Research & Reviews: Journal of Microbiology and Biotechnology

e-ISSN: 2320-3528
p-ISSN: 2347-2286

Analysis of the Combined Effects of Aeration and Agitation Rate on Dextransucrase production by *Leuconostoc lactis* KU665298 in a Laboratory Fermenter using Response Surface Methodology

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Research Article

Received date: 08/03/2018

Accepted date: 22/08/2018

Published date: 30/08/2018

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Keywords: Dextransucrase, RSM, Agitation speed, aeration rate

ABSTRACT

Dextransucrase is a glucosyltransferase which synthesizes high molecular weight glucose polymer (dextran) from sucrose. Dextran has diverse applications in pharmaceutical, food and fine chemical industries. The combined effects of the agitation and aeration rates on the production of dextransucrase in laboratory fermenter were systematically investigated with *Leuconostoc lactis* KU665298 by applying RSM. Dextransucrase production from *L. lactis* was found to improve after the scale up of production process from shake flask (3.6 U/mL) to fermenter level (5.27 U/mL) at an agitation of 200 rpm and aeration of 0.85 vvm. The volumetric oxygen transfer coefficient (K_La) was used as the basis for the evaluation of aeration efficiencies. K_La value for most befitting fermentation condition aiding maximum dextransucrase production was 0.15 min^{-1}. This is the first report in which RSM has been applied to design fermentation batches of different combinations of aeration and agitation rate in a laboratory fermenter to produce dextransucrase.

INTRODUCTION

Dextransucrase (EC 2.4.1.5) is an extracellular glucosyltranferase, belonging to glycoside hydrolases family (GH 70) [1,2]. This extracellular inducible enzyme catalyzes the synthesis of high molecular weight glucose polymer called dextran by transferring D-glucosyl units from sucrose to dextran polymer chain and releases low caloric sugar fructose [3-6]. Dextran is an elongated chain polymer of D-glucose largely linked with α (1-6) linkage and side chains having α (1-2), α (1-3), and α (1-4) linkages depending upon the producing strain [7-9]. Microorganisms belonging to the genera *Lactobacillus*, *Leuconostoc* and *Streptococcus* of *Lactobacillaceae* and *Streptococcaceae* families are the main producers of dextransucrase [10]. The thriving importance of dextransucrase is based on the wide applications of dextran. Dextran has acknowledgeable industrial applications in medical, pharmaceutical, food, textile and chemical industries, depending on its solubility and molecular mass [11-14]. Dextrans of low molecular weight have been recognised as a blood plasma substitute for the last 60 years while the high molecular weight dextrans can be applied as viscosifying, stabilizing, gelling, sweetening and emulsifying agents in food production [13,15,16]. Cross linked dextran known as sephadex is widely used for purification of proteins in research [17]. In addition to this, silver nanoparticles were also prepared using dextran as both reducing and stabilizing agent [18]. Recently, dextran has been used in coating of magnetic nanoparticles (MNPs) for their biomedical applications such as a contrast agent in magnetic resonance imaging (MRI) and gene therapy [19,20].

In the presence of appropriate acceptor (glucose, maltose, isomaltose, etc.) dextransucrase can also be used to synthesize low molecular weight oligosaccharides (such as leucrose) and other useful carbohydrates such as the

e-ISSN: 2320-3528
p-ISSN: 2347-2286

antioxidant 1,5-anhydro-D fructose. Oligosaccharides synthesized using dextransucrase are used as neutraceuticals, stabilizers and prebiotics. Recently, a novel truncated dextransucrase (DSR-S1-ΔA) was created for the synthesis of useful oligosaccharides by transglycosylation with different acceptors [21].

Keeping in view the importance of dextransucrase, it became necessary to produce the enzyme at a large scale. In the present study, dextransucrase production was carried out in laboratory fermenter with *L. lactis*. Central composite design (CCD) of response surface methodology (RSM) has been opted to study the combined effects of aeration and agitation rate on dextransucrase production by *L. lactis* in a laboratory fermenter. The dissolved oxygen (DO) concentration in a fermentation broth has a profound effect on the performance of aerobic fermentation systems. Therefore, the volumetric oxygen transfer coefficient (K_La) was considered as most important scale up factor and calculated by gassing out method. The main aim of the study was to find out the optimum combination of aeration and agitation rate and the volumetric oxygen transfer coefficient (K_La) of bioreactor for the maximum dextransucrase production from *L. lactis*.

MATERIAL AND METHODS

Experimental Design

A CCD of RSM was chosen to study the combined effects of agitation speed (rpm), and aeration rate (vvm) on *L. lactis* growth and production of dextransucrase. Parameters were represented at two levels, high (+1) and low (-1) i.e. agitation speed at 300 and 100 rpm; aeration rate of 0.75 and 0.25 vvm. A total of 13 experimental runs were designed by CCD with different treatments of agitation and aeration rate (Table 1). The experiments were conducted in lab scale fermenter and enzyme activity (U/mL) was taken as the response in each run. The statistical software "Design-Expert 10.0" (Stat-Ease) was used to analyze the experimental results.

