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Preliminary Evaluation of Aeration Strategy on Recombinant Thermostable Lipase Production in Stirred Tank Fermenter

Fadzlirahimi Ismaila[1*], Khairol Ismailb[2], Adam Leow Thean Chorc[3], Raja Noor Zaliha Raja Abdul Rahmand[4], Rosfarizan Mohamade[5], and Abu Bakar Sallehf[6]

[1,2]Technology Transfer and Entrepreneur Development Centre, MARDI Head Quarters, Persiaran MARDI-UPM, 43400 Serdang, Malaysia

[3]Enzyme and Microbial Technology Research Centre, Faculty of Biotechnology and Biomolecular Sciences, University Putra Malaysia, 43400 Serdang, Malaysia

[4]Department of Microbiology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 Serdang, Malaysia

[5]Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 Serdang, Malaysia

[6]Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 Serdang, Malaysia

**Email:* fbi@mardi.gov.my

***Abstract*—** This study was focused on the aeration effect on thermostable T1 lipase production and fermentation performance by recombinant E. coli. Observation of fermenter aeration strategies such as impeller speed, airflow rate, and dissolved oxygen tension (DOT) throughout the fermentation was carried out and compared. 89.82 U/ml of lipase activity was observed at 1.5 L/min (1 vvm) airflow rate with 250 rpm impeller speed. When impeller speed increased from 250 to 350 rpm, an increase in lipase activity up to 93.03 U/mL was observed. No improvement in lipase production was observed at controlled DOT. More studies on aeration effect needed in order to improve higher lipase production.

***Keywords*—** recombinant E. coli, thermostable lipase, stirred tank fermenter, aeration, DOT, impeller speed, airflow rate, batch fermentation.

I. INTRODUCTION

Aeration is a crucial factor in microbial fermentation processes, especially those carried out in stirred tank fermenter (STF). Aeration affects bacterial growth, metabolism, and consequently, protein production. The level of dissolved oxygen in the fermentation broth, which is influenced by aeration, plays a significant role in determining the performance of the fermentation process. Some works suggested that aeration was more significant in affecting recombinant protein yields and productivity compared to temperature and agitation (Hypatia et al., 2012).

As most E. coli strain is aerobic mesophile, culturing in a fully equipped fermenter with optimal aeration is the most suitable approach to enhance growth and protein production. In E. coli growth, the amount of carbon source and level of oxygen available play an important role in the metabolic fluxes associated with its growth. Oxygen is a growth-limiting factor and below a critical value affects the growth rate but at the same time can have inhibitory effects when present in excess. Similarly, low levels of dissolved carbon dioxide are reported to stimulate growth, meanwhile increasing the levels have progressive inhibitory effects. The levels of dissolved oxygen and carbon dioxide are affected by the consumption or production respectively, and the transfer rate between phases. In aerobic systems, fermenters can work in optimal conditions for gas-liquid transfer by employing agitation and aeration (Alba and Calvo, 2000). Controlled aeration conditions will influence mass transfer by affecting bubble size, air hold up and turbulence within the vessel as well as biomass production and recombinant expressions systems (Deniz et al., 2014).

The increasing industrial demand for lipases has led to the searching of special ability lipases in compromising various processing environment. It uses covered leather, feed, food, cosmetic, detergent and pharmaceutical sector (Rajak et al., 2025). Thermostable lipase is favored by the industry since it can be used under high temperature catalysis process exceeding up to 50℃ (Yasser and Ahmed, 2008; Febriani et al., 2020). Recent study also shows some lipase has successfully degrade plastic waste such as polyethylene (Elizabeth and Onoruoiza, 2025).

Scaling-up studies with fermenter operation is complex and costly process towards developing commercial production of an enzyme (Nadal-Rey et al., 2021). Bioprocessing with fermenter operation has make significant contribution to economy of many countries (Sadino-Riquelme et al., 2023). In fact, the industry has started since 1985 by Quorn Foods pioneering the production of Mycoprotein from Fusarium venenatum (Moresi, 2025). To find the optimal method that yields high protein synthesis in an affordable and useful way, various factors, including physical, nutrition, and fermentation mode, can be adjusted (Abdel-Fattah et al., 2012; Ismail et al., 2018). In order to establish subsequent commercial production, this pilot investigation measured the production of recombinant thermostable T1 lipase in a 2 L stirred tank fermenter using various aeration strategies.

