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Thermal Hydrolysis of Wastewater Sludge Followed by Fungal Fermentation for Organic Recovery and Hyphae Fiber Production

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ABSTRACT

Wastewater sludge creates a difficult environmental problem for many large cities. This study developed a three-phase innovative strategy for sludge treatment and reduction, including thermal hydrolysis, fungal fermentation, and anaerobic digestion. Increasing the temperature during the treatment from 140 to 180 °C significantly improved the sludge reduction and organic release efficiencies ($p < 0.05$, one-way analysis of variance (ANOVA) for the triplicate experiments at each temperature). After two cycles of thermal hydrolysis, the overall volatile solid reduction ratios of the sludge were 36.6%, 47.7%, and 58.5% for treatment at 140, 160, and 180 °C, respectively, and the total organic carbon (TOC) conversion efficiency reached 28.0%, 38.0%, and 45.1%, respectively. The highest concentrations of carbohydrates and proteins were obtained at 160 °C in sludge liquor, whereas the amount of humic substances significantly increased for the treatment at 180 °C ($p < 0.05$, one-way ANOVA for the triplicate experiments at each temperature) due to the Maillard reaction. Fungal fermentation of the hydrolyzed sludge liquor with *Aspergillus niger* converted the waste organics to valuable fiber materials. The biomass concentration of fungal hyphae reached 1.30 and 1.27 $g{\cdot}L^{-1}$ in the liquor of sludge treated at 140 and 160 °C, corresponding to organic conversion ratios of 24.6% and 24.0%, respectively. The fungal hyphae produced from the sludge liquor can be readily used for making papers or similar value-added fibrous products. The paper sheets made of hyphae fibers had a dense structure and strong strength with a tensile strength of 10.75 $N{\cdot}m{\cdot}g^{-1}$. Combining fungal fermentation and anaerobic digestion, the overall organic utilization efficiency can exceed 75% for the liquor of sludge treated at 160 °C.

1. Introduction

Wastewater sludge is a by-product of the wastewater treatment process. The sludge yield coefficient is typically close to 0.5 in municipal wastewater treatment plants (WWTPs) using activated sludge [1–3]. In the conventional activated sludge process, 30%–50% of the organic pollutants, 30%–45% of the nitrogen (N), and more than 80% of the phosphorus (P) in the influent eventually end up in the sludge [4]. Because of its huge volume, high cost of disposal, offensive nature, and serious environmental risks, wastewater sludge has become a major environmental and social problem, especially for large cities [5,6]. Currently, the major approaches of sludge treatment and disposal include landfill, incineration, anaerobic digestion, and land applications [7]. The cost of sludge treatment and disposal can exceed 50% of the total cost of wastewater treatment [8]. Poor sludge dewaterability and the lack of effective utilization of the sludge are the two key challenges in the management of wastewater sludge [9–11]. The typical sewage sludge contained rich organic and nutrient substances including about 40% proteins, 14% carbohydrates, and 10%–25% lipids [12]. However, current treatments do not recover the large amounts of organic and nutrient resources in sludge. Therefore, it is imperative to develop efficient and sustainable strategies to mitigate the sludge problem.

In recent years, thermal hydrolysis has been increasingly used as an effective method for sludge treatment. Thermal hydrolysis

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can result in rapid sludge reduction together with the improved sludge dewaterability [13]. Although the thermal hydrolysis technology has many advantages (e.g., improved dewaterability, organic dissolution, and microbiological sterilization), it is still essential to improve the thermal hydrolysis efficiency and to find better utilizations for the products. Researchers have developed various means for the enhancement of sludge thermal hydrolysis, such as optimizing the operating conditions (e.g., temperature, residence time, and solid content) and adding chemicals [14,15]. Multicycle thermal hydrolysis treatment would improve the overall efficiency of organic dissolution [16]. However, few studies have examined to what extents the multiple thermal hydrolysis can enhance the sludge reduction and material release, let alone investigating the biodegradability and uses of the products of thermal hydrolysis.

Anaerobic digestion for methane production is the most popular technology for the recovery and use of the hydrolyzed organics of the sludge, and it has been adopted by WWTPs via the Cambi thermal hydrolysis process (THP) and BioThelys technologies [8,17]. The operational experience of full scale WWTPs has shown that the thermal hydrolysis of sludge increases the net electricity production of biogas by over 20% [17,18]. However, the market value of biogas has been continuously decreasing in recent years due to the much increased global natural gas production. It is therefore sensible to explore alternative strategies for resource recovery, for example, to convert the organic carbon in sludge to other more valuable products. Compared to anaerobic digestion, fungal fermentation can produce a large amount of fungal hyphae. The hyphae grow fast and interconnect to form a three-dimensional (3D) network of fibers that can be easily collected from the suspension [19]. Such fungal growth in sludge liquor transforms waste organic carbon into valuable fibrous materials. Fungal hyphae can be used as the raw microfibers in the fabrication of various functional products such as paper, textiles, biosorbents, carriers for catalysts, and carbon electrodes for energy storage [20–22]. Besides, these biomaterials are sustainable, biocompatible, and biodegradable, and have thus attracted growing research attention [23]. In addition, the supernatant of thermally hydrolyzed sludge has been fully sterilized with a very high organic content, which can be used as a suitable substrate for specifically designed fungal fermentation. However, to the best of our knowledge, no previous studies have applied fungal fermentation to sludge liquor to produce hyphae fibers for organic recovery and utilization.

This study developed a novel integration of the thermal hydrolysis of sludge and fungal fermentation of the sludge liquor to reduce the sludge and recover organic resources. The sludge reduction, dewaterability, organic release, and the characteristics of the sludge liquor and the treated sludge were comprehensively evaluated for each cycle of the thermal hydrolysis process. Before the conventional use of the hydrolyzed products for anaerobic digestion, fungal fermentation was applied to the sludge liquor to induce hyphae growth and produce value-added microfiber materials. The effectiveness and performance of the fungal fermentation were investigated, and the properties of the microfiber products were characterized.

