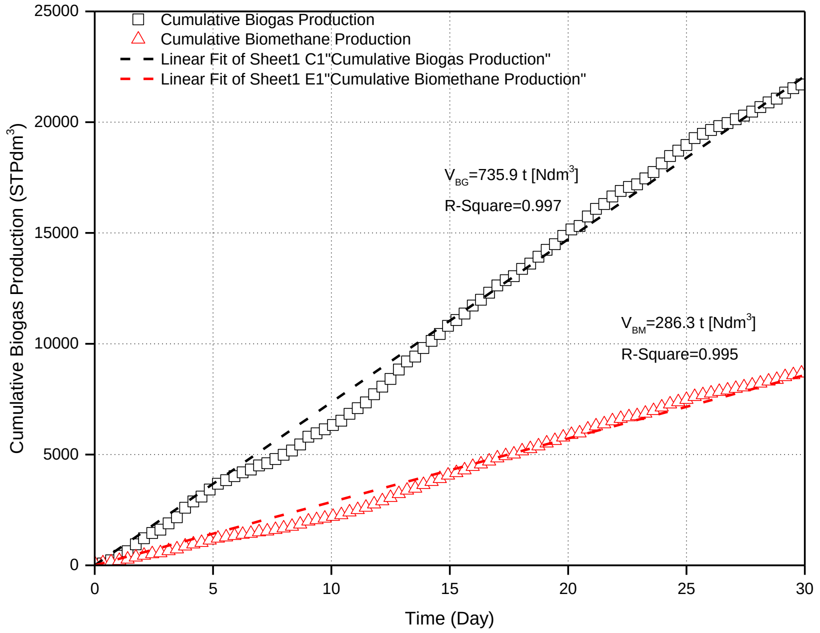

Figure 50. Cumulative biogas and biometan production in the 1 000 dm^3 bioreactor during mesophilic digestion of algae mix and cattle manure in ration 1:3

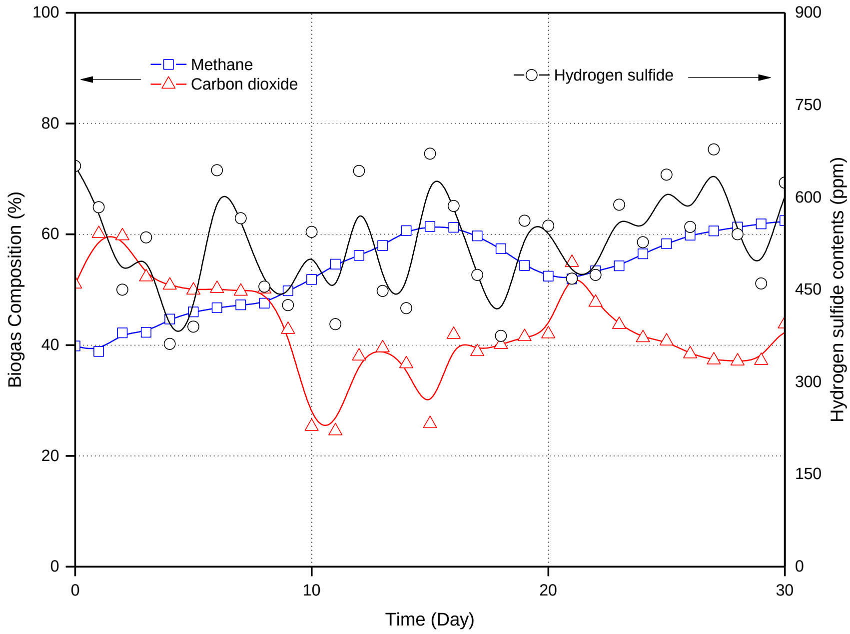

Figure 51. Concentration of methane. carbon dioxide and hydrogen sulphide during mesophilic digestion of algae mix and cattle manure in ration 1:3