Microorganism and Preparation of Inoculum

A dextransucrase producing culture was isolated from sugarcane baggase and identified as *Leuconostoc lactis* KU665298 by 16s rRNA sequencing. The medium components and different physical parameters were previously optimized in shake flask level and used for bench scale fermentation (data not shown). The culture of *L. lactis* KU665298 was maintained on medium (pH 8.0), containing (%, w/v): Sucrose 2.0, peptone 1.5, beef extract 2.5, K_2HPO_4 2.5, $MgSO_4$ 0.02, $MnSO_4$ 0.001, NaCl 0.001, $CaCl_2$ 0.001, $FeSO_4$ 0.001 and agar 2.0. The stored culture of *L. lactis* KU665298 was transferred to autoclaved seed medium of same composition excluding agar for the preparation of the inoculum. The flasks containing *L. lactis* KU665298 inoculated seed medium were incubated at 25°C in an orbital shaker at 150 rpm and the exponential phase cell mass (21 h age) were used as inoculum for the production of dextransucrase. The size of the inoculum was in accordance with the previously optimized value (6.5%, v/v) for maximum dextransucrase production.

Sterilization and Inoculation of Production Medium for Different Fermentation Batches

Fermentation batches at 8 L working volume based on the different treatments in the experimental design were performed in a 14 L laboratory fermenter (BIOFERM-LS2, Scigenics India Pvt. Ltd.) at a constant temperature of 25°C. The initial medium pH was set at 8.0 and was not controlled during the course of fermentation. In addition to pH and temperature, the fermenter was well equipped with agitation, aeration, dissolved oxygen and antifoam sensors as well as controls. In situ sterilization of 7.8 L production medium [(%, w/v) sucrose 2.0, peptone 1.5, beef extract 2.5, K_2HPO_4 2.5, $MgSO_4$ 0.02, $MnSO_4$ 0.001, NaCl 0.001, $CaCl_2$ 0.001, and $FeSO_4$ 0.001] supplemented with 2 mL silicone oil as antiform agent was carried out at 121°C for 15 min. The sterilized production medium was inoculated with the inoculum (6.5%, v/v) for each experimental run through the inoculation port by peristaltic pump attached to an inoculation bottle. After inoculation, periodical sampling (at 2 h interval) was carried out and samples were analyzed for the growth of *L. lactis* cells and the production of dextransucrase. The pH and DO of the fermentation broth during the entire course of cultivation were recorded with the help of DO and pH probe for each fermentation batch.

Dextransucrase Assay

Dextransucrase converts sucrose into dextran and D-fructose. Dextransucrase activity was evaluated by measuring the reducing sugar released from sucrose using the oxide-reduction technique of the dinitosalacylic acid reagent [22]. In the presence of reducing sugars, the 3,5-dinitrosalicylic acid reduces to 3-amino-5-nitrosalicylic acid, resulting in color change (yellow to Red colouration) which is quantified by measuring absorbance at 540 nm.

e-ISSN: 2320-3528
p-ISSN: 2347-2286

Definition of Dextransucrase Activity

The enzyme activity was expressed in terms of units. One unit (U/ml) of dextransucrase activity was defined as the amount of enzyme that liberates 1 μmol of reducing sugar (fructose) per min per ml at 25°C in 25 mM sodium acetate buffer (pH 5.0) in a 2.0% (w/v) sucrose solution.

Determination of Volumetric Oxygen Transfer Coefficient (K_La)

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

$dC_L/dt = K_La\,(C^*-C_L)-Q_{o2}X \rightarrow$ Eq.1

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) ($mMO_2g^{-1}h^{-1}$)

Rearranging the equation 1:

$C_L=C^*-1/K_La\,(Q_{o2}X+dC_L/dt) \rightarrow$ Eq.2

In order to determine K_La, the aeration to fermenter has been stopped temporally when fermentation was in active stage, and decrease in dissolved oxygen concentration (C_L) was measured as a function of time for determination of oxygen uptake rate ($Q_{o2}X$). Aeration was established again 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.* $dC_L/dt + Q_{o2}X$ were correlated.

Role of K_La on Growth and Enzyme Production by *L. lactis* KU665298

The effect of K_La was determined by comparing the K_La values of different fermentation batches of *L. lactis* KU665298 with respect to the biomass and dextransucrase production. The results were demonstrated by K_La versus growth and enzyme activity curve.

RESULTS AND DISCUSSION

In the present study, combined effects of aeration rate and agitation speed on cell growth and dextransucrase production from *L. lactis* was studied during the course of fermentation by adopting a statistical tool called response surface methodology. A CCD of RSM was chosen to study the combined effect of agitation speed (rpm), and aeration rate (vvm) towards dextransucrase production. The CCD contained a total of 13 experimental runs (**Table 1**). The optimal value for the two components as obtained from the maximum point of the model was calculated to be as agitation speed (200 rpm) and aeration rate (0.85 vvm). Previously, a combination of the conventional method and RSM has also been applied in process optimization for production of 2,3-butanediol from maltodextrin by metabolically engineered *Klebsiella oxytoca*. The optimal conditions of pH, aeration rate, agitation speed and substate concentration were 6.8, 0.8 vvm, 400 rpm and 150 g/L, respectively [23].

Table 1: Different experimental runs designed by CCD with different treatments of agitation and aeration rate and dextransucrase production by *L. lactis* in each experimental run.

