Enzymatic hydrolysis of cellulose containing wastes of beer industry

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One of the main objectives of the sustainable development concept suggested by the UN conference "Environment and the Development" (Rio de Janeiro, 1992), is gradual replacement of mineral fuel resources, which at modern rate of production can be exhausted in nearest 50-60 years.[1] The burning of mineral fuel results in CO_2 dispersion in the atmosphere, which causes such global ecological problems as greenhouse effect, global change of the climate, loss of the biological diversity. Besides pollutents from burning mineral fuels cause such regional ecological problems as: deterioration of human health, pollution of reservoirs and reduction of soil fertility.[2] The modern tendency of refusal from ecologically more risky atomic energy stimulates search of alternative kinds of power resources. Therefore it is necessary to pay attention to reception of energy on the basis of renewable kinds of raw materials. The commercial production of fuels from lignocellulosics still requires significant efforts on both technical and economic levels. Utilization of cellulose containing wastes allows to reduce the cost of substrate, which mainly accounts for the expenditure of bioethanol production. Among the technical problems needed to be resolved pretreatment is considered as having crucial value.[3] In this context the pretreatment of waste (of rice and barley pellets) has been performed by three methods and enzymatic hydrolysis carried out to compare its reaction ability.

Cellulose containing solid wastes were kindly supplied by Ochokovskoy (Moscow, Russia), Klinskoy (Klin, Russia) and Three Coins (Colombo, Sri Lanka) beer production plants and represented light brown short-fibrous seed covers of barly and rice accordingly. Humidity after drying in air makes 7,25 %, 6.25% and 8.75% for barley and rice spittle of three plants accordingly. Humidity was determined before pretreatment by a standard technique of humidity definition in vegetative raw materials. The hemicellulose content was determined by cold and hot water extraction and it makes 20,2 %, 16.7% and 19.2% accordingly. The content of cellulose was determined by nitrate-alcohol method of Kuschner[4]. It makes 18 %, 20% and 25% of waste weight accordingly. The defined composition of raw materials shows theoretically an opportunity of reception 198g, 200g and 250g of glucose, and also 202g, 167g and 192g xylose from 1 kg of waste accordingly. It enables theoretically utilize 38.9%, 36.7% and 44.2% of cellulose containing waste of these beer poduction plants. The analysis of hindering components to hydrolysis shows the presence of 26,13 %, 36.5% and 19% lignin, and also 17 %, 11.75% and 14.75% pitch substances accordingly. Pitch substances were extracted by the method of TAPPIT-6-59. Content of lignin was determined by the russian standerd method 4845-54 and Komarov's method.[4]

Cellulose containing wastes of bear industry were pretreated by following three methods. (1) Crushing in the grinder equipment SAR within 10 sec, with rotation speed of 5000rmp/min was carried out in the Institute of Theoretical and Experimental Biophysics of Russian Academy of Sciences (Pushino). (2) Deep crushing was carried out in an equpment with the speed of substrate submission 50kg/h (Efficiency 6.5 kWt) in the All Russian Science and Technical Institute of Protein Synthesis (Moscow). (3) The steam explosion was performed in the absence of SO_2 in an extrusion equipment intended for grain processing in the federal research and production centre "The Instruments" (Moscow). The installation has productivity of 50 kg/hours, established efficiency 5,5 kWt and frequency of rotation 485 rpm/min. The pretreated matter was stored in sealed plastic bags at room temperature and initially has brown colour.

Following cellulase complexes were used to carryout the hydrolysis: E1 - Asp. B20 (activity on C? C: 297 ?/g; protein 15 %); E2 - Celloviridine * 1-60 (activity on CMC: 2178 ?/?; protein 21 %); E3 - B1 mix (activity on CMC: 5000?/g; protein: 41 %); E4 - ACE (activity on CMC: 2134 ?/ml; protein: 13 g/l). There general cellulase activity was determined by method of filter paper Watman ? 1.

Optimal E/S ratios for static hydrolysis of waste pretreated by above three methods were determined for each enzyme. Hydrolysis was carried out in plastic eppendorf tubes with capacity of 10 ml at temperature 45^0? . Medium ? ? level was established at 4,7 with 4ml of aacetate buffer solution. Substrate accounts for 5.88-18.7% ODM (w/w) of the reaction medium. Eppendorf tubes sealed to avoid evaporation were incubated without shaking 1-72 hours. Samples were taken each 1, 2, 3, 4, 6, 12 and 44 hours of incubation and centrifuged 5 min at 13000 rpm using laboratory centrifuge MPW - 310 (Poland). Samples were analyzed for enzyme activity and glucose content. Enzyme activity was determined by filterpaper method and glucose content by the glucose oxidase method.[5]

Conclusion

With the purpose of cellulose decristalization, increase of the reaction surface area and the degree of polymerization pretreatment of raw materials is carried out. Such a pretreatment also allows simultaneously to increase the degree of cellulose conversion, minimize the hinderence from lignin, lower energy waste for pretreament and to minimize environment pollution at implementation.[6] Within the first hour maximum speed was recorded in the variant where the E/S ratio is 1:3. The hydrolyzing ability of cellulase complexes decreases according to next order: E1>E3>E2>E4. Within 13-14 hours 100% of cellulose conversion have been observed in the variant 1:1, where substrate was pretreated by steam explosion. Substrate pretreated by deep crushing reach 100% conversion of cellulose within 25 hours and that for the substrate pretreated by grinding was 48-56 hours. Such a pretreatment allows to reach initial speeds of hydrolysis from 5g/l?h (grinding) up to 14 g/l?h (crushing and steam explosion). Continuous mechanical stirring of viscous reaction medium during the whole hydrolysis process can also be considered as drawback because of its high energy consumption and following denaturation of enzyme complex.[7] Suggested mechanical methods of pretreatment and the method of steam explosion, which carried out in absence of SO_2 do not have an influence on medium pH stability. In these conditions rapid stop of glucose formation has been observed within the first 1-2 hours. Kinetic curves of enzymetic activity change show that the maximum speeds of reactions were reached within the first hour of incubation period, which were decreased followingly. The analysis of curves in inverse coordinates show, that the inhibition is of noncompetitive t???.

References

[1] Deleage JP, Souehou C. Energy: an Interdisciplinary Theme for Environmental Education. UNESCO-UNEP International Environmental Education. 1986, p. 18.

[2] Leggett J. Global Warming The Green Peace Report. 1990, p. 271.

[3] Dekker RFH. The Utilization of Autohydrolysis Exploded Hardwood (Eucalyptus regnans) and Softwood (Pinus radatia) Sawdust for the production of cellulolytic enzymes and fermentable substrates. Biocatalysis. 1987, p. 63-75.

[4] Obolenskaya AB, Elnitckaya ZP, Leonovich AA. Laboratory Works of Wood and Cellulose Chemistry. Moscow, Ecology. 1991, p 320.

[5] Fischer J, Chromy V, Voznicek J. Biochem. Clin. Bohemoslov, 1981. p 48.

[6] Sinitsyn AP, Gusakov AV, Chernoglasov VM. Bioconversion of Lignocellulosic Materials, Moscow. 1995, p 219.

[7] Pristavka AA, Rabinovich ML, Kodituwakku PAD, Kozlov YP, Zacchi G. Applied Biochemistry and Microbiology. 1998, p 5.