Mixing Studies in Loop Bioreactors for Production of Biomass from Natural Gas

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ABSTRACT: *The mixing behavior of the gas-liquid phase in three loop bioreactors was investigated. A gas-induced External Airlift Loop Bioreactor (EALB), a forced-liquid Vertical Tubular Loop Bioreactor (VTLB) and a forced-liquid Horizontal Tubular Loop Bioreactor (HTLB) were used for mixing studies as well as biomass production from natural gas. The effect of design parameters, riser to downcomer cross sectional area ratio (A_r/A_d), height to diameter ratio (H/D), length to diameter ratio (L/D) and volume of gas-liquid separator (S); as well as operational parameters, i.e. superficial gas velocity (U_{sG}) and superficial liquid velocity (U_{sL}) on mixing time were studied. It was found that liquid circulation (pumping) had an important effect on mixing time. VTLB, because of providing an effective countercurrent flow between gas and liquid streams, demonstrated the best mixing time performance. HTLB, as the second, provides a moderated mixing time output. EALB, since circulates no forced liquid, presents less mixing ability (gas moves liquid). It was observed from experimental results that mostly superficial gas velocity has an obvious effect on EALB. Accordance to mixing time data, a region that was independent on bioreactor type was explored that happened in high gas superficial velocity. In that zone, mixing time was not reliant on bioreactor variety and varies with the variation of operational and design parameters only. Some empirical correlations for mixing time in terms of A_r/A_d, H/D, L/D, U_{sG}, U_{sL} and volume of gas-liquid separator were obtained and expressed separately which can be used for design and scale up. The best biomass production occurred in the VTLB for gas mixture of 40% methane and 60% air.*

KEY WORDS: *Mixing time, Loop bioreactors, EALB, VTLB, HTLB, Biomass, Natural gas.*

INTRODUCTION

In order to avoid the mechanical stirring, different types of bioreactors have been extended; the majority of these bioreactors are loop bioreactors [1- 4]. Loop bioreactors are characterized by a definitely directed circulation flow that can be driven in fluid or fluidized systems by propeller or jet drive and mainly in gas-liquid systems, furthermore by airlift drive or liquid pump. They are especially appropriate for fluid systems requiring

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high dispersion priority. On the other hand, their simple constructions and operation results in low investment and operational cost [5]. Loop bioreactors are used for application in chemical engineering and biotechnology due to their advantages of good mass, high fluid circulation, short mixing times and low shear rates [6]. Moreover, loop bioreactors have shown an acceptable performance for the production of biomass from natural gas due to their unique hydrodynamic characteristic [1,3,4].

Despite of low solubility, flammability and non–high purity in natural sources, methane is a good candidate for biomass production because of its non-toxicity, selectivity and volatility [1]. Bacteria which are able to utilize methane as the sole source of carbon and energy have been known since 1906 [7]. In addition, some studies have indicated a potential use of methane oxidizing bacteria as a source of protein either for food supplement or as fodder [8-11]. Methanotrophic bacteria, or methanotrophs, were defined by their ability to use methane as their sole source of carbon and energy, although some will also grow on methanol [7]. Methanotrophs are a subgroup of bacteria known as methylotrophs [9,12]. These bacteria are used for biomass production (bio–protein) and for bioconversion of organic compounds, such as amino–acids, enzymes, vitamins, bio–polymer and others [12,13]. The oxidation of methane by aerobic methanotrophs is initiated by Methane MonoOxygenases (MMOs) [13,14]. The two known pathways used by methanotrophic bacteria for the synthesis of multi-carbon compounds from methane are the serine and riboluse monophosphate [12,13]. Past investigations on methane fermentation, are mostly devoted to the metabolic pathways of methane oxidation and the taxonomy and physiology of methane-utilizing organisms [12,15,16]. Although loop bioreactors are used for fermentation of other carbon sources and some technical data are available in this regard [1,17-20], data have not been provided for significant factor such as mixing behavior for methane fermentation in loop bioreactors.