Run	Agitation Speed (rpm)	Aeration Rate (vvm)	Dextransucrase production (U/ml)
1	200	0.5	4.01
2	200	0.5	3.48
3	100	0.25	2.29
4	300	0.25	3.76
5	200	0.85	5.27
6	100	0.75	3.65
7	59	0.5	2.79
8	200	0.5	3.47

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p-ISSN: 2347-2286

9	200	0.146	3.36
10	200	0.5	3.75
11	200	0.5	3.86
12	341	0.5	2.32
13	300	0.75	2.77

The results were analysed by using ANOVA (analysis of variance), suitable for analysis of the designed experiment. The model F-value is calculated as ratio of mean square regression and mean square residual. The Model F-value of 6.72 implies the model is significant (**Table 2**). Model P-value (Prob>F) was found to be very low (<0.0133), which signifies the relevance of the model. The smaller the magnitude of the P, the more significant will be the corresponding coefficient. Values of P less than 0.05 generally indicate model terms are significant. “Lack of fit” is not significant which proves our model to be fit i.e. significant.

Table 2: ANOVA for response surface quadratic model analysis of variance.

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	
Model	6.25	5	1.25	6.72	0.0133	Significant
A-Agitation	6.971E-004	1	6.971E-004	3.748E-003	0.9529	
B-Aeration	1.18	1	1.18	6.34	0.0400	
AB	1.38	1	1.38	7.42	0.0296	
A2	3.02	1	3.02	16.23	0.0050	
B2	0.34	1	0.34	1.83	0.2184	
Residual	1.30	1	0.19			
Lack of Fit	1.08	3	0.36	6.40	0.0524	Not significant
Pure Error	0.22	4	0.056			
Cor Total	7.55	12				

Quadratic process order proved best and processed for further analysis due to low standard deviation (0.43) and high R-squared value (0.8275), respectively (**Table 3**). A negative "Pred R-Squared" implies that the overall mean may be a better predictor of response than the current model. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Our ratio of 8.139 indicates an adequate signal. This model can be used to navigate the design space. RSM based on CCD has also been applied to optimize aeration and agitation rate for lipid and gamma linolenic acid by a filamentous fungus in a 5-L bioreactor. Second order polynomial model was opted to evaluate the effects. Quadratic model indicated the significant interactive effect of agitation and aeration on lipid production [24].

Table 3: Quadratic fitted model analysis.

Std. Dev.	0.43	R-Squared	0.8275
Mean	3.44	Adj R-Squared	0.7043
C.V. %	12.52	Pred R-Squared	-0.0615
PRESS	8.01	Adeq Precision	8.139
-2 Log Likelihood	6.98	BIC	22.37
		AICc	32.98

3D graph was generated for regression analysis of CCD design, using pair wise combination of two factors for dextransucrase production. **Figure 1** represents the interaction between agitation speed and aeration rate where the

e-ISSN: 2320-3528
p-ISSN: 2347-2286

shape of the response surface indicates the effect of these two variables. The increase in the agitation speed from 100 rpm to 200 rpm and prolonged aeration rate from 0.25 vvm to 0.85 vvm led to increase in dextransucrase activity. However, further increase in agitation speed enzyme activity consequently decreases, although the increase in aeration rate from mean point results in increase in enzyme activity. The maximum dextransucrase activity obtained by performing suggested experiment was 5.27 U/mL which is more than predicted value 4.699 U/mL calculated by ANOVA and shown in Predicted *vs.* Actual plot (**Figure 2**). In the perturbation plot (**Figure 3**), the effect of agitation speed and aeration rate at the optimum run conditions in the design space were compared. A nearly steep curvature line in case of agitation speed (A) showed that the response of the enzyme activity was very sensitive to this factor, while the relatively less steep curvature lines of aeration rate (B) indicated lower sensitivity to change. Aeration cu rvature line shows that aeration has improved response above mean point and nearly constant response below mean point. In case of agitation curvature line show negative response above and below mean point and maximum response observed at mean point.

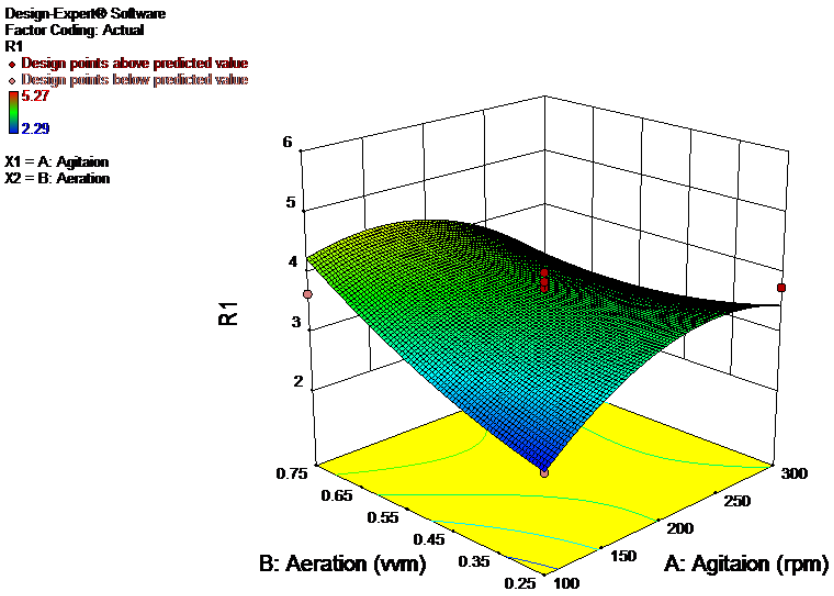

Figure 1: 3-D Response surface plot.

