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Optimization of fermentation conditions for oil production by strain 6-18

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Abstract. By investigating the effects of carbon source, nitrogen source, carbon-nitrogen ratio and inorganic salts on oil production of strains, the optimum carbon source, nitrogen source, carbon-nitrogen ratio, types and concentration of inorganic salts were determined. By investigating the effects of liquid loading, culture temperature and initial value of medium on oil production of strains, the optimum culture conditions were determined.

1. Introduction

Acetyl CoA plays a leading role in lipid synthesis in microbial cells, and the formation of acetyl CoA is influenced by many factors, such as nitrogen sources, isocitrate dehydrogenase activity and so on. All the conditions that can affect these factors will directly or indirectly affect the final oil production, mainly carbon source, nitrogen source, carbon-nitrogen ratio, temperature and so on [1].

Microbial strains can convert excess carbohydrates into lipids when carbon sources are abundant and other nutrients are deficient. There are many carbon sources for cultivating oil-producing microorganisms, such as glucose, sucrose, starch saccharifying liquid, molasses, lactose, starch factory wastewater, pulp industry wastewater and wood hydro lysate, etc. [2]. But the most suitable carbon source for cell growth and oil synthesis is glucose. The biomass of bacteria produced with glucose as carbon source is high, and the oil production is also high. Nitrogen source promotes cell growth and lipid accumulation can be observed under severe nitrogen deficiency. Nitrogen sources mainly include corn pulp, amino acids, nitrates, ammonia salts and urea [3]. Nitrogen source is conducive to the growth of bacteria, so low C/N ratio is required in the prophase of culture, a large number of bacteria can be obtained, and high C/N ratio is required in oil production stage to accumulate more fat. Such changes in culture conditions can be achieved by adding appropriate concentration of sterile glucose solution before harvesting at the later stage of logarithmic growth of bacteria [4]. Temperature can regulate the fatty acid composition of microbial lipids, which is caused by an adaptive response of cells to changes in external temperature. Usually, the melting point of unsaturated fatty acids is lower than that of saturated fatty acids, and the short-chain fatty acids are lower than that of long-chain fatty acids [5]. Therefore, when strains grow from high temperature to low temperature, the contents of unsaturated fatty acids and short-chain fatty acids in cell membranes increase, mainly palm oleic acid or oleic acid, while the average chain length increases with the increase of temperature. These changes are to ensure the normal fluidity and permeability of cell membranes. Most of the optimum temperatures for lipid production are around 25 °C, but the content of unsaturated fatty acids increases at low temperatures. Moreover, the optimum time for cell to synthesize lipid is related to the strain of lipid-producing bacteria, the growth



stage of microorganisms and the length of culture time [6]. For example, the optimum culture time of *Aspergillus niger*, *Aspergillus oryzae*, *Rhizopus*, *Rhodotorula* and *Saccharomyces cerevisiae* is 3, 7, 5 and 6 days, respectively. The oil content of oil yeast was less in logarithmic phase, increased sharply at the end of logarithmic phase and reached the maximum at the beginning of stable phase.

2. Material and Methods

According to the characteristics that fat can be dissolved in organic solvents such as ether and petroleum ether, and the volatility of organic solvents, the fat in oil sample can be extracted repeatedly by reflux evaporation and distillation, then the organic solvent can be removed by evaporation, and the weight of the residual fat in the extraction bottle can be weighed, so the content of crude fat in the sample can be obtained. The quality of oils and fats was determined by Soxhlet extraction method, that is, washing the extracting bottle and baking it in an oven at 105°C before weighing it accurately, then 2-5g dry bacteria were grounded into powder, wrapped in two layers of filter paper, put into the extraction tube of Soxhlet extractor, soaked in petroleum ether with boiling range of 30-60°C for about 8 hours, then removed the extraction bottle, and continued to volatilize in the water bath to remove residual petroleum ether. It is then baked at 105 °C to a constant weight and weighed accurately. The poor quality of the extraction bottle after the advance extraction is the quality of the extracted oil.

3. Result and Discussion

3.1. Effect of Culture Conditions on Oil Production by Strain 6-18

3.1.1. Determination of Growth Curve of Strain 6-18. The most commonly used methods for measuring microbial growth are volume measurement, dry weight measurement and turbidimetry. In this experiment, turbid metric method was used to determine microbial biomass.

