

SCREENING NEW ISOLATES OF *Acetobacter* AND *Gluconobacter* BACTERIA FOR HIGH GLYCEROL CONCENTRATION CULTURE FOR DIHYDROXYACETONE PRODUCTION IN MINIMAL MEDIA

Pafan Phansi and Siwarutt Boonyarattanakalin*

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Abstract

Recently, the isolates of *Acetobacter orientalis*, *Gluconobacter frateurii*, and *Gluconobacter thailandicus* have been identified for their ability to biotransform glycerol to dihydroxyacetone (DHA). In order to increase the production of DHA, this study aimed to investigate the effect of different concentrations of initial glycerol on bacterial growth and DHA productivity by *Acetobacter orientalis*, *Gluconobacter frateurii*, and *Gluconobacter thailandicus* isolates. The bacteria isolates were evaluated for their growths in various initial glycerol concentrations of 40, 50, 55, and 60 g/L; and their DHA production. The quantitative screening was performed by shake-flask fermentation at different initial concentrations of glycerol at 40, 50, 55, and 60 g/L. The cultured media were analyzed for DHA concentration and yields. In bioreactor, the feasibility of a large-scale biosynthesis of DHA in low-cost media was studied by using only glycerol as a carbon source and inorganic salts as supplemented nutrients for industrial production of DHA. *Gluconobacter thailandicus* BCC14436 could biotransform glycerol to DHA and generate a high DHA concentration and yield, when compared to other acetic acid bacteria. *G. thailandicus* BCC14436 produced the highest DHA concentration (p) at 56.08 ± 2.82 g/L with a DHA product yield (y_{sp}) of $95.57 \pm 4.80\%$ of DHA moles/glycerol moles at the highest glycerol concentration of 60 g/L in a shake-flask screening. In bioreactor batch fermentation (BBF), the DHA concentration (p) of 61.69 ± 0.81 g/L with a DHA production yields (y_{sp}) of $99.61 \pm 0.37\%$ were achieved at the 90th hour. This study reveals the possible use of *G. thailandicus* BCC14436 for an industrial application of DHA production for the first time.

Keywords: Dihydroxyacetone, Glycerol, *Gluconobacter*, *Acetobacter*

Sirindhorn International Institute of Technology, Thammasat University, P. O. Box 22, Thammasat Rangsit Post Office, Pathum Thani, 12121, Thailand. Tel.: +66 (0) 2986 9009 p2305; fax: +66 (0) 2986 9112-3; E-mail: siwarutt@siit.tu.ac.th

* Corresponding author

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Introduction

Glycerol is an abundantly available by-product derived from transesterification of vegetable oil or animal fat with an alcohol to produce biodiesel. Approximately, 10 wt.% of crude glycerol is generated during the process (Johnson and Taconi, 2007). Due to the growth of biodiesel demand, the amount of glycerol has been greatly increased by biodiesel production boom to generate renewable fuel. Biodiesel plants become the principal producers of glycerol to supply more than the demand of glycerol worldwide. The oversupply of glycerol in the market negatively impacts the prices of crude glycerol and refined glycerol which are declined to \$0.01 lb⁻¹ and \$0.05 lb⁻¹ respectively in 2013 (Liu *et al.*, 2013). Utilizations of crude glycerol without further purification would be cost-effective. The operating cost of purifying glycerol would lead to higher costs in glycerol valorization processes. Consequently, the discharge of crude glycerol into the environment would offer more economic feasibility than refining for manufacturing (Da Silva *et al.*, 2009). As a result, the discharged glycerol waste causes environment burden. Therefore, an effective management of glycerol surplus is necessary to prevent an environmental damage and would increase the glycerol value.

An alternative solution for sustainable managing of an excessive glycerol can be done by transforming glycerol to value-added products. Glycerol valorizations add significant value to glycerol waste in economical perspective and reduce the glycerol disposal into the environment. The applications of glycerol derivatives are common in various industries such as cosmetic, pharmaceutical, chemical synthesis, and food industries. Glycerol bioconversions to value-added products are more attractive than chemical conversions because glycerol bioconversions are more environmental friendly and have low operating costs. Glycerol can be transformed by bioprocessing to various value-added products such as dihydroxyacetone, 1,3-propanediol, succinic acid, propionic acid,

ethanol, citric acid, and polyhydroxalcanoate (Da Silva *et al.*, 2009).

