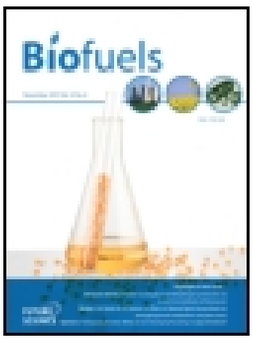

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Hydrothermal liquefaction of municipal wastewater sludge and nutrient recovery from the aqueous phase

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ABSTRACT

The hydrothermal liquefaction of municipal sludge was investigated under isothermal conditions (255 °C, 45 min) with TiO_2 as a catalyst. In this study, we used two separation methods (an organic solvent-assisted extraction method and the Soxhlet extraction method) for the production of bio-crude oil. The maximum yield of bio-crude oil was 20.7 wt. % reported with the Soxhlet extraction method. The aqueous phase was examined for TN, TP, COD, and TOC to determine the suitability of this phase for microalgae cultivation. Four strains of oleaginous microalgae were cultivated in the aqueous phase. The results show that the growth of microalgae in the aqueous phase was lower compared to the control medium; this may be due to the high COD value. Microalgae and yeast co-cultivation increases biomass and lipid productivity using nutrients in the aqueous phase.

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Hydrothermal liquefaction; sludge; biofuel; aqueous phase; microalgae; yeast

Introduction

Population growth is linked to the increased global demand for energy and the depletion of petroleum sources. Currently, petroleum sources satisfy almost 84% of the world's energy requirements, and petroleum consumption is estimated to increase by 50% in the coming years [1]. The use of biofuels reduces the emissions of particulate matter, CO and total hydrocarbons, thereby making the process biodegradable and less toxic [2–5].

Municipal sludge has a chemical composition of 20–30% proteins, 6–35% lipids and 8–15% carbohydrates [6]. Although sewage sludge contains important organic and inorganic constituents, it is often disposed of as an undesirable substance [7,8]. The increase in population leads to the production of a large amount of municipal sludge. This raises concerns about increasing contamination by metals and pathogenic microorganisms, the risk to human health, and its unpleasant odor [9].

Common municipal sludge disposal methods include landfilling, agricultural use and incineration [6]. In waste treatment plants, an activated sludge process is used in which microorganisms remove micro-pollutants [10]. The activated sludge process is expensive and time consuming; e.g. aerobic digestion takes 20 or more days, and anaerobic digestion takes 20–30 days. The conversion of municipal sludge by hydrothermal liquefaction (HTL) into bio-crude oil can replace non-renewable energy and reduce the volume of waste as well as eliminating pathogens and harmful constituents [11]. Therefore, alternative solutions for municipal sludge disposal become more important. The selection of a municipal sludge management strategy is based on energy requirements which depend mainly on nitrogen, phosphorus and the use of carbon as an energy source [12]. In this study, we have met these two parameters by producing hydrocarbon-rich crude oil and recycling the nutrient-rich aqueous phase through microalgae cultivation.

HTL is a process dependent on temperature (200–400 °C) and pressure (5–15 MPa), in which wet biomass can be converted into liquid fuels [13]. There is no need for an energy-consuming drying step, and the wet feedstock can be used directly [14]. HTL of organic waste leads to the generation of four products: gases, bio-crude oil, bio-char and the aqueous phase [13]. Previous studies have reported that the ideal temperature for maximum bio-oil and bio-char yield is in the range of 300–350 °C [15]. During HTL of bio-solids, low temperatures (250–300 °C) favor the formation of solids, whereas moderate temperatures (300–350 °C) and high temperatures (>350 °C) favor the formation of liquids and gases, respectively [16]. The HTL process based on high temperature (300–500 °C) has been reported in previous studies [17], but limited studies have been reported on HTL conducted at low temperature. Titanium oxide (TiO_2) is the main catalyst used in HTL in industrial and technical research due to its high thermal stability [18]. In this study, low-temperature HTL of municipal sewage sludge was performed and the Soxhlet extraction method was used for the recovery of bio-crude oil. Furthermore, the use of the aqueous phase for the cultivation of microalgae was investigated.