The mixing characteristics of loop bioreactors represent a very important factor for design, operation and comparison of bioreactors [5, 21]. Mixing time is generally defined as the time required by a mixed liquid to reach a specified degree of homogeneity after a tracer pulse has added to it. Mixing time is a direct index of homogeneity of the component concentration, e.g. microorganisms, dissolved gases and substrates, in a broth. A thorough knowledge of the mixing behavior and the factors that influences it is of particular importance for modeling and scaling up from a laboratory to an industrial scale [6]. A high mixing effect is obtained with an ideal stirred tank reactor type, whereas the demand of high driving concentration difference for the gases-transfer is best fulfilled with an ideal tube reactor type. In loop bioreactors, combination of them has been observed [5].

This work was undertaken in order to investigate and compare the liquid phase mixing characteristics of three loop bioreactors using the tracer injection. The effect of the geometrical and operational parameters on liquid mixing for a gas-induced External Airlift Loop Bioreactor (EALB), a forced-lLiquid Vertical Tubular Loop Bioreactor (VTLB) and a forced-liquid Horizontal Tubular Loop Bioreactor (HTLB) were examined extensively. In addition, some equations which correlate experimental laboratory data were developed.

EXPERIMENTAL SECTION

Microorganism and growth medium

The microorganism (a *Methylomonas* spp.) used in this work was isolated from an oil field in Iran during the research work on our previous investigation on a bubble column bioreactor [10]. The growth medium was named as Methane Salt Broth (MSB) which has been optimized by *Yazdian et al.* (2005) [10]. The carbon source in MSB medium is methane.

Gas mixture

Five streams of mixed gases were used for evaluation of biomass production. Inlet gas flow rates of air and methane were adjusted so that it would provide mixtures from 20 v% to 60 v% air (five streams with ten v% interval). When oxygen is present in a gas (such as air), the proportion of methane to air is normally in the range of 5 to 15 volumes of methane to 95 to 85 volumes of air, respectively [22] to form a flammable mixture; however, since gases were spareged right away after mixing and passed for a single time through the liquid phase, all experiments were carried out safely. Furthermore, in the rest of the experiments (with pure gases that are not combustible), air and methane were used independently in the experiments. Hence, the composition of gas mixtures used in these experiments was completely safe and combustion-free.

Table 1: Characteristics of the loop bioreactors used for mixing studies.

Characteristics	Unit	Reactor type		
		EALB	VTLB	HTLB
D	m	-	-	0.03
D_d	m	0.03	0.03	-
D_r	m	0.03, 0.06	0.03	-
D_s	m	0.11, 0.18	0.11, 0.18	0.11, 0.18
A_r / A_d	#	1.00, 4.00	1.00	-
h_s	m	0.10	0.10	0.10
H/D	#	-	45, 67	-
L/D	#	-	-	37, 54
$S=V_s / V_d$	#	0.61, 1.65	0.61, 1.65	0.61, 1.65
N	#	6	6	6
D_o	m	0.10	0.10	0.10
LP	#	-	MP	MP

Loop bioreactors

Three experimental loop bioreactors (laboratory scale made of glass), which operated with air and water, were used. The EALB and the VTLB configurations consisted of two vertical columns connected at the top (separator) and the base by horizontal piping. In addition, there is a liquid pump just the bottom of the downcomer in the VTLB. The HTLB consists of two long horizontal parts, short vertical downstream and upstream tubes, a top part which is placed right above the upper end of the downstream and a U-shape bend. The geometrical characteristics of the devices are given in Table 1 and illustrated in Fig. 1. The air was distributed by a perforated tube through a gas pump in different zones of the loop bioreactors. The difference in hydrostatic pressure between the two regions in the EALB results in circulation of the liquid. However, in the VTLB and HTLB, the liquid medium was circulated by a liquid magnetic pump. All experiments were carried out at 30 (±0.5) °C. This was done by a Temperature Loop Controller (T.L.C.) placed inside the dissolved methane detector and connected to an electrical heater positioned at the top of the loop bioreactors. Also, a cooling system was used for removing the microbial heat produced during the fermentation process.