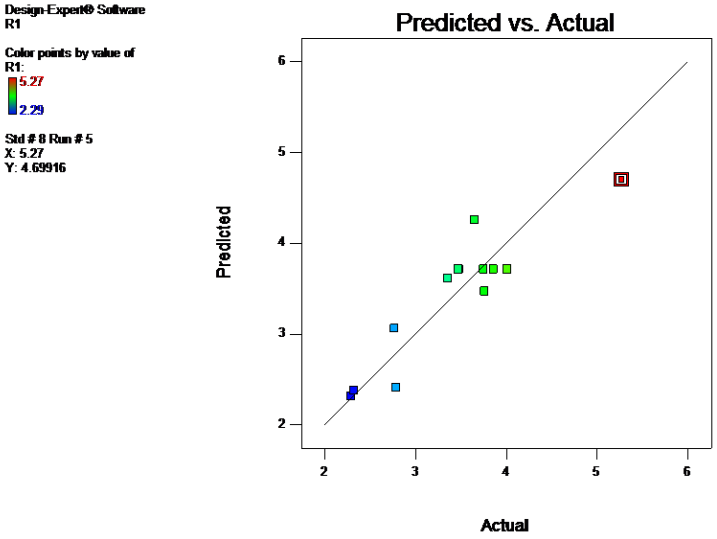

Figure 2: Predicted *vs.* Actual plot for different experimental runs in CCD.

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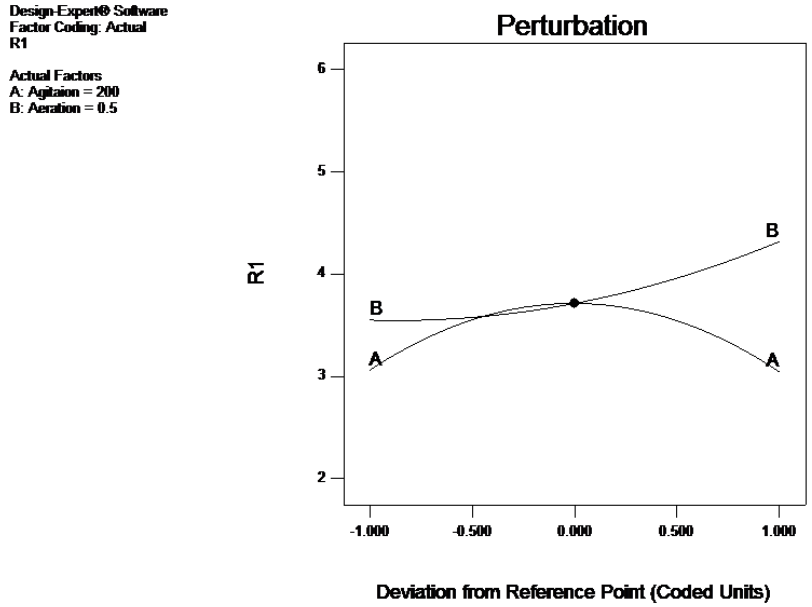

Figure 3: Perturbation plot showing the optimum value for different variable.

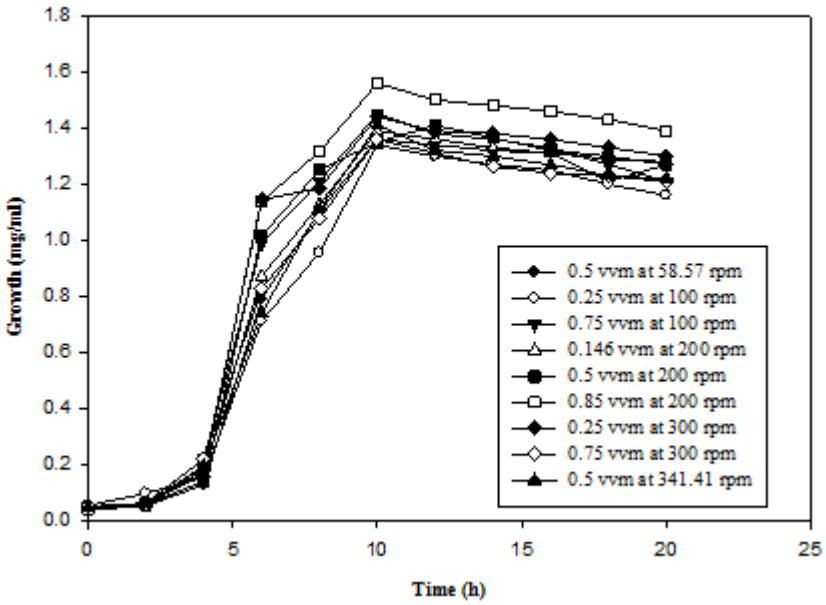

Figure 4a: Effect of agitation speed and aeration rate on cell mass production by *L. lactis* in fermenter.

