# Bioethanol from plum waste by SSF process catalyzed by immobilized yeast cells

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## INTRODUCTION

The fourfold increase in the price of oil over the last six years has created a situation that has not been experienced by oil-importing economies since the energy crisis of the 1970s. Technologies for motor biofuel production from plant raw material, which were developed 20–30 years ago, have once again become the subject of researcher and investor interest.
The presence of a lot of natural reserves of renewable organic materials as vegetable biomass creates prerequisites for the development of new technologies and for the improvement of old technologies of production of biofuel using organic materials.

Bioethanol is a kind of alter fuel (Gray, 2006; Fulton, 2007; Hahn-Hägerdal, 2006) could be obtain by treatment of different materials containing cellulose which is waste products of industry, agriculture and municipal economy (Bohlmann, 2006; Mojovic, 2005; Sun, 2002; Lin, 2006).
Ethanol already contributes substantially to the world's transportation fuel supply. Ethanol made from sugarcane provides about half of the fuel for passenger cars and light-duty vehicles in Brazil (Pessoa, 2005). In the United States, ethanol made primarily from the corn starch corn is used extensively as an oxygenated fuel extender (Sheehan, 1999).
Various cellulose-containing raw materials, including agricultural and industrial wastes, could be used for bioethanol production.

Enzymatic hydrolysis of cellulose could be combined with conversion of the obtained glucose - such process is named a simultaneous saccharification and fermentation of raw material. Simultaneous saccharification and fermentation processes are capable of improving hydrolysis rates, yields, and product concentrations as compared to separate hydrolysis and fermentation systems, because the continuous removal of the sugars by the yeasts reduces the end-product inhibition of the enzymes (Linde, 2007; Shen, 2008).

In this work the possibility of conversion of plum waste to sugars with application of different enzymatic preparations and their following transformation to ethanol under the actions of immobilized into polyvinyl alcohol yeast cells *S. bayanus* was investigated.

## MATERIALS AND METHODS

The *Saccharomyces bayanus* cells (AEB group, Switzerland) were used in the work. The cells were kept at 4°C in refrigerator. To accumulate cell biomass for biocatalyst production, the cells were cultivated in the following medium (g/L): glucose - 10; yeast extract - 2.0; NaCl - 1.0; $(NH_4)_2SO_4$- 2.0; $MgSO_4x7H_2O$ - 1.0; $KH_2PO_4$- 1.0. Cultivation was done under aerobic conditions at 26°C for 17 h on a shaker with constant agitation (180 rpm). Yeast biomass was separated by centrifugation





(9000 rpm, 15 min) and immobilized into polyvinyl alcohol cryogel (PVA CG) according to previously patented procedure (Efremenko, 2008).
The process of enzymatic hydrolysis was carried out at pH and 50°C for 6 h.

Simultaneous saccharification and fermentation reaction mixtures contained plum waste, enzymes preparation (0.1 mg/g substrate), and immobilized yeast cell ($2.0\times10^6$ cells/ml). pH was adjusted to 5.0 with 0.05 M phosphate buffer. Reactions (20 ml) were carried out in tubes with 50 ml working volume on an orbital shaker at 180 rpm. Simultaneous saccharification and fermentation process was carried out under anaerobic conditions at 35°C for 72 h. After end of process the samples were analyzed for ethanol content. All analyses were carried out in triplicate and the average value of each controlled parameter was calculated.

Ethanol was estimated by HPGC using nitrogen as a carrier gas and hydrogen gas in the flame detectors, both at a flow rate 32 ml/min.

## DISCUSSION

It was shown that cellulose, hemicellulose and pectin-containing compounds are the main components of plum wastes. That fact confirmed the necessity of pretreatment of the raw material by the complexes of hydrolytic enzymes, containing cellulases, hemicellulases and pectinases to increase concentration of sugars, witch could be fermented into ethanol.
Enzymatic treatment of raw material was realized by industrial enzymes and their mixtures, table 1.

| Enzyme preparations | Composition |
|---|---|
| PCA-Pel | Pectinliase from *Penicillium canescens* |
| Celloviridin | industrial cellulase |
| B221-151 | Cellulase from *P. verruculosum* |
| EgII-B1 | Cellulase from *P. verruculosum* + endoglucanase |
| EgII-PCA | hemicellulase from *P. canescens* + endoglucanase |
| Tandem (EgII, bGl) | Endoglucanase+ b-glucosidase |
| Triple preparation | Pectinliase + endoglucanase + b-glucosidase |
| Control | With out enzymes |

