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Algal Remediation of Wastewater Produced from Hydrothermally Treated Septage

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Abstract: Hydrothermal carbonization (HTC) is a promising technology to convert wet wastes like septic tank wastes, or septage, to valuable platform chemical, fuels, and materials. However, the byproduct of HTC, process liquid, often contains large amount of nitrogen species (up to 2 g/L of nitrogen), phosphorus, and a variety of organic carbon containing compounds. Therefore, the HTC process liquid is not often treated at wastewater treatment plant. In this study, HTC process liquid was treated with algae as an alternative to commercial wastewater treatment. The HTC process liquid was first diluted and then used to grow *Chlorella* sp. over a short period of time (15 days). It was found that the algae biomass concentration increased by 644 mg/L over the course of 10 days, and which subsequently removed a majority of the nutrients in the HTC process liquid. Around 600 mg/L of algal biomass was collected in the process liquid at the end of treatment (day 15). Meanwhile, chemical oxygen demand (COD), total phosphorous, total Kheldjal nitrogen, and ammonia were reduced by 70.0, 77.7, 82.2, and 99.0% by fifteen days compared to the untreated wastewater, respectively. This study demonstrates that HTC process liquid can be treated by growing algae creating a potential replacement for expensive synthetic nutrient feeds for algal production.

Keywords: hydrothermal treatment; *Chlorella* sp.; wastewater remediation; septage; algae; nitrogen removal

1. Introduction

Hydrothermal carbonization (HTC) is a wet treatment process conducted at 160–260 °C and at autogenous pressures. It is commonly used to produce high value solid products (e.g., fuels, fertilizers, materials, etc.) from wet wastes such as lignocellulosic biomass, human and animal wastes, etc. [1–3]. HTC is an attractive choice for these wet wastes as it does not require cost intensive drying prior to the treatment unlike pyrolysis, gasification, and combustion. Our recent research on HTC of septic tank wastes known as septage, as well as other's studies on sewage wastes and manures, have shown concentration of phosphorus in hydrochar (carbon rich solids that are produced during HTC of biomass) solids and control of the nitrogen distribution (as protein fragments, nitrates, and ammonia), which are favorable for hydrochar as solid fertilizer [4–6]. However, one detrimental result of this HTC processing is a nutrient laden process liquid that must be remediated.

Previous research has investigated using anaerobic digestion (AD) or wet air oxidation (WAO) of wastes to remediate the nutrients (or, pollutants) from the wastewater [7,8]. AD has been shown to

reduce the phosphorus contained in hydrothermal wastes by almost 50%, but requires a treatment time of up to 12 weeks [9]. WAO can reduce the amount of oxidizable carbon in the liquid but does not adequately reduce the amount of ammonia in the liquid [8]. Using existing wastewater treatment options to handle septic tank wastes would be expensive (with disposal costs potentially exceeding $1000 USD/dry ton) and counterproductive to the environmentally beneficial use of wastes. Therefore, this work was undertaken to investigate alternative options for hydrothermal process liquid remediation.

One possible solution to the process water remediation problem is the integration of an algal growth process using the liquid from the hydrothermal treatment. Decades of previous research have been completed on microalgae scale-up and industrial applications. However, the field still requires key breakthroughs to achieve economical application. Generally, the greatest challenge facing industrial scale use is the successful combination of key principles of engineering, biology and biochemistry [10]. Among these, the supply and management of nutrients in the growth media is a key issue. As microalgae metabolize nitrogen, they favor the uptake of the most reduced form, ammonium [11–13]. In practical applications, this leads to an acidification of the medium and culture crash. Balancing nitrogen species and supplying phosphorous is not only a biological challenge, it is very costly.

Economic barriers must be overcome in order to implement industrial microalgae systems. The use of synthetic nutrients makes microalgae aquaculture too expensive for large-scale viability. Previous research efforts unique to hydrothermal processes has included the recycling of algal hydrothermal wastes into algae feed streams, however this still requires a feed stream of nutrients to sustain growth [14]. The expense of nutrient feedstocks has motivated researchers to explore the integration of cheaper nutrient streams such as from wastewater [15]. Using wastewater as a nutrient source for micro algal cultivation decreased the overall algal production cost by 30% and by 44% when algae was cultured in raceway ponds and thin layer cascade reactors respectively [16]. However, utilizing wastewater as a microalgae nutrient stream has its own drawbacks—one such challenge in nutrient purity and control [11,17].