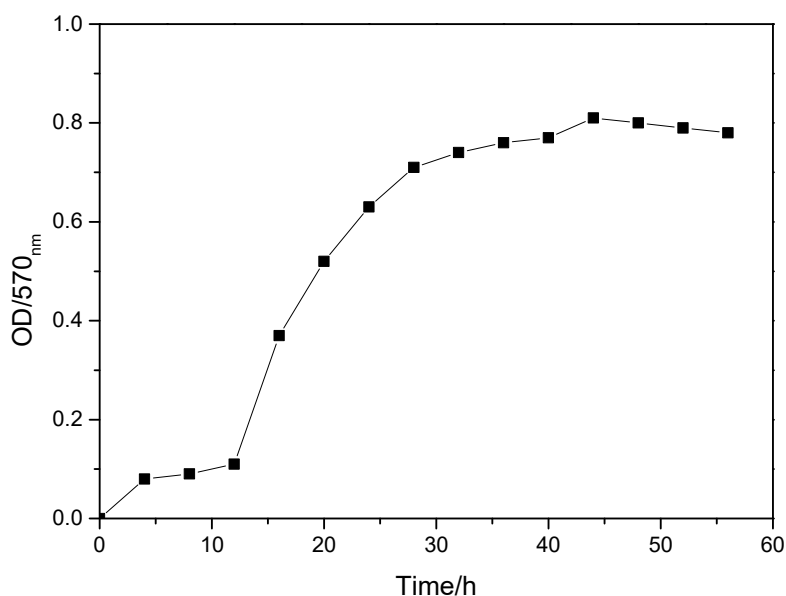


Figure 1. Growth Curve of Strain 6-18.

The results showed that the growth of bacteria was relatively slow, and it entered a stable growth stage after 40 hours and a late growth stage before 8 hours. In view of shortening the fermentation time as far as possible, it is advisable to cultivate seeds for 24 hours.

3.1.2. Effect of inoculation rate on oil production. Inoculation could significantly affect the length of microbial growth delay. The demurrage period is short and vice versa. Therefore, this part of the study on the vaccination volume, the general vaccination volume is large, the results are shown in Figure 2.

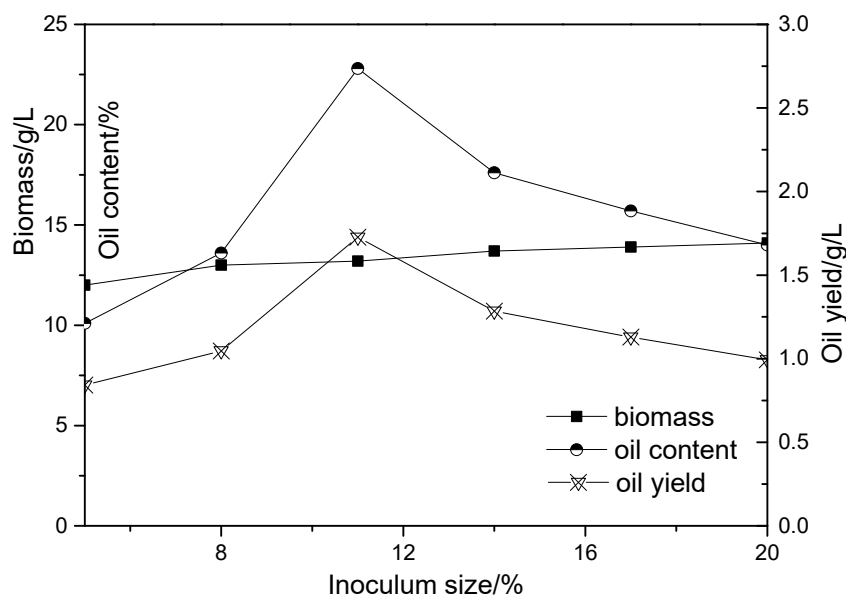


Figure 2. Effect of Inoculation on Oil Production by Fermentation.

The results showed that the amount of inoculation had little effect on biomass, but had a great influence on the accumulation of strain oil, and the inoculation amount had the best effect on oil production.

