



Wastewater sludge as raw material for microbial oils production



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HIGHLIGHTS

- Different types of wastewater sludge were used to cultivated oleaginous microbes.
- Secondary sludge medium gave high lipid accumulation in microbes than other sludge.
- Suspended solids concentrations have great impact on lipid accumulation.
- Carbon to nitrogen ratio is important for lipid accumulation in microbes.

ARTICLE INFO

Article history:

Received 3 April 2014

Received in revised form 18 August 2014

Accepted 19 August 2014

Keywords:

Biodiesel
Wastewater sludge
Oleaginous microorganism
Sludge derived oil

ABSTRACT

Biodiesel is a promising alternative energy source as it is renewable, biodegradable, and environmental friendly. This study deals with oils production from wastewater sludge. Primary, secondary, and mixed municipal sludge and pulp and paper secondary sludge collected in Québec City, Canada has 5–11% oil content (based on weight) in initial sludge. The oil content is in the order of pulp and paper secondary sludge > municipal primary sludge > municipal mixed sludge > municipal secondary sludge. Different types of sludge were also used for lipid production by *Pichia amethionina* sp., *Galactomyces* sp., and *Trichosporon oleaginosus*. The results showed that maximum lipid content was obtained in municipal secondary sludge among all types. Further, the effect of initial sludge suspended solids concentration (10–30 g/L) on lipid accumulation was investigated. The maximum lipid content of 30.17% and 32.42% w/w dry weight, was reached by *P. amethionina* sp. and *Galactomyces* sp., respectively, at 25 g/L suspended solids concentration, and that of 37.69% w/w dry weight by *T. oleaginosus* at 30 g/L suspended solids concentration all with municipal secondary sludge. The results showed that carbon to nitrogen ratio had more obvious effect on lower suspended solids concentration (10 g/L) than on the higher one (30 g/L) in lipid accumulation of the three strains.

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1. Introduction

The predication of fossil resource depletion and the growing environmental concerns drive the development of alternative renewable energies. Biodiesel is gaining significant attention due to its renewable, biodegradable, non-toxic, and burning clean characteristics. However, biodiesel is not competitive with petro diesel in terms of the production cost [1]. The major cost (70% of total) of biodiesel production is from the utilization of feedstock oils and fats [2–4]. Vegetable oils and animal fats are the main source of biodiesel production in industry. The cost of these two types of oils is gradually growing due to the competition with food industry and kitchen. Moreover, the long life cycle (at most twice a year) and

large land taken make the production unfavorable and inefficient. The fact forces to seek replacements.

Waste cooking oils and fats are cheaper (<10 cents) comparing with original vegetable oils and animal fats. In USA, the daily waste cooking oil production is around 100 million gallons [5]. Converting waste cooking oils to biodiesel would efficiently reduce biodiesel feedstock cost as well as provide solution of waste cooking oil disposal. Talebian-Kiakalaie et al. [6] have reviewed biodiesel production from waste cooking oils through various technologies including membrane reactor, reactive distillation column, reactive absorption, ultrasonic and microwave irradiation process by using different catalysts. It was reported that the biodiesel production cost would reduce up to 90% of that produced from edible oils [6,7]. Waste cooking oils have shown great benefit for biodiesel production. However, several problems are present in biodiesel production from waste cooking oils. Firstly, it is a significant challenge to collect the waste cooking oils as these are most from

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households and no systematic collection method. In addition, waste cooking oils contain water and food particles. It requires a refining process before employing for biodiesel production. Moreover, it has high content of free fatty acid (FFA) which causes soap formation and consequently low biodiesel yield and difficulty of biodiesel recovery [6]. Additionally, waste cooking oils has high saturation rate which leads to a poor cold flow property [8–10].

Oleaginous microorganisms have been found to be very comparable alternative oil source due to their fast growth rate (several hours to several days), large lipid contents (up to 70% on dry microorganism weight basis), more amenable to genetic manipulation for further improvement of lipid profiles, and less land requirement as compared to oilseed crops and animals [11–16]. In addition, the feedstock oil properties are critical in biodiesel production as it determines the biodiesel properties. By comparing the feedstock properties, it can be learnt that microbial oil has similar properties as traditional feedstock oil (plant oil and animal fat) [17–20]. Moreover, as mentioned previously, microbial oil is abundant and sustainable. Therefore, using microorganisms as oil/fat source would be a favorable way in biodiesel production.

Utilization of heterotrophic oleaginous microorganisms for biodiesel production has been widely studied [21,22,12,23–25]. Glucose as carbon source tends to lead to high lipid accumulation in microorganisms [26,27]. However, it would result in high lipid production cost and make the microbial oil uncompetitive with edible oils for biodiesel production [28]. It drives a great interest in using organic waste as raw material for microbial lipid production. A recent report has reviewed the agricultural and industrial wastes employed for microbial lipid production [29]. Studies have exhibited that organic wastes were promising replacement of the expensive raw material glucose [30,31]. When considering to building a lipid production plant, the raw material should be abundant, easy to obtained, and has reliable supply.

