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Short communication

A novel biological treatment of hydrothermal carbonization wastewater by using *Thraustochytrium striatum*

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ABSTRACT

Hydrothermal conversion (HC) is a promising thermochemical technology to produce biofuels and bioproducts from biomass. However, the disposal of HC aqueous product (HC-AP) is one of the biggest challenges. This research investigated a new biological method using a marine protist, *Thraustochytrium striatum* to treat HC-AP from hydrothermal carbonization of municipal solid waste. A full factorial experiment was carried out to examine the effects of operation conditions on HC-AP treatment regarding cell growth, cellular lipid accumulation, and removals of total organic carbon (TOC)/total nitrogen (TN)/total phosphorus (TP)/recalcitrant compounds. Among four factors, only dilution rate and nitrogen concentration had significant effects on all responses, while salinity and pH were insignificant. Under the optimum conditions, *T. striatum* achieved 1.27 g/L dry cell mass and 14 % cellular lipid content while removing 82 % TOC, 53 % TN, 94 % TP, and ~89 % refractory compounds. This research offers a new biological platform for HC-AP treatment and valorization.

1. Introduction

Hydrothermal conversion (HC) [e.g., hydrothermal carbonization (HTC) and hydrothermal liquefaction (HTL)] has been considered as a promising thermochemical technology to produce biofuels and bioproducts from various types of biomass (e.g., lignocellulose, municipal solid waste (MSW), and microalgae) given its good scalability, inherent simplicity, and no requirement of dewatering [1–4]. During HC, biomass can be transformed into multiple coproducts, including hydrochar, bio-oil, gas, and an aqueous product (HC-AP). The bio-oil is an energy-dense fraction which can be further refined to drop-in liquid fuels, and hydrochar can be used as carbon materials (e.g., nanocarbon) and solid fuel, while the HC-AP is usually left behind as wastewater which contains a large quantity of organic compounds and nutrients. The sustainable disposal of HC-AP is one of bottlenecks in the scale-up of HC, which has been historically overlooked [5]. Hundreds of chemicals were identified in HC-AP depending upon the types of biomass and operation conditions [4–7], mostly including carboxylic acids, saccharides, hydrocarbons, phenols, ketones, amino acids, *N*-heterocyclic compounds, and alcohols [8–10]. Such complex compounds render HC-AP toxicity and inhibition toward both organisms and the environment (e.g., land and water) [11,12]. Thus, the HC-AP would cause serious pollutions to the environment without proper treatment. In addition, the HC-AP could contain ~80 % nutrients (e.g., N and P) and 30−50 wt% organic carbon of the feedstock [4,13]. Such high organic carbon and nutrient contents transferring to HC-AP represent a significant reduction of feedstock conversion efficiency and energy yield of HC. Therefore, the concerns about economic and environmental sustainability of HC require the treatment of HC-AP and/or valorization of HC-AP into value-added products [14,15].

Given its organic compositions and nutrients, HC-AP could serve as a nutrient (e.g., carbon, nitrogen, and micro/macro nutrients) source to support microbial growth. As such, biological approaches have been studied to treat and upgrade HC-AP, such as anaerobic digestion (AD) and microalgal cultivation because they are simple, cost-effective, eco-friendly, energy-efficient, and adaptable to complex components of HC-AP [1,5]. However, the existence of refractory and toxic compounds in the HC-AP often causes inhibitions to the biological treatment for which extensive dilution, long residence time, pH neutralization, and/or pre-treatment (e.g., chemical oxidation, adsorption, and extraction) are always needed [4].