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Table 1. Proximate and ultimate analyses of the sludge.

Proximate analysis (%, dry basis)	
Ash	42.02 ± 0.1
Volatile solid	53.01 ± 0.2
Fixed carbon[a]	4.97 ± 0.1
Elemental (%, dry basis)	
C	45.2 ± 0.2
H	7.3 ± 0.1
N	9.3 ± 0.2
P	6.1 ± 0.1
O[b]	32.1 ± 0.2
HHV	20.04 ± 0.1 (MJ/kg)

HHV: high heating value.
[a]Calculated by difference; Fixed carbon % = 100% – (VS % + Ash %).
[b]Oxygen calculated by difference.

Materials and methods

Materials

Fresh sewage was collected from a primary settling tank constructed at the Uttaranchal University for municipal wastewater treatment using microalgae. Municipal wastewater contains solid residues of wastes from kitchens, hotels, restaurants and animals. In this study, we used one yeast strain (*Saccharomyces cerevisiae* UUIND1) and four strains of oleaginous microalgae (*Chlorella minutissima*, *Scenedesmus abundans*, *Chlorella singularis* UUIND5 and *Chlorella sorokiniana* UUIND6).

Proximate analysis of municipal wastewater sludge

The sludge was heated overnight at 105 °C; then the dry weight (wt. %), moisture, volatile solids and ash content were determined after heating the sludge at 550 °C for 4 h [19] (Table 1). The moisture content was estimated as 85.01 ± 0.2%. The elemental composition of the dried sludge was analyzed using an elemental analyzer (Table 1). FTIR spectroscopy with wavelengths of 4000–400 cm^{-1} was used to determine the chemical groups in the sludge feedstock. Traditional biomass (e.g. wheat straw, rapeseed straw, sawdust, etc.) has a high calorific value compared to sewage sludge [20].

Experimental setup and procedures

HTL was carried out in custom-made 50-mL stainless steel (SS-316) reactors. A muffle furnace (ACMAS Technologies Pvt-Ltd) was used for heating, at a rate of 5 °C/min. The reactor was charged with wet sludge and TiO_2 (powder, 325 mesh) in a ratio of 10:1. HTL experiments were carried out according to the modified method given by Kumar et al. [21]. Briefly, the reactor was heated to 255 °C (pressure 4.5 MPa) at a rate of 5 °C/min with a holding time of 45 min. After this heating, the reaction was cooled and then the reactor was opened immediately. The gaseous phase was vented off. The water phase and solid mixture were separated, using two methods, and the yields obtained by each method were compared.

In the first method, a Soxhlet apparatus was used as described by Kumar et al. [21]. The solid phase was first acidified with sulfuric acid (1.3 M) to pH 1–2 and then dried. Acetone was used as the solvent and bio-crude oil was extracted from the solid. The acetone solvent was recovered from the bio-crude oil by heating it to 60 °C. The extracted crude oil was designated bio-crude oil-1 (Figure 1). The extracted oil was mixed and weighed, and its elemental composition was determined with an element analyzer (Thermo Fisher). It was analyzed by gas chromatography–mass spectrometry (GC-MS; Agilent, Santa Clara, CA, USA) using the procedure of Kumar et al. [21].

