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*Article*

# Production of 1,3-Dihydroxyacetone by *Gluconobacter nephelii* in Upflow Aerated Bioreactors with Agro-industrial Wastes as External Nitrogen Source

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**Abstract.** Cultivation of recently identified *Gluconobacter nephelii* to produce 1,3-dihydroxyacetone (DHA) was conducted using the growth medium containing the external nitrogen source from agro-industrial wastes. Cell cultivation in 250 mL glass flasks using 80 g/L glycerol and corn steep liquor as nitrogen source yielded the highest biomass (1.75 ± 0.23 g/L) and DHA (49.5 ± 3.14 g/L) concentrations as compared to other nitrogen sources (i.e., glutamic mother liquor and soybean meal hydrolysate). Subsequent cultivation in larger bioreactors (32 L) with corn steep liquor as the preferred nitrogen source indicated that internal-loop airlift bioreactor yielded significantly higher biomass and DHA concentrations than flat-panel airlift and bubble column bioreactors. Further optimization of internal-loop airlift photobioreactor revealed that DHA production in *G. nephelii* depended on the aeration rates, with the maximum DHA concentrations (46.09 ± 1.01 g/L) obtained when maintaining the aeration rates at 0.64 vvm.

**Keywords:** DHA, *Gluconobacter*, agro-industrial waste, airlift, glycerol, corn steep liquor.

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## 1. Introduction

1,3-Dihydroxyacetone ($C_3H_6O_3$) is a high valued chemical, which is used extensively in cosmetic industry especially for the making of sunless tanning [1-3]. It was also served as the building block for the production of fine chemicals such as 1,2-propylene glycerol or lactic acid [2,4]. Due to wide range of applications, the demand for DHA was predicted to increase significantly, thereby requiring the production process to be both technically and economically feasible [5]. It has been suggested that the biological production of DHA is more efficient than the chemical pathways due to lesser expense required for process safety and easier product separation, and importantly DHA compounds produced biologically are more acceptable by pharmaceutical and cosmetic industries than those produced via chemical synthesis [6]. Various microorganisms are able to produce DHA from different substrates, for examples *Komagataeibacter* (*Acetobacter*) *xylinum*, *Ogataea* (*Hansenula*) *polymorpha*, *Klebsiella aerogenes*, *Escherichia coli*, *Pichia membranifaciens*, *Saccharomyces cerevisiae*, *Gluconobacter frateurii* and *Gluconobacter oxydans* [3,6-12]. Among reported species, *G. oxydans* has received the most attention due to relatively high DHA yield and fewer byproducts [13]. Recently, new strain of acetic acid bacteria in genus *Gluconobacter* was isolated from tropical fruits rambutan and identified as *Gluconobacter nephelii*, yet limited information on growth and DHA production by *G. nephelii* was available [14,15].

Oxygen availability was one of the factors that influenced growth and DHA production in *Gluconobacter* so that providing well-mixed condition and adequate oxygenation in cultivating systems have become necessary for the success of biological DHA production [16-18]. Aerated stirred tank bioreactors have been generally employed in the cultivation of *Gluconobacter* at various scales. In spite of the advantages such as low construction cost and ease of operation, aerated stirred tank bioreactors often experience mass and heat transfer limitation when attempting to scale up or culture at high cell density [5]. Airlift bioreactors were suggested as the possible alternatives to stirred tank bioreactors because they exhibited relative high gas-liquid mass transfer, good liquid mixing, low liquid shear force as a result of no mechanical moving parts and low operating cost [19-22]. Airlift bioreactors are available in different configurations such as internal-loop column, external-loop column, cone-shape, cone-shape with baffle inside, and flat-panel, with the selection criteria depending on types of bioprocess, microorganisms, growth media, sterilization options, scaling up, ease of construction and operation, space limitation and budget [3,19,23-25]. Unfortunately, only one report on DHA production in airlift bioreactor is available. In that work, a laboratory scale (2 L) internal-loop airlift bioreactor subjected to increasing aeration rates from 0.9 to 2.1 vvm was employed to grow *G. oxydans*, resulting in DHA concentrations approximately 35 g/L and subsequently 153 g/L after optimizing glycerol feeding method [3].

On the separated note, growth medium is the major expense in biological processes, in some cases accounting for as high as 60% of the total operating cost [26,27]. The growth medium for *Gluconobacter* usually contains expensive ingredients such as peptone and yeast extract, which are external source of nitrogen [28]. Out calculation of growth medium cost for *G. oxydans* revealed that peptone and yeast extract accounted for approximately 55% of medium total cost. One of the possible options for reducing medium cost is using external nitrogen source from agro-industrial byproducts or wastes [26,28]. Corn steep liquor, glutamic mother liquor and soybean meal are common agro-industrial wastes in Thailand. The costs of these materials per kg in Thailand are approximately 5 to 196 times cheaper than those of yeast extract and peptone [29].