II. METHODOLOGY

A. Microorganism and medium

Previously engineered Escherichia coli BL 21(DE 3) plays (PGEX / T1 S) harboring thermostable T1 lipase gene was used in this trial. An overnight single colony was inoculated into 150 mL LB broth and incubated at a temperature of 30°C with 250 rpm agitation for 10h. The fermentation medium used was commercial Luria-Bertani (LB) medium with the addition of 0.1g/L ampicillin (Amp) and 35 mg/L chloramphenicol (Chlo) as the selection marker.

B. Submerged fermentation

A 2 L Biostat® B (B Braun, Biotech Int, Germany) stirred tank fermenter with a working volume of 150 mL was used throughout this study. The fermenter was equipped with impellers, pH, temperature, airflow, and dissolved oxygen tension (DOT) control systems. The temperature within the fermenter vessel was controlled by employing the external double wall or thermostat jacket. From previous work, temperature was set at 37°C throughout the fermentation process in all trials (Ismail, F. et al., 2023). Airflow was adjusted in a range of 0.5 to 2.0 L/min (0.33 – 1.33 vvm).

Impeller speeds were varied from 150 to 550 rpm by two six-bladed Rushton turbine impellers. Optimum impeller speed was then used in the further experiment in this study. Steam-serializable polarographic probe (METTLER TOLEDO) was used to measure the DOT levels. Dissolved oxygen in the culture broth was controlled automatically at 20%, 40%, 60% 80%, and 100% of air saturation by setting the DCU. DOTs were controlled constantly throughout the fermentation hour by automatic changing of impeller speed from a range of 5 rpm to 900 rpm.

Cultures in the fermenters were induced with 0.025 M isopropyl-ß-D-thiogalactosidase (IPTG) after cell absorbance reached ~0.75 at 600 nm. Samples were taken every 2 hours throughout 12 h fermentation.

C. Lipase activity assay

Lipase activity was assessed as before, using Kwon and Rhee colorimetric method with some modifications (Leow et al., 2004). The enzyme activity was determined by measuring the amount of free fatty acids from the standard curve of free fatty acids. 1 unit of activity is defined as the amount of enzyme that causes the release of 1 µmol of free fatty acids per minute under assay conditions.

III. RESULTS AND DISCUSSIONS

A. Influence of Different Air Flow Rate Influx

Typical time courses of 24 h lipase fermentation by recombinant E. coli are shown in Figure 1. Impeller speed was set at 250 rpm and DOT level was not controlled. The strain grew well in all airflow rates investigated. The fast cells growth pattern was observed with 1.5 and 2.0 L/min airflow rates, while cells growth profiles with 0.5 and 1.0 L/min airflow rates seem similar at the exponential phase.

DOT level was observed to drop to 0% after 4h of fermentation for all airflow rates studied, as a sign of rapid use and insufficient of oxygen by growing cells (Boruta et al., 2023). The oxygen that presents earlier in the fermentation broth is usually used up for the synthesis of membrane components (Deniz et al., 2014). It then started to increase at 8 h for 1.0 and 2.0 L/min of airflow rates.

While for 0.5 and 1.5 L/min, 0% of DOT levels were prolonged to 16 h and 12 h respectively. As depicted in Table 1, the highest cells growth rate (µ) was observed at 1.5 L/min airflow rate, while the lowest value was observed at both 1.0 and 2.0 L/min airflow rates (0.21 h-1). Lipase production was observed to increase with the increase of controlled airflow rate.

