Abstract

Due to the finite stocks of phosphate rock and low phosphorus (P) use efficiency (PUE) of traditional mineral P fertilizers, more sustainable alternatives are desirable. One possibility is to culture microalgae in wastewater to recover the P and then convert the microalgae biomass into slow-release fertilizers through hydrothermal carbonization (HTC). Therefore, this study aimed to recycle P from wastewater to agricultural field using microalgae and HTC technology. *Chlorella vulgaris* (CV) and *Microcystis sp.* (MS) were cultured in poultry farm wastewater with an initial concentration of 41.3 mg P kg^{-1}. MS removed 88.4% P from the wastewater, which was superior to CV. CV- and MS-derived hydrochars were produced at 200 or 260°C, in solutions using deionized water or 1*wt*% citric acid. The MS-derived hydrochar using 1 *wt*% citric acid solution at 260 °C (MSHCA260) recovered the highest amount of P (91.5%) after HTC. The charring promoted the transformation of soluble and exchangeable P into moderately available P (Fe/Al-bound P), and using citric acid solution as feedwater increased the P recovery rate and formation of Fe/Al-bound P. With the abundant moderately available P pool, hydrochar amendment released P more slowly and enhanced the soil P availability more persistently than chemical fertilizer did, which helped to improve PUE. In a wheat-cultivation pot experiment, MSHCA260 treatment improved wheat PUE by 34.4% and yield by 21.6% more than chemical fertilizer did. These results provide a novel sustainable strategy for recycling P from wastewater to crop-soil systems, substituting the mineral P fertilizer, and improving plant PUE.

Keywords: hydrochar; microalgae technology; phosphorus fractionation; phosphorus use efficiency; sustainable development; wheat

1. Introduction

Phosphorus (P) is an essential plant nutrient and makes up around 0.2% of plant dry weight (Václavková et al., 2018; Adegbeye et al., 2020). Nevertheless, soil P exists in pools of low availability and thus becomes one of the major factors limiting crop growth, affecting approximately 30% agricultural fields worldwide (Xu et al., 2019; B. Li et al., 2020). Consequently, a vast amount of P fertilizers is required for agricultural production. However, P-based synthetic fertilizers rely on P extracted from phosphate rock which is a finite non-renewable resource that might be depleted in 50-100 years (Withers et al., 2020). In addition, crops take up only 30-45% of the supplied P from synthetic P fertilizer (Shen et al., 2011; Oita et al., 2020). The P that is not incorporated into the plants is washed into waterbodies through leaching and runoff, causing environmental issues (Pan et al., 2018; Lee et al., 2020). Therefore, it is crucial to seek alternatives to chemical fertilizers and to develop methodologies that improve P use efficiency (PUE) by crops, while minimizing the negative environmental impacts.

Wastewater contains plentiful P that requires removal prior to discharge into watercourse. Microalgae have been shown to grow rapidly in such wastewater, efficiently removing P (Cabanelas et al., 2013; Subramaniyam et al., 2016; Huo et al., 2020). Microalgae are capable of absorbing inorganic P in excess through storage within their cells in the form of polyphosphate granules (Delgadillo-Mirquez et al., 2016)(Solovchenko et al., 2019). Previous studies reported that microalgae can accumulate large quantities of P (up to 2-4% of their cell dry weight), and thus have potential to be applied as fertilizer after appropriate processing (Cabanelas et al., 2013; Santos and Pires, 2018; Luo et al., 2019). Therefore, reclaiming P from wastewater streams with microalgal cultures is a sustainable and environmental-friendly solution to the shortage of phosphate rock. In the last decade, direct application of dried microalgae as an alternative to chemical P fertilizer has been evaluated (Ray et al., 2013; Mukherjee et al., 2015; Schreiber et al., 2018). A major concern is that the polyphosphate-rich biomass releases the phytoavailable P too slowly into soil to satisfy the demands of growing plants. Moreover, microalgal toxins, such as microcystin and cyanotoxin, potentially threatens both soil microbial activity and plant growth if microalgae are directly applied to soil (Machado et al., 2017). These factors have driven the researchers to explore additional treatments to enhance the fertilizer values of microalgal biomass prior to its use in an agricultural context.

One such potential tool for increasing PUE is the application of biochar (Anyaoha et al., 2018; Bornø et al., 2018; Fei et al., 2019; H. Li et al., 2020). Pyrolysis is the thermal treatment of biomass in absence of air at temperatures of 400-600°C, converting dry biomass into pyrochar (Foong et al., 2020). Hydrothermal carbonization (HTC) converts wet biomass to hydrochars at lower temperature (180-260 °C) (Hao et al., 2018; Cui et al., 2020). The higher hydrothermal temperature might lead to the increased generation of noxious compounds in hydrochars, including phenols and organic acids (Hao et al., 2018). Compared with pyrolysis, HTC is generally more energy-efficient and, since it is carried out in water, wet microalgae can be directly processed without prior dehydration (Lachos-Perez et al., 2017). More importantly, the hydrolysis reaction occurring in HTC process can promote the degradation of polyphosphate into orthophosphate, with over 90% P present as orthophosphate in sewage sludge- or manure-derived hydrochars (Heilmann et al., 2014; Huang and Tang, 2016; Idowu et al., 2017). In addition, the predominant chemical P fraction in hydrochars is iron (Fe)/ aluminum (Al)-bound P (Huang and Tang, 2016; Wang et al., 2017), which is considered a moderately labile P pool for plants and acting as a buffer for available P in soil (Yao et al., 2013; Heilmann et al., 2014; Fei et al., 2019).

