Korean J. Chem. Eng., **30**(4), 787-789 (2013)
DOI: 10.1007/s11814-013-0020-0

RAPID COMMUNICATION

Utilization of cane molasses for docosahexaenoic acid production by *Schizochytrium* sp. CCTCC M209059

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(*Received 14 July 2012 • accepted 11 February 2013*)

Abstract−Cane molasses (CM), an agro-industrial by-product, was first examined for docosahexaenoic acid (DHA) production by *Schizochytrium* sp. Cell dry weight as 21.94 g/L at treated CM cultivation was similar to that at pure glucose (26.7 g/L) cultivation. Batch fermentation at different initial CM concentration showed that DHA percentage could reach 47.51% at 10 g/L CM but only 37.90% at 70 g/L CM. By analyzing the fermentation process, monosodium glutamate might be a positive agent for effective DHA production. Finally, monosodium glutamate and malic acid were introduced to the fed-batch fermentation for effective DHA production.

Key words: Docosahexaenoic Acid, *Schizochytrium* sp., Cane Molasses, Lipid, Monosodium Glutamate

INTRODUCTION

Docosahexaenoic acid (DHA, C22:6), which is a member of ω-3 polyunsaturated fatty acids (PUFAs), is believed to be an important structural component of neural and retinal tissues [1] and is widely used in infant additives and medicine industry. Fish oil and microorganism are two conventional DHA sources. Among numerous microorganisms, *Schizochytrium* sp., a heterotrophic marine thraustochytrids, is noteworthy and often considered to be a highly promising commercial strain for DHA production [2] due to fast growth rate and high productivity. However, microalgal DHA is limited by high production costs in comparison to current fish oil prices [3]. To lower the cost of microalgal DHA, high productivities must be pursued by culture optimization and the use of inexpensive medium components. Recently, several fermentation processes have been studied for bio-production of DHA from cheap raw materials, such as crude glycerol [4], coconut water [5], and sweet sorghum juice [6].

Cane molasses (CM), an agro-industrial by-product that contains abundant sugar, amino acids, organic acids, inorganic compounds, and vitamins, is often used as a carbon source in the production of different primary metabolites [7] and secondary metabolites [8].

To our knowledge, CM has only been used in one recent study to produce PUFAs by a kind of mold, *Mucor recurvus* sp. [9]. However, utilization of the molasses in marine microorganism fermentation has not been investigated. Therefore, in this study, the application of pretreated CM in DHA production by *Schizochytrium* sp. was well investigated. In addition, monosodium glutamate (MSG) and malic acid were introduced to fermentation to solve the defect of low DHA percentage using CM.

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EXPERIMENTAL

1. Microorganism

Schizochytrium sp. CCTCC M209059, which was preserved in the China Centre for Type Culture Collection (CCTCC) [10], was used in the present study.

2. Culture Conditions

The main culture medium contained 40 g/L glucose (or fructose, sucrose) and 0.4 g/L yeast extract, which were dissolved in artificial sea water. This medium also contained trace elements in a prepared solution (2 ml/L) and vitamin solution (2 ml/L). The compositions of trace element solution and vitamin solution were as indicated in our previous study [10]. In the fermentation culture, *Schizochytrium* sp.HX-308 cells were grown at 25 °C, 160 rpm. The initial pH of about 5.5 did not need to be adjusted at all. In batch fermentation, the cultivation was stopped when total sugar solution was exhausted. In fed-batch fermentation, 4 mL of 40% sugar solution was supplied before the total sugar concentration less than 10 g/L.

3. Pretreatment of Cane Molasses

CM, which was obtained from the Guilin sugar refinery (Guangxi, PR China), contained [11] 45% (w/w) sucrose, 10% (w/w) converted sugars (5% glucose and 5% fructose), 3.3% (w/w) other carbohydrates, 1.5% (w/w) crude protein, 0.5% (w/w) crude fat, 5.1% (w/w) ash, 2.6% (w/w) salt, 6.9% (w/w) metal ions, and 25% (w/w) water. The crude molasses was diluted with distilled water to obtain 200-300 g/L total sugar concentration and the pH was adjusted to pH 1.0 with 5 M H_2SO_4, and heated at 100 °C for 2 h. After centrifugation at 8,000 rpm for 15 min, the supernatant was adjusted to pH 6.5 with 10 M NaOH for further use [7]. After treatment, sucrose in cane molasses could be converted to glucose and fructose.