Measurements

Mixing times

There have been some attempts in predicting the mixing time using Bo (Bodenstein) number [23-25]. In these works, the mixing time was given as a function of circulation time and Bo number. However, since each section of loop bioreactors have their own dispersion characteristics, Bo number may vary from part to part and could not be considered as an overall criterion for mixing time measurement [24,26-28]. Considering this complexity, it is better to determine mixing time by geometrical characteristics of the reactor and operational parameters [6,27-39]. The mixing time is defined as the time required to achieve a specific percentage of concentration homogeneity (in our case 95% of final response) within the reactor after the addition of a quantity of tracer. Mixing time (t_m) was determined using tracer response techniques when air was introduced. These are based on the fact that if a pulse of tracer (a dye) is injected to the flow, a decaying sinusoidal type of response is detected at the downstream of the injection point [5,19,25]. Tracer (0.5 ml, Brilliant Blue G, λ=595 nm) was injected to the loop bioreactors. Amounts of optical density were recorded in a spectrophotometer (VARIAN CARRY 50 CONC, Australia); until the response

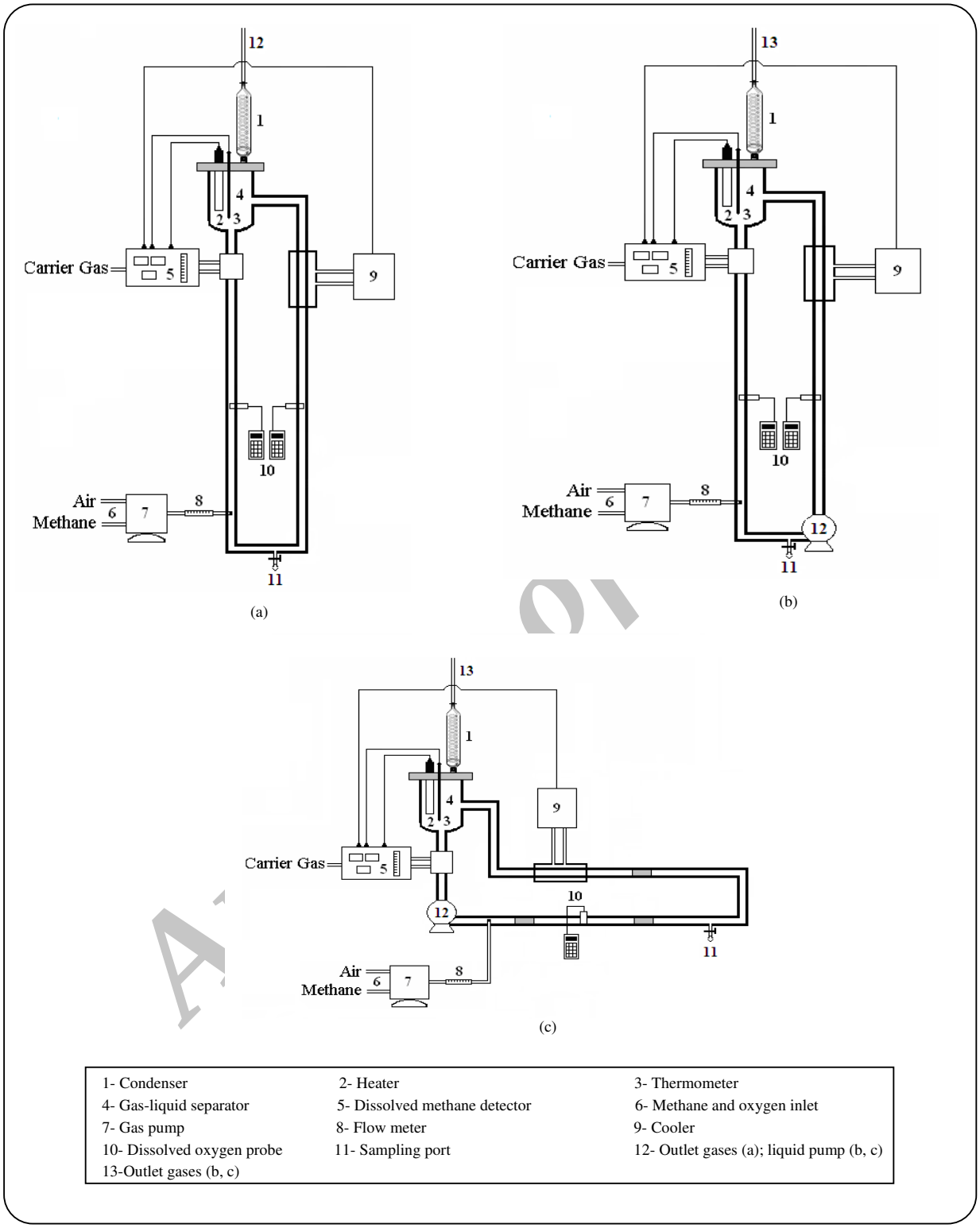

Fig. 1: A schematic diagram of loop bioreactors (a: gas-induced EALB; b: forced-liquid VTLB; c: forced-liquid HTLB).

of the pulse was completely damped (each experiment was carried out in triplicate). Thereafter, mixing time was determined by changing geometrical and operational parameters in mentioned loop bioreactors.

