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# Effect of different aeration and polyethylene glycol concentration on growth of *Escherichia coli* DH5α in a 1L bioreactor

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## Abstract

Large culture volume in bioreactor necessitates aeration for providing sufficient oxygen for cell growth. Thus, extend of aeration and amount of anti-foam needed for suppressing foam formation are key parameters determining the success of bioreactor fermentation. Specifically, while aeration provides more oxygen for powering cellular metabolism and could lead to faster growth rate and more efficient metabolism, it also introduces greater shear stress, mixing and foam formation. On the other hand, anti-foaming agents such as polyethylene glycol (PEG) could exert a toxicity effect on cells as well as introducing increased osmolarity and viscosity that could hamper cell growth. In this preliminary study, effect of different PEG concentrations and extent of aeration on growth of *Escherichia coli* DH5α (ATCC 53868) in a 1L bioreactor at 37 $^{o}$C with LB Lennox growth medium was investigated. Experiment results revealed that *E. coli* DH5α growth in bioreactor at 1 VVM aeration with 1 g/L PEG was faster than that in a 250 mL glass shake flask, and with greater secretion of alkaline metabolites. Similar optical density obtained between bioreactor and shake flask cultivation pointed to the maximized utilization of growth medium nutrients for biomass formation. Increase in bioreactor aeration to 3 VVM at 1 g/L PEG, however, resulted in increased secretion of acidic metabolites into the culture broth while allowing similar maximal optical density to be obtained compared to aeration of 1 VVM at 1 g/L PEG. This indicated that *E. coli* DH5α was able to adapt to physiological impacts from increased aeration and highlighted that no significant metabolic energy was diverted from biomass formation. Finally, increase in PEG concentration to 10 g/L from 1 g/L did not introduce additional toxicity effect given that growth profile of *E. coli* DH5α under the two PEG concentrations overlapped each other. However, observations of reduced secretion of acidic metabolites at the outset of growth in 10 g/L PEG pointed to physiological impacts that did not affect growth rates and biomass formation. Collectively, *E. coli* DH5α was able to tolerate enhanced aeration of 3 VVM and 10 g/L PEG anti-foam without significant detrimental impacts on growth rates and biomass formation.

***Keywords:*** polyethylene glycol, aeration, optical density, pH, *Escherichia coli*, bioreactor, shake flask, biomass formation, growth rates, metabolites,

***Subject areas:*** bioengineering, biotechnology, microbiology, biochemistry, cell biology,



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**Highlights**

1) Aeration increases oxygen transfer rate and help improve growth rates and biomass formation. However, increased aeration in a bioreactor typically result in foam formation, which necessitates the use of anti-foam for control.

2) Effect of increased aeration (3 VVM) and elevated polyethylene glycol (PEG) anti-foam (10 g/L) on growth of *Escherichia coli* DH5α (ATCC 53868) in LB Lennox medium at 37 °C in a 1L bioreactor was investigated in this study.

3) Comparison of growth in 1L bioreactor and 250 mL shake flask revealed faster growth rate during growth of *E. coli* DH5α in the bioreactor. Increased secretion of alkaline metabolites during growth in bioreactor marked a possible physiological adaptation to high shear conditions.

4) Enhanced aeration of 3 VVM compared to 1 VVM with 1 g/L PEG exerted physiological stress on *E. coli* DH5α and resulted in increased secretion of acidic metabolites. However, greater aeration did not reduce biomass formation; thereby, suggesting that *E. coli* DH5α could have adapted to high shear stress and mixing.

5) Finally, 10 g/L PEG did not exert additional toxicity on *E. coli* DH5α compared to 1 g/L PEG, where similar growth profiles for optical density were obtained. However, there was increased secretion of alkaline metabolites by *E. coli* DH5α during growth at 10 g/L PEG.

6) Collectively, in demonstrating good growth under high aeration and PEG concentrations, results revealed that *E. coli* DH5α could adapt to growth under high shear rate as well as elevated osmolarity and viscosity conditions.

