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An Investigation of Hydrolysis yield in Thermophilic Anaerobic Digestion of Cattle Manure

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Abstract:*One of the vital essential fees related to the operation of organic wastewater cure is thedealing with and disposal of sludges generated specially in the conversion of soluble organics asmeasured through BOD/COD into both carbon dioxide (aerobic) or methane (anaerobic), waterand bacterial cells. The motive of the study is to toughen the biogas construction cost and yield for the period ofanaerobic digestion of cattle manure.Hydrolysis being an anaerobic digestion-limiting step, a literature study was once applied onthe approaches to fortify it by way of various the experimental conditions. Bioaugmentation, addition ofsurfactant and decreasing the pH to 7.0 had been anticipated to enhance biogas creation.Progress of selected organisms used to be studied for their addition into reactors. Two reactorshad been operated under pH-manipulate at 7.0 but the test had to be stopped when you consider that of theacid addition that used to be excessive.In the meantime, experiments were made to design an efficient protocol for extracting proteinsfrom the reactor digestate and evaluating produced hydrolytic enzymes depending of theconditions. It was shown that proteins are regularly present in the liquid fraction.*

Keywords-Aerobic biological treatment, anaerobic biological treatment, anaerobic digestor,bioaugmentation.

I. INTRODUCTION

Biogas production through anaerobic digestion (AD) is an environmentalfriendly process utilizing the increasing amounts of organic waste producedworldwide. A wide range of waste streams, including industrial and municipalwaste waters, agricultural, municipal, and food industrial wastes, as well asplant residues, can be treated with this technology. It offers significantadvantages over many other waste treatment processes. The main product ofthis treatment, i.e., the biogas, is a renewable energy resource, while the byproduct,i.e., the digester residue, can be utilized as fertilizer because of itshigh nutrient content available to plants (Ward et al., 2008). The performanceof the AD process is highly dependent on the characteristics of feedstock aswell as on the activity of the microorganisms involved in differentdegradation steps (Batstone et al., 2002). The conversion of organic mattersinto biogas can be divided in three stages: hydrolysis, acid formation, andmethane production. In these different stages which are however carried outin parallel, different groups of bacteria collaborate by forming an anaerobicfood chain where the products of one group will be the substrates of anothergroup. The process proceeds efficiently if the degradation rates of thedifferent stages are in balance (Yong et al., 2015)

There is an increasing interest in bioenergy production across the world forenvironmental as well as economic and social reasons. The production ofbiogas contributes to the production of renewable and sustainable energysince biogas works as a flexible and predictable alternative for fossil fuels.

The main political driving forces linked to the biogas system has a countryspecificvariation (Huttunen et al., 2014). Within the European Union,well-developed biogas industry can be found in Germany, Denmark, Austria,and Sweden followed by the Netherlands, France, Spain, Italy, the UnitedKingdom, and Belgium. In these countries, with a strong agro-sector,reduction of nutrient emissions and renewable energy production are equallystrong driving forces supporting biogas production. In other countries, likePortugal, Greece, and Ireland, as well as in many of the new East-Europeanmember states, the biogas sector is currently under development, due to theidentified large potential for biomass utilization there.

II. RELATED WORK

Organisms, enzymes and reactions are highly dependent on pH and have different pHoptima. Therefore, selection of one optimal value for the whole sequence of processesinvolved in AD is

difficult. However, for AD the optimum pH mean is 7(Chen et al., 2008).Moreover, ammonia concentration depends on pH and ammonia is known as the principalinhibitor of AD (Zeeman et al., 1991), the decrease of its concentration likely results in ahigher hydrolysis rate. Furthermore, there is a relief of ammonia-induced inhibition at lowerpH. Braun et al. (1981) showed that lowering the pH from 8 to 7.4 during anaerobic digestionof liquid piggery manure resulted in a reduction of the concentration of ammonia from 316mg l-1to 84 mg l-1and an increased biogas production. Zeeman et al. (1985) observed thatdecreasing the pH from 7.5 to 7.0 during thermophilic anaerobic digestion of cow manureresulted in four times increased methane production

