

World Congress on Oils and Fats & 28th ISF Congress

27 - 30 September 2009 • Sydney Australia



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**POTENTIAL OF DOCOSAHEXAENOIC ACID (DHA) PRODUCTION
FROM HETEROTROPHIC MARINE MICRO-ALGAE
OF *SCHIZOCHYTRIUM LIMACINUM*
USING GLYCEROL FROM BIODIESEL PRODUCTION**



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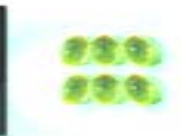
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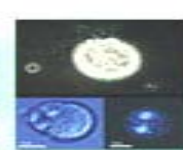
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1. Introduction

Docosahexaenoic acid (DHA)

- ❖ DHA ($C_{22:6} n - 3$) is main kind of long chain omega (ω)-3 polyunsaturated fatty acids (PUFAs).
- ❖ Medically established therapeutic capabilities against cardiovascular diseases, cancers, schizophrenia, and Alzheimer's.
- ❖ It can be produced from various microorganisms such as marine bacteria, autotrophic and heterotrophic algae, fungi and mosses.



1. Introduction

Schizochytrium limacinum

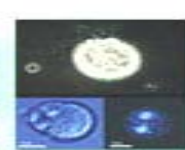
It is a heterotrophic marine microalgae

This microbe contains large amounts of DHA

DHA can be extracted from these algae biomass

The biomass can be used directly as a fish feed additive in the aquaculture industry

It has reported that, this strain can grow well in glucose and pure glycerol. Its supported good cell growth and DHA yield.
(Yokochi *et al.*, 1998)



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objective

To investigate the potential of using **Crude glycerol** obtained from biodiesel plant for DHA production by *Schizochytrium limacinum*

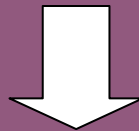


2. Methods

2.1 Algal strain and cultivation condition

S. limasinum ATCC -1381

American Type Culture
Collection (ATCC, USA)



- Cultivate at 25 °C, pH 6.5 on orbital shaking at 150 rpm in the dark
- Keep controlling as aerobic condition



2.2 Medium composition for inoculums

Modified Wu's basal medium

• Glucose	20	g l ⁻¹
• Yeast extract	4	g l ⁻¹
• NaCl	25	g l ⁻¹
• MgSO ₄ ·7H ₂ O	5	g l ⁻¹
• KH ₂ PO ₄	1	g l ⁻¹
• CaCl ₂	0.2	g l ⁻¹
• KCl	1	g l ⁻¹
• Trace mineral	1	mL



2.3 Effect of aeration rates

Cultures were grown in 500 mL Erlenmeyer flasks containing different medium volumes at 15%, 30%, 45%, 60% and 75%, respectively.

2.4 Effect of carbon concentrations

Glucose was replaced by crude glycerol concentrations (25, 50, 75, 100, 125 and 150 gL⁻¹) at the optimal for medium volumes.

After
harvesting,
biomass
and DHA were
analyzed



2.5) Cell growth and lipid analysis

The biomass was determined by cell dry weight. The culture was harvested and washed with distilled water twice by centrifugation, and then dried at 70 °C in a vacuum oven

The fatty acids methyl esters (FAME) were prepared from dried algal biomass by modified from Indarti *et al.*, (2005)

FAMEs was analyzed by gas chromatography (GC-17A, Shimadzu, Japan)

