Journal of Environmental Protection and Ecology 12, No 4, 1850–1859 (2011)

FEASIBILITY OF USING THE IMMOBILISED YEAST BIOREACTOR FOR THE EFFICIENT PRODUCTION OF BIO-ETHANOL

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Abstract. The demand for ethanol is increasing every day because ethanol is the only renewable fuel that is produced in commercial quantity. Improvements in technology for ethanol production are necessary for this increase. Otherwise production of ethanol is limited by the available feedstocks and processing technology. Corn is the primary feedstock for ethanol production in batch or continuous bioreactors. Both batch and continuous systems have operational restrictions with maintaining a good growth of yeast and preventing contamination with bacteria. Continuous fermentation systems offer important economical advantages in comparison with traditional systems. Fermentation rates are significantly improved, especially when continuous fermentation is combined with cell immobilisation techniques to increase the yeast concentration in the bioreactor. Hence the technique holds a great promise for the efficient production of fermented beverages, such as beer, wine and cider as well as bio-ethanol. The feasibility of using the immobilised yeast bioreactor was addressed by laboratory testing. The immobilised yeast bioreactors are packed with bio-carrier which is a porous solid that provides a large surface area for attachment of the yeast cells. Cell immobilisation is an effective method of improving the efficiency of substrate utilisation and productivities of various fermentation processes. However, in order to use this technology in fuel ethanol production, the immobilised carrier must be cheap and cell immobilisation should be achievable with minimum additional cost. In addition, the carrier should be also biodegradable and renewable considering the nature of intended purpose of its fermentation product. Spent grain is a by-product of brewing and can be treated in order to be a cheap and helpful support for yeast immobilisation.

Keywords: immobilised yeast bioreactor, continuous fermentation, bio-ethanol, spent grain.

AIMS AND BACKGROUND

The primary commercial process for production of bio-ethanol is direct fermentation. Direct fermentation processes are both batch and continuous. The older and more traditional processes are batch. The use of immobilised microbe bioreactors will provide a continuous system[1,2]. Continuous fermentation systems offer important economical advantages in comparison with traditional systems. Fermentation rates are significantly improved, especially when continuous fermentation is combined with cell immobilisation techniques to increase the yeast concentration in the

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bioreactor. Hence the technique holds a great promise for the efficient production of fermented beverages, such as beer, wine and cider as well as bio-ethanol[3].

Traditional fermentation systems use freely suspended yeast cells in a batch bioreactor. The reactor is filled with unfermented medium and the whole reactor volume ('batch') is gradually fermented and subsequently removed from the reactor. By contrast, continuous fermentation systems have a continuous flow of unfermented medium into the bioreactor and a corresponding continuous flow of fermented product out of the system. The continuous bioreactor operates at steady-state with a content that is equal to the finished product that flows out of the system. A relatively slow inflow and little internal heterogeneity between the points of in- and outflow in the reactor are needed to avoid direct mixing of the unfermented inflow and the finished product[4,5]. The primary disadvantage to the batch fermentation process is the long lag phase during which time the yeast activates its ability to synthesise enzymes. Both batch and continuous processes are subject to contamination by bacteria which can reduce yield and result in time when the unit is not operational.

The basic objective of this study consists of potential ethanol production improvements. This involved designing and installing a laboratory unit as a continuous bioreactor for ethanol production. To determine the feasibility and advantages of using this unit as a continuous ethanol bioreactor, needed work was performed in laboratory conditions in a continuous model for ethanol fermentation system referred as immobilised yeast bioreactor[6].

EXPERIMENTAL

IMMOBILISED YEAST BIOREACTOR

The immobilised yeast bioreactor is a tubular reactor with this parameters: working volume 0.28 l, outer diameter 50 mm, length 270 mm, and weight 600 g (Fig. 1).

Fig. 1. Immobilised yeast bioreactor for continuous production of bio-ethanol

The medium was pumped by a peristaltic pump and introduced close to the top of the reactor that worked up flow, through a tube that discharged the medium in the bottom. The effluent was collected close to the top of the reactor by a tube, with the help of a suction pump. The length of this tube controlled the liquid level in the reactor (Fig. 2). The tube has a jacket that is used for temperature control, through which circulate water from a thermostatic bath, used to control the reactor temperature at 30 ± 0.5ºC. The reactor usually worked in up-flow operation, but it can also work in down flow. The medium was pumped by peristaltic pump and another peristaltic pump was used for the recirculation stream. The reactors and tubes were washed and sterilised prior to operation.