The hydrothermal processing of septage wastes has been shown to produce process liquid with high amounts of nitrate and ammonia (nearly 2 g Nitrogen/L), with the ratio of the two being controllable through treatment temperature [4]. Additionally, this process liquid has other nutrients that are attractive for algal growth, such as phosphorous and multiple micronutrients [4]. This study looked at utilizing algae to remediate process liquid from the hydrothermal treatment of septage, and to evaluate the process liquid as an alternative to synthetic algal growth media.

2. Materials and Methods

2.1. Septage Feedstock Preparation

Septage feedstocks were sampled from a local septic tank in Athens, OH. These samples were then treated hydrothermally at temperatures ranging from 180–220 °C and for residence times of 30–120 min. Complete details on sample characterization and treatment methodology can be found elsewhere [4]. The samples chosen for remediation via algal treatment for this specific study, after dilution by a factor of ~10 with deionized (DI) water, had approximate values of total nitrogen (TN) content of 48 ± 1 mg/L, total phosphorus (TP) content of 1.1 ± 0.1 mg/L, and COD of 720 ± 10 mg O_2/L.

2.2. Algae Sepcies Identification

The culture used was identified using DNA sequence data of the internal transcribed spacers (ITS 1 & 2) of the ribosomal operon. A portion of the culture was placed in a 1.5 µL tube and centrifuged to remove water. Cells were disrupted using a Tissuelyser LT (Qiagen Inc., Valencia, CA, USA). DNA was extracted using NucleoSpin Plant II DNA kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's protocol. A polymerase chain reaction (PCR) was conducted using

an Applied Biosystems 2720 Thermocycler (Applied Biosystems, Foster City, CA) and IllustraTM PuReTaq Ready to Go PCR Beads (GE Healthcare, Piscataway, New Jersey, USA) with 19 µL dH2O, 2 µL of each primer and 2 µL template DNA. Primers (360FE 5′-CGGAGARGGMGCMTGAGA-3′ and 26R-1 5′-GTTAGTTTCTTTTCCTCCTCCGC-3′) were used to amplify a region of the ribosomal operon 18S rDNA-ITS1-5.8S rDNA- ITS2-26S rDNA. The thermocycler conditions were as follows: An initial denaturing at 95 °C for 1:00, followed by 35 cycles of 93 °C for 0:30, 50 °C for 0:30, and 72 °C for 1:00. The PCR products were purified using the UltraCleanTM PCR Clean-up DNA purification kit (Mo Bio, Carlsbad, CA, USA) and sequenced with the 26R primer at the Ohio University Genomics Facility. DNA fragment was visualized with SequencherTM version 5.2.4 (GeneCodes Corp, Ann Arbor, MI, USA) and the final sequence was submitted to GenBank (MN082368). The region was BLAST™ searched on GenBank and the closest matches were KJ676124 and KJ676125 with 98.06% identity. These sequences were from two UTEX cultures (UTEX2168 and UTEX2248) of *Chlorella* sp. Although UTEX2168 has the designation of Scenedesmus sp. on GenBank, the UTEX website confirmed it as *Chlorella* sp. [18]. Therefore, the culture was designated *Chlorella* sp.

2.3. Algae Growth and Sampling

Microalgae *Chlorella* sp. was obtained from a bioreactor at the Institute for Sustainable Energy and Environment (ISEE) at Ohio University. To introduce the algae to the HTC septage liquid, the treated septage media was diluted to a uniform concentration of nutrients, with exact values being shown in Table 1. The algae were sampled from the bioreactor and washed with DI water. This washing was done to prevent the nutrients in the bioreactor from influencing the experimental set up. DI water was then used to remove the algae from the filter. A 40 mL solution of washed algae was transferred to a 1 L flask which contained 400 mL of the treated septage media. The flask was put on a shaker and the speed was adjusted to 160 rpm. Blue light was used as a light source throughout the experiment, as it promotes growth better than red or green light, with a 14:10 light: Dark cycle and a photosynthetic active radiation (PAR) of 108.422 µmol/m^2/s measured by a Stellar Net IR Black-Comet spectrometer [19]. Compressed air was bubbled into the media at 3 psig (10mL/sec at 20–25 °C). The biomass concentration was estimated by measuring optical density at 750 nm as stated by Melinda J. Griffiths et al. using a HACH DR 6000 spectrophotometer [20]. A correlation was established between biomass concentration as total suspended solids (TSS) and the optical density at 750 nm (OD_{750}) as shown in Equation (1). The coefficient of 1482.3 is the slope of biomass compared to absorbance at λ(nm) = 750. The total suspended solids (TSS) were measured according to APHA Standard Methods for the Examination of Water and Wastewater (method 2540 D) [21].

