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Different types of pretreatment of lignocellulosic wastes for methane production

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Biotechnological processes for anaerobic digestion of lignocellulosic substrates were performed in a laboratory bioreactor. Bioreactors used were operating at 35°C to follow the process of methane production. Cattle manure and wheat straw were involved in a ratio of 65:35 as a substrate, as well as only wheat straw as a sole substrate. We report on performing two pretreatment techniques to the substrates - chemical with ammonium hydroxide (NH_4OH) and polyethylene glycol (PEG) and biological – employing white-rot basidiomycetes (*Trametes hirsuta*) before starting the process of anaerobic digestion. The biological method of pretreatment gave the highest cumulative biogas yield for the substrate wheat straw and cattle manure. Chemical pretreatment lead to higher specific biogas yield when only wheat straw was used as a substrate. Both methods were easy to perform and lead to increased biomethane yield in comparison to that obtained with the participation of untreated substrates.

Key words: substrate pretreatment, anaerobic digestion, biomethane yield

INTRODUCTION

Anaerobic digestion of lignocellulosic biomass provides an excellent opportunity to convert abundant bioresources into renewable energy [1]. Anaerobic digestion is a process of organic matter mineralization by microorganisms into biogas (mainly methane and carbon dioxide) and digestate in the absence of oxygen [2]. The products obtained are of arising energetic and ecological significance. Biodegradation of organic wastes is a complex biotechnological process, which is performed by a specific microbial community. It combines the breakdown of wastes with opportunities to obtain the energy carrier methane. The chemical composition of the substrates used has a direct impact on the efficiency of their conversion. Microbial digestion of lignocellulose contained in the agricultural wastes is difficult to accomplish and slow because of the presence of lignin therein. This requires preliminary physical, chemical or biological treatment. To enhance the enzymatic and microbial accessibility, preliminary treatment was identified as an obligatory step before accomplishment of the whole complex biotechnological process [3]. Generally lignocelluloses have a stable structure, insoluble in water and resistant to both mechanical and enzymatic effects. Over the years, a great variety of pretreatment methods have been exploited [4]. Because of the simultaneous presence of lignin and crystalline cellulose, the water molecules can not penetrate into the lignocellulose fibers. To optimize the anaerobic digestion of wheat straw and manure and make the biogas process more profitable, several pretreatment techniques were evaluated. Pretreatment technology is a prerequisite to facilitate the release of monomers from a lignocellulosic biomass prior to enzymatic biodegradation [5]. Recently, some methods have been tried with ionic liquids, but they were still expensive and unpractical [6]. Alkaline hydrolysis results in the reduction of degree of polymerization and loosening of the intermolecular bonds connecting the lignin and hemicelluloses, thus increased porosity of the material and increased specific surface area of the lignocelluloses was realized [7]. Involving wood decaying fungi in a pretreatment process that can break down all major wood components (cellulose, hemicelluloses and lignin) more or less simultaneously, would result in substrates more accessible to biodegradation. Much efforts are aimed at developing a pre-treatment technology, which is eco-friendly and cost effective, aiming at increasing the porosity of the substrate, removing lignin or hemicellulose, and reducing the overall crystallinity in the structure to facilitate the biological conversion of biomass into bioenergy and biobased products [8]. Lignocellulosic substrates are suitable for continuous biogas production, because of their high potential methane yield [9, 10].

The aim of this study was the development of an effective technology for anaerobic digestion of lignocellulosic wastes by selecting the most effective method for pretreatment of wheat straw added to cattle manure to realize higher degree of anaerobic digestion of substrate and obtaining maximum methane yield.

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EXPERIMENTAL

The bioreactor working volume was 3 dm^3, equipped with a system for monitoring and control and continuous stirring. Released gas volume from both bioreactors was measured using a graduated cylinder in the gas holder working on a water displacement principle. Concentration of methane was measured with an automatic gas analyzer "Dräger" equipped with an IR-sensor for methane.

Certain parameters characterizing the process were followed during its realization:

Total solids were measured by dehydration of a certain volume of culture liquid at 105 °C; volatile solids - by burning at 575° C [11].

