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Effect of k_La and Fed-batch Strategies for Enhanced Production of Xylitol by *Debaryomyces nepalensis* NCYC 3413

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Authors' contributions

Author KH performed experiments, data acquisition, analysis and drafted the manuscript. Author SNG conceived, designed, coordinated the study, performed the data analysis and drafted the manuscript. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: To evaluate the effect of volumetric oxygen transfer coefficient on the production of xylitol by *Debaryomyces nepalensis* and to enhance the yield and productivity of xylitol by fed-batch fermentation using xylose as substrate.
Place and Duration of Study: All experiments were performed at the Applied and Industrial Microbiology Laboratory, Indian Institute of Technology Madras, from March 2013 to May 2014.
Methodology: Batch cultivations were carried out in a 7.5 L fermentor under various oxygen transfer coefficients in the range 12 to 39.6 h^{-1} in order to understand the effect of oxygen on xylitol production. Fed-batch studies were performed in 2.5 L bioreactor with a working volume of 1 L. The cultures were initially grown as batch cultures. Feed containing xylose and nitrogen source was added to the medium intermittently.
Samples were periodically collected at regular intervals of time and the concentrations of xylose, xylitol and glycerol were determined by HPLC.
Results: Maximal xylitol yield (0.64 g/g) and productivity (0.43 g/L·h) were obtained at k_La 13.68

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h^{-1}. The effect of pH was also studied at this k_La. A pH value of 6.0 was found to be favorable for xylitol accumulation. Fed-batch fermentation involving feeding of xylose and nitrogen source was used for xylitol production by *D. nepalensis*. Within the fed-batch phase, the yield of xylitol was 0.83 g/g and the productivity was increased to 0.83 g/L.h with a final product concentration of 90 g/L.
Conclusion: Higher k_La favors biomass production whereas product formation was favored at lower k_La. Fed-batch process resulted in enhancement of final product concentration by 73%.

Keywords: Debaryomyces nepalensis; xylitol; volumetric oxygen transfer coefficient; correlation; Fed-batch fermentation.

1. INTRODUCTION

Lignocelluloses are abundant and inexpensive sources of energy, projected as potential feed stocks for the production of bioethanol. But the conversion of lignocellulose to ethanol is a complex and expensive process, which requires several pretreatment and purification processes. One interesting approach to counteract the high cost is to integrate ethanol production with the production of commercially valuable chemicals [1]. The production of value added co-products along with ethanol not only improves the process economics but also permits the complete utilization of available resource. The U.S. Department of energy screened 12 potential compounds that can be derived from biomass which includes several organic acids and polyols such as xylitol and arabitol [2].

Xylitol is a naturally occurring polyol with sweetness similar to sucrose. It is recommended as an alternative sweetener for diabetics, since its metabolism does not require insulin [3]. Among the most notable features of xylitol are noncariogenicity, low caloric value, capability of reducing the incidence of otitis media in children and enhancing bone mineralization [4]. Xylitol is also used as a building block for the synthesis of biodegradable polymers [5] and other commercial derivatives such as xylaric acid and glycols [2]. Xylitol is an appropriate product that can be derived from biomass since xylose is one of the major sugars obtained from hydrolysis of lignocellulose. Moreover, due to its unique properties, xylitol received worldwide interest and the market share is expected to increase rapidly [6].

Conventionally, xylitol is manufactured by chemical hydrogenation of xylose. But, the requirement of pure xylose and tedious purification steps are the major drawbacks associated with the chemical process. On the other hand, microbial production offers some advantages like mild operating conditions, non-requirement of pure xylose and high yield when compared to chemical process. Hence significant research has been carried out in exploiting microbes for xylitol production and yeasts were reported as major producers. Although, *Candida* sp. are shown to be the best xylitol producers among yeasts, the fact that *Candida* sp. are notorious opportunistic pathogens, restricts their use in bioproduction of xylitol which has major application in food industry [6-8]. *Debaryomyces nepalensis* has been previously reported to efficiently utilize hemicellulose-derived sugars and produce industrially important metabolites like xylitol, arabitol and ethanol in appreciable amounts. The organism is halotolerant and osmotolerant, showing tolerance to high salt and sugar concentrations [9]. Salt tolerance is a desirable feature for fermentations using lignocellulose as substrate, since pretreatment of lignocellulose releases large amounts of inorganic salts that are inhibitory to microbes [10]. Moreover, tolerance to osmotic stress can also provide cross tolerance to other inhibitors of lignocellulose hydrolysates [11]. Based on these observations, *D. nepalensis* can be considered an ideal strain for xylitol production.

Xylitol is an intermediate of xylose metabolic pathway. In *D. nepalensis*, the reduction of xylose to xylitol is mediated by an NADPH-dependant xylose reductase. Xylitol is then oxidized to xylulose by an NAD-dependant xylitol dehydrogenase. This disparity in cofactor preferences results in cofactor imbalance and accumulation of xylitol [12]. Regeneration of NAD occurs through electron transport system with oxygen as final electron acceptor. Thus, oxygen plays a key role in creating redox imbalance and hence an important parameter affecting xylitol production.