Biochar can also improve soil health by increasing soil electrical conductivity (EC), organic matter content, surface area, and nutrient availability (Bornø et al., 2018; Yu et al., 2019; Chu et al., 2020c). The microporous structures, surface functional groups, and intrinsic minerals of hydrochar could improve the capacity of nutrients adsorption and retention in soil (Yu et al., 2019; Chu et al., 2020a, 2020c), potentially avoiding P loss and improving plant PUE. Also, remarkable alterations of the microbial community structure in biochar-amended soil have been reported (Ye et al., 2019; Lu et al., 2020), possibly by affecting phosphatase activity secreted by soil microorganisms and consequently, by P solubilization. These beneficial properties, plus the increased moderately labile P pool present within microalgae-derived hydrochars, are likely to improve the PUE, nutrients retention, and crop growth.

This study aims to achieve P recycling from wastewater to food through the recovery of P from wastewater using microalgae, converting the biomass into hydrochar by HTC, and applying the microalgae-derived hydrochars to a crop-soil system. The specific objectives of this work included 1) investigating the fate of P from wastewater to hydrochar and then to the crop-soil system; 2) screening the most suitable microalgae-derived hydrochar to improve the PUE compared to traditional synthetic P fertilizer.

2. Methods and materials

2.1. Microalgal cultivation and harvest

Chlorella vulgaris strain CCAP 211/12 and *Microcystis sp*. strain CCAP 1450/13 were used in this study and purchased from Culture Collection of Algae and Protozoa (CCAP), Scottish Marine Institute, Scotland. The wastewater was collected from the poultry farm at Nottingham Trent University's Brackenhurst Campus and filtered before culturing microalgae. The trials of P removal from wastewater by culturing microalgae were carried out in 3 L borosilicate bioreactors in the Integrated Water Energy and Food facility, Nottingham Trent University, UK. The culturing conditions were: constant aeration (4 mL s^{-1}), photoperiod of 14h:10h light:dark cycles, at a controlled temperature of 25 ± 1°C under cool white fluorescent light of 10000 lux intensity. The chemical characterization of wastewater is shown in **Table S1**. The initial total P (TP) concentration in the wastewater was 41.3 mg L^{-1}. Three replicates were conducted for each microalgal strain. The dry weight of microalgae was gravimetrically assessed every two days according to standard method 2540-D (APHA, AWA, WPCF 1992) and the biomass in wastewater reached the stationary phase after 14 days. Also, TP of wastewater was analyzed every two days using an auto analyzer (AQ400, SEAL Analytical GmbH, Germany) in order to monitor the P removal rate. At the end of culture total nitrogen (TN) were measured colorimetrically as nitrate after the water samples had been oxidized and total organic carbon (TOC) were measured using an organic carbon analyzed by an organic carbon analyzer (TOC-C_{CSN}), Shimadzu). Afterwards, the microalgae were collected by flocculation. The methods of flocculation were the same as detailed in our previous study (Li and Pan, 2013), and are included in the Supplementary Information. The flocculation efficiency of both CV and MS was more than 95% (**Fig. S1**).

2.2. Microalgae-derived hydrochars preparation

HTC of microalgae was conducted in a 600 mL Teflon lined stainless steel hydrothermal reactor (Parr Instruments, Moline, IL, USA), using a solid:liquid ratio of 1:9 (w/w). The wet microalgal biomass was directly mixed with the feedwater and the final solid/liquid ratio was calculated based on the moisture content. Eight types of hydrochars were produced using two different microalgae under two different feedwaters (deionized water and 1 *wt*.% citric acid solution) and two different reaction temperatures (200 and 260 °C). For each run, the reactor was heated to 200 or 260 °C at 3 °C min^{-1}, and held at the final temperature for a duration of 2 h. The pressures originating from feedwater alone at the respective reaction temperatures were not monitored. The reactor was rapidly cooled down to room temperature using a recirculating condensing engine. The solid and liquid products were initially separated by centrifugation and fully gravity filtered through a 0.45 μm membrane filter. The total solid recovery rate was recorded.

2.3. Characterization of microalgae-derived hydrochars

The pH of the hydrochars was analyzed using a solid/deionized water ratio of 1:2.5 (w/v). The specific surface area (SSA) and porosity were measured using a NOVA 1200 analyzer (Anton Paar QuantaTec Inc., Graz, Austria), and were calculated by the Brunauer-Emmett-Teller method (Yu et al., 2019). Total C, H, N, and S contents were determined using an Elemental Analyzer (EL III; elemental Analysensysteme GmbH, Germany). Concentrations of metallic elements, including K, Al, Ca, Fe, and Mg were determined by firstly digesting the hydrochars using HNO_3 (61%) with hydrogen peroxide and then analyzing the digests using inductively coupled plasma-optical emission spectrometry (ICP-OES), as described in a previous study (Chu et al., 2019).

The sequential extraction of the microalgae-derived hydrochars were carried out to evaluate the fractions of P present, following previous studies (Hedley et al., 1982; Bornø et al., 2018) as shown in **Fig S2**. P fractionation in chars can be separated into soluble P, exchangeable P, alkaline-dissolved P and organic P, acid-dissolved P and organic P, and residual P fractions. The solids were separated from the extract after each batch of extraction via centrifugation at 8000 *g* for 5 min, and the supernatant was filtered using a 0.45 μm membrane filter. The P concentrations in extracts were analyzed colorimetrically by auto-analyzer. TP concentrations of hydrochars were calculated by summation of all the P fractions. The P recovery rate was calculated according to the following formula:

$P_{recover} = (P_{total} \times \lambda / P_{feedstock}) \times 100\%$;

where P_{total} is the TP content in the hydrochar, $P_{feedstock}$ the TP content in the feedstock, and λ represents the yield of the hydrochar.

