Assessing hydrothermal liquefaction for the production of bio-oil and enhanced metal recovery from microalgae cultivated on acid mine drainage

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Abstract

The hydrothermal liquefaction (HTL) of algal biomass is a promising route to viable second generation biofuels. In this investigation HTL was assessed for the valorisation of algae used in the remediation of acid mine drainage (AMD). Initially the HTL process was evaluated using *Arthrospira platensis* (Spirulina) with additional metal sulfates to simulate metal remediation. Optimised conditions were then used to process a natural algal community (predominantly *Chlamydomonas sp.*) cultivated under two scenarios: high uptake and low uptake of metals from AMD. High metal concentrations appear to catalyse the conversion to bio-oil, and do not significantly affect the heteroatom content or higher heating value of the bio-oil produced. The associated metals were found to partition almost exclusively into the solid residue, favourable for potential metal recovery. High metal loadings also caused partitioning of phosphates from the aqueous phase to the solid phase, potentially compromising attempts to recycle process water as a growth supplement. HTL was therefore found to be a suitable method of processing algae used in AMD remediation, producing a crude oil suitable for upgrading into hydrocarbon fuels, an aqueous and gas stream suitable for supplementing the algal growth and the partitioning of most contaminant metals to the solid residue where they would be readily amenable for recovery and/or disposal.

1. Introduction

Declining water quality is an issue of increasing importance worldwide. In particular, water contamination by heavy metals from domestic or industrial sources can have a significant impact on the biodiversity of aquatic ecosystems and human health [1]. Mining operations have long been recognised as one of the major anthropogenic sources of metals to the aquatic ecosystem [2]; acid mine drainage (AMD) in particular causes persistent and severe pollution and affects most countries with historic or current mining industries [3]. Although chemical compositions and pH vary from site to site, AMD tends to contain elevated concentrations of dissolved metals such as Fe, Al, Zn, Sn and Pb [3, 4]. Because of the longevity of AMD (many mines in Europe continue to release metals centuries after closure) [5], treatment presents substantial long term liabilities for mine operators and for governments that inherit orphan sites. As a result, there has been a growing interest in more efficient and cost-effective remediation technologies.

Microbial remediation of metal contaminated wastes has gained increasing popularity over the last few years [6]. Algae in particular have been demonstrated to sequester metals via biosorption and intracellular uptake [7]; the uptake of metals is strongly dependent on the provision of adequate light, temperature and nutrients for algal growth [7]. The use of both living and dead biomass has been explored, with living algal cells found to be particularly efficient at remediating water with low metal concentrations [8, 9]. Although these methods are highly effective in lowering metal concentrations in AMD sufficiently, large volumes of secondary waste are created in the form of metal-contaminated biomass and sediments [10]. Use of the biomass as a fuel and recovery of the associated metals from the AMD could relieve this threat, as well as presenting a revenue stream to offset operational costs of both the AMD treatment and the biofuel production itself. Additionally the elevated temperature of the AMD could potentially enhance algal growth. While recovery of the metals is possible through the complete drying and direct combustion of the algae for power

generation, with the absorbed metals being recoverable from the ash [11], a potentially more efficient alternative is to process the biomass through hydrothermal liquefaction (HTL).

HTL utilises water at sub-/near-critical conditions (200–380 °C, 50–280 bar) as both the reaction medium and solvent for a host of reactions, converting algal biomass into a bio-oil, alongside an aqueous phase, a solid residue and a number of gaseous products. HTL can be used to process biomass at a concentration of ca. 5–25 % with water, with one study estimating that the energy consumption of biomass preparation was reduced by 88 % if the input slurry generated is used without drying steps [12]. The temperatures used in HTL are well within the range of those encountered in many conventional oil refinery operations [13], and as such, HTL processing of algal biomass is energy-efficient and potentially scalable. For example, the life cycle performance of laboratory, pilot- and full-scale scenarios, demonstrated significant improvements in GHG emissions with respect to gasoline and corn ethanol, and a potential Energy Return on Investment (EROI) of around 2.5 for the full-scale scenario [14], subject to the optimisation of a closed-loop system incorporating energy and nutrient recycling.

HTL comprises hundreds of simultaneous reactions, including the decarboxylation of carbohydrates to sugars and fragmentation to aldehydes, hydrolysis of lipids to fatty acids and subsequently longer-chain hydrocarbons, and depolymerisation and deamination of proteins. In addition, repolymerisation of the reactive fragments into larger oil compounds is also favourable [15]. Most liquefactions under optimised conditions have resulted in bio-oil yields around 30–45 % [16, 17], regardless of algae strain, although, notably, Li *et al.* obtained yields of 55 % for *Nannochloropsis* sp. under HTL at 260 °C for 60 min and at 25 % total solid (TS) loading, and 82.9 % for *Chlorella* sp. (220 °C, 90 min, 25 % TS) [18]. The numerous reactions occurring under HTL conditions lead to a bio-oil containing a diverse range of chemical compounds, the main constituents of which have been found to be C5-C16 cyclic nitrogen compounds, C15–C33 branched and unbranched hydrocarbons, branched oxygenates, aromatic compounds, and heterocycles [18]. Elevated heteroatom (O and N) contents with respect to mineral crude oil are typical of algal bio-

oils, which give rise to undesirable fuel properties, such as high acidity and viscosity, and the diverse chemical compositions can negatively affect combustion performance, storage stability and economic value [19, 20].

The higher heating value (HHV) of the oils usually fall between 25–35 MJ kg^{-1}, with higher lipid levels in the biomass corresponding to higher bio-oil HHV. Although this constitutes a significant increase with respect to the starting biomass, it still falls short of the energy content of mineral oil (41–48 MJ kg^{-1}). Although the bio-oil it is not suitable for use as a transport fuel without further modification, potentially it can be refined in a similar manner to crude oil to give a range of fuels, including gasoline, diesel and aviation kerosene [21].

The HTL reaction can also be accelerated by metal catalysts and a number of investigations have examined their effect. As algae are complex mixtures of proteins, carbohydrates, lipids and alternative metabolites, additional metals rarely effect the algae uniformly across species [22]. For example Biller et. al. demonstrated that Na_2CO_3 promoted the decomposition of carbohydrates more effectively, although overall the catalyst had no significant positive effect on the bio-oil yields or overall efficiency of the process [23]. This is in direct contrast to other studies that used alternative algal strains [24, 25]. Similar variation has been observed for K and Li homogeneous salts [17, 23], where reaction temperature appears to be the major contributing factor involved in the yield and product distribution [22].

