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ETHANOL PRODUCTION FROM GLUCOSE AND XYLOSE BY IMMOBILIZED *Zymomonas mobilis* CP4(pZB5)*

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DISCLAIMER

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SUMMARY

Fermentation of glucose-xylose mixtures to ethanol was investigated in batch and continuous experiments using immobilized recombinant *Zymomonas mobilis* CP4(pZB5). This microorganism was immobilized by entrapment in κ-carrageenan beads having a diameter of 1.5-2.5 mm. Batch experiments showed that the immobilized cells co-fermented glucose and xylose to ethanol and that the presence of glucose improved the xylose utilization rate. Batch fermentation of rice straw hydrolyzate containing 76 g/L glucose and 33.8 g/L xylose gave an ethanol concentration of 44.3 g/L after 24 hours, corresponding to a yield of 0.46 g ethanol/g sugars. Comparable results were achieved with a synthetic sugar control. Continuous fermentation runs were performed in a laboratory scale fluidized-bed bioreactor (FBR). Glucose-xylose feed mixtures were run through the FBR at residence times of 2 to 4 hours. Glucose conversion to ethanol was maintained above 98% in all continuous runs. Xylose conversion to ethanol was highest at 91.5% for a feed containing 50 g/L glucose-13 g/L xylose at a dilution rate of 0.24 h^{-1}. The xylose conversion to ethanol decreased with increasing feed xylose concentration, dilution rate and age of the immobilized cells. Volumetric ethanol productivities in the range of 6.5 to 15.3 g/L-h were obtained.
Keywords: Ethanol, recombinant *Zymomonas mobilis*, xylose fermentation, immobilization, fluidized-bed bioreactor.

INTRODUCTION

Lignocellulosic biomass is a low-cost renewable resource that can be used to meet the projected increase in fuel ethanol demand. These feedstocks are comprised of cellulose, hemicellulose and lignin. Lignocellulosic hydrolyzates produced either chemically or enzymatically contain both pentoses and hexoses. Glucose derived from cellulose is the major hexose sugar while the pentoses derived from hemicellulose are comprised of xylose and arabinose. While glucose can be fermented to ethanol by a number of microorganisms, the conversion of xylose and arabinose to ethanol is more difficult. Since pentoses, which are predominantly comprised of xylose, can account for 8-28% of the raw material (1), their efficient conversion to ethanol is necessary for an economical process. Advances in metabolic engineering have led to the construction of several xylose-fermenting microorganisms. These include recombinant bacterial strains of

Escherichia coli (2-4), *Klebsiella oxytoca* (5, 6), *Zymomonas mobilis* (7) and a recombinant *Saccharomyces* yeast (8).

Current batch processes for ethanol production from glucose and xylose using these free- cell biocatalysts have a low volumetric productivity and require long fermentation times. Development of high-productivity processes and reactors can potentially reduce capital costs for commercial fuel ethanol production. The use of continuous systems having high biocatalyst loading along with some form of biocatalyst retention mechanism can improve ethanol productivities compared to traditional batch systems. Some of these methods include cell recycle by filtration, sedimentation, entrapment in membranes, or entrapment in gels. With glucose alone as the fermentation substrate, the volumetric ethanol productivities for continuous systems with high conversion have been compared (9). The fluidized-bed bioreactor (FBR) was shown to achieve higher productivities compared to a free cell continuous stirred tank reactor (CSTR), immobilized cell CSTR, hollow fiber reactor and a packed bed reactor with immobilized cells (9). For bioreactor feeds containing xylose or mixtures of glucose and xylose, the volumetric ethanol productivities for different continuous systems are shown in Table 1. For xylose fermentation, species of the naturally occurring yeast *Pichia stipitis* have been immobilized and used in different bioreactor configurations as shown in Table 1. Since the fermentation rate of xylose to ethanol is significantly lower than the rate of glucose fermentation to ethanol, correspondingly lower volumetric ethanol productivities are achieved in comparison to feeds containing glucose only. There are no reports in the literature so far dealing with the application of a FBR using immobilized cells of a recombinant strain for ethanol production from glucose and xylose feed mixtures.

An FBR shows plug flow or multistage characteristics and has advantages over a well-mixed reactor. A higher reaction rate is maintained along the reactor length because of the overall higher substrate concentration and localization of product inhibition to the exit section. An FBR also provides effective mass transport compared to packed-bed reactors by overcoming channeling and carbon-dioxide buildup.

The FBR has been used for ethanol production from glucose (9) and from industrial dry-milled corn starch (15). Economic impact of the use of FBR for ethanol production from glucose has been estimated to be 6 cents/gallon (16). For the dry-milled corn starch to ethanol process using the FBR process technology, a cost savings up to 3 cents/gallon has been estimated (17). In both cases, cost savings were realized due to higher ethanol yields, lower operating costs and lower capital costs for the continuous FBR process with an immobilized *Z. mobilis* biocatalyst compared to a conventional yeast batch process. These potential cost savings by using the FBR have provided the incentive to extend this bioreactor configuration to ethanol production from lignocellulosic feedstocks.

In this investigation, a recombinant *Z. mobilis* strain was immobilized by entrapment in κ-carrageenan and used in batch and continuous experiments for the conversion of glucose-xylose mixtures to ethanol. Batch fermentation kinetics using these immobilized cells on synthetic glucose-xylose mixtures and on lignocellulosic hydrolyzates are presented. Results of continuous fermentation runs in the FBR are also reported. The impact of glucose to xylose feed ratio, residence time in the FBR, and long term use of the biocatalyst are discussed.

MATERIALS AND METHODS

Microorganism

Z. mobilis CP4(pZB5) which contains the *E. coli* genes for xylose assimilation (xylose isomerase, xylulokinase) and pentose metabolism (transketolase, transaldolase) on the plasmid pZB5 was obtained from the National Renewable Energy Laboratory, Golden, Colorado. The stock culture was maintained in medium containing 10 g/L yeast extract, 2 g/L KH_2PO_4, 10 mg/L tetracycline and 25% (w/w) glycerol at -70°C.

Batch fermentation

Cells were grown in a 75 L fermentor (New Brunswick Scientific Co., Edison, New Jersey) at 30°C and pH 5.5 (maintained using 2.5 M NaOH). A working volume of 50 L was used. The seed culture (4 L) was prepared in two fernbachs. The seed culture medium consisted of 50 g/L glucose, 10 g/L xylose, 10 g/L yeast extract, 2 g/L KH_2PO_4 and 10 mg/L tetracycline. The medium without tetracycline was sterilized at 121°C for 20 minutes and allowed to cool before antibiotic was added. Each fernbach was inoculated with 1.5 ml of stock culture. The seed culture was incubated at 30°C and 50 rpm for 36 hours before it was used to inoculate the fermentor. The composition of the fermentation medium was the same as that of the seed medium. The cells were allowed to reach their late exponential growth phase and then harvested using a Sharples centrifuge (Sharples Equipment Division, Philadelphia, Pennsylvania). The cell pastes were stored at 4°C until ready for use in the immobilization step.

