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Oxygen transfer rate modulates the dextransucrase production by *Acetobacter tropicalis*

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Abstract

Dextransucrase is an important class of extracellular enzymes with great industrial importance. Dextransucrase synthesizes the high molecular weight glucose polymer dextran by transferring D-glucosyl moieties to a growing glucan chain. Keeping in view the potential of dextransucrase, bench scale production of *Acetobacter tropicalis* cells with high dextransucrase activity was carried out in laboratory fermenter at 7 L scale. The growth of *A. tropicalis* has been affected by the supply of oxygen during the fermentation. Therefore, the optimum combination of aeration rate and agitation speed for dextransucrase production from *A. tropicalis* was found to be 0.5 vvm and 450 rpm. The volumetric oxygen transfer coefficient, K_La values were found to affect the growth of *A. tropicalis* and production of dextransucrase. In the present study, the growth and dextransucrase production increased with increase in K_La upto an optimum limit and then started decreasing with increase in K_La. Maximum biomass (1.41 mg/ml) and maximum dextransucrase activity (15.8 U/ml) was obtained at K_La value of 0.28 min^{-1}. The maximum dextransucrase activity obtained in laboratory fermenter was 15.8 U/ml which was greater than the enzyme level obtained at shake flask level (11.6 U/ml).

Keywords: dextransucrase; *Acetobacter tropicalis*; dextran; fermentation; agitation; aeration; dissolved oxygen; dissolved oxygen concentration (C_L); volumetric oxygen transfer coefficient (K_La).

Introduction

Dextransucrase (sucrose: 1,6-α-D-glucan 6-α-glucosyl-transferase, EC 2.4.1.5) is the enzyme that catalyzes the synthesis of dextran from sucrose. Dextran (C6H10O5) n is a polysaccharide consisting of glucose monomers linked mainly by α(1→6) glycosidic linkages in the linear chain (95%) and 5% α(1→2), α(1→3) and/or α(1→4) branch linkages [1, 2]. Dextransucrase synthesizes dextran by transferring D-glucosyl moieties to a growing glucan chain. Dextransucrase is an important class of extracellular enzymes with great industrial importance and the Leuconostoc mesenteroides NRRL B-512F dextransucrase has been used to produce commercial dextran [3]. Dextransucrase was produced mainly by various *Leuconostoc* and *Streptococcus* species [4, 5] and by the mold *Rhizopus* spp. [6]. Dextransucrase was also found to be secreted by *Lactobacillus*, *Acetobacter* and *Pediococcus pentosaceus* [7].

Dextrans are useful in various industries because of their inertness, porous structure and gelling properties [8]. Moreover, the presence of 95% linear linkages makes dextran water soluble. Dextrans have been used as food syrup stabilizers, matrix of chromatography columns, blood plasma substitutes, antithrombogenic agents, treatment for iron deficiency anaemia, drug carriers [9, 8]. Dextransucrase also catalyzes the transfer of glucosyl units from sucrose into acceptor molecules. This results in the synthesis of glucooligosaccharides [10, 11] and the release of fructose. Fructose ($C_6H_{12}O_6$) is a low caloric sugar and has extensively been used in food industry. It was possible to synthesize oligosaccharides with α (1→2) branch linkage with this enzyme [12, 13]. These molecules are highly resistant to the attack of digestive enzymes and have also been proven to stimulate the growth of beneficial bacteria of the intestinal microflora [14, 15]. Dextransucrase can also be used to catalyze many oligosaccharides [16] and other useful carbohydrates such as the antioxidant 1,5-anhydro-D-fructose [17].

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In batch fermentation with *Leuconostoc mesenteroides* NRRL-B512(F), decrease in nitrogen/carbon ratio slows down the rate of dextransucrase production (18). Temperature, pH and sucrose concentration highly affects the production of dextransucrase by *L. mesenteroides* NRRL-B512(F) [19, 20]. High sucrose concentration does not inhibit the cell growth, however at concentration higher than 40 g/dm^3 separation of products from cells become difficult. The dextran and fructose mixture obtained by fermentation of sucrose rich media by *L. mesenteroides* NRRL-B512(F) were separated by using simulated moving bed (SMB) chromatography [21].

