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Airlift bioreactor under bio-consumption environment: Mass transfer study of metabolic gases

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ABSTRACT

The current study focused on tracking the biogas pathways in the internal loop airlift bioreactor under conditions of metabolic consumption. The focus of the investigation was simultaneously set on the wastewater produced by the dairy industry. Two stages were used to conduct this investigation. Using the Taguchi model, an optimization study of the bioreactor's operating conditions was conducted as the first step. The experimental findings demonstrated a distinct impact of the biogas flow rate on the overall mass transfer coefficient compared with the other operating parameters (liquid type and volume). By analyzing the S/N ratio and ANOVA analysis, the best level was determined for each parameter studied. The optimal factors were also identified (flow rate = 50, volume = 5.5, type of sol. = whey). These optimization values were then applied in real bacteria bio-consumption. The biogas consumed by the organism was also reduced from 50 to 25 liters/hour, thus saving 50% of the energy consumed.

Keywords: mass transfer, airlift, bioreactor, metabolic gases, dairy wastewater.

INTRODUCTION

Biological processes are a special vital environment that an organism needs to survive within complex and interconnected chemical, physical and thermodynamic pathways. These processes are occupied by natural chemicals necessary for the microorganism's metabolism, represented by nutritious mineral salts, as well as relatively large amounts of water [Li et al., 1995; de Mello et al., 2024]. In addition, biological gases such as oxygen and carbon dioxide are essential for microorganisms to use in metabolic reactions. As a result, modifications to gas levels, whether positive or negative, can have a significant impact on how well the process works [Çalik et al., 2004; García-Ochoa et al., 2000]. It is important to ensure that the gas source supplies the culture medium with sufficient quantities to meet the needs of these metabolic reactions. [Garcia-Ochoa and Gomez, 2009]. To improve the performance of the bioreactor, it is necessary to study various factors such as the reactor's geometry, characterization of liquid media, operating conditions [García-Ochoa et al., 2000]. The type of reactor is considered the cornerstone on which the biological process is based. Therefore, choosing and designing the reactor to suit the needs of different microorganisms is a loophole that has baffled scientists and researchers [Fu et al., 2003]. Airlift bioreactors (ALR_S) are the most widely used and popular in fermentation processes and microorganism cultivation (algae, bacteria, etc.) as well as in research fields. This importance comes as a result of their properties that are compatible with fluid dynamics and do not waste energy through the use of pumping sources that reduce wasting the gas consumption out the reactor on the one hand and increase the mixing process and mass transfer on the other hand [Chaffin et al. 2024]. Despite the continued development and design of this type of reactor, there are still many aspects in this type of reactor that may need to be addressed in order to develop the design and efficiency of the process [Al-Mashhadani, 2017], especially the reactor geometry, because it has an important and effective role in the design of the airlift reactor [Kilonzo et al., 2007], as the design and engineering of the airlift bioreactor (ALR_S) affect the flow of fluids at different stages. The first stage is the gas flow from the bottom of the reactor (which is the area containing the distributor) to the draft tube, called the riser area, and the second stage is the gas flow from the top of the draft tube to the bottom of the reactor again, called the downcomer area [Mechanics et al., 1999; Merchuk et al., 1994].Transferring gas from the gas bubble to the liquid phase and then to the cells of living organisms takes a long time because this vital process takes place in several stages and steps. Each stage may limit the amount of mass transfer from the gas phase to the liquid phase, in addition to other vital reactions that occur inside the biological cell itself, which are considered the most important determining factors. This transfer stage may be accompanied by an opposite stripping stage. For example, in fermentation processes, the process of transferring oxygen gas into the reactor and then the biological cell is accompanied by a stripping process for carbon dioxide gas and goes through the same stages that the dissolution and transfer of oxygen went through, except that the transfer of carbon dioxide is accompanied by the reaction process with water, forming carbonic acid and its ions [Gouveia et al., 2003]. In general, oxygen is necessary for cells to breathe. When these organisms are in a liquid solution, oxygen can be supplied via three different mechanisms: the velocity at which it moves from a gas to a liquid, the rate at which microbes consume it, and the rate at which it is absorbed. Comprehending these variables is essential for formulating and creating efficient bioreactors [Garcia-Ochoa and Gomez, 2009]. The rate at which oxygen is transferred depends on two factors - the liquid lateral mass transfer coefficient (K_L) and the total surface area specified for mass transfer (a) [Al-Hemiri and Selman 2011; Akita and Yoshida 1973] . However, measuring these two factors independently is not possible. Therefore, they are combined and referred to as the volumetric mass transfer coefficient (K_{La}) [Galaction et al., 2004; Kawase et al., 1992; Van'T Riet, 1979]. This factor has been extensively studied for this type of bioreactor. However, these studies were often conducted under abiotic operating conditions. Consequently, these studies lack much of the information necessary for the operation or design of these reactors [Aiba et al., 1973; Andrew, 1982; Ho & Oldshue, 1987]. The current research aims to develop a hypothesis to verify the pathways and patterns of biogases in the airlift bioreactor and under the biological environment. The present study also examined how acidity affects an organism's ability to grow or function in the whey solution, with the goal of utilizing Taguchi experimental design to maximize the most crucial variables.