Immobilized biocatalyst preparation

Bead preparation was initiated by dissolving 40 grams κ-carrageenan (FMC Corporation, Rockland, Maine) in 600 ml of de-ionized water at 75°C. The dissolved gel was then placed in a water bath at 35°C. To this was added 40 g (wet weight) of recombinant *Z. mobilis* CP4(pZB5) cell paste. The final solution volume was brought up to 1 L with de-ionized water. In order to increase the density of the beads, 30 g of Fe_2O_3 was added to the gel solution. Bead formation was achieved using a previously developed technique in which the heated gel material was forced through a small nozzle using a peristaltic pump (18). A vibration transducer was attached to the flexible delivery tube. By observing the nozzle exit stream under stroboscopic light, the vibrational frequency was tuned to produce monodispersed droplets having a diameter of 1.5-2.5 mm. The gel beads were collected in a stirred vessel containing 0.3 M KCl and were allowed to cure for 24 h at 4°C before use.

Lignocellulosic hydrolyzates

Batch experiments were conducted with immobilized biocatalyst beads on lignocellulosic hydrolyzate supplied by Arkenol, Inc. (Mission Viego, CA). This hydrolyzate was obtained by the concentrated acid hydrolysis of rice straw by the patented Arkenol process (19). The concentrations of glucose and xylose in the hydrolyzate were 107 g/L and 37 g/L, respectively. The hydrolyzate was supplemented with 10 g/L yeast extract, 2 g/L KH_2PO_4, 3.73 g/L KCl and 10 mg/L tetracycline. The initial pH of the medium was 5.8.

Fluidized-Bed Bioreactor (FBR)

The FBR, as shown in Fig. 1, was a jacketed glass column with 5.1 cm inside diameter and 47 cm in length. The volume of the FBR was 0.9 L. The FBR was sterilized at 121°C for 20 minutes before the biocatalyst beads were loaded. The bulk volume occupied by the beads was 550 ml. The pH in the upper part of the FBR was controlled at 5.0 using 1 M NaOH. The base injection was placed very close to the pH probe in order to avoid pH overshoot. The temperature of the FBR was maintained at 30°C.

Recombinant *Z. mobilis* CP4(pZB5) grew within the beads for the first 2-3 days. For the continuous experiment starting with a 50 g/L glucose and 10 g/L xylose feed, this was accomplished by pumping feed solutions containing 50 g/L glucose (A. E. Staley Co., Decatur, Illinois), 10 g/L xylose (Sigma Chemical company, St. Louis, MO), 3.73 g/L KCl , 2 g/L $KH2PO_4$, 5 g/L Difco yeast extract and 10 mg/L tetracycline through the FBR at residence times of 3-4 h. For the continuous experiment starting with a 40 g/L glucose and 20 g/L xylose feed, the FBR was started with pumping feed solutions containing these sugar concentrations. The feed was sterilized by autoclaving at 121°C for 1 h. Following initial growth within the biocatalyst beads by the microorganism, feed solutions containing appropriate concentrations of glucose and xylose were pumped through the FBR to give residence times in the range of 2-4 h. Other components of these feed solutions were the same as described immediately above. These feeds also were sterilized by autoclaving at 121°C for 1 h. All the feed solutions contained 3.73 g/L KCl for stabilization of the biocatalyst beads. The feed lines were changed when the empty feed reservoir was replaced. For each set of experimental conditions, at least six residence times were allowed for the FBR to reach steady state before samples were analyzed for glucose, xylose and ethanol, acetic acid and lactic acid.

Analytical Methods

Glucose, xylose, ethanol, acetic acid and lactic acid were analyzed using a high performance liquid chromatography (HPLC) system consisting of a Waters 410 RI detector (Waters Corporation, Milford, MA), a Waters 717 Plus Autosampler (Waters Corporation, Milford, MA) and an Alltech 425 HPLC pump (Deerfield, IL). The column was an Aminex HPX-87H (BioRad Laboratories, Hercules, California) column. The mobile phase was 5 mM H_2SO_4 pumped at a flow rate of 0.6 ml/min. Data acquisition and analysis were performed using the Waters Millennium software. Glucose and lactic acid were also analyzed by a YSI Biochemistry Analyzer (YSI Incorporated, Yellow Springs, OH).

Cell loading in the biocatalyst beads was measured by a dissolving known volume of beads in 5% (w/v) sodium citrate solution with stirring. After the beads were completely dissolved, the solution was filtered through a 0.2 μm Millipore filter. The filter was dried in an 80°C oven and weighed to calculate the cell dry weight. The protein content of the beads was determined using the procedure as described in an earlier work (15).

RESULTS AND DISCUSSION

Batch fermentation studies

The immobilized *Z. mobilis* CP4(pZB5) biocatalyst beads were used in batch fermentation studies on glucose, xylose and glucose-xylose mixtures. All these experiments were conducted in 125 ml shake flasks containing 60 ml fermentation media and 20 ml beads. The media contained different glucose and xylose concentrations, 10 g/L yeast extract, 2 g/L KH_2PO_4, 3.73 g/L KCl, and 10 mg/L tetracycline. The temperature was maintained at 30°C and the agitation speed at 75 rpm. Fermentation of 32 g/L glucose (Fig. 2a) was completed in approximately 10 hours and an ethanol concentration of 16 g/L was obtained giving an yield of 0.50 g ethanol/g glucose, corresponding to 98% of the theoretical yield. Fermentation of 20.4 g/L xylose (Fig. 2b) was slower, and an ethanol concentration of 7.6 g/L was achieved after 48 hours. The residual xylose concentration was 3.68 g/L. Based on the consumed sugar, the ethanol yield was calculated to be 0.45 g ethanol/g xylose, corresponding to 88% of the theoretical yield. Fig. 2c shows the batch fermentation of a 17.1 g/L glucose and 22.2 g/L xylose mixture. Co-fermentation of the sugars to ethanol was observed, although the rate of glucose utilization (2.86 g/L-h) is about three times higher than the rate of xylose utilization (0.92 g/L-h). An ethanol concentration of 18.2 g/L was obtained after 24 hours, giving a yield of 0.47 g ethanol/g sugars, corresponding to 92% of the theoretical yield. Comparing the xylose utilization profile to that in Fig. 2b, it is clear that the presence of glucose in the medium improves the xylose utilization rate. This observation has also been made in free-cell experiments (9). During xylose fermentation alone, the xylose utilization rate was 0.37 g/L-h. During fermentation of the glucose-xylose mixture, a significantly higher xylose utilization rate of 0.92 g/L-h was obtained. Table 2 summarizes the batch fermentation results on individual sugars and different glucose-xylose mixtures. In these experiments, the initial cell loading in the immobilized biocatalyst beads was of the order of 3 g dry cell weight/L of beads. Ethanol yields from the glucose-xylose mixtures are in the range of 0.46-0.48 g/g, that corresponds to 90-94% of the theoretical yield (0.51 g/g). The co-fermentation pattern and the enhancement of xylose utilization in the presence of glucose was also observed during the batch fermentation of the other sugar mixtures shown in Table 2.

