Pre-treatment of mscanthus sinensis with Bacta-sile to aid anaerobic digestion

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Abstract: The investigation of the biodegradability and methane potential of bacterial pre-treated miscanthus sinensis has been carried out. One percent solution of Bacta-sile: A silage promoter was used to pre-treat miscanthus sinensis at 25℃. The anaerobic digestion experiments were carried out at 25℃ and 35℃ in batch experiments. The organic loading rates (OLR) varied between 1.25 g and 7 g in different batch reactors. The results showed that the highest methane concentration was 57% from digester 1 while the lowest methane produced was 38% from digester 3. The low methane production from digester 3 was attributed to temperature changes and poor organic loading rate. Bacterial pretreatment aided biodegradation of miscanthus at 25℃. Operating temperature of 25℃ had a great effect on digestion experiments resulting to longer required Hydraulic Retention Time (HRT).

Keywords: anaerobic digestion, methane (CH_4), carbon dioxide (CO_2), cyanobacteria, Hydraulic Retention Time (HRT), Organic Loading Rate (ORL)

DOI: 10.3965/j.ijabe.20130601.008

Citation: Abiodun O. Jegede. Pre-treatment of mscanthus sinensis with Bacta-sile to aid anaerobic digestion. Int J Agric & Biol Eng, 2013; 6(1): 82－89.

1 Introduction

The energy crop market providing feedstock for power stations and anaerobic digesters is expanding day by day. This expansion could be regarded as a response to various heat and power projects being developed across the world that make use of energy crops[1]. Miscanthus is a C_4 perennial grass, which has the potential of producing huge annual yields with little input of nutrient. Miscanthus is native to Asia (Eastern) and its cultivation in Europe is increasing. Miscanthus could reach a height of three meters and yield more than 20 tones of dry matter/acre/annum, (20 t of dm/ac/year) under a perfect weather after three years[2]. It belongs to the Kingdom: *Plantae*, Order: *Poales*, Family: *Poaceae* and Genus: *Miscanthus*. Miscanthus has C_4 photosynthesis pathway and it exhibits a usual combination of high water, nitrogen and light use effectiveness. The crop does not require large amount of water for optimal growth, because of its high productivity[3]. Jones[4] reported that there are about 17 various species of miscanthus. The major types of miscanthus are miscanthus giganteus (m giganteus), miscanthus sinensis (m. sinensis) and miscanthus sacchariflorus (m. sacchariflorus).

M. sinensis has coloured foliage and is a clumping plant. M. sinensis could grow as tall as 1.5 m. This type of miscanthus has a wide range of inflorescence with different colours such as pale pink, buff and deep blue. It is usually grown in the temperate regions of the world as garden plants (ornamental plants). In some parts of North America m. sinensis is an invasive species. M. sinensis are propagated vegetatively by separating the rhizome into small fractions by mechanical method for replanting or by stem slicing through tissue culture because miscanthus are triploid type crops[5]. It is usually harvested in spring between January and April preceding new rhizomes re-growth in April, because daily

Received date: 2012-11-23 **Accepted date:** 2013-03-11

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temperature are usually more than 10℃[6]. It could be harvested by using mown or with a harvester typically used for harvesting forage maize. Miscanthus can store carbon in both above and below ground materials. Studies from 15 European states show that about 12 million tons of carbon in a year could be sequestered by miscanthus[5].

Miscanthus net heat content on dry basis is 17 MJ/kg and it has been used in the following ways; as energy crop: used in co-firing in coal power plants, production of second generation liquid biofuels e.g. ethanol and also for bio-oil production; Paper production; in Horticulture: serves as raw material in plant pots production; for animal bedding. Past works and experiments on anaerobic digestion of miscanthus are scares. However, experiments and studies have been conducted on other species have been reported.

Koch et al.[7] reported that grass silage were fermented as mono substrate at mesophilic conditions, (38℃) using a loop bioreactor. The average biogas generation of about 0.5 m^3/kg of volatile solids, having concentration of methane (CH_4) of about 52% was achieved at a loading rate of 3.5 kg of volatile solids per cubic meters a day (3.5 kg/VS m^3 d). It was reported that the level of degradation was above 60% based on volatile solids and about 75% according to the chemical oxygen demand (COD). The system performed stable despite the high concentration of ammonium NH_4 of approximately 4 g/L for the total operation session of 310 days[7]. However, the specific types or species of the grass used were not mentioned. The system was inoculated with substrates from a working biogas plant operating at mesophilic conditions.