The volumetric oxygen transfer coefficient (K_La) was considered as one of the most important scale up factors in fermentations. The dissolved oxygen (DO) concentration in a fermentation broth has a profound effect on the performance of aerobic fermentation systems. Schneider et al, [22] reported the highest yield under an aeration of 1 vvm where the oxygen level was stayed between 40-80 % of saturation during the fermentation. Lazic et al. [23] found that pH 5.5 and 0.05 vvm of air, was most favourable conditions for dextran production by *Leuconostoc mesenteroides* and it also reduced the fermentation time. The aim of this study was to optimize the effect of agitation speed and aeration rate on dextransucrase production by *Acetobacter tropicalis* and to determine the role of oxygen transfer rate on the enzyme production.

Materials and methods

Chemicals

All the chemicals used in the present study were either procured from Sigma Aldrich (U.S.A.) or HiMedia Laboratories Pvt. Ltd., Mumbai and were of high purity analytical grade. The media constituents were of bacteriological grade.

Microorganism and maintenance of culture

Acetobacter tropicalis was procured from the Department of Biotechnology, Himachal Pradesh University, Shimla. *A. tropicalis* was maintained on modified MRS (Mann Rogosa Sharpe's) [24] agar plates and agar slants having the following composition (%, w/v): Sucrose 2.0, peptone 1.0, $MnSO_4$ 0.025, Sodium Acetate 0.5 and agar 2.0 (pH 7.0) or in 20% (v/v) glycerol stocks and subculturing was done periodically after 3 days with the same medium at 25 °C.

Inoculum and medium preparation for bench scale fermentation

The optimum conditions for production and effect of oxygen transfer rate on cell mass and dextransucrase activity by fermentation at a scale of 7 L were investigated for *A. tropicalis.* The medium components and different physical parameters were optimized in shake flask level and the optimized parameters were used for bench scale fermentation (data not shown). For the development of a laboratory inoculum, the seed was prepared in conical flasks containing the seed medium (%, w/v; Sucrose 2.0, peptone 1.0, $MnSO_4$ 0.025, Sodium Acetate 0.5; pH 7.0). The seed medium was inoculated with organism *A. tropicalis*, and incubated at 25°C for 12 h on a rotary shaker (150 rpm). The production medium (pH 7.0) contained (%, w/v) Sucrose 2.0, peptone 1.0, $MnSO_4$ 0.025, Sodium Acetate 0.5. The fermenter was loaded with production medium with additionally contained 0.01% (v/v) silicone oil as antifoam agent (Himedia). The medium was sterilized *in situ* with the help of steam supplied through the jacket of fermenter.

Dextransucrase assay

Dextransucrase converts sucrose into dextran and D-fructose. Dextransucrase activity was determined spectrophotometrically by measuring the reducing sugar released from sucrose (Miller, 1959) [25]. The dextransucrase from *A. tropicalis* was found to be extracellular in nature and hence the supernatant was used for the enzyme assay. The assay mixture (2ml) contained enzyme solution (10μL) and 2 % sucrose (2.0 ml) prepared in acetate buffer (25 mM, pH 5.5). The reaction mixture was incubated at 37°C for 30 min. The reaction was stopped by keeping the reaction mixture in boiling water bath (100°C) for 10 min. A set of control was also run. From the reaction mixture, 1 mL was discarded and 1ml DNSA (dinitosalacylic acid) reagent was added to remaining 1 ml reaction mixture. . The reaction mixture was kept in boiling waterbath for 20 min for colour development (Yellow to Red colouration). The absorbance of colour developed was measured at 540 nm in a spectrophotometer (LABINDIA). Activity of the dextransucrase from the supernatant of *A. tropicalis* was expressed in terms of units (U/ml). The dextransucraes unit has been defined as the amount of enzyme that liberates 1 μmol of reducing sugar (fructose) per min per ml at 37 °C under standard assay condition.