2. Materials and methods

2.1. Experimental materials

Wastewater sludge was obtained at regular intervals from a full-scale municipal WWTP in Shenzhen, China. The WWTP used a secondary activated sludge process without primary sedimentation, and the sludge samples were excess sludge discharged from the secondary sedimentation tanks. The typical characteristics of the raw sludge are summarized in Table 1.

The fungal fermentation process used *Aspergillus niger* (CCTCC AF 2014010), which was purchased from the China Center for Type Culture Collection (CCTCC). *Aspergillus niger* is known for its strong environmental adaptability, growth of hyphae, and easy formation of mycelial pellets.

2.2. Thermal hydrolysis treatment of the wastewater sludge

The thermal hydrolysis of wastewater sludge was carried out in an electrically heated autoclave reactor with an inner volume of 1.0 L (GSA-1; Senlong, China). For each treatment, 600 g of the sludge mixture was placed in the reactor. The temperature program was as follows: pre-heating from room temperature to 100 °C, holding for 1 h, and then sequentially heating to a preset final temperature that was maintained for 1 h. The residence time of 1 h was chosen based on the treatment condition of thermal hydrolysis previously reported by others for a satisfactory sludge reduction and organic recovery result [24–26]. During the heating process, the sludge in the reactor was continuously mixed by stirring at (200 ± 2) revolutions per minute (rpm). After the thermal hydrolysis treatment, the condensate water was pumped quickly through the reactor for rapid cooling to decrease the temperature to below 50 °C within 3 min. The reactor was then opened and cooled down naturally. A previous study suggested that the condition of 4000 rpm can remove free water from the sludge by centrifugation while the removal of bound water would require a more stringent conditions and higher energy input [27]. The sludge mixture was centrifuged for solid–liquid separation or dewatering at 4000 rpm for 10 min. Samples were extracted from the supernatant and dewatered sludge, and the supernatant liquor was collected and then processed for fungal fermentation.

The wastewater sludge was subject to two cycles of thermal hydrolysis treatment and the potential organic recovery from the sludge was evaluated after each cycle. After the first thermal hydrolysis treatment cycle and the subsequent dewatering of the sludge by centrifugation, deionized (DI) water was added to the dewatered sludge until it again reached the weight of 600 g, and then the sludge mixture was re-suspended before the second cycle of treatment. The temperature program for the second thermal hydrolysis cycle was to heat from room temperature to a preset final temperature that was maintained for 1 h. After thermal hydrolysis, the reactor was cooled down by water cooling within 3 min, the sludge was centrifuged, and the supernatant liquor was collected. The sludge mixtures after the first and second cycles of thermal hydrolysis were named TH1 sludge and TH2 sludge. Three final temperatures, that is, 140, 160, and 180 °C, were tested for the thermal hydrolysis of the sludge. The schematics of the two-cycle thermal hydrolysis treatment of wastewater sludge and the sludge supernatant for subsequent biological utilization

Table 1
Typical characteristics of the sludge mixture for the experimental study.

Parameter	Value
Total solid (TS) content ($g\cdot L^{-1}$)	64.39 ± 4.16
Total volatile solid (TVS) content ($g\cdot L^{-1}$)	37.68 ± 2.03
Total chemical organic demand (TCOD) ($g\cdot L^{-1}$)	52.24 ± 0.71
Total organic carbon (TOC) ($g\cdot L^{-1}$)	18.96 ± 0.74
pH	6.96 ± 0.02
Ash (dry wt%)	41.40 ± 0.81
Carbon (C) (dry wt%)	30.24 ± 0.33
Hydrogen (H) (dry wt%)	5.10 ± 0.07
N (dry wt%)	4.42 ± 0.09
Sulfur (S) (dry wt%)	0.70 ± 0.03
P (dry wt%)	1.84 ± 0.02

are shown in Fig. S1 in Appendix A. The thermal hydrolysis treatment was conducted in triplicates on each sludge sample for each temperature and treatment cycle.

The analyses of the treated sludge and supernatant included the volume, weight, and various physical and chemical parameters such as solid content, organic concentration, organic composition, and molecular weight distribution. The dewatering ability of the sludge samples was also evaluated. The efficiency of the release of particular components during the thermal hydrolysis of the sludge was determined using the following equation:

$$\text{Conversion efficiency (\%)} = \frac{V_s \cdot C_s}{M \cdot \mathrm{TS} \cdot X_i} \times 100\% \tag{1}$$

where V_s (L) represents the volume of the supernatant removed from the sludge by centrifugation, C_s (g·L^{-1}) denotes a chemical concentration in the supernatant, M (g) is the total amount of the sludge mixture (600 g) before each treatment, TS (wt%) is the solid fraction of the sludge mixture, and X_i (dry wt%) is the proportion of the chemical component (dry weight based), such as the organic carbon, in the sludge. The dewaterability of the sludge mixture was evaluated based on the water content of the dewatered sludge after centrifugation at 4000 rpm for 10 min. The efficiency of volume reduction of the sludge after thermal hydrolysis and dewatering by centrifugation was calculated using the following equation:

$$\text{Volume reduction efficiency (\%)} = \frac{V_i - V_s}{V_i} \times 100\% \tag{2}$$

where V_i (L) is the volume of the sludge mixture before centrifugation.

2.3. Fungal fermentation

The supernatant of the sludge that had been treated with thermal hydrolysis was used as the organic substrate for the fungal fermentation. Before the fungal fermentation experiment, the sludge supernatant was centrifuged at 12 000 rpm for 10 min to further remove all particulate matters. This was conducted to enable a more accurate measurement of the amount of fungal production during the fermentation process. Ninety milliliters of the supernatant were placed into a 250 mL conical flask. After sterilization at 121 °C for 30 min, 5 mL of *Aspergillus niger* was inoculated into the solution liquor in the flask. The fungal fermentation experiment was carried out for up to 7 days in an incubator at 28 °C with a shaker at 150 rpm. Three replicates of the fermentation were carried out on each supernatant liquor sample. At the end of the fungal fermentation, all of the *Aspergillus niger* mycelium pellets were collected with a 0.45 μm membrane filter, and the amount of fungal biomass was measured after drying at 105 °C. The organic and nutrient concentrations in the liquid after the fungal fermentation were used to calculate the organic reduction and utilization efficiencies and the fungal biomass yield. The collected mycelium pellets were then used to make hyphae paper sheets as a fibrous product. In addition, the liquid after fungal fermentation was further processed with anaerobic digestion to test the potential of biogas production. The details of the hyphae paper making and anaerobic digestion tests are given in the Appendix A (the characteristics of the seed sludge for anaerobic digestion are presented in Appendix A Table S1).