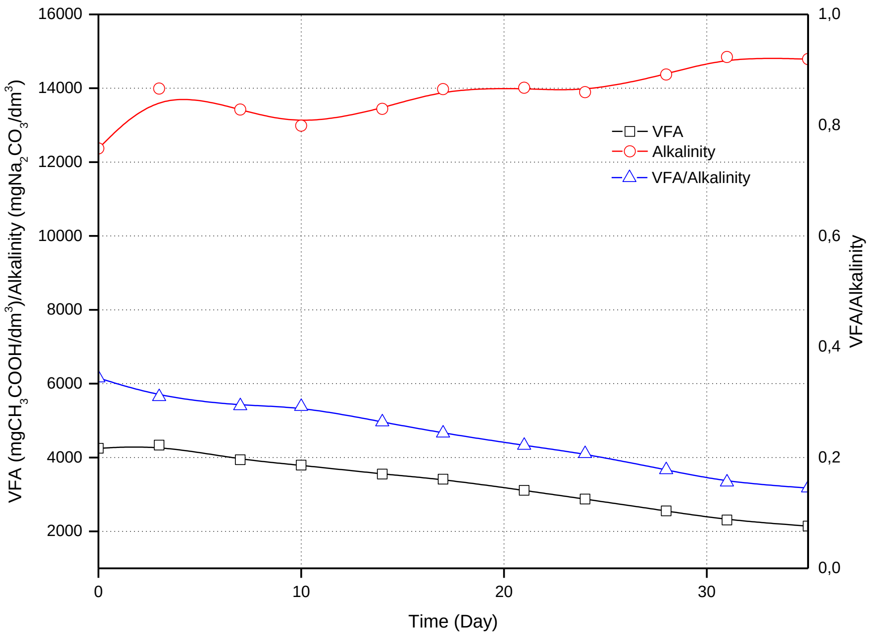

Figure 52. Changing of VFA. alkalinity VFA/alkalinity ratio during mesophilic digestion of algae mix and cattle manure in ration 1:3

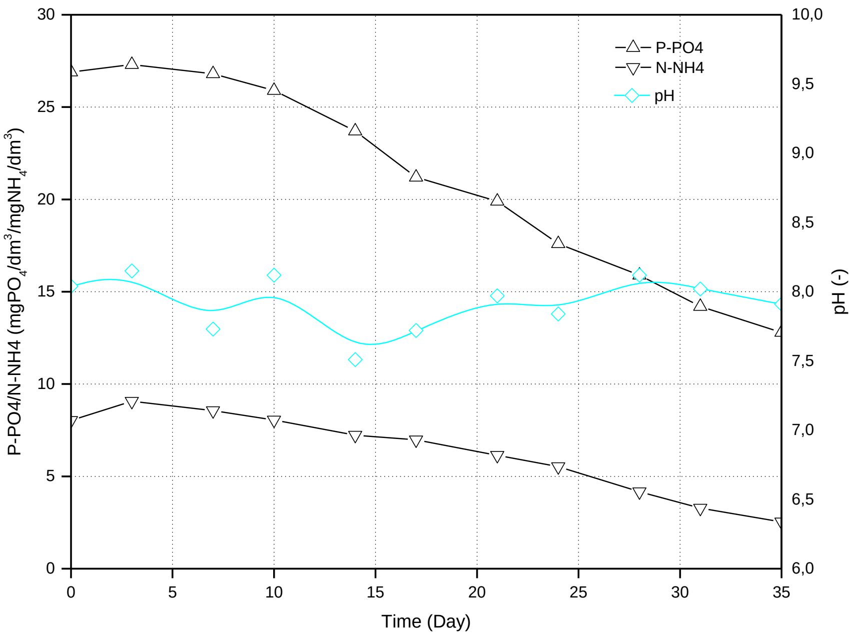

Figure 53. Change of phosphorus and ammonia concertation and pH during algae mix and cattle manure in ration 1:3 of mesophilic digestion in the 1 000 dm^3 bioreactor

6.2.3 Mixture of combined mechanical and acid pretreatment of algae and cattle manure in ratio 1:3 in the 1 000 dm^3 reactor