2.4. Soil incubation experiment

The soil used in the incubation experiment was collected from the top soil of Embleys farm in the UK (0-15 cm; 29% clay, 42% silt, 29% sand). The soil had the following basic properties: pH 7.7, organic matter content 2.1%, EC 0.52 mS cm^{-1}, cation exchange capacity (CEC) 2.42 cmol kg^{-1}, total N 1.2 g kg^{-1}, TP 0.63 g kg^{-1}, total K 3.2 g kg^{-1}, Olsen-P 12.1 mg kg^{-1}. Soils and hydrochars were air-dried, sieved through 2 mm mesh, and mixed to ensure a relatively homogeneous distribution. 100 g of the top soil were placed in the 200 mL transparent plastic jars for soil incubation experiments. The jars were covered with loose lids to allow air circulation but to minimize water evaporation. Treatments were as follows: Untreated soil (no chemical fertilizers or hydrochars were applied), control (chemical fertilizers were applied), CV (dried powder of *Chlorella vulgaris*), CVHCA200 (CV-derived hydrochar using 1 *wt*%

citrate acid solution as feedwater; 200 °C HTC), MS (dried powder of *Microcystis sp.*), MSHCA260 (MS-derived hydrochar using 1 *wt*% citric acid solution as feedwater; 260 °C HTC). CVHCA260 and MSHCA260 were selected because they had the highest P recovery rate of the *Chlorella vulgaris*- or *Microcystis sp.*-derived hydrochars. The chemical fertilization control contained 500 mg N kg soil^{-1} in the form of NH_4NO_3, 100 mg P kg soil^{-1} in the form of KH_2PO_4, and 300 mg K kg soil^{-1} in the form of K_2SO_4. The application rate of hydrochar was 0.5 *wt*% of the soil; chemical fertilizers were added at rates equivalent to the N, P, and K rates used in the chemical fertilization control. Each treatment comprised four replicates. Incubation lasted for 120 days in an illuminated incubator at 25 °C. The soils were sampled at 0, 10, 30, 50, 80, and 120 days. During the incubation period, deionized water was added every two days to maintain a field water-holding capacity at 60% (w/w).

2.5. *Wheat pot experiment*

The experiments used 5L plastic pots, each of four kilograms of air-dried soil sieved to pass through a 2 mm mesh. A filter paper was placed at the bottom of the pots to prevent soil loss. Before cultivating wheat, hydrochars were mixed with soil and the pots were incubated for four weeks in a greenhouse under moderately moist conditions (60% field water-holding capacity). Wheat seeds were pre-germinated in a petri dish covered with a filter paper and kept in the dark for three days. After the preincubation period, five germinated wheat seedlings were carefully transplanted to each pot and thinned to one after one week. The design of treatments was the same as described in the soil incubation experiment (2.4). Each treatment comprised four replicates.

The wheat plants were harvested at the tillering (20 days after transplantation) and maturation stages (120 days after transplantation). In order to satisfy the requirement of sampling at two different growth stages, two batches of experiments were conducted at the same time. Rhizosphere soil samples were collected by carefully cleansing the soil from the roots (Chu et al., 2017; Sha et al., 2020). The soil samples were divided into two parts: one portion was freshly prepared for the determination of enzyme activity and soil microbial C and P content, and another portion was air-dried for analysis by sequential P fractionation. In fresh soil samples, the concentration of microbial biomass C (MBC) and P (MBP) were investigated using the chloroform fumigation-extraction method (Brookes et al., 1982). Acid and alkaline phosphatase activities in the soil samples were determined as described in a previous study (Bornø et al., 2018). In dried soil samples, the soil pH was analyzed in a slurry of 1:2.5 (w/v, soil to water) using a pH-meter. Soil organic matter (SOM) was measured using the potassium dichromate oxidation method. Soil total N (TN) was determined by initially digesting with H_2SO_4 (98%) and then using the Kjeldahl method (Chu et al., 2016a). CEC was measured using the compulsive exchange method with 1.0 M ammonium acetate extraction at pH 7.0 (Brookes et al., 1982). The analysis of soil P fractionation was same for the hydrochars, as described above (2.3).

2.6. Statistical analyses

All statistical analyses were performed using SPSS version 23.0 (SPSS Inc. Chicago, IL, USA). One-way analysis of variance (ANOVA) was used to evaluate the significant difference at a $P < 0.05$ probability level with Duncan's multiple range test.

