Bioethanol production from secondary bioresources of the pulp and paper industry

Olga Bolotnikova[1,2], *Nadezhda* Kvasha[1,3*], and *Ekaterina* Malevskaia-Malevich[1]

[1]The North-West Institute of management - branch of the Russian Presidential Academy of National Economy and Public Administration (RANEPA), Saint-Petersburg, Russia
[2]Petrozavodsk State University, Petrozavodsk, Russia
[3]Bonch-Bruevich Saint-Petersburg State University of Telecommunications, Saint-Petersburg, Russia

Abstract. The prospects for obtaining bioethanol based on secondary bioresources of the pulp and paper industry are discussed in order to close economic cycles. The transformation of sugars of acidic hydrolyzate of deciduous wood into bioethanol by batch culture of a selected strain of the yeast Saccharomyces cerevisiae with a combination of various xylose-assimilating yeasts was studied. The ethanol yield from hexose`s part was 46% from fermented sugars, its concentration reached to 9,0±0,6 g l-1. The use of pentose sugars under microaerobic conditions (concentration of dissolved oxygen 0.5-3.0%) gave the efficiency of ethanol production up to 26.7 - 35.5% from fermented sugars. The ethanol concentration in terms of the pentose`s fraction was 3.9-4.5 g l-1 (the yeast Pachysolen tannophilus); 5.2 g l-1 (the yeast Candida tropicalis); 5.6 g l-1 (the yeast Candida shehatae). The total amount of ethanol obtained from both hexose and pentose parts after distillation was 4.2-4.6 g (5.2-5.7 ml) with alcohol by volume 96%.

1 Introduction

The pulp and paper industry (PPI) in Russia occupies a significant place in global production, which is explained by an interconnected set of historical and geographical reasons. In terms of the scale of the raw material base of the pulp and paper industry, Russia ranks first in the world, having more than 20% of the world's forest fund [1]. At the same time, from the standpoint of the currently dominant sustainable approach [2], the pulp and paper industry can be characterized as unsustainable. Despite its strategic position, pulp and paper accounts for less than 1.5% of the total Russian industrial output [3]. However, it has such a synergistic set of characteristics as significant resource and capital intensity, a significant level of waste generated, impact on the environment and human health. Taking into account the emerging externalities, the costs of conditionally free natural resources and ecosystem services, there is an imbalance in achieving the full efficiency of this type of economic activity. The solution to this problem is provided by the implementation of a sustainable circular mechanism focused on models of closed economic cycles [4].

* Corresponding author: nadia_kvasha@rambler.ru

Modern pulp and paper industry is mainly associated with various methods of biochemical processing of cellulose-containing raw materials (wood, secondary resources, agricultural waste, straw, husk, textile waste, etc.). A significant proportion of pulp and paper waste, which can become sources of secondary raw materials, is also bioresources. The organization of recycling processes in the economy based on biotechnologies is defined as biorefining [5]. Currently, the most significant secondary raw material that can be obtained in the course of the technological process of pulp and paper mill is bioethanol.

The main factors determining the type and amount of pulp production waste are pulping technology; type of raw materials used; purification technologies (mainly water); product range. The share of cellulose in the feedstock varies within 30-55% for different species and places of wood growth. For species growing on the territory of Russia, which are currently used as raw materials for pulp and paper, the excess of cellulose content in coniferous species over deciduous species is typical. Since wood and other cellulose-containing raw materials also contain other polymers (in particular, hemicellulose and lignin), their ratio with cellulose in softwood and hardwood is reversed. In addition, the composition of non-cellulose organic waste of pulp and paper industry significantly depends on the type of wood. Conifers contain more lignin (28-32%), but less hemicelluloses (21-26%) than hardwoods of the North-West region of Russia (17-23% and 22-33%, respectively, and sometimes higher). The share of monosaccharides in hardwood can be 1.5-2 times higher than that of softwood. With all this, hardwoods contain more pentoses and less hexoses than conifers. When cooking deciduous wood growing on the territory of the North-West region of Russia (mainly birch), 90% of sugars include pentoses, almost completely represented by xylose [1].