MATERIAL AND METHODS

An airlift bioreactor with a capacity of 10 liters and dimensions of 28×18×30 cm (in length, width, and height, respectively) was used to match the dimensions of the diffuser. The diffuser, in the form of a flat ceramic plate, had dimensions of 18×7.8×5.2 cm in length, width, and height, respectively. The test was conducted using distilled water and whey solution.

Whey solution

In this investigation, whey solution was preparation using the cheese production method, as this solution is a major by-product of cheese manufacturing and is like dairy factory wastewater. It was used because it contains a mixture of organic and inorganic substances, including fatty acids and proteins. and other organic nutrients that help the organism in the biological process and in the process of consuming oxygen [Poništ et al., 2021]. The whey was made using the separation method, by separating the cheese from the whey (as a by-product). The solution was prepared by adding 40 g of pasteurized milk per liter of water, and to make the whey separate from the milk, 37.5 g of yogurt and 3 g of citric acid were added to each liter of water, since the yogurt and citric acid help the milk particles to stick together. It produces cheese that is separated using a muslin cloth. Table 1 shows the characteristics of the whey solution.

Experimental work

The Characteristics of mass transfer using a microbubble diffuser were verified. It was placed in the middle of the airlift bioreactor bottom at 2 cm from the draft tube for easy gas circulation. It was connected by a pipe from a nozzle at the top of the reactor attached to a flow meter to measure and

Table 1. Characteristics of a whey solution

Characteristics	Values
Turbidity (NTU)	79.6-99.2
PH	5.35-5.84
COD (mg/L)	12420-13100
TDS (mg/L)	2.14-2.51

study the flow rate. The pH meter and dissolved oxygen (DO meter) were used, and their probes were inserted through nozzles in the lid of the air-lift bioreactor. While the heater was used to stabilize the temperature with 30 ± 1 °C, as shown in Figure 1. A pH meter was used to stabilize the pH of the solutions. Dissolved oxygen readings were taken every 5 seconds until the readings stabilized.

Inhibiting the activity of the organism

Whey is a solution abundant in many minerals and proteins that help the microorganism grow and increase its activity in addition to the aeration factor that stimulates the organism and puts it in permanent movement and growth. This causes oxygen to be in a state of constant biological consumption, which may affect the dissolution readings in the stage of optimization of the Taguchi approach. Therefore, the microorganism's growth was inhibited and stopped in a whey solution by using hydrochloric acid at a concentration of 1 M. The solution's acidity was adjusted to the extent that prevented the organism's growth. the solution was kept with an acidity of 2–3 and at a temperature of 10 °C for use more than once.

Design of experiments using the Taguchi method

When investigating more than one variable some errors must occur. When mentioning more than one parameter this indicates that many experiments have been conducted which increases the time taken and thus raises the cost. Therefore, the Taguchi approach was used which is characterized by its simplicity and efficiency in organizing and improving variables with the fewest possible number of experiments[Ibrahim and Salman, 2022]. This study aims to achieve the highest volumetric mass transfer coefficient using a rectangular airlift bioreactor with a microbubble pumping technique through a diffuser. Equation 1 is used to calculate the value of the volumetric mass transfer coefficient (KLa) [Zevenhoven, 2012]:

$$KLa = \frac{\frac{C[O_2]^* - C[O_2]_0}{C[O_2]^* - C[O_2]_t}}{t_2 - t_1} \tag{1}$$

where: $\frac{C[O_2]^* - C[O_2]_0}{C[O_2]^* - C[O_2]_t}$ is plotted against $t_2 - t_1$ and the slope representing KLa is extracted. $C[O_2]^*$ (mg/L) is dissolved oxygen concentration after stabilization (in steady state), $C[O_2]_0$ (mg/L) is the initial dissolved oxygen concentration, $C[O_2]_t$ (mg/L) is the dissolved oxygen concentration at any time ($C[O_2]_1$, $C[O_2]_2$, $C[O_2]_3$, ...).

