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Strategies for recovery and recycling of nutrients from municipal sewage treatment effluent and hydrothermal liquefaction wastewaters for the growth of the microalga *Scenedesmus* sp. AMDD

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ABSTRACT

Developing reliable processes for recycling nutrients obtained from hydrothermal liquefaction of algae could improve the sustainability and scalability of algae based biofuels. In this study, hydrothermal liquefaction (HTL) wastewater was obtained from continuous liquefaction experiments in plug flow reactor and two strategies were evaluated to recycle both phosphorous and nitrogen for the growth of Scenedesmus sp. AMDD. The first strategy involved using HTL wastewater as a source of nitrogen while minimizing the algae growth inhibitors present in wastewater using hydrothermal gasification and activated carbon adsorption. The second strategy focused on recovering nitrogen as ammonia and phosphorous as struvite from HTL wastewater and recycling only the nutrients thereby decoupling the HTL wastewater and most inhibitors from nutrient recycle process. Water samples obtained from these two strategies were analyzed using various techniques (elemental analysis, gas chromatography, and hydrogen NMR) and potential growth inhibitors were identified as nitrogen containing heteroaromatics. The second approach relied solely on activated carbon treatment to remove these heteroaromatics. Although comparable growth rates were obtained using the first approach after strong dilution, algae growth and biomass yields obtained using the latter approach was much more robust as indicated by less variability, similar rates and biomass yields as compared to the synthetic medium. Apparent nitrogen and phosphorous removal rates for this case were > 99% and 68 ± 5.7%, respectively. This approach of decoupling nitrogen and phosphorous nutrients from HTL water offers a flexible, reliable, and scalable process for recycling nutrients without the inhibitors in HTL water and an important step towards the commercial production of algae for biofuels.

1. Introduction

Algae as a source of biomass for renewable fuels and chemicals offers several advantages: significantly higher productivity compared to terrestrial plants [1], does not compete with the food supply, and can be grown on land and water not suitable for agriculture. These attributes make algae a promising feedstock for the production of biofuels. However, there are several economic and resource constraints on the commercial production of algae for biofuels. One resource constraint is the availability of nitrogen and phosphorus which are essential nutrients for the growth of algae. On a dry mass basis algae consists of around 7% nitrogen and 1% phosphorus [1]. Several scalability assessments have shown that the existing supply of nitrogen and phosphorus are insufficient for production of significant quantities of algal biofuels [2–6]. For example as estimated in the study by Chisti [2], production of 82 million tons of algal biomass would produce biofuel that would replace < 3% of the equivalent US fuel produced from petroleum, yet would require some 5.4 million tons of nitrogen (around 44% of current US usage) and 1.1 million ton of phosphorus. Supply of nutrients through wastewater, although attractive, would only cover a fraction of this nutrient supply [5]. So the production of algae for biofuel at commercial scale would require technologies that could minimize the use of nutrients through nutrient reuse and recycling.

Hydrothermal liquefaction (HTL) is a promising technology in this regard. It is the conversion of wet algae (around 10–30 wt%) in hot

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liquid water at around 250–374 °C and 4–22 MPa to a bio-crude oil with a high heating value in the range of 35–40 MJ Kg^{-1} [7,8]. Depending on the specifics of the process and conditions up to half of the nitrogen and majority of the phosphorus (85–100%) end up in the HTL aqueous phase [9]. So HTL not only obviates the need for an energy intensive drying step, which would improve the overall energy efficiency (compared to technologies that require drying the algae), but also affords an opportunity to recycle a significant portion of the nutrients present in the HTL aqueous phase. Advancements in nutrient recycling and reuse from HTL water could significantly impact the scalability of algae based biofuels. One of the major challenges with recycling HTL water nutrients however arises from the presence of various organic compounds in the HTL aqueous phase, which can poison the algae and inhibit their regrowth. Some of these inhibitory compounds include: phenols, furans, toluene, benzene, amino-phenol, pyridines and 2-pyrrolidinone, among others [9]. Studies have reported in the literature [10–12] that HTL water requires significant dilution to reduce the concentration of these inhibitors. In some cases, even after dilution, sustained growth rates were only observed with species that could adapt to these inhibitors [13]. This could potentially limit the varieties of algae processed through HTL for biofuel production.

This work describes strategies to recycle both nitrogen and phosphorus while minimizing the inhibition of algae cultures caused by specific organics present in the HTL water aqueous phase. Two strategies are demonstrated and compared for recycling HTL water nutrients:

a) Using HTL water as a source of nitrogen and phosphorus after minimizing the organics in HTL water phase that could potentially inhibit the growth. Organics in HTL water were minimized by catalytic hydrothermal gasification followed by activated carbon adsorption (Fig. 1).

b) Recovering the nitrogen as ammonia and phosphorus as struvite from HTL water and using these recovered nutrients for algae growth thereby trying to avoid most organics present in HTL water (Fig. 2).

The study also looks at organic contaminants in HTL wastewater using various analytical techniques that could be inhibiting the growth of *Scenedesmus* sp. AMDD.

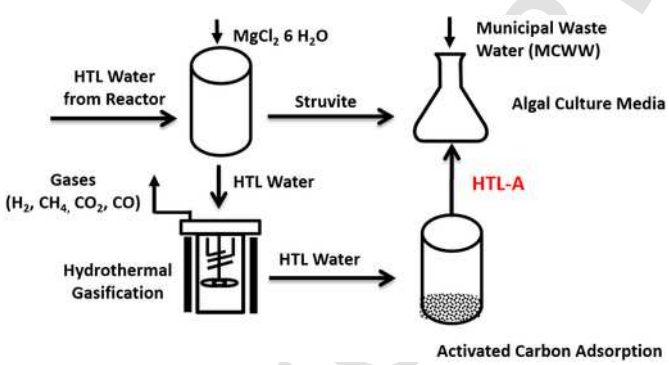

Fig. 1. Process A developed for pre-treatment of hydrothermal liquefaction (HTL) wastewaters for use as an algal growth medium. Struvite was precipitated through addition of $MgCl_2{\cdot}6H_2O$ as described in Materials and Methods. Catalytic hydrothermal gasification was conducted in a 250 mL batch autoclave using a molybdenum based catalyst at 375 °C with a residence time of 180 min. The resulting HTL water with reduced organics was treated with 1 g of DARCO activated charcoal, filtered with 5C Advantec filter paper (< 5 μm pore size) followed by treatment with HYDRODARCO C and HYDRODARCO M activated carbons This resulting water was referred to as HTL-A. It was then added as an amendment to Mill Cove wastewater (MCWW) at a final concentration of 0.2%, 0.4% and 0.8% (v/v) and used for algal growth.

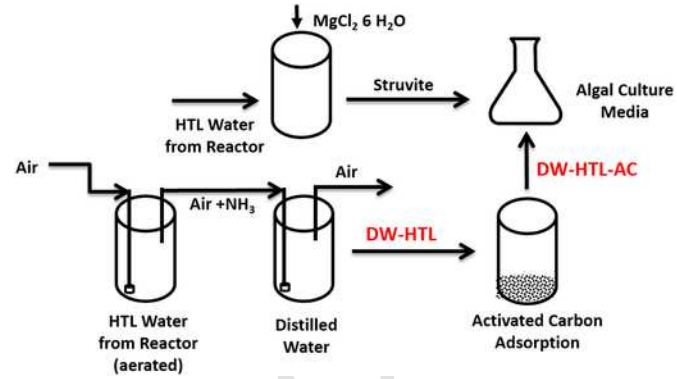

Fig. 2. Process B developed for pretreatment of hydrothermal liquefaction (HTL) wastewaters for use as an algal growth medium based on ammonia stripping and struvite recovery. Struvite was precipitated through addition of $MgCl_2{\cdot}6H_2O$ as described in Materials and Methods. A 0.1 L volume of raw hydrothermal liquefaction wastewater was sparged with air at a rate of around 0.15 L min^{-1} using a rotameter. The ammonia stripped by air was recaptured by sparging it through DI water. The water obtained was referred to as DW-HTL. This mixture was then diluted with distilled water to 4% of its original volume and either used directly for algal growth or cleaned twice with DARCO activated carbon (cleaned water referred to as DW-HTL-AC) and then used for algal growth.