Dihydroxyacetone (DHA) is a well-known chemical building block and an active ingredient in sunless tanning products. A skin pigment change can be triggered by Millard reaction through an oxidation of amino acids histidine and tryptrophan of peptides in stratum corneum (Fesq *et al.*, 2001; Garone *et al.*, 2015). Apart from an aesthetic purpose, DHA is applicable for a treatment of a depigmentation disorder of a skin, where DHA provides even and long lasting skin pigmentation (Fesq *et al.*, 2001). In addition, DHA can be used as a chemical building block for synthesizing other useful chemicals such as dihydroxyacetone phosphate (Schümperli *et al.*, 2007) and poly(carbonate-ester)s (Weiser *et al.*, 2011). Among the possible chemicals that are made from glycerol, the price of DHA is relatively more expensive than other chemicals; thus biotransformation of glycerol to DHA would greatly enhance the glycerol value more than purifying crude glycerol (Kumar *et al.*, 2015).

Biosynthesis of DHA from glycerol is carried out by acetic acid bacteria (AAB). AAB are gram-negative obligate aerobic bacteria found in sugary, alcoholic, and acidic environment (Mamlouk and Gullo, 2013). These microorganisms are able to metabolize carbohydrate and produce the corresponding products such as aldehydes, ketones, and organic acids (Mamlouk and Gullo, 2013). The strains possess an enzyme known as glycerol dehydrogenase locating at cytoplasmic membrane. The enzyme catalyzes the incomplete oxidative reaction of glycerol and excrete DHA to the outside of the cell (Deppenmeier *et al.*, 2002). Recently, *Gloconobacter oxydans* has been extensively used by industrial production of DHA because of their great ability to completely convert glycerol to DHA (Hu *et al.*, 2010; Ma *et al.*, 2010). The culture media containing glycerol as a sole carbon source and yeast extract were used for microbial fermentation of DHA by many acetic acid bacteria such as *Acetobacter suboxydans*,

Acetobacter xylinum, *Gluconobacter oydans*, and *Gluconobacter frateurii* (Z.-C. Hu *et al.*, 2010; Liu *et al.*, 2013; Isara Poljungreed *et al.*, 2017; Issara Poljungreed and Boonyarattanakalin, 2017, 2018). However, the culture media containing yeast extract, organic nitrogen and phosphorous are more expensive than the culture media containing inorganic salts such as inorganic nitrogen and phosphorus. The use of yeast extract could result in a high production cost of DHA production. The recent study revealed the DHA production by *G. frateurii* BCC36199, in the low-cost minimal media containing only glycerol and inorganic salts, provided more advantages in terms of an economical aspect compared to the conventional culture media, due to the inexpensive inorganic salts (Issara Poljungreed and Boonyarattanakalin, 2017, 2018). Therefore, the use of the low-cost culture media would be preferred for the more cost-effective production of DHA.

Major challenges of microbial production of DHA are from improper initial glycerol concentration, accumulative of DHA product, and limitation of oxygen supply (Claret *et al.*, 1992; Ma *et al.*, 2010). Many studies focused on improvements of DHA productivity by modifying fermentation processes (Z.-C. Hu *et al.*, 2011), genetic modifications (Ma *et al.*, 2010), determining the optimum culture medium for cell growth (Wethmar and Deckwer, 1999), and studying the new potential microorganisms for DHA synthesis (Liu *et al.*, 2013). *Acetobacter* and *Gluconobacter* have been found to be able to convert glycerol to DHA (Mamlouk and Gullo, 2013).

