Optimization of parameters of alcohol fermentation of xylose-containing inedible substrates using the yeast Pachysolen Tannophilus

Yuliya Bazarnova[1,*], *Olga* Bolotnikova[2], *Natalia* Michailova[3], and *Jing* Pu[4]

[1]Peter the Great St.Petersburg Polytechnic University, Russia
[2]Petrozavodsk State University, Republic of Karelia, Russia
[3]St. Petersburg State Technological Institute (Technical University), Russia
[4]University, School of Agriculture & Biology, China

Abstract. This work presents economical and ecological advances of microbiological utilization of inedible sources of plant biomass, procedure which is associated with bioethanol obtaining. We study influence of forced aeration and initial concentration of biomass of xylose-assimilating yeast P. tannophilus Y-1532/B2 on ethanol output from various xylose-containing substrates during periodical fermentation. The highest ethanol output is observed for OTR values equal to 5.0-8.0 mMole/l×h and yeast seeding density equal to 0.25 g a.d.s./g of substrate sugars. We show the possibility for intensification of ethanol obtaining technology from xylose-containing substrates. This was made using traditional biotechnological approaches of fermentation productions by optimization parameters of forced aeration of the fermentation medium and density of P. tannophilus biomass seeding. The obtained results might be used as initial parameters for calculation of laboratory regulations of complex microbiological utilization of secondary inedible sources of plant biomass of various origin and content. Industrial implementation of this technology will allow one to increase the economical coefficient of ethanol production from secondary inedible sources of plant biomass for 59.7-96.8% due to fermentation of D-xylose.

1 Introduction

Dynamic development of world civilization cannot be performed without searching for renewable energy suppliers, which are alternatives to oil and coal [1-11]. Depletion of its strategic reserves serves as a powerful catalyst for interest to the processes of microbiological conversion of inedible sources of plant biomass [12-14]. Ethanol, in contrast to other forms of bioenergy, is inexpensive and low-toxic for environment [15, 16]. Tradition producer of ethanol - the yeast Saccharomyces cerevisiae - cannot assimilate D-xylose. As it is known, 20-92% of all hemicellulose sugars of annual and plurannual plants

* Corresponding author: j.bazarnowa2012@yandex.ru

fall to share D-xylose. This makes impediments for complex conversion of various plant wastes to the technical ethanol [17].

The situation was principally changed after discovering the xylose-assimilating yeast. These yeasts in contrast to bacteria and mycelial fungi, are of an active fermentation type of sugars catabolism, and possess a number of technological advantages [18]. It is known that xylose-assimilating yeast P. tannophilus are characterized by the unique physiological plasticity, including the following: resistance to changes in temperature, medium pH, concentration of D-xylose; increased tolerance to presence of ethanol and toxic impurities in the fermentation medium and common components of industrial wastes of lignocellulose [19].

Nevertheless, now there is no exact information about parameters, which are optimum for alcohol fermentation of xylose-containing substrates by strains of this species.

The aim of the present work was to analyze the influence of the aeration and initial concentration of the P. tannophilus Y-1532/B2 yeast seeding on production of ethanol from xylose-containing substrates of various compositions.

2 Materials and methods

We studied parameters of forced aeration, which promote maximum economical output of alcohol from D-xylose. For this purpose we determined the rate of oxygen dissolving in the fermentation medium (OTR, mMole/l h) by sulfite method [20, 21].

The experiment was carried out in the Erlenmeyer round-bottomed flasks of various dimensions and in laboratory fermentation unit Biostat M (Braun, Germany) of 2 l volume (its filling factor is 0.75). This fermentation unit has a turbine mixture and a barbator, compressor with regulated supply of sterile air, reduction gear for variation of the mixing unit rate, detectors for measuring pH and pO2.

Medium pH value was maintained by automatic supply of NaOH solution. Various conditions of forced aeration were created by variation the filling degrees of flasks and fermentor by nutrient medium with 2.0% D-xylose as a single source of carbon. Also we varied rotation speed of the round thermostating shaker (the fermentor mixing unit).

We studied influence of the aeration conditions at initial concentration of P. tannophilus yeast biomass equal to 1.00 0.05 g a.d.s./l. Initial concentration of D-xylose was (20.00 3.00) g/l. The nutrient medium content and experiment conditions are described in details in [22]. The substrate inhibition was prevented by addition in fermentation medium low concentrations of P. tannophilus Y-1532/B2 biomass [23], which was grown at optimum temperature and pH [24].

