Study on the Fermentation Conditions of the Microbial Oils

Xie Ying[a], Wu Chenyu[b], Liu Shuang[c], Wu Yanshuang[d] and Zhang Zhen[e]

College of Biology and Food Engineering, Jilin Institute of Chemical Technology, Jilin 132022, China

[a]xieying3704@163.com, [b]923415524@qq.com,[c]1427104883@qq.com, [d]2642530993@qq.com, [e]771559239@qq.com

Keywords: microbial oils, oil contents, fermentation conditions, biodiesel

Abstract. T-5 strains of Mortierella were screened from soil sample, which had a high lipid contents. Through the orthogonal experiment, the optimization conditions of lipid were that temperature was 28°C, pH was 6.0, glucose concentration was 100g/L and inorganic nitrogen source concentration was 1g/L. The results of Gas chromatography analysis showed that the fatty acid was composed of 25.10% of Palm Acid, 25.17% of oleic acid, 20.99% of linoleic acid and 4.78% of stearic acid, which were similar to the composition of plant fatty acid, so it can be used as a feedstock for biodiesel production.

Introduction

Microbial oils were produced by microbial biosynthesis in vivo, which was a new way to develop oil resources. Because Oil-producing microbes had the advantages of the rapid proliferation of cells, short production cycles, rich raw materials of microbes, and not subject to seasonal climate change, Oil-producing microbes also can be used as biodiesel oil source[1,2]in addition to be used to produce edible oils and fats instead of the animal and vegetable fats and oils, especially the health functional oil. Biodiesel can be produced from a variety of animal and vegetable oil by esterification or transesterification process, the fatty acid composition of microbial oils was similar to vegetable oil, which not only can make up for the deficiency of animal and plant resources[3], but also can use agricultural waste as raw material[4,5,6]. In recent years, because of the impact of crude oil prices and environmental pressures, bio-diesel industry was paid more attention. With the development of industrial biotechnology, fermentation of microbial oils from raw materials to process were constantly achieved new progress. Recently, reports from United States national renewable energy laboratory noted that fermentation of microbial oil may be the important research in the bio-diesel industry and the bio-economy [7].

In this study, T-5 strains of Mortierella were screened from soil sample, which had a high lipid contents. Its fermentation conditions will be optimized and lipids will be analyzed to see if it is similar to the fatty acid composition of vegetable oils. It can lay a foundation for efficient utilization of straw cellulose hydrolysate and industrial production of microbial oil.

Materials

Strain. Oil-producing fungal strains of Mortierella sp. are from soil sample in Jilin Institute of Chemical Technology, which were named T-5.

Medium and major reagent. Nitrogen-limited medium: Glucose 40g/L, $(NH_4)_2SO_4$ 1.5 g/L, KNO_3 0.5g, KH_2PO_4 7g/L, NaH_2PO_4 2 g/L. It will sterilize 20min at 121°C. Ether and other reagents were purchased from North of Tianjin medical chemical reagent factory.

Method

Optimization of lipid fermentation conditions. Orthogonal test was designed to consider the effects of carbon source, mixed inorganic nitrogen source, the initial pH and temperature on fermentation. After fermentation was complete and other conditions did not change, lipid contents were determined.

Determine of lipid contents. The high fat contents of strains were inoculated into 50 mL Martin medium, then they were shaking cultured at 28°C and 180 r/min for 24h. Then 5% inoculum concentration inoculated into a fermentation medium, and shaking cultured for 7 days. The culture solution was centrifuged at 3500 r/min and the supernatant was discarded, and after the pellet was washed three times by centrifugation and was dried to constant weight at 105°C, then dry cell weight was measured. Lipid was extracted by Soxhlet extraction method[8], oil production rate was calculated: Oil ratio = fat mass / cell dry weight × 100%.

Analyzing of fatty acid in lipid. Lipid was weight 0.5g, and 1% sulfuric acid-methanol 20 mL was added, then they were heated at 70 °C in water bath for 30 min, 20 mL hexane were added in order to extract methyl ester production. The supernatant was removed, and 10mL n-hexane were added to clean once, then supernatants were merged into the bottle. Lipid components were detected by GC-MS-2010-plus gas chromatography - mass spectrometry.