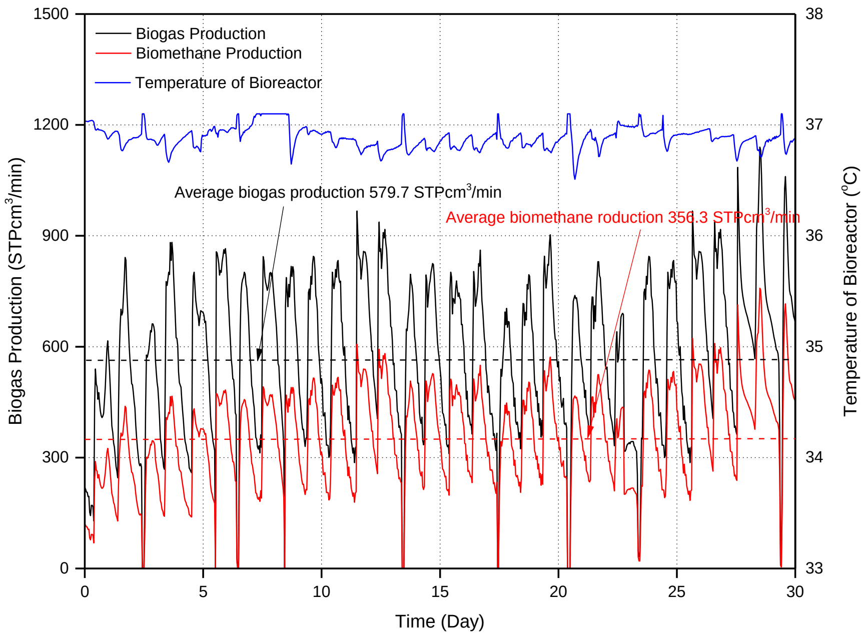

Figure 54. Production of biogas and biomethane and temperature changes in the 1 000 dm^3 bioreactor during mixture of combined mechanical and acid pretreatment of algae and cattle manure in ratio 1:3 mesophilic digestion

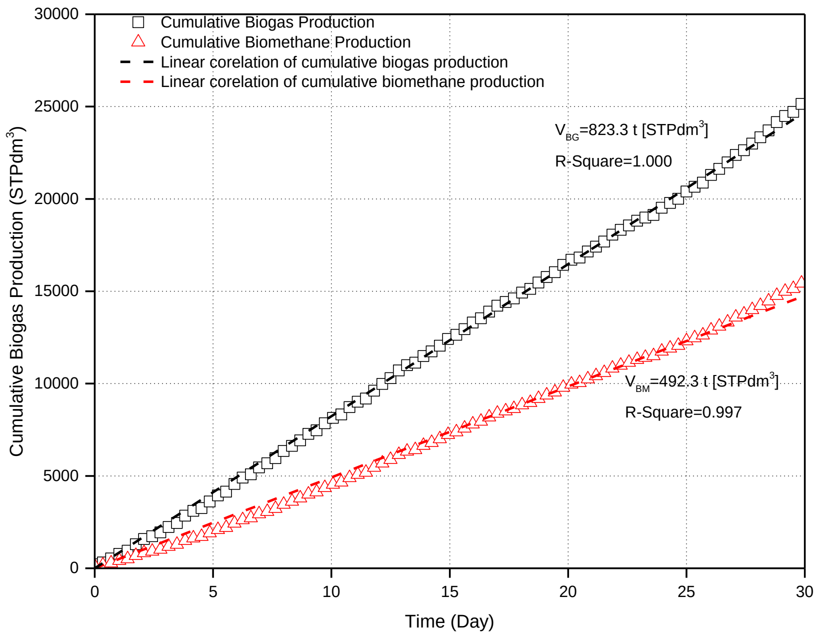

Figure 55. Cumulative biogas and biometan production in the 1 000 dm^3 bioreactor during mesophilic digestion of mixture of combined mechanical and acid pretreatment of algae and cattle manure in ratio 1:3

