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Mixing efficiency studies in an airlift bioreactor with helical flow promoters for improved reactor performance

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Abstract

Cultivation of aerobic microorganisms typically requires rapid, but gentle and simultaneously energy efficient mixing in bioreactors to prevent high gradients between local concentrations. The mixing efficiency in a pilot scale airlift bioreactor with helical flow promoters was investigated with varying air inputs. Helical flow promoters were helical tubes attached to riser or downcomer, or both and besides acting as flow promoter they were used as additional gas spargers. Mixing time, circulation time, dimensionless mixing time and Bodenstein number were used as assessment parameters to analyze the mixing efficiency of different airlift setups with helical flow promoters. Introducing air through the helical flow promoter in the downcomer zone prolonged the circulation time, but shortened the mixing time and decreased the Bodenstein number. Low Bodenstein number indicated an improved axial dispersion. Mixing was more related to dispersion in the airlift reactor with helical flow promoter in the downcomer zone whereas in a regular airlift reactor mixing was related to liquid circulation speed.

Keywords

Airlift bioreactor; ALR; Bodenstein number; helical flow promoter; mixing; mixing time

Abbreviations

ALR, airlift reactor; *BCR*, bubble column reactor; *Bo*, Bodenstein number; *CSTR*, continuous stirred tank reactor *D*, aeration through helical flow promoter in downcomer; *D-helix*, helical flow promoter in downcomer; *HALR*, airlift reactor with helical flow promoter(s); *HFP*, helical flow promoter; *lpm*, liter per minute, gas flow rate; *MLR*, multiple linear regression; *R*, aeration through helical flow promoter in riser; *R-helix*, helical flow promoter in riser; *slpm*, standard liters per minute (1 atm, 25 °C), gas flow rate; *t_c*, circulation time; *t_{mix}*, mixing time; *t_{dim}*, dimensionless mixing time; *U_{gs}*, superficial gas velocity caused by sparger aeration.

1. Introduction

An efficient and sustainable bioprocess favors moderate energy consumption, cheap feedstock and culture media as well as high production rate and an easily harvestable product. Additionally, phenomena inside the fermentation tank should receive more attention for its efficient operation and reliable production protocol. Therefore, in an aerobic fermentation system, sufficient oxygen mass transfer and efficient mixing in bioreactors is an important prerequisite to prevent high gradients between local nutrient concentrations. Insufficient aeration and agitation might cause significant losses in product yield by formation of unwanted by-products or even cell death that of course have a negative impact on the process economy [1-6].

Simultaneous mixing and oxygen mass transfer in an energy efficient manner is a problem to be solved in aerobic cell cultivations, especially at an industrial scale. Companies in

fermentation mostly uses continuous stirred tank reactors (CSTRs). Typically, in CSTRs mixing is improved by increasing the stirrer speed which leads to high energy consumption and further effects the overall process economy [2]. Thus, it is very important to find an optimum efficient liquid-phase mixing to maintain constant conditions even in a large-scale reactor and to provide favorable environment for growing cells [7]. In stirred tank reactors, it is difficult to optimize mixing and mass transfer at the same time; impellers with low power number provide higher dispersion mixing intensities while impellers with high power number provide better mass transfer performance [8]. If mixing time in the reactor is significantly longer than the oxygen uptake rate, the system is vulnerable to spatial variation in dissolved oxygen concentration [9]. A high local substrate concentration can cause oxygen depletion as well. For example in *E.coli*, high substrate concentration increased the respiratory activity, which led to a faster local oxygen consumption [10]. A short mixing time in the reactor is advisable, as it indicates physiological similarity in the different locations of the reactor. A quick response of the system to distribute additions to the process such as nutrients or pH equalization substances are expected from an ideal bioprocess [8].

Pneumatically agitated airlift reactors (ALRs) can provide energy efficient mixing for aerobic cultures. The widely recognized advantages of ALRs are fast mixing and transfer phenomena (both mass and heat) and the lack of mechanical agitation, which in turn means low shear stress. [11] The simplicity of a pneumatically agitated reactor gives an obvious advantage especially in large-scale operation where mechanical problems associated with mixing often arise [7]. Moreover, according to Humbird et al. [12] pneumatically agitated bubble column reactors (BCRs) are always less expensive to operate than CSTRs and as ALRs are modified BCRs with better mixing capacity [13], ALRs are very interesting reactor type for cultivation of aerobic cultures.

The liquid pool of an ALR is divided into distinct zones. The gas sparged into the riser part of a reactor causes gassed and ungassed zones in the reactor. This means different gas hold-ups and results in different bulk densities of the fluid in different parts of the ALR. The difference in fluid density causes circulation of fluid in the reactor [3]. Internal type of airlift reactors are like simple bubble columns but they have an internal baffle or a draft-tube, which split the reactor into riser and downcomer [3,14]. The principal independent variable in an airlift bioreactor system is the gas input rate. Parameters, such as mixing, gas holdup, liquid velocity, bubble size and mass transfer, needed to characterize the flow in an airlift reactor, are all functions of it [15]. Increasing gas input rate from sparger enhances ALR performance until a certain point related to reactor geometry is reached, after which an increase in gas flow rate causes flooding *i.e.* the reactor receives more gas than it can effectively disperse.