## Introduction

Aeration typically generates a lot of foam in a bioreactor, especially at high perfusion rates exceeding 1 VVM (volume of air per volume of liquid per minute). Such foam and bubbles pose a significant source of stress for bacterial cells given the energy released when the bubbles burst during cell cultivation in the bioreactor. Additionally, formation of foam also increases the risk of contamination since foam that reaches the top plate of the bioreactor could serve as a conduit for the movement of microbial contaminants into the culture broth. Thus, from the biotechnology process perspective, it is imperative to assess the vulnerability of each production strain to different levels of physiological stress arising from exposure to foam from different levels of aeration.

However, increase in aeration also provides more oxygen for microbial respiration, in particular, the oxidative phosphorylation pathway, which potentially could improve cell growth rate, biomass formation, and production titer of target compounds. Thus, a trade-off exists between increase in aeration and the ill effects of foam generation and bursting. Anti-foam such as polyethylene glycol (PEG) are commonly used in reducing foam formation in bioreactor during



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aeration. Use of polyethylene glycol, however, may exert species-specific toxicity effects on the cells;[1] thus, a need exists for understanding the effect of different concentrations of polyethylene glycol anti-foam on the growth and metabolism of cells in a bioreactor. Other studies have also implicated the use of anti-foam on bioprocess productivity.[2]

Using *Escherichia coli* DH5α (ATCC 53868) as model organism, this study sought to understand the effect of different aeration rates and different concentrations of polyethylene glycol on the growth of the bacterium in a 1L bioreactor at 37 °C. Specifically, LB Lennox was chosen as growth medium given its widespread use in microbial cell culture and propensity for generating foam during high intensity aeration in a bioreactor. Optical density and pH would be tracked during culture of *E. coli* DH5α in 500 mL of LB Lennox medium in a 1L bioreactor for understanding how aeration affected cell growth and the nuances of the trade-off between aeration induced improvement in cell growth and physiological stress arising from high shear and breakage of foam.

## Materials and Methods

### *Materials*

LB Lennox medium was purchased from Difco and used as is, its composition is [g/L]: Tryptone, 10.0; Yeast extract, 5.0; NaCl, 5.0. Polyethylene glycol (molecular weight ~8000 Da) was purchased from Sigma-Aldrich and used as is.

### *Culture of Escherichia coli DH5α in shake flask*

Stock cultures of *Escherichia coli* DH5α (ATCC 53868) were prepared in 40% glycerol and stored at -70 °C before use. One glycerol stock culture was used in inoculating 100 mL of LB Lennox medium in a 250 mL glass shake flask with cotton plug. This is known as the seed culture. Incubation was at 37 °C and 230 rpm rotational shaking in a temperature controlled incubator. After 8 hours of incubation, 1 mL of inoculum was withdrawn from the seed culture and used in inoculating the experiment cultures containing 100 mL of LB Lennox medium in 250 mL glass shake flasks. Three biological replicates were performed. Incubation conditions were 37 °C and 230 rpm rotational shaking in a temperature controlled incubator (Yih Der LM-570RD, Taiwan). At appropriate time points, aliquots were withdrawn from the cultures for optical density and pH measurement.

### *Culture of Escherichia coli DH5α in 1L bioreactor*

*E. coli* DH5α glycerol stock culture was used in inoculating 100 mL of LB Lennox medium in a 250 mL glass shake flask with cotton plug as the seed culture. The seed culture was incubated at 37 °C and 230 rpm rotational shaking in a temperature controlled incubator. After 13 hours of incubation, 5 mL of seed culture was used as inoculum for 500 mL of LB Lennox medium in a 1L



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bioreactor with temperature controlled at 37 °C and 400 rpm stirring. Depending on experimental conditions, either 1 g/L or 10 g/L of polyethylene glycol (PEG) was used. Aeration was either 1 VVM or 3 VVM (volume of air per volume of liquid per minute). At appropriate time points, aliquots were withdrawn for optical density and pH measurement.

