To Study Yeast Growth Kinetics in a Specially Designed External Loop Airlift Bioreactor

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Abstract

Two types of airlift fermenters, conventional (UT-ALF) and modified (CDT-ALF) were investigated to evaluate their performance with respect to baker's yeast growth. The riser tube of conventional external loop airlift fermenter is replaced by a converging-diverging tube. The new reactor is called modified airlift reactor.

The results were compared for the two types of airlift fermenter (UT-ALF and CDT-ALF). Growth rates (dx/dt) were determined from experimental data for both the reactors under identical operating conditions and compared. CDT-ALF always shows higher growth rate compared to UT-ALF under any operating condition. Maximum growth was reported in CDT-ALF at 50 gm/l initial glucose conc. and 1.0 vvm air flow rate which was 20 % higher than UT-ALF. Yield (YX/S) was found to be 0.51which is theoretically very near to maximum achievable value.

Keywords: *Yeast cell, Bioreactor, Growth rate, Airlift fermenter (ALF), Culture, Air flow rate.*

Symbols:

ALF	*Air-lift fermenter*
CDT	*Converging – diverging tube*
UT	*Uniform tube*
KLa	*Volumetric mass transfer coefficient (1/ hr.)*
D.O	*Dissolved oxygen*
DNS	*Dinitrosalicylic acid*
O D	*Optical density*
vvm	*Volume per volume per minute*
dx/dt	*Growth rate*
dmax	*Maximum dx/dt*
tmax	*Time to achieve dmax*

1. Introduction

Oxygen supply is a critical factor for many aerobic fermentation processes [1]. Initial glucose concentration in batch culture is another crucial parameter which control cell mass

growth. Higher initial sugar concentration will lead to alcohol production. Yeast is an aerobic organism. The growth behaviour [2] of this yeast can be roughly subdivided into oxidative growth will cell yield (high oxygen uptake rates) and fermentative growth with ethanol production. If initial sugar conc. is low, final cell conc. becomes low.
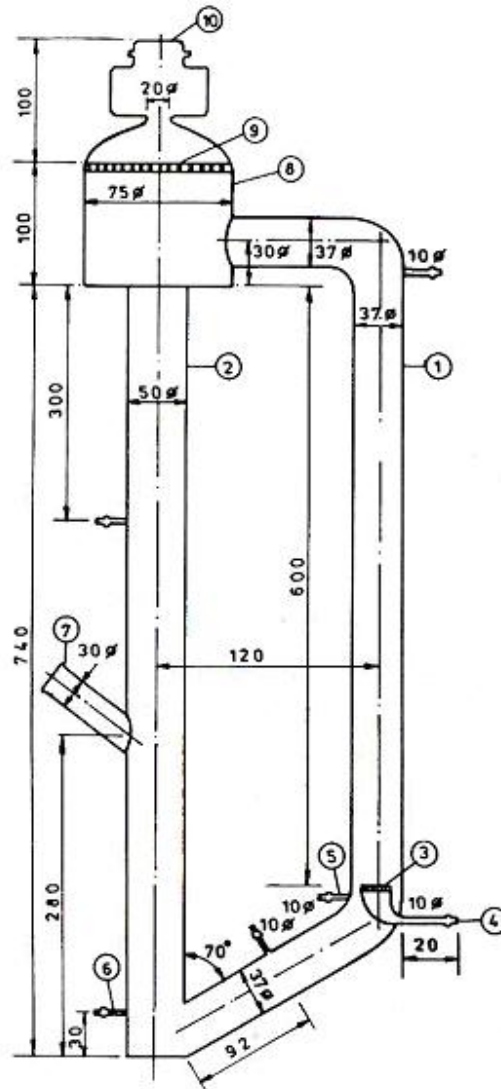

Figure 1. Conventional External Loop Uniform Tube Air-Lift Fermenter (UT-ALF).