Therefore, the objectives of this works aimed to evaluate the feasibility of using *G. nephelii* to produce DHA by using the growth medium that contained external nitrogen source from agro-industrial wastes (i.e., corn steep liquor, glutamic mother liquor and soybean meal hydrolysate). Moreover, the study intended to compare the performance of different bioreactors in the cultivation of *G. nephelii* to produce DHA, and finally to determine the effects of aeration rates on DHA production.

## 2. Materials and Methods

### 2.1. Preparation of Culturing Strain

*Gluconobacter nephelii*, previously isolated from rambutan, was obtained from the Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Science, Chulalongkorn University [30]. Inocula (3 mL) were transferred into 125-mL glass flasks, which already contained sterile growth medium (27 mL)

with the composition described as follows: 20 g/L glycerol, 10 g/L yeast extract, 10 g/L peptone, 5 g/L glucose and 100 mL/L potato extract [30]. Glass flasks and growth medium were autoclaved at 121 °C for 20 mins before use. Initial pH of culture suspension was adjusted to 5.0 by adding 2.0 M NaOH. Flasks containing microbial seeding were maintained in a temperature controlled shaker at 115 rpm and 30 °C for 24 h.

### 2.2. Preparation of Agro-industrial Wastes

Corn steep liquor from Friendship Corn Starch Limited (Thailand) and glutamic mother liquor from Ajinomoto (Thailand) were centrifuged at 10,000×*g* for 10 min to obtain clear supernatant. Soybean meal samples, purchased from local market, were grinded and submerged in 1 M HCl at the weight ratio of solids to acid solution at 1:6 at 50 °C for about 20 h, then solids were separated from liquid by filtration and the pH of clear supernatant was adjusted to neutral by adding 2 M NaOH [31]. Prepared liquid samples from agro-industrial wastes were stored in the refrigerator at 3 °C to preseved quality. Liquid samples (100 mL) from each agro-industrial waste were analyzed for CHN contents, which revealed the nitrogen mass fractions of 4.28%, 5.87% and 2.3% for corn steep liquor, glutamic mother liquor and soybean meal hydrolysate, respectively.

### 2.3. Optimal Glycerol Concentrations

Microbial inocula (3 mL) from section 2.1 were mixed in 125 mL glass flask, which contained the growth medium (27 mL) having the composition as described earlier [30]. Glycerol concentrations in the growth medium, chosen as experimental variable, were varied from 20 to 120 g/L. Glass flasks were kept in the temperature controlled shaker at 115 rpm and 30 °C. The initial pH of cell suspension in flasks was adjusted to 5.0 by the addition of 2 M NaOH. Liquid samples (1 mL) from glass flasks were collected every 6 h interval and analyzed for the concentrations of DHA, glycerol and biomass.

### 2.4. Selection of Nitrogen Source from Agro-industrial Wastes

Agro-industrial wastes (i.e., corn steep liquor, glutamic mother liquod and soybean meal hydrolysate) were evaluated as the external nitrogen source to substitute peptone and yeast extract in the growth medium for *G. nephelii*. Initial nitrogen concentrations in control and treatment flasks were prepared at 2.1 mg N/L, which were equal to nitrogen concentrations in the growth medium for *G. nephelii* as describe in section 2.1 [30]. For control flasks (i.e., C), microbial inocula (3 mL) from section 2.1 were cultured in 125 mL glass flasks containing 27 mL sterile growth medium [30]. Corn steep liquor (1.48 mL), glutamic mother liquor (1.44 mL) and soybean meal hydrolysate (1.92 mL) were mixed with microbial inocula (3 mL) and sterile growth medium (27 mL) in the absence of peptone and yeast extract in 125 mL flasks, which were referred to as T1, T2 and T3, respectively. For the remaining treatment flasks (i.e., T4, T5 and T6), the same amounts of corn steep liquor (1.48 mL), glutamic mother liquor (1.44 mL) and soybean meal hydrolysate (1.92 mL) were also mixed in 125 mL glass flasks containing microbial inocula (3 mL) and growth medium (27 mL) excluding glycerol. Table 1 summarizes the details of control and treatment flasks in this section. Initial glycerol concentrations were prepared according to the results from section 2.3. All flasks were maintained in the temperature controlled shaker at 115 pm and 30 °C while the initial pH of cell suspension was adjusted to 5.0. Liquid samples (1 mL) from each flask were collected every 6 h interval and analyzed for biomass and DHA concentrations.

