

Features of using solid cellulose-containing domestic wastes for production of bioethanol

T.A. Bolotnikova¹, E.B. Aronova¹, J.G. Bazarnova¹, O.I. Bolotnikova^{1,2,*}, A.I. Ginak³

¹Peter the Great St. Petersburg Polytechnic University, Polytechnicheskaya Str. 29, Saint Petersburg, 195251, Russian Federation

²Petrozavodsk State University, Lenina Str. 33, Petrozavodsk, 185910, Republic of Karelia, Russian Federation

³Saint-Petersburg State Institute of Technology, Moskovskiy Av. 26, Saint Petersburg, 190013, Russian Federation

Abstract. In this paper, we consider the process of producing biogas with a high methane content when used as a co-substrate for fermentation of plant residues of microalgae. Microalgae *Chlorella sorokiniana* are a valuable source for obtaining valuable components such as lipids, pigments, proteins, chlorophyll and others. After the extraction of valuable components, residual biomass is formed, which requires further disposal. In this experiment, the digestion process is carried out using an inoculant — lyophilically dried activated sludge from sewage treatment plants in Hamburg in the amount of 450 ml and residual biomass of the microalga *Chlorella sorokiniana* in the amount of 2.1 g. The studies were carried out in the Anaerobes Test system AMPT-II system. Fermentation produces 205 ml of methane gas.

1 Introduction

The problem of household wastes disposal is becoming increasingly acute from year to year. Almost half of the volume is waste paper and cardboard. Just one family of four people on the average throws out about 100 kg of paper and packaging wastes per year. The period of the disintegration in natural conditions is from 3 months to a year [1]. The traditional burning of paper wastes is extremely harmful to the environment [2]. Therefore, the development of efficient recycling technologies for such materials is one of the aims for the state policy of developed countries in the field of ecology and waste management.

Using different microorganisms fermenting D-glucose as the main component of cellulose is the main topic of discussion. Bioethanol is widely used not only in various industries, but also as an additive to boiler fuel for internal combustion engines, which meets modern environmental requirements and has a positive economic effect [1]. Enzymatic hydrolysis of abundant paper and paperboard wastes to glucose-containing substrates has significant cost and requires special technologies [3]. Cheapening of this process can be achieved through the use of mineral acids [4]. Similar methods of cellulose destruction were widely used in the USSR in the hydrolysis industry. It was at that time when soft methods for wood hydrolysis were developed, which made possible to reduce the anthropogenic impact on the environment [5]. However, cellulose hydrolysates obtained in this way are unsuitable for ethanol conversion by bacteria, which metabolic activity depends on pH level of the medium [5]. At the same time, the special strains of *Saccharomyces cerevisiae*

yeast to utilize acid wood hydrolysates are known [6]. Therefore, the purpose of this work was to optimize the parameters of paper and paperboard wastes acid hydrolysis and to evaluate the ethanol yield from these substrates with the help of the special strain of *Sacch. cerevisiae* "Omskie".

2 Methods

For the experiment, paper and cardboard were taken from the house bin in quantities of 1 kg of each sample. These samples were dispersed in a hydraulic diluent to a homogeneous pulp with fragments of size: 150-200×4 mm (newsprint) and 100×5 mm (cardboard). The dispersion was carried out at atmospheric pressure and +95°C. H₂SO₄ was added to the resulting homogeneous mass to a final concentration of 70%. The mixture was stirred, incubated for 2 hours at +60-70°C. A part of the acid fraction was removed and water was added to a final concentration of H₂SO₄ of 40%. This solution has been incubated for 2 hours under the previous conditions (hydraulic module of 8.7).

The preparation of glucose-containing substrates obtained by the acid method for ethanol conversion was carried out during sequential neutralization with milk of lime, distillation vacuum (+80°C, pressure at the top of the column 0.7 MPa), continuously purged with air for 60 minutes to pH=4.8.

The glucose-containing mixture was cooled down to the temperature of +25°C, diluted with water to the final concentration of H₂SO₄ of 10%, neutralized with 25% NH₄OH to pH=6.0. The neutralized solution was filtered and evaporated by 50% (Table 1). The concentration of

* Corresponding author: olga-bolotnikova@rambler.ru

reducing sugars in the bottom product was determined by Fehling's test.

Table 1. Obtaining of the bottom product.

Characteristics	Stage
Atmospheric pressure, +95°C	The dispersion
2 hours, H ₂ SO ₄ (70%); 2 hours, H ₂ SO ₄ (40%).	Acid hydrolysis
Milk of lime	Neutralization
+80°C, pressure at the top of the column 0.7 MPa	Distillation vacuum
60 minutes to pH=4.8	Air purge
Cooling to a temperature of +25°C,	Cooling
To a final concentration of H ₂ SO ₄ of 10%,	Dilution with water
25% NH ₄ OH to pH=6.0.	Neutralization
Evaporation by 50%	Filtration, evaporation

The bottom product was cooled (+40°C), diluted with water to a concentration of reducing substances of 10-13 g/l, and 0.02% (NH₄)₂SO₄ and 0.01% ((CaH₂PO₄)₂×H₂O+2CaSO₄×2H₂O) were added. This wort was fermented with a batch culture of *Sacch. cerevisiae* "Omsk" (Collection of industrial microorganisms, VNIHydrolysis, St. Petersburg). Ethanol fermentation conditions were: 100 ml round-bottom Erlenmeyer flasks with 100 ml of wort on a thermostatic rotary shaker for 24 hours at +30-32°C. After 18-24 hours 10 ml of yeast suspension from these flasks were transferred to a 500 ml flasks containing 100 ml of the wort which were incubated under similar conditions. Yeast biomass was used for anaerobic fermentation in an amount of 10.0±0.5 g a.d.m. (absolutely dry matter)/l.