Sterilization and inoculation of production medium

The fermentation was carried out in 14 L laboratory fermenter (BIOFERM-LS2, Scigenics India Pvt. Ltd.) at 7 L working volume in batch mode. As the fermenter has inbuilt facility of sterilization (*in situ* sterilizable), 7 L production medium [(%, w/v) Sucrose 2.0, peptone 1.0, $MnSO_4$ 0.025, Sodium Acetate 0.5.] was loaded into the fermenter. The sterilization of the production medium was carried out at 121°C for 15 min. After the completion of sterilization cycle, the temperature of the medium was adjusted to 25°C by cooling. The sterilized production medium was inoculated with 1% (v/v), 12 h old seed culture through the inoculation port by peristaltic pump attached to a feed bottle. The pH, temperature and dissolved oxygen were monitored throughout the operation. However, temperature was maintained at 25°C for the entire course of fermentation with the help of inbuilt heater and chiller. After inoculation, periodical sampling (at 1h interval) was carried out and samples were analyzed for growth and dextransucrase production by *A. tropicalis* cells.

Growth and production of dextransucrase

The growth of *A. tropicalis* cells and activity of dextransucrase was measured under different conditions of agitation and aeration by adopting the analytical procedures. The effect of these variables on pH, dissolved oxygen (DO, % saturation), cell mass and dextransucrase activity was observed.

Effect of agitation on the growth and production of dextransucrase activity by Acetobacter tropicalis

Agitation rate is important for maintaining homogeneity in bioreactor and also helps in oxygen transfer. The effects of agitation on production of biomass, dextransucrase activity, DO and pH by *A. tropicalis* was investigated by varying the agitation rate (150, 300, 450 and 600 rpm). The fermentation was carried out at 25oC temperature keeping the aeration rate 0.5 vvm. Samples at regular interval of 1 h were withdrawn and analyzed for cell growth and dextransucrase activity. The pH and DO of the fermentation broth during the entire course of cultivation were monitored with the help of DO and pH probe. The suitability of the agitation rate was determined on the basis of the results obtained.

Effect of aeration rate on growth and production of dextransucrase activity by Acetobacter tropicalis

The effect of aeration rate on the growth and production of dextransucrase by *A. tropicalis* and change in pH and dissolved oxygen (DO) profile of the fermentation broth was investigated under varying aeration rate (0.50 and 1.00 vvm) at 300 and 450 rpm.

Determination of volumetric oxygen transfer coefficient (K_La)

Dissolved oxygen concentration depends on the relative rate of oxygen transfer and utilization.

Rate of oxygen transfer (nO_2,T) is given by following equation:

$nO_2,T = K_La\ (C^*\_\ C_L)$ ----------Equation 1

The volumetric oxygen transfer coefficient (K_La) is defined by following equation:

$K_La = n_{O2},T\ /C^*\_\ CL$ ---------- Equation 2

Dynamic method or gassing out method for K_La determination was used which is based upon the dynamic oxygen balance equation:

$dCL/dt = K_La\ (C*\_\ CL) - Q_{02}X$ ---------- Equation 3

K_La = Volumetric oxygen transfer coefficient

C* & C_L = Saturation and actual dissolved oxygen concentration in the liquid medium, respectively.

Q_{o2} = Rate of oxygen consumption per unit mass of cells (cellular respiration) ($mMO2g^{-1}h^{-1}$)
Rearranging the equation 3:

$CL = C* - 1/\ K_La\ (Q_{02}X + dCL/\ dt)$ ------- Equation 4

For this, when fermentation was in active respiration, aeration was stopped temporally and decrease in dissolved oxygen concentration (C_L) was measured as a function of time for determination of oxygen uptake rate ($Q_{02}X$). Aeration was established and increase in the dissolved oxygen concentration was also measured as a function of time. Punctual differential was obtained from gassing curve and C_L vs. $dC_L/dt + Q_{02}X$ were correlated.

Role of K_La on growth and enzyme production by Acetobacter tropicalis

The effect of K_La was determined by comparing the K_La values of different fermentation batches of *A. tropicalis* with respect to the biomass and dextransucrase production. The results were demonstrated by K_La versus growth and enzyme activity curve.