Primary an aerobic environment must be created to enable the growth of the yeast. Ethanol production and yield will be determined using this immobilised yeast bioreactor with corn as feedstock. The corn is processed in dry milling.

Fig. 2. Laboratory batch bioreactor used for our experiments

The bioreactor product will be distilled using a single column distillation unit. The yield will be determined by the amount of product recovered in the distillation system overhead.

To produce ethanol is used conventional and high-gravity fermentation. The last technique, still experimental, minimised water use in ethanol production. Potential savings would come from the reduced cost of water and wastewater cleanup, as well as from reduced energy use. This process would involve less heating and cooling per liter of ethanol.

YEAST IMMOBILISATION AND SUPPORT PREPARATION

For ethanol production was used yeast strain *Saccharomyces cerevisiae*, type SW 35. Yeasts were cultivated in the sterilised Erlenmeyer with a synthetic medium containing malt extract (3 g), yeast extract (3 g), peptone (5 g), glucose (10 g) and water (1 l). The inoculum cultures were incubated for about 48 h at 30ºC and 120 rpm. These conditions promote biomass growth and a concentrated yeast culture was obtained for bioreactor inoculation.

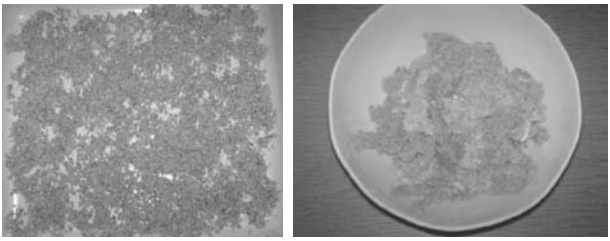

Fig. 3. Yeast support procedure treatment

Yeast support was prepared from dry spent grains by an acid/base treatment method. First is hydrolysed the starchy endosperm present in spent grains by dispersed in 3%(v/v) HCl solution at 60°C for 190 min. The mixture after this was cooled, washed with water and dried. The solids were homogenised by shaking (120 rpm) in a basic solution 2% (w/v) NaOH at 30°C for 24 h. The support was ready for use after being washed several times with water until neutral pH and dried (Fig. 3). From 100 g spent grain was prepared nearly 10 g carrier.

Cell immobilisation by retention is an effective method of improving the efficiency of substrate utilisation and productivities of various fermentation processes. The concept of cell immobilisation provides a promising strategy for the use of yeast cells in a bioreactor for easy scale up and industrial production of bio-ethanol. However, in order to use this technology in fuel ethanol production, the immobilised carrier must be cheap and cell immobilisation should be achievable with minimum additional cost. In addition, the carrier should preferentially be also biodegradable and renewable considering the nature of intended purpose of its fermentation product.

Nevertheless, immobilisation can not risk decreasing the renewal rate of microbial cells, very important in long-term operation. In fact, the aim is to achieve apart a great amount of biomass per mass (or volume) unit of support, they must also have other important features, namely: (1) a high mechanical and chemical stability, (2) they must be non toxic and biocompatible with the cells, and (3) they must promote a high diffusion coefficient both for substrates and fermentation products. This goal can be enough for using the immobilised cells as biocatalysts, as long as substrates and reaction products can diffuse through the support without strong mass transfer resistances. This means that in a long-term continuous fermentation it is essential to preserve cell viability. To favour the ethanol synthesis we are mainly interested in anaerobic conditions, but it is also necessary to guarantee a small growth rate of yeast cells and favour its renewal. Therefore, it is not only important to trap viable cells but also to allow old ones to be released and dragged by the effluent stream.

FEEDSTOCK MEDIUM (SWEET WORT PRODUCTION)

Sweet wort is produced by dry milling which has higher yields of ethanol compared to wet milling in a special vessel as shown in Fig. 4.

