sustainability

MDPI

Article

Treatment and Valorization of Agro-Industrial Anaerobic Digestate Using Activated Carbon Followed by *Spirulina platensis* Cultivation

Ángela Sánchez-Quintero [1], Marie-Ange Leca [1], Simona Bennici [2], Lionel Limousy [2], Florian Monlau [1,3] and Jean-Baptiste Beigbeder [1,*]

[1] APESA, Pôle Valorisation, 64121 Montardon, France
[2] Institut de Science des Matériaux de Mulhouse (IS2M—UMR CNRS UHA 7361), Axe Transports, Réactivité, Matériaux pour des Procédés Propres (TRM2P), Université de Haute Alsace (UHA), 68093 Mulhouse, France
[3] Total Energies, PERL—Pôle D'Etudes et de Recherche de Lacq, Pôle Economique 2, BP 47–RD 817, 64170 Lacq, France
* Correspondence: jeanbaptiste.beigbeder@apesa.fr

Abstract: The increased production of biogas through the anaerobic digestion (AD) process has raised several concerns regarding the management of liquid digestate, which can present some environmental risks if not properly handled. Among the different techniques to treat AD digestate, microalgae and cyanobacteria cultivation has emerged as a sustainable approach to valorizing digestate while producing valuable biomass for production of biofuels and high value bioproducts. However, the intrinsic parameters of the liquid digestate can strongly limit the microalgae or cyanobacteria growth as well as limit the uptake of residual nutrients. In this study, the detoxification potential of activated carbon (AC) was evaluated on agro-industrial liquid digestate prior to *Spirulina platensis* cultivation. Different doses of AC, ranging from 5 to 100 g/L, were tested during adsorption experiments in order to determine the adsorption capacity as well as the removal efficiency of several compounds. Experimental results showed the high reactivity of AC, especially towards phosphate (PO_4-P), total phenol (TP) and chemical oxygen demand (COD). At a dosage of 50 g/L, the AC pretreatment successfully achieved 54.7%, 84.7% and 50.0% COD, TP and PO_4-P removal, corresponding to adsorption capacity of 94.7 mgDCO/g, 17.9 mgTP/g and 8.7 mgPO_4-P/g, respectively. Even if the AC pretreatment did not show significant effects on *Spirulina platensis* growth during toxicity assays, the AC adsorption step strongly participated in the digestate detoxification by removing hardly biodegradable molecules such as phenolic compounds.

Keywords: cyanobacteria; *Spirulina platensis*; activated carbon; detoxification; anaerobic digestate; nutrients; circular economy

check for updates

Citation: Sánchez-Quintero, Á.; Leca, M.-A.; Bennici, S.; Limousy, L.; Monlau, F.; Beigbeder, J.-B. Treatment and Valorization of Agro-Industrial Anaerobic Digestate Using Activated Carbon Followed by *Spirulina platensis* Cultivation. *Sustainability* **2023**, *15*, 4571. https://doi.org/10.3390/su15054571

Academic Editors: Micol Bellucci, Simone Rossi and Francesca Casagli

Received: 21 January 2023
Revised: 24 February 2023
Accepted: 28 February 2023
Published: 3 March 2023

Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Anaerobic digestion (AD) is the biological process where organic matter is converted into biogas, mainly composed of methane and carbon dioxide. This biological process is based on the breakdown of organic materials by microorganisms in the absence of oxygen, under regulated conditions [1]. According to the European Biogas Association (EBA), the overall EU methane production may increase by at least fivefold by 2050 [2]. Along with the biogas, the AD process generates a co-product, referred as digestate, which is usually found as a brown-dark liquid/solid slurry in the case of agricultural digestate. Today, AD digestate is mainly used as biofertilizer for soils in agriculture, as it is a source of a large quantity of nutrients, such as nitrogen (800–1600 mg N/L) [3] and phosphorus (200 mg PO_4^{3-}/L) [4]. Digestate is a simple product to handle and apply, and it may be used successfully as a mineral fertilizer alternative. The organic portion of digestate, on the other hand, can contribute to soil organic matter turnover, altering biological, chemical and

physical soil characteristics as a soil amendment [5]. With the predicted growth tendency of the AD sector, the production of liquid digestate is expected to strongly increase, and other treatment techniques must be developed to valorize this AD by-product.

Traditional processing of digestate requires large amounts of energy and poses environmental dangers such as eutrophication [6]. Aquatic microalgae and cyanobacteria may provide an innovative, interesting and alternative digestate treatment method [7]. These photosynthetic microorganisms are used for their ability to remove heavy metals as well as some toxic organic compounds while producing compounds of interest [8]. The utilization of chemically based nutrients for microalgae growth can account for up to half of the cost of the culture [9]. The combination of on-site liquid digestate treatment with microalgal production has the potential to significantly reduce the costs associated with the preparation of culture media [6]. In photoautotrophic conditions, CO_2 or bicarbonate ions can be used as inorganic carbon sources for microalgal growth [10]. Thus, coupling the AD process with microalgae or cyanobacteria cultivation could contribute to nutrient bioremediation from liquid digestate as well as CO_2 capture from biogas [11].

Among a large number of microalgae and cyanobacteria species, several studies have demonstrated the opportunity of cultivating *Spirulina* using AD digestates as sustainable and low-cost source of nutrients [12–16]. The relatively large size and filamentous structure of *Spirulina* facilitate its harvesting [17], which can considerably reduce the overall biomass production costs [18]. In addition, *Spirulina* contains a large variety of compounds of interests such as proteins, carbohydrates vitamins, minerals and pigments for applications in the food industry [19], cosmetics [20] and the fuel sector [21].

However, several limiting factors such as high concentrations of ammonia and chemical oxygen demand (COD), risk of bacterial contamination and strong turbidity limit the direct utilization of raw AD digestate for algae or cyanobacteria cultivation [22]. Due to its slightly alkaline pH, which favors the free-ammonia form of nitrogen (potentially toxic for microalgae and cyanobacteria development), digestate presents one difficulty for the culture of such microorganisms [23]. Pretreatments such as filtration [24], volatilization [25], absorption [26], autoclaving [27] and dilution [28] have been investigated to reduce the overall toxicity of the AD digestate [6]. Nevertheless, dilution was utilized in the majority of the investigations to diminish the inhibitory effect produced by total ammonia nitrogen, free ammonia nitrogen and turbidity [29].