Chemical and physical pretreatments are used to improve the hydrolysis rate of otherwastes like wood or straw. However, addition of organisms producing hydrolytic cellulolyticenzymes would be more cost-effective (Angelidaki et al., 2000) because they will producetheir own enzymes, add new degradation pathways for manure and improve the finalhydrolysis-rate (Schwarz et al., 2001).For bioaugmentation, organisms that grow under thermophilic and anaerobic conditions,and that produce enzymes that are not already present in the digesters should be selected.Clostridium josui and Clostridium stercorarium were selected, since both of them are knownfor producing hydrolytic enzymes, being thermophilic and anaerobic.

Since hydrolysis is limited by the available surface area of cellulose, increasing surface areashould improve the hydrolysis. Helle et al. (1993) showed that surfactants increasedhydrolysis rate by 67%, probably by lowering the nonactive binding sites that decrease theeffectiveness of enzymes. Several tests conducted by Eriksson et al. (2002) indicated that amajor obstacle in the enzymatic conversion of lignocellulose is the adsorption of significantamounts of enzyme on exposed lignin surfaces without being able to degrade it. Surfactantsprevented unproductive binding of cellulases to lignin, by binding lignin in the lignocellulosefibers to the hydrophobic part of the surfactant by hydrophobic interactions. Then, addingsurfactants in digesters should increase the available substrate and its hydrolysis rate byhydrolytic enzymes.

Rhamnolipids are surfactants that can be produced either by chemical synthesis or by meansof microbial cultivation; it is ecologically well acceptable and biodegradable (Mohan et al.,2006). The use of rhamnolipids for solid substrate fermentation resulted in a better cellulaseand xylanase activity, the last one being 119.6% higher than the control (Liu et al., 2006).Zhang et al. (2009) tried to explain mechanisms of the stimulatory effect of rhamnolipids onrice straw hydrolysis. Rhamnolipids increased the activity and stability of hydrolytic enzymesand prevented unproductive binding of enzymes to lignin.

III. MATERIALS AND METHODS

The inoculation of the serum bottles was performed under anaerobic conditions, in ananaerobic bag, filled with nitrogen. After breaking the ampoule, 0.5 ml of medium wasadded to suspend the biomass; then the solution was transferred into a serum bottle andpressurized with some nitrogen from the anaerobic bag. Cultures were incubated overnightat their optimal temperature, C. josui at 45 °C (Sukhumavas et al., 1988) and C. stercorariumat 65 °C (Madden, 1983).

Growth curve: Culture growth was checked with OD measurements at 600 nm in duplicates with aspectrophotometer.Samples were taken every two hours in order to make a growth curve and determine theexponential growth phase. After 24 hours at their optimal growth temperature, cultureswere still sampled for 4 days.A growth curve was made for C. stercorarium at 65°C and 52°C in order to know when theyreached their maximal OD600 for their enrichment and addition in reactors.

Amplification: 600 ml of medium was prepared and inoculated with C. stercorarium cultures to have astarting OD600 of 0.1. Following the growth curves, C. stercorarium cultures were harvestedafter 33h

cultivation and put in the fridge at 4°C.Because of some encounter problems in C. josui cultivation, 600 ml bottles were inoculatedwith an OD600 below 0.1 and they were left in incubation until their OD600 was sufficient forthe reactors inoculation.