In an earlier study, we reported the optimization of production medium for xylitol using statistical approaches [13]. Since oxygen is considered a critical parameter in the biological production of

xylitol, the influence of oxygen availability on xylitol production by *D. nepalensis* was studied in a bioreactor in terms of k_La. A fed-batch fermentation strategy was used that combines high yield with high productivity and final product concentration.

2. MATERIALS AND METHODS

2.1 Microorganism and Inoculum Preparation

Debaryomyces nepalensis NCYC 3413 was maintained on a solid YEPP medium containing yeast extract 10 g/l, peptone 20 g/l and pectin 5 g/l at pH 7.0 and incubated at 30°C for 26 h and later stored at 4°C. A single colony was transferred from an overnight-grown culture plate into the YEPD medium (50 ml) containing yeast extract 10 g/l, peptone 20 g/l and dextrose 20 g/l and incubated for 12 h at 30°C and 180 rpm.

2.2 Media Composition and Culture Conditions

Semi-synthetic medium containing xylose, 100 g/l; $(NH_4)_2SO_4$, 3 g/l; $MgSO_4$, 0.1 g/l; K_2HPO_4, 10 g/l; Na_2HPO_4, 3 g/l; yeast extract, 1 g/l; $CaCl_2{\cdot}2H_2O$, 147 mg/l; $FeCl_3$, 10 mg/l; $MnSO_4{\cdot}H_2O$, 3.38 mg/l; $ZnSO_4{\cdot}7H_2O$, 8 mg/l; $CuSO_4{\cdot}5H_2O$, 0.5 mg/l; citric acid, 6.9 mg/l. The initial pH of the medium was adjusted to 6.0 using H_3PO_4 and NaOH.

2.3 Bioreactor Studies

Batch cultivations were carried in a 7.5 L stirred tank bioreactor (New Brunswick Scientific, Bioflo 110, Eppendorf Inc., USA) containing 3 L of production medium. The bioreactor was equipped with temperature, agitation, aeration, pH controllers, and two Rushton turbines with six flat-blades. The temperature and pH of the fermentation were maintained at 30ºC and pH 6.0, respectively. The production medium was inoculated with 8% (v/v) of seed culture. Dissolved oxygen concentration of cultures was measured using a polarographic electrode and expressed as percentage of O_2 saturation. The oxygen transfer rate was studied as the volumetric oxygen mass transfer coefficient (k_La). The aeration and agitation rates were varied to obtain different k_La values. Samples were periodically collected at regular intervals of time in order to determine xylose, xylitol, glycerol and biomass concentration.

2.4 Fed-Batch Studies on Xylitol Production

Fed-batch studies were performed in 2.5 L bioreactor with a working volume of 1 L. The cultures were initially grown as batch cultures. The aeration rate and agitation speed were fixed at 0.5 vvm and 350 rpm, respectively. Feed was added to the medium intermittently, when the xylose concentration in the medium reached 10 g/L. Xylose was added to the fermenter to achieve a concentration of 50 g/L. A mixture of $(NH_4)_2SO_4$ and yeast extract was also fed to the fermenter along with xylose to achieve an initial concentration of 3 and 1 g/L, respectively. The temperature, pH, aeration, and agitation were controlled at the same values as batch cultures. Samples were collected at regular intervals for measurement of cell growth, residual xylose and produced xylitol.

2.5 Determination of k_La

The static gassing out method was used to determine k_La values. Dissolved oxygen concentration was measured using electrode. The DO level was lowered to 0 % saturation by passing N_2 through the system. This was followed by passing air at various rates and the increase in DO concentration with time was monitored. The k_La values were calculated using the following equation

$$dC_L/dt = k_La\ (C^* - C_L) \quad (1)$$

$$ln(1 - C_L/C^*) = -k_La\ *t \quad (2)$$

The slope of $ln(C^* - C_L/C^*)$ *vs* t gives k_La

2.6 Estimation of k_La using Correlations

The k_La values obtained at different aeration and agitation rates were used to obtain a correlation between k_La, agitation speed and air flowrate. Prediction of mass transfer coefficient is usually based on correlation between k_La and power input per unit volume as given in the literature

$$k_La = a\ (P/V_L)^x\ (V_S)^y \quad (3)$$

Where P/V_L is the power requirement per unit volume and V_s is the superficial gas velocity.

Since the accurate measurement of power input is difficult, Power number, i.e. N^3D^2 was used to obtain an exponential correlation with k_La. A

correlation was developed between k_La, agitation speed and air flow rate. The k_La values for different agitation speeds and each given air flow rate were fitted by a straight line forcing the interception to zero. The slopes (k_La/N) obtained in the previous step were fitted using the air flow logarithm [14].

2.7 Modeling of Growth and Substrate Utilization in Bioreactor

The relationship between biomass concentration, substrate concentration and time during batch fermentations in bioreactor was given by a logistic equation which can be described as follows:

$$\frac{dX}{dt} = kX\left(1 - \frac{X}{X_m}\right) \quad (4)$$

$$\frac{dS}{dt} = -\frac{1}{Y_{X/S}}\left[kX\left(1 - \frac{X}{X_m}\right)\right] \quad (5)$$

Where $X_m$is the maximum attainable biomass and k is the carrying capacity

2.8 Analytical Methods

Growth was evaluated by measuring the optical density of culture at 600 nm (A_{600}). As standardized previously for *D. nepalensis*, absorbance 1.0 at 600 nm corresponds to 0.335 g cell dry weight per litre culture. Samples collected at regular intervals were analyzed for metabolite production and substrate utilization. The cells were separated from culture medium and the supernatant was stored at 4°C for further analysis. The concentration of xylose, xylitol and glycerol were estimated by HPLC (Jasco) equipped with refractive index detector and Aminex HPX-87H column (Bio-Rad, Richmond, USA) at 45°C with 0.01N H_2SO_4 solution as the mobile phase at a flow rate of 0.6 ml/min.

