HUNGARIAN JOURNAL
OF INDUSTRIAL CHEMISTRY
VESZPRÉM
Vol. 39(2) pp. 321-324 (2011)

BIOETHANOL PRODUCTION FROM PAPER SLUDGE PRETREATED BY SUBCRITICAL WATER

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The depletion of oil reserves, energy dependency and rising greenhouse gas emissions urge for alternatives in all of the affected sectors. The transport sector contributes to 25% of global anthropogenic greenhouse gas emission and its energy dependency is 98%. It has to be emphasised that in the transport sector such a general solution as petrol nowadays is unlikely to be found in the future. The evaluation of alternatives depends on a lot of factors. Biofuels might serve as alternatives if they are produced in a sustainable way. Paper sludge is a waste stream of the paper industry and has a high cellulose content, which makes it appropriate for bioethanol production.

The aim of this paper is to show an alternative for bioethanol production from paper sludge. Paper sludge is pre-treated by subcritical water which is followed by an enzymatic hydrolysis and a fermentation step. Optimum condition for the pre-treatment and the enzymatic hydrolysis were determined previously. This paper aims at showing the optimum conditions for the fermentation step. Experiments were performed in shake flasks.

Keywords: bioethanol, paper sludge, pretreatment, optimum consitions

Introduction

Alternative transport fuels have raised attention all over the world. It has to be emphasised that among the different alternatives there is no "best" option nowadays. The evaluation of the different alternatives depends on a lot of factors. Concerning biofuels the food versus fuel debate seems to be solved with the appearance of second generation biofuels and with biofuels produced from waste streams. However it is still a question whether biofuel production of these feedstocks is cost competitive and sustainable. The aim of this paper is to show experimental results of bioethanol production from paper sludge as feedstock.

Paper sludge is a residue of the paper industry. In Japan 5.5 million ton paper sludge is produced yearly. Due to its high cellulose content it might be an appropriate feedstock for bioethanol production. Cellulose is a polysaccharide hard to access. Several methods have been investigated in the literature to make the cellulose content more easy to access. In these experiments subcritical water and enzymatic hydrolysis were used as pretreatment methods. Optimum conditions for the pretreatment were determined previously. The pretreatment steps were followed by a fermentation step. [1, 2]

Materials and methods

Microorganism and culture media

The yeast strain used was Saccharomyces cerevisiae ATCC 9763. The composition (per dm^3) of the liquid nutrient medium used in the experiments was as follows D(+)glucose, 50 g; $(NH_4)_2SO_4$, 7.5 g; KH_2PO_4, 3.5 g; $MgSO_4 \cdot 7H_2O$, 0.75 g; $CaCl_2 \cdot H_2O$ 1 g; yeast extract 5 g; bacto peptone 5 g; $ZnSO_4 \cdot 7H_2O$ 0.04 g; $MnSO_4 \cdot 7H_2O$ 0.009 g; $CuSO_4 \cdot 5H_2O$, 0.008 g in distilled water.

The composition of the precultivation medium was as follows 5% YPD, 0.04% adenine in distilled water.

Yeast cells were stored on YPD agar slants at 4 °C. YPD agar medium was made up of 5% YPD and 1.5% agar dissolved in distilled water.

Composition of paper sludge

Paper sludge has a varying composition. The composition always depends on the paper type produced at the factory. Average composition of paper sludge is shown in *Table 1* and *2*.

Table 1: inorganic components in paper sludge as a percentage of the total inorganic material

Component	%
$CaCO_3$	6.07
$Mg_3Si_4O_{10}(OH)_2$	23.97
$Al_4Si_4O_{10}(OH)_8$	16.15
TiO_2	14.19
Al_2O_3	24.15
Fe_2O_3	1.28
P_2O_5	0.41
unknown	13.78

Table 2: organic components in paper sludge as a percentage of the total organic material

Component	%
ash	38
tree resin	1.45
lignin	5.28
α-cellulose	24.23
β-cellulose	18.83
γ-cellulose	9.73
unknown	2.48

Analytical methods

Ethanol and glucose concentrations were measured by HPLC (Shimadzu LC-20AB prominence liquid chromatograph) using an RID detector (Shimadzu RID 10A), Shodex sugar KS-G G804181 and Shodex sugar KS-802 H806003 columns at 80 °C, distilled water as mobile phase at a flow rate of 1 ml/min.

Dry cell weigth was determined photometrically at 600 nm using a Jasco V-550 UV-VIS specrophotometer. A calibration curve between dry cell weight and absorbance was set previously.

Fermentation experiments

Liquid nutrient medium composition was used as described in microorganism and culture media. pH was adjusted with HCl and NaOH solutions. Inoculum levels or pH values were altered, but only 1 parameter was changed at one time.

Experiments were carried out in shake flasks.

The precultivation medium was incubated for 24 hours at 30 °C and 100 rpm.

The main cultivation medium was incubated for 48 hours at 30 °C and 100 rpm.