4. Performance Analysis of Fermentation Broth

One milliliter broth was used for sugar composition analysis; glucose and glutamate were measured enzymatically by using a bio-

analyzer simultaneously (SBA-40C, Institute of Biology, Shandong Academy of Sciences, China). Total sugar content was analyzed according to the method of 3,5-dinitrosalicylic acid (DNS) assay [12]. Fructose content was then calculated by subtraction.

5. Cell Dry Weight, Total Lipids and Fatty Acid Analysis

Ten milliliters broth was used to determine cell dry weight (CDW) by gravimetric method. The methods of lipid extraction and fatty acid methyl esters (FAMEs) analysis were according to our previous study [13].

RESULTS AND DISCUSSION

1. Use of Different Carbon Source by *Schizochytrium* sp.

Glucose, fructose, sucrose, untreated CM and treated CM were studied as the initial carbon source for *Schizochytrium* sp. (Fig. 1). At the end of the fermentation, fructose and glucose could yield a considerable amount of cell dry weight (CDW) (23.74 g/L and 26.7 g/L, respectively) and lipids (6.7 g/L and 7.3 g/L, respectively), showing effective utilization of these two sugars. Sucrose could not be catabolized by *Schizochytrium* sp., and the CDW of 7.1 g/L might be ascribed to the utilization of nitrogen source, such as yeast extract and sodium glutamate. CDW and lipid content reached to 17.69 and 2.39 g/L when cells were cultivated in untreated CM, respectively, which were 25% and 65.67% less than that when using glucose as carbon source. This might be explained by the impossibility of sucrose utilization.

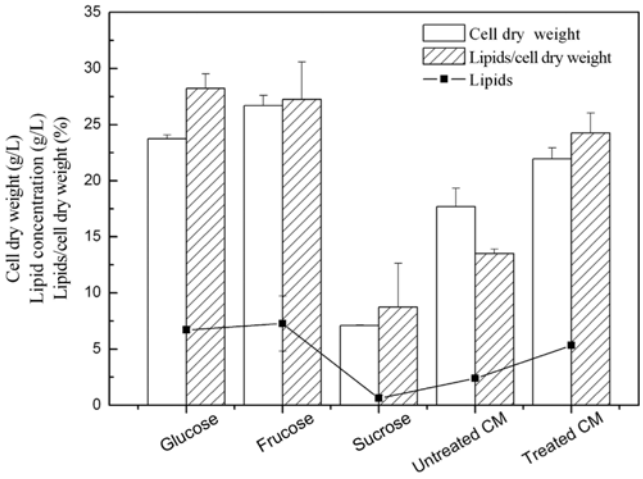

Fig. 1. Fermentation results of *Schizochytrium* sp. using various carbon sources.

Sucrose in CM could be converted to glucose and fructose after hydrolysis. CDW and lipid content reached to 21.94 g/L and 5.32 g/L in treated CM cultivation, which was 24.02% and 122.59% higher than that of untreated CM cultivation, showing treated CM possesses considerable potentiality for glucose substitution.

2. Batch Fermentation at Different Initial Cane Molasses Concentration

Growth, lipid content and fatty acid composition at different CM concentrations by *Schizochytrium* sp. were investigated (Table 1). When cells were cultivated in 10, 15, 20 g/L CM, total sugar was exhausted in 18 h with 7-9 g/L MSG left. CDW, lipid content and the percentage of lipids in CDW increased with the increase of the initial sugar concentration. At 35 and 70 g/L CM, fermentation period was 30 h and 54 h, respectively, not showing any increase compared with that at the same concentration of pure glucose.

Interestingly, fatty acid composition changed significantly with the variation of the initial total sugar concentration. Saturated fatty acids, especially C14:0 and C16:0 increased from 1.56% and 24.11% at 10 g/L CM to 13.09% and 29.13% at 70 g/L CM, while PUFAs, such as eicosapentaenoic acid (EPA) and DHA, decreased from 5.17% and 47.51% at 10 g/L CM to 1.16% and 37.90% at 70 g/L CM. This indicated that lower initial sugar concentration (below 20 g/L) could get higher PUFAs content, while higher concentration showed the opposite trend. This might be related to the residual MSG at the end of the fermentation [14]. In addition, DHA percentage in 35 and 70 g/L treated CM was only 36.12% and 37.90%, much lower than that of using the same concentration of pure glucose as carbon source. This might be ascribed to the disparity of fructose and glucose metabolism, resulting in the deficiency of NADPH for PUFAs accumulation.