3.1.3. Effect of Fermentation Temperature on Oil Production.

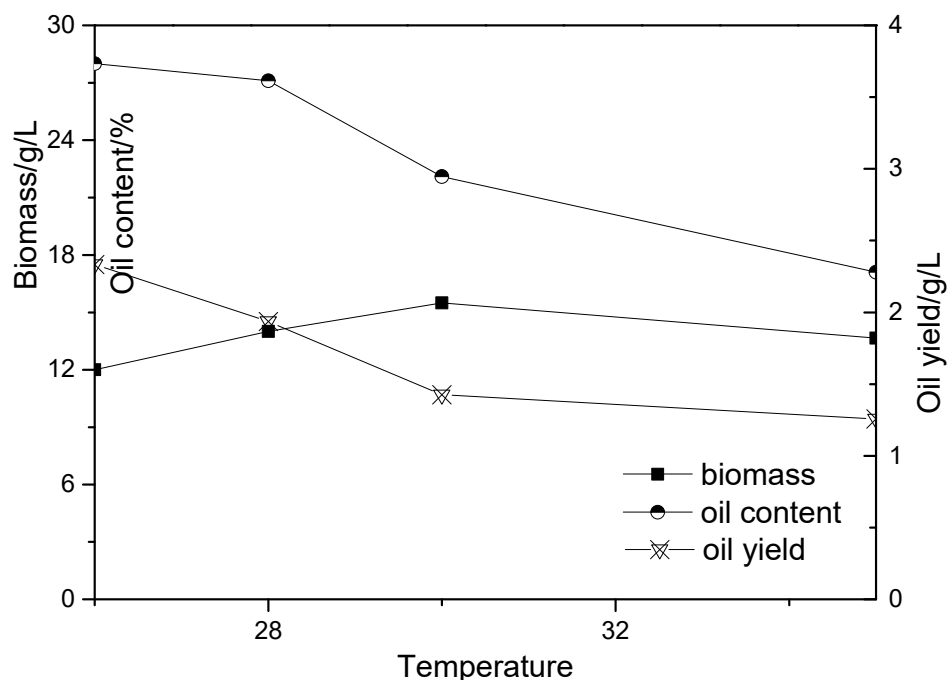


Figure 3. Effect of Temperature on Oil Production.

Temperature has a great influence on microbial lipid synthesis. Appropriate temperature promotes the synthesis of microbial lipid, and too high or too low temperature will hinder the synthesis of cell lipid. *Cryptococcus* spp. seeds were inoculated in the basic lipid-producing medium at 26, 28, 30 and 35 °C for 96 hours. The results were shown in Fig. 3.

3.2. Effect of Medium Composition on Oil Production by Strain Fermentation

3.2.1. Effect of Carbon Source on Oil Production. The synthesis of fungal oils begins with the synthesis of saturated fatty acids from acetyl CoA, which is mainly derived from the oxidation and decomposition of exogenous carbon sources. Microbial strains can convert excess carbohydrates into lipids when carbon sources are abundant and other nutrients are deficient, so the selection of carbon sources is very important. In the basic fermentation medium, 4% glucose, sucrose, lactose, molasses and dextrin were used as carbon sources, while the other components remained unchanged. Shake flask fermentation was carried out for 4 days under the optimized culture conditions. The results were shown in Table 1.

Table 1. Effect of carbon source on oil production

Carbon source	Biomass (g/L)	Oil content (%)	Oil yield (%)
glucose	14.33	43.57	6.24
sucrose	15.55	46	7.22
lactose	6.11	10	0.61
molasses	9	0	0
soluble starch	10	0	0
dextrin	5.79	6.67	0.38

Table 1 shows that sucrose as carbon source is the highest biomass and lipid content, while glucose is slightly lower than sucrose. As glucose is widely used in industrial production, glucose is selected as carbon source.

3.2.2. Effect of nitrogen source on Oil Production. The effects of different inorganic nitrogen sources on lipid production by fermentation were investigated. The results are shown in Table 2.

Table 2. Effect of inorganic nitrogen source on oil production

Nitrogen source	Concentration (g/L)	Biomass (g/L)	Oil content (%)	Oil yield (%)
ammonium sulphate	0.2	10.77	42.05	4.53
	0.5	11.05	45.87	5.07
	1	11.44	44.86	5.13
	2	11.44	41	4.69
	3	12.22	38.07	4.65
	4	11.89	37.83	4.5
ammonium nitrate	0.2	10.91	17.21	1.88
	0.5	11.62	23.87	2.77
	1	7.89	42	3.31
	2	0.11	39.6	3.61
	3	12.67	28	3.55
potassium nitrate	0.2	9.07	54.9	4.98
	0.6	10.06	51.32	5.16
	1	14.33	49.83	7.14
	2	16.5	47.2	7.79
	3	13.33	43	5.47
	4	9.83	41	4.23
sodium nitrate	0.2	10.83	53.56	5.8
	0.6	11.67	47.29	5.52
	1	14.5	37.58	5.45
	2	14.83	31.16	4.62

Table 2 shows that using potassium nitrate as nitrogen source is not only conducive to the growth of bacteria, but also conducive to the accumulation of lipids. The yield of lipids reaches 7.79g/L. Strain 6-18 can utilize nitrate, which indicates that the strain has enzymes that can catalyze the reduction of nitrate ion to ammonium ion. During the experiment, it was found that only inorganic nitrogen source was added, the strain grew slowly at first, and the delay time was long. Therefore, the effect of organic

nitrogen source on the lipid production by strain fermentation was also investigated. The results are shown in Table 3.