2. Materials and methods

2.1. Microalgae biomass

The *Chlorella* sp. microalgae used for hydrothermal liquefaction experiments was obtained from buyalgae.com. As per the specifications provided by the supplier, biomass consisted of 65.1%, 6.4%, 1.2% and 6% crude protein, total fat, phosphorus and ash, respectively. An elemental analysis carried out in our laboratory determined the following composition: 49.5%, 7.6%, 10% and 0.7% carbon, hydrogen, nitrogen and sulphur, respectively, by weight. Magnesium chloride hexahydrate (certified ACS) and sodium hydroxide (certified ACS) were obtained from Fischer Scientific. HYDRDRACO C and HYDRDRACO M activated carbon were donated as samples by Cabot Corp (Boston, MA).

2.2. Hydrothermal liquefaction water recovery

HTL water used in the experiments was obtained from hydrothermal liquefaction of *Chlorella* sp. (20–30 wt%) in a continuous plug flow reactor (0.45 L) system at 350 °C, 2900–3000 psig pressure with a feed rate of around 1 L h^{-1}. The unit consisted of a feed tank, high pressure piston driven pumping system, a preheater/heat recovery system, three-zone furnace, reactor, product separation and collection tanks, and instrumentation and controls. Aqueous phase from the HTL product mix was separated and recovered from bio-crude without using any solvents and stored at 4 °C until used for further processing.

2.3. Struvite precipitation

Struvite precipitation experiments were performed in 400 mL beakers at room temperature. To drive struvite formation and precipitation, 1 g of magnesium chloride hexahydrate (MCH) was added to approximately 250 mL of HTL water at pH 8 and stirred for 30 min at 110 rpm. After an additional 5 min to allow for precipitate settling, a sample of the clarified supernatant was taken for analysis of residual dissolved P. Additional 1 g quantities of MCH were added and the process was repeated until the quantity of residual P in the HTL stabilized, indicating that no further P could be recruited into the formation of struvite. We also examined the effect of pH on struvite recovery by adding sodium hydroxide to HTL water to raise the pH to 9,

10 or 11 and repeated the precipitation process as described above. To obtain struvite for microalgae growth experiments, 4 g of MCH were added to 250 mL of HTL water at pH 8 and stirred at 110 rpm for 30 min. The precipitate was filtered using a 5C Advantec filter paper and air dried. Dried struvite samples were stored in vials at room temperature until use. Struvite was subsequently re-dissolved in either municipal wastewater effluent or distilled water as an additional source of phosphorus and, to a lesser extent nitrogen, and used for microalgae growth trials as described below.

2.4. HTL Water processing and cleanup for algae growth

Hydrothermal liquefaction (HTL) water obtained from liquefaction experiments for use in microalgae growth trials underwent a variety of different treatment processes as described below. In Process A, 200 mL of HTL water was treated initially with 1 g of powdered DARCO activated charcoal in a beaker with stirring for 30 min and then filtered using a 5C Advantec filter paper (< 5 μm pore size). Preliminary experiments using this HTL water as a source of nutrients indicated that additional processing would be required as growth was severely retarded by inhibitors (data not shown). In order to reduce the organic content of the HTL water and recover more energy products, catalytic hydrothermal gasification was conducted in a 250 mL batch autoclave using a molybdenum based catalyst at 375 °C with a residence time of 180 min. The resulting HTL water (110 mL) with reduced organics (around 40–42% reduction in C content) was treated with 1 g of DARCO activated charcoal, followed by treatment with HYDRODARCO C and HYDRODARCO M activated carbons using 85–90 mL of water and around 0.5 g of activated carbon each time (Fig. 1). Process A treated HTL water was used for all experiments in growth trial 1.

2.5. Ammonia recovery

In a separate set of experiments, 100 mL of HTL water from which struvite was previously recovered was sparged with air at 0.15 L min^{-1}. The headspace enriched with ammonia was sparged into an adjacent vessel containing 0.25 L of DI water. The DI water was refreshed three times during the course of the experiment to maintain the concentration gradient for ammonia dissolution. The three 0.25 L lots of DI with ammonia were combined and used as a source of nitrogen for algae growth experiments. The solution was sampled and assayed at regular intervals for dissolved ammonia. Ammonia enriched distilled water was either used directly for growth or was treated with DARCO activated carbon as explained above before use for growth trials. This mixture was used for all experiments described in growth trial 2.

2.6. Analysis of hydrothermal liquefaction Water

The elemental carbon determination in HTL water samples was performed using Elementar 'Vario' EL cube elemental analyzer. The calibration standard used was sulfanilamide. The sample size was approximately 15–20 mg. Samples were analyzed in triplicate and the data presented are averages of three independent analyses.

GC–MS analysis was performed using Agilent technologies 7890A GC System with a 5975C inert XL mass selective detector. A DB-23 column was used with an injection volume of 1 μL at a 5:1 split ratio. The injector was maintained at 250 °C with the following temperature program: 50 °C hold for 5 min, 25 °C/min ramp to 175 °C, 4 °C/min ramp to 230 °C and hold at 230 °C for 36 min.

Hydrogen NMR spectra were acquired on a Varian Unity Inova NMR spectrometer at a proton frequency of 399.920 MHz using a 5 mm indirect detection probe. The acquisitions were done on neat water samples; a glass capillary filled with deuterated dichloromethane (CD_2Cl_2) was inserted into the NMR tubes for locking, shimming and chemical shift reference (5.31 ppm). The very large water peak was suppressed by a pre-saturation sequence. The acquisition parameters were kept constant for all samples: gain 2, vertical scale: 4000, pulse angle: 45°, relaxation delay: 20, acquisition time: 3 s, number of scans: 16. The probe was tuned and matched for every sample. The processed spectra were integrated for specific chemical shift regions.

2.7. Preparation of microalgae growth media using processed HTL Water and municipal wastewater effluent

The freshwater chlorophyte microalgae *Scenedesmus* sp. AMDD was used for all growth experiments presented in this paper. This particular strain was originally isolated from a soil sample but representatives of this genus are frequently isolated from active wastewater ponds suggesting that they are readily adaptable to these environments and therefore useful models for wastewater studies [14–16]. Samples of municipal wastewater effluent were obtained from the Mill Cove Waste Water (MCWW) secondary treatment plant in Bedford, Nova Scotia. The growth of this strain in MCWW was previously described in detail elsewhere [16]. For trial 1, algal growth was carried out in MCWW alone, MCWW amended with struvite or MCWW amended with both struvite and HTL water pretreated according to Process A at dilutions of 0.2, 0.4 and 0.8% (v/v) which raised the concentration of N from 0.76 mM in MCWW alone to 1.6, 2.4 and 3.9 mM, respectively, (Table 1). Struvite was added from a concentrated solution at levels sufficient to raise the concentration of P to 0.58 mM from 0.03 mM in MCWW alone (Table 1). No further amendments were made to these formulations. For growth trial 2, ammonia enriched distilled water prepared from the aeration of HTL water according the Process B as described above was added as a 4% (v/v) amendment to distilled water. This level of dilution was chosen so that the final N concentration (3.2–3.6 mM, Table 2) was similar to the MCWW-Struvite-0.8% HTL treatment. Following from that, sufficient struvite was added to raise the P concentration to 0.24 mM, which resulted in N:P ratios between 13 and 16, which are more appropriately balanced for algal growth. Cultures used for growth trial 2 were further amended with 0.1 mM KCl and trace metals. Cultures based on modified Bold's basal medium were included in growth trial 2 as a positive control. For all cultures, the pH was adjusted to 7.0 through the dropwise addition of HCl.