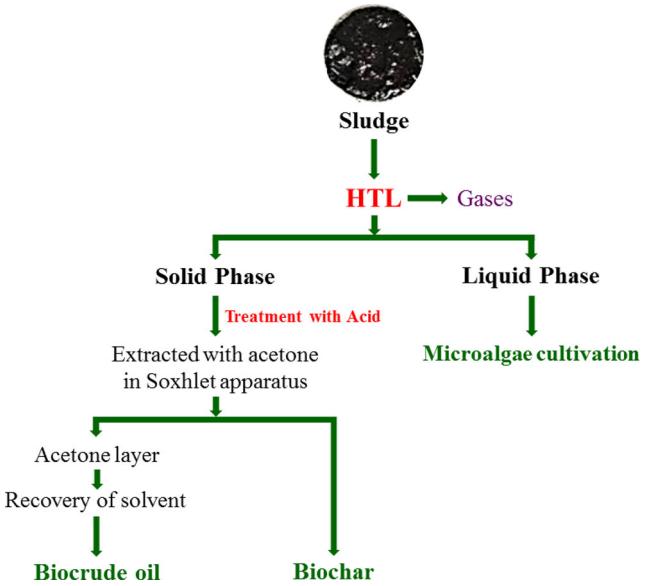

Figure 1. Soxhlet-based separation and extraction procedure of bio-crude oil from sludge. HTL: hydrothermal liquefaction.

In the second separation method, acetone was added to the solid phase to recover the oil. This procedure was repeated 3 times. The aqueous phase was treated with diethyl ether. The total bio-crude oil obtained was measured gravimetrically and designated bio-crude oil-2.

Calculation methods

For both approaches, the percentage bio-crude oil yield, high heating value (HHV) and energy recovery (ER) were calculated using the following equations [17,21]:

$$Bio-crudeoil = \frac{\text{Bio-crude mass}}{\text{Sample mass} \times \text{Slurry wt. \% solids}} \quad (1)$$

$$Bio-crude\ oil\ (wt\ \%) = Biooil/feedstock \times 100 \quad (2)$$

$$HHV\ (MJ\ kg^{-1}) = 0.3383 \times C + 1.442 \times (H - O/8) \quad (3)$$

$$Energy\ Recovery\ (\%) = \frac{Yield\ of\ bio-crude\ oil\ \% \times HHV\ bio-crude\ oil\ \%}{HHV\ sludge} \quad (4)$$

Water quality analysis of HTL aqueous phase

The aqueous phase of HTL was diluted with DM water to 1 L and filtered through Whatman Type 3 filter paper for water quality analysis. Total nitrogen (NO^{3-}, NO^{2-} and NH^{4+}) and total phosphorus (PO^{4-}) were analyzed using a HACH DR 5000 analyzer. A TOC analyzer was used to determine total organic carbon.

Table 2. Algal biomass (mg/L) cultivated in the aqueous phase at different concentrations.

Microalgae	100×	200×	400×	BBM	200× + 1% BBM	200× + 5% BBM
Scenedesmusabundans	100 ± 02	440 ± 02	No growth	902 ± 01	604 ± 01	640 ± 02
Chlorellaminutissima	197 ± 02	323 ± 01	No growth	784 ± 01	519 ± 02	765 ± 02
Chlorella sorokiniana	74 ± 01	2349 ± 02	73 ± 02	763 ± 02	469 ± 02	712 ± 01
Chlorella singularis	No growth	162 ± 01	40 ± 01	801 ± 01	382 ± 01	523 ± 03

Table 3. Elemental composition of bio-crude oil on a dry basis.

Elemental analysis (wt. %)	
C	62.11 ± 0.1
H	10.24 ± 0.2
N	8.72 ± 0.1
O[a]	18.22 ± 0.3
S	0.71 ± 0.2
HHV	32.45 ± 0.1 (MJ/kg)
ER	33.51 ± 0.2

[a]Oxygen calculated by difference.

Cultivation trials

Due to the high COD, it is necessary to dilute the aqueous phase of HTL with distilled water before using for microalgae cultivation. In this study, the co-cultivation of yeast and microalgae was also investigated. The growth trial was performed in two 100× and 200× dilutions with/without 1–5% Bold's Basal Medium (BBM) in a 500-mL flask with a 200-mL working volume of the four microalgae strains and single yeast strain. Microalgae culture assays in the HTL aqueous phase were compared with standard control BBM [22]. The concentrations in the aqueous phase of 200× + 5% of BBM for all strains of microalgae showed growth capability, but the growth was low compared to the control medium (BBM). Microalgae and yeast co-cultivation in a 3:1 ratio at 200× + 5% BBM + 0.5% glucose was selected for further study (Table 2). Microalgae strains were cultivated for 14 days under a photoperiod of 16 h light: 8 h dark under visible light (200 μmol m^{-2} s^{-1}). The growth rate was determined by absorbance using a spectrophotometer (Shimadzu 133 model # 1700) at 730 nm.