The aim of the present research work is to study the mixing conditions in the ALR occupied with helical flow promoters (HFP). Different types of HFPs in ALRs have been previously studied by Gluz and Merchuk [16], Schlötelburg et al. [17], and most recently by Zheng et al. [18]. HFP is an additional part that causes a helical motion pattern in the liquid flow. HFPs have been previously added at the upper part of riser or downcomer [17]. Zheng et al. [18] used helical sieve plates as HFPs for the full length of the riser. In the current study, HFPs were made of plastic tube and were added into riser and downcomer or only into downcomer, for the full length of the mentioned reactor part. The reactor studied in the current research was first introduced in Räsänen et al. [19] and their results showed that the HFP with extra aeration in downcomer zone of ALR significantly improved the oxygen mass transfer rate in a reactor. The hypothesis in this study was to improve the overall mixing by using HFPs. Mixing time t_{mix} , circulation time t_c and dimensionless mixing time t_{dim} were used to evaluate the overall mixing in a reactor and

Bodenstein (Bo) number was used to evaluate axial dispersion in the reactor. In this article, ALR with HFPs is called HALR to distinguish it from regular ALR.

2. Materials and methods

2.1. Experimental setup of airlift reactor with helical flow promoters

A cylindrical, draft-tube airlift reactor was fabricated by using polymethyl methacrylate (plexiglass, PMMA) occupied with helical flow promoters (Fig 1). Räsänen et al. [19] first introduced the HALR. The examined reactor setups had HFP in both, the riser (R-helix) and the downcomer (D-helix). One of the examined reactor setup had HFP only in the downcomer. HFPs could also be used for an extra aeration as HFP-gas spargers. The examined setups are listed in Table 1, in which the aeration rates through HFPs are indicated with numbers 0, 5 and 10 (slpm, 1 atm, 25 °C). R and D indicate R-helix and D-helix, respectively. Each experiment setup was examined at four different sparger aeration rates between 60 and 120 lpm. Airflows through HFPs were controlled by using two mass flow meters (MC-20SLPM-D, Alicat Scientific, USA) and airflow through the gas sparger (diameter 140 mm) was controlled with a rotameter. The gas sparger (diameter 140 mm) was made up of POM (polyoxymethylene) with cross-shaped design, located inside the draft-tube 10 cm above the bottom. Four perforated tubes, each with ten holes ($\text{\O} 0.5$ mm) were connected to the round core section ($\text{\O} 50$ mm) of the sparger. The regular ALR without HFPs served as a reference configuration. The working volume of the reactor was 0.116 m^3 .

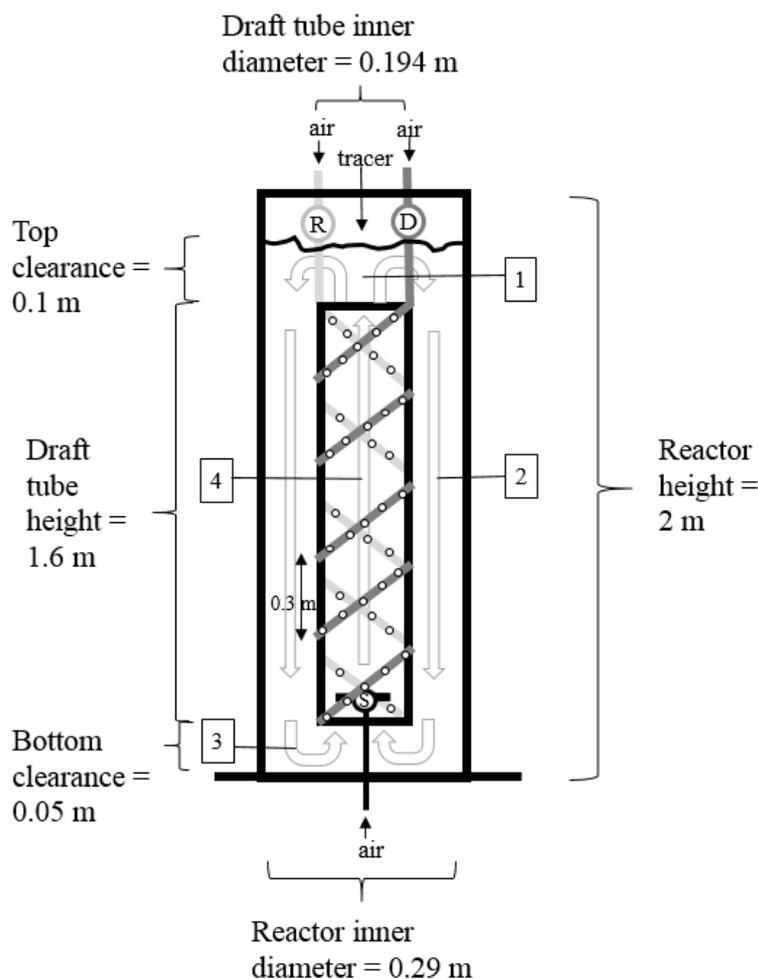


Figure 1. The airlift reactor with helical flow promoters (plastic hose, \O_o 15 mm): (R) = helical flow promoter/gas sparger in the riser, *i.e.* on the inner surface of the draft tube; (D) = helical flow promoter/gas sparger in downcomer, *i.e.* on the outer surface of the draft tube; (S) = bottom sparger; arrows indicate the liquid flow inside the reactor; pH electrodes' locations: gas separator (1); middle downcomer (2); base (3); middle riser (4).