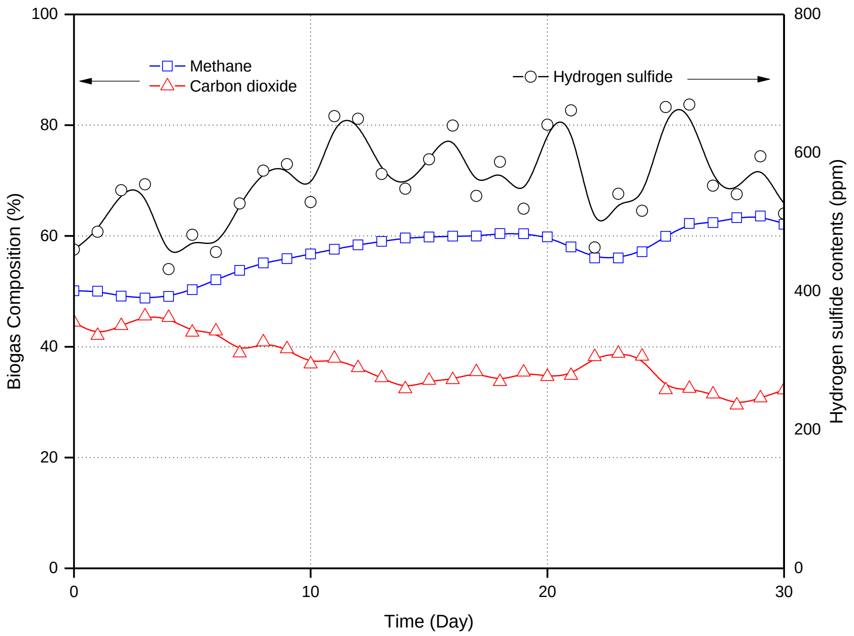

Figure 56. Concentration of methane. carbon dioxide and hydrogen sulphide during mesophilic digestion of mixture of combined mechanical and acid pretreatment of algae and cattle manure in ratio 1:3

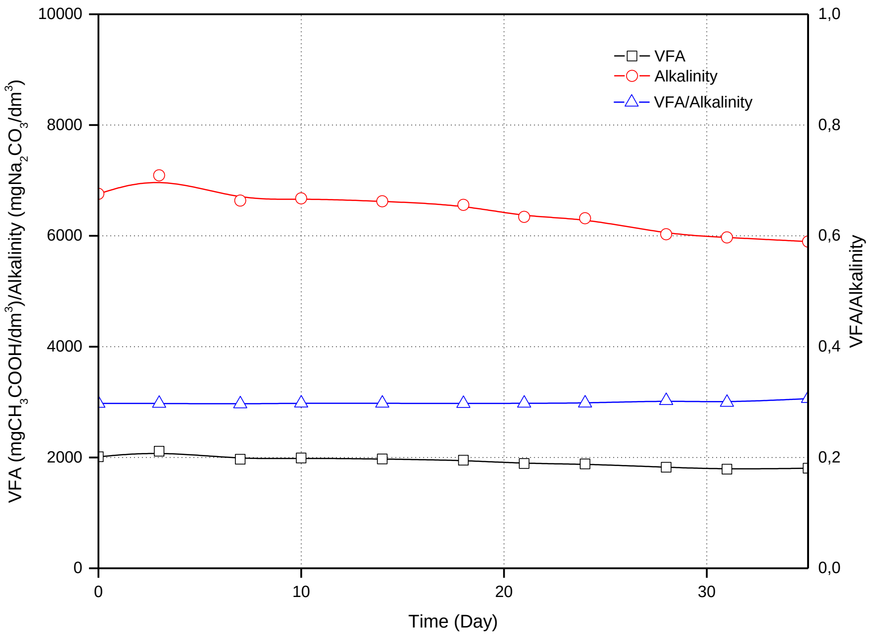

Figure 57. Changing of VFA. alkalinity VFA/alkalinity ratio during mesophilic digestion of mixture of combined mechanical and acid pretreatment of algae and cattle manure in ratio 1:3