3. Results

3.1. Growth of microalgae and P removal in the wastewater

The microbial growth curves were plotted showing the values of biomass (as dry weight) in wastewater versus time (in days) (**Fig 1**). CV and MS both exhibited exponential growth and reached a stationary phase after 8 days. After 14 days culture, the biomass (dry matter) of MS reached the maximum value (1.14 g_{dw} L^{-1}), 8.5% higher than that of CV (**Table S2**). The average biomass productivities of CV and MS were 0.068 and 0.071 g_{dw} L^{-1} d^{-1}, respectively. The plot of P removal in the wastewater versus time (in days) is also shown in **Fig 1**. From 0 to 8 days, the P concentration in the MS culture declined from 41.3 to 4.8 mg L^{-1}, with a daily P removal rate of 2.95 mg L^{-1} day^{-1}, and in the CV culture from 41.3 to 8.8 mg L^{-1}, with a daily P removal rate of 2.32 mg L^{-1} day^{-1}(**Table S2**). After 8 days, P removal gradually reached the stationary phase. After 14 days culture, the maximum P removal rate by MS was 10.7 mg L^{-1} day^{-1}, which was 23.4% higher than that of CV (**Table S2**). Overall, after 14 days culture, MS and CV removed 88.4% and 78.7% P, respectively, from an initial concentration of 41.3 mg P L^{-1}; both microalgae were demonstrated to be able to remove and enrich P from wastewater efficiently although MS was superior to CV in this respect. In addition to the P removal, after 14 days the TN concentration in CV and MS culture declined from 321.6 mg L^{-1} to 182.1 and 160.4 mg L^{-1}, TOC from 375.2 to 53.2 and 34.8 mg L^{-1}, respectively, suggesting that with the fast growth in the wastewater the microalgae possibly absorbed and assimilated the N and C at a high rate as well.

3.2. Basic physiochemical characteristics of hydrochars

The physiochemical characteristics of the microalgae and microalgae-derived hydrochars are displayed in **Table 1**. The microalgae-derived hydrochars using deionized water as feedwater all exhibited an alkaline pH after processing. Using citric acid as feedwater markedly neutralized the alkalinity of hydrochars from 7.2-8.5 to 5.7-6.6. With hydrothermal temperature decreasing from 260 °C to 200 °C, the lower pH was observed in hydrochars, irrespective of microalgae strain. Transforming the microalgae into hydrochars decreased the C, H, N, and S content, irrespective of microalgae strain (**Table 1**). As a consequence of the vaporization, degradation, and dissolution processes of labile fractions occurring during HTC, elements including C, H, N, and S were partially lost to feedwater, whereas conservative elements such as P and metals were retained in the hydrochars (**Table 1 and 2**). The C concentration in hydrochars using citric acid as feedwater ranged from 53.9-66.2%, which is 1.5-6.3% higher than that in hydrochars where deionized water was used as feedwater, while H and N concentration decreased, resulting in higher C/N and lower H/C ratio.

Moreover, the hydrochars using citric acid as feedwater showed a markedly higher concentration of metals, including Al, Ca, Fe, and Mg, irrespective of microalgal strain processed (**Table 1**). The increased abundances of these elements were possibly beneficial for P bonding in hydrochars. Additionally, as a metal with high mobility, K in hydrochars showed an opposite trend to other metals. Using citric acid as feedwater during HTC reduced K accumulation in hydrochars compared to those when using deionized water. In addition, different reaction conditions during HTC changed the adsorptive capacity of hydrochars (**Table 1**). When compared to the raw microalgae, hydrochars markedly increased the SSA and porosity. The SSA for hydrochars using citric acid as feedwater during HTC ranged from 5.8-6.7 m^2 g^{-1}, which was 16.6-18.2% higher than that in hydrochars using only deionized water as feedwater.

3.3. Recovery rate of P in hydrochars

As displayed in **Table 2**, the charring process resulted in an increased P content in the hydrochars. The P content in CV-derived hydrochars ranged from 3.4±0.2 – 4.3±0.4 %, which was 23.1-67.9% higher than that of raw CV, and in MS-derived hydrochars ranged from 4.2±0.3 – 5.8±0.4%, which was 14.7-72.2% higher than that of raw MS. Moreover, with increasing hydrothermal temperature from 200 to 260°C the TP increased from 3.2-4.1% to 3.5-4.1% in CV-derived hydrochars, and from 3.9-5.4% to 4.5-6.2% in MS-derived hydrochars. In addition, TP in MS-derived hydrochars varied from 3.9-6.2%, which was 21.9%-31.9% higher than that in CV-derived hydrochars. This result might be attributed to the higher P uptake by MS in wastewater (**Fig. 1**).

With hydrothermal temperature increasing from 200 °C to 260 °C, in contrast with P recovery, the solid recovery rate of hydrochars declined from 40.7-49.8% to 37.3-45.4% in CV-derived hydrochars, and from 44.6-58.8% to 42.1-55.2% in MS-derived hydrochars. This result might be ascribed to the degradation of polymeric materials (such as hemicellulose and cellulose) at higher temperatures during HTC. Moreover, using citric acid solution as feedwater during HTC markedly increased the hydrochar yield and TP, irrespective of microalgal strain. The solid recovery rate of microalgae-derived hydrochars using citric acid solution ranged from 41.1-62.0%, but that of hydrochars using deionized water ranged from 32.3-46.3%. Also, for CV, P content in CVHCA200 and CVHCA260 was 9.4-27.0% higher than that in CVHW200 and CVHW260; for MS, P concentration in MSHCA200 and MSHCA260 was 21.6-

28.2% higher than that in MSHW200 and MSHW260. The highest P recovery rate for CV and MS were both attained by using citric acid as feedwater, 72.3% in CVHCA260 and 91.5% in MSHCA260.

3.4. Fractionation of P in hydrochars

The results of the sequential P fractionation of the microalgae and microalgae-derived hydrochars are presented in **Fig. 2**. The charring process significantly reduced soluble, exchangeable, and residual P fractions, but increased largely the Fe/Al-bound and Ca-bound P fractions. The soluble and exchangeable P generally correspond to the phytoavailable P. This phytoavailable P constituted 34.0% of the *Chlorella vulgaris* and 27.9% of the *Microcystis sp.*, whereas less than 20% was detected in derived hydrochars (e.g., 8.8% in MSHCA260) (**Table S5**). Additionally, with increasing hydrothermal temperature from 200 to 260 °C, the soluble P fraction decreased sharply, irrespective of microalgal strains.