The HFPs were made of food compatible reinforced plastic hose (\O_o 15 mm, \O_i 10 mm). HFP in the riser (R-helix) was revolved inside the draft tube with a pitch of 30 cm and holes of $\text{\O} 0.6$ - 0.7 mm made with needle after every 2.5 cm. HFP

in the downcomer (D-helix) also had a pitch of 30 cm and it was revolved around the outer surface of a draft tube. The D-helix contained holes made with $\text{\O} 0.6$ mm needle after every 5 cm. HFPs open ends were sealed with plastic plugs.

2.2. The pH tracer method for mixing time, circulation time and axial dispersion measurement

The pH method is the best quantitative method to study the mixing properties of a reactor with volumes more than 100-200 liters, with relatively accurate measurements (pH change of 0.5-2 units) [20]. The method used for measurements was described by Sanchez-Miron, et al. [21]. Initially, pH of the working fluid (tap water) in the reactor was lowered to 2 by adding appropriate amount of 32 % (w/v) HCl. The initial pH of the tap water was approximately 7. The reactor was then bubbled with air, at superficial gas velocity of 0.03 m/s for at least 20 min to remove possibly present carbonates with buffering capacity in the form of carbon dioxide. The pH was increased afterwards to 4.5 by using 6 M NaOH. HCl (32%, 40 ml) was added as Dirac pulse by a syringe in the middle and right above the dispersion in the gas separator of the reactor in all the experiments (Fig. 1).

The usage of HCl and NaOH will accumulate NaCl in the working fluid. The transition concentration for bubble coalescence of NaCl solution is between 0.145-0.175 M [22, 23]. The working fluid in the reactor was removed with regular intervals and the peak concentration of NaCl caused by the acid and base additions in the working fluid was evaluated to be 0.04 M before replacing it with fresh tap water. Thus, the salt concentration did not have effect on the bubble coalescence during the experiments. The addition of the tracer acid (40 mL per test) was minor to the working volume, only 0.034%, thus it did not have effect on the overall bubble coalescence in the experiments either.

The pH changes with addition of HCl were measured by using four pH-sensors (2x Hamilton EasyFerm 200, 2x Hamilton Easyferm 120) located in (1) gas separator, (2) downcomer, (3) base, (4) riser (Fig. 1). All the measurement data was collected every third second by Biostat Q DCU (Type 84101/2, B. Braun Biotech International, Germany) with tailored software (eLabs Oy, Finland).

The mixing properties of HALR in ten different helix aeration setups with four different sparger aeration rates (Table 1) were performed by using tap water as a working fluid. Regular ALR was serving as a reference setup. All the experiments were performed at least in triplicate and results mentioned are average \pm standard deviation. All measurements were done at ambient temperature (20 ± 2 °C) and pressure (1 atm).

Table 1. Experimental setups for HALR and for regular ALR.

Setup	Name	Airflow, Gas sparger, lpm	Airflow, R-helix, slpm	Airflow, D-helix, slpm	Total ariflow, lpm
1	ALR	60, 80, 100, 120	-	-	60-120
2	ALR+D10	60, 80, 100, 120	-	10	70-130
3	ALR+R0+D0	60, 80, 100, 120	0	0	60-120
4	ALR+R5+D0	60, 80, 100, 120	5	0	65-125
5	ALR+R10+D0	60, 80, 100, 120	10	0	70-130
6	ALR+R0+D5	60, 80, 100, 120	0	5	65-125
7	ALR+R0+D10	60, 80, 100, 120	0	10	70-130
8	ALR+R5+D5	60, 80, 100, 120	5	5	70-130
9	ALR+R5+D10	60, 80, 100, 120	5	10	75-135
10	ALR+R10+D5	60, 80, 100, 120	10	5	75-135
11	ALR+R10+D10	60, 80, 100, 120	10	10	80-140

Number after R and/or D means the air diffused in slpm through the mentioned HFP

2.3. Data analysis

2.3.1. Mixing, circulation and dimensionless mixing times

Mixing time was defined as the time interval between tracer addition and the point after which pH fluctuated less than $\pm 5\%$ from its final value (Fig. 2) [11, 20]. Generally, the mixing time (t_{mix}) is supposed to be dependent on sparger aeration rate (Eq. 1) [24]:

$$t_{mix} = aU_{gs}^b \quad (1)$$

U_{gs} = superficial gas velocity in the riser caused by the sparger aeration; a and b = experimentally determined constants.

Circulation time t_c was the time interval between two peaks in pH measurement data (Fig. 2) [3].

