Comparison of modified airlift reactor with conventional airlift reactor

T.K. Ghosh[1], Shounak Banerjee[1], C.B. Majumder[2], and Tai-hoon Kim[3,]*

[1] Department of Biotechnology, Heritage Institute of Technology
Kolkata –700107, India
tapanswatighosh@gmail.com

[2] Department of Chemical Engineering
Indian Institute of Technology, Roorkee – 247-667, India
chandfch@iitr.ernet.in.

[3] Hannam University, Daejeon, Korea
taihoonn@empal.com

Abstract

Air supply is a crucial parameter for many aerobic processes. Specific growth rate (μ) was determined for both the reactors (conventional UT-ALF and modified CDT-ALF) under identical operating conditions. The conventional uniform tube external loop airlift fermenter (UT-ALF) was modified keeping the volume and height same. Only the riser part of the conventional external loop airlift fermenter was replaced by a irregular geometry in the form of converging-diverging tube. The modified system is thus named converging-diverging tube airlift fermenter (CDT-ALF), which possesses a novel geometry comprised of walls with sinusoidal waves that mimic baffles in an effort to promote mixing at low Reynold's number (Re). This geometry provides a unique hydrodynamic environment suitable also for the cultivation of mammalian cells and tissues.

The results from UT-ALF and CDT-ALF were compared. At any operating condition CDT-ALF showed higher μmax (maximum specific growth rate) compared to UT-ALF. The highest (μmax) was observed for CDT-ALF corresponding to an initial sugar concentration of 50 kg/m3 and air flow rate of 1.0 vvm. This was 29 % higher compared to UT-ALF under identical operating conditions. So, operating cost may be minimized to a great extent if commercialized.

Keywords: Airlift bioreactor, Aeration, Aerobic process, Animal cell Culture, Specific , growth rate.

---

* Corresponding Author.

Symbols :

ALF	Airlift fermenter
A_d	Cross sectional area of down comer
A_r	Cross sectional area of riser
CDT	Converging-diverging tube
K_La	Volumetric mass transfer coefficient, $hr.^{-1}$
R_e	Reynold's number
UT	Uniform tube
vvm	volume per volume per minute
μ	Specific growth rate, $hr.^{-1}$
μ_{max}	Maximum sp. Growth rate ($hr.^{-1}$)

1. Introduction :

Oxygen solubility in fermented media is a critical factor for many aerobic fermentation processes.Initial glucose concentration in batch culture is another crucial parameter which controls cell mass growth. Higher initial sugar concentration will lead to alcohol production may hamper yeast growth . Yeast is an aerobic organism. The growth behavior [1,2,3,4,5] of this can be roughly divided into oxidative growth with cell yield and high oxygen uptake rate, and fermentative growth with ethanol production.. If initial sugar concentration is low, final cell concentration becomes low, to the contrary at increased initial glucose concentration the Crabtree-effect will arise. However, higher cell mass growth is possible in airlift reactor, if higher K_La is maintained. As the geometry is converging and diverging at regular intervals, the system has natural tendency to achieve turbulent flow regime at low Reynold's number (Re) and vortex formation. This natural phenomenon provides high mass transfer at low 'Re' i.e at low air flow rate [6,7,8]. A novel wavy walled bioreactor enhanced mixing at controlled shear stress was used to culture Chondrocytes in suspension. Results suggested that the novel wavy wall bioreactor generates a hydrodynamic environment distinct from those traditionally used to cultivate engineered cartilage [9]. Baier reported that interfacial surface is highly active for interfacial mass transfer due to local vortex formation [10]. Curran reported that delivery of oxygen to cell in an annular flow bioreactor is enhanced by the force convective transport offered by Taylor vortex flows. Oxygen transport in Taylor vortex flows was significantly greater [11] Mass transfer enhancement of more than four times was observed at low Reynold's number for dissimilar rough tubes [12]. This paper deals with the performance of the (CDT-ALF) with respect to air flow rate and initial sugar concentration on specific growth rate of yeast was studied.

2. Experimental

Figure 1 represents uniform tube external loop airlift fermenter (conventional). In general, an airlift fermenter comprises three major parts--- riser, downcomer and gas-liquid separator. Air is purged at the bottom of the riser through a sintered glass plate. The riser part was replaced by an irregular geometry in the form of converging-diverging tube. The modified system is called converging-diverging tube airlift fermenter. In both the systems height and volume of the riser are equal. Working volume of the reactor is 0.002 m^3

Figure 2 represents converging-diverging tube external loop airlift fermenter. The major dimensions of the two ALF systems are presented in Table 1.

The ALF systems and bulk medium were sterilized inside an autoclave separately. Sterilized reactors were aseptically filled with inoculated medium. The sterilized air was introduced through a sparger of sintered plate as in Figure 3. The fermentation temperature was maintained at room temp. i.e 30 to 32 ^{0}C

Samples were collected from both the reactors at an interval of 30 minutes always through the sample port with the help of a peristaltic pump. In each sample, cell mass concentration and residual sugar concentration were determined by standard method. Range of variables investigated are shown in table 2.

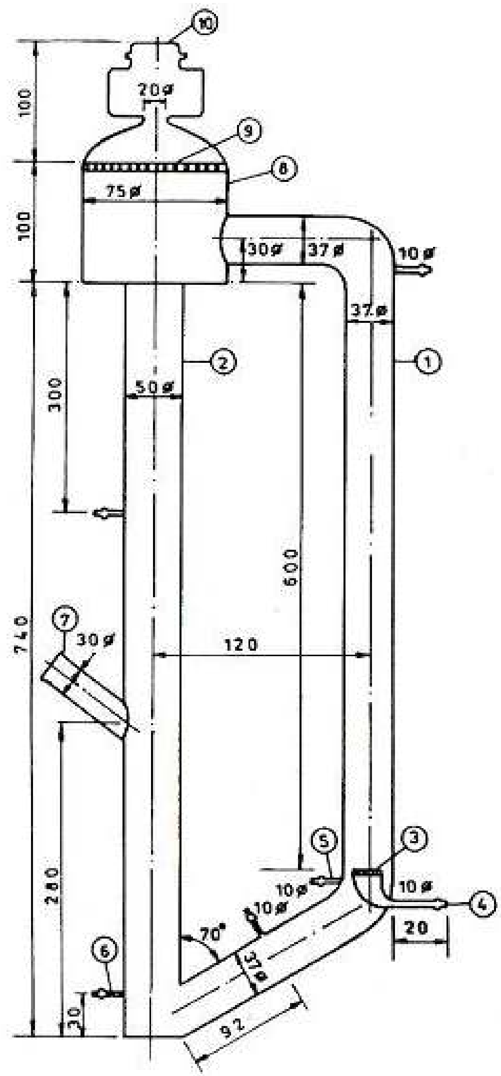

Figure: 1 Conventional External Loop uniform tube Air-Lift Fermenter (UT-ALF)

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

All dimensions are in mm.