Furthermore, the diluting procedure uses a significant amount of freshwater, having several negative environmental impacts. Another innovative technique for digestate pretreatment would be the use of different adsorbents such as zeolite [30], clay [31] and biochar [32] preceding a biological treatment such as algal or cyanobacteria cultivation. Activated carbon (AC) can adsorb heavy metals [33] and inorganic impurities [34] along with residual organic matter. As an all-purpose sorbent, AC has been mostly employed as a detoxification agent in the literature [35].

In this context, this study aims at using AC as an alternative and sustainable pretreatment of AD liquid digestate prior to *Spirulina platensis* cultivation. This pre-treatment could contribute to the turbidity reduction along with the detoxification of the digestate, especially for recalcitrant molecules such as phenols, which are not easily degraded by conventional biological process. In the first part of the study, a series of detoxification tests was performed to evaluate the optimal dose of AC (from 5 to 500 g/L) for removing several pollutants such as phenols and COD. Then, cyanobacterium cultivation assays were carried out to compare the growth properties of *Spirulina platensis* in the raw digestate as well as the pre-treated digestate, diluted at three different dilution rates.

2. Materials and Methods

2.1. Anaerobic Digestate

The digestate used in this study was collected at the "XL-Methalandes" territorial biogas plant located in Hagetmau (Nouvelle-Aquitaine, France). The industrial plant is composed of two digesters of 7500 m^3 operating under mesophilic conditions and generat-

ing an average methane production of 562 Nm^3/h. The feedstock is mainly composed of agricultural residues, animal manure and food wastes. The digestate was directly sampled from the digester tank and subsequently filtered at 50 µm to remove large particles. After filtration, it was stored at −20 °C before testing the different adsorbents. The principal characteristics of the liquid digestate are summarized in Table 1 (cf. Section 2.4.3 for the analytical methods).

Table 1. Composition of liquid AD digestate after filtration.

Parameters	Values
pH	8.5 ± 0.0
Conductivity (mS/cm)	22.97 ± 0.6
COD (mg/L)	8650 ± 265
NH_4-N (mg/L)	4405 ± 79
PO_4-P (mg/L)	869 ± 5
Total phenol (mg/L)	1054 ± 43

2.2. Adsorbent Treatment

The activated carbon CYCLECARB® 401 was provided by the company Chemviron (A Kuraray Company, Calgon Carbon Corporation), who carried out the thermal activation of the material at temperatures higher than 800 °C. In this study, the digestate pretreatment experiments were performed by mixing 20 mL of digestate at different concentrations of adsorbent: 5, 10, 25, 50 and 100 g/L. The mixture was kept stirred at 250 rpm at room temperature for 2 h. At the end of the experiment, the biphasic system was centrifuged at 10,000 rpm for 5 min, and the supernatant was filtered on a 1.6 µm filter before being analyzed (cf. Section 2.4: Analytical Procedures).

2.3. Cyanobacterium Cultivation Assays

The Paracas strain of *Spirulina platensis* (*S. platensis*) was purchased from the company Teramer (France). After reception, the strain was cultivated and maintained for several days in a bubble column photobioreactor located in a greenhouse and supplied with BG11 media (diluted twice with demineralized water) together with 8 g/L of $NaHCO_3$. After 45 days of cultivation, the *S. platensis* culture reached an optical density (at 680 nm) of 1.6, before being used as inoculum.

The cyanobacteria cultivation assays were performed in 250 mL tubes using a working volume of 200 mL. The inoculum volume was fixed to 20% of the total working volume. The treated and raw digestates were diluted with demineralized water and the algal inoculum to reach appropriate dilution factors of 20×, 40× and 60×. To evaluate if any nutrients were transferred from the inoculum to the culture media, a negative control was incubated in the same conditions with only demineralized water. A positive control using BG11 culture media was investigated in the same conditions. Artificial light was used to illuminate the tubes at an average intensity of 60 $\mu mol/m^2/s$ using light/dark cycles of 16/8 h. The cultures were kept under agitation by continuously injecting air at a flowrate of 0.5–1 L/min.

2.4. Analytical Procedures

2.4.1. Microalgal Growth

During the cultivation assays, *S. platensis* growth was continuously monitored by measuring the optical density at 680 nm with a UV-visible spectrophotometer (Jenway, UK). The specific growth rate (μ, d^{-1}) was determined using the following equation:

$$\mu = \ln (OD_f/OD_i)/(t_f - t_i) \quad (1)$$

where OD_f and OD_i were measured at the end (t_f) and at the beginning of the exponential phase (t_i), respectively.

The algal dry weight was measured by vacuum filtration of a specific volume of algal culture sample using a pre-weighed glass microfiber filter with a retention size of 0.7 µm (MF300, Fisherbrand, Toronto, ON, Canada). After drying the filter 24 h at 105 °C, the dry weight was finally calculated by weight difference. A calibration curve was prepared by measuring the optical density (OD) at 680 nm as well as the dry weight (DW, g/L) of solutions with different quantities of *S. platensis*. The linear regression showed a coefficient of determination (R^2) of 0.9910 with the following equation:

$$DW = 0.6052 \times OD + 0.0003 \quad (2)$$

The biomass concentration, expressed in g/L, was determined by selecting the final optical density obtained after 10 days of cultivation and converting it into dry weight with Equation (2). The biomass productivity, expressed in mg/L/d, was calculated from the difference between the initial biomass concentration (after inoculation) and the final biomass concentration, divided by the cultivation duration.

2.4.2. Adsorbent Characterization

The AC adsorbent was characterized using different techniques, including elemental analysis by X-ray fluorescence; N_2, CH_4 and CO_2 adsorption by volumetric analysis and pH measurement in aqueous suspension (Table 2).

Table 2. Characteristic of the commercial AC adsorbent used in this study.

Parameters	Values
Physical properties	
pH	10.77 ± 0.03
Particle size (µm)	<300
N_2—specific surface (m^2/g)	894.6 ± 2.0
N_2—total pore volume (cm^3/g)	0.358
CO_2—specific surface (m^2/g)	495.5 ± 8.5
CO_2—micropore volume (cm^3/g)	0.081
Chemical properties (wt. %)	
C	94.490
O	2.587
Mg	0.086
Al	0.882
Si	0.693
P	0.018
S	0.415
Cl	0.046
K	0.052
Ca	0.198
Fe	0.262
Zn	0.002
Sr	0.007
Na	0.197

A wavelength dispersion X-ray fluorescence (WDXRF) spectrometer (Zetium, Panalytical, Almelo, The Netherlands) was used to perform the XRF measurements on pellets made of 0.1 g of the sample and 0.2 g of binder boric acid (H_3BO_3).