1. Riser 2.Down-comer 3.Gas sparger with 25φ sintered glass 4. Gas inlet 5. Sampling port 6. Drain 7. Probe point 8. Gas liquid separator 9. Perforated plate 3φ on 10 mm pitch 10. Gas outlet
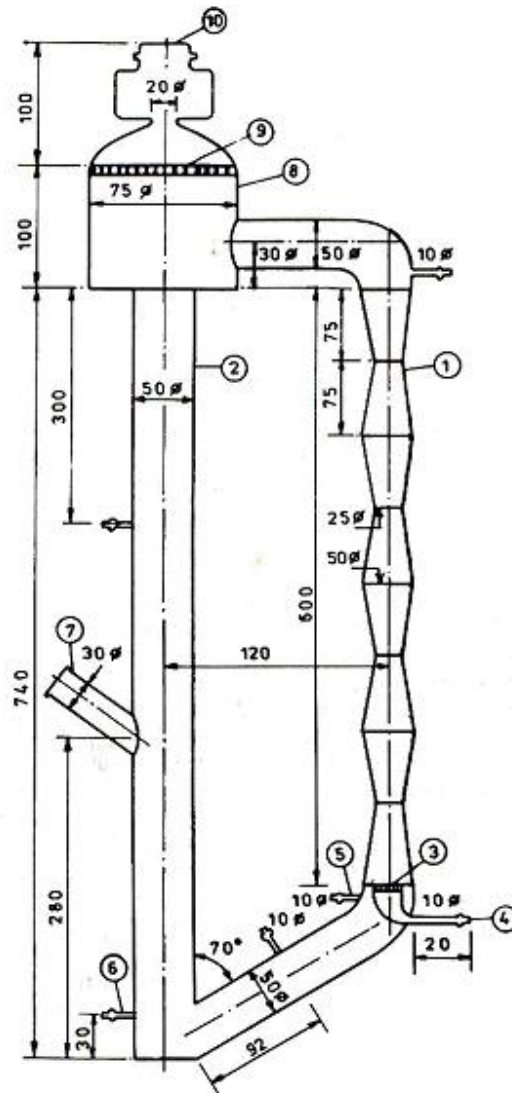All dimensions are in mm.

Figure 2. External Loop Converging-Diverging Tube Air-Lift Fermenter (CDT-ALF).

1. Riser 2. Down-comer 3. Gas sparger with 25ϕ sintered glass 4. Gas inlet
5. Sampling port 6. Drain 7. Probe point 8. Gas liquid separator
9. Perforated plate 3ϕ on 10 mm pitch 10. Gas outlet
All dimensions are in mm.

To the contrary at increased initial glucose concentration the crabtree-effect will arise. Airlift fermenters were used extensively in the past for commercial production. To improve performance of UT-ALF (Uniform tube airlift fermenter) as in figure 1, the riser part was replaced by an irregular geometry in the form of a converging diverging geometry. The modified system is named converging diverging tube airlift fermenter (CDT-ALF) as in figure 2 [3, 4].