Microalgae were used for recycling HC-AP, but heavy dilution

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(10∼1000 X) was usually necessary to mitigate the inhibition of HC-AP [16–19]. Du et al. [20] cultivated *Chlorella vulgaris* in HC-AP of algae and found that *C. vulgaris* effectively degraded total nitrogen (TN) (46–60 %), total phosphorus (TP) (86–95 %), and chemical oxygen demand (COD) (50–61 %) at 50–200 X dilution of HC-AP with maximum cellular lipid content of 11.2 % at both 50 X and 100 X dilution rates. The AD has also been employed to treat HC-AP, but it often suffers from inhibition by inhibitors (e.g., ammonia, furan, and phenol) and low organics degradation efficiency so that long lag phase, long residence time and/or extensive dilution (5∼1000 X) are required [21–23]. AD of HC-AP from corn silage and corn straw took long residence time of 91 and 33 days to achieve 80 and 65 % COD removal at 20 X and 5 X dilution rate, respectively due to the inhibitors (e.g., aromatic compounds and furfurals) in HC-AP [24,25]. A dilution rate of 1000 X was used in AD of the HC-AP from wastewater algae to reach 44∼61 % COD removal in 16–46 days [23].

Thraustochytrium striatum, a unicellular marine stramenopile protist, has been found in various habitats (e.g., plant detritus, salt marshes, rocky shores, fecal pellets of zooplankton, etc.) [26]. Such complex habitats render *T. striatum* an unique capability of tolerating environmental conditions (e.g., pH, temperature, and salinity) fluctuations [27]. A novel mutant of *T. striatum* (*T. striatum* HBS) recently has been developed in our lab. It is able to metobolize a variety of organics, including organic acids and aromatic compounds (e.g., acetic acid and lignin-derived phenolic compounds) [28]. In addition, this strain can produce lipids (e.g., oleic acid and palmitoleic acid) for value-added bioproducts even under extremely harsh conditions such as black liquor (a byproduct from paper or biorefinery industry) [29].

Therefore, *T. striatum* HBS was investigated for treating HC-AP in this research with the objectives being to study the effects of operation conditions (dilution rate, nitrogen source concentration, salinity, and pH) on HC-AP treatment effectiveness in terms of cell growth, lipid accumulation, and removal of total organic carbon (TOC)/TN/TP/aromatic compounds. This study offers important results about a new biological approach for HC-AP treatment and a "green" pathway to valorize HC-AP to value-added products (e.g., lipid) using *T. striatum*.

2. Materials and methods

2.1. Preparation of hydrothermal carbonization wastewater

A MSW mixture of known compositions (paper, plastic, metal, glass, and food) was created as the feedstock for HTC. The simulated MSW mixture included (dry weight basis): 16.4 % plastic (shredded plastic bottles), 17.6 % food (rabbit food pellets), 45.5 % paper (shredded discarded office paper), 9.6 % glass (crushed glass bottles), and 10.9 % metal (shredded aluminum cans) [30]. The solid content of the MSW mixture was increased to 20 % by adding deionized water (DI). HTC of the simulated MSW feedstock was conducted in a 500-mL pressure reactor (PARR 4570 Series) at 280 °C for 10 min. The process included a heating phase, reaction phase (when subcritical conditions and desired temperature were met), and cooling phase. After maintaining the desired temperature and time under autogenous pressure conditions, the reactions were quickly quenched by introducing tap water into the reactor through a cooling coil. The cooled resulting mixture was placed in a beaker and the pH of the HC-AP was measured. The mixture was then vacuum filtered through a 1.5-μm glass microfiber filter. The filtrate was collected as HC-AP for wastewater treatment research. This reaction was done in triplicate.

2.2. Microorganism culture preparation

T. striatum HBS was developed under nitrogen stress from a marine protist, *T. striatum* ATCC 24473. The *T. striatum* HBS culture was stored on agar media of artificial sea water (ASW). The ASW was composed of 30 g/L of NaCl, 10.8 g/L of $MgCl_2{\cdot}6H_2O$, 0.7 g/L of KCl, 1.0 g/L of $CaCl_2{\cdot}2H_2O$, and 5.4 g/L of $MgSO_4{\cdot}7H_2O$ [31]. The seed culture was cultivated in the standard GYP medium in 100 % ASW for 5 days. The GYP medium contained glucose (30 g/L), yeast extract (6 g/L), and peptone (6 g/L). Filtration through a 0.22-μm filter was used to sterilize all cultivation media. The cultivations were carried out in the dark at 25 °C and 150 rpm. The cell growth was monitored periodically by OD_{660} (optical density at the wavelength of 660 nm) while dry cell mass (DCM) was also analyzed [31].