Acetobacter orientalis, *Gluconobacter frateurii*, and *Gluconobacter thailandicus* have been reported for their ability to convert glycerol to DHA. The bacteria can oxidize glycerol into DHA by glycerol dehydrogenase (Tanasupawat *et al.*, 2004; Kommanee *et al.*, 2012). These strains could be new promising bacteria that may be applied for an industrial bio-production of DHA. Therefore, the study aims to evaluate the bacterial strains that can tolerate higher initial glycerol concentrations and generate higher DHA concentration and yield for more productive process. Moreover,

the current microbial productions of DHA relied on some specific bacteria such as *Gluconobacter oydans*, *Clavispora lusitaniae* G10, *Gluconobacter* sp. CGMCC No. 5146 are under patent protection, which restricts the research and development of these bacteria for commercial applications in the production of DHA (Bo and Yurong, 2014; Ohrem and Westmeier, 1998; Tong *et al.*, 2013). This study would identify the new bacterial isolates that have never been investigated for their DHA production before.

Materials and Methods

Microorganisms

Acetic acid bacteria (1 isolate of *Acetobacter* and 7 isolates of *Gluconobacter*, listed in Table 1), investigated in this study, were purchased from the National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Thailand. The microorganisms were stored at -20°C in glucose-ethanol media (GE) containing D-glucose (2%, w/v), ethanol (0.5%, w/v), peptone (1.2%, w/v), yeast extract (0.3%, w/v) and glycerol (20%, w/v). The microorganisms were activated by culturing on a Glucose-Ethanol-Calcium carbonate Agar (GECA) medium (20 g/L of glucose, 5 g/L of ethanol, 1.2 g/L of peptone, 3 g/L of yeast extract, and 7 g/L of calcium carbonate) at 30°C for 24 h. The proliferation of microorganisms was done by incubating them in an organic media containing glycerol (30 g/L), peptone (10 g/L) and yeast extract (5 g/L) at 30°C and oscillating speed of 250 rpm for 24 h.

Qualitative Screening of DHA Production in Different Glycerol Concentrations (40, 50, 55, and 60 g/L)

The microorganisms (1 isolate of *Acetobacter* and 7 isolates of *Gluconobacter*) were cultured in inorganic media. The media contained glycerol (30 g/L) and other supplementary inorganic salts: (NH₄)₂SO₄ (3.1 g/L), K₂HPO₄ (1.67 g/L), KH₂PO₄ (1.31 g/L), CaCO₃ (0.1 g/L) and MgSO₄·7H₂O (0.375 g/L). The

Table 1. Qualitative screening of DHA production by acetic acid bacteria cultured in various glycerol concentrations

Genus	Species	BCC	Fehling's solution Test			
			Glycerol Conc. 40 g/L	Glycerol Conc. 50 g/L	Glycerol Conc. 55 g/L	Glycerol Conc. 60 g/L
<i>Gluconobacter</i>	<i>frateurii</i>	36199	FFD700 (+)	FFD700 (+)	FFD700 (+)	FFD700 (+)
<i>Gluconobacter</i>	<i>frateurii</i>	15771	FFD700 (+)	FFD700 (+)	FFD700 (+)	FFD700 (+)
<i>Gluconobacter</i>	<i>frateurii</i>	15856	FFD700 (+)	FFD700 (+)	FFD700 (+)	DBDB70 (w)
<i>Gluconobacter</i>	<i>thailandicus</i>	14436	FFD700 (+)	FFD700 (+)	FFD700 (+)	FFD700 (+)
<i>Gluconobacter</i>	<i>thailandicus</i>	14438	FFD700 (+)	FFD700 (+)	FFD700 (+)	FFD700 (+)
<i>Gluconobacter</i>	<i>thailandicus</i>	14454	FFD700 (+)	FFD700 (+)	FFD700 (+)	FFD700 (+)
<i>Gluconobacter</i>	<i>thailandicus</i>	14432	FFD700 (+)	FFD700 (+)	FFD700 (+)	FFD700 (+)
<i>Acetobacter</i>	<i>orientalis</i>	49190	DBDB70 (w)	DBDB70 (w)	DBDB70 (w)	DBDB70 (w)