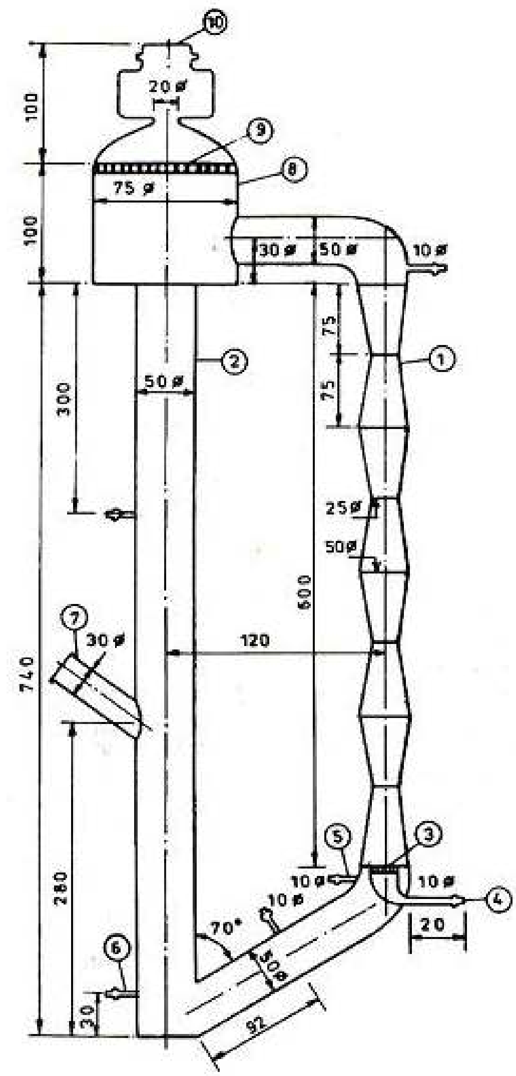

Figure: 2 External Loop Converging-Diverging Tube Air-Lift Fermenter (CDT-ALF)

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.

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

	UT-ALF	CDT-ALF
1. Riser height	0.6000	0.6000
2. Down comer height	0.7400	0.7400
3. Riser diameter		
for CDT-ALF		
Dmax	0.0500	
Dmin	0.0250	
For UT-ALF		
Dave		0.0375
4. Down comer diameter	0.0500	0.0500
5. Distance between riser and downcomer	0.1200	0.1200
6. Diameter of top connector	0.0500	0.0500
7. Diameter of bottom connector	0.0500	0.0500
8. Diameter of gas-liquid separator	0.0750	0.0750
9. Height of gas-liquid separator	0.2000	0.2000
10. Diameter of sintered glass sparger	0.0250	0.0250
11. Volume of the fermenter (m^3)	0.0020	0.0020

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

Table 2. Range of variables studied:

Air flow rate	0.5 to 3.0 vvm.
Fermentation time	8 to 21 h
Glucose concentration	10 to 50 kg/m^3

3. Materials and methods

Baker's yeast *S. cerevisiae* (NCIM 3190) was selected for the present investigation. Glucose was used as substrate for yeast growth. . The cell mass concentration was determined by a turbidity meter, which was calibrated earlier by a standard method using known concentration (dry wt.) of yeast suspension. Residual sugar concentration was estimated quantitatively by Muller method. Figure : 3 describe the experimental Set-up.

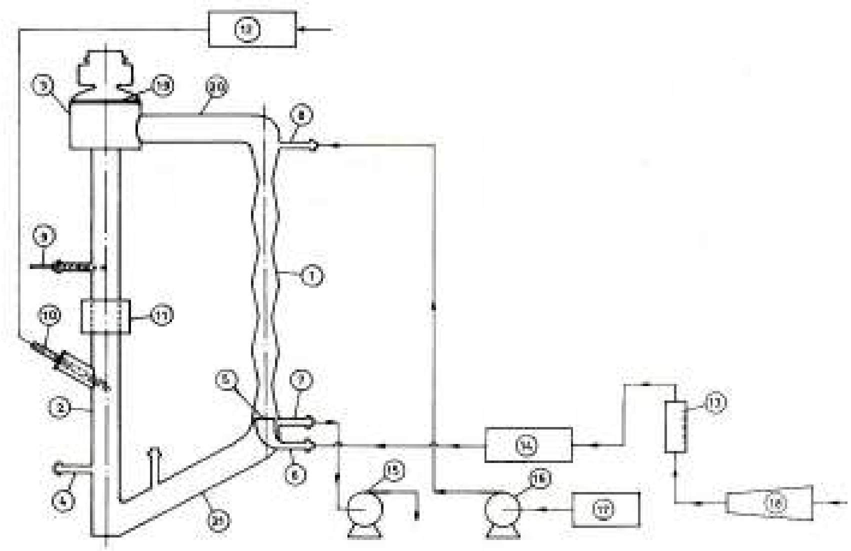

Figure : 3 Experimental Set-up

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.