In the study of Mahnert et al.[8], three ensiled and fresh grass species were examined in batch lab scale experiments to estimate their optimal biogas generation potential at mesophilic conditions (35℃). The species are cocksfoot (Dactylic glomerata), perennial ryegrass (Lolium prorenne) & meadow foxtail (Alopecurus pratensis). The methane and biogas output based on volatile solids were examined to be between 0.31 – 0.36 m^3/kg of volatile solids and 0.65 – 0.86 m^3/kg of VS. Furthermore, the grass and cattle waste were digested in a second experiment which was a semi-continuous system to determine their biogas generation potentials at organic loading rates of 0.7 kg and 1.4 kg of volatile solids per cubic meters a day. Digestion experiments were carried out in continuous stirred bioreactors at mesophilic conditions (35℃), with the three fresh species as single substrates, cattle waste and a co-digestion of both mixtures. At an organic loading rate of 0.7 kgvs/m^3 d and 1.4 kgVS/m^3 d the biogas outputs obtained were 0.61 m^3/kg vs and 0.56 m^3/kg vs, respectively, for the grass as a single substrate. The effect of the organic loading rate on biogas output was very minimal for cattle slurry and co-digestion experiments. For the co-digestion experiments, biogas yield was proportional to the quantity of volatile solids from grass in the cattle slurry mixture.

In this study, m. sinensis were anaerobically digested to investigate the methane potential. Bacta-sile original, a composition of bacteria and enzymes was used as bacterial pre-treatment agent to investigate the effects on biodegradation and methane production of m. sinensis during digestion experiments. Different types of pre-treatment techniques are usually applied to aid biodegradability of complex biomass because of their physiology which could resist or delay hydrolysis.

2 Materials and methods

2.1 Digestion experiment overview

Anaerobic digestion of m. sinensis were carried out in three batch experiments in lab glass bottles with sizes 280 mL and 330 mL and working volume between 170 mL and 300 mL. Digester feedings were done occasionally and recorded. The digestion experiments were carried out inside an incubator at temperature 25℃ and 35℃.

2.2 Substrate preparation: m. sinensis

The m. sinensis used were obtained from the Botanic Gardens, Edinburgh, United Kingdom. The grass was cut into small sizes of less than 0.5 cm×4 cm scissor before silaging. The miscanthus were later ensilaged in a two-litre container and kept at room temperature for three weeks.

2.3 Pre-treatment of substrates

The miscanthus were ensilaged in a two-litre plastic container and kept at room temperature before use for

approximately three weeks. Some quantity of the miscanthus after two weeks ensilaging were treated with 1% solution of Bacta-sile original (a composition of bacteria: *pediococcus, enteroccus and lactobacillus*, Enzymes: *cellulase, hemi-cellulase and amylase*) for approximately two weeks at 25℃. The Bacta-sile original was obtained from Scotmin, United Kingdom.

2.4 Experimental setup

2.4.1 Digester setup

The laboratory-scale reactors with the volumes of 280 mL and 330 mL and working volume between 250 mL and 300 mL were equipped with only one opening for the withdrawal of gas with the use of a 5 mL syringe when sampling was required. The cap is equipped with fittings and septum to prevent gas leakage. Syringes with size 2 mL were inserted into the reactors to serve as pressure relieve mechanism in case there is a pressure built up in the laboratory scale reactors.

The digesters were placed in an incubator (Stuart Scientific, Orbital incubator S150), maintained at 25℃ and later 35℃ for four days.

The incubator is equipped with shaking mechanism and shaking was set between 60 rev/min and 100 rev/min to avoid accumulation of scum in the digesters and to ensure proper temperature distribution. Adequate mixing will prevent digester contents of developing localized pockets of temperature variations.