Performance of the airlift reactors with respect to growth rate (dx/dt) and effect of airflow rate and initial sugar concentration on yeast growth were studied [5, 6].
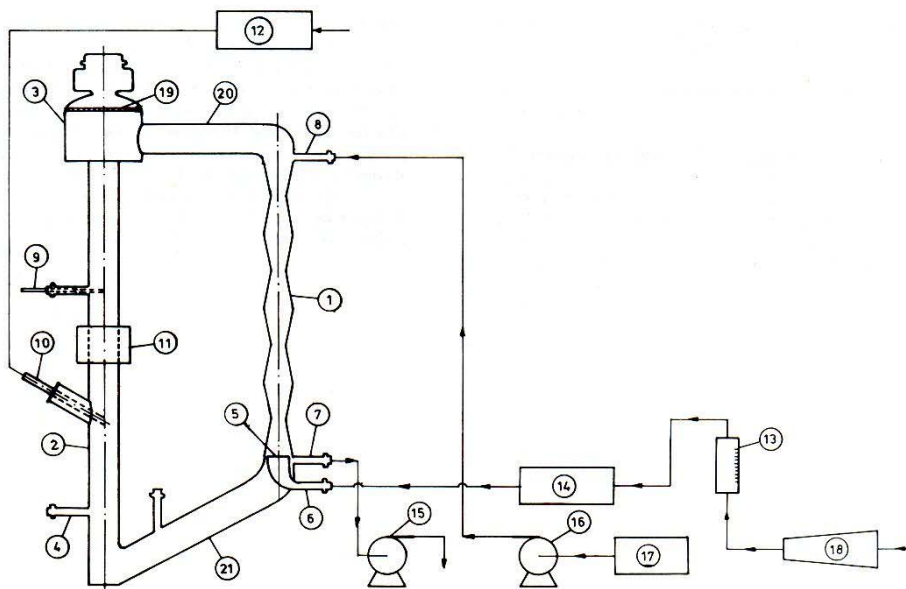

Figure 3. Experimental Set-up.

1. Riser   2. Down-comer   3. Gas liquid separator   4. Drain port   5. Sintered plate separator   6. Air inlet port 7. Sampling   port   8. Feeding port   9. Thermometer   10. Dissolve oxygen probe   11. Heating coil   12. D.O probe display   13 Rot meter   14. Air filter   15. Peristaltic pump for sampling   16. Peristaltic pump for feeding fresh medium   17. Sterilized medium reservoir   18. Oil free compressor   19. Perforated glass plate   20. Top connector   21. Bottom   connector.

2. Experimental Set-up

Figures 1 and 2 represent two types of airlift fermenters (ALF) with which investigations were performed. These are conventional uniform tube airlift fermenter (UT – ALF) figure 1 and converging – diverging tube air lift fermenter figure 2. In general, an airlift fermenter system comprises three major parts ----- i) riser ii) down-comer and iii) gas–liquid separator. The main differences between the two systems are in the construction of the riser part only. In case of conventional ALF (UT-ALF), the riser is made of uniform tube, whereas, the modified ALF has converging – diverging tube as riser. In both the cases the height and volume of the risers is maintained equal. The major dimensions of the two ALF systems are presented in table 1.

Table 1. Details of Air-lift fermenter (ALF) used.

	UT-ALF	CDT-ALF
1. Riser height	0.600.0	0.600.0
2. Down comer height	0.740.0	0.740.0
3. Riser diameter		
For CDT-ALF		
D_{max}		0.050.0
D_{min}		0.025.0
For UT-ALF		
Dave	0.037.5	
4. Down comer diameter	0.050.0	0.050.0
5. Distance between riser and downcomer	0.120.0	0.120.0
6. Diameter of top connector	0.050.0	0.050.0
7. Diameter of bottom connector	0.050.0	0.050.0
8. Diameter of gas-liquid separator	0.075.0	0.075.0
9. Height of gas-liquid separator	0.200.0	0.200.0
10. Diameter of sintered glass sparger	0.025.0	0.02.0
11. Volume of the fermenter (m^3)	0.0020	0.0020

Material of construction: Borosil glass, All dimensions are in meter

The experimental set-up has been schematically shown in figure 3. The experimental set-up has been described as follows. Item (1) represents the riser tube. Riser is connected with the down-comer (2) which has gas liquid separator (3) at its top. To maintain desired temp. A suitable heating coil (11) with thermostat is provided with the down-comer. A thermometer (9) is used to measure the temp. of water inside down-comer. A Dissolved oxygen (D O) probe has also been inserted in the down-comer which has given connection with the display (12) of the probe. To drain the liquid a port (4) has been provided at the bottom of the down-comer. A sintered glass air sparger (5) is connected at the bottom of the riser to disperse the filtered air from oil free compressor (18) which was metered through a rotameter (13) and air filter (14). Item (7) is the sampling port which is connected with a peristaltic pump (15) to discharge sample for analysis.