To test the quality, an analysis of the signal-to-noise ratio S/N (highest to best) (HB) was used to improve the performance of the process. Equation 2 [Ibrahim and Salman, 2022] shows the relationship used to extract the S/N ratio.

$$[S/N] = -10\ log[\frac{1}{n}\sum_{i=1}^{n}\frac{1}{y^2{}_i}] \tag{2}$$

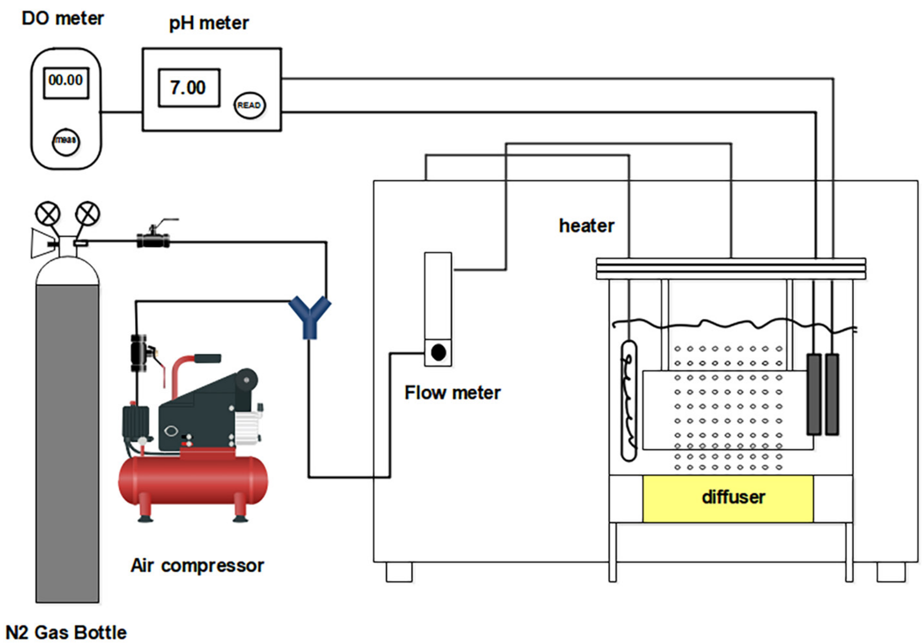

Figure 1. Schematic diagram of the airlift bioreactor experimental setup

where: S/N is the signal-to-noise ratio, and n is the number of observations, and y_i is (i)th observed value of the response variable.

In this study, three factors were verified: flow rate (coded G1), volume (coded G2), and type of solution (coded G3), shown in Table 2 which shows the levels of the three factors that will be studied and improved. The first and second factor has four levels, while the third factor has two levels.

Based on the design of the Taguchi approach the orthogonal matrix that was followed, which is shown in Table 3, will be L_{16}, as the ($4^2 \times 2^1$) array will reduce the number of experiments from 144 to 16 experiments, and this means saving time and cost.

RESULATS AND DISCUSSION

Inhibiting the activity of the organism

Since whey is a medium full of organic materials, proteins, and sugars, the emergence and growth of the organism when the appropriate conditions are available is very easy. To stop and inhibit this growth, a comparison was made between the ability of antibiotics and acids to inhibit its activity. As shown in Figure 2. the organism's behavior and its oxygen consumption in the control is very high until it is consumed. While antibiotics have demonstrated little ability to inhibit the activity of living organisms, it has been proven that antibiotics can create antibiotic-resistant bacteria [Kupolati et al., 2020; Bian et al., 2024; Zhou et al., 2024] . Therefore, acidity was relied upon to inhibit the activity of living organisms due to the availability of the substance used (1 M of HCL) and the ease of storing and reusing the solution. Also, exposure of the organism to severe acidity (i.e. a high concentration of H^+ ions) affects the structure of molecules, as it works to break up the hydrogen bonds that connect the DNA strands and thus affects the synthesis of ATP in cellular respiration, which requires a proton, which in turn depends on the concentration of H^+ ions, that is the solution must be acidic (4–7) for the organism to

Table 2. The levels of the parameters and their codes

Parameters	Coded	Level 1	Level 2	Level 3	Level 4
Flow rate	G1	5	20	35	50
Volume	G2	5.5	7	8.5	10
Type of sol.	G3	Distilled water	Dairy wastewater	--	--

Table 3. The coded and real values of L_{16} orthogonal array

Exp No.	Coded values			Real values		
	G1	G2	G3	Flow rate	Volume	Type of sol.
1	1	1	1	5	5.5	D.W
2	1	2	1	5	7	D.W
3	1	3	2	5	8.5	Whey
4	1	4	2	5	10	Whey
5	2	1	1	20	5.5	D.W
6	2	2	1	20	7	D.W
7	2	3	2	20	8.5	Whey
8	2	4	2	20	10	Whey
9	3	1	2	35	5.5	Whey
10	3	2	2	35	7	Whey
11	3	3	1	35	8.5	D.W
12	3	4	1	35	10	D.W
13	4	1	2	50	5.5	Whey
14	4	2	2	50	7	Whey
15	4	3	2	50	8.5	D.W
16	4	4	2	50	10	D.W