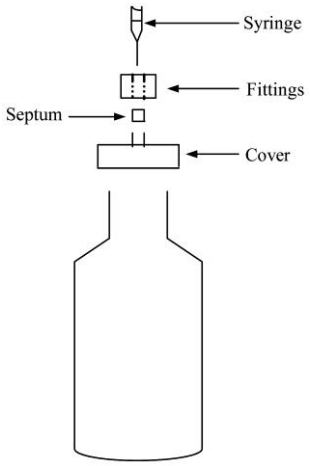

Figure 1 Schematic diagram of the digester

2.4.2 Inoculum

The inoculum (sludge) used for the experiments was obtained from a food anaerobic digester. The inoculum was kept in the freezer for two months before used. 50-70 mL of the sludge was used to inoculate the digesters at start-up.

2.4.3 Digesters start-up and operation

Trace elements; metals and selenite solution were added to all the digesters to provide necessary nutrients for the micro-flora during digestion and distilled water was used throughout the experiments. The details of the trace element are: $FeCl_2.4H_2O$, 2g: H_3BO_3, 0.05g; $ZnCl_2$, 0.05 g; $CuCl_2.2H_2O$, 0.038 g; $MnCl_2.4H_2O$, 0.05 g; $(NH_4)_6Mo_7O_{24}.4H_2O$, 0.05 g; $AlCl_3$, 0.05 mg; $CoCl_2.6H_2O$, 0.05 g; $NiCl_2.6H_2O$, 0.092 g; ethylenediaminetetraacetate, 0.5 mL; concentrated HCl, 1 mL; $Na_2SeO_3.5H_2O$, 0.1 g.

Digester 1 (D1) was set up and fed with silage – ensilage miscanthus without promoter (bacta-sile) 2.5 g TS, 0.25 mL of trace element and 70 mL of inoculum. The digester volume was 330 mL with working volume of 270 mL and the pH value measured was 7.83. After 35 days without methane production, the total solids (TS) in the reactor were withdrawn and 3 g of silage with promoter – bacta-sile were fed into the digester. The pH value after the addition of new substrate was measured and found to be 6.56.

Digester 2 (D2) was also started with 1.25 g of silage -without promoter, 70 mL of inoculum, 0.25 mL of trace elements and the pH value was measured to be 8.02. The reactor volume was 330 mL and the working volume was 270 mL. On day 35 after no methane production was detected from the reactor, the total solid from the reactor was withdrawn and 7 g TS of silage-with promoter *-bacta-sile Original* was fed in to the reactor and the pH value measured was 6.57. On day 47 the operating temperature of the reactor was increased from 25℃ to 35℃.

Digester 3 (D3) was set up and 3 g TS of silage without promoter was fed into the digester with 50 mL of the remaining inoculum and 0.25 mL of trace element was added. The reactor volume was 280 mL with working volume of approximately 250 mL and the pH value was measured to be 8.27. On day 12, the operating temperature was increased from 25℃ to 35℃. Digester 3 was operated throughout without feeding of miscanthus pre-treated with bacta-sile. Increasing of the temperature in digesters 2 and 3 was done to study the

effect of the temperature change on both digesters and to compare with D1.

2.5 Analytical procedures for digestion experiments

2.5.1 Total Solids (TS) determination

The total solid, the dry portion of a material, is the inorganic and organic fixed solids, e.g. minerals. The total solids of the substrate used for this study was determined by the use of a weighing balance.

2.5.2 pH measurement

In this study, the pH meter used was made by Thermo Orion, Fisher brand model Hydrus 400. The pH values of the contents of the digesters were measured at the beginning of the experiments and occasionally during feeding and sampling.

2.5.3 Nutrients measurement (Nitrogen and Phosphorus)

There are some basic levels of nutrients required by anaerobic micro-flora for anaerobic digestion and the basic ones are nitrogen and phosphorus. In addition, after digestion digestate usually contains some important nutrients – such as: Mg, N, P, Na, K etc., that makes it applicable for use as bio-fertilizer. Two of these elements, namely nitrogen and phosphorus were determined using HARCH spectrophotometer DR/4000 series, manufactured by Camlab. The measurements were done according to instructions in HACH DR/4000 Spectrophotometer handbook/manual.

2.5.4 Chemical Oxygen Demand (COD)

The COD of a system is a vital tool to estimate the equivalent oxygen in an organic matter of a substrate that is capable to oxidize by a chemical oxidant that is strong. The COD of the contents of the reactors were measured before and after digestion.

