

# USE OF ALKALINE FISH GLYCERIN AND SPENT BLEACHING EARTH FROM OMEGA- 3 OIL REFINING FOR BIOGAS PRODUCTION



# Use of Alkaline Fish Glycerin and Spent Bleaching Earth from Omega-3 Oil Refining for Biogas production

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This report presents scientific work. Results should be kept in their context. The author is not responsible for the use of data in other context than this study and its objectives.

Cover picture: taken from ReSource International headquarters Hafnarfjörður – Iceland

## Executive summary

The purpose of the present study was to examine the biogas potential from anaerobic digestion of alkaline fish glycerin (AFG) and spent bleaching earth (SBE) from omega-3 fish oil refining. Both fractions show promising use in biogas production feedstock. However literature review on SBE from fish oil refining was limited to one publication. Publication with AFG did not exist at our knowledge but a publications on a similar product, crude glycerin from biodiesel production, seems to be more developed.

In order to increase practical knowledge and be efficiently consulting its customers, it is proposed to MBP to increase documentation of existing biogas plants using SBE and AFG as substrate. A survey model was designed in order to collect valuable information when MBP or its consultants are contacting or visiting biogas plant operators. Increase knowledge database could also help RSI to develop further advising work and collaboration with MBP on business development.

A second part of the project was the laboratory analysis and anaerobic digestion of SBE and AFG. The substrates were tested in co-digestion with cattle manure (CM) both in batch assays and in continuous stirred-tank reactors (CSTR) with different proportions. Batch assays were made in order to determine biogas potential of the substrates while CSTR reactors could determine long term inhibition effects and show more realistic biogas potential for the full-scale process.

Batch assays shows that CM with 5% SBE and 10% SBE had respectively biogas yield of 37,5 mL/g<sub>substrate</sub> and 44,8 mL/g<sub>substrate</sub>. This is respectively an increase by 137% and 163% comparing to CM alone in the same configuration. CM with 1,5% AFG and 3,0% AFG had respectively biogas yield of 41,3 mL/g<sub>substrate</sub> and 47,9mL/g<sub>substrate</sub>. This is respectively an increase by 162% and 188% comparing to CM alone in the same configuration.

Continuous anaerobic digestion was tested in three separated CSTR reactors of three liters working volume and a Hydraulic Retention Time (HRT) of 20 days. All reactors where started-up using CM alone. After 112 days, reactor number 2 (R2) was fed with a mixture of CM+5% SBE and R3 with CM+1,5% AFG. R1 was fed all along the experiment with CM only and was used as a reference. In R2 after introduction of the SBE in the feedstock, the biogas production increased directly to a higher production rate compared to the reference. However the biogas production in R3 decreased over the first 20 days, but after that started producing at even higher rate than R2. Once in stabilized state, R2 had a biogas production rate 180% higher than the reference and R3 was producing 355% more than the reference.

After 76 days feedstock in R2 was increased to CM+10% SBE and R3 to CM+3%AFG. This time both reactor (R2 and R3) showed increased production with no lag time. Once in stabilized state, R2 had a biogas production rate 219% higher than the reference and R3 was producing 554% more than the reference.

During the continuous test, R3 presented some heavy foaming on the surface of the effluent bottle coming from the reactor surface. This foaming effect characteristic of glycerin in anaerobic digestion (AD), is to be considered in a full-scale anaerobic digestion plant as it could lead to the failure of the process.

In conclusion SBE and AFG presents a great potential for biogas production however a more thoroughly documentation from full-scale installations and more laboratory analysis could help to develop special handling technical solutions.

# Definitions

**Biofuel:** A biofuel is a fuel that contains energy from geologically recent carbon fixation. These fuels are produced from living organisms.

**Biogas:** Biogas typically refers to a mixture of gases produced by the breakdown of organic matter in the absence of oxygen. Typically biogas is composed of methane (CH<sub>4</sub>); carbon dioxide (CO<sub>2</sub>) and trace elements such as Hydrogen sulfide (H<sub>2</sub>S).

**Biomethane:** Biomethane or natural gas refers to methane gas (CH<sub>4</sub>) concentrated from biogas by removal of the CO<sub>2</sub>, water and trace elements. Final concentration of the methane is usually around 95% CH<sub>4</sub> and is suitable for vehicle combustion engines.

**COD – Chemical Oxygen Demand:** The amount of chemical oxidant required to breakdown the waste, also an indicator of the concentration of organics in the substrate.

**Digestion:** The breakdown of sludge and other waste biologically by microorganisms. Results in by-products such as methane gas (CH<sub>4</sub>), carbon dioxide (CO<sub>2</sub>), sludge solids and water. Aerobic digestion requires oxygen, anaerobic digestion the absence of oxygen.

**Effluent (or digestate):** The final output flow of a biogas plant/anaerobic digestion.

**Feedstock (or substrate):** The input material for a biogas plant/anaerobic digestion.

**Hydraulic retention time:** is a measure of the average length of time that a soluble compound remains in a constructed bioreactor

**pH:** A measure of acidity or alkalinity of water, or any given substance. The scale is 1 to 14 with 7 being neutral. Over 7 is alkaline or caustic, under 7 is acid or base. Common biological processes are more efficient in pH range close to neutral.

**Total solids (TS):** is the fraction of solids (dissolved and suspended) within the substrate. Equivalent to Dry Matter (DM).

**Upgrading:** Biogas upgrading refers to the process of removal of CO<sub>2</sub>, water and trace elements (H<sub>2</sub>S) from biogas. The final product is biomethane and can be used as a biofuels for combustion engines.

**Volatile solids (VS):** Fraction from the loss on ignition of total solids. VS fraction is often considered as the organic fraction of substrates in opposition of the mineral fraction (ashes after ignition).

**Wet weight (w/w):** The weight of any quantity of a substance before it is dried.

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# 1. Introduction

The present description introduces the project „ Use of Alkaline Fish Glycerin and Spent Bleaching Earth from omega-3 Oil Refining for Biogas production“. Both fractions show promising use in biogas production feedstock. However, practical knowledge is limited and it is proposed to further investigate benefits from the use of these fractions into anaerobic digestion (AD) in order to support development of future biogas projects.

## 1.1. Goal and scope

The purpose of the study was to examine the biogas potential of alkaline fish glycerin (AFG) and spent bleaching earth (SBE) from omega-3 fish oil refining. The substrates were tested in co-digestion with cattle manure (CM) both in batch assays and in continuous stirred-tank reactors (CSTR) with different proportions. Batch assays were made in order to determine biogas potential of the substrates while CSTR reactors could determine long term inhibition effects and show more realistic biogas potential for the full-scale process.

A literature review is also presented in order to gather existing knowledge in a condensed form. In addition the review includes a presentation of anaerobic digestion plants where crude glycerin and/or SBE are used as substrate.

## 2. Literature review

### 2.1. Generation process

The process of extracting and refining omega-3 fatty acids for human consumption is a multi-step process generating a multitude of by-products (Figure 1). During the neutralization step, unstable fatty acids are removed by alkaline solutions that results in soapstock. In bleaching, a type of clay (montmorillonite clay) is used to remove pigments from the oil, which become an oil-rich by-product called spent bleaching earth (SBE). In winterization, further unstable fatty acids are filtered, without any chemical addition. The by-product here is stearin, a waxy substance. In deodorization, the valuable omega-3 fatty acids are extracted by distillation. In esterification, ethyl esters are produced which are again distilled to concentrate any remaining omega-3 oils. Alkaline fish glycerin (AFG) is the by-product of this stage [1], [2].