Cellulose concentration in the samples was measured according to the photometric method proposed by Updegraff [12].

Two types of pretreatment were performed:

1) Chemical pretreatment: 28% NH_4OH, polyethylene glycol-4000 (3%) and water were involved; a ratio of 1:0.5:20 was used for straw: NH_4OH: H_2O. The mixture underwent heating at 90 °C for 5h in a water bath. This was followed by rinsing till neutral pH.

2) Biological pretreatment was accomplished by white-rot basidiomycetes - *Trametes hyrsuta,* isolated from soil samples from Bulgaria. The inoculum was prepared by inoculation of 50 mL potato dextrose medium (glucose, 20.0 g/L) with 3 mycelial disks (diameter of 0. 5 cm, from a 7 day-old culture from potato dextrose agar). Incubation was without agitation at 28 °C for 7 days. The obtained biomass was transferred onto sterile 5 g of wheat straw and incubated statically for 10 days.

As a criterion for the effectiveness of pretreatment served the residual concentration of cellulose, the total organic matter in dry wheat straw and composition and volume of the biogas released.

RESULTS AND DISCUSSION

Several of the substrates used today for biogas production are slowly degraded and only partially digested in the process of anaerobic biodegradation. This was the reason to apply chemical and biological pretreatment of wheat straw in our experiments before introduction of the pretreated substrate into the bioreactor. Experiments were carried out for untreated and pretreated substrates for comparison and evaluation of the different pretreatment techniqies - chemical and biological (*Trametes hyrsuta*), (Fig.1).

Fig. 1. Digital image of *Trametes hyrsuta*

The results presented in Table 1 show the changes in the chemical composition of the substrate before and after the corresponding pretreatment method.

Among the biological methods for pretreatment, the use of wood degrading fungi is one of the most effective and widely applied methods (Fig. 2). The hyphae of the fungi can secrete many ligninolytic enzymes which catalyze oxidative reactions during lignin depolymerization [13]. The peroxidases produced by them weaken and/or destroy the bonds linking cellulose and lignin.

Fig. 2. Schematic presentation of biological pretreatment of wheat straw and introduction into the bioreactor.

Table 2 presents the changes in the organic solids after cultivation in an anaerobic digester for biogas production when cattle manure and wheat straw or only wheat straw were used as substrates. The degree of biodegradation of the substrate was calculated. The data obtained indicate that the biological pretreatment improves the rate of biodegradation. From the reports available, it is evident that white rot fungi can be used to remove lignin from lignocellulosic substrates and by shortening the incubation time and optimization of the delignification process better utilization of the substrate can be realized [14]. Our results clearly indicate that the biological pretreatment improves the rate of biodegradation. The percentage of biodegradation in the variant with biological pretreatment (54.2 %) is the highest one, compared with all other variants.

The daily production of biogas was followed for a period of 15 days when cattle manure and wheat straw were used as a substrate (Fig. 3 a) and 20 days when only wheat straw was involved in the anaerobic biodegradation (Fig. 3 b). The daily production of biogas applying the two methods of pretreatment was different, as well as the lenghth of the process during which biogas was released as a result of digestion. It is seen that the amount of biogas after biological pretreatment is the greatest one in comparison to the control option, followed by the chemical treatment for both substrates tested.

The effect of pretreatment appeared to be less expressed when only wheat straw was used as a substrate, considering the rate of biodegradation. It was calculated to be 36.9% at biological pretreatment and 16.2% when chemical pretreatment was applied.