2.3. Treatment of HC-AP with T. striatum HBS

A series of full factorial experiments were performed to study the effects of operation conditions on HC-AP treatment, including dilution rate, nitrogen supplement, salinity, and pH. Given the potential toxicity of HC-AP on microorganisms, dilution has been used as one of detoxification measures to mitigate inhibition on biological treatment of HC-AP [19]. The difference of feedstock chemical compositions and HC operation conditions can cause significant variation of nitrogen content of HC-AP. The nitrogen content may become a limiting factor for microbial growth during HC-AP treatment, for which external nitrogen supplement may be needed to achieve desired carbon to nitrogen (C/N) ratio. In this study, ammonia chloride was used to adjust C/N ratio. *T. striatum* grows the best at 100 % seawater salinity so that the salinity of HC-AP would need to be increased by adding salts. The salinity was adjusted by using ASW and measured in conductivity (Accumet XL500 pH/ISE/Conductivity Benchtop Meter, Fisher scientific, Waltham, MA). In addition, pH is one of key factors influencing biological treatment of HC-AP, e.g., AD and most microalgae prefer pH around 7.0 while the pH of HC-AP can vary widely between 3.7 and 10. Our research indicated that *T. striatum* HBS can grow almost equally well in pH 3–10 [28]. Therefore, using *T. striatum* HBS to treat HC-AP in this study may not need pH adjustment because the pH of HC-AP was 4.3. The four factors with two levels were selected as follows: (1) dilution rate [0 (raw HC-AP without dilution) and 10 X (HC-AP/ASW = 1:9, v/v)], (2) nitrogen supplement (0 nitrogen supplement and nitrogen supplement to increase the TN to 0.3 g/L), (3) salinity [0 ASW added (raw HC-AP) and adding ASW to achieve seawater salinity], and (4) pH [4.3 (pH of raw HC-AP) and 7.0 by adding 1 M NaOH]. Ammonia chloride was used to supplement the nitrogen source for HC-AP for creating a suitable C/N ratio (i.e., 5.0) to support *T. striatum* HBS growth. Before used, HC-AP media were filtered through a 0.22-μm filter for sterilization. With 10 % (v/v) inoculation size, the culture was incubated at 25 °C and 150 rpm in the dark. The cell growth was monitored periodically during cultivation by measuring OD and DCM.

2.4. Analysis of TOC, TN, TP, and cellular lipids of T. striatum cells

A TOC analyzer (Shimadzu TOC-L, Columbia, MD) was employed to determine the TOC of HC-AP before and after *T. striatum* HBS treatment. Prior to TOC measurement, a RICCA organic carbon standard was used to build a standard curve. TN and TP were analyzed by using TNT 828 kit and TNT 843 kit, respectively on a Hach spectrophotometer (DR 3900, Loveland, CO). Direct transesterification was employed to analyze the cellular lipids of *T. striatum* cells. The total lipid was expressed as total fatty acids (TFAs) measured as the sum of fatty acid methyl esters (FAMEs) by following the methods used by Xiao [31].

2.5. Chemical compounds analysis of HC-AP

The chemical compounds in HC-AP before and after treatment were analyzed on a GC–MS (Agilent 6890N–5973N MSD, Agilent Technologies, Santa Clara, CA). The analytical column was Ultra 2 (5 %-phenyl)-methylsiloxane column (Agilent Technologies, Santa Clara, CA). The oven temperature was initially kept at 80 °C for 2 min and increased to 300 °C at the rate of 10 °C/min. The carrier gas was helium with the flow rate of 1.5 mL/min. The full scan mode was used for the mass

spectrometer. During *T. striatum* HBS treatment, aliquots of 1 mL were withdrawn from flasks and extracted using 10 X (v/v) ethyl acetate for twice. The extracted samples underwent derivatization with MSTFA (with 1 % TMCS) at 60 °C for 45 min [32]. The sample was then ready for analysis on the GC–MS with the injection volume of 1 μL and the split ratio of 20:1.