Ethanol inhibition kinetics of xylose fermentation

In order to evaluate the extent of ethanol inhibition on xylose fermentation, batch experiments with different initial ethanol concentrations in the fermentation media were conducted using the immobilized *Z. mobilis* CP4(pZB5) beads. The initial xylose concentration in all cases was 20 g/L. Xylose utilization profiles as a function of time and initial ethanol concentration are plotted in Fig. 3. This figure shows that the rate of xylose utilization declined with increasing ethanol concentrations. When there was no initial ethanol present in the medium, the xylose utilization rate was 0.44 g/L-h. At an initial ethanol concentration of 52.8 g/L, the biocatalyst beads were able to utilize xylose, although at a slower rate of 0.20 g/L-h.

Batch fermentation of lignocellulosic hydrolyzates

Batch studies were conducted with the immobilized *Z. mobilis* CP4(pZB5) beads on lignocellulosic hydrolyzate. The results were compared with those obtained in control

experiments, where synthetic solutions of glucose and xylose at similar concentrations were used. The fermentation media contained the appropriate sugar concentrations, 10 g/L yeast extract, 2 g/L KH_2PO_4, 3.73 g/L KCl and 10 mg/L tetracycline. Fig. 4a shows the results on hydrolyzate containing 76 g/L glucose and 33.8 g/L xylose. An ethanol concentration of 44.3 g/L was achieved after 24 hours, corresponding to a yield of 0.46 g ethanol/g sugars. Glucose was completely consumed while the residual xylose was 13.3 g/L, giving a xylose conversion of 60.7%. Samples taken after 24 hours showed that the rate xylose utilization became very slow and its concentration only dropped to 12 g/L after 74 hours. Fig. 4b shows the results obtained on a sugar control with 82.7 g/L glucose and 39.5 g/L xylose. Since the initial sugar concentrations were higher than the hydrolyzate, a higher ethanol concentration of 52.1 g/L was obtained after 24 hours, corresponding to a yield of 0.49 g/g. The residual xylose concentration was 16.8 g/L, giving a conversion of 57.5%. In this case also, xylose utilization almost ceased after 24 hours. The decrease in xylose utilization rates was clearly due to ethanol inhibition, similar to the observation discussed previously. From the above results it can be concluded that at these sugar concentrations, comparable results were obtained on the hydrolyzate and sugar control.

An experiment was also performed at lower initial sugar concentration by a 50% dilution of the hydrolyzate and sugar control. Fig. 5a shows the results obtained on hydrolyzate media containing 39 g/L glucose and 16.5 g/L xylose. An ethanol concentration of 24 g/L was achieved after 7 hours, corresponding to a yield of 0.49 g/g. The residual xylose after 7 hours was 6.3 g/L. After 24 hours, the xylose concentration dropped to 2.6 g/L, thereby giving a xylose conversion of 84.2%. The pH of the medium dropped from an initial pH of 5.8 to 3.8, and hence could be the cause for incomplete xylose utilization. The results on the sugar control containing 43.4 g/L glucose and 19.7 g/L xylose are shown in Fig. 5b. In this case, an ethanol concentration of 25.2 g/L was obtained after 7 hours, corresponding to a yield of 0.48 g/g. Ethanol concentration increased to 28 g/l after 24 hours, at which time the residual xylose dropped to 0.7 g/L, giving a xylose conversion of 96%. The pH of the medium dropped from an initial pH of 5.8 to only 4.2 in this case.

Continuous Fermentation Studies in the Fluidized-Bed Bioreactor

Different compositions of glucose-xylose mixtures were fed continuously through the FBR at residence times between 2 to 4 hours. The feed compositions and the steady state effluent concentrations of glucose, xylose and ethanol are shown in Table 3. Based upon daily base consumption, the dilution of effluent by base was in the range of 1-3%. These base dilution effects have been corrected for in Table 3. However, the concentration profiles shown in Figures 6 and 7 are real time data and have not been corrected for base dilution.

Fig. 6 shows the concentration profiles of glucose, xylose and ethanol during a continuous fermentation starting with a 49.9 g/L glucose and 12.95 g/L xylose feed. At a dilution rate of 0.24 h^{-1}, an average steady state ethanol concentration of 26.9 g/L was obtained at a yield of 0.44 g ethanol/g sugars (or 86% of theoretical yield). Glucose and xylose conversions were 99.8% and 91.5%, respectively. At a dilution rate of 0.36 h^{-1}, glucose conversion was maintained at 99% while xylose conversion dropped to 79.4%. Ethanol concentration achieved in this case was 26.3 g/L at 88% of theoretical yield. At a higher dilution rate of 0.49 h^{-1}, glucose conversion

was essentially complete at 98.4% and xylose conversion was 81%. The ethanol concentration dropped slightly to 25.4 g/L at 86% of theoretical yield. Thus, maximum uptake of xylose occurred at the lowest dilution rate of 0.24 h^{-1}.

The concentration profiles for a new continuous FBR run started with a 37.0 g/L glucose and 22.2 g/L xylose feed are shown in Fig. 7. At a dilution rate of 0.25 h^{-1}, an average steady state ethanol concentration of 25.8 g/L was obtained at 90% of theoretical yield. Glucose and xylose conversions were 99.5% and 88%, respectively. Thus, at a similar residence time in the FBR, the xylose conversion dropped when the feed xylose concentration was increased. At higher dilution rates of 0.39 h^{-1} and 0.49 h^{-1}, xylose conversions decreased to 72.3% and 45.7 %, respectively. The corresponding glucose conversions were 98.3% in both the cases.
After completing the experiments with the above feed, the run was continued by switching the feed to 68.8 g/L glucose and 23.1 g/L xylose. At a dilution rate of 0.25 h^{-1}, an average steady state ethanol concentration of 34.5 g/L was obtained at 88% of theoretical yield. Glucose conversion was 99%, but xylose conversion fell to 36.4%. At higher dilution rates of 0.5 h^{-1} and 0.39 h^{-1}, the xylose conversion dropped to 22.9% and 9.6%, respectively. Since the feed glucose concentration was higher in this experimental run, the steady state ethanol concentration in the effluent stream was also correspondingly higher. This higher ethanol concentration could decrease the rate of xylose utilization in the FBR, as indicated by results of batch ethanol inhibition kinetics. In order to obtain a higher xylose conversion, a longer residence time in the FBR would be necessary. This can be achieved by using a longer column length. Results of the above continuous runs are summarized in Table 3. Volumetric ethanol productivities achieved in the continuous FBR runs were in the range of 6.5 to 15.3 g/L-h. Lactic acid and acetic acid were detected in the effluent from the FBR during these runs at concentrations up to 4 g/L and 0.3 g/L, respectively.