2.5.5 Gas sampling and analysis

The gas samples from the reactors were analyzed basically for methane and carbon dioxide concentrations by Gas Chromatograph (GC) 600 series manufactured by GOW-MAC Instruments Co., equipped with a thermal conductivity detector (TCD), and helium gas was used as the carrier gas. The oven temperature was 50℃ and the column length is 100 μL. The gas chromatograph was calibrated with 55% methane and 45% carbon dioxide from Cryoserve. The standard curves area from the gas chromatograph was integrated to give the standards for the methane and the carbon dioxide. Five millilitres glass syringe with 0.4 mm × 25 mm was used to withdraw gas sample from the reactors and injected to the GC column. About 0.5 mL is usually injected into the GC. Gas sampling and tests were done at an average of three times in a week. The syringe has a closing mechanism to prevent the sample gas from escaping.

3 Results and discussion

3.1 Digester 1 (D1)

Figures 2 and 3 showed the results of the methane concentration and volume changes from D1. 2.5 g of silage- miscanthus without treatment with bacta-sile (promoter) was used to start D1 and the pH measured was 7.83. For about 35 days into the operation of the digester there was no methane production and the pH value was found to be 6.57. The total solid content of the digester was withdrawn and 3 g of silage which has been treated with 1% solution of silage promoter was fed to the digester on day 35. The following day that is day 36 methane gas was detected at 12% concentration. The methane production increased and attained a maximum concentration of 57% with an accumulated volume of 11.4 mL at the end of the digestion experiment. The COD of the content of the digester was 4 500 mg/L at the beginning of the digestion experiment and 5 900 mg/L when 3 g TS of substrate was added. The COD reduced to approximately 2 000 mg/L at the end of the anaerobic digestion process. The digestate of the digester was also found to have good concentrations of nitrate and phosphate at 506 mg/L and 70 mg/L respectively at the end of the experiment.

The inability of the miscanthus without silage promoter pretreatment to digest and produce methane at 25℃ could be associated with the morphology of miscanthus. Miscanthus is made up of mainly lignin, hemi-cellulose and cellulose. The bonds among lignin, cellulose and hemi-cellulose have not been broken down to release cellulose for anaerobic digestion. In addition, the digestion experiment was performed at 25℃ which would rather delay hydrolysis stage of the anaerobic digestion of cellulose. The addition of miscanthus treated with the promoter showed from the results that the

bacteria and the enzymes present in the promoter have been able to degrade the bonds and the links among lignin, hemicelluloses and cellulose to accelerate digestion of cellulose. The pre-treatment process aided the hydrolysis stage of the digestion process. However, details of mechanism for the stage could not be accounted for, which would rather need further investigation.

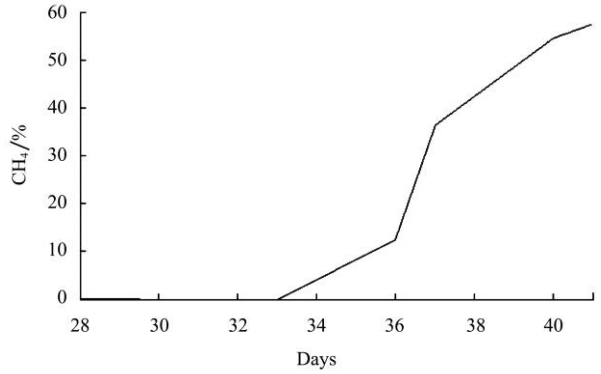

Figure 2 Methane concentration changes from Digester 1 (D1)

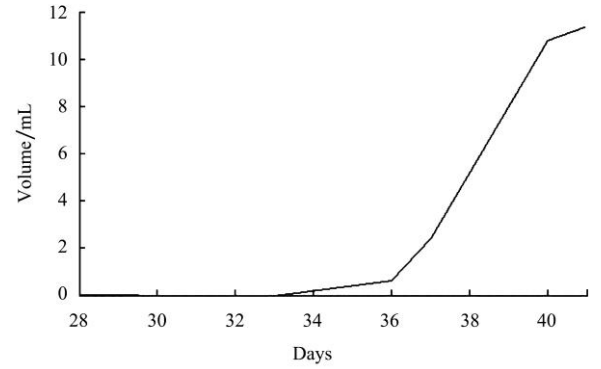

Figure 3 Volume changes from Digester 1 (D1)