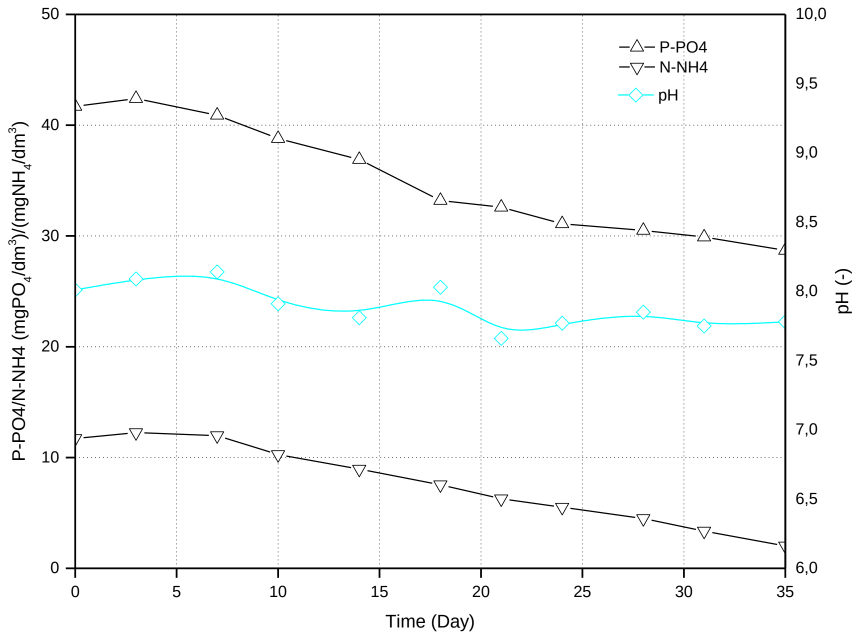

Figure 58. Change of phosphorus and ammonia concertation and pH during mixture of combined mechanical and acid pretreatment of algae and cattle manure in ratio 1:3 of mesophilic digestion in the 1 000 dm^3 bioreactor

6.2.4 Mixture of Hydrothermally Pretreated at 95°C for 60 minutes Algae and Cattle Manure in Ratio 1:3 in the 1 000 dm^3 Reactor

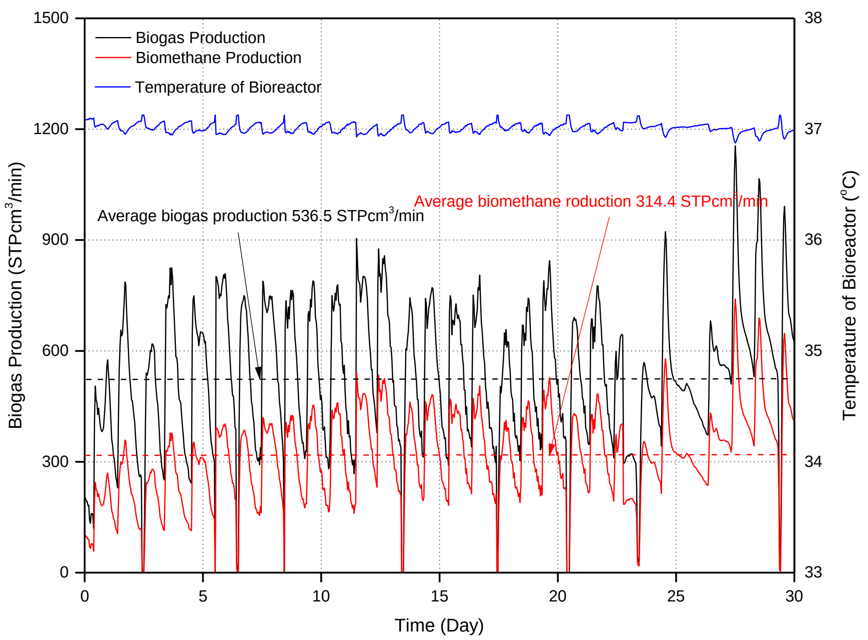

Figure 59. Production of biogas and biomethane and temperature changes in the 1 000 dm^3 bioreactor during mixture of hydrothermally pretreated at 95°C for 60 minutes algae and cattle manure in ratio 1:3 mesophilic digestion