The feed was then switched to 26.4 g/L glucose and 31.1 g/L xylose and was run at a dilution rate of 0.25 h^{-1} for 5 days. Glucose conversion was again high at 98.5% but xylose conversion was only 15.2%. These results indicate that there was activity loss towards xylose fermentation either due to these dilution rates or due to microbial activity loss. In order to verify this, we returned to the initial feed condition of a 34.2 g/L glucose and 18.4 g/L xylose mixture and the results were compared to the initial results. Glucose conversion remained greater than 99%, but xylose conversion dropped from 87.8% to 1.6%. The cell loading in the biocatalyst beads was 3 g (dw)/L at the start of this continuous FBR run. At the end of this month long run, the cell loading was found to increase more than ten-fold and determined to be 38 g (dw)/L. The increase in cell loading in the beads was confirmed by a protein assay. The protein content in the fresh beads and beads at the end of the continuous FBR run was determined to be 0.31 g/L and 3.0 g/L, respectively.

The feed used for these continuous FBR runs contained 10 mg/L tetracycline for maintenance of the plasmid. Therefore, plasmid instability may be ruled out as the cause for the decline in xylose utilization as a function of time. In prior FBR experiments for ethanol production from glucose using immobilized *Z. mobilis* (9), it has been observed that the cells grew within the beads rapidly during the first week of operation. Following this, the cell density within the beads reached a steady state value. Therefore, during long term operation of the FBR, the cells were presumably in non-growth phase. Under these conditions, it was possible that the decline in

xylose utilization rates was due to the instability of the xylose metabolism enzymes in this recombinant strain under non-growth conditions. The batch fermentation data indicate that this effect is not directly due to immobilization itself. This phenomenon is presently under investigation. When confirmed, this effect will negatively impact the use of this strain for long term non-growth immobilized co-fermentation. However, it will not significantly impact its use in a simultaneous saccharification and co-fermentation (SSCF) process where the cells continue to grow. The testing of other recombinant *Z. mobilis* strains ATCC 31821(pZB5) and ATCC 39676(pZB4L) for xylose fermentation under immobilized non-growth conditions is currently in progress. FBR tests of other recombinant xylose fermenting organisms are also planned.

CONCLUSIONS

Batch and continuous fermentation studies of lignocellulosic sugars to ethanol were conducted using immobilized recombinant *Z. mobilis* CP4(pZB5). Co-fermentation of glucose and xylose was observed in batch experiments. The results showed that the presence of glucose in the fermentation medium improved the xylose utilization rate. Ethanol inhibition was observed to limit the xylose utilization rate during batch fermentation of the Arkenol rice straw hydrolyzate and sugar control. Continuous fermentation studies conducted in the FBR with different glucose-xylose feed mixtures showed that while the glucose conversion to ethanol was maintained above 98% in all runs, the xylose conversion decreased with increasing feed xylose concentration, dilution rate and effluent ethanol concentration. Long term continuous FBR runs are being planned with other recombinant xylose fermenting organisms.

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FIGURE CAPTIONS

Figure 1. Schematic of experimental set-up for continuous FBR runs.

Figure 2. Batch fermentation of A) glucose, B) xylose and C) glucose-xylose mixture by immobilized *Z. mobilis* CP4(pZB5).

Figure 3. Xylose concentration profiles as functions of initial ethanol concentration during batch fermentation studies with immobilized *Z. mobilis* CP4(pZB5).

Figure 4. Batch fermentation results on A) Arkenol hydrolyzate and B) sugar control using immobilized *Z. mobilis* CP4(pZB5).

Figure 5. Batch fermentation results on diluted A) Arkenol hydrolyzate and B) sugar control using immobilized *Z. mobilis* CP4(pZB5).

Figure 6. Continuous fermentation results in FBR with immobilized *Z. mobilis* CP4(pZB5) starting with 50 g/L glucose, 13 g/L xylose feed. Dilution rates (1/h) are indicated above the arrows in the figure.

Figure 7. Continuous fermentation results in FBR with immobilized *Z. mobilis* CP4(pZB5) starting with 50 g/L glucose, 13 g/L xylose feed. Dilution rates (1/h) are indicated above the arrows in the figure.

Table 1. Volumetric ethanol productivities from xylose and glucose-xylose mixtures using different bioreactor configurations.

Bioreactor	Microorganism	Feed	Volumetric ethanol productivity (g/L-h)	Reference
Free-cell CSTR	Recombinant *Z. mobilis* 39676(pZB4L)	8 g/L glucose, 40 g/L xylose	1-2	10
High cell density CSTR	Co-culture of *Saccharomyces diastaticus* and *Pichia stipitis*	35 g/L glucose, 15 g/L xylose	1-4	11
Immobilized cell CSTR	Pichia stipitis	50 g/L xylose	2-3	12
Packed bed with immobilized cells	Pichia stipitis	50 g/L xylose	2-4	13
Pulsed-packed bed with immobilized cells	Pichia stipitis	50 g/L xylose	2-4	14

Table 2. Batch fermentation results on glucose-xylose mixtures using immobilized *Z. mobilis* CP4(pZB5) biocatalyst beads.

Glucose (g/L)	Xylose (g/L)	Total sugars (g/L)	Ethanol (g/L)	Residual xylose (g/L)	Xylose conversion (%)	Yield[@] (g/g)	Fermentation time (hours)	Productivity (g/L-h)
32	0	32	16	-	-	0.50	10	1.6
0	20.4	20.4	7.6	3.7	81.9	0.45	48	0.16
0	43	43	10.4	18.2	57.7	0.42	72	0.14
28.2	10.7	38.9	17.7	0.6	94.4	0.46	24	0.74
17.1	22.2	39.3	18.2	0.8	96.4	0.47	24	0.76
6.9	34.5	41.4	18.2	2.6	92.5	0.47	48	0.38
53.7	21.8	75.5	35.1	2.7	87.6	0.48	48	0.73
33.6	43.9	77.5	31.8	9.6	78.1	0.47	48	0.66

Table 3. Steady state data from continuous FBR runs with immobilized *Z. mobilis* CP4(pZB5) and glucose-xylose feed mixtures.