$$biomass\ concentration\left(\frac{mg}{l}\right) = 1482.3 \times OD_{750} \quad (1)$$

Table 1. Change in Nutrient Concentration During Algal Remediation; Nutrient removal occurs mainly during the first few days, with growth occurring after day 5. '-' denotes below detection limit.

Day	Biomass (mg/L)	Total N (mg/L)	TKN (mg/L)	NO_3-N (mg/L)	NH_3-N (mg/L)	COD (mg/L)	Total P (mg/L)
0	-	48.0 ± 0.3	38.7 ± 1.7	9.3 ± 1.3	14.5 ± 3.2	716 ± 4	1.05 ± 0.13
5	195.7	16.8 ± 2.1	11.6 ± 1.9	5.2 ± 0.2	0.23 ± 0.03	320 ± 20	0.31 ± 0.02
10	644.3	11.8 ± 2.7	9.5 ± 2.6	1.7 ± 0.2	0.24 ± 0.11	340 ± 60	0.18 ± 0.08
15	602.8	9.1 ± 2.5	6.9 ± 2.4	1.2 ± 0.1	0.13 ± 0.03	230 ± 80	0.24 ± 0.17

2.4. Raw and Remediated Process Water and Algal Sample Characterization

A Hach DR6000 UV/VIS spectrometer was used for the determination of chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), total nitrogen, nitrate, and ammonia in liquid samples. Test kits 827, 830, 835, 844 and 823, all of the Hach-TNT product line, were used to measure total

nitrogen, ammonia, nitrate, phosphorous, and COD, respectively. All samples were filtered through a 0.2 micron filter before analysis. An inductively coupled plasma-optical emission spectroscopy (ICP-OES from Thermo Scientific iCAP 6000 series) was used to determine phosphorous concentration. Ultimate analysis of the solid products was performed using a Thermo Scientific CHNS (Flash-2000 model) with BBOT (2,2′-(2,5-Thienediyl)bis [5-(2-methyl-2-propanyl)-1,3-benzoxazole]) standards. Additionally, control experiments were conducted to ensure that any change in concentrations were not a result of evaporation. This was done by completing the full experimental procedure without adding algae to the initial septage wastewater sample. It was confirmed that all changes in concentrations were due to the algae growth, and not the bubbling of the air through the process water.

3. Results and Discussion

Chlorella sp. was selected for treatment of the process water samples due to its ability to quickly use the nutrients found in the hydrothermal process liquid [14]. The algae were allowed to grow for 15 days in the HTC process liquid, just beyond the point where the growth peaks and begins to decline. This duration ensures that the algae have fully utilized the available nutrients. A shorter duration would result in unutilized nutrients, while a longer duration would cause death of the algae and this would increase the COD of the wastewater. Note that the initial HTC liquid samples were diluted to avoid limiting the growth of the algae due to the high levels of salts found in the original sample [4]. Figure 1 shows the growth curve of the algae trials (on the primary axis) and the normalized values ($Normalized\ concentraiton = \frac{concentration}{initial\ concentration}$) for COD, total nitrogen, TKN, ammonia, nitrate, organic nitrogen and phosphorus content on the secondary axis. Table 1 shows these values as well in mg/L.

As seen in Figure 1, after the addition of the algae sample to the process liquid, there is a short period of acclimatization with no significant growth, but there is significant nutrient uptake. This delay of growth during nutrient uptake has been observed by Xin et al. as well during the removal of nitrate and phosphate from model wastewater using other micro algae (Scenedesmus sp. LX1) [22]. This could be due to the algae focusing on nutrient uptake compared to growth processes. There is very little additional growth after day 10, with biomass concentrations peaking around day 12 and 13 at 670 mg/L. The slight decrease that occurs on day 11 may be due to the metabolic processes of the algae shifting to accommodate the decreased nutrients. The biomass concentration begins to decline after day 13, suggesting inadequate nutrients to sustain the algae. These biomass yields are about half the maximum observed for *Chlorella* species in other wastewaters which had 3–5 times as much total nitrogen and 100 times as much phosphorous with similar removal efficiencies of 80% [23–25]. Though the biomass concentration is lower, the nutrient utilization (biomass grown/mass of nutrients consumed) is much higher. This could be due to the algae in nutrient-rich environment prioritizing growth of algae over nutrient uptake, while algae in nutrient deficient environments prioritize nutrient uptake before the growth of algae.