However, the charring process significantly improved the Fe/Al-bound P fraction by 2.1-3.4 and 1.7-3.8 fold for CV and MS, and the Ca-bound P fraction by 2.7-4.7 and 1.4-2.5 -fold for CV and MS, respectively (**Fig. 2**). Fe/Al-bound P was the largest P fraction in microalgae-derived hydrochars, ranging from 32.8-52.7%. In addition, using citric acid solution as feedwater during HTC significantly increased the Fe/Al- and Ca-bound P fractions. The Fe/Al- and Ca-bound P in CVHCA200, CVHCA260, MSHCA200, and MSHCA260 ranged from 15.2-30.5% and 5.8-12.6%, which was 23.6-64.0% and 7.4-90.9% higher than those in CVHW200, CVHW260, MSHW200, and MSHW260, respectively. Using citric acid solution as feedwater probably better facilitated the bonding between those metallic elements present and P.

Overall, HTC promoted the P transformation from readily available and recalcitrant fractions to potentially available fractions and using citric acid as feedwater sharpened such transformation. Fe/Al-bound P occupied 46.2% and 51.5% in CVHCA260 and MSHCA260, but only 19.5% and 20.1% in the raw CV and MS (**Table S5**), suggesting that P release in hydrochars will be possibly more sustainable for satisfying the long-term demand of plant growth.

3.5.P release from microalgae-derived hydrochars to soil

The raw microalgae and microalgae-derived hydrochars were incubated in the soil for 120 days, in order to investigate the release capacity of phytoavailable P, as shown in **Fig. 3**. The unfertilized soil and soil applied with chemical fertilizers were regarded as control groups. CVHCA260 and MSHCA260 were selected in this experiment because of their highest TP (**Table 2**) and highest moderately labile P pool (**Fig. 2**) among the respective microalgae-derived hydrochars. At 10 days after incubation, the available P concentration in soils treated with chemical fertilizer was remarkably and consistently higher than that in other treatments. However, after 50 days, the two hydrochar treatments, CVHCA260 and MSHCA260, significantly and persistently improved the concentration of soil available P compared to the chemical fertilizer group. At 120 days after incubation, the soil available P concentration under CVHCA260 and MSHCA260 treatment was 47.7% and 56.3% higher than that for the chemical fertilizer group, respectively. In addition, from 10 to 30 days the available P concentration in soils treated with CV and MS were consistently higher than those treated with CVHCA260 and MSHCA260, however, an opposite trend was observed from 30 to 120 days after incubation. At 120 days after incubation, soil available P concentration under CVHCA260 treatment was 10.9% higher than that under CV treatment, and under MS260 treatment was 21.0% higher than that under MS treatment. From 50 to 120 days, the highest soil available P concentration was consistently detected in the MSHCA260 treatment, although a decreasing trend was detected for all the groups.

3.6. Rhizosphere soil properties

The properties of rhizosphere soils amended with microalgae or microalgae-derived hydrochars at the ripening stage of wheat are shown in **Table S6**. CVHCA260 and MSHCA260 significantly reduced soil pH by 0.4-1.6 units compared to the control. CV, MS, CVHCA260, and MSHCA260 significantly improved the SOM by 19.8%, 12.3%, 25.8%, and 26.6% respectively compared to that in control. Also, amendment by MSHCA260 significantly improved the CEC compared to the control and the two microalgae-derived hydrochars significantly improved the CEC compared to the control, and to the CV- and MS- amended soils. Moreover, with addition of CV and MS, soil total N was maintained at a similar level to that of the control, however, amendment by CVHCA260 and MSHCA260 significantly reduced soil TN by 33.3% and 42.9%, respectively, compared to the control. Because of effects on SOM and TN, CV and MS addition significantly increased soil C/N ratio compared to control, by 62.3% and 92.4% for CVHCA260 and MSHCA260 respectively.

The incorporation of microalgae and microalgae-derived hydrochars to soil affected microbial activity, as shown in the results of soil phosphatase activity in **Fig. S3** and MBC and MBP content in **Fig. S4**. The introduction of C from microalgae or microalgae-derived hydrochars into soil significantly improved the soil MBC, irrespective of the growth stages, compared to the control. However, soil MBP displayed different results. At the tillering stage, no significant difference was detected for MBP, while at the ripening stage, CV and MS addition significantly reduced MBP by 26.2% and 33.9% compared to the control, and more significantly lower MBP were detected in CVHCA260 and MSHCA260 treatment (reduced 71.9% and 79.5%). Different treatments affected the phosphatase activity in rhizosphere soil. No significant differences were detected for acidic phosphatase activity among treatments. However, alkaline phosphatase activity significantly improved either at tillering or ripening stages for CV, MS, CVHCA260, and MSHCA260 treatments when compared to the control; alkaline phosphate activity in the rhizosphere soil under CVHCA260 and MSHCA260 treatments was 39.5-42.9% higher than that under CV and MS treatment at the tillering stage, and 51.7-56.0% higher at the ripening stage.

