Hydrothermal carbonisation of anaerobic digestate for hydro-char production and nutrient recovery

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Abstract

This study investigates the potential of hydrothermal carbonisation (HTC) for fractionating anaerobic digestate of sewage sludge into carbon-rich hydrochar and nutrient-rich aqueous phase (AP). AP is subsequently used to supplement cultures of the alkali halophilic microalgae *D. tertiolecta* (CCAP 19/30), to convert sodium bicarbonate into sodium carbonate solution as part of an integrated biogas purification system.

HTC at 200°C gave the highest hydrochar yields (78%) and solid carbon retentions (75%), indicating high carbon capture potential. In contrast, the essential growth nutrients nitrogen, phosphorus and sulphur were partially solubilised, resulting in HTC-AP concentrations between 11 times (S) and 50 times (P) higher than those in artificial growth medium. Trace nutrient concentrations in the AP were 10 to 80 times higher compared to the artificial medium, with minimal heavy metal solubilisation.

Dunaliella tertiolecta grew successfully and without inhibition at HTC-AP concentrations up to 2% (produced at 200°C). AP-supplemented cultures achieved higher cell concentrations (up to 10.0 x 10^6 cells mL^{-1}), biomass content (maximum of 1.14 ± 0.06 g L^{-1}) and bicarbonate-to-carbonate conversion (83% and 80%, for 1% and 2% of HTC-AP) than the control cultures. Therefore, HTC-AP appears to be a suitable artificial growth medium substitute for cultivating alkali-halophilic microalgae to regenerate carbonate and produce algae biomass, providing an added-value product.

Keywords

Hydrothermal carbonisation, nutrient recovery, anaerobic digestion, carbon capture microalgae cultivation, hydrochar

1. Introduction

Hydrothermal carbonisation (HTC) has attracted significant interest for the treatment and separation of wet biomass residues (*e.g.,* sewage sludge or anaerobic digestate) into stabilised hydrochar, nutrient-rich aqueous phase (AP) products and reaction gases [1,2]. Unlike pyrolysis-based treatments, HTC operates at lower temperatures (180 – 250 °C), avoids the need for energy-intensive drying, and reduces the formation of toxic organic by-products, reducing the cost of downstream processing [3]. During HTC, water acts both as solvent and catalyst, and enables the recovery of inorganic biomass compounds (nutrients) into the liquid phase [4]. Conventional methods of sewage sludge and anaerobic digestate treatment often require energy intensive drying followed by incineration or landfilling, which also results in loss of valuable nutrients. Direct spreading on land requires pasteurisation and is often problematic due to the presence of pollutants and trace elements in residues.

The carbon-rich hydrochar could be used as soil improver, bio adsorbent for environmental remediation, novel carbon nanomaterial, or solid fuel source [5,6]. Moreover, stabilised hydrochar can act as a carbon sink, presenting a potential route for long-term carbon sequestration to offset emissions from other sectors [7–9]. However, widespread adoption of this technology has been limited by the treatment and disposal of the HTC-AP, which contains high amounts of ammonium nitrogen, organic carbon, as well as solubilised minerals and metals from the biomass feed. Due to its high nutrient content, the HTC-AP could be used as a natural fertiliser [10], but direct land application is limited by the presence of potential toxic and inhibitory components such as cyclic oxygen and nitrogen compounds and heavy metals. Therefore, different methods have been proposed for the recovery of these nutrients, including chromatographic separation, membrane filtration, nano filtration or bio electrochemical systems (BES) [11].

Alternatively, the HTC-AP could be used directly as a nutrient source for microalgae or cyanobacteria cultures as part of an integrated biorefinery concept to yield valuable metabolites and bulk algal biomass for conversion into fuels and chemicals [12–14]. As well as utilising the essential nutrients from the HTC-AP, algae can metabolise the organic waste stream components to enable safe disposal of the treated water stream. A potential application is our recently developed algae-based biogas purification and carbon capture process, which combines anaerobic digestion (AD) of biomass with a two-stage CO_2 absorption and algae cultivation system to yield net carbon-negative biomethane fuel and algae by-product [15]. The system employs sodium carbonate to absorb biogas CO_2, before the resulting bicarbonate solution is used as a carbon source for algae growth to regenerate the CO_2 absorbent [16–18]. HTC of the anaerobic digestate can be used to convert the non-digestible biomass fraction into hydrochar for long-term carbon capture whilst recovering essential nutrients for algae growth.

67 The efficiency of the HTC process for the production of hydrochar and recovery of nutrients
68 to the AP depends both on the quality of the feedstock (*e.g.*, waste biomass or AD-digestate),
69 and the reaction time and temperature [19]. High nutrient recovery to the water phase is
70 desirable if the AP is used as fertiliser but must be balanced against the formation of water-
71 soluble organics, which could inhibit algae growth. Several studies have already demonstrated
72 the cultivation of different microalgae strains in the HTC-AP from anaerobic digestate derived
73 from agricultural wastes [20], food wastes [14], and sewage sludge [20,22]. However, all
74 studies have been conducted in freshwater media, whereas our algae-based biogas purification
75 and carbon capture process operates at high salt loading, requiring alkali-halophilic strains such
76 as *D. tertiolecta* (CCAP 19/30). Another key consideration is the distribution of carbon to the
77 hydrochar, AP and gas phase, respectively, to achieve high carbon capture and prevent the
78 accumulation of organic compounds within the algae photobioreactor.

79 Therefore, the current study focuses on the hydrothermal carbonisation of anaerobic digestate
80 (derived from sewage sludge) into carbon-rich hydrochar and nutrient-rich AP. The alkali-
81 halophilic microalgae *D. tertiolecta* (CCAP 19/30) was selected for growth studies in the HTC-
82 AP, due to its ability of converting sodium bicarbonate into the carbonate absorbent required
83 for biogas purification. HTC treatment of dewatered digestate was conducted at three different
84 temperatures to assess the distribution of carbon, macro- and micronutrients to the different
85 product phases. *D. tertiolecta* CCAP 19/30 was cultivated with different concentrations of
86 HTC-AP to evaluate the suitability of this HTC-AP for substituting the artificial growth
87 medium.

88 **2. Materials and methods**

89 **2.1 Materials and algae species**

90 The inoculum (from a long-term operating anaerobic digester), and organic feedstock (sewage
91 sludge) were collected from the Wanlip Sewage Treatment Works (Wanlip STW) plant
92 (Leicester, UK). Inoculum and sewage sludge feedstock were stored at 5 °C prior to use. *D.*
93 *tertiolecta* (CCAP 19/30) was obtained from the Culture collection of Algae and Protozoa
94 (CCAP, Scotland, UK). All reagents, compounds, and Inductivity Coupled Plasma (ICP) metal
95 standards were purchased from Fisher Scientific (UK).