3.2 Digester 2 (D2)

Figures 4 and 5 showed the results of the methane concentration and volume changes from D2. At start-up, D2was fed with 1.25 g TS of miscanthus without bacteria pre-treatment and the pH was measured to be 8.02. After 35 days without methane production, the whole total solids (TS) in digester 6 were withdrawn and 7 g of TS of pre-treated miscanthus with bacta-sile was fed to the reactor on day 35. The chemical oxygen demand (COD) of digester content was also done and estimated to be 3 623 mg/L at the beginning of the experiment. The COD when 7 g TS of substrate was added to the digested was estimated to be 12 650 mg/L and reduced to 7 360 mg/L at the end of the experiment. The nitrate and phosphate concentrations were also found to be 930 mg/L and 120 mg/L, respectively.

The highest methane concentration of approximatley 38% and an acumulated methane volume of 3 mL was recorded on day 37 at operating temperature of 25℃. The methane production was fluctuating throughout the digestion experiments and dropped to around 11% after the operating temperature was increased to 35℃. The pH of the digester was slightly acidic with pH 5.52 at the end of the digestion experiment indicating the reduction of methanogenesis bacteria which resulted into low degradation of the substrate. Methane producing bacteria are sensitive to temperature change and probably caused the reduction of the methanogenesis bacteria. The temperature change would also have probably caused a shock to the microbes, since they can respond to a few changes in centigrade.

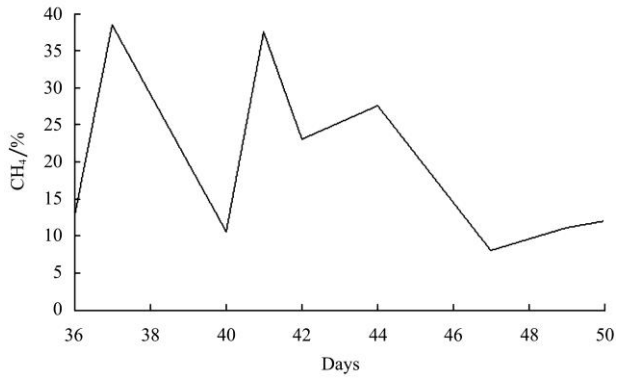

Figure 4 Methane concentration changes from Digester 2 (D2)

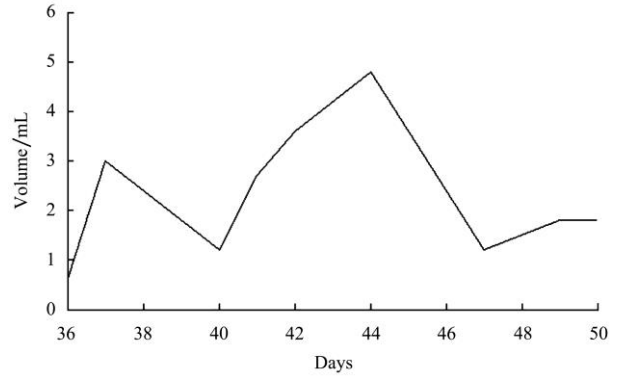

Figure 5 Volume changes from D 2

3.3 Digester 3 (D3)

Figures 6 and 7 showed the results of the methane concentration and volume changes from D3. The digestion experiment with D3 was performed to see the effect of temperature increase on miscanthus without bacterial pre-treatment. Three grams of TS of

miscanthus were digested. The pH value and COD before digestion were 8.27 and 6 500 mg/L, respectively. The temperature was increased from 25℃ to 35℃ on the 12th day and prior to this there was no methane gas detected in the digester. The COD reduced to 2 300 mg/L at the end experiment from 6 500 mg/L. The nitrate and phosphate concentrations of the digestate at the end of the anaerobic digestion experiment were discovered to be 480 mg/L and 63 mg/L.

After the increment on day 12, methane was detected and peaked at 53% with accumulated volume of 5 mL for three days after which the digestion experiment was ended. It could be seen that the increment in temperature increases the rate of reaction in the digester leading to methane production by methane producing micro-flora. This result indicates the strong dependence of anaerobic microbes on temperature, because methane production started after the operatiing temperature of the digester was increased from 25℃ to 35℃. Methane producing bacteria are active in the mesophilic temperature range from 30℃ to 35℃ and at thermophilic range which is from 50℃ to 60℃.