The test results; FFD700 (Gold): highly positive result (+), DBDB70 (Goldenrod): weakly positive result (w), and 4876FF (RoyalBlue1): negative result (-) reported in RGB color code system.

different glycerol concentrations of 40, 50, 55, and 60 g/L were used for screening the bacteria. The isolates were maintained at 30°C and oscillating speed at 250 rpm for 72 h. The fermented media at 72 h were examined for DHA accumulation by using the Fehling's solution (Z. C. HU and ZHENG, 2009). DHA is a reducing sugar and is oxidized by Cu²⁺ to turn the culture solution from colorless to yellow precipitate (Z. C. HU and ZHENG, 2009). The presence of the reducing sugar in the fermented media is detected as a yellow to red precipitate.

A positive control was the unfermented inorganic media with DHA standard solution and a negative control was the unfermented inorganic media.

Determination of DHA Product in the Cultured Media with Different Glycerol Concentrations (40, 50, 55, and 60 g/L) for Screening Bacteria with High DHA Production

The culture media was analyzed by high-performance liquid chromatography (HPLC) to determine the concentrations of DHA and glycerol. The cultured media of different glycerol concentrations of 40, 50, 55, and 60 g/L were collected at 72 h. The DHA product in the cultured media was presented as DHA concentration (p , g/L) and yield (y_{sp} , %). The

DHA yield (y_{sp} , % of DHA moles/glycerol moles) was calculated by Equation 1.

$$y_{sp} = (\text{DHA}_{final} \text{ moles} / \text{glycerol}_{initial} \text{ moles}) \times 100 \quad (1)$$

Optical density OD₅₆₀ measurement of bacterial growth

A bacterial growth was investigated by measuring an optical density (OD) of the fermented media during fermentation. The OD measurement was carried out by using UV spectrophotometer (Genesys 10 UV) at the absorbance of wavelength 560 nm (Liu *et al.*, 2013). The OD₅₆₀ of the fermented media was monitored every 6 h to follow the bacterial growth. The unfermented media was used as a blank in this experiment.

DHA Production in a 7-L Bioreactor Batch Fermentation (BBF)

The culture medium used in a 7-L bioreactor batch fermentation (BBF) contained glycerol (60 g/L), (NH₄)₂SO₄ (3.1 g/L), K₂HPO₄ (1.67 g/L), KH₂PO₄ (1.31 g/L), CaCO₃ (0.1 g/L) and MgSO₄·7H₂O (0.375 g/L). A batch fermentation was carried out at 30°C with 4 L of culture medium in a 7-L stirred bioreactor (BioFlo/CelliGen115, New Brunswick Scientific, Edison, NJ, USA). *G. thailandicus* BCC14436 inoculum of 133

mL was used by batch fermentation. The batch fermentation was operated by maintaining dissolved oxygen of 90% at 30°C with aeration rate of 2 vvm and agitation speed of 800 rpm. The pH was controlled at 4.5 by using NaOH at the concentrate of 1 M. The microbial production of DHA was cultivated for 120 h. The fermented media of 5 mL was harvested every 6 h and kept at -20°C for analyzing of DHA concentrations and yields.

Results and Discussion

Qualitative Screening for DHA Production in Microbial Fermentation of Glycerol

Media with the initial glycerol concentrations of 40, 50, 55, and 60 g/L were fermented by bacteria of genera *Acetobacter* (1 isolate) and *Gluconobacter* (7 isolates). The fermented media were preliminarily examined by Fehling's reagent test for selecting the bacteria with the ability to generate DHA. The examination was simple and took a short time to determine the results. The method was done as a preliminary screening to eliminate certain bacterial isolates that gave negative results. The negative control was the unfermented medium without DHA, which would turn from a colorless to a blue color by Fehling's test reagent. The positive control was the unfermented medium with the added DHA, which would turn from a colorless to a yellow color by Fehling's test reagent. The test results reported as negative, weakly positive, and highly positive results for DHA production if the fermented media turned blue, yellow-green, and yellow colors, respectively, with the Fehling's test reagent.

