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Comparison of two microbial oil extraction technologies

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Abstract. Microbial oil production has many advantages such as rapid proliferation of microbial cells and short production cycle. The raw materials needed for growth are abundant and inexpensive such as starch, sugar, especially the waste which can be utilized in food or paper industry. 170 oil-producing strains were screened by Sudan black staining observation using fat grains as screening index, among which, 6 high lipid yield yeast strains were identified by residual sugar as indirect index. By comparing the oil-producing ability of this strain, it was determined that the first strain was a good strain for oil production by fermentation. The biomass, oil content and oil yield of the strain were 11.58 g/L, 36.68%, 4.25 g/L, respectively. Through analysis on 26S rDNA sequence and observation on morphological characteristics, culture characteristics and physiological and biochemical characteristics, this strain belonged to *Cryptococcus* spp.

1. Introduction

It is a complex and powerful work in screening oil-producing microorganism strains; hence, a simple, fast and efficient screening and identification method is very necessary. Generally speaking, there are two methods for the identification of microbial oil content: semi-quantitative method and Sudan black staining method [1]. The advantages of this method are simple operation and short time, but it is difficult to distinguish strains with little difference in oil content. The other is quantitative detection method, oil extraction method. This method is more accurate and could detect the oil content of the strain better. There are four common methods for oil extraction: Soxhlet extraction, supercritical CO₂ extraction, organic solvent extraction and acid-heat extraction. Supercritical CO₂ extraction has high efficiency in oil extraction, but it requires high equipment. Because of the limited laboratory conditions, this method is not used [2]. Although organic solvent method is simple and feasible, it cannot effectively extract intracellular lipids because of its poor cell breaking ability, so the effect of oil extraction is not good. Soxhlet extraction method has good effect on oil extraction, and acid-heat method is simple to operate [3]. So this chapter mainly studies Soxhlet extraction method and acid-heat method, and compares the two extraction methods, and establishes a fast and simple method for oil extraction.



2. Material and Methods

2.1. Experimental materials

Cryptococcus SP-18 was isolated and preserved in our lab before use. Activated and preserved strains were cultured at 28 for 48 hours at constant temperature. A ring of yeast was inoculated into the seed medium. The strains were cultured at 28 for 24 hours at 180 r/min at constant temperature and oscillation. The cultured seed liquid was inoculated into the fermentation medium at 28 C for 96 hours at 180 r/min.

2.2. Experimental methods

Soxhlet Extraction for Oil and Fat: Accurately weigh 3G dry bacteria and put them in a mortar for grinding. Put the grinded samples into the weighed filter paper bag (named W₁), seal the mouth of the bag, put them in the oven for drying for 3h, then cool them to room temperature and put them in the weighing bottle for weighing (W₂). The filter paper package containing the sample was put into the extracting cylinder with long tweezers and injected with anhydrous ether with 1.67 times the siphon volume at one time, so that the sample package was completely immersed in the ether. Connect all parts of the extractor, connect the condensate water flow, extract in the constant temperature water bath, adjust the water temperature between 70-80 C, make the condensation droplets of ether beads (120-150 drops/min or reflux more than 7 times/h), extract the ether in the extracting cylinder and check the oil-free with filter paper droplets (about 6-12 hours). After extraction, the filter paper bag was removed with long tweezers and volatilized ether in the ventilated place. The ether in the extraction bottle was recovered separately. After utilization, the filter paper bag was placed in the oven for drying for 2 hours and cooled until constant weight (W). Oil yield can be seen in formula (1):

$$\text{oil yield(\%)} = \frac{W_2 - W}{W_2 - W_1} \times 100\% \quad (1)$$

Among which, W₂-weighing bottle and filter paper bag and drying sample weight (g)

W-weighing bottle and filter paper bag and drying residue weight after extraction (g)

W₁-weighing bottle and filter paper package weight (g)