2.8. Conditions for growth of microalgae in HTL and municipal wastewaters

For both growth trials, three replicates of the *Scenedesmus* sp-AMDD strain were cultivated in batch cultures for either 11 or 14 days in 250 mL Erlenmeyer flasks (150 mL of culture) under the following growth conditions: 175 rpm shaking (Innova 2100, New Brunswick Sci-

Table 1
Nitrogen and phosphorus concentrations and N:P ratios of Mill Cove wastewater (MCWW) alone and supplemented with struvite and hydrothermal liquefaction (HTL) wastewater at various concentrations according to Process A. Values reported were obtained from a single representative sample.

Growth Media	N (mol L^{-1})	P (mol L^{-1})	Initial N:P Ratio
MCWW[1]	0.76×10^{-3}	0.03×10^{-3}	25.33
MCWW-Struvite[2]	0.90×10^{-3}	0.6×10^{-3}	1.5
MCWW-Struvite-0.2% HTL[3]	1.7×10^{-3}	0.6×10^{-3}	2.8
MCWW-Struvite-0.4% HTL[3]	2.4×10^{-3}	0.6×10^{-3}	4.04
MCWW-Struvite-0.8% HTL[3]	4.0×10^{-3}	0.60×10^{-3}	6.7

[1] MCWW is the growth media using only Mill Cove Waste Water.
[2] MCWW-struvite is the growth media obtained by amending MCWW with struvite at levels sufficient to raise the concentration of P to 0.58 mM
[3] MCWW-Struvite-0.2% HTL, MCWW-Struvite-0.4% HTL, MCWW-Struvite-0.8% HTL are the growth media obtained by amending MCWW with struvite and HTL wastewater at dilutions of 0.2, 0.4, and 0.8% (v/v), respectively.

Table 2
Apparent N and P removal, growth rate and final biomass yield during growth of Scenedesmus sp. AMDD in Mill Cove wastewater (MCWW) alone and supplemented with struvite and hydrothermal liquefaction wastewater at various concentrations according to Process A. Apparent nutrient removal rates were calculated as the difference between the initial concentrations of N or P in the media and the residuals detected in the culture media on the final day of cultivation. Data reported as mean ± standard deviation, n = 3.

Growth media	Apparent N removal (%)	Apparent P removal (%)	Specific growth rate (d^{-1})	Final biomass yield (g dw L^{-1})
MCWW[1]	95.3 ± 0.3	> 99	1.6 ± 0.1	0.8 ± 0.1
MCWW-Struvite[2]	87.3 ± 14.1	45.5 ± 8.3	1.1 ± 0.1	0.6 ± 0.4
MCWW-Struvite-0.2% HTL[3]	77.5 ± 35.1	50.6 ± 14.6	1.1 ± 0.1	0.7 ± 0.6
MCWW-Struvite-0.4% HTL[3]	70.4 ± 29.8	57.7 ± 18.7	1.2 ± 0.1	0.9 ± 0.9
MCWW-Struvite-0.8% HTL[3]	54.4 ± 39.3	57.2 ± 18.9	1.2 ± 0.1	0.6 ± 0.5

[1] MCWW is the growth media using only Mill Cove Waste Water.
[2] MCWW-Struvite is the growth media obtained by amending MCWW with struvite at levels sufficient to raise the concentration of P to 0.58 mM.
[3] MCWW-Struvite-0.2% HTL, MCWW-Struvite-0.4% HTL, MCWW-Struvite-0.8% HTL are the growth media obtained by amending MCWW with struvite and HTL wastewater at dilutions of 0.2, 0.4, and 0.8% (v/v), respectively.

entific, Edison, USA) under continuous illumination of 175 μmol photons $m^{-2} s^{-1}$ white light in a plant growth chamber (Conviron, Winnipeg, Canada) at 22 °C. The cultures were bubbled continuously with air supplemented with up to 5% (v/v) with CO_2. Culture samples were taken daily using aseptic technique and assayed for algal cell numbers using a Coulter counter (Multisizer 3, Beckman-Coulter, Canada). On the last day, cultured biomass was harvested by centrifugation and lyophilized for later analysis. On the first and last day of cultivation, 10 mL of culture was sampled aseptically, filter sterilized, frozen and stored until subsequently thawed for nutrient analysis.

2.9. Gravimetric biomass analysis

Final biomass yield of the cultures was determined by gravimetric analysis of filtered samples taken on the last day of cultivation as described in the study by Zhu et al. [15]. Briefly, pre-weighed 25 mm glass microfiber filters (Whatman GF/F, UK) were used to filter 10–20 mL of microalgae culture. The filters were then washed with the same volume of dH_2O. Washed filters were freeze-dried using a Freezone 4.5 Litre Benchtop Freeze Dryer (Labconco, Kansas City, USA) and the dried filter weight was determined on a 0.001 mg resolution balance (Mettler Toledo XP6, Mississauga, Canada). The biomass productivity was reported in units of mg L^{-1} d^{-1} according to Griffiths and Harrison (2009).

2.10. Nutrient analysis

Total organic carbon, free ammonium and phosphate concentrations in the algal cultures were measured on the first day and residual free ammonium and phosphate concentrations in the samples were determined on the last day of cultivation using commercially available, spectrometry-based assay systems (TOC-MR for total organic carbon; TNT832 and TNT843 for ammonium and phosphorus assay, respectively; Hach Co., Loveland, CO) using a portable spectrometer (DR 2800, Hach Co.).

3. Results and discussion

Two different approaches were studied to recycle nutrients from residual HTL water. In Process A (Fig. 1), P was separated from the HTL water by precipitating dissolved phosphate as struvite. The remaining water containing unrecovered phosphorous, most of the ammonia and organics was used for the production of gaseous products as outlined in the Materials and Methods section. The water recovered after this step was filtered through activated carbon to minimize the inhibitory compounds before being used as a source of nutrients for the growth of *Scenedesmus* sp. AMDD (water sample HTL-A; see Fig. 1 for description of Process A). In Process B, P was also recovered by struvite precipitation while ammonia was stripped from unprocessed HTL water through aeration and redissolved into distilled water as described in Materials and Methods (see Fig. 2). The growth of *Scenedesmus* sp. AMDD on nutrients recycled from HTL water by these two strategies and the corresponding analyses of the water samples resulting from these two approaches are discussed below.

3.1. Nutrients recovery

3.1.1. Phosphorus recovery into struvite

Depending on the initial concentration of algae in the feedstock, concentrations of phosphate in the HTL aqueous phase ranged from 7700 to 9800 mg L^{-1}. These concentrations are orders of magnitude higher than what is seen in most municipal and industrial wastewaters [18]. In various wastewater treatment plants, the spontaneous precipitation of phosphate (generally as struvite) in pipes has been known to cause operational issues [19]. Approaches studied to avoid this have involved intentional precipitation in systems and processes designed specifically for precipitation of phosphate as struvite. Struvite is a white crystalline substance consisting of magnesium, ammonium and phosphorus in equal molar concentrations ($MgNH_4PO_4$. $6H_2O$; see Fig. 3). It has the potential to be used as a slow release eco-friendly fertilizer. The recovery of these nutrients from HTL aqueous phase as struvite would minimize issues with plant operations treating wastewater and the recovered struvite could be reused as a nutrient for algae growth.