Fig. 4. Sweet wort production vessel

In the dry mill, the milling step consists of grinding the corn and adding water to form the mash. Unlike in dry milling, where the entire mash is fermented, in wet milling only the starch is fermented. In dry milling, the mash, which still contains all the feed co-products, is cooked (a diagram that respect different rest times at 60 and 73–75^{o}C to activate natural enzymes) and an enzyme is added. In both systems a second enzyme is added to turn the starch into a simple sugar, glucose, in a process called saccharification. In modern dry mills, saccharification has been combined with the fermentation step in a process called simultaneous saccharification and fermentation. Traditional processes for bio-ethanol production from starch are expensive and processes to realise efficient production of bio-ethanol from starch at low cost are required. There are two main reasons for the high costs, one being that as the yeast *Saccharomyces cerevisiae* can not utilise starchy materials, large amounts of amylolytic enzymes need to be added. On the other hand, the starchy materials need to be cooked at a high temperature to obtain a high ethanol yield. Wort is then fermented into ethanol by yeast. The mash must be cooled to at least 30^{o}C before the yeast is added.

ANALYSIS

Effluent samples were collected periodically for analysis. Ethanol and sugars concentrations were measured. The samples analysed were immediately centrifuged (universal centrifuge at 3500 rpm for 15 min) to remove biomass, then filtered through 0.2-μm pore membranes and frozen for later analysis. Ethanol was measured by distillation according to the standard method EBC. pH was measured with a pH-meter.

Calculations and data presentation. In a continuous process, productivity (g l^{-1} h^{-1}) is calculated as the product concentration in g l^{-1} liquid multiplied by the dilution rate (h^{-1}). In a batch process, productivity is calculated as the product concentration in g l^{-1} liquid divided by the fermentation time (h). Dilution rate (feed flow per reactor volume per h) is based on total volume of the continuous reactor. Residence time (h) in the reactor can be calculated by inversing the dilution rate (h^{-1}).

RESULTS AND DISCUSSION

Experiments were carried out in the immobilised yeast bioreactor using for continuous operation occupied 40% of the net reactor volume, in order to allow for bed expansion due to both CO_2 production and biomass growth. Over a period of 3 days, after steady state had been reached, the flow rate was increased in order to study reactor performance at different dilution rates (*D*), ranging from 0.2 to 1.2 h^{-1}. The results are summarised in Fig. 5.

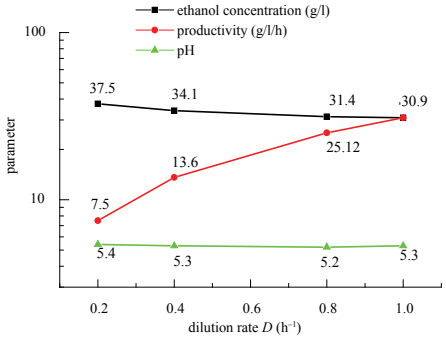

Fig. 5. Impact of dilution rate on productivity and ethanol concentration

Due to the low flow rates used, large volume of samples could not be collected that is why effluent samples were analysed once a day. The data in Fig. 4 show that it took 3–5 days to reach the first steady state. In biological systems, a start-up period is required in order to develop a significant concentration of biomass that explains this long period, also due to the low dilution rate used. The following steady states were reached much faster. Along the 7 days this continuous fermentation lasted, the change in ethanol concentration in the effluent was not significant. For dilution rate up to 1 h^{-1} there were noticed a lot of problems during bioreactor running. The main reason is the increase of biomass observed during the long-term fermentation that, certainly, influenced ethanol production. Biomass built up in this small size reactor and the flow through the bed became unstable, as operation duration increased. CO_2 produced could not escape easily. It formed big slugs and when these burst the support was often swept away blocking the reactor outlet. So, hydrodynamic and flow problems forced to stop

the continuous experiment at $D = 1.2\ h^{-1}$, before steady state had been reached. Although ethanol concentration remained nearly constant during the continuous fermentation, ethanol productivity reached 30.9 g l^{-1} h^{-1} at $D = 1\ h^{-1}$, that was a good result compared to batch fermentation.

The larger flow rates used enabled to collect enough effluent to be analysed for ethanol content by distillation. The highest ethanol concentration 37.5 g l^{-1} was obtained at $D = 0.2\ h^{-1}$. While the dilution was increased from 0.2 to 1 h^{-1}, the outlet ethanol concentration decreased, as expected, to 30.9 g l^{-1}. For further improvement of the productivity the dilution rate must be increased and, simultaneously, a larger volume of support was used to increase biomass concentration in the reactor, but this explains why the experiment was sometimes interrupted in 50% of the runs.