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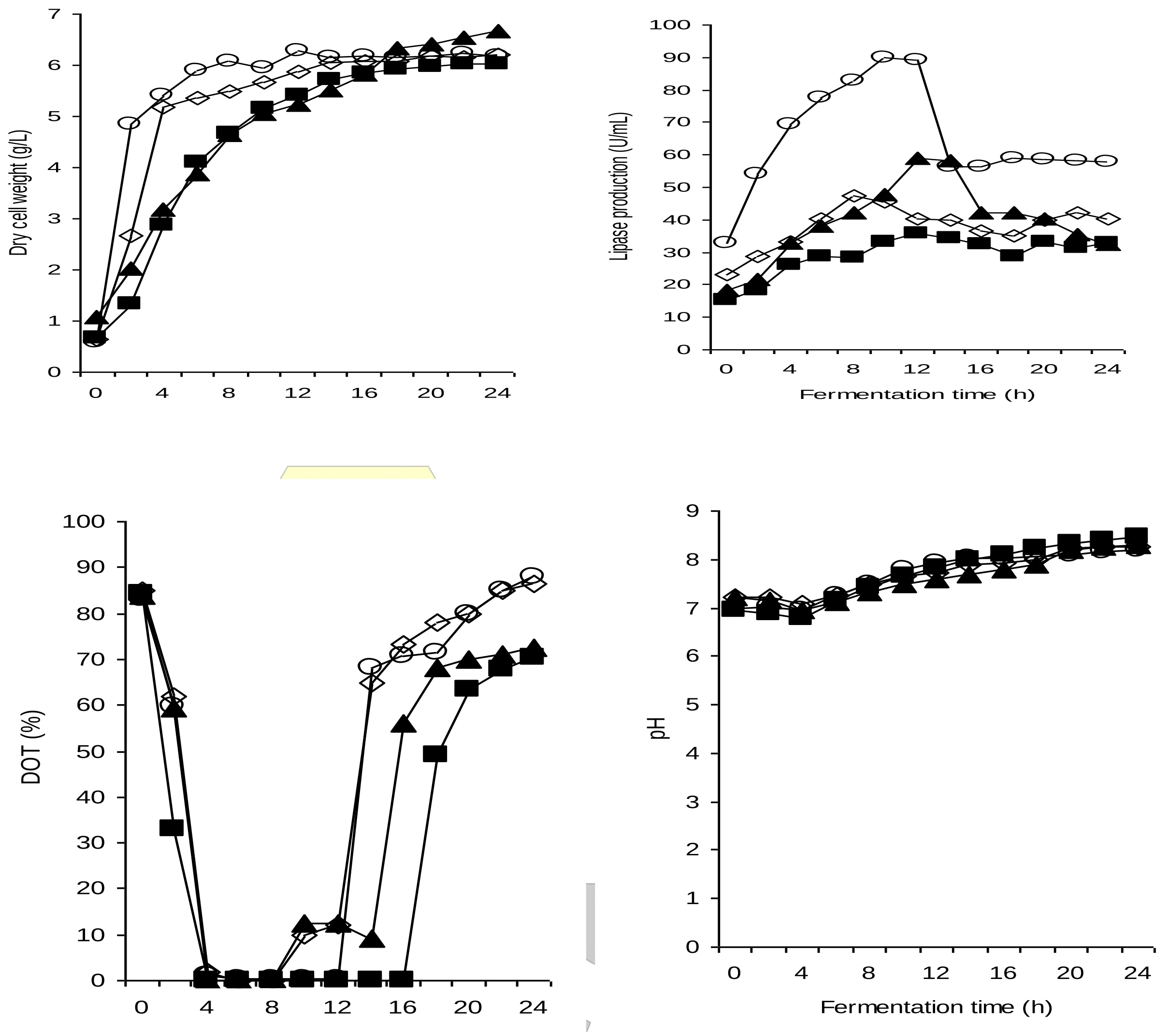

Figure 1: *Effect of different airflow rates on lipase production by recombinant E. coli in batch submerged fermentation using 2 L fermentor. ■ – 0.5; ▲ – 1.0; ○ – 1.5; ◊ - 2.0 L/min.*

The highest value of lipase production was observed at 1.5 L/min with 89.81 U/mL lipase activities but only assembled lower enzyme production when airflow rates increased and maintained at 2.0 L/min. Even though the Xmax value of 1.0 L/min airflow rate is slightly higher than 1.5 L/min airflow rate, higher lipase production and shorter production time have resulted in the highest Pr value for 1.5 L/min airflow rate. This may be suggested that enzyme production may be associated with expression systems instead cell mass. Several works on recombinant E. coli cultivation have been reported to use a 1.5 L/min of aeration rate for foreign protein expression (Gosset et al. 1993; Hahm et al., 1995). The results of this study suggest that intermediate levels of aeration with appropriate agitation speed increased lipase production. Appropriate airflow rate produces smaller size air bubbles, which then increase the contact surface area and oxygen uptake rate by the cells (Wang et al., 2023). A higher oxygen uptake rate may affect the overall metabolic state of E. coli cells and so the protein-synthesizing system (Hahm et al., 1995). In addition, a lower airflow rate (0.5 and 1.0 L/min) may not be sufficient to overcome transport resistance and supply the required oxygen to the biomass (Veenanadig et al., 2000).

