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Research Article

High Cell Density Cultivation of *Escherichia coli* DH5α in Shake Flasks with a New Formulated Medium

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Received date: November 16, 2021; Accepted date: November 22, 2021; Published date: November 25, 2021

Citation: Wenfa Ng (2021) High Cell Density Cultivation of Escherichia coli DH5α in Shake Flasks with a New Formulated Medium. *J, Biotechnology and Bioprocessing* 2(10); DOI: 10.31579/2766-2314/065

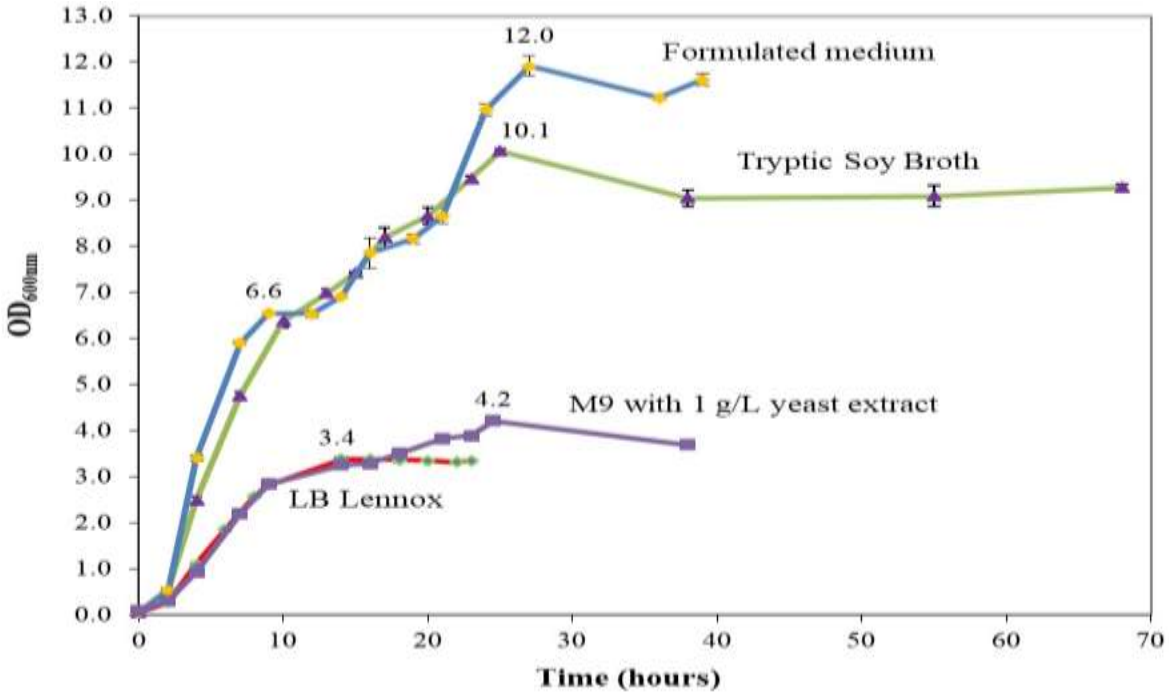
Abstract

High cell density cultivation necessitates cell division and biomass formation, the mechanisms of which remain poorly understood, especially from the cellular energetics perspective. Specifically, the sensing of energy abundance and the channelling of nutritional energy into biomass formation and cell maintenance remains enigmatic at the sensory, effector and decision levels. Thus, optimization of cell growth remains an iterative trial and error process where the principal parameters are growth medium composition and incubation temperature. In this study, a new semidefined formulated medium was shown to be useful for high cell density cultivation of *Escherichia coli* DH5α (ATCC 53868). Comprising K_2HPO_4, 12.54; KH_2PO_4, 2.31; D-Glucose, 4.0; NH_4Cl, 1.0; Yeast extract, 12.0; NaCl, 5.0; $MgSO_4$, 0.24; the medium possessed a high capacity phosphate buffer able to moderate pH fluctuations during cell growth known to be detrimental to biomass formation. With glucose and NH_4Cl providing the nutrients for initial growth, followed by a lag phase of 3 hours, a maximal optical density of 12.0 was obtained after 27 hours of cultivation at 37 ºC and 230 rpm. Yeast extract provides a secondary source of carbon and nitrogen. Maximal optical density obtained in formulated medium was higher than the 10.1, 4.2, and 3.4 obtained in Tryptic Soy Broth, M9 with 1 g/L of yeast extract, and LB Lennox, respectively. Cultivation of *E. coli* DH5α in formulated medium with 6 g/L of glucose resulted in a longer lag phase of 8 hours and a longer time (68 hours) to attainment of maximal optical density, which marked the upper limit of glucose concentration beyond which biomass formation would be reduced. Specifically, glucose concentration above 6 g/L markedly reduced biomass formation possibly due to the environmental stress arising from low pH in the culture broth. Glucose concentration below 4 g/L, on the other hand, reduced biomass formation through a smaller pool of nutrients serving as biomass building blocks. Deviation from 1:1 molar ratio between glucose and NH_4Cl was not detrimental to biomass formation and growth rates. Collectively, a semi-defined formulated medium could increase optical density of *E. coli* DH5α beyond that of LB Lennox and Tryptic Soy Broth, and may find use in cultivation of cells for applied microbiology research.

Keywords: cell growth, biomass yield, *Escherichia coli*, shake flask, cell maintenance, environmental stress, metabolic programme, low pH, growth medium, optimization

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

Graphical Abstract



Short description

Large amount of cells are typically required for characterization studies or applied microbiology research. However, growth of *Escherichia coli* DH5α (ATCC 53868) in common growth media typically results in low biomass yield. Mechanisms underlying low biomass yield remain poorly understood, and optimization of biomass yield requires iterative trial and error experimentation with growth medium composition and incubation temperature as parameters. This study set out to develop a medium capable of high cell density aerobic cultivation of *E. coli* DH5α in shake flasks.

Experiment results revealed that a new semi-defined formulated medium with a high capacity phosphate buffer system could deliver an optical density higher than that available from growth of *E. coli* DH5α in Tryptic Soy Broth, M9 with 1 g/L yeast extract and LB Lennox medium. In addition, growth rate of *E. coli* DH5α in the formulated medium was also faster than that in other growth media tested. Overall, optimization experiments revealed that excess glucose beyond 6 g/L should be avoided for *E. coli* DH5α cultivation, given that it depressed broth pH and reduced biomass formation. On the other hand, deviation from a 1:1 molar ratio between glucose and ammonium chloride concentrations did not negatively impact on biomass formation and growth rates. Collectively, modulation of pH variation by phosphate buffer system provided conducive conditions for biomass formation during growth of *E. coli* DH5α in the new formulated medium.

