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Application of a Compatible Xylose Isomerase in Simultaneous Bioconversion of Glucose and Xylose to Ethanol

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Abstract Simultaneous isomerisation and fermentation (SIF) of xylose and simultaneous isomerisation and cofermentation (SICF) of glucose-xylose mixture was carried out by the yeast *Saccharomyces cerevisiae* in the presence of a compatible xylose isomerase. The enzyme converted xylose to xylulose and *S. cerevisiae* fermented xylulose, along with glucose, to ethanol at pH 5.0 and 30°C. This compatible xylose isomerase from *Candida boidinii*, having an optimum pH and temperature range of 4.5-5.0 and 30-35°C respectively, was partially purified and immobilized on an inexpensive, inert and easily available support, hen egg shell. An immobilized xylose isomerase loading of 4.5 IU/(g initial xylose) was optimum for SIF of xylose as well as SICF of glucose-xylose mixture to ethanol by *S. cerevisiae*. The SICF of 30 g/L glucose and 70 g xylose/L gave an ethanol concentration of 22.3 g/L with yield of 0.36 g/(g sugar consumed) and xylose conversion efficiency of 42.8%.

Keywords: xylose isomerase, *Saccharomyces cerevisiae*, *Candida boidinii*, xylose, ethanol fermentation, bioconversion

INTRODUCTION

Lignocellulose is made up of three major components: cellulose, hemicellulose and lignin. The bioconversion of glucose, the breakdown product of cellulose, to ethanol is a well developed technology. On the other hand, bioconversion of the pentose sugar, D-xylose, still remains a biochemical challenge especially when it is present with glucose. The pentosan fraction in lignocellulose predominantly consists of xylose, which amounts to as much as 40% and generally goes unutilized because it is more difficult to be assimilated by xylose-fermenting yeasts as compared to glucose [1-3]. A number of approaches have been employed by various research groups for bioconversion of glucose and xylose to achieve high ethanol concentration, yield and productivity. A detailed account of them including the advantages and drawbacks can be found in a recent review by the authors [4].

Xylose can be converted to its ketoisomer xylulose by two different pathways. Yeasts and fungi use a two - step oxidoreduction reaction to convert xylose to xylulose [5]. Most of the bacteria and actinomycetes employ xylose isomerase (EC 5.3.1.5) pathway for catabolism of xylose. Xylulose is converted by xylulokinase to xylulose-5-phosphate which then enters the pentose phosphate pathway to produce ethanol.

In the present approach, *Saccharomyces cerevisiae* along with xylose isomerase from *Candida boidinii* were used for conversion of both glucose and xylose to ethanol. This was because *S. cerevisiae* could ferment glucose giving a high concentration of ethanol at pH 5.0 and temperature 30°C. It can also ferment the keto isomer of xylose, *i.e.*, xylulose to ethanol. The temperature and pH optima of xylose isomerase depend on its source and in almost all cases have been found to range between 50-90°C and 6.5-7.5 respectively [6,7]. However, *Candida boidinii* seems to be an exception in that it is an yeast which produces xylose isomerase, optimally active at pH 4.5-5.0 and temperature 30-35°C [8]. Its enzyme is thus compatible with the conditions required for fermentation of glucose by *S. cerevisiae*.

The present paper reports the results of immobilization of xylose isomerase and its use in simultaneous isomerisation and fermentation (SIF) of xylose and simultaneous isomerisation and cofermentation (SICF) of glucose-xylose mixture by *S. cerevisiae* to ethanol.

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MATERIALS AND METHODS

Microorganisms

Saccharomyces cerevisiae (NRRL-2358), an ethanologenic strain and *Candida boidinii* (DSM-70034), a xylose isomerase producer, were used.

Culture Maintenance

Saccharomyces cerevisiae and *Candida boidinii* were

maintained on agar slants containing malt extract-0.3%, yeast extract-0.3%, glucose-1% and peptone-0.5% at pH 6.6, at 4°C after growing them at 30°C.

Media for Production of Ethanol by *S. cerevisiae*

The basal medium for fermentation of ethanol consisted of: peptone - 0.5%, yeast extract - 0.5%, KH_2PO_4 - 0.5%, $MgSO_4 \cdot 7H_2O$ - 0.2% at pH 5.0. The sugars, glucose and xylose, were filter-sterilized and added separately to the medium in varying proportions as described in Results. The fermentation of sugars was carried out by using the yeast inoculum at 1% level.

The seed inoculum of *S. cerevisiae* was prepared in the above medium consisting of 1% glucose and grown for 18 h at 200 rpm and 30°C. The cell concentration used as inoculum was 2×10^8 cells/mL.