Results and discussion

Effect of agitation and aeration rate on the growth and dextransucrase production by Acetobacter tropicalis

The fermentation was carried out at controlled temperature of 25°C with optimized medium (pH 7.0) containing (%, w/v) Sucrose 2.0, Peptone 1.0, $MnSO_4$ 0.025, Sodium acetate 0.5 at 0.5 vvm aeration rate but at different agitation speed (150, 300, 450 and 600 rpm). The effect of varying agitation rate was studied on production of dextransucrase in the cells of *Acetobacter tropicalis*. The agitation improves the rate of oxygen transfer from bulk gas to the medium, which has ultimately been utilized by the growing microorganism. The increase in the agitation speed from 150 rpm to 450 rpm proved to be beneficial for the growth of the *A. tropicalis* (Figure 1 a). The maximum cell mass (1.41 mg/ml) of *A. tropicalis* was obtained at 7 h of fermentation at 450 rpm, which was higher than the cell mass attained under 300 rpm at 8 h of fermentation (1.25 mg/ml). However, at 150 rpm agitation the growth rate was much slower and this caused an early attainment of the stationary phase. Further increase in agitation rate (600 rpm) decreases the growth. This might be due to the shearing forces operative at high agitation rate. The 450 rpm of agitation speed at 0.5 vvm aeration was found to be the most optimum for the cultivation of *A. tropicalis*. The maximum dextransucrase activity of 15.77 U/ml was obtained in 7th h at 450 rpm agitation (Figure 1 b) followed

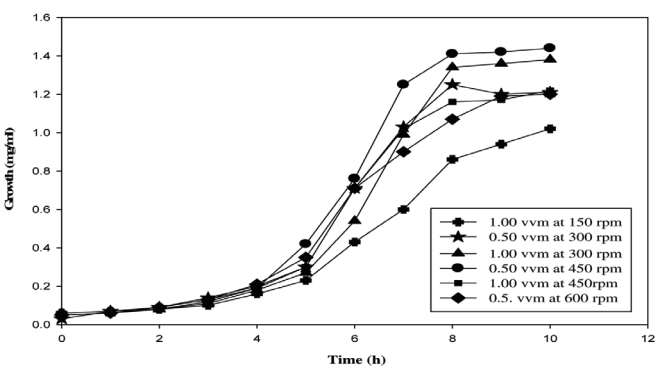

Figure 1a Effect of agitation speed and aeration rate on cell mass production by *Acetobacter tropicalis* in fermenter.

by agitation rate of 300 rpm at 8th h of fermentation (13.35 U/ml). In case of 150 rpm and 600 rpm, the maximum dextransucrase activity was found to be 9.83 U/ml and 11.93 U/ml respectively at 8th h of fermentation.

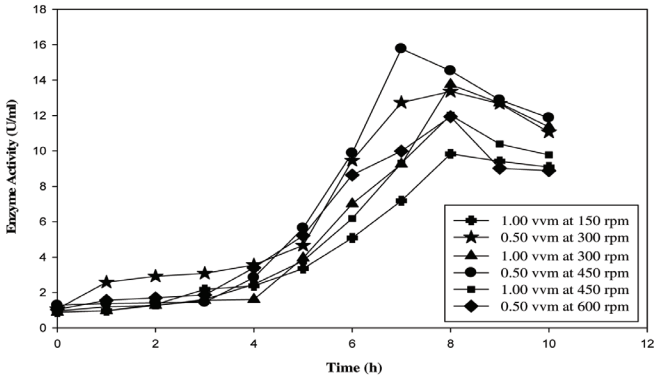

Figure 1b Effect of agitation speed and aeration rate on dextransucrase activity by *Acetobacter tropicalis* in fermenter.