The qualitative screening results were performed on a total of 8 bacterial isolates of acetic acid bacteria as shown in supporting information (Table 1). The fermented cultures by *G. frateurii* (3 isolates) and *G. thailandicus* (4 isolates) gave highly positive results for a qualitative screening in initial glycerol concentrations of 40, 50, and 55 g/L. When the initial glycerol concentration was increased to 60 g/L, the *G. frateurii* (2 isolates) and *G. thailandicus* (4 isolates) cultures still gave

highly positive results but the *G. frateurii* TBRC15856 culture gave weakly positive result.

In contrast to most of bacterial isolates, the culture by *A. orientalis* showed weakly positive results in the qualitative screening in all initial glycerol concentrations. Since all of the bacterial isolates showed at least weakly positive results, they would be further evaluated quantitatively for the DHA production.

According to the qualitative screening results, most of the cultures by *Gluconobacter* isolates expressed highly positive results in the qualitative screening in all initial glycerol concentrations. Apart from these genera, *Acetobacter* genera such as *A. suboxydans*, *A. xylinum*, and *A. indonesiensis* have been reported for their ability to convert glycerol to DHA (Underkofler and Fulmer, 1937; Nabe *et al.*, 1979; Kommanee *et al.*, 2012). However, the qualitative screening results of *A. orientalis* implied that the isolate did not perform well in converting glycerol to DHA.

Quantitative Screening for High DHA Production in Different Glycerol Concentrations of (40, 50, 55, and 60 g/L)

The cultured media of 8 isolates of acetic acid bacteria, including the isolate of *A. orientalis*, the 3 isolates of *G. frateurii*, and the 4 isolates of *G. thailandicus* were further evaluated for their ability to generate DHA by HPLC. Cultured media samples were analyzed for DHA concentrations (p), and yields (y_{sp}) were calculated by using Equation 1. Table S1 (Supplementary Material) shows DHA concentrations and yields of the quantitative screening of *A. orientalis*, *G. frateurii*, and *G. thailandicus* isolates in the initial glycerol concentrations of 40, 50, 55, and 60 g/L. *G. thailandicus* gave the highest amount of DHA concentrations and yields in all initial glycerol concentrations. *G. frateurii* produced a high DHA concentration and production yields in the initial glycerol concentrations of 40 g/L but these isolates performed poorly on DHA production, when initial glycerol concentrations were increased to 50, 55, and 60 g/L. In addition, isolate of *A. orientalis* produced the smallest amount of DHA

concentrations and yields in comparison to *G. frateurii* and *G. thailandicus*. The quantitative screening results of *A. orientalis* isolate was similar to the qualitative screening results, which showed weakly positive results of DHA concentration in the cultured media of *A. orientalis*. Therefore, the isolate of *A. orientalis* is not a suitable isolate to be applied for the DHA production in a bioreactor.

The initial glycerol concentration plays a significant role on biosynthesis of DHA. An unsuitable initial glycerol concentration could lead to low bacterial growth and DHA productivity (Claret *et al.*, 1992). The initial glycerol concentration of 30 g/L was an optimal concentration for *G. oxydans* to convert glycerol to DHA, while an initial glycerol concentration over 30 g/L could negatively result in low DHA productivity of the bacteria (Stasiak-Różańska *et al.*, 2014). According to the quantitative screening results, the *A. orientalis* isolate BCC49190 did not satisfactorily utilize glycerol and generate DHA resulting in low DHA concentrations and production yields. Although *G. frateurii* isolates performed well in DHA production at relatively low concentrations of initial glycerol, the isolates produced lower levels of DHA at the higher initial glycerol concentrations (Figures 1 and 2). Thus, the *G. frateurii* isolates were not selected for further study of the DHA production in a bioreactor. On the other hand, the *G. thailandicus* isolates yielded higher DHA production at the higher glycerol concentrations (Figures 1 and 2). This is an important unique advantage of *G. thailandicus* isolates over *G. frateurii* isolates. If the fermentation can be done at higher initial glycerol concentration, the DHA productivity would be higher. In particular, the *G. thailandicus* isolate BCC14436 performed the best in biotransformation of glycerol to DHA with the initial glycerol concentrations of 40, 50, 55, and 60 g/L. *G. thailandicus* BCC14436 gave high DHA concentrations (p) of 34.18 ± 0.50 , 44.47 ± 2.65 , 52.16 ± 4.68 , and 56.08 ± 2.82 g/L with the DHA product yields (y_{sp}) of 87.36 ± 1.27 , 90.94 ± 5.42 , 96.96 ± 8.70 , and $95.57 \pm 4.80\%$ of DHA, when the isolate