Media for Growth of *Candida boidinii* for Xylose Isomerase Production

Candida boidinii cells were grown according to the procedure of Vongsuvanlert and Tani [8]. The basal media was composed of NH_4Cl - 0.4%, KH_2PO_4 - 0.1%, K_2HPO_4 - 0.1%, $MgSO_4 \cdot 7H_20$ - 0.05%, yeast extract - 0.2%, and peptone - 0.3%, pH - 5.0.

The inoculum was prepared by growing cells in the basal medium containing 0.5% xylose for 24 h at 30°C and 200 rpm.

Partial Purification of Xylose Isomerase

The *Candida boidinii* cells were grown and harvested. They were then subjected to French Press and the cell debris was removed by centrifufation. The supernatant was used as cell-free extract which was first subjectd to protamine sulfate treatment. The precipitate formed as a result of this treatment was removed by centrifugation. The supernatant was then subjected to ammonium sulfate precipitation. The precipitate formed (that contained the enzyme) was then dissolved in minimum volume of buffer. It was dialysed overnight and subsequently treated with $MnCl_2$.

Preparation of Immobilized Xylose Isomerase

The partially purified xylose isomerase was used for immobilization on hen egg shells [9]. The shells were broken into small pieces, and kept in a boiling water bath for 15 min. The shells were then washed several times with acetone and dried in an oven at 60°C. The pieces were then crushed to 100 mesh size. To 1 g of ground shell, 9 mL of partially purified enzyme was added and stirred for 15 min. To this solution, glutaraldehyde was added slowly with shaking to a final concentration of 1.2% (v/v) and the mixture incubated at 5-10°C for 4 h. The bound enzyme was separated by centrifugation and washed thoroughly with 0.02 M potassium phosphate buffer, pH 6.8 and suspended in 10 mL of buffer.

Simultaneous Isomerization and Fermentation (SIF) of Xylose

Fermentation of media containing xylose was carried out in the presence of immobilized xylose isomerase, by *S. cerevisiae*. The concentration of xylose used were 20 and 70 g/L.

Simultaneous Isomerisation and Co-fermentation (SICF) of Glucose and Xylose

Fermentation of a mixture of glucose and xylose was carried out in the presence of immobilized xylose isomerase by *S. cerevisiae*. The combination of glucose and xylose concentrations were chosen such that the total sugar concentration was 100 g/L. Depending upon the type of pretreatment the lignocellulosic residues are subjected to, one would expect various concentrations of glucose and xylose in the hydrolysate. Accordingly, two sets of glucose-xylose concentrations were selected which contain low xylose (glucose - 80 g/L and xylose - 20 g/L) as well as high xylose (glucose - 30 g/L and xylose - 70 g/L) in glucose-xylose mixture.

D-Xylose Isomerase Assay

D-xylose isomerase activity was determined by measuring the formation of xylulose from xylose. The standard reaction mixture was composed of 500 μL of 0.05 M sodium acetate buffer, pH 5.0; 50 μL of 0.01 M $MnCl_2$ and 100 μL of enzyme extract. The mixture was equilibrated for 5 min at 30°C. Fifty microliter of 0.1 M D-xylose solution, was added to the reaction mixture and incubated for 10 min. The reaction was stopped by adding 50 μL of 50% TCA [10]. The xylulose formed was estimated by cysteine carbazole method [11].

The activity of xylose isomerase is expressed in terms of international units (IU) where 1 IU is defined as the amount of enzyme required to produce 1 μmole of xylulose per minute at 30°C, pH 5.0.

Estimation of Fermentation Products by HPLC

Analysis of glucose, fructose, xylose, xylulose, xylitol, arabitol, glycerol, acetic acid and ethanol was done by HPLC, using HPX-87H pre-packed column. The column and the mobile phase lines of the instrument were thoroughly washed with 5 mM sulfuric acid in Milli-Q water. The flow rate of the mobile phase was 0.5 mL/min and the temperature was maintained at 30°C. A refractive index (RI) detector was used for the detection of sugars. The peak areas and retention times (RT) of various products obtained in test samples were compared with those of known standards run under the same conditions to identify and quantify them.

Protein Estimation: The protein concentration was measured by Folin-Lowry method [12].

Reproducibility: All the data reported are the mean of at least three experiments. The mean values differed from the individual values by not more than 2%.

RESULTS AND DISCUSSION

Immobilization of Xylose Isomerase and Characterization of the Immobilized Preparation with Respect to pH and Temperature

The cell free extract having specific activity of 0.027 IU/(mg protein) was partially purified as mentioned in Materials and Methods. The $MnCl_2$ - treated enzyme with specific activity of 0.1 IU/(mg protein) was thus 3.7 fold purified (Table 1).