3.7. P fractionation in rhizosphere soil of wheat

The results of the sequential P fractionation of rhizosphere soil from a wheat pot experiment are shown in **Fig. 4**. The results of labile and stable P pools in the rhizosphere soil are shown in **Fig. S5**. Notably, the addition of CV, MS, CVHCA260, and MSHCA260 significantly reduced soluble P by 4.9-, 3.8-, 3.5-, and 3.6-fold, and exchangeable P by 47.4%, 82.6%, 80.5%, and 68.3%, respectively, at the tillering stage. However, an opposite varying trend was observed at the ripening stage; the addition of CV, MS, CVHCA260, and MSHCA260 increased soil labile P pool (sum of soluble and exchangeable P fraction) by 1.7-, 1.6-, 1.8-, and 2.1-fold compared to the control, respectively (**Fig. S5**). Compared to CV and MS, MSHCA260 treatment resulted in a higher labile P pool at the ripening stage, suggesting that addition of CVHCA260 could maintain a higher level of soil available P for a longer period.

Fe/Al-bound P is defined as the moderate P pool because the P bound to Fe and Al (hydr)oxides is not directly absorbed by plants but gradually becomes soluble. The significantly higher Fe/Al-bound P concentrations were detected at the tillering stage in the soils treated with CV, MS, CVHCA260, and MSHCA260, whereas the opposite trend was detected at the ripening stage (**Fig. 4C**). In contrast with these treatments, the Fe/Al-bound P in the control became higher as plants matured, suggesting that, at an early growth stage of wheat, the readily available P from chemical P fertilizer became gradually bound to Fe and Al (hydr)oxides. Despite the highest Fe/Al-bound P concentration (**Fig. 2**) among all hydrochars, the lowest soil Fe/Al-bound P fraction was detected in the MSHCA260 treatment at the ripening stage (**Fig. 4C**).

The sums of Ca-bound P and residual P fractions were defined as the stable P pool, because Ca-bound P usually corresponds to apatite and residual P corresponds to recalcitrant P-containing clay mineral (Hedley et al., 1982). Unlike the labile and moderately available P pool, soil Ca-bound and residual P pool kept relatively stable after the addition of CV, MS, CVHCA260, or MSHCA260 (**Fig. 4D and 4E**). No significant difference was detected for soil Ca-bound P or residual P fraction among treatments except at the ripening stage, where addition of MSHCA260 significantly reduced the soil Ca-bound P fraction compared to the control.

3.8. PUE and yield of wheat

The results of PUE and yields of wheat grain are shown in **Fig. 5**. CV, CVHCA260, and MSHCA260 treatment significantly improved the plant PUE by 32.4%, 35.3%, and 34.4% compared to the control (**Fig. 5A**). Compared to the raw CV, amendment by microalgae-derived hydrochars did not significantly improved the PUE. However, among four treatments only MSHCA260 significantly improved the wheat grain yield by 21.6% (**Fig. 5B**). In addition, despite statistically insignificant difference, CVHCA260 treatment improved the grain yield by 14.5% compared to the control.

4. Discussions

4.1. Recovery of P from wastewater and conversion to microalgae-derived hydrochars

With the development of “Enhanced biological P removal”, microalgae-based techniques are attracting increased attention because of the luxury uptake of P by microalgae, accumulating P up to 2-4% of their cell dry weight (Cabanelas et al., 2013; Santos and Pires, 2018; Luo et al., 2019). The cost of wastewater treatment must be counterbalanced with efficacy of P removal and production of microalgal biomass, which further produces a significant economic benefit to society (Prasad et al., 2014; Solovchenko et al., 2019). **Table S4** compared the P removal efficiencies obtained in this study with the results in previous studies. This comparison showed that with the increased initial P concentration in influent wastewater the P removal efficiency became higher. In the present study, MS and CV removed the P from wastewater (with initial P concentration of 41.3 mg L^{-1}) at 2.95 and 2.32 mg L^{-1} day^{-1}; in previous studies 8.39 mg L^{-1} day^{-1} P removal rate was observed from the wastewater at initial P concentration of 128.2 mg L^{-1} (Luo et al., 2019) and 0.55 mg L^{-1} day^{-1} P removal rate from the wastewater at initial P concentration of 8.0 mg L^{-1} (Tao et al., 2017). These results was likely attributed to stimulated biosynthesis and storage of polyphosphate to cope with the external stress of excessive P concentration (Mujtaba et al., 2017; Shen et al., 2017; Powell et al., 2009; Solovchenko et al., 2016, 2019). Given that in this study P concentration in the wastewater kept declining as the microalgae was cultured under steady state, when treating wastewater by using microalgae in the real wastewater treatment facilities the continuous influent of external P source might be helpful for P luxury uptake by microalgae.

Polyphosphate is the dominant P speciation in microalgae (Powell et al., 2009; Solovchenko et al., 2019) and generally recalcitrant to degradation, making it largely unavailable for plants. HTC has been demonstrated to be able to promote degradation by polyphosphate hydrolysis in feedwater. Hence, in the present study the enriched P in microalgae was transferred to hydrochars by HTC. The P concentrations in microalgae-derived hydrochars ranged from 3.2-6.2% (**Table 2**), which were notably higher than hydrochars derived from animal manure and crop residuals (<3%) (Heilmann et al., 2014; Wang et al., 2017; Fei et al., 2019). This result demonstrated that luxury P uptake by microalgae from wastewater played an important role in producing the P-rich hydrochars. In addition, with increasing hydrothermal temperature from 200 to 260°C, the hydrochar yields were observed to decrease but the P recovery rate increased, irrespective of feedwater or microalgal strain (**Table 2**). A similar trend has been reported in the HTC treatment of swine manure (Heilmann et al., 2014), wetland plants (Cui et al., 2020), and sewage sludge (Huang and Tang, 2016). The cracking of biopolymers and P precipitation during HTC might be responsible for the higher P accumulation in higher temperature-derived hydrochars (Dai et al., 2015; Ekpo et al., 2016). The composition of feedwater was demonstrated to be an important factor for hydrochar yield and P recovery rate. Using 1% citric acid solution as feedwater significantly improved these parameters, irrespective of reaction temperature or microalgae strain. Using citric acid solution as feedwater likely promoted the bonding between metal cations and phosphate. Use of acidic feedwater has previously been demonstrated to promote the release of metal cations in hydrochars (Idowu et al., 2017; Yuan et al., 2018; Cui et al., 2020) and in the transformation of organic P to inorganic P (Heilmann et al., 2014; Wang et al., 2017).