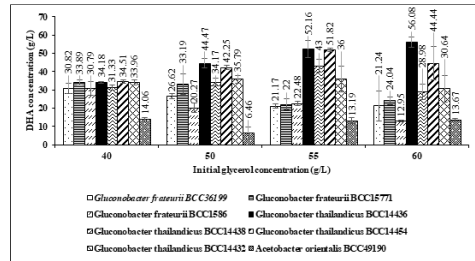


Figure 1. DHA concentrations in the cultures by 8 isolates of acetic acid bacteria in different glycerol concentrations of 40, 50, 55, and 60 g/L. The culture media were maintained at 30°C and oscillating speed at 250 rpm for 72 h before DHA quantitative analysis

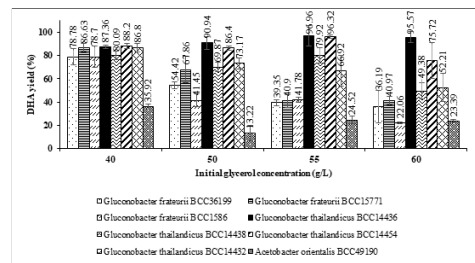


Figure 2. DHA production yield (%) by each culture of 8 isolates of acetic acid bacteria in different glycerol concentrations of 40, 50, 55, and 60 g/L. The culture media were maintained at 30°C and oscillating speed at 250 rpm for 72 h before DHA quantitative analysis

was cultivated in an initial glycerol concentration of 40, 50, 55, and 60 g/L, respectively. The results revealed that the *G. thailandicus* isolate BCC14436 could generate high DHA concentrations and tolerate to various initial glycerol concentrations of 40, 50, 55, and 60 g/L compared to other isolates. Therefore, *G. thailandicus* BCC14436 was selected for a further investigation of bioconversion glycerol to DHA in a bioreactor.

The quantitative results of the DHA production in various initial glycerol concentrations of 40, 50, 55, and 60 g/L revealed that *G. thailandicus* BCC14436 produced the most

Table 2. DHA concentrations and DHA production yields in a bioreactor batch fermentation of *G. thailandicus* BCC14436 with the initial glycerol concentration of 60 g/L

Time (h)	Biomass concentrations (OD ₅₆₀)	DHA	DHA production yields
		concentrations <i>p</i> ^a (g/L)	<i>y</i> _{p/s} ^a (% of DHA moles/ glycerol moles)
0	0.02 ± 0.02	0.00 ± 0.00	0.00 ± 0.00
6	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
12	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
18	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
24	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
30	0.02 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
36	0.07 ± 0.05	1.56 ± 1.57	2.51 ± 2.51
42	0.08 ± 0.07	2.26 ± 2.26	3.62 ± 3.62
48	0.15 ± 0.09	4.62 ± 3.11	7.41 ± 4.96
54	0.20 ± 0.13	10.07 ± 7.47	16.15 ± 11.90
60	0.42 ± 0.05	18.95 ± 4.99	30.52 ± 7.77
66	0.42 ± 0.07	21.75 ± 6.25	35.03 ± 9.76
72	0.47 ± 0.03	30.85 ± 1.21	49.80 ± 1.49
78	0.52 ± 0.02	36.74 ± 8.47	59.20 ± 13.12
84	0.59 ± 0.02	53.19 ± 5.90	85.98 ± 10.34
90	0.57 ± 0.05	> 55	> 95
96	0.58 ± 0.03	> 55	> 95
102	0.58 ± 0.03	> 55	> 95
108	0.57 ± 0.04	> 55	> 95
114	0.55 ± 0.02	> 55	> 95
120	0.56 ± 0.03	> 55	> 95