3. Materials and methods

3.1 Experiments on batch growth

The airlift fermenter system without medium was sterilized by heating *in a horizontal autoclave at a* temperature of 121 ^{0}C for 15 minutes. The bulk medium was sterilized batch *wise and then inoculated with a* strain of baker's yeast. For batch operation both the reactors were aseptical*y filled with inoculated medium* with the help of peristaltic pumps. The culture volume in each of the reactor was two liters approximately. The sterilized air was introduced through a sparger of sintered glass plate. The f*ermentation temperature in the* reactor was maintained at 29 $\pm$ 1 ^{0}C.

Range of variables studies:

Air flow rate	0.5 to 3.0 vvm
Total time	08 to 22 hrs.
Glucose conc.	10 to 70 gm/ L.

Both the reactors were operated under identical operating conditions. *Samples were collected through* the sampling port no.7 at an interval of one hour with the help of a per*istaltic pump (15). The* residual glucose conc. was determined quantitatively by a colorimetric method.

Materials
Baker's yeast S. cerevisiae (NCIM 3190) was selected for the investigation.
Composition of media for yeast culture

Glucose	10 ----- 70 gm/L
Ammonium sulphate	3.5 gm/L
Magnesium sulphate	1.5 gm/L
Yeast extract	0.5 gm/L
K_2Hpo_4	7.0 gm/L
Na_2Hpo_4	2.0 gm/L
$CaCl_2$, $6 H_2O$	0.2 gm/L
PH	4.5 – 5.0

Methods:

Glucose estimation
Reagents
   Composition of DNS (dinitrosalicylic acid): 10 grams of DNS, 2gms of phenol and 10 gms of sodium hydroxide were dissolved in one liter distilled water with stirring. To the aliquots of this reagent, sodium sulfite, at the rate of 0.05 % was added just prior to the use of the reagent.
d) Rochelle salt solution (40 %): 40gms of Rochelle salt was dissolved in 100 ml distilled water.

Procedure:

   Glucose was estimated quantitatively by Miller method. Fermented bro*th was*centrifuged and 0.5 ml clear supernatant was taken for glucose estim*ation.*   Three ml DNS reagent and 0.5 ml distilled water were added to it, heated in a boiling water bath for three minutes*, and then cooled in* running tap water.   21 ml water was added to it.   So, total volume was 25ml.   Then optical density (O.D) was measured by spectrophotometer. With the help of a glucose standard c*urve unknown glucose conc. was* determined by known O D value.

4. Result

   Initial glucose concentration and initial cell mass concentration (dry wt.) were maintained almost identical in both the reactors. Both the reactors were operated under identical operating condition.   Samples were collected every one hour interval. Cell

mass concentration (dry weight, X gm/l) and residual glucose concentrations were determined experimentally with standard method.

Range of variable studied:
Air rate   0.5   to   3.0 vvm
Initial glucose concentration   10 gm/I   to   50 mg/l
Maximum dx/dt (d $_{max}$) were calculated from each   x vs t plot