Table 1. Details of control and treatment flasks in the experiment to select agro-industrial wastes as external nitrogen source in the growth medium of *G. nephelii*.

| Experiment | Contents of growth medium | | |
|---|---|---|---|
| | Glycerol | Nitrogen source | Other compositions |
| C | Section 2.3 | Peptone and yeast extract | [29] |
| T1 | Section 2.3 | Corn steep liquor | [29] |
| T2 | Section 2.3 | Glutamic mother liquor | [29] |
| T3 | Section 2.3 | Soybean meal hydrolysate | [29] |
| T4 | - | Corn steep liquor | [29] |
| T5 | - | Glutamic mother liquor | [29] |
| T6 | - | Soybean meal hydrolysate | [29] |

### 2.5. Bioreactor Description

Internal-loop airlift bioreactor was constructed from two co-axial transparent acrylic cylinders (i.e., outer cylinder: diameter 20 cm, thickness 0.5 cm, height 135 cm; inner diameter: diameter 10 cm, thickness 0.5 cm, height 90 cm), resulting in total working volume of 32 L (Fig. 1(a)). Sterile air, obtained by passing compressed air through Gelman Acrodisc filtered paper with average pored size of 0.45 μm, was introduced into the internal-loop airlift bioreactor through an aquarium air diffuser located at the center bottom of inner cylinder (i.e., draft tube). Bubble column bioreactor was also built from transparent acrylic cylinder (i.e., inner diameter 20 cm, thickness 0.5 cm, height 135 cm), resulting in total working volume of 32 L (Fig. 1(b)). Aquarium air diffuser was installed at the bottom of cylinder. Sterile air for bubble column bioreactor was obtained by filtering compressed air through Gelman Acrodisc paper (average pored size 0.45 μm). For flat-panel airlift bioreactor as shown in Fig. 1(c), it was assembled from two rectangular transparent acrylic slabs (i.e., height 50 cm, length 55 cm and thickness 0.5 cm) to create a parallel walls with distance 20 cm apart. Rectangular baffle (i.e., height 30 cm, length 55 cm and thickness 0.5 cm), located at the middle between two parallel slabs, was mounted to bioreactor walls about 5 cm above the curve bottom. The total working volume of flat-panel airlift bioreactor was 32 L. Aquarium air diffusers were placed at the bottom along the length of riser section to induce liquid circulation within bioreactor. Sterilized air was obtained by passing compressed air through Gelman Acrodisc filtered paper (average pored size 0.45 μm).

### 2.6. Cultivation of *G. nephelii* in Bioreactors

Cell suspension of *G. nephelii* (300 mL), prepared according to the procedure described in section 2.1, was transferred to a 5 L stirred tank bioreactor to combine with the growth medium (2.7 L) containing the same compositions as indicated in section 2.1 [30]. Initial glycerol concentrations and external nitrogen source from agro-industrial wastes were prepared according to the results of section 2.3 and 2.4, respectively. Stirred tank bioreactors were operated in batch mode under room temperature (30 to 32 °C) and constant aeration to maintain aerobic condition. Cell cultures in stirred tank bioreactor were maintained at pH 5.0 by an automatic additon of 2 M NaOH. Cell cultivation in stirred tank bioreactors was conducted until obtaining biomass concentration about 10 g/L, then the entire cell suspension (3 L) was transferred and mixed with sterile growth medium (29 L) containing glycerol and agro-industrial wastes in larger bioreactors previously described in section 2.5. In this section, three bioreactor designs (i.e., internal-loop airlift, bubble column and flat-panel airlift) and aeration rates ranged from 0.12 to 0.84 vvm (i.e., vvm = volumes of air per min/effective volume of bioreactor) were chosen as experimental variables. All bioreactors were placed under room temperature (30 to 32 °C) while the pH of cell suspension was maintained at 5.0 by an automatic addition of 2 M NaOH. The batch cultivation of *G. nephelii* was conducted for 72 h for all bioreactors. Liquid samples (10 mL) were obtained from each bioreactor at 6 h interval and analyzed for biomass and DHA concentrations.