The partially purified enzyme was immobilized due to its inherent advantage of being able to use the immobilized enzyme without much loss in its activity. The covalent linkage method was preferred over other methods such as adsorption and ionic interactions because the chances of detachment of enzyme from the support are minimized if the enzyme is to be used under rather harsh environment conditions like that of lignocellulose hydrolysate. The partially purified xylose isomerase was, therefore, immobilized on to hen egg shell, an inexpensive and inert support. This support has been used by other researchers for the immobilization of other enzymes like catalase and invertase [9,13].

The enzyme retained 47.5% of its original activity in its immobilized form. The specific activity of immobilized xylose isomerase was 0.047 IU/(mg protein) as compared to specific activity of 0.1 IU/(mg protein) of the non-immobilized enzyme. Apart from a good retention of activity, this support can be easily separated from the fermentation media. Literature reports reveal that a majority of enzymes lose more than 50% of their activity on immobilization [14].

The activity of xylose isomerase was assayed at various pH, *i.e.* 3.0-8.0. Sodium citrate buffer (0.05 M) was used to assay the enzyme activity in the pH range of 3.0-5.5 and 0.05 M potassium phosphate buffer, in the pH range of 6.0-8.0. The immobilized enzyme showed a marginal shift in optimum pH towards the acidic side. The optimum activity of the non-immobilized enzyme was at pH 4.5-5.0 whereas the optimum activity of the immobilized enzyme was at pH 4.0-5.0 (Fig. 1(a)). Similarly, the activity of xylose isomerase in the non-immobilized form was optimum at 30-35°C whereas that of the immobilized enzyme at 25-30°C (Fig. 1(b)). The pH and temperature optima of immobilized en-

Table 1. Partial purification of xylose isomerase produced by *Candida boidinii* DSM 70034

Steps	Vol. (mL)	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Purification fold	Yield (%)
Cell free extract	1000	710	25500	0.027	1.0	100
Protamine sulfate	1000	620	8260	0.075	2.70	87
Ammonium sulfate precipitation (30-80%)	500	275	2815	0.097	3.59	38.7
Dialysis	500	269	2810	0.095	3.51	37.8
$MnCl_2$ treatment	480	240	2400	0.100	3.70	33.8

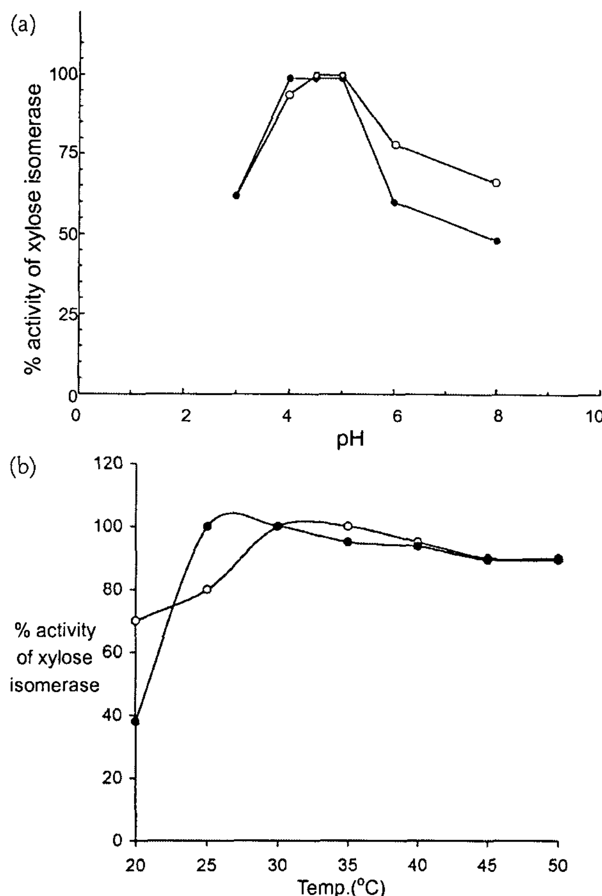

Fig. 1. Effect of pH on xylose isomerase activity (a) temperature was kept constant at 30°C 0.05 M sodium citrate buffer was used for reaction at pH 3.0, 4.0, 4.5 and 5.0. 0.05 M potassium phosphate buffer was used for reaction at pH 6.0 and 8.0. (b) pH was kept constant at 5. O: non-immobilized enzyme, ●: immobilized enzyme.

zymes tend to undergo a change because of a change in the stereospecific conformation of the protein as a result of covalent linkage. Xylose (glucose) isomerase from various sources such as *Lactobacillus brevis* and *Bacillus coagulans* has been immobilized on a variety of supports such as microcrystalline cellulose and DEAE Sephadex where a minor shift in pH and temperature optima has been reported [15,16]. The changes in the pH and temperature optima of the immobilized xylose isomerase in the present study were also not drastic and were within the range required for optimal fermentation of glucose and xylose by *S. cerevisiae.*