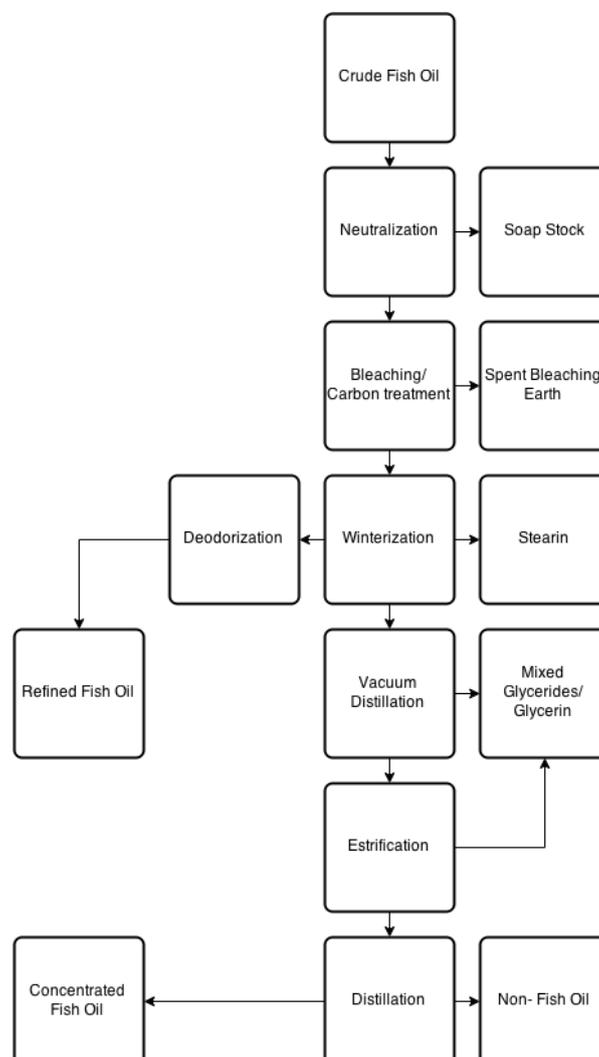


Figure 1: An example of fish oil refining flowchart.

### **2.1.1. Spent Bleaching Earth (SBE)**

Bleaching clay is used to remove contaminants, color pigments and solids from the fish oil during a filtering process. After filtering, the oil rich clay is then called SBE [2], [3]. SBE contains around 40% wet weight (w/w) oil which is difficult to recover for further use. Therefore, using SBE for producing biogas in a digestion process is seen as a viable option [1].

There may be some concern when using SBE as a biogas substrate [3]:

- The minerals in SBE can cause damage to equipment, especially by sedimentation at the bottom of the digesters. Hence, sufficient mixing is required to maintain the solids suspended in the digesters to be able to efficiently utilize the digester volume.
- Digestate from the biogas plants using SBE as a substrate may contain heavy metals and organic pollutants, this should be taken into consideration when applying it as a fertilizer to agricultural lands.
- Because of the high dry matter content of SBE, it has to be mixed with a very fluid material to be able to pump the substrate and mix it in the digester.
- SBE has a high oxidation potential so proper attention is required to avoid self-ignition during storage or transportation.

### **2.1.2. Alkaline Fish Glycerin (AFG)**

AFG generation results from the esterification process (glycerin is pumped out of the reactors after ester has been washed out) [4]. It is a readily digestible, and can also be easily stored over a long period of time. At room temperature AFG is in solid form and needs to be heated up in order to be pumped [5].

Pure glycerin also referred as glycerol has the chemical formula  $C_3H_5(OH)_3$ . It is also a common by-product from bio-diesel production through transesterification. Among the impurities that might cause difficulties in anaerobic metabolism are substances such as long chain fatty acids, chloride and sulphates [6]. These substances are, however, usually associated with bio-diesel production, and not fish oil refining.

## **2.2. Treatment technologies**

SBE and AFG can be utilized in different technologies illustrated in Figure 2Figure 1. As mentioned earlier, SBE contains mainly oil, colored pigments, heavy metals and other trace components. The nature of the contamination of SBE depends on the purpose it was used for. Because of its high oil content (20-40%) It would be beneficial to use it as an energy source instead of landfilling it. It is possible to use SBE as addition to animal feed [7] (oil fraction extracted) , in brick and cement production [8], for soil improvement [9], composting and incineration. SBE can be recycled and regenerated for further utilization. Boukerroui *et al.* [10] thermally treated SBE at 500 °C for 1 hour followed by washing with a solution of HCL (1 M.) The regenerated bleaching earth showed similar adsorption properties as did a commercial virgin one. It can also be used as feed stock for biodiesel and biogas (see chapter 3) production which are renewable fuel with a low emission profiles. Using SBE as substrate for biodiesel production could be beneficial when the crude oil price is high as Huang *et al.* [11] reported.

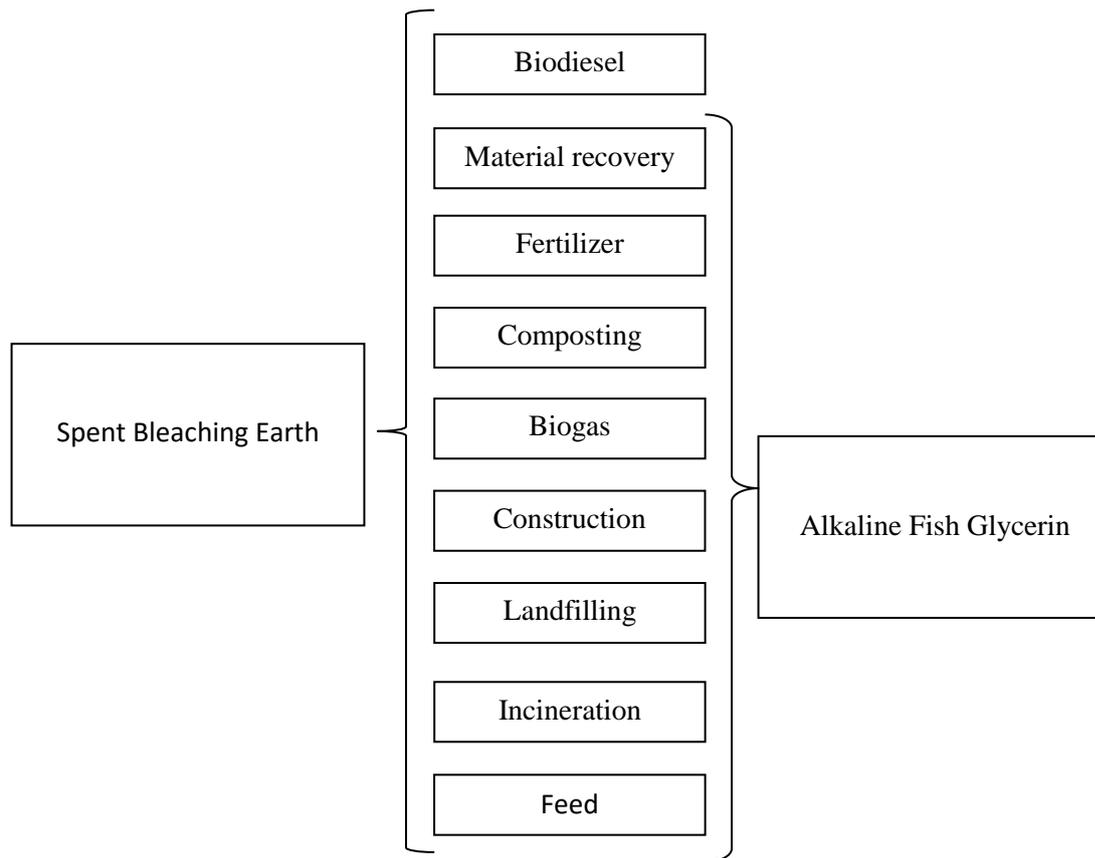


Figure 2: Example of utilization of Spent Bleaching Earth (SBE) and Alkaline Fish Glycerin (AFG).

### 2.3. Biogas production

The increase in fish oil processing and the expansion of the renewable energy market imply that these by-products could play a part in the future of biofuel. Fish oil refining by-products are rich in lipids and proteins and have a great potential for energy production. Such substrates have the advantage of giving high methane yields, and can be attractive as substrates in an anaerobic digestion process. Thus, anaerobic digestion could be a good approach for SBE and AFG utilization and energy generation. Anaerobic digestion of this biodegradable waste will provide a solution for reducing environmental impact from the consumption of fossil fuels. Plant nutrients such as nitrogen and phosphorus are retained in the digestate after anaerobic digestion, which can be used as a bio-fertilizer in agricultural production provided it meets the required standards [12].

The AD process consists of four metabolic stages which are shown in Figure 3. In hydrolysis, the insoluble organic polymers are broken down and become available for the other bacteria. Acidogenic bacteria produce carbon dioxide, hydrogen, ammonia and organic acid from sugars and amino acids. Then acetic acids are formed by acetogenic bacteria. Eventually, these products are transformed into methane by methanogenic micro-organisms [13].

