Asian Journal of Chemistry Vol. 20, No. 7 (2008), 5153-5156

Extraction of Microbial Lipids by Using Supercritical Carbon Dioxide as Solvent

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Production of microbial oils by fermentation process using oleaginous organisms is one of the potential alternative sources of lipids. *Rhodotorula gracilis*, an oleaginous yeast is reported to accumulate 50-60 % of its biomass as lipids. The oil in the form of discrete globules within the cells, is usually extracted using organic solvents after disruption of the rigid cell wall. The efficiency of extraction of lipids by using supercritical carbon dioxide from untreated and acid treated cells was studied in this experiment. The fatty acid profile of the oil obtained was also investigated by gas chromatographic analysis.

Key Words: Oleaginous yeast, Supercritical extraction, Fatty acid profile.

INTRODUCTION

The potentials of certain microorganisms to produce lipids similar to vegetable oils have been well documented[1-3]. Lipids generally includes triglycerides, fatty acids, sterols, terpenes, carotenoids, tocopherol and other fat soluble vitamins. All microorganisms produce lipids, but the term oleaginous is attributed to those organisms which have been reported to producing at least 20 % and above of their biomass as lipids. Oleaginicity seems to be confined to eukaryotic organisms like algae, yeasts and fungi. In oleaginous microorganisms, the oil is accumulated intracellularly and is protected by rigid cell wall. The disruption of the cell wall is very much essential for effective penetration of the organic solvents for extraction. The methods employed for extraction for plant lipids, animal lipids and bacterial lipids, are not effective for yeasts due to the rigid cell wall. There is need for disruption of the cell wall before solvent extraction by chemical, mechanical or enzymatic methods[4,5].

Supercritical extraction is a unit operation that exploits the dissolving power of fluids at temperature and pressure above their critical point. At the critical point, the properties of liquid and vapour become identical. A supercritical fluid exhibits physico-chemical properties between those of liquids and gases, which enhance its role as a solvent[6]. At present, supercritical

extraction has been applied industrially in decaffination of coffee powder and in the recovery of hops extract. The efficiency of extraction of lipids from untreated and acid treated cells were studied in this experiment.

EXPERIMENTAL

The oleaginous microorganism *Rhodotorula gracillis* CFR-1 was used in all the experiments. The organism was maintained on potato dextrose slants and subcultured for every one month.

Fermenter medium and cultivation: The seed inoculum medium consisted of molasses (270 mL), magnesium sulphate (0.8 g), calcium chloride (0.8 g), potassium dihydrogen phosphate (1.5 g), ammonium sulphate (1.63 g) and pH was adjusted to 5 before sterilization. The medium was sterilized at 10 psi pressure for 15 min. The medium was inoculated with 2 d old culture slants and incubated for 24 h at 30 ºC on a rotary shaker at 200 rpm.

The fermenter medium composed of molasses (1.3 L), magnesium sulphate (4 g), calcium chloride (4 g), potassium dihydrogen phosphate (7.5 g) and ammonium sulphate (16.33 g). The pH was adjusted to 5 and then the medium was sterilized in an autoclave at 15 psi for 1 h. The organism was grown in a 15 L fermenter (New Bruswick, USA) with a working capacity of 10 L. The fermenter medium was inoculated with the inoculum along with trace element mixture and vitamin solution at 30 ± 1 ºC, aeration (1 vvm), agitation (350 rpm) and silicone oil was used as the anti-foaming agent.

Harvesting of biomass and extraction: The biomass was harvested and cells were separated by centrifugation and washed free from molasses. Two samples of about 20 g (50 mL) of cell suspension were taken in two 250 mL conical flasks. The samples were mixed with 50 mL of HCl (1 N) and 50 mL of distilled water, respectively. The mixtures were kept for 12 h at 30 ºC on a shaker at 200 rpm. The mixtures were filtered and the residue was collected, dried and weighed accurately for supercritical extraction.

The extractor was a high pressure vessel made of acid proof steel with a capacity of 4 L. The exit was connected to the seperator vessel (S) through a valve. The carbon dioxide under extraction pressure was expanded into the separator, where the oil and the gas separates out. The separator was heated with the hot water from the heat exchanger to heat adiabatically expanded carbon dioxide to the required temperature. The pressure, temperature and frequency of the gas flowing through the flow meter were recorded. The solvent pressure was maintained at 210 bar pressure at 55 ºC, the flow rate was between 6-6.5 Kg/h. The oil obtained in each case was estimated gravimetrically.

Preparation of methanolic HCl: 95 mL of absolute dry methanol was taken in a dry beaker, 5 mL of acetyl chloride was added along the sides of the beaker slowly in ice cold conditions.

Preparation of methyl esters: The oil was treated with petroleum ether (40-60) in the ratio 3 mg of oil with 0.1 mL of ether. 0.1 mL of the above mixture was mixed with 5 mL of methanolic HCl and refluxed on a water bath for 2 h. The refluxed mixture was extracted and washed with hexane in a separating funnel and dried by treating with anhydrous sodium sulphate. The solvent was evaporated by nitrogen flushing and the esters were extracted with chloroform.

Gas chromatographic analysis: The esters were analyzed in Shimadzu gas chromatorgraphy GC-9A. Samples were separated on 6" × 1/8" stainless steel column packed with 10 % Alltech (cs-10 in chromosorb) 80/100 mesh. The carrier gas was nitrogen at a flow rate of 40 mL/min and the oven temperature was held at 160 ºC. The volume of the sample injected was 1 mcL. Peaks were identified by comparison of their retention times with those authentic samples of methyl esters of fatty acids from sigma chemicals.

RESULTS AND DISCUSSION

The results obtained were tabulated in Tables 1 and 2. The yields of oil recovered by supercritical fluid extraction was found to be low compared to the conventional liquid extraction by Soxhlet which is 42.102 % (w/w). From the data obtained it is clear that the cell wall degradation is necessary even for the supercritical extraction. The fatty acid profile appear to be similar in both the cases. The oil extracted was checked after certain time intervals and the process was stopped when there was no more oil obtained. The rate of extraction was higher in case of acid treated samples, while it took 6 h for complete extraction in case of untreated samples.

TABLE-1
EXTRACTION OF LIPIDS BY SUPERCRITICAL CARBON DIOXIDE

Time (min)	CO_2 flow rate (Kg/h)	Yield (%) w/w	Nature of raw material	Efficiency
360	6.09	4.22	Untreated	10.03
300	6.71	27.25	Acid treated	64.72

TABLE-2
FREE FATTY ACID PROFILE OF THE OIL OBTAINED BY SUPERCRITICAL EXTRACTION USING CARBON DIOXIDE AS SOLVENT

Treatment	Myristic acid	Palmitic acid	Stearic acid	Leic acid	Linoleic acid	Linolenic acid
	Relative percentage (% w/w)					
Untreated	1.26	34.08	2.82	48.16	11.54	2.00
Acid treated	1.52	29.58	5.20	53.84	9.82	–

The supercritical extraction process was able to extract 64.7 and 10 % of the oil present in the cell from acid treated and acid untreated cells.

ACKNOWLEDGEMENTS

The authors are thankful to the management of Dayananda Sagar Institutions and CFTRI, Mysore, for their support and encouragement.

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(*Received*: 25 May 2007; *Accepted*: 9 April 2008) AJC-6513