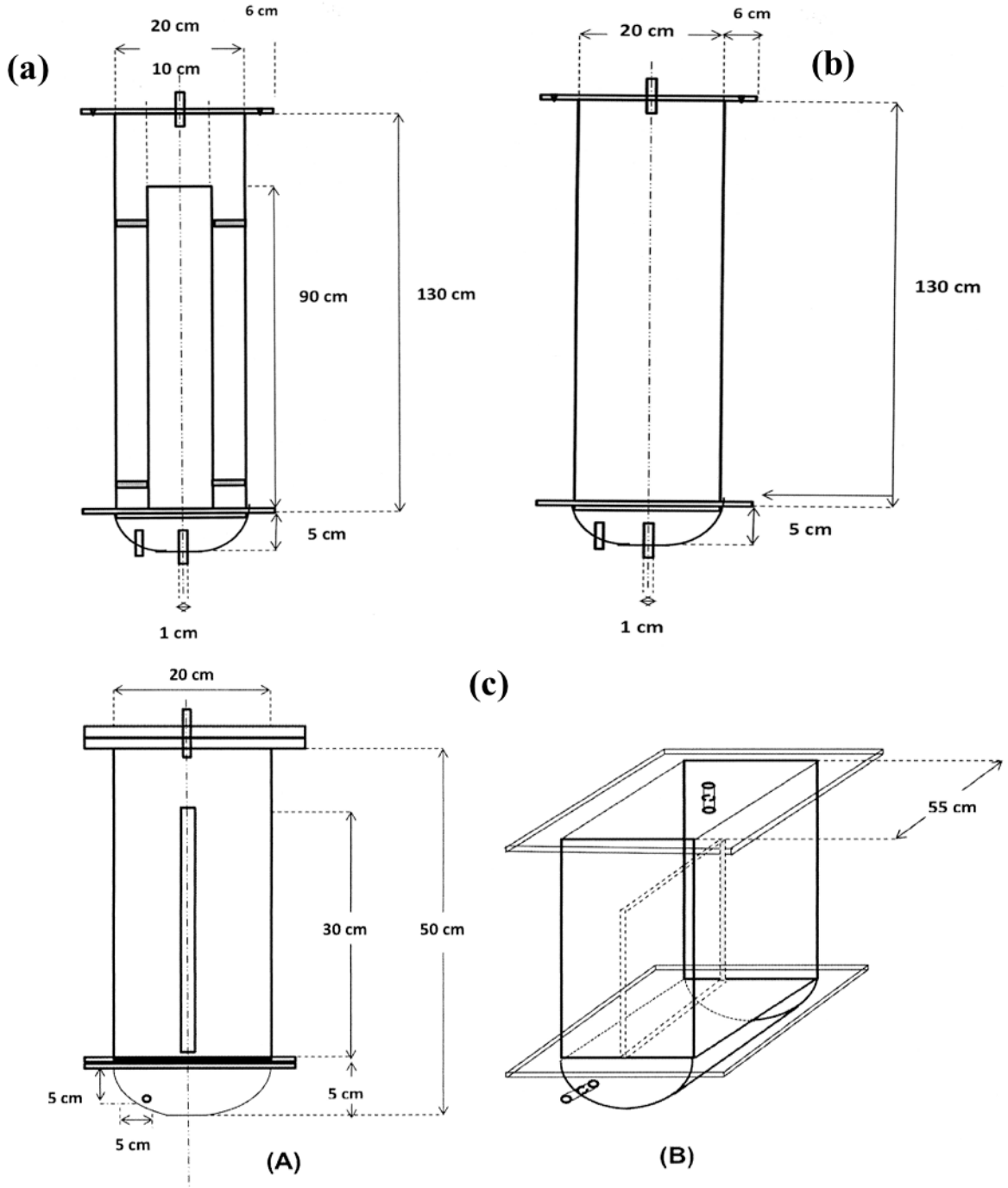


Fig. 1. Schematic drawing (not to scale) of bioreactors for the cultivation of *G. nephelii* to produce DHA: (A) internal-loop airlift bioreactor (B) bubble column bioreactor and (C) flat-panel airlift bioreactor.

### 2.7. Analytical Methods

Liquid samples were centrifuged at 10,000×*g* for 10 min to obtain clear supernatant, which was subsequently used to determine DHA concentrations according to diphynylamin assay [32,33]. Glycerol concentrations were determined using HPLC according to the precedure described elsewhere [34]. Biomass concentrations was measured gravimetrically given the linear relationship to the optical density at 660 nm [33]. Experimental data were statistically compared by using one-way ANOVA and performing Tukey HSD post-hoc test with the significance level of 0.05 [35]. Yield of glycerol conversion to cellular

biomass (i.e., $Y_{X/S}$) was calculated according to $Y_{X/S} = -\Delta X/\Delta S$ where $\Delta S$ and $\Delta X$ are the amounts of glycerol consumption and biomass production during the cultivation, respectively. Yield of glycerol conversion to DHA ($Y_{DHA/S}$) was calculated according to $Y_{DHA/S} = -\Delta DHA/\Delta S$ where $\Delta DHA$ is the amount of DHA production during the cultivation.