96 **2.2 Anaerobic digestion of sewage sludge and pre-processing of AD-digestate**

97 Anaerobic digestion (AD) of sewage sludge was carried out in 10 L continuous stirred tank
98 reactors (9 L working volume), inoculated with 8.5 L of microbial culture and 0.5 L of fresh
99 sewage sludge. Reactors were fed daily by removing 0.5 L of AD-digestate and replacing it
100 with fresh sewage sludge, maintaining hydraulic retention time (HRT) of 18 days, and organic
101 loading rate (ORL) of 3.5g VS L^{-1} day^{-1}. Reactors were maintained at 37 °C under continuous
102 vertical stirring (100 rpm). After establishing stable biogas production, AD-digestate removed
103 from the reactors was collected over a period of five consecutive days, mixed and centrifuged
104 (10000xg, 20 min, room temperature) to increase the solid loading of the material. The
105 centrifuged AD-digestate was divided into equal fractions and stored at 5 °C until further
106 processing.

107 **2.3 Hydrothermal carbonisation (HTC) of AD-digestate**

108 HTC reactions were conducted using a 300 mL high-pressure stainless-steel reactor (Model
109 4560, Parr Instrument). Each treatment used approximately 100 g of centrifuged AD-digestate
110 with a total solid content of 11.27%. Once the desired reaction temperature was reached (200,
111 220 or 240 °C), the temperature was maintained for 1 hour, before cooling the reactor in

ambient air. Reaction gases were collected into an inverted measuring cylinder to determine the HTC gas yields. Hydrochar and reaction water were separated by gravity filtration using Whatman filter paper (Cole-Parmer, UK), followed by further washing of the hydrochar with deionised water. The solid residue was dried at 105 °C and stored at -20 °C, while the AP and wash water fractions were stored at 4 °C for further use and analysis. The HTC treatment of solid AD-digestate at each temperature was performed in duplicate (n = 2).

2.4 Analysis of AD-digestate and HTC products

The total solid (TS) and moisture contents were determined by drying the samples in a convection drying oven at 105 °C (± 1 °C), followed by cooling in a desiccator for 10 minutes to determine the relative weight loss [23]. The ash content was determined by heating the dried samples inside a muffle furnace (550 °C, 3 h) following the National Renewable Energy Laboratory procedure [24]. The volatile solid content (VS) was calculated as the difference between the TS and ash content of each sample. Each sample was analysed in triplicate (n=3). The total carbon (C), hydrogen (H) and nitrogen (N) content of the dry AD-digestate and hydrochar samples was measured by elemental analysis (Thermo Scientific^{TM} FLASH 1112 series CHN analyser). Volatile oxygen content was calculated by subtracting the ash, carbon, hydrogen and nitrogen content from 100.

The S, P and metal contents of the solid (hydrochar and solid AD-digestate) and liquid samples (HTC-AP) were determined on an Inductivity Coupled Plasma–Optical Emission Spectrometer (ICPE-9000, Shimadzu) following the operating procedure described by Zand *et al.* [25]. While the aqueous samples were analysed directly, the solid samples were digested in acid in a temperature and pressure-controlled microwave assisted digestion system (CEM MARS 5 with XP-1500 vessels). Briefly, 0.5 g of oven-dried sample was mixed with 10 mL of a 1:3 mixture of concentrated nitric acid (70% trace analysis grade, Fisher Scientific) and hydrochloric acid

(36% trace analysis grade, VWR International). Following microwave digestion (20 min), the samples were made up to 50 mL with deionised water. Multi-element calibration solutions were prepared at different concentration levels (1 – 1000 µg L^{-1}) from the single element (1000 mg L^{-1}) ICP grade standards (Fisher, UK), to produce four-point calibration curves for each element (r^2 = 0.9999).

The total carbon (TC) and total inorganic carbon (TIC) contents in the AP were measured using a Rosemount Dohrmann DC-190 high temperature TOC Analyser (Teledyne Tekmar, USA), by direct injection of the sample (50 µL). The total organic carbon (TOC) was calculated as the difference between the TC and TIC. Ammonium (NH_4^+), nitrate (NO_3^-) and phosphate (PO_4^{3-}) concentrations were determined using Hach-Lange colorimetry test cuvettes (LCK302, LCK338, LCK350, Hach-Lange, Germany).

2.5 Culturing of *Dunaliella tertiolecta* with HTC-AP

D. tertiolecta (1 x 10^5 cells mL^{-1}) from the exponential phase of cell growth (6^{th} day of culture) were inoculated into 200 mL of Modified Johnson medium (J/I) (7.4 mM $MgCl_2.6H_2O$, 2.0 mM $MgSO_4.7H_2O$, 2.7 mM KCl, 1.4 mM $CaCl_2.2H_2O$, 9.9 mM KNO_3, 0.29 mM KH_2PO_4, 8.9 µM $FeCl_3.6H_2O$, 4.8 µM Na_2EDTA, 9.9 µM H_3BO_3, 0.31 µM $(NH_4)_6Mo_7O_{24}.4H_2O$, 0.24 µM $CuSO_4.5H_2O$, 0.21 µM $CoCl_2.6H_2O$, 0.29 µM $ZnCl_2$ and 0.20 µM $MnCl_2.4H_2O$), supplemented with 11.68 g L^{-1} NaCl (0.2 M) and 1.21 g L^{-1} Tris-HCl (3.0 mM), adjusted to pH of 8 (Roy *et al*., 2021). Cultures were grown in glass flasks (0.25 L) placed in an orbital shaker incubator with continuous shaking (100 rpm) at 20 °C (± 2 °C) under light-emitting diode (LED) irradiation (12 kLux, mixture of cool and warm white) with a light: dark cycle of 16:8 h.

Cultures were mixed with 34 g L^{-1} of sodium bicarbonate ($NaHCO_3$), to monitor the performance of the cultures to regenerate carbonate for CO_2 absorption [15]. To evaluate the

efficiency of cell growth within the HTC-AP, small concentrations of HTC water (1 – 3%) obtained at HTC temperatures of 200 °C (1%, 2%, 3%), 220 °C (1%) and 240 °C (1%) were added to the J/I medium prior to inoculation. The cultures were sampled every two days (1.2 ml samples) for cell counting, chlorophyll and carotenoid assay analysis. At the end of the growth cycle (12 days), cells were harvested by centrifugation (4000xg, 4 °C, 10 min) and dried in a convection oven for 24 h at 105 ± 1 °C to determine culture biomass content. All experiments were carried out in biological triplicate (n=3).

2.6 Determination of growth rate, doubling time, and cell density

Cell numbers in the cultures were counted using an improved Neubauer Haemocytometer (Weber, UK). Diluted cell suspensions were treated with 8 µL of 2% formalin solution, before 0.010 mL of sample was slowly added so that it spread evenly into each chamber. The total number of cells was calculated using Equation 1. Specific growth rates (µ) and doubling times (t_d) of all cultures were calculated according to the Equations 2 and 3 [15,26].