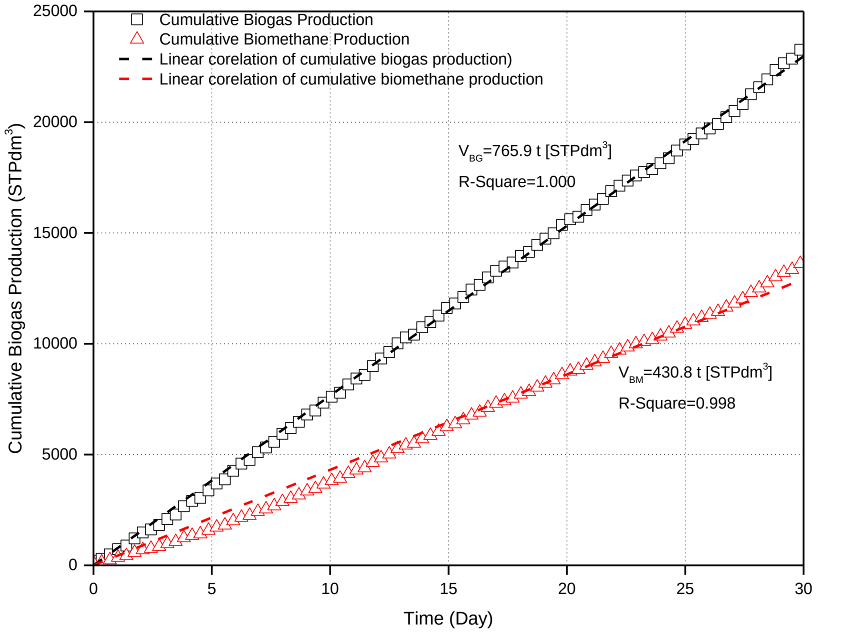

Figure 60. Cumulative biogas and biometan production in the 1 000 dm^3 bioreactor during mesophilic digestion of mixture of hydrothermally pretreated at 95°C for 60 minutes algae and cattle manure in ratio 1:3

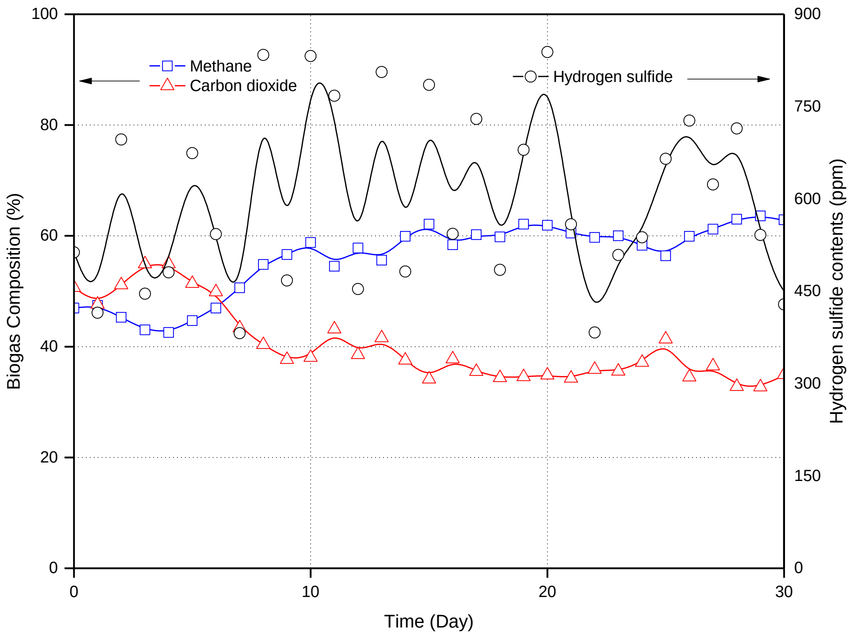

Figure 61. Concentration of methane. carbon dioxide and hydrogen sulphide during mesophilic digestion of mixture of hydrothermally pretreated at 95°C for 60 minutes algae and cattle manure in ratio 1:3