3. Results

3.1 Effect of aeration rates

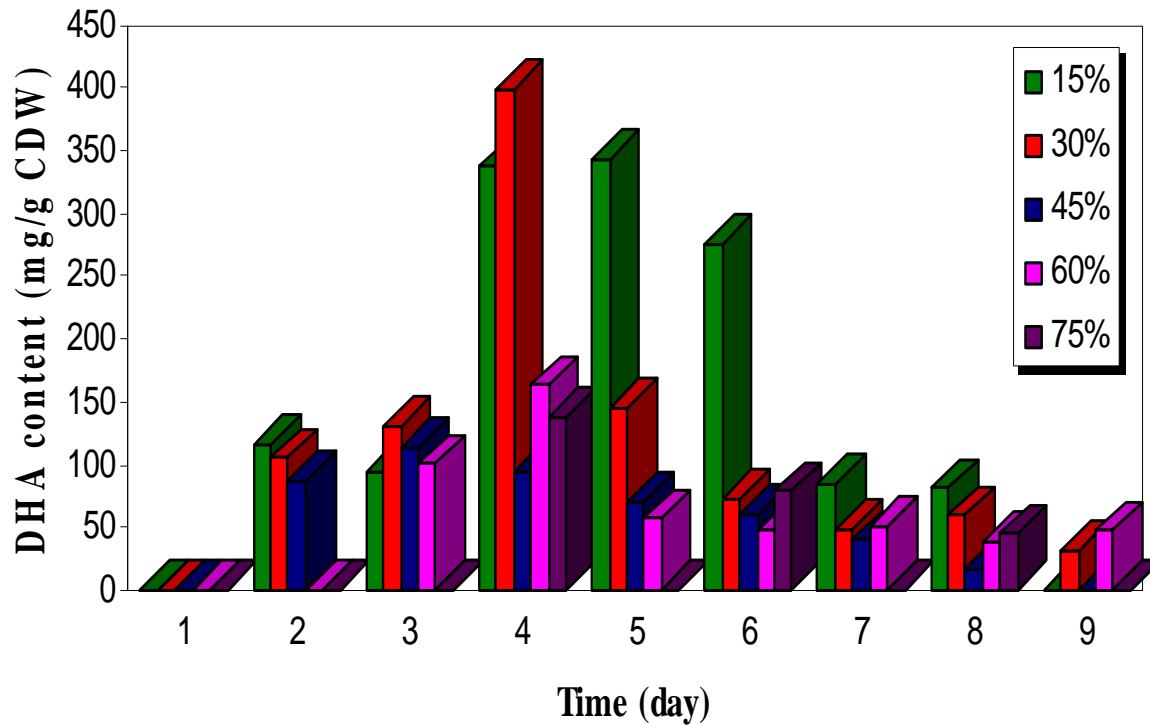
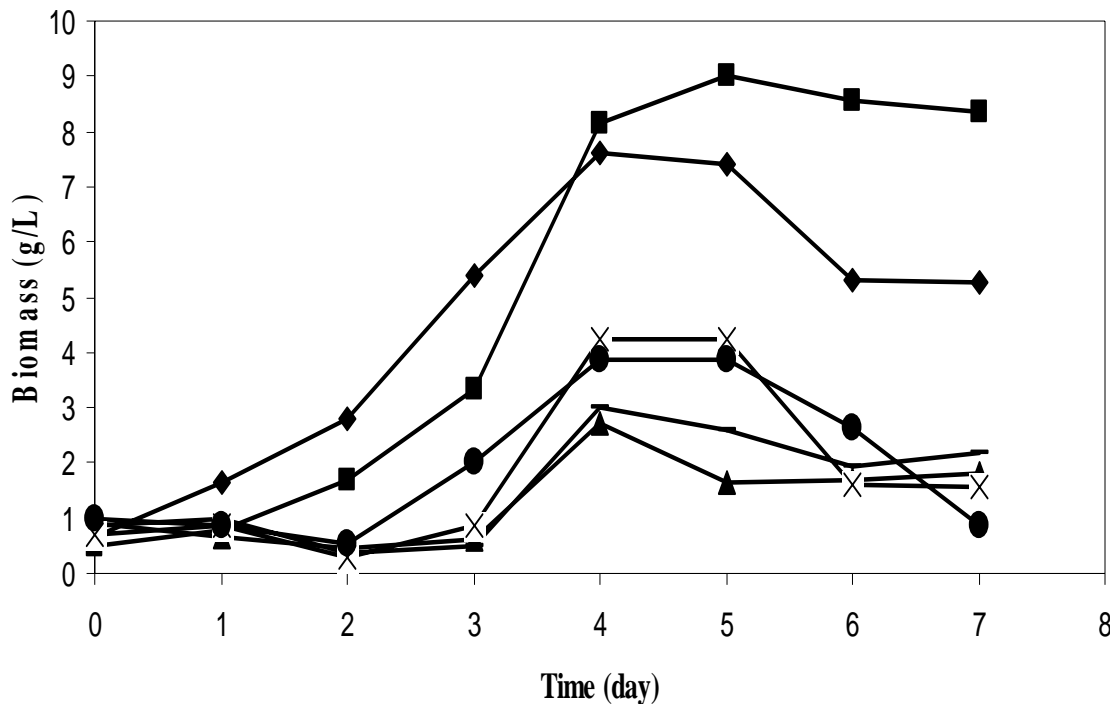


Fig. 1. DHA production ($\text{mgG}^{-1}\text{CDW}$) from the fermented of 5 different medium volumes cultured in 2% glucose medium at 25°C

(15 %; 30 %; 45%; 60 % and 75 % medium volumes)

The result shown that, when the range of medium volume increase from 15 to 75% (about 75 to 375 mL perflask) the 7 gL^{-1} maximum biomass within 4 day and the highest DHA production of 400 mgG^{-1} (about $2,900 \text{ mgL}^{-1}$) were obtained within 3 day for the 30% medium volume. The cell growth well at lower medium volume.