The precipitation and recovery of phosphate as struvite from wastewater depends on several factors such as: pH of the solution, the molar ratio of participating ions, mixing intensity, and the presence of other ions such as Ca^{2+}, Fe^{3+}, SO_4^{2-} etc. Since HTL water has very high concentrations of both phosphate and ammonium, struvite precipitation was studied by varying the pH and magnesium concentration while keeping the mixing rate high to minimize its affects. The pH was varied from 8 (typical pH of HTL water) to 11 with magnesium concentrations up to 3000 mg L^{-1} (based on amount of Mg^{2+} source added to the solution) giving magnesium to phosphate molar ratios up to around 1.55 (Fig. 3A). Under the experimental conditions studied, there was no apparent advantage observed by varying the pH as far as phosphate recovery was concerned, which was typically always about 75%. For all the pH values studied, phosphate recovery increased with increasing magnesium concentration up to approximately 2000 mg L^{-1}, after which no further increased were observed (Fig. 3A). Since there was no apparent advantage of altering the pH on phosphate recovery, struvite for algae growth experiments was obtained by using HTL water without modifying the pH at a magnesium concentration around 2000 mg L^{-1}. The struvite crystals obtained from HTL water at pH 8 were analyzed using x-ray diffraction. As evident from the close match between the XRD pattern (Fig. 3C) of the crystals and reference data (XRD powder pattern 00–015-0762) the crystals were pure struvite.

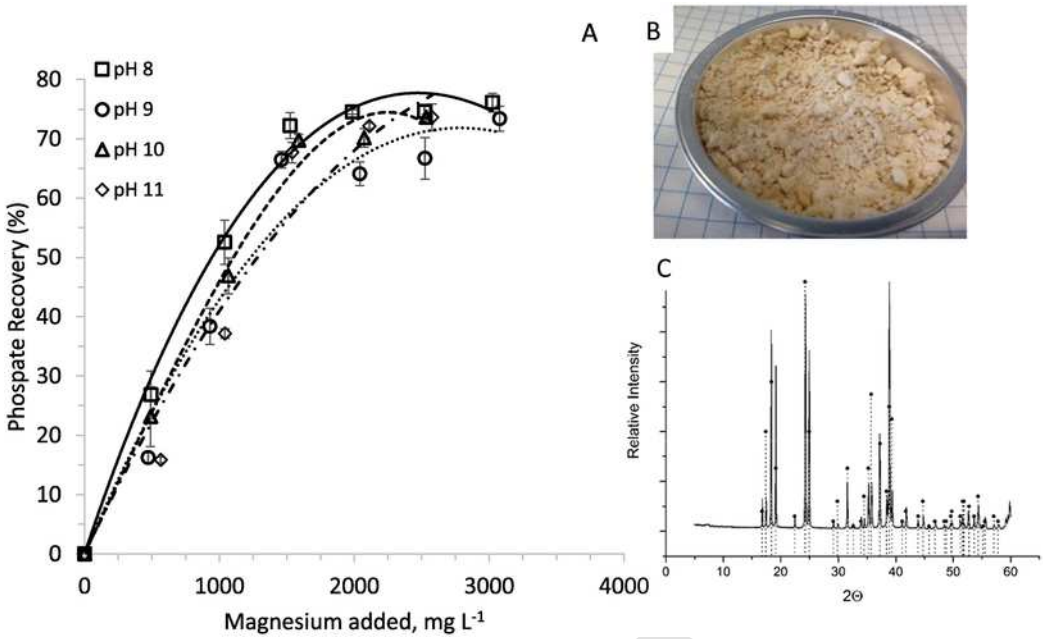

Fig. 3. A. Effect of magnesium dichloride hexahydrate addition and pH on the recovery of phosphate into struvite precipitate in samples of hydrothermal liquefaction wastewater. Data reported as mean ± standard deviation, n = 2. B. Dried struvite recovered from hydrothermal liquefaction wastewater. C. XRD pattern for the precipitated product obtained from hydrothermal liquefaction wastewater (solid line) and that of standard struvite (dashed lines, XRD powder pattern 00-015-0762).

3.1.2. Ammonia recovery into distilled Water

Algal biomass used in this study composed of approximately 10% N (w/w), mainly in the form of proteins. During HTL following various conversion steps like depolymerization, hydrolysis, decarboxylation, deamination, etc., some of the N ends up in various heterocyclic compounds like pyrizines, pyridines, amides, amines, etc. in the water or the oil phase, while a significant portion is evolved as ammonia that ends up in the aqueous phase. Under the conditions studied, the ammonia concentration in the HTL water ranged from 11,600–13,900 mg L^{-1} depending on the concentration of algae slurry used for feedstock. This would correspond to roughly 40–50% of the algal biomass N ending up as ammonia in the HTL water. Some of the methods available to remove ammonia from wastewaters include steam stripping, air stripping, biological nitrification and denitrification, reverse osmosis and ion exchange. Air stripping is generally preferable because of its low cost and high ammonia removal efficiency [20,21]. In industrial applications of this process, stripped ammonia is usually absorbed by an acidic solution forming a mineral fertilizer. For the particular case of nutrient recapture as studied here, the stripped ammonia from the HTL wastewater could be reassimilated by the algae for growth. The basic pH of HTL wastewater (pH = 8.0) lends itself well for this type of recovery.

Under the conditions at which experiments were performed around 90% of the ammonia in HTL water was removed in 136 h (Fig. 4). Overall around 75% of the ammonia dissolved in HTL water was recaptured in the DI water. The rates, recoveries, and reabsorption of the ammonia recovery process would depend on factors such as pH, temperature, flow rates, mass transfer coefficients, etc. [20]. These factors could be optimized to further improve these recoveries and rates of ammonia transfer and reabsorption. However, the objective of this study was to demonstrate the feasibility of the nutrient recycle approaches, so these factors were not studied.

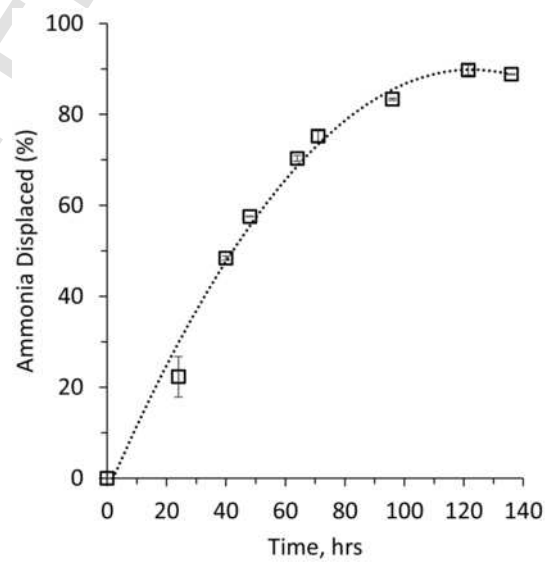

Fig. 4. Ammonia stripping from hydrothermal liquefaction (HTL) wastewater by aeration. Dissolved ammonia in HTL wastewater was volatilized by vigorous aeration for 136 h. The headspace above the aerated HTL water was directed under positive pressure through plastic tubing into an adjacent vessel and bubbled into a volume of distilled water to capture the stripped ammonia. Data reported as mean ± standard deviation, n = 2.