Significance of the work

Formulation of growth medium remains an art, where few design rules exist. More importantly, each medium may be specific to particular microbial species from the perspective of optimizing cell growth and biomass formation. This study demonstrated the utility of a new semi-defined formulated medium in enabling high cell density aerobic cultivation of *Escherichia coli* DH5α (ATCC 53868) in shake flasks. Specifically, the formulated medium could support a higher optical density compared to Tryptic Soy Broth, LB Lennox, and M9 with 1 g/L of yeast extract. More importantly, the series of optimization experiments revealed that the optimal range of glucose concentration for *E. coli* DH5α might be between 4 and 6 g/L, where glucose concentration beyond 6 g/L resulted in reduced biomass formation. Additionally, a molar ratio of 1:1 between glucose and ammonium chloride did not affect biomass formation and growth rates. In summary, this study did not yield general design principles for growth medium formulation, but experiment results illuminated the detrimental impacts of excess glucose on biomass formation.

Highlights

1) Cellular decision to divide into new daughter cells remain enigmatic, particularly from the energetics perspective, where we know little about the partitioning of cellular energy between cell maintenance and biomass formation.

2) To increase biomass formation for downstream applications, current methods remain the iterative trial and error optimization of growth medium composition and incubation temperature.

3) A new semi-defined formulated medium was shown to be effective in high cell density cultivation of *Escherichia coli* DH5α (ATCC 53868) at 37 °C and 230 rpm in aerobic shake flasks with maximal optical density of 12.0, which was higher than 10.1, 4.2, and 3.4 in growth of the bacterium in Tryptic Soy Broth, M9 with 1 g/L of yeast extract, and LB Lennox medium.

4) Specifically, yeast extract provided a secondary carbon and nitrogen source in addition to glucose and ammonium chloride, with a high capacity phosphate buffer reducing fluctuations in broth pH known to reduce biomass formation.

5) Optimal glucose concentration for maximal biomass formation appeared to be between 4 and 6 g/L, where 6 g/L of glucose resulted in a longer lag phase (8 hours versus 3 hours) and longer time to maximal optical density (68 hours versus 27 hours) compared to 4 g/L of glucose during growth of *E. coli* DH5α in shake flasks.

6) Experiments revealed that glucose concentration beyond 6 g/L reduced biomass formation due to a low broth pH, and is the key parameter for medium formulation.

7) Additionally, deviation from a 1:1 molar ratio between glucose and ammonium chloride did not negatively impact on biomass formation and growth rates.

Introduction

Lack of biomass formation is a critical problem of many commercially available media in cultivation of common bacteria for applied microbiology studies. Such studies typically require relatively large amount of biomass for various characterization studies and experiments. For example, growth of *Escherichia coli* DH5α (ATCC 53868) in LB Lennox medium at 37°C typically generated optical density of ~3, which translated to only a small amount of biomass, insufficient for use in experiments such as investigating the adsorption of heavy metals on *E. coli*, which require large amount of biomass.

The problem of lack of biomass formation in specific growth medium for particular bacterium likely stems from multiple factors: for example, (i) inability of the growth medium in supplying the necessary nutrients for biomass formation, and (ii) incompatibility of the medium with respect to the bacterium's metabolism, where a metabolic sensor in the cell might assess that the presented medium lacked critical components necessary for promoting biomass formation. Typically, energy derived from nutrients could be channelled to either cellular maintenance or biomass formation, but the proportion of energy directed to either pathway is likely species specific with unknown mechanisms. Thus, the holy grail in microbial cell culture is in tailoring growth medium composition to the specific microbial physiology and metabolism of the microbial species. However, our understanding of the relationship between microbial growth and growth medium composition remains limited and require iterative growth experiments for understanding the growth characteristics of particular microbe, given differing genetic, epigenetic and posttranscriptional regulation in individual species and strains [1]. Hence, the goal of designing a growth medium for a particular microbial species remain inaccessible with current knowledge of microbial growth in different growth media even though attempts have been made in understanding the connection between growth medium used and a metabolic network of a cell [2-6] as well as using phylogeny to predict type of growth medium suitable [7]. Specifically, while a multitude of media are available for the cultivation of various microorganisms, growth medium formulation remains an art, where specific design rules for an optimal medium with respect to a particular species of microbe are lacking. Thus, trial and error approaches remain the norm in the formulation of medium for bacterial culture [8].

In this study, a growth medium was formulated for enhancing the biomass formation of *Escherichia coli* DH5α during growth at 37 °C and 230 rpm rotational shaking in shake flasks. Specifically, maximal optical density obtained was 12.0 over 27 hours of cultivation with a diauxic growth phase where *E. coli* DH5α switched primary carbon source from glucose to yeast extract. More importantly, maximal cell concentration obtained was 3.5 times that of LB Lennox medium. pH variation of the culture broth during growth of *E. coli* DH5α was modulated by a high capacity phosphate buffer system which reduced the environmental stress of low pH on the bacterium. Overall, the formulated semi-defined medium was suitable for high cell density aerobic cultivation of *E. coli* DH5α.

Materials and Methods

Materials

LB Lennox medium and Tryptic Soy Broth were purchased from Difco and Merck, respectively and used as is. Composition of LB Lennox

medium was [g/L]: Tryptone, 10.0; Yeast extract, 5.0, NaCl, 5.0. Composition of Tryptic Soy Broth was [g/L]: Pancreatic digest of casein, 15.0; Papaic digest of soya bean, 5.0; NaCl, 5.0. Composition of M9 medium with 1 g/L of yeast extract in [g/L]: D-Glucose, 4.0; NH_4Cl, 1.0; Na_2HPO_4, 6.78; NaH_2PO_4, 3.0; NaCl, 0.5; Yeast extract, 1.0. Composition of formulated medium was in [g/L]: K_2HPO_4, 12.54; KH_2PO_4, 2.31; D-Glucose, 4.0; NH_4Cl, 1.0; Yeast extract, 12.0; NaCl, 5.0; $MgSO_4$, 0.24.