Analysis of Combined Effects of Agitation and Aeration Rate on *L. lactis* Cell Biomass, Dextransucrase Production and Dissolved Oxygen Concentration of Fermentation Broth

The need of oxygen during the fermentation has to be statisfied by the correct set up of aeration and agitation leading to the transfer of a sufficient amount of oxygen to each cell [25]. The correct combination of agitation and aeration rate was also essential for the growth and hyper production of dextransucrase by *L. lactis*. There was an early attainment of the stationary phase in the fermentation batch with 59 rpm agitation and 0.5 vvm aeration rate. This might be due to the oxygen limitation for the growing cells of *L. lactis*. Further, two fermentation batches were designed with 0.25 and 0.75 vvm aeration rate and at 100 rpm agitation. Higher biomass (1.34 mg/mL) was observed with 0.75 vvm aeration as compared to (1.061 mg/mL) 0.25 vvm aeration. Cell biomass of 1.20, 1.22 and 1.56 mg/mL was recorded at 200 rpm agitation speed with aeration rates of 0.146, 0.5 and 0.85 vvm, respectively (**Figure 4a**). A further increase in agitation speed from 200 to 341 rpm resulted in decrease of *L. lactis* growth. This might be due to the shearing forces operative at high agitation rate. Therefore, 200 rpm of agitation speed at 0.85 vvm aeration was found to be the most optimum for

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p-ISSN: 2347-2286

the cultivation of *L. lactis*. Dextransucrase activity of 3.36 U/mL was recorded with 0.146 vvm aeration rate at 200 rpm agitation speed. The enzyme activity with 0.25 and 0.75 vvm aeration at 100 rpm agitation speed was found to be 2.29 U/ml and 3.65 U/ml, respectively. However, at 0.5 vvm aeration, dextransucrase activity of 2.79, 4.01 and 2.32 U/mL was attained with 59, 341 and 200 rpm agitation, respectively. The maximum dextransucrase activity of 5.27 U/ml was obtained with 0.85 vvm aeration at 200 rpm agitation speed at 10th h of fermentation, which coincide with the maximum cell biomass of *L. lactis* (**Figure 4b**). An Applikon®, 3 L volume bioreactor has been used to study the effects of aeration and agitation on xylitol production by employing a 22 factorial experimental design with a central point. Interaction of the factors (both aeration and agitation) had a significant effect on xylitol production. The highest yield was obtained with agitation at 100 rpm and, aeration at 0.1 vvm in semi anaerobic conditions [26]. A study by Saad et al. [24] showed that the optimal values of aeration rate and agitation speed were 0.32 vvm, and 599 rpm for lipid production and 1.0 vvm and 441.45 rpm for gamma linolenic acid production by Cunninghamella bainieri 2A1 in a 5-L bioreactor. A full factorial design was employed to evaluate the agitation and aeration rate in the production of dextransucrase by *L. mesenteroids* FT045B and maximal dextransucrase activity of 3.99 U/mL was obtained with agitation speed 100 rpm and aeration rate 1 vvm [27].

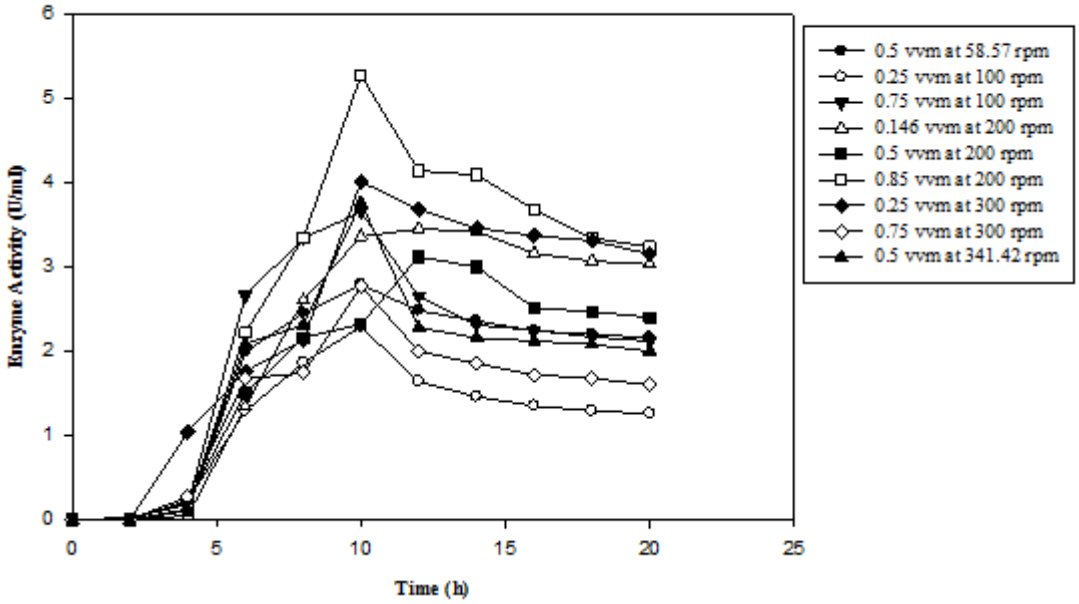

Figure 4b: Effect of agitation speed and aeration rate on dextransucrase activity of *L. lactis* in a laboratory fermenter.