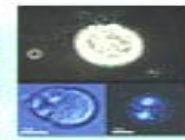
3.2 Effect of carbon concentrations



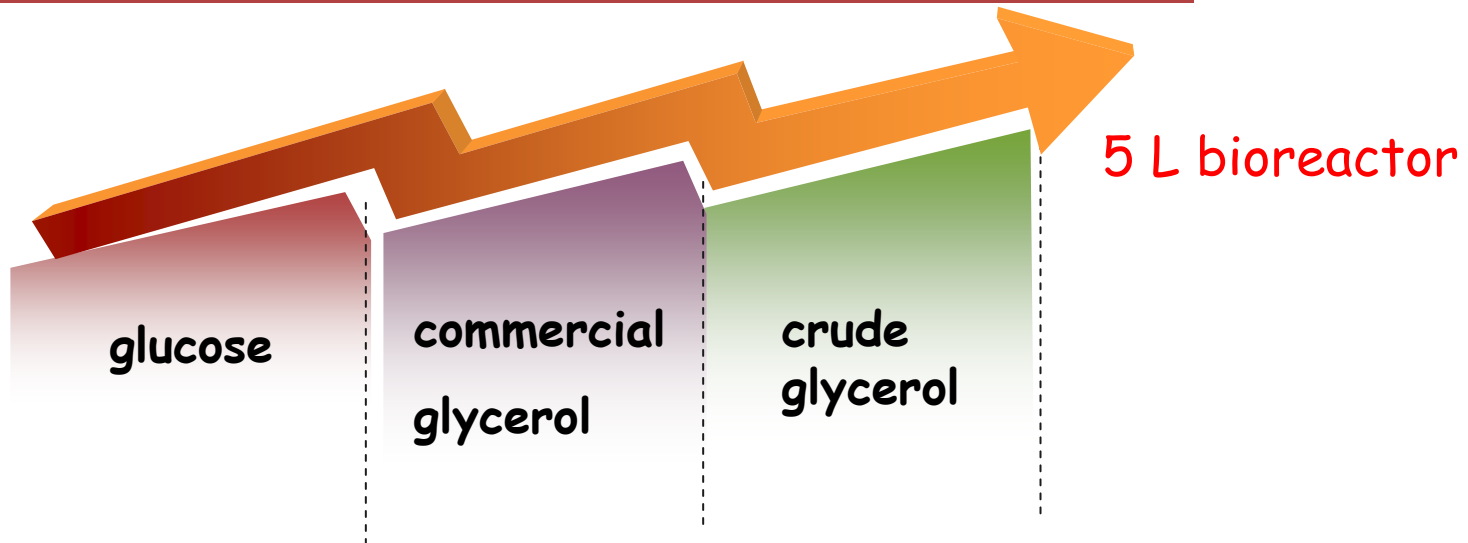
As initial crude glycerol concentrations increased resulting an increased in the biomass production. The highest biomass of 9 gL^{-1} within 120 hrs when crude glycerol was used up to 50 gL^{-1} .

In addition, when increased crude glycerol more than 50 gL^{-1} , the result shown that the algal growth become decreased.

Fig. 2. Cell growth curves of glycerol waste 6 difference concentrations at 30 % medium volume. Symbols represent the biomass (◆ 25 g; ■ 50 g; △ 75 g; / 100g; × 125 g; • 150 g)



4. Discussion



It is proved that the crude glycerol obtained from biodiesel production has a potential to use as carbon source to produce a higher value fine chemical of DHA via marine micro-algae cultivation. The optimal cultivating condition obtained in this study will be further used to consider in production of DHA in 5 L bioreactor under batch fermentation condition. In addition, variations of carbon sources such as glucose, commercial glycerol and crude glycerol will be compared and evaluated how effects on the DHA production and the degree of intracellular DHA to maintain the normal membrane of the strain.



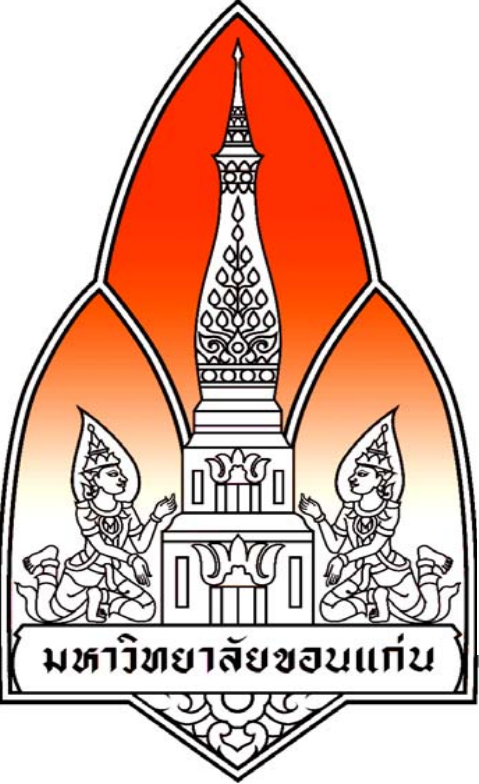
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5. Acknowledgements

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Thank You !

