

Production of Lipids from Municipal Sewage Sludge by Two Stage Extraction Process

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In this investigation, municipal sewage sludge was used as a lipid feedstock. Two stage extraction processes was developed to obtain good yield of lipid content. The maximum lipid yields 32.5% was achieved from chloroform: methanol solvents through optimum conditions of 2:1 ratio, 50°C temp for 30 min. The lipid content was characterized by TLC analysis. The lipid properties were analyzed and proved as a lipid. Hence, municipal sewage sludge serves as a valuable raw material for lipid production.

Keywords : lipids, municipal waste, sludge, solvent extraction

INTRODUCTION

Municipal sludge has become serious environmental problem because of its disposal (Fenfen *et al.*, 2012). Municipal sludge consists of lipids which include triglycerides, diglycerides, monoglycerides, phospholipids and free fatty acids. The microorganisms were present in sewage sludge contains cell membrane, primarily composed of phospholipids (Kargbo, 2010). The municipal sewage sludge is formed during treatment of waste water from residential areas and small scale industries. This sludge consists of microorganisms and a variety of organic matter which includes lipids, greases, fats and long chain fatty acids (Girisha *et al.*, 2014). The Primary sludge is a source of low-quality fats and grease whereas the

secondary sludge is composed mainly of microbial cells and suspended solids produced during the aerobic biological treatment (Fortuny *et al.*, 2009). The quantity of lipid is high in primary sludge (Fenfen *et al.*, 2012).

The main component of waste water is the residual remaining oil such waste water should not be discharged (Suehara *et al.*, 2005). Municipal waste water plant produces more sludge is rich in microbial source which utilize the organic and inorganic compounds in the water as a source of energy, carbon and nutrients. Activated sludge is produced during the biological treatment of industrial and municipal wastewaters. The microorganism consists of lipid which can be extracted by solvents such as methanol, n-hexane etc., (Dufreche *et al.*, 2007).

Lipids are generally soluble in organic solvents; hence solvents are used to extract lipids from sludge. Some solvents such as ethanol, ethyl ether, petroleum ether, n-hexane and methanol have been used for extraction of lipids (Fenfen *et al.*, 2012).

Therefore, a two stage extraction process, using various solvents to extract lipid from sewage sludge. This lipid content rich in municipal sewage sludge may be used as a raw material for biodiesel production in future. Later, optimization of the extraction process yields % using chloroform; methanol solvent was established. Besides, lipid properties were analyzed according to standard analysis of lipids.

MATERIALS AND METHODS

Chemicals

Methanol (99.9% purity), n-hexane (99.9% purity), chloroform (99% purity), diethyl ether (99% purity), ethanol (99.9% purity), sulphuric acid (95-97% purity) and glacial acetic acid (99.9% purity) were purchased from Southern India Chemicals, Chennai, Tamilnadu, India.

Collection and Preparation of Sewage Sludge Sample

The municipal sewage sludge was collected from the Municipal Waste Water Treatment Plant located in Koyambedu, Chennai, Tamilnadu, India. The primary sludge was taken from primary clarifier and the secondary sludge was collected from secondary clarifier. The secondary sludge is also called as activated sludge and mainly composed of microbial cells

(protozoa, rotifiers and aerobic bacteria) and suspended solids.

The sludge and water contents were separated through gravity settling process. The sludge was filtered and dried. The dried sample was taken and then powdered using mortar and pestle. (Sparks *et al.*, 2007).

Extraction of Lipids

Lipids were extracted from the dried sludge using solvents. The extraction was done in the mode of single and two stage process using different solvents such as chloroform, methanol, diethyl ether, n-hexane and ethanol.

Two Stage Extraction Process: Primary and Secondary Sludge

A thermostatic glass reactor at atmospheric pressure was used for two stage extraction process. Three trials were carried out for each solvents and process conditions. Dried sludge (10 gm in each) was loaded for effective extraction using four different solvents. The extraction was executed for 6 h with solvent system as 50 ml of chloroform methanol mixture (2:1 ratio), 50 ml diethyl ether, 50 ml of n-hexane and 50 ml of ethanol. These samples were heated at 50°C for 30 min and transferred to a separating funnels and allowed for overnight for phase separation. The solvent was removed under vacuum at 400 mbar in a rotary evaporator.