Acidic-Thermal Method: Accurate weighing of 0.5g dry bacteria, adding appropriate concentration of hydrochloric acid, shaking and mixing, after a period of time at room temperature, boiling water bath, rapid cooling after boiling water bath, pouring into the separating funnel, adding appropriate amount of anhydrous ethanol to shake, adding a certain volume of organic solvent extraction, standing for a period of time, collecting organic layer in the weighed test tube (m₁), heating to volatilize organic solvents, weighing (m₂). Oil yield see formula (2):

$$\text{oil yield(\%)} = \frac{m_2 - m_1}{0.5} \times 100\%$$

Among which, m₂-quality of test tube after extraction and drying;

m₁-quality of clean test tube

3. Result and Discussion

3.1. Comparison of two extraction methods

Soxhlet extraction is a common method of oil extraction. Because of its purity and less impurities, it is often used to extract oil roughly, but its efficiency is relatively low. Each Soxhlet extractor can only extract one sample, and it takes a long time for 12 hours. The bacteria used need to be dried and ground. The quality of each bacteria used is at least 2 g, if it is low at 2g, the experimental error will be increased, resulting in inaccurate experimental results. Therefore, soxhlet extraction method is not suitable for the determination of lipid content in the screening of oil-producing microorganisms.

Table 1. Comparison of two oil extraction methods

extraction method	Sample requirements	Instrument requirements	Extraction time	Minimum sample size required
Soxhlet extraction	Dry bacterium powder	complex	12	3
Acid heat method	dry or wet	simple	3	0.5

The acid-heat method is to treat the cell wall with hydrochloric acid firstly before destroying the cell wall further to make the oil easy to leach and extracting the oil with organic solution. The acid-heat method can process more than 20 samples at a time, and it takes only 4-6 hours. The dry and wet bacteria can be used. The extraction and analysis can be carried out with 0.5 g of dry bacteria each time. The operation is simple and easy. The comparison of the two extraction methods is shown in Table 1, and the yield of oil extracted by the two extraction methods is shown in Table 2.

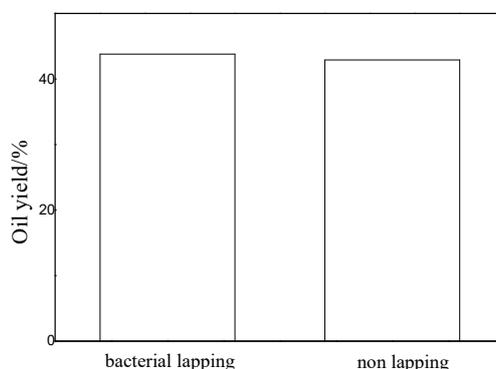
Table 2. Comparison of two oil extraction methods

extraction method	Acidic-thermal extraction of oil yield (%)	Oil yield by Soxhlet extraction (%)
1	46.21	33.59
2	45.38	34.41
3	44.87	32.61
Average	45.48	33.54

3.2. Optimization of extraction process of oil by acid-heat method

The technological conditions of oil extraction by acid-heat method were preliminarily studied. 0.5g bacterium was weighed and the cell wall was treated with 4 mol/L hydrochloric acid. On this basis, several influencing factors of oil extraction by acid-heat method were studied.

3.2.1. Bacterial pretreatment. The samples used for extracting oil by acid-heat method are both wet and dry, but the moisture content of wet bacteria is more, and the effect of extracting oil is not as good as that of dry bacteria, so the bacteria are dried first. After drying, effect of hydrochloric acid on its treatment and oil yield may be affected. Therefore, bacteria are ground into powder to investigate effect of grinding and non-grinding dry bacteria on extraction of oil by acid-thermal method. The results are shown in Figure 1.