Differing reaction temperatures and feedwater composition in the HTC process affected the P fractionation in the resulting hydrochars (**Fig. 2**). The charring of microalgae significantly reduced the soluble, exchangeable, and residual P fractions, but increased the Fe/Al-bound and Ca-bound P fractions. Similar results have been reported where 44.3% readily available P was detected in the raw sewage sludge but only 7.5% was detected in the derived hydrochars (Fei et al., 2019). Spectroscopic methods have demonstrated inorganic orthophosphate to be the primary P speciation in sewage sludge- and animal manure-derived hydrochars using (Heilmann et al., 2014; Huang and Tang, 2015, 2016). During HTC some orthophosphate dissolved into the feedwater and was lost, whereas most other P species bonded and adsorbed with various metals to increase retention on the hydrochars (Zhang et al., 2016). A larger proportion of microalgal P was chemisorbed by Fe/Al (hydr)oxides compared to that by Ca-containing compounds, which is comparable with hydrochars derived from sewage sludge (Huang and Tang, 2016; Fei et al., 2019), but extremely different from animal manure and other plant biomass where Ca-bound P is dominant (Heilmann et al., 2014; Dai et al., 2015; Bornø et al., 2018; Cui et al., 2020). Because PAC was used as a reagent to flocculate and collect the microalgae after culturing in wastewater (**Fig S1**), a larger amount of $AlCl_3$ was possibly remained in the microalgae and promoted the formation of the Al-bound P fractions, as reflected in the Al concentration of hydrochars (0.9-2.8%). Notably, using 1% citric acid solution as feedwater significantly increased the Fe/Al-bound P fraction in these hydrochars, irrespective of microalgal strains or reaction temperature, achieving 46.2% and 51.5% in CVHCA260 and MSHCA260. Importantly, these Fe/Al-bound P fractions can be desorbed in soil and slowly released as phytoavailable species (Yao et al., 2013; Heilmann et al., 2014; Fei et al., 2019), avoiding the P leaching or runoff due to overly fast dissolution, which occurs with chemical P fertilizer (Koppelaar and Weikard, 2013; Sha et al., 2018; Liu et al., 2020). The Fe/Al-bound P fractions are considered as moderately available and act as a buffer for available P in soils (Wang et al., 2014; Zhang et al., 2016; Cui et al., 2020). The loss of a readily-available P pool during the HTC was more than made up for by a much larger increase in Fe/Al-bound P that eventually can be slowly released into the soil to support plant growth.

4.2. Application of microalgae-derived hydrochars to a crop-soil system as slow-release P fertilizer

Soil incubation and wheat pot experiments both revealed that the microalgae-derived hydrochars supplied the soil with a pool of slowly-releasable P, and consequently, the plants with more sustainable P nutrition than did traditional chemical P fertilizer. In the soil incubation experiment, amendment with CVHCA260 and MSHCA260 persistently improved the soil available P from 50 to 120 days (**Fig 3**). In the wheat pot experiment, CVHCA260 and MSHCA260 amendment significantly improved the soil soluble and exchangeable P fractions (**Fig. 4A and 4B**). The abundant Fe/Al-bound P pool in hydrochars could be an important reason for the observed slow-release of P. Sewage sludge-derived hydrochars have been demonstrated to transform the available P fraction from raw sludge to an Fe/Al-bound P fraction after HTC, possessing a strong capacity to release P in electrolyte solution (Huang and Tang, 2016; Fei et al., 2019). Similar results were also reported in the persistent increase in the soil labile P pool, following amendment with crop residue-derived biochars (Xu et al., 2016; Bornø et al., 2018). These results suggest that chemical P fertilizer was beneficial for increasing soil P availability at an early stage of plant growth whereas microalgae and microalgae-derived hydrochars were able to supply P to plants over a longer term. However, prior to utilization by plants the initial pulse of fast-release P from a chemical P fertilizer could possibly be lost by leaching, runoff, and assimilation by soil organisms, as reflected by the significantly higher soil MBP in the control (**Fig. S4**).

As the wheat grew, it is possible that the pool of moderately available P treated with microalgae-derived hydrochars gradually transformed to the labile P pool. This effect is similar with the application of organic and slow-release fertilizer (Chu et al., 2016b; Václavková et al., 2018). Root activity, leading to the exuding of organic acids into soil, and phosphatase excreted by soil microorganisms might have driven such transformation (Shen et al., 2018). In the present study, the amendment of CVHCA260 and MSHCA260 greatly improved the alkaline phosphatase activity in the rhizosphere both at tillering and ripening stages (**Fig. S3**). HTC promoted the hydrolysis of macromolecules from microalgae cells, such as polyphosphate and proteins, to produce a large amount of low weight molecules in the resulting hydrochars (Bornø et al., 2018; Yu et al., 2019; Chu et al., 2020b). It is speculated that these low weight molecules were readily assimilated by soil microorganisms and thus increased microbial activity or, possibly caused a shifted in the composition of the microbial community, resulting in increased levels of alkaline phosphatase, concomitantly increasing soil available P. Moreover, the charring process significantly improved the SSA and porosity (**Table 1**). Higher SSA and increased porous volume levels are extremely important for improving nutrient retention in soil because they can facilitate higher mass transfer fluxes and adsorption loading of soil nutrients (Bornø et al., 2018; Chu et al., 2020a; Lu et al., 2020), which might be another reason for the maintaining higher soil available P over a long term. Previous studies reported that the addition of bentonite hydrochar composites to soil, which have generally possessed SSA and porous volume than hydrochars, significantly improved soil N retention and plant N use efficiency (Chu et al., 2020a).