Feed glucose g/L	Feed Xylose g/L	Dilution rate 1/h	Effluent glucose g/L	Effluent xylose g/L	Effluent ethanol g/L	Yield g/g	Productivity g EtOH/L-h	Xylose conversion%	Glucose conversion%
49.9	12.9	0.24	0.1	1.1	26.9	0.44	6.5	91.5	99.8
49.1	12.6	0.36	0.6	2.6	26.3	0.45	9.5	79.4	98.8
49.1	12.6	0.49	0.8	2.4	25.4	0.44	12.4	81.0	98.4
37	22.2	0.25	0.2	2.7	25.8	0.46	6.5	87.8	99.5
35.4	22.4	0.39	0.6	6.2	22.2	0.44	8.7	72.3	98.3
35.4	22.4	0.49	0.6	12.1	19.6	0.44	9.6	45.7	98.3
68.8	23.1	0.25	0.7	14.7	34.5	0.45	8.6	36.4	99.0
74.6	20.9	0.39	1.2	18.9	33.2	0.44	12.9	9.6	98.4
68.8	23.1	0.5	1.3	17.8	30.5	0.42	15.3	22.9	98.1

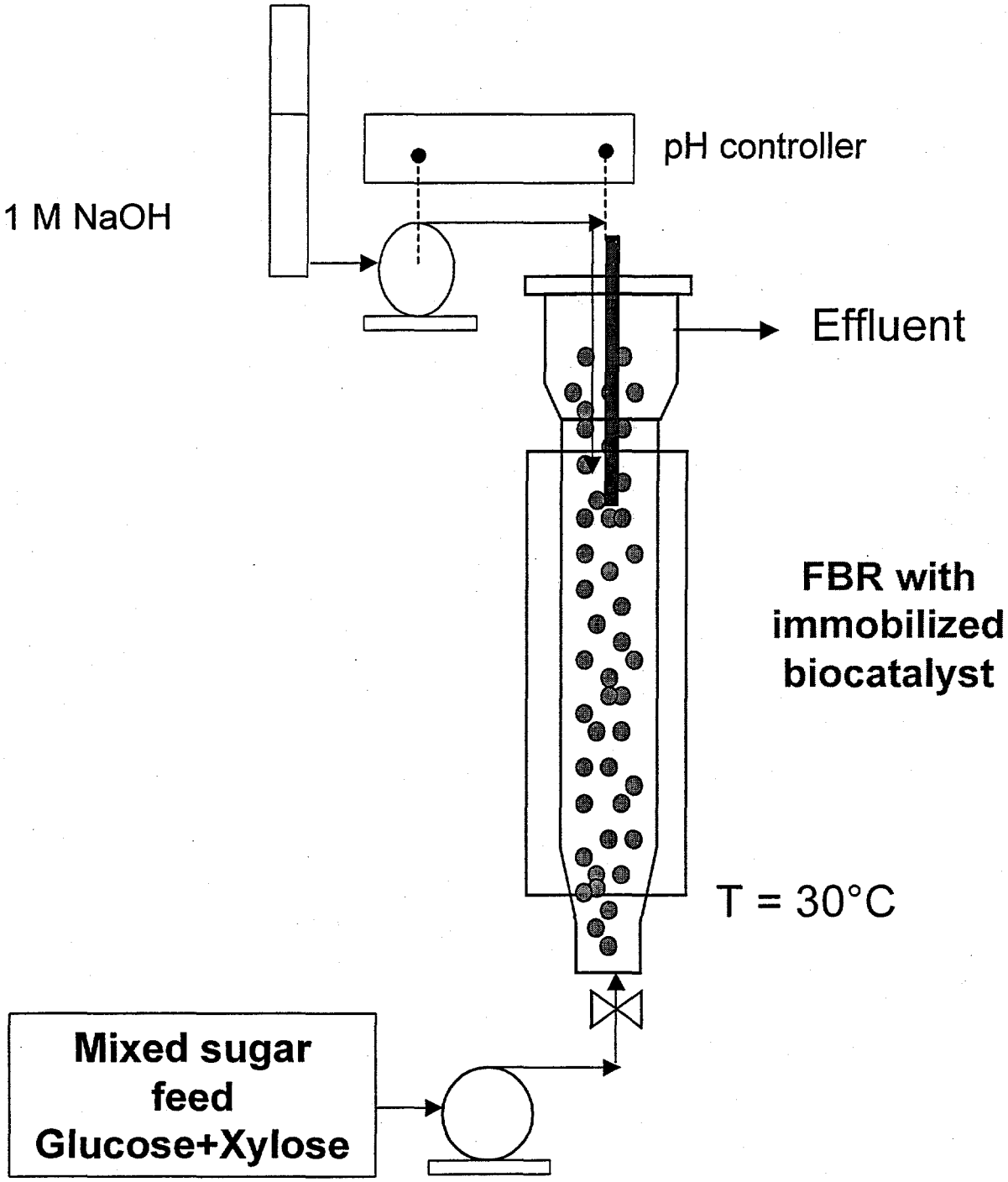

Figure 1. Schematic of experimental set-up for continuous FBR runs.