Currently, there is a shortage of spruce raw materials traditional for the North-West region of Russia; therefore, it is being replaced by hardwood and secondary raw materials, including vegetable origin. This, in turn, exacerbates the problems of processing the pentose part of the hemicellulose components of the organic waste of pulp and paper industry, which are not assimilated by industrial strains of microorganisms. The involvement of a secondary resource obtained from the pentose part of sugars in the circular ecosystems of pulp and paper requires the search for new biocatalysts.

The purpose of this work is to evaluate the fundamental possibility of obtaining bioethanol based on sugars, hardwood substrates and other plant bioresources as a substitute raw material for pulp and paper mills in the North-West region of Russia.

Research objectives:

- To study the alcohol conversion of hardwood substrate by batch culture of the selection strain S. cerevisiae in combination with various xylose-assimilating yeasts.
- Consider differentiated approaches to detoxification of hexose and pentose fractions.
- Based on experimental data, calculate the theoretically expected yields of alcohol from secondary non-food sources of plant biomass of various origin and composition.

2 Literature review

A detailed analysis of the Russian pulp and paper industry in Russia was carried out in the work of Mironov A. [6]. The author notes its huge economic potential both in the domestic and international markets, which is not fully realized. This fact is explained by the significant obsolescence of technologies, that is, the technological underdevelopment of the pulp and paper complex. The authors [7] in their study note the severity of the problem of pulp and paper waste, since the industry occupies one of the leading places in the formation of persistent organic pollutants that accumulate, while having a negative impact on the environment.

Quite a lot of scientific works have already been devoted to the study of the circular model of industry [4, 8, 9]. Green growth theory argues that innovative technologies, models and mechanisms that completely decouple GDP growth from resource use and carbon emissions [10] should provide the compatibility of economic growth with the ecology of the planet.

For the pulp and paper industry, the shift of circular principles to the field of biotechnologies is actualized. The typology of biotechnologies, including taking into account Russian peculiarities, is being developed, for example, in the study [11]. There are sufficiently deep studies of the fundamental issues of biorefining, as well as some practical aspects of this model [5, 12]. At the same time, the scientific literature lacks a sufficient number of theoretical, methodological and practical developments aimed at establishing the directions and forms of biorefining of secondary resources released in pulp and paper industries.

Research in the field of obtaining green ethanol both from pulp and paper mill waste and, in principle, from wood and plant biomass is being carried out quite widely [13, 14, 15]. At the same time, the need to conserve resources and ensure efficiency requires a deeper study of some issues.

The possibilities of bioconversion of nonfood sources of plant materials with the help of microorganisms are discussed, for example, in the works of Møller F. et al. [16, 17, 18]. Among the works in the field of microbiological conversion of waste from wood processing enterprises of various profiles, one can distinguish studies of Cheng H. and Wang L [19].

3 Research methods

Strains of yeast S. cerevisiae "Omskie", P. tannophilus Y-1532 and Y-1533, C. tropicalis Y-1633, C. shehatae Y-1632 were used (Museum of Microorganisms, All-Russian Research Institute of Hydrolysis, St. Petersburg). Yeast biomass for ethanol fermentation was obtained in two stages. First, the inoculum was grown in liquid YEPD medium composition (S. cerevisiae) or YEPX medium (xylose-assimilating yeasts) in Erlenmeyer flasks, then the yeasts were grown with laboratory fermenter Biostat M [16].

Hardwood substrate was obtained with separate sampling of pentose and hexose sugar fractions (Table 1).

Table 1. Sugar composition of hardwood substrate.