2.6. Data analysis

JMP Pro 12 (SAS Institute, Cary, NC, USA) was used to do the analysis of variance (ANOVA, $\alpha = 0.05$ and $p_{critical} = 0.05$). All experiments were performed in three biological replicates, unless specified, otherwise.

3. Results and discussion

3.1. T. striatum HBS cultivation in HC-AP

The TOC, TN, TP, and pH of the HC-AP from HTC of simulated MSW were 8.14 g/L, 0.13 g/L, 0.8 g/L, and pH = 4.3, respectively. Berge et al. [30] who did HTC on the same simulated MSW used in this study, achieved higher TOC (16 g/L) and pH (4.6), which could be because they used lower temperature (250 °C) but much longer residence time (20 h) than those (280 °C and 10 min) used in this research. Although higher temperature usually results in higher TOC, longer residence time could also cause more TOC [21]. In doing HTC of MSW pulp (steamed and separated organics from MSW) at 250 °C for 2 h, TOC and TN were 4.9 and 0.92 g/L, respectively in HC-AP [33]. Based on this comparison, temperature seems to be more significant on the generation of TOC in HC-AP [21]. Low TN in our HC-AP could be because there was low protein content (~5 wt%) in the raw simulated MSW [34]. Low pH 4.3 of the HC-AP in this study could be caused by the organic acids that were produced by the decomposition of carbohydrates (e.g., cellulose and hemicellulose in paper and food fibers in the simulated MSW) [35].

Carbon and nitrogen sources are two major nutrients of substrates for microbioal growth and metabolism. Of HC-AP from organic feedstocks, there are various readily digestible organic carbon sources, such as saccharides and organic acids (e.g., levulinic, lactic, acetic, and formic acids) generating from cellulose/hemicellulose breakdown and mono-aromatic and polyaromatic compounds deriving from lignin polymerization [36]. Because of the low protein content in the simulated MSW, the TN of HC-AP in this study is too low (0.13 g/L) to support the growth of *T. striatum*. Therefore, ammonia chloride was used as an external nitrogen source supplement for *T. striatum* cultivation in the HC-AP. In addition, dilution was studied to mitigate inhibition resulted from some potential inhibitors (e.g., furfural, 5-HMF, levoglucosan, and aromatic and *N*-heterocyclic compounds) from dehydration/degration of carbohydrates, lignin, protein, and so on during HTC of organic matter [4,37].

Table 1 shows the *T. striatum* DCM results across four factors combination (dilution rate, nitrogen source supplement, salinity, and pH). The range of DCM after 10-day treatment was from 0.76 to 1.26 g/L. Fig. 1 shows the time course of *T. striatum* growth in HC-AP under different treatment conditions. A lag phase was found in the first 4 days of growth followed by a log phase for 6 days. After 8~10 days of treatment, *T. striatum* grew from 0.75 to 1.27 g/L in HC-AP with 10 X dilution, 0.3 g/L NH_4Cl as a nitrogen supplement, and salinity of 100 % ASW without pH adjustment (DNAh and DNAH in Fig. 1). After this stage, the cell growth leveled off, indicating the stationary phase. Under the same conditions but without salinity adjustment, *T. striatum* grew only slightly less with 1.10 g/L of DCM. For other treatment conditions, there is no significant cell growth observed, indicating that dilution and nitrogen supplement are essential for *T. striatum* cultivation in HC-AP. Interestingly, *T. striatum* grew robustly in HC-AP even at low pH value of 4.3, which is similar to the reported result by Li et al. [28]. The