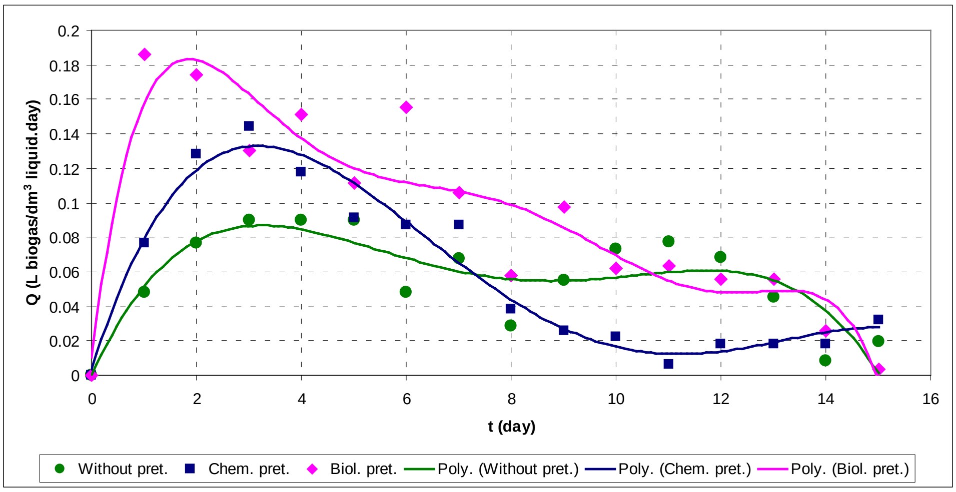
Table 3 presents the yields of biogas and methane obtained from the consumed substrate. The most efficient synthesis of biogas was observed after biological prereatment (cumulative biogas yield). An important feature is the percentage of methane in the biogas released. The obtained results showed that both chemical or biological pretreatment techniques applied to the substrate increase the percentage of methane in the biogas.

Table 1. Chemical composition of wheat straw before and after pretreatment.

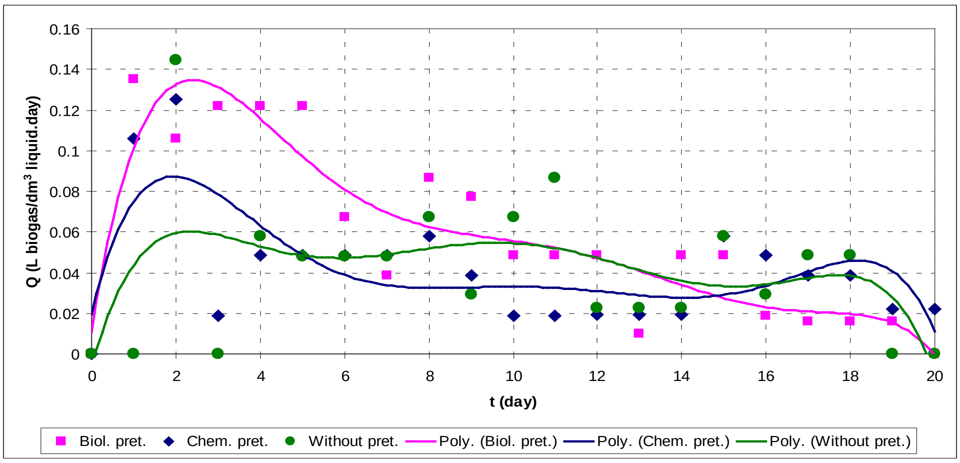
Substrate	Pretreatment		Total Solids, %	Volatile Solids, %	Cellulose, %
Wheat straw	Chemical	before	93.7	95.2	41.7
		after	91.8	90.1	36.2
	Biological	before	93.7	95.2	41.7
		after	92.1	93.5	31.8
Cattle manure	Without pretreatment		22.3	83.2	17.4

Table 2. Changes of organic matter after cultivation in a bioreactor for biogas production

Substrate	Method of pretreatment	VS, g/dm^3			Rate of biodegradation, %
		Influent	Effluent	Utilization g/dm^3	
Cattle manure+wheat straw	No pretreatment	41.3	26.6	14.7	35.6
Cattle manure+wheat straw	Chemical	32.8	16.1	16.7	50,9
Cattle manure+wheat straw	Biological	31.2	14.3	16.9	54.2
Wheat straw	No pretreatment	27,5	24.2	3.3	14.85
Wheat straw	Chemical	22.2	18.6	3.6	16.2
Wheat straw	Biological	27.3	17.2	10.1	36.9



a



b

Fig. 3. Effect of the method of pretreatment on the daily synthesis of biogas (cattle manure and wheat straw-a and only wheat straw-b)