B. Aeration Mixing with Different Impeller Speed

The time course of lipase fermentation by E. coli with different impeller speeds is shown in Figure 2. All fermentations to investigate the effect of impeller speed were initially carried out without any airflow rate and DOT control. The highest lipase production (93.03 U/mL) which gave overall productivity of 18.61 U/mL.h was achieved with 350 rpm impeller speed. The performance of fermentation at an impeller speed of 350 rpm was slightly similar to 250 rpm. High Xmax and Pmax values were observed which resulted in high productivity. This may be suggested that these may be the optimal range of agitation for high lipase production by this recombinant. At higher agitation, 450 and 550 rpm, lipase production was decreased to 29.70 and 22.34 U/mL, respectively, which then gave overall productivity of 7.43 and 3.72 U/mL.h, respectively. The ability of cells to synthesize lipase, as indicated by the value of Pmax/Xmax was reduced at higher agitation (Table 1). A greater reduction in lipase production (30.57 U/mL) was observed at low agitation speed (150 rpm). In addition, the t value (10 h) was also almost double than 350 rpm agitation.

Table 1: Fermentation performance for different aeration control on thermostable T1 lipase production.

Strategy		Xmax (g/L)	Pmax (U/ml)	Pmax/Xmax (U.L/g)	μ (h-1)	t (h)	Pr (U/ml.h)
Airflow rate (L/min)	0.5	6.04	35.9	5.94	0.4	12	2.99
	1	6.66	58.9	8.84	0.21	12	4.91
	1.5a	6.63	89.82	13.54	0.55	8	11.22
	2	6.02	47.3	7.86	0.21	8	5.91
Impeller speed (rpm)	150	5.87	30.57	5.21	0.31	10	3.06
	250a	6.63	89.82	13.54	0.55	8	11.22
	350	6.49	93.03	14.33	0.87	5	18.61
	450	5.84	29.7	5.08	0.46	4	7.43
	550	6.37	22.34	3.51	0.61	6	3.72
DOT (%)	20	5.52	20.59	3.73	0.585	9	2.28
	40	5.12	23.62	4.61	0.548	8	2.95
	60	5.07	21.42	4.22	0.475	9	2.38
	80	6.09	53.37	8.76	0.441	11	4.85
	100	6.17	23.54	3.65	0.651	5	4.71