Although the agitation has more profound effect in improving the dissolved oxygen concentration than aeration, some negative factors were also associated with the agitation speed that can hamper the net benefit an aerobe can have. An increase in the agitation rate beyond 450 rpm proved to be ineffective in enhancing the dextransucrase production, and in fact resulted in decrease in dextransucrase activity. The dissolved oxygen profiles of the fermentation broth under different rates of agitation reveals that depletion in the dissolved oxygen was insignificant at the higher rate of agitation (Figure 1 c). In contrast to the decline in DO from 100% to 1.2% and 1.1% during first 6 h and 5 h of the course of fermentation at 150 rpm and 300 rpm respectively, the DO level at higher agitation rate (450 rpm) first dropped below 1.2% during first 6 h and then started to increase from 8 h and reached to 20% at 10th h of fermentation. Further increase in agitation speed (600 rpm) dropped the DO level to 1.3% at 6 h and started to increase and reached 33.7% at 10th h of fermentation.

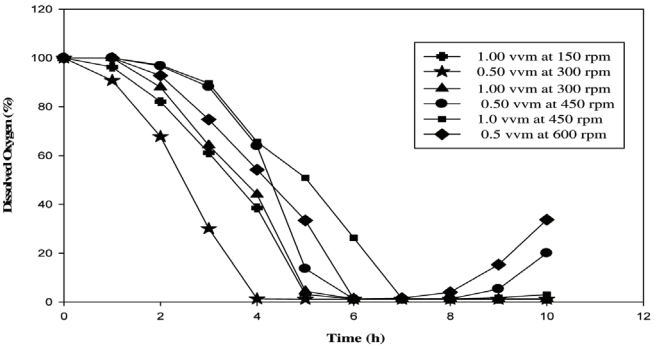

Figure 1c Effect of agitation speed and aeration rate on dissolved oxygen during growth of *Acetobacter tropicalis* in fermenter.

The pH of the fermentation broth was also found to decrease during the course of fermentation in all the cases (Figure 1 d). The decrease in the pH of the fermentation broth was found to be associated with the growth of *A. tropicalis*. The reduction in culture broth pH from 7.0 to 5.0 might be due to the acid metabolite production by the utilization of carbohydrate substrate. It can be inferred from these results that rate of agitation was helpful in maintaining the higher dissolved oxygen level which subsequently help in growth and dextransucrase production by *A. tropicalis.*

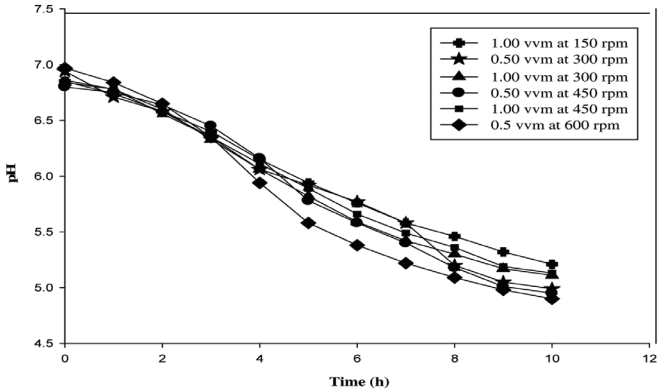

Figure 1d Effect of agitation speed and aeration rate on the final pH of fermentation broth during growth of *Acetobacter tropicalis* in fermenter.

For aerobic organisms, rate of aeration is important as it affects the metabolic and physiological process. Therefore, it was essential to find out the optimum combination of rate of aeration and agitation speed for the production of dextransucrase by *A. tropicalis*. Hence these cells were grown at varying aeration rate (0.5 vvm and 1.0 vvm) at the agitation speed of 300 and 450 rpm. The growth of *A. tropicalis* was affected by the supply of oxygen during the course of fermentation. The maximum growth of *A. tropicalis* was obtained at 8th h of fermentation (1.41 mg/ml) at 0.5 vvm aeration rate and 450 rpm. The cell mass production increased up to 8th h of incubation and after that it became constant at 0.5 vvm aeration as well as at 1.0 vvm (Figure 1 a). The maximum biomass (1.34 mg/ml) at 1.0 vvm aeration rate and 300 rpm was also obtained at 8th h of fermentation and remain nearly constant with further incubation. In case of 300 rpm agitational speed, 1.0 vvm of aeration resulted in maximum biomass as compared to 0.5 vvm (1.25mg/ml). In contrast, at 450 rpm maximum growth yield was obtained at 0.5 vvm (1.41mg/ml) as compared to 1.0 vvm (1.16mg/ml). Similarly, maximum dextransucrase production (15.77 U/ml) was observed at 7th h of fermentation at 0.5 vvm aeration and 450 rpm followed by 13.75 U/ml at 8th h at 1.0 vvm at 300 rpm. Further increase in aeration (1.0 vvm) at 450 rpm resulted in decrease of the dextransucrase activity to 12.02 U/ml. Hence it could be concluded that with increase in aeration rate, there was increase in dextransucrase activity upto optimum level and then decrease drastically with increase in further aeration (Figure 1 b). Lazic et al, [23] found that pH 5.5 and 0.05 vvm of air, were the favourable conditions for dextransucrase production by *Leuconostoc mesenteroides* and these optimal conditions also reduced the fermentation time. High activity of dextransucrase was attained when fermentation of *Leuconostoc mesenteroides* FT045 B was carried out in medium containing 3 and 4% of sucrose at 25°C, 132 rpm and 0.15 vvm aeration, the dextransucrase production obtained was nearly 11.0