3. RESULTS

3.1 Effect of Aeration Rate on Xylitol Production in Bioreactors

Oxygen is a crucial factor in xylitol fermentation and microaerobic conditions was shown to favor xylitol accumulation. The effect of oxygen on the metabolism of *D. nepalensis* was studied. Different levels of oxygen availability were maintained in the reactor by varying the aeration and agitation rates. Initially, the agitation rate was fixed at 400 rpm and xylitol production was studied at four different aeration rates (0.33, 0.4, 0.5 and 0.75 vvm). The rate of xylose consumption was almost similar in all the cases, but slightly higher at 0.4 vvm, implying that aeration had negligible effect on substrate consumption (Fig. 1a). As could be seen in Fig. 1b, a maximum of 37.6 g/L of xylitol was obtained at 0.5 vvm and at 0.4 vvm. Although the volumetric productivities are similar at these two aeration rates, i.e, 0.35 g/L.h, the yield at 0.5 vvm is 0.5 g/g, which is slightly higher than that obtained at 0.4 vvm. Upon increasing the aeration rate to 0.75 vvm, there is an increase in biomass production with a concomitant decrease in xylitol yield (Figs. 1b and 1c). At 0.33 vvm, the yield and productivity of xylitol were low and there was an increased formation of glycerol (Fig. 1d).

3.2 Effect of Agitation Rate on Xylitol Production in Bioreactors

In the next step, the aeration rate was fixed at 0.5 vvm and the agitation rates were varied (300, 350, 400 and 500 rpm). The rate of xylose utilization was slightly low at 300 rpm and complete utilization happened only at the end of 196 h (Fig. 2a). The maximum xylitol concentration achieved was 54 g/L at 300 and 350 rpm (Fig. 2b). But the rate of product formation was high in case of 350 rpm, with productivity and yield reaching 0.43 g/L.h and 0.64 g/g, respectively. The corresponding k_La value was 13.68 h^{-1}. Under these conditions, more than 60% of xylose consumed was used for xylitol formation. The amount of glycerol formed was also very low at 300 and 350 rpm, indicating efficient utilization of xylose for xylitol production at these agitation rates (Fig. 2d). When the agitation rate was increased to 500 rpm, an increase in biomass formation was evident (Fig. 2c) and further increase in agitation rate to 700 rpm resulted in the formation of 45 g/L of biomass with low levels of xylitol (9.5 g/L) (data not shown).

3.3 Correlation for Prediction of k_La Values

Volumetric oxygen transfer coefficient is affected by both aeration and agitation. Figs. 3a & 3b depict the relationship between k_La, aeration rate and agitation speed. The logarithmic relation obtained from Fig. 3b can be used to estimate kLa at different aeration

