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Research Article

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Experimental studies on determination of volumetric oxygen transfer coefficient in stirred tank bioreactor using fungal broths isolated from textile effluent

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ABSTRACT

The treatment of textile waste water was conducted by using biological aerobic process. The design features of bioreactors may be categorized on the basis of mass transfer aspects and the mechanical aspects of bioreactor. The differences in geometric dimension attributes often result in dissimilar mass transfer, shear dynamics and mixing inside the bioreactor, that lead to disparities in cell growth. Experiments to evaluate the mass transfer characteristics were conducted to determine the nutrient requirements of the culture. The aim is to study the k_La of the bioreactor of different scales which is used as a main parameter for scaling up of cell culture process. The fungal used for the studies are Asperigillus Terrus and Asperigillus fumigates which are isolated from the textile waste sludge. The results of these characterization studies were used to define the scale up strategy for process.

Key words: Bioreactor, k_La, Asperigillus *Terrus, Asperigillus Fumigates.*

INTRODUCTION

The analysis of bioreactor performance determine by means of mixing time and mass transfer coefficient (1). The k_La values are dependent on factors such as agitation rate, gas velocity, bubble size, temperature, gas sparging system and cell morphology (2). The cells are expanded in shake flasks (1L) by sub culturing the cells every 2-3 days once they attain the transfer criteria established for these steps. These cells are then used to be used to inoculate the 5L bioreactor. Cells are grown in this bioreactor for 2-3 days. To understand the effect of these geometrical differences on hydrodynamic properties of the vessels, characterization experiments were performed. The data from these experiments was used to decide the starting parameters for operation of the large-scale bioreactor when scaled up. Scale-up is a complex problem in industries. Scale-up methods based on aeration efficiency have been traditionally used in the bioreactor field. The parameters for scale-up are volumetric mass transfer coefficient, Power consumption per unit volume, Impeller tip velocity, mixing time. The oxygen transfer rate is dependent upon the volumetric mass transfer coefficient and the driving force, $C^*- C_L$, (where C_L, is the dissolved oxygen concentration and C* is the oxygen saturation concentration in the liquid phase at the gas-liquid interface). K_La estimation was done using the static gassing out method (3). As explained earlier, the volume of the bioreactor was going to be high because of the higher dilution. Hence there is a large volume of culture that is away from the impellor. Hence estimating the k_La for different volume was done to provide an understanding of mass transfer to set both the starting conditions and the final conditions in the batch. The rate of oxygen transfer from air bubbles to liquid in a batch stirred bioreactor was given by the relationship

$$\frac{dC_L}{dt} = k_L a\,(C^* - C_L) \qquad (1)$$

The oxygen transfer rate (OTR) was determined using the static gassing out method (4). The change in dissolved oxygen concentration, C_L in the liquid phase was detected using a polarographic oxygen probe. At different combinations of airflow rates and stirrer speeds as described earlier, the dissolved oxygen concentration, C_L profile with respect to time was graphed. The k_La was then determined by the slope obtained from the semi logarithmic plot of time, the dissolved oxygen saturation concentration in the liquid. The solubility of oxygen in the broth is a function of the media composition, temperature and pressure. The dependency with temperature and pressure can be quantified accurately enough by applying Henry's law. However, the dependency with the medium composition is rather difficult to describe and is normally neglected. The medium composition maintained was K_2HPO_4 (1.6), Na_2HPO_4 (0.6), NH_4NO_3 (1.0), NaCl (0.5), $MgSO_4.7H_2O$ (0.1), $CaCl_2$ $2H_2O$ (0.1) in g/l. The relative magnitudes of the various mass-transfer resistances depend on the composition and rheological properties of the liquid, mixing intensity, bubble size, interfacial adsorption. At steady state there is no accumulation of oxygen at any location in the bioreactor; therefore, the rate of oxygen transfer from the bubbles must be equal to the rate of oxygen consumption by the cells. The efficiency of gas-liquid mass transfer depends to a large extent on the characteristics of bubbles in the liquid medium. Bubble behavior strongly affects the value of k_La, some properties of bubbles affect mainly the magnitude k_L, whereas others change the interfacial area. Small bubbles have correspondingly slow bubble-rise velocities; consequently they stay in the liquid longer, allowing more time for the oxygen to dissolve. Small bubbles therefore create high gas hold-up, defined as the fraction of the fluid volume in the reactor occupied by gas. Because the total interfacial area for oxygen transfer depends on the total volume of gas in the system as well as on the average bubble size, high mass-transfer rates are achieved at high gas hold-ups. Under normal operating conditions, a significant fraction of the oxygen in fermentation vessels in contained in the gas hold-up. The temperature of aerobic fermentations affects both the solubility of oxygen and the mass-transfer coefficient k_L. Increasing temperature causes C^* to drop, so that the driving force for mass transfer is reduced. At the same time, diffusivity of oxygen in the liquid film surrounding the bubbles is increased, resulting in an increase in k_L. For temperatures 10^0C to 40^0C increase in temperature is more likely to increase the rate of oxygen transfer. Above 40^0C the solubility of oxygen drops significantly, adversely affecting the driving force and rate of mass transfer. The bioreactor maintained at 35^0C the volumetric oxygen mass-transfer coefficient k_La is usually high. Oxygen transfer is influenced by the presence of cells in fermentation broths; the nature of the effect depends on the species of organism, its morphology and concentration. Cells with complex morphology generally lead to lower transfer rates. Cells interfere with bubble break-up and coalescence; cells, proteins and other molecules which adsorb at gas-liquid interfaces also cause *interfacial blanketing.* Then concentrations of cells, substrates and products change throughout batch fermentation, hence the value of k_La can also vary. Shear rate is essential because microorganisms, bio-flocs and other suspended solids are susceptible to damage that is dependent on the prevailing shear rate and associated shear stress (5).