Cell density (Cells mL^{-1}) = Number of cells per square x dilution factor x 10^4 (1)

Specific growth rate: $\mu = \frac{(\ln n_2 - \ln n_1)}{(t_2 - t_1)}$ (2)

Doubling time: $t_d = \frac{\ln 2}{\mu}$ (3)

Where n_2 and n_1 were the numbers of cells at time t_2 and t_1

2.7 Determination of total chlorophyll content

After harvesting fresh cells (1 mL culture) by centrifugation (4000xg, 4 °C, 10 min), acetone solution (85%v/v, 1 mL) was added to the pellet and homogenised in extracting solution using a vortex mixer. After separation of the supernatant by centrifugation (14000xg, 4 °C, 10 min),

180 the absorbance was taken at 480, 647, and 664 nm against a blank of 85% acetone solution
181 using a spectrophotometer (Jenway 6305, UK). Total chlorophyll content in the extract (µg
182 mL^{-1}) was calculated using equations 4 – 7 [15].

183 Carotenoid (Car) (µg mL^{-1}) = 4 x (Abs_{480}) (4)

184 Chlorophyll a (Chl a) (µg mL^{-1}) = (12.25 x Abs_{664}) – (2.55 x Abs_{647}) (5)

185 Chlorophyll b (Chl b) (µg mL^{-1}) = (20.31 x Abs_{647}) – (4.91 x Abs_{664}) (6)

186 Total Chlorophyll (µg mL^{-1}) = (Chl a) (µg mL^{-1}) + (Chl b) (µg mL^{-1}) (7)

187 Where, Abs_{480}, Abs_{647} and Abs_{664} are the absorbance of the acetone extract measured at 480,
188 647, 664 nm respectively.

189 **2.8 Determination of $NaHCO_3$ and Na_2CO_3 content**

190 Carbonate/bicarbonate contents were determined according to Jain and Mehta (1980). Total
191 alkalinity (complete conversion of $NaHCO_3$ and Na_2CO_3 into CO_2, Equation 8) was determined
192 by mixing 1 mL of centrifuged culture supernatant (3000xg, 15 min) with 3 mL of
193 decarbonised water (boiled to remove dissolved CO_2) prior to titration with 0.1M HCl to a
194 methyl orange end point (pH 4.3). The $NaHCO_3$ content was determined by mixing another
195 1 mL of centrifuged culture supernatant with 3mL of decarbonised water and 4 mL of 0.1M
196 NaOH before adding 2 mL of 0.48M $BaCl_2$ solution and phenolphthalein indicator (2 drops).
197 The mixture was titrated with 0.1M HCl to the indicator endpoint (loss of pink colour at pH
198 8.3). The net amount of alkali used (m-n) corresponds to the bicarbonate (HCO_3^-) content of
199 the sample, resulting in the formation of carbonate, which is subsequently precipitated as
200 barium carbonate (Equations 9 and 10). The carbonate content was thus determined by
201 subtracting the bicarbonate content from the total alkalinity of the sample [15].

202 Alkalinity: $pNa_2CO_3 + nNaHCO_3 + (2p + n)\ HCl$

203 $\rightarrow (2p + n)\ NaCl + (p + n)\ CO_2 + (n + p)\ H_2O)$ (8)

204 $NaHCO_3$: $(n+p)\ BaCl_2 + m\ NaOH + n\ NaHCO_3 + p\ Na_2CO_3$

205 $\rightarrow (n+p)\ BaCO_3 + 2(n+p)\ NaCl + (m-n)\ NaOH + nH_2O$ (9)

206 Where m>n, amount $BaCl_2$ > n+P

207 Titration: $(m-n)\ NaOH + (m-n)\ HCl \rightarrow (m-n)\ NaCl + (m-n)\ H_2O$ (10)

208 **3. Results and Discussion**

209 **3.1 Feedstock characterisation**

210 The anaerobic digestate used in this study was collected from a continuous sewage sludge fed
211 AD reactor, as described above. During AD, 60.5% of volatile solids were converted into
212 biogas, causing a significant reduction in both the VS and TS concentrations in the AD-
213 digestate (Table 1). In contrast, the concentrations of non-volatile solids remained the same,
214 resulting in an increase in the ash content of the dried solids from 17.4 % to 33.9%. The high
215 ash content of the digestate results in relatively low concentrations of carbon (35.2%) and
216 hydrogen (5.1%), while the nitrogen content of the digestate (4.3%) is slightly higher than in
217 the sewage sludge (4.20 ± 0.06), as during AD, unlike hydrogen and carbon, most of the
218 nitrogen in the sewage sludge remains in the anaerobic digestate phase (rather than the gas
219 phase). As previously reported, most of this nitrogen is in the form of proteins and other cell
220 components from the microbial AD community, together with unconverted ammonium present
221 in sewage sludge [28].

222 To increase the solid concentrations of the AD-digestate, it was centrifuged prior to the HTC
223 experiments to remove excess water. Low solid loadings can result in reduced product yields,
224 increase the energy requirements of the reaction, and increase the amount of water that needs

225 to be treated after the reaction. The final solid loadings of 10.8% are in the range of typical
226 hydrothermal conditions. Centrifugation of the digestate had little impact on its ash content and
227 elemental composition, indicating minimal loss of solids to the supernatant (liquid) phase
228 (estimated as 3.2% based on difference in TS and VS content).

229 *Table 1 Properties of feedstock used for hydrothermal carbonisation*

Parameter	Sewage sludge (Feed stock)	AD-digestate	**Centrifuged digestate**
Moisture content (% WW)	92.39 ± 0.42	96.24 ± 0.02	89.22 ± 0.72
TS (% WW)	7.61 ± 0.42	3.76 ± 0.02	10.78 ± 0.72
Volatile solid (% WW)	6.29 ± 0.43	2.48 ± 0.01	7.34 ± 0.19
Ash content (% DW)	17.43 ± 0.96	33.92 ± 0.17	32.26 ± 3.66
C (%)	43.51 ± 0.89	35.19 ± 0.61	35.48 ± 0.37
H (%)	6.59 ± 0.15	5.14 ± 0.08	5.24 ± 0.04
N (%)	4.20 ± 0.06	4.25 ± 0.06	4.90 ± 0.02
O (%)	27.54 ± 0.92	21.56 ± 0.59*	22.12 ± 1.85*

230 *WW – wet weight, DW – dry weight*

231 ***Oxygen calculated by difference***

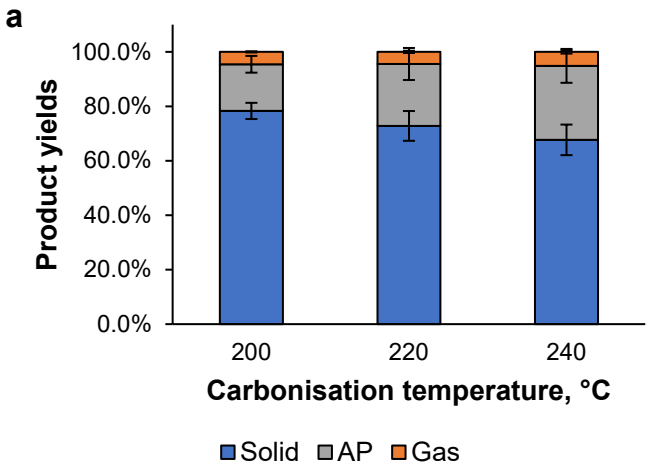
232 In addition to the CHN content, the concentrations of other growth nutrients (P, S, Na, K, Ca,
233 Mg, Fe, B, Zn, Co, Cu, Mo) present in the modified Johnson medium, as well as metals (Al,
234 Cr, Cd, Pb, Sr, Ni, Bi, Ba) were determined to investigate their partitioning to the AP during
235 HTC reactions. Amongst these, the highest concentrations were observed for Ca (40.5 mg g^{-1}),
236 P (37.7 mg g^{-1}), Fe (20.8 mg g^{-1}), sulphur (17.9 mg g^{-1}) and Al (16.5 mg g^{-1}), while the
237 concentrations of heavy metals (Bi, Pb, Cr, Cd) were all below 0.1 mg g^{-1} (Table 2).