Surface area and porosity measurements were carried out using Micromeritics ASAP2420 equipment (Norcross, GA, USA). Around 100–200 mg of sample was degassed under secondary vacuum at 300 °C for 12 h. After that, samples were placed at 100 °C for 2 h directly on the analysis port to remove surface water and eventual residual volatile organic species before being analyzed. Analysis was carried out on CO_2 adsorption at 0 °C (with temperature control being achieved with an ice-water bath) and N_2 at −196 °C (in a liquid nitrogen bath). The apparent specific surface area was calculated by applying the Brunauer, Emmett and Teller (BET) equation. Adsorption tests were carried out up to a relative pressure (P/P°) of

0.03 for CO_2 to examine the ultramicro- and micro-pore region, and up to 1 (P/P°) for nitrogen, to explore the micro- and meso-pore region. The pore size distribution was determined using the density functional theory (DFT) method.

The pH of the activated carbon was measured by suspending 1 g of sample into 10 mL of deionized water. After 1 h of stirring, the agitation was stopped and the solid phase decanted. The pH of the limpid solution was measured using a calomel electrode–glass electrode system.

2.4.3. Digestate Composition

After applying the treatment, the digestate was analyzed to evaluate the adsorption capacity of the AC at various concentrations. pH and conductivity were measured using a pHmeter and a conductivity meter, both from the brand WTW. The color of the solution was quantified by measuring the OD at 450 nm using a microplate reader (Hepoch, BioTek Instruments, USA). Conventional reagent kits from Spectroquant were used to determine the COD (APHA 522 D), phosphate (APHA 4500-P C) and ammonium (APHA 4500-NH3 F) concentrations following standard protocols (APHA, 1995 [36]). Total phenols concentration was evaluated with a slightly modified Folin–Ciocâlteu method, as described previously [37].

2.4.4. Adsorption Performance

The removal efficiency (R) as well as the adsorption capacity (Q) of the AC were calculated based on the following equations:

$$R\ (\%) = (C_i - C_f)/C_i \times 100 \quad (3)$$

where C_i and C_f are the initial and final concentration of pollutants quantified in the solution, respectively.

$$Q\ (mg/g) = (C_i - C_f) \times V/M \quad (4)$$

where V and M are the volume of digestate and the weight of adsorbent used during the detoxification experiment.

2.5. Statistical Analysis

All the detoxification tests as well as the cultivation assays were performed in triplicate, and results were expressed as mean value with the associated standard deviation. A one-way analysis of variance (ANOVA) followed by Tukey's post hoc analysis was conducted to detect statistical differences between the different experimental groups, using a 95% level of confidence. The statistical analysis was performed using the OriginPro 2022 software, version 9.9.0.220.

3. Results and Discussion

3.1. Adsorption Tests with Activated Carbon

Liquid digestate generated from AD processes is a complex by-product with the presence of various recalcitrant compounds, which often inhibit the cyanobacteria proliferation. Therefore, adsorption pre-treatment could be used to reduce the overall digestate toxicity in order to promote cyanobacteria growth and nutrient uptake. However, the selection of appropriate adsorption conditions should be deeply studied to achieve high detoxification responses together with relatively low operating costs.

The different concentrations of AC presented various responses to the overall digestate detoxification performance (Figure 1). In general, the adsorption capacity was relatively high at low adsorbent dosage and decreased while increasing the adsorbent concentration for COD, phosphate and phenol parameters. The opposite behavior was observed in the case of removal efficiency for the same parameters. For instance, COD removal varied from 23.6 to 64.2% while increasing the adsorbent concentration from 5 to 100 g/L (Figure 1A). However, the statistical analysis indicated that increasing the adsorbent concentration from 50 to 100 g/L did not present any significant effect on the COD removal efficiency

(*p*-value > 0.05). The highest phosphate removal of 54.5% and adsorption capacity of 29.6 mg/g were achieved with the use of 100 and 5 g/L of AC, respectively (Figure 1B). As observed for the COD, the phosphate adsorption capacity decreased with the quantity of AC used during the detoxification tests. In addition, the AC was highly reactive with the phenolic compounds present in the digestate. Even at the lowest adsorbent concentration of 5 g/L, 39.6% of the initial phenol concentration was removed after 2 h of reaction, corresponding to an adsorption capacity of 83.5 mg/g (Figure 1C). The phenol removal efficiency was then increased with higher concentration of AC. At 50 g/L of AC, up to 84.7% phenol uptake from the digestate was achieved, which was found to be at the same significance level as the treatment performed at 100 g/L (87.8% of phenols removed).

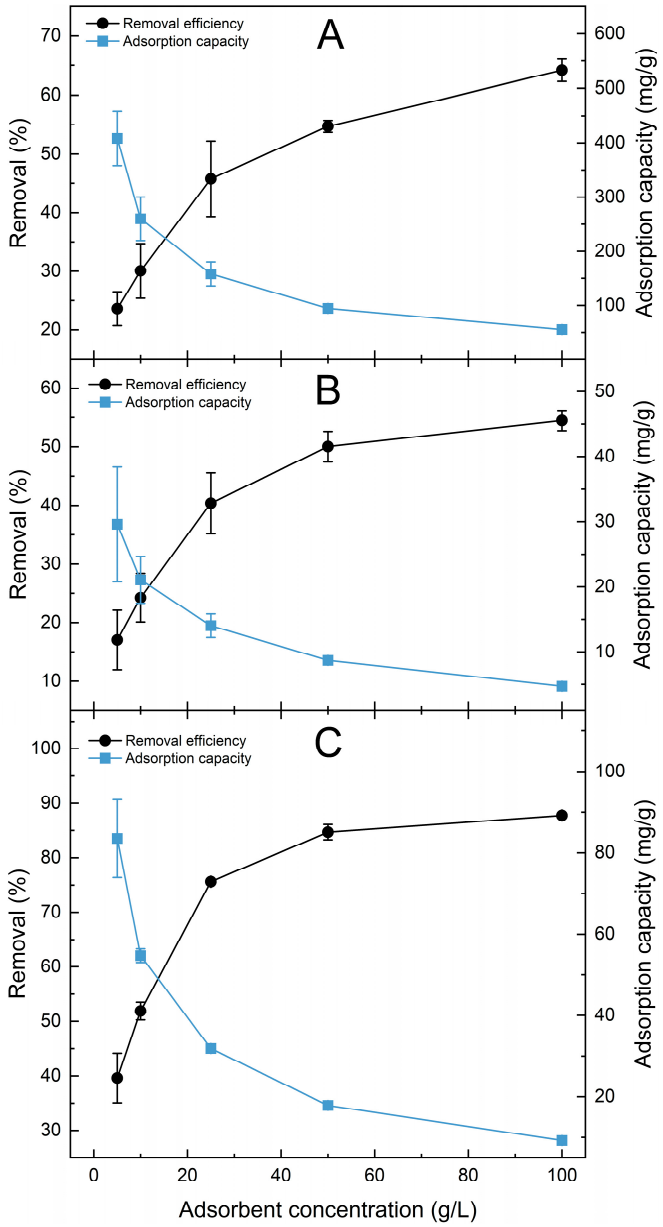

Figure 1. Adsorption capacity and removal efficiency calculated for COD (**A**), PO_4-P (**B**) and total phenol (**C**) parameters when treating the agro-industrial digestate with several concentration of commercial activated carbon.