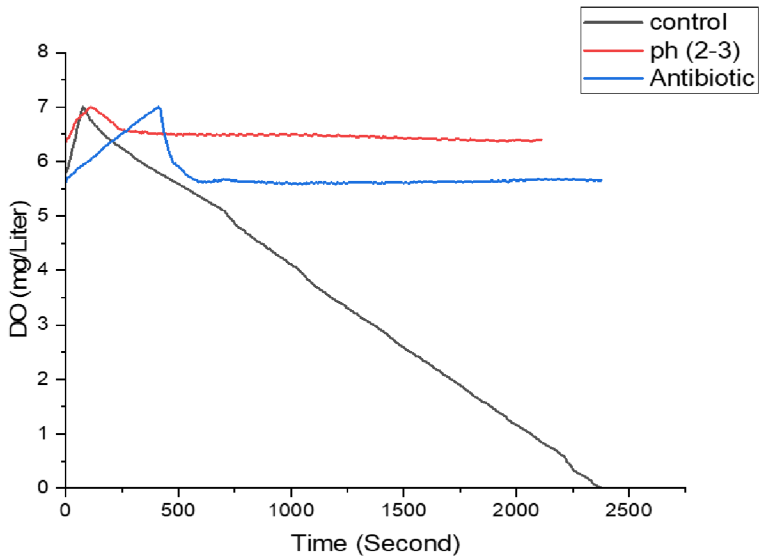

Figure 2. Testing the ability of the antibiotic and acidity to inactivate the organism

grow. Any increase or decrease in H^+ ions will inhibit the activity and spoil it [Keenleyside, 2019]. Therefore, the solution was kept at an acidic level (2–3), which showed its effectiveness in inhibiting the activity of the organism more than antibiotics, as the solution was used more than once without any growth of the organism.

Multiple regression equation and the signal-to-noise ratio

Using the Minitab19 program, a regression equation was obtained that shows the relationship between the volumetric mass transfer coefficient and the studied parameters, as the correlation coefficient R^2 showed a perfect fit of the model with a rate equal to 94.9%. Equation 3 shows the regression equation.

$$KLa = 0.0523 + 0.001277\,x_1 - 0.01324\,x_2 + \\ + 0.000847\,x_2^2 - 0.000073\,x_1x_2 \quad (3)$$

Figure 3. shows the relationship between the predicted and experimental values for L16 orthogonal matrix of the volumetric mass transfer coefficient KLa was extracted from Equation 3. These values are explained in Table 4, where it was observed that the predicted values are close to the experimental values. Table 4 also shows the signal-to-noise ratio values that were extracted from Equation 2

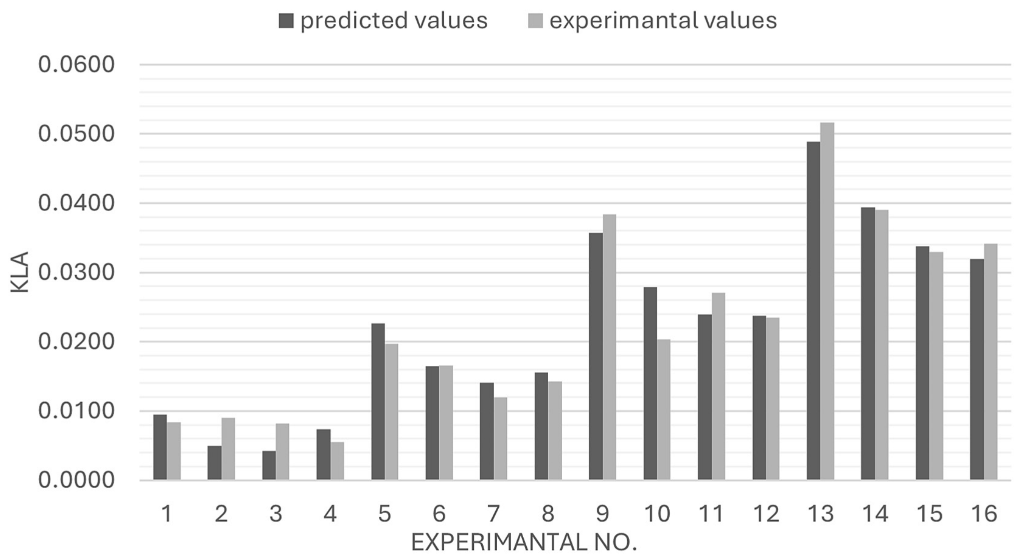

Figure 3. Comparison between experimental and predicted values of KLa

Table 4. A table showing the signal-to-noise ratio

Exp No.	Coded values			Real values			EXP KLa	PRED KLa	S/N RATIO
	G1	G2	G3	Flow rate	Vol.	Type of sol.			
1	1	1	1	5	5.5	D.W	0.0084	0.0095	-41.5144
2	1	2	1	5	7	D.W	0.009	0.0050	-40.9151
3	1	3	2	5	8.5	Whey	0.0082	0.0042	-41.7237
4	1	4	2	5	10	Whey	0.0055	0.0073	-45.1927
5	2	1	1	20	5.5	D.W	0.0197	0.0226	-34.1107
6	2	2	1	20	7	D.W	0.0166	0.0164	-35.5978
7	2	3	2	20	8.5	Whey	0.012	0.0141	-38.4164
8	2	4	2	20	10	Whey	0.0143	0.0155	-36.8933
9	3	1	2	35	5.5	Whey	0.0384	0.0357	-28.4164
10	3	2	2	35	7	Whey	0.0203	0.0279	-33.8501
11	3	3	1	35	8.5	D.W	0.0271	0.0239	-31.3406
12	3	4	1	35	10	D.W	0.0235	0.0237	-32.5786
13	4	1	2	50	5.5	Whey	0.0517	0.0489	-25.7302
14	4	2	2	50	7	Whey	0.039	0.0394	-28.1787
15	4	3	2	50	8.5	D.W	0.033	0.0338	-29.6297
16	4	4	2	50	10	D.W	0.0342	0.0320	-29.3195