Growth of E. coli DH5α in different growth media

E. coli DH5α glycerol stock culture was prepared in 40% glycerol and stored at -70 ºC until use. One glycerol stock culture was used in inoculating 100 mL of LB Lennox medium in 250 mL glass conical flask for initiating a seed culture at 37 ºC and 230 rpm rotational shaking in a temperature controlled incubator. After 24 hours of incubation, 1 mL of seed culture was used as inoculum into 100 mL of a specified medium in 250 mL glass conical flask as experiment cultures. Three biological replicates were performed for each experiment. Experiment cultures were incubated at 37 ºC and 230 rpm rotational shaking in a temperature controlled incubator (Yih Der LM-570RD, Taiwan).

Measurement of optical density and pH

At appropriate time points, 5 mL of culture was withdrawn from the experiment cultures for measurement of optical density and pH. Specifically, samples were placed in a quartz cuvette of pathlength 10 mm (volume: 3.5 mL), and optical density measured using a UV-Visible spectrophotometer (Shimadzu Biospec Mini) at 600 nm. Samples were diluted with deionized water if the optical density exceeded 1. On the other hand, pH of samples were measured with an Orion 9156 BNWP pH probe outfitted to a Mettler Toledo Delta 320 pH meter.

Results and Discussion

Components	Concentration (g/L)
NH_4Cl	1.00
Yeast extract	12.00
NaCl	5.00
$MgSO_4$	0.24

Components	Concentration (g/L)
K_2HPO_4	12.54
KH_2PO_4	2.31
D-Glucose	4.00

Table 1: *Composition of formulated medium*

A formulated medium with yeast extract and a high capacity phosphate buffer system was formulated to enable the high cell density aerobic cultivation of *E. coli* DH5α at 37 ºC and 230 rpm rotational shaking in 250 mL shake flasks (Table 1). Specifically, K_2HPO_4 and KH_2PO_4 comprise the phosphate buffer system, while yeast extract provided a secondary source of carbon and nitrogen for further biomass formation. NaCl was added to tune osmolarity of the medium, and $MgSO_4$ provided a source of the essential element Mg and sulphur. Glucose and ammonium chloride are the primary carbon and nitrogen sources respectively. Yeast extract is a secondary source of carbon and nitrogen. It also provides vitamins and growth factors for the bacterium.

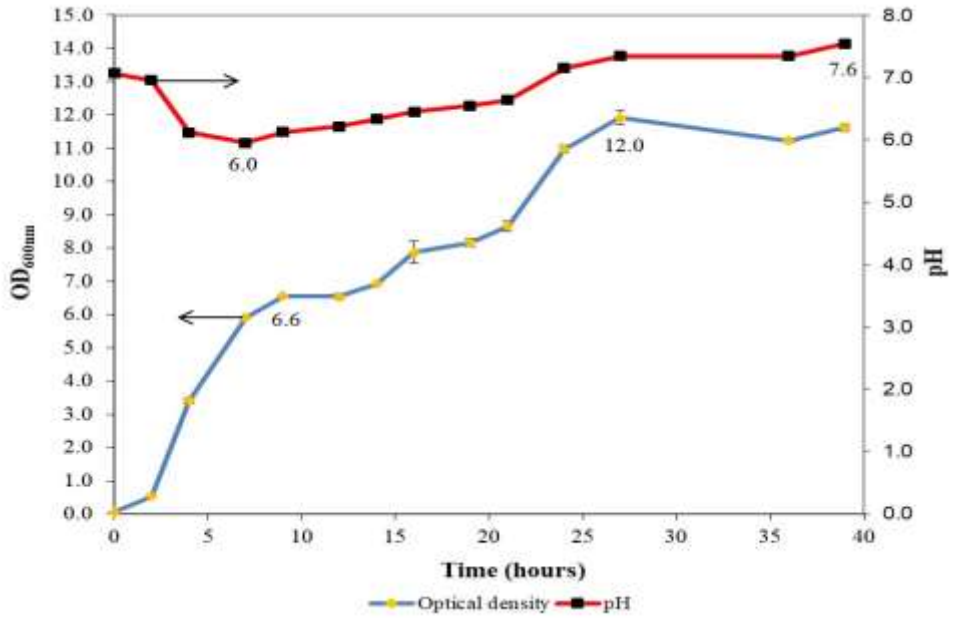


Figure 1: *Growth of Escherichia coli DH5α in formulated medium with 89 mM of phosphate buffer, 4 g/L of glucose and 1 g/L of ammonium chloride at 37 oC and 230 rpm rotational shaking in 250 mL shake flasks.*

Growth of *E. coli* DH5α was rapid with the attainment of optical density (at 600 nm) of 6.6 after 9 hours of cultivation. This marked the start of the lag phase which lasted for 3 hours, where *E. coli* DH5α synthesized new enzymes and altered its metabolic programme for using the carbon and nitrogen sources present in yeast extract for biomass formation. During the initial phase of growth, glucose and ammonium chloride served as the primary carbon and nitrogen source of the bacterium. Broth pH decreased from 7.1 to 6.0 during this initial phase of growth due to the secretion of acidic metabolites. This was followed by a gradual increase in broth pH to 7.6 at the end of cultivation. Due to the presence of a high capacity phosphate buffer system in the medium, pH variation in the culture broth was reduced significantly and helped reduce the environmental stress on the bacterium. After the first lag phase, the bacterium experienced a second lag phase at 16 hours into the cultivation, possibly due to the depletion of a secondary carbon source. Following the end of the second lag phase of 3 hours, optical density increased to a maximal value of 12.0 at 27 hours into the cultivation.