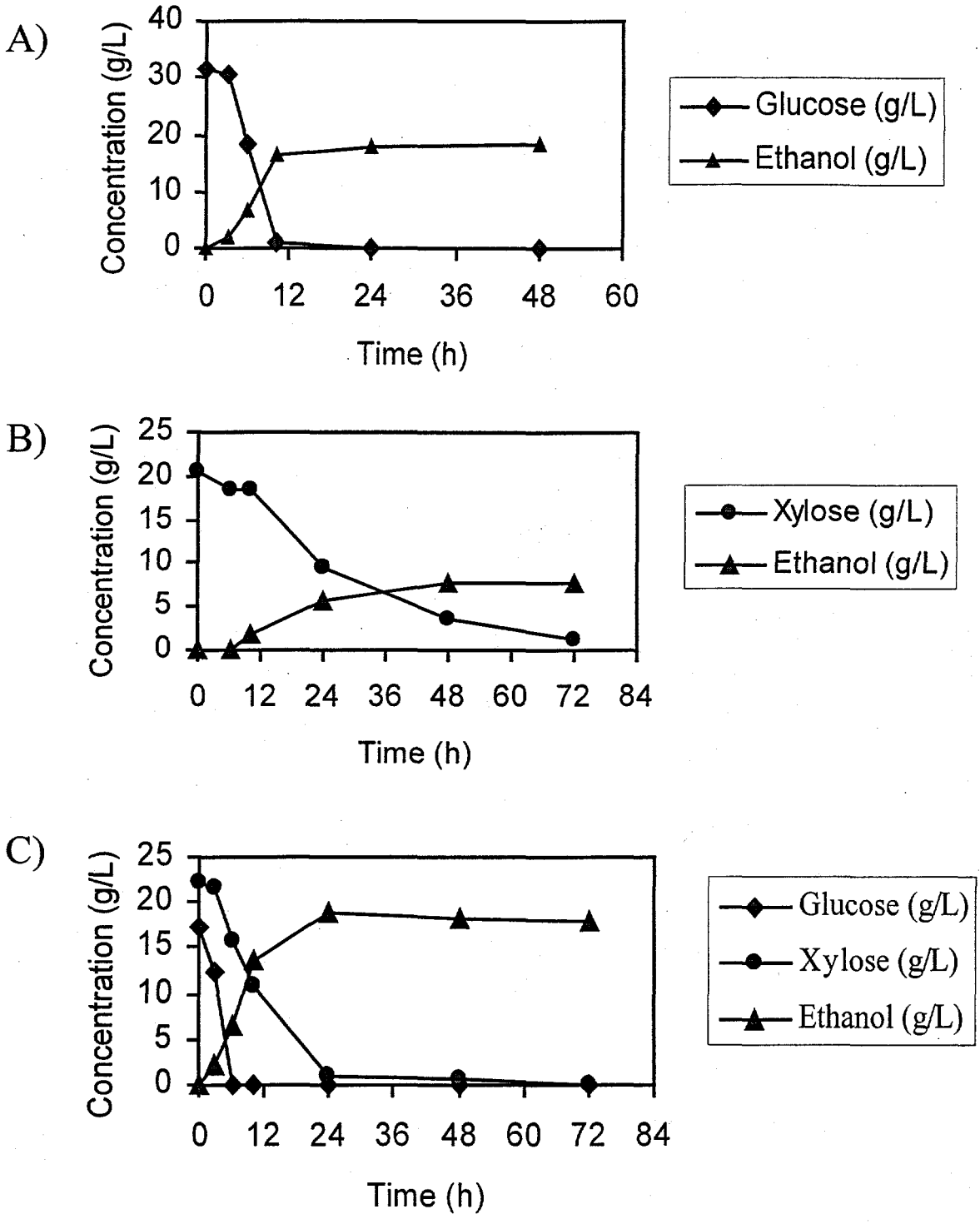

Figure 2. Batch fermentation of A) glucose, B) xylose and C) glucose-xylose mixture by immobilized *Z. mobilis* CP4(pZB5).

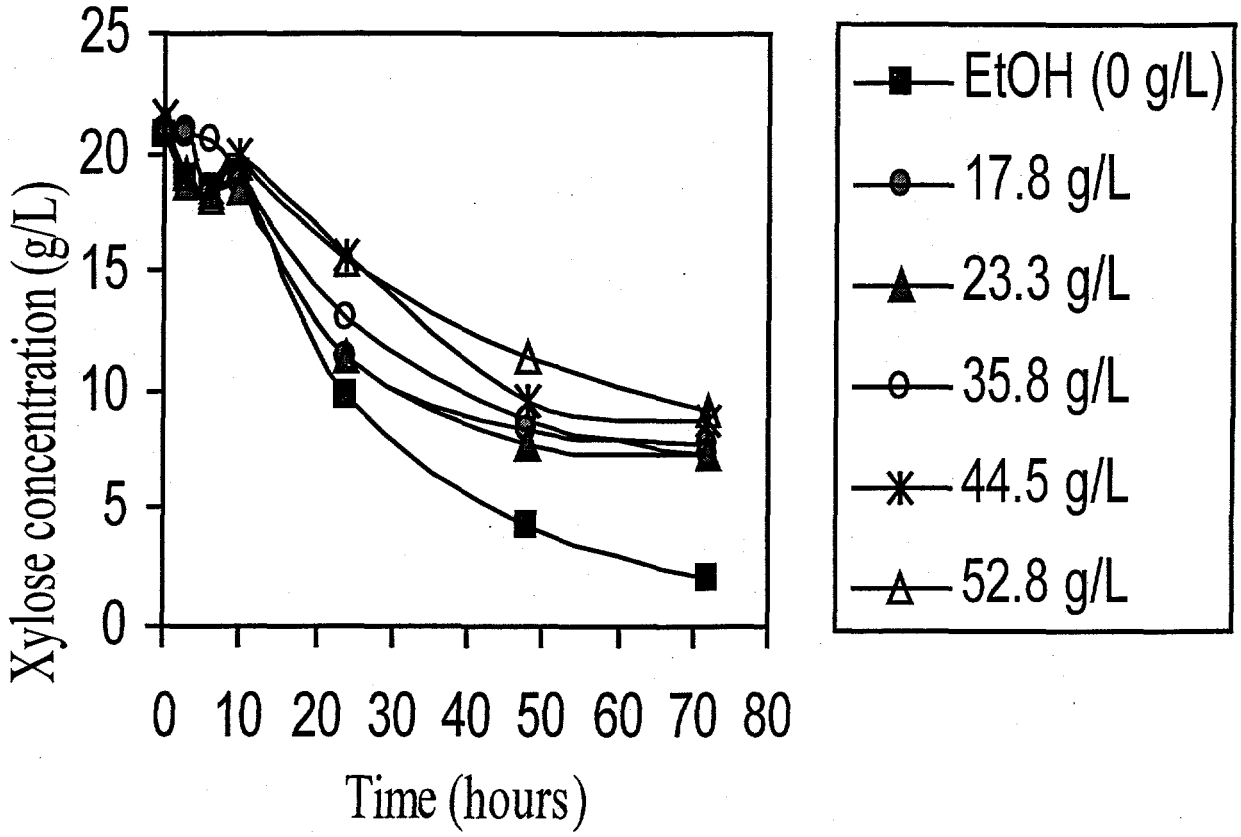

Figure 3. Xylose concentration profiles as functions of initial ethanol concentration during batch fermentation studies with immobilized Z. mobilis CP4(pZB5).