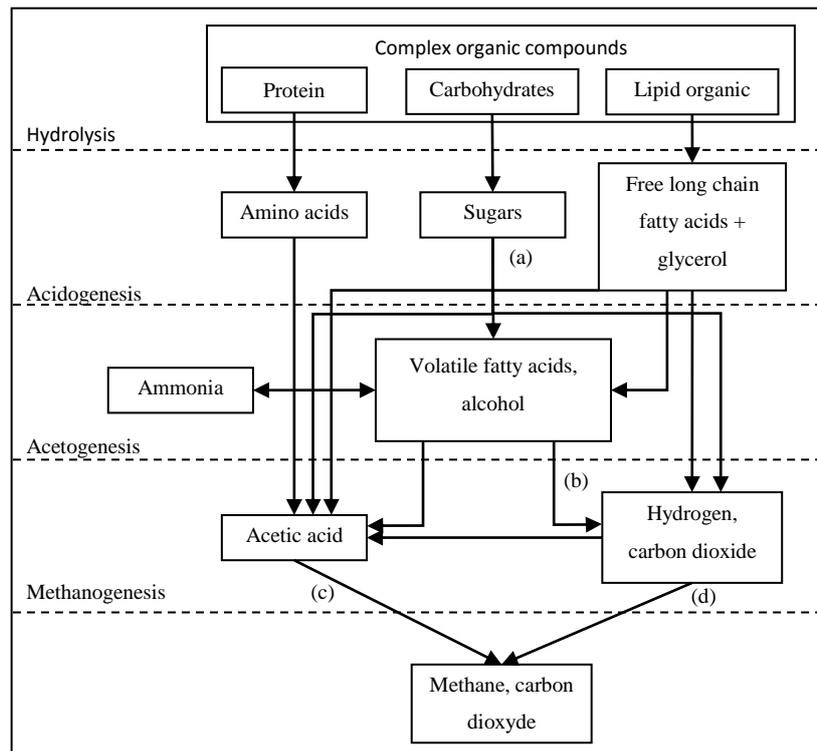


Figure 3: Anaerobic digestion of complex organic matters.

The process in optimized conditions results in the production of biogas which is a mixture of methane (CH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>). Depending on the substrate use and the process, the biogas can contain also hydrogen sulfide (H<sub>2</sub>S) and water vapor. If the biogas is upgraded i.e. cleaned from CO<sub>2</sub>, H<sub>2</sub>S and water, it can be used as natural gas on gas network or directly as vehicle fuel. The biogas can also be used in large combustion engines for production of electricity and heat.

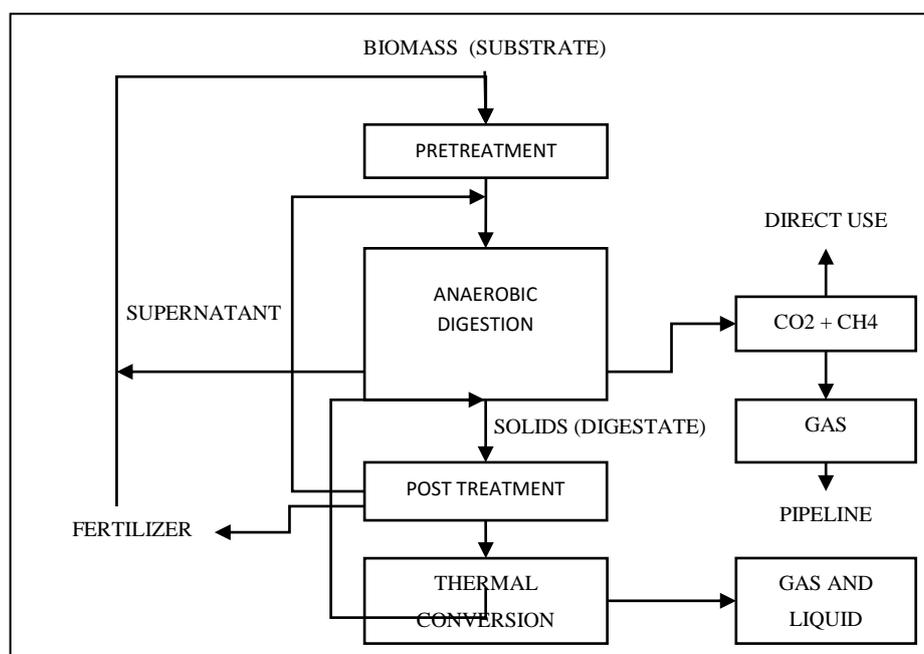


Figure 4: Typical process for production of biogas with pre-treatment, anaerobic digestion and gas utilization.

## 2.4. Full-scale AD use

### 2.4.1. Denmark

The following chapter describes a study case for spent bleaching earth (SBE) handling and treatment in the anaerobic digestion plant at Vesttec in Denmark. Information have been collected with the help of David Magnussen and Carsten Jensen (MBP Group) in year 2013.

The SBE is unloaded into an 80 m<sup>3</sup> pre-mixing tank and blend with degassed manure. The mixture is agitated vigorously with a stirrer powered by a tractor for homogenization and the tank is kept at 45-50°C. Another stirring is applied the day after the first mix. It is important according to the plant manager that the mixing tank should not be oversized.



*Figure 5: Product unloaded in receiving tank.*

The plant has two process tanks, one for very liquid products and another one for high solids content materials in which the SBE is digested (100m<sup>3</sup>). The SBE mix is pumped from the top of the working volume so that material will fall by gravity to the bottom. As oil content does not separate from the bleaching earth it is believed that biological degradation occurs directly on the SBE during the falling phase.

The AD tank needs to be equipped with a sand removal system at the bottom so that residual will not build up at the bottom, and so it is not necessary to stop process for cleaning and maintenance operation.

In this plant there is no sanitization (heat treatment). The product is heat treated from the sender in Norway. It is recommended not to put SBE through a heating treatment, because it will attach to the pipes and slow down the heating process.



Figure 6: Spent Bleaching Earth blended with degassed manure from the manure tank.

#### 2.4.2. Biogas plant surveying

As described in Chapter 4.1, there are several aspects that are presented as critical for the correct handling of specific substrates. ReSource International is proposing MBP to develop a knowledge database where experience with by-products sold is registered. Furthermore, registration should not concern only MBP's by-products but also general information about the biogas plant, its ownership, its operation, technical details and use of other substrates in the process.

Thus, by providing more consistent data in an organized form it will be possible to use data and information to develop new markets, support customers and develop R&D work. Some example of data that could be collected are presented in table below:

Table 1: Example of pertinent information input for the development of a biogas knowledge database.

Type of ownership
Type of operator
Total treatment capacity
Biogas use
Substrate handling
Subsidies (energy and feedstock)
Gate fee structure
Pre-treatment
Type of infrastructure
Interview with operator
Accident history
Digestate handling
Photos

## 3. Materials and methods

In this chapter is presented how test on anaerobic digestion of SBE and AFG have been carried through at RSI's laboratory facilities.

### 3.1. Substrates and inoculum

#### 3.1.1. Spent bleaching earth (SBE) and Alkaline fish glycerin (AFG)

SBE and AFG were collected from omega-3 oil refining company Lýsi hf. in Reykjavík, Iceland. The SBE was collected directly from the container under the filtering unit inside the plant in January 2015. However the AFG was not available at that time due to a general power shut down in Reykjavik district. AFG is in solid form at room temperature and needs to be heated up before to be in a pumpable form. Sampling of AFG was only possible in the beginning April 2015.

#### 3.1.2. Cattle manure (CM)

The CM was collected from the dairy farm Leirárgarðar located in Hvalfjarðarsveit, part of Reykjavik area and transferred to RSI facilities in Reykjavík. The first collection of manure was done in January 2015 and the second collection in May 2015. Cattles were fed during this period of time with the same feed and it is unlikely that manure present different chemical compositions. Before loading the digesters for the batch and continuous tests, CM was filtered through a 2 cm sieve to remove large materials (e.g. bedding material, long fibers and other foreign objects). Foreign materials such as animal bedding and stone can have a significant impact on the AD process by blocking pumping and stirring system for example [14].

#### 3.1.3. Inoculum

A mesophilic anaerobic granulated sludge obtained from the wastewater treatment plant of Vífilfell Coca Cola in Reykjavík was used as inoculum. The inoculum was pre-incubated at 37°C for degassing to ensure depletion of the biodegradable organic matter until no significant gas production was observed. The inoculum was homogenized in a blender before use.

### 3.2. Substrates analyses and preparation

Characteristics of the substrates were determined using standard methods. The studied substrates were characterized by total solids (TS) content, volatile solids (VS) content and pH prior to the tests. All analyses were performed in triplicate, with the average values reported. TS content was determined by drying samples at 105°C in a furnace until the samples were completely dried. AFG could not be dried and remained liquid after the TS measurement. VS content was determined with incineration of samples (at 550°C for 2 hours) and subtracting the remaining ash weight from the dried weight (Standard Method 2540 Solids). VS is therefore expressed as the volatile fraction of the solid fraction. The different fractions of an organic substrate are presented in a schematics view in Figure 7. To measure the pH of the SBE and AFG, the samples were diluted with de-ionized at ratio of 1:3. PH was measured in the raw substrates and after mixing the substrates in the ratios used in the

batch assays (prior to incubation) and after the batch test (Standard method 9040C). pH was also monitored during the continuous tests.