Heterogeneous catalysts potentially present a more attractive option for ease of separation, especially considering the temperatures necessary for HTL processing. For example, Duan and Savage examined a variety of common industrial catalysts (Pd/C, Pt/C, Ru/C, Ni/SiO$_2$-Al$_2$O$_3$, sulfided CoMo/γ -Al$_2$O$_3$ and a zeolite) under hydrogen and inert conditions in batch reactions. Under these conditions they found that generally the bio-oil yield was increased substantially, in some cases from 34% up to 57% of the total biomass [26].

To create an economical biorefinery it is necessary to consider upstream factors, as well as final product quality. Despite the advantages conferred by HTL, cultivation of algal biomass is still a

relatively energy-intensive process, and requires high inputs of water, nutrients and CO_2 [27]. As well as optimising bio-oil yields; maximising carbon efficiency, efficient water / nutrient recycling and ensuring an inexpensive source of CO_2 are crucial to the success of algal biofuel production [28].

In this investigation the suitability of using HTL to process metal contaminated algal biomass was assessed. Firstly, Spirulina (*Arthrospira platensis)* with representative levels of metal sulfates were processed in a batch HTL system. Finally, two algal cultures cultivated on AMD were converted under the optimal conditions to assess the viability of encompassing a combination of AMD remediation and biofuel production (figure 1). Here, we aim to determine how metals affect the yield and composition of the HTL reaction products (the solid, aqueous, oil and gaseous phases) and assess the viability and usefulness of these products for exploitation as a biofuel, metal remediation and for the recycling of nutrients to promote further microalgal growth.
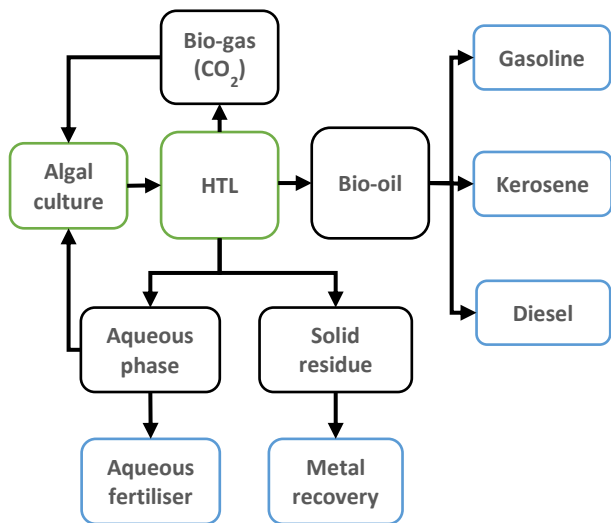

Figure 1 Proposed HTL biorefinery concept encompassing algal remediation of AMD

2. Materials and Methods

2.1 Materials

Spirulina powder (*Arthrospira platensis*) was obtained from Bulk Powders (Colchester, UK). The dried biomass contained 63% protein, 20% carbohydrate, 6% fat and 11% miscellaneous biochemical content. Metal sulfates (99%+), ($FeSO_4 \cdot 7H_2O$, $MgSO_4 \cdot 7H_2O$, $ZnSO_4 \cdot 7H_2O$, $PbSO_4$ and $SnSO_4$) were obtained from Sigma Aldrich and used without further purification. The HCl and HNO_3 (both trace metal grade) were purchased from Fisher Chemicals.

2.2 Methods

2.2.1 Hydrothermal liquefaction (HTL) batch reactions

Batch liquefaction was conducted in accordance with previous literature precedent [23]. The reactor, connected to a pressure gauge, needle valve, and spring-loaded relief valve, contained a total internal volume of *ca.* 50 ml. The reactor body was heated inside a vertical tubular furnace, with the temperature of the reaction mixture monitored using a thermocouple connected to data logging software. The reactor was loaded with approximately 4.000 g of dry biomass, 0–1500 mg metal sulfates, and 20 ml deionized water, and heated within the furnace, pre-heated to 550 °C, until the specified reaction temperatures were reached, 310 °C (15 min) – 350 °C (35 min), then removed from the furnace and allowed to cool to room temperature. Mixing was provided by convection in the reactor, temperature profiles of the reaction are given in the supporting information. In order to determine experimental error and test the repeatability of experimental results, three repeat runs of HTL of pure Spirulina at both 310 °C and 350 °C were used to assess the variation in the experimental set-up. The reaction pressure required for the hydrothermal liquefaction reaction was generated *in situ* through the expansion of the reactor fluids and partial vaporisation of the water. The reaction pressure varied from 120 bar at 310 °C to 180 bar at 350 °C

2.2.2 Gas analysis

After cooling, gaseous products were released *via* the needle valve into an inverted, water-filled measuring cylinder to measure gaseous fraction volume. Gas phase yields were calculated using the ideal gas law, assuming an approximate molecular weight of 44 g mol^{-1} (the molecular mass of CO_2, which makes up approx. 96–98 % of the gaseous product). A sample from each gas phase was separated and analysed using a gas chromatograph (Agilent 7890A) containing an HP-Plot-Q capillary column (using helium as the carrier gas), and fitted with an Agilent 5975C MSD detector. The samples were loaded at 35 °C, hold time 7 min, ramped to 150 °C at 20 °C min^{-1}, hold time 0 min, ramped to 250 °C at 15 °C min^{-1}, hold time 16 min.

2.2.3 Aqueous phase analysis

The aqueous phase was decanted from the reactor contents and filtered through a 0.22 µm filter. The dissolved product yield in the water phase was determined gravimetrically from a 2.5 ml aliquot, dried at 60 °C for 12 hours. The concentration of ammonium ions in the water phase was determined spectrophotometricaly using a Randox Urea analysis test kit (Merck, Milipore). The sample was diluted with deionised water to a concentration of 1 % prior to analysis. Subsequently 10 µl of sample was reacted for 5 min with 1000 µl of a urease reactant, followed by the addition of 200 µl of sodium hypochlorite solution to induce the colour change. After 10 min, sample absorbance was measured at 600 nm and urea concentration calculated relative to a standard solution. From this, ammonium ion concentration was calculated. Total nitrogen content analysis was carried out using a Merck-Millipore Spectroquant Total Nitrogen Cell Test kit and photometer, based on the Koroleff method of persulphate digestion to transform organic and inorganic N compounds into nitrate. Each sample was diluted to 0.1% prior to analysis. 10 ml of diluted sample was digested for 1 h at 120 °C, then allowed to cool to room temperature and reacted with a benzoic acid derivative form a nitro compound.