The ratio between t_{mix} and t_c is called the dimensionless mixing time (Eq. 2) [3]. Dimensionless mixing time (t_{dim}) measures the required number of liquid circulations to achieve the desired homogeneity level.

$$t_{dim} = t_{mix}/t_c \quad (2)$$

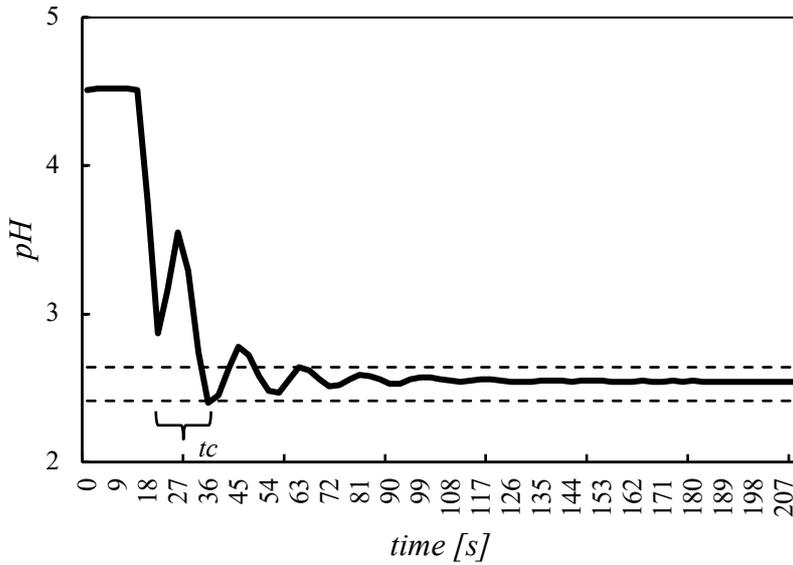


Figure 2. Example of pH tracer curve. Dotted lines present the $\pm 5\%$ values from the final pH value. t_c is the time interval between the two first peaks in the tracer curve.

2.3.2. Bodenstein number

The Bodenstein (Bo) number was derived from pH measurement data with the following steps. pH data was converted to H_3O^+ concentrations to obtain mean residence time and its variance of the added tracer. Bo number was analyzed for the first circulation of liquid phase (with tracer) [3]. Bo number for the reactor was an average of the results from four different pH probes in individual reactor zones (Fig 1).

Mean residence time t_s was obtained from the relationship presented in Eq. 3 [26]:

$$t_s \cong \frac{\sum t_i C_i(t_i) \Delta t_i}{\sum C_i(t_i) \Delta t_i} \quad (3)$$

C_i = existing tracer concentration at time t_i (g/l); Δt_i = interval between two measurements (s).

Variance (Var) was obtained as stated in Eq. 4:

$$Var \cong \frac{\sum t_i^2 C_i(t_i) \Delta t_i}{\sum C_i(t_i) \Delta t_i} - t_s^2 \quad (4)$$

The dimensionless variance, Var_{dim} (Eq. 5)

$$Var_{dim} = Var/t_s^2 \quad (5)$$

The Bodenstein number for open boundary conditions [26] can be determined from Eq. 6:

$$Var_{dim} = 2/Bo + 8/Bo^2 \quad (6)$$

Liquid phase Bo number is a measure of degree of dispersion when liquid circulates once through the reactor. Thus, Bodenstein number is the ratio between bulk movement of the fluid (convection) and the axial dispersion [21]. For plug flow system $Bo \approx \infty$ and for complete back mixing $Bo = 0$ [27, 28]. Therefore, smaller Bo number represents better dispersion properties. According to Chisti [3], a reactor can be considered as perfectly mixed when $Bo < 0.1$, while it observes plug flow characteristics when $Bo > 20$.

2.4. Estimation of critical parameters

Sparger aeration rate (S), D-helix aeration rate (D) and the R-helix aeration rate (R) are variable parameters when studying the HALR setups (Table 1). Hence, among these variable parameters, statistically critical ones for t_{mix} , t_c , t_{dim} and Bodenstein number of HALR setups, were estimated using multiple linear regression analysis with confidence level of 0.95 (MLR analysis with MODDE 8.0, Umetrics, Sweden).

3. Results and discussion

3.1. 95% mixing time

The overall expression was that t_{mix} (time needed to achieve 95% homogeneity) was decreased with an increase in superficial gas velocity (Fig. 3). This observation was in accordance with many previous studies [21, 28-32]. t_{mix} was shorter in regular ALR than in any of the HALR setups without air (0 slpm) diffused through the D-helix (Fig. 3a). The difference between ALR and HALR setups increased with an increased sparger aeration rate. With moderate amount of air (5 slpm) diffused through D-helix (Fig 3b), mixing times in HALR setups had more fluctuation and the difference to regular ALR was not evident as in Fig. 3a, until the highest tested sparger aeration rate.

The trends of t_{mix} of HALR setups with strong D-helix aeration (10 slpm) (Fig. 3c) had lower slopes and had even more fluctuations compared to moderate D-helix aeration. It can be suggested that with an increased D-helix aeration rate, t_{mix} becomes less dependent on the sparger aeration rate. The regular ALR had significantly faster mixing only at the strongest tested sparger aeration rate. The reason behind the behavior of the HALR setups with D-helix aeration might be due to an enhanced dispersion in the downcomer caused by small air bubbles diffused towards downward flowing liquid. Dispersion and thus not uniform liquid flow is most probably the reason behind the larger deviation in the results with HALR setups in question. Air bubbles diffused through D-helix were smaller than the bubbles diffused through the sparger and entering the downcomer from the riser [19] and it has been suggested that small bubbles enhanced mixing in airlift bioreactor [33]. However, according to t_{mix} , the HFPs seemed to hinder the mixing if air was not diffused through the D-helix (Fig. 3a).