On the other hand, small variations of pH were observed during the AC treatment (Table S1). Starting from 8.50, the pH of digestate was slightly higher when increasing the

AC dose, with the highest value of 8.67 observed with 100 g/L of AC. Furthermore, the AC did not react with the ammonium present in the AD digestate, with an average removal efficiency of 2.7% and no statistical differences among the different concentrations of AC (Table S2). Finally, the different AC treatments were efficient in removing the color of the liquid digestate from 5.3 to 48.9% of the initial color (Table 3). The maximum OD removal of 48.9% was obtained after 2 h of treatment using 50 g/L of AC. This condition was found to be statistically higher than all the other AC concentrations ($p < 0.05$) and equally effective as compared to 100 g/L of AC ($p > 0.05$).

Table 3. Optical density (OD) variation and reduction efficiency quantified during the digestate pretreatment using various doses of AC. For the OD reduction, different letters indicate statistical differences among different AC concentrations ($p < 0.05$).

AC Concentration (g/L)	OD 450 nm	OD Reduction (%)
0	3.5 ± 0.0	-
5	3.3 ± 0.1	5.3 ± 2.7 ^{c}
10	3.2 ± 0.1	10.2 ± 2.1 ^{c}
25	2.3 ± 0.2	35.3 ± 6.6 ^{b}
50	1.8 ± 0.1	48.9 ± 4.0 ^{a}
100	2.0 ± 0.2	44.4 ± 5.2 ^{ab}

Several studies reported the use of AC treatment to detoxify waste streams polluted with hazardous compounds such as micropollutants [38], phenol [39] and phosphorus [40]. Removing phenolic compounds is a critical detoxification step since such organic molecules can negatively affect microalgae and cyanobacteria metabolism when exposed to a certain concentration. Parsy et al. [41] recently reported the effect of various phenol concentrations on six microalgae strains (*Chlorella* sp., *Dunaliella* sp., *Nannochloropsis* sp., *Tetraselmis* sp., *Picochlorum* sp. and *Coccomyxa* sp.) and one cyanobacterium (*Synechococcus* sp.) when performing microplate toxicity assays. For most of the investigated strains, the half maximal effective concentration (EC_{50}) ranged from 58.1 to 500 mg/L of phenol, except for *D. salina* and *C. simplex*, which had higher resistance. Similarly to our study, Lütke et al. [39] obtained a slightly lower adsorption capacity of 73.95 mg/g when treating a solution with 500 mg/L of phenol. The adsorption was performed at 25 °C and 150 rpm for a contact time of 24 h using 1 g/L of AC produced from black wattle bark residues. Authors highlighted the possibility of reusing the adsorbent after performing a desorption step at 300 °C for 2 h [39]. As suggested by Mattson et al., the phenol adsorption mechanisms on AC are due to the formation of a donor/acceptor complex between the phenols and the carbonyl-based functional groups, where the latter represent an electron-donating group and the phenols represent an electron acceptor [42]. In another study, Marazzi et al. [25] tested different doses of a commercial AC (1 to 40 g/L) to remove the high turbidity of the liquid fraction of digestate. By measuring the optical density at 680 nm, results indicated that an AC dosage of 40 g/L combined with 10 min of reaction generated an OD diminution of 88%. Besides the experimental conditions, such as the pH, the temperature and the coexisting ions in the solution, the phosphate adsorption by carbon materials is generally driven by the surface chemistry of the sorbent, the porosity of the carbon matrix, the presence of specific functional groups, the high surface area and the presence of modification elements. Modifying the commercial AC was demonstrated to largely improve the phosphate adsorption capacity. The AC used in this study contained 0.26 wt. % of iron, which probably contributed to the phosphate ion adsorption due to the creation of efficient adsorption sites [43].

After evaluating the experimental results of the AC detoxification tests, it was concluded that a dosage of 50 g/L was sufficient to achieve an overall pretreatment of the AD digestate prior to the cyanobacteria cultivation step, as compared to lower AC concentrations. In addition, increasing the dosage to 100 g/L did not show significant effects on all

the investigated responses, and its utilization would considerably affect the operating costs of the pretreatment step.

3.2. S. platensis Cultivation and Growth Properties

The large diversity of macro- and micronutrients present in the AD digestate can be favorable to cyanobacteria metabolism. Even if the AC pretreatment considerably reduced several parameters, including COD, turbidity and phenol content, the ammonium concentration of the raw (4405 mg/L) and pretreated digestate (4220 mg/L) were still above the threshold concentration supported by *Spirulina* cyanobacteria (100–150 mg/L) [44–46]. In this context, a series of cyanobacteria toxicity assays were performed using both raw and pretreated digestate diluted at three different dilution rates: 20×, 40× and 60× (Table 4). The undiluted digestates, treated or not with AC, were not tested in this experiment since preliminary cultivation assays showed they were too toxic to *S. platensis* culture (data not shown). Thus, the aim of the experiment was to compare the growth performance between the two types of digestate diluted at different dilution rates as well as two control groups. The OD at 680 nmwas continuously measured over 10 days in order to evaluate the growth profiles of all the conditions.

Table 4. Physical–chemical properties of the raw digestate (RD) and the treated digestate (TD) as well as the different diluted media used for the cultivation assays (values estimated based on RD and TD analysis).