The values for the signal-to-noise ratio appear in Table 5, as well as its drawing, as shown in Figure 4. It shows us the highest value for the S/N ratio, and based on the theory that higher is better, it was noted that the most influential factor in the process is the flow rate, followed by the effect of the volume of both solutions used.

By using the Taguchi approach (Minitab 19), the optimal factors that can be used in experiments containing the organism factor were discovered, and as shown in Table 4, the optimal factors are (flow rate = 50), (volume = 5.5). also, whey is the ideal medium for the growth of organisms.

Analysis of variance (ANOVA)

ANOVA analysis was used to determine the consistency of the results of the process and to ensure that each factor used in the process affects the process or not. It also works to compare the factors to show if there are statistically significant differences between them [Razmi and Ghasemi-Fasaei, 2018; Salahi and Mohammadi, 2011]. the value of F shows the effect and importance of each factor on the process. If the value of F > 1, this indicates the impact of this factor on the process[Abbas and Abbas, 2022; Googerdchian et al., 2018] The P value indicates that the study of this factor was carried out in controlled conditions. The P value must be less than 0.05 for each factor at a 95% confidence level [Salman, 2019; Salahi and Mohammadi, 2011] .As shown in Table 6, the results of the ANOVA analysis show that the flow rate factor (contribution ratio 59.80%) has a significant impact on the results of the volumetric mass transfer coefficient, followed by the volume (contribution ratio 9.61%) and then the type of solution (contribution ratio 1.3%). The F test shows that the flow rate has a

Table 5. Response table for signal-to-noise ratios

Level	Flow rate	Volume	Type of sol.
1	-42.34	-32.42	-34.38
2	-36.25	-34.64	-34.79
3	-31.52	-35.28	–
4	-28.21	-36.00	–
Delta	14.12	3.58	0.41
Rank	1	2	3

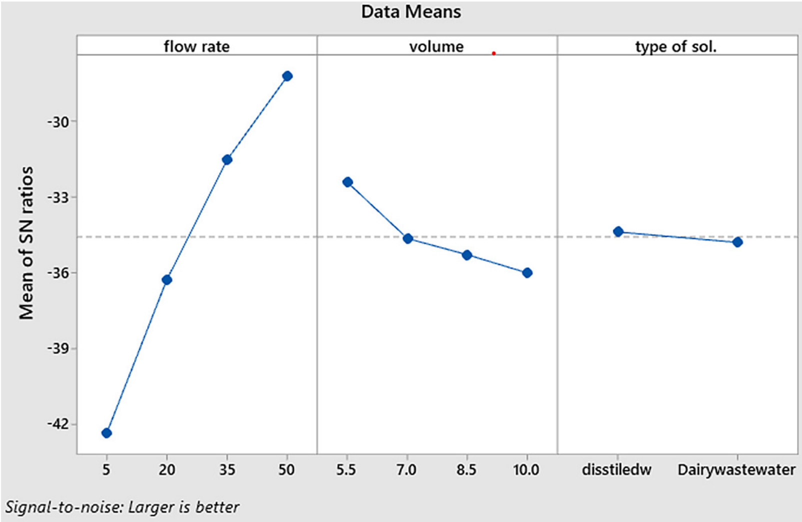

Figure 4. Main effects plot for S\N ratio (larger is better)

Table 6. Values of analysis of variance ANOVA

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value	P-Value
Flow rate	3	0.002301	83.77%	0.002301	0.000767	38.84	0.000
Volume	3	0.000268	9.75%	0.000268	0.000089	4.52	0.039
Type of sol.	1	0.000020	0.73%	0.000020	0.000020	1.01	0.343
Error	8	0.000158	5.75%	0.000158	0.000020		
Total	15	0.002746	100.00%				
Model summary							
S	R-sq						
0.0044433	94.25%						

significant effect on the process, with a value of (25.31) and the same applies to the P test.