**Table 1. Composition of enzyme preparations used for conversion of plum wastes**

It was established (table 2) that as a result of hydrolysis of plum wastes under the action of enzymatic preparations the concentration of reducing sugars increased from 0.6 up to 10 g/l, here the most output of glucose and fructose was observed in the case of individual preparation of

cellulases of Celloviridin (28,2 and 18,6 g/l, respectively), and also mixtures of preparation of pectinliase with Celloviridin (25,9 and 18,6 g/l, respectively).
It should be noted, that standard of raw material without enzymatic treatment initially contained high concentration of reducing sugars 36,5 g/l, it testified to expedience of the use of plum waste, as raw material for the production of fuel ethanol.

The obtained hydrolysates of plum waste were used in two process of ethanol production in parallel – simultaneous saccharification and fermentation and separate hydrolysis and fermentation, respectively. In both cases the process was realized for 72 h at 35$^{o}$C. The initial concentration of immobilized cells was close to $2.0\times10^{6}$ cells/ml.
It was established (table 3) that concentration of ethanol was higher (up to 9.19 g/l) in 10 investigated sapmles when the SSF process of plum wastes was used for ethanol production., as compared to SHF process.

In the case of SHF process the maximum concentration of the final product was obtained in hydrolysates after their treatment by mixture of enzymes PCA-Pel and Celloviridin (18 g/l), EgII-B1 (17 g/l) and PCA-Pel + EgII-B1 (16.7 g/l).

It was shown (table 3) that ethanol yield was 95-97 % from theoretical level during realization of two process of ethanol production (SSF and SHF) when the mixture of hemicellulase and endoglucanase was used.

| № | Enzyme | Fructose, g/l | Glucose, g/l |
|---|---|---|---|
| 1 | PCA-Pel | 17.5 | 24.2 |
| 2 | Celloviridin | 18.6 | 28.2 |
| 3 | B221-151 | 17.8 | 25.3 |
| 4 | EgII-B1 | 16.0 | 21.8 |
| 5 | EgII-PCA | 15.7 | 22.8 |
| 6 | Tandem (EgII, bGl) | 16.6 | 24.7 |
| 7 | Triple preparation | 12.9 | 18.5 |
| 8 | PCA-Pel + Celloviridin | 18.6 | 25.9 |
| 9 | PCA-Pel + B221-151 | 16.1 | 21.6 |
| 10 | PCA-Pel + EgII-B1 | 18.1 | 24.8 |
| 11 | PCA-Pel + EgII-PCA | 15.4 | 21.9 |
| 12 | PCA-Pel + Tandem | 15.3 | 21.7 |
| 13 | Control | 14.7 | 21.8 |

**Table 2. Concentration of sugars in plum hydrolyzates after their treatment with various enzymatic preparations**

| № | SHF | | SSF | |
|---|---|---|---|---|
| | Ethanol, g/l | Ethanol yield, % | Ethanol, g/l | Ethanol yield, % |
| 1 | 4.65 | 21.79 | 13.84 | 64.85 |
| 2 | 16.34 | 68.24 | 12.23 | 51.08 |
| 3 | 7.74 | 35.15 | 8.71 | 39.55 |
| 4 | 17.19 | 89.05 | 15.01 | 77.76 |
| 5 | 15.77 | 80.18 | 15.97 | 81.20 |
| 6 | 14.71 | 69.72 | 18.46 | 87.49 |
| 7 | 15.38 | 95.81 | 15.59 | 97.12 |
| 8 | 18.03 | 79.30 | 10.75 | 47.28 |
| 9 | 10.72 | 55.66 | 12.56 | 65.21 |
| 10 | 16.73 | 76.17 | 16.79 | 76.44 |
| 11 | 14.70 | 77.11 | 14.83 | 77.79 |
| 12 | 13.36 | 70.55 | 13.37 | 70.60 |
| 13 | 16.06 | 85.92 | 16.06 | 85.92 |

**Table 3. Ethanol concentration and yield from theoretic level obtained during SSF and SHF processes of plum waste**





## CONCLUSIONS

Thereby, the fundamental possibility and efficiency of use of combined process of enzymatic hydrolysis and fermentation for production of bioethanol from plum wastes were obtained. The pretreatment of substrate in the presence of enzymes cellulases and endoglucanase was preferable when SSF process was used.

In the case of SHF process the best results were obtained when enzymes pectinliase and cellulase were used, it confirmed reasonability of use of enzymes with wide spectrum of activity for hydrolysis of this substrate.

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