	Raw Digestate				**Treated Digestate with 50 g/L of AC**			
Parameters	RD	RD20	RD40	RD60	TD	TD20	TD40	TD60
COD (mg/L)	8650	433	216	144	3917	196	98	65
PO_4-P (mg/L)	869	43	22	14	434	22	11	7
NH_4-N (mg/L)	4405	221	110	74	4220	211	106	71
Total phenols (mg/L)	1054	53	26	18	161	8	4	3

Similar growth tendency of *S. platensis* was observed in both the raw digestate (RD) and the treated digestate (TD) conditions tested at three different dilution factors (Figure 2A,B). Higher growth responses were generated when increasing the dilution factor of the cultivation media for both the raw and pretreated digestate. Similarly, the lag phase of *S. platensis* was affected by the dilution factor of the raw and pretreated digestate, with a longer lag phase observed the more the digestate was concentrated. For example, the lag phase of the untreated digestate diluted 20 and 40 times lasted 4 and 2 days, respectively. However, the extended lag phase could be potentially reduced by acclimating the inoculum to the anaerobic digestate, as proposed by Japar et al., who improved growth performance of different microalgal species with a specific acclimatization to anaerobic digested palm oil mill effluent [47]. The positive control achieved the strongest cyanobacteria proliferation with no lag phase, followed by the highest optical density of 2.4 achieved after 10 days of cultivation (Figure 1A). On the other hand, *S. platensis* cells were not able to grow in the negative control, with only a small variation of the optical density at the beginning of the experiment (Figure 1A).

As observed previously, the growth rate of the BG11 media was significantly higher than all the other conditions, with a value of 0.22 d^{-1} ($p < 0.05$). The statistical analysis indicated that there were no statistical differences between the pre-treated and the raw digestate for all the dilution factors investigated (Figure 3). Since the pretreatment did not show any major effects in the growth responses, the procedure was stopped after 10 days of cultivation, even though several cultures were still in the exponential phase. The very limited growth observed in the case of the negative control (0.09 d^{-1}) indicated that the cyanobacteria inoculum, introduced at the beginning of the experiment, did not provide any nutrients to the *S. platensis* growth. Consequently, the cyanobacteria development observed in the other conditions performed with AD digestate was directly linked with the utilization of nutrients such as nitrogen and phosphorus.

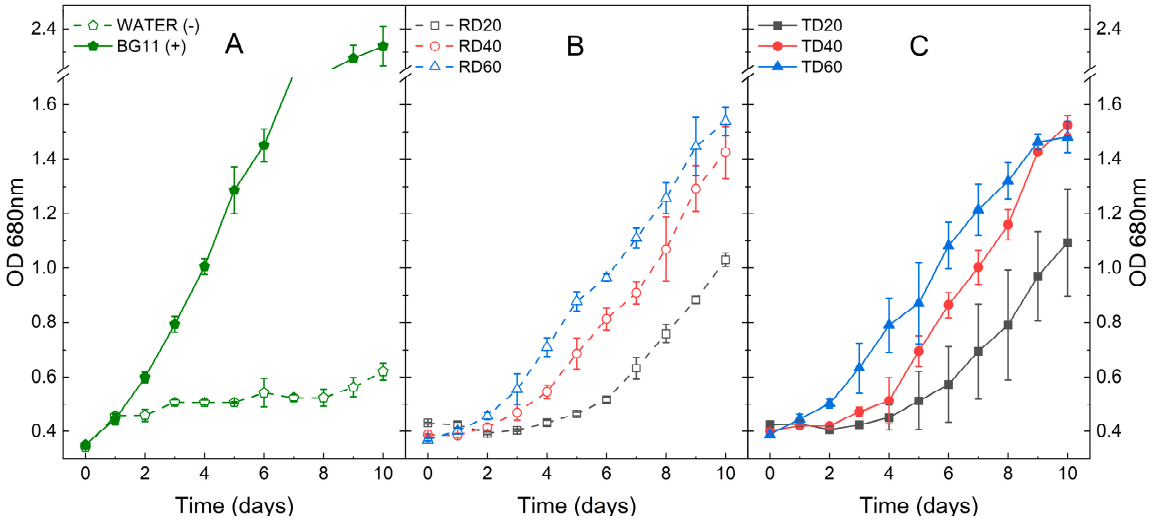

Figure 2. Growth curves of *Spirulina platensis* using both control groups (**A**) as well as the raw digestate (**B**) and the treated digestate (**C**) diluted at different dilution factors (20×, 40× and 60×). The positive and negative control refer to the use of BG11 synthetic media and demineralized water, respectively.

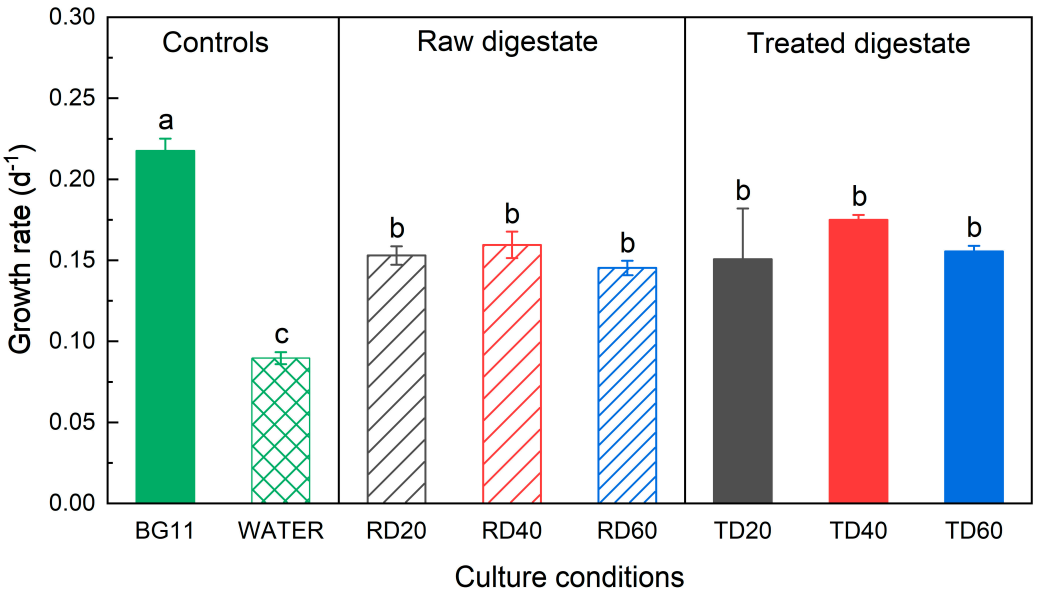

Figure 3. *Spirulina platensis* specific growth rates calculated for all the investigated conditions, including the control groups and the raw (RD) and treated digestate (TD) diluted at three dilution factors (20×, 40× and 60×). For all the culture conditions, different letters above bars indicate statistical differences ($p < 0.05$).