Phosphate concentration in the aqueous phase was determined using the Merck-Millipore Spectroquant test kit and photometer system. Prior to analysis, each sample was diluted by a factor

of 5–1000, depending on estimated phosphate content, and reacted with the reagents provided. Aqueous samples (6 ml), diluted in 11.6 ml deionised water were acidified with 0.4 ml 67% v/v HNO_3 prior to analysis using Perkin Elmer Optima 2100 ICP-OES to determine the Fe, Zn and Mg content.

2.2.4 Crude bio-oil analysis

To separate the remaining bio-oil and solid residue phase, the reactor was washed repeatedly using chloroform until the solvent was clear, the solution was filtered, and any residual bio-oil washed off the filter paper. The solvent was evaporated using a rotary evaporator set to 40 °C. To determine the energy content, approximately 200 mg bio-oil was weighed into a steel crucible and analysed using an IKA C1 bomb calorimeter to determine energy content. The ash content was determined by the mass difference of the crucible prior to and after energy content analysis. Bio-oil samples were analysed on a Carlo Erba Flash 2000 Elemental Analyser to determine CHN content. Elemental analyses were carried out in duplicate for each sample, and average values are reported.

2.2.5 Solid residue analysis

The solid residue yield was calculated from the mass of the retentate collected on the filter paper after drying for 12 hours in an oven at 60 °C. The filter paper was weighed immediately on removing from the oven, both before and after use, to minimise errors associated with absorption of atmospheric moisture. Solid residue samples were digested in *aqua regia*. Briefly, 6 ml of HCl (37 %; Fisher Tracemetal grade) was added to approximately 100 mg residue. After any initial reaction had subsided, 2 ml concentrated HNO_3 (Fisher Trace metal grade) was added and the digest covered and left at room temperature for 15 min. The digest was then heated to 95°C for 60 min, cooled and made up to 50 ml with ultra-pure water. Filtered digestates were analysed using an Agilent 7700 Series ICP-MS to determine P, Pb, Sn, Mg, Zn and Fe content. SEM analysis of the solid residue was carried out using a JEOL JSM-6480LV system. Elemental composition analysis was carried out using INCA software. Samples were analysed on a Carlo Erba Flash 2000 Elemental Analyser to determine CHN content.

2.2.6 Culturing of AMD-1 and AMD-2 cultures

A mixed community of microalgae (predominantly *Euglena-* and *Chlamydomonas*-like morphologies) isolated from the mine drainage of a former tin mine in the UK were grown in AMD supplemented with nitrates and phosphates (see supporting information). Following scale up in conical flasks (3 l), the biomass for HTL was generated in a bubble column with artificial illumination. Bubble columns were constructed using PVC components; 110 mm clear polycarbonate tubing, with a working culture volume of 10 l. Light was supplied via 36 W Grolux fluorescent tube and 36W 865 daylight fluorescent tube providing 80 μmol photons $m^{-2}s^{-1}$. Cultures were aerated by constant bubbling at 3 l min^{-1}. Cultures were grown at 20 °C in cycles of 16 h of light: 8 h dark photoperiods (16:8h).

The AMD-1 culture was grown on a synthetic acid mine drainage medium (sAMD) supplemented with both phosphate and nitrate salts (a full description is given in the supporting information). The AMD-2 culture was grown on AMD supplemented with phosphate and nitrate salts (see supporting information). Cell counts were conducted via flow cytometry daily and stationary growth phase biomass was harvested by centrifugation.

2.2.7 Culturing the AMD-1 algae with HTL aqueous phase

The photobioreactors were held at room temperature, which fluctuated between 16 ^{o}C to 22 ^{o}C, under full aeration and were inoculated with 1 l of the AMD algae cultured in a sAMD medium (see supporting information), with no additional nitrates or phosphates but with the addition of the aqueous phase from the hydrothermal processing of Spirulina at 350 °C, (diluted 1:100 v/v with deionised water). The starting inoculum was ~ 10^{5} cells ml^{-1}.

3. Results and discussion

3.1 HTL of Spirulina and metal sulfates

Initially, the effect of additional metals present with the algal biomass on the HTL process was examined using the commercially available cyanobacterium Spirulina (*Arthrospira platensis*) with the addition of a range of metal sulfates under batch HTL process conditions. The metal concentrations used (described in Table 1) were based on data collected from AMD from a former tin mine between January and March 2014. The main metal contaminants found in this mine water were Fe, Zn and Mg, with lower amounts of Sn and Pb also being present. To examine the effect of the metals on the HTL process, the five separate metal sulfates were added to the Spirulina biomass at concentrations found in the AMD. Two distinct scenarios were investigated, the first with the main metals Fe, Zn and Mg being present at a low concentration in order to assess the effect of a minimum uptake scenario, where the algae display minimal adsorption of the metals present in dilute AMD streams. In the second scenario the concentration of metals was higher, mimicking a situation where the algae had successfully been used to remediate concentrated AMD metal effluent streams (high uptake scenario).

Table 1 The metal salts used in conjunction with Spirulina to mimic metal contaminated algae used in remediation of AMD. The low uptake scenario mimics a situation where there is poor metal uptake from the AMD, the high uptake scenario mimics the best case, where all the metal present in the AMD has been absorbed

Metal / Scenario	Representative salt(s)	Metal concentration in the reactor / mg L^{-1}
Pb	$PbSO_4$	10
Sn	$SnSO_4$	210
Low uptake (Mg/Zn/Fe)	$MgSO_4$	17
	$FeSO_4$	43
	$ZnSO_4$	90
	Total	150
High Uptake (Mg/Zn/Fe)	$MgSO_4$	2665
	$FeSO_4$	5820
	$ZnSO_4$	14,396
	Total	22,855
Mg	$MgSO_4$	2665
Zn	$ZnSO_4$	5820
Fe	$FeSO_4$	14,396

3.1.1 Effect of additional metal sulfates on the bio-oil yield and HHV of Spirulina

The Spirulina/metal mixtures were processed in a batch reactions at either 310 °C or 350 °C. The resulting oil was extracted, weighed and the N content and energy density assessed (figure 2). In the majority of cases lower processing temperatures generated higher bio-oil yields than those obtained at *ca.* 350 °C. This corresponds directly with previous literature findings [29-31]. There was also a positive correlation between bio-oil yields and increasing total metal content (Figure 2a), and is potentially due to the catalytic effect of the metals present. $FeSO_4$ is commonly employed as a catalyst in coal liquefaction for example [32], and significant improvements in oil yield when

liquefying pine wood in supercritical ethanol using 5% w/w $FeSO_4$ have also been reported [33]. Similarly, the addition of both $MgSO_4$ and $ZnSO_4$ also increased oil yields, though neither metal has previously been investigated as a potential HTL catalyst.