Figure 5 also shows the remaining plots and the effect of all parameters on the efficiency of the model, as all factors used in the process are important for the success of the process [Al-Alawy and Al-Ameri, 2017]

Effect of influencer parameters

The flow rate is considered one of the most principal factors affecting the airlift bioreactor, as the higher the flow rate, the better the mass transfer rate, especially in the process of mass transfer between gas and liquid [Garcia-Ochoa and Gomez, 2009]. The flow rate factor is also considered one of the important factors that must studied when designing the airlift bioreactor, as studies have shown that the design of the draft tube especially when designed in analysis models such as the CFD model, affects the flow dynamics and contributes to enhancing gas retention and reactor efficiency [AL-Mashhadani et al., 2015; Coimbra et al., 2024; Lingwei et al., 2024]. As shown in Figure 6 and Figure 7 it was observed that the highest volumetric mass transfer coefficient achieved at a flow rate of 50 L/hr. This is due to the use of a bubble diffuser that converts the bubble from MB to FB when the flow rate increases. Therefore, the flow rate can control the bubble size. mass transfer in distilled water is extremely easy because it does not contain ions and impurities that hinder the mass transfer process. bubbles also increase the driving force to transfer mass from gas to liquid [Jassim and Abdulkhaleq, 2014; Al-Dulaimi and Al-Yaqoobi, 2021; Al-Yaqoobi et al., 2024]. As for whey, changing the medium changes the bubble size, as the type of solution affects the bubble size and thus increases the transfer of the substance in whey, despite it containing many molecules and proteins that can hinder the transfer process [Al-Yaqoobi and Zimmerman, 2022]. The volume and height of the solution play an important role in mass transfer