238 **3.2 Carbonisation of AD-digestate**

239 The HTC reaction temperatures used in this study (200 °C, 220 °C and 240 °C) are based on
240 the range of typical HTC temperatures (180 – 250 °C), which generally represent a compromise
241 between carbon retention to the hydrochar, and volatilisation of hydrogen and oxygen to
242 produce a stable solid residue. HTC reaction products were separated into three separate
243 phases: solid hydro-char, AP, and gas products. As HTC gas products are known to consist
244 predominantly of CO_2 (with minor amounts of CO, H_2 and CH_4) the gas yields were calculated
245 by assuming a molecular weight of 44 g mol^{-1} [29]. AP yields are difficult to measure directly
246 due to the loss of volatile organics during AP drying, and hence were calculated as the
247 difference between biomass feed and solid and gas yields (Figure 1a). As expected, hydrochar
248 was recovered as the major reaction products, with yields up to 78.3% (± 3.0%) at the lowest
249 reaction temperature of 200 °C. In line with previous studies [30,31], solid yields reduced as
250 the reaction temperature was increased (72.8% ± 5.5% and 67.7% ± 5.6% at 220 °C and
251 240 °C, respectively), which may be associated with the slow reaction kinetics at the lower
252 temperature [32]. Gas yields remained constantly low between 4.4% and 5.1% (± 0.2 – 0.6%),
253 suggesting reaction gases are mostly formed below 200 °C. Therefore, the reduction in solid
254 yields at higher temperatures is expected to correspond to the increased production of water-
255 soluble reaction products, as observed previously [33].

256 In addition to the overall mass balance, the carbon and nitrogen distributions to the three
257 product phases were determined. Unlike the overall mass balance, C and N distribution to the
258 AP were measured directly using the concentrations obtained from TC and colorimetric (NH_4^+
259 and NO_3^-) analysis, and the volume of recovered water. The carbon distribution closely matches
260 the overall mass distribution, with a slight reduction in solid (1.9 – 3.9%) and gas recovery (1.0
261 – 1.2%) and a corresponding increase in the AP yields (Figure 1b). Hydrothermal conditions

preferably solubilise smaller compounds with higher H/C and O/C ratios, leading to lower hydrogen and oxygen retention in the solid. Therefore, the differences between the total mass balance and the carbon balance indicate a high retention of inorganic minerals within the solid hydrochar product, corresponding to low metal loadings in the AP product. The overall carbon mass balance closure ranges from 94.2% to 99.9%, indicating minimal losses throughout the extraction process. However, the water recovery from the reactor ranged from only 51.2% to 59.7%, demonstrating significant water losses during product collection and separation, presumably through evaporation. As these losses did not affect the overall carbon balance, the water-soluble carbon products appear to be relatively non-volatile and concentrate in the retained water.



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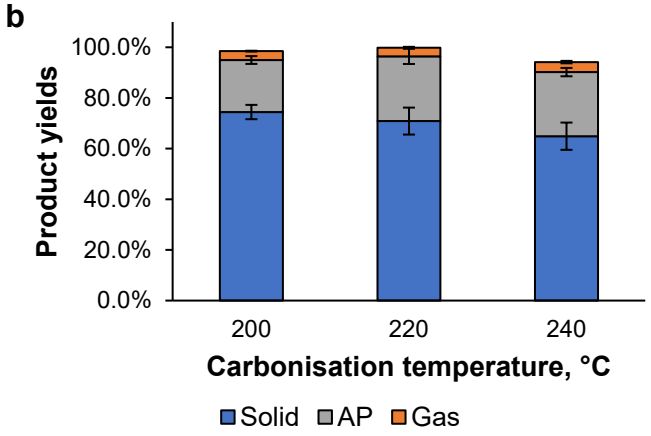

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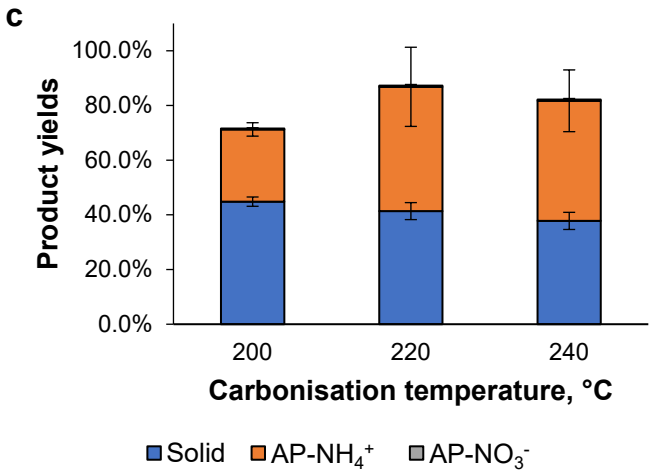

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275 *Figure 1: Product yields from hydrothermal carbonisation of digestate. (a) total mass*

276 *balance; (b) carbon distribution; (c) nitrogen distribution.*

277 Unlike carbon, the nitrogen recovery to hydrochar accounted for only 37.8 – 44.8% (± 1.7 –

278 3.1%) of nitrogen in the digestate, reducing with increasing reaction temperature (Figure 1c).

This result is consistent with previous studies showing that nitrogen retention in the hydrochar continues to reduce up to temperatures of 270 °C [34,35]. Proteins in the digestate feed start to degrade at 150 °C via hydrolysis and cracking reactions to form ammonia (which dissolves in water to form ammonium, NH_4^+) and organic nitrogen compounds, which mostly distribute to the AP [36]. Ammonia in the HTC-AP accounts for 26.4% to 45.5% (± 1.1 – 3.9%) of initial nitrogen, while the yields of nitrate are low (< 0.5%) at all temperatures. Although biomass degradation could also cause the formation of nitrites (NO_2^-), their yields tend to be even lower than those of nitrates and were not determined in this study [35]. Overall nitrogen balance closure significantly increases from 71.6% at 200 °C to 87.3% at 220 °C, consistent with the decomposition of organic nitrogen compounds in the AP to form ammonia. The missing nitrogen may also be attributed to the evaporation of ammonia during the extraction process, in line with the significant losses of water during the extraction. Process scale-up and continuous processing of the reaction products are expected to significantly reduce these losses, leading to improved nitrogen recovery to the AP.