*Measurement of optical density and pH*

Optical density was measured at 600 nm with a Shimadzu Biospec-Mini UV-Visible spectrophotometer with a quartz cuvette of 10 mm pathlength (volume: 3.5 mL). If the optical density exceeded 1, appropriate dilution with deionized water was used prior to measurement. pH was measured with an Orion 9156 BNWP probe fitted to a Mettler Toledo Delta 320 pH meter.

## Results and Discussion

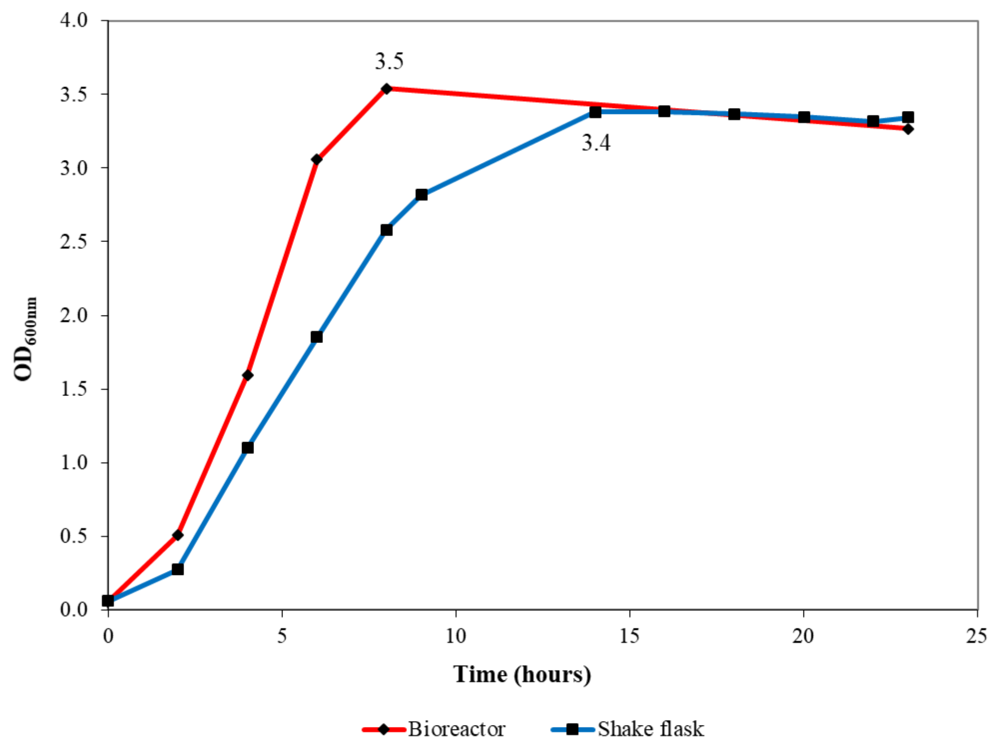


**Figure 1a:** Comparison of growth of *Escherichia coli* DH5α in LB Lennox medium at 37 °C in shake flask and 1L bioreactor (with 1 g/L polyethylene glycol). Aeration did improve rate of biomass formation.

Higher aeration available in bioreactor did improve biomass formation in *E. coli* DH5α during growth in bioreactor compared to 250 mL shake flask with cotton plug (Figure 1a).



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Specifically, rate of growth of *E. coli* DH5α was higher during growth in bioreactor compared to shake flask and the maximal optical density obtained was also higher (3.5 versus 3.4). While the shake flask culture system did allow oxygen transfer through the cotton plug, rate of oxygen transfer was expected to be lower than 1 VVM (volume of air per volume of liquid per minute) than that available in the bioreactor. Hence, while not measured, it could be extrapolated that the oxygen transfer rate in a 250 mL cotton plug shake flask with 100 mL of medium was lower than 1 VVM; thereby, constraining the growth rate of *E. coli* DH5α.