4.1 Effect of air flow rate on dx/dt when initial glucose concentrations were 10 gm/l and 30gm/l

The results depicted in figure 4 shows that as the airflow gradually increased (0.5 to 3.0 vvm) the d$_{max}$ (maximum dx/dt) were decreased accordingly. But at any operating condition CDT-ALF always shows higher d$_{max}$ compared to UT-ALF. This difference is very less at high air rate. (3.0 vvm) Maximum d$_{max}$ was reported for the lowest air rate of 0.5 vvm.
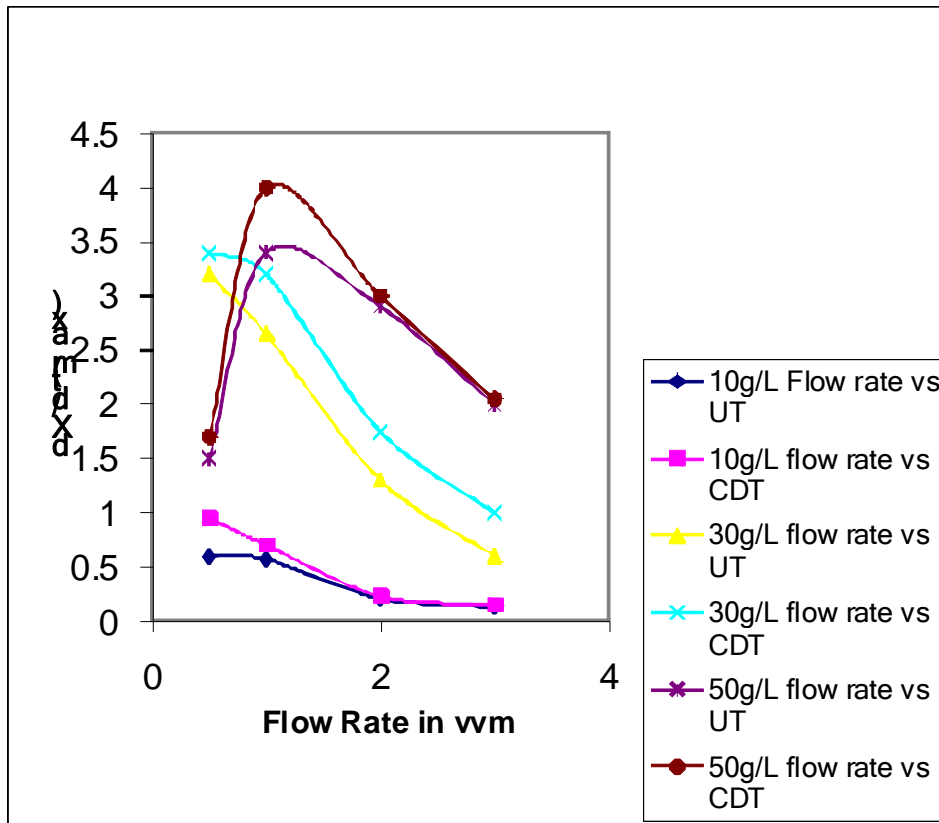

Figure 4. Effect of air flow rate on dx/dt max.

4.2 Effect of air flow rate on dx/dt when initial glucose concentrations was   50 gm/l

Figure 4 also illustrates the dependence of d$_{max}$ on air rate when   initial glucose concentration was increased to 50 gm/l. This pattern is quite different compared to previous one. As air rate increased from 0.5 to 1.0 vvm d$_{max}$ also increased to its highest value and goes down as air rate further increased from 1.0 to 3.0 vvm. One

remarkable observation was that maximum d_{max} was reported for 1.0 vvm air rate. CDT-ALF reported much higher d_{max} compared to UT-ALF at 1.0 vvm.