The solvent removed from the first stage was used in second stage extraction process to increase a high yield of lipid content. This procedure was also done for extract of lipid from secondary sludge. The

samples were withdrawn from the two stage extraction process during the progress at predetermined time intervals to find out the yield of lipid content; the mixture was allowed to separate into two layers. The upper phase consists of lipids and the lower layer contains sludge waste with other impurities. The lower layers were taken into further extraction process. The lipid was collected from upper layer and washing with distilled water three times. Residual water was then removed by evaporation at 100°C. The final purified lipid was taken for TLC analysis and lipid properties examination.

The yield of lipid was calculated using the following formula

$$\text{yield of lipid (\%)} = \frac{\text{grams of lipid produced}}{\text{grams of dried sludge used in extraction}} \quad (1)$$

In this process, the various solvents were used to investigate their effects on lipid yield.

Determination of Quantity of Lipids

Solvents were evaporated from the extracts obtained from sludge by single

and double stage extraction at 70 degree C and the lipids was separated and weighed.

Process Flow Sheet

Process Flow Sheet of the experiment is depicted in Figure 1.

QUALITATIVE ANALYSIS OF LIPIDS

Spot Test

Lipids obtained by evaporating solvents were used as sample. A spot of the lipid obtained by extraction was placed on a white paper and the translucence was observed.

Ethanol Emulsion Test

Take 2ml of lipids in a test tube and add 2ml of ethanol to it. Changes in the test tubes were observed.

Confirmation of Lipids by Thin Layer Chromatography

Clean the glass slide with ethanol and coat with aqueous slurry of silica gel. Heat the slide at 110°C for 1 hour. Allow it to