Components	Concentration, % Hexose part	Pentose part
D-glucose	1.95±0.10	0.76±0.04
D-mannose	0.05±0.01	0.60±0.03
D-galactose	0.05±0.01	0.10±0.01
D-xylose	0.07±0.01	1.44±0.07
L-arabinose L-rhamnose	0.04±0.01 0.02±0.01	0.16±0.01 0.04±0.01

To prepare for fermentation, the hexose fraction was subjected to two-stage neutralization with limewater (pH=3.5), ammonia solution (pH=4.3-4.5) and continuously stirring with air (+85°C). Then it was cooled to a final temperature of +45°C and purged with air in 60 minutes. While the final concentrations of inhibitors were: 0.03±0.01 (furfural), 0.13±0.01 (oxymethyl furfural), 0.40±0.02 (volatile organic acids), 1.78±0.09 (ligno-furan complexes). Подготовленное таким образом сусло обогащали (NH4)2SO4 – 0.02% и (CaH2PO4)2×H2O+2CaSO4×2H2O - 0.01%. The wort prepared in this way was enriched with (NH4)2SO4 – 0.02% and (CaH2PO4)2×H2O+2CaSO4×2H2O - 0.01%. Pentose

fraction of hydrolyzate was spargered with steam to the final concentrations of volatile inhibitors: 0.03±0.01 (furfural), 0.08±0.01 (oxymethyl furfural), 0.15±0.01 (volatile organic acids). Substances of the lignofuran complex were oxidized by spargered this substrate with air to the final concentration 1.22±0.06% [9]. The resulting substrate was neutralizing with limewater (pH=4.3-4.5). The nutrients were contributed with the following concentrations: (NH4)2SO4 – 0.05%; K2HPO4 – 0.02%; MgSO4 – 0.01%. Before fermentation, the wort based on hexose and pentose fractions was diluted 2 times with H2O.

Fermentation of hexoses was carried out at the initial concentration of 2.0% of S. cerevisiae yeast in 750 ml Erlenmeyer flasks containing 400 ml of prepared wort at + 32-34°C for 24 hours. Fermentation of pentoses was carried out at 2.0% initial concentration of the xylose-assimilating yeasts in 250 ml Erlenmeyer flasks with 100 ml of pentose-containing hydrolysis wort at + 30-32°C with concentration of dissolved oxygen of 0.5-0.3% for 24 hours. At the end of the process, the distiller's wort was decanted. Then 100 ml of mash based on the pentose fraction of hardwood hydrolyzate was combined with 400 ml of mash from the hexose fraction of this hydrolysate. This mixture was subjected to distillation and rectification. The concentration of monosaccharides and ethanol obtained after distillation was determined by the procedures of gas chromatography using a Vista 600 device (Varian, USA) according to [16].

4 Research results

It is widely known that the utilization of complex multicomponent substrates by xylose-assimilating yeasts under aeration conditions favoring the formation of ethanol from D-xylose (the main pentose of non-edible sources of plant biomass) is accompanied by the phenomenon of diauxic cell growth [20]. Therefore, ethanol conversion of hexose and pentose sugar fractions was carried out using various biocatalysts. At the first stage, hexose sugars were fermented into ethanol by a batch culture of S. cerevisiae "Omskie" in accordance with the technological parameters of hydrolysis production [21]. The metabolic activity of this selection strain was not suppressed at residual concentrations of inhibitors (see above) after the standard method of hardwood hydrolyzate detoxification. The concentration of ethanol in the mash after fermentation of hexose sugars was 9.0±0.6 g l^{-1} (the volumetric rate of formation was 0.38 g l^{-1} h^{-1}, the economic coefficient was 0.46 g g^{-1}).

At the second stage of research, pentose part of sugars from the acid hardwood hydrolyzate was fermented into ethanol by various strains of the xylose-assimilating yeasts. Their alcohol-forming activity was inhibited by furfural, oxymethyl furfural, and volatile organic acids to a greater extent than alcohol-forming activity of S. cerevisiae "Omskie" [22]. Therefore, pentose fraction of the hardwood hydrolyzate was prepared for microbial conversion by a modified method, which provided a deeper extraction of the indicated inhibitors (see above). This technique made it possible to avoid the diauxic growth of D-xylose assimilation, increase the concentration and economic coefficient of ethanol formation during 24 hours of the batch micro-aerobic fermentation. The total yield of ethanol with alcohol by volume 96% from the hexose and pentose sugar fractions of the hardwood hydrolyzate corresponded to 4.2-4.6 g (5.2-5.7 ml) (Table 2).