2.4. Analytical methods

The sludge and supernatant samples were filtered through a glass-fiber membrane (pore size: 0.45 μm) before the liquid-phase components were analyzed. The typical parameters including the total chemical organic demand (TCOD), chemical organic demand (COD), total phosphorus (TP), ammoniacal nitrogen (NH_4^+–N), total solid (TS) content, and total volatile solid (TVS) content were determined according to the standard methods (see Ref. [28]). The solution pH was measured with a pH meter (PHB-4; INESA, China). The concentrations of total organic carbon (TOC) and total nitrogen (TN) were measured with a total organic carbon analyzer (TOC-L; Shimadzu, Japan). The carbohydrate content was determined following the phenol–sulphuric acid method using glucose as the standard (see Ref. [29]). The protein and humic acid contents were analyzed using the modified Lowry method [30]. The molecular weight distribution of the organic products in the supernatant of the sludge derived from the thermal hydrolysis was measured by gel permeation chromatography (GPC) (GPC-20A; Shimadzu, Japan). The elemental analysis (carbon (C), hydrogen (H), nitrogen (N), and sulfur (S)) of the sludge samples was performed with an elemental analyzer (Vario EL; Elementar, Germany). To determine the ash content of the raw and treated sludge, the dry samples were ignited in a muffle furnace at 575 °C for 4 h before analysis. Alterations in the chemical structural features of the sludge after the thermal hydrolysis treatment were analyzed using the Fourier transform infrared (FTIR) spectroscopy, following the method described in previous studies [31]. The morphology of the fungal hyphae and paper products were examined using scanning electron microscopy (SEM) (Supra 55 Sapphire; Carl Zeiss Microscopy GmbH, Germany).

In this study, the experiments of thermal hydrolysis, fungal fermentation, and anaerobic digestion were conducted in triplicates for each treatment condition. The measurement for each parameter of a sample was also performed in technical triplicates. The effects of the thermal hydrolysis conditions on the results of sludge reduction, fungal fermentation, and anaerobic digestion were analyzed statistically with the one-way analysis of variance (ANOVA) using SPSS (v 17.0) (IBM Corp, USA). The p-value less than 0.05 was considered to be significant for the statistical tests.

3. Results and discussion

3.1. Organic release from the sludge by thermal hydrolysis

The waste sludge was effectively treated by thermal hydrolysis, and the resulting release of organics into the supernatant would enable organic utilization and recovery. The liquid-phase properties of the sludge supernatant after the two cycles of thermal hydrolysis are presented in Fig. 1. Due to the complex nature of the sludge, a wide variety of products was expected from the thermal hydrolysis reactions. The lump-sum parameter of the TOC was used to represent the organic content in the supernatant liquor, and the efficiency of organic release from the sludge was calculated using Eq. (1). For the first cycle of thermal hydrolysis, the organic release efficiency increased remarkably with an increase of the reaction temperature from 140 to 180 °C ($p < 0.05$). The average TOC concentrations of the TH1 filtrate were 5 590, 7 548, and 10 280 mg·L^{-1} for the treatments at 140, 160, and 180 °C, respectively, which corresponded to conversion efficiencies of 20.1%, 27.8%, 34.1% for the organic carbon content in the raw sludge. The organic conversion efficiency, or the amount of organic released, for the TH2 filtrate, was considerably lower than that for the TH1 filtrate ($p < 0.05$), although the additional dissolved TOC still increased as the reaction temperature increased (Fig. 1(b)). For the complex organics in sludge, dissolution of such organics as humic substances would be difficult within 1 h or so of the thermal hydrolysis treatment. For the biomass-rich sludge, most cellulose components such as cell walls would not be hydrolyzed and dissolved. Moreover, a series of secondary reactions would also take place during the thermal hydrolysis process, including dehydration, dehydrogenation, decarboxylation,

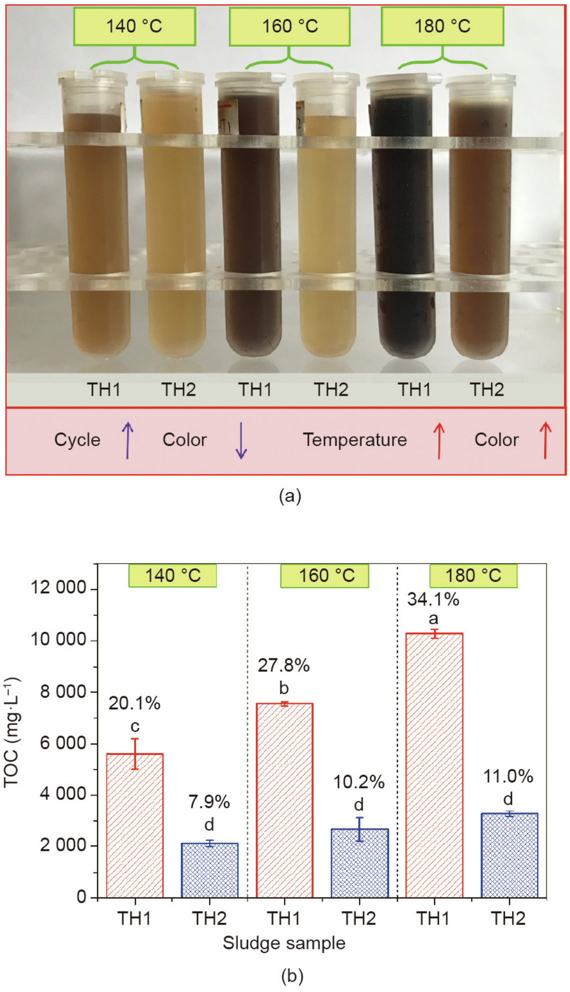


Fig. 1. Effects of the thermal hydrolysis temperature and treatment cycle on the release of organics from the sludge, with the organic conversion efficiencies stated: (a) photographs of the sludge liquor filtrates obtained, and (b) TOC concentrations of the sludge liquor samples and the related conversion ratios based on the raw sludge. TH1: first cycle of the thermal hydrolysis treatment; TH2: second cycle of the thermal hydrolysis treatment. Different lowercase letters above the bars in (b) indicate significant differences between the different treatment at $p < 0.05$.

