The Using Of Enzymes For Degradation Of Cellulose Substrate For The Production Of Biogas

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enzymatic hydrolysis, cellulose substrate, glucose

Abstract
The main purpose of this article is to describe different kinds of cellulose degradation processes such as enzymatic hydrolysis. As the cellulose substrates the sawdust, leaves and cartons were used. Anaerobic degradation processes utilized for the biogas production can be accelerated by this way.

1. Introduction
As substrates for biogas production waste materials which contain polysaccharides, lipids and proteins can be used. Plant origin substrates contain mainly cellulose (Fig. 1), hemicellulose and lignin [1]. The structure of cellulose consists of parallel glucan chains and is stabilized by hydrogen bonds.

![Cellulose structure](image)

Fig. 1. Cellulose structure

The main structural unit of cellulose is D – glucose [3]. Cellulose is resistant to different types of degradation [2]. The cellulose substrate contains various representation of lignin, which influences the resistance of substrate and also increases the cost of pretreatment. Usage of hay, straw and recycled paper as a substrate containing lignocelluloses for increasing biogas production is currently being tested and interesting topic.

A mechanical pretreatment is the first step in the pretreatment of lignocelluloses substrate. In next steps is possible to degrade cellulose by chemical or enzymatic hydrolysis [5, 8]. The chemical hydrolysis by H₂SO₄ and HCl leads to breaking cellulose into cellotetrose and cellobiose [2, 4, 8]. By Fenton reaction is possible to degrade cellotetrose and cellobiose to glucose, a simple aldehydes and ketones. Direct way to degrade cellulose into glucose is enzymatic hydrolysis.

Enzymatic hydrolysis of cellulose takes place with a mixture of enzymes – cellulases [1, 5-9], at 36 – 37 °C. Cellulases break down cellulose in three ways. Endoglucanases convert chains of cellulose from
the inside, creating new ends of shorter chains [1]. Exoglucanases separated from non-reducting ends of new chains celllobiotic units. In consequent steps cellotetrose and cellobiose are decomposed to glucose in liquid phase by the enzyme β-glucosidase [1]. Cellulases are produced by different types of bacteria and filamentous fungi [5,7]. Research of different tribes of filamentous fungi isolated from waste waters from paper production, which are able to produce cellulose in large scale seems to be interesting nowadays [5,7]. Lignocellullase substrate pre-treated in this way can be used to increase the quality and quantity of produced biogas.

2. Experimental part

Cellulose substrate

Cellulose substrate was inactivated with lime (Fig. 2). Total solids were 43% and volatile solids were 44%.

Principes of glucose determination

Oxidation of glucose with oxygen was preformed with enzymatic catalysis by glucose oxidase for hydrogen peroxide and gluconate. Formed hydrogen peroxide was determinated by oxidation copulation with substituted phenol and 4- aminoantipyrine and cotalised with peroxidase.

Determination of glucose

Operating solution was mixed with serum or sample, standard glucose solution and distilled water (control solution) in the ratio 100:1 or 150:1 in three tubes. Incubation was preformed for 30 min. at 15-25 °C or for 15 min. at 37°C. The incubation mixture must be protected from direct light. Within 40 min. after incubation the absorbance of sample ($A_1$) and absorbance of sample ($A_2$) was measured and compared with control solution.

Calculation

Glucose (mmol.l⁻¹) = $a_1/A_2$

where $a$ is glucose contentration in standard solution [mmol.l⁻¹].

Enzymatic hydrolysis

In various experiments with enzymes different amounts of enzyme mixture for cellulose substrate degradation were used (Fig. 3). The effectiveness of individual experiments on the measurement of COD value (mg.l⁻¹) and glucose concentration was determined (g.l⁻¹). Procedures and results in degradation of the substrate are given in experiments No. 1-3, and Fig. 4-5.
Fig. 3. Enzymatic hydrolysis

3. Results and discussion

Enzymatic hydrolysis decomposes cellulose to cellotetrose and cellobiose in first step. Subsequently cellotetrose and cellobiose are degraded to glucose, lower aldehydes and ketones (Fig. 4). Products of enzymatic hydrolysis depend on the type and composition of the substrate, the reaction time, the type and quantity of the enzymes and the reaction temperature. Glucose itself can be enzymatic degraded to aldehydes and ketones.

Compound of enzymes was used for the degradation of cellulosic substrate. Time dependence of glucose production resulting from substrate degradation is shown in figure 5. From each experiment is appeared that the production of glucose is affected by the amount of enzymes used, quantity of substrate, reaction temperature and time. The highest glucose yield was achieved in the experiments no. 3. In experiment No. 2 and 3. a decrease in glucose concentration after 24 hrs. was observed, which was caused by the amount of used enzymes (Tab. 1, Fig. 4).
Fig. 4. Expected mechanism of cellulose degradation by enzymatic hydrolysis

Fig. 5. Time dependance of glucose production by enzymatic degradation of cellulose substrate.

Tab. 1. Time dependance of glucose production by enzymatic degradation of cellulose substrate in experiments no. 1-3.

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Glucose (g.l⁻¹)</th>
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<tbody>
<tr>
<td></td>
<td>E 1</td>
</tr>
<tr>
<td>6</td>
<td>0.1</td>
</tr>
<tr>
<td>24</td>
<td>0.2</td>
</tr>
<tr>
<td>48</td>
<td>0.22</td>
</tr>
<tr>
<td>72</td>
<td>0.26</td>
</tr>
</tbody>
</table>
Tab. 2. Time dependence of COD value in experiments no. 1-3 of glucose production by enzymatic degradation of cellulose substrate.

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>COD (mg.l⁻¹)</th>
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<tbody>
<tr>
<td></td>
<td>E 1</td>
</tr>
<tr>
<td>6</td>
<td>200</td>
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<tr>
<td>24</td>
<td>360</td>
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<tr>
<td>48</td>
<td>430</td>
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<tr>
<td>72</td>
<td>600</td>
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Fig. 6. Comparation of experiments no. 1-3 by COD value.

4. Conclusion
From results it can be concluded that lignocellulosic substrates can be successfully modified by enzymatic hydrolysis. We dealt with the use of enzymatic hydrolysis in experiments No. 1-3. It is obtained possible to decompose cellulose substrate to glucose by enzymes. Yield of glucose is depends on quantity of substrate used, amount of used enzymes used, reaction temperature and time (Fig. 5). Substrate is decomposed to glucose and lower aldehydes for prolonged exposure to enzymes. Efficiency and progress of enzymatic hydrolysis is affected by content of lignin in substrate. Cellulose is covered by lignin and thereby increase the resistance of cellulose to enzymatic degradation. It is therefore possible to combine the procedures for remove lignin (e.g., chemical hydrolysis) and subsequently enzymatic hydrolysis or microbial degradation can be used. The effectiveness of enzymatic hydrolysis can be regulated or completely inhibited by temperature increasing. Enzymatic hydrolysis is one of the processes by which we can pretreated cellulose substrates such as sawdust, leaves, hay, straw, recycled paper and thus accelerated the processes of degradation under anaerobic conditions. Products of enzymatic degradation of substrates are glucose and lower aldehydes, which are
highly degraded in anaerobic processes. Substrates treated in this way are easier and faster biodegradable and can be used to increase biogas yield and quantity.

5. References

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