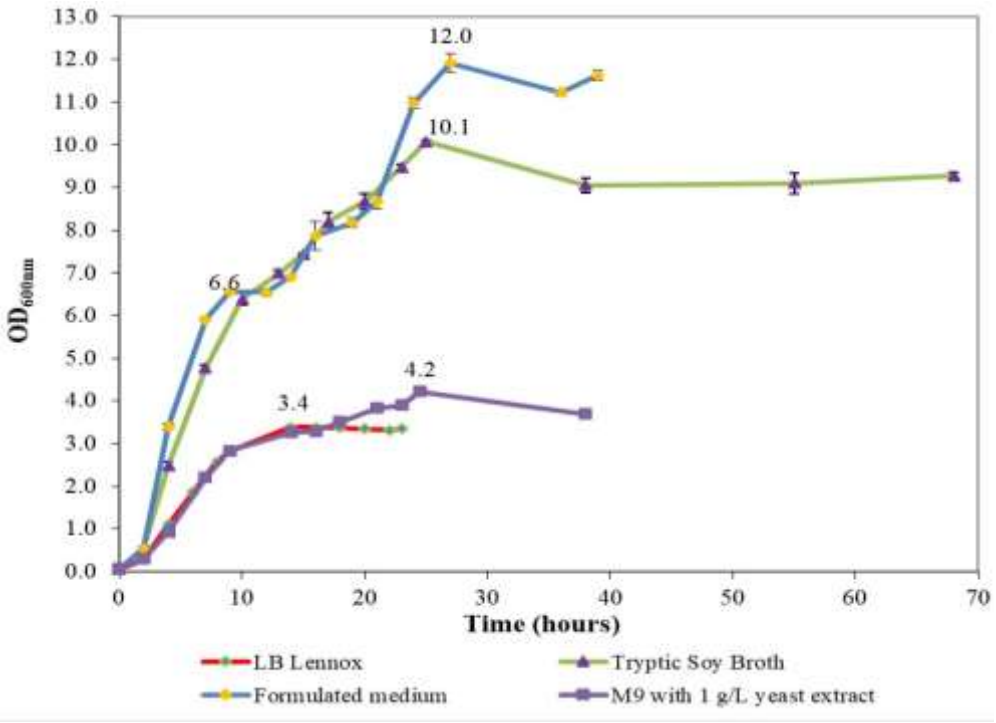


Figure 2a: *Growth of E. coli DH5α in different growth media at 37 oC and 230 rpm rotational shaking, revealing that the formulated medium provided a platform for high cell density aerobic cultivation of the bacterium.*

Biomass formation was enhanced during the cultivation of *E. coli* DH5α in formulated medium compared to Tryptic Soy Broth, M9 with 1 g/L yeast extract, and LB Lennox medium. Specifically, the maximal optical density obtained during growth in formulated medium was 12.0 compared to 10.1, 4.2 and 3.4 for Tryptic Soy Broth, M9 medium with 1 g/L yeast extract, and LB Lennox, respectively (Figure 2a). Growth rate of *E. coli* DH5α was also faster in formulated medium compared to that in the other media. Overall, *E. coli* DH5α grew more slowly in M9 medium with 1 g/L of yeast extract and LB Lennox medium, and the two media were not suitable for the generation of large amount of biomass typically required for applied microbiology studies. But interestingly, the growth profile of *E. coli* DH5α at the outset of growth was identical for both LB Lennox medium and M9 medium with 1 g/L of yeast extract.

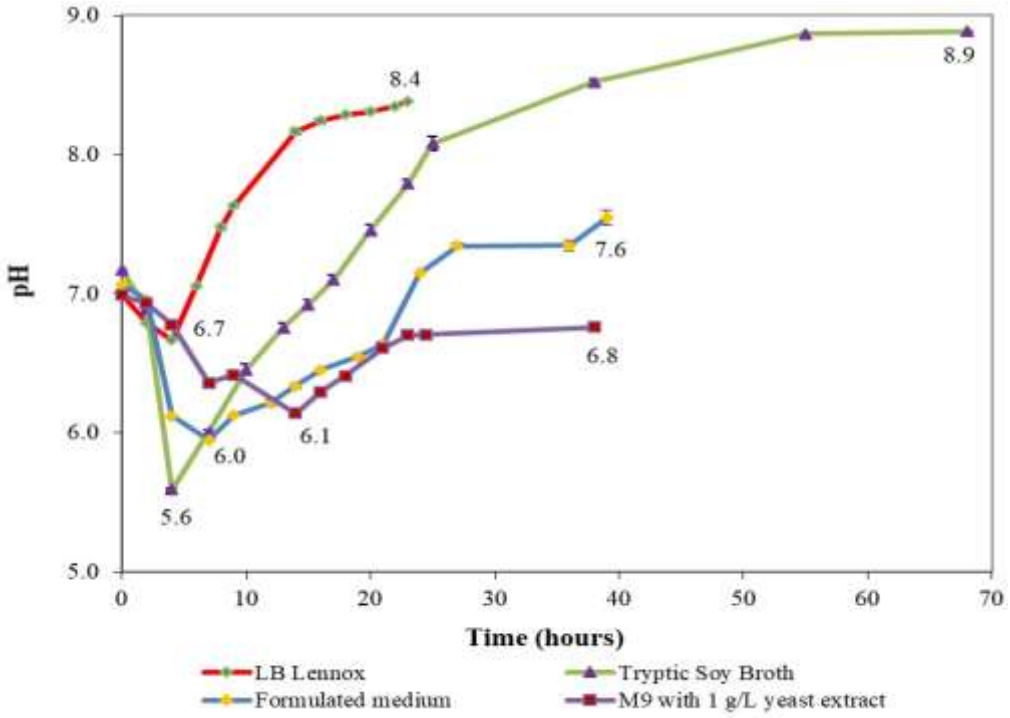


Figure 2b: *Variation in pH during E. coli DH5α growth in different growth media. Note the large fluctuation in pH during growth of the bacterium in LB Lennox and Tryptic Soy Broth, which are unbuffered media compared to the formulated medium and M9 medium with 1 g/L yeast extract.*

pH is known to affect cell viability and growth; thus, pH variation should not be too drastic during microbial cultivation. Hence, a high capacity phosphate buffer system was added to the composition of the formulated medium to reduce pH fluctuation during cultivation of bacteria. From Figure 2b, it could be seen that pH variation during *E. coli* DH5α's growth in formulated medium was significantly less than that in Tryptic Soy Broth. Specifically, pH decreased from 7.1 to 6.0, followed by a gradual rise to 7.6 at the end of the cultivation period in the formulated medium. This was significantly less drastic compared to that in Tryptic Soy Broth where pH decreased from 7.2 to 5.6, followed by a rise to 8.9 at the end of culture. Cells grown in formulated medium secreted acidic metabolites into the medium during growth, followed by a change in metabolism that culminated in the net secretion of alkaline metabolites. pH fluctuation in the formulated medium was buffered by the high capacity phosphate buffer present. Smaller pH fluctuation during growth reduced the environmental stress on the cells and may have contributed to higher optical density obtained in the formulated medium.