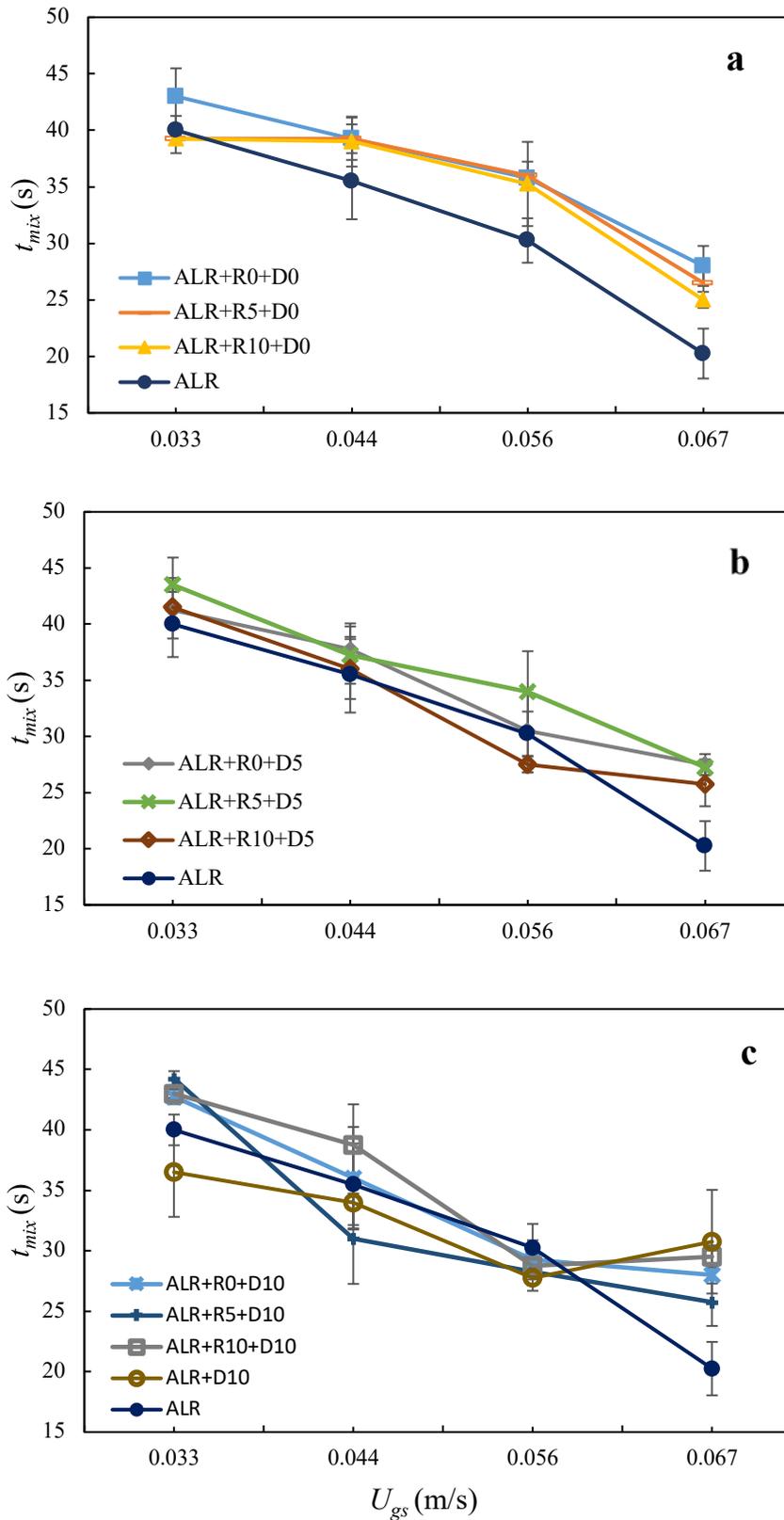


Figure 3. Mixing time (t_{mix}) in the HALR and in the regular ALR as a function of superficial gas velocity caused by sparger aeration rate (U_{gs}). (a) HALR setups without D-helix aeration; (b) HALR setups with 5 slpm D-helix aeration; (c) HALR setups with 10 slpm D-helix aeration. t_{mix} of the regular ALR is presented as a reference in each subfigure.

The MLR analysis was performed to find statistically significant factors affecting t_{mix} in HALR. According to Table 2, sparger and D-helix aeration rates had significant effect on t_{mix} in the HALR. MLR coefficients for both factors (S and D) were negative, which means that they shortened t_{mix} with an increase in aeration rates. The R-helix aeration rate did not show significant effect on t_{mix} , neither with combined nor with second order terms. This might be due the effect of the small air bubbles diffused through R-helix is damped with the larger air bubbles (in both size and number) diffused through the sparger. The coefficient of the sparger aeration rate was over nine times

higher than the coefficient of the downcomer aeration rate. Therefore, impact of sparger aeration on t_{mix} was stronger than impact of D-helix aeration. This confirmed the observations from the Fig. 3.

Table 2. Results of the MLR analysis of the mixing time (t_{mix}), circulation time (t_c), and dimensionless mixing time (t_{dim}), for the HALR. S stands for sparger aeration, R for riser helix aeration and D for downcomer helix aeration.