RESULTS AND DISCUSSION

Mixing times

The mixing characteristics of loop bioreactors of different configurations can be compared by considering the time required to achieve a certain degree of mixing (in this study 95%). The mixing time may also be used as an operation and scale up parameter, its variation being dependent on the operational and geometrical conditions. To predict or compare the mixing times in the loop bioreactors which were being designed or made, as a function of the operating variables and geometry, the specific mixing time, denoted as the mixing time per unit liquid volume (t_m/V), is usually used. These specific mixing time concept has also been used previously by *Rousseau & Bu'Lock* (1980); *Popovic & Robinson* (1993) and *Gavrilescu & Tudose Radu* (1997) [6,30,40]. Here, we determine the mixing time with the same method and define a geometrical parameter such as S to interpret the effect of the volume of the separator, H/D to illustrate the influence of VTLB height and L/D to present the impression of HTLB length. S, H/D and L/D obtain two values (see Table 1).

Like all the authors, which investigated the influence of the gas velocity, the same results have been observed in this study. In all devices investigated, the mixing time decreases with an increasing aeration rate. In the EALB, two regimes of mixing time were observed. At low gas velocities, mixing time decreased sharply, while at higher velocities mixing time was almost constant (Fig. 2). Especially in laboratory systems, the mixing time becomes less efficient at higher gas velocities. These values of gas velocity correspond to the transition from the circulating to the turbulent regime. Mixing time decreased with increasing values of both gas and liquid flow rates in the VTLB and HTLB too. Our experiments showed that there are two zones of the dependency of mixing time on gas velocity (Fig. 3 and Fig. 4). For different values of gas velocity (in this study), mixing time was sensitive at low liquid velocities; while at higher liquid velocities, mixing time was less affected by high values of aeration rates. In the VTLB, at low gas

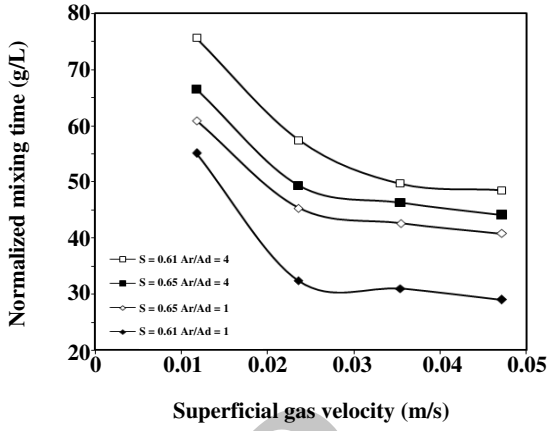

Fig. 2: Normalized mixing time versus superficial gas velocity in the gas-induced external airlift loop bioreactor.

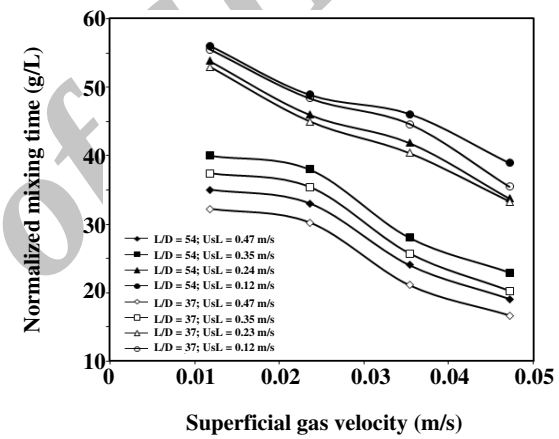

Fig. 3: Normalized mixing time versus superficial gas velocity in the forced-liquid horizontal tubular loop bioreactor.