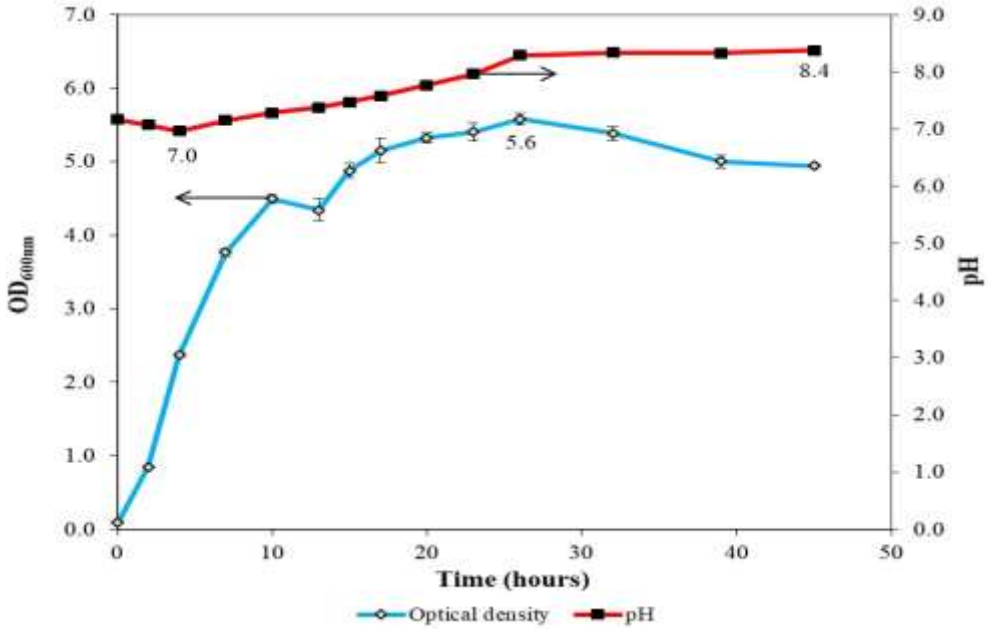


Figure 3: *Growth of E. coli DH5α in formulated medium without glucose and ammonium chloride at 37 oC and 230 rpm rotational shaking. Note that 12 g/L of yeast extract could support significant biomass formation.*

Since yeast extract offers a secondary source of carbon and nitrogen for the growth of *E. coli* DH5α, growth experiment was conducted to assess the relative amount of biomass that could be supported by 12 g/L of yeast extract in the formulated medium in the absence of glucose and ammonium chloride. Experiment results revealed that a maximal optical density of 5.6 (at 26 hours of incubation) could be supported by 12 g/L of yeast extract (Figure 3). pH of the broth decreased slightly from 7.2 to 7.0 followed by a gradual increase to 8.4. thereby, indicating that growth on yeast extract as primary carbon and nitrogen sources likely activated metabolic pathways different from growth on glucose and ammonium chloride (Figure 2b).

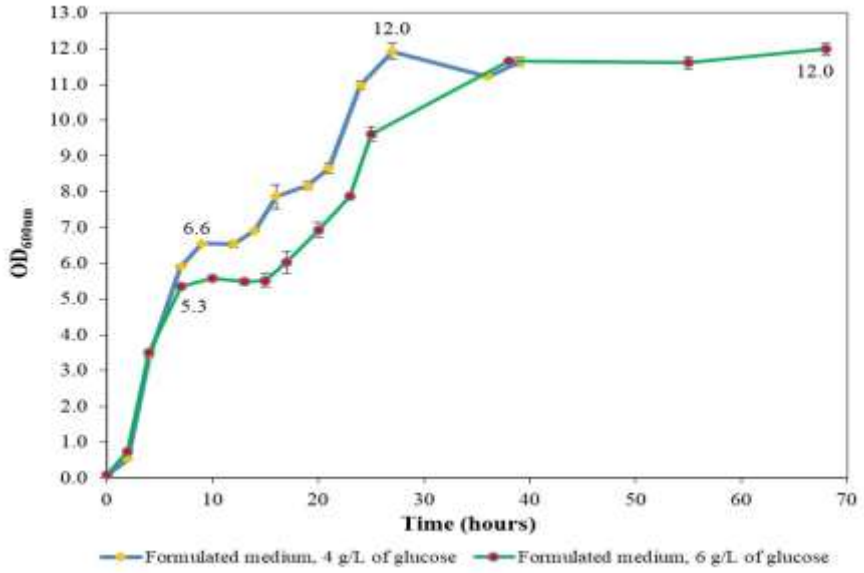


Figure 4a: *Comparison of growth of E. coli DH5α in formulated medium with 4 g/L of glucose and 1 g/L of ammonium chloride, and formulated medium with 6 g/L of glucose and 1.5 g/L of ammonium chloride. Note that growth of E. coli DH5α in formulated medium with 6 g/L of glucose came with a longer lag phase and slower attainment of maximal cell density of 12.0 g/L, which was the same as that obtained by E. coli DH5α during growth in formulated medium with 4 g/L of glucose.*

Growth of *E. coli* DH5α in formulated medium with 6 g/L of glucose and 1.5 g/L of ammonium chloride resulted in a longer lag phase of 8 hours and a longer time period to attainment of maximal optical density of 12.0 at 68 hours (Figure 4a). For comparison, lag phase during *E. coli* DH5α growth in formulated medium with 4 g/L of glucose and 1 g/L of ammonium chloride was 3 hours, and only 27 hours was needed to attain maximal optical density of 12.0. Growth in the two versions of formulated medium that differed in concentrations of glucose and ammonium chloride exhibited the same general profile with glucose and ammonium chloride fuelling the first phase of growth before lag phase, followed by growth on yeast extract.

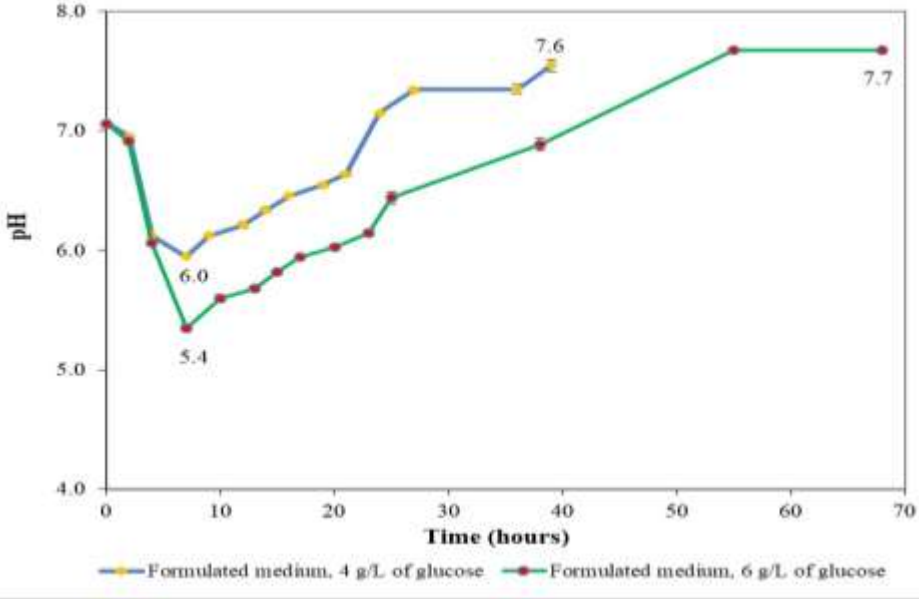


Figure 4b: *Variation of pH during growth of E. coli DH5α in formulated medium with 4 g/L of glucose and 1 g/L of ammonium chloride, and formulated medium with 6 g/L of glucose and 1.5 g/L of ammonium chloride.*