The experimental results were used to calculate the theoretically expected yields of ethanol from secondary non-edible sources of various origin and composition plant biomass in accordance with the data [21]:

I. In cellulose fraction fermenting:

1000×0.3940×0.70×0.82×0.94×0.46×0.97=94.8 kg of ethanol (117.7 l of alcohol having 96% alcohol by volume), where: 0.3940 - coefficient considering the content of hardly hydrolysable polysaccharides; 0.70 - coefficient considering the output of reducing substances in hydrolysis; 0.82 - coefficient considering the output of monosaccharides (the

ratio of reducing substances); 0.94 - coefficient considering the output of hexoses (the ratio of the monosaccharides sum); 0.46 - coefficient of the conversion of hexoses into ethanol; 0.97 - coefficient considering the losses during distillation and rectification.

Table 2. Efficiency of Bioethanol Formation from Hardwood Substrates with Different Xylose-Assimilation Yeasts.

Xylose-assimilating yeast strain	E T H A N O L			Total alcohol yield from hexoses and pentoses, g**
	Pentose part			
	g×l^{-1}	g×g^{-1}	%**	
P. tannophilus Y-1532	3.90±0.30	0.27±0.01	26.7	4.20±0.30
P. tannophilus Y-1533	4.50±0.40	0.31±0.02	30.7	4.40±0.20
C. tropicalis Y-1633	5.20±0.30	0.36±0.02	35.5	4.50±0.30
C. shehatae Y-1632	5.60±0.40	0.38±0.02	38.2	4.60±0.40

* - % from fermentable sugars;
** - based on 96 vol.% ethanol.

II. In hemicellulose fraction fermenting:

1000×0.2554×0.95×0.78×0.98×0.32×0.97=57.5 kg of ethanol (71.4 l of alcohol having 96% alcohol by volume), where: 0.2554 - coefficient considering the content of easily hydrolysable polysaccharides; 0.95 - coefficient considering the output of reducing substances in hydrolysis; 0.78 - coefficient considering the output of carbohydrates (the ratio of reducing substances); 0.98 - coefficient considering the content of sugars, which are fermented to ethanol by special yeast strain; 0.32 - coefficient of the conversion of hexoses into ethanol; 0.97 - coefficient considering the losses during distillation and rectification.

The calculations show that the economic coefficient of the alcohol formation from secondary non-food-based biomass sources can be increased by: 39.4-53.9% (hydrolysates from oak wood and birch wood, respectively) and 59.7-96.8% ((hydrolysates from straw and oat hulls, respectively), Figure 1 and 2.

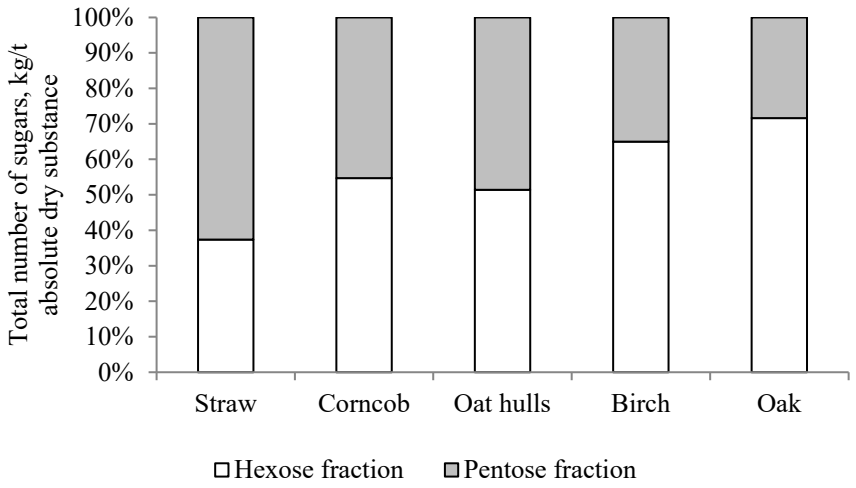

Fig. 1. The ratio of hexose and pentose sugars to the total number of sugars of various xylose-containing substrates.