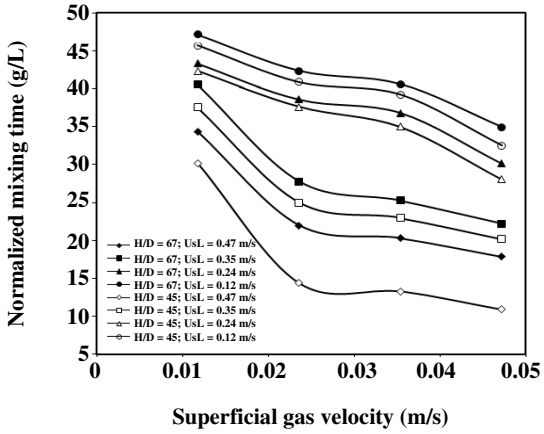

Fig. 4: Normalized mixing time versus superficial gas velocity in the forced-liquid horizontal tubular loop bioreactor.

velocities mixing time was highly sensitive to different amounts of liquid circulation rates. However, in the HTLB, mixing time was sensitive to low gas velocity especially at low liquid flow rates.

Figs. 2-4, show that when the amount of A_r/A_d, L/D and H/D decreased from 4 to 1, 54 to 37 and 67 to 45, respectively, the mixing time decreased, too. These are expectable and also have been shown by many investigators that observed a decrease in the mixing time with decrease of known geometrical parameters such as A_r/A_d, L/D and H/D ratio [29, 31-34, 36, 41-43]. Also, no discernible effect of the separator volumes on mixing time was observed (0.61 and 1.65 for S). However, in the EALB, separator volume showed its role considerably in compare to the VTLB and HTLB. It is necessary to find the minimum critical value of S for optimum operation of the mentioned loop bioreactors in further studies. The effect of design factors on mixing time in the investigated loop bioreactors (particularly in the EALB) can therefore be attributed mainly to the effect of design parameters (e.g., A_r/A_d) on the recirculation of liquid velocity, which proves to be the physical parameter which most strongly affects the recirculation rates in the none-forced liquid loop bioreactors. Therefore, the mixing time can be considered as a measure of the macro-scale mixing by convective mechanisms [6].

Based on our experimental results, some new correlations for normalized mixing time (t_m/V) are presented in Eqs. (1-3). All the experimental data on the mixing time obtained in the EALB were correlated using the above considerations, by SIGMA-PLOT, resulting in the following dependence ($R^2 = 0.92$).

$$\frac{t_m}{V} = 12.30 U_{sGr}^{-0.35} . (1+\frac{A_r}{A_d})^{0.30} . (1+S)^{-0.37} \qquad (1)$$

Eq. (1) determines mixing time by only three parameters in the EALB. This could be an advantage for our correlation compared to other correlations, which use four or even more parameters [6,38]. Depending on the gas velocity range the exponent for U_{sgr} has the values from -0.075 [29] to -0.60 [32]. However, the data of most authors show that this value is between -0.30 and -0.50. Weiland (1984) [34] showed the A_r/A_d had greater influence on the mixing time in the smaller reactor (up to 5 L). This fact has been clearly shown in Fig. 2. The influence of A_r/A_d in external loop reactors

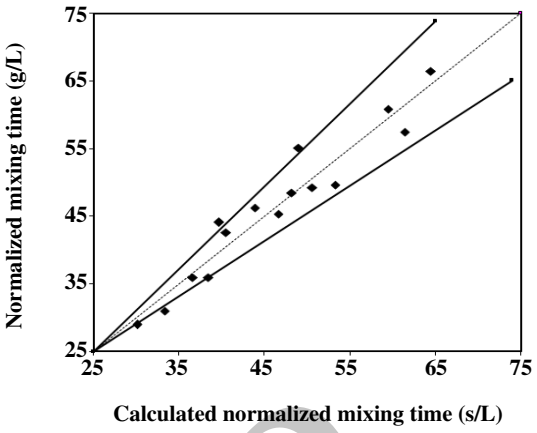

Fig. 5: Comparison between calculated and experimental values of normalized mixing time in the gas-induced airlift loop bioreactor.