EXPERIMENTAL SECTION

MICROORGANISM AND CULTURE MAINTENANCE

In this study the fungal screening was performed. About 10 strains was isolated among them the two fungal strain shows the maximum ability in decolorizing the dye. These dual fungal strains used for mass transfer studies and was identified on the basis of morphological characteristics and 16S rDNA sequences were analyzed at NCBI server were *Asperigillus Terreus* (KJ522845) and *Asperigillus Fumigates* (KJ522846) **(Fig. 1, 2)** isolated from textile effluent collected from Common Effluent Treatment Plant, Kanchipuram, was used in this study (6). The strain was grown and maintained on nutrient agar slant at 4ºC. Inoculum was prepared by suspending the spores from slant. Cells were grown in 250 ml Erlenmeyer flasks containing 100 ml medium containing glucose - 1.0 mg/l, yeast extract- 0.2 mg/l, peptone - 0.5 mg/l. The cell suspensions were aseptically transferred to for further studies (7).

MEASUREMENT OF DISSOLVED OXYGEN

The concentration of dissolved oxygen CA_L in fermenters is normally measured using a *dissolved-oxygen electrode.* The most common type of DO electrode is the polarographic electrode. The *electrode response time* can be measured by quickly transferring the probe from a beaker containing medium saturated with nitrogen to one saturated with air. It can be measured by making a step change in oxygen partial pressure in the measurement medium and measuring the sensor response.