4. Results and Discussion

4.1 Dependence of specific growth rate (μ) on air flow rate and initial glucose concentration of 10 kg/m^3 and 30 kg/m^3

Initial glucose concentration was 10 kg/m^3 for both the reactors. At time t = 0 both the reactors had the same initial cell mass concentration. Samples were collected from both the reactors under identical operating conditions. Air flow rate was varied from 0.5 to 3.0 vvm . The pattern depicted in Figure 4 shows that how specific growth rate (μ) changes with time. Specific growth rate (μ) gradually increases with time, attain; its maximum value (μ_{max}) then goes down slowly. This pattern is almost same for all the experimental conditions. Figures 4 and 5 also indicate that as the air flow rate gradually increased (0.5, 1.0, 2.0 and 3.0 vvm)the μ_{max} decreased accordingly and the time to attain μ_{max} increased. Lag time and total fermentation time also increased with air flow rate.

Same pattern was repeated when air flow rate is increased from 0.5---3.0 vvm at initial sugar concentration of 30 kg/m^3 as shown in Figure 5. Very high lag time was observed at 3.0 vvm air flow rate. From the result as depicted in Figure 5 it was observed that μ_{max} decreased slowly when air flow rate was increased from 0.5 to 3.0 vvm.

Highest μ_{max} was reported at the lowest air flow rate of 0.5 vvm. (Figure 5a) From the graphical presentation of the data for the two reactor systems it is found that higher μ_{max} was always achieved in CDT airlift fermenter compared to that in UT airlift fermenter at any operating condition.

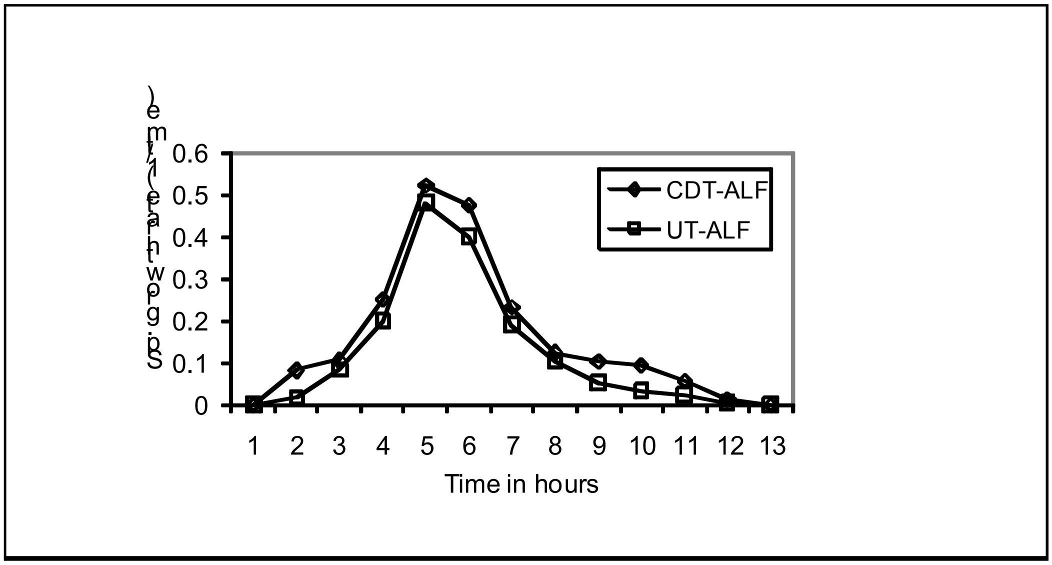

Figure 4a) Effect of air flow rate on specific growth rate at initial glucose concentration of 10

gm/l and air flow rate 0.5 vvm

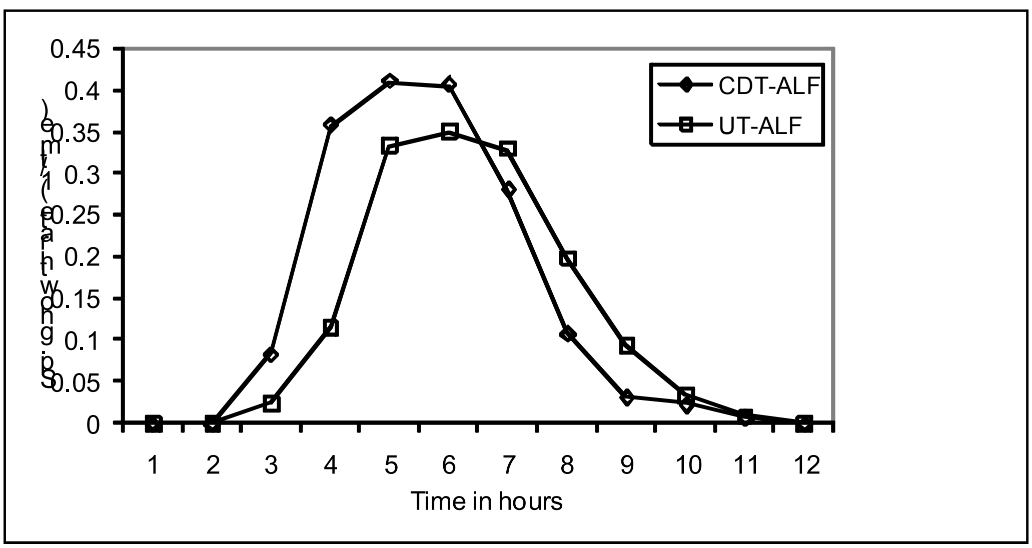

Figure 4b) Effect of air flow rate on specific growth rate at initial glucose concentration of 10 gm/l and air flow rate 1.0 vvm.