Effect of Xylose Isomerase on Fermentation of Glucose by *S. cerevisiae*

Due to its activity on glucose, the enzyme xylose isomerase has been more commonly termed as glucose isomerase also. Xylose isomerase from different sources show different affinity patterns towards xylose and

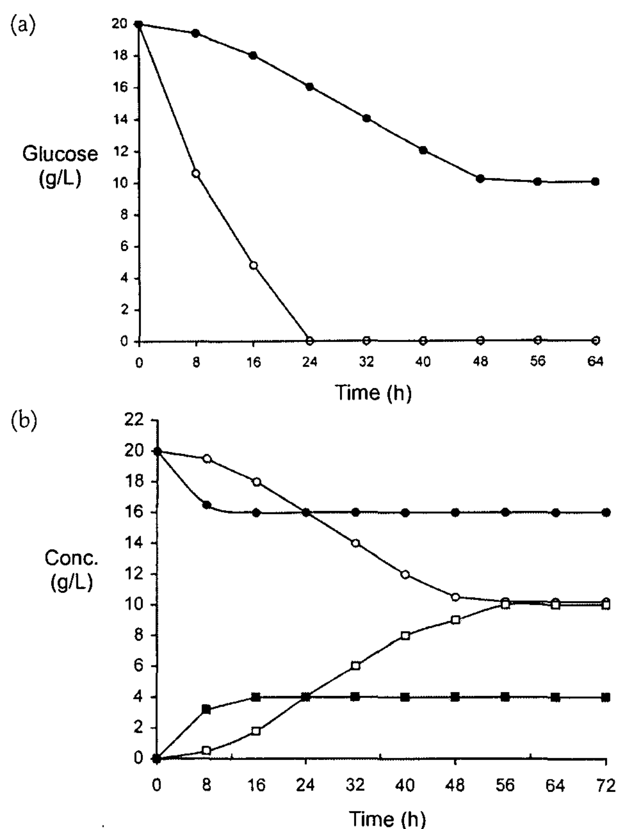

Fig. 2. (a) Glucose isomerization by xylose isomerase and fermentation by *S. cerevisiae*, O: fermentation, ●: isomerization; (b) effect of xylose isomerase on glucose and xylose: 20 g/L each O: glucose, ●: xylose, □: fructose, ■: xylulose.

glucose. Some may have a lower K_m for xylose and a higher one for glucose and vice-versa [17-19]. The affinity of xylose isomerase from *C. boidinii* to glucose and xylose and the maximum velocity of both the reactions, *i.e.* glucose ↔ fructose and xylose ↔ xylulose was determined from Lineweaver Burk plot. The immobilized xylose isomerase had a higher affinity for xylose as its K_{mapp} for xylose was 0.02 M as compared to 0.04 M for glucose. The V_{mapp} of the enzyme for xylose and glucose was found to be 0.119 μmol/min/mg and 0.625 μmol/min/mg respectively (data not shown).

Further, it was observed that whereas the fermentation of glucose to ethanol by *S. cerevisiae* was complete in 24 h, the rate at which glucose to fructose isomerisation by xylose isomerase took place was much slower. In 24 h, only 20% of glucose was isomerised to fructose and after 56 h, the equilibrium ratio (49:51) of glucose to fructose was obtained (Fig. 2(a)). As opposed to the isomerisation of glucose to fructose, the isomerisation of xylose to xylulose was much faster giving the equilibrium ratio (80:20) of xylose to xylulose in 16 h (Fig. 2(b)). It was also observed that the yeast fermented fructose at the same rate as glucose (results not shown). The yield based on glucose consumption would not be as high [0.48 g/(g glucose consumed)] if fructose (the isomerised product of glucose) was also not fermented to ethanol. Further, fructose was not detected during the course of glucose fermentation, implying thereby that it was utilized by the yeast as soon as it was formed. Thus the isomerisation of glucose to fructose by xylose isomerase did not affect the fermentation of glucose to ethanol by the yeast.

Table 2. Effect of immobilized xylose isomerase dosage on xylose fermentation by *S. cerevisiae*

XI activity (IU/g initial xylose)	Xylose at the end of fermentation (g/L)	Ethanol conc. (g/L)
2.63	39	0.75
3.10	36.5	2.20
3.56	33	4.70
4.03	30	5.60
4.50	27	7.50
5.12	28	7.50

Initial xylose concentration of 50 g/L was used in all the experiments.