Notes:
The number of sugars was calculated per ton of absolute dry substance [21].

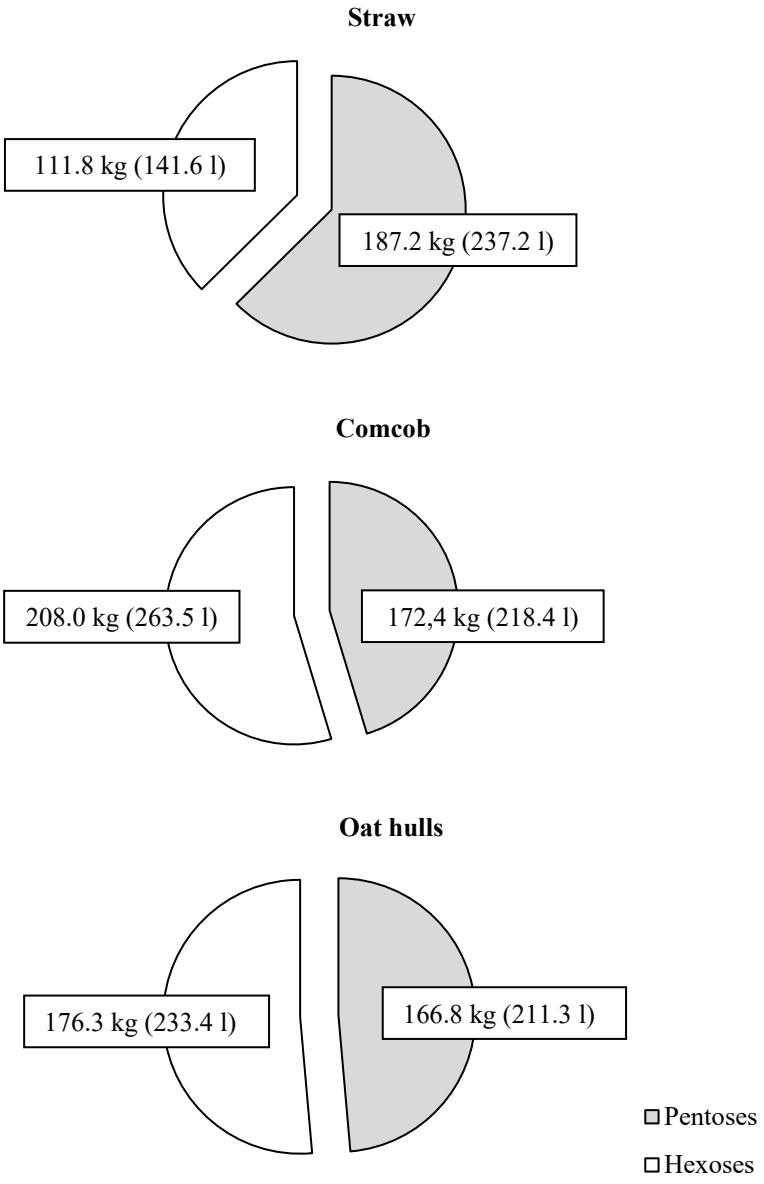

Fig. 2. Theoretical ethanol yield during yeast bioconversion of pentose and hexose sugar fractions of various xylose-containing wastes.

Notes:

The amount of ethanol was calculated in kilograms (kg) and liters (l) per ton of absolute dry substance [21].

5 Conclusion

The principal possibility of complex sugar-to-ethanol bioconversion of acid hardwood hydrolyzate using the selection strain S. cerevisiae and various xylose-assimilating yeasts has

been established. Theoretical calculations clearly illustrate a significant increase in the yield of hydrolysis alcohol due to the fermentation of D-xylose, the main pentahydric sugar. To implement the proposed fermentation method, the technological scheme of traditional fermentation industries is applicable. The only difference will be the need for alcoholic fermentation of pentose sugars in a microaerobic mode. However, this problem can be solved without a fundamental change in existing technologies.