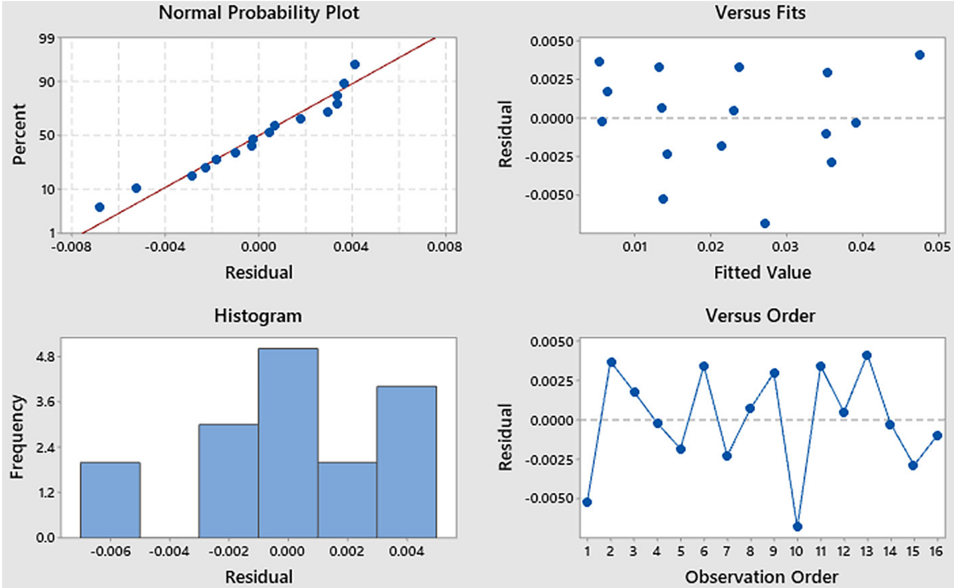

Figure 5. Residual plots for KLa

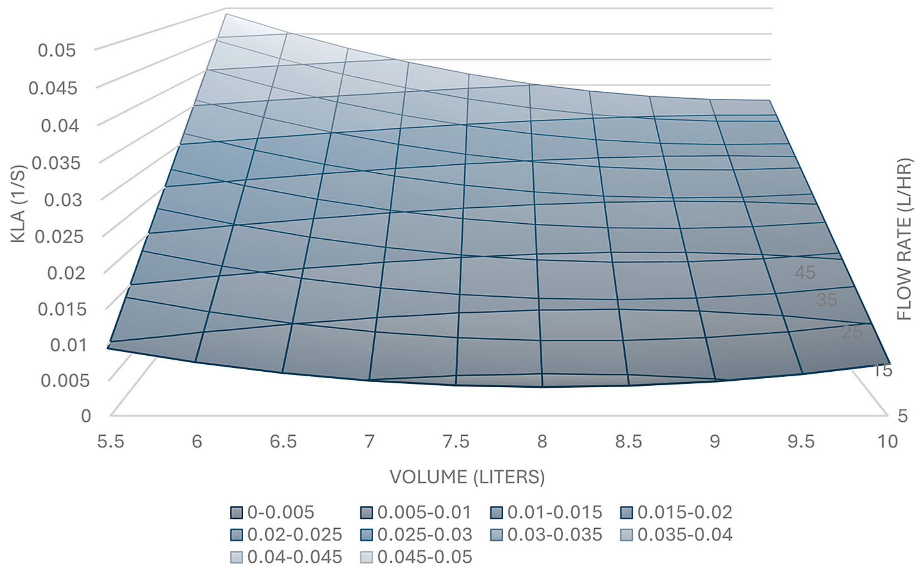

Figure 6. Surface plot for KLa vs flow rate and volume

in the airlift bioreactor and its effect on the volumetric mass transfer coefficient. distilled water achieves the highest volumetric mass transfer coefficient at a volume of 10 L, which is the highest volume achieved by the bioreactor, while in whey, the highest volumetric mass transfer coefficient is achieved at a volume of 5.5 L, which is the lowest volume achieved by the bioreactor, As shown in Table 4, This is due to the absence of impurities and ions that hinder the transfer process in distilled water. Also, at large volumes (higher height), gas circulation is better for distilled water. As for whey, its content of molecules and proteins works to accumulate these particles in the larger volume, so the smaller volume is more effective for the transfer of material in whey, as well as its need for less energy to move and circulate the gas [Garcia-Ochoa and Gomez, 2009].

The organism's consumption of oxygen

Aeration and the amount of gas entering the bioreactor are important matters that have to be studied and taken care of to achieve good nutrition for the organism, as any increase or decrease in oxygen may lead to the accumulation and

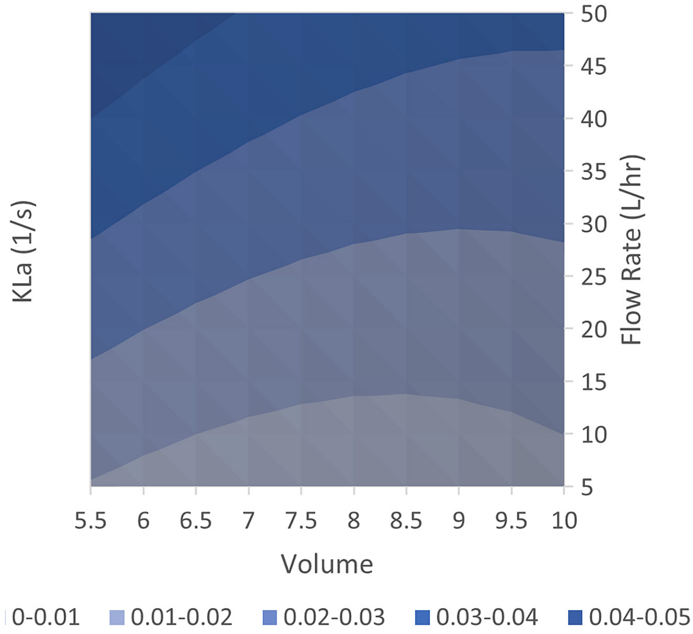

Figure 7. Contour plot of KLa vs flow rate and volume

clustering of cells in the lower part of the bioreactor, and thus the gas and nutrients do not reach the cells, and thus the cells die [Mulakhudair et al., 2022]. Therefore, experimental work was conducted by using the optimal factors obtained from the (MINITAB 19) program, through which the minimum flow rate will be used for the growth of the organism and thus save energy. As shown in Figure 8. shows a clear difference between the time taken for oxygen to dissolve and the time taken for the microorganism to consume oxygen, as the organism consumes oxygen in 43 minutes to consume it completely, while the oxygen dissolves in 8 minutes until reaching a steady state, and this indicates the loss of energy and oxygen, as 50 L/h of oxygen does not go completely to the cells of the organism, and thus the cells do not grow well enough to be able to consume oxygen at the same time it dissolves. In order not to waste energy and to feed the cells of the organism well, three values of flow rates (5, 15, 25) L/h were studied. Figure 9a and Figure 9b shows that using the lowest available flow rate (5, 15) L/h during continuous aeration does not give any clear response to the organism's complete consumption of oxygen, whereas at a flow rate of 5 L/h, the dissolved oxygen reaches

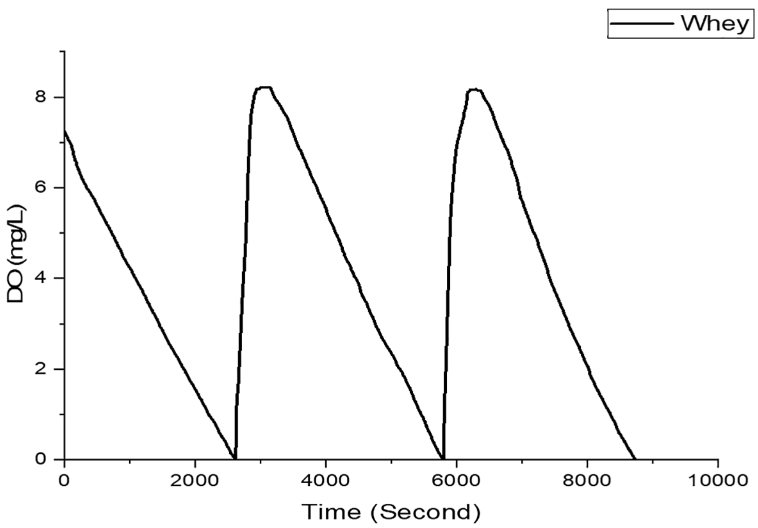

Figure 8. Figure showing the dissolution of oxygen in a solution and the organism's consumption of oxygen at a flow rate of 50 L/h