The dissolved oxygen profiles of the fermentation broth under different sets of agitation and aeration reveals that depletion in the dissolved oxygen was insignificant at the higher rate of agitation and aeration (**Figure 4c**). There was a decline in DO from 100% to below 3% during first 8-10 h and then started to increase and reached to 100% at 10-12th h of fermentation. The effects dissolved oxygen and agitation on the production of serratiopeptidase (SRP) was investigated in a 5-L fermenter with 2-L working volume. The dissolved oxygen concentration, pH, biomass and SRP yield, were continuously measured during the course of the fermentation runs. The maximum SRP production of 11,580 EU/ml was obtained with an agitation of 400 rpm and aeration of 0.075 vvm, which was 58% higher than the shake-flask level [28]. 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 *L. lactis*. Optimum conditions for xylanase production by Penicillium citrinum in a 5-L laboratory-scale fermenter were found to be 5.0 pH, 30°C temperature, 400 rpm agitation, 1.0% aeration and 10% DO level. Combination of all the parameters resulted in 2.5 times higher enzyme activity compared to shake flask conditions [29].

Course of Fermentation of *L. lactis* for the Maximum Biomass and Dextransucrase Production

During the present study, 0.85 vvm aeration at 200 rpm agitation speed was found to be the most optimum for the production of dextransucrase by *L. lactis* in a laboratory fermenter. The fermentation by *L. lactis* cells for dextransucrase production was carried out in the optimized medium (pH 8.0) at 0.5 vvm aeration and 450 rpm agitation speed. The cells of *L. lactis* started growing exponentially after an initial lag period of 2 h and attained a stationary phase of growth after 10 h fermentation. The maximum cell mass (1.56 mg/mL) of *L. lactis* was observed at 10 h of fermentation. The maximum dextransucrase activity obtained was 5.27 U/mL at 10 h of incubation which coincided with the maximum growth of *L. lactis* (**Figure 5**). Hence, the dextransucrase production by *L. lactis* has been found to be growth dependent.

e-ISSN: 2320-3528
p-ISSN: 2347-2286

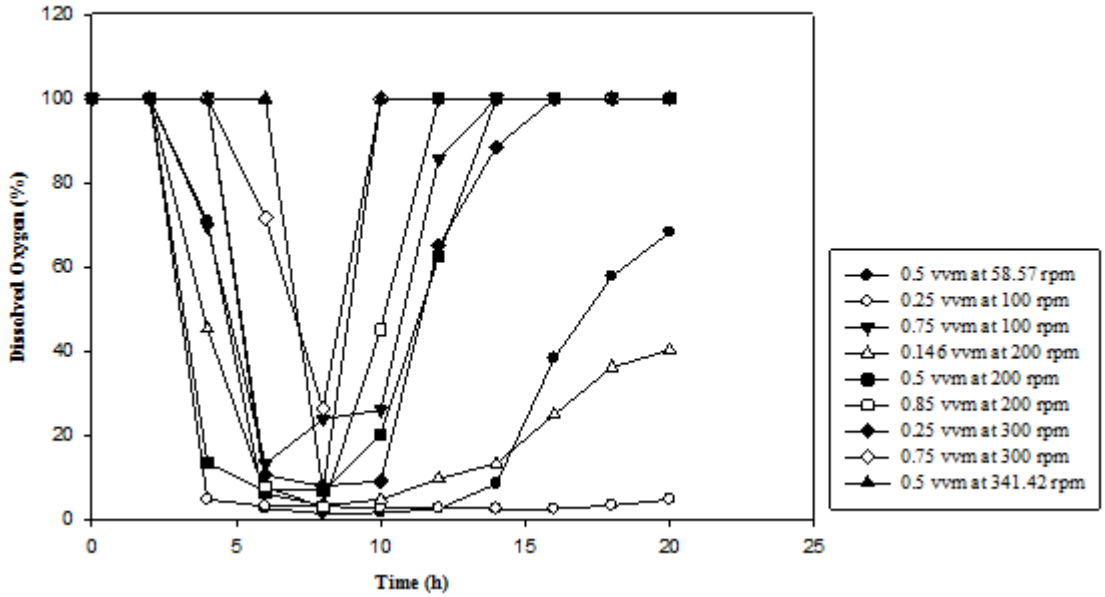

Figure 4c: Effect of agitation speed and aeration rate on dissolved oxygen during growth of *L. lactis* in laboratory fermenter.