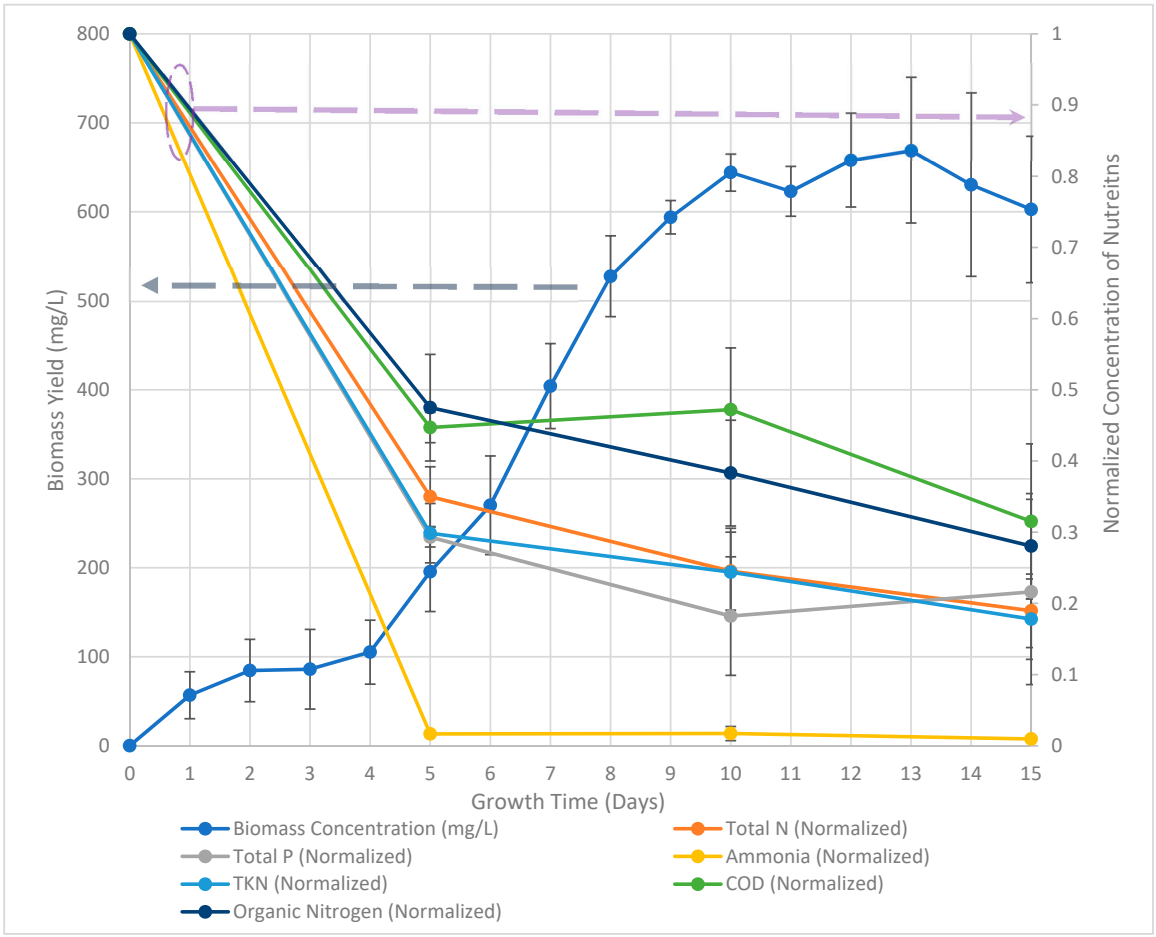

Figure 1. Algae Growth and Water Remediation; growth is shown to be slowed during the acclimatization period (days 0–5), while nutrient uptake occurs primarily during these days.