3.3 Characterisation of hydrochar

HTC of the centrifuged digestate resulted in a significant increase in solid ash content from 32.3% to between 47.9 and 49.4% (Table 2). The remaining volatile matter (VM) content (51 – 52%) is similar or higher than those obtained during previous HTC studies of AD-digestates (34.5% - 52%) [29,37,38], but lower than the values from direct carbonisation of sewage sludge (57.4 – 63.0%) [31,39]. These differences can be explained by variations in reaction conditions (*e.g.*, reaction time, temperature, and solid loading), as well as differences in the composition of the feed material. For example, anaerobic digestion of sewage sludge results in a significant decrease in VM content, as the volatile material is converted into biogas, increasing the ash content of these samples prior to HTC.

303 Carbon content in the hydrochar remained relatively constant, regardless of HTC temperature,
304 (33.7% – 34.5%), only slightly lower than the carbon content in the centrifuged digestate
305 (35.5%). These results show the preferential retention of carbon (over H, N and O) within the
306 reducing solid organic fraction, indicating the formation of larger, carbon-rich compounds,
307 such as polycyclic aromatics. In contrast, HTC caused a much higher relative decrease of the
308 hydrogen, nitrogen and particularly oxygen contents, resulting in a significant reduction in the
309 H/C (0.147), N/C (0.138) and O/C (0.624) ratios of the centrifuged digestate. The H/C ratios
310 of the hydrochar reduced with increasing carbonisation temperature (0.128, 0.125 and 0.119 at
311 200, 220, and 240 °C, respectively), consistent with previous results for the carbonisation of
312 wood [30], digestate [32] and active sewage sludge [31]. This may indicate an increase in the
313 rate of the dehydration reaction (removal of hydroxy groups to form H_2O), or preferential loss
314 of shorter hydrocarbons with higher H/C ratios at higher temperatures. The N/C ratios remained
315 relatively constant (0.080 – 0.083), suggesting that most of the excess nitrogen is solubilised
316 at temperatures below 200 °C, while additional nitrogen losses are proportional to the overall
317 decrease in hydrochar yields at increased HTC temperatures. Finally, the O/C ratios increase
318 from 0.290 to 0.326 as the reaction temperature is raised from 200 °C to 240 °C. The oxygen
319 content of the digestate feed is mostly reduced via decarboxylation (release of CO_2),
320 decarbonylation (release of CO) and dehydration reactions (removal of hydroxy groups to form
321 H_2O) at temperatures above 150 °C [31]. Therefore, the increase in O/C ratio with increased
322 HTC temperature suggests that most of these deoxygenation reactions occur below 200 °C,
323 consistent with the constant gas yields observed at all studied HTC temperatures. Instead, the
324 remaining oxygen appears to incorporate preferentially into heavy char products, resulting in
325 the release of shorter hydrocarbons with high H/C and low O/C ratios.

326

327 *Table 2: Elemental compositions of AD-digestate and solid HTC reaction products*

Parameter	Centrifuged Digestate	HC200	HC220	HC240
		Proximate and ultimate analyses (% w/w)		
Ash content	32.26 ±3.66	49.35 ± 0.61	47.89 ± 0.03	48.08 ± 0.07
***C**	35.48 ± 5.10	33.72 ± 0.57	34.53 ± 1.12	34.01 ± 0.12
***H**	5.24 ± 0.057	4.34 ± 0.062	4.33 ± 0.157	4.07 ± 0.012
***N**	4.89 ± 0.035	2.80 ± 0.003	2.78 ± 0.044	2.73 ± 0.026
P	3.77	5.64	5.16	4.47
S	1.79	2.09	1.76	1.43
****O**	22.12 ± 1.85	9.79	10.47	11.10
		Metal concentration (mg g^{-1})		
Al	16.51	24.27	23.07	23.21
B	0.157	0.251	0.228	0.137
Ba	0.443	0.708	0.642	0.586
Bi	0.089	0.78	0.241	0.080
Ca	40.5	53.9	55.6	51.5
Cd	0.005	0.008	0.006	0.005
Co	0.010	0.015	0.012	0.013
Cr	0.073	0.114	0.114	0.107
Cu	0.445	0.464	0.587	0.574
Fe	20.8	35.34	34.9	32.6
K	7.27	4.56	4.48	4.41
Mg	5.63	14.0	11.4	10.6
Mn	0.269	0.580	0.479	0.404
Mo	0.054	0.320	0.145	0.052
Na	2.51	1.12	1.33	1.38
Ni	0.042	0.107	0.108	0.092
Pb	0.087	0.175	0.136	0.119
Si	0.88	0.647	0.630	0.492
Sr	0.206	0.312	0.287	0.264
Zn	0.767	0.152	0.229	0.974

328 **From Elemental CHN analysis*

329 ***Oxygen calculated by difference*

330 Comparing ICP analysis results for the three hydrochar products to centrifuged digestate shows
331 a substantial increase in the concentrations of most metals in the reaction solids, in line with

the increase in ash content. Based on the ICP analysis of the AP, the calculated recoveries of Cu, Al, Ca, Fe, Ba, Mn, and Sr to the hydrochar exceed 98% at all three reaction temperatures. The lowest metal recoveries are seen for Cd (50.2 – 61.0%), Bi (56.1 – 57.7 %), Mo (62.3 – 64.8%) and Co (70.8 – 73.7%), but all four elements are present at very low initial concentrations (<0.1 mg g^{-1}). The high metal recoveries are consistent with literature results and can be explained by the presence and formation of water-insoluble metal oxides, minerals (*e.g.*, carbonates and phosphates), and interaction of different minerals to form metal complexes [40–43]. Accordingly, the recoveries of phosphorus (93.2 – 94.1%) and sulphur (80.9 – 82.9%) are also high. Phosphorus can form a range of different molecular moieties (for example, organic phosphates, orthophosphate, phosphonate and polyphosphate) which may associate with the minerals present in the digestate feed to form water-insoluble precipitates, or be adsorbed onto the rough and uneven surfaces of the hydrochar [42,44,45]. Relatively low recoveries are seen for sodium (69.6 – 72.5%) and potassium (65.1 – 69.1%), reducing slightly with an increase in reaction temperature, in line with the overall decrease in hydrochar yields. Both elements form a large number of water-soluble salts and are an important component of the algal growth medium used in this study.