experimentally was investigated by *Bello et al.,* (1984) [33]. Their results showed that mixing time increased with the increase of this ratio, with an exponent of 0.26. *Joshi et al.,* (1990) [36] theoretically predicted the value of 0.37 for this exponent. *Sukan & Vardar-Sukan* (1987) [35] investigated the effect of gas-liquid separator on the mixing time in the loop bioreactor (750 cm^3). They concluded that there exits an optimum gas-liquid separator. It would be interesting to compare their experiments with the experiments in the greater volume. *Weiland* (1984) [34] found that gas-liquid separator volume had strong influence on the mixing time. His results presented as t_m versus gas-liquid separator volume, gave the slope of -0.39 (the exponent of third variable in Eq. (1) is near to Weiland's). Fig. 5 compares the experimental values of mixing time and its calculated amounts determined from Eq. (1). Values estimated with Eq. (1) agreed with the experimentally measured data with less than 12% error. This maximum deviation only states in which ranges the measured and calculated data cover each other. Its range of applicability is between 0.01-0.05 m/s for superficial gas velocities. A_r/A_d is between 1-4. Also, the amount of separator volume to downcomer volume ratio is 0.61 to 1.65.

The dependence of the mixing time on the operational and geometrical velocities expressed by the following equation in the HTLB, too:

$$\frac{t_m}{V} = 4.01 U_{sG}^{-0.34} . U_{sL}^{-0.41} . (\frac{L}{D})^{0.11} . (1+S)^{-0.05} \qquad (2)$$

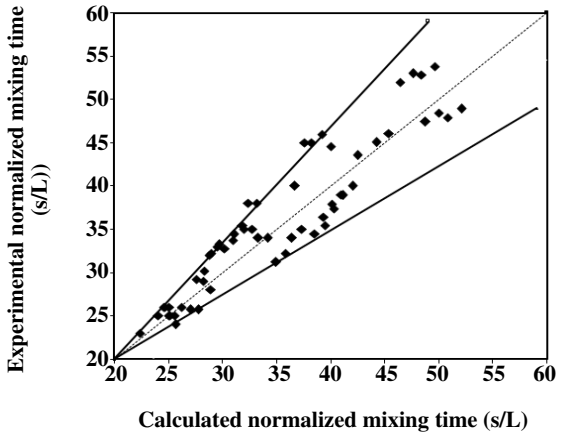

Fig. 6: Comparison between calculated and experimental values of normalized mixing time in the forced-liquid vertical tubular loop bioreactor.

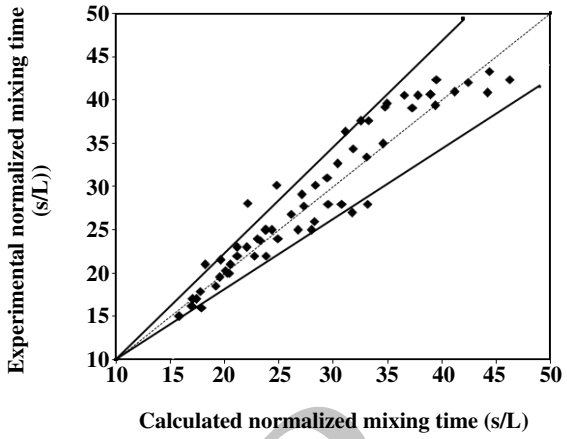

Fig. 7: Comparison between calculated and experimental values of normalized mixing time in the forced-liquid horizontal tubular loop bioreactor.

Eq. (2) determines mixing time by four uncomplicated parameters in the HTLB. Its range of applicability is between 0.01-0.05 m/s and 0.1-0.5 m/s for superficial gas and liquid velocities, respectively. L/D is between 37-54. Also, separator volume to downcomer volume ratio is 0.61 to 1.65. Eq. (2) correlated 90% of the data with less than 15% error. Fig. 6 compares the experimental values of mixing time and its calculated amounts determined by Eq. (2).

On the basis of our experimental results in the VTLB, a new correlation for normalized mixing time (t_m/V) is presented by Eq. (3).

$$\frac{t_m}{V} = 1.06 U_{sG}^{-0.42} . U_{sL}^{-0.48} . (\frac{H}{D})^{0.29} . (1+S)^{-0.09} \qquad (3)$$

Eq. (3) determines mixing time by four simple parameters in the VTLB. Based on Eq. (3), the mixing time can be predicted using gas and liquid velocities and construction characteristics of the bioreactor only. We hope that these correlations could be used for rapid and simple estimation of the mixing time in the VTLB. The power of S shows this fact, on the other hand, that the separator type could have the least effect on mixing type. Eq. (3) correlated 90% of the data with less than 14% error. Fig. 7 compares experimental values of mixing time and its calculated amounts determined by Eq. (3). Its range of applicability is between 0.01-0.050 m/s and 0.1-0.5 m/s for superficial gas and liquid velocities, respectively. H/D is between 45-67. Also, separator volume to downcomer volume ratio is 0.61 to 1.65.