^a data presented as average ± standard deviation of two independent experiments

DHA at the higher glycerol concentrations. The bioconversion of glycerol to DHA at the initial glycerol concentration of 60 g/L would be a more economical process than using the lower initial glycerol concentration. The resulting higher DHA concentration would facilitate its purification process. Therefore, *G. thailandicus* BCC14436 was selected for further studying of DHA production at the initial glycerol concentrations of 60 g/L in a large-scale bioreactor.

A large scale DHA biosynthesis of *G. thailandicus* BCC14436 was carried out by culturing in the initial glycerol of 60 g/L in a 7-L bioreactor batch fermentation (BBF). Table 2 shows biomass concentration (OD₅₆₀, Figure 3a), DHA concentration (*p*) (g/L), and DHA production yield (*y*_{sp}, % of DHA moles/glycerol moles) of DHA production of *G. thailandicus* BCC14436 in BBF. The glycerol consumption of isolate of *G. thailandicus* BCC14436 in BBF was slower in the first 30 h as shown in Figure 3(b). At the 54th h of culturing, the glycerol consumption significantly increased and was at constant after 90 h. Glycerol was completely consumed

by *G. thailandicus* BCC14436 after 90 h. During the cultivation of *G. thailandicus* BCC14436 in BBF, DHA concentration was slightly produced at the 30th h and dramatically increased after the 54th h of culturing as shown in Figure 3(b). DHA concentration (*p*) of 61.69±0.81 g/L with a DHA production yields (*y*_{sp}) of 99.61±0.37% were achieved at the 90th h (Figure 3(c)).

The biomass concentration of *G. thailandicus* BCC14436 in BBF gradually increased after 30 h and reached the greatest OD₅₆₀ of 0.59±0.02 at the 84th h of culturing as shown in Figure 3(a). The biomass concentration of *G. thailandicus* BCC14436 was relatively stable, with no significant change observed, after the 84th h of culturing. Although, a low biomass concentration (OD₅₆₀) of *G. thailandicus* BCC14436 was found, a high DHA concentration of 61.69±0.81 g/L and yields of 99.61±0.37% were obtained. The kinetic of glycerol bioconversion to DHA could be affected by a high initial concentration of initial glycerol. As a result, the bacteria might take a longer time to adapt themselves in the high glycerol environment