3.4 Composition of HTC-AP

The AP produced by HTC treatment of AD-digestate at different temperature was dark in colour and the recovered amount of water increased with increasing reaction temperature (Table 3). While its inorganic carbon content increased with increasing reaction temperature (0.80 to 1.71 g L^{-1}), the organic carbon content reduced by a similar amount (16.01 to 14.68 g L^{-1}), giving an approximately constant total carbon concentration. These results can be explained by the increased solubility and hydrolysis of biochemical compounds such as polysaccharides, cellulose, hemicellulose and lignin at temperatures above 190 °C into sugars, sugar derivatives and other intermediates [32,46]. Further reactions of these intermediates

cause the formation of a wide range of water-soluble organic compounds, including acetic acid, propionic acid, butanoic acids, aldehydes, furans, pyrroles, pyrazines, pyridines, 3-methylbenzofurane and 5-methyl-2-furancarboxyaldehyde [39], as well as deoxygenated compounds with low water solubility, which partition to the hydrochar phase. While some of these organic products could act as a carbon source for mixotrophic algae growth, compounds such as phenols, furans, and phenolics are known growth inhibitors and may be toxic to algae growth [10]. Colorimetric analysis of the AP showed that most of the inorganic nitrogen was in the form of ammonium (NH_4^+), while the formation of nitrates (NO_3^-) was low. Ammonia can be formed by the cleavage of peptide bonds, deamination and ring opening reactions and commonly accounts for > 95% of total AP nitrogen [3,35]. Raising the reaction temperature from 200 to 220 °C resulted in a significant increase in ammonium concentrations from 3.89 to 7.36 g L^{-1}, consistent with earlier studies [34,46,47]. At 240 °C, AP ammonium concentration slightly reduced to 5.80 g L^{-1}, potentially due to increased evaporation of ammonia. In contrast, the modified Johnson medium used for the cultivation of *D. tertiolecta* contains mostly nitrates (0.614 g L^{-1}) corresponding to a nitrogen concentration of 0.18 g L^{-1}. Based on these results, the nitrogen concentration in the HTC AP is 16.7 to 31.6 times higher than the nitrogen concentration in the modified Johnson medium and could therefore substitute a significant portion of this macronutrient.

The total phosphorus concentration (determined by ICP analysis) in the HTC-AP remained relatively constant between 0.434 and 0.459 g L^{-1}. However, as the water recovery increases at higher temperatures, the overall P recovery to the AP increased slightly from 5.9% at 200 °C to 6.7% at 240 °C, consistent with previous studies on HTC of AD-digestate [34,46]. Unlike total phosphorus, the phosphate (PO_4^{3-}) concentration (by colorimetric analysis) was found to increase from 0.71 g L^{-1} at 200 °C to 1.28 and 1.20 g L^{-1} at 220 and 240 °C, respectively, corresponding to 51%, 96% and 85% of total phosphorus in the AP. Phosphorus in the HTC

382 AP can be attributed to the decomposition of complex organic phosphorus containing
383 compounds (for instance, phospholipids, DNA and phosphates monoesters), forming PO_4^{3-} and
384 organic phosphorus compounds [28]. Therefore, the results suggests that most of the AP
385 phosphorus was solubilised at the lowest HTC temperature, while organic intermediates
386 continued to react to form phosphates as the final reaction product. Despite the relatively low
387 overall recovery of phosphorus to the HTC-AP, the P concentrations are ~50 times higher than
388 the phosphorus concentrations in the Johnson medium. This shows that small quantities of the
389 HTC-AP should be sufficient to meet the phosphorus demand for the algae culture.

390 The sulphur concentration in the AP reduced from 0.71 to 0.55 g L^{-1} when increasing the
391 reaction temperature from 200 to 240 °C, corresponding to a 2.2% reduction in overall sulphur
392 recovery. Most of the sulphur (80.9 to 82.9%) partitioned into the hydrochar, more than twice
393 the sulphur retention observed during HTC of sewage sludge (40%) [36]. Nonetheless, these
394 values exceed the sulphur concentrations in the modified Johnson medium by a factor of 8.5 to
395 11.0 and should therefore be sufficient to meet the algae culture sulphur requirements.

396

Table 3: Concentrations of growth nutrients and trace metals in HTC-AP products and modified Johnson medium

Parameter	AP200	AP220	AP240	Johnson medium
Water recovery (%)	53.3	51.2	59.7	*n/a*
Total organic carbon (g L^{-1})	16.06	15.36	14.68	*n/a*
Total inorganic carbon (g L^{-1})	0.80	1.37	1.71	4.86*
	From colorimetric analysis (g L^{-1})			
NH_4^+	3.89 ± 0.33	7.36 ± 3.13	5.80 ± 1.41	0.034
NO_3^-	0.16 ± 0.08	0.27 ± 0.17	0.21 ± 0.12	0.614
PO_4^{3-}	0.71 ± 0.28	1.28 ± 0.62	1.20 ± 0.60	0.028
	From ICP analysis (mg L^{-1})			
S	710	595	545	64.1
P	453	434	459	8.98
Na	140	129	132	4598
K	482	443	460	504
Ca	26.4	20.5	24.3	56.1
Mg	5.75	22.6	59.5	228
Fe	29.3	12.1	6.00	0.497
Mo	2.20	2.15	2.20	0.21
Mn	0.35	0.35	0.45	0.011
B	8.70	7.20	7.30	0.107
Zn	1.45	0.85	1.05	0.019
Co	0.35	0.30	0.30	0.012
Cu	bdl	bdl	bdl	0.015
Al	5.90	6.60	7.25	*n/a*
Cr	0.80	0.60	0.40	*n/a*
Cd	0.25	0.2	0.25	*n/a*
Pb	1.10	1.05	1.10	*n/a*
Sr	0.40	0.35	0.35	*n/a*
Ni	0.80	0.70	0.65	*n/a*
Bi	4.15	4.15	4.15	*n/a*
Ba	0.50	0.75	0.40	*n/a*

* Inorganic carbon concentration based on bicarbonate feed concentration of 34 g L^{-1}

400 The highest metal concentrations in the AP were found for potassium (443 – 460 mg L^{-1}) and
401 sodium (129 – 140 mg L^{-1}), which are both essential components of Johnson medium.
402 Relatively high concentrations were also found for Ca (20.5 – 26.4 mg L^{-1}) and Mg (5.75 –
403 59.5 mg L^{-1}), but in both cases the concentrations are lower than in the growth medium. In
404 contrast, the concentrations of other trace nutrients are between 10 (Mo) to 80 (B) times higher
405 than in the medium, except for copper, which could not be detected by ICP analysis. The
406 concentrations of heavy metals were found to be very low, mostly below 1 mg L^{-1}, except for
407 Bi, which reached concentrations of ~4 mg L^{-1}.