The dependence of mixing time on gas and liquid flow rates was studied in forced-liquid loop bioreactors has been studied by *Chisti & Jauregui-Haza* [2], *Fadavi & Chisti* [44,45]. They concluded that mixing time decreased with both increasing gas and liquid flow rates (both the gas and liquid velocities contribute to promote mixing in the bioreactor). Furthermore, *Papagianni et al.* [25] used Verlaan's equation to estimate mixing time. The mixing times calculated with the equation of Verlaan et al. [24] were very close to those obtained from the experimental data for a forced-liquid tubular loop bioreactor. They suggested that the relative mixing time (t_m/t_c) is a factor that can be used with confidence in characterization of the mixing in forced-liquid TLBs. Many investigators observed a decrease in the mixing time with decrease of known geometrical parameters such as L/D, H/D, A_r/A_d ratio and horizontal connection pipe length between riser and downcomer in loop reactors [34,44,45] as well.

Fig. 8 illustrates the comparison of mixing time versus superficial gas velocity for different loop bioreactors. The shortest mixing time was achieved in the VTLB. In the HTLB, t_m was more than in the forced-liquid VTLB. However, mixing times in the HTLB and VTLB were less than in the EALB; because of the increased liquid velocity in the forced-liquid loop bioreactors. Therefore, the experimental results showed that the longest mixing times were obtained in the EALB. According to experimental outcomes, the forced-liquid circulation (in the VTLB and HTLB) led to a shortening

Table 2: Mixing time correlations and experimental results for different loop bioreactors.

Characteristics	Correlation	RED*(s)	R^2
Gas-induced EALB	$12.30U_{sGr}^{-0.35}.(1+\frac{A_r}{A_d})^{0.30}.(1+S)^{-0.37}$	29-75	0.92
Forced-liquid HTLB	$4.01U_{sG}^{-0.34}.U_{sL}^{-0.41}.(\frac{L}{D})^{0.11}.(1+S)^{-0.05}$	16-55	0.90
Forced-liquid VTLB	$1.06U_{sG}^{-0.42}.U_{sL}^{-0.48}.(\frac{H}{D})^{0.29}.(1+S)^{-0.09}$	10-47	0.90

**RED: Range of experimental data (mixing time, [second])*

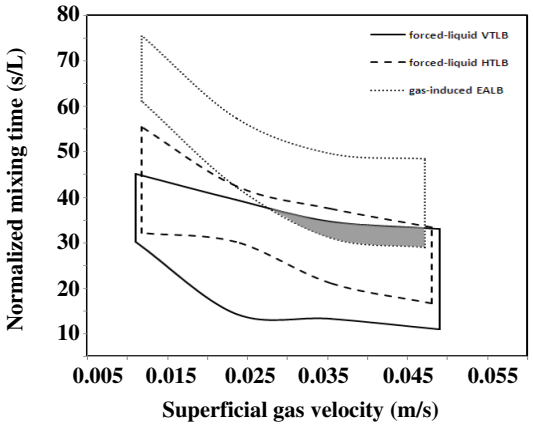

Fig. 8: Comparisons of mixing time versus superficial gas velocity for loop bioreactors (forced-liquid VTLB, forced-liquid HTLB and gas-induced EALB).

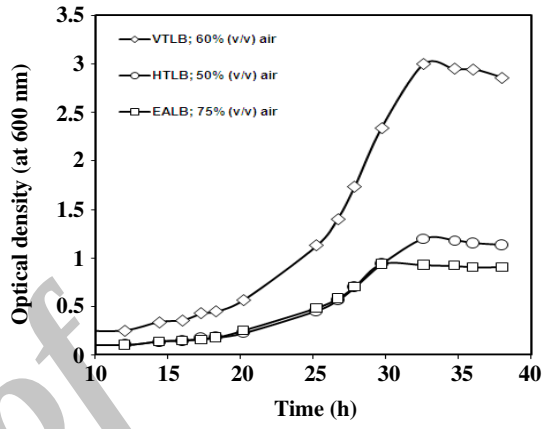

Fig. 9: Optical density of MSB culture versus time at 600 nm for different loop bioreactors in optimum ratio of air to methane.