DSU/mL [26]. Highest dextransucrase yield was obtained under 1 vvm aeration where the oxygen level was stayed between 40-80 % of saturation [22] Further, Monsan et al. [27] found highest dextransucrase activity in moderate conditions of stirring and aeration.

Course of fermentation by Acetobacter tropicalis for the production of dextransucrase

During the present study, 0.5 vvm aeration at 450 rpm agitation speed was found to be the most optimum for the production of dextransucrase by *Acetobacter tropicalis* in a laboratory fermenter. The fermentation by *A. tropicalis* cells for dextransucrase production was carried out in the optimized medium (pH 7.0) at 0.5 vvm aeration and 450 rpm agitation speed. The cells of *A. tropicalis* started growing exponentially after an initial lag period of 3 h. The stationary phase of growth was attained after 8 h of incubation. The maximum cell mass (1.41 mg/ml) of *A. tropicalis* was observed at 8 h of fermentation. The maximum enzyme activity obtained was 15.8 U/ml at 7 h of incubation which does not coincide with the maximum growth of *A. tropicalis* (Figure 2). The culture pH reduced from 7.0 to 5.0 at 10th h of growth. The increase in cell mass of *A. tropicalis* leads to the rapid utilization of oxygen which reaches to minimum at 6 h of fermentation. The concentration of dissolved oxygen was remained minimal till 8 h of incubation. Once the *A. tropicalis* cells reached the stationary phase, the dissolved oxygen concentration started increasing and attained 20% saturation at 10h of incubation. Landon [28] studied effect of stirring on dextransucrase production for *Leuconostoc mesenteroides* NRRL B512 (F) and suggested some limitations in difussion through cell membrane in static culture. Recently, dextransucrase production by a new bacterial strain *Weissella confusa* Cab3 was scaled up in lab scale bioreactor resulting in further enhancement of enzyme activity (22.0 U/ml) than shake flask level of 17.54 U/ml [29].The dextransucrase activities in excess of 21.9 U/ml have also been obtained by culturing *L. mesenteroides* NRRL B512(F) under non-aerated fed-batch fermentation conditions [30]. However, Santos et al, [20] found that aeration does not have an important effect in fermentation by *L. mesenteroides* NRRL B512(F). It affects positively the strain growth but the dextransucrase activity decreased with aeration.

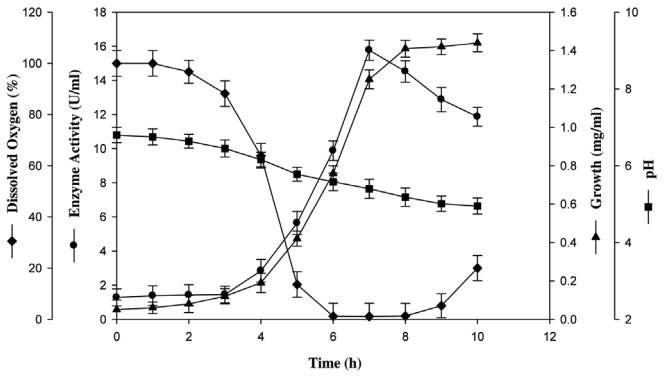

Figure 2 The course of fermention by *Acetobacter tropicalis* for the production of dextransucrase.