Determination of algal dry cell weight and total lipid

The algal biomass dry cell weight (DCW, g L^{-1}) was measured by drying microalgae biomass at 60 °C for 12 h in a hot air oven. The following equation was used to determine the productivity of the microalgae biomass:

$$Microalgal\ biomass\ productivity = \frac{Final\ DCW - Initial\ DCWt}{Cultivation\ time} \quad (5)$$

The total lipids were extracted from the dry biomass of microalgae using the methods of Chatsungnoen and Chisti [23]. Lipid productivity and yield were calculated using the following equations:

$$\text{Lipid content } \% = \text{Total lipids (g)}/\text{DCW (g)} \quad (6)$$

$$\text{Lipid productivity} = \text{Biomass productivity} \times \text{Lipid content } (\%)/100 \quad (7)$$

Data analysis

Data analysis was carried out by repeating the experiments in triplicate ($n = 3$). Data are presented as mean ± standard deviation with $p < 0.05$.

Results and discussion

Characterization of municipal wastewater sludge by FTIR

The FTIR spectrum of municipal wastewater sludge is shown in Figure S1 (Supplementary material). The bands at 3500–3000 cm^{-1} correspond to the OH stretching of alcohol and acids. The strong peaks at 3133, 2851 and 1539 cm^{-1} show the hydrocarbons (C-H) in the organic matter of the sludge [14]. The region of the band at 1637 cm^{-1} represents the amide groups. The spectral band at 1400 cm^{-1} of nitrite (N-O stretch) and 2345 cm^{-1} indicates nitrile (C≡N stretch) in the sludge [24]. Similar band patterns of sewage sludge were reported by Tian et al. [14]. The region of the bands from 1191 to 1000 cm^{-1} can be attributed to the OH vibration of hydrocarbons, mineral compounds and silicate compounds present in the sludge [25].

Bio-crude oil yield, analysis and energy recovery

The results indicate that a bio-crude oil yield 5 times higher was obtained from the Soxhlet separation method. Therefore, this first method was selected for further evaluation. The bio-crude oil yield was 20.7 wt. % with TiO_2 and 14.01 wt. % without the catalyst. Qian et al. [26], reported a bio-crude oil yield from sludge of 26.8 wt. % at 399 °C with 60 min of holding time (isothermal) and 27.5 wt. % at 499 °C with 1 min of holding time (fast HTL). Wang et al. [17] reported a yield of 47.45 wt. % of bio-crude oil at 270 °C with a holding time of 60 min using ethanol:water (1:1) and with $CuSO_4$ as a catalyst. In this study, we treated the solid phase with H_2SO_4 for the production of bio-crude oil. Research suggests that the high solids content increases the bio-crude oil in the HTL process and maintains an almost constant energy ratio [27].

In the GC-MS analysis, bio-crude compounds were identified using the NIST library. Eleven compounds were recognized on the basis of retention area % > 1. The compounds identified in the GC-MS analysis of bio-oil extracted from the sludge at 255 °C were mainly 2-pentene, 3,4-dimethyl, vanillin, phenol, 2-methoxy-4-(1-propenyl), dodecanoic acid, tetradecanoic acid, pentadecanoic acid, n-hexadecanoic acid, 9-octadecenoic acid, oleic acid, octadecanoic acid, and cis-vaccenic acid, with area % values of 0.21, 1.41, 1.61, 2.38, 9.63, 1.03, 59.73, 3.22, 5.22, 5.40, and 8.19, respectively (Figure S2, Supplementary material). The maximum compounds were similar to the compounds of HTL from sludge reported by Liu et al. [14]. They may differ from other compounds reported in sludge due to differences in sludge composition and GC-MS analysis procedures. The chemical composition and characteristics of bio-crude oil depend mainly on the feedstock and the reaction conditions of HTL [28]. The elemental composition of bio-crude oil on a dry basis was observed for C, H, N, O (oxygen by

Table 4. Algal biomass and lipid yield in the aqueous phase at 200× + 5% yeast.