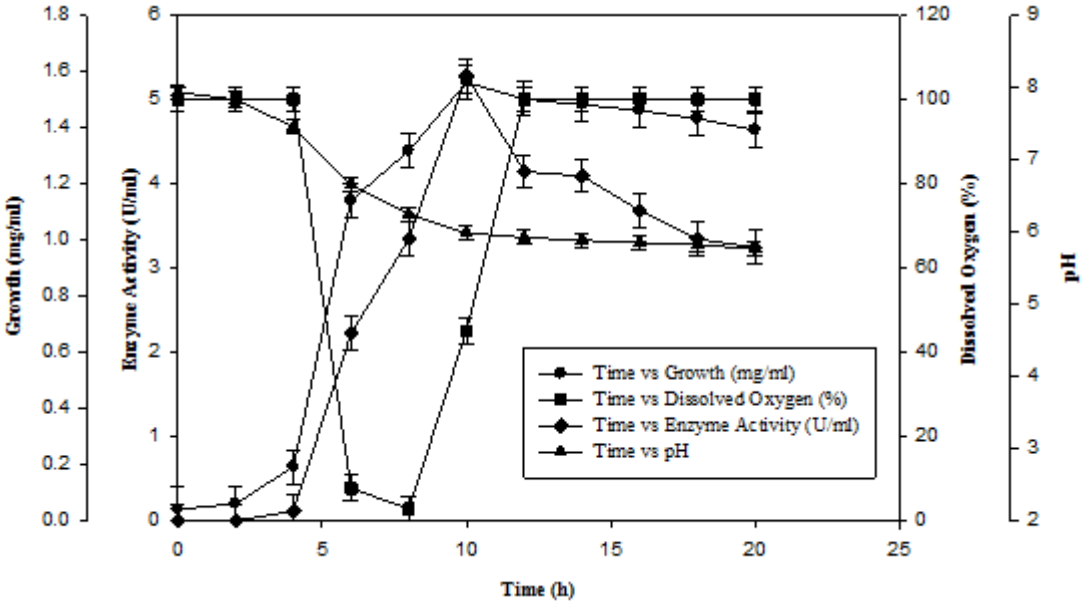

Figure 5: The course of cultivation of *L. lactis* for the production of dextransucrase.

The culture pH reduced from 8.0 to 6.0 at 10th h of growth. The pH of the broth decreased slowly up to the 12th h but once the cells reached their stationary phase of growth, the pH became more or less constant. This decrease in pH during the course of fermentation may be due to the acid metabolite production by the utilization of carbohydrate substrate in the initial phase of growth. The increase in cell mass of *L. lactis* leads to the rapid utilization of oxygen which reached to minimum at 8 h of fermentation. The concentration of dissolved oxygen has remained minimal till 10 h of incubation. Once the *L. lactis* cells reached the stationary phase, the dissolved oxygen concentration started increasing and attained 100% saturation at 12 h of incubation. Vengadaramana et al. [30] scaled up the production of α-amylase from 25 mL to 2 L and maximum amylase activity of 51.17 U/mL was recorded with 300 rpm agitation and 1.2 vvm aeration rate at 28 h of fermentation. The influence of agitation speed and aeration rate on dextransucrase production from *L. mesenteroides* B/110-1-1 in 5 L vessel has also been studied. Maximum growth rate and enzyme production were achieved between 6 to 7 h of fermentation. The optimal $K_L a$ value obtained was 30.85 h^{-1} at 0.15 vvm aeration rate and 225 rpm agitation speed [31]. Veljkovic et al. [32] reported that the oxygen transfer rate of 1.0 mmol l^{-1} h^{-1} was optimal for extracellular dextransucrase production by *L. mesenteroides* in batch fermentation without pH control. However, a

e-ISSN: 2320-3528
p-ISSN: 2347-2286

high dextransucrase activity of 21.9 U/ml has been reported in non-aerated fed-batch fermentation conditions by culturing *L. mesenteroides* NRRL B-512(F) [33]. Further, Scale-up of the this non-aerated process was carried out up to a 1000 dm^3 scale with enzyme broths and two batches of enzyme produced was used for dextran production [34]. High dextransucrase production of 11.0 DSU/mL from *L. mesenteroides* FT045 B was recorded with 132 rpm agitation and 0.15 vvm aeration at 25°C temperature using 3 to 4% sucrose [35].

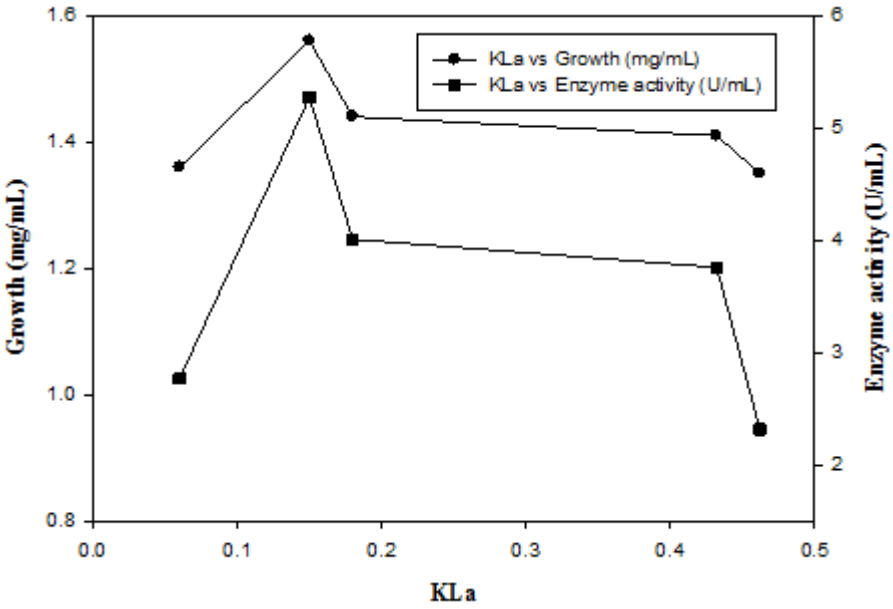

Figure 6: Effect of K_La on growth and dextransucrase production by of *L. lactis*.