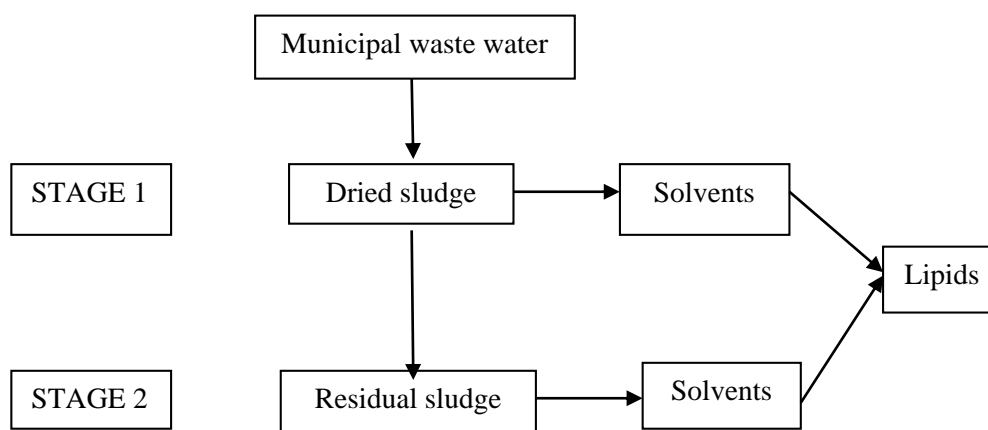


Fig. 1: Process Flow Sheet

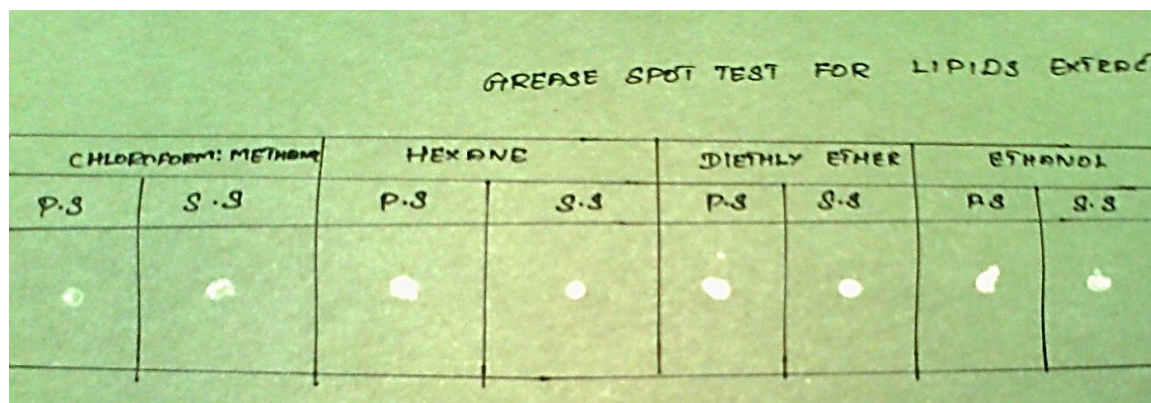


Fig. 2: Grease spot test for lipids

cool for sometimes and then spot the lipid sample over it. Keep the slides in solvents and allow it to move through the gel. Allow it to air dry for some time and then spray 50% sulfuric acid, then heat for 10 minutes. The formations of colored spots were observed.

RESULTS AND DISCUSSIONS

Extraction of Lipids

Lipids were obtained from primary sludge and secondary sludge by first stage extraction with solvents. The yield of lipids was calculated by weighing the lipids after evaporating the solvents.

Grease Spot Test

Transluminescence was observed when the lipids were spotted on the paper. Lipids obtained by evaporating solvents were used as sample. A spot of the lipid obtained by extraction was placed on a white paper. Transluminescence was observed when the lipids were spotted on the paper (Figure 2). This test shows the transluminescence property of lipids. The oil obtained from that primary sludge and

secondary sludge have this property.

Ethanol Emulsion Test

The lipid samples extracted from primary and secondary sludges with all the solvent extraction systems were tested in this method. 2ml of lipids were taken in test tubes and 2ml of ethanol was added to it. Changes in the test tubes were observed. Emulsion was formed when the lipid samples were dissolved in ethanol. This test shows the presence of lipids in the samples.

Thin Layer Chromatography

The lipids samples were dissolved in solvents. Silica gel was prepared as slurry and poured on glass plates and heated at 110°C for 10 minutes. Then 10 µl of samples were spotted on silica plates and kept in a solvent phase (petroleum ether: diethyl ether: water-80:19:1). When the solvent phase reaches 3/4th the plates were taken out and air dried. 50% sulfuric acid was used as coloring agent. Brown colored spots were obtained on silica gel when the lipids spotted, heated and kept in solvent phase. This test confirmed the

Table 1: Quantity of Lipids Extracted from Sludge

Solvents	Lipids Extracted from Primary Sludge (g)		Total (g)	Lipids Extracted from Secondary Sludge (g)		Total (g)
	Stage 1	Stage 2		Stage 1	Stage 2	
Chloroform: Methanol	2.75	0.50	3.25	1.90	0.55	2.45
N-hexane	1.83	0.62	2.45	1.65	0.45	2.00
Diethyl ether	1.72	0.60	2.32	1.70	0.45	2.15
Ethanol	1.10	0.40	1.50	1.26	0.50	1.76

presence of lipids in all the eight samples of lipids obtained from sludges. Brown colored spots were obtained on silica gel when the lipids spotted, heated and kept in solvent phase. 50% sulfuric acid was used as coloring agent.

Quantity of Lipids Extracted from Sludge

The quantity of lipids obtained from primary sludge in single stage extraction of Chloroform and methanol mixture was 27.5% and it was increased to 32.5% in second stage extraction. The amounts of lipids extracted from primary sludge with other solvents were comparatively lower than the mixture of solvents. In case of n-hexane the yield of lipids in single stage was 18.3% and 24.5% in further increase of stages of extraction from primary sludge. On extracting lipids from primary sludge by single stage extraction and double stage extraction with diethyl ether, the yield was 17.2% and 23.2% respectively. The amount of lipids extracted with ethanol from primary sludge was 11% in first stage and increased to 16% in second stage.