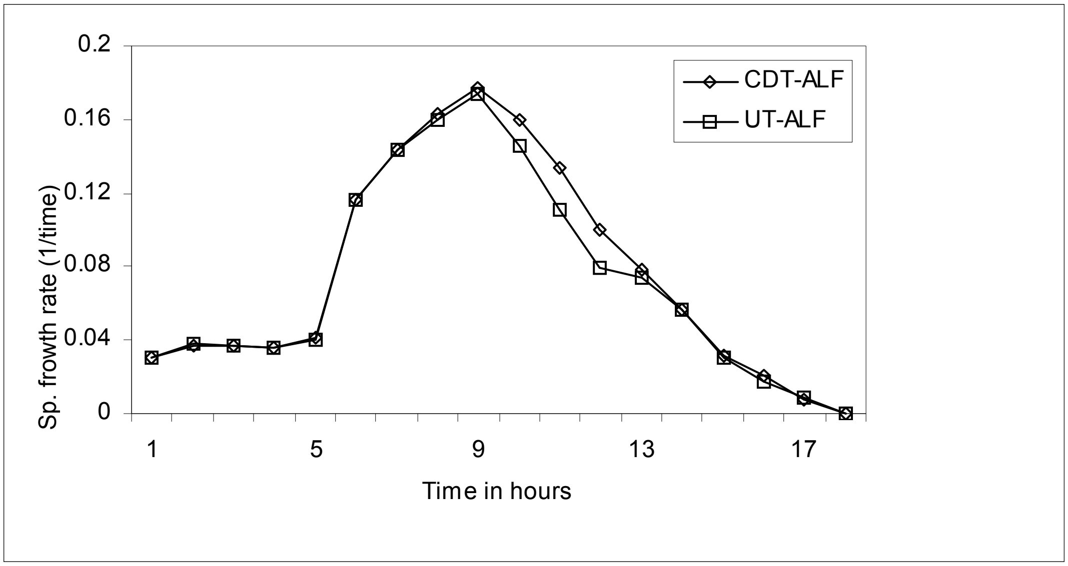

Figure 4c) Effect of air flow rate on specific growth rate at initial glucose concentration of 10

gm/l and a flow rate 2.0 vvm

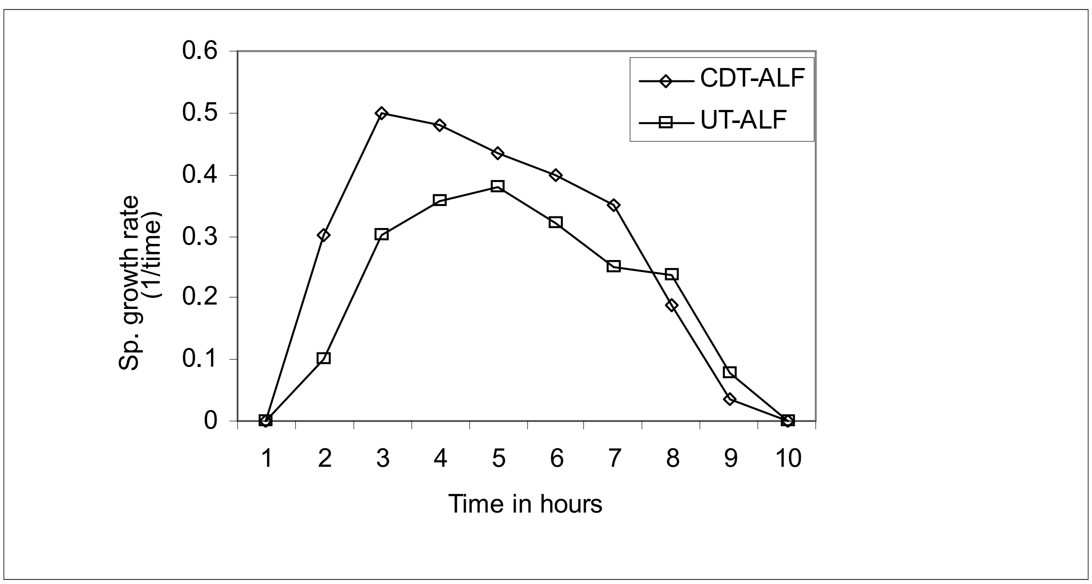

Figure 5a) Effect of air flow rate on specific growth rate at initial glucose concentration of 30

gm/l and air flow rate 0.5 vvm.

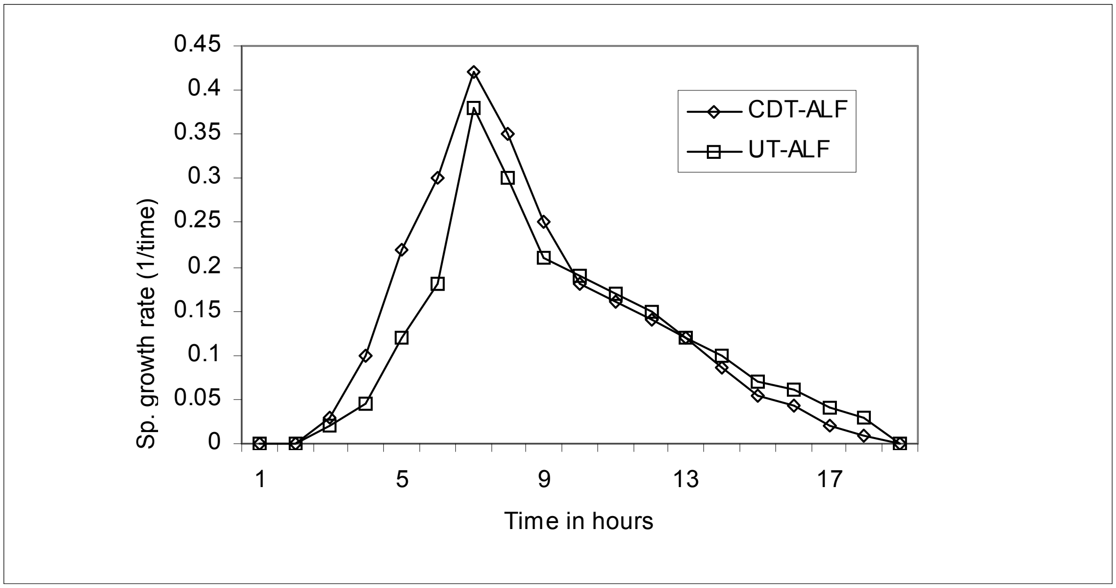

Figure 5b) Effect of air flow rate on specific growth rate at initial glucose concentration of 30 gm/l and air flow rate 1.0 vvm.