Determination of Volumetric Oxygen Transfer Coefficient (K_La)

The availability of dissolved oxygen for microbial consumption depends upon relative rate of oxygen transfer and utilization. The volumetric oxygen transfer coefficient (K_La) is a critical parameter which was employed to analyse the oxygen transfer capabilities of bioreacter. The K_La varies with both the intensity of agitation and scale of aeration. The values of K_La during the cultivation of *L. lactis* were determined by dynamic oxygen balance equation. The DO concentration, C_L, has to be greater than the critical value of DO in order to avoid oxygen limitation. The total rate of microbial oxygen comsumption, $Q_{o2}X$ depends upon the microorganism and its growth rate in addition to pH, temperature, and medium components.

The maximum K_La (0.462 min^{-1}) was obtained for the fermentation batch with aeration rate of 0.5 vvm and agitation speed of 200 rpm whereas the minimum K_La, 0.06 min^{-1} was obtained at 0.75 vvm and 300 rpm. The K_La was the basis for evaluation of efficiencies of aeration and agitation for the production of serratiopeptidase by Serratia marcescens in a stirred tank bioreactor. K_La for the fermentation run with the maximum serratiopeptidase production at 400 rpm and 0.075 vvm aeration was 11.3 h^{-1} [28]. A study was conducted recently to evaluate the effects of K_La on the production of dihydroacetone from crude glycerol. It was observed that a high K_La of 52.05 h^{-1} (for baffled flask culture) and 82.14 h^{-1} (in the fed batch culture) showed a dramatic increase in dihydroxyacetone production [36].

Role of K_La in Growth and Enzyme Production by *L. lactis*

The K_La is a critical scale up factor in fermentation. The growth rate and dextransucrase activity of *L. lactis* was found to be affected by value of K_La. In the present study, maximum biomass (1.56 mg/ml) and dextransucrase activity (5.27 U/mL) was obtained at 0.15 min^{-1} K_La. A sudden decrease in biomass and enzyme production was observed with further increase in K_La from 0.18 to 0.462 min^{-1} (**Figure 6**). These outcomes propose that oxygen transfer upto a certain level benefit the growth and dextransucrase production by *L. lactis* in a bioreactor However, much higher K_La resulted in declination of growth and in turn reduction in dextransucrase activity of *L. lactis* due to oxygen toxicity or shear developed by higher oxygen transfer rate. The combined effect of aeration rate, agitation speed, and K_La on growth and dextransucrase production has been summarized in **Table 4**. Ghoshal et al. [29] investigated the effect of agitation and aeration rate on oxygen transfer coefficient (K_La) for the xylanase production by *P. citrinum* in laboratory scale stirred tank reactor. It was inferred that agitation had more pronounced effect than aeration. Role of oxygen transfer coefficient in production of dextransucrase by *Acetobacter tropicalis* was studied in 7-L laboratory fermenter. Maximum *A. tropicalis* cell biomass (1.41 mg/mL) and dextransucrase activity (15.8 U/mL) were attained in a fermentation run with 0.5 vvm aeration and 450 rpm agitation. Growth of *A. tropicalis* and production of dextransucrase were found to increase with

e-ISSN: 2320-3528
p-ISSN: 2347-2286

increase in K_La upto an optimum level and then started to decrease with much high K_La. The K_La for the fermentation batch supporting maximum dextransucrase activity by A. tropicalis was 0.28 min^{-1} [37]. However, high dextransucrase activity of 31 U/mL was obtained when a novel culture of *L. mesenteroides* T_3 (isolated from water kefir grain) was grown under static culture conditions at 23°C [38].

Table 4: Summary of results obtained during interactive experimental design.

Batch	Agitation (rpm)	Aeration (vvm)	Enzyme activity (U/ml)	Biomass (mg/ml)	K_La (min^{-1})
1.	58.57	0.5	2.79	1.36	---
2.	100	0.25	2.29	1.34	---
3.	100	0.75	3.65	1.45	---
4.	200	0.146	3.36	1.39	---
5.	200	0.5	2.32	1.35	0.462
6.	200	0.85	5.27	1.56	0.15
7.	300	0.25	4.01	1.44	0.18
8.	300	075	2.77	1.36	0.06
9.	341.42	0.5	3.76	1.41	0.432

CONCLUSION

An appropriate combination of agitation and aeration rate is necessary for growth and dextransucrase production by *L. lactis* KU665298 in a fermenter and K_La was found to be a critical factor. Different fermentation batches were designed to enhance the production of dextransucrase from *L. lactis* at minimum cost and time. Dextransucrase production from *L. lactis* has been improved by 1.46 fold after the scale up and production time was lowered to 10 h which favor the bioprocess economics. This suggests that dextransucrase production by *L. lactis* holds the potential for the commercial production of dextran and other useful oligosaccharides.

ACKNOWLEDGMENTS

The author Nisha Devi gratefully acknowledges the senior research fellowship from Department of Biotechnology, Govt. of India, New Delhi.

CONFLICT OF INTEREST

The authors have no financial conflicts of interest to declare.

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