As observed previously, diluting the raw and the treated digestate presented positive effects on *S. platensis* growth, more specifically on the biomass production response (Table 5). The highest biomass concentration and productivity were obtained at a dilution factor of 40, with no statistical differences between the raw and the treated digestate. For instance, biomass productivity of 63 and 68 mg/L/d were generated for the raw digestate and the digestate treated with 50 g/L of AC, both diluted 40 times. In addition, employing a higher dilution rate of 60× did not show any significant positive responses to the biomass concentration or productivity parameters ($p > 0.05$). Finally, the positive and negative controls achieved the highest and lowest growth responses, with 115 and 17 mg/L/d, respectively.

Table 5. Biomass concentration and productivity obtained after *Spirulina platensis* cultivation assays performed in 250 mL tubes with the raw digestate (RD) as well as the treated digestate (TD) diluted at three dilution factors (20×, 40× and 60×). Cultures carried out in BG11 and demineralized water represent the positive and negative controls, respectively. For each parameter, different letters indicate statistical differences among the groups ($p < 0.05$).

	Controls		**Raw Digestate**			**Treated Digestate with 50 g/L of AC**		
Parameters	BG11	Water	RD20	RD40	RD60	TD20	TD40	TD60
Biomass concentration * (g/L)	1.36 ± 0.13^{a}	0.38 ± 0.02^{e}	0.62 ± 0.02^{d}	0.86 ± 0.07^{bc}	0.93 ± 0.04^{b}	0.66 ± 0.15^{cd}	0.92 ± 0.02^{b}	0.91 ± 0.03^{b}
Biomass productivity (mg/L/d)	115 ± 9^{a}	17 ± 0^{d}	36 ± 1^{cd}	63 ± 8^{b}	71 ± 4^{b}	40 ± 1^{c}	68 ± 0^{b}	66 ± 1^{b}

* Measured after 10 days of cultivation.

In the present study, *S. platensis* was able to proliferate using an initial ammonium-nitrogen (NH_4^+-N) concentration between 221 and 71 mg/L depending on the dilution factor and the pretreatment condition. In general, higher dilution of the raw and the pretreated digestate generated higher growth. Similarly, Jiang et al. reported higher *Spirulina subsalsa* cultivation responses when diluting complex wastewater from the monosodium glutamate production process. The high concentration of ammonia (120 mg/L) detected in the raw effluent inhibited *Spirulina subsalsa* growth, and a 50% (*v*/*v*) dilution with modified Zarrouk medium was necessary to support any algal proliferation [44]. In another study, slightly different behavior was observed when cultivating *Arthrospira platensis* in seawater supplemented with four different ratios of digestate (2.5–15%, *v*/*v*) in a fed-batch system [16]. The highest biomass production of around 1300 mg/L was obtained after 7 days of cultivation using 5% (*v*/*v*) of digestate. According to Markou et al., the supplementation with 2.5% (*v*/*v*) of digestate did not provide enough essential nutrients to *Spirulina*, whereas digestate concentration higher than 5% (*v*/*v*) limited the light availability due to the strong turbidity of the digestate.

4. Conclusions

The commercial activated carbon used in this study presented significant detoxification properties towards several compounds initially present in the agro-industrial digestate, including color, phenol, COD and phosphates. In general, increasing the AC dose generated higher removal efficiencies but considerably reduced the adsorption capacity of the material. On the other hand, AC adsorption agent showed negligible effects on ammonium nitrogen removal, with almost no variations of the initial concentration for all the investigated adsorbent dosages ($\leq$100 g/L). Consequently, the dilution of the pretreated digestate was mandatory (at least 20 times) in order to reduce the ammonium toxicity and promote *S. platensis* proliferation during the cultivation assays.

Even if the AC pretreatment did not improve the *S. platensis* growth response and biomass productivity, the anaerobic digestate was strongly detoxified by removing hardly biodegradable compounds such as phenolic compounds and other organic compounds. Based on the experimental results, the AC detoxification strategy has the potential to be applied to a wide range of liquid effluents such as manure, digestate and wastewater.

The integration of AC pretreatment together with other techniques such as ion exchange (IEX) material (resin, zeolite, clay, etc.) for ammonium removal could be a solution for the valorization of AD digestate with sustainable cyanobacteria cultivation media without employing freshwater input. However, such an approach should be carefully studied to ensure that it is scalable and economically viable.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su15054571/s1, Table S1. Initial and final pH measured during digestate pre-treatment using various doses of activated carbon (AC). For the variation of pH, different letters indicate statistical differences among different AC concentrations ($p < 0.05$). Table S2. Ammonium concentration variations observed during digestate pre-treatment using various doses of

activated carbon (AC). The same letter indicate no statistical differences were observed among the different AC doses ($p > 0.05$).