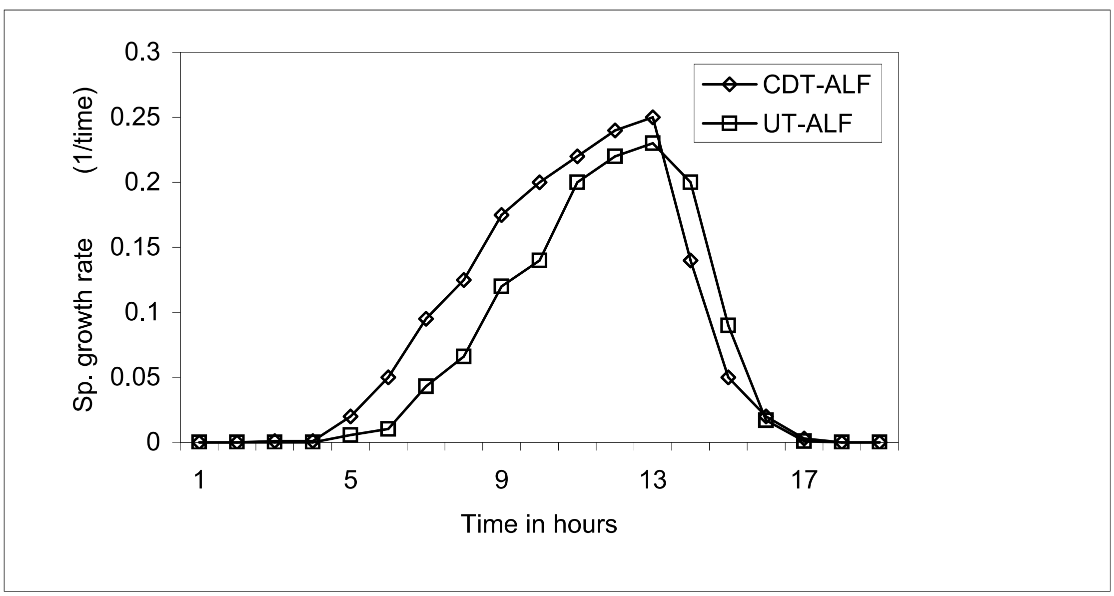

Figure 5c) Effect of air flow rate on specific growth rate at initial glucose concentration of 30 gm/l and air flow rate 2.0 vvm.

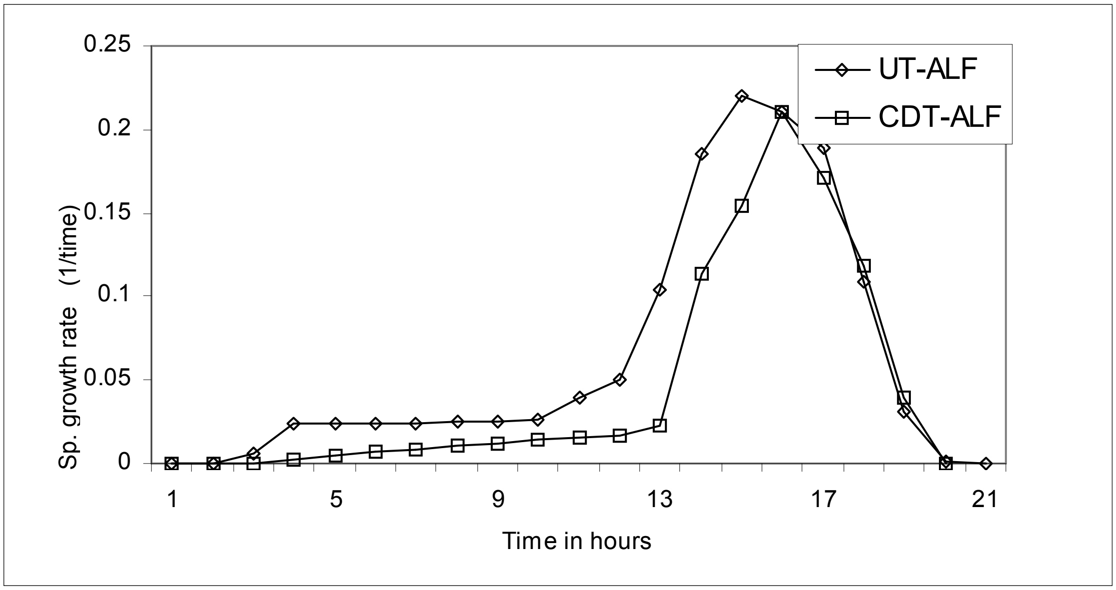

Figure 5d) Effect of air flow rate on specific growth rate at initial glucose concentration of 30 gm/l and air flow rate 3.0 vvm.

4.2 Effect of air flow rate on specific growth rate (μ) when initial glucose concentration was increased to 50 kg/m^3

The results as presented in Figure 6 also show the same pattern as depicted in Figure 4 and Figure 5, i.e μ increases with time, attains its maximum value then goes down slowly. Only one remarkable exception was observed when air flow rate increased from 0.5 to 1.0 vvm, μ_{max} increased but total fermentation time decreased. The highest μ_{max} was obtained in CDT-ALF corresponding to an air flow rate of 1.0 vvm, which is 29 % higher compared to that in UT-ALF. But for all the previous results highest μ_{max} was recorded for 0.5 vvm air flow rate. Results are summarized in Table 3. Air flow rate above 1.0 vvm , μ_{max} decreases gradually for both the reactor systems. Above 50 kg/m^3 of initial sugar concentration Crabtree effect arises. Values of maximum specific growth rate (μ_{max}) corresponding to a glucose concentration of 30 kg/m^3 and 50 kg/m^3 are presented in table 3.