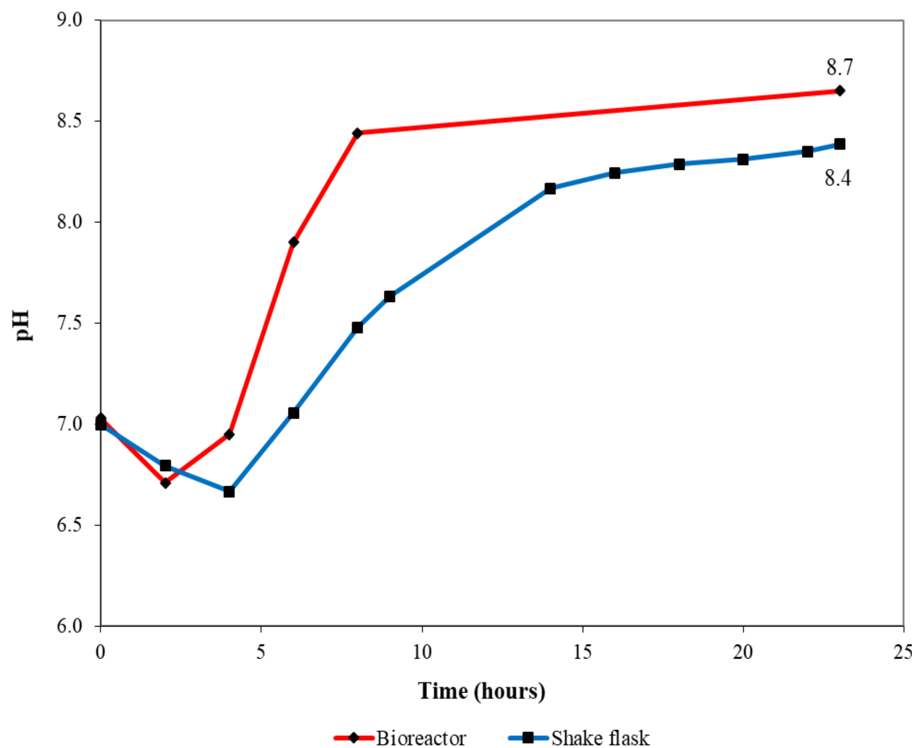


**Figure 1b:** Variation in pH of *E. coli* DH5α grown in LB Lennox medium in shake flask and a 1L bioreactor at 37 $^{o}$C. Greater aeration in a bioreactor resulted in a pH profile that revealed the net secretion of more alkaline metabolites into the culture broth.

Accompanying faster growth rate in the bioreactor was a pH profile that revealed *E. coli* DH5α secreted more alkaline metabolites into the culture broth compared to growth in shake flask (Figure 1b). This indicated that greater oxygen availability that comes from higher aeration likely resulted in faster metabolism that generated greater biomass formation and secretion of alkaline metabolites into the medium. In general, pH declined from the onset of growth in both bioreactor and shake flask most probably due to the net secretion of acidic metabolites into the culture broth. However, pH subsequently increased to 8.7 and 8.4 for growth in a bioreactor and shake flask, respectively. Specifically, the turning point at which pH started to increase occurred earlier during cultivation in a bioreactor compared to a shake flask.