3.2. Microalgae growth on nutrients recycled from hydrothermal liquefaction aqueous residues

Preliminary growth trial experiments suggested that the amendment of MCWW with as little as 1% (v/v) HTL aqueous phase contained a sufficiently high level of inhibitors to reduce the specific growth rate of the alga Scenedesmus sp. AMDD to between 0.23 and 0.47 d^{-1} from the control value of 1.55 d^{-1} observed in unamended MCWW, despite pre-treatment with activated carbon. Likewise, biomass accumulation in MCWW cultures amended with 1% HTL

was reduced to between 0.03 and 0.05 g dw L^{-1} from 1.2 g dw L^{-1} in unamended control cultures. Three strategies were developed that were aimed at reducing the toxicity of HTL water. These included 1) catalytic hydrothermal gasification on the HTL water to reduce the organics load and to form energy products, 2) separating the phosphorus from the toxic HTL water by precipitating, recovering and drying struvite for later reuse and 3) increasing the number of activated carbon treatments using different functional groups to target different classes of inhibitory organic compounds. Employed together, these constituted the Process A pretreatment of HTL water for use in growth trial 1 (Fig. 1, Table 1). Growth in MCWW without amendment was typical of this strain in secondary wastewater effluent with virtually complete removal of N and P (Table 2, [16]).

Amendment of MCWW with struvite increased the P concentration nearly 20 fold but unexpectedly this did not result in increased growth rates or biomass yields compared to MCWW alone (Table 1). Indeed, both the initial exponential growth rate and the final biomass yield were reduced in MCWW-struvite, compared to unamended MCWW (Table 2). The apparent removal efficiencies of both N and P in MCWW-struvite decreased to 87% and 46%, respectively (Table 2). Growth in Process A treated HTL water was similar to MCWW-struvite (Table 2). Although the final biomass yields in MCWW-struvite and MCWW-struvite-HTL were comparable to unamended MCWW, they were highly variable in all treatments with the highest variability observed in MCWW-struvite-0.4% HTL (Table 2). This suggests the presence of growth inhibitors in both the struvite and in HTL water. The high variability suggested that the inhibitors could be unevenly distributed in the culture media, causing unpredictable variability in the replicate algal cultures.

In growth trial 2, Process B treated HTL water was used (Fig. 2). Ammonia concentrations obtained in the distilled water were comparable to the concentrations assayed in MCWW-struvite-0.8% HTL, although phosphorus concentrations were only approximately 40% of those obtained in MCWW amended with struvite (Tables 1 and 3). Activated carbon treatment had no effect on N levels in DW-$HTL_{growth\ media}$ (Table 3). Unless DW-$HTL_{growth\ media}$ was treated with activated carbon, there was little to no growth observed (Table 4). After reduction of organics using activated carbon as explained (DW-HTL-$AC_{growth\ media}$), growth of algae was comparable to the positive control (Bold's basal medium; Table 4). In growth media DW-HTL-$AC_{growth\ media}$, N was practically all removed by algal growth while P was reduced by 68.3% (Table 4).

3.3. Analyses of HTL water samples

Some of the compounds typically present in the HTL aqueous phase include organic acids, alcohols, phenols, amides, pyrazines and pyridines, among others. HTL water and treated HTL water samples were analyzed by elemental analysis to determine percent carbon content, and by gas chromatography-MS (GC–MS) and hydrogen NMR to determine type of organics and their relative quantities in each of the water samples.

Table 3
Nitrogen and phosphorus concentrations and N:P ratios of distilled water enriched in N and P from stripping of hydrothermal liquefaction wastewater and struvite addition, respectively, according to Process B. Included are the same parameters from a modified Bold's medium used as the positive control. Values reported were obtained from a single representative sample analysis.

Growth media	N (mol L^{-1})	P (mol L^{-1})	Initial N:P Ratio
DW-$HTL_{growth\ media}$[1]	3.6×10^{-3}	0.2×10^{-3}	18.0
DW-HTL-$AC_{growth\ media}$[2]	3.2×10^{-3}	0.2×10^{-3}	16.0
Modified Bold's Basal	9.7×10^{-3}	1.7×10^{-3}	5.7

[1] DW-$HTL_{growth\ media}$ is growth media obtained using ammonia enriched distilled water from the stripping of hydrothermal liquefaction wastewater as a source of nitrogen and using struvite as a source of phosphorous.
[2] DW-HTL-$AC_{growth\ media}$ is the growth media obtained using ammonia enriched distilled water after treating it with activated carbon and using struvite as a source of phosphorous.

Table 4
Apparent N and P removal, growth rate and final biomass yield during growth of Scenedesmus sp. AMDD in distilled water enriched in N and P from stripping of hydrothermal liquefaction wastewater and struvite addition according to Process B. Apparent nutrient removal rates were calculated as the difference between the initial concentrations of N or P in the media and the residuals detected in the culture media on the final day of cultivation. Data reported as mean ± standard deviation, n = 3.

Growth media	Apparent N removal (%)	Apparent P removal (%)	Specific growth rate (d^{-1})	Final biomass yield (g dw L^{-1})
DW-$HTL_{growth\ media}$[1]	44.0 ± 5.8	−20.4 ± 11.1	0.7 ± 0.1	0.1 ± 0.1
DW-HTL-$AC_{growth\ media}$[2]	> 99	68.3 ± 5.7	1.2 ± 0.1	1.0 ± 0.1
Modified Bold's Basal	63.2 ± 31.1	6.4 ± 5.0	1.2 ± 0.1	1.0 ± 0.3

[1] DW-$HTL_{growth\ media}$ is growth media obtained using ammonia enriched distilled water from the stripping of hydrothermal liquefaction wastewater and using struvite as a source of phosphorous.
[2] DW-HTL-$AC_{growth\ media}$ is the growth media obtained using ammonia enriched distilled water after treating it with activated carbon and using struvite as a source of phosphorous.

3.3.1. Elemental analysis

The carbon contents as determined by elemental analysis of water samples HTL, HTL-A, DW-HTL, and DW-HTL-AC were 2.95 ± 0.03, 0.83 ± 0.04, 0.12 ± 0.01 and 0.09 ± 0.01 wt%, respectively (data reported as mean ± standard deviation, n = 3). The carbon content of 2.95 wt% in the original HTL water from the reactor would amount to around 33% of the original carbon in the algae ending up in the water phase. Reforming and subsequent cleanup of the HTL water reduced the original carbon content by around 70% (sample HTL-A). The water samples obtained after dissolving the stripped ammonia (DW-HTL) still had some carbon content due to the entrainment of some volatile organics from the HTL water equivalent to about 4% of the carbon in the sample HTL water. Treatment of this water sample (DW-HTL) with activated carbon reduced the organic carbon content by a further 25%.