## 3. Results and Discussion

### 3.1. Optimal Glycerol Concentrations

*G. nephelii* fed with the growth medium containing different initial glycerol concentrations from 20 to 120 g/L exhibited similar growth patterns by displaying the lag period for about 6 h, following by the period of rapid cell growth in the next 18 to 24 h that led to the increase of cell biomass from neligible levels to about 1 to 2 g/L, and finally reaching the stationary period. Similar concentration profiles were observed for DHA that is the significant DHA production occurred during the period of rapid cell growth before reaching relatively constant levels. The concurrent increase of cell biomass and DHA concentrations suggested that DHA is the growth associated product [28]. As shown in Fig. 2, average biomass and DHA concentrations depended on the initial glycerol concentrations in the growth medium with the highest biomass (1.96 ± 0.01 g/L) and DHA (55.1 ± 1.89 g/L) concentrations observed when using 80 g/L initial glycerol. Increasing glycerol concentrations from 20 to 80 g/L resulted in the increase of biomass concentrations to the maximum before biomass concentrations decreased when applying higher glycerol concentrations than 80 g/L. The similarity was noticed for DHA concentrations, which initially increased with increasing glycerol concentrations and followed by the decreasing trend after glycerol concentrations exceeded 80 g/L. The increase of biomass and DHA concentrations was likely the results of providing sufficient amounts of essential substrates to support growth and DHA metabolic pathways. Despite the limited data for *G. nephelii*, results of previous works on other related bacterial species in genus *Gluconobacter* seemed to indicate the declining growth and lower DHA production when maintaining excessive glycerol concentrations in the growth medium. For examples, adverse effects on growth of DHA production of *G. oxydans* were demonstrated when increasing glycerol concentrations over 100 g/L while subsequent work reported the rapid reduction of both biomass and DHA concentrations in *G. frateruii* after applying biodiesel-derived glycerol exceeding 100 g/L [6,36-38]. Product inhibition by DHA was also unlikely in this experiment since the obtained DHA concentrations were significantly lower than inhibitory thresholds repored between 65 and 85 g/L for *G. oxydans* [36,37]. Finally, biomass yields ($Y_{X/S}$) and DHA product yields ($Y_{DHA/S}$) were determined in the range from 1.7% to 6.3% and 92% to 98%, respectively, which are comparable to the findings from previous works [6,37].

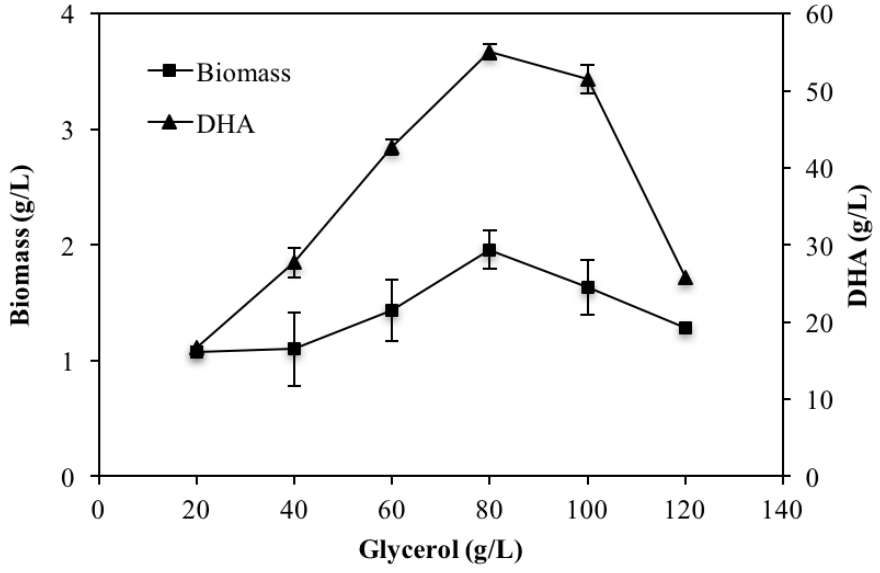


Fig. 2. Effects of applying different initial glycerol concentrations in the growth medium on cell biomass and DHA concentrations during the batch cultures of *G. nephelii*.