Determination of Optimum Loading and Stability of Immobilized Xylose Isomerase in SIF and SICF by *S. cerevisiae*

The fermentation of 50 g xylose/L was carried out in the presence of varying dosages of immobilized xylose isomerase. It was found that at an optimum xylose isomerase dosage of 4.5 IU/(g initial xylose), a maximum ethanol concentration of 7.5 g/L was obtained (Table 2). Since xylose isomerase showed affinity for glucose also, although a low one, this affinity could affect the optimum xylose isomerase loading in a glucose - xylose mixture. However, the fermentation of glucose - xylose mixture (50 g/L each) in the presence of different xylose isomerase loadings showed that there was no change in the optimum loading of xylose isomerase which was the same [4.5 IU/(g initial xylose)] as for xylose fermentation (data not shown).

Stability of Immobilized Xylose Isomerase

In order to determine the stability of the immobilized preparation of xylose isomerase, the fermentation of xylose (20 g/L) was carried out using an optimum xylose isomerase loading of 4.5 IU/(g initial xylose). After the first fermentation run of 72 h, the immobilized preparation was separated from the fermentation media and washed. This washed preparation was assayed for xylose isomerase activity to detect loss in enzymatic activity, if any. It was found that the immobilized enzyme retained its original activity up to 3 consecutive runs of 72 h each, after which it showed 14.3% loss in activity (Table 3). Thus, the immobilized preparation could be reused up to 3 times without losing any significant activity.

Table 3. Effect of repeated use of immobilized xylose isomerase on xylose fermentation by *S. cerevisiae*

Run	Xylose consumed (g/L)	Ethanol concentration (g/L)	Activity left IU/g support	Activity loss of enzyme after the run (%)
1	10.5	3.5	0.77	0
2	10.4	3.5	0.77	0
3	10.2	3.45	0.75	2.6
4	8.8	2.8	0.66	14.3

Initial activity of immobilized preparation : 0.77 IU/g support
Initial concentration of xylose : 20 g/L
Reaction conditions : Temp. = 30°C, pH = 5.0
Duration of each fermentation run : 72 h.

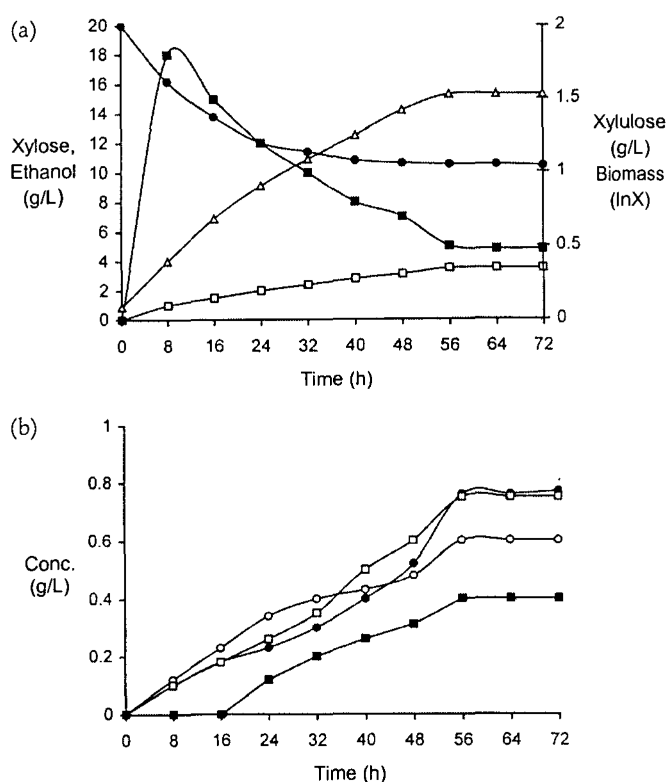

Fig. 3. Time course of fermentation of xylose (20 g/L) using xylose isomerase and *S. cerevisiae*. Xylose isomerase loading: 4.5 IU/(g initial xylose); (a) profile of main products ●: xylose, □: ethanol, ■: xylulose, △: biomass; (b) profile of by-products O: xylitol, ●: arabitol, □: glycerol, ■: Acetic acid.