Author Contributions: Á.S.-Q.: Investigation, methodology, data curation, writing—review and editing; M.-A.L.: investigation, methodology, writing—review and editing; S.B.: formal analysis, data curation, writing—review and editing; L.L.: formal analysis, data curation, writing—review and editing; F.M.: methodology, writing—review and editing, project administration, supervision; J.-B.B.: Investigation, methodology, data curation, writing—review and editing, project administration, supervision. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Nouvelle Aquitaine region (FRANCE) as well as the MICA Carnot Institut through the PYRAMID project.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Acknowledgments: The authors are grateful to the Nouvelle Aquitaine region and the MICA Carnot Institut for funding this study.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Méthanisation et Biogaz. Available online: https://atee.fr/energies-renouvelables/club-biogaz/methanisation-et-biogaz (accessed on 3 November 2022).
2. Technical Analysis of the 2018 and 2021 ICCT Reports on the Role of Biomethane as a Renewable Energy Source. European Biogas Association. Available online: https://www.europeanbiogas.eu/technical-analysis-of-the-2018-and-2021-icct-reports-on-the-role-of-biomethane-as-a-renewable-energy-source/ (accessed on 3 November 2022).
3. Ayre, J.M.; Moheimani, N.R.; Borowitzka, M.A. Growth of Microalgae on Undiluted Anaerobic Digestate of Piggery Effluent with High Ammonium Concentrations. *Algal Res.* **2017**, *24*, 218–226. [CrossRef]
4. Li, K.; Liu, Q.; Fang, F.; Luo, R.; Lu, Q.; Zhou, W.; Huo, S.; Cheng, P.; Liu, J.; Addy, M.; et al. Microalgae-Based Wastewater Treatment for Nutrients Recovery: A Review. *Bioresour. Technol.* **2019**, *291*, 121934. [CrossRef] [PubMed]
5. Makádi, M.; Tomócsik, A.; Orosz, V. Digestate: A New Nutrient Source—Review. *Biogas* **2012**, *14*, 295–310. [CrossRef]
6. Xia, A.; Murphy, J.D. Microalgal Cultivation in Treating Liquid Digestate from Biogas Systems. *Trends Biotechnol.* **2016**, *34*, 264–275. [CrossRef] [PubMed]
7. Monlau, F.; Sambusiti, C.; Ficara, E.; Aboulkas, A.; Barakat, A.; Carrère, H. New Opportunities for Agricultural Digestate Valorization: Current Situation and Perspectives. *Energy Environ. Sci.* **2015**, *8*, 2600–2621. [CrossRef]
8. Abdel-Raouf, N.; Al-Homaidan, A.A.; Ibraheem, I.B.M. Microalgae and Wastewater Treatment. *Saudi J. Biol. Sci.* **2012**, *19*, 257–275. [CrossRef]
9. Levine, R.B.; Costanza-Robinson, M.S.; Spatafora, G.A. Neochloris Oleoabundans Grown on Anaerobically Digested Dairy Manure for Concomitant Nutrient Removal and Biodiesel Feedstock Production. *Biomass Bioenergy* **2011**, *35*, 40–49. [CrossRef]
10. Hanifzadeh, M.; Sarrafzadeh, M.-H.; Nabati, Z.; Tavakoli, O.; Feyzizarnagh, H. Technical, Economic and Energy Assessment of an Alternative Strategy for Mass Production of Biomass and Lipid from Microalgae. *J. Environ. Chem. Eng.* **2018**, *6*, 866–873. [CrossRef]
11. Yang, W.; Li, S.; Qv, M.; Dai, D.; Liu, D.; Wang, W.; Tang, C.; Zhu, L. Microalgal Cultivation for the Upgraded Biogas by Removing CO2, Coupled with the Treatment of Slurry from Anaerobic Digestion: A Review. *Bioresour. Technol.* **2022**, *364*, 128118. [CrossRef]
12. Hultberg, M.; Lind, O.; Birgersson, G.; Asp, H. Use of the Effluent from Biogas Production for Cultivation of Spirulina. *Bioprocess Biosyst. Eng.* **2017**, *40*, 625–631. [CrossRef]
13. Cicci, A.; Bravi, M. Production of the Freshwater Microalgae Scenedesmus Dimorphus and Arthrospira Platensis by Using Cattle Digestate. *Chem. Eng. Trans.* **2014**, *38*, 85–90. [CrossRef]
14. Massa, M.; Buono, S.; Langellotti, A.L.; Castaldo, L.; Martello, A.; Paduano, A.; Sacchi, R.; Fogliano, V. Evaluation of Anaerobic Digestates from Different Feedstocks as Growth Media for Tetradesmus Obliquus, Botryococcus Braunii, Phaeodactylum Tricornutum and Arthrospira Maxima. *New Biotechnol.* **2017**, *36*, 8–16. [CrossRef] [PubMed]
15. Matos, Â.P.; Vadiveloo, A.; Bahri, P.A.; Moheimani, N.R. Anaerobic Digestate Abattoir Effluent (ADAE), a Suitable Source of Nutrients for Arthrospira Platensis Cultivation. *Algal Res.* **2021**, *54*, 102216. [CrossRef]
16. Markou, G.; Diamantis, A.; Arapoglou, D.; Mitrogiannis, D.; González-Fernández, C.; Unc, A. Growing Spirulina (Arthrospira Platensis) in Seawater Supplemented with Digestate: Trade-Offs between Increased Salinity, Nutrient and Light Availability. *Biochem. Eng. J.* **2021**, *165*, 107815. [CrossRef]
17. Kanchanatip, E.; Su, B.-R.; Tulaphol, S.; Den, W.; Grisdanurak, N.; Kuo, C.-C. Fouling Characterization and Control for Harvesting Microalgae Arthrospira (Spirulina) Maxima Using a Submerged, Disc-Type Ultrafiltration Membrane. *Bioresour. Technol.* **2016**, *209*, 23–30. [CrossRef]