Table 3. Effect of organic nitrogen source on oil production

Nitrogen source	Concentration (g/L)	Biomass (g/L)	Oil content (%)	Oil yield (%)
corn steep liquor	1	14.45	47.3	6.28
	1.5	16	46.67	6.83
	2	15.8	44.8	7.46
	2.5	17.17	49.4	7.1
	3	18.7	41.4	8.48
yeast extract	0.5	15.3	40	6.13
	1	16.94	48.06	8.09
	1.5	15.17	41.61	6.31

It can be seen from the table that corn syrup and yeast extract are the nitrogen sources, the oil content is not different, but corn syrup is more conducive to the growth of bacteria, and the oil yield is 8.48g/L. Comparing the effects of potassium nitrate and corn syrup on oil production of strains, it was found that potassium nitrate was beneficial to oil accumulation and corn syrup was beneficial to bacterial growth. If corn syrup and potassium nitrate were used as compound nitrogen sources, the oil production of strains might be increased.

3.2.3. Effect of Inorganic Salts on Oil Production.

Table 4. Effect of inorganic Salts on oil production

inorganic salt type	Concentration (g/L)	Biomass (g/L)	Oil content (%)	Oil yield (%)
magnesium sulphate	0.1	15.3	49.72	7.62
	0.3	14.3	47.5	6.8
	0.5	16.8	47.8	8.04
	1	18.2	52.3	9.6
	1.5	20	56.9	11.4
	2	21.33	33.9	7.2
	2.5	19.3	49.6	9.6
zinc sulfate	0.001	20.8	47.4	9.88
	0.002	20	47.5	9.5
	0.003	21.5	49.5	10.66
	0.004	20	47.43	9.5
manganese sulfate	0.001	23.7	50.7	11.99
	0.002	23.7	55.8	14.3
	0.003	24.8	46.9	11.7
	0.004	21	56.9	11.9

Increasing the use of inorganic salts and trace elements properly can increase the oil production rate and yield of fungi. Certain metal ions, as essential trace elements of bacteria, participate in redox enzymatic reactions, and play a role in oxygen transport. They are very important for dehydrogenase activity in unsaturated fatty acid synthesis, and also have a certain effect on the growth of bacteria. Magnesium sulfate, zinc sulfate and manganese sulfate were added to the basic fermentation medium for 4 days at 28 C. The results are shown in Table 4. Table 4 shows that adding appropriate amount of magnesium sulfate is not only beneficial to the growth of bacteria, but also beneficial to the accumulation of lipid in bacteria. When the concentration was 2g/L, the biomass was the highest, but the oil content was very low, and the overall oil yield was not high. When the concentration is 1.5g/L, the grease accumulation is the highest and the grease yield is the highest. Therefore, the concentration of magnesium sulfate is determined to be 1.5g/L. Zinc sulfate had little effect on the growth of bacteria, but it obviously inhibited the synthesis of lipids, resulting in a significantly lower yield of lipids than the blank. Therefore, it is not suitable to be added to the medium. Manganese sulfate promotes the

growth of bacteria and is beneficial to the accumulation of lipids in bacteria. The yield of lipids reaches 14.3g/L at 0.002g/L. The amount of manganese sulfate added is determined to be 0.002g/L.

4. Conclusion

Effects of medium composition on the growth and lipid production of strain 6-18 were investigated by single factor and multi-factor orthogonal experiments. Glucose was the best carbon source, potassium nitrate was the best nitrogen source, and corn syrup was the best compound. Potassium hydrogen phosphate, magnesium sulfate and manganese sulfate promoted oil production, while zinc sulfate inhibited oil production. Sodium hydrogen phosphate had no significant effect on oil production. The optimum medium for oil production by strain fermentation consists of 120 g/L glucose, 1 g/L potassium nitrate, 2.5 g/L corn syrup, 2 g/L potassium hydrogen phosphate, 1.5 g/L magnesium sulfate heptahydrate and 2 mg/L manganese sulfate.

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