N content of the bio-oil was reduced from 9.7% in the Spirulina feed to 7% in the bio-oil produced by HTL without additional metals. All samples displayed lower bio-oil N levels at higher processing temperatures. The metal concentration had little effect on the N content of the bio-oil though slightly elevated levels were observed in the process when Sn and Mg were present. Despite the N content of the bio-oil being lower than in unprocessed Spirulina, the presence of metals did not reduce the N content to a suitable level for combustion and a further hydrogenation upgrading stage would be necessary to produce hydrocarbon fuels from this process.

Consistently higher energy density was observed at the higher processing temperatures, consistent with findings in the literature [17]. Interestingly, increased concentrations of Mg, Zn and Fe caused a decrease in bio-oil energy content (Fig 2(c)). This is likely the result of the higher yields of bio-oil observed under these conditions. For example, *Nannochloropsis* sp. processed at 350 °C yielded 34 % oil with a HHV of 38.1 MJ kg^{-1}, while 46 % oil was obtained at a processing temperature of 310 °C, though with a much lower HHV of 27.7 MJ kg^{-1} [15]. This is presumably due to the increased partitioning of oxygenated organic components into the oil phase.
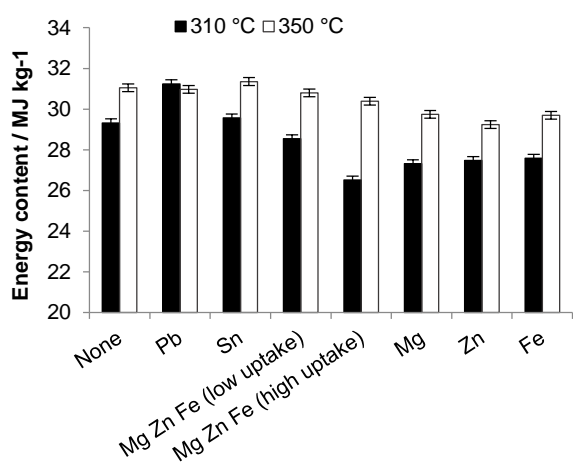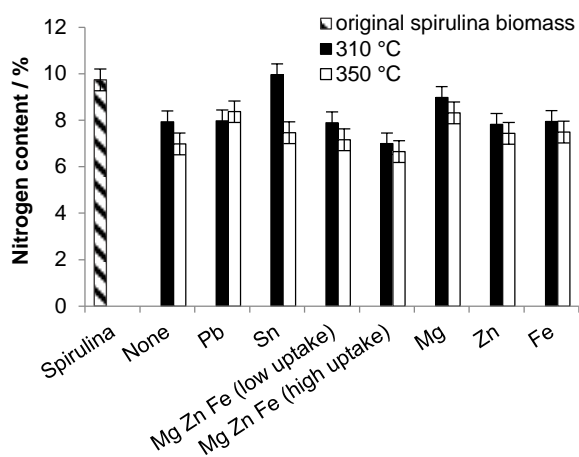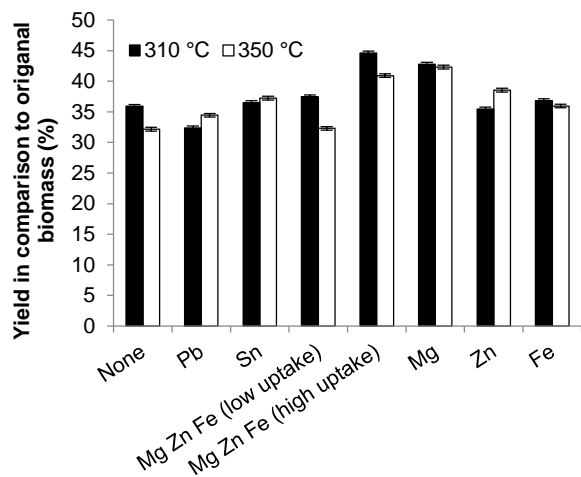
Fig. 2 Bio-oil produced by hydrothermal liquefaction of Spirulina with additional metal sulfates, where a) total bio-oil yields calculated as % of the original starting biomass (excluding additional metals), b) total N content in the bio oil and c) The energy content of the bio-oil.

3.1.2 Assessment of the aqueous phase from the HTL of Spirulina and metal sulfates

On processing of the algal biomass the light organic compounds and some alternative inorganic elements are deposited in the aqueous phase. To assess this residue, the aqueous phase was dried in an oven at 60 °C for 12 hours and the residue weighed (figure 3a). The organic residue in the aqueous phase was consistently reduced at higher processing temperatures, this organic material was presumably partitioning into the bio-oil phase and correlates with the increasing bio-oil yields. Recycling of algal nutrients (such as N, P, K, Fe) would be required for an economically viable algal biorefinery. While the AMD provides most metallic species in abundance, AMD is generally phosphate and nitrogen limited. The total N, NH_4^+ and phosphate content of the aqueous phase samples were therefore examined (figure 3b). Nutrient partitioning into the aqueous phase was influenced both by the reaction conditions and the levels of metal in the sample. The total nitrogen content in the aqueous phase was similar irrespective of temperature though higher processing temperatures increased the total amount of NH_4^+ slightly. A high level of N was retained in the aqueous phase irrespective of metal loading and could be used as a useful source of NH_4^+ for nitrogen limited AMD.

Reasonable partitioning of phosphorus into the aqueous phase also occurred and was stable irrespective of the temperature employed. However, while up to 1920 mg l^{-1} of P was observed at the low metal loadings, on addition of high levels of metals to the system the phosphate levels detected in the aqueous phase dropped dramatically. At the highest metal loading, 22,855 mg l^{-1} of total metals, phosphate levels in the aqueous phase dropped to 13.9 mg l^{-1}, from 1860 mg l^{-1} in the aqueous phase from processing of pure Spirulina.

The limitation of aqueous P concentrations is potentially due to the formation of insoluble metal phosphates (particularly iron and zinc phosphates) and their partitioning into the solid residue. Despite additional metals being in excess, small levels of phosphate (up to 15 mg l^{-1}) were still detected in the aqueous residue. Zinc, however, was a notable exception to this. The aqueous phase

from a sample processed with 5820 mg l^{-1} Zn still contained 460 mg l^{-1} of phosphate when processed at 310 °C, and 720 mg l^{-1} when processed at 350 °C.

Only trace levels of Sn and Pb were detected in the aqueous phase of the water from the HTL processing of pure Spirulina, and did not increase with the addition of extra Sn or Pb sulfate to the system. At the levels examined Sn and Pb partition almost entirely out of the aqueous phase, demonstrating that HTL is a suitable processing method that partitions these contaminants into the solid residue allowing the aqueous phase to be recycled.