in the mixing time about 49% compared to the EALB. This behavior indicated that mixing processes was predominately controlled by the macro-circulation of the liquid phase within the loops and to a lesser extent by the axial dispersion due to ascending bubbles [21]. Due to the presented data, a region (it was shown in gray color in Fig. 8) that was independent on bioreactor type was explored that occurred in high gas superficial velocity that ranged between 0.03 m/s to 0.05 m/s. In that zone, mixing time was not reliant on bioreactor variety and changed with variation of operational and design parameters only. The experimental results for mentioned loop bioreactors (based on correlations and range of experimental data) are compared in Table 2.

Biomass production

In order to investigate the performance of the mentioned loop bioreactors in terms of biomass production and according to mixing time data, some experiments were designed [46-48]. In all experiments operational and design parameters were fixed at the values mentioned above. However, different mixtures of air and methane were fed. Fig. 9 illustrates the scenario of biomass growth based on optimum ratio of air to methane for EALB, VTLB and HTLB. All experiments were started by seven volume percent inoculum of active *Methylomonas* culture and carried out in triplicate. Fig. 9 shows that the best biomass production happened in the VTLB for gas mixture of 40% methane and 60% (maximum optical density: 3; doubling time: 96 min).

CONCLUSIONS

Mixing time was essentially correlated with the various combinations of design parameters (A_r/A_d, H/D, L/D and volume of gas-liquid separator (S)) as well as operational parameters (U_{sG} and U_{sL}) in the gas-induced external airlift loop bioreactor (EALB), forced-liquid vertical tubular loop bioreactor (VTLB) and forced-liquid horizontal tubular loop bioreactor (HTLB). Mixing time increased with increasing design parameters such as

A_r/A_d, H/D and L/D; and decreased (improved) by increasing the rate of aeration and liquid pumping. Mixing time in the EALB has shown a significant influence by increasing the rate of aeration; whereas other two loop bioreactors (VTLB and HTLB) that circulate liquid, responded better mixing time by increasing the amount of pumping. Generally, those that circulate liquid resulted in lesser (better) mixing time. As Fig. 8 illustrates, the shortest mixing time was achieved in the VTLB. VTLB, not only affords a forced liquid flow, but also provides a countercurrent contact between gas and liquid flow. The horizontal loop bioreactor circulates the bubbles by the force of pumped liquid too; however, since it makes bubbles flow in horizontal direction, it creates a moderate mixing time. The conventional EALB, in this comparison, demonstrated the poorest mixing time performance. To show the maximum difference, as Fig. 8 and Table 2 evidence, the VTLB can perform a mixing time around 49 percent better than EALB. According to mixing time data, a region that was independent on bioreactor type was explored that happened in high gas superficial velocity that ranged between 0.03 m/s to 0.05 m/s. In that zone, mixing time was not reliant on bioreactor variety and varies with variation of operational and design parameters only.

This research has been devoted to covering mentioned variables to obtain a generally applicable equation for the loop bioreactors design in order to produce biomass from natural gas in optimum conditions. Based on mixing time data, the best biomass production occurred in the forced-liquid VTLB for gas mixture of 40% methane and 60% air.

Notation

A_r/A_d	Riser to downcomer cross sectional area ratio
Bo	Bodenstein number
D	Bioreactor diameter
D_d	Downcomer diameter
D_o	Hole size in sparger
D_r	Riser diameter
D_s	Separator diameter
EALB	External airlift loop bioreactor
h	Hour
h_s	Liquid level in separator
H/D	Height to diameter ratio
L/D	Length to diameter ratio
LP	Liquid pump
MP	Magnetic pump
N	Holes number in sparger
RED	Range of experimental data
S	Separator to downcomer volume ratio
s	Second
t	Time
t_m	Mixing time
U_{sG}	Superficial gas velocity
U_{sGr}	Superficial gas velocity in riser
U_{sL}	Superficial liquid velocity
V	Bioreactor volume
V_d	Downcomer volume
V_s	Separator volume

Greek letters

λ	Wavelength

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