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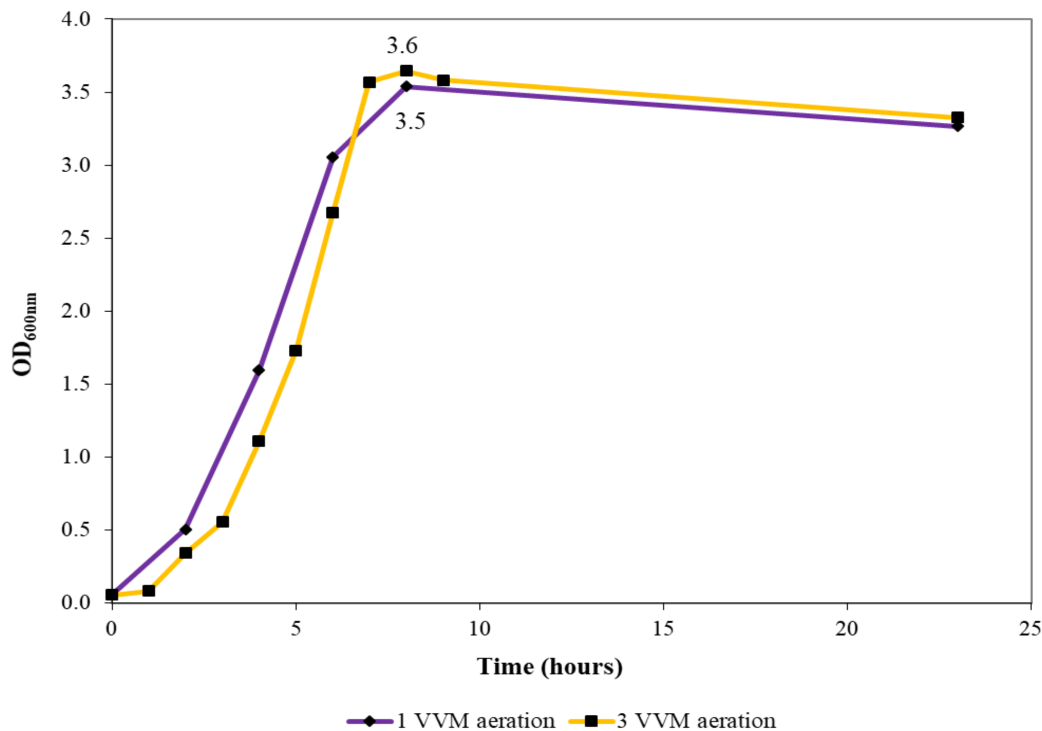


**Figure 2a:** Growth of *E. coli* DH5α in bioreactor under 1 VVM and 3 VVM aeration in LB Lennox medium at 1 g/L polyethylene glycol anti-foam. Cultivation conditions were 37 $^{o}$C and 400 rpm stirring. Greater aeration did increase the length of the lag phase, but did not reduce maximal optical density obtained; thereby, highlighting that effects from physiological stress arising from greater fluid shear and mixing did not affect biomass formation.

Increased aeration posed a detrimental impact on the growth of *E. coli* DH5α that manifested as a longer lag phase, but maximal optical density obtained was not affected (Figure 2a). Specifically, lag phase was longer when *E. coli* DH5α was cultivated at 3 VVM aeration with 1 g/L of polyethylene glycol anti-foam compared to 1 VVM aeration at the same anti-foam concentration in LB Lennox medium in a 1L bioreactor. Physiological stress arising from greater fluid shear and mixing that accompanied increased aeration likely increased the lag phase of *E. coli* DH5α as the bacterium seek to adapt to greater shear stress. However, maximal optical density and growth rate were not affected due probably to the ability of the bacterium at recovering from the physiological stress imposed.



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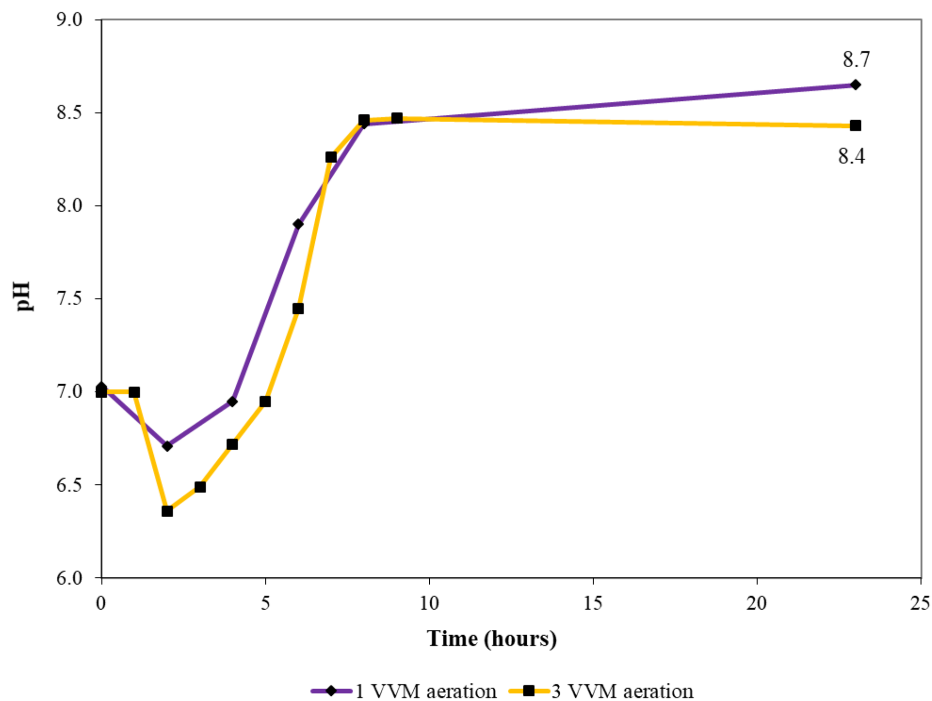