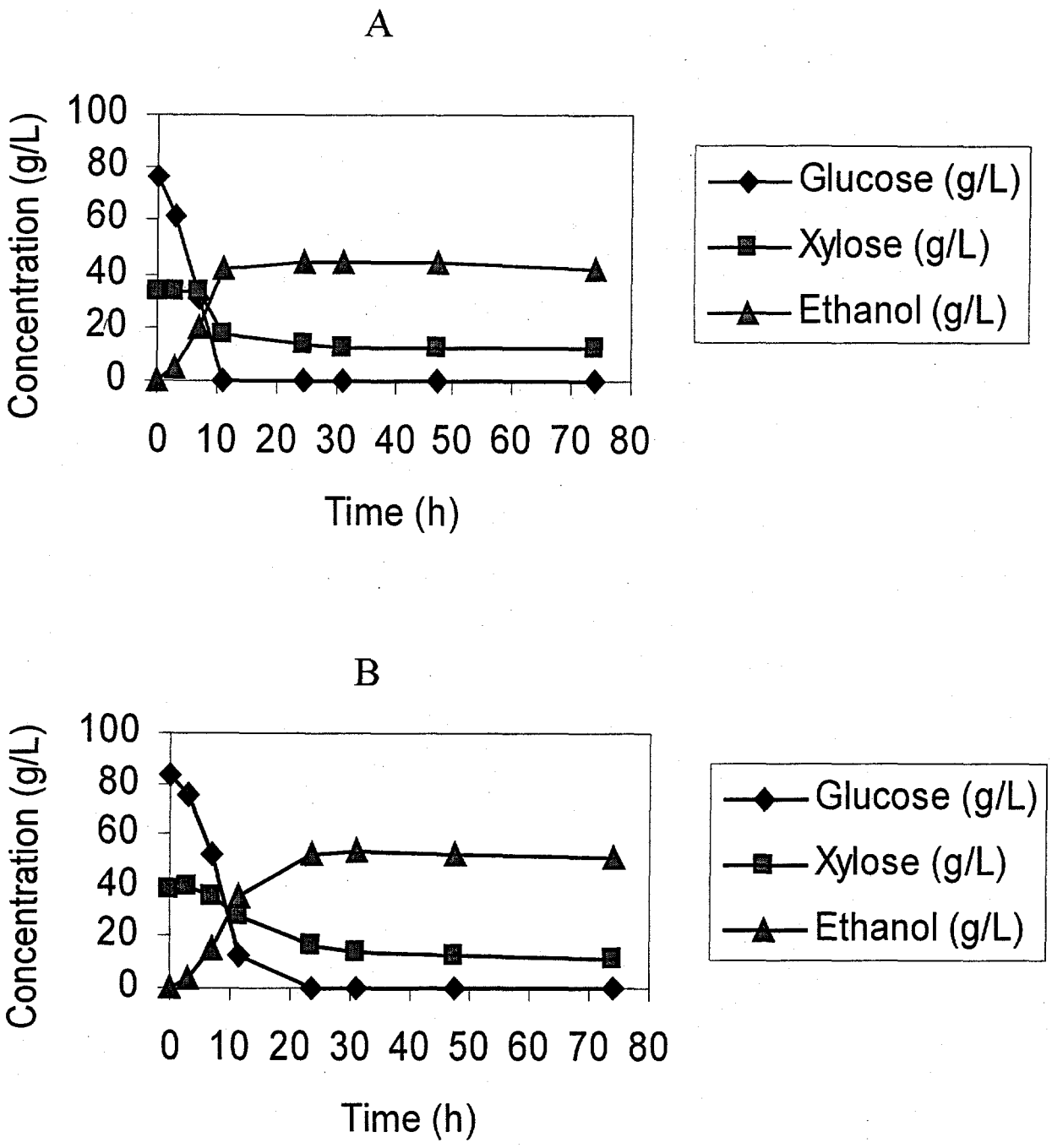

Figure 4. Batch fermentation results on A) Arkenol hydrolyzate and B) sugar control using immobilized *Z. mobilis* CP4(pZB5).

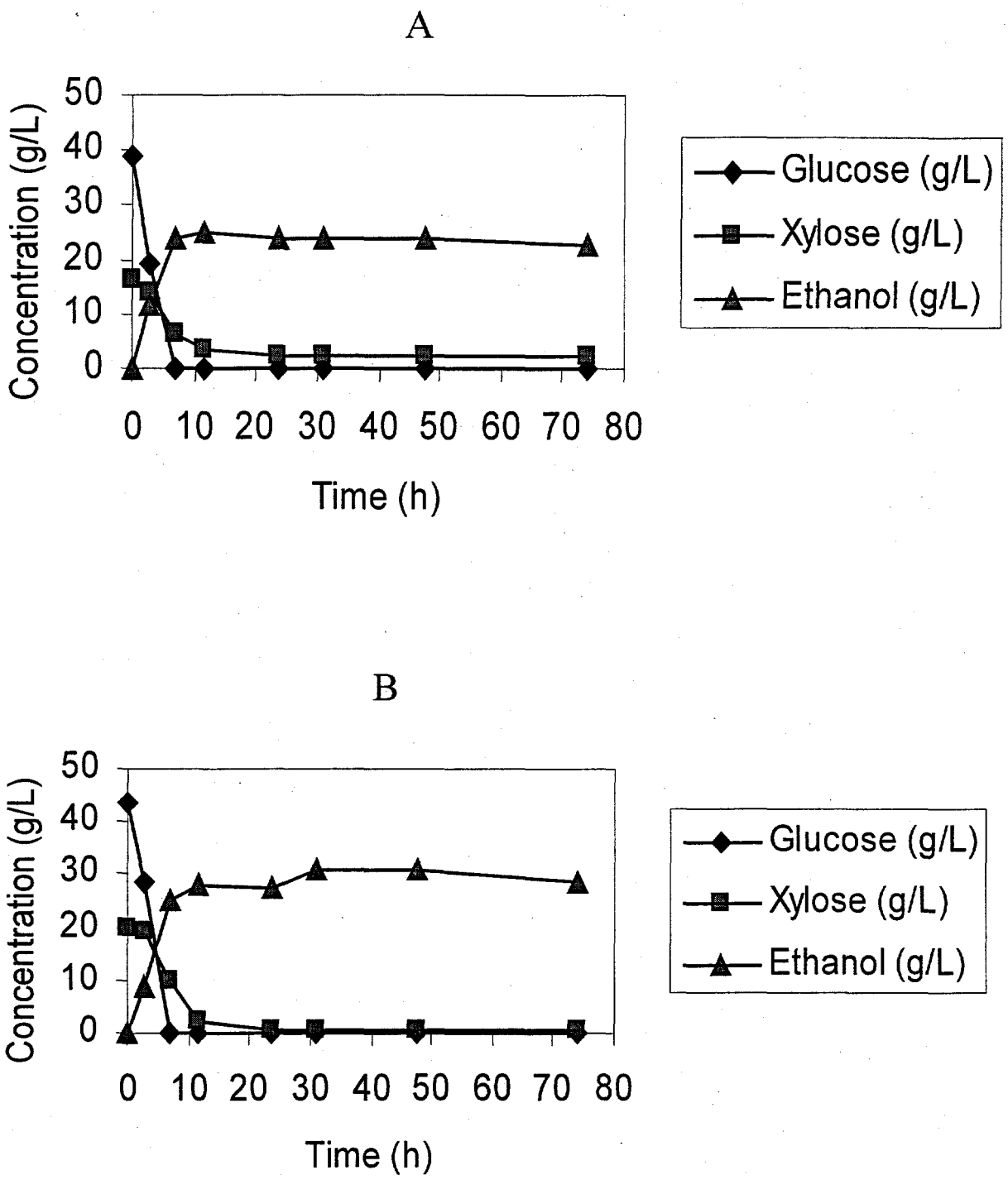

Figure 5. Batch fermentation results on diluted A) Arkenol hydrolyzate and B) sugar control using immobilized *Z. mobilis* CP4(pZB5).