Similar pH profiles were obtained during growth of *E. coli* DH5α in formulated medium with 4 g/L of glucose and 6 g/L of glucose, respectively (Figure 4b). Specifically, growth of *E. coli* DH5α in formulated medium with 6 g/L of glucose resulted in a greater reduction in pH at the onset of the cultivation compared to that during growth of the bacterium in formulated medium with 4 g/L of glucose. Overall, pH decreased from the start of the culture, followed by a gradual rise to alkaline pH values in both cultures. For example, growth of *E. coli* DH5α in formulated medium with 4 g/L of glucose resulted in a decrease in pH from 7.1 to 6.0 after 7 hours of incubation, followed by a gradual rise to 7.6 at the end of the cultivation period. On the other hand, growth of the bacterium in formulated medium with 6 g/L of glucose first initiated a decrease in pH from 7.1 to 5.4, which was followed by a rise to pH 7.7 at the end of the culture at 68 hours. What was most interesting was that the pH and optical density profiles of the two media overlapped at the start of the culture period prior to attainment of minimal pH. Furthermore, rate of increase of pH at the latter part of the culture was similar in cells cultivated in the two growth media. Overall, environmental stress from low pH might have affected biomass formation resulting in a longer time period to attainment of maximal optical density during growth of *E. coli* DH5α in formulated medium with 6 g/L of glucose.

What differed was the presence of higher glucose concentration of 6 g/L that delayed the attainment of maximal cell density in *E. coli* DH5α. Thus, glucose concentration of between 4 and 6 g/L might be the optimal range for *E. coli* DH5α in the context of optimizing biomass formation. However, glucose concentration of 6 g/L in formulated medium might be the upper limit by which there would be no decrease in biomass formation. But, greater amount of time was needed to achieve maximal optical density during culture of *E. coli* DH5α in formulated medium with 6 g/L glucose. Taken together, *E. coli* DH5α growth in formulated medium with 4 g/L of glucose was not retarded in the same way that lower broth pH reduced biomass formation during the bacterium's growth in formulated medium with 6 g/L of glucose.

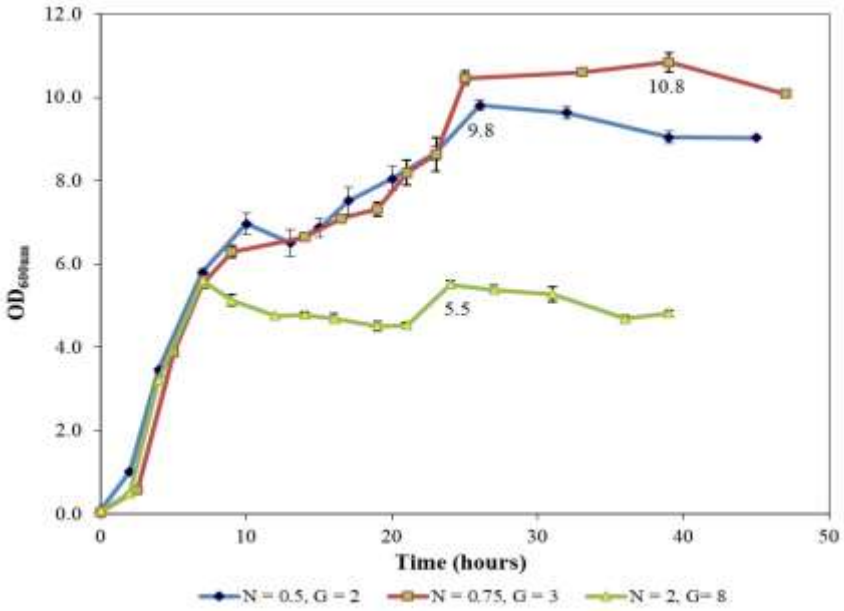


Figure 5a: *Cultivation of E. coli DH5α in formulated medium with different concentrations of glucose (G) and ammonium chloride (N) but with a 1:1 molar ratio between the two medium components at 37 oC and 230 rpm rotational shaking.*

Glucose concentration of 4 g/L and ammonium chloride concentration of 1 g/L was shown, through systematic experiments in this study, to be optimal for biomass formation, resulting in an optical density of 12.0 at 27 hours of incubation. Specifically, by keeping a 1:1 molar ratio between glucose and ammonium chloride, concentrations of glucose and ammonium chloride was varied systematically. Experiment results revealed that high glucose concentration of 8 g/L resulted in low broth pH correlated with reduced biomass formation possibly due to environmental stress from low pH (Figure 5a). On the other hand, glucose concentration below 4 g/L resulted in reduced biomass formation due to lower amount of nutrients, but did not significantly affect the culture broth pH profile (Figure 5b).

Irrespective of the amount of glucose and ammonium chloride provided in the formulated medium, initial growth phase of *E. coli* DH5α was similarly rapid (Figure 5a). This suggested that glucose was metabolized first prior to yeast extract. However, glucose concentration beyond 6 g/L led to reduced biomass formation. Specifically, in the case where glucose was 8 g/L and ammonium chloride 2 g/L, maximal optical density obtained was 5.5, which was significantly lower than that obtained with formulated medium with glucose of 4 g/L and ammonium chloride of 1 g/L. On the other hand, glucose concentration of less than 4 g/L resulted in reduced biomass formation due to lower nutritional content of the medium. For example, growth profile of *E. coli* DH5α in formulated medium with glucose of 2 g/L and ammonium chloride of 0.5 g/L was closely matched with that of *E. coli* DH5α in formulated medium with glucose of 3 g/L and ammonium chloride of 0.75 g/L. However, greater amount of ammonium chloride and glucose likely enabled greater biomass formation that resulted in a higher optical density of 10.8 in formulated medium with glucose of 3 g/L and ammonium chloride of 0.75 g/L compared to optical density of 9.8 in formulated medium with glucose of 2 g/L and ammonium chloride of 0.5 g/L.