decarbonylation, and condensation polymerization. These reactions led to a certain extent of aromatization and carbonization of the organic matter in sludge, as suggested by the results of FTIR analysis. All these factors limited the conversion of solid organics to soluble organics during the thermal hydrolysis process, and the difficulty of organic solubilization increased with the cycle of thermal hydrolysis.

After the two cycles of sludge thermal hydrolysis, the accumulated organic conversion efficiencies were 28.0%, 38.0%, and 45.1% on average for the thermal reactions at 140, 160, and 180 °C, respectively. Thus, the temperature was clearly the key factor affecting the efficiency of organic release from the sludge. Accordingly, the TS content and volatile solid (VS) content of the sludge decreased significantly after the thermal hydrolysis treatment (Appendix A Fig. S2). The average TS reduction efficiencies achieved with the TH1 sludge were 15.8%, 21.2%, and 28.3% for the treatments at 140, 160, and 180 °C, respectively, whereas the VS reductions reached 26.0%, 34.4%, and 45.8% compared to the raw sludge. After the second thermal hydrolysis cycle, the accumulated VS reductions were 36.6%, 47.7%, and 58.5% for the treatments at 140, 160, and 180 °C, respectively. These results suggest that thermal hydrolysis of the sludge provides benefits such as improving the sludge dewaterability, reducing the sludge volume, and dissolving solid organics for further fermentation and utilization. On the other hand, thermal hydrolysis would increase the sludge treatment cost and energy consumption. However, by heat exchange which is commonly practiced in large-scale applications, the heating energy requirement can be effectively reduced. Moreover, with the biogas production from anaerobic sludge digestion, a net energy output of the sludge treatment process can be achieved [26].

The dissolution and release of TP, TN, and NH_4^+–N from the sludge under the different thermal hydrolysis conditions were also analyzed (Appendix A Table S2). The highest TP concentration (52.5 $mg·L^{-1}$) was recorded for the TH1 filtrate treated at 140 °C. The amount of TP released in this study was much lower than that reported in some previous studies, which found TP concentrations as high as 500 $mg·L^{-1}$ [32]. After two cycles of thermal hydrolysis, only 4.5% of the TP in the sludge was dissolved into the supernatant at 140 °C. However, the results of this study were similar to a previous report of a rather low TP solubilization (1.5%) from the sewage sludge after thermal hydrolysis [33]. Interestingly, unlike the trend in TOC release, the TP release efficiency decreased as the thermal hydrolysis temperature increased. Brooks [34] noted a similar trend in TP release efficiency, that is, the amount of dissolved TP decreased when the reaction temperature exceeded 130 °C. The low TP release from the sludge into the supernatant was probably due to the precipitation of calcium and magnesium with phosphates in the reactor [33,35]. For the release of nitrogen, the TN and NH_4^+–N concentrations in the supernatant increased obviously as the temperature increased ($p < 0.05$). During the first thermal hydrolysis cycle, the TN concentration of the TH1 filtrate increased from 1585 to 2812 $mg·L^{-1}$ when the reaction temperature increased from 140 to 180 °C. After two thermal hydrolysis cycles, the accumulated TN conversion efficiency from the sludge reached 53.8%, 71.5%, and 84.2% for treatments at 140, 160, and 180 °C, respectively. Apparently, increases in reaction temperature resulted in more hydrolysis of proteins, as evidenced by the lower protein concentration (3272 $mg·L^{-1}$) at 180 °C than at 160 °C (4084 $mg·L^{-1}$) in the supernatant of the hydrolyzed sludge.

3.2. Chemical characteristics of the liquid-phase products

With the disintegration and degradation of microbial cells, extracellular polymeric substances (EPS), and other organic compounds during the thermal hydrolysis process, a large proportion of the organics in the waste sludge was converted into organic polymers and macromolecules. The representative products such as carbohydrates, proteins, and humic substances released into the liquid phase of the sludge during the different thermal hydrolysis conditions are shown in Fig. 2. The carbohydrate concentrations of the TH1 filtrate reached 1741, 2117, and 1672 $mg·L^{-1}$ for the treatments at 140, 160, and 180 °C, respectively. Similar to carbohydrates, the highest concentration of proteins was also recorded at 160 °C (4084 $mg·L^{-1}$). The soluble organics, including carbohydrates and proteins, released from the sludge can supply nutrient substrates for microbial growth [36,37]. Consistent with the trend of the total organic release over the two cycles of thermal hydrolysis treatment, the dissolved carbohydrate and protein concentrations decreased noticeably in the second treatment cycle.

The humic substances, with a mixed aliphatic and aromatic structure, might include carbonyl, alcoholic, phenolic, enolic hydroxyl, and carboxylic groups [38]. The dissolution of humic substances from sludge was strongly affected by the temperature of the thermal hydrolysis process. The humic concentration increased from 2366 to 9323 $mg·L^{-1}$ as the temperature increased from 140 to 180 °C, especially for the first cycle. Humic-like substances were apparently the primary type of organic components in the supernatant treated at 180 °C. In fact, the supernatant turned