Determination of volumetric oxygen transfer coefficient (K_La)

In many aerobic fermentation systems, the rate of oxygen transfer to the cells is the limiting factor which determines the rate of biological conversion. The volumetric oxygen transfer coefficient (K_La) is an important parameter which is used to compare the oxygen transfer capabilities of various aerobic bioreactors. Dissolved oxygen concentration depends on the relative rate of oxygen transfer and utilization. Both the agitation speed and aeration rate affect the value of K_La. The DO concentration, C_L, has to be greater than the critical value of DO in order to avoid oxygen limitation. Aerobic biological reactors are designed to maximize the value of K_La in order to keep the value of C_L above the critical level. The critical dissolved oxygen concentration is in order of 5-10% of the solubility of oxygen. The values of K_La during the cultivation of *A. tropicalis* were determined by C_L versus $Q_{o2}X$ + [(dCL/ dt)] curve (Figure 3). The maximum K_La, 0.38 min^{-1} was obtained for the fermentation batch with aeration rate of 0.5 vvm and agitation speed of 600 rpm whereas the minimum K_La, 0.11min^{-1} was obtained at 0.5 vvm and 450 rpm. The values of K_La for 1.0 vvm at 300 rpm, 0.5 vvm at 450 rpm and 1.0 vvm at 600 rpm were 0.18 min^{-1}, 0.28 min^{-1} and 0.29 min^{-1} respectively. The fermentation kinetics of *Leuconostoc mesenteroides* in a 5 L bioreactor for the production of dextransucrase was studied and K_La obtained was 30.85 h^{-1} at 0.15 vvm aeration rate and 225 rpm [31]. In case of ß-glucuronidase production by recombinant E. coli, highest enzyme production was obtained at 300 rpm agitation and 2 vvm aeration having K_La value 1.686 h^{-1} [32]. The best condition obtained for pectate lyase production from Paenibacillus polymyxa N10 were 150 rpm agitation, 0.5 vvm aeration with K_La value 37.08 h^{-1} [33]. Sachidanandham et al, [34] determined K_La as a function of impeller speed and the rate of aeration, and observed an inhibitory effect on the production of protease using Bacillus amyloliquefaciens by increasing the impeller speed (thus increasing K_La) beyond 300 rev min^{-1} in a 10 litre batch bioreactor. Belo and Mota [35] reported an increase in K_La (due to an increase in impeller speed) and its negative effect on recombinant cytochrome

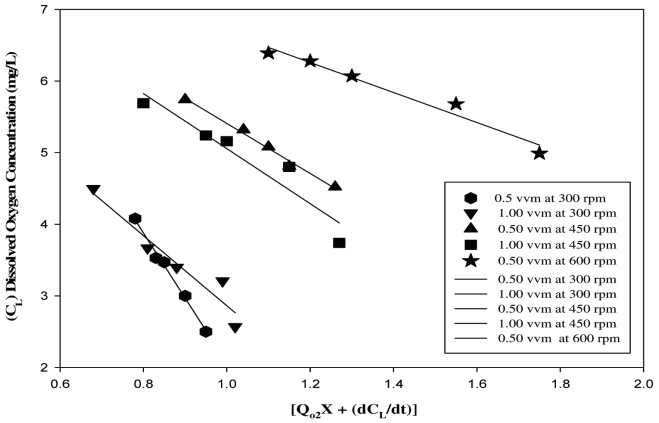

Figure 3 C_L versus $Q_{02}X$ + [(dCL/ dt)] curve for determination of volumetric oxygen transfer coefficient (KL*a*) of Acetobacter sp. under varying conditions of fermentation.

b5 production in E. coli. Pham et al, [36] reported an increase in xylanase production rate with an increase in K_La, but the final concentration of the enzyme was not affected by the increase in K_La.