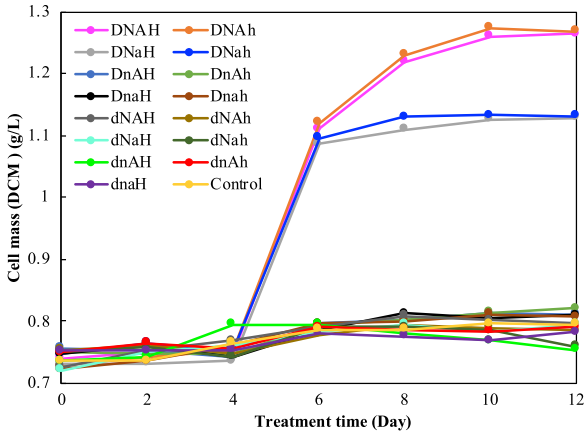

Fig. 1. Time course of *T. striatum* growth in HC-AP under different treatment conditions. "D" = 10X dilution, "d" = no dilution, "N" = 0.3 g/L NH_4Cl, "n" = no NH_4Cl addition, "A" = salinity of 100 % artificial sea water, "a" = no salinity adjustment, "H" = initial pH = 7, "h" = initial pH4.3, and "control" = raw HC-AP.

Table 1
DCM and removals of TOC, TN, and TP after HC-AP treatment with *T. striatum* for 10 days.[a]

Treatment condition	DCM (g/L)		TOC removal (%)		TN removal (%)		TP removal (%)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DNAH	1.26	0.05	81.52	1.15	52.99	2.25	93.75	3.98
DNAh	1.27	0.07	80.96	2.29	52.62	0.74	93.10	3.95
DNaH	1.12	0.01	80.36	3.41	52.23	2.95	92.41	1.31
DNah	1.13	0.01	80.42	1.14	52.27	0.74	92.48	3.92
DnAH	0.81	0.01	1.97	0.03	1.28	0.07	2.27	0.03
DnAh	0.81	0.03	1.65	0.07	1.07	0.06	1.90	0.08
DnaH	0.80	0.04	1.86	0.05	1.21	0.09	2.14	0.03
Dnah	0.81	0.05	1.12	0.05	0.73	0.02	1.29	0.02
dNAH	0.80	0.02	0.89	0.03	0.58	0.03	1.02	0.04
dNAh	0.78	0.04	0.67	0.01	0.44	0.01	0.77	0.01
dNaH	0.78	0.03	0.75	0.01	0.49	0.03	0.86	0.01
dNah	0.78	0.03	0.62	0.02	0.40	0.02	0.71	0.04
dnAH	0.76	0.02	0.84	0.04	0.55	0.02	0.97	0.05
dnAh	0.78	0.02	0.69	0.03	0.45	0.02	0.79	0.01
dnaH	0.76	0.05	0.72	0.02	0.47	0.01	0.83	0.01
dnah (raw HC-AP as a control)	0.79	0.03	0.79	0.03	0.51	0.01	0.91	0.01

[a] "DCM" = dry cell mass, "TOC" = total organic carbon, "TN" = total nitrogen, "TP" = total phosphorus, "HC-AP" = hydrothermal conversion aqueous product, "SD" = standard deviation, "D" = 10X dilution, "d" = no dilution, "N" = 0.3 g/L NH_4Cl, "n" = no NH_4Cl addition, "A" = salinity of 100 % artificial sea water, "a" = no salinity adjustment, "H" = initial pH = 7, and "h" = initial pH4.3.

capability of tolerance to low pH renders *T. striatum* a great potential for treating acidic HC-AP (e.g., from feedstock rich in lignocellulosic biomass) without the need of neutralization or extensive dilution. Statistical analysis shows that only dilution rate and nitrogen source supplement had significant effects ($p < 0.05$) on DCM, whereas salinity and pH were not significant (Table **S1**). Dilution rate had the most significant effect on DCM than other factors based on the number of F-value, which could indicate the inhibition of *T. striatum* growth by some inhibitors (e.g., furans and phenolic compounds) in HC-AP. In addition, the deficiency of trace elements (e.g., Fe^{2+} and Zn^{2+}), minerals (e.g., K and P), and/or vitamin could also cause *T. striatum* to grow less and slower in the HC-AP than that in the standard GYP medium used for the inoculum preparation in Section 2.2 [38], i.e., *T. striatum* achieved the growth rate of about 0.72 g/day and DCM of 4.3 g in 6 days in the GYP medium in comparison with data in Fig. 1.