**Figure 2b:** pH profile of growth of *E. coli* DH5α at 37 ºC in LB Lennox medium in a bioreactor under 1 VVM and 3 VVM aeration. 1 g/L of polyethylene glycol was used as anti-foam. Higher aeration intensity resulted in the secretion of more acidic metabolites.

Observations of pH variation during growth of *E. coli* DH5α in LB Lennox medium under 1 VVM and 3 VVM aeration in the bioreactor revealed that the bacterium secreted less alkaline metabolites into the culture broth at 3 VVM aeration compared to 1 VVM (Figure 2b). Additionally, final pH of the culture broth was lower (8.4) during growth at 3 VVM aeration compared to that at 1 VVM aeration (8.7). During the initial phase of growth, greater aeration at 3 VVM resulted in the net secretion of more acidic metabolites into the culture broth compared to growth at 1 VVM aeration. Taken together, under physiological stress arising from shear flow and mixing that accompanied greater intensity of aeration, *E. coli* DH5α secreted more acidic metabolites into the medium followed by a reduced secretion of alkaline metabolites.



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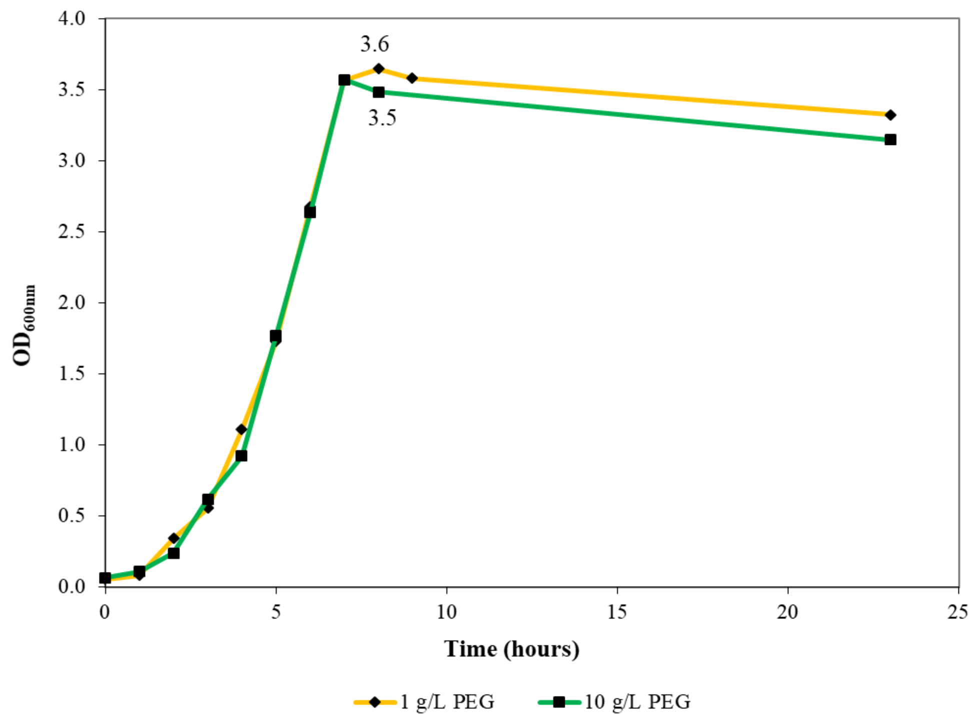


**Figure 3a:** Growth profile of *E. coli* DH5α in LB Lennox medium with 3 VVM aeration in a bioreactor together with either 1 g/L or 10 g/L polyethylene glycol anti-foam. Experiment results indicated that 10 g/L polyethylene glycol did not exert additional toxicity effect on the bacterium.