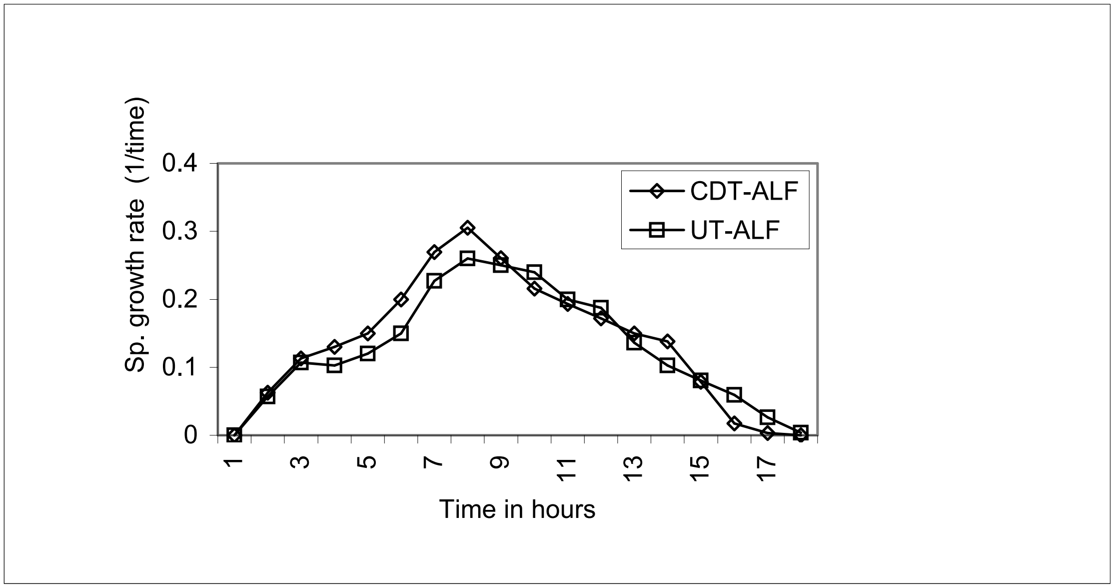

Figure 6a) Effect of air flow rate on specific growth rate at initial glucose concentration of 50 gm/l and air flow rate 0.5 vvm.

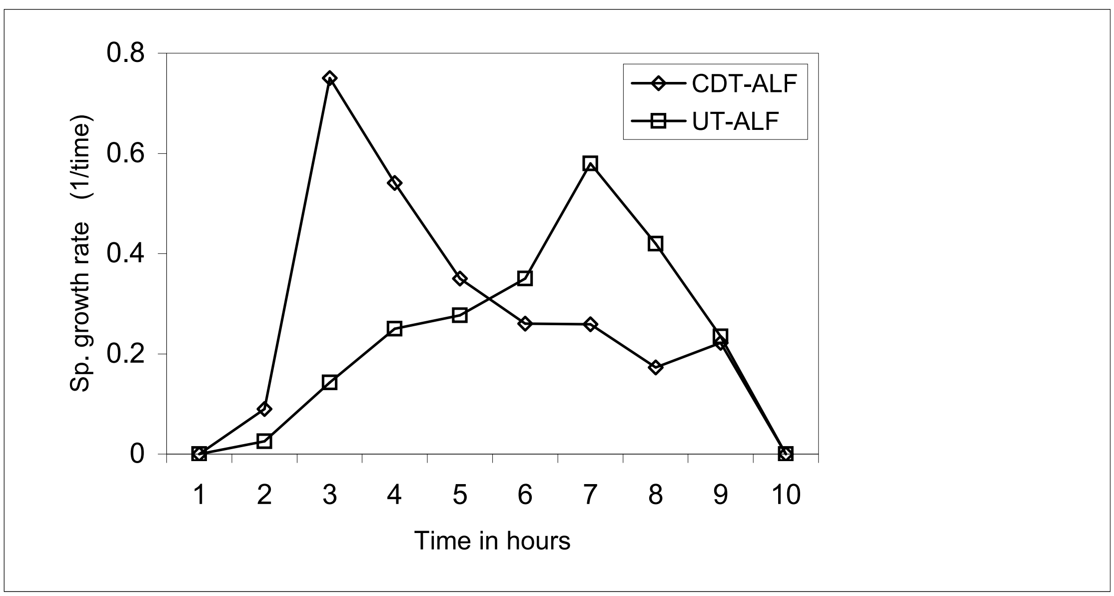

Figure 6b) Effect of air flow rate on specific growth rate at initial glucose concentration of 50 gm/l and air flow rate 1.0 vvm.

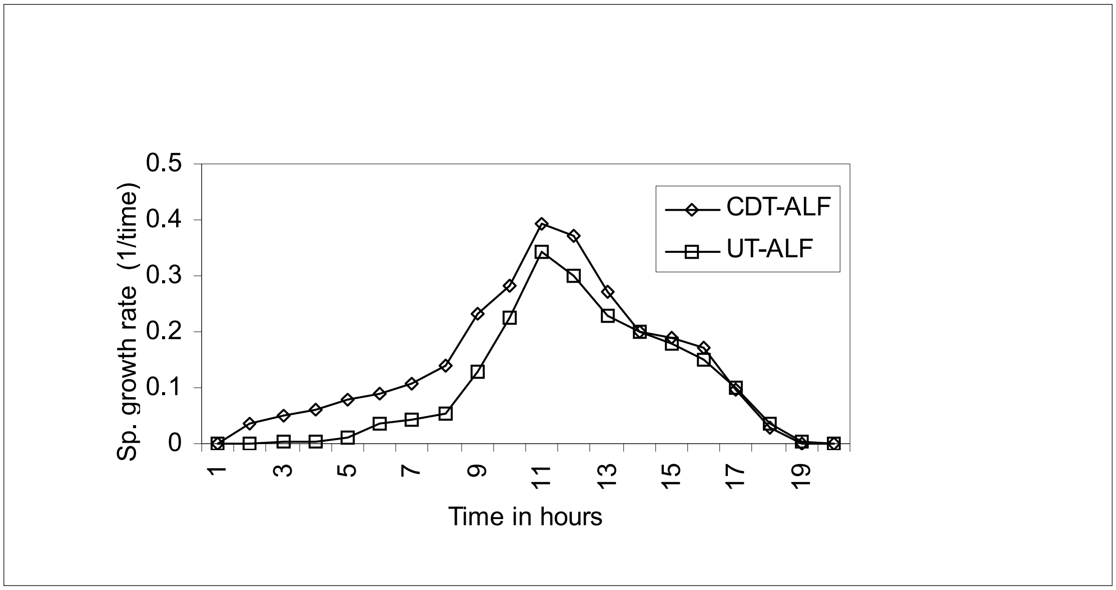

Figure 6c) Effect of air flow rate on specific growth rate at initial glucose concentration of 50 gm/l and air flow rate 2.0 vvm.