Initial glucose concentration was in all cases 50 g/l.

Samples were taken at 0, 3, 6, 9, 12, 18, 21, 24, 36, 48 hours of the fermentation

Medium scale fermentation on paper sludge as substrate was performed in a fermentor with a working volume of 7 dm^3.

Mathematical methods

For modeling microbial kinetics the following equations were used.

$$\frac{dx}{dt} = \mu_{max}\left(\frac{S}{K_{sx}+S}\right)x$$

$$\frac{dS}{dt} = -\left(\frac{1}{Y_{x/s}}\frac{dx}{dt}\right) - \left(\frac{1}{Y_{P/S}}\frac{dP}{dt}\right)$$

$$\frac{dP}{dt} = q_{max}\left(\frac{S}{K_{SP}+S}\right)x$$

These model functions represent inhibition free substrate limitation kinetics. Biomass and product formations were based on the Monod equation where the specific production rates depend on the concentration of the limiting substrate (glucose) and on some parameters such as saturation constant and specific rate constants [2].

Numerical calculations were performed by MATLAB Simulink® software package. The main purpose of the numerical calculation was to estimate the parameters of the equations above in order to describe the fermentation process as close as possible to the measurements. The kinetic parameters were estimated by Nelder-Mead simplex algorithm, where the target function was to minimize the error between the calculated and the measured biomass, substrate and product function-values. The error were defined as the sum of the squares of the differences between the experimental and the calculated data.

Results and discussion

Experimental results

Two types of experiments were carried out using glucose and pretreated paper sludge as substrates for ethanol production. The aim of the experiments carried out on glucose as substrate was to determine the optimum conditions for the microorganism Saccharomyces cerevisiae ATCC9763. The effect of different pH values and different inoculum levels was examined. Glucose consumption, ethanol production and cell growth was determined at 5 different pH levels (pH=3.5; pH=4; pH=4.5; pH=5; pH=5.5) and at 5 different inoculum levels (2%; 6%; 10%; 20%; 60%). The determination of the pH range at which the microorganism can work is needed, because the optimum pH for enzymatic hydrolysis and fermentation steps is likely to be different. The determination of the effect of the initial inoculum levels is required to determine the maximum ethanol production in the shortest time possible. This might be preferable on indusatrial scale, where maximum product formation should be reached as soon as possible and therefore production costs can be reduced.

After detemining the optimal conditions for the fermentation using glucose as substrate paper sludge was used as substrate. Since the enzymatic hydrolysis was performed in acetic acidic buffer, first the acetic acid had to be removed from the medium, because it was an inhibitor for the fermentation. This was done by vacuum evaporation. After that the conditions used for paper sludge were the optimal conditions determined on glucose as substrate.

Optimum conditions for the microorganism were pH=5 with 10% inoculum. pH dependence and inoculum dependence are shown in *Fig. 1* and *2*.

As it can be seen on Fig. 1 glucose consumption is the fastest at pH=5, in this case all the glucose present in the reaction mix is consumed by 12 hours and the maximum ethanol concentration is reached in 12 hours. Experiments at other pH values (pH=3.4; 4; 4.5; 5.5) show slower glucose consumption, all of the glucose is consumed only 3 hours later (at 15 hours) compared to experiments carried out at pH=5. Maximum ethanol concentration is less than that measured at pH=5.

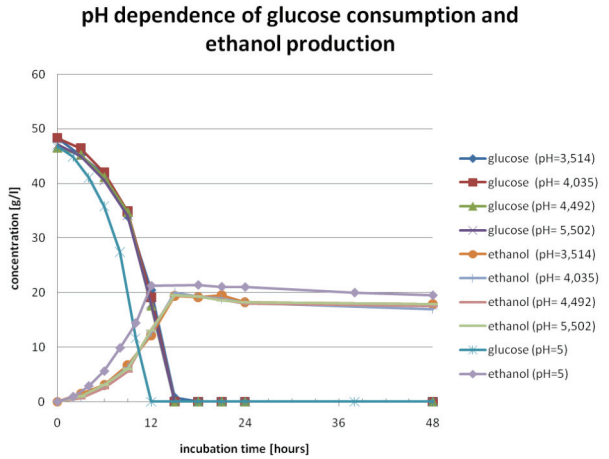

Figure 1: pH dependence of glucose consumption and ethanol production

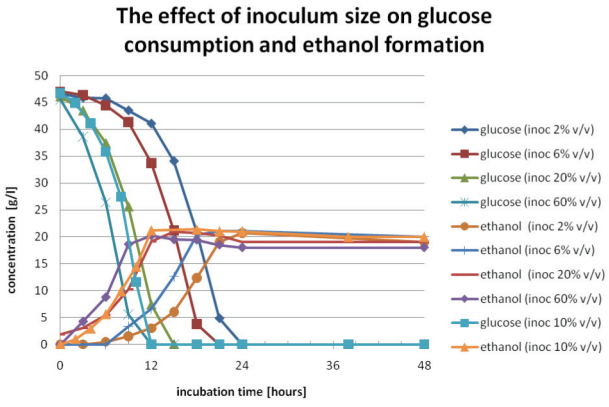

Figure 2: The effect of inoculum size on glucose consumption and ethanol formation

Fig. 2 shows that maximum ethanol yield (19.3 g/l) is reached in the shortest time using 60% inoculum. However this maximum ethanol yield is less than that reached with 10% inoculum (21.38 g/l).