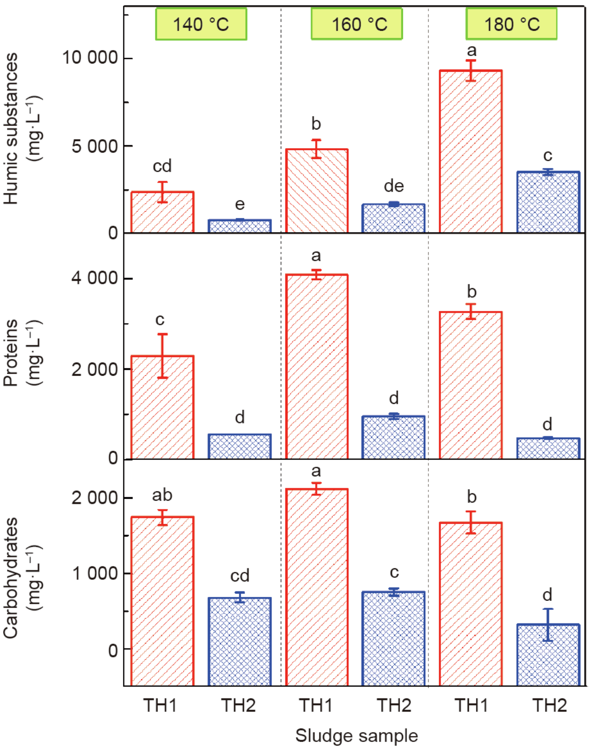


Fig. 2. Effects of the thermal hydrolysis temperature and treatment cycle on the release of soluble carbohydrates, proteins, and humic substances from the sludge. Different lowercase letters above the bars indicate significant differences between the different treatment at $p < 0.05$.

black and had an unpleasant odor when treated at 180 °C. Meanwhile, as the humic concentration increased at 180 °C, the carbohydrate ($p < 0.05$) and protein ($p < 0.05$) contents decreased significantly compared to the treatment at 160 °C. It has been recognized that at high temperatures, Maillard reactions occur between carbohydrates or proteins or both, which generate Amadori compounds or melanoidins [10,14]. Humic-like substances have a much lower biodegradability than carbohydrate- and protein-types of organics. It is apparent that the thermal hydrolysis products obtained at high temperatures, such as humic substances and Maillard products, would not be easily assimilated and used by microbiological processes. In other words, the thermal hydrolysis of sludge at a high temperature (e.g., 180 °C) is unfavorable for the intended purpose of organic utilization and recovery.

The molecular weight (MW) distributions of the organic products in the supernatant of the treated sludge were analyzed (Fig. 3). The results of the GPC method showed that the supernatant was dominated by small molecules with MWs less than 1 kDa, followed by those between 1 and 10 kDa. Previous studies on the molecular sizes of the liquid-phase products of anaerobic sludge after thermal liquefaction were somewhat larger than that, ranging from 10 to 40 kDa [39]. The MW distributions of the supernatants of the sludge obtained in the present study under different thermal hydrolysis conditions showed little difference, suggesting that the chemical bonds between the polymers and macromolecules (e.g., proteins and carbohydrates) in the sludge can be broken down by thermal hydrolysis at temperatures of 140 °C or higher.

3.3. Fungal fermentation and the hyphae product

Given the thermal hydrolysis temperature of 140 °C or higher, the treatment can achieve complete microbial sterilization of the sludge. The organic-rich sludge liquor would supply a suitable medium solution for fungal fermentation. In other words, this would provide a favorable condition with the suitable (sterilized) substrate to enable a purposely projected pure-culture fermentation to produce more valuable products. Fungal hyphae are a type of biofiber materials that can be more valuable than biogas generated by anaerobic digestion. Considering the potential economic benefit, fungal fermentation was first applied on the hydrolyzed sludge liquor for more value-added products before the conventional anaerobic digestion. Herein, the supernatant liquor, or the filtrate, of the sludge produced by the thermal hydrolysis was used to ferment fungus *Aspergillus niger*. Fungal growth was successfully achieved and mycelium pellets of fungal hyphae were observed in the supernatant solution (Fig. 4). The highest fungal growth rates

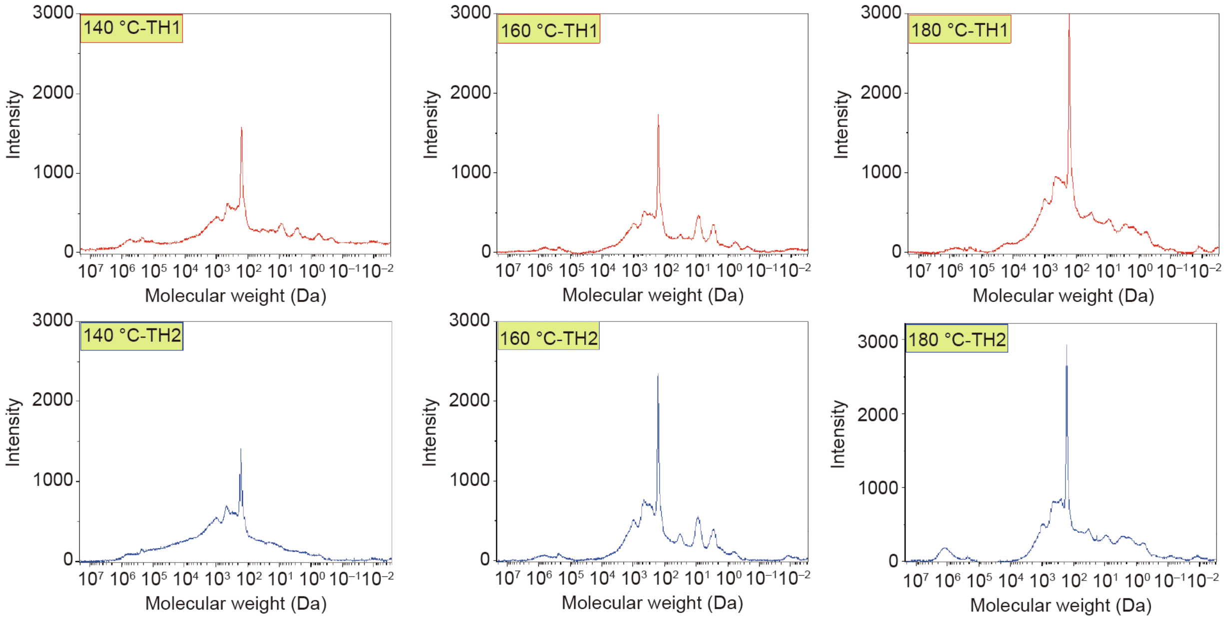
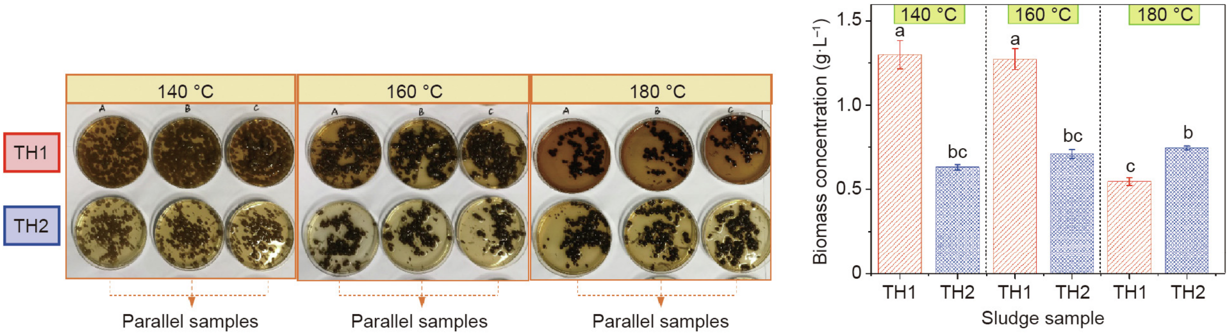