The proposed method can be recommended for implementation at adjacent pulp and paper biotechnological complexes within the framework of a circular biorefining model that organizes the recycling of organic waste from pulp production.

The main factor constraining, in our opinion, the industrial implementation of the D-xylose bioconversion process is the low productivity of ethyl alcohol by xylose-assimilating yeast. Therefore, further research should be directed, first, to improving the industrial characteristics of xidose-assimilating producer strains. This is possible only with an integrated approach to this problem, based on a combination of genetic and biotechnological research methods.

The study was funded by the Russian Science Foundation grant No. 23-28-00619: «Methodology for ensuring the efficiency of circular economic systems in the region (on the example of the North-West of Russia)», https://rscf.ru/project/23-28-00619/.

References

1. V.K. Dubovy, A.S. Smolin, E.G. Smirnova, P.M. Keizer, *Technological processes and equipment of the PPI: textbook. manual* (HSE SPbGUPTD, SPb, 2022)
2. A. Babkin, E. Malevskaia-Malevich, N. Kvasha, E. Eliseev, E3S Web of Conferences **291**, 01002 EDP Sciences (2021)
3. O.V. Kostenko, Fundamental research **9**, 38-43 (2019)
4. N.V. Kvasha, E.D. Malevskaya-Malevich, The economy of the North-West: problems and prospects of development **4(71)**, 38-43 (2022)
5. M.K. Awasthi, R. Sindhu, R. Sirohi, V. Kumar et al., Renewable and Sustainable Energy Reviews **158**, 112122 (2022)
6. A. Mironov, *Pulp and paper industry in Russia. Trends, performance results and their implications for regional development* (Literes, Msc, 2022)
7. E.M. Tretyakova, Ya.V. Petrukhin, Vector of Science of Togliatti State University **1**, 43-45 (2012)
8. S.K. Ghosh, Circular economy: global perspective 1-23 (2020)
9. Y. Geng, J. Sarkis, R. Bleischwitz, Nature **565(7738)**, 153-155 (2019)
10. J. Hickel, G. Kallis, New political economy **25(4)**, 469-486 (2020)
11. O.V. Kudryavtseva, E.Yu. Yakovleva, M.V. Vilt, Bulletin of the University **13**, 123-132 (2014)
12. S.K. Maity, Renewable and Sustainable Energy Reviews **43**, 1427-1445 (2015)
13. B.K. Moldazhanov, World science **11(20)** (2018)
14. T. Raj et al., Bioresource technology **344**, 126292 (2022)
15. A. Joshi et al., Sustainable biofuels 43-72 (2021)
16. O. Bolotnikova, J. Bazarnova, E. Aronova et al., E3S Web of Conferences **140**, 02008 (2019)
17. O.I. Bolotnikova, N.P. Mikhailova, Yu.G. Bazarnova et al., Izvestiya vuzov. Applied Chemistry and Biotechnology **9(4(31)),** 679-693 (2019)

18. F. Møller, E. Slentø., P. Frederiksen, Biomass Bioenergy **60**, 41-49 (2014)
19. H. Cheng, L. Wang, *Lignocelluloses feedstock biorefinery as petrorefinery substitutes*, in Matovic, M.D. (Ed), Biomass Now - Sustainable Growth and Use. InTech, Croatia, Rijeka, 347-388 (2013)
20. M. Oktaviani, W. Mangunwardoyo, E. Hermiati, Biomass Conversion and Biorefinery 1-13 (2021)
21. V.I. Sharkov, S.A. Sapotnitsky, O.A. Dmitrieva, I.F. Tumanov, *Technology of hydrolysis productions* (Lesnaya prom-st, MSC, 1973)
22. O.I. Bolotnikova, N.P. Mikhailova, A.I. Ginak, Izvestiya SPbGTI (TU) **39(65)**, (2017)