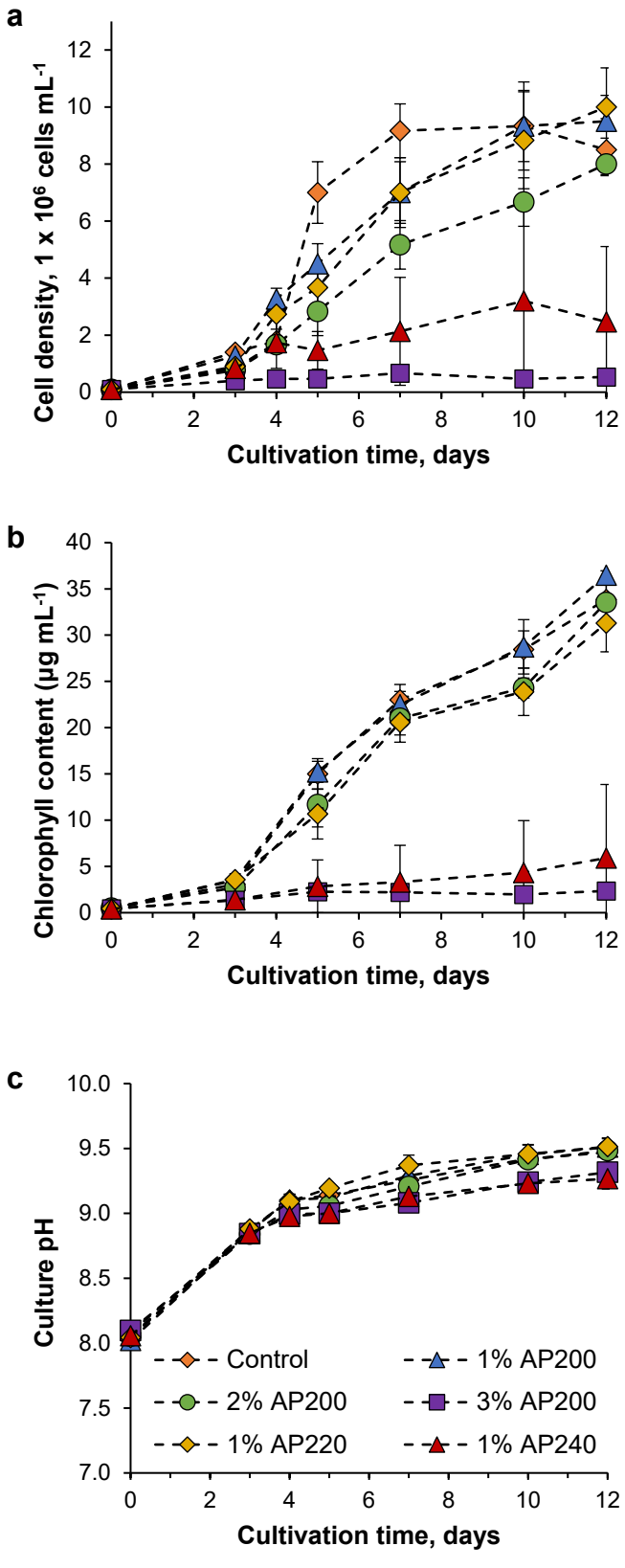
408 Metal concentrations (except Fe and Cr) in the AP generally increased with increasing reaction
409 temperature, indicating increased transformation of metals into dissolved ions under
410 hydrothermal carbonisation [40]. Similar trends were previously observed for Cd and Pb [48],
411 Cr [41] and K, Ca, Al and Mn [49].

412 **3.5 Cultivation of *Dunaliella tertioleta* in HTC-AP**

413 The presence of high concentrations of C, N, P, K, S, and metals (Ca, Mg, Fe) in the HTC-AP
414 demonstrates that nutrients are solubilised and transferred from the AD-digestate to the water
415 phase during carbonisation treatment. Nutrient concentrations in the HTC aqueous phases were
416 several folds higher (up to 80-fold) than required for cultivating *D. tertiolecta*, based on the
417 composition of modified Johnson medium (culture medium, Table 3). Therefore, the diluted
418 HTC-AP may be suitable for cultivating this strain, similar to earlier studies with freshwater
419 algae [50–52]. Adding low concentrations of HTC-AP has minimum impact on the colour of
420 the growth medium and is therefore unlikely to impact light attenuation, affecting algae growth.
421 However, the HTC-AP also contains high concentrations of organic carbon (up to 16.1 g L^{-1}),
422 which may inhibit algal growth. Previous studies employed high dilutions of the AP products
423 (50 – 200 fold) to reduce the inhibitory effect of toxic compounds for algae culturing

[14,22,50–52]. Therefore, initial screening studies were conducted to explore the compatibility of *D. tertiolecta* with the AP obtained at the three different HTC temperatures at concentrations up to 3% in modified Johnson medium (Figure 2).

Growth curves for all cultures (including the control) displayed an initial lag phase of up to three days, which may be due to the cell's acclimatisation to the culture medium [13]. The highest exponential growth rates (0.95 ± 0.13 d^{-1}) were obtained for cells grown with 1% AP200, slightly higher than the growth rates obtained in the control. Increasing the AP concentration from 1% to 3% or increasing HTC temperature from 200 to 240 °C, both resulted in a significant reduction in growth rate and final cell densities, indicating significant growth inhibition. Cells grown with the control medium reached the stationary phase after seven days, with a maximum cell density of 8.50×10^6 cells mL^{-1}, before cell concentrations started to decline after 10 days of cultivation, potentially due to nutrient limitations [13]. In contrast, cell densities in cultures grown with 1% and 2% of AP200 and 1% AP220 continued to increase after 7 days, with maximum cell densities obtained with 1% AP220 (10.0×10^6 cells mL^{-1}). As the AP products were added to the algae growth medium, total nutrient concentrations in the mixed media were increased, which may explain the higher maximum algae concentrations compared to the control.



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443

444 *Figure 2: Cultivation of Dunaliella tertiolecta with different concentrations of HTC-AP (a)*

445 *Algae growth curves; (b) Chlorophyll content in the cultures; (c) Culture pH.*

The highest biomass contents were found in cultures grown with 1% AP200 (1.14 ± 0.06 g L^{-1}) and 1% AP220 (1.11 ± 0.08 g L^{-1}), higher than those in the control (1.01 ± 0.04 g L^{-1}), demonstrating that the addition of HTC-AP to the culture medium can improve the production of biomass (Table 4). Cell concentrations are in line with previous growth studies of *Chlorella* sp. (0.64 – 1.5 g L^{-1}) [13,22,50,52], although most of these studies diluted the AP in water, rather than growth medium. Ash content, total chlorophyll and carotenoid content were relatively similar in all cultures, except for the cultures grown with 3% AP200 and 1% AP240, which displayed much higher ash content and lower chlorophyll and carotenoid contents.

Table 4 Proximate analysis of D. tertiolecta biomass grown with HTC-AP

	Control	**1% AP200**	**2% AP200**	**3% AP200**	**1% AP220**	**1% AP240**
Growth rate (μ, d^{-1})	0.80 ± 0.17	0.95 ± 0.13	0.63 ± 0.17	0.26 ± 0.05	0.71 ± 0.08	0.62
Biomass content (g L^{-1})	1.01 ± 0.04	1.14 ± 0.06	1.05 ± 0.04	0.28 ± 0.08	1.11 ± 0.08	0.355
Ash content (%)	20.74 ± 0.31	21.13 ± 5.14	20.19 ± 5.08	47.29 ± 8.06	19.15 ± 4.94	23.00 ± 5.98
Chlorophyll content (mg g^{-1} biomass)	33.59 ± 2.62	31.95 ± 0.51	31.91 ± 2.88	2.34 ± 0.20	28.29 ± 3.43	16.65
Carotenoid content (mg g^{-1} biomass)	8.47 ± 1.15	7.64 ± 0.44	8.04 ± 1.12	1.24 ± 0.20	6.63 ± 0.86	3.60

To evaluate the health of the different algae cultures, the accumulation of chlorophyll in each culture was determined (Figure 2b). Cultures with higher chloroplastic pigments in the cells can absorb more light energy, which is subsequently converted into chemical energy resulting in higher cell densities [53]. In line with the growth curves, the fastest increase in chlorophyll content was observed for the control and 1% AP200 cultures, followed by the 2% AP200 and 1% AP220 cultures. In contrast, much lower chlorophyll concentrations were detected for cells in the 1% AP240, and particularly the 3% AP200 media, explaining the low cell concentrations obtained for these cultures, and indicating significant inhibition for these experiments.