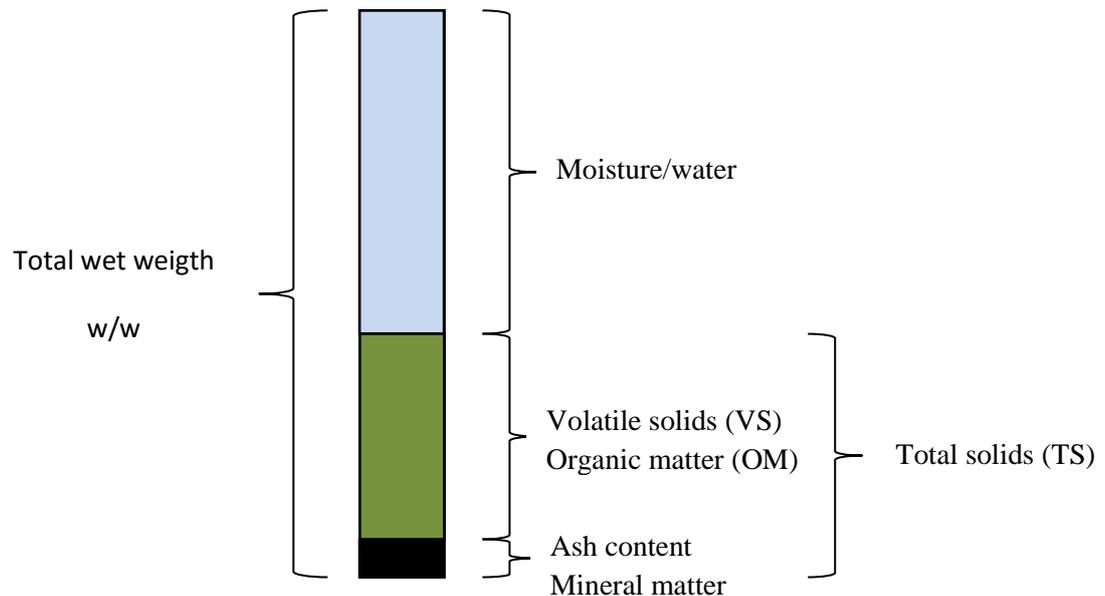


Figure 7: Physical substrate characterization. Water, total solids, volatile solids and ash fraction of an organic substrate.

### 3.3. Experimental setup

#### 3.3.1. Batch test

Biodegradability and biogas potential of the substrates were determined using batch biomethane potential tests (BMP). The preparation of the batch tests was carried out according to the method described by Ward [2]. The batch test comprised of 500 ml serum bottles (digesters) that were filled up to a volume of 250 ml. The experimental design of the batch test is presented in Figure 8 and Figure 9. Each digester was inoculated with homogenized inoculum followed by the addition of CM and percentage wet weight of SBE or AFG (25 g of each experimental substrate was added to each digester). All the samples were prepared in triplicates. To determine the effect of co-digestion of CM, SBE and AFG on biogas potential, digesters with only inoculum and CM were prepared. Furthermore in order to accurately present the amount of gas produced by the substrates, additional digesters containing only inoculum were included to account for background gas production from residual material in the inoculum. Then the gas produced from the inoculum was subtracted from the total gas produced by the digesters.

Figure 10 shows the batch test setup. The pH of each digester was measured at room temperature prior to starting the batch test. The digesters were sealed with rubber stoppers and aluminum caps. Then the headspace in the digesters was flushed out with N<sub>2</sub> gas through the rubber stopper for 2 min in order to create complete anaerobic conditions. Digesters were placed in an incubator at a mesophilic temperature (37°C) until gas production was no more than 1% of the total gas produced. To prevent accumulation of sludge, ensure homogeneous conditions, and improve the contact between microorganisms and substrate [15], manual mixing was applied after each gas volume measurement.

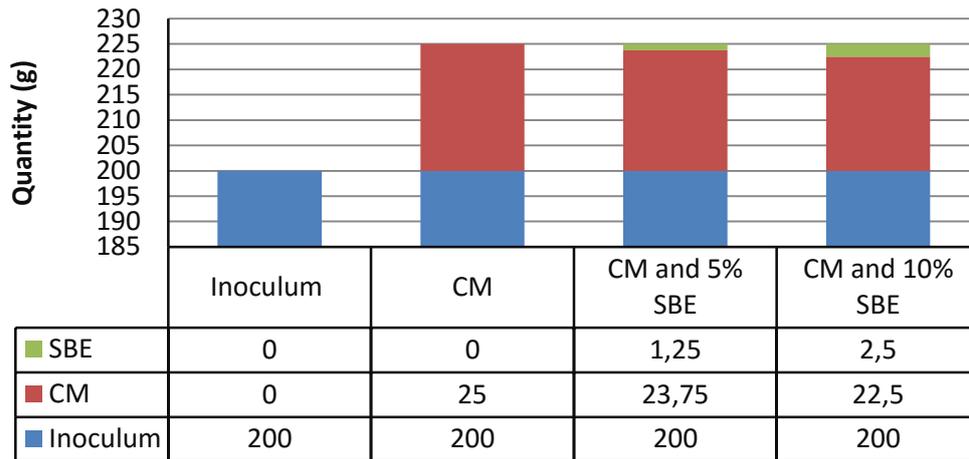


Figure 8: Experimental conditions used for cattle manure (CM) and spent bleaching earth (SBE) batch test.

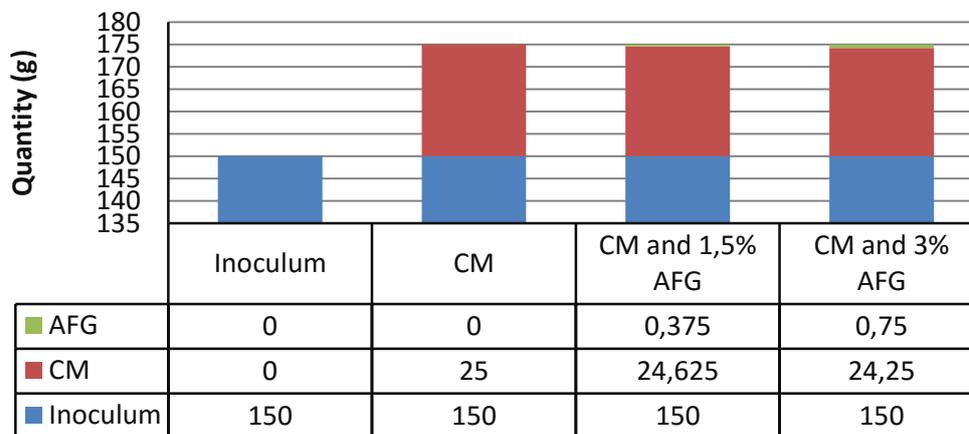


Figure 9: Experimental conditions used for cattle manure (CM) and alkaline fish glycerin (AFG) batch test.



Figure 10: Incubator with the batch test digesters.

The volume of biogas produced was measured by the water displacement method ( [2], [16] ) every 3 to 4 days in the first weeks (when the gas production was high) and then once a week or less for the remaining period. The biogas measurement system consisted of three cylinders which were standing in a container and filled with the acidified water (pH=2). The cylinders were closed on top and open at the bottom. The rubber-stopper caps sealing the digesters were pierced with a syringe needles

which allowed the biogas produced in the digesters to traverse across a pipe connection to the cylinders and accumulate on the top. The water in the cylinders was pushed back by the gas due to pressure difference until the pressures equalize with atmospheric pressure. The distance between the top of the cylinders and the water level was used to measure the volume of the produced biogas. Another pipe was connected to the valves on top of the cylinders on one side, and to a vacuum pump on the other side to purge the accumulated gas and draw the water up again into the cylinders.