The bioreactor was inoculated and run in batch mode for 2 days, to increase cell density, before continuous operation was started. In these set of experiments, in up-flow, sometimes a recirculation stream was used to improve biomass fluidisation. In the first 2 runs shown in Table 1, operation without support was tested.

Table 1. Impact of support volume used on bioreactor performance

Experiment	1	2	3	3	4
$V_{support}/V_{reactor}$	no support	1	0.3	0.4	0.6
Dilution rate D (h^{-1})	0.4	0.4	0.4	0.4	0.4
Ethanol concentration (g/l)	18.4	–	34.2	37.4	41.8
Productivity (g/l/h)	7.36	–	13.68	14.96	16.72
Operation time in days	4[a]	2[b]	6	7	4[c]

[a] Washing out of bioreactor from the third day; no steady state reached; [b] blockage of the run, no steady state reached; [c] no steady state reached.

The reactor worked for 3 days at a dilution rate of 0.2 h^{-1} and, at steady state, the ethanol concentration was 36.5 g l^{-1}. In run 2 the flow rate was increased to $D = 0.4\ h^{-1}$; however, a few hours later a decrease in activity was registered. Impact of support volume used on alcohol concentration and productivity is presented in Fig. 6. The ethanol content in the effluent was low and the biomass had been washed out. The problems faced to attain biomass retention without immobilisation, even with a flocculent strain, confirm this is unpractical and the use of supports is required. Once again, the first dilution rate tested was 0.2 h^{-1}. After 7 days in continuous operation, the effluent analysis for ethanol showed reproducibility confirming steady state had been reached at a concentration of 40.7 g l^{-1}. At this stage a big increase in biomass was already noticeable; however, biomass built up as operating conditions were changed to $D = 0.4\ h^{-1}$. The reactor had been running at this dilution rate for 4 days when operation had to be stopped, due to the strong accumulation of biomass, some of it probably non active due to mass

transfer resistances. Alcohol and substrate concentrations in the beginning and in the end of continuous fermentation are presented in Fig. 7.

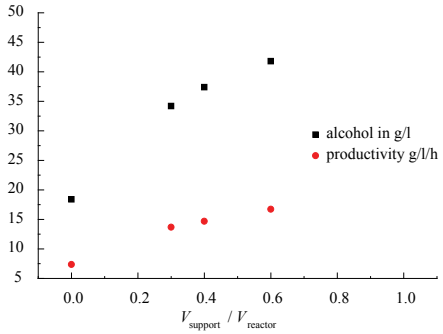

Fig. 6. Impact of support volume used on alcohol concentration and productivity

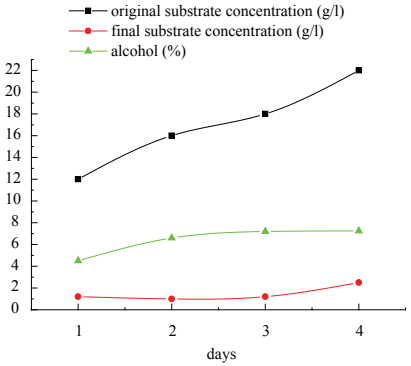

Fig. 7. Alcohol and substrates concentration in the beginning and in the end of continuous fermentation

For a long-term continuous operation with this strain, it is much better not to fill up the reactor with support. Reactor operation was restarted with 40% of the volume filled with spent grain support at a dilution rate of 0.4 h^{-1}.

The ethanol concentration in the effluent increased steadily, till a constant productivity of 14 g l^{-1} h^{-1} was achieved. In order to maximise productivity, the dilution rate was increased as well as the volume of spent grain, which reached 60% of the reactor volume. This strategy led to a productivity of 18 g l^{-1} h^{-1} but this result could not be confirmed due to another operation interruption, once again caused by biomass accumulation.

Alcohol concentration is proportional with original substrate concentration until a limited value. The extensive increasing of original substrate concentration is not effective, because there is a high final substrate concentration which in economical terms means lost of money or low yield. The maximum substrate

concentration value is related closely with the type of microorganisms used and their alcohol tolerance. The problems related with substrate inhibition must be considered, too.

