Journal of Environmental Management 198 (2017) 90–98

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Research article

# Thermo-chemo-sonic pre-digestion of waste activated sludge for yeast cultivation to extract lipids for biodiesel production

P. Selvakumar, P. Sivashanmugam*

*Department of Chemical Engineering, National Institute of Technology, Tiruchirappalli, Tamil Nadu, 620015, India*

ARTICLE INFO

*Article history:*
Received 21 December 2016
Received in revised form
24 February 2017
Accepted 20 April 2017

*Keywords:*
Municipal waste activated sludge
Economical feedstock
Ultrasonication
Oleaginous yeast
Microbial lipid
Biodiesel

ABSTRACT

The low cost biosynthesis of microbial lipids are an efficient feedstock to replace plant based oil for biodiesel production. The present study objective is to explore the effect of thermo-chemo-sonic pre-digestion of municipal Waste Activated Sludge (WAS) to cultivate oleaginous *L. starkeyi* MTCC-1400 as a model organism to produce high yield biomass and lipid. Higher Suspended Solids (SS) reduction (20 and 15.71%) and Chemical Oxygen Demand (COD) solubilization (27.6 and 22.3%) were achieved at a Specific Energy (SE) input of 5569 kJ/kg for WAS digested with NaOH and KOH, respectively. The maximum biomass of 17.52 g $L^{-1}$ and lipid 64.3% dwt were attained in NaOH pre-digested sample. The analyzed lipid profile exhibited high content of palmitic acid (45.6%) and oleic acid (38.7%) which are more suitable for biofuel production. Thus, these results strongly motivate the use of pre-digested WAS as an efficient and economical substrate for biodiesel production.



## 1. Introduction

The biodegradable nature of Fatty Acid Methyl Esters (FAMEs) along with its less toxic nature compared to fossil fuels makes it one of the most attractive renewable fuels. Its low emission profile and its compatibility with current commercial diesel engines coupled with persisting refueling technology make it distinct among other similar fuels existing in the market. Also, its ability to provide the energy density on par with diesel together with its exceptional lubricating properties makes it unique (Willson et al., 2010; Zhang et al., 2013).

The production costs of other biofuels are comparatively high because of the influence of its expensive raw materials basically derived from common lipid feedstocks like soybean, coconut oil, sunflower, palm, canola, and rapeseed making up around 70–85% of the overall biodiesel production cost (Mondala et al., 2009; Kargbo, 2010; Siddiquee and Rohani, 2011). Consistent increase in the shortage of agricultural lands over the years too has affected the cost of biofuels leading to a fuel crisis, which is directly proportionate to the shortage of food supply (Kwon et al., 2012). This necessitates a profuse search for new sources of raw materials which are cost-effective, non-edible and abundant. At this juncture, the advantages of the microbial oil with its shorter life cycle, requirement of minimal labour, easier to scale up of its production, and its usage without affecting the environmental conditions outplay the proficiency of animal fats and vegetable oils (Lopes da Silva et al., 2011).

Oleaginous yeast can be stored up to 70% of lipids in their dry biomass and this can be achieved to convert biodiesel using transesterification reaction with methanol in the presence of an appropriate catalyst (Guerzoni et al., 1985; He et al., 2010). The pattern of lipid accumulation in the microorganism has been well studied. It is known that the oleaginous species for their growth and lipid (Triglycerides) formation mainly utilize the carbon or similar sources with limited other nutrients usually nitrogen in cultivation medium. In addition to this, oleaginous species hold a significant amount of carbohydrates, proteins and other nutrients in addition to their normal lipids thereby increasing their industrial value (Sanchez et al., 2003). However, the high cost and demand of raw materials with sufficient nutrient sources for continuous microbial cultivation have always been a major issue for industrial development and its applications (Tao et al., 2010).

In the recent years, the number of wastewater treatment plants increased due to industrialization and urbanization. These plants are generating a huge amount of sludge which is rich in organic components such as proteins, carbohydrates, fibres and other

* Corresponding author.
*E-mail addresses:* selvaa26kumar@gmail.com (P. Selvakumar), psiva@nitt.edu (P. Sivashanmugam).

http://dx.doi.org/10.1016/j.jenvman.2017.04.064
0301-4797/

nutritional sources (Seiichi Inoije et al., 1996). According to the nutritional values, the waste activated sludge can be used for microbial cultivation to extract lipids, which will serve as an inexpensive raw material for the production of biodiesel. However, waste activated sludge needs appropriate pre-digestion for converting complex organics into simpler which can be efficiently utilized by microbe to convert lipids. To facilitate the sludge digestion, the various sole and combined disintegration methods such as thermal, thermochemical, biological and ultrasonic have been applied with high energy inputs, as reported in the literature (Uma et al., 2012; Kavitha et al., 2013; Gayathri et al., 2015). Based on the above background analysis, the present study aimed to develop a novel combinative (thermo-chemo-sonic) method with low energy consumption for effective pre-digestion of municipal waste activated sludge to cultivate the yeast for lipid extraction.

Till date, no attempt has been made for biotransformation of thermo-chemo-sonic pre-digested municipal waste activated sludge into valuable feedstock namely lipids for biodiesel production. Hence, the core objectives of this study are to (i) investigate and optimize the impact of operational parameters (physical and chemical) for thermo-chemo-sonic pre-digestion of waste activated sludge (ii) determine the energy utilization for effective sludge pre-digestion (iii) optimize the effect of different process parameters on biomass and lipid production using pre-digested municipal waste activated sludge as the nutritional source.