Gases were sampled directly from the valves installed on the cylinders. The quality of the biogas was measured with a gas analyzer for methane and carbon dioxide content. The biogas produced contained nitrogen from the initial flushing of the digesters. In order to calculate the actual percentage of methane and carbon dioxide, it was assumed that these two gases together are equivalent to 100 % of the biogas volume. The final percentage of methane was calculated by the equation below [2]:

$$\% CH_4 \text{ (actual)} = \frac{\% CH_4(\text{measured})}{\% CH_4(\text{measured}) + \% CO_2(\text{measured})} \times 100$$

### 3.3.2. Continuous tests

Continuous tests were conducted in three liters anaerobic digesters (R1, R2 and R3). In total three anaerobic digesters were used during the experiments. Each reactors is equipped with a stirring system being activated for ten seconds every three minutes. The temperature is kept constant at 37°C within the reactors using silicone heating blankets connected with PLC controllers and temperature probes.

Feeding of the reactors was done by injecting through the feeding port the feedstock with a 50mL syringe. The feeding port equipped with a valve is connected to a pipe which goes to the bottom of the reactor to make sure that new feedstock is in direct contact with activated sludge. The digestate is released by overflow tube to an Erlenmeyer flask when new liquid is injected in the reactor allowing therefore to keep a constant volume of liquid in the reactor. Through the same tube the biogas is extracted from the reactor and is collected in a volumetric gas flow meter as designed by Angelidaki et al.[17]. The process diagram is illustrated in Figure 11.

A valve at the bottom of the reactors was used to take samples for pH measurement. After each measurement the volume of liquid used was reinjected into the reactor.

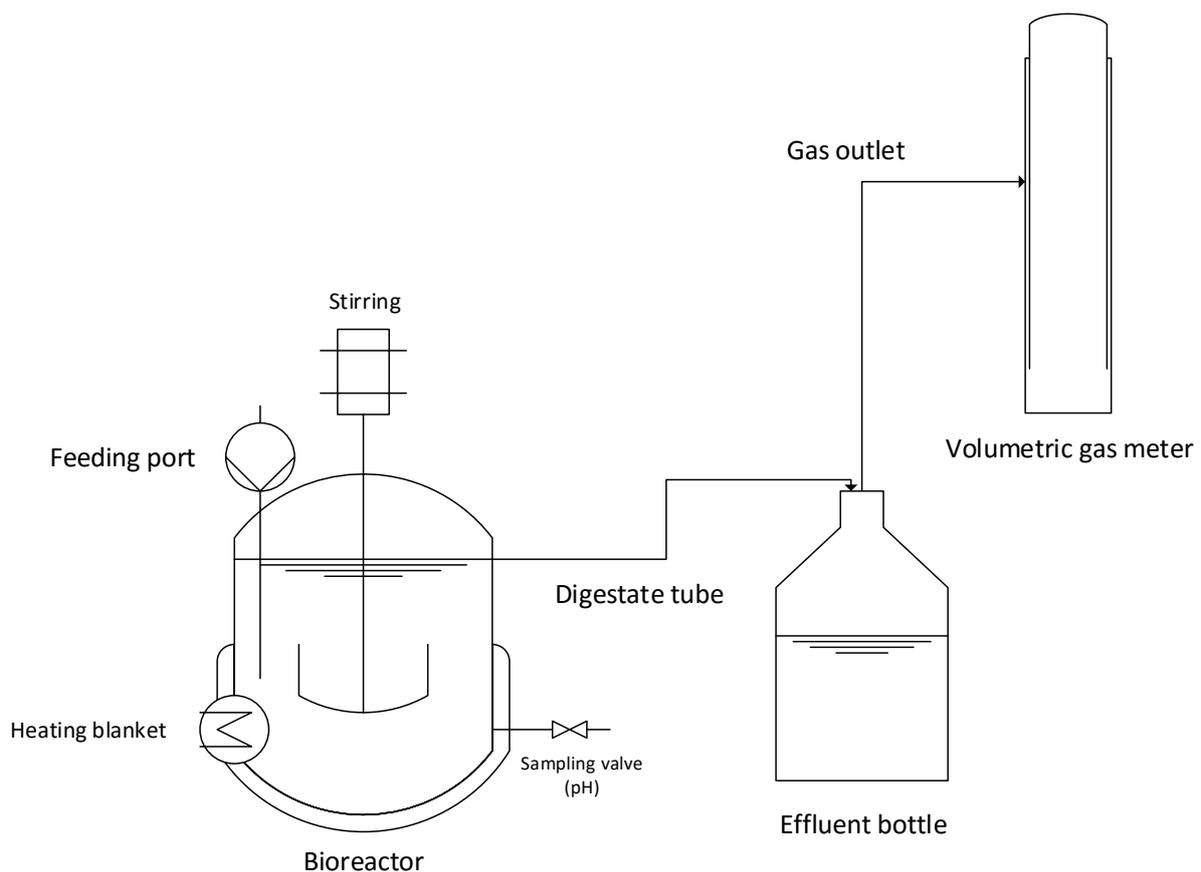


Figure 11: Diagram of the continuous anaerobic digestion system experimental setup.

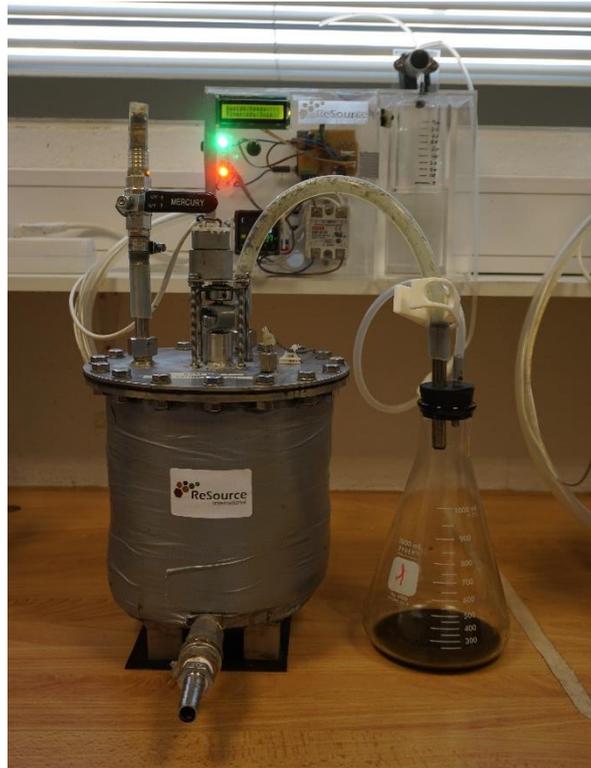


Figure 12: Semi continuous experimental setup.

The three reactors were operated simultaneously. After an initial phase of 112 days, the feeding in reactor 2 (R2) and reactor 3 (R3) was changed with respectively CM+5% SBE and CM+1,5% AFG. Co-substrates concentrations are in % of the total substrate wet weight (w/w).

After a period of 76 days from the first change in feeding, the fraction of SBE in R2 was increased to 10% and the fraction of AFG in R3 was increased to 3%. The Hydraulic Retention Time (HRT) was 20 days with an organic loading rate (OLR) of 1,5 gVS/L/day based on feeding with only cow-manure.

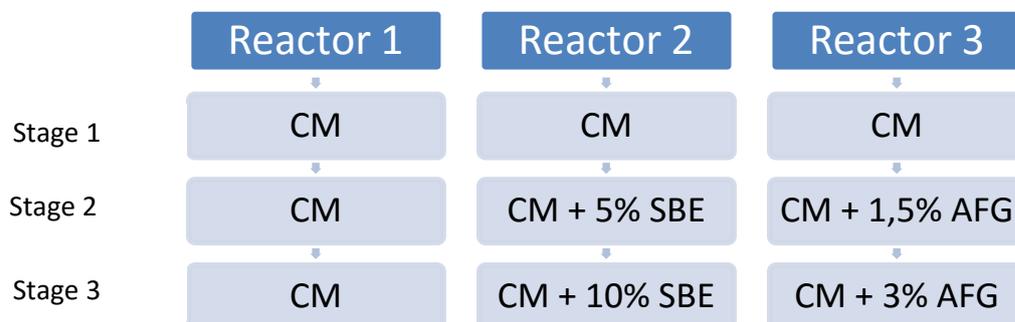


Figure 13: Feeding strategy for comparative study of co-digestion of cow manure (CM) with Spent Bleaching Earth (SBE) and Alkaline Fish Glycerin (AFG).

## 4. Result and discussion

### 4.1. Substrate analyses

The characteristics of the inoculum, CM, SBE, AFG alone and the tested mixtures are shown in Table 2 and Table 3. The high TS value of SBE (88%) and its relatively low VS (51%) is because, SBE contains a high portion of inorganic and indigestible matters which is mainly clay. The VS fraction of the SBE is considered to be mainly fish oil residues. SBE sample had the appearance of thick middle dried black clay.