Cultivation of *E. coli* DH5α in LB Lennox medium with 3 VVM aeration but differing concentrations of polyethylene glycol (molecular weight ~8000 Da) did not result in differences in growth behaviour. Specifically, growth profile obtained during growth at 1 g/L and 10 g/L polyethylene glycol (PEG) overlapped each other and similar maximal optical density were obtained (Figure 3a). Thus, PEG did not exert concentration-dependent toxicity effect on *E. coli* DH5α, even at 10 g/L PEG. Hence, PEG (molecular weight ~8000 Da) is an appropriate anti-foam for use with the cultivation of *E. coli* DH5α in a bioreactor.



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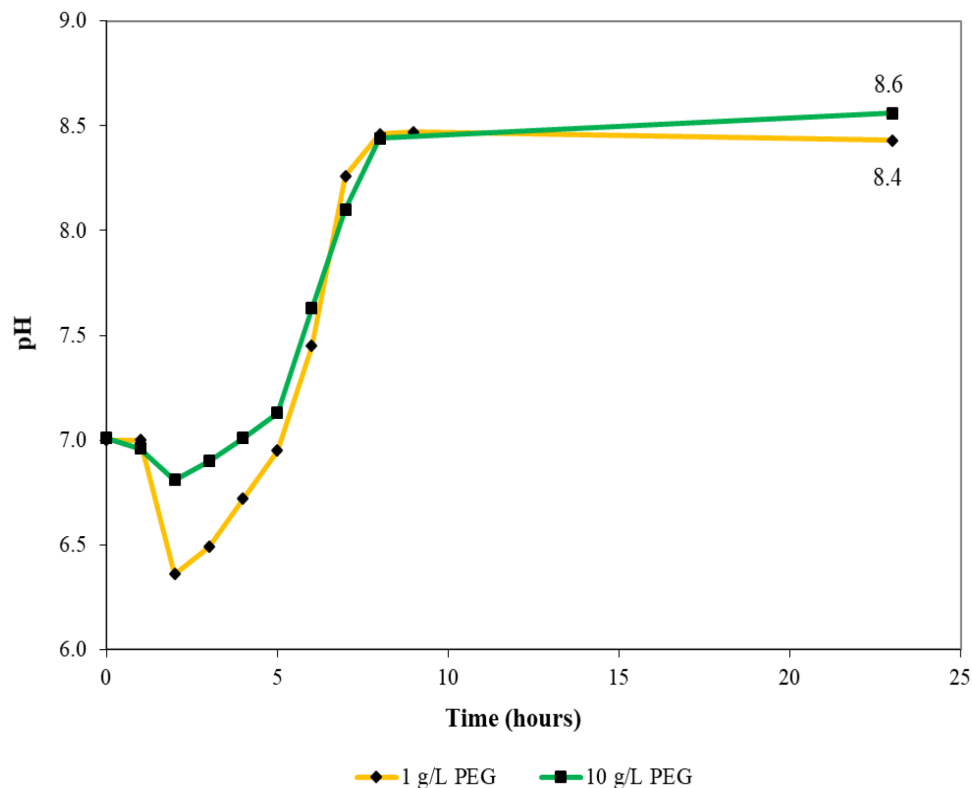


**Figure 3b:** pH variation with growth of *E. coli* DH5α in LB Lennox medium at 3 VVM aeration and 37 $^{o}$C incubation with either 1 g/L or 10 g/L PEG anti-foam. Exposure to 10 g/L PEG reduced the secretion of acidic metabolites at the onset of growth and did not affect the final pH of the culture broth significantly.