The corresponding normalized concentrations curves show that during the acclimatization period the algae take in a large amount of the available nutrients. By the time the algae concentration has reached 200 mg/L (day 5 for all trials), 80% of the total consumed nutrients (at day 15) have already been removed from the process liquid. This corresponds to a 50% reduction in all listed concentrations. This rate of remediation is greater for ammonia. By day 5 over 98% of the available ammonia has been consumed, with only 0.23 mg/L of nitrogen as ammonia remaining. This preferential uptake of ammonia compared to nitrate consumption was previously detailed by Kim et al. [26].

The *Chlorella* sp. reduced the total nitrogen content in the HTC liquid from 48.0 ± 0.3 to 11.8 ± 2.7 mg/L by day 10. The overall removal of total nitrogen is higher in this HTC process liquid compare to municipal wastewater for the same *Chlorella* sp. [23,27]. Ammonia and other TKN's are consumed first by the algae, with concentrations falling from 14.5 ± 3.2 and 38.7 ± 1.7 to 0.23 ± 0.03 and 11.6 ± 1.9 mg-N/L, respectively, by day 5. Nitrate is consumed secondarily, with an initial drop from 9.3 ± 1.3 to 5.2 ± 0.2 and then 1.7 ± 0.2 mg-N/L at days 0, 5 and 10, respectively. In regards to the application of this remediation methodology to other hydrothermal process liquid, those containing nitrates will take longer compared to those containing primarily TKN's.

The COD profile exhibited a slight increase from day 5 to 10, followed by another decline at day 15, from 320 ± 20 to 340 ± 60 and finally to 230 ± 80 mg O_2/L, respectively. One possible explanation for this increase is the metabolic processes of the algae reducing CO_2 as they progress through the available nutrients and grow [26]. The decline in COD on day 15 may also be due to the slight decline of the biomass concentrations shown in Figure 1. Algae death and metabolic changes can both excrete additional organic compounds into the solution, resulting in an increase in COD.

Li et al. [24] proposed that resource competition with bacteria may result in such observations as well. Though the hydrothermal processing itself would be expected to kill all bacteria found in the septage, the experimental methodology was not completed in such a way that would totally prevent possible contamination by external bacteria not present in the initial algae sample [28]. This may have resulted in increased competition from bacteria for resources, causing the lower growth compared to other studies of *Chlorella* [23,24]. Overall COD removal observed was lower than that observed in other wastewaters treated with *Chlorella* sp., possibly due to the high organic carbon content present in hydrothermal process water [1]. This level of COD reduction was also lower than other treatment options such as aerobic membrane bioreactors [29].

In additional to the liquid sample characterizations, the solid analyses of the dried algae were performed to show how the organic and nutrient matters from HTC process liquids are being utilized. The harvested algae sample had a solid nitrogen content of 2.07 ± 0.27 and carbon content of 39.3 ± 0.8 mass% (dry basis). This carbon removal only accounts for 10% of the reduction in COD. The carbon content is directly comparable to other *Chlorella* species grown under similar conditions. However, the nitrogen content is far lower than those grown under excess nutrients (which can exceed 5% nitrogen on a dry basis), which is expected of this feedstock, as the nutrient concentrations were significantly lowered throughout the experimental process [22,30]. This indicates that while nitrogen (as either nitrate or ammonia) may be the limiting nutrient, it is not so sparse as to completely halt growth. The initial uptake of all nitrogen species observed in the first few days may also be a factor in limiting the accessibility of nitrogen to n^{th} generation algae.

4. Conclusions

This study has demonstrated that algal remediation of hydrothermal process water is effective at reducing the nutrient content of the HTC process liquid. When evaluating this method for the remediation of hydrothermal process liquid, 5 days of treatment may be sufficient for drastically reducing the cost of subsequent wastewater treatment if the algae can be removed by either filtration or centrifuge. Future studies may focus on adjusting HTC process conditions to optimize ammonia/TKN content for fast growth, and nitrate for sustained growth. Previous works have already demonstrated that this adjustment can be a simple as changing the treatment temperature from 180 to 260 °C [4]. Further optimization of both wastewater remediation and algal growth can be investigated by examining acclimatizing algal populations in process water with high ammonia content, and then transferring mature algal populations into process wastes with low ammonia and high nitrate concentrations.

