Biotechnological complex for processing of plant raw materials and agricultural wastes

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Abstract. The work is devoted to the application of biotechnological complex for the processing of plant raw materials and agricultural waste. The complex includes equipment for raw material preparation, acid and enzymatic hydrolysis. The research is focused on studying the kinetics of these processes and optimising process parameters to maximise the yield of valuable products. Experimental results show significant differences in the efficiency of acid and enzymatic hydrolysis. Acid hydrolysis, especially at high temperatures and pressures, shows high conversion of biomass to reducing sugars, but leads to the formation of by-products. In contrast, enzymatic hydrolysis provides more selective cleavage and fewer by-products, although it requires more expensive enzymes. Comparison with traditional wheat processing methods confirms the advantages of the proposed integrated technology, which provides a significant increase in the yield of target products such as glucose-fructose syrup and starch. The developed complex can be used for research and training of specialists in the field of biotechnology, contributing to the development of innovative solutions in the agro-industrial sector.

1 Introduction

There are several main directions in solving the problem of rational utilisation of plant raw materials and waste. One of them is the utilisation of renewable sources of raw materials, such as plant biomass, for the production of biofuels, bioplastics and other valuable products. This way, on the one hand, reduces dependence on fossil fuels and greenhouse gas emissions and, on the other hand, favours the development of the agricultural sector and its economic sustainability. However, it is worth noting that the production of first generation biofuels, for example, based on cereal crops, may lead to competition with food production and, consequently, to an increase in food prices. Another important area is the use of biotechnological methods to process agricultural waste. This involves the hydrolysis of cellulose and starch to simple sugars, which can then be used to produce various bioproducts, which in turn correlates with research on biomass processing using fly larvae [1]. One of the main advantages of this approach is the possibility to utilise a significant amount of waste such as straw, cake and other agricultural residues, reducing their negative impact on the environment. Nevertheless, the application of these methods is often associated with certain technical difficulties, such as the need to use expensive enzyme preparations or acids, as well as problems with the efficiency and scalability of the processes, which emphasises the importance of finding efficient and affordable ways of improving soil fertility and preserving organic matter in the soil [2]. In light of these problems and perspectives, the presented research aimed at developing a biotechnological complex for processing plant raw materials and agricultural waste is particularly relevant, especially given the importance of legal regulation in the field of organic agriculture [3]. It is such an integrated approach, focused on deep processing of biomass using modern biotechnology, is a promising way to create sustainable and environmentally friendly agro-industrial production. In addition, the development and implementation of such a complex will reduce dependence on imported technologies, as well as increase the competitiveness of the domestic biotechnology industry. Research in this area is becoming more and more relevant, as the volume of agricultural waste requiring utilisation is steadily increasing. The aim of the work is to study the possibility of using biological methods for adaptation to climate change [4], as well as the development and testing of a single laboratory complex, including equipment for the preparation of raw materials, hydrolysis and fermentolysis. This complex is designed to study the kinetic parameters of the processes of processing plant raw materials and wastes, which will optimise technological regimes and increase the efficiency of production of valuable bioproducts. The developed complex is a teaching and research laboratory, which is designed to train qualified personnel in the field of biotechnology and biochemistry. This is an important step in promoting research and development of

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innovative technologies in the field of plant raw material processing in the Russian Federation.

2 Method and materials

In this work, a set of experimental studies aimed at studying the processes of processing of plant raw materials and agricultural wastes in order to obtain valuable bioproducts was implemented. Experimental work was carried out on a specially designed laboratory complex, including a number of apparatuses of raw materials, which provided their necessary preparation for further processing. Several apparatuses were used to study the processes of hydrolysis. The first of them is a laboratory hydrolyser of low-temperature hydrolysis with a volume of 6 litres. In this apparatus acid hydrolysis processes were carried out at overpressures up to 0.35 MPa and temperatures up to 145 °C. The hydrolyser was heated by a ceramic-insulated spiral heater with automatic maintenance of the set temperature. Sampling in the process of hydrolysis was carried out through a mesh filter. The second apparatus is a hydrolyser for high-temperature hydrolysis, which allows to carry out the processes of chemical hydrolysis at temperatures from 100°C to 180°C and overpressure up to 1.2 MPa. To study the processes of enzymatic hydrolysis, a special unit was used, which allowed to control the temperature and provide stirring of the reaction mixture, which affects the quality of products [5]. The apparatus used in the process of enzymatic hydrolysis allowed to conduct experiments in optimal conditions for the action of enzyme preparations. The process parameters such as temperature, pressure, concentration of reagents and reaction products were monitored and measured at all stages of the research. In the course of experiments, the change in the concentration of reducing agents during the hydrolysis process was determined, as well as the parameters of the kinetics of enzymatic hydrolysis, which made it possible to evaluate the efficiency of different processing modes and find the optimal conditions.