3.3.2. GC–MS analyses

GC–MS analysis was performed to identify some of the compounds in the different water samples obtained after subsequent conversion and/or clean-up steps. Table 5 lists the major compounds identified that had a GC–MS library match quality > 80. Some of the organics identified in the HTL water sample include: methylpyrazine, dimethylpyrazines, methylpyridines, methylcyclopentanone, benzeneethanamine, etc. Similar compounds have also been reported in other studies [12,22]. It should also be noted that GC–MS may not be able to detect all compounds due to factors such as: low concentrations, complexity of mixtures, co-elution with other compounds, or due to specific columns/operating conditions being used for analysis. GC–MS analysis of water sample HTL-A (obtained after conversion of organics and activated carbon treatment) only detected acids such as acetic acids, propionic acids, etc. at relatively high area percentages indicating the detection of only a few compounds. Most of the organics detected in Sample DW-HTL (obtained after dissolving the stripped ammonia) are in the form of heteroaromatics like pyrazines, pyridines, and their derivatives. These organics were also detected in the original

Table 5
Chemical compounds identified by GC–MS in hydrothermal liquefaction water and water samples obtained after different processing and cleanup steps. The numbers in brackets after each compound indicate peak quality and relative area percent. Values reported were obtained from a single representative sample analysis of HTL, HTL-A, DW-HTL, and DW-HTL-AC.

HTL[1]	HTL-A[2]	DW-HTL[3]	DW-HTL-AC[4]
Pyridine, 2-methyl- (96, 0.3)	Acetic acid (91, 37.7)	Pyrazine, ethyl- (94, 2.5)	Carbonic acid, dimethyl ester (80, 30)
Pyridine, 3-methyl- (93, 0.3)	Propionic acid (90, 8.6)	1,3-Diazine (90,5.8)	
Pyrazine (91, 1.5)	Butanoic acid, 3-methyl-(80,5.3)	Pyrazine, methyl- (90,7.5)	
Pyrazine, methyl- (91, 3.8)		Pyridine, 2-methyl-(90,0.7)	
Pyrazine, ethyl- (90, 1.1)			
Pyrazine, 2,6-dimethyl- (78, 1.6)			
Pyrazine, 2,3-dimethyl- (86, 0.7)			
2,5-Pyrrolidinedione, 1-ethyl- (91,3.6)			
2-Pyrrolidinone, 1-methyl- (90,2.6)			
Pyrrolidine, 1-acetyl- (87,1.3)			
2-Cyclopenten-1-one, 2-methyl-(90, 0.3)			
2-Cyclopenten-1-one, 3-methyl- (89,0.6)			
Cyclopentanone, 2-methyl- (89, 0.3)			
1,4-Benzenediamine, N,N′-diethyl-(83,0.6)			
Benzeneethanamine (81,1.1)			

[1] TL is the original water obtained from the hydrothermal liquefaction reactor without any further processing.
[2] HTL-A is the water obtained after hydrothermal gasification and activated carbon treatment of the HTL water as described in Materials and Methods section.
[3] DW-HTL is the ammonia enriched distilled water obtained from recapturing stripped ammonia from hydrothermal liquefaction wastewater.
[4] DW-HTL-AC is the water obtained after treating DW-HTL with activated carbon compounds detected in the water sample after activated carbon treatment of the above sample (DW-HTL-AC) was carbonic acid dimethyl ester.

HTL water. The treatment of this water (DW-HTL) with activated carbon appears to have removed all of the hetroaromatics as the only.

3.3.3. Proton NMR analyses

Proton NMR was used to compare the different types of organic compounds in water samples. When combined with data from the GC–MS analysis, this provided additional information as to the presence or absence of specific types of compounds in these samples. The proton NMR spectra were acquired using quantitative parameters (see Materials and Methods section) which allowed the quantitative comparison of different samples for specific types of protons. ^{1}H NMR spectra obtained from different water samples are shown in Fig. 5. ^{1}H NMR spectra acquired were split into five regions: two regions associated with the aromatic protons and three with the aliphatic protons. The two aromatic regions were from 8 to 9 ppm and 6.5–8 ppm and the aliphatic regions were from 0.7 to 1.8 ppm, 1.8–3.0 ppm and 3.0–4.5 ppm. Types of protons associated with these regions are listed in Table 6 along with the absolute proton intensities of each region obtained by integrating the proton spectra from different water samples. HTL water without any treatment contains protons in all the regions of the spectrum representing a wide variety of compounds present in the water sample. Some of these signals could represent compounds like pyrizines, pyridines, cyclopentanones and their derivatives (as identified in the GC–MS) with the following associated proton signals: pyrazines and their derivatives in heteroaromatic region (8.0–9.0) and their side chains (Ar-CH-) in the Hα region (1.8–3.0) and alkyl region (0.7–1.8); Pyrrolidinone and their derivatives in regions of saturated alkyl chains (0.7–1.8), Hα to heteroatom region (1.8–3.0) and CH_x group attached to the heteroatoms (3.0–4.5); and Cyclopentanone and its derivatives in the regions of 0.7–1.8 and 1.8–3.0 representing saturated alkyl chains and CH attached to the heteroatom, respectively. For this sample only around 4.4% of the total proton intensity is in the aromatic region with around 1.7% in the heteroaromatic region. Processed water samples (HTL-A, DW-HTL, and DW-HTL-AC) exhibited fewer proton peaks with lower intensities than HTL water depending on the specific process used for conversion and extent of contaminant removal. Total proton intensities for the samples HTL-A, DW-HTL, and DW-HTL-AC were 15%, 2%, and 1% respectively, of the intensity obtained from sample HTL indicative of reduced amounts of the different types of contaminants in the samples in the following order of.

Decreasing concentration: HTL > HTL-A > DW-HTL > DW-HTL-AC. Water sample HTL-A showed proton intensities in all the regions but at a much lower intensity than sample HTL. The singlet at around 2.0 for samples HTL and HTL-A could potentially be associated with compounds like acetic acid and their derivatives which were identified during GC–MS of sample HTL-A. The sample also contained around 2.5% of its total proton intensities in the aromatic and heteroaromatic region with most of it (86%) in the heteroaromatic region. The peak in the heteroaromatic region (8.0–9.0) is a singlet with no other peaks indicating a single symmetric heteroaromatic compound as a possible source of this proton peak. A symmetrical heteroaromatic like pyrazine could be a potential source of the above mentioned singlet. As determined using GC–MS analysis the presence of pyrazines and their derivatives have been detected in some HTL water samples. The presence of any heteroaromatics in sample HTL-A was not detected in the GC–MS analysis which could be due to a number of reasons as discussed above. Water samples that were used to redissolve the stripped ammonia (DW-HTL and DW-HTL-AC) had only a few impurities as indicated by various analyses (% C using elemental analysis, GC–MS, and low intensity proton signals in q-NMR). These impurities would constitute the volatile compounds that were carried over by air stripping and redissolved in DI water. The heteroaromatic proton intensity for samples HTL-A and DW-HTL were very similar at 32.5 and 30.5, respectively. Treatment of DW-HTL with activated carbon removed all the aromatic and heteroaromatic compounds as indicated by the absence of proton intensities in the aromatic regions: 6.5–8.0 and 8.0–9.0. The only proton peaks in this sample were in the aliphatic regions with much lower total proton intensity, equivalent to 1% of the original HTL water sample. GC–MS analysis of the water sample indicated carbonic acid dimethyl ester (DMC) as a potential impurity in this sample. A singlet in the aliphatic region 3.0–4.5 as seen in Fig. 5 could be associated with DMC or a similar compound. Other aliphatic compounds in small quantities may also be present as evident from proton peaks in aliphatic regions of 0.7–1.8 and 1.8–3.0. The growth influence of these contaminants in different water samples on the growth of algae is discussed in the following section.