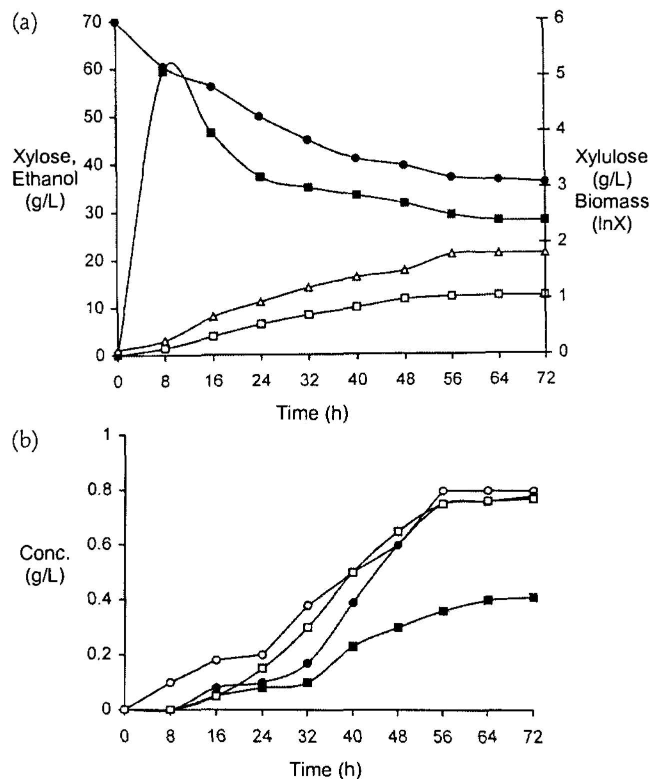

Fig. 4. Time course of fermentation of xylose (70 g/L) using xylose isomerase and *S. cerevisiae*. Xylose isomerase loading : 4.5 IU/(g initial xylose); (a) profile of main products ●: xylose, □: ethanol, ■: xylulose, △: biomass; (b) profile of by-products O: xylitoal, ●: arabitol, □: glycerol, ■: Acetic acid.

Table 4. Fermentation parameters of conversion of xylose and glucose-xylose mixture to ethanol by *S.cerevisiae*

Initial concentration of glucose (g/L)	Initial concentration of xylose (g/L)	Sugar utilization efficiency (%)	Ethanol conc. (g/L)	Yield g/(g sugar consumed)	Overall productivity (g/L/h)
--	20	45	3.5	0.39	0.06
--	70	43.5	12	0.40	0.22
80	20	89.0 (45)	42.4	0.47	0.76
30	70	62.7 (42.8)	22.3	0.36	0.40

Xylose isomerase loading : 4.5 IU/g initial xylose
Duration of fermentation : 72 h
The yield and productivity were calculated at 56 h.
The figures in parentheses indicate % xylose utilization efficiency considering 100% glucose utilization.

Simultaneous Isomerization and Fermentation (SIF) of Xylose

The time course of xylose fermentation carried out at of 20 and 70 g/L concentrations is shown in Fig. 3 and 4. With 20 g/L xylose, ethanol concentration of 3.5 g/L, yield of 0.39 g/(g xylose consumed) and xylose utilization efficiency of 45% was obtained. The overall productivity was 0.06 g/L/h (Table 4). In the case of fermentation of 70 g xylose/L, the ethanol concentration increased to 12.5 g/L and overall productivity to 0.22 g/L/h. The yield and xylose utilization efficiency did not vary much as compared to the fermentation of 20 g xylose/L (Table 4). It was observed that xylose isomerisation occurred till 48 h afterwhich it became constant. The utilization of xylulose took place as a result of its formation through xylose isomerisation. The fermenta-

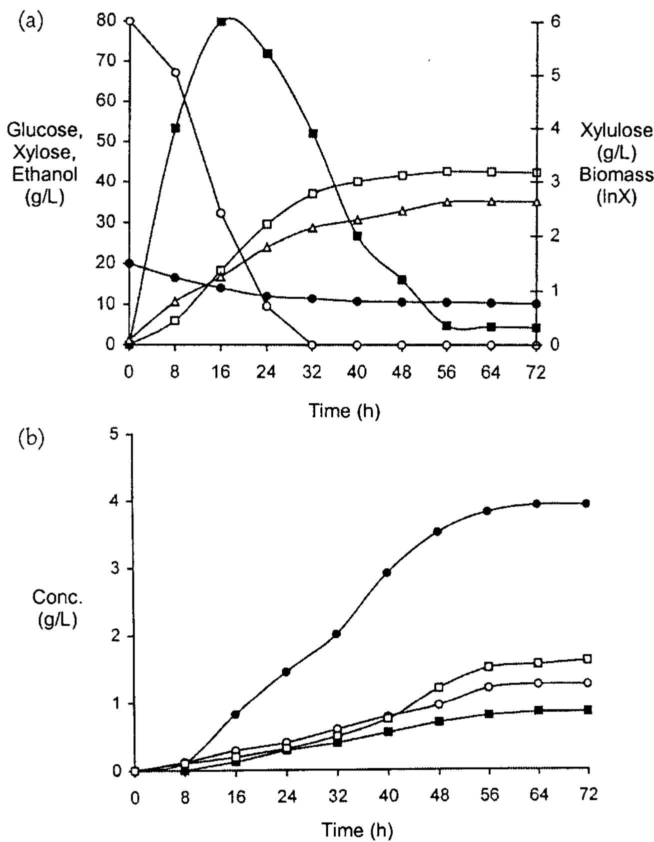

Fig. 5. Time course of fermentation of a mixture of glucose (80 g/L) and xylose (20 g/L) using xylose isomerase and *S. cerevisiae*. Xylose isomerase loading : 4.5 IU/(g initial xylose); (a) profile of main products O: glucose, ●: xylose, □: ethanol, ■: xylulose, △: biomass; (b) profile of by-products O: xylitol, ●: arabitol, □: glycerol, ■: acetic acid.