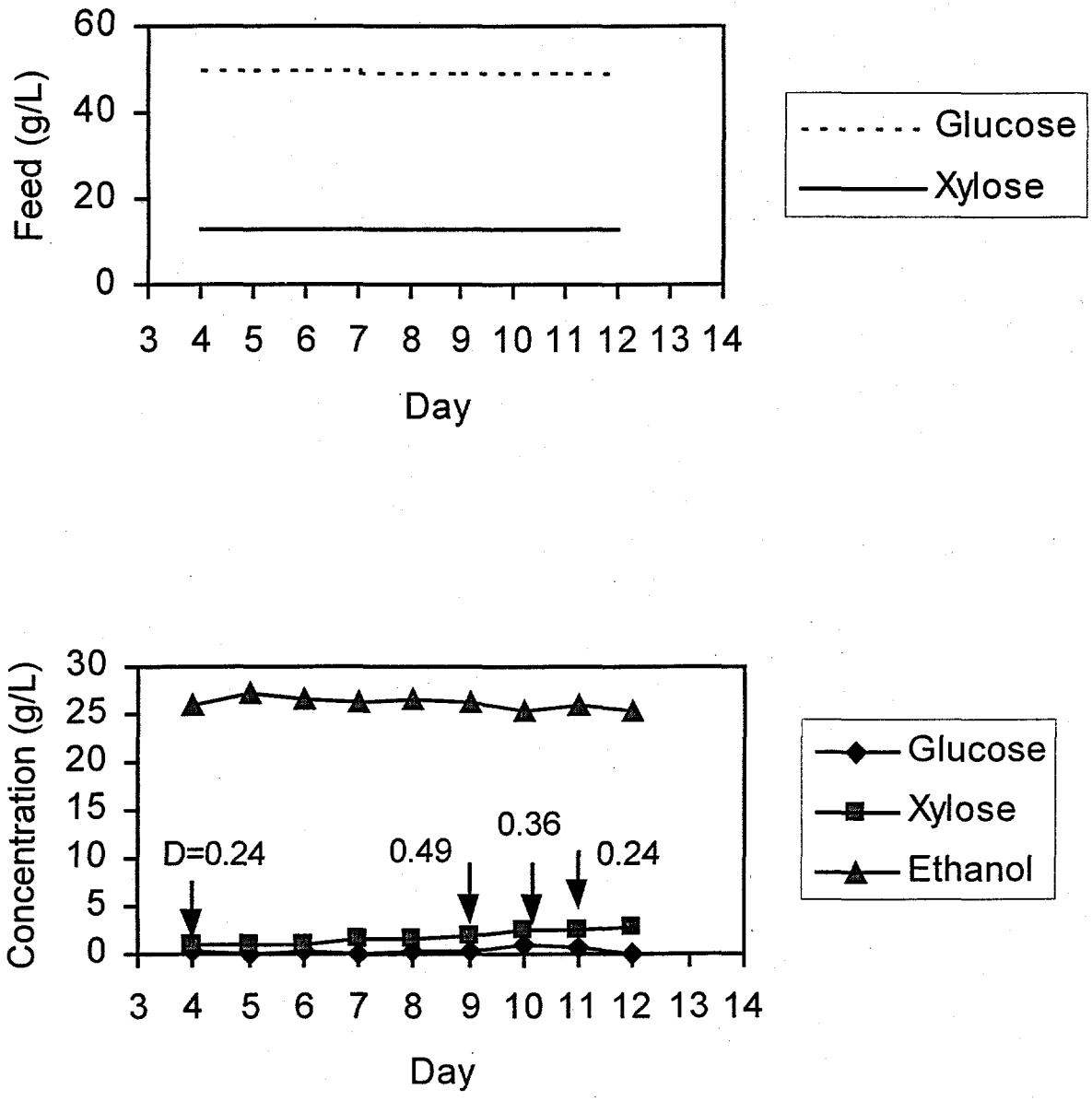

Figure 6. Continuous fermentation results in FBR with immobilized *Z. mobilis* CP4(pZB5) starting with 50 g/L glucose, 13 g/L xylose feed. Dilution rates (1/h) are indicated above the arrows in the figure.

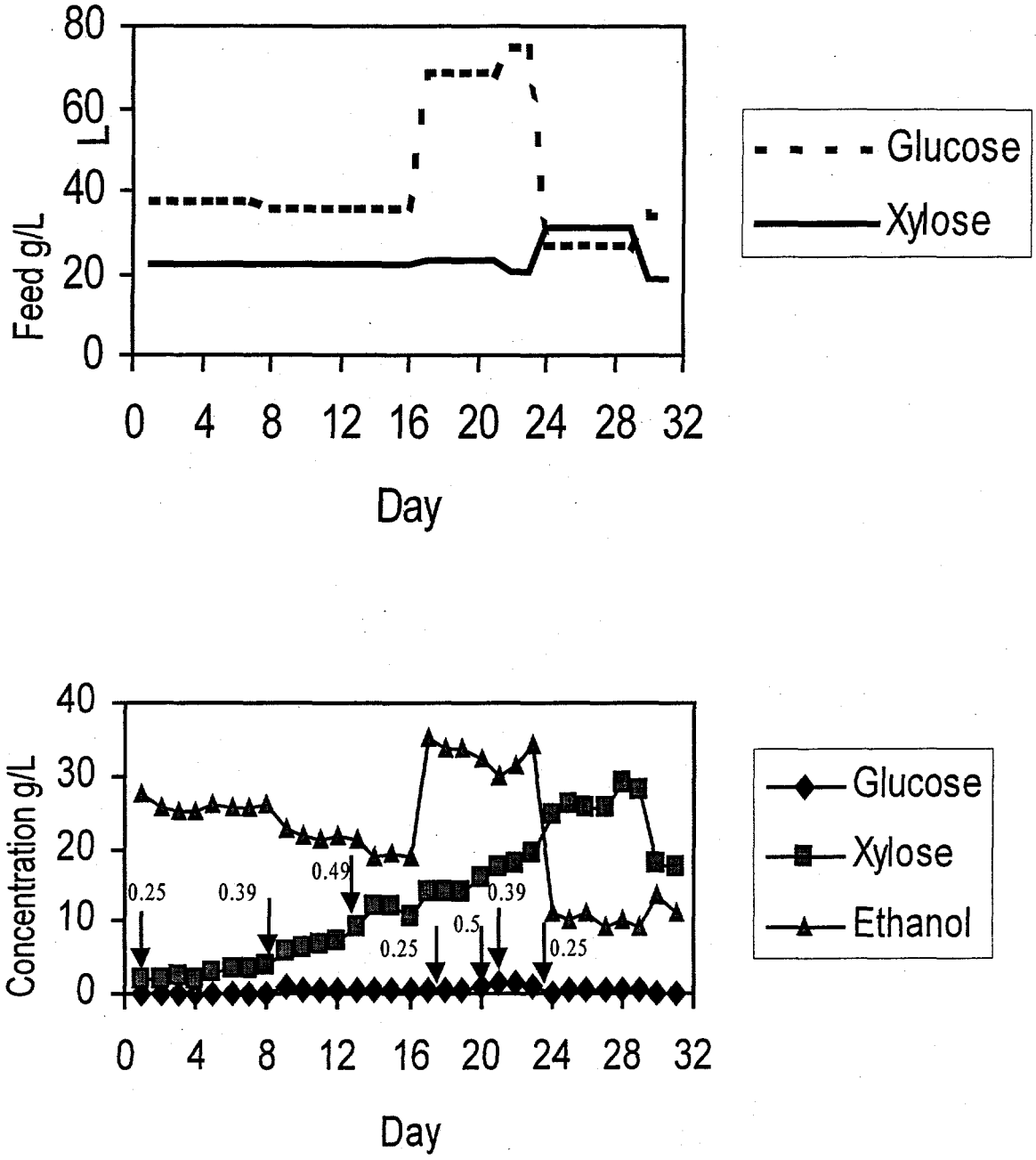

Fig. 7. Continuous fermentation results in FBR with immobilized *Z. mobilis* CP4(pZB5) starting with a 37 g/L glucose, 22 g/L xylose feed. Dilution rates (1/h) are indicated next to the arrows in the figure.