AFG had both high TS and VS content with 98% and 97%, respectively. In contrary to SBE, AFG contains a high portion of organic and biodegradable matters. AFG contains very little water it has a high viscosity at room temperature, however, it decreases at higher temperatures (for example in the digesters with mesophilic temperature). It should be noted that, heated storage containers are needed when using AFG as substrate in biogas plants, otherwise the material will be too viscous to be pumped.

Tables 1 and 2 show the pH measurements in SBE, AFG, CM, inoculum and the experimental ratios of the substrates after mixing. SBE was acidic (pH 2.87) in deionized water suspension whereas AFG was alkaline (pH 11.87). As can be seen, adding the SBE to CM slightly lowered the pH, but the optimum range for AD is around pH 6.8 – 7.2 [15] so it was not inhibitory for the test. The pH in samples containing AFG were higher than the optimum range, hence, HCL was added to the digesters prior to incubation to adjust the pH at a value close to 7 to prevent inhibition.

*Table 2 - Characteristics of the substrates and mixtures. Total solids (TS) and volatile solids (VS) of individual and mixed materials for cow-manure (CM) and Spent Bleaching Earthe (SBE) batch test.*

Material	TS (%)	VS (% of TS)	pH prior to incubation	pH after incubation
<b>Inoculum</b>	2.2	91.7	7.1	-
<b>CM</b>	8.4	84.1	7.22	-
<b>SBE</b>	87.6	51.3	2.87	-
<b>Inoculum and CM</b>	2.8	87.9	6.95	7.45
<b>CM and 5% SBE</b>	3.1	82.6	7.01	7.54
<b>CM and 10% SBE</b>	3.4	78.8	6.99	7.56

*Table 3 - Characteristics of the substrates and mixtures. Total solids (TS) and volatile solids (VS) of individual and mixed materials in for cow-manure (CM) and Alkaline Fish Glycerin (AFG) batch test.*

Material	TS (%)	VS (% of TS)	pH prior to incubation	pH after incubation
<b>Inoculum</b>	2.1	91.4	8.06	-
<b>CM</b>	6.7	84.3	7.63	-
<b>AFG</b>	97.5	96.5	11.87	-
<b>Inoculum and CM</b>	2.6	88.5	7.15	7.23
<b>CM and 1.5% AFG</b>	2.7	88.6	7.16	7.25
<b>CM and 3% AFG</b>	2.9	89.3	7.20	7.28

## 4.2. BMP Batch test

In this part biogas production results from the batch tests are presented. The accumulated biogas production is presented in volume of biogas produced per gram w/w substrate (mL/g) and in volume of biogas produced per gram VS (ml/g<sub>vs</sub>). Both units are presented in the literature. Biogas yield per gram of VS indicates the degradability of substrates and the efficiency of the process whereas biogas yield per gram of substrate is of interest in estimating biogas production in relation to the size of the digester, more practical for day to day operation.

### 4.2.1. SBE

Figure 14 and Figure 15 show the accumulated biogas yields of the mixtures of SBE and CM. Table 3 shows the biogas yields (the maximum production by anaerobic digestion) in term of substrate weight and biogas in term of VS.

Figure 14 shows that the biogas yields (ml/g wet weight) increased considerably as the proportion of SBE increased up to a maximum of 10 % w/w. An addition of 10% SBE enhanced the biogas yield for about 65% when compared to the yield of CM alone.

Higher SBE proportions in the mixtures may cause inhibition, because of the presence of long chain fatty acids [2]. Inhibition can lower the biogas and methane production in biogas plants. Additionally, because of high dry matter content of SBE, it is likely that high SBE content will create problems with pumping and mixing the material into the digesters and its sedimentation in the digesters during the AD process, especially in continues systems.

*Table 4: Ultimate biogas yields of the tested mixtures of cattle manure (CM) and spent bleaching earth (SBE).*

Substrate	Biogas yield	Yield increase	Biogas yield	Yield increase
	(ml/g)	%	(ml/gVS)	%
CM	27,39	-	521,58	-
CM + 5% SBE	37,47	137%	619,3	119%
CM + 10% SBE	44,77	163%	625,95	120%

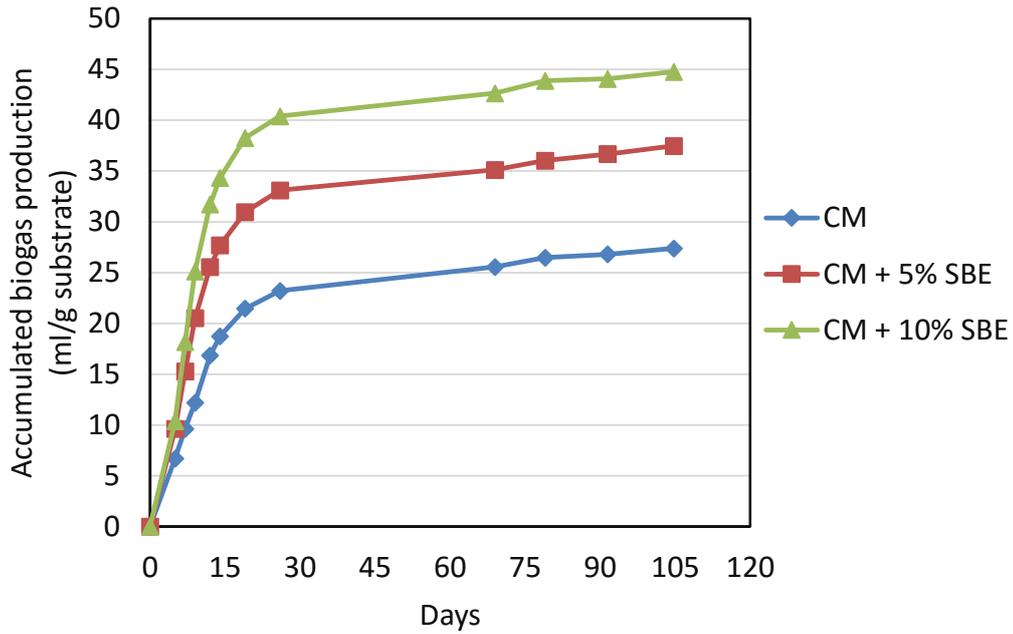


Figure 14: Accumulated biogas production of the mixtures of cattle manure and spent bleaching earth in ml biogas per gram substrate.

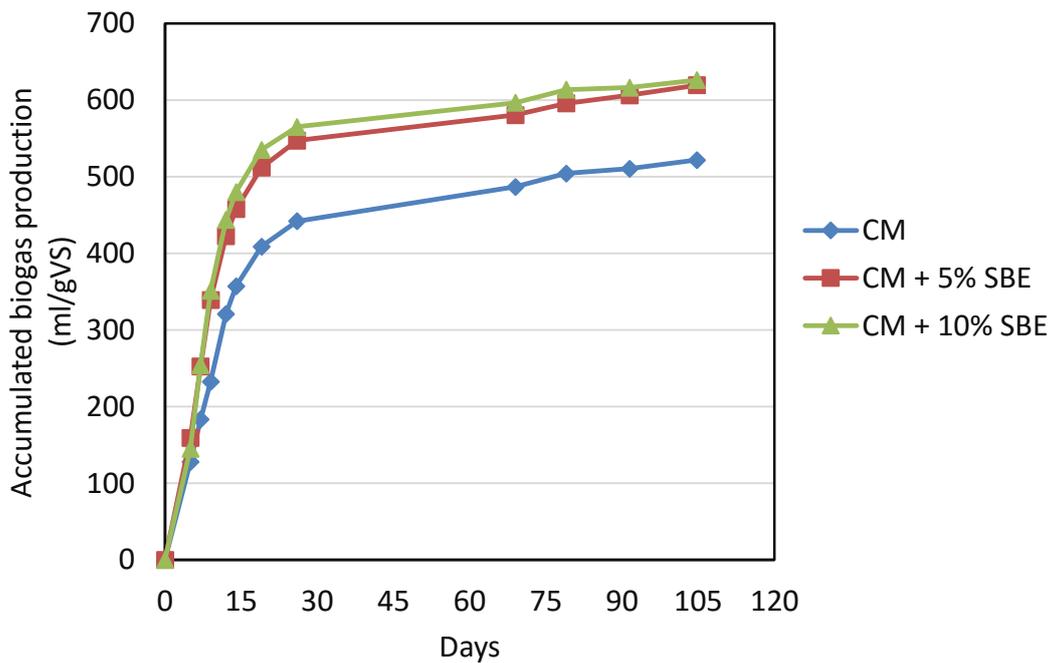


Figure 15: Accumulated biogas production of the mixtures of cattle manure and spent bleaching earth in ml biogas per gram volatile solids.