3.4. Potential inhibitors and their influence on growth

Other studies have identified phenols and phenolics, cyclic ketones, heavy metals, pyrazines, pyrroles and amines as the major organic compounds found in HTL aqueous phase residues [11]. In particular, heterocyclic aromatics such as pyrazine and pyrrole are commonly de-

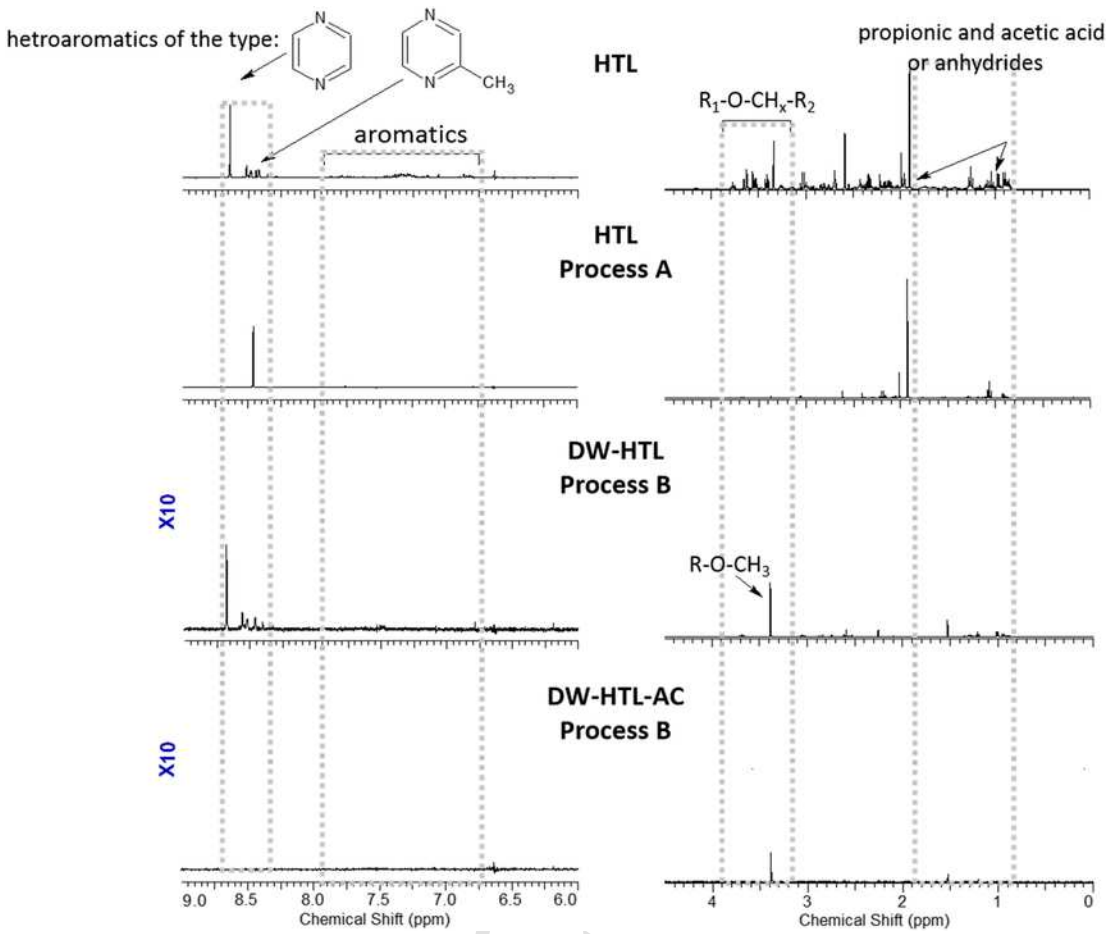

Fig. 5. ^{1}H NMR of neat water samples with pre-saturation of the water peak compare regions for protons associated with molecules of the type as indicated in the dashed boxes. Note the y-axis signal for process B water samples (DW-HTL and DW-HTL-AC) are magnified by a factor of 10. HTL is the original water obtained from the hydrothermal liquefaction reactor without any further processing. HTL-Process A is the water obtained after hydrothermal gasification and activated carbon treatment of the HTL water. DW-HTL is the ammonia enriched distilled obtained from recapturing stripped ammonia from hydrothermal liquefaction wastewater as described in Materials and Methods section. DW-HTL-AC is the water obtained after treating DW-HTL with activated carbon.

tected in oil fractions and aqueous waste streams after HTL of microalgae biomass (this study, [12,13,23–25]).Various compounds within these broad classes are either known to be or are potential inhibitors of algal growth. Several studies have reported poor growth on HTL waste streams unless heavily diluted [13]. Pyridine and picoline are known to inhibit chlorophyll formation and growth in *Chlorella vulgaris* quite potently (IC_{50} = 0.1% v/v) [26]. The effects of cyclic ketones on algal growth are uncertain, however one study found that cyclohexanone was toxic to the growth of six different species of marine microalgae [27]. In this study, robust algal growth on recycled HTL nutrients was well correlated with the reduction or elimination of these various organic compounds.

3.5. Comparison of nutrient recovery and reuse strategies

Process A represents a more conventional approach for the recycling of nutrients from HTL water. The main disadvantage with this approach however is the need for extensive processing to remove or reduce the complex assemblage of organic compounds from the water, some of which are potent inhibitors of algal growth. Hydrothermal gasification process used in process A may also represent a significant energy and economic penalty. Despite extensive processing, residual inhibitors remained which ultimately limited the efficiency of nutrient recycling. Process B differs from the conventional approach of Process A mainly by the separation of ammonia from HTL water by air-stripping, thereby decoupling the N recycling from HTL water recycling. This has the advantage of separating the N from the inhibitory compounds dissolved in the HTL water and simplifies the water clean-up and processing step resulting in a much lower concentration of these inhibitors and more stable and predictable growth. Although comparable growth rates using Process A water could be obtained after strong dilution, the presence of residual inhibitors caused poor growth in some cultures leading to unpredictable results and culture crashes. In addition, the N decoupling via Process B offers more flexibility for N storage in a biorefinery, similar to the strategy for P recycling in which P is stored temporarily as a stable struvite precipitate for later use as an algal growth nutrient. This approach would also makes it feasible to use HTL processing as a source of fertilizers for higher plants.

4. Conclusions

Ammonia and phosphorus nutrients partitioned into the aqueous waste streams generated by hydrothermal liquefaction of algal biomass can be recycled to support additional rounds of microalgae growth. However, the recycling strategy employed bears importantly on the efficiency with which these nutrients can be reused in support of algal growth. In this study, we demonstrate that a complete decoupling of nutrients from the HTL water offers a more flexible approach for reuse. Phosphorus was decoupled from HTL through its precipitation as struvite, which was subsequently redissolved to the desired concentration

Table 6
Regions of ^{1}H NMR spectra, corresponding protons associated with each region and absolute proton intensities in these regions for different water samples. Absolute proton intensity in a particular region is indicative of its abundance in that region and of molecules associated with them. Values reported were obtained from a single representative sample analysis of HTL, HTL-A, DW-HTL, and DW-HTL-AC.