The yields of lipids on first stage extraction from secondary sludge were 19%, 16.5%, 17% and 12.6% with respective solvents. The amount of lipids obtained on increasing the extraction of lipids were 24.5%, 20%, 21.5% and 17.6% respectively. (Table 1)

Comparison of Yield of Lipids from Sludge

The extraction with a polar solvent help in destroying the cellular membrane and when it is followed by a non polar solvent, it will favors the lipids extraction within the cell (Dufreche *et al.*, 2007). The yield of lipids from primary sludge by using chloroform and methanol as co-solvent was higher when compared to use of single solvents. The lipids extracted from dried form of primary sludge were 32.5% by using this mixture of solvents. On comparing the amount of lipids extracted from secondary sludge the solvent mixture provides high quantity of 24.5%. The amount of lipids were obtained from primary sludge with hexane was 25.3% and from blended sludge was 21.9% (Olkiewicz *et.al*, 2012).

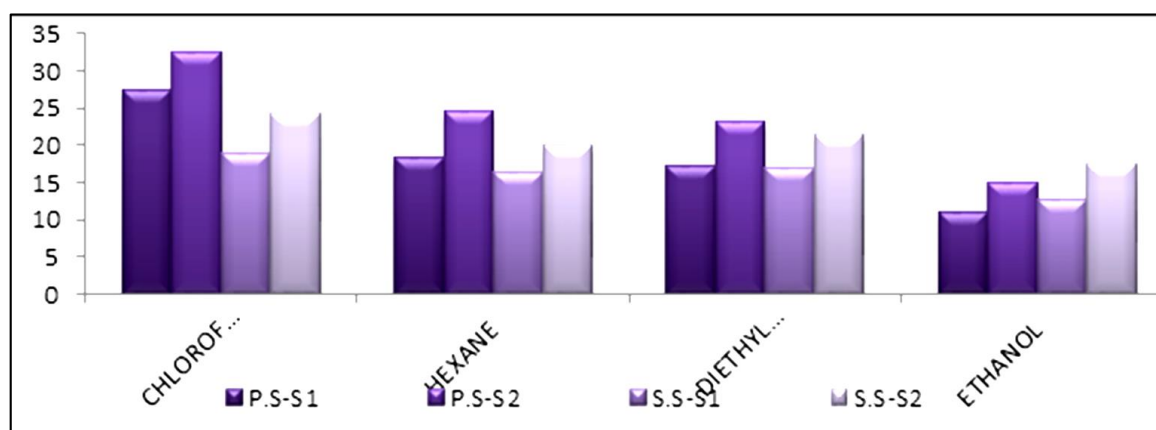


Fig. 3: Yield of Lipids from Sludges

The use of single solvent ethanol for the extraction of lipids from primary sludge and secondary sludge yields lower when compared to other solvents. The yield was only 15% and 17.6% respectively. The mixed solvent double stage extraction has higher efficiency when compared to single solvent extraction in two stages (Fenfen *et al.*, 2012). The results have showed that the solvents diethyl ether and *n*-hexane has moderate efficiency. The extraction tests yielded different lipid quantities depending on the extracting solvent. 4 %wt of the starting sludge mass was recovered as lipids when diethyl ether was used, whereas the recovered amount greatly increased when using *n*-hexane (8 %wt.) and methanol (14 %wt.) (Rebeca *et.al.*). They stated that methanol was the most effective solvent to maximize the recovering of the lipid fraction in the starting sewage sludge.

On comparing the results of yield of lipids from municipal sewage sludge, the use of chloroform with a co-solvent methanol in the ratio of (2:1) was found to

be effective and extract high quantity of lipids. (Fig: 3)

CONCLUSION

The municipal sewage sludge produced during waste water treatment has capacity to serve as the source of lipids. The polar and non-polar solvents were used for the extraction process. A mixture of chloroform and methanol produced maximum amount of lipids from primary as well as from the secondary sludge. From the results, it was clearly shown that the yield can be increased by increasing the number of stages of extraction.

In case of single solvent extraction with two stages, *n*-hexane produced high quantity of lipids when compared to other solvents such as diethyl ether and ethanol. Among the two types of sludge, primary sludge yields maximum amount of lipids than secondary sludge. Hence, the mixed solvent system with a polar and non-polar solvent can produce high quantity of lipids by two stage extraction system from primary sludge.

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