**Figure 1.** Effect of dry cell morphology on extraction of oils by thermal-acid method

The experimental results show that the yield of oil extracted by acid-heat method is not significantly increased by using powder dry bacteria. Block dry bacteria can quickly dissolve into suspension in hydrochloric acid solution without affecting the acidification of hydrochloric acid. Therefore, there is

little difference between the yield of oil and bacteria grinding or not. In order to save experimental cost and time, non-body grinding is chosen.

3.2.2. Determination of the dosage of hydrochloric acid. 0.5 g dry bacteria were weighed and acidolysed with 4 mol/L hydrochloric acid. The volume of hydrochloric acid would also have a great influence on the extraction of oil by acid-heat method. The amount of hydrochloric acid was investigated. The results are shown in the figure 2.

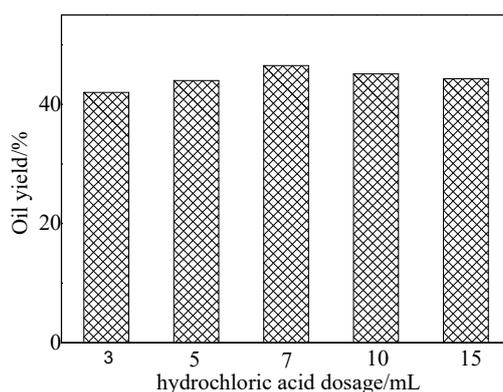


Figure 2. Effect of hydrochloric acid volume on extraction of oils by thermal-acid method

The experimental results show that too little hydrochloric acid, incomplete cell wall breakage, oil is not easy to leach, will reduce the oil yield, hydrochloric acid dosage is too high, may make the leached fatty acid hydrolysis, oil yield will also be reduced. When the dosage of hydrochloric acid was 7 mol/L, the grease yield was the highest.

3.2.3. Determination of pH after acid hydrolysis. After the bacteria were treated with hydrochloric acid, before heat treatment, the pH environment of the bacteria solution may have some influence on the extraction of oil. The effect of acidity, neutrality and alkalinity of pH on the extraction of oil by acid-heat method was investigated. The results are shown in Figure 3.

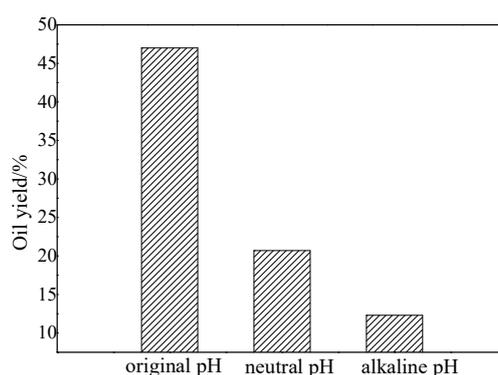


Figure 3. Effect of hydrochloric acid volume on extraction of oils by thermal-acid method

The experimental results show that in acid environment, it is more conducive to acid-thermal extraction of oil, neutral and alkaline environment will hinder the extraction of oil, reduce the yield of oil extraction.

3.2.4. Determination of acid heat time. The acid-heat time has a great influence on the extraction of oil by acid-heat method. The effect of acid-heat time is investigated. The results are shown in Figure 4.

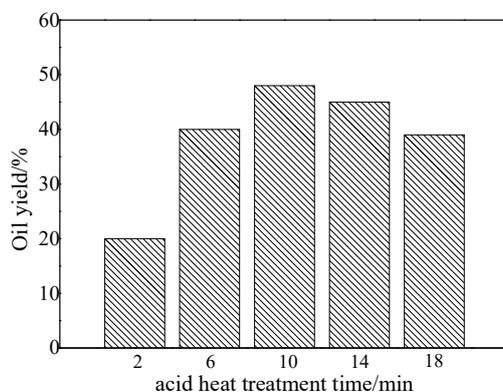


Figure 4. Effect of processing time on extraction by thermal-acid method

The experimental results show that the oil yield increases with the increase of acid-heat time, but decreases with the increase of acid-heat time after a certain time. Insufficient acid-heat time leads to incomplete cell wall breakage, and too long acid-heat time leads to oil decomposition reaction, which reduces oil yield. When the acid-heat time is 10 minutes, the grease yield is the highest.