δ (ppm)	Protons associated with	Absolute intensity			
		HTL [1]	HTL - A [2]	DW-HTL [3]	DW-HTL-AC [4]
0.7–1.8[1]	Saturated alkyl chains typically made of CH, CH_2 and CH_3 groups	3056.1	417.0	79.0	39.9
1.8–3.0[2]	Mostly from CH aliphatic with Hα to functional groups such as C═C, C═O, Ar—CH	4316.0	977.8	44.3	11.9
3.0–4.5[3]	Mostly from CH group attached to heteroatom	2144.1	68.6	58.7	56.9
6.5–8.0[4]	Aromatic	446.5	5.2	14.8	0
8.0–9.0	Heteroaromatic	175.0	32.5	30.5	0

[1] HTL is the original water obtained from the hydrothermal liquefaction reactor without any further processing.
[2] HTL-A is the water obtained after hydrothermal gasification and activated carbon treatment of the HTL water.
[3] DW-HTL is the ammonia enriched distilled water obtained from recapturing stripped ammonia from hydrothermal liquefaction wastewater.
[4] DW-HTL-AC is the water obtained after treating DW-HTL with activated carbon.

into municipal wastewater. Ammonia was decoupled from HTL water through air-stripping and redissolution in distilled water. At scale, it would not be feasible to use distilled water for ammonia recovery and a source of nitrogen-poor but non-potable water would have to be identified for ammonia and struvite redissolution. This advance towards recovering and recycling both nitrogen and phosphorous without inhibitors would significantly improve the overall sustainability and scalability of an algae biorefinery.

Conflict of interest

All authors declare no conflict of interest.

Author contributions

D Singh and P J McGinn developed the concept, designed the study, interpreted the data and prepared the manuscript. K C Park. G Robertson, L Scoles, and W Ma executed the experiments, analyzed data, and contributed towards parts of the manuscript.

Uncited reference

[17]

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Statement of informed consent, human/animal rights

No conflicts, informed consent, human or animal rights applicable.

References

[1] R.H. Wijffels, M.J. Barbosa, An outlook on microalgal biofuels, Science 329 (2010) 796–799.
[2] Y. Chisti, Constraints to commercialization of algal fuels, J. Biotechnol. 167 (2013) 201–214.
[3] R. Pate, G. Klise, B. Wu, Resource demand implications for US algae biofuels production scale-up, Appl. Energy 88 (2011) 3377–3388.
[4] B.K. Shurtz, B. Wood, J.C. Quinn, Nutrient resource requirements for large-scale microalgae biofuel production: multi-pathway evaluation, Sustainable Energy Technol. Assess. 19 (2017) 51–58.
[5] J. Peccia, B. Haznedaroglu, J. Gutierrez, J.B. Zimmerman, Nitrogen supply is an important driver of sustainable microalgae biofuel production, Trends Biotechnol. 31 (2013) 134–138.
[6] T. Wang, H. Yabar, Y. Higano, Perspective assessment of algae-based biofuel production using recycled nutrient sources: the case of Japan, Bioresour. Technol. 128 (2013) 688–696.
[7] D.C. Elliott, P. Biller, A.B. Ross, A.J. Schmidt, S.B. Jones, Hydrothermal liquefaction of biomass: developments from batch to continuous process, Bioresour. Technol. 178 (2015) 147–156.
[8] D.C. Elliott, T.R. Hart, A.J. Schmidt, G.G. Neuenschwander, L.J. Rotness, M.V. Olarte, A.H. Zacher, K.O. Albercht, R.T. Hallen, J.E. Holladay, Process development for hydrothermal liquefaction of algae feedstcoks in a continuous-flow reactor, Algal Res. 2 (2013) 445–454.
[9] L. Leng, J. Li, Z. Wen, W. Zhou, Use of microalgae to recycle nutrients in aqueous phase derived from hydrothermal liquefaction process, Bioresour. Technol. 256 (2018) 529–542.
[10] P. Biller, A.B. Ross, S.C. Skill, A. Lea-Langton, B. Balasundaram, C. Hall, et al., Nutrient recycling of aqueous phase for microalgae cultivation from the hydrothermal liquefaction process, Algal Res. 1 (2012) 70–76.
[11] U. Jena, N. Vaidyanathan, S. Chinnasamy, K.C. Das, Evaluation of microalgae cultivation using recovered aqueous co-product from thermochemical liquefaction of algal biomass, Bioresour. Technol. 102 (2011) 3380–3387.
[12] L. Garcia Alba, C. Torri, D. Fabbri, S.R.A. Kersten, D.W.F. Wim Brilman, Microalgae growth on the aqueous phase from hydrothermal liquefaction of the same microalgae, Chem. Eng. J. 228 (2013) 214–223.
[13] M. Bagnoud-Velásquez, U. Schmid-Staiger, G. Peng, F. Vogel, C. Ludwig, First developments towards closing the nutrient cycle in a biofuel production process, Algal Res. 8 (2015) 76–82.
[14] A. Brenner, A. Abeliovich, Water purification: algae in wastewater oxidation ponds, In: Handbook of Microalgal Culture: Applied Phycology and Biotechnology, 2013, pp. 595–601.
[15] O. Hammouda, A. Gaber, N. Abdel-Raouf, Microalgae and wastewater treatment, Ecotoxicol. Environ. Saf. 31 (1995) 205–210.
[16] K.C. Park, C. Whitney, J.C. McNichol, K.E. Dickinson, S. MacQuarrie, B.P. Skrupski, et al., Mixotrophic and photoautotrophic cultivation of 14 microalgae isolates from Saskatchewan, Canada: potential applications for wastewater remediation for biofuel production, J. Appl. Phycol. 24 (2012) 339–348.
[17] C.J. Zhu, Y.K. Lee, Determination of biomass dry weight of marine microalgae, J. Appl. Phycol. 9 (1997) 189–194.
[18] L. Peng, H. Dai, Y. Wu, Y. Peng, X. Lu, A comprehensive review of phosphorus recovery from wastewater by crystallization processes, Chemosphere 197 (2018) 768–781.
[19] J.D. Doyle, S.A. Parsons, Struvite formation, control and recovery, Water Res. 36 (2002) 3925–3940.
[20] B. Liu, A. Giannis, J. Zhang, V.W.C. Chang, J.Y. Wang, Air stripping process for ammonia recovery from source-separated urine: modeling and optimization, J. Chem. Technol. Biotechnol. 90 (2015) 2208–2217.
[21] L. Zhang, Y.W. Lee, D. Jahng, Ammonia stripping for enhanced biomethanization of piggery wastewater, J. Hazard. Mater. 199-200 (2012) 36–42.
[22] C. Gai, Y. Zhang, W.T. Chen, Y. Zhou, L. Schideman, P. Zhang, et al., Characterization of aqueous phase from the hydrothermal liquefaction of Chlorella pyrenoidosa, Bioresour. Technol. 184 (2015) 328–335.
[23] N. Sudasinghe, B. Dungan, P. Lammers, K. Albrecht, D. Elliott, R. Hallen, et al., High resolution FT-ICR mass spectral analysis of bio-oil and residual water soluble organics produced by hydrothermal liquefaction of the marine microalga Nannochloropsis salina, Fuel 119 (2014) 47–56.
[24] D.R. Vardon, B.K. Sharma, G.V. Blazina, K. Rajagopalan, T.J. Strathmann, Thermochemical conversion of raw and defatted algal biomass via hydrothermal liquefaction and slow pyrolysis, Bioresour. Technol. 109 (2012) 178–187.
[25] W. Yang, X. Li, Z. Li, C. Tong, L. Feng, Understanding low-lipid algae hydrothermal liquefaction characteristics and pathways through hydrothermal liquefaction of algal major components: crude polysaccharides, crude proteins and their binary mixtures, Bioresour. Technol. 196 (2015) 99–108.
[26] B.B. Singh, R. Chandra, Comparative chronic toxicity of pyridine, α-picoline, and β-picoline to Lemna minor L. and Chlorella vulgaris B, Bull. Environ. Contam. Toxicol. 75 (2005) 482–489.
[27] I.L. Hook, S. Ryan, H. Sheridan, Biotransformation of aliphatic and aromatic ketones, including several monoterpenoid ketones and their derivatives by five species of marine microalgae, Phytochemistry 63 (2003) 31–36.