The methane concentrations for samples with SBE were between 67 to 69%, whereas for the samples having only CM was 65%.

#### 4.2.2. AFG

The results presented in Figure 16 and Figure 17 show the accumulated biogas of the experimental mixtures of AFG. The yield increased with addition of 1.5% AFG to the mixture. This is because AFG has both higher proportion of VS per kg substrate and methane yield per gram VS than CM (Table 3). While they tend to decrease as the proportion of AFG increased to 3%. Biogas yields per g substrate (w/w) increased as proportion of AFG increased in the mixture.

The final biogas yields per gram substrate and gram VS are shown in Table 5. As shown in Table 5, the optimal addition of AFG appeared to be 1.5 % for biogas per g VS, although, the yields produced by addition of 3% AFG were still higher than that achieved by CM alone.

Based on the results presented in this study, it can be concluded that adding AFG (more than 1.5%) as a co-substrate to a biogas plant will decrease the overall yield in terms of VS. From the results presented it can be calculated that a biogas plant that is solely treating CM can increase its volumetric biogas production by two fold upon addition of 3% AFG.

The methane concentration in the biogas was quite similar across all AFG ratios tested, being between 60 and 63 % methane.

*Table 5: Biogas and methane yields of the tested mixtures of cattle manure (CM) and alkaline fish glycerin (AFG).*

<b>Substrate</b>	<b>Biogas yield (ml/g wet weight)</b>	<b>Yield increase %</b>	<b>Biogas yield (ml/g VS)</b>	<b>Yield increase %</b>
<b>CM</b>	25,53	-	543,95	-
<b>CM + 1.5% AFG</b>	41,29	162%	744,53	137%
<b>CM + 3% AFG</b>	47,94	188%	704,04	129%

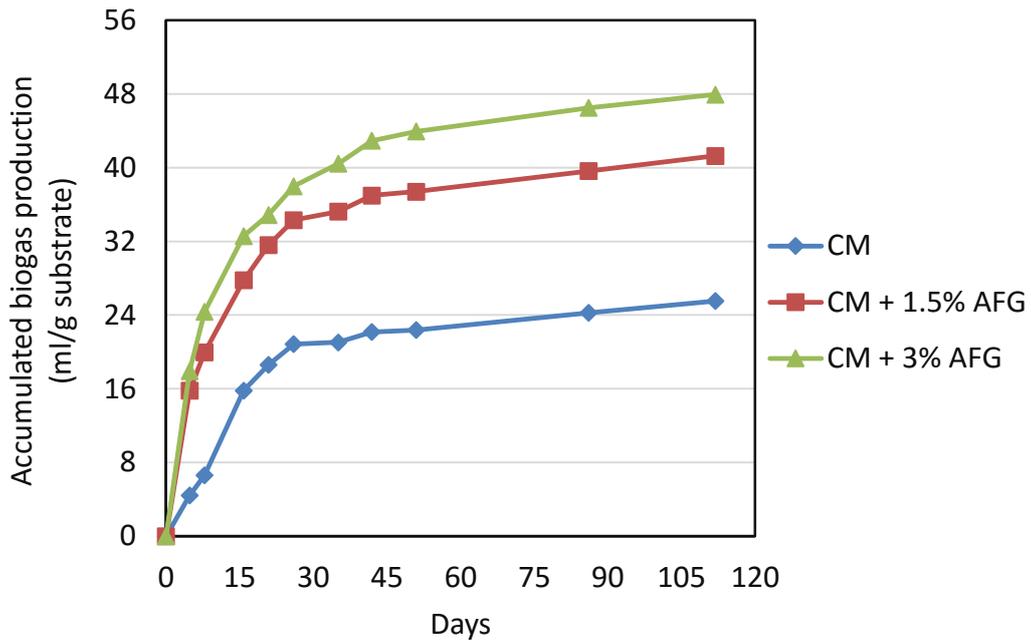


Figure 16: Accumulated biogas production of the mixtures of cattle manure (CM) and Alkaline Fish Glycerine (AFG) in ml biogas per gram substrate.

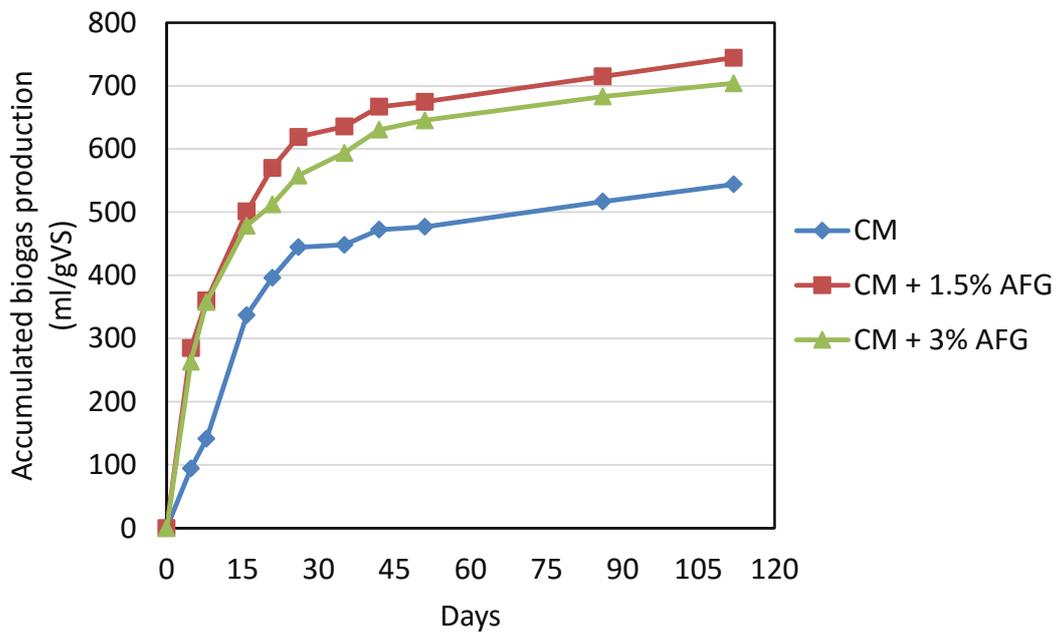


Figure 17: Accumulated biogas production of the mixtures of cattle manure (CM) and Alkaline Fish Glycerine (AFG) in ml biogas per gram volatile solids.

Table 6 summarizes the results of biogas and methane production from other studies using CM, SBE and AFG as feedstock for biogas production. CM in our study had higher methane potential (320 ml/g VS) than that achieved by Normak et al. [18] and Hamilton et al. [19] being around 240 ml/g VS. Campos et al. [20] studied the co-digestion of pig slurry with olive bleaching earth (OBE). Maximum methane yield was achieved with 5% OBE. Addition of OBE up to 12.5% resulted in process inhibition because of LCFAs accumulation. The potential of co-digestion of SBE from omega -3 oil refining industry with cattle manure was studied by Ward [2]. Cattle manure with 2.5 to 12.5% (w/w) of SBE was used. Methane yield per g<sub>VS</sub> decreased with increasing proportion of SBE in the substrate. The highest methane yield achieved was when 7.5% SBE was added and the lowest was with 12.5% SBE. The methane production potential of oil-clay was studied by Valtavaara [3]. Methane production potential was high ranging from 532 to 664 ml CH<sub>4</sub>/g VS. However it was not indicated from which industry the SBE was coming from.

A study conducted on biogas production from co-digestion of glycerin from biodiesel with pig manure [21] showed highest methane production achieved with 80% pig manure. It was almost 125% more methane than when pig manure was mono-digested. To the best of our knowledge, there is no study using glycerin from fish oil refining as substrate and further study could be topic of publications in a scientific journal.