3 Results and discussion

Within the framework of the conducted research, a set of experimental works aimed at studying the possibilities of effective processing of plant raw materials and agro-industrial wastes into valuable bioproducts was realised. The experimental work consisted of several stages, each of which was aimed at studying a certain aspect of the biomass processing process.

Initially, the preparation of plant raw materials was carried out, which included grinding and, if necessary, drying. This was followed by the hydrolysis process, which was carried out using both acid and enzymatic methods. In acid hydrolysis, two types of hydrolysers were used: low-temperature and high-temperature.

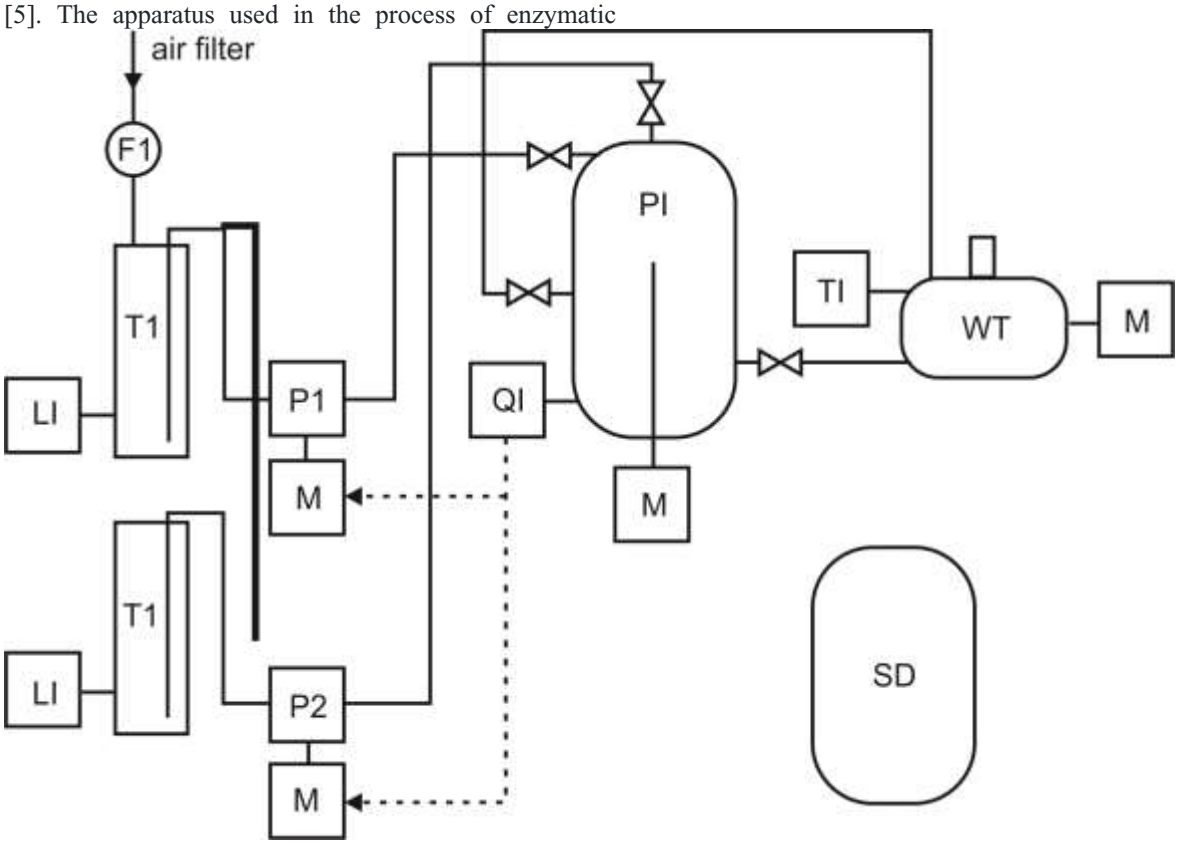


Figure 1. Schematic diagram of the enzymatic hydrolysis unit in use

Laboratory unit of enzymatic hydrolysis used in the work (Figure 1), is designed to study and carry out processes of enzymatic hydrolysis of plant raw materials. The main element of the unit is a fermentolyser (P-1) - a reactor with a heat exchange jacket and a mechanical stirrer, designed to carry out the process of enzymatic hydrolysis. The unit is equipped with a stirrer drive (SD), which provides stirring of the reaction mixture and allows measuring the stirrer shaft rotation speed, as well as voltage and current in the electric motor circuit. To ensure sterility of the process, a sterilising air filter (F1) is available. To maintain the required pH level, the unit is provided with tanks for acid (E-1) and alkali (E-2) supply, as well as peristaltic pumps (H-1, H-2) for their dosed supply to the fermentolyser. A water ultrathermostat (T-1) is used to maintain the set temperature in the jacket of the fermentolyser. The process is also equipped with a temperature compensated pH sensor (QI) to monitor the acid-alkaline balance and visual level gauges (LI) to monitor the liquid level. A thermometer (TI) is also present to monitor the temperature of the reaction mixture. All these elements combine to provide the necessary control and maintenance of conditions for optimal performance of the enzymatic hydrolysis process. In the low temperature hydrolyser, operating at temperatures up to 145 °C and pressures up to 0.35 MPa, acid hydrolysis was carried out using organic acids such as citric acid. In a high-temperature hydrolyser capable of operating at temperatures from 100 °C to 180 °C and pressures up to 1.2 MPa, hydrolysis was carried out under more stringent conditions. The solutions obtained after hydrolysis, were hydrolysate containing reducing agents such as sugars. In enzymatic hydrolysis, specially selected enzyme preparations were used to ensure the cleavage of cellulose and hemicellulose to simple sugars. The process was carried out in a fermentolyser with controlled parameters such as temperature and pH. Process parameters were monitored at all stages of the experimental study and samples were taken to evaluate microbial growth and analyse the biochemical composition. During the experimental studies, detailed data were obtained to characterise the efficiency of different methods of processing plant raw materials, including acid and enzymatic hydrolysis, as well as their variations. In particular, acid hydrolysis, especially under high temperature and pressure conditions, was found to exhibit a significant ability to convert biomass to reducing agents, making it an attractive primary processing step. However, it should be noted that this method, despite its high conversion rate, is accompanied by the formation of by-products such as furfural and other organic compounds that may inhibit subsequent biotechnological processes and require additional