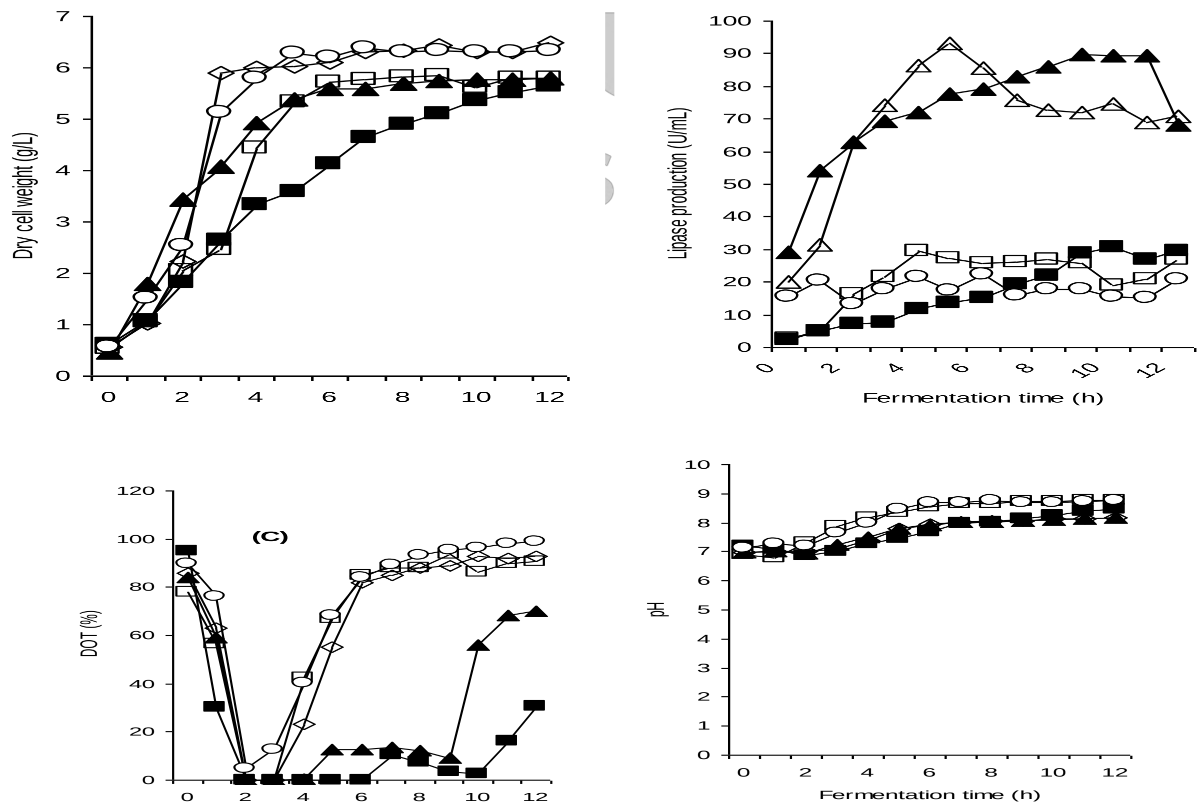

Figure 2: Effect of different impeller speed on lipase fermentation by recombinant E. coli in batch submerged fermentation using 2 L fermenter. ■ *– 150;* ▲ *– 250;* ◊ *– 350; £ – 450; O – 550*

Both biomass concentration and lipase production were influenced by agitation speed at 350 r.p.m. Variation in agitation speed results in a change in oxygen transfer rate, which in turn affected the rate and extent of cell growth and lipase production. Appropriate mixing conditions of air might be the reason for the achievement of maximum production.

Good mixing conditions will greatly allow oxygen from the air to disperse thoroughly in the broth. Garcia de Fernando et al. (1991) reported that the growth of microorganisms and enzyme production is significantly affected by agitation and aeration.

As reported by Gulati et al. (2000), it may also be due to increased oxygen transfer rate, the increased surface area of contact with the media components, and better dispersibility of the inducer. Elibol and Ozer (2000) reported that the increase in production (or reduction in fermentation time) due to improved oxygen transport was common in their work for both lipase productivity and cell growth.

This was anticipated since, in a mechanically agitated vessel, agitations affect the rate of oxygen transfer. Gulati et al. (2000) also reported that the critical demand for oxygen was higher for lipase production than for growth.

Lower lipase production at higher impeller speed may be suggested that the enzyme system responsible for lipase expression may be damaged by a high shear rate. This finding was consistent with that of Pourrat et al. (1988) who reported that greater aeration and agitation could result in the excessive breakdown of the enzyme, thus decreasing the production of extracellular protease. High agitation speed can also cause a drop in enzyme production due to cell lysis or excessive cell permeability.

Reduction of specific growth rate and lipase production in fermentation at lower impeller speed (0.31 h-1 and 30.57 U/mL, respectively) may be due to non-homogeneity of oxygen supply and imperfect mixing, which in turn, limiting the nutrient and oxygen transfer to the culture.

Growth-inhibiting acidic by-products of incomplete substrate oxidation such as acetic acid was also produced in response to oxygen limitation (Korz et al., 1995).

This result was quite similar to other previous bacterial fermentation work. Kulkarni and Gadre (2002) used 400 rpm of agitation speed to produce alkaline lipase from Pseudomonasfluorescens NS2W.

Lipase production of Aspergillus terreus was increased to 14 200 U/L and a reduction in time to 54 h rather than 96 h was observed at an agitation speed of 300 rpm (Gulati et al., 2000).

Elibol and Ozer (2000) reported that the overall productivity of Rhizopus arrhizus lipase increased with the increasing aeration rate and agitation speed and the effect of agitation were stronger than the aeration rate. Additionally, Demirtas et al. (2003) has confirmed previous observations that agitation has a stronger effect on mass transfer coefficient (KLa) and hence oxygen transfer rate (OTR) compared to aeration.

C. Influence of Dissolve Oxygen Tension (DOT) Control Strategy

The dissolved oxygen concentration shows the rate of oxygen transfer from the gas phase to the liquid, on the rate at which oxygen is consumed by the culturing cells for growth, maintenance, and production (Deniz et al., 2014).