	Coefficient	P-value
t_{mix}		
Constant	33.99	0
S	-7.538	<0.0001
D	-0.844	0.026
R	-0.406	0.279
S*S	0.094	0.892
D*D	0.469	0.47
R*R	0.156	0.81
S*D	-0.582	0.248
S*R	-0.119	0.812
D*R	0.719	0.119
t_c		
Constant	17.881	0
S	0.108	0.108
D	1.802	0
R	0.241	0.001
S*S	-0.005	0.966
D*D	-0.32	0.003
R*R	0.148	0.161
S*D	-0.322	<0.0001
S*R	0.15	0.068
D*R	-0.109	0.144
t_{dim}		
Constant	1.912	0
S	-0.442	<0.0001
D	-0.257	<0.0001
R	-0.049	0.021
S*S	-0.003	0.935
D*D	0.082	0.024
R*R	-0.008	0.827
S*D	0.051	0.071
S*R	-0.012	0.661
D*R	0.061	0.019

The sparger and D-helix aeration rates were significant factors, thus the HALR setups were combined according to D-helix aeration rate (Table 3). Equation (1) for t_{mix} prediction was fitted for the setup combinations presented in Table 3. In case of volumetric oxygen mass transfer coefficient prediction, considering separately the air pumped into the downcomer through the D-helix yielded better data fits [19]. Thus, t_{mix} was less affected compared to oxygen mass transfer by D-helix aeration. This might also depend on the larger sparger aeration step increase (20 lpm) between measurement points in the current study. However, different coefficient values (Table 3) revealed that the reactor geometry (in the current study the HFPs and the aeration through them) had an impact on the mixing time. R^2 values for setup combinations with moderate or high D-helix aeration were good (>0.97 both) and R^2 value for the regular ALR was sufficient (0.868). These equations can be used for t_{mix} prediction within the measurement range with these particular HALR setups.

Table 3. Coefficients for the mixing time correlation Eq. 1.

$t_{mix}=aU_{gs}^b$	a	b	R^2
ALR	2.75	-0.799	0.868
ALR+R0/5/10+D0*	7.87	-0.494	0.751
ALR+R0/5/10+D5**	4.89	-0.637	0.979
ALR+R0/5/10+D10***	4.04	-0.696	0.978
ALR+D10	11.81	-0.331	0.702

*setups 3-5 in Table 1; **setups 6, 8, 10 in Table 1; ***setups 7, 9, 11 in Table 1.

3.2. Circulation time

MLR analysis was performed for t_c data in order to find significant factors affecting liquid circulation in HALR (Table 2). The result showed that, both D- and R-helix aeration, along with combined effect of D-helix and sparger aerations, and second order D-helix aeration term have statistical significance on t_c . The positive coefficients for HFP aerations (R- and D-helix aeration rates) mean that, with increase in aeration t_c is prolonged. However, the impact of R-helix aeration was only approximately 11 % of D-helix aeration. The interaction effect of the sparger and D-helix aeration had negative coefficient, hence shortened t_c . Yet, the coefficient of the D-helix aeration rate is largest, meaning that it has strongest impact on t_c in HALR. It had also lowest p-value indicating the highest statistical significance. Gluz and Merchuk [16] made similar observation that HFP in the downcomer slightly increased t_c .

The reasons for longer t_c in HALR setups with aeration through D-helix than in regular ALR or HALR setups with only R-helix aeration, was most probably small gas bubbles diffused through the D-helix which. Those gas bubbles, on the other hand, reduced the density difference between riser and downcomer zone and hence reduce the driving force for the liquid circulation, thus leading to increase in circulation time. On the other hand, those small gas bubbles caused more dispersion in the HALR and thus lead to better mixing and decrease in mixing time. After studying t_{mix} and t_c , the effect of the D-helix aeration seems to distinguish the studied HALR from ALRs found in the literature as it is generally stated that faster circulation is an indicator of faster mixing [21, 34, 35]. In the current study, it was found that as D-helix aeration is present, t_{mix} decreases according to sparger aeration rate (Fig 3.) while t_c stays almost constant regardless increase in sparger aeration.

3.3. Dimensionless mixing time

Dimensionless mixing time measures the required number of liquid circulations to achieve desired homogeneity level in the ALR and HALR. MLR analysis for t_{dim} (the ratio of t_m and t_c) in HALR showed that sparger and D-helix aerations are statistically the most significant factors (Table 2), having negative coefficients. This means that they decrease the value of t_{dim} with increased aeration rates. The finding that t_{dim} was dependent on gas velocity was in agreement with [3, 31], R-helix aeration had a minor negative impact and the combined effect of HFP aerations (D^*R) had positive impact (Table 2) on t_{dim} , but both of them were less significant statistically than sparger or D-helix aeration rates.

t_{dim} for the regular ALR decreased as the sparger aeration was increased (Fig. 4). The decrease in t_{dim} was logical, as it was found that t_{mix} in ALR was decreased with increased sparger aeration rate (Fig. 3) and according to Equation 2, t_{dim} can be decreased either with decrease in t_{mix} or with increase in t_c . Decline in the slope of t_{dim} (Fig. 4) seemed to be the closest to linear for regular ALR indicating linear dependence on sparger aeration rate. However, t_{dim} values of HALR setups with D-helix aeration (setups 2, 6-11 in Table 1) were smaller than t_{dim} values of regular ALR within the measurement range. The MLR results (Table 2) showed that the mentioned HALR setups had increased t_c because of D-helix aeration while their t_{mix} was mostly affected by sparger aeration. t_c in HALR was not affected by the sparger aeration rate. Again, t_{dim} can be reduced either by decreasing t_{mix} or by increasing t_c and because with the same sparger aeration rate, setups with D-helix aeration have longer t_c than the regular ALR, t_{dim} of HALR with D-helix aeration was smaller. These results mean that the required number of liquid circulations to achieve desired homogeneity was smaller in HALR with D-helix aeration, than in regular ALR, mainly due to an increase in t_c of the HALR. Contrary, when air was not diffused through D-helix (setups 3-5 in Table 1), the required number of liquid circulations *i.e.* t_{dim} was higher than in regular ALR. This was mainly due the slower t_{mix} as t_c were in the same range with the regular ALR.