Only trace levels of Fe, Zn and Mg were observed in the aqueous phase at the low uptake scenario. In the high uptake scenario, some partitioning into the aqueous phase was observed (figure 3d). Fe, present in the reaction at a concentration of ca. 14,000 mg l^{-1}, partitioned almost entirely out of the aqueous phase. Less than 5% of the Zn added to the system partitioned into the aqueous phase, though 37–39 % Mg partitioned into the aqueous phase in the presence of Fe and Zn. The high recovery of Mg in the aqueous phase could be beneficial, as a lack of micro/macronutrients such as Mg has been shown to be a major contributing factor in the reduction of algal growth on HTL process water [34].
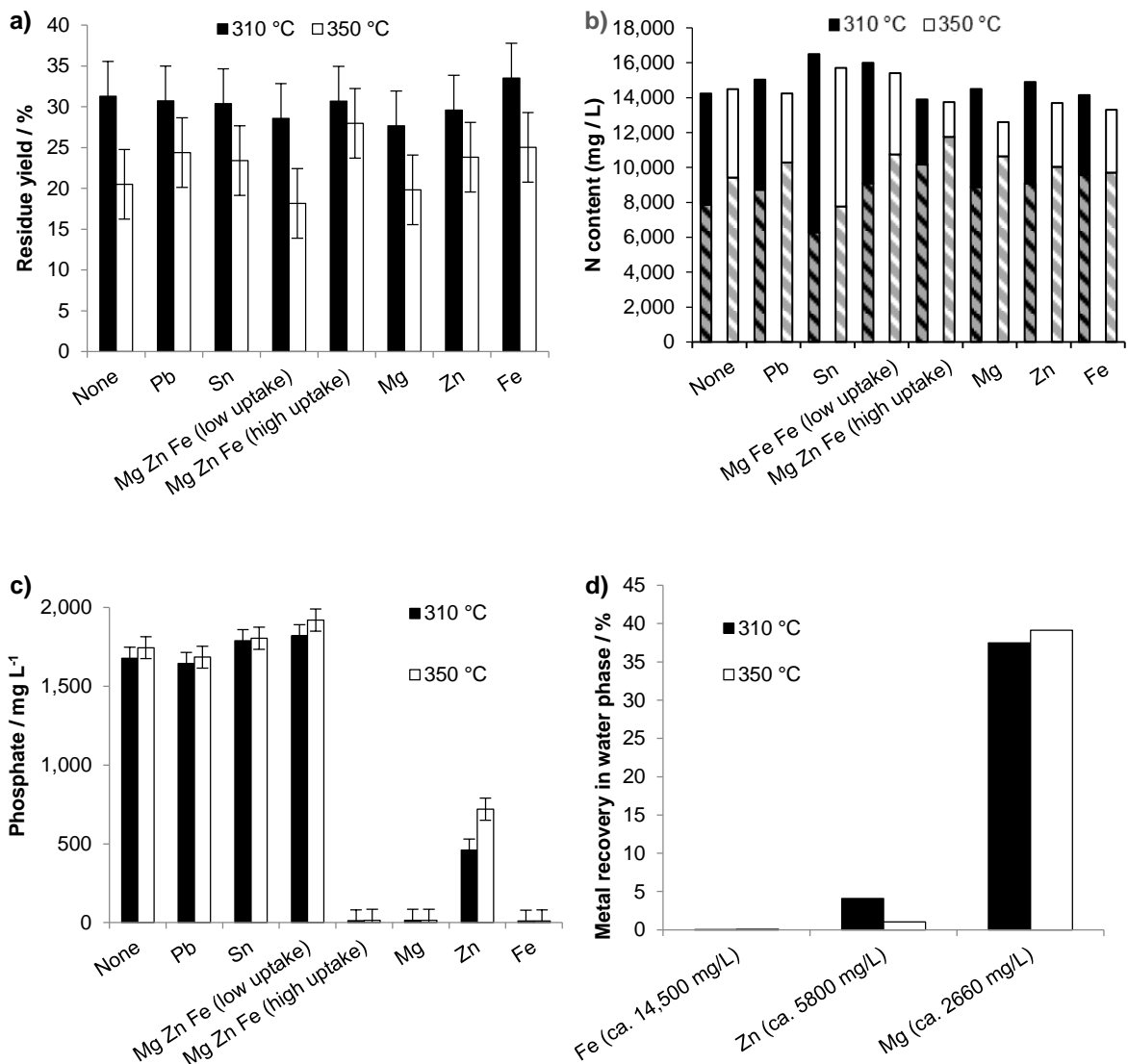
Fig. 3 Analysis of the aqueous phase produced by hydrothermal liquefaction of Spirulina with additional metal sulfates where a) is the yield of residue on removal of the water, b) is the total N content in the water phase, the grey shaded area represents the amount of nitrogen present as NH_4^+ c) is the total phosphate content in the aqueous phase and d) gives the level of metals present in the aqueous phase on HTL of the combined Mg, Zn, Fe (high uptake) sample (total metal content 22,855 mg l^{-1}).

3.1.3 Effect of metal sulfates on the gas phase produced from the HTL of Spirulina

Gas yields of around 7.3-16.2 % were obtained for all samples (table 3), gas yields generally increased with increased temperature and were found to be higher in the presence of metal loadings and particularly Mg, Zn and Fe. GC-MS analysis demonstrated that the gas phase was

predominantly composed of CO_2 (between 96% - 99%), and the remaining fraction composed of volatile organics, particularly propene, isobutylene, 1-butene and 1,2-dimethylcyclopropane (full analysis is given in the supporting information).

Lower processing temperatures tended to increase yields of CO_2 and decrease VOC content. Similar results have been noted previously where *Nannochloropsis* sp. yielded a gas phase with elevated hydrocarbons on increasing temperatures, although the most abundant species were determined to be methane and C_2 hydrocarbons, rather than C_3–C_5 species [35]. This suggests that the use of lower processing temperatures would be beneficial for gas phase recycling to supplement algal cultivation, as relatively high-purity CO_2 is required to culture the algae.

Table 3. Analysis of the gas fraction produced from the hydrothermal liquefaction of Spirulina with additional metal sulfates. Gas yield is given as a percentage of the original biomass, on the assumption that the volume is entirely composed of CO_2.