### 3.2. Selection of Nitrogen Source from Agro-industrial Wastes

Three agro-industrial wastes including corn steep liquor, glutamic mother liquor, soybean meal hydrolysate were evaluated as the nitrogen source to substitute peptone and yeast extract in the growth medium of *G. nephelii* with the results displayed in Fig. 3. The highest biomass (1.96 ± 0.01 g/L) and DHA (55.1 ± 1.89 g/L) concentrations were associated with control flasks, which contained the growth medium comprising of peptone and yeast extract. Among agro-industrail wastes evaluated, corn steep liquor (i.e., T1) yielded the highest biomass (1.75 ± 0.23 g/L) and DHA (49.5 ± 3.14 g/L) concentrations as compared to glutamic mother liquor (i.e. T2) and soybean meal hydrolysate (i.e., T3). Biomass and DHA concentrations associated with glutamic mother liquor were determined at 1.25 ± 0.02 and 13.2 ± 0.36 g/L, respectively, while the use of soybean meal hydrolysate produced biomass and DHA concentrations at 1.11 ± 0.05 and 16.5 ± 1.39 g/L, respectively. According to the results presented, the use of corn steep liquor produced comparable biomass and DHA concentrations to those from control flasks, suggesting that it could be employed to substitute peptone and yeast extract in the original growth medium for *G. nephelii*. Previous works also reported the use of corn steep liquor as the nitrogen source for *Gluconobacter*. For examples, Hu et al. concluded that corn steep liquor was equally preferable to peptone and yeast extract as external nitrogen source in the growth medium for *G. oxydans* after obtaining insignificant differences in DHA concentrations (i.e. ≈ 35 g/L) while Lui et al. demonstrated that *G. frateruii* produced the highest DHA concentrations (72.6 ± 2.2 g/L) when corn steep liquor was employed as compared to other nitrogen sources [3,40]. The effectiveness of corn steep liquor in promoting growth and DHA production might be linked to its compositions and the method of processing raw materials (i.e., corn). Valuable organic ingredients in raw materials such as reducing sugars, essential minerals and vitamins that were required to support growth and DHA synthesis might leach into liquid phase during the wet milling process of corn starch production. Another previous work also demonstrated that corn steep liquor could provide the complete source of nitrogen, phosphorus, vitamins and other trace elements in microbial growth medium [39]. Lower effectiveness of glutamic mother liquor was perhaps related to its composition, which contained mostly lysine and insignificant amount of carbon source [29]. Despite achieving lower effectiveness in promoting cell growth and DHA production as compared to corn steep liquor, there have been no reports on the use of glutamic mother liquor or soybean meal hydrolysate as substituted nitrogen source in the growth medium for *Gluconobacter*. Moreover, the absence of glycerol in growth media for T4, T5 and T6 led to ineffective production of biomass and DHA. The obtained results clearly suggested that the presence of other carbon sources in agro-industrial wastes was unable to influence growth and DHA production in *G. nephelii*. This finding agreed with previous results that identified glycerol as the main substrate for growth and DHA metabolic pathways in the related strain *G. oxydans* [13].

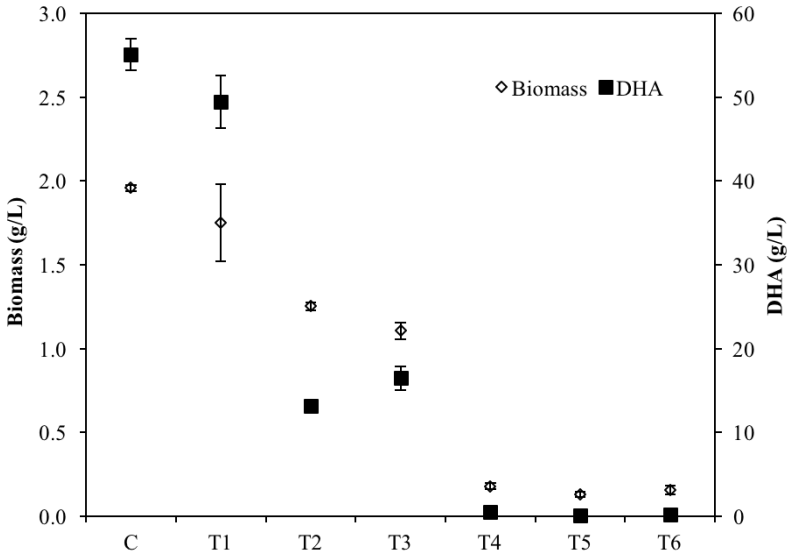


Fig. 3. Biomass and DHA concentrations during the batch cultivation of *G. nephelii* with different nitrogen sources in the growth medium: control flasks (C) were supplied with nitrogen sources from peptone and yeast extract; treatment flasks T1, T2 and T3 were supplied with nitrogen sources from corn steep liquor, glutamic mother liquor and soybean meal hydrolysate, respectively; and T4, T5 and T6 were supplied with corn steep liquor, glutamic mother liquor and soybean meal hydrolysate, respectively, but without glycerol.