Fig. 3. Molecular weight distributions of the liquid-phase products in the supernatant of the sludge after thermal hydrolysis under different conditions.

were achieved using the TH1 filtrate of the sludge treated at 140 and 160 °C; the fungal biomass concentrations were 1.30 and 1.27 $g \cdot L^{-1}$, respectively. The corresponding organic conversion ratios of the TH1 filtrate for fungal growth were 24.6% and 24.0%, respectively, for these two treatment conditions. Thus, nearly a quarter of the waste organic carbon in the supernatant of the hydrolyzed sludge was converted into fungal biomass. Although the TH1 filtrate obtained in the 180 °C condition had a higher organic content than that obtained at 140 °C (cf. Fig. 1), the amount of fungal growth was much lower ($p < 0.05$). The high humic content in the sludge liquor obtained at 180 °C apparently hindered the fungal growth. It is obvious that the composition of organic substrates in the solution had a profound effect on the fungal growth, and that thermal hydrolysis of the sludge excess 180 °C was unfavorable for the fungal fermentation of the sludge liquor. The amount of fungal growth in the supernatant obtained from the second cycle of thermal hydrolysis (140 and 160 °C) was considerably lower than that from the first cycle ($p < 0.05$) because of the reduced supply of organic substrates. Nonetheless, the organic conversion ratios for fungal growth for these samples were still more than 20%. The fungus biomass yield was 0.87 and 0.69 grams of biomass produced per gram pf organic carbon degraded (g-biomass/g-$C_{degradation}$) for the TH1 filtrate of the sludge treated at 140 and 160 °C, respectively. As shown in Appendix A Fig. S3, tests using glucose and peptone, which are a more ideal substrate for fungal fermentation, under a similar condition produced biomass concentrations of 2.08 $g \cdot L^{-1}$ with a fungus biomass yield of 0.90 g-biomass/g-$C_{degradation}$. Thus, the biomass concentration produced by the fungal fermentation of the sludge supernatant could achieve over 60% of the amount produced using the glucose-based growth media. This suggests that treatment of the sludge by thermal hydrolysis followed by fungal fermentation for fungus hyphae growth can be an effective strategy for converting waste organics into valuable organic products.

During the fungal fermentation process, fungal hyphae were proliferated from small spots of about 1 mm to large mycelium pellets of 3–5 mm in diameter (cf. Fig. 5). The SEM images show that the mycelium pellets had a fibrous structure. The hyphae fibers entwined with each other to form a tight 3D network with features such as good stability and large specific surface areas. The hyphae collected from fungal fermentation can provide high-quality fibrous materials for value-added products. The fungal



(a) (b)

Fig. 4. Performance of fungal fermentation after 7 days of the supernatant liquors of the sludge after two cycles of thermal hydrolysis treatment at different temperatures. (a) Photos of the fungal hyphae pellets grown in the different sludge liquors; (b) biomass concentration of *Aspergillus niger* in the suspensions (fungal growth conditions: in an incubator shaker at 28 °C and 150 rpm for 7 days). Different lowercase letters above the bars in (b) indicate significant differences between the different treatment at $p < 0.05$.