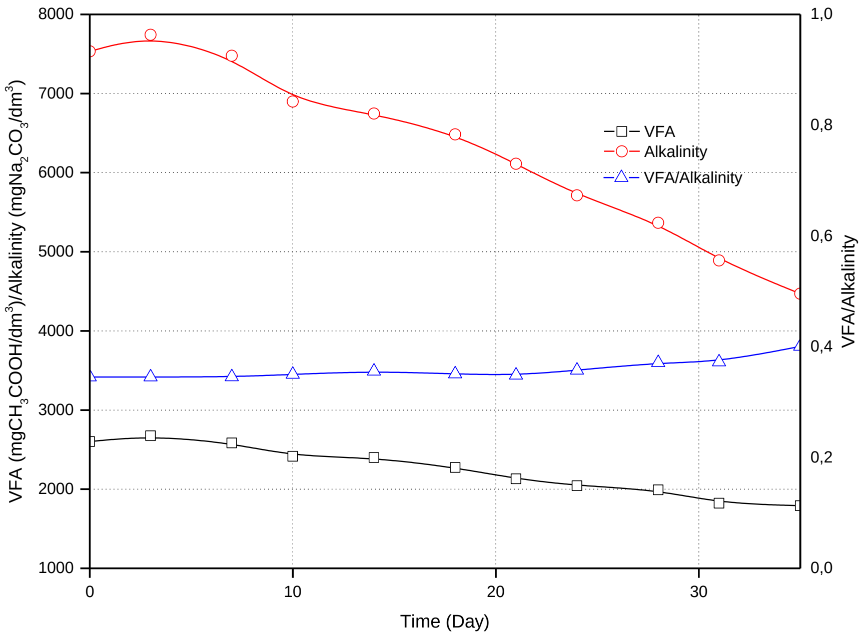

Figure 62. Changing of VFA. alkalinity VFA/alkalinity ratio during mesophilic digestion of mixture of hydrothermally pretreated at 95°C for 60 minutes algae and cattle manure in ratio 1:3

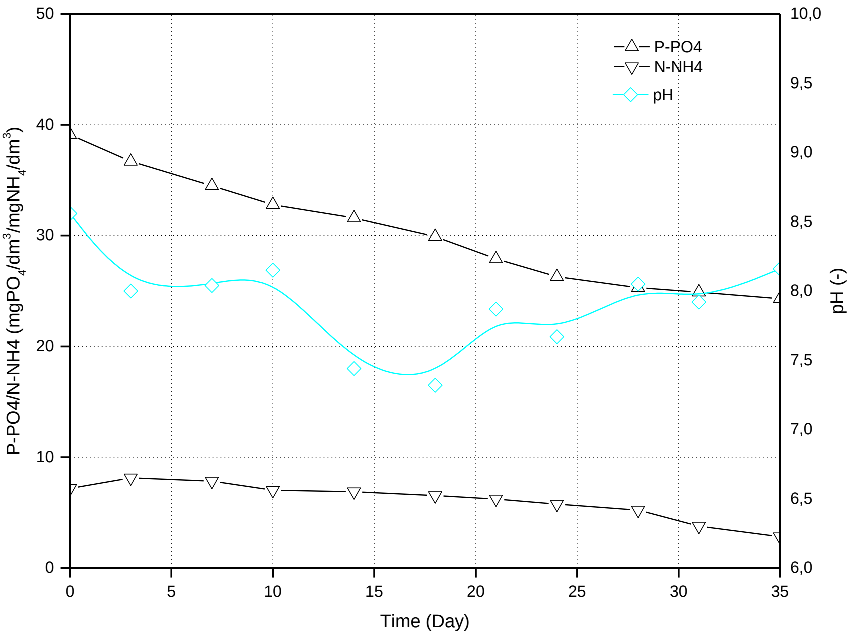

Figure 63. Change of phosphorus and ammonia concertation and pH during mixture of hydrothermally pretreated at 95°C for 60 minutes algae and cattle manure in ratio 1:3 of mesophilic digestion in the 1 000 dm^3 bioreactor

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