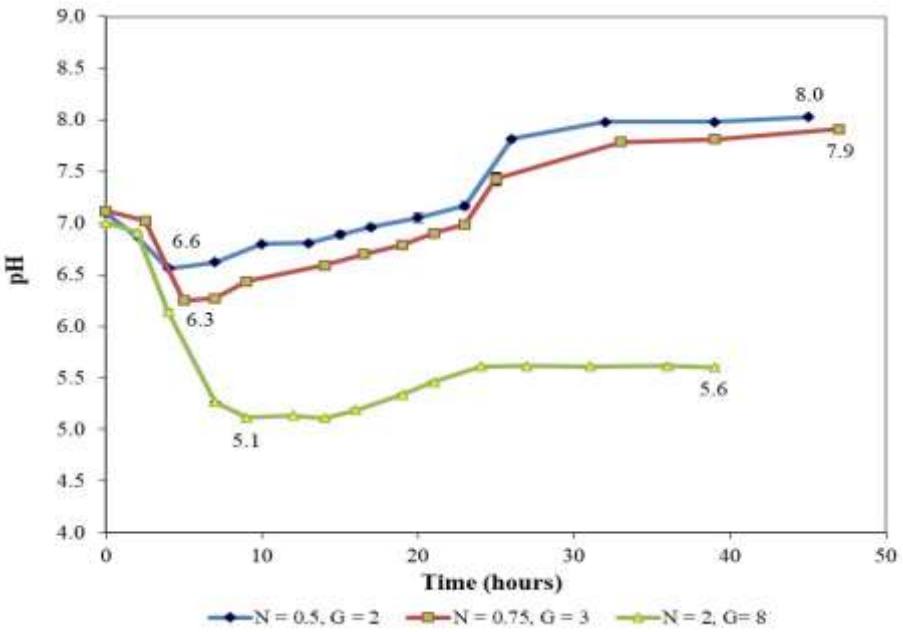


Figure 5b: *Variation of pH during growth of E. coli DH5α in formulated medium with different concentrations of glucose (G) and ammonium chloride (N). Note that excess glucose concentration in the formulated medium led to lower biomass formation due likely to a sharp fall in the culture broth pH.*

Observations of pH variation with culture time during growth of *E. coli* DH5α in formulated medium with different concentrations of glucose and ammonium chloride revealed that culture broth pH decreased with higher concentration of glucose in the medium. Specifically, pH of formulated medium with glucose of 8 g/L and ammonium chloride of 2 g/L had a minimal pH of 5.1 compared to 6.3 and 6.6 for formulated medium with glucose of 3 g/L and 2 g/L, respectively (Figure 5b). More importantly, similar pH profiles were obtained for *E. coli* DH5α grown in formulated medium with glucose of 2 g/L and 3 g/L. Specifically, pH first decreased followed by a gradual increase to alkaline value at the end of cultivation. The rate of increase of pH during latter part of the culture was similar for *E. coli* DH5α grown in formulated medium with 3 g/L of glucose and 0.75 g/L of ammonium chloride and formulated medium with 2 g/L of glucose and 0.5 g/L of ammonium chloride.

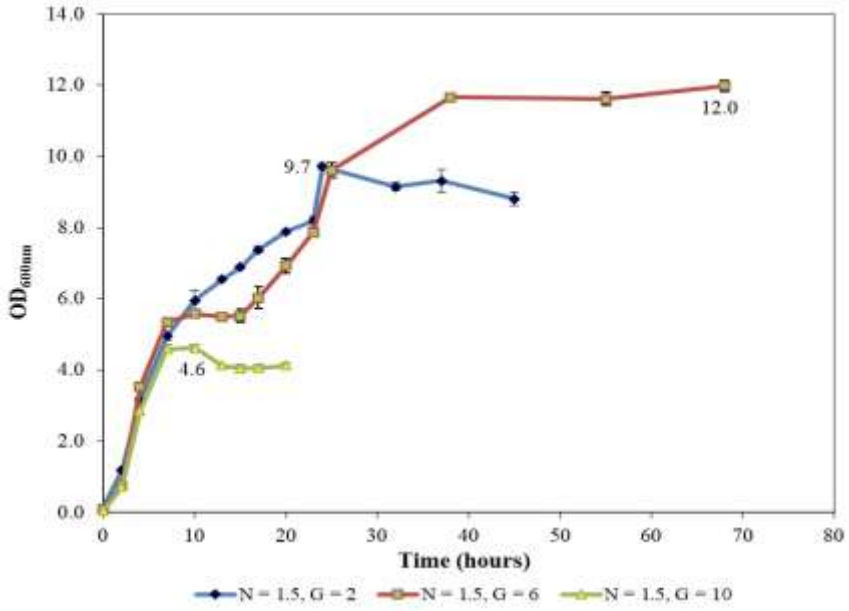


Figure 6a: *Growth of E. coli DH5α in formulated medium with different concentrations of glucose (G) and ammonium chloride (N) aiming to understand the effect of deviation from a 1:1 molar ratio between glucose and ammonium chloride on biomass formation.*

Experiment results revealed that deviation from a 1:1 molar ratio between glucose and ammonium chloride did not significantly affect biomass formation and growth rate (Figure 6a). Specifically, the growth profiles of *E. coli* DH5α at the onset of growth coincided with each other during growth in formulated medium with different concentrations of glucose and ammonium chloride that deviated from the 1:1 molar ratio between

glucose and ammonium chloride. This indicated that biomass formation and rate of growth were not affected by the molar ratio between glucose and ammonium chloride. Rather, excess glucose in the medium (such as 10 g/L of glucose) would result in a significant reduction in biomass formation. For example, the maximal optical density of *E. coli* DH5α cultivated in formulated medium with 10 g/L of glucose was 4.6 compared to 9.7 during growth in formulated medium with 2 g/L of glucose. Environmental stress from low culture broth pH likely impacted on biomass formation.

