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Nutrient recovery from municipal sludge for microalgae cultivation with two-step hydrothermal liquefaction

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ABSTRACT

Coupling of hydrothermal treatment methods with microalgae cultivation within a municipal wastewater treatment process can allow internal recycle of chemicals to improve overall waste treatment efficiency and provide biofuel as by-product. In this work, municipal sludge was treated with high temperature water in two steps to selectively recover nutrients and cellulose-rich fractions to avoid Maillard reactions. In the first step, nitrogen and phosphorous compounds were recovered in the liquid product and cellulose was recovered in the solid residue. In the second step, the cellulose-rich solid residue (ca. 60 wt%), was treated with sulfuric acid to obtain glucose. Cultivation of a mixotrophic microalgae (*Euglena gracilis*) and a heterotrophic microalgae (*Aurantiochytrium*) with the liquid product and the isolated glucose product gave positive growth indicating that the proposed two-step hydrothermal liquefaction process allows production of oils and recycle of nutrients such as sugars, nitrogen and phosphorous derived from municipal sludge. Hydrothermal treatment is an effective solid reduction technique and is a feasible method for recycling nutrients for microalgae cultivation.

1. Introduction

Microalgae has a large potential for producing chemicals and liquid fuels due to its fast growth rate and high lipid content [1–4]. Microalgae strains such as *Aurantiochytrium* and *Botryococcus braunii* produce hydrocarbons that can be used as biofuels [5–7]. However, the high potential of algae as a chemical and fuel source has yet to be realized in mass production [8,9]. One of the major problems of mass cultivation of microalgae is the supply of nutrients such as nitrogen and phosphorous [8–11]. Analyses show that if scale up of microalgae oil feedstock production were implemented, the demand for nutrients would seriously compete with agricultural commercial fertilizer use and lead to sustainability problems [12,13]. To achieve sustainability, effective and economical methods to recycle nutrients from wastewater [14–16], microalgae [17–21] and lipid extracted microalgae [22] have to be considered.

Wastewater in municipal water treatment plants contains nitrogen and phosphorus compounds that are essential nutrients for microalgae cultivation. Autotropic microalgae can be cultivated with wastewater [16], however, the use of faster-growing heterotrophic microalgae requires a carbon source nutrient. Municipal sludge can provide the required carbon source for heterotrophic microalgae since it is rich in proteins and polysaccharides (cellulose). These natural polymers, proteins and polysaccharides, when converted into amino acids and sugars, can be used as nutrients for algae cultivation. However despite the high potential of municipal sludge as a nutrient source, many wastewater treatment facilities apply anaerobic digestion to produce biogas, that is followed by sludge dewatering and incineration or landfill [23,24].

In this work, hydrothermal treatment of the solid portion of municipal sludge is considered for obtaining nutrient sources for microalgae. The key point of originality described herein is the coupling of hydrothermal treatment with microalgae cultivation within the wastewater process to allow internal recycle of nutrients and to improve overall efficiency. Moreover, the methods employed herein reduce the amount of sludge that needs to be incinerated, which is as an important constraint for next generation municipal waste treatment processes in Japan [25].

High temperature water is a term used to denote a liquid state condition that is generally below 350 °C. High temperature water at its autogenous pressure undergoes dissociation compared with its state at room temperature [26]. Acid catalyzed reactions such as hydrolysis can be promoted by simple control of temperature without catalyst [27] so that the downstream processing is simplified. Under

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hydrothermal treatment, the liquid or solid effluent becomes sterilized as high temperature conditions destroy bacteria or other species that may compete with microalgae cultures. When incineration is used as an integral part of the wastewater treatment plant as required by regulations in Japan, waste heat may be used for generating the hydrothermal conditions.

Hydrothermal treatment of municipal wastewater has been studied for microalgae cultivation, where it has been demonstrated that filtered wastewater combined with algae residue can be used as nutrients for algae cultivation [28]. Thus, it can be inferred that liquid nutrients can also be obtained from the solid portion of municipal sludge with hydrothermal treatment. However, the hydrothermal treatment of the solid portion of municipal sludge to obtain nutrients for algae cultivation has not been examined. In this work, internal recycle of nutrients for microalgae cultivation was studied through hydrothermal treatment of the solid portion of municipal sludge as an integral part of the waste treatment process. In the study, heterotropic microalgae *Aurantiochytrium* [5–7] and mixotrophic microalgae *Euglena gracilis* [29–31] were used because both of these forms are being cultured to maximize oil production [5].

The objective of this study was to develop a method for converting the solid portion of municipal sludge into nitrogen and carbon liquid nutrients that can be used for microalgae cultivation. In this study, preconditioned municipal sludge was treated with water at temperatures from 225 to 275 °C. The yields of nutrients in the obtained effluent, such as nitrogen- and phosphorous-containing compounds and sugars were evaluated to understand the liquefaction phenomenon of municipal sludge during hydrothermal treatment. Cultivation experiments with microalgae *Aurantiochytrium* and microalgae *Euglena gracilis* were conducted with liquid nutrients obtained from the liquid and solid effluents of the hydrothermal treatment. Estimation of the reduction of solid materials provided by hydrothermal treatment is made.