3. Fed-batch Fermentation for Effective DHA Production

Our previous study [10] and reports in other studies [15,16] showed that malic acid and MSG could reinforce the NADPH synthesis and benefit the PUFA production. According to Wedding's study, malic acid could induce a structure change of malic enzyme, from the dimer to the more active tetramer or ocatamer forms, which enhance the NADPH generating reaction to ensure enough NADPH for DHA

Table 1. Fermentation results at different initial concentration of treated cane molasses

Initial sugar concentration (g/L)	Cell dry weight (g/L)	Total lipids (g/L)	Lipids/cell dry weight (%)	Fermentation time (h)	Rudimental MSG (g/L)	Relative fatty acid content (%)[a]				
						C14:0	C16:0	EPA	DPA	DHA
10 g/L CM	5.01±0.071	0.47±0.29	9.24±5.66	18	7.5±0.71	1.56±0.60	24.11±4.69	5.17±0.40	14.34±0.69	47.51±3.32
15 g/L CM	11.48±0.76	2.32±0.90	20.47±9.18	18	8.5±0.71	5.05±0.04	28.49±0.99	2.55±0.05	13.46±0.28	43.68±0.78
20 g/L CM	14.59±0.86	3.88±0.16	26.61±0.51	18	8.5±0.71	5.78±0.17	28.88±0.24	2.16±0.04	13.18±0.16	42.86±0.08
35 g/L CM	14.94±3.37	5.32±0.24	36.37±6.60	30	0	10.31±0.50	35.85±5.79	0.88±0.18	11.19±0.97	36.12±3.80
70 g/L CM	35.32±2.5	14.56±1.2	41.22±2.1	54	0	13.09±1.4	29.13±1.8	1.16±0.02	12.08±0.12	37.90±1.1
35 g/L Glu	20.23±0.21	7.39±0.93	36.56±4.99	30	0	9.70±0.42	25.14±0.04	1.03±0.22	13.35±0.08	47.27±1.65
70 g/L Glu	33.88±1.34	13.95±1.41	41.27±5.79	54	0	12.57±1.29	26.21±1.75	1.35±0.55	12.70±0.30	42.39±1.22

[a]C14:0 represents myristic acid; C16:0 represents hexadecoic acid; EPA represents eicosapentaenoic acid; DPA represents docosapentenoic acid; DHA represents docosahexaenoic acid

Table 2. Fermentation results when adding monosodium glutamate and malic acid in fed-batch fermentation

		Control	MSG[a] addition	Malic acid addition
Cell dry weight (g/L)		29.65±3.04	36.60±2.67	28.38±2.37
Lipid content (g/L)		7.73±1.77	2.67±0.28	6.20±1.05
Lipids/Cell dry weight		26.09%	7.29%	21.87%
Relative fatty acid content (%)[b]	C14:0	13.78±1.50	12.66±0.91	11.99±1.63
	C16:0	28.49±2.17	24.02±3.27	28.30±2.73
	EPA	1.43±0.41	1.72±0.37	1.42±0.06
	DPA	11.19±0.04	12.51±0.93	11.96±0.31
	DHA	38.71±1.42	42.39±2.65	42.20±2.62

[a]MSG represent monosodium glutamate

[b]C14:0 represents myristic acid; C16:0 represents hexadecoic acid; EPA represenst eicosapentaenoic acid; DPA represents docosapentenoic acid; DHA represents docosahexaenoic acid

production [17]. In addition, glutamate could enhance G6PDH activity, and provide more NADPH for polyunsaturated fatty acid production [18]. So, in order to solve the problem of low DHA percentage using CM as carbon source, MSG and malic acid were introduced to the fermentation broth when feeding CM nutrient. Table 2 summarizes the effects of MSG and malic acid addition on CDW, lipid content, percentage of lipid on biomass, and fatty acid profiling. MSG addition enhanced CDW and DHA percentage of total fatty acids from 29.65 g/L and 38.71% to 36.6 g/L and 42.39%, but showed negative effect on lipid content; while malic acid addition could also enhance DHA percentage of total fatty acids from 38.71% to 42.20%, but showed no significant impact on cell growth and total lipid production.

SUMMARY

DHA production from cane molasses by *Schizochytrium* sp. was developed for the first time. This study has demonstrated that hydrolyzed CM could be used as an economical and feasible carbon source for DHA production by *Schizochytrium* sp. with slightly negative effect on DHA percentage of total fatty acids. Nevertheless, MSG and malic acid addition in fed-batch fermentation could minimize this effect.

ACKNOWLEDGEMENTS

This work was financially supported by the National Science Foundation for Distinguished Young Scholars of China (No. 21225626), the National Basic Research Program of China (no. 2009CB724700), the Nature Science Foundation of Jiangsu Province (no. BK2012424), the National Science and Technology Pillar Program (no. 2011BAD 23B03), and the National High Technology Research and Development Program of China (no. SS2012AA021704).

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