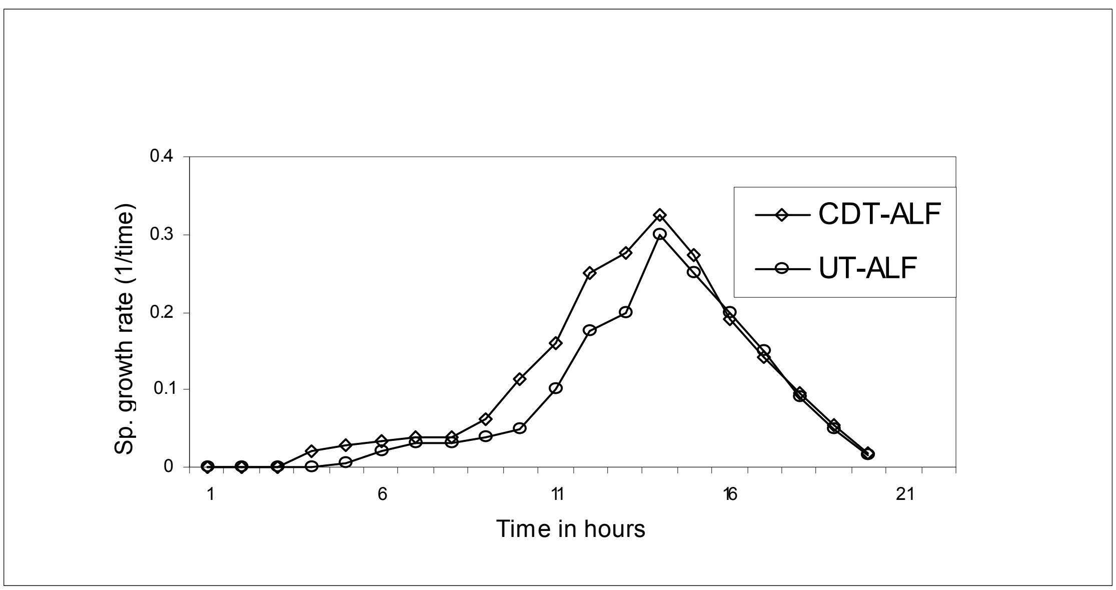

Figure 6d) Effect of air flow rate on specific growth rate at initial glucose concentration of 50 gm/l and air flow rate 3.0 vvm.

Table 3. Values of maximum specific growth rate (μ_{max}) corresponding to a glucose Concentration of 30 kg/m^3 and 50 kg/m^3

Initial glucose Concentration	Air Rate (vvm)	CDT-ALF	UT-ALF	% higher
30 kg/m^3	0.5	0.48	0.38	25
50 kg/m^3	1.0	0.75	0.58	29

5. Discussion

Results presented in Figure 4 show that specific growth rate (μ) increases with time and attain its maximum value, then goes down slowly. At any operating condition CDT always shows higher μ_{max} than UT. At higher air flow rate (3.0 vvm) the difference in μ_{max} is less compared to lower air flow rate.In batch cultivation μ_{max} gradually decreased with the increase of air flow rate as depicted in Figure 4 and Figure 5. With the increase of air flow rate volumetric mass (oxygen) transfer coefficient (K_La) decreases in CDT-ALF [7,8] causing low oxygen availability in the media. This may be the reason why low μ_{max} is reported at higher air flow rate.

At higher airflow rate liquid re-circulation velocity and turbulence increase resulting higher shear force. Yeast cells need more energy to survive under such condition. Higher maintenance energy is needed to adjust to the adverse environment causing low μ_{max} . To supply the required maintenance energy at higher turbulent condition, higher metabolic by-product is essential which in turn inhibits cell growth. For that reason low μ_{max} is reported at higher air flow rate.

Yeast cells take some time to acclimatize to the new environment. The time of acclimatization is called lag time. Higher turbulence in the fermented media due to higher air flow rate will force yeast cell to take more time to acclimatize to the adverse condition. As a result lag time and total fermentation period increase with increase in air flow rate. At any operating condition CDT always shows higher μ_{max} than UT.

Converging-diverging geometry will produce vortex formation in the system which will provide high mass transfer [11,12,14]. R. Srinivasan et al reported that oxygen mass transfer is enhanced by the forced convective transport affected by Taylor vortex flows [14,15]. Due to converging diverging geometry liquid velocity oscillates with varying amplitude which causes pressure fluctuation [16] in the system. As a result the system switched over to turbulent flow regime at low Re. This phenomenon provides high mass transfer at low air flow rate.

5.1 Effect of air flow rate on μ_{max} at sugar conc. 50 kg/m^3 and above.

Results depicted in Figure 6 shows the same pattern as discussed earlier. One remarkable observation from Figure 6b, could be the increase of μ_{max} with the increase of airflow rate up-to 1.0 vvm and attains its maximum value at 1.0 vvm [17]. This observation is just opposite to our previous observation. Dissolved oxygen concentration in fermented medium is a critical parameter for yeast cell growth. Viscosity of the fermentable media increases at higher glucose concentration. As a result at high glucose concentration oxygen mass transfer rate should be low at low air flow rate. Cells are thus unable to grow due to low oxygen availability causing low μ_{max} at low air flow rate. When air flow rate increases oxygen transfer rate also increases due to increase in turbulence in the media. Oxygen concentration decreased significantly with increasing sugar concentration of the media as reported by Baburin [18]. The degree of turbulence is directly proportional to the air flow rate and inversely proportional to the medium viscosity. So, to achieve reasonable dissolved oxygen concentration at high glucose concentration higher air flow rate is essential. The results presented in Figure 6 commensurate with this logic given above. So, μ_{max} was increased when air flow rate changed from 0.5 to 1.0 vvm due to increased dissolved oxygen concentration in the media. When air flow rate further increased μ_{max} goes down from its maximum value[19,20]. D.Y.Ryu reported that aeration rate above 1.0 vvm caused a significant drop in cell yield and product content in a draft type airlift fermenter [17].