Results and Discussion

Optimization of lipid fermentation conditions. Lipid fermentation experiment of the strain was designed by L_9 (3^4) orthogonal experiment, which was used to consider the effect of glucose amount (A), inorganic nitrogen amount (B), initial pH (C) and temperature (D) on lipid fermentation, optimized fermentation condition was determined used 4 factors and 3 levels orthogonal and lipid contents as test data by Soxhlet extraction method, the results are shown in Table 1. In Group 9 test, the 7 is the best combination, the best four factors are A3B1C3D2. With the increase of glucose, production of lipid increase and metabolism proceed with enough carbon. With the decrease of inorganic nitrogen source, lipid production rate increase. The process of lipid production is from the reaction of acetyl CoA carboxylase carboxylation. Fatty acid long chain was synthesized after many times of fatty chain extension and saturated or unsaturated. When nitrogen depleted, adenosine monophosphate deaminase activity of lipid producing microbes increases, a large number of the adenosine monophosphate (AMP) transform into inosine monophosphate (IMP) and ammonia, prompt the formation of acetyl-CoA and increase lipid production rate, so when the carbon-nitrogen ratio are relatively large, lipid production rate is high. When the pH is 5.5, the metabolically is the most active, and lipid production rate is the highest. When the temperature is 28°C, lipid production rate is the highest, too high or too low temperature will hinder the synthesis of fat cells. Lower temperature will effect enzyme activities. The melting point of unsaturated fatty acids is lower than saturated fatty acids, the melting point of short-chain fatty acids is lower than long-chain fatty acids. The contents of unsaturated fatty acids and short chain fatty acids increase, mainly including palmitic acid and oleic acid increase, so the temperature is not too high.

According to range analysis, the order of range is $D_B > D_A > D_C > D_D$, that is the order of four factors in the stage of lipid fermentation is inorganic carbon amount > glucose amount > initial pH > fermentation temperature. The range of inorganic nitrogen is largest, indicating its influence for lipid fermentation is larger than the other three factors. So, in the lipid fermentation process, the amount of inorganic nitrogen source is more important for oil-producing rates.

Verify the optimization condition of lipid fermentation. The optimum level of lipid fermentation is A3B1C3D2 by the orthogonal experiment. That is process conditions are that amount of glucose is 100 g/L, amount of inorganic nitrogen is 1 g/L, initial pH is 6.0, fermentation temperature is 28°C. At the process conditions, verifying the optimization condition of lipid fermentation is carried out, the result of lipid content is 30.56%, it showed that the optimum level of lipid fermentation from orthogonal experiment is the optimum conditions, which is the best condition in the actual operation.

Analysis of fatty acid in lipid. The composition of lipid were detected by GC-MS- 2010-plus gas chromatography-mass spectrometry, the main compositions of the T-5 are palmitic acid, oleic acid, linoleic acid, stearic acid(Figure 1 and Table 2), which were similar to vegetable fatty acid, it can be used as raw material for biodiesel.

Table 1 Results and analysis of lipid fermentation by orthogonal experiment

	A Glucose (g/L)	B Inorganic nitrogen(g/L)	C initial pH	D Temperature (°C)	Lipid content (%)
1	50	1	5.0	25	25.00
2	50	2	5.5	28	21.35
3	50	3	6.0	30	21.58
4	70	1	5.5	30	24.37
5	70	2	6.0	25	23.35
6	70	3	5.0	28	22.75
7	100	1	6.0	28	28.83
8	100	2	5.0	30	26.00
9	100	3	5.5	25	22.86
I j	67.93	78.20	73.75	71.21	
II j	70.47	70.70	68.58	72.93	
III j	77.69	67.19	73.76	71.95	
*k*j	3	3	3	3	
I j/*k*j	22.64	26.07	24.58	23.74	
II j/*k*j	23.49	23.57	22.86	24.31	
III j/*k*j	25.90	22.40	24.59	23.98	
*D*j	3.26	3.67	1.73	0.57	

Note: I j, content of lipid in level 1; II j, content of lipid in level 2; III j, content of lipid in level 3; kj, number of the same level; I j / kj average of test in level 1; II j / kj, average of test in level 2; III j / kj, average of test in level 3; *D*j, range value.

Table 2 Fatty acid composition of T-5 strain

Number	RT/min	Name	Molecular formula	Molecular weight	Composition of fatty acid (%)
1	32.614	methyl palmitate	$C_{17}H_{34}O_2$	270	25.10
2	35.051	methyl linoleate	$C_{19}H_{34}O_2$	294	20.99
3	35.118	Methyl oleate	$C_{19}H_{36}O_2$	296	25.17
4	35.431	Methyl stearate	$C_{19}H_{38}O_2$	298	4.78

Summary

Four factors and three levels orthogonal experiment were designed including variables of amount of glucose, inorganic nitrogen, pH and temperature, and the optimum conditions for lipid fermentation are determined, including amount of glucose is 100 g/L, inorganic nitrogen is 1 g/L, initial pH is 6.0, fermentation temperature is 28 °C. The results of range analysis showed that the effects of four factors on lipid fermentation are amount of inorganic nitrogen > amount of glucose > initial pH>fermentation temperature. The optimum fermentation conditions for lipid are verified, and the results showed lipid contents were generally higher, which was the best condition in the actual operation.