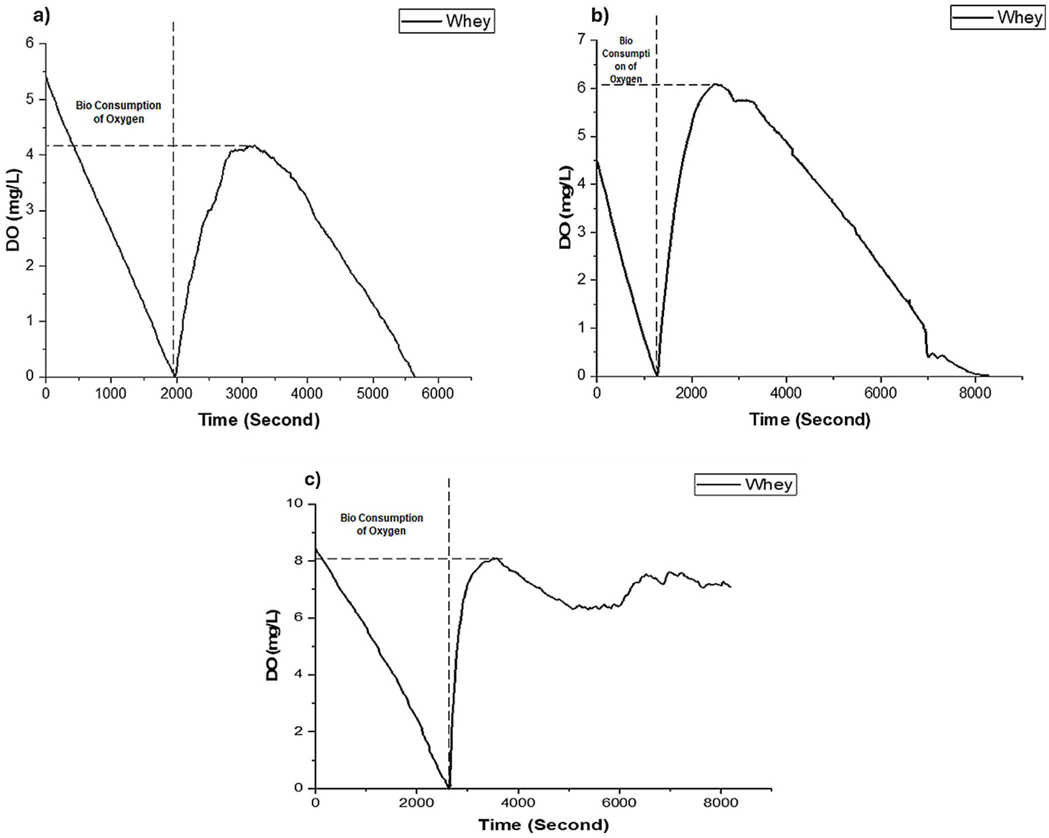

Figure 9. Organism's consumption of oxygen at a flow rate: a) 5 L/h, b) 15 L/h, c) 25 L/h

a steady state at 4 mg/L and then begins to gradually decrease to zero. As for at a flow rate of 15 L/h, the dissolved oxygen reaches a steady state at 6 mg/L and begins to decrease as well, and this indicates the insufficiency of the pumped gas and thus its failure to reach all the cells of the organism The required response was observed at a flow rate of 25 L/h, whereas the dissolved oxygen reaches a steady state at 8 mg/L and then the values remain stable at these limits without a decrease in concentration, and where this increase and decrease in the concentration of dissolved oxygen in a stable manner with the continuous aeration factor at a flow rate of 25 L/h, as shown in Figure 9c. indicates that cells do not accumulate at the bottom of the bioreactor, and thus enough gas and nutrients reach the cells of the organism. Through this study, gas was provided from 50 L/h to 25 L/h, i.e. saving 50% of the gas used and feeding the organism at the same time, thus saving a lot of energy, effort, and money

CONCLUSIONS

The airlift bioreactor was used for its efficiency and ability to use the gas stream to circulate the liquid, but solving the problem of gas reaching the organism is still a dilemma that puzzles scientists. Therefore, the airlift bioreactor was used in the design of the airlift bioreactor. It was noted that ANOVA analysis and the S/N ratio show the most influential factor in the process is the flow rate then the volume in both solutions. The percentage shares of each tested factor, according to the ANOVA analysis in Mini Tab, were for flow rate – 80.59%, volume – 9.61% and solution type – 1.3%. The optimal conditions were also identified as flow rate was 50 L/h, volume – 5.5 L, and type of sol. – whey. Share of 50% of the gas consumed by the organism was saved by reducing the flow rate from 50 to 25 L/h. Thus, the studied model can be applied to studying the mass transfer characteristics of the airlift bioreactor.

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