Figure 3 shows a typical time course of batch fermentation of recombinant lipase. Controlled DOT levels were achieved through agitation/ impeller speed. Agitation speed shows a parallel increase to DOT level with the highest agitation speed achieved was around 900 rpm for constant 100% saturation of DOT level. Agitation speed profiles of all variables studied were also in like a similar pattern, which increased to maximum level after 2 h of fermentation time. It is necessary, as to maintain oxygen concentration at the active exponential growth phase.

It then started to decrease after about 3 h of fermentation time to as low as 10 rpm when cell growth entered the stationery and death phase. High cell mass was observed at 80% and 100% controlled DOT, with 6.09 and 6.17 g/L respectively.

This may be due to sufficient oxygen supplies to the culture cells. This may also suggest that recombinant cells in this study were mildly affected by shear stress with high impeller speed. However, all cases of controlled DOT studied shows no further improvement in lipase production when lipase.

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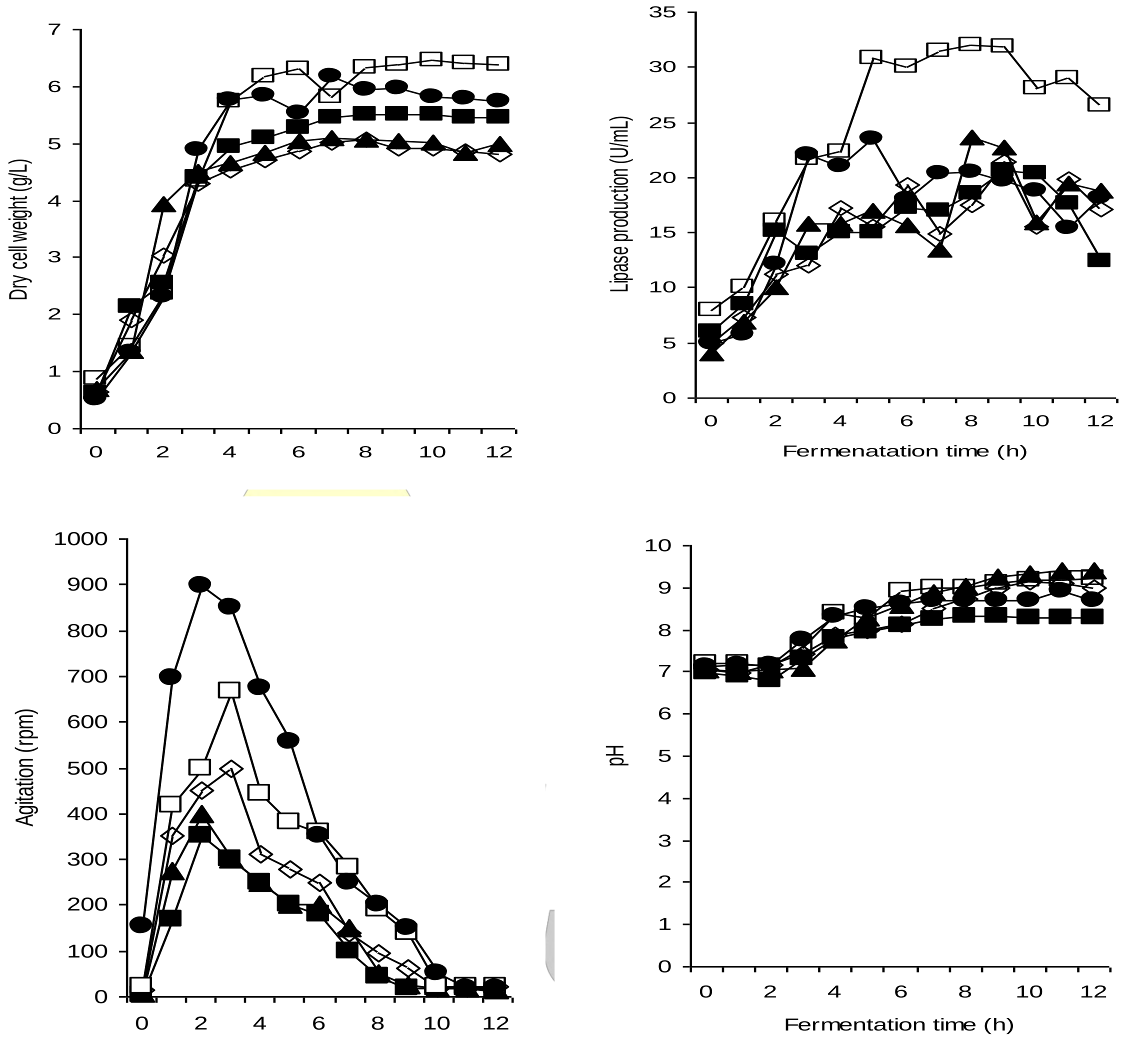