Time to achieve d_{max} was recorded from experimental data. Results as presented in figure 5 shows that time (t_{max}) to achieve d_{max} increased gradually as air rate increased from 0.5 to 3.0 vm. for initial glucose concentration 10 gm/l and 30 gm/l When glucose concentrati0n was increased from 30 to 50 gm/l a complete different pattern was observed. As air flow increased from 0.5 to 1.0 vvm time to achieve d_{max} reduced drastically. When air rate increased further (1.0 to 3.0 vvm) time to achieve d_{max} increased much higher than previous value. At 0.5 vvm (5o gm/l) CDT-ALF takes less time compared to UT to achieve d_{max} Results are presented in figure 5.
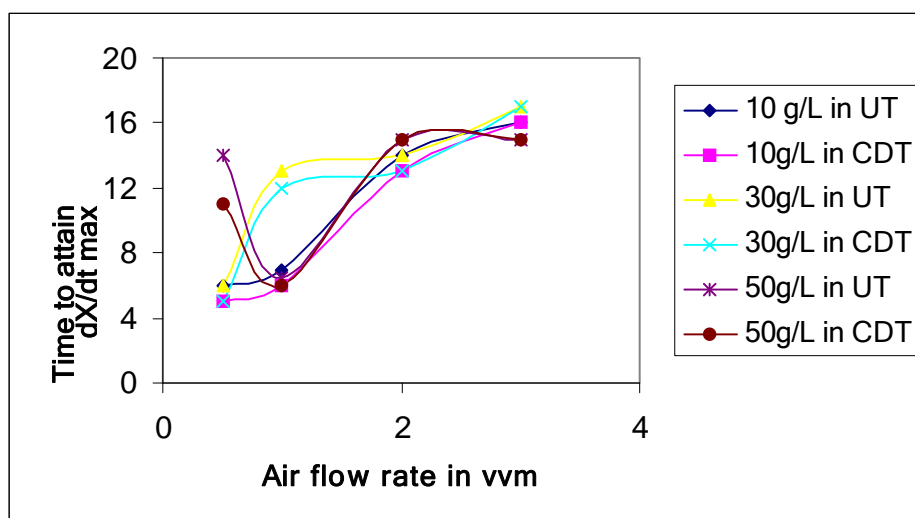

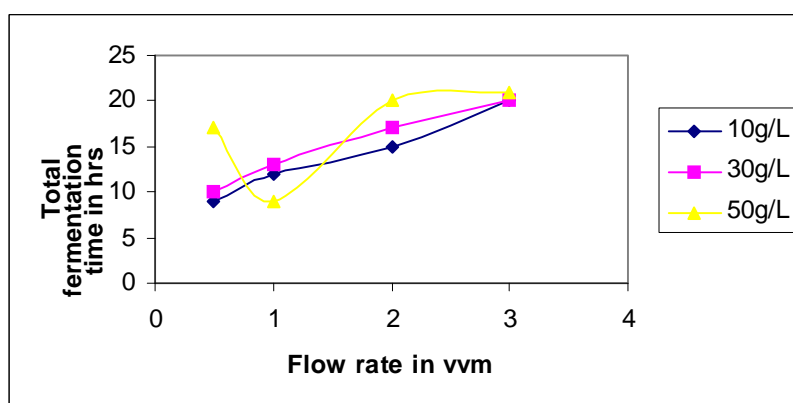Figure 5. Effect of air flow rate on time to achieve dx/dt max.

Figure 6. Effect of air flow rate on total fermentation time.

Total fermentation time also recoded for each set of experiment. Results depicted in figure 6 shows that as air rate increased gradually from 0.5 to 3.0 vvm total fermentation time also increased accordingly. A remarkable result was recorded when initial glucose concentration was increased from 30 to 50 gm/l as in figure 6. According to the figure 6 as air flow increased from 0.5 to 1.0 vvm total fermentation

time reduced drastically. Total fermentation once again increased more than 100 % when air rate further increased from 1.0 to 2.0 vvm. Total fermentation times were almost equal in UT and CDT.

5. Discussion

5.1. Effect of airflow rate on d_{max} at 30 gm/l initial glucose concentration

In batch cultivation as air flow rate increased d_{max} gradually decreased (figure 4). In airlift fermenter with the increase of airflow rate liquid re-circulation velocity also increased resulting in higher share force. This may be the reason for low d_{max} at high air rate. Hong Sun et al studied the effects from shear stress on morphology and growth [7]. D. Taherzadeh et al reported that hydrodynamic conditions have a significant impact on the bio-film life cycle [8]. At high turbulence yeast cells need more energy to survive. As a result higher maintenance energy is required to sustain causing low growth rate (low d_{max}). Moreover, higher metabolic byproduct is must to generate required maintenance energy. This metabolic byproduct inhibits cell growth. As a result low d_{max} and very high total fermentation time were reported at higher air rate. Time (t_{max}) to achieve d_{max} also is directly proportion to air flow rate (figure 5 and figure 6).