### 3.3. Cultivation of G. nephelii in Bioreactors

Three bioreactor designs including internal-loop airlift, flat-panel airlift and bubble column were employed to culture *G. nephelii* to produce DHA under different aeration rates. In this section, the initial glycerol concentrations in growth medium were prepared at 80 g/L and corn steep liquor were selected as the external nitrogen source according to the results of section 3.1 and 3.2, respectively. During the cultivation, significant amount of foam formation was observed in internal-loop airlift and bubble column bioreactors when maintaining aeration rates exceeded 0.32 vvm. Due to this operational difficulty, simple mechanical foam breakers (i.e., plastic paddles mounted to small motor) were placed on top of cylindrical columns of both bioreactors, leading to significant reduction of foam on water surface up to the aeration rates of 0.84 vvm. Nonetheless, the range of aeration rates (i.e., 0.12 to 0.84 vvm) used in this experiment was still lower than the values reported in earlier publications, which indicated the range of aeration rates from 0.5 to 2.5 vvm during the batch cultures of *G. oxydans* [3,40]

Batch cultivation of *G. nephelii* in different bioreactor designs was carried out for 72 h for a given aeration rate with the results illustrated in Fig. 4. For the range of aeration rates considered, biomass and DHA concentrations varied from 2.23 ± 0.04 to 4.58 ± 0.32 g/L and 19.66 ± 0.19 to 46.09 ± 1.01 g/L, respectively, for cell cultivation in internal-loop airlift bioreactors. Biomass concentrations from flat-panel airlift and bubble column bioreactors were determined in the range from 1.88 ± 0.12 to 4.37 ± 0.52 g/L and 1.91 ± 0.05 to 4.3 ± 0.37 g/L, respectively, which were slightly lower than the results from internal-loop airlift system over the range of aeration rates applied. In contrast, DHA concentrations from internal-loop airlift bioreactor were significantly higher ($p < 0.05$) than the results from remaining bioreactors, which yielded DHA concentrations in the range from 11.83 ± 0.76 to 35 ± 0.89 g/L for flat-panel airlift bioreactor and 14.51 ± 0.51 to 39.1 ± 1.89 g/L for bubble column. Better performance of internal-loop airlift bioreactor as compared to other bioreactor configurations might be linked to better distribution of air bubbles and well-definded liquid flow patterns in bioreactor. Based on our observation, air bubbles dispersed fully in the riser section of internal-loop airlift bioreactor while they were unable to diffuse into the section near edges and corners of the flat-panel system so that cells residing in the stagnant zone of flat-panel airlift bioreactor were likely to receive lesser oxygen, hence resulting in lower DHA production. *G. nephelii* cultured in internal-loop airlift bioreactor probably experienced relatively unchanged hydrodynamic condition due to well-established liquid flow patterns. This is in contrast to cells in bubble column bioreactor in which their movement in bulk liquid was random as a result of constantly changing liquid

motion. The established liquid circulation in internal-loop airlift bioreactor was cited as one of the reasons for higher growth and astaxanthin production by *H. pluvialis* as compared to the cultivation in bubble column bioreactor [20].