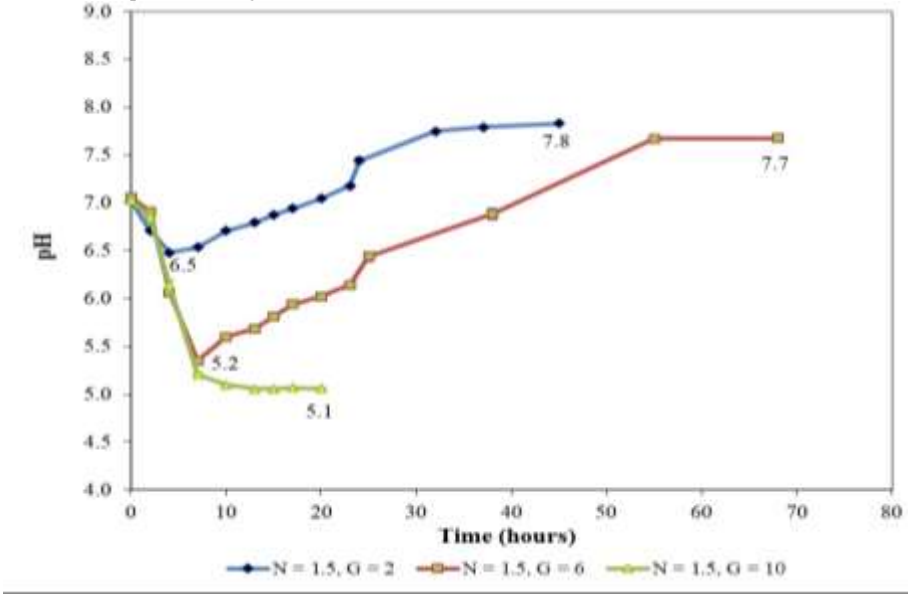


Figure 6b: *pH variation during growth of E. coli DH5α in formulated medium with different concentrations of glucose (G) and ammonium chloride (N) for assessing the effect of deviation from a 1:1 molar ratio between glucose and ammonium chloride on biomass formation.*

Comparison of pH profiles of *E. coli* DH5α growth in formulated medium with different glucose and ammonium chloride concentrations revealed that growth of the bacterium in 2 g/L of glucose and 1.5 g/L of ammonium chloride, and 6 g/L of glucose and 1.5 g/L of ammonium chloride generated similar pH profiles, which indicated that deviation from a 1:1 molar ratio between glucose and ammonium chloride did not bring forth significant changes to the pH profile (Figure 6b). Specifically, pH first decreased followed by a gradual rise to a higher value in both cases with the rate of pH increase during latter part of the culture similar during culture in the two growth media. However, higher glucose concentration such as in the formulated medium with 6 g/L of glucose resulted in a greater decline in pH. Specifically, minimal pH was 5.2 compared to 6.5 during growth of *E. coli* DH5α in formulated medium with 6 g/L of glucose compared to 2 g/L of glucose. Cultivation of *E. coli* DH5α in formulated medium with 10 g/L of glucose resulted in significant reduction in biomass formation due to a low culture broth pH of 5.1 which did not rise to a higher value at the end of the culture. Specifically, growth likely ceased for *E. coli* DH5α in formulated medium with 10 g/L of glucose upon reaching the lowest pH. Considering that the pH profiles at the onset of cultivation all coincided with each other, metabolism of glucose likely occurred first as it is a preferred carbon source of *E. coli*.

Finally, comparing the growth performance of *E. coli* DH5α in formulated medium with 2 g/L of glucose and 0.5 g/L of ammonium chloride and that in formulated medium (Figure 5a) with 2 g/L of glucose and 1.5 g/L of ammonium chloride (Figure 6a) revealed that the same maximal optical density of ~9.7 was obtained. This indicated that the formulated medium is glucose limited rather than nitrogen limited given that yeast extract is an abundant source of nitrogen for growth of *E. coli* DH5α.

Conclusion

Cellular decision making to undergo cell division and biomass formation remain poorly understood, particularly from the energetics perspective. Specifically, the relative partitioning of energy from nutrients into energy for cellular maintenance and biomass formation remain enigmatic with regards to the sensing, effector and decision mechanisms. Thus, to obtain high cell concentration for subsequent applied microbiology studies or biotechnology production, optimization of growth medium remained the only available approach in biotechnology for realizing high cell density cultivation of a particular microorganism. In this study, a semi-defined formulated medium with a high capacity phosphate buffer system was shown to be useful for high cell density cultivation of *E. coli* DH5α in a shake flask culture system. Specifically, compared to commercial LB Lennox medium, the formulated medium could support an optical density about 3.5 times that of LB Lennox. More importantly, growth in the medium likely did not generate inhibitory compounds. Glucose concentration in the formulated medium had an important role in controlling the metabolism of cells and biomass formation potential. Specifically, glucose concentration between 4 and 6 g/L is likely optimal with respect to biomass formation. However, formulated medium with 6 g/L of glucose and 1.5 g/L of ammonium chloride resulted in a longer lag phase and slower path to maximal optical density compared to formulated medium with 4 g/L of glucose and 1 g/L of ammonium chloride which had a lag phase of 3 hours and attained the same maximal optical density of 12.0 in 27 hours of cultivation compared to 68 hours. Thus, environmental stress from low culture broth pH likely reduced *E. coli* DH5α fitness at cell growth and biomass formation.

Glucose concentration either lower or higher than the optimal range of 4 and 6 g/L would result in reduced biomass formation. Specifically, excess glucose in the medium was correlated with low culture broth pH, which exerted significant environmental stress on the cells that reduced biomass formation. In cases with excess glucose concentration such as 10 g/L, *E. coli* DH5α could not recover from the environmental stress and cease biomass formation. On the other hand, glucose concentration lower than 4 g/L had lower nutritional content, which led naturally to reduced biomass formation. More importantly, deviation from a 1:1 molar ratio between glucose and ammonium chloride concentration in the medium

did not result in significant changes to growth profile and growth rate. What was more important was the amount of glucose present in the medium, where departure from the optimal range could result in reduced biomass formation either due to lack of nutrients or generation of excessive amount of acidic metabolites that depressed broth pH. The latter leading to environmental stress to cells that reduced biomass formation. Overall, presence of a relatively high concentration of yeast extract of 12 g/L meant that the formulated medium is limiting in carbon source and not nitrogen, a fact corroborated by experiments where similar maximal optical density of *E. coli* DH5α was obtained in different versions of formulated medium with the same concentration of glucose but different concentrations of ammonium chloride.

Collectively, a formulated medium was shown to be useful for high cell density cultivation of *E. coli* DH5α in shake flasks at 37 ^{o}C and 230 rpm rotational shaking. Large population of cells generated could be maintained in the medium, which suggested that growth inhibitory compounds were not secreted during growth. Overall, the medium could find use in the research settings where large population of cells are needed for downstream experiments aimed at utilizing the cells for various biotechnological applications such as biosorption removal of heavy metals.

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DOI: 10.31579/2766-2314/065