Table 3. Efficiency of biogas and methane synthesis

Substrate	Method of pretreatment	Utilization of VS (ΔVS), g/dm^3	Cumulative biogas yield (Q_Σ), ml/l working volume	Specific biogas yield (Q_{sp})*, ml/g ΔVS	Methane, %
Cattle manure+wheat straw	No pretreatment	14.7	1103	75.0	52
Cattle manure+wheat straw	Chemical	16.7	1027	61.5	57
Cattle manure+wheat straw	Biological	16.9	1388	82.1	58
Wheat straw	No pretreatment	3.5	833	238	48
Wheat straw	Chemical	3.6	863	239.7	51
Wheat straw	Biological	10.1	1195	118.3	49

*-$Q_{sp} = Q_\Sigma/\Delta VS$

The values of pH were followed during the whole process, and an increase from 5.5 to 7 was established, accompanied by an increase in methane yield, which is in accordance with the statement of Kheiredine and co- workers [15], who reported that the optimum pH for degradation in an anaerobic process is in the neutral range. This fact was also stated by Sánchez and co-workers [16].

The abundance of lignocellulosic substrates makes them a potential feedstock for biofuel production but their conversion is a major hurdle. They have to be pretreated physically, chemically, or biologically to be easily used by fermenting organisms for methane production. Alkaline pre-treatment of lignocellulosic material aims to remove part of the lignin and hemicellulose by solubilization and to enhance the accessibility of the cellulosic part for cellulolytic enzymes [17], together with the effect of the amphyphilic substance polyethylene glycol which also helps in making the substrate highly water soluble [18] and favors the increase in the methane yield. In agreement with this are the results obtained after application of chemical preliminary treatment.

For most of the parameters followed in this study the biological pretreatment appeared to be the most effective. These results are due to the fact that many microorganisms in nature are able to attack and degrade lignin, thus making easy access to cellulose. Such organisms are abundantly found in the forest and include the wood decaying fungi [19]. They possess enzyme systems to attack, depolymerize and degrade the polymers in lignocellulosic substrates [20]. The application of fungi for delignification is an environmentally friendly technology that can be applied for lignocellulosic biofuel production, which was confirmed by our investigations and the results obtained.

CONCLUSIONS

Biotechnological exploitation of lignocellulosic wastes is promising for sustainable and environmentally-friendly energy production because of the abundant availability of these renewable sources.

Finding the most appropriate substrate and method of pretreatment could permit enhancement of biodegradation and increase in biomethane yield.

The biological method of pretreatment gave the highest cumulative biogas yield when wheat straw and its mixture with cattle manure were used as substrate. A ratio between the cattle manure and wheat straw (65:35) was the most appropriate one and together with application of biological treatment of the straw improved the efficiency of biogas synthesis.

On the other hand, chemical pretreatment lead to higher specific biogas yield when only wheat straw was the substrate.

Most significant was the fact that both methods of pretreatment helped for obtaining an increased percentage of methane in the biogas released.

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РАЗЛИЧНИ НАЧИНИ НА ПРЕДВАРИТЕЛНО ТРЕТИРАНЕ НА ЛИГНОЦЕЛУЛОЗНИ ОТПАДЪЦИ ЗА ПРОДУКЦИЯ НА МЕТАН

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(Резюме)

Осъществени са биотехнологични процеси на анаеробна деградация на лигноцелулозни субстрати в лабораторен биореактор. Използваните биореактори работиха при 35°C и беше проследен процеса на продукция на метан. Оборски тор и пшенична слама се използват като субстрат в съотношение 65:35, както и само пшенична слама като единствен субстрат. Ние докладваме за прилагането на две техники на предварително третиране на субстратите – химическо с амониев хидроксид (NH_4OH) и полиетилен гликол (PEG) и биологическо – с участието на дървесина-разлагащи базидиомицети (*Trametes hirsuta*) преди стартиране на процеса в анаеробния реактор. Биологичният метод на претретиране доведе до най-висок кумулативен добив на биогаз при субстрат оборски тор и пшенична слама. При химическото претретиране се получи по – висок специфичен добив на биогаз, когато само пшенична слама се използва за субстрат. И двата метода са лесни за осъществяване и водят до увеличен добив на биометан в сравнение с този, получен с участие на нетретирани субстрати.