3.2.5. Determination of acid number. After treating bacteria with hydrochloric acid, one heat treatment may lead to incomplete oil extraction and affect oil yield. Therefore, several heat treatments were carried out to investigate the effect of heat treatment times on bacteria. The results are shown in the figure 5.

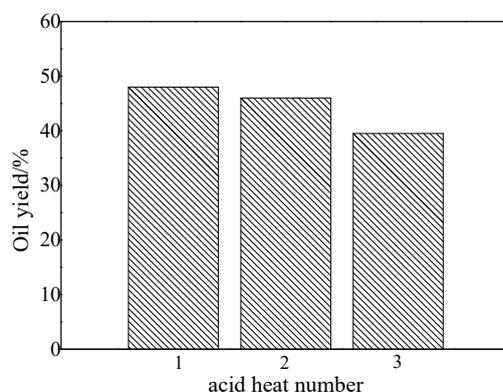


Figure 5. Effect of processing times on extraction of oils by thermal-acid method

The experimental results show that heat treatment of bacteria for many times is not conducive to the extraction of oil, and will reduce the yield of oil. The possible reason is that more heating times, intracellular lipid decomposition reaction, resulting in the reduction of oil. Therefore, only one heat treatment was carried out on the bacteria.

3.2.6. Determination of the dosage of organic solvents. The effects of the amount of organic solvent on the extraction of oil by acid-thermal method were investigated by using petroleum ether and ether of equal volume. The results are shown in figure 6. The experimental results show that the oil yield will not increase if there are too few organic solvents and too many organic solvents. On the contrary, the extraction cost will increase. When the amount of organic solvents is 15 ml, the oil yield will reach the maximum. When the amount of organic solvents is 20 mL, the oil yield will not increase any more. Therefore, 15 mL organic solvents are used to extract oil.

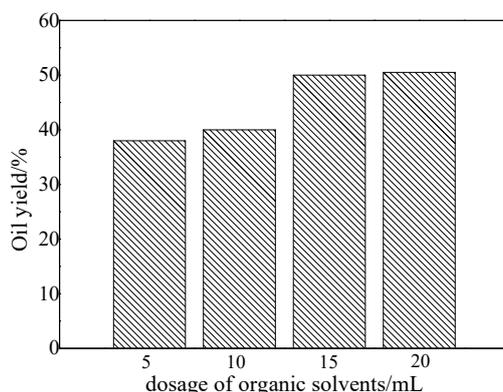


Figure 6. Effect of organic solvent volume on extraction of oils by thermal-acid method

3.2.7. Determination of extraction time. The extraction solution was put into a triangular bottle, sealed with plastic paper, and extracted in a shaker at a certain speed. The effect of extraction time on the yield of microbial oil was investigated. The results are shown in figure 7.

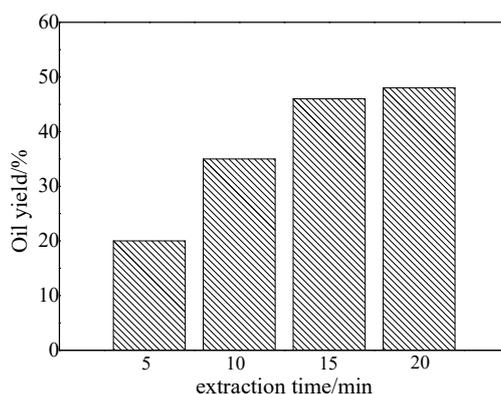


Figure 7. Effect of Extraction time on extraction of oils by thermal-acid method

Extraction time is too short, organic solvent extraction oil is not complete, affecting the oil yield. If the extraction time is too long, the oil yield will not be increased. When the extraction time is 20 minutes, the oil yield is the highest.

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