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References

1. Toufiq Reza, M.; Freitas, A.; Yang, X.; Hiibel, S.; Lin, H.; Coronella, C.J. Hydrothermal carbonization (HTC) of cow manure: Carbon and nitrogen distributions in HTC products. *Environ. Prog. Sustain. Energy* **2016**, *35*, 1002–1011. [CrossRef]
2. Zhang, G.; Ma, D.; Peng, C.; Liu, X.; Xu, G. Process characteristics of hydrothermal treatment of antibiotic residue for solid biofuel. *Chem. Eng. J.* **2014**, *252*, 230–238. [CrossRef]

3. Jambaldorj, G.; Takahashi, M.; Yoshikawa, K. Liquid Fertilizer Production from Sewage Sludge by Hydrothermal Treatment. Available online: http://www.esi.nagoya-u.ac.jp/h/isets07/Contents/Proceedings/FinalManuscript/Session05/1121Ganchimeg.pdf (accessed on 19 June 2019).
4. McGaughy, K.; Reza, M.T. Recovery of Macro and Micro-Nutrients by Hydrothermal Carbonization of Septage. *J. Agric. Food Chem.* **2018**, *66*, 1854–1862. [CrossRef] [PubMed]
5. Zhuang, X.; Huang, Y.; Song, Y.; Zhan, H.; Yin, X.; Wu, C. The transformation pathways of nitrogen in sewage sludge during hydrothermal treatment. *Bioresour. Technol.* **2017**, *245*, 463–470. [CrossRef] [PubMed]
6. Ekpo, U.; Ross, A.B.; Camargo-Valero, M.A.; Fletcher, L.A. Influence of pH on hydrothermal treatment of swine manure: Impact on extraction of nitrogen and phosphorus in process water. *Bioresour. Technol.* **2016**, *214*, 637–644. [CrossRef] [PubMed]
7. Wirth, B.; Mumme, J.; Erlach, B. Anaerobic treatment of waste water derived from hydrothermal carbonization. In Proceedings of the 20th European Biomass Conference and Exhibition, Milan, Italy, 18–22 June 2012; pp. 683–692.
8. Baroutian, S.; Smit, A.M.; Andrews, J.; Young, B.; Gapes, D. Hydrothermal degradation of organic matter in municipal sludge using non-catalytic wet oxidation. *Chem. Eng. J.* **2015**, *260*, 846–854. [CrossRef]
9. Li, W.; Guo, J.; Cheng, H.; Wang, W.; Dong, R. Two-phase anaerobic digestion of municipal solid wastes enhanced by hydrothermal pretreatment: viability, performance and microbial community evaluation. *Appl. Energy* **2017**, *189*, 613–622. [CrossRef]
10. Scott, S.A.; Davey, M.P.; Dennis, J.S.; Horst, I.; Howe, C.J.; Lea-Smith, D.J.; Smith, A.G. Biodiesel from algae: challenges and prospects. *Energy Biotechnol.—Environ. Biotechnol.* **2010**, *21*, 277–286. [CrossRef] [PubMed]
11. Wang, J.; Curtis, W.R. Proton stoichiometric imbalance during algae photosynthetic growth on various nitrogen sources: toward metabolic pH control. *J. Appl. Phycol.* **2016**, *28*, 43–52. [CrossRef]
12. Scherholz, M.L.; Curtis, W.R. Achieving pH control in microalgal cultures through fed-batch addition of stoichiometrically-balanced growth media. *Bmc Biotechnol.* **2013**, *13*, 39. [CrossRef]
13. Hellebust, J.A.; Ahmad, I. Regulation of Nitrogen Assimilation in Green Microalgae. *Biol. Oceanogr.* **1989**, *6*, 241–255.
14. Du, Z.; Hu, B.; Shi, A.; Ma, X.; Cheng, Y.; Chen, P.; Liu, Y.; Lin, X.; Ruan, R. Cultivation of a microalga Chlorella vulgaris using recycled aqueous phase nutrients from hydrothermal carbonization process. *Adv. Biol. Waste Treat. Bioconversion Technol.* **2012**, *126*, 354–357. [CrossRef] [PubMed]
15. Abdel-Raouf, N.; Al-Homaidan, A.A.; Ibraheem, I.B.M. Microalgae and wastewater treatment. *Saudi J. Biol. Sci.* **2012**, *19*, 257–275. [CrossRef] [PubMed]
16. Acién Fernández, F.G.; Fernández Sevilla, J.M.; Molina Grima, E. Costs Analysis of Microalgae Production. In *Biofuels from Algae*, 2nd ed.; Pandey, A., Chang, J.-S., Soccol, C.R., Lee, D.-J., Chisti, Y., Eds.; Elsevier: Amsterdam, The Netherlands, 2019; pp. 551–566.
17. Abinandan, S.; Shanthakumar, S. Challenges and opportunities in application of microalgae (Chlorophyta) for wastewater treatment: A review. Renew. *Sustain. Energy Rev.* **2015**, *52*, 123–132. [CrossRef]
18. UTEX. Available online: https://utex.org/pages/search-results?q=2168 (accessed on 18 June 2019).
19. Blair, M.F.; Kokabian, B.; Gude, V.G. Light and Growth Medium Effect on Chlorella Vulgaris Biomass Production. *J. Environ. Chem. Eng.* **2014**, *2*, 665–674. [CrossRef]
20. Griffiths, M.J.; Garcin, C.; van Hille, R.P.; Harrison, S.T.L. Interference by pigment in the estimation of microalgal biomass concentration by optical density. *J. Microbiol. Methods* **2011**, *85*, 119–123. [CrossRef] [PubMed]
21. American Public Health Association. *Standard Methods for the Examination of Water and Wastewater*, 21st ed.; American Public Health Association/American Water Works Association/Water Pollution Control Federation: Washington, DC, USA, 2005; ISBN 978-0-87553-047-5.
22. Xin, L.; Hong-Ying, H.; Ke, G.; Ying-Xue, S. Effects of different nitrogen and phosphorus concentrations on the growth, nutrient uptake, and lipid accumulation of a freshwater microalga Scenedesmus sp. *Bioresour. Technol.* **2010**, *101*, 5494–5500. [CrossRef] [PubMed]
23. Wang, L.; Min, M.; Li, Y.; Chen, P.; Chen, Y.; Liu, Y.; Wang, Y.; Ruan, R. Cultivation of Green Algae Chlorella sp. in Different Wastewaters from Municipal Wastewater Treatment Plant. *Appl. Biochem. Biotechnol.* **2010**, *162*, 1174–1186. [CrossRef]