*Table 6 - Summary of the results from other studies using cattle manure, spent bleaching earth and glycerin as feedstock for biogas and methane production.*

<b>Substrates</b>	<b>Biogas (ml/g wet weight)</b>	<b>Biogas (ml/gVS)</b>	<b>Methane (ml/g wet weight)</b>	<b>Methane (ml/gVS)</b>	<b>Reference</b>
Cattle manure	-	-	-	247	[18]
Dairy manure	-	-	-	240	[19]
Pig slurry + 5 and 12.5% olive bleaching earth	32-37	-	-	208-340	[20]
Cattle manure + 2.5 to 10.5% spent bleaching earth (from fish oil refining)	-	-	24-30	287-327	[2]
Spent bleaching earth	-	-	-	560-664	[3]
20 to 100% pig manure + glycerin (from biodiesel)	-	-	-	73-250	[21]

### 4.3. BMP Continuous test

Accumulated biogas production per liter of bioreactor is presented below in Figure 18. After initialization of Stage 2, production of biogas in R3 (AFG) has reduced drastically during a period of 40 days (2HRT). After that the production of biogas started with a production rate two times more than R2 (SBE) and four times more than R1 (CM) (See Table 7). As shown in Figure 19, pH value during experiment was in average 7,6. However pH in R3 felt down to 7,0 with initialization of Stage 2 and recovered after 50 days. It is possible that the sudden introduction of AFG has unbalanced the anaerobic digestion process and acidified the reactor liquid which could have then been inhibited. After 2 HRT the system has recovered while pH value went back to its initial range.

It is relevant to notice that during the transition between Stage 2 and Stage 3, the production rate of biogas in R3 and the pH does not show any inhibition effect. Thus the microorganism system might have adapt with time to glycerin load. Biogas production rate in Stage 3 is 2 times higher in R2 than in R1 and almost 6 times higher in R3 than R1.

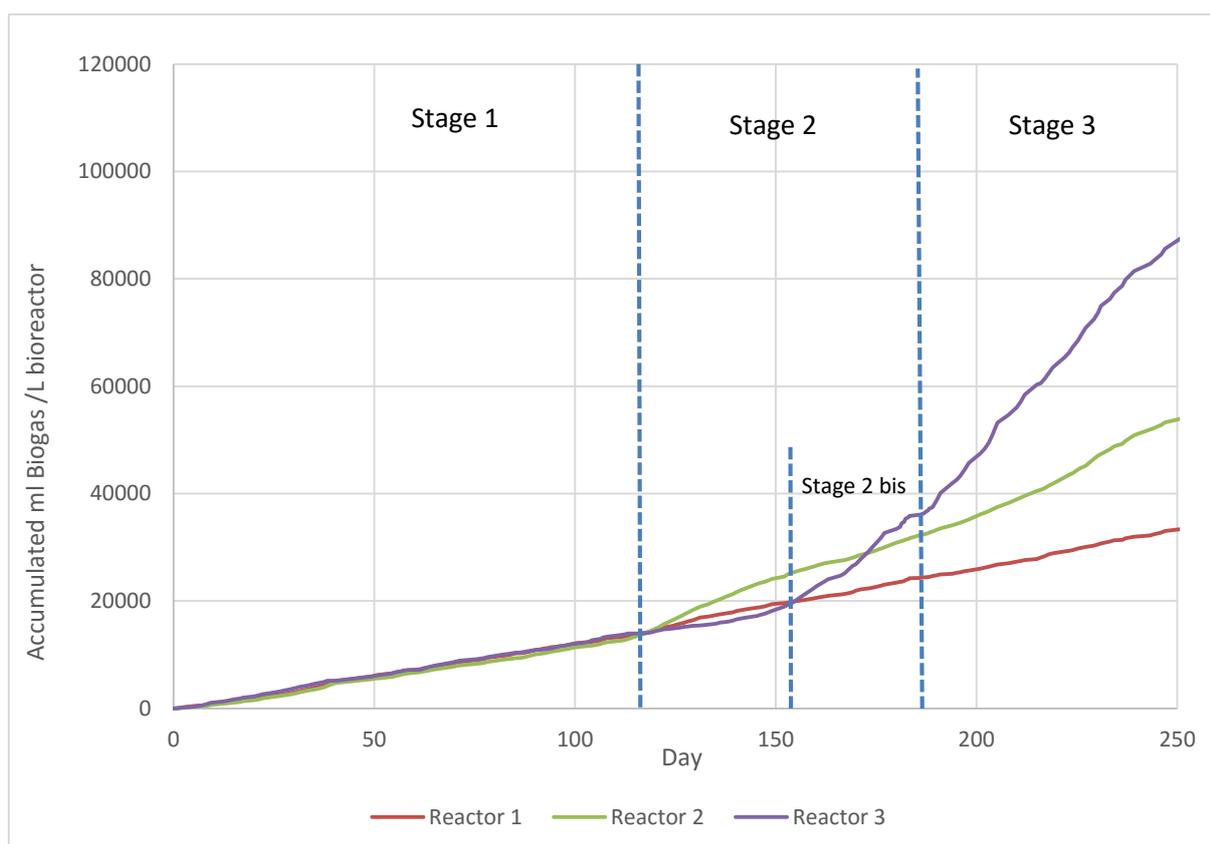


Figure 18: Accumulated biogas production in ml per liter of bioreactor

Table 7 - Production rate in m3 biogas per m3 bioreactor per day

Production rate (m3/m3/day)	Day	Reference		
		R1	R2	R3
Stage 1	0-112	0,119	0,113	0,123
Stage 2	112-188	0,147	0,264	-
Stage 2 – bis *	152-188	-	-	0,522
Stage 3	188-264	0,143	0,313	0,793

\* Stage 2 – bis describes production rate of R3 after inhibition overpassing

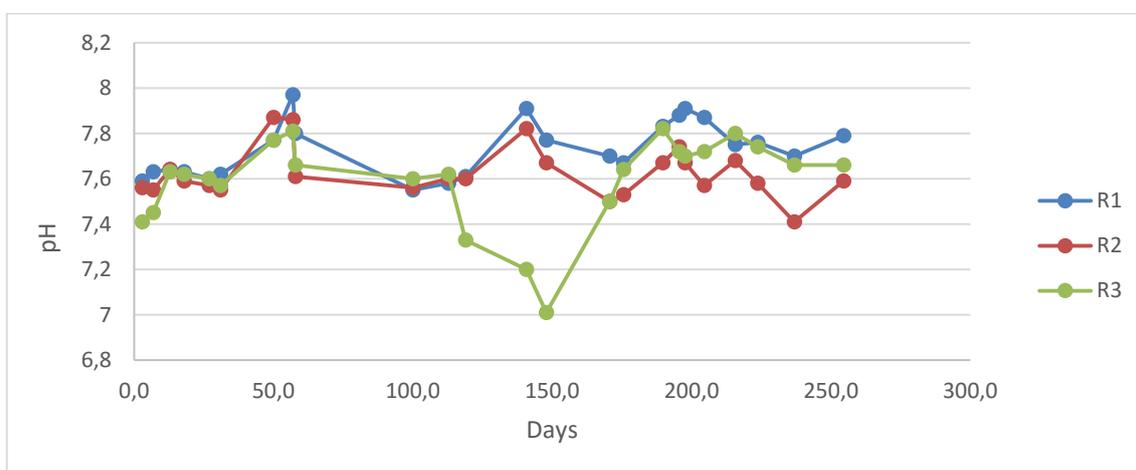


Figure 19: pH value within reactor R1, R2 and R3

Heavy foaming at the surface of the digestate bottle of R3 indicates foaming from the surface of the digester. This foaming was reported in the literature for biogas production from glycerin [22]. It is therefore recommended if glycerol is used in a full-scale biogas plants to monitor the formation of foam and to use foam killing methods for example.

## 5. Conclusion

In conclusion, this study showed that SBE and AFG are suitable feedstocks for biogas production in co-digestion with CM. Tests in batch and continuous mode anaerobic digestion showed large increase of biogas production in co-digestion with CM.

Performance between batch and continuous test for SBE were of the same order. However with AFG, batch tests did not perform as good as in the continuous test. In addition, an adaptation phase was observed with the introduction of AFG that was not observed during the second increase in AFG in the feedstock later in the test. Thus it is determined that in this case the anaerobic digestion had to adapt in order to fully digestate the substrate.

Such results should be taken into consideration while introducing AFG into an AD system and a step wise increase with good process monitoring is advised. For handling SBE, the physical properties of the material and its large non-organic fraction (clay) must be considered. Premature pumping system failure and accumulation at the bottom of the anaerobic digester could lead to failure of the whole process.

Literature on anaerobic digestion of SBE is really limited and non-existent for AFG. There is a need for more tests to get statistical values and determine the adaptation effect of the micro-organisms. Use of AFG might depends also a lot on co-substrate and further study on the C/N ratio is required.

RSI advices MBP to develop further the documentation on full-scale anaerobic digestion plant with a special focus on the handling methods, process monitoring and co-digested substrates.

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