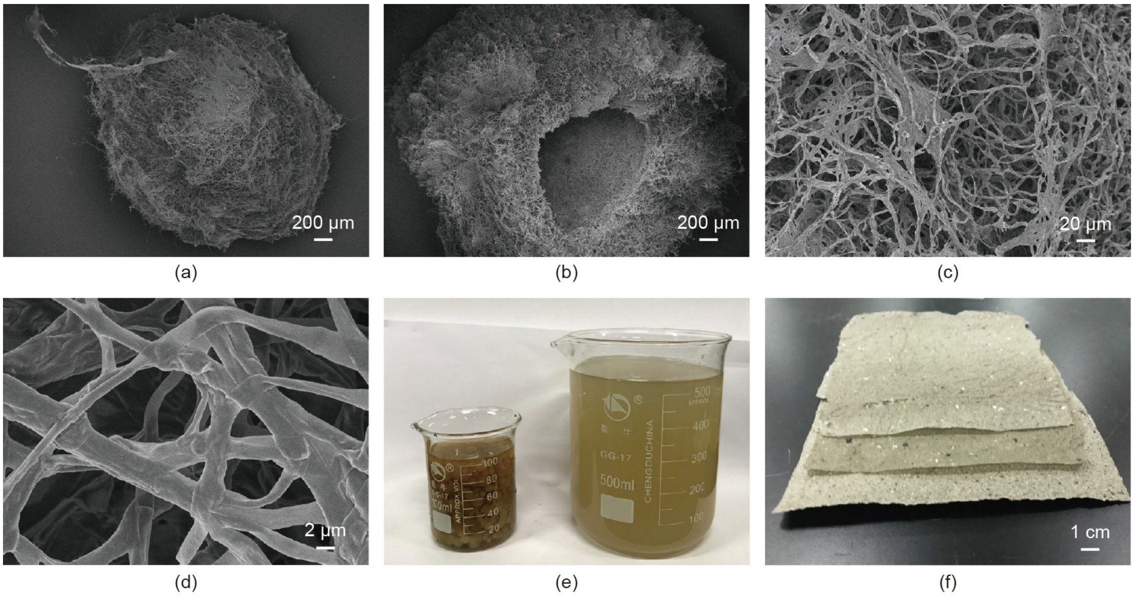


Fig. 5. Morphology of fungal hyphae collected after fungal fermentation of the sludge liquor and their paper-like products: (a, b) the overview of the mycelium pellets of the fungal hyphae observed by SEM, (c, d) SEM images of the 3D network structure of the fungal hyphae, (e) the suspension of fungal hyphae, and (f) the papers or foams of different thicknesses made of the hyphae fibers.

hyphae can be used to make paper sheets, packaging and filling materials, or as the raw substrate for carbon fibers. Figs. 5(e) and (f) show the paper made of fungal hyphae collected from the sludge supernatant. The paper-making process with the fungal hyphae can be referred to the typical paper-making process, which consisted mainly of hyphae collection, defibering, paper-making, pressing, and drying (cf. Appendix A Fig. S4). As an example, the as-produced hyphae paper had a grammage of 104.2 $g\cdot m^{-2}$. The paper sheets showed a dense fibrous microstructure under the SEM (Appendix A Fig. S5), and the representative physical characteristics of the paper product are listed in Appendix A Table S3. The hyphae paper had a ring crush strength of 2.59 $N\cdot m\cdot g^{-1}$ and a tensile strength of 10.75 $N\cdot m\cdot g^{-1}$. The paper sheet could be well bent and folded, exhibiting an excellent mechanical property. By varying the amount of hyphae fibers used, the thickness of the fibrous products can be controlled to produce paper sheets or soft foams for different needs. This paper-making with fungal hyphae presents an environmentally friendly transformation and recovery of organic carbon from wastewater sludge for the biosynthesis and fabrication of value-added products. In addition to the normal use, the hyphae papers can be carbonized to produce high-value carbon fibers or used as N-rich precursors for nitrogen-doped electrode materials [22].

The residual supernatant solution from the fungal fermentation was used for anaerobic digestion to produce biogas. For the residual supernatant of the sludge treated at 140, 160, and 180 °C (first cycle), the total biogas yields were 302.9, 312.3, and 298.1 mL per gram of COD, respectively (Appendix A Table S4). The results imply that the residual supernatant after fungal fermentation can still produce biogas through anaerobic digestion. Collectively, by both steps of biological fermentation processes, that is, fungal fermentation (24.1%) and anaerobic digestion (51.4%), an overall organic utilization efficiency of more than 75% was achieved for the supernatant of the sludge treated by thermal hydrolysis at 160 °C (Appendix A Fig. S6). This innovative strategy, including thermal hydrolysis, fungal fermentation, and anaerobic digestion, for sludge treatment can effectively realize sludge reduction and waste organic utilization. As given in Fig. S6, the yields of hyphae fibers and biogas from the hydrolyzed sludge liquor (160 °C) could reach 14.32 g and 62.5 L (about 48.0 g) per kilogram of dry sludge, respectively. Although the yield of biogas from the conventional anaerobic digestion (78.1 L or 60.0 g per kilogram of dry sludge in 160 °C) was higher, the hyphae fiber growth prior to anaerobic digestion would produce valuable fibrous products (e.g., paper sheets with a value of around \$20 $USD\cdot kg^{-1}$) or high-quality precursors for more expensive functional materials (e.g., carbon fibers with a market value of over \$70 $USD\cdot kg^{-1}$). This route of organic carbon recovery would add more value to the products than biogas production (i.e., methane with a value equivalent to \$0.2 $USD\cdot kg^{-1}$ or less). Take the treatment of 1 t wet sewage sludge (80% water content) as an example, the route of fungal fermentation prior to anaerobic digestion could recover 2.86 kg hyphae fibers and 9.6 kg biogas (about 70% methane), whereas the anaerobic digestion route could only obtain 12.0 kg biogas (about 70% methane). The primary economic analysis suggests that the potential benefits of this new process can be up to \$58.54 USD per tonne of wet sludge, much higher than that of the anaerobic digestion process (\$1.68 USD per tonne of wet sludge). Nonetheless, further studies remain to be carried out to demonstrate the long-term operation and cost-effectiveness of the integrated system for sludge treatment and resource recovery.

3.4. Characteristics of the treated sludge and improved sludge dewaterability

The solid phase of the sludge after two-cycle thermal hydrolysis treatment was also analyzed and compared to the raw sludge. As shown in Appendix A Fig. S7, almost all of the organic elements including C, H, N, S, and oxygen (O) decreased after the thermal hydrolysis, whereas the ash content increased [14]. The percentage of ash increased from 41.5% to 51.3% after the first thermal hydrolysis treatment at 160 °C and further increased to 56.2% after the second treatment. Increases in temperature led to reduced amounts of volatile substances in the solid phase of the sludge. It has been suggested that the H/C and O/C molar ratios might provide information about the reaction pathways of organics during the thermal hydrolysis process [40,41]. The H/C molar ratios of the sludge was around 2.0, and was not very altered by the thermal hydrolysis treatment. In contrast, the O/C molar ratios of the TH1 sludge decreased from 0.45 (raw sludge) to 0.41, 0.41, and 0.32 after treatment at 140, 160, and 180 °C, respectively. The low O/C molar ratios could be attributed to the decarboxylation reactions during the treatment [42], which appeared to be more significant at 180 °C.