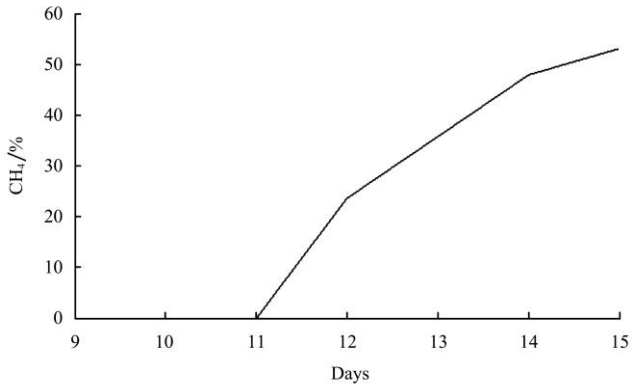

Figure 6 Methane concentration changes from D3

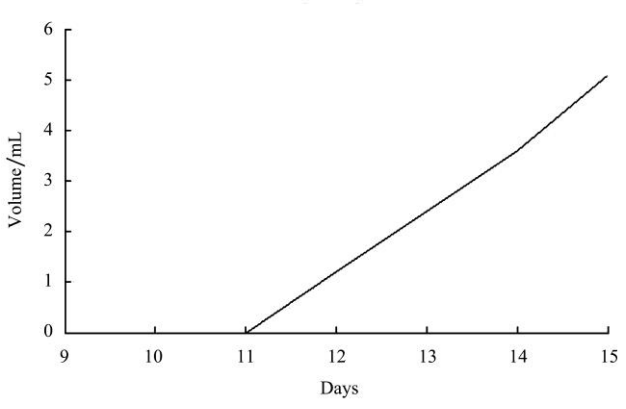

Figure 7 Volume changes from D3

3.4 Effect of temperature

Temperature is an important factor in an anaerobic digestion process. All the digesters did not start producing methane until about three to four weeks of retention time at 25℃. The delay could be attributed to the operating temperature of the digesters as the anaerobic microbes are temperature dependents, the higher the temperature the higher the rate of reaction and the shorter the HRT. 25℃ was chosen for the experiment to investigate the methane potential at this temperature range, because setting up anaerobic digesters in countries with cold climate means additional energy input for the digesters which could have effects on the economics of the anaerobic digesters.

After the temperature was increased there were significant changes in D1 and D3, but D2, remained unstable till the experiments were concluded. The failure of D2 to remain stable after temperature increase could be attributed to the organic loading rates (OLR) and the inability of the bacteria to quickly adapt to the temperature change.

The miscanthus without bacta-sile pre-treatment digested in D3 yielded good results at 35℃, the substrate stayed 12 days in the digester at 25℃ without methane production. However, the longer time of ensilaging would have contributed to the partial fermentation of the grass before anaerobic digestion. All the silage used were kept in a plastic bottle under anaerobic conditions and the one fed into D3 were kept for about two months compared to others used in D1 and D2, which were preserved for two weeks. The longer period of ensilaging could be a contributing factor that aided the digestion of the substrate fed to D3.

3.5 Biogas production, methane concentration and loading rate

Biogas production and methane concentration varied with the different organic loading rates in the digestion experiments. 12% methane concentration was recorded from D2 on day 50 at an operating temperature of 35℃. The drop in methane production is as a result of instability of the digester after the addition of 7 g of miscanthus pre-treated with bacta-sile. The drop could also be attributed to the inability of anaerobic microbes to

respond to the increase in temperature.

D1 produced the highest methane concentration at 57% while D2 recorded 53% methane concentration. The result from D1 which was fed with miscanthus treated with bacta-sile could be compared with D3 fed with silage without bacta-sile pretreatment. These results could be viewed from two perspectives. It could be argued that is economical to apply bacterial pre-treatment to complex biomass at 25℃ to achieve 57% methane concentration compared to 53% from D3 which requires additional energy because of the need to increase the temperature by 10℃. There would be need to evaluate the cost of production and the energy required for the production of the bacterial enzymes used for the pretreatment and the cost of operating the digester at 35℃ to obtain same results.