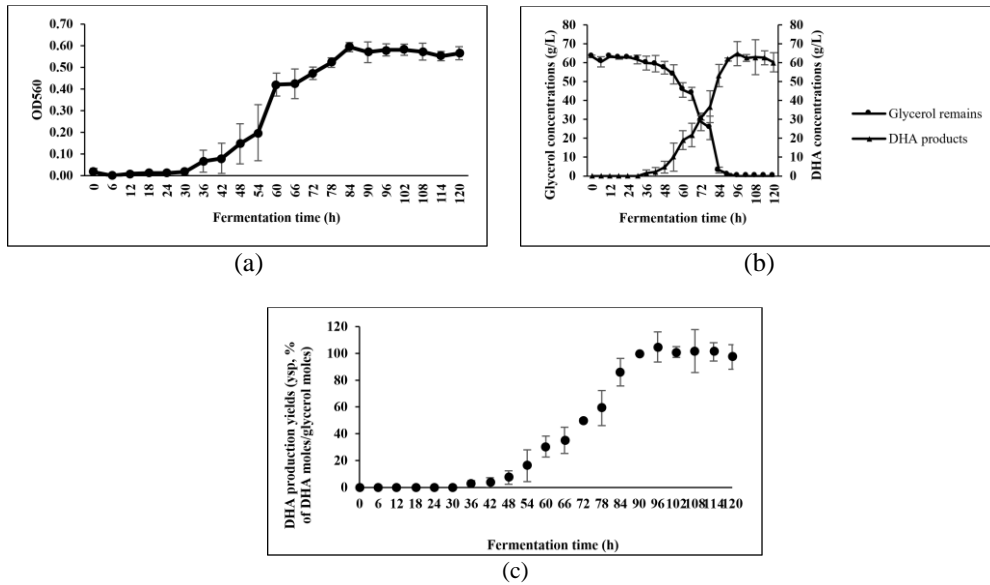


Figure 3. Kinetics of bioconversion glycerol to DHA; (a) optical density (OD₅₆₀), (b) glycerol and DHA concentrations, (c) DHA production yields by *G. thailandicus* BCC14436 in a bioreactor batch fermentation (BBF). The culture medium contained glycerol (60 g/L). The fermentation was carried out at 30°C with 4 L of culture medium in a 7-L stirred bioreactor, 90% dissolved oxygen with aeration rate of 2 vvm, and agitation speed of 800 rpm. The pH was controlled at 4.5 by using NaOH (1M)

(Claret *et al.*, 1992). As expected, the minimal media promotes the production of DHA, which is the desired production, rather than the biomass production. Consequently, most of the glycerol was converted to DHA (Issara Poljungreed and Boonyarattanakalin, 2017, 2018).

According to DHA production in BBF of *G. thailandicus* BCC14436, the low-cost minimal media was used by this experiment in order to study the economical and technical feasibility of the DHA production. The minimal media mainly contained glycerol and inexpensive inorganic salts as the sources for nitrogen, phosphorus, and other minerals. Glycerol, the sole carbon source in the media, is inexpensive substance because of its aforementioned abundance and could be obtained from the waste of biodiesel production. Recently, the study of DHA production of *G. frateurii* BCC36199 in the minimal media was achieved to produce a high DHA concentration of 28.64 g/L with a DHA production yields of 94.75% in a minimal

medial contained only glycerol (30 g/L) as a sole carbon source (Issara Poljungreed and Boonyarattanakalin, 2018). The study suggested that glycerol and inorganic salts are suitable for microbial production of DHA because most glycerol was completely utilized mainly for the DHA production rather than for biomass production. This finding is similar to the results of DHA production of *G. thailandicus* BCC14436 in BBF, which revealed a high DHA concentrations and yields from a low biomass concentration. These results would also facilitate the purification of DHA because the other possible byproducts such bacterial proteins would be minimal.

The BBF of isolate of *G. thailandicus* BCC14436 in the low-cost minimal media developed in this study produced a high DHA concentration (p) and yields (y_{sp}). The study indicated that *G. thailandicus* BCC14436 could tolerate higher initial glycerol concentrations and completely convert glycerol to DHA with high DHA production yields. Moreover, the BBF of DHA production is simple, and relies

on the low-cost minimal media as a starting material. Therefore, the DHA production of the new isolate, *G. thailandicus* BCC14436, in the low-cost minimal media could be efficiently applied for industrial production of DHA.

Conclusions

All isolates of acetic acid bacteria; *Acetobacter* and *Gluconobacter* genus were evaluated for their glycerol biotransformation into DHA. *G. thailandicus* BCC14436 could tolerate high initial glycerol concentrations and able to utilize glycerol to produce DHA. The scale-up of DHA production of *G. thailandicus* BCC14436 was carried out in a 7-L stirred bioreactor batch fermentation (BBF) in the low-cost minimal media that contained only glycerol as a carbon source and only inorganic salts as supplementary nutrients. *G. thailandicus* BCC14436 completely bio-transformed glycerol to DHA with the highest DHA concentration of 61.69 ± 0.81 g/L with the maximum DHA production yields (y_{sp}) of $99.61 \pm 0.37\%$ at the 90th h of the fermentation. The results of this study explore, for the first time, the application of *G. thailandicus* BCC14436 in a bioconversion of glycerol into DHA. The developed BBF relies on an inexpensive minimal medium containing only glycerol as a sole carbon source and inorganic salts as nitrogen and phosphorus sources.

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