

## **Direct Transesterification of *Rhodococcus* Biomass for Production of Fatty Acid Methyl Esters**

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**Abstract:** *With increasing the world population demand for fuels for varieties of process is also increasing tremendously. This demand cannot be fulfilled for longer period of time because of limited availability of conventional fuels. Generation of renewable energy source can be an alternate solution. Production of biodiesel from varieties of sources is one of the alternate. It is a mixture mono alkyl ester produced by transesterification of triglycerides. Numbers of sources are used as feedstock for the production of biodiesel. These sources include animal based, plants based, microalgal and microbial oils. Here an attempt was made to produce FAMES using Rhodococcus strain. Rhodococcus was first forced to accumulate maximum lipids and then it was used for production of FAME by direct transesterification of biomass. In the whole process we have omitted lipid extraction which is crucial with other kind of sources. Production of FAMES was confirmed by gas chromatographic analysis.*

**Keywords:** *Rhodococcus, FAMES, Biodiesel, direct transesterification*

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### **1. INTRODUCTION**

Rudolf diesel has inspired and invented biodiesel. It was his first engine which was run peanut oil in 1893 and then after engine revolution was started. [1], [2] Biodiesel is mainly produced by either animal based feedstocks or plant based feedstocks. Both these kind of sources have certain advantages and disadvantages. One of the biggest disadvantages is extraction of lipids from these sources. [3] It is very time consuming and costly process. An alternate of that scientists have focused on microalgae. It is comparatively easy to grow and extract the lipids from microalgae but the major limitation is its cultivation time. [4], [5] Now a day researchers have focused on microorganisms.[6]–[8] Microorganisms are can grow much faster than microalgae and also able to accumulate lipids within them if provided with suitable environmental conditions. Reports have said that microbes are able to accumulate more that 60.0% of lipid within them. These lipids are triacylglycerol which could be easily converted to fatty acid methyl esters. [9] Selection of suitable method for production of biodiesel is also an interesting subject of research. In the reaction known as transesterification, triglycerides are allowed to react with alcohol in presence of either acid or alkali catalyst. Most preferred acid catalysts are HCl and H<sub>2</sub>SO<sub>4</sub> whereas NaOH and KOH are highly preferred alkali sources. Choice of catalyst is highly depend on the feedstock. [10]–[12] Here an attempt was made to produce biodiesel from Rhodococcus. The strain was first forced to accumulate the lipid at maximum possible and then used for production of FAMES. Here direct acid transesterification of microbial biomass was done without extraction of lipids. Gas chromatographic analysis done to confirm the presence of FAMES and obtained peaks were compared with standard FAMES.

### **2. MATERIAL AND METHOD**

#### **2.1. Strain and Media**

Isolated Rhodococcus strain use as a culture. LB broth containing yeast extract 10g/l, NaCl 10g/l, peptone 5 g/l, glucose 40 g/l, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1.4g, MgSO<sub>2</sub>.H<sub>2</sub>O 1.0 g/l, trace element solution 1.0 ml/l, phosphate buffer (1M) 35.2 ml/l, solution A 1.0 ml/l was used as production media. Trace element solution contain FeSo<sub>4</sub>.7H<sub>2</sub>O 0.5g, ZnSo<sub>4</sub>.7H<sub>2</sub>O 0.4g, MnSo<sub>4</sub>.H<sub>2</sub>O 0.02g, H<sub>3</sub>Bo<sub>3</sub> 0.015g, NiCl<sub>2</sub>.6H<sub>2</sub>O 0.01g, EDTA 0.25g, CoCl<sub>2</sub>.6H<sub>2</sub>O 0.05g, CuCl<sub>2</sub>.2H<sub>2</sub>O 0.005g per litre.

Solution A contains only NaMoO<sub>4</sub>.2H<sub>2</sub>O 2.0g/l. Phosphate buffer, trace element solution, and solution A were autoclaved separately and added to the sterile media aseptically.

## 2.2. Preparation of Seed Culture

Loop full cells from stock culture grown into 50 ml nutrient broth for 1 day at 37°C on rotary shaker (120 rpm) for overnight.

## 2.3. Culture Condition

Experiment was carried out in 250 ml flask containing 50 ml medium. Initial population of microbes were set to 1 X 10<sup>6</sup> cells (according to McFarland' Constant) and then allowed to grow under various environmental conditions. Two major parameters those affect the lipid accumulation were also studied. These parameters were incubation time and rate of aeration. To determine the optimum accumulation time microbes were incubated for 1 - 6 days. Production of biomass and lipids were determined at the regular interval of 24 hrs. To determine the effect of aeration microbes were grown at static, 120 rpm and 200 rpm. Biomass and Lipids were determined after optimum incubation time. During the whole experiment, concentration of glucose present in the broth after incubation was also determined by DNS method. [13], [14] Lipid was extracted by Bligh and Dyer method. Lipid was only extracted to determine the accumulation capability of microbes otherwise it is not required of directly used for transesterification.