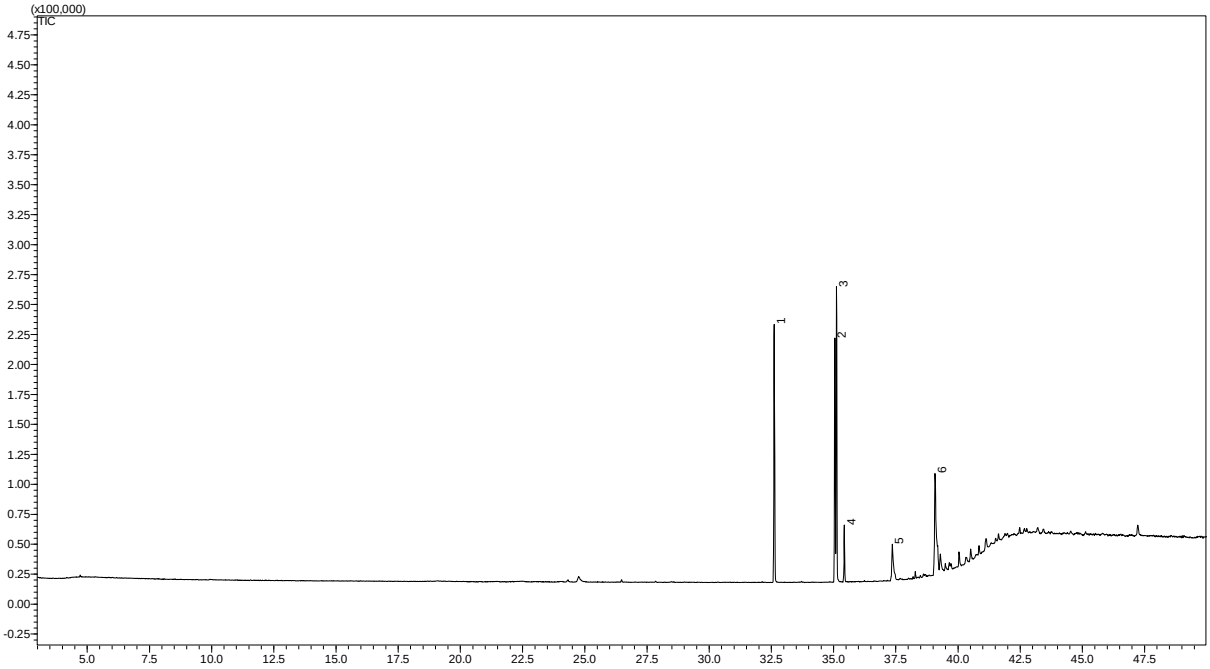

Figure 1 TIC for Adipic acid monomethyl ester

The lipid composition of T-5 strains were analyzed, the results showed that methyl palmitate was 25.10%, methyl linoleate was 20.99%, methyloleate was 25.17%, methylstearate was 4.78%, which was similar to vegetable oil.

Acknowledgements

This work was financially supported by science and technology planning project(2013030)of Jilin Institute of Chemical Technology.

References

[1] C.H.Chang: Ecological Engineering Vol. 27(2006), p. 49-57

[2] H.D.Jiang, Y.Y. Ling, S.S.Yang: Bioresource Technology Vol. 96(2005), p. 1633-1644

[3] Y.H.Li, B.Liu, B.Z.Zhao: Chinese Journal of BiotechnologyVol. 22(2006), p. 650−656

[4] Z.Yan, J.Chen: Journal of Cereals & Oils Vol. 3(2004), p. 43-46

[5] S. Papanikolaou, M. Komaitis, G. Aqqelis: Bioresource Technology Vol. 95(2004), p. 287-291

[6] A.D.Song, C.Feng, H.Xie: Food and Fermentation Industries Vol. 32(2006), p. 93-96

[7] Z.B.Zhao, Y.Y.Hua, B.Liu: Progress In Biotechnology Vol, 25(2005), p. 1-6

[8] F.L.Liu, X.G.Dai: Methods of food physical and chemical analysis(1991).