	Scenedesmus abundans		*Chlorella minutissima*		*Chlorella sorokiniana*		*Chlorella singularis*	
Growth medium	Biomass (mg/L)	Lipid (%)	Biomass (mg/L)	Lipid (%)	Biomass (mg/L)	Lipid (%)	Biomass (mg/L)	Lipid (%)
Aqueous phase	640 ± 0.2	28.0 ± 0.2	765 ± 0.2	24.6 ± 0.2	712 ± 0.2	22.6 ± 0.1	523 ± 0.1	17.0 ± 1.1
Control (BBM)	892 ± 0.4	21.0 ± 1.1	874 ± 0.2	22.9 ± 1.3	713 ± 0.2	22.0 ± 3.2	826 ± 0.1	19.2 ± 0.2

BBM: Bold's Basal Medium.

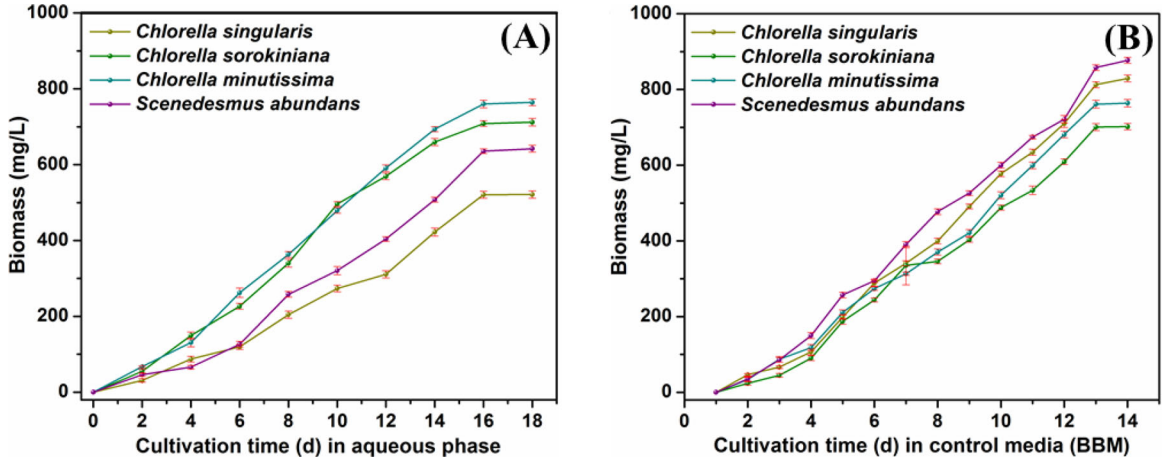

Figure 2. Growth of different microalgae cultures in aqueous phase of HTL 200× with 5% yeast and control medium (Bold's Basal Medium, BBM). The error bars represent the standard deviation around each point. HTL: hydrothermal liquefaction.

difference), and S as 62.11 ± 0.1, 10.24 ± 0.2, 8.72 ± 0.1, 18.22 ± 0.3, and 0.71 ± 0.2 in wt. %, respectively. The HHV and ER of the bio-crude oil were 32.45 MJ/kg and 33.51%, respectively. The bio-crude oxygen content was 8.62% (calculated by weight difference) (Table 3). The carbon content of the bio-crude oil was 62.01%. High contents of C and H increase the HHV of bio-crude oil, while high contents of O and H control the heating value [29].