Observation of pH variation during growth of *E. coli* DH5α in LB Lennox medium at 3 VVM aeration with 10 g/L of PEG anti-foam revealed that there was reduction in secretion of acidic metabolites at the onset of growth compared to the case during growth of the bacterium in 1 g/L PEG (Figure 3b). *E. coli* DH5α growth in 10 g/L PEG also resulted in a higher final pH of the culture broth (8.6) compared to that achieved during growth at 1 g/L PEG (8.4). Thus, exposure to 10 g/L PEG did exert metabolic effect on *E. coli* DH5α that manifested in the relative amount of acidic and alkaline metabolites secreted; however, metabolic changes did not result in changes to rate of biomass formation and maximal optical density obtained. This highlighted that *E. coli* DH5α could adapt sufficiently to the osmotic shock and physiological stress of growth in a more viscous growth medium supplemented with 10 g/L PEG without detrimental impact on biomass formation.



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**Conclusions**

Aeration increases the oxygen available to cells, which promote respiration and use of the efficient oxidative phosphorylation pathway for energy generation and growth. This naturally manifest in a higher rate of growth and greater biomass formation. Observations of enhanced growth rate and slightly greater biomass formation in *E. coli* DH5α cultivated in LB Lennox medium at 37 $^{o}$C in a 1L bioreactor with 1 VVM aeration compared to growth in 250 mL glass shake flask with cotton plugs revealed that aeration offered a beneficial effect on *E. coli* DH5α growth. More importantly, it also revealed that natural rate of transfer of oxygen through the cotton plug of aerobic shake flasks could not reach 1 VVM. Hence, bioreactors with capability of adjusting aeration remain the mode of choice for commercial production of useful compounds from microbial cells, given enhanced growth rate and biomass formation.

Comparison of growth under 1 VVM and 3 VVM aeration in LB Lennox medium revealed that physiological stress from enhanced aeration at 3 VVM resulted in a longer lag phase but did not result in a reduction in maximal optical density obtained or slower growth rate. On the other hand, observations of pH variation during growth at 1 VVM and 3 VVM aeration revealed that *E. coli* DH5α did exhibit physiological and metabolic adaptations to increased shear stress and mixing from enhanced aeration. This came about through the secretion of more acidic metabolites at the outset of growth followed by reduced secretion of alkaline metabolites.

Finally, tests with elevated PEG (molecular weight ~8000 Da) anti-foam for controlling foam formation arising from enhanced aeration of 3 VVM revealed that PEG did not exert additional toxicity on *E. coli* DH5α at 10 g/L PEG compared to 1 g/L PEG based on biomass yield. However, elevated PEG of 10 g/L did result in reduced secretion of acidic metabolites by *E. coli* DH5α at the onset of growth compared to growth under 1 g/L PEG. Hence, while 10 g/L PEG likely exerted physiological impacts on *E. coli* DH5α through the combination of osmotic shock and more viscous fluid environment for swimming motility, relatively little metabolic energy was diverted away from biomass formation.

Collectively, *E. coli* DH5α exhibited enhanced growth rate during growth in a 1L bioreactor compared to a shake flask in LB Lennox medium at 37 $^{o}$C. While enhanced aeration of 3 VVM increased shear stress on the bacterium, biomass formation and growth rate in *E. coli* DH5α were not significantly affected. On the other hand, increased secretion of acidic metabolites at the outset of growth under 3 VVM aeration suggested physiological adaptations to increased fluid shear. Finally, elevated concentrations of PEG at 10 g/L did not exert additional toxicity on *E. coli* DH5α compared to 1 g/L PEG with biomass yield as evaluation parameter. Rate of biomass formation was also not affected. However, reduction in secretion of acidic metabolites at the outset of growth revealed physiological adaptations by the bacterium towards increased osmotic stress and fluid viscosity from higher PEG concentration in the culture medium.



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## Conflicts of interest

The author declares no conflicts of interest.

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