With the persistently improved soil available P pool in the rhizosphere, microalgae-derived hydrochar treatments significantly improved plant PUE (**Fig. 5A**). Notably, however, compared to CV, CVHCA260 and MSHCA260 were observed not to significantly improve plant PUE. A possible reason might be that improved P availability due to hydrochar addition exceeded the demand of plant growth. In the soil incubation experiment, the soil labile P pool of soils treated with hydrochars were still far higher than those of most arable soils (< 20 mg kg^{-1}) and even in excess of the recommended P application rate (40-50 mg kg^{-1}) in agricultural fields (Sha et al., 2018; Václavková et al., 2018; H. Li et al., 2020). Therefore, in future studies, a reduced application rate of hydrochars will be attempted. In addition, despite all four treatments increasing PUE, only MSHCA260 significantly increased the yield of wheat grain (**Fig. 5B**), perhaps because as well as improved soil P availability, increases soil C/N, SOM, CEC could also be contributing (**Table S6**). These factors are beneficial for nutrient mineralization and retention in soil and root morphology (Shen et al., 2011; Xu et al., 2014; Lu et al., 2020), which might also help promote wheat yield production. In addition, a slightly alkaline soil (pH 7.7) was used in the present study and the CHVCA260 and MSHCA260 addition lowered the soil pH to 6.4 and 6.6, respectively (**Table S6**). The lowering soil pH was beneficial for dissolving Ca-bound P and facilitating hydrochars to provide more adsorption sites between Fe/Al and P compounds. In the present study only deionized water and 1% citric acid were used as feedwater. In case the feedwater with higher pH is attempted to produce hydrochars, e.g., 2-5% citric acids, would be acidic and thus possibly aggravate the soil acidification. Although lowering soil pH might be helpful for the increase of Fe/Al-bound P pool, the soil acidification is also harmful for root respiration and growth, as shown in the previous study that poplar sawdust-hydrochar with pH of 3.7 inhibited rice growth and yield greatly (Yu et al., 2019). Further studies could carry on to investigate the effects of different feedwater pH on P recovery in hydrochars.

In recent years the synergistic effects of hydrochar application together with chemical fertilizer on crop yield have been widely reported (Bornø et al., 2018; Yu et al., 2019; Chu et al., 2020a, 2020c). However, importantly, this study for the first time demonstrated a substitutive role of hydrochars over chemical P fertilizer to improve crop production.

5. Conclusions

The microalgae, CV and MS, both showed a strong ability for the removal of P from P-rich wastewater, and MS was superior to CV in this respect. After 14 days culture in wastewater, MS removed 88.4% P from wastewater at 2.65 mg L^{-1} day^{-1}. Then 91.5% P were recovered from the raw MS to the hydrochar MSHCA260. The P-enriched microalgae-derived hydrochars behaved as a slow-release P fertilizer to satisfy the long-term demand of growing wheat. MSHCA260 amendment improved the plant PUE by 34.4% and yield production by 21.6%. The findings from this study can be used to develop sustainable and eco-friendly strategies to recycle P from wastewater to agricultural fields for food production, which has the positive dual effects of saving the cost of wastewater treatment and the production of a valuable slow-release P fertilizer to alleviate the possible future shortage of phosphate rock. A limitation of the present study is that the microalgal culture was conducted at lab-controlled conditions and steady state. The large-scale outdoor experiments are worthy of conducting to investigate the P removal rate and biomass production of microalgae with continuous influent P source and actual light intensity, temperature and air flow. Also, the microalgae-derived hydrochars did not improve significantly the PUE compared to the raw microalgae. Thus, further work is required to carry out before the large-scale outdoor trials. Further modifications such as loading metal cations to ameliorate the P chemisorption can be attempted in addition to using citric acid solution as HTC feedwater.

Conflicts of interests

The authors declare no competing financial interest

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Author contributions

QC designed the experiments; GP and LX acquired the funding and supervised the research; QC, TL, BY, and MC performed the experiments; QC analyzed the data; YF and LY visualized the work; QC wrote the manuscript; TL, RM, LY, and MC reviewed and edited the manuscript; QC, GP, and LX finalized the manuscript.

Supplementary Information

Detailed information about the materials and methods for microalgae flocculation, chemical properties of wastewater, mass content and relative abundance of different P fractions in raw microalgae and microalgae-derived hydrochars, the properties of rhizosphere soils amended with microalgae or microalgae-derived hydrochars at ripening stage of wheat, flocculation efficiency and zeta potential for flocculating microalgae, overview of the sequential P fractionation procedure performed on hydrochar and soil samples, acid and alkaline phosphatase activity in the rhizosphere soil, microbial biomass C and P in the rhizosphere soil, labile and stable P pool in the rhizosphere soil, are presented in the **Supplementary Information**.

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