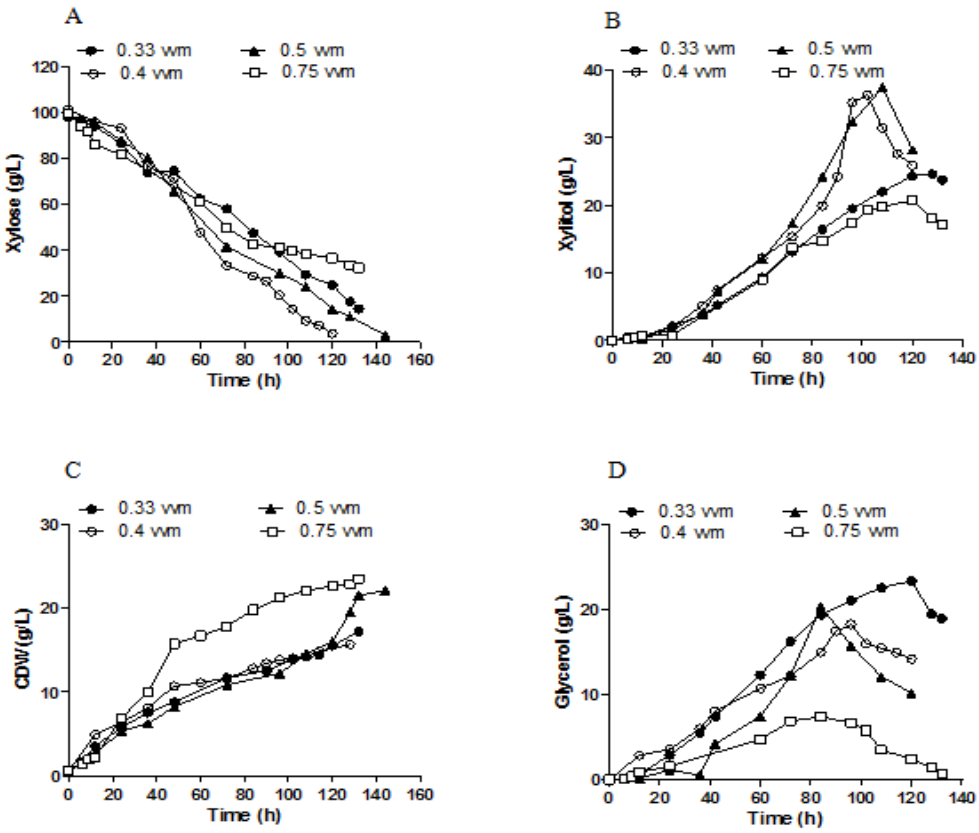

Fig. 1. Influence of aeration rate on the production of xylitol by *D. nepalensis*. The culture was agitated at a speed of 400 rpm and temperature, pH were maintained at 30ºC and 6.0, respectively. The aeration rate was varied between 0.33 vvm and 0.75 vvm. Concentration profiles of a) xylose, b) xylitol, c) biomass and d) glycerol

and agitation rates. An exponential correlation was established between k_La and the power number (N^3D^2) as shown in Fig. 3c. The exponent obtained in this study is 0.30 which falls within the range reported in literature (0.16-0.68).The yield of xylitol and biomass during batch cultivation at different aeration and agitation rates were also correlated to k_La. As shown in Fig. 3d, there is an increase in biomass yield with increasing k_La suggesting a linear relationship, whereas xylitol yield followed a different trend with respect to k_La, resembling inhibition kinetics. Xylitol formation was reduced at higher k_La values and the inhibition pattern appeared to be analogous to substrate inhibition.

3.4 Effect of pH on Xylitol Production in Bioreactor

Batch production of xylitol was performed at three different pH values 6.0, 6.5 and 7.0. The results showed that the effect of pH on xylitol production was not very significant in the range of pH tested (Fig. 4). The substrate utilization and xylitol production profiles were almost similar at all the pH values (Figs. 4a and 4b). But the yield of xylitol was slightly higher at pH 6.0. Hence pH 6.0 was considered optimum for xylitol production by *D. nepalensis*. The effect of pH was more pronounced on glycerol yield and glycerol formation was highest at pH 6.0 and further increase in pH resulted in poor yields (Fig. 4d). An opposite behavior was evident with biomass formation, where a high pH was found to be favorable (Fig. 4c). Optimum pH for xylitol production, as mentioned in the literature, was generally found to be within the range 5.0-7.0 [15,18].

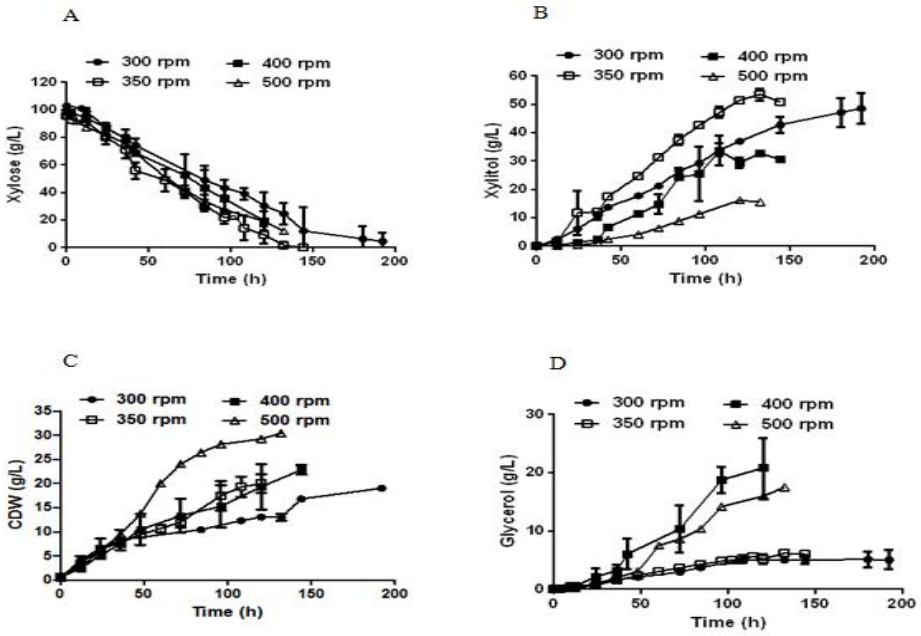

Fig. 2. Effect of agitation speed on xylitol production by *D. nepalensis*. The fermentation broth was agitated at various agitation rates (300-500 rpm) and aeration rate was maintained at 0.5 vvm. Time course profiles of a) xylose, b) xylitol, c) biomass, and d) glycerol

