Bioethanol production from agricultural wastes

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Summary

In this study, different strains of Saccharomyces cerevisiae have been screened for the ability of bioethanol production. Yeasts were grown in synthetic liquid medium containing two different substrates: sucrose at different concentrations (10 to 400g/l) and cane molasses (120g/l of sucrose). The screening was made in batch regime and the growth rates, ethanol and biomass productions were determined. The results indicate a flocculent yeast strain – F as the more suitable microorganism to produce ethanol, presenting the highest value of growth rate ($0.49h^{-1}$) and ethanol yield (0.40g/g) with 120g/l of sucrose concentration. In addition, ethanol production was also studied in a continuous process with the selected yeast strain (F strain), with sucrose and cane molasses (120g/l) at different dilution rates (0.05-0.5 h^{-1}). Data showed that when dilution rate raised to $0.4h^{-1}$ the highest sugar conversion, 85% and 90%, were achieved with an ethanol production of 40.5g/l and 50.8g/l for sucrose and molasses, respectively.

1. Introduction

Ethanol has many desirable features as a petroleum substitute and could help make a smoother transition from a petroleum-based to a bio-based sustainable and environment friendly economics [1, 2]. Most of the ethanol produced in the world is currently obtained from agricultural products such as starch biomass, molasses or cane juice, by hydrolysis of starch and fermentation of sugars. Starch and sugar-based ethanol is often referred as a first-generation biofuel.

Recently, lignocellulosic biomass such as agricultural and forestry residues and herbaceous energy crops can serve as low cost feedstocks for production of fuel ethanol and other value-added commodity.chemicals. These residues are much more social and economical interesting than using sugar crops such as sugar cane, sugar beet or sweet shorgum [3] and the chemical properties of the components of lignocellulosic residues make them a substrate of enormous biotechnological value [4]. However, development of efficient pretreatment and cost-effective enzymatic conversion of any lignocellulosic biomass to fermentable sugars is a key issue. Moreover, the technology for ethanol production from non-food plant sources (second generation biofuel) is being developed rapidly so that large-scale production will become a reality soon [5].

Fermentation of biomass involves significantly greater challenges, owing to the necessity of converting pentose as well as multiple hexose sugars to ethanol. The production of ethanol by continuous fermentation is a more productive process, compared to batch ones [6, 7], as higher productivities are achieved. There are several methods to increase cells density inside a bioreactor; cell immobilization [8], settling techniques [9], as well as utilizing the natural ability of several microorganisms for flocculation [10] are some of them. As main advantages from the alternative processes,

flocculation systems present low associated capital and operational costs and design simplicity.

In this work, we present results of high cell density fluidized reactors and its implications on cell physiology and how to integrate them on a continuous culture model, using flocculent yeast strains for ethanol production.

2. Material and Methods

2.1. Microrganisms strains

Saccaharomyces cerevisiae strains were obtained from: INETI Collection Microorganisms CCMI 396, DER 24; Deutsche Collection Microorganisms DSMZ 2548, Collection Microorganisms NCYC 1119, an isolated strain F from corn fibre hydrolysates (DVT).

2.2. Batch growth experiments

S. cerevisiae strains were kept on malt extract agar slants at 4ºC and the medium used for the growth of the inocula was YMAgar (Difco). In batch growth experiments the culture medium was: 120 g/L sucrose, 3.5 g/L peptone, 3 g/L yeast extract, 2 g/L KH_2PO_4, 1 g/L $MgSO_4.7\ H_2O$ and 1 g/L$(NH_4)_2SO_4$. The pH was adjusted to 5.0 and the medium was sterilized at 121ºC for 15 minutes**.** For fermentation studies a defined molasses medium (diluted to yield a sugar load of 120 g/L) was used.

2.4. Sugar and Ethanol Determination

Sugars (S) and ethanol (P) were determined by HPLC using a refractive index as a detector and a Sugar-Pak column was used at 70ºC with MilliQ water containing EDTA Ca^{2+} (5mM) as the mobile phase; the flow rate was 0.5 mL/min and the injection volume was 20 µL.

2.5. Bioreactor for Continuous Experiment

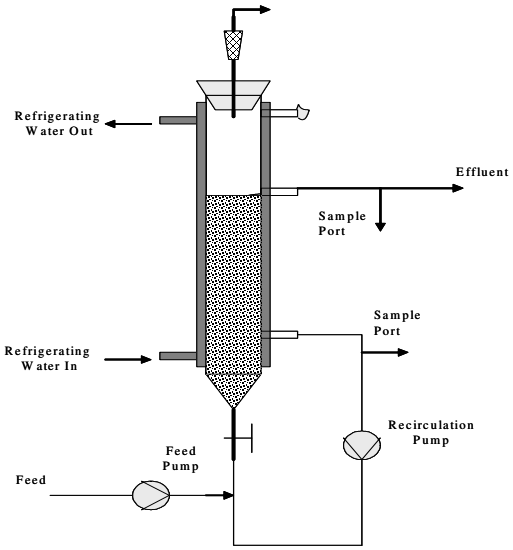

Fig 1 - *Schematic representation of the experimental apparatus used in continuous culture.*

Experiments were performed in a 1 L-glass tubular bioreactor (ID 60 mm, H 800 mm) (Fig. 1). The temperature was maintained at 28 ºC through a jacketed water bath. The pH was monitored but not controlled. The bed of free cells was fluidized by the

generated gas during fermentation and by the recirculation of cells through a peristaltic pump. This reactor is shown schematically in Figure 1. For the model development, only steady-state values were taken into account for statistic calculations. To fit-in measured variables and operational parameters during steady-states the software package SCAN (Minitab Inc.) was used. Some methods of linear regression were tested (OLS, PCR, PLS), using percentage of consumed sugar, dilution rate, ethanol concentration and biomass concentration in the outflow as inputs to predict the biomass concentration within the broth.