Analysis of aqueous phase of sludge HTL

The biomass component affects the reaction and the characteristics of the aqueous phase during the HTL process [30]. The aqueous phase of the sludge was black in color. High amounts of COD (31401 ± 03 mg L^{-1}) and TOC (18490 ± 01 mg L^{-1}) were reported in this study. The pH, TN, and TP were found to be 7.9, 3517 ± 02 mg L^{-1}, and 1826 ± 01 mg L^{-1}, respectively. The organic matter of the feedstock dissolved in water leads to a high TOC [31]. Previous studies reported that the COD values of the aqueous phase of HTL ranged from 40 to 160 g L^{-1} for the non-lignocellulosic biomass, and the TOC values generally ranged from 2 to 35 g L^{-1} [30,32]. Nitrogen and phosphorus are the two main inorganic components of the aqueous phase of HTL, and these can be considered potential nutrients for microalgae cultivation [30]. The percentage of N content depends on the protein content of the feedstock used [33]. A high concentration of ammonia (1.9–12.7 g L^{-1}) was reported in sludge, algae, etc. The TP of the aqueous phase of HTL was generally in the range of 0.5–18.9 g L^{-1} [34].

Effect of the aqueous phase and co-cultivation of yeast and microalgae on growth, biomass and lipid productivity

The co-cultivation of microalgae and yeast (3:1) in a 200× aqueous phase + 5% BBM + 0.5% glucose achieved maximum algal biomass and lipid productivity in all microalgae strains. The growth of microalgae in the aqueous phase alone was lower compared to the control medium; this may be due to the high COD value and the presence of a catalyst that affects the intracellular metabolism of microalgae (Figure 2). The aqueous phase of HTL lasted the logarithmic phase of the microalgae from day 7 to day 16; this may be due to some traces of TiO_2. The highest lipid yield of 28.0 ± 0.2% was recorded in *Scenedesmus abundans* followed by *Chlorella minutissima* (24.6 ± 0.2%), grown in the aqueous phase (Table 4). Under stress conditions, there was an increase in the lipid productivity of the microalgae [35]. Oxidative stress in microalgae leads to the formation of reactive oxygen species which are responsible for the accumulation of lipids inside the microalgae cells . Cordova et al. [36] used aqueous-phase HTL for yeast cultivation to produce lipids.

Effects of nitrogen and phosphorus on microalgae

Growth kinetics and lipid accumulation in microalgae cells depend mainly on the N/P ratio present in the growth medium . The total phosphorus present in the aqueous phase of HTL was 1826 ± 01 mg L^{-1}, and the total nitrogen content was 3517 ± 02 mg L^{-1}. The HHV was observed to be 28.12%. The rate of growth of microalgae is directly related to the removal of nitrogen and phosphorus from wastewater [37]. In this study, *Chlorella minutissima* showed the maximum growth rate and therefore the maximum removal of nitrogen (91%) and phosphorus (85%) (Table S1, Supplementary material), while *Scenedesmus abundans* showed the lowest removal of P (81%) and N (84%). Nitrogen limitation in the growth medium leads to a greater accumulation of lipids in microalgae cells [35,38]. Microalgae are able to absorb N from ammonium, nitrate, nitrite, urea, etc. [39]. Ruiz-Marin [40] reported that *C. vulgaris* and *S. obliquus* showed preferences for ammonium to use as N source in wastewater. Patel et al. [41] reported

that *Monoraphidium* sp., *Scenedesmus* sp., and the marine *Tetraselmis suecica* grow well at high P load in waste water, but do not increase the biomass yield. Rodolfi et al. ([42]) reported that with a sufficient phosphorus supply, continuous light and CO_2 enrichment, a significant increase in biomass productivity of microalgae occurred as compared to the control.

Conclusion

HTL of municipal sewage sludge was investigated for energy recovery and recycling of organic nutrients from the aqueous phase. HTL of sludge at 255 °C for 45 min with TiO_2 resulted in a bio-crude yield of 20.7%, HHV of 28.12% and energy recovery of 34.27%. The growth of microalgae in the aqueous phase was lower compared to the control medium due to the high COD and catalyst. Co-cultivation of microalgae and yeast increases biomass and lipid productivity using aqueous-phase nutrients. The highest lipid yield, 28.0 ± 0.2%, was recorded in *Scenedesmus abundans* when co-cultured with yeast in the aqueous phase (200× + 5%).

Disclosure statement

No potential conflict of interest was reported by the authors.

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