Wastewater sludge is naturally produced in large quantity all over the world. It contains abundant nutrients that are essential for the growth of microorganism [32,33]. Studies have revealed that wastewater sludge could be used as medium for the growth of microorganisms such as *Acidithiobacillus ferrooxidans*, *Lipomyces starkeyi*, *Sinorhizobium meliloti*, and *Bacillus thuringiensis* [34–37]. The use of wastewater sludge as raw material to cultivate oleaginous microorganisms would reduce the cost of lipid production and mitigate the sludge disposal pressure. Angerbauer et al. [38] have investigated lipid accumulation in *L. starkeyi* with mixed (primary and secondary) sludge and observed that the mixed sludge could be used for lipid production in the microorganism. Other researchers have studied waste activated sludge as nitrogen source and glucose as carbon source for lipid accumulation in *Cryptococcus curvatus* [39–41]. The results were encouraging.

Wastewater sludge compositions vary according to the sludge type (primary, secondary, mixed) and wastewater source. To the

best of our knowledge, the effects of wastewater sludge component on lipid accumulation in microorganisms have not been reported. The aim of the work is to investigate lipid content in original wastewater sludge and the ability of wastewater sludge as a culture medium to produce microbial oil. Sludge components and initial suspended solids concentration impact on lipid accumulation in microorganism were demonstrated. Glucose and glycerol was utilized to study lipid accumulation enhancement with the addition of carbon source supplement.

2. Materials and methods

2.1. Strains

Oleaginous yeast *Pichia amethionina* sp. SLY, *Trichosporon oleaginosus* ATCC 20509, and fungus *Galactomyces* sp. SOF were used for lipid accumulation study. *P. amethionina* sp. and *Galactomyces* sp. were isolated from municipal secondary sludge and soil in our lab. Biolog system (BIOLOG Inc., Hayward, USA) was used for investigating metabolic potential of the strains according to their ability to utilize different carbon sources. Identified strains were grown on the tryptic soy agar plates for 24 h at 30 ± 1 °C and then preserved at 4 °C for further study. *T. oleaginosus* ATCC 20509 was subcultured and streaked on malt extract agar plates, incubated for 24 h at 30 ± 1 °C and then preserved at 4 °C for further study.

2.2. Basic medium

P. amethionina sp. SLY and *Galactomyces* sp. SOF were cultivation with N-limit synthetic medium (C/N ratio of 50) 40 g/L glucose, 1.0 g/L $(\text{NH}_4)_2\text{SO}_4$, 7 g/L KH_2PO_4 , 2 g/L NaH_2PO_4 , 1.5 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and 1.0 g/L yeast extract. *T. oleaginosus* was grown in a basic medium containing (gram per liter) 40 glucose, 2.7 KH_2PO_4 , 0.95 Na_2HPO_4 , 0.404 NH_4Cl , 0.2 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 yeast extract, EDTA 0.1, 0.04 $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.0055 $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.0052 citric acid- H_2O , 0.001 $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, and 0.00076 $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ [26].

2.3. Wastewater sludge as medium

Different types of wastewater sludge were used for microbial oil production study to investigate sludge component effect on lipid accumulation in microbes. The primary (PWS), secondary (SWS), and mixed (MWS) wastewater sludge samples were obtained from a municipal wastewater treatment plant, Communauté Urbain de Québec (CUQ), and the pulp & paper secondary wastewater sludge (PPSWS) was collected from White Birch Paper Industry located in Québec, Canada. The sludge was first concentrated by allowing it to undergo gravity settling at 4 °C for 24 h. The resulting solution had a suspended solids concentration (SS) around 20 g/L. To achieve high SS concentrations (up to 30 g/L), the sludge was centrifuged with SORVALL RC 5C Plus centrifugation at 5000 rpm for 15 min. The supernatant and concentrated sludge were stored for further utilization.

To study different types of sludge effect on the lipid accumulation, sludge with 30 g/L SS concentrations was employed. Municipal secondary sludge was utilized to investigate suspended solids concentration effect on lipid accumulation. The suspended solids concentrations, 10–30 g/L, were obtained by mixing the supernatant and concentrated sludge.

2.4. Carbon source supplement for lipid accumulation enhancement

Municipal secondary sludge with 10–30 g/L SS concentration was used in the experiment. Glucose and glycerol were added to the sludge to achieve the C/N ratio of 50–200.

Table 1
The characterization of sludge.

Properties	Primary wastewater sludge	Secondary wastewater sludge	Mixed wastewater sludge	Pulp & paper secondary wastewater sludge
TS (g/L)	27.58 ± 1.18	25.36 ± 0.95	25.65 ± 0.56	26.02 ± 1.48
TSS (g/L)	24.2 ± 1.02	20.7 ± 0.39	22.1 ± 1.37	22.9 ± 0.93
VSS (mg/L)	18.8 ± 0.24	15.5 ± 1.02	16.4 ± 0.47	20.5 ± 1.03
TC (g/kg TS)	483 ± 12.01	421 ± 15.19	451 ± 9.60	567 ± 14.61
TN (g/kg TS)	33.82 ± 0.69	49.91 ± 2.38	37.14 ± 1.11	15.14 ± 1.82
TP (g/kg TS)	34.13 ± 1.47	28.76 ± 0.83	31.59 ± 0.66	17.26 ± 0.29
pH	5.61 ± 0.01	6.42 ± 0.01	5.89 ± 0.03	6.52 ± 0.01

TP: total phosphorus; TN: total nitrogen; TC: total carbon; TS: total solid; TSS: total suspended solid; VSS: volatile suspended solid.

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