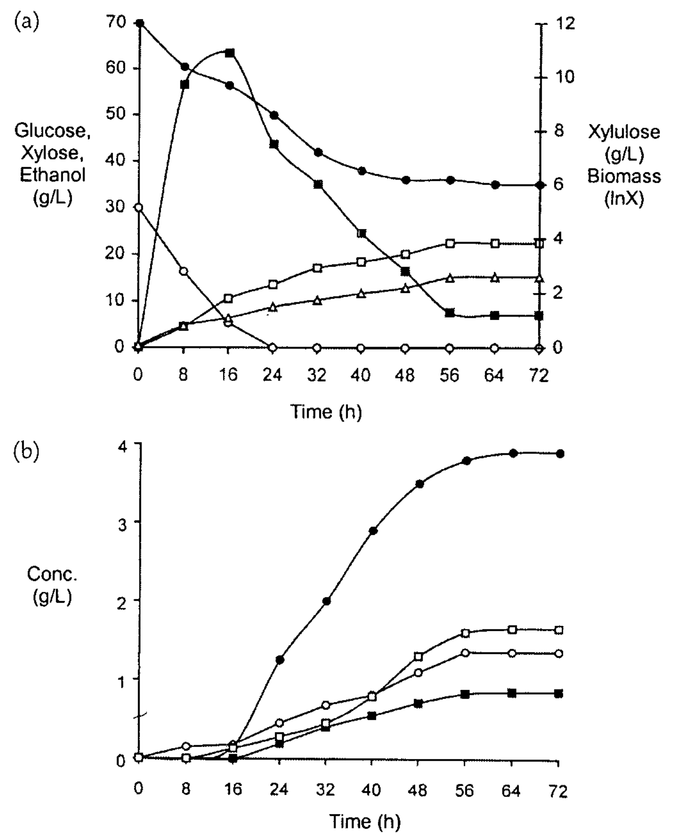

Fig. 6. Time course of fermentation of a mixture of glucose (30 g/L) and xylose (70 g/L) using xylose isomerase and *S. cerevisiae*. Xylose isomerase loading : 4.5 IU/(g initial xylose); (a) profile of main products O: glucose, ●: xylose, □: ethanol, ■: xylulose, △: biomass; (b) profile of by-products O: xylitol, ●: arabitol, □: glycerol, ■: acetic acid.

tion of xylulose occurred steadily till 56 h in both the cases, after which there was no further consumption of xylulose. This plateau formation at 56 h coincided well with that of formation of main product ethanol, the biomass and by-products (xylitol, arabitol, glycerol and acetic acid), all of which were also built up till 56 h (Figs. 3 and 4). The concentration of each byproduct was less than 0.8 g/Land 4.0 g/Lin fermentations with 20 and 70 g xylose/L respectively.

Tsao *et al.* [21] have reported the fermentation of xylose using commercial xylose isomerase (Sweetzyme Q, Novo Industries, Denmark) and *Schizosaccharomyces pombe* at pH 6.0 and 40°C. From 120 g/L xylose, an ethanol concentration of 17.5 g/L was obtained. The sugar utilization efficiency was 50% and the yield 0.29 [21]. The low yield obtained by them was probably due to high diversion of xylulose towards by-product formation.

On the other hand, when fermentation of glucose was carried out at different concentrations, *viz.*, 20, 50, 80 and 100 g/L, it was observed that the glucose utilization efficiency was 100% in all the cases. The ethanol yield was 0.48 g/(g glucose consumed) except for 100 g glucose/L where it was 0.46. The overall productivity was as high as 1.1 g/L/h. Thus, the fermentation parameters of SIF of xylose were quite low as compared to those obtained from fermentation of glucose.