purification steps, which increases the overall cost of the process. Thus, in our experiments, using high-temperature hydrolysis of straw at 170°C and 1 MPa pressure in the presence of sulphuric acid, an average of 380 grams of reducing agents per kilogram of dry feedstock was obtained. This corresponds to a cellulose and hemicellulose conversion of about 65-70%, which is quite high. At the same time, the content of glucose as the target sugar averaged 80% of the total content of reducing agents, which corresponds to 304 grams of glucose per kilogram of raw material. The remaining 20% was mainly other monosaccharides such as xylose and arabinose, as well as oligosaccharides and their degradation products. It should be noted that under such severe hydrolysis conditions, the formation of by-products such as 5-hydroxymethylfurfural (5-HMF) was also observed, the amount of which, in some cases, reached 2-3% of the total content of reducing agents, which confirms the need for additional purification measures. In addition, the analysis of energy costs showed that high-temperature hydrolysis is a more energy-consuming process, requiring a significant amount of heat to maintain the necessary temperature and pressure, which should also be taken into account when assessing the economic feasibility of the method. In contrast, enzymatic hydrolysis demonstrated the ability to selectively convert cellulose and hemicellulose to sugars under more moderate conditions, which is its undoubted advantage. Thus, using a complex of cellulases and hemicellulases, a high degree of conversion was achieved at relatively low temperatures in the range of 50-60°C and atmospheric pressure. According to my data, enzymatic hydrolysis of wheat waste using Trichoderma reesei cellulase for 24 hours at 55°C produced an enzyme-lysate with a reducing agent content varying from 180 to 220 grams per kilogram of dry raw material, depending on the enzyme batch and pretreatment. The glucose content of the fermentolysate, meanwhile, reached an average of 90% of the total reducing agents content, which ranges from 162 to 198 grams of glucose per kilogram of dry raw material. It is important to note that enzymatic hydrolysis does not produce as many by-products as acid hydrolysis, which simplifies the subsequent processing steps. However, it should be taken into account that enzyme preparations are generally more expensive than acids used in chemical hydrolysis, which should also be taken into account in economic analyses. In addition, enzymatic hydrolysis requires more precise control of process conditions, such as pH and temperature, to ensure optimal enzyme activity. It has been shown that by using precision farming techniques and taking into account various factors, yields can be significantly improved [6-8]. Analysing the data obtained, significant differences in product yields can be seen between conventional and alternative technology for processing 1 million tonnes of wheat. Thus, the traditional technology, focused on the production of ethanol and native starch, shows a protein yield of 68.5 thousand tonnes, ethanol yield of 270 thousand tonnes, and no GFS. The alternative technology, focused on complex processing, shows a protein yield similar to the traditional one, 68.5 thousand tonnes, while the ethanol yield is significantly reduced to 65.5 thousand tonnes, and the HFC yield is 354.3 thousand tonnes, which is significantly higher than the traditional processing method. In addition, the yield of native starch in the alternative technology is 161.5 thousand tonnes, which is many times more than in the traditional approach, where there is none. Thus, alternative technology, using modern biotechnological