3.2. Effects of treatment conditions on the removal of TOC, TN, and TP from HC-AP

As shown in Table 1, the removal ranges across all treatment conditions were 0.6~81.5 % for TOC, 0.4~53.0 % for TN, and 0.8~93.8 % for TP, respectively. As shown in Fig. 2, the optimum treatment conditions (10 X dilution, 0.3 g/L NH_4Cl, 100 % salinity, and initial pH = 4.3) achieved the highest removal of TOC, TN, and TP simultaneously, i.e., *T. striatum* removed up to 81.5 % of TOC, 53.0 % of TN, and 93.8 % of TP under the optimum condition. Since additional nitrogen (i.e., ammonia chloride) was supplemented to balance C/N of HC-AP, it was hardly to distinguish what percentage of the total nitrogen removal is from the original nitrogen of HC-AP. Further study will be conducted on the nitrogen removal by using high-nitrogen HC-AP generated from HC of high-nitrogen feedstocks (e.g., microalgae). Similar to DCM, only dilution rate and nitrogen supplement had significant effects on the removals of TOC, TN, and TP (Table **S1**). Favorable TOC, TN, and TP removals in this study show that *T. striatum* has a superior potential to be a new effective biological technique to treat HC-AP in addition to AD and microalgae.

Table **S2** shows the Pearson correlation coefficients among DCM and removals of TOC/TN/TP during the HC-AP treatment with *T. striatum*. The coefficients will provide scientific insights between key parameters during HC-AP treatment. There were positive relationships observed between DCM and removals of TOC/TN/TP with R = 0.96~0.99 and $P < 0.01$. These results suggested that *T. striatum* be able to grow in HC-AP with appropriate dilution rate and nitrogen supplement, leading to effective removals of TOC, TN, and TP from HC-AP concurrently.

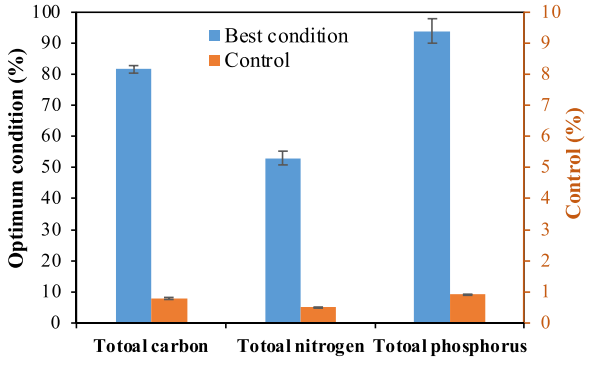

Fig. 2. TOC, TN, and TP removal from HC-AP via *T. striatum* treatment (Optimum condition refers to 10X dilution, 0.3 g/L NH_4Cl, 100 % salinity, and initial pH = 4.3, and control (y axis on the right) refers to raw HC-AP).