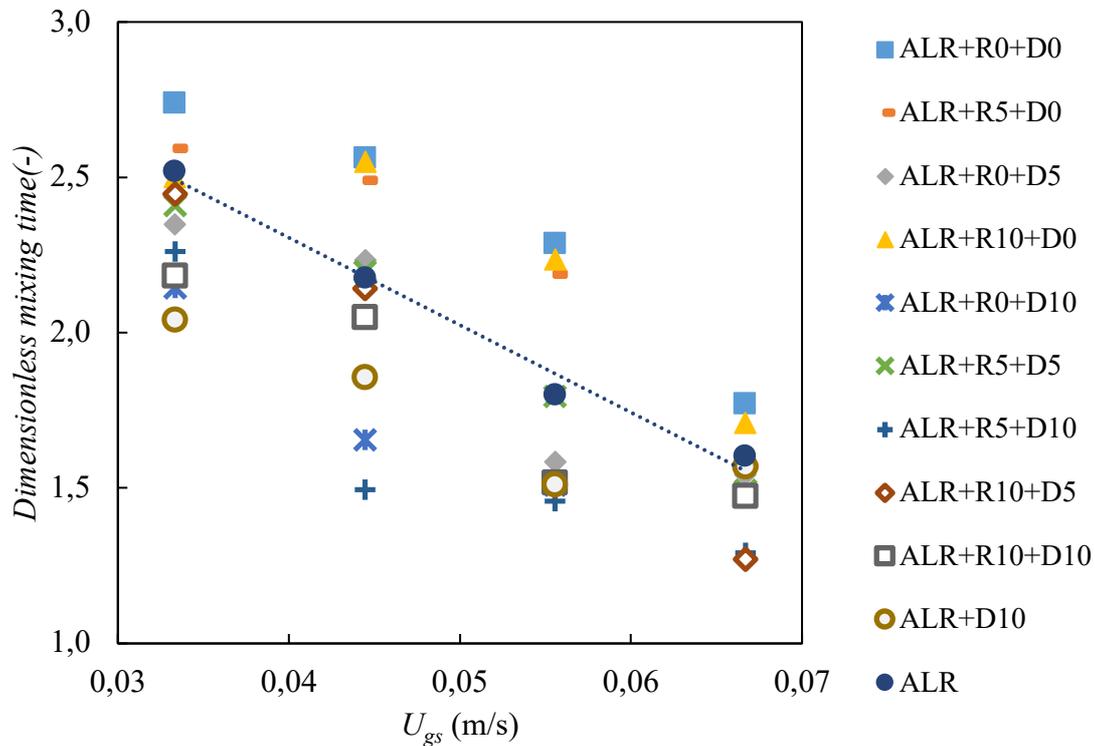


Figure 4. Dimensionless mixing time in the HALR and in the regular ALR as a function of superficial gas velocity caused by the sparger aeration (U_{gs}). Dotted line indicates the trend for regular ALR.

In the current study, the air diffused through D-helix decreased the circulation speed but at the same time, it seemed to improve the mixing when considering the number of liquid circulations needed to achieve desired homogeneity. Gavrilescu and Tudose [36] found the same in the study with external circulation ALR; the required number of circulations to achieve desired degree of mixing were diminished with impeded circulation. In addition, the HALR setups without D-helix aeration showed faster circulations with slower mixing when compared with setups with D-helix aeration. These findings

suggested the possible effect of axial dispersion on mixing phenomena in HALR with D-helix aeration.

3.4. Bodenstein number

Bodenstein number was used to evaluate the liquid flow behavior *i.e.* axial dispersion since t_c independently tells about liquid velocity. According to MLR analysis, D-helix aeration rate and the second order term of R-helix aeration rate showed significant impact on Bo number in HALR (Table 4). MLR coefficients for both were negative, meaning that the Bo number was declined with an increase in aeration through HFPs. The significance of the D-helix aeration rate was greater than the significance of the second order term of R-helix aeration. Thus, the D-helix aeration rate was the most significant parameter to effect on the Bo number. The average Bo number for the HALR was ca. 28, which meant that the liquid flow was more like a plug flow with superimposed dispersion than a mixed tank. HALR setups containing both HFPs were combined based on the D-helix aeration rate; ALR+R0/5/10+D, ALR+R0/5/10+D5 and ALR+R0/5/10+D10 (see Table 3) and the Bo number results for those combinations are shown in Fig. 5.

Table 4. Results of the MRL analysis for Bodenstein number in HALR. S stands for sparger aeration, R for riser helix aeration and D for downcomer helix aeration.