In cultivation processes the dissolved oxygen (D.O) concentration is generally accepted as critical parameter [9]. Dissolve oxygen concentration (D.O) is treated as medium component (limiting substrate) for yeast cell growth [9]. According to the result presented earlier [10] CDT-ALF always gave higher volumetric mass transfer coefficient ($K_L a$) compared to UT-ALF, and in CDT-ALF $K_L a$ decreased gradually with increased air rate. This may be the reason why in CDT-ALF d_{max} were always higher compared to UT-ALF (figure 4).

In case of CDT-ALF with the increase of airflow rate $K_L a$ decreases resulting low oxygen availability in the medium. Again with the increase of air rate liquid re-circulation velocity increased causing high shear force and high turbulence [11,12]. All these effects are detrimental to cell mass growth which has been observed during experiment. Low d_{max} and high total fermentation time at higher air rate (figure 4 and figure 6).

However, in case of UT-ALF, with the increase of airflow oxygen transfer increases [10] and this phenomenon should go in favour of increased d_{max}. But from experimental result it was reported that with the increase of airflow d_{max} gradually decreased. The reason for such contradiction may be attributed to the fact that with the increase of air rate shear force increases in any system and this effect might be superseding the positive effect of higher oxygen concentration in the media for cell mass growth in UT-ALF.

Therefore, in both fermenter systems at higher airflow rate d_{max} becomes equally low. For the above mentioned reason total fermentation time and time (t_{max}) to achieve d_{max} also proportional to air rate for 10 and 30 g/l initial sugar concentration. (figure 5 and figure 6).

5.2. Effect of airflow rate on d_{max} at 50 gm/l initial glucose concentration

With reference to the figure 4 it was reported that d_{max} increases with air flow rate. Maximum d_{max} was reported at 1.0 vvm. This observation is contrary to the previous observation (figure 4). At high glucose concentration viscosity of the media increases. As a result at low airflow oxygen transfer rate decreases. As the air rate increases oxygen transfer rate also increases due to the increase of turbulence in the media.

Baburin reported [13] that the oxygen concentration significantly decreased with increasing sugar concentration of the media. To the contrary, the degree of turbulence is directly proportional to the air flow rate vis-à-vis the liquid re-circulation rate, it is inversely proportional to the viscosity. So, at higher glucose conc. (50 g/l) to achieve reasonable oxygen concentration for better yeast growth airflow rate should be high. For the above mentioned reason d_{max} is very high at 1.0 vvm (figure 4). Results depicted in figure 5 and figure 6 also shows that time to achieve d_{max} and total fermentation time were much less at 1.0 vvm compared to 0.5 vvm at 50 gm/l initial sugar conc. The reason for the same just mentioned. When air flow rate was 1.0 vvm d_{max} was maximum but total fermentation time and time to achieve d_{max} were much lower compared to 0.5 vvm due to increase in dissolved oxygen concentration in the media at low dissolved oxygen concentration fermentation system switches over to anaerobic condition. Experimental results support this logic because at 0.5 vvm d_{max} was much lower compared to 1.0 vvm but total fermentation time and time to achieve d_{max} was much higher at 0.5 vvm compared to 1.0 vvm. When glucose conc. was further increased crabtree effect was observed. According to this effect fermentative growth with ethanol production starts due to higher initial glucose conc. C. Liu et al. studied growth and productivity of tobacco line of hairy roots in an airlift reactor [14}. A new photobioreactor for continuous microbial production in hatcheries based on external-loop airlift system was studied by K. Loubiere et al. [15].

6. Conclusion

Proposed modification of the airlift fermenter reported much batter performance with reference to yeast growth. So, operating cost may be minimized to a great extent if commercialized properly.

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