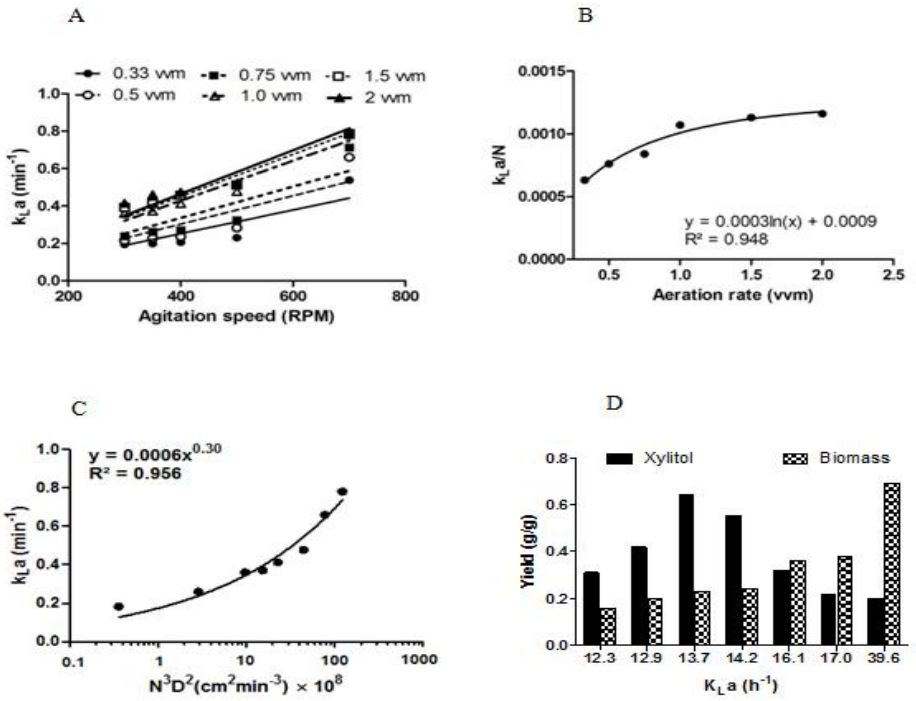

Fig. 3. Influence of aeration and agitation rates on volumetric oxygen transfer coefficient. k_La values were measured using gassing out method in the production medium applying various air flow rates and agitation rates. k_La values at different a) agitation speeds and aeration rates, b) Correlation of k_La/N with air flow rate and c) k_La values versus power numbers in xylitol production medium d) Effect of k_La on xylitol and biomass yields

3.5 Modeling of Growth and Substrate Utilization in Bioreactor

Under optimal aeration and agitation conditions, *Debaryomyces nepalensis* exhibited a typical growth pattern. The cells entered exponential phase following inoculation, without any lag phase and continued in that phase till 96 h. The cell concentration reached a maximum of 20 g/L at 96 h and from there on stationary phase commenced, during which the specific growth rate was close to zero. A logistic growth model was used to describe the growth and substrate utilization, taking X_m as 22 g/L from batch experiments yielding maximum xylitol (eq 4 and 5). Fig. 5 depicts the logistic fit of growth and xylose consumption, where the solid lines represent the estimated response for the model. The model appears to be suitable to describe the data, as indicated by the determination coefficients (R^2 = 0.957; 0.978).

3.6 Fed-batch Production of Xylitol by *D. nepalensis*

In order to enhance the xylitol production by *D. nepalensis*, experiments were performed under fed-batch mode. Fermentation was initially started as a batch process and throughout the fermentation, the temperature and pH were maintained at 30ºC and 6.0, respectively. The culture was agitated at 350 rpm and the aeration rate was maintained at 0.5 vvm. Fed-batch fermentations for xylitol production are usually carried out in two stages. The first phase is characterized by high growth rate followed by xylitol production phase. Since the growth rate of *D. nepalensis* was high at lower xylose concentrations, fed-batch fermentation were performed with an initial xylose concentration of 50 g/L. Fig. 6 shows the profiles of biomass and xylitol during batch and fed-batch phases. As seen in Fig. 6a, at the end of the batch phase, 14 g/L of biomass was obtained which corresponds to a yield of 0.38 g/g and the amount of xylitol obtained was 21.8 g/L. Xylose was consumed rapidly during this phase and the concentration of xylose depleted to 15 g/L by 30 h. The yield and productivity of xylitol during this phase were 0.6 g/g and 0.72 g/L. h, respectively. The fed-batch phase was started by adding a feed containing xylose and mixed nitrogen sources ($(NH_4)_2SO_4$ and yeast extract) intermittently. The feed was added to achieve a concentration of 50 g/L of xylose, 3 g/L of $(NH_4)_2SO_4$ and 1 g/L of yeast extract. Biomass and glycerol production was negligible during this phase. In the fed-batch phase, the concentration of xylitol continued to increase and reached a maximum of 43 g/L corresponding to a yield to 0.65 g/g. The productivity of xylitol decreased to 0.58 g/L. h and the final product concentration was also remarkably low.