The sludge was further analyzed using the FTIR to investigate the changes in the organic functional groups after the thermal hydrolysis. In the FTIR spectra given in Appendix A Fig. S8, the typical peak at 3421 cm^{-1} was assigned to the –OH stretching vibration [43]. The intensity of –OH became weaker with increases in the thermal hydrolysis temperature and treatment cycles, indicating that thermal hydrolysis enhanced the dehydration of the sludge. The two peaks at 2922 and 2852 cm^{-1} were attributed to the asymmetric and symmetric –C–H stretching of methylene groups, respectively [10]. The peaks of aliphatic $-CH_x$ became more intense for the thermally hydrolyzed sludge. This is consistent with the result of Wang and Li [10], who found that the relative intensity of aliphatic compounds became stronger after thermal hydrolysis. The decrease of the relative intensities was recorded at 1654 and 1560 cm^{-1} after the thermal hydrolysis treatment. These two peaks corresponded to the stretching vibration of –C=O in ketone and amide groups and the asymmetric stretching of –C=O in carboxylic groups, respectively [40]. The results suggest that decarboxylation reactions occurred during the thermal hydrolysis process, which was consistent with the change in the O/C ratio and the large amount of humic substances found in the liquid phase of the sludge. The intensity of aromatic C=C at 1458 cm^{-1} and aromatic C–H structure at 913 cm^{-1} increased consistently after the thermal hydrolysis. These alterations suggest that the sludge underwent carbonation and aromatization during thermal hydrolysis, especially at high temperatures and after multiple treatments [44]. A reduction in the relative FTIR intensity was recorded at 1406 cm^{-1}, which was assigned to the N–O binding in nitrite [45]. This was apparently caused by the dissolution of proteins and amino acids from the microbial cells into the liquid solution, leading to a decrease in the absorbance of N–O. The strong and broad band at around 1032 cm^{-1} was attributed to the superposition of C–O–R (R: different substituent groups such as aliphatic ethers and alcohol) and –Si–O stretching [40]. At elevated thermal hydrolysis temperatures and increased numbers of treatment cycles, the peak broadened, probably due to a reduction in C–O–R groups resulted from the dissolution of organic compounds in the sludge.

The dewaterability of the sludge improved significantly after the thermal hydrolysis treatment ($p < 0.05$). The solid contents of the sludge after dewatering by simple centrifugation at 4000 rpm for 10 min were compared (Fig. 6). For the raw wastewater sludge, dewatering was difficult, and the dewatered sludge had a solid content of 13.4%. In contrast, the solid content of the dewatered TH1 sludge increased to 16.7%, 18.1%, and 18.9% for treatments at 140, 160, and 180 °C, respectively. Correspondingly, the efficiency of volume reduction of the thermal hydrolysis sludge after centrifugation reached 69.8%, 72.8%, and 73.2% for the 140, 160, and 180 °C cases, respectively (Appendix A Fig. S9). Compared to the raw sludge with a volume

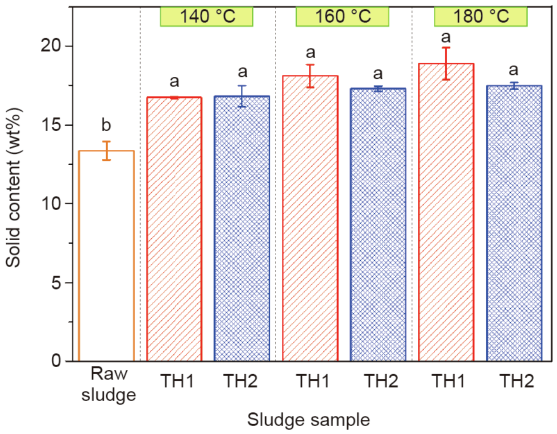


Fig. 6. Solid content after dewatering by centrifugation of the sludge treated by thermal hydrolysis. Different lowercase letters indicate significant differences between the different treatment at $p < 0.05$.

reduction efficiency of 51.9%, the thermal hydrolysis treatment significantly increased the volume reduction of the sludge by 35%–41% under the same centrifugation condition. For the thermal hydrolysis temperature higher than 160 °C, the improvement in sludge dewaterability and volume reduction with the increase of the thermal temperature became marginal, suggesting that 160 °C was sufficient for the purpose of sludge dewatering. These results are consistent with previous reports that a threshold temperature higher than 150 °C is necessary for efficaciously dewatering sludge [14]. Besides landfill, it is reported that the dewatered sludge after thermal hydrolysis can be converted into biogas via high-solid anaerobic digestion or into biosolid fertilizers via composting for beneficial land applications [46–48]. For the land application, the sludge does not need the process of chemical stabilization that would have to be commonly applied to the sewage sludge without thermal hydrolysis. Overall, 160 °C is apparently the optimal temperature for thermal hydrolysis of the wastewater sludge for the improvement of sludge dewaterability and recovery of usable organic resources from the sludge.

4. Conclusions

This study developed an innovative sludge treatment strategy, including thermal hydrolysis, fungal fermentation, and anaerobic digestion, to achieve sludge reduction, resource recovery, and dewaterability improvement. The overall VS reduction ratio and the TOC conversion efficiency of the sludge reached 47.7% and 38.0%, respectively, after two cycles of thermal hydrolysis at 160 °C. Fungal fermentation was utilized with *Aspergillus niger* to convert nearly a quarter of the waste organics in the sludge liquor to hyphae fibers or valuable fibrous products. As a value-added product, the paper made of hyphae fibers had a dense structure and good strength. Combining fungal fermentation for hyphae fibers and anaerobic digestion for biogas production, the overall efficiency of organic utilization was more than 75% for the sludge liquor obtained from the thermal hydrolysis treatment. The potential economic benefit of fungal fermentation followed by anaerobic digestion of the hydrolyzed sludge liquor can be much greater than that of only anaerobic digestion. These findings present a new sludge treatment method that integrates thermal hydrolysis and fungal fermentation to realize effective sludge reduction and waste organic valorization.

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Compliance with ethics guidelines

Jia-jin Liang, Bing Li, Lei Wen, Ruo-hong Li, and Xiao-yan Li declare that they have no conflict of interest or financial conflicts to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.eng.2020.09.002.

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