approaches, allows not only to increase the yield of valuable products, but also to significantly reduce losses and increase the economic efficiency of processing of plant raw materials. At the same time, it is important to take into account the role of microorganisms for maintaining soil fertility and waste processing [9, 10]. According to the obtained data, the comparison of traditional and alternative technology of wheat processing shows that the alternative method, including deep processing of grain, allows to significantly increase the yield of target products, such as glucose-fructose syrup (GFS) and starch, as well as protein products. For example, in the traditional processing of 1 million tonnes of wheat, the yield of ethanol is 270,000 tonnes, and there is no GFS, while the alternative technology produces 65,500 tonnes of ethanol and 354,200 tonnes of GFS. In addition, the alternative method allows to increase the yield of native starch by 16 times, as well as, to increase the yield of protein. These data confirm that the use of modern biotechnological approaches based on deep processing of plant raw materials is a promising direction for increasing the efficiency of biomass utilisation and reducing the amount of waste. At the same time, it is important to take into account the need to reduce the environmental impact, including by reducing greenhouse gas emissions [11, 12]. The analysis of the obtained results shows that the laboratory complex used in this work allows us to study various parameters of the process of processing of plant raw materials, as well as to select the optimal technological modes for obtaining the target products. The data obtained during the experimental work correlate with the results of other similar studies conducted both in Russia and abroad. For example, studies on straw processing to produce bioethanol have shown that the use of acid hydrolysis allows obtaining a high yield of sugars, while enzymatic hydrolysis provides a more selective breakdown of cellulose and hemicellulose. At the same time, a number of studies aimed at obtaining pectin from beet pulp have shown that the most optimal is the use of acid hydrolysis with the use of citric acid. In general, a comparative analysis of the results obtained and the data of other researchers indicates the need for an integrated approach to the processing of plant raw materials, based on the use of various methods of hydrolysis and fermentolysis, to obtain the widest possible range of target products and reduce the volume of waste. In addition, the economic feasibility of each method should be considered individually, depending on the specific type of raw material and the desired end product. At the same time, it is important to carry out analyses taking into account geo-ecological factors [13, 14] and using GIS-technologies [15].

4 Conclusion

The study demonstrates the effectiveness of the developed laboratory complex for studying the processes of processing of plant raw materials and waste. The complex, which includes equipment for preparation of raw materials and carrying out both acid and enzymatic hydrolysis, allows us to effectively study the kinetics of these processes and optimise the technological parameters to achieve maximum yield of target products. Experimental results showed significant differences in the efficiency of acid and enzymatic hydrolysis. Acid hydrolysis, especially at high temperatures and pressures, provides a high degree of biomass conversion into reducing sugars, but is accompanied by the formation of by-products requiring additional purification. Enzymatic hydrolysis, on the other hand, is characterised by high selectivity and less formation of by-products, although it requires the use of more expensive enzyme preparations. Comparison with traditional methods of wheat processing confirms the advantages of the integrated approach, which provides a significant increase in the yield of valuable products such as glucose-fructose syrup and starch. The developed complex is a valuable tool both for scientific research and for training of specialists in the field of biotechnology of plant raw material processing, contributing to the development of innovative technologies in the agro-industrial complex of Russia. The results obtained confirm the relevance and importance of further research in this area for the creation of sustainable and environmentally friendly agro-industrial production.

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