24. Li, Y.; Chen, Y.-F.; Chen, P.; Min, M.; Zhou, W.; Martinez, B.; Zhu, J.; Ruan, R. Characterization of a microalga Chlorella sp. well adapted to highly concentrated municipal wastewater for nutrient removal and biodiesel production. *Bioresour. Technol.* **2011**, *102*, 5138–5144. [CrossRef]
25. Cabanelas, I.T.D.; Ruiz, J.; Arbib, Z.; Chinalia, F.A.; Garrido-Pérez, C.; Rogalla, F.; Nascimento, I.A.; Perales, J.A. Comparing the use of different domestic wastewaters for coupling microalgal production and nutrient removal. *Bioresour. Technol.* **2013**, *131*, 429–436. [CrossRef]
26. Kim, S.; Lee, Y.; Hwang, S.J. Removal of nitrogen and phosphorus by Chlorella sorokiniana cultured heterotrophically in ammonia and nitrate. *Int. Biodeterior. Biodegrad.* **2013**, *85*, 511–516. [CrossRef]
27. Lau, P.S.; Tam, N.F.Y.; Wong, Y.S. Effect of algal density on nutrient removal from primary settled wastewater. *Environ. Pollut.* **1995**, *89*, 59–66. [CrossRef]
28. Ducey, T.F.; Collins, J.C.; Ro, K.S.; Woodbury, B.L.; Griffin, D.D. Hydrothermal carbonization of livestock mortality for the reduction of pathogens and microbially-derived DNA. *Front. Environ. Sci. Eng.* **2017**, *11*, 9. [CrossRef]
29. Gander, M.; Jefferson, B.; Judd, S. Aerobic MBRs for domestic wastewater treatment: a review with cost considerations. *Sep. Purif. Technol.* **2000**, *18*, 119–130. [CrossRef]
30. Bhola, V.; Desikan, R.; Santosh, S.K.; Subburamu, K.; Sanniyasi, E.; Bux, F. Effects of parameters affecting biomass yield and thermal behaviour of Chlorella vulgaris. *J. Biosci. Bioeng.* **2011**, *111*, 377–382. [CrossRef] [PubMed]