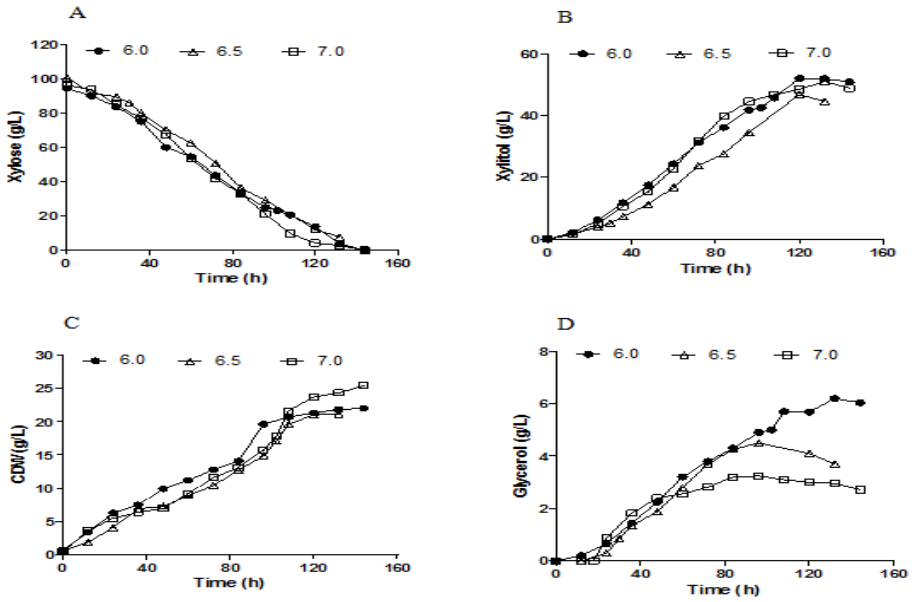

Fig. 4. Effect of pH on the production of xylitol by *D. nepalensis* NCYC 3413. Batch cultivations were performed at different pH values and an aeration rate of 0.5 vvm and 350 rpm. Time course profiles of a) xylose consumption, b) xylitol, c) biomass, and d) glycerol production

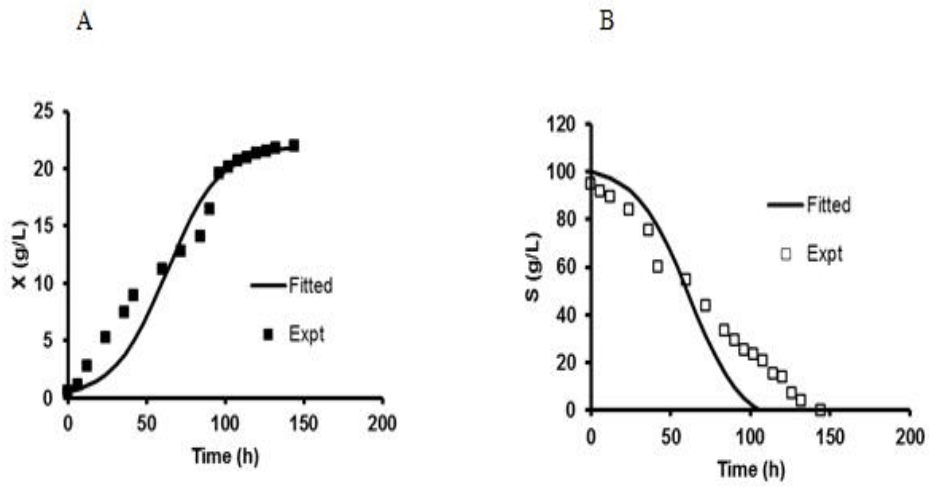

Fig. 5. Comparison between experimental and model predicted values of biomass and substrate. Batch cultivation of *D. nepalensis* was performed at an aeration rate of 0.5 vvm and agitation speed of 350 rpm. A logistic growth equation was used to simulate the experimental data of a) biomass formation and b) substrate utilization. The experimental data obtained at optimum k_La was used for simulation

To improve the yield and final product concentration, the xylose concentration during fed batch was increased to 100 g/L instead of 50 g/L (Fig. 6b). At the end of batch phase, 20 g/L and 20.8 g/L of biomass and xylitol produced were produced, respectively. In the fed-batch phase, consumption of xylose was slow and only 55% was utilized. The yield of xylitol increased to 0.47 g/g during fed-batch phase which can be ascribed to low biomass production. Although the yield in the fed-batch phase was better than that obtained in the batch phase, it is significantly low when compared to the previous experiment (Fig. 6a). Ultimately, 40 g/L of xylitol was produced with a productivity of 0.3 g/L h. Moreover, the amount of glycerol continued to increase at a slow rate in the fed-batch phase. This shows that cells were stressed by high osmotic pressure and low oxygen levels upon feeding xylose in large amounts. Consequently, the metabolism was slowed down and a drastic reduction in productivity of xylitol was observed.

From the shake flask experiments, it was evident that xylitol production rate was highest at a xylose concentration of 100 g/L (data not shown). Based on this, a fed-batch process was started with 100 g/L of xylose during batch phase. During the batch phase, the cells consumed xylose rapidly for both biomass and xylitol production. At the end of the batch phase, a maximum of 20 g/L and 50 g/L of biomass and xylitol was produced, respectively (Fig. 6c). The product yield of 0.59 g/g and productivity of 0.76 g/L.h was obtained during the batch phase. Fed-batch phase took off following the addition of feed containing xylose and nitrogen source as described earlier. To avoid hyperosmotic stress, the feed was added to achieve a concentration of 50 g/L of xylose. During the fed-batch phase, little or no biomass formation was observed. As shown in Fig. 6c, the cell dry weight values were constant over the entire fed-batch phase. As a result, xylose is majorly consumed for xylitol production. Hence the yield of xylitol increased to 0.83 g/g in the fed-batch phase and the final product concentration was 90 g/L. The maximum theoretical yield of xylitol was estimated to be 0.917 g/g [15]. The yield obtained in this study during fed-batch phase was 0.83 g/g which is nearly 90% of the theoretical maximum. The productivity during fed-batch phase was 0.83 g/L.h which was only slightly higher than that obtained during batch phase. Further addition of feed resulted in drastic reduction of xylitol production. This suggests that nutrient depletion resulted in lower conversion efficiency. In addition, the high concentration of xylitol seemed to inhibit growth and product formation. A repeated fed-batch fermentation, where a portion of medium is replaced by fresh production medium, can improve the productivity by reducing product inhibition.