Metal	Gas Yield (%)		CO_2 (%)		Volatile organic carbon (%)	
	310 °C	350 °C	310 °C	350 °C	310 °C	350 °C
None	9.8	13.4	98.7	97.4	1.32	2.64
Pb	8.6	9.5	98.4	96.8	1.64	3.18
Sn	9.1	14.6	98.5	98.4	1.49	1.62
Mg Zn Fe (low uptake)	9.8	7.3	99.0	95.9	1.05	4.13
Mg Zn Fe (high uptake)	15.4	15.6	99.4	97.6	0.6	2.44
Mg	13.1	10.8	99.3	97.3	0.72	2.73
Zn	10.7	11.8	97.8	97.5	2.21	2.50
Fe	13.8	16.2	99.0	96.7	1.01	3.27

3.1.4 Assessment of the solid residue produced from the HTL of Spirulina with metal sulfates

There was only a minor decrease observed in the solid residue yields on increasing the temperature (figure 4a). Adding catalytic levels of metals also did not change the solid residue substantially. However, with increasing metal concentrations, more metals reported to the residue fraction thereby increasing apparent yields. This strongly suggests that the majority of the metals partitioned into the solid residue phase. Only around 2% of the carbon from the original biomass partitioned into the solid residue on HTL of the original Spirulina (Figure 4b). Whilst this value remained more or less constant following the addition of small amounts of additional metals (data not shown) up to 10% of the carbon from the biomass partitioned into the solid residue when processing of the high uptake scenario at the lower reaction temperature. Mg, Zn and Fe all elevated the presence of carbon in the solid residue. In general, the carbon concentration in the solid residue appeared to be at the highest, at high metal loadings and lower temperatures, suggesting that the production of large asphaltene type organics are catalysed by the metals present, though a large decrease in the carbon content was observed at higher temperatures in the presence of iron, suggesting that the iron is catalytically breaking these compounds down.

The increase in the solid phase yield is also due to the partitioning of other elements, such as oxygen, in the solid residue (figure 5). In all cases, the solid residue phase was composed predominantly of inorganic matter, with carbon only making up 3.5–11.8 % of the solid residue by mass.
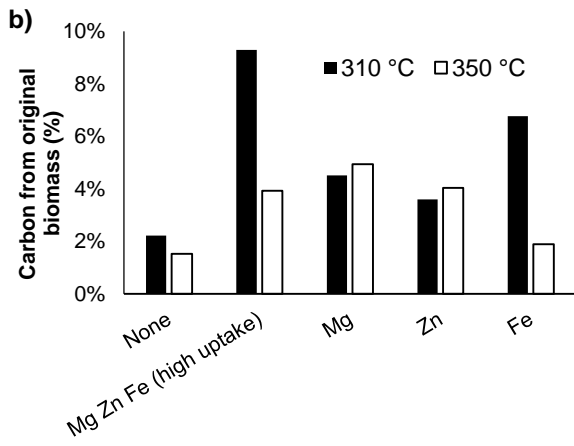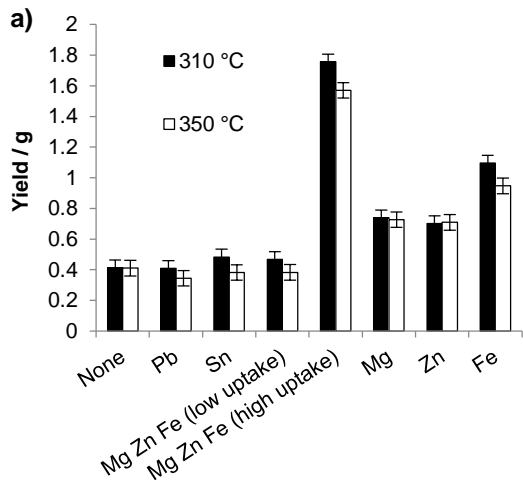
Fig. 4 a) Total solid residue yield for Spirulina processed with additional metal sulfates b) the % of carbon from

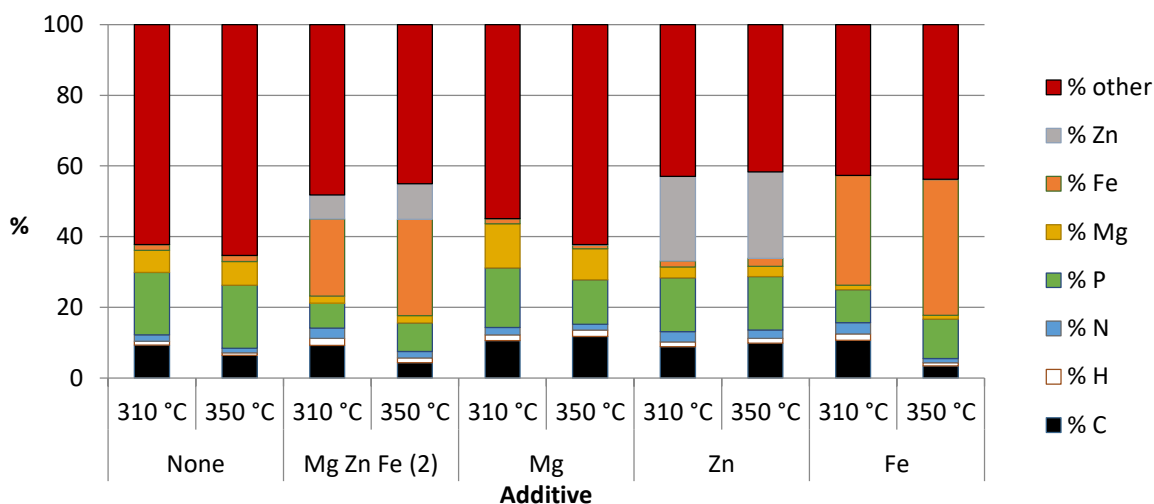the original biomass that partitioned into the solid residue phase

Fig. 5 *Elemental composition of the solid residue produced from the hydrothermal liquefaction of Spirulina, showing the high uptake scenario and the individual Mg, Zn and Fe sulfates. The full dataset is given in the supporting information*

This data, coupled with the low level of metals detected in the aqueous phase, demonstrates that the vast majority (up to 100 %) of the added Fe, Zn, Sn and Pb are present in the solid residue.

The solid residue was also examined by SEM (see supporting information). On addition of metals, the physical appearance of the solid residue altered significantly, and the metals were detected in solid residue from metal-containing reactions at levels not observed in the solid residue from Spirulina liquefaction alone. The results are in good agreement with data obtained through ICP-MS, suggesting that the metals in the solid residue phase are predominantly oxides. The low magnesium content of the mixed-metal additive sample is in agreement with the high magnesium levels detected in the aqueous phase.