Oxygen transfer from bulk gas phase (air) to bulk liquid phase (fermented media) depends on operating conditions . At low air flow rate system may switched over to anaerobic condition which will help to produce ethanol in the media [21,22]. As a result low μ_{max} is observed at low air flow rate[23]. When glucose concentration was increased above 50 kg/m^3 Crabtree effect was observed.

6. Conclusion :

Experimental investigation have been carried out with conventional and modified airlift fermenters. In the modified system maximum specific growth rate was attained at the lower air flow rate. Hence CDT-ALF system is more promising to be used as fermenter.

7. Acknowledgement : The entire graphical representation was done by Shounak Banerjee, . 3rd. year Biotechnology student.

8. References:

[1] B. Bilgen, I.M.C. Mateu, I.M and G.A. Barabino, " Characterization of mixing in a novel wavy-walled bioreactr for tissue engineering, " Biotech. Bioengg., 92, 2005 pp 907—919.

[2] J. Klein J. Maia, A.A. Vicente, L. Domingues, J.A. Teixeira, and M. Jurascik, " Relationships between hydrodynamics and rheology of flocculating yeast suspensions in a high-cell-density airlift bioreactor,"Biotech. Bioengg. , 89 , 2005, pp 393—399

[3] K. Loubiere, E. Olivo, G. Bougaran, J. Pruvost, R. Robert and J. Legrand, " A new photobioteactor for continuous microbial production in hatcheries based on external-loop airlift and swirling flow,'' 102, 2009, pp 132 – 147.

[4] N. Muller, P. Girard, D.L. Hacker, M. Jordan, and F.M. Wurm, " Orbital shaker technology for the cultivation of mammalian cells in suspension," Biotechnol and Bioengg., 89, 2005, 400 – 406.

[5] K. H. Bellgardt, w. Kuhlmann, and H.D.Meyer, " Modelling and control of Biotechnical processes", Pergamon press , Oxford, Vol 1, 1983, pp 67—74.

[6] Narayan, C. M., in : Ph.D. thesis , Indian Institute of Technology, kharagpur, India 1983.

[7] T K Ghosh, in : Ph.D thesis, Indian Institute of Technology, Kharagpur,
India, 1992.

[8] Ghosh, T.K, B.R. Maiti and B.C. Bhattacharyya, " Studies on mass transfer characteristics of a modified airlift fermenter," Bioprocess Engineering , 9, 1993, pp 239 – 244.

[9] E.M. Bueno, B. Bilgen, R.L. Carrier and G.A. Barabino, " Increased rate of chondrocyte aggregation in a wavy- walled bioreactor, " Biotechnol. and Bioeng., 88, 2004 pp 767—777.

[10] G. Baier, M.D. Graham, and E.N Lightfoot , " Mass transfer in a novel two-fluid Taylor vortex extractor," AIChE, 46, 200, pp 2395 – 2407.

[11] S. J. Curran, and R.A. Black , " Oxygen transfer and cell viability in a annular flow bioreactor comparison of laminar couette and Taylor vortex of laminar flow regime", Biotech. Bioengg., 89, 2005, pp 766-774.

[12] S. Tantirige and O. Trass , " Mass transfer at geometrically dissimilar rough surfaces," Canadian Journal of Chem. Engg. 62, 1984 pp 490—496.

[13] G.L. Miller, " Use of dinitrosalicylic acid reagent for determination of reducing sugar," Anal. Chem., 31 1959, pp 426—428.

[14] A. Koynov, .J. G. Khinast and G. Tryggvason, " Mass transfer and chemical reactions in bubble swarms with dynamic interfaces," AIChE, 51 (2005) 2786-2800

[15] R. Srinivasan, S. Jayanti, and A. Kannan, "Effect of Taylor vortices on mass transfer from rotating cylinder," AIChE, 51, 2005, pp 2885—2898.

[16] V.P. Chilekar, M.J.F. Warnier, J V D Schaaf , B.F.M. Kuster , J.C. Schouten. and J.R.V. Ommen, "Bubble size estimation in slurry bubble column from pressure fluctuations, " AIChE 51, 2005, pp 1924—1937.

[17] J.C. Merchuk, N. Ladwa, A. Cameron, M. Bulmer and A. Pickett, "Effect of Rheological properties and mass transfer on plant cell bioreactor performance : Production of tropane alkaloids," Biotech. Bioengg., 42, 1993, pp 1181 – 1189.

[18] Baburin et al. Chem. Abs. 96(1) 4932 (K) 1982.

[19] A. Contreras, F. Garcia, E. Molina, and J.C. Merchuk," Interaction between CO2 mass transfer, light availability, and hydrodynamic stress in growth of phaeodactylum tricomufum in a concentric tube airlift photobioreactor,'' Biotechnol. Bioeng., 60, 1998, pp 317 – 325.

[20] P.G. Verschuren, and J.C. Van der Heuvel, " Substrate controlled development of anaerobic acidifying aggregates at different shear rates in a gas lift reactor," Biotechnol. Bioeng., 77, 2002, pp 306 – 315. 77 (2002) 306—315.

[21] F. Lei, L. Olsson, and S.B. Jorgensen, " Experimental investigation of multiple steady states in aeribic continuous cultivations of saccharomyces cerevisiae,'' Biotecjnol. Bioeng., 82, 2003, 766 – 777.

[22] F. Kensy, H.F. Zimmermann, I. Knabben, T. Anderlei, H. Trauthwein, U. Dingerdissen and J. Buchs, " Oxygen transfer phenomena in 48-well microtiter plates : Determination by optical monitoring of sulfite oxidation and verification by real-time measurement during microbial growth," Biotechnol. Bioeng, 89, 2005, pp 698—708.

[23] E. A. Sandoval-Basurto, G. Gosset, F. Bolivar and O.T.Ramirez, " Culture of Escherichia Coli under dissolved oxygen gradients simulated in a two compartment scale-down system : Metallic response and production of recombinant protein, " Biotechnol. Bioeng. 89, 2005, pp 453—463.