Simultaneous Isomerisation and Cofermentation (SICF) of Glucose and Xylose

Glucose-xylose mixture [80 g glucose/L and 20 g xylose/L, and 30 g glucose/L and 70 g xylose/L] was fermented by *S.cerevisiae* in the presence of optimum loading of immobilized xylose isomerase (Figs. 5 and 6). It was observed that the fermentation of glucose was complete in all the cases within 24 h and the utilization of glucose was 100%. As was the case with fermentation of xylose alone (Figs. 3 and 4), the isomerisation of xylose to xylulose and the fermentation of xylulose to ethanol took place simultaneously. This was inferrred from the data which revealed that the amount of xylulose was always less than the difference between the initial amount of xylose and the amount of xylose present at any given point of time. Further, although glucose and xylulose were co-fermented, the fermentation

of xylulose seemed to be partially repressed in the presence of glucose. It was found for example, that there was an accumulation of 11 g xylulose/L at 16th hour of SICF (Fig. 6) whereas the concentration of the same was only 3.7 g/L in SIF (Fig. 4). It may be mentioned here that there was probably no competetion for the transport of the two sugars, as glucose has been reported to enter the cell by facilitated diffusion and xylulose by a fast, non-active mechanism [22]. Further, since the yeast did not show a diauxic pattern of glucose and xylulose utilization, glucose did not cause complete inhibition of xylulose utilization by the yeast. However, there are reports contrary to the above observation which indicate that in a mixture of glucose and xylulose, glucose was preferentially utilized; only after glucose reached sufficiently low levels, xylulose utilization occurred [23].

The fermentation of a mixture of 80 g glucose/L and 20 g xylose/L gave a sugar utilization efficiency of 89%; the ethanol concentration was 42.4 g/L and the yield was 0.47 g/(g sugar consumed). The overall productivity in this case was found to be 0.76 g/L/h. The sugar utilization efficiency decreased to 62.7% in the case of a mixture of 30 g glucose/L and 70 g xylose/L. There was also a decrease in the ethanol concentration and yield to 22.3 g/L and 0.36 g/(g xylose consumed) respectively (Table 4). Thus, an increase in concentration of xylose in the glucose - xylose mixture resulted in decreased concentration, yield and productivity of ethanol. In both the cases it was observed that xylulose utilization reached a plateau at 56 h. The build up of by-products also reached a plateau at 56 h. The concentration of each byproduct was less than 0.8 g/L in the case of 80 g glucose/L and 20 g xylose/L mixture, whereas it was up to 4.0 g/L in the case of 30 g glucose/L and 70 g xylose/L mixture. The cessation of fermentative activity after 56 h in both SIF and SICF processes seems to be, at least partially, due to the inhibition of xylose isomerase activity by the byproduct glycerol (see below). It is not known whether the depletion of any key nutrient from the medium was also responsible for this phenomenon.

In order to determine whether the isomerisation of xylose to xylulose was affected by the components of fermentation media, the activity of the immobilized xylose isomerase preparation was assayed in the presence of each media component separately and in combination. None of the media components inhibited the activity of xylose isomerase (Table 5). But among the by-products, *viz.*, xylitol, arabitol, glycerol and acetic acid, glycerol exerted inhibition on xylose isomerase. The nature of inhibition was non-competitive and the inhibition constant, K_i, for glycerol was found to be 50 mM (data not shown). The nature of inhibition was also reversible because when the same enzyme preparation was washed and assayed for its enzymatic activity, it regained its original activity. Thus, glycerol was partially responsible for low utilization efficiency of xylose observed in both SIF and SICF processes. Further, literature reports show that the fermentation of xylulose to ethanol by *S. cerevisiae* takes place optimally at 35°C [24]. Also, sodium tetraborate has been reported to shift the equilibrium constant of the xylose:xylulose reaction to favour the formation of xylulose such that the ratio of xylose:xylulose is 20:80 as opposed to 80:20 in the absence of borate [25]. The impact of these parameters on improvement of ethanol concentration and yield in SIF and SICF processes shall form the subject matter of a separate communication.

Table 5. Effect of media components and products on the activity of xylose isomerase

No.	Components	Xylose isomerase activity (IU/g support)	Remaining activity (%)
1.	Yeast extract (5 g/L)	0.77	99.2
2.	Peptone (5 g/L)	0.75	96.6
3.	KH_2PO_4 (5 g/L)	0.76	97.9
4.	All combined (1+2+3)	0.75	96.6
5.	Xylitol (2.5 g/L)	0.75	96.6
6.	Arabitol (5 g/L)	0.75	96.6
7.	Glycerol (2 g/L)	0.33	42.5
8.	Acetic acid (1.5 g/L)	0.74	95.3
9.	Ethanol (5 g/L)	0.77	99.2
10.	All combined (5+6+7+8+9)	0.36	46.3

Original activity of xylose isomerase - 0.776 IU/(g support).

Thus, the approach used in this study showed the possibility of bioconversion of both glucose and xylose by using a compatible xylose isomerase from *Candida boidinii* by the ethanologenic yeast, *Saccharomyces cerevisiae*. However, the fermentation efficiency of xylose remains to be optimized by incorporating process improvements such as those mentioned above for obtaining high ethanol concentration in SICF process.

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