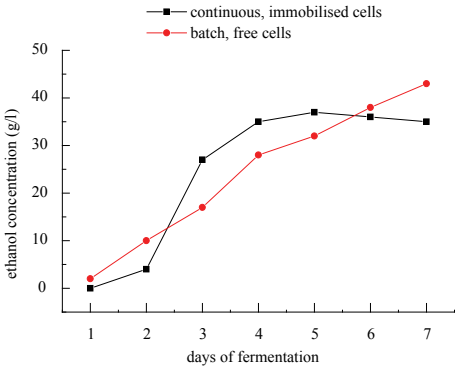

Fig. 8. Ethanol concentration versus fermentation time for free cell and immobilised cell system

As we can see in Fig. 8, the ethanol production in the continuous system is faster and after the third day is stable for a long time. In the batch system it needs 2 days more to get the same ethanol concentration as in continuous system (higher volume), meanwhile the cycle of batch fermentation is over with the continuous system we can produce more and more.

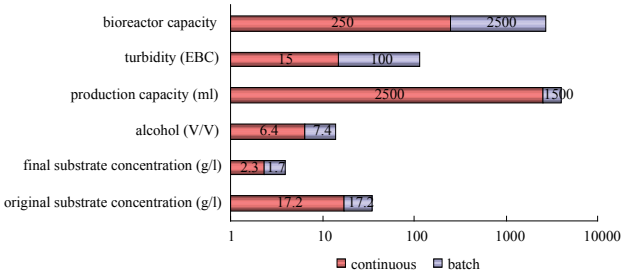

Fig. 9. Batch and continuous parameters for a residence time 72 h in 30°C

The data in Fig. 9 show that although the batch bioreactor capacity is 10 times higher for the same time (7 days) we can produce nearly double volume of product. The high turbidity in batch fermentation 100 (EBC – European Brewing Convention) means that there is a lot of biomass in the batch, in engineering terms means special filtration techniques for yeast removing. Batch system use more deeply the substrate (final extract 1.7 g/l) compared to continuous system which leave a higher unconsumed extract (2.3 g/l) in the final product.

CONCLUSIONS

The data gathered in these runs confirms that continuous fermentation with yeasts can reach high ethanol productivities, once a high cell density is achieved. Spent grains are proved to be able to trap the biomass and allow non-stop continuous mode for a long time, before becoming clogged. Nevertheless it was confirmed that, in the range of dilution rates used, ethanol productivity increases with dilution rate.

Continuous fermentation offers important advantages, such as higher conversion rates, faster fermentation rates, improved product consistency, reduced product losses and environmental advantages. An important aspect of continuous fermentation is the high volumetric efficiency, which is usually obtained by increased yeast cell concentrations in the reactor compared to traditional batch systems. Immobilising yeast cells on several support types can provide high cell densities in the bioreactor, which, in combination with high flow rates, leads to short residence times. These economic benefits are the driving force for a global research effort aimed at studying and implementing continuous fermentations. The immobilised yeast bioreactor also provides a continuous process which needs a much smaller equipment compared to the standard batch system.

REFERENCES

1. M. D. BREDWELL, P. STRIVASTAVA, R. M. WORDEN: Reactor Design Issues for Synthesis-Gas Fermentations. Biotechnology Progress, **15**, 834 (1999).
2. H. C. CHEN, D. G. MOU: Pilot-scale Multi-stage, Multi-feeding, Continuous Ethanol Fermentation using Non-sterile Cane Molasses. Biotechnology Letters, **12** (5), 367 (1990).
3. W. M. INGLEDEW: Continuous Fermentation in the Fuel Alcohol Industry: How Does the Technology Effect Yeast? Ch. 11. In: The Alcohol Textbook (Eds K. Jacques, D. Kelsall, T.P. Lyons). 4th ed. Nottingham Press UK, 2003, 135–143.
4. D. R. KELSALL, T. P. LYONS: Practical Management of Yeast: Conversion of Sugars to Ethanol. Ch. 10. In: The Alcohol Textbook (Eds K. Jacques, D. Kelsall, T.P. Lyons). 4th ed. Nottingham Press UK, 2003, 121–133.
5. K. T. KLASSON, M. D. ACKERSON, E. C. CLAUSEN, J. L. GADDY: Bioreactor Design for Synthesis Gas Fermentations. Fuel, **70**, 605 (1991).
6. P. L. SPATH, D. C. DAYTON: Preliminary Screening – Technical and Economic Assessment of Synthesis Gas to Fuels and Chemicals with Emphasis on the Potential for Biomas-derived Syngas. National Renewable Energy Laboratory, 2003, 58–68.

Received 18 September 2009
Revised 7 April 2010