18. Barros, A.I.; Gonçalves, A.L.; Simões, M.; Pires, J.C.M. Harvesting Techniques Applied to Microalgae: A Review. *Renew. Sustain. Energy Rev.* **2015**, *41*, 1489–1500. [CrossRef]
19. Soni, R.A.; Sudhakar, K.; Rana, R.S. Spirulina–From Growth to Nutritional Product: A Review. *Trends Food Sci. Technol.* **2017**, *69*, 157–171. [CrossRef]
20. Ragusa, I.; Nardone, G.N.; Zanatta, S.; Bertin, W.; Amadio, E. Spirulina for Skin Care: A Bright Blue Future. *Cosmetics* **2021**, *8*, 7. [CrossRef]
21. Sumprasit, N.; Wagle, N.; Glanpracha, N.; Annachhatre, A.P. Biodiesel and Biogas Recovery from Spirulina Platensis. *Int. Biodeterior. Biodegrad.* **2017**, *119*, 196–204. [CrossRef]
22. Chong, C.C.; Cheng, Y.W.; Ishak, S.; Lam, M.K.; Lim, J.W.; Tan, I.S.; Show, P.L.; Lee, K.T. Anaerobic Digestate as a Low-Cost Nutrient Source for Sustainable Microalgae Cultivation: A Way Forward through Waste Valorization Approach. *Sci. Total Environ.* **2022**, *803*, 150070. [CrossRef] [PubMed]
23. Barzee, T.J.; Yothers, C.; Edalati, A.; Rude, K.; Chio, A.; El Mashad, H.M.; Franz, A.; Zhang, R. Pilot Microalgae Cultivation Using Food Waste Digestate with Minimal Resource Inputs. *Bioresour. Technol. Rep.* **2022**, *19*, 101200. [CrossRef]
24. Rude, K.; Yothers, C.; Barzee, T.J.; Kutney, S.; Zhang, R.; Franz, A. Growth Potential of Microalgae on Ammonia-Rich Anaerobic Digester Effluent for Wastewater Remediation. *Algal Res.* **2022**, *62*, 102613. [CrossRef]
25. Marazzi, F.; Sambusiti, C.; Monlau, F.; Cecere, S.E.; Scaglione, D.; Barakat, A.; Mezzanotte, V.; Ficara, E. A Novel Option for Reducing the Optical Density of Liquid Digestate to Achieve a More Productive Microalgal Culturing. *Algal Res.* **2017**, *24*, 19–28. [CrossRef]
26. Attene, L.; Deiana, A.; Carucci, A.; De Gioannis, G.; Asunis, F.; Ledda, C. Efficient Nitrogen Recovery from Agro-Energy Effluents for Cyanobacteria Cultivation (Spirulina). *Sustainability* **2023**, *15*, 675. [CrossRef]
27. Park, J.; Jin, H.-F.; Lim, B.-R.; Park, K.-Y.; Lee, K. Ammonia Removal from Anaerobic Digestion Effluent of Livestock Waste Using Green *Alga scenedesmus* sp. *Bioresour. Technol.* **2010**, *101*, 8649–8657. [CrossRef] [PubMed]
28. Rajagopal, R.; Mousavi, S.E.; Goyette, B.; Adhikary, S. Coupling of Microalgae Cultivation with Anaerobic Digestion of Poultry Wastes: Toward Sustainable Value Added Bioproducts. *Bioengineering* **2021**, *8*, 57. [CrossRef]
29. Uggetti, E.; Sialve, B.; Latrille, E.; Steyer, J.-P. Anaerobic Digestate as Substrate for Microalgae Culture: The Role of Ammonium Concentration on the Microalgae Productivity. *Bioresour. Technol.* **2014**, *152*, 437–443. [CrossRef]
30. Jiménez-Castañeda, M.E.; Medina, D.I. Use of Surfactant-Modified Zeolites and Clays for the Removal of Heavy Metals from Water. *Water* **2017**, *9*, 235. [CrossRef]
31. Alshameri, A.; He, H.; Xin, C.; Zhu, J.; Xinghu, W.; Zhu, R.; Wang, H. Understanding the Role of Natural Clay Minerals as Effective Adsorbents and Alternative Source of Rare Earth Elements: Adsorption Operative Parameters. *Hydrometallurgy* **2019**, *185*, 149–161. [CrossRef]
32. Yang, X.; Zhang, S.; Ju, M.; Liu, L. Preparation and Modification of Biochar Materials and Their Application in Soil Remediation. *Appl. Sci.* **2019**, *9*, 1365. [CrossRef]
33. Singh, S.; Wasewar, K.L.; Kansal, S.K. Chapter 10–Low-Cost Adsorbents for Removal of Inorganic Impurities from Wastewater. *Inorg. Pollut. Water* **2020**, 173–203. [CrossRef]
34. Moyo, M.; Chikazaza, L.; Nyamunda, B.C.; Guyo, U. Adsorption Batch Studies on the Removal of Pb(II) Using Maize Tassel Based Activated Carbon. *J. Chem.* **2013**, *2013*, e508934. [CrossRef]
35. Ma, H.; Guo, Y.; Qin, Y.; Li, Y.-Y. Nutrient Recovery Technologies Integrated with Energy Recovery by Waste Biomass Anaerobic Digestion. *Bioresour. Technol.* **2018**, *269*, 520–531. [CrossRef]
36. APHA. *Standard Methods for the Examination of Water and Wastewater*, 19th ed.; American Public Health Association: Washington, DC, USA, 1995.
37. Blainski, A.; Lopes, G.C.; de Mello, J.C.P. Application and Analysis of the Folin Ciocalteu Method for the Determination of the Total Phenolic Content from *Limonium brasiliense*, L. *Molecules* **2013**, *18*, 6852–6865. [CrossRef] [PubMed]
38. Hagemann, N.; Schmidt, H.P.; Kägi, R.; Böhler, M.; Sigmund, G.; Maccagnan, A.; McArdell, C.S.; Bucheli, T.D. Wood-Based Activated Biochar to Eliminate Organic Micropollutants from Biologically Treated Wastewater. *Sci. Total Environ.* **2020**, *730*, 138417. [CrossRef]
39. Lütke, S.F.; Igansi, A.V.; Pegoraro, L.; Dotto, G.L.; Pinto, L.A.A.; Cadaval, T.R.S. Preparation of Activated Carbon from Black Wattle Bark Waste and Its Application for Phenol Adsorption. *J. Environ. Chem. Eng.* **2019**, *7*, 103396. [CrossRef]
40. Kumar, P.; Sudha, S.; Chand, S.; Srivastava, V.C. Phosphate Removal from Aqueous Solution Using Coir-Pith Activated Carbon. *Sep. Sci. Technol.* **2010**, *45*, 1463–1470. [CrossRef]
41. Parsy, A.; Guyoneaud, R.; Lot, M.-C.; Baldoni-Andrey, P.; Périé, F.; Sambusiti, C. Impact of Salinities, Metals and Organic Compounds Found in Saline Oil & Gas Produced Water on Microalgae and Cyanobacteria. *Ecotoxicol. Environ. Saf.* **2022**, *234*, 113351. [CrossRef] [PubMed]
42. Mattson, J.A.; Mark, H.B.; Malbin, M.D.; Weber, W.J.; Crittenden, J.C. Surface Chemistry of Active Carbon: Specific Adsorption of Phenols. *J. Colloid Interface Sci.* **1969**, *31*, 116–130. [CrossRef]
43. Almanassra, I.W.; Kochkodan, V.; Mckay, G.; Atieh, M.A.; Al-Ansari, T. Review of Phosphate Removal from Water by Carbonaceous Sorbents. *J. Environ. Manag.* **2021**, *287*, 112245. [CrossRef]
44. Jiang, L.; Pei, H.; Hu, W.; Ji, Y.; Han, L.; Ma, G. The Feasibility of Using Complex Wastewater from a Monosodium Glutamate Factory to Cultivate Spirulina Subsalsa and Accumulate Biochemical Composition. *Bioresour. Technol.* **2015**, *180*, 304–310. [CrossRef] [PubMed]

45. Ogbonna, J.C.; Yoshizawa, H.; Tanaka, H. Treatment of High Strength Organic Wastewater by a Mixed Culture of Photosynthetic Microorganisms. *J. Appl. Phycol.* **2000**, *12*, 277–284. [CrossRef]
46. Li, X.; Li, W.; Zhai, J.; Wei, H.; Wang, Q. Effect of Ammonium Nitrogen on Microalgal Growth, Biochemical Composition and Photosynthetic Performance in Mixotrophic Cultivation. *Bioresour. Technol.* **2019**, *273*, 368–376. [CrossRef] [PubMed]
47. Japar, A.S.; Takriff, M.S.; Mohd Yasin, N.H. Microalgae Acclimatization in Industrial Wastewater and Its Effect on Growth and Primary Metabolite Composition. *Algal Res.* **2021**, *53*, 102163. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.