	Coefficient	P-value
Bo		
Constant	27.946	0
S	0.421	0.38
D	-2.491	<0.0001
R	0.55	0.208
S*S	1.121	0.161
D*D	0.136	0.856
R*R	-1.487	0.049
S*D	0.892	0.134
S*R	-0.89	0.1034
D*R	-0.048	0.929

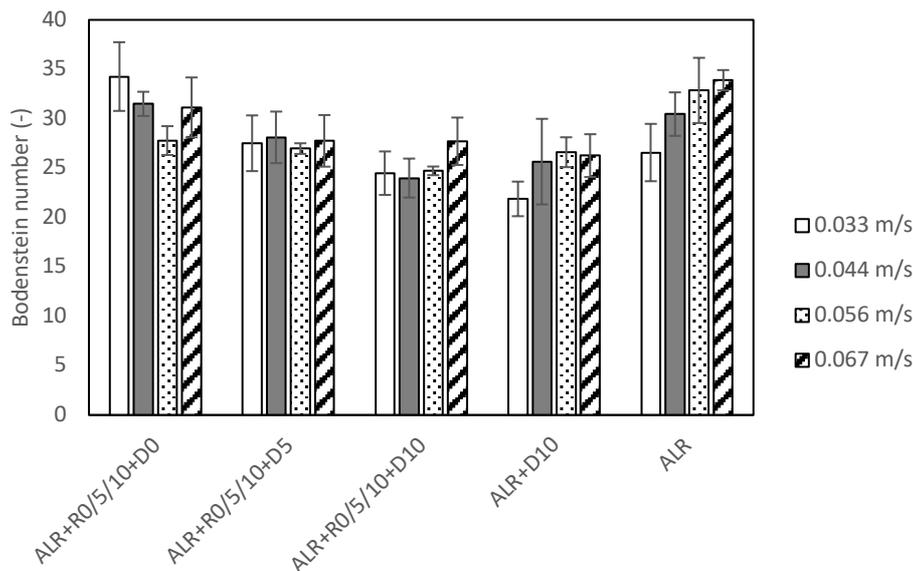


Figure 5. Bodenstein number for HALR and ALR as a function of the superficial gas velocity caused by the sparger aeration rate (U_{gs}).

The Bo number was 20-35 for all HALR setups as well as for the regular ALR (Fig. 5). For regular ALR; Bo number is increased with the increased sparger gas flow rate, meaning that the axial dispersion is weaker as the sparger gas flow rate is increased. These results are in accordance with Chisti [3] who stated that in general Bo number for the ALR reactor as a whole increases with gas flow rate. Kilonzo et al. [31] also made similar observation. In their study Bo number of internal loop ALR increased consistently with increasing superficial gas velocity. This phenomenon did not show with HALR setups. On the contrary, Bo numbers were rather stable over the studied sparger aeration range according to the D-helix aeration rate.

In regular ALR, t_{mix} was decreased with increased sparger aeration, and at the same time Bo number was increased. This means that axial dispersion was not significantly linked to faster mixing. Thus, faster mixing in the regular ALR was mainly caused by faster circulation and less by axial dispersion. Contrastingly, almost constant Bo numbers (Fig. 5) and decreased mixing times (Fig. 3) with increased t_c for the HALR setups, indicated that the mixing phenomenon was rather dependent on the dispersion than on the circulation speed in HALR. Due to increased turbulence, t_{mix} did not increase with increased t_c . Results of t_{dim} supports the finding, too. t_{dim} for HALR setups with D-helix aeration was smaller than for regular ALR. Smaller t_{dim} means lower number of liquid circulations needed to achieve desired homogeneity level in the liquid, *i.e.* mixing was more dependent on other phenomenon than liquid circulation.

4. Conclusions

The main objective of the work was to evaluate the mixing efficiency in draft tube sparged airlift reactor with novel HFP-gas spargers. Mixing time t_{mix} , circulation time t_c and dimensionless mixing time t_{dim} were used to evaluate the overall mixing in a reactor and Bodenstein (Bo) number was used to evaluate axial dispersion in the reactor.

The main discoveries from current study were that mixing in HALR was mainly dependent on the sparger aeration rate. The stronger the sparger aeration, the shorter the t_{mix} . Concurrently, the D-helix aeration had a minor accelerating impact on t_{mix} while t_c was mainly decelerated by the D-helix aeration rate. These together mean that in the HALR, faster circulation time did not mean faster mixing. t_{dim} showed that HALR with D-helix aeration required reduced number of liquid circulations to achieve desired homogeneity compared with a regular ALR. The Bodenstein number of HALR was mostly dependent on D-helix aeration so the HFP aeration improved axial dispersion compared with a regular ALR. Thus, D-helix aeration extended t_c , but resulted in shorter t_{mix} because of improved dispersion.

The mass transfer results from previous study [19] with the same reactor type showed that HFPs improved the volumetric oxygen transfer coefficients of the HALR significantly when compared with regular ALR. The improvement could be reached even without additional energy consumption, because HALR's oxygen mass transfer capacity can be enhanced by diffusing a fraction of total aeration through D-helix. Compared with CSTR, the energy consumption for the same volumetric oxygen transfer coefficient were only 17% with HALR [19]. These results together with the results from the current study considering mixing phenomena mean that in case of HALR, mixing and oxygen mass transfer can be optimized at the same time, unlike in conventional CSTRs [8]. The HALR type of bioreactor would provide a fascinating, economic alternative to conventional stirred tank reactors. Furthermore, it would be beneficial to study the performance of the HALR with highly aerobic, high cell density cultivation, to see the performance with living biomass.

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