Role of K_La on growth and enzyme production by Acetobacter tropicalis

The volumetric oxygen transfer coefficient (K_La) is one of the most important scale up factors in fermentations. K_La value affected the growth rate and enzyme activity in *A. tropicalis* for the production of dextransucrase. In the present study, the growth of *A. tropicalis* increased with increase in K_La from 0.11 to 0.28 min^{-1} and thereafter decreased suddenly with further increase in K_La (Figure 4). Maximum biomass (1.41 mg/ml) was obtained at 0.28 min^{-1} K_La. Similar pattern was observed in case of dextransurase production by *A. tropicalis*. Initially enzyme activity increased with increase in K_La upto a limit and then decreased (Figure 4). Maximum dextransucrase activity (15.77 U/ml) was obtained at 0.28 min^{-1} K_La. This exactly coincides with the profile obtained for the growth of *A.tropicalis*. So, it could be concluded from the above data that higher oxygen transfer rate adversely affect the growth of *A. tropicalis* which in turn result in less dextransucrase production. Maximum production of proteases (15.28 UP/ml) by *Staphylococcus aureus* mutant RC128 was obtained when the bioreactor was operated at 300 rpm and at 2 vvm with a volumetric oxygen transfer coefficient (K_La) of 175.75 h^{-1} (37) In case of glucose oxidase production by recombinant Saccharomyces cerevisiae, Kapat et al. [38] concluded that K_La alone does not facilitate the specifc production of enzyme. It is the appropriate combination of impeller speed and aeration rate which is more important. Thus, the optimal DO concentration should be considered as a scale up parameter for production process instead of K_La. The major findings of the dextransucrae production by *A. tropicalis* in a laboratory fermenter have been summarized in Table 1.

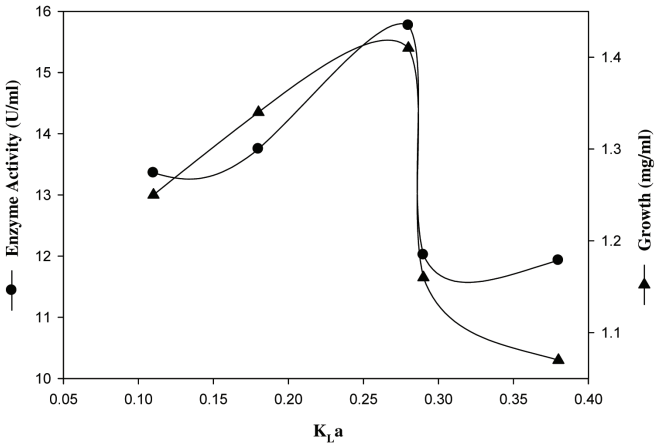

Figure 4 Effect of KLa on growth and dextransucrase production by *Acetobacter tropicalis*

Table 1 Interactive effect of K_La on growth and dextransucrase production by Acetobacter tropicalis

Batch	*Agitation (rpm)*	*Aeration (vvm)*	*Enzyme activity (U/ml)*	*Biomass (mg/ml)*	*KLa (min^{-1})*
1.	150	1.0	9.83	0.86	---
2.	300	0.5	13.35	1.25	0.11
3.	300	1.0	13.75	1.34	0.18
4.	**450**	**0.5**	**15.77**	**1.41**	**0.28**
5.	450	1.0	12.02	1.16	0.29
6.	600	0.5	11.93	1.07	0.38

Conclusion

It could be concluded from the present study that oxygen transfer rate plays a vital role in the production of dextransucrase from *Acetobacter tropicalis*. *A. tropicalis* growth and dextransucrase production increased with increase in K_La upto an optimum limit and then started decreasing with increase in K_La. The values of K_La can be used as a scale up factor for the large scale production of dextransucrase by *A. tropicalis*. Maximum enzyme activity coincided with maximum growth of *A. tropicalis*, which suggests that dextransucrase production was growth associated in nature. The enzyme activity was increased 1.36-fold and fermentation time for dextransucrase production was also lowered. Dextransucrase produced by *A. tropicalis* holds the potential for the commercial production of dextran which has various applications in the biopharmaceutical and food industries.

Acknowledgements

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Conflicts of Interest

The authors wish to express that they have no conflict of interest

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