The results observed with 20% inoculum differ slightly from those obtained using 10% inoculum. The application of 10% inoculum is however less cost intensive, therefore this has been chosen as optimum condition.

After the optimum conditions for the microorganism have been found the experiments were performed using pretreated paper sludge as substrate. Initial glucose concentration of the paper sludge hydrolysate was 10.9 g/l on average. Maximum ethanol concentration was 4.1 g/l (yield: 82%) on average which is in agreement with the experimental results carried out on glucose as substrate.

Medium scale experiments were performed in a fermentor with a working volume of 7 l. Initial glucose concentration was 16.53 g/l and maximum ethanol production was 6.8 g/l which is in agreement with the shake flask experiments on glucose as substrate and on paper sludge as substrate.

Simulation results

Data gained from the shake flask fermentation experiments on glucose as substrate with different initial pH values and different inoculum levels were used to model glucose consumption, ethanol production and biomass production by Saccharomyces cerevisiae. Three parameter rate equations were used. The fit of the perfomance curves to the experimental data was tested.

Parameters were optimized to find the maximum ethanol concentration.

Optimized parameter simulation results for experiments carried out at pH=5 are shown on *Figs 3, 4, 5*.

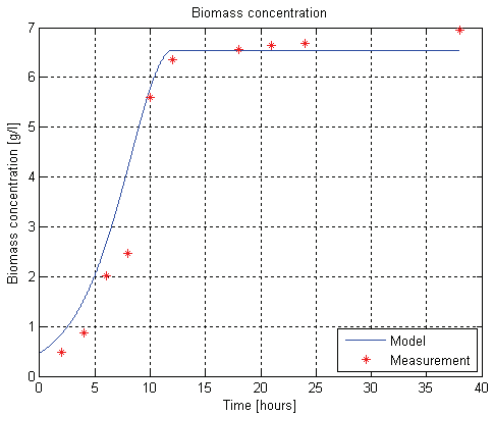

Figure 3: modeled and measured parameters of biomass concentration

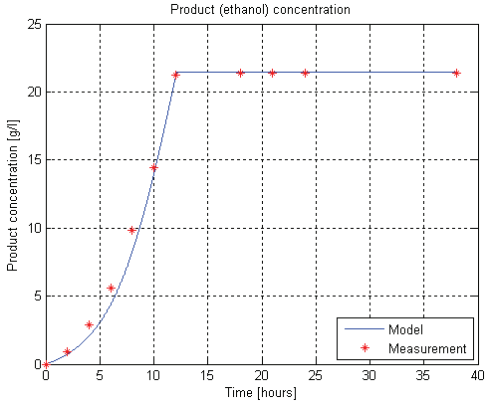

Figure 4: modeled and measured parameters of ethanol production

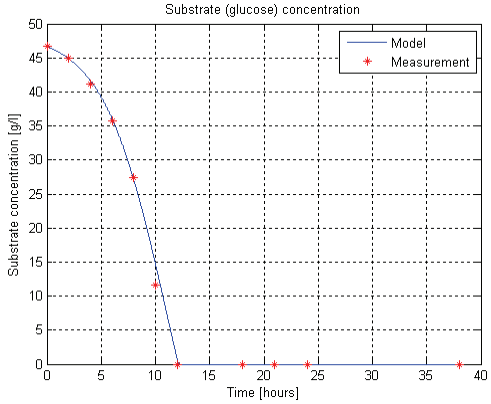

Figure 5: modeled and measured parameters of glucose consumption

Conclusion

Paper sludge is a waste stream with high cellulose content which makes it appropriate for bioethanol production. The combined pretreatment with subcritical water and enzymatic hydrolysis provides glucose for the the further fermentation step.

The aim of this paper was to find optimum conditions for the fermentation. Therefore experiments were carried out on pure glucose as substrate. Optimum conditions were found to be at intitial pH=5 and with an inoculum level of 10%.

Optimization was also performed by modeling and the parameters for optimal conditions (maximum ethanol concentration) were determined.

Experiments on paper sludge were performed with the optimum conditions preciously determined. These experiments were in agreement with those performed on glucose as substrate. Ethanol yields obtained were always above 5% which is the minimum conentration needed for ditillation. Paper sludge was found to be an alternative for bioethanol production.

ACKNOWLEDGEMENTS

Experiments were carried out in Japan at Shizuoka University. The authors gratefully acknowledge the support of the Suzuki Foundation.

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