Figure 3: *Effect of different controlled dissolve oxygen tension (DOT) on lipase fermentation by recombinant E. coli in batch submerged fermentation using 2 L fermenter.* ■ *– 20%;* ▲ *– 40%; ◊ – 60%; £ – 80%;* ● *– 100% DOT.*

Activity was slightly similar in range (20 U/mL), except for 80% saturation, when compared to aeration and impeller speed studies. At constant 80% saturation, lipase activity was 32 U/mL, with enzyme productivity of 4.85 U/mL.h (Table 1). It was slightly higher than that at constant 100% saturation, which was 4.71 U/mL.h. Excess oxygenation result was much lower than that observed in the previous uncontrolled DOT level (93.3 U/mL) with 18.61 U/mL.h of productivity. This can be suggested that controlled DOT levels using agitation may affect the enzyme expression system. Dissolve oxygen was reported to have various effects on heterologous gene expression in recombinant bacteria.

An increase in the DO level had caused an increase in β-lactamase expression in Streptomyces lividans. Contrary to a previous report by Ryan et al. (1989) where β-lactamase expression in recombinant E. coli strain was found to decrease with an increase in the DO level. Deniz et al., (2014) also reported that excess oxygenation resulted in low ethanol production by E.coli KO11. Results from this study also somehow contradict to some previous work with E. coli strain. Korz and co – workers (1995) reported that constant DOT level of E. coli TG1 in simple fed – batch technique was controlled at 20% saturation by increasing the stirrer speed. Falk et al., (1991) also reported that high levels of aeration decrease lipase production in Staphylococcus carnosus. Kleman et al. (1991) however reported that 70% saturation was the desired level of dissolve oxygen concentration for E. coli fed – batch fermentation, using glucose as carbon source.

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De León (2003) reported that DOT strongly affects fermentation performance and the negative effect on its limitation on maximum cell concentration. However, information on the effect of DOT on recombinant protein accumulation by E. coli is relatively scarce. Furthermore, no general rules can be derived from available information since various proteins and host/vector systems as well as different expression systems reported to behave differently (Bhattacharya and Dubey, 1997; De León et al., 2003). This diversity of results can also be related to the different working conditions used by different researchers, in particularly the mode of dissolve oxygen control in the fermenter system.

These include the maximum agitation speed and airflow rate control, which in this case was not been set. While in fungus cultivation, reported earlier by Gulati et al., (2000), lipase production of 12 000 U/L was achieved in 60 h when dissolve oxygen (DO) was maintained above 20% saturation for A. terreus cultivation. It was also observed that increasing the DO level (40% saturation) did not further enhance lipase production. This indicated that maintaining DO level above certain critical/limiting value was important for lipase production rather than the saturation percentage of DO. Giuseppin (1984) reported that Rhizopus delemar lipase production was dependent on the oxygen concentration in the culture medium. Increasing oxygen concentration enabled cells to maintain better growth viability and resulted in increased protein production (Wang et al., 2023).

IV. CONCLUSION

Thermostable lipase production from this recombinant remains important when mixing and aeration conditions are close to those commonly reported for E. coli. Mixing condition through impeller speed study seems more important, since it shows more significant results than aeration. Good mixing condition will not only increased oxygen transfer rate, but also increased the overall homogeneity in the cultivation system. It thus increases the surface area of contact with the media components and better dispersibility of inducer. Highest lipase production observed in impeller speed study confirmed these facts. Although controlled aeration and DOT showed low enzyme productivity, important findings with both controlled strategies have to be put in interest. Fed batch operation is urged since it has been reported to increase cell mass and lipase production by recombinant E. coli (Nelofer et al., 2013).

While using tools such as response surface methodology (RSM) is also strongly recommend in optimizing culture parameter since many enzymes production has been reported improved (Mohammad et al., 2019; Sundaramahalingam et al., 2022).

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