## 2. Materials and methods

### 2.1. Materials

The yeast strain was maintained on Yeast Peptone Dextrose (YPD) agar purchased from Himedia chemicals, Mumbai, India. NaOH (Sodium hydroxide) and KOH (Potassium hydroxide) with high purity were procured from Sigma–Aldrich (Bommasandra, Bengaluru, India) and used for pre-digestion of municipal WAS. Mixed component of chloroform and methanol purchased from Merck Chemicals Ltd., Mumbai, India was used for lipid extraction process. Other chemicals and solvents used in this study purchased were of analytical grade from Sigma Aldrich or Merck.

### 2.2. Yeast strain, culture conditions and inoculum preparation

Sample of L. *starkeyi*MTCC-1400 obtained from the Microbial Type Culture Collection (MTCC) Chandigarh, India, is used as a model strain for the purpose of this study. The yeast strain was sustained on YPD agar slants at 4 °C and it was sub-cultured twice in a month (Sherman, 2002). For stock culture, one loop of strain was inoculated in 10 mL of YPD broth and then incubated at 26 °C for 48 h for activation. Inoculum was prepared by 1 mL of stock culture was inoculated aseptically into 50 mL of enrichment medium consisting of (g/L): yeast extract, 10; glucose, 20; peptone, 10 and malt extract, 3 maintained under aerobic conditions at 30 °C with pH 6 in a reciprocal Shaker at 120 rpm. For the yeast cultivation process, 10% v/v of inoculum was loaded. All these media were autoclaved before use at 121 °C for 15 min with 15 psi (Xin Zhao et al., 2008).

### 2.3. Sludge collection and handling

Waste activated sludge was collected after partial thickening in the secondary clarifier by flotation from the MWTP in Madurai, Tamil Nadu, India, which has a capacity to process around 172.50 MLD of wastewater per day. Collected sample was immediately taken to the laboratory and stored at 4 °C before using it for the present study (Magdalena Olkiewicz et al., 2015).

### 2.4. Optimization of thermo-chemical pre-digestion

The sample was pre-digested through various processes and methods to make it pertinent for microbial cultivation. 150 mL of sludge was transferred into a 250 mL glass beaker and 1 N of NaOH and KOH were used to adjust the desired pH level of the sample. The samples in beaker were continuously stirred using a slow-speed mechanical stirrer (RQ-121/D). In order to reach the desired temperature, the beakers were submerged in a thermostatic water bath with the varied operating temperature from 50 to 90 °C. The samples were collected and analyzed at regular time interval. The experimental conditions of thermo-chemical digestion were optimized based on these above crucial factors.

### 2.5. Optimization of combined thermo-chemical-sonic pre-digestion

The sample with the optimized condition of thermo-chemical digestion was further subjected to sonic treatment. The combined digestion (thermo-chemo-sonic) was performed in a probe system (Sonicator (advance), Lark Innovative Fine Teknowledge, Chennai, India) that emits 25 kHz frequency through a tip with a diameter of 6 mm. For each digestion test, 150 mL of sample was filled in a 250 mL of stainless steel beaker and the ultrasonic probe was dipped into the sample to a depth of 1 cm. The desired temperature was maintained by placing the beaker in a water bath (Yiying et al., 2009). The samples were collected and analyzed at regular time interval to study the effect of the combined digestion process. The degree of Digestion (DDCOD) of both thermo-chemical and the combined digestion (thermo-chemo-sonic) were measured by COD solubilization which can be calculated as (Jeongsik et al., 2003).

$$\text{COD solubilization}(\%) = \left(\frac{\text{SCOD}}{\text{TCOD}}\right) \times 100 \quad (1)$$

### 2.6. Growth kinetics and biomass productivity

The yeast strain was cultivated in both control (undigested WAS) and different pre-digested sample medium. 200 mL of control and each pre-digested WAS sample was filtered and sterilized for cultivation. To each medium, 1.75% (w/v) of glucose was added as a carbon source to achieve the optimum C/N ratio of 150. Then, 10% (v/v) of inoculum was added when it reached the room temperature and each sample was incubated at the optimized condition of different parameters namely pH, temperature and rpm. A 0.5 mL sample was taken from each cultivation medium at 24 h time intervals for the measurement of specific growth rate by Optical Density (OD) at 600 nm using Spectrophotometer. The obtained data were plotted against time and were used to estimate the growth kinetics. Growth kinetic parameters were obtained in triplicates for each medium and the specific growth rate (μ) was calculated as

$$\text{Specific growth rate}(\mu) = \ln\left(\frac{\text{ODt2}/\text{ODt1}}{t2 - t1}\right) \quad (2)$$

where, $OD_{t1}$ and $OD_{t2}$ are the cell concentration at initial ($t_1$) and the final ($t_2$) of the logarithmic growth phase.

For biomass productivity ($P_{dwt}$), 1.0 mL aliquots of culture were collected from each medium at the end of the exponential phase and cells were harvested by centrifugation using cooling centrifuge (MPW-352/R/RH Centrifuge, MPW MED Instruments, Poland) for 10 min at 8000 rpm at 4 °C. Then, the pellets were washed with double distilled water, freeze-dried and dry weight was determined