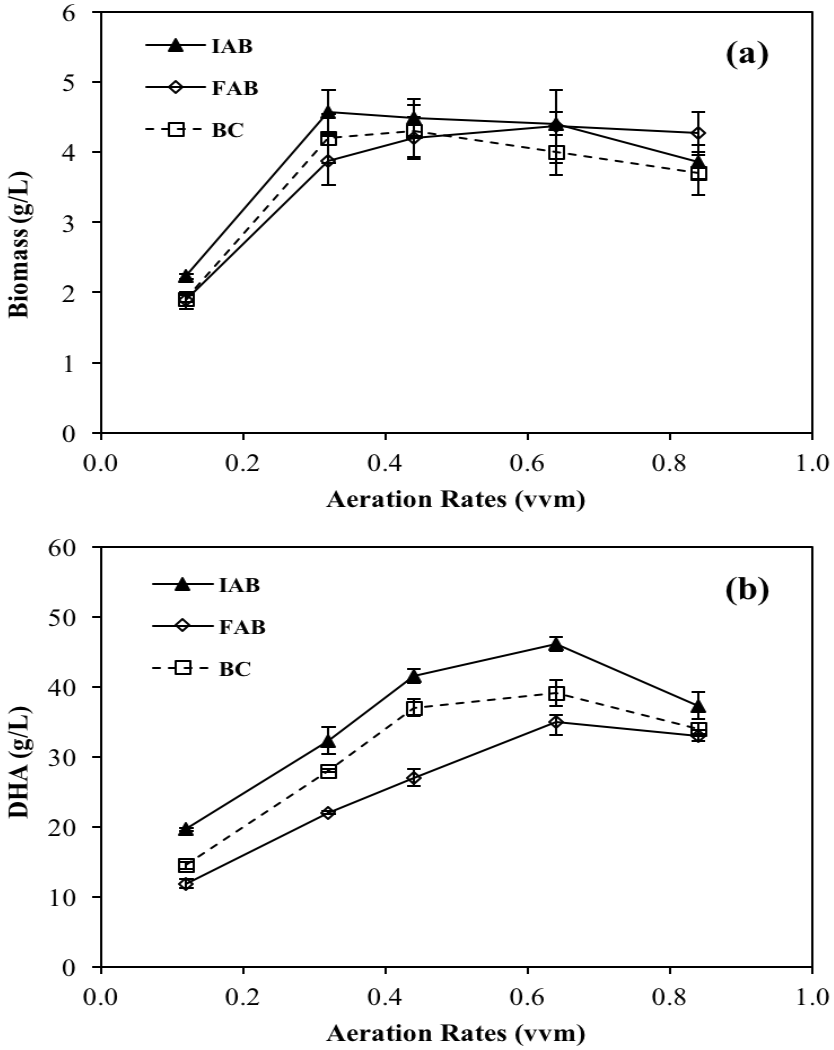


Fig. 4. Biomass and DHA concentrations during the batch cultivation of *G. nephelii* in internal-loop airlift bioreactor (i.e., IAB), flat-panel airlift bioreactor (i.e., FAB) and bubble column bioreactor (i.e., BC) subjected to increasing aeration rates.

It should also be pointed out that the range of DHA concentrations (i.e., 11.83 ± 0.76 to 19.66 ± 0.19 g/L) when maintaining the aeration rates at 0.12 vvm in bioreactors were lower than the results of flask cultivation, which yielded DHA concentrations as high as 49.5 ± 3.14 g/L. Since *Gluconobacter* is the oblique aerobic species, it was possible that at this aeration rates (i.e., 0.12 vvm) oxygen availability in bioreactors might become the limiting factor on DHA production after performing cell cultivation in larger bioreactor (i.e., 32 L). By increasing aeration rates from 0.12 to 0.84 vvm, the effects of oxygen limitation on DHA production could be reduced as can be seen by the significant rise of DHA concentrations in all bioreactor designs. For internal-loop airlift bioreactor, DHA concentrations increased from 19.66 ± 0.19 to 46.09 ± 1.01 g/L when the aeration rates were increased from 0.16 to 0.64 vvm. Further increase of aeration rates to 0.84 vvm resulted in lower DHA concentrations measured at 37.28 ± 1.90 g/L. Similar DHA concentration profiles were observed for the remaining bioreactors, where the maximum DHA concentrations were determined at 35 ± 0.89 and 39.1 ± 1.89 g/L for flat-panel airlift and bubble column bioreactors, respectively, when maintaining aeration rates at 0.64 vvm. Despite the requirement for aerobic condition during DHA production, limited data were available on the influence of aeration rates on DHA

production in *Gluconobacter* [3]. Along with the results presented in current work, only few publications demonstrated the dependency of DHA concentrations on aeration rates in the upflow oxygenated bioreactor systems. In those works, DHA concentrations increased significantly after increasing aeration rates in bubble column and airlift bioreactors up to 1.5 vvm, resulting in DHA concentrations approximately 35 to 39 g/L [3,40]. Finally, DHA yield ($Y_{DHA/S}$) associated with the optimal operating condition (i.e., 0.64 vvm) were determined at 91% for this work.

## 4. Conclusions

Series of batch cultivation were conducted to culture *G. nephelii* to produce DHA. Initial glycerol concentrations in growth medium should be prepared at 80 g/L while corn steep liquor was identified as the preferred nitrogen source as compared to glutamic mother liquor and soybean meal hydrolysate. Subsequent cultivation of *G. nephelii* in larger bioreactors revealed that internal-loop airlift bioreactor yielded higher DHA concentrations as compared to flat-panel airlift and bubble column bioreactors. Increasing aeration rates up to 0.84 vvm were able to decrease oxygen-limitation on DHA production. The maximum DHA concentrations measured at 46.09 ± 1.01 g/L were associated with internal-loop airlift bioreactor when maintaining the aeration rates at 0.64 vvm.

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