We studied influence of P. tannophilus Y-1532/B2 biomass seeding on production of ethanol. For this purpose all fermentations were carried out at initial sugars concentrations equal to (36.1 2.0) g/l and OTR value equal to 8.0 mMole/l h.

The amount of D-xylose and hexose (D-glycose, D-galactose and D-mannose) in the plant biomass were detrmined according to recommendation from [25, 26]. The ethanol output was calculated using the data from [27].

Statistical analysis of the experimental results was carried out according to [28] using the standard computer program MS Excel. Maximum error for each experimental point was not more than 5.0%.

3 Results and Discussion

3.1 Influence of aeration condition of fermentation of D-xylose

It is known, that oxygen is a key factor, which regulates the direction of D-xylose catabolism in a yeast cell.

According to the experimental data, during 96 hours of anaerobic fermentation the culture P. tannophilus Y-1532/B2 used only a minor part of D-xylose. Increasing of OTR up to 2.0 mMole/l h has already activated this process, whereas the rate of D-xylose consumption remained low. Intensive aeration (140 mMole/l h) drastically increased the kinetics of D-xylose consumption by the yeasts, while decreasing the fermentation period. The primary biotechnical characteristics of ethanol production from D-xylose at various conditions of forced aeration are presented in Table 1.

The obtained results show that a sharp interval of OTR values from 5.0 to 8.0 mMole/l h is optimum for alcohol production by the yeast P. tannophilus Y-1532/B2. However, growth and development of periodical culture P. tannophilus Y-1532/B2 were preserved even in such conditions. This proves the outstanding significance of additional factors for increasing ethanol production from D-xylose.

3.2 Influence of the initial concentration of the P. tannophilus yeast seeding on the alcohol production from the mixed xylose-containing substrates

It is known that initial concentration of yest-saccharomycetes (the seeding density) has a direct influence on efficiency of the hexose alcohol fermentation [29]. Substrates for alcohol fermentation are obtained from plant biomass wastes and usually contain hexoses apart from D-xylose. So production of ethanol in a model medium was studied according to quantitative and qualitative composition of the carbon sources, which is similar to hydrolyzate of hard wood at various initial concentrations of the P. tannophilus Y-1532/B2 yeast. The composition of the nutrition medium is described in details in [30].

The density of the yeast biomass seeding, obtained in optimum conditions [23] in grams of absolutely dry substance (g a.d.s.) was calculated per mass unit (g) of substrate sugars. The OTR value was selected in such a way that the ethanol output is maximum. The experimental data presented in Table 2 shows that increase in density of xylose-assimilating yeast seeding up to a certain "threshold" value activated the alcohol production and simultaneously decreased the duration of the fermentation process. So, at initial concentration of P. tannophilus Y-1532/B2 biomass equal to 0.11 g a.d.s./g of sugars of the model substrate, the productivity and alcohol output increased for 35% and 26%, respectively. These values obtained its' maxima at the yeast seeding density equal to 0.25 g a.d.s./g of sugars. After this, the further increase of initial concentration of the yeast P. tannophilus Y-1532/B2 didn't have any drastic influence of efficiency of the alcohol fermentation of the model hexose-pentose substrate.

Consequently, it was shown that usage of the widespread approach for fermentation production resulted in increase of economic alcohol output from the model xylose-containing substrate. The used substrate is similar to the hydrolyzate of hard wood according to composition of the carbon sources.

4 Conclusions

The obtained results prove the principal possibility for intensification of ethanol production from xylose-containing substrates. This was made using traditional biotechnological

approaches of fermentation productions by optimization parameters of forced aeration of the fermentation medium and density of P. tannophilus biomass seeding.

The obtained results might be used as initial parameters for calculation of laboratory regulations of complex microbiological utilization of secondary inedible sources of plant biomass of various origin and content. According to the theoretical calculations presented in Table 3, development and practical implementation of this technology will allow one to increase the economical coefficient of ethanol production from secondary inedible sources of plant biomass for 59.7-96.8% due to fermentation of D-xylose.

The industrial implementation of this technology allows the Russian Federation to become a leader at the world market of production of biofuel, which is relatively cheap and low-toxic for the environment. Also it will create favourable economically-feasible conditions for involving various wastes of plant biomass in industry.

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