After fermentation the yeast biomass was separated in a laboratory centrifuge in 5-10 minutes at 5000 rpm. Fractional distillation of ethanol-containing mash was carried out on a distillation unit with a deflector (200 mm). On epuration column ethanol raw was purified to separate the ether-aldehyde fraction. Ethanol concentration was determined in the distillate obtained after distillation of the test sample by gas chromatography under conditions [7]. The experimental results were statistically processed using Student's test at a significance level of 0.05 with the MS Excel computer program.

3 Results and Discussion

Today, cellulose-containing waste is a valuable source of recyclable materials, which are widely used to obtain a number of national economic products [8]. However, household waste paper and paperboard of poor quality usually contain bitumen, wax, paraffin, glue and other impurities, clogging nets and cloth of paper machines, sticking to the surface of the drying cylinders [9]. Such contaminated household waste, unsuitable for re-extraction of cellulose, is subjected to acid degradation. Therefore, samples of paper and cardboard from the house garbage container were first subjected to thermomechanical processing (so-called cold method).

This ensured not only their crushing to garbage, but also the dispersion of contaminants to sizes that did not significantly affect the process of acid hydrolysis of cellulose.

It is known that the technological parameters of concentrated and diluted acid hydrolysis of wood differ significantly [10]. The undoubted advantages of the first method are the high efficiency of the breaking the β-glycosidic bonds and, the mild temperature treatment of plant materials. At the same time, significant amounts of acid, the duration and high consumption of gypsum to neutralize the sugar-containing substrate, prior to its microbiological conversion, reduce the commercial attractiveness of such hydrolysis for the disposal of household cellulose-containing waste [10]. The main advantage of dilute acid hydrolysis is the minimal temporary loss. However, the extreme temperature regime, as well as the impossibility of complete degradation of cellulose macromolecules, cast doubt on the environmental friendliness of this method [11]. Therefore, the destruction of ground samples of waste paper and cardboard to glucose-containing substrates was carried out by the combined method, mixing the elements of concentrated and diluted hydrolysis. By the end of 4 hours of such treatment, the fibrous component of municipal solid waste was 95% converted into a glucose-containing solution. The combined method greatly simplifies the technological scheme and, is characterized by a sufficiently high efficiency and, lower financial costs achieved by reducing corrosion of equipment and reducing the neutralization of acid hydrolysates. In addition, it allows to reduce the volume of water required for acid hydrolysis, and avoid using significant external pressure as a factor that increases the efficiency of the destruction β-1,4-glycosidic bonds in cellulose macromolecules. These features well illustrate the environmental safety of the developed method.

It is widely known that yeast, in comparison with bacteria and mycelial fungi, is characterized by greater metabolic activity, acidophilicity and fermentative type of sugar catabolism [12]. Special strains *Sacch. cerevisiae* have already been constructed in the USSR for the disposal of acid wood hydrolysates and by-products of hydrolysis plants. These strains are traditionally used as ethanol producers [6].

The choice of the strain of *Sacch. cerevisiae* "Omskie" as a biocatalyst made it possible to prepare wort for fermentation using traditional industrial methods (neutralization, vacuum rectification, continuous air purging, neutralization and filtration) [13]. Towards the end of 24-hour anaerobic fermentation the concentration of RB in the wort decreased to 2.5%, and the yield of ethanol was 52 ml/kg (paper waste) and 34 ml/kg (cardboard waste). Theoretical calculations show that scaling the process discussed above will make possible to obtain up to 150 L of rectified ethanol per ton of absolutely dry cellulose-containing household waste, which corresponds to the standard for the hydrolysis wood industry [14].

4 Conclusion

During the experiment, optimal conditions were selected for the conversion of household cellulose-containing garbage into glucose-containing substrates. The fundamental possibility for the utilization of household cellulose-containing garbage to bioethanol was proved. This method greatly simplifies the typical technological process of acid hydrolysis of cellulose, reduces its toxic effect on the environment. The use of a special biocatalyst allows optimization of the financial costs of preparing acidic cellulose hydrolysates for ethanol bioconversion through the use of standard detoxification technology. The well-developed network of pulp and paper mill industry in the Russian Federation will become a good technological potential for processing a variety of household and industrial wastes into a target product of great economic importance.

This work was supported by the grant No 18-44-100001 from Russian Foundation for Basic Research (RFBR).

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