3.3. Specific chemical compounds removal and cellular lipid accumulation of T. striatum

GC–MS analysis showed that HC-AP contained a variety of chemicals (e.g., organic acids, furans, phenols, O-heterocyclic compounds, ketones, and aldehydes), and their quantities varied a lot among each other. Of such compounds in HC-AP, the top 8 most degradable compounds include glycolic acid, sorbic acid, succinic acid, methylsuccinic acid, 5-HMF, catechol, isophthalic acid, and terephthalic acid with removals of 88.6, 88.8, 86.5, 84.5, 36.1, 55.5, 77.1, and 33.3 %, respectively (Fig. 3). Catechol, isophthalic acid, and terephthalic acid could be derived from lignin and 5-HMF could be derived from cellulose of the simulated MSW. These compounds can inhibit microbial growth [4,19,21,22]. Many organic acids were identified, including glycolic, sorbic, succinic, and methylsuccinic acids which could lead to low pH of HC-AP [39]. Nakai et al. [40] found that polyphenols (e.g., catechol and hydroquinione) at a concentration as low as 7.5 mg/L inhibited *Microcystis aeruginosa* (a microalga) because of the formation of reactive radicals resulted from polyphenols auto-oxidation. Si et al. [41] reported that AD bacteria removed most furfural and 5-HMF and partial aromatic compounds of HC-AP. Most aromatic compounds are hardly biodegradable because of their superior stability resulted from the inertness of C—C and C–H bonds of these hydrocarbons and the resonance energy of aromatic rings [42]. Traditional AD treatment cannot effectively degrade aromatic compounds due to oxygen deficiency [41]. This study shows that *T. striatum* has a promising potential to metabolize a broad spectrum of organic acids, especially the aromatic compounds of HC-AP, which was also reported by Li et al. [29]. Few *N*-heterocyclic compounds were observed in HC-AP before and after *T. striatum* treatment, which could ease the biological treatment of HC-AP because many *N*-heterocyclic compounds found in the HC-AP are toxic to microorganisms (e.g., bacteria and microalgae) and synergistic toxicity among such compounds may also occur [12,13,43–45]. The possible reason is that HC-AP in this study was generated from HTC of simulated MSW that contained low level of nitrogen-rich compounds, such as proteins and amino acids [19]. In addition, HTC operation conditions (e.g., long residence time and high temperature) can also influence the generation of *N*-heterocyclic compounds, whereases the residence time for HTC in this research were relatively low despite relatively high temperature, resulting in low *N*-heterocyclic in the HC-AP. Based on our previous research [46], *T. striatum* can assimilate various organic and inorganic nitrogen sources, including yeast extract, peptone, sodium nitrate, MSG, urea, and ammonia chloride. Therefore, the HTC conditions could be manipulated to control the nitrogen fate in the HC-AP [47], e.g., nitrate, nitrite, ammonium, and *N*-heterocyclic compounds to generate more digestible nitrogen compounds for *T. striatum* if *N*-heterocyclic compounds is found to inhibit *T. striatum*.

Cellular TFA content and profile of *T. striatum* cultivated in HC-AP are shown in Fig. 4. *T. striatum* accumulated 14 % (g/100 g DCM) under the optimum treatment condition which is approximately 3 times higher than that under the control condition. This lipid content of *T. striatum* is higher than 11.2 % of *C. vulgaris* cultivated in HC-AP of algae at similar TOC concentration [20]. The TFA under the optimum condition consisted of 8.5 % of C16:X, 4.0 % of C18:0, 64.6 % of C18:1, and 23.0 % of C18:3. In contrast, the TFA produced under the control condition showed different profile pattern, i.e., C18:2 instead of C18:0 or C18:3 was produced by *T. striatum*. In general, more types of C18 FAs produced under the optimum condition, while C18:0 and C18:3 were not detected under the control condition. Moreover, the proportions of saturated FAs were higher under the optimum condition. Such results suggest that different HC-AP treatment conditions lead to different metabolisms in converting substrates to FAs. For the control, *T. striatum* only showed marginal removal of TOC/TN/TP and major toxic compounds, which is consistent with slight cell growth. Notably, even in the severely harsh HC-AP, *T. striatum* can still produce comparable lipids per unit carbon source as other lipid-producing microorganisms [7,20].