3.6 pH and COD

The pH of an anaerobic digester is a major determining factor of its performance. Methanogenic bacteria work best at pH range of 6.5-7.5. All the digesters had pH of approximately neutral values when the experiments were concluded except for D2 which was unstable and recorded a drop in methane production which had a pH of 5.82. The digester probably started becoming acidic after the addition of 7 g of substrate there-by reducing the population of methanogenic bacteria that perform best at pH 6.5-7.5. The failure of D2 could be attributed to the declining population of methane producing bacteria as a result of the loading rate.

The COD removal is also an important indicator to know the performance of an AD. The average COD from the digesters reduced significantly at an average of 50% at the end of the experiment.

In addition, the COD added when substrates were added to the digesters during operation were accounted for. Therefore the removal of COD in the all digesters at an average 55% at the end of the digestion experiments

3.7 Digestate as fertilizer

Digestate and biogas are the two main products that are produced during an anaerobic digestion process. If these two products are well managed the process could have good economy. Apart from the organic matter content, the digestate also has nutrients that can affect the soil quality positively, stimulate microbial activities and also help in improving the water-holding potential if applied as fertilizer. Some of the nutrients are N, K, P and Mg[9]. Although the nutrients required in anaerobic digestion process are divided into two- macronutrients and micronutrients. Macronutrients are required in relative large amounts by bacteria, for example, nitrogen and phosphorus. Micronutrients, such nickel and cobalt are required in small amounts by most bacteria. The two macronutrients that are usually of great interest in any biological treatment process are phosphorus and nitrogen. These are available to anaerobic bacteria including methane-producing bacteria as orthophosphate-phosphorus (HPO4-P) and ammonical-nitrogen (NH_4^+-N)[10]. The application of the digestate as bio-fertilizer will also increase crop yields, increase soil quality and grain quality[11].

However, there are possibilities of heavy metals concentration in the digestate, which is the reason why digestate, which must be used as organic fertilizer, must be screened and certified. In this study only nitrogen and phosphorus in form of nitrate and phosphate concentrations were determined in all the digesters and they were found to be rich in the two nutrients (nitrogen and phosphorus) because the amounts of nutrients available. At the end of the digestion experiments, the COD removed in D1-3 are 3 900 mg/L, 5 290 mg/L and 4 200 mg/L. The measured nitrogen and phosphorus from D1 are 506 and 70 mg/L; those from D2 are 930 and 120 mg/L; from D3 are 480 and 63 mg/L. These amounts exceed the minimum nutrients requirement for anaerobic digesters as described by Gerardi[10] and which concentrations will affect soil quality by stimulating microbial activity, increasing soil water-holding capacity and improving soil structure[11].

4 Conclusions

The m. sinensis was prepared and used in two forms, silage and silage treated with bacta-sile original. At 25℃, the silage without promoter was unable to break down quickly because of the morphology and the duration of ensilaging.

Methanogenesis was encouraged rapidly when silage treated with bacta-sile was fed into the digesters at 25℃. The pre-treatment could have a positive impact on the degradation of the complex biomass. A maximum methane potential of about 57% was recorded with the silage treated with promoter at 35℃. D3 containing silage without pre-treatment was able to undergo methanogenesis at a short period of time at operating temperature of 35℃ and yielded 53% CH_4. This result could be attributed to temperature since anaerobic micro-flora is temperature dependent.

All the experiments had a long hydraulic retention time which can be attributed to the 25℃ operating temperature for most of the study. Miscanthus treated with bacta-sile yielded 57% CH_4 at 35℃, 3 g of TS added. Untreated miscanthus yielded 53% CH_4 at 35℃, 3 g TS added. The untreated miscanthus at 25℃ was unable to digest quickly because digestion and degradation was delayed. At an high loading rate of 7 g TS of miscanthus, the digestion process was unstable reducing methane potential to 12% even at 35℃.

It is advisable to pre-treat miscanthus with silage promoter to accelerate digestion and to reduce the energetic cost in AD. Operating temperature of 25℃ is moderate for AD, but longer retention time should be expected. Further detailed studies are therefore necessary to ascertain and to unravel the details of the delay of the hydrolysis stage and the mechanisms involve in degradation after pretreatment procedures.

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