3. Results and Discussion

3.1. Growth and ethanol production screening for different yeasts strains

The ability of five yeasts strains (CCMI 396, DER 24, DSMZ 2548, NCYC 1119 and F) to grow and flocculate in sucrose medium and molasses (120 g/L) and to produce ethanol was studied and compared. Strain F presented the highest values of growth rate (0.49 h^{-1}) and ethanol yield (0.40 g/g) and was selected as the most promising flocculent strain for ethanol production.

3.2 Continuous experiments

The flocculent yeast strain selected was used for continuous culture. Its capacity for flocculation permitted to obtain a fluidized biomass bed inside the bioreactor without using a solid support and thus lowering the costs of pumps and support. With a bed of free cells there is an increased contact between biomass and culture medium, which facilitates the uptake of the feeding substrate. In addition, as cells tend to sediment at the bottom of the bioreactor, wash out is not observed at dilution rates near the maximum specific growth rate of the microorganism (approximately 0.49 h^{-1}).

The biomass concentration in the outflow showed an increased instability as the dilution rate was being increased. An explanation for this may be the fact that when dilution rate increased the rate of formation of gas increased as well, and the produced bubbles may have dragged cells toward the upper side of the bioreactor, forcing them to go out in the effluent. Nevertheless the concentration of outgoing cells was in average below 5 g/L. During the various steps of the experiments, the residual sugar has varied, although it has always been underneath 30 %. At the earlier stage, when dilution rate was 0.7 h^{-1} and recirculation velocity was about 4 cm/s, almost all sugar was consumed (96 %).

After increasing dilution rate to 0.23 h^{-1}, there was a significant decrease in the extent of sugar conversion and approximately 23 % of sugar remained intact. In this time the concentration of feeding sugar switched to 120 g/L and four days later the recirculation velocity was raised (till 7 cm/s). This produced a better mixture level inside the bioreactor as the consumed sugar percentage achieved nearly 90 %. Increasing again dilution rate (now for a value near the maximum specific growth rate of the flocculent microorganism) no significant effect was observed concerning sugar uptake.

During the experiments, the yield of ethanol production ($Y_{P/S}$) was very close to the stoichiometric one (0.50 g/g), considering only the steady-state periods of the process; also the yield of biomass formation ($Y_{X/S}$) was low, as desired, and the ethanol yield was varying around 0.45 g/g during the running. In this way, except for that mentioned period, ethanol concentrations in the outflow ranged between 45 and 50 g/L.
Ethanol productivity was calculated as follows:

$$r_P = P \times D \qquad (1)$$

A linear increase in the ethanol productivity was observed with an increase in dilution rate (see Figure 2). The best result attained was 22 g/Lh for a dilution rate of 0.42 h^{-1}. Microbiological monitoring was performed in a regular way and no persistent contamination was found. This is due to the environmental conditions within the broth: low pH (2.50-3.90) and high ethanol concentration (around 0.6 % (v/v)). Also cells viability was followed and it was obtained for all the experiment more than 90 % of viable cells in the recirculation flow.

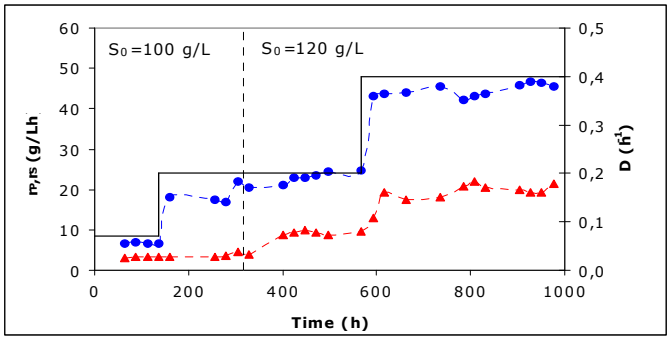

Fig. 2- Continuous fermentation: ethanol productivity (r_P, ▲) and sugar consumption rate (r_S, ●), along time running for different dilution rates (D, —).

4. Conclusions

The good results obtained with our bioreactor can lead us to believe that higher productivities can still be achieved if we can use even higher dilution rates. As biomass is flocculated and sediments easily, there is no problem with cells wash out. The problem of washing biomass out may arise for hydrodynamic reasons, as the gas dragging force on cells flocs. This has been solved in a scale-up reactor design by installing a degassing zone on the top of the bioreactor, allowing cells to sediment before leaving with the effluent (to be published).

The model developed for estimating biomass concentration within the broth shows good capability of predicting that variable thus being a useful tool in monitoring the fermentation process with flocculated cells. To validate the model larger sets of measured variables would be needed, as well as accurate measures of the biomass concentration. Nevertheless there is strong indication that the model can be used as an inferential sensor for biomass with this bioreactor in the tested conditions.
In conclusion this work shows that higher ethanol productivity (20 g/Lh) is obtained with flocculating yeast fluidized continuous reactors compared to those achieved by traditional processes: 2-5 g/Lh for batch reactors; 6-8 g/Lh for CSTR with free cells and 10-16 g/Lh for CSTR with immobilized cells what makes this process very attractive for large-scale ethanol production and a crucial factor for its economical viability.

In this context, the bioethanol production using this process with agro wastes available in the region (e.g. wheat straw) is being also carried out after the application of an

efficient pretreatment (acid hydrolysis) and cost-effective enzymatic conversion of the lignocellulosic biomass to fermentable sugars.

5. Acknowledgments

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