D. tertiolecta is known for its high tolerance to heavy metals and its ability to remove metals from the culture medium [54–56]. Therefore, given the low concentrations of metals in the diluted HTC-AP products (< 0.1 mg L^{-1}) and similar metal concentrations in the AP200, AP220 and AP240 products (two of which did not display inhibition), it is unlikely that the observed inhibition can be attributed to the presence of heavy metals. Similarly, the concentrations of ammonium in the mixed media were low (0.117 g L^{-1} for 3% AP200 and 0.058 g L^{-1} for 1% AP240) and comparable to the concentration of the 1% AP220 experiment (0.074 g L^{-1}), therefore unlikely to be the cause of the observed inhibition [52]. Instead, the inhibition is likely to be caused by the presence of organic compounds such as hydroxy methyl furfural (HMF), furfural, phenol or formic acid, produced during the carbonisation of biomass >190 °C [39,44,57,58]. HMF and furfural (~1 mM) can delay the growth of algae, whilst concentrations above 7 mM can completely inhibit algal growth by blocking electron transfer in the photosynthetic electron transport system [14,59]. Although the total organic carbon content in the HTC-AP from the current study was relatively independent of temperature, higher temperatures can increase the conversion of water-soluble intermediates, such as amino acids and sugars, into more toxic secondary products, increasing their inhibitory effect on algae cultivation. The results from the growth studies suggest that the concentrations of these inhibitors are significantly lower in the AP200 and AP220 products but increasing their concentration above a certain threshold (3%) still results in significant growth inhibition.

3.6 Effect of HTC-AP supplementation on carbonate regeneration by *Dunaliella tertioleta*

The main objective of culturing *D. tertiolecta* in our recently developed algae-based biogas purification and carbon capture process [15] is the conversion of sodium bicarbonate ($NaHCO_3$) into sodium carbonate (Na_2CO_3) to regenerate the CO_2 absorbent required for

biogas purification. First, bicarbonate ions are used by the algae to produce CO_2 required for algae growth ($HCO_3^- + H^+ \rightarrow CO_2 + H_2O$). The consumption of protons causes the culture pH to increase, resulting in the deprotonation of bicarbonate to regenerate the original carbonate ions ($HCO_3^- + OH^- \rightarrow CO_3^{2-} + H_2O$).

Based on our earlier study, cultures were supplemented with a bicarbonate concentration of 34 g L^{-1}, corresponding to a carbon loading of 4.86 g L^{-1}. Carbonate and bicarbonate concentrations were monitored throughout the growth cycle and the final carbon distribution was determined after 12 days of cultivation, by multiplying the algae yield with the algae carbon content (Figure 3). As expected, bicarbonate concentrations reduced in all six cultivation experiments, together with an increase in carbonate concentrations. The highest bicarbonate conversions were obtained for cultures grown with 1% and 2% AP200 (83% and 80%, respectively), higher than the conversion achieved in the control. In contrast, conversions were significantly reduced in the cultures grown in 3% AP200 and 1% AP240, indicating a clear link between bicarbonate conversion and algae carbon yields. Despite the big differences in algae growth between the cultures with different AP loadings, trends in culture pH were very similar, increasing from around 8.0 at the start of culture to final values between 9.3 to 9.5 (Figure 2c). These trends explain the partial conversion of bicarbonate to carbonate, even for the cultures where algae growth was poor.

Carbonate yields ranged between 43.6% for 1% AP220 to 53.5% for the control, while the carbonate yields of the cultures in 1% and 2% AP200 and 1% AP240 were within 0.5% of the theoretical yields of 50%. Algae accounted for up to 14.1% of the converted bicarbonate carbon, resulting in a significant discrepancy between the converted bicarbonate carbon and carbon partitioned to algae and carbonate products of up to 49.1% for the culture in 3% AP200. This unaccounted carbon may be attributed to the loss of excess CO_2 to the atmosphere,

indicating the importance of maintaining fast algae growth to achieve a high degree of carbon capture.

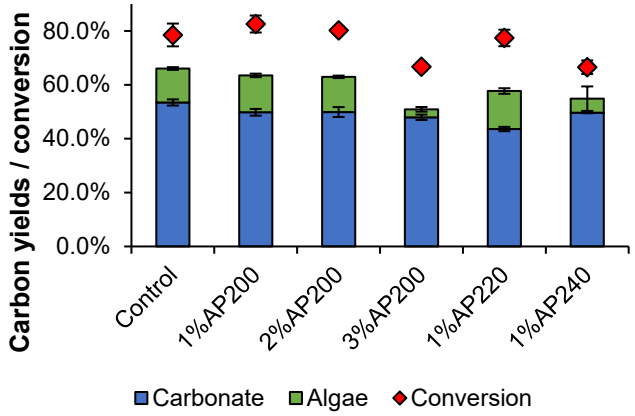

Figure 3: Bicarbonate conversion and carbon distribution to carbonate and algae products from cultivation of Dunaliella tertiolecta with different concentrations of HTC-AP

4. Conclusion

This study investigated the potential of hydrothermal carbonisation to fractionate sewage sludge derived AD digestate into carbon-rich hydrochar and nutrient-rich aqueous phase, to supplement the cultivation of *D. tertiolecta* as part of an integrated biogas purification system. HTC resulted in high hydrochar mass and carbon yields of up to 78% and 75%, respectively, together with high retention of metals within the solid, leading to low heavy metal concentrations in the HTC-AP. Although most of the phosphorus and nitrogen partitioned to the solid phase, AP concentrations were up to 11 times (S) and 50 times (P) higher than their concentrations in artificial algal growth medium. Similarly, concentrations of trace nutrients were between 10 and 80 times higher than in the artificial growth medium. In contrast, HTC solubilised the majority of nitrogen, leading to HTC-AP nitrogen concentrations between 17 to 32 times those required for algae growth.

528 *D. tertiolecta* was successfully grown without inhibition with HTC-AP concentrations of up
529 to 2%, achieving increased cell concentrations and biomass concentrations compared to the
530 control. These cultures also displayed the highest rates of carbonate regeneration, indicating
531 the feasibility of HTC-AP as nutrient source for integrated biogas purification via alkali-
532 halophilic microalgae. However, significant inhibition was observed for samples obtained
533 above 220°C and at HTC-AP concentrations above 3%, attributed to the presence of organic
534 by-products such as furfurals and phenols, which are known to inhibit algae growth. Further
535 studies are required to optimise HTC reaction conditions (reaction time, reaction temperature,
536 solid loading) and monitor the fate of organic pollutants during microalgae culture.

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