3.2 HTL of algal cultures used in the remediation of acid mine drainage

Following the positive assessment of the HTL for the model compounds, the HTL process was applied to a natural algal community selected for cultivation on AMD. Two scenarios were examined. In both cases, the same starting algal community was used. The first scenario, AMD-1, the algae were

cultured in a low pH, synthetic AMD broth. In AMD-2, the algal culture was grown in real AMD taken from a former tin mine. 310 °C was selected as the temperature for processing as this was shown to give optimised bio-oil yields. The initial elemental composition of the AMD-1 algae were similar to the Spirulina, though due to the increased metal uptake, the AMD-2 biomass was notably different (table 4). Overall, the mass fractions of the four main product components from liquefaction were similar for AMD-1 algae and Spirulina, though the AMD-2 algae produced a far higher amount of solid residue on liquefaction (figure 6).

Table 4 Main elemental composition of the algae (w/w % dry mass) demonstrating low metal uptake (AMD1) and the algal community demonstrating high metal content (AMD2) compared to the Spirulina sample with no additional metal sulfates.

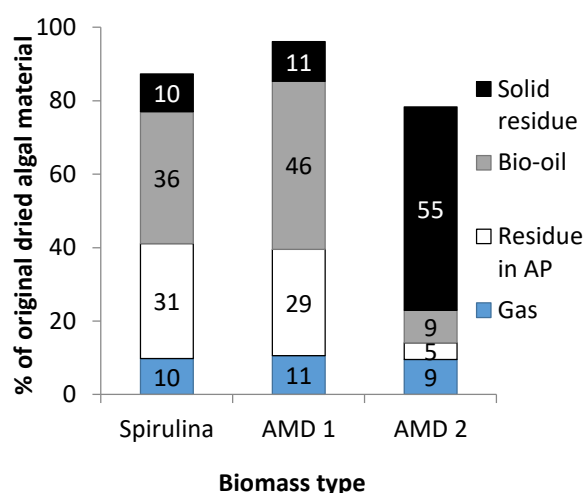|	C %	H %	N %	P %	Mg %	Zn %	Fe %	Sn %	Pb %	other %
Spirulina	43.47	5.76	9.74	2.06	0.39	0.00	0.13	0.0001	0.0003	38.46
AMD-1	47.58	6.95	8.95	2.25	0.32	0.27	0.46	0.0014	0.0117	33.23
AMD-2	17.56	3.37	2.89	2.42	0.10	0.04	23.44	0.0003	0.0015	50.18

Fig. 6 Mass balance for the algae used in the remediation of acid mine drainage on being processed by HTL. The percentages given were calculated from the weight of the original starting material on drying (biomass and any metals remediated from the AMD).

Table 5. Yields of bio-oil from the hydrothermal processing of the algae used in the remediation of acid mine drainage compared with Spirulina

	Bio-oil yield / % of total dry mass	% C	% H	% N	% other	HHV / MJ kg^{-1}
Spirulina	35.9	70.8	9.1	7.9	12.1	29.32
AMD-1	45.7	68.8	7.9	7.1	16.2	29.70
AMD-2	8.9	68.6	7.2	7.5	16.5	28.73

Bio-oil yields (calculated as a percentage of the original dry algal biomass) were higher for the AMD-1 algae than for Spirulina with no additives, comparable to yields obtained with additives at the highest metal loading. This increase is likely to be due partly to the comparatively higher C and H content of the biomass, and partly due to catalytic effects from absorbed metals in the biomass. The AMD-2 biomass, with a far lower carbon content, gave a correspondingly lower yield of 8.8 % bio-oil, with the solid residue making up 55.3 % of the product by weight. Interestingly, although the yield was extremely low, the composition and energy content of the bio-oil were similar to that of the AMD-1 biomass (table 5). This suggests that the volume of remediated metals will not have a large effect on the quality of the bio-oil, and the quality of the bio-oil should be relatively uniform irrespective of any seasonal or geographical changes in the metal content being remediated.

AMD-1 has a significantly higher level of carbon partitioning into the oil (84 %) than either Spirulina or AMD-2 (73 % and 42 %, respectively) at the same processing conditions, demonstrating that initial biomass concentration cannot be the only factor affecting bio-oil yields. The AMD-1 algae contained 0.46 wt. % Fe and 0.27 wt. % Zn, in addition to 0.32 % Mg, which is also present in Spirulina at similar levels.

Table 6 Elemental composition of the solid residue produced by the hydrothermal liquefaction of algae with a low metal uptake (AMD1), and a high metal uptake (AMD2), used in the remediation of acid mine drainage. The solid residue is given as a percentage of the orginal total dry algal biomass.

Feedstock	Solid residue yield / %	C %	H %	N %	P %	Mg %	Zn %	Fe %	Sn %	Pb %	other %
Spirulina	10.31%	9.34	1.12	1.78	17.65	6.28	0.03	1.59	0.00015	0.00047	62.21
AMD-1	10.85	28.54	2.86	4.47	13.79	3.75	3.02	7.33	0.01	0.12	36.10
AMD-2	55.34	10.45	1.46	1.68	4.48	0.14	0.11	50.1	0.00	0.01	31.54

For the AMD-2, the decrease in bio-oil yield was matched by a large increase in the solid residue yield, with solid residue comprising 55 % of the product by weight, compared with ca. 10 % for pure Spirulina and AMD1 (table 6). Elemental analysis of the solid residue reveals that the CHN ratios are similar to those detected in the solid residue from HTL of both Spirulina with and without metal additives, but distinct from those observed for AMD1 algal culture. Both metals and phosphorus are present at higher mass fractions in the solid residue phase than in the starting biomass. The AMD1 solid residue contained moderate levels of Zn and Mg (3 % and 3.75 %, respectively), and somewhat less Fe (7 %) than expected from the model.Sn and Pb were also detected at low levels. The AMD2 algal solid residue contained predominantly Fe (50 %) with little contribution from other metals (0.11 % Zn and 0.14 % Mg).
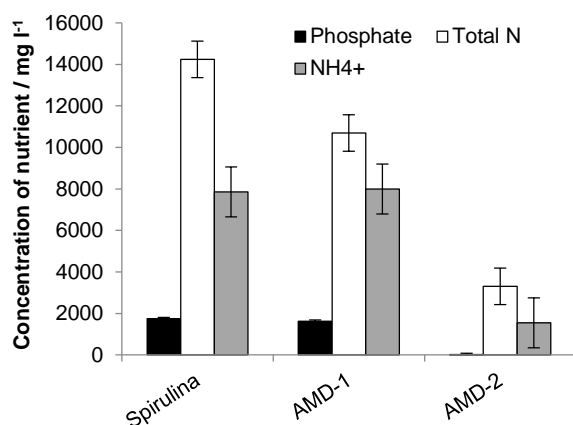
Fig. 7 Nutrient content of the aqueous phase on the hydrothermal processing of algal cultures used in the remediation of acid mine drainage.