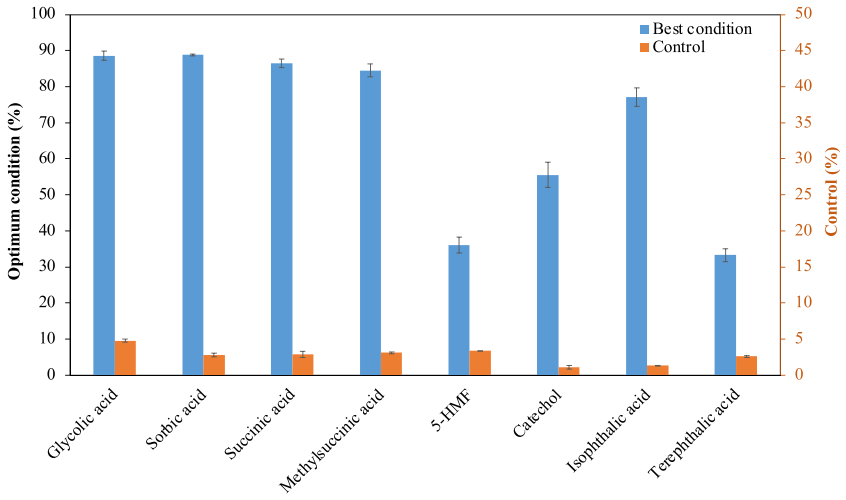

Fig. 3. Top 8 chemical compounds removal from HC-AP via *T. striatum* treatment (Optimum condition refers to 10X dilution, 0.3 g/L NH_4Cl, 100 % salinity, and initial pH = 4.3, and control (y axis on the right) refers to raw HC-AP).

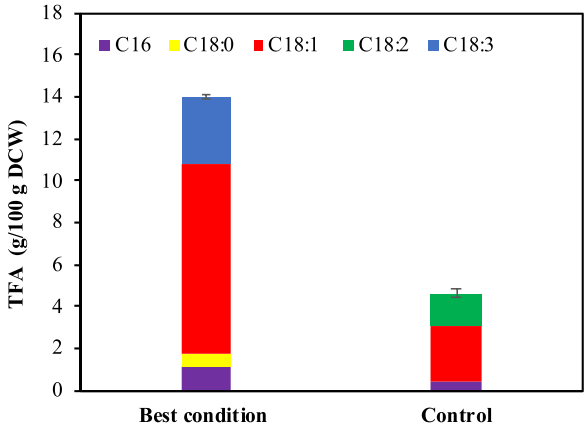

Fig. 4. Total fatty acid (TFA) content and profile of *T. striatum* after HC-AP treatment (Optimum condition refers to 10X dilution, 0.3 g/L NH_4Cl, 100 % salinity, and initial pH = 4.3, and control refers to raw HC-AP).

Salinity is insignificant on DCM and removal of TOC/TN/TP, but it is expected to have a significant impact on TFA production according to our previous research [46] on the effect of salinity on TFA production by *T. striatum* which showed that high salinity promoted *T. striatum* to accumulate more lipids. The possible reason is that *T. striatum* changed FAs in cell membranes for resistance to osmotic pressure and maintenance of the membrane fluidity in response to different salinity [48].

4. Conclusions

A new biological approach was studied for treating HC-AP from HTC of simulated MSW with *T. striatum*. Among factors of dilution rate, nitrogen supplement, salinity, and pH, only dilution rate and nitrogen supplement had significant effects on DCM and removals of TOC/TN/TP. Under the optimum treatment condition (10 X dilution, 0.3 g/L NH_4Cl, 100 % ASW salinity, and pH = 4.3), *T. striatum* reached 1.27 g/L DCM, accumulated 14 % TFA (g/100 g DCM), and removed 81.5 % TOC, 53.0 % TN, and 93.8 % TP, while degrading some recalcitrant organic acids and furans. *T. striatum* is a promising microorganism for biological treatment of HC-AP to enhance the sustainability of HC of biomass.

Data availability

Data will be made available on request.

Authorship contribution statement

Ke Zhang: Formal analysis, Methodology, Data Curation, Investigation, Writing-Original draft preparation. **Kameron J. Adams:** Investigation. **Sandeep Kumar:** Resources, Writing-Review & Editing. **Zhi-Wu Wang**: Resources, Writing-Review & Editing. **Yi Zheng:** Conceptualization, Methodology, Writing-Review & Editing, Supervision, Project administration.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.procbio.2021.12.005.

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