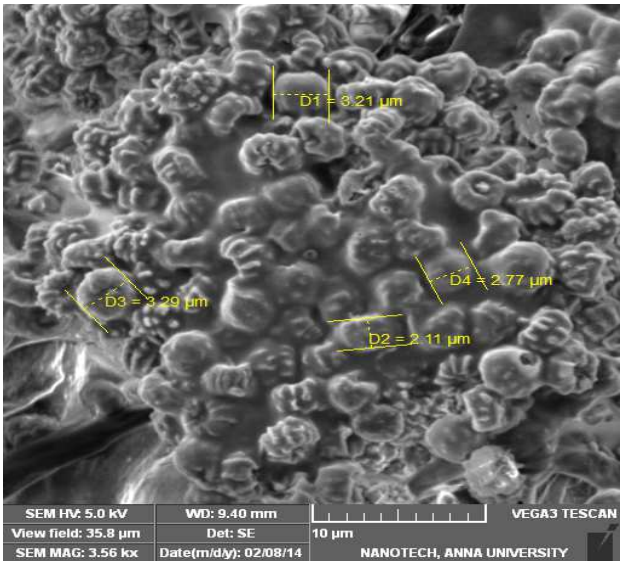

Fig 1 SEM image of Asperigillus Terreus

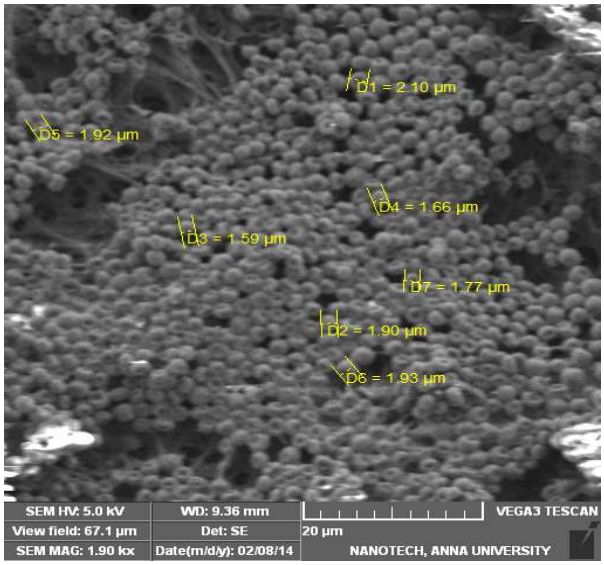

Fig 2 SEM image of Asperigillus Fumigates

STATIC GASSING-OUT TECHNIQUE

Bioreactor k_La was done by static gassing out method (8, 9). In this technique, the oxygen concentration of the solution is lowered by gassing the liquid out with nitrogen gas, so that the solution is scrubbed free of oxygen. The deoxygenated liquid is then aerated and agitated and the increase in dissolved oxygen was monitored using the dissolved oxygen probe. Assuming that the liquid is well mixed and there is no oxygen uptake. Integrating equation (1) we get,

$$\int_{C_L^0}^{C_L} \frac{dC_L}{C^*\text{-}C_L} = k_L a \int_0^t dt \qquad (2)$$

$$k_L a = \frac{1}{t} \ln\left(\frac{C^* - C_L^0}{C^* - C_L}\right) \qquad (3)$$

$$ln\,(C^* - C_L) = \ln(C^* - C_L^0) - t(k_L a) \qquad (4)$$

DETERMINATION OF MIXING TIME

Mixing time is the time required to achieve a predefined level of homogeneity in the bioreactor. Mixing time studies were conducted by adding sodium hydroxide (0.3N) solution at the top of the bioreactor and measuring the change in pH. The time taken for the pH to stabilize is noted as the mixing time for the bioreactor at the given agitation speed (10).

RESULTS AND DISCUSSION

The oxygen transfer coefficient was calculated by plotting a graph of $ln\,(C^* - C_L)$ against time (11, 12). The slope of the graph is $k_L a$, and the intercept of $\ln(C^* - C_L^0)$. The kLa value obtained for 1l/min of air sparging was 3.6 to 7.2 hr^{-1} for the agitation of 70 to 150 rpm. (**Fig 3a**) Similarly for 2.5 l/min at 70 to 150 rpm was 7.2 to 12.6 hr^{-1} (**Fig 3b**) and 5 l/min 9.0 to 13.32 hr^{-1} (**Fig 3c**) the values were found to have a higher K_La values due to baffled configurations which may have reflected in them having comparatively lower K_La values. Since mixing time is not playing a major role in the process. Process mainly dependent on k_La and this clearly defines shear stress which can damage the cells. Even though mixing time cannot be directly used to scale up processes because of the sheer sensitivity, it is performed to understand the mixing difference between scales, so that accordingly feed flow rates can be modified to avoid any pH and osmolality shock.

Table 1 Values of k_La at various runs

Run No.	Air Sparging rate (l/min)	Agitation (rpm)	K_La (h^{-1})	R^2
1	1	70	3.6	1
2	1	100	4.32	0.954
3	1	150	7.2	0.962
4	2.5	70	7.2	0.981
5	2.5	100	9.36	0.97
6	2.5	150	12.6	0.979
7	5	70	9	0.978
8	5	100	10.8	0.96
9	5	150	13.32	0.948