In the aqueous phase, the total N is lower for AMD-1 algae than Spirulina, and lower again for the AMD-2 algae, likely to be caused by the comparatively lowered N content of the starting biomass. A similar pattern was also seen for NH_4^+. Phosphate analysis of the aqueous phase revealed that the phosphate content of the process water from liquefaction of AMD-1 biomass was similar to that of pure Spirulina, whereas the process water from AMD-2 contained extremely low phosphate levels, presumably a combination of the comparatively lower starting concentration of biomass and the high levels of iron and magnesium in the original starting material leading to precipitation of Zn and Fe phosphates.

Elevated levels of iron were present in the process water produced from the HTL of the AMD-2 algae. Little Fe partitioning to the aqueous phase is observed in the Spirulina model even at relatively high loadings, but the AMD-2 algae material seemingly contained a sufficiently high initial Fe concentration for this to occur. Magnesium recovery in the aqueous phase was also high, although no zinc was recovered. This is in agreement with the Spirulina model: even at high loadings, little zinc recovery in the aqueous phase is observed, and Mg partitions to the aqueous phase relatively readily.
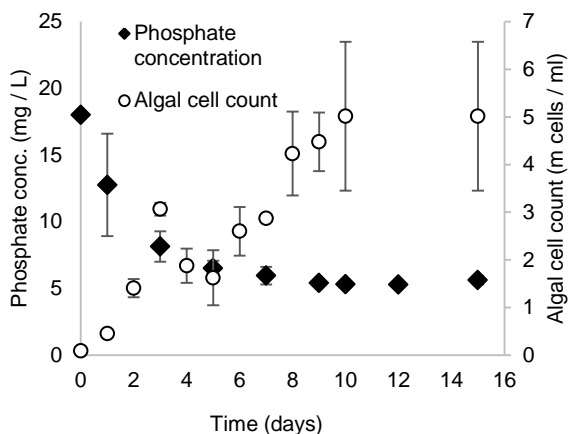
Figure 8. Cell count and phosphate use of AMD-1 cultured in a 10l bubble column bioreactor cultured on sAMD with the supplementary aqueous phase produced from the HTL of the Spirulina biomass. The bioreactor was run in duplicate.

As the acid mine drainage only contains trace levels of phosphate and ammonium, it must be supplemented by these elements to ensure algal growth. The effectiveness of the HTL output streams to supplement microalgal culture was established by combining the aqueous phase produced from the HTL of Spirulina with the sAMD media (Figure 8). Previous work demonstrated that a dilution factor 1:100 for HTL water was a general good compromise for a reasonable biomass concentration, high grow rate and low toxicity. Chemical analysis showed that the diluted HTL aqueous phase had a total nitrogen and phosphate concentration of 200 mg l^{-1} and 18 mg l^{-1} respectively, thus representing a suitable source of these elements.

 The algal culture was then grown over 15 days in a 10 l bubble column bioreactor at an average temperature of 18 °C. The culture grew well, with 4×10^6 cells ml^{-1} obtained after 7 days from inoculation, demonstrating that the HTL phase was not unduly toxic or nitrogen limited, in addition the algae were able to utilise around 75% of the phosphate provided from the HTL process. The overall data demonstrate that the nutrients lacking in the remediation can be provided by the HTL process, rather than needing additional fertilizer input.

The gas yields from both algae were similar to those obtained from Spirulina (around 10 % by mass), with composition remaining unaffected giving 98.9 % and 99.3 % CO_2 for the AMD-1 and AMD-2 biomass, respectively, which should be amenable for use to further stimulate algal growth. The main VOCs components of the gaseous products from AMD-1 were similar to the gas products identified from Spirulina liquefied at 310 °C, though AMD-2 only produced propene and isobutylene in significant quantities (see supporting information).

4. Conclusions

The results presented demonstrate that HTL could be used to process algae used in the remediation of acid mine drainage. On liquefaction of Spirulina bio-oil oil yields were increased slightly on addition of the metals, and this did improve the carbon recovery in the oil phase, though the HHV was reduced. A corresponding decrease in the total organic content of the aqueous phase was identified. The oil produced from both low metal and high metal systems, were remarkably similar irrespective of the lack of biomass in the AMD-2 sample.

One of the key parts of the proposed biorefinery is the ability to salvage metals captured from AMD from the solid residue phase for purification or disposal. In this work, metals were found to partition almost exclusively into the solid residue phase, clearly an advantage for potential metal recovery. This occurred with the notable exception of magnesium, which was recovered at roughly 30–50 % in the aqueous phase when also in the presence of Fe and Zn. However, elevated levels of magnesium in the aqueous phase should also be beneficial for process water recycling, although continual recycling could result in a gradual accumulation of magnesium beyond useful concentrations. In the AMD algal samples, high metal partitioning in the solid residue phase was also observed, allowing for either effective disposal or recovery.

Another important factor influencing the success of the biorefinery is nutrient recycling from the process water for algal cultivation. Unfavourably, high metal loadings drove partitioning of phosphates from the aqueous phase into the solid phase, presenting a potential setback to the

recycling of process water as a growth supplement for further algal cultivation. It seems likely that the phosphate would need to be recovered from the solid residue phase through further chemical processing. Nevertheless, the phosphate that did partition into the aqueous phase could be metabolised by the algae effectively.

Processing at lower temperatures yielded a gas phase composed of higher purity CO_2 with lower VOC levels, which is favourable both for partial gas phase recycling into algal cultivation and lowers the gas phase toxicity for environmental release. On liquefaction of the algal communities used to remediate AMD, the CO_2 produced was around 99% pure, and while higher value volatile components, such as propylene, were observed, they were present at too low levels to justify extraction and purification.

In general, processing temperatures were found to have a more substantial influence than metal loadings over product yields and properties. The use of lower temperatures also resulted in higher nitrogen levels in the aqueous phase, though lower NH_4^+ content. However, at higher processing temperatures of around 350 °C bio-oil energy content was improved, and nitrogen levels were somewhat lowered.

HTL processing was found to be a suitable method of processing algae used in the remediation of acid mine drainage. The oil is of a high enough quality for further hydrogenation into fuels, whereas the majority of the metals are almost exclusively recovered in the solid residue phase. There is also scope for the recovery of NH_4^+ and limited P from the aqueous phase to supplement algal growth and aid in the further remediation of metal rich effluent.

Acknowledgements

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