## 2.4. Transesterification

After incubation biomass was collected by centrifugation the pellet were dried in oven at 55°C for overnight. To the dried biomass 20 volume of methanol containing concentrated sulphuric acid to a final concentration of 0.2 mol/L was added and mixed vigorously for 1 hour hr at 70°C. After completion of reaction the mixture was allowed to cool at room temperature. To the reaction mixture hexane was added and mixed. Upper hexane layer was collected and used for further analysis [15].

## 3. RESULTS AND DISCUSSION

### 3.1. Effect of Incubation Time

When lipids concentrations by microbes grown for different time intervals were compared, it was found that as the time increases accumulation also increase.(Table 1) initially no significant accumulation of lipids were observed but after incubation of 120 hrs it has increase at an exponential rate. This might be because of availability of enough nitrogen in the media. As it is known nitrogen is the major constituent for the synthesis of proteins and other biomolecules, since it is present in sufficient quantity in media, enzymes required for cell division and other metabolic activities were produced initially but as incubation time increases, availability of nitrogen in the media decreases resulted in to inhibition of production of certain enzymes required for cell division. Under this unfavourable condition microbes generally do not divide and start accumulating available carbon source in the media and store them as lipid droplets as reserve energy material. These lipid droplets are triglyceride which will be ultimately converted into FAMES upon transesterification. [7], [16], [17] When the concentration of glucose was determined it was found that as the incubation time increases the concentration of glucose decreases. However, it is not comparable with the concentration of lipid accumulated as it acts as key material for many other metabolic processes.

**Table1.** Lipid accumulation by *Rhodococcus* at various time interval

Sample	Time (hours)	Lipid %	Sugar (mg/ml)
1	48	1.298 ± 0.21	11.79 ± 0.11
2	72	1.3 ± 0.12	11.34 ± 0.14
3	96	1.38 ± 0.12	10.49 ± 0.09
4	120	2.8 ± 0.14	8.59 ± 0.10
5	144	7.4 ± 0.31	7.02 ± 0.07

### 3.2. Effect of Aeration

Rate of aeration has shown significant effect on lipid accumulation. When microbes were grown at static, 120 rpm and 200 rpm it was found that higher the rate of aeration increase the lipid

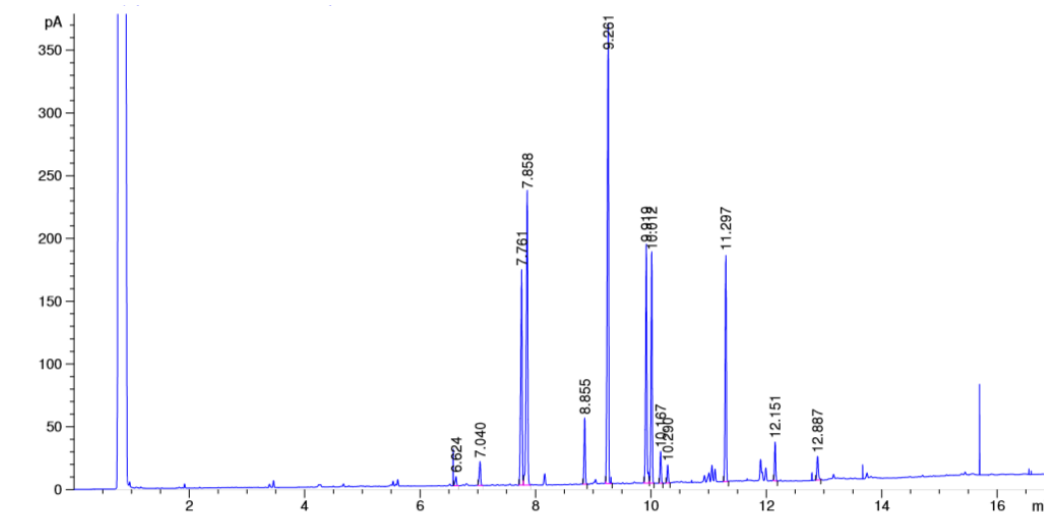
accumulation. (Table 2) This might be because of rapid availability of dissolved oxygen in the media which has favoured accumulation of lipids. [17]

**Table2.** Lipid accumulation by *Rhodococcus* at various aeration condition

Environmental Condition	Lipid %
Static	2.01 ± 0.12
120 rpm	4.16 ± 0.21
200 rpm	7.4 ± 0.31

### 3.3. Gas Chromatographic Analysis

Gas analysis has shown number of various FAMES present in the sample, these include C14:0, C16:0, C16:1, C18:0, C18:1, C20:0, C22:0 and C24:0 fatty acids. All these fatty acid contributes more than 80.0% of total FAMES present in the sample. Presence of long chain fatty acids and mono unsaturated fatty acids are indication of good quality of product.



**Fig1.** Gas chromatogram of FAMES produced from *Rhodococcus*

## 4. CONCLUSION

From overall study it was concluded that *Rhodococcus* can accumulate lipid in higher concentration if grown under specific environmental conditions. However there is a possibility to accumulate more lipid than the present study if concentration of carbon and nitrogen are optimized.

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