Protein quantification

Fraction preparation

Sävsjödigestate samples were centrifuged at 7,000 g for 10 min then at 15,000 g for 30 minin order to separate as much as possible liquid (supernatant) and solid fraction.The solid fraction was resuspended in two different buffers in order to separate the enzymes bound to the solids:

- Buffer I : [Na2HPO4 100 mM, NaCl 0.5 M] + [NaH2PO4 100 mM, NaCl 0.5 M]. Thesecond solution was mixed to the first one to pH 7.8.
- Buffer II : [Na2HPO4 100 mM, NaCl 0.5 M, TEAB 50 mM, SDS 4%] + [NaH2PO4 100 mM,NaCl 0.5 M, TEAB 50 mM, SDS 4%]. The second solution was mixed to the first one topH 7.8.

The addition of SDS and TEAB was supposed to increase the solubilization of proteins boundto solids.After being suspended in buffers for 1 hour, solutions were centrifuged at 15,000 g for 30minutes, to separate newly solubilized enzymes from the solids, and the supernatant waskept and used for analyses.Samples from the same digestate were centrifuged at 7000 g for 10 min, their supernatantwas weighted and centrifuged again at 15000 g for 30 min to determine the solid and liquidpercentage in the digestate.

Reactor operation

8 reactors were operated mimicking the conditions of the full scale digester in Sävsjö.Reactors were heated at 52°C and stirred at 100 revolutions per minutes (rpm) during all theexperiment. Performance of reactors was evaluated based on analysis of total solids, volatilesolids, biogas production rate and composition.Reactors were fed every day from Monday to Friday, 250 ml of digestate were removed and250 ml of manure (from Sävsjö) were added.Once a week, digestate was analyzed following methods of Sluiter et al. (2005 & 2008) forthe TS and VS analyzes and reactors stirred up at 200 rpm for 30 min, likewise for every newbatch of manure. Gas samples were also taken once a week in every reactor to be analyzedwith a biogas analyzer from Agilent Technologies (490 micro GC).Reactors were operated for 44 days with two pH-controlled at 7.0. All analyses were doneduring that time. Due to problems with the acid addition, all reactors were stopped andstarted again with all new conditions at the end.

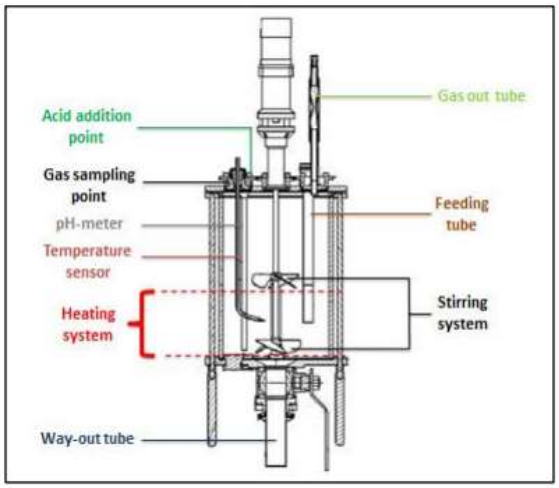

Figure 1: Reactor scheme

pH

In two reactors, the pH was controlled at 7.0 with a pH-meter. Since during anaerobicdigestion pH was only expected to increase pH control was only made by addition of 2 Mhydrochloric acid as soon as the pH went above the settled range (6.95-7.05).However, a problem occurred with the pH control, addition of acid was excessive and pH inthe reactor was below 7.0. To counterbalance it, sodium hydroxide (NaOH) 3M was added.

IV. CONCLUSION

Anaerobic digestion is a problematic system that needs to be expanded with a purpose to be utilized

inbiogas creation fee-quite simply. Hydrolysis being one of the crucial limiting steps,bioaugmentation, pH manipulate and addition of surfactants have been chosen to beef up thehydrolysis yield.The hydrolytic organisms C. Josui and C. Stercorarium develop beneath anaerobic andthermophilicconditions, they have been selected for his or her enzymes creation and used for thebioaugmentation of digesters. Cultivation confirmed some sporulation after their exponentialsegment or beneath non-most desirable stipulations, that is why they have to be harvested in the course of theirexponential phase to preclude spores formation.

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