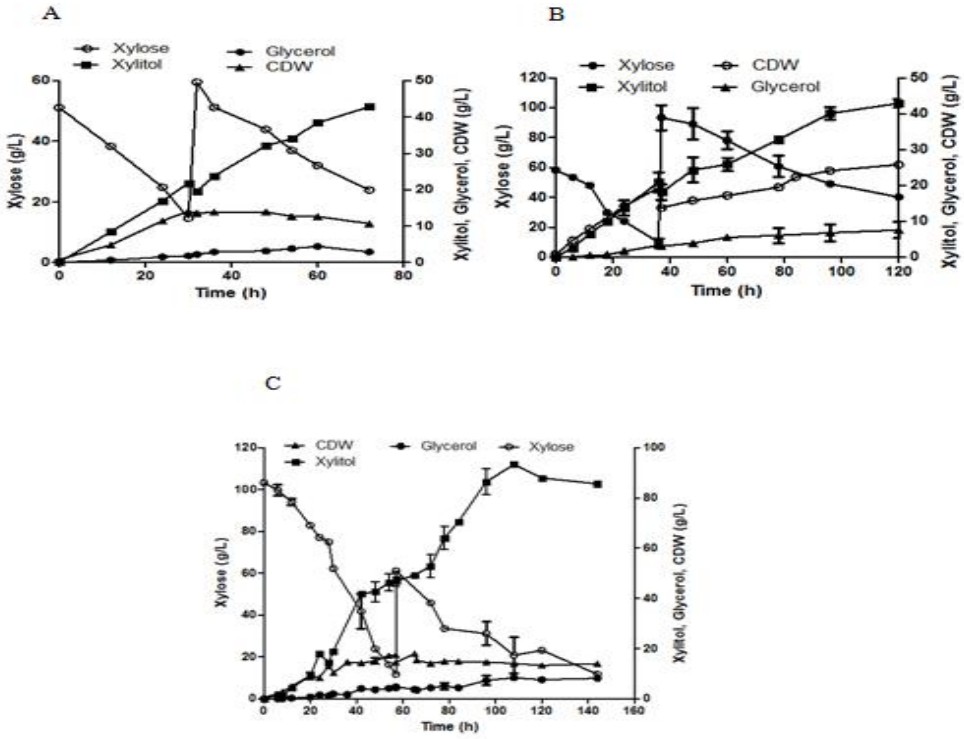

Fig. 6. Time course profiles of xylose, xylitol, glycerol and CDW during fed-batch cultivation of *D. nepalensis* a) with 50 g/L xylose in the first phase and 50 g/L xylose in the fed-batch phase b) with 50 g/L xylose in the first phase and 100 g/L xylose in the fed-batch phase and c) with 100 g/L xylose in the first phase and 50 g/L xylose in the fed-batch phase. The aeration and agitation rates were maintained at 0.5 vvm and 350 rpm, respectively. The presented values are the average of two independent fermentations

4. DISCUSSION

In order to improve the product yields, it is necessary to understand the relationship among substrate consumption, biomass and product formation. Microbial growth and product formation are influenced by numerous physiological and nutritional factors. In addition to these, the metabolic state of the cell also has a significant impact on product formation. It is known that many mathematical models with varying degrees of complexity were presented in the literature describing the influence of substrate and product on the rate of growth. The present study describes (i) the effect of aeration and agitation on the growth and xylitol production by *D. nepalensis* (ii) fed-batch fermentation to enhance the yield and productivity of xylitol.

Among the several physical and physiological parameters influencing xylitol production, oxygen is of prime importance because its availability is indirectly related to the redox status of the cell. Although many reports signifying the influence of oxygen on xylitol production are available, the data is difficult to compare since the oxygen availability was represented differently and there is no consensus on the impact of aeration and agitation rates on xylitol yield [16–19]. Oxygen plays an important role in determining the distribution of carbon flux between biomass and xylitol formation. Under micro-aerobic conditions, NADH regeneration is impaired resulting in a high NADH/NAD ratio, which inhibits xylitol dehydrogenase and increases xylitol production [18]. On the other hand excess of oxygen accelerates the respiratory chain activity and rapid regeneration of NAD^+ leads to loss of xylitol and concurrent biomass accumulation. Many reports have confirmed that maximal xylitol accumulation happens under microaerobic conditions [18-23]. The results in the present study also confirm that at a higher k_La, a major portion of xylose was used for biomass formation with very low yield of xylitol; whereas xylitol production was enhanced at lower k_La values. Our results are in agreement with various published reports.