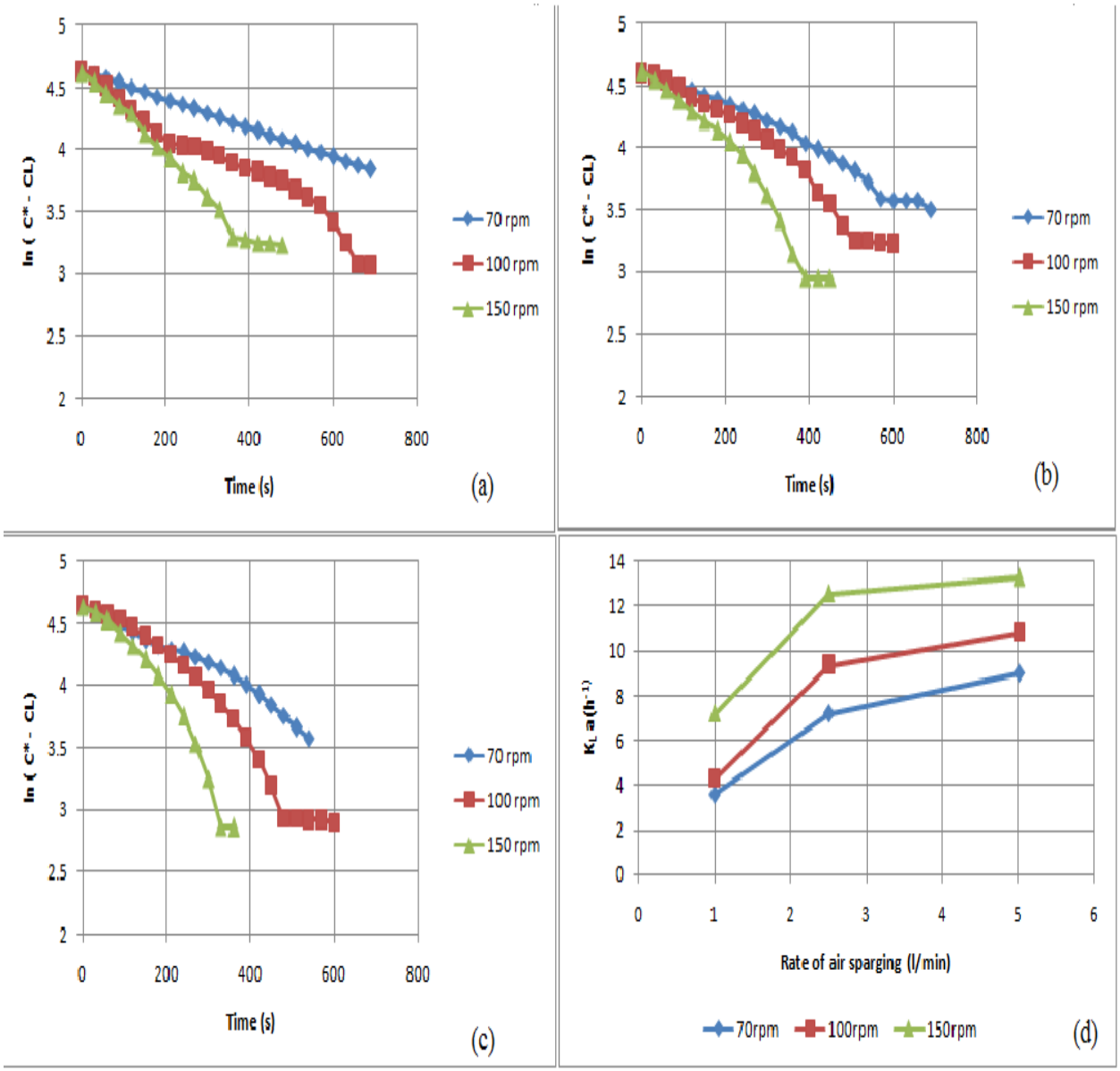

Fig 3 Values of k_La at various air sparging rates (a) 1 l/min (b) 2.5 l/min (c) 5 l/min (d) comparison

CONCLUSION

The results of the experimental run showed that the process set points used in the experimental run worked well to propagate the inoculums. The air flow rates and agitation were optimized based on the pH and pCO_2 profiles. The following process trends were found to be comparable to that of the 5L batches. However, based on the process

performance of the seed and production stage, it was found that higher flow rate and higher rate of mixing leads to cell death, the cell growth was poor and cell viability was found to be lower than desired range.

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