2. Experimental

An overview of the methods used in this work is given in Fig. 1. First, preconditioning of the municipal sludge was performed as a step to sterilize the solids, facilitate analyses, and to insure uniformity of solids for all of the performed studies. Then, hydrothermal treatment was conducted on preconditioned municipal sludge samples. The products of the hydrothermal treatment were separated into liquid product (L_{HT}) and solid residue (S_{HT}) by filtration. Acid hydrolysis was conducted on the S_{HT} to obtain an acid saccharified liquid product (L_{AS}). Then, the L_{HT} and L_{AS} were used as nutrient sources for microalgae cultivation.

2.1. Materials and methods

Water was purified with a distillation apparatus (Yamato Co., model WG-220) that produced water having a conductivity of 5.5 μS·m^{-1}. Glucose (>98.0%, Wako chemicals) was used for a standard to construct calibration curves for HPLC analyses and also as a nutrient for the microalgae cultivation experiments. Sulfuric acid (>95.0%, Wako chemicals) was used in the acid hydrolysis treatment of the solid residues. Calibration of the total organic carbon and total nitrogen analysis was conducted with potassium hydrogen phthalate (0.1 mol/L, Nacalai Tesque Inc.) and acid potassium nitrate (>99%, Wako chemicals). Amino acid mixture standard solution, Type AN-2 (Wako chemicals) was used as standard for the amino acid analyses. Chemicals used for preparing GTY and STY cultures for *Aurantiochytrium* 18W-13a strain were, D (+)-glucose (>98.0%, Wako chemicals), tryptone (BD Biosciences, San Jose, CA), yeast extract (BD Biosciences, San Jose, CA), D (−)-sorbitol (>98.0%, Wako chemicals) and artificial seawater (coral pro salt, Red Sea USA, Houston, TX). Chemicals and procedures used for preparing AF-6 cultures for *Euglena gracilis* (NISE-48) are given in the literature [32,33].

Municipal sludge was obtained from Minami Gamo wastewater treatment facility located in Sendai, Japan [24]. The municipal sludge received was a 7 to 3 weight ratio blend of primary sludge and excess sludge that was dewatered to a moisture content of 80 wt% (moisture content of 76.7%: solid content 23.3%). The solid portion of the sludge had a combustible content of 85.7% and an ash content of 14.3% on a dry basis.

2.1.1. Microalgae and standard cultures and preculture methods

Euglena gracilis (NISE-48) and *Aurantiochytrium* 18W-13a strains used in the cultivation experiments were purchased from National Institute for Environmental Studies, Japan (NIES), and National Institute of Technology and Evaluation, Japan (NITE) respectively.

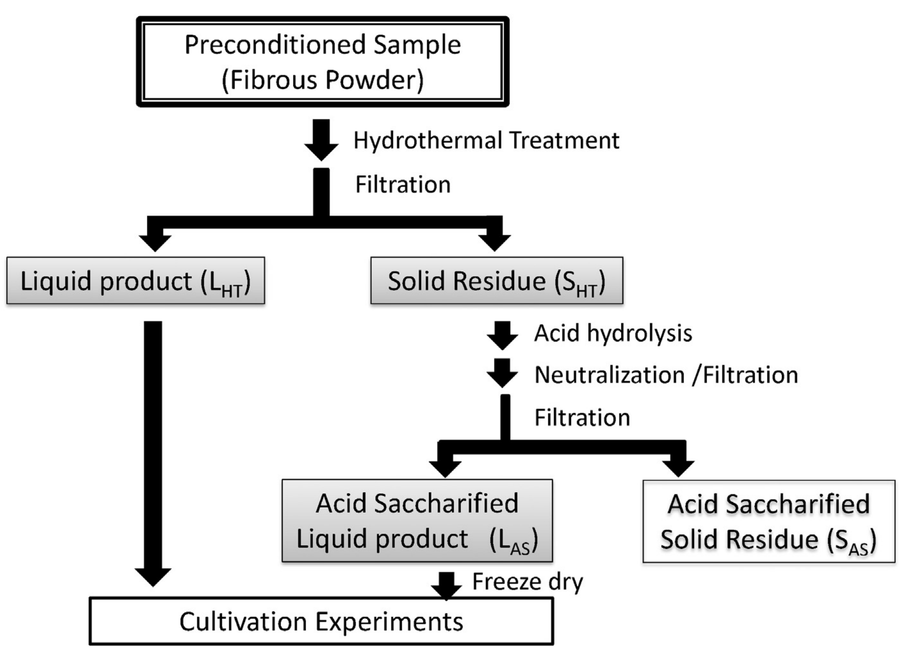

Fig. 1. Experimental flow of hydrothermal treatment of municipal sludge, acid hydrolysis of solid residue (S_{HT}) and cultivation experiments, using hydrothermal liquid product (L_{HT}) and acid saccharified liquid product (L_{AS}).