It was reported that at an intermediate agitation rate of 200 rpm, a major fraction of xylose (83 %) was utilized for xylitol production by *D. hansenii* UFV-170 and the yield obtained was 0.76 g/g. Increasing the agitation rate to 300 rpm led to increased formation of cell mass [24]. Redox potential has been reported as a parameter to monitor oxygen availability for xylitol production in *C. parapsilosis* KFCC 10875. It was evident from their study that low redox potential favored xylitol accumulation, whereas biomass formation dominated at high redox potential [22]. Similarly, an intermediate oxygen transfer rate was found favorable for xylitol accumulation in a metabolic engineered strain of *D. hansenii* [20]. Oxygen is a key substrate in many aerobic microbial processes. In the present study, the effect of oxygen was also studied in terms of volumetric oxygen transfer coefficient (k_La), since k_La is an important scale-up criterion. Correct prediction of k_La values and knowledge of various factors affecting k_La are crucial for process design. Several empirical correlations were proposed in the literature explaining k_La as a function of power input per unit volume (P/V) and superficial gas velocity (V_s). However, in the absence of P/V, many researchers have used the Power Number N^3D^2 to represent the power input. A similar correlation based on power number was used in this study to explain the dependence of k_La on operating variables. The exponent obtained in our study was 0.3. The Power Number correlation was used to evaluate the effect of impeller speed on k_La using distilled water and the exponent of N^3D^2 was 0.42. But in the presence of biomass support particles, the exponent value was reduced to 0.31 [25]. Yagi and Yashida reported an exponent value of 0.74 for Newtonian fluids [26]. The value of the exponent was reported to be in the range 0.43 – 0.68 for pure water [27], whereas an exponent value of 0.24 was reported during the growth of *P. aeruginosa* in complex medium [28]. The variations in exponent value could be due to the difference in the properties of liquids employed in different studies and the range of impeller speeds considered. The correlation given by Yagi and Yoshida [26] was obtained at higher agitation speeds in the range 250 – 600 rpm, whereas Sivaprakasam et al. [28] used a lower range of 50 – 200 rpm.

Apart from dissolved oxygen, pH was shown to be another parameter that strongly affects growth and product formation. An acidic pH is usually favorable for yeast growth, but they often have a wide tolerance range. *D. nepalensis* was shown to tolerate a pH range of 3.0-11.0 [29]. The optimum pH for xylitol production was found to be 5.5 for *D. hansenii* [30]. In this study, *D. nepalensis* showed optimal pH of 6.0 for xylitol production. It has also been reported that pH range of 4-6 was suitable for xylitol production [31]. At low pH the maintenance requirement in the cell is high and as a result xylitol productivity decreases. An exception for this rule is *C. tropicalis* DSM 7524, for which the optimum pH is 2.5. Similarly, alkaline pH is also inhibitory since it affects the uptake of xylose [30].

A logistic growth model was used to describe the growth of *D. nepalensis* at optimal k_La value. Logistic model is generally used to describe and analyze the effect of factors other than nutrient limitation on microbial growth and also to predict microbial growth [32–35]. Growth of *D. nepalensis* in bioreactor under conditions of limited oxygen supply was simulated using the logistic function. The R^2 value of 0.957 shows the validity of logistic model to predict biomass formation during xylitol production. A modified logistic function which includes the substrate term was used to model the substrate consumption kinetics. This model appeared to explain the substrate utilization data well, as shown by the R^2 value of 0.978.

Xylitol production in yeast is induced by high xylose concentration. But, as mentioned earlier, at high xylose concentrations growth and product formation are inhibited due to osmotic stress [36]. Fed-batch culture is an excellent fermentation strategy to overcome substrate and product inhibitions and has been reported using several yeast strains. Since oxygen is a critical parameter for xylitol accumulation, batch experiments were performed to determine optimal rates of aeration and agitation. To further improve the yield and productivity, fed-batch process was designed based on the optimal aeration agitation rates obtained from batch experiments. Since xylitol production is favoured by micro-aerobic conditions, we used the same optimal conditions obtained in the batch process. The fed-batch strategy used in this study resulted in a high yield (0.83 g/g) and productivity (0.83 g/L h). The yield obtained was superior to that reported in fed-batch fermentation using *C. magnolia* (0.72 g/g). But in the latter case, productivity was high owing to the use of a high cell density culture in repeated fed-batch fermentation [37]. Using *C. tropicalis* KFCC-10960, Oh and Kim achieved a relatively high xylitol yield (0.93 g/g) and productivity (3.98 g/L

h). But such high yield appears due to the presence of glucose in the medium as a co-substrate. When D-glucose is supplemented in the feed, it is used for cell growth and only little amount of D-xylose is used for cell growth and maintenance. Consequently, a high xylitol yield is possible [36,38].

5. CONCLUSION

In conclusion, we reported xylitol production by a novel yeast strain, *Debaryomyces nepalensis.* Aeration and agitation conditions were found important variables affecting the efficiency of xylitol production. The influence of oxygen availability was investigated to determine the optimal aeration and agitation conditions. Xylitol production was favored with reduced oxygen availability (lower k_La value). Fed-batch fermentation, under optimal aeration and agitation rates, resulted in improvement of final xylitol concentration and yield, thus signifying the potential of the strain for industrial application. But fed-batch culture did not significantly improve the productivity of xylitol. However, changing the aeration and substrate feeding strategies might improve productivity and further investigation in this direction is required in order to maximize the productivity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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