

Article

Large-Scale Waste Bio-Remediation Using Microalgae Cultivation as a Platform

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Abstract: Municipal and agricultural waste treatment is one of the key elements of reducing environmental impact with direct effects on the economy and society. Algal technology has been tested to enable effective recycling and valorisation of wastewater nutrients including carbon, nitrogen and phosphorus. An integrated evaluation and optimisation of the sustainability of an algal bio-refinery, including mass and energy balances, carbon, water and nutrient use and impact analysis, was assessed. A bio-refinery approach of waste remediation using algal cultivation was developed at Swansea University, focusing on nutrient recovery via algal biomass exploitation in pilot facilities. Mass cultivation (up to 1.5 m³) was developed with 99% of nitrogen and phosphorus uptake by microalgal cultures. *Nannochloropsis oceanica* was used as a biological model and grown on three waste sources. The compounds obtained from the biomass were evaluated for animal feed and as a potential source of energy. The bioremediation through algal biotechnology was examined and compared to alternative nutrient recovery passive and active methods in order to know the most efficient way of excess nutrient management. Conclusions emphasise the high potential of algal biotechnology for waste remediation and nutrients recovery, despite the need for further development and scalable applications of this new technology.

Keywords: algal biotechnology; waste remediation; environmentally friendly solution; environmental pollution; struvite; N and P uptake; waste management; microalgae; mass cultivation

1. Introduction

The Water Framework Directive (WFD) was implemented in 2000 in Europe and aims to bring all inland water bodies to a standard denoted as having a ‘good ecological status’ [1]. Additionally, the Habitats Directive requires water discharged into inland water to have nutrients removed [2]. The phosphorous, nitrate and ammonium content of water bodies are the focus for water treatment regulations. EU regulation states that the maximum discharge limit of phosphorous is 2 mg L^{−1} for a population density less than 100,000 inhabitants and 1 mg L^{−1} for populations above 100,000 inhabitants. In addition, the influent phosphorous concentration levels that enter a waste water treatment plant (WWTP) must be reduced by a minimum of 80% [2]. In the Latin American countries; Chile, Bolivia and Ecuador, the water discharge limits are 40–80 mg L^{−1} for NH₃-NH₄ and 10–15 mg L^{−1} for TP [3–6]. In China, according to [7] and [8], the discharge limits for NH₃-N are 8 mg L^{−1}, 20 mg L^{−1} for TN and 1 mg L^{−1} for TP.

To treat these waters, methods ranging from ‘passive’ low energy forms to ‘active’ energy intensive can be implemented. Most of these biological processes are however considered as ‘under development’ and constant research and optimisation is needed to improve the nutrient uptake efficiencies of these technologies [9,10]. Wastewater treatment using microalgal cultivation (passive method) currently requires cost effective technical/biological development; for instance, using new methods such as

membrane technology [11]. Algal biotechnology has been successfully used as a bioremediation method [12]; however, this research usually focuses on small scale laboratory testing with little research on growth of algae on real wastewater streams [9], this issue will be addressed in the study.

Regarding active methods to deal with N & P remediation, physicochemical approaches are currently used world-wide [13]. These methods use nutrient precipitation to obtain solids from wastewater and require additional chemicals that can cause pollution [9]. Struvite has been identified as an alternative to phosphate recovery processes [5]. Struvite is a chemical precipitation method, using additional magnesium salt that forms a crystalline P- product which can be easily dried and transported [14]. This can be used to fertilise soils and there is a fledgling market for slow release phosphorus-rich fertilisers [15]. Struvite requires an alkaline environment and the addition of elemental magnesium which is difficult to contain and a rare mineral [13]. Scale of struvite method also needs to be developed to make it an ecologically and financially effective technology [9].

It has been estimated that the cost of controlling and removing scale from wastewater treatment plants in the United States is \$40,000 per million m³, per day [16]. Utilising the properties of wastewaters to naturally form scale, and creating technologies that can actively encourage and harvest this process has the potential for more cost-effective waste remediation. On an industrial scale, fluidised bed reactors are used to recover struvite; these systems provide large surface areas for crystallisation. When concentrations of magnesium, phosphate and ammonium are associated in a ratio of 1:1:1 and the pH and energy levels/mixing is optimum, between 70% and 90% uptake rates of nutrients [17] are common. The cost of building a pilot scale fluidised bed reactor is around £40,000 (Envirogen Technologies (USA)) which is around the same cost as building a pilot scale (>2000 L) bioreactor for microalgae cultivation.

Fluidised bed reactors (for the struvite method) are flow-through systems which have been developed to increase nutrient removal, but the standard systems, which are batch fed, have shown only 20–50% phosphorous removal efficiencies [18]. The majority of costs associated with recovery of nutrients via struvite is associated with increasing and maintaining the pH which can cost up to \$3500/ton-P [19]; this cost is not incurred with algal production. Algal systems with higher rates of remediation efficiencies (up to 99%) can also be flow-through with a continuous growth regime with addition of nutrients and biomass harvested daily. The greatest cost associated with algal production are the artificial lighting systems (especially in the winter) when grown in photobioreactors (PBRs). This cost can be reduced greatly if the bioreactor design is optimized according to location latitude with the aim to increase natural light exposure. If algal growth facilities are colocated with flue gas producing industries or energy production, the cost of the CO₂ and the artificial light support system required for algal growth has the potential to be decreased significantly. Additionally, for wastewater algal treatment, the high-rate algal ponds (HRAP) or algal turf scrubbers (ATS) are used [13,20]. These systems have low CAPEX (capital expenditure); however, water and space usage is higher in comparison with PBRs. Remediation processes in HRAP and ATS are usually less efficient in comparison with the PBR algal remediation. Furthermore, the productivity of algal cultures in PBRs are higher for monospecific biomass; this will be important for the further biomass utilisation [21].

From an ecological perspective, the water usage for PBRs needs to be reduced, especially drinking and irrigation water [22]. For this reason, *Nannochloropsis oceanica* (*N. oceanica*) was the model species in this study. This marine species can grow in seawater from the ocean [23] or waste brine water [9,24]. Brine (highly concentrated NaCl water) is the main residue produced during the desalination process through reverse osmosis. This is one of the new waste challenges that will be addressed in the near future [25].

This study aims to evaluate the bioremediation performances by *N. oceanica* of three sources of wastewater. *N. oceanica* was cultivated in a large-scale (1500 L) PBR. Nutrient (ammonium and phosphorus) uptake and retention time were assessed and compared to alternative methods. Microalgal and struvite nutrient recovery systems and their remediation efficiency were mainly investigated in this study. The potential for the development of a circular economy based on nutrient recycling from

wastewaters by microalgae was investigated as well as the use of microalgal biomass as a source of energy and for product development (e.g., biofertilizer, animal feed).

2. Materials and Methods

2.1. Experimental Design

The aim of the study was to optimise the cultivation process of *Nannochloropsis oceanica* grown in a 1500 L PBR with waste-based media as feedstock. *N. oceanica* was chosen as a marine species because during media preparation for inoculation, the wastewater can be mixed with brine from a desalination plant. The initial salinity concentration for all experiments was 30 IU by adding seawater salt to the wastewater. The injected inoculum of culture (in exponential growth) was 10% of the total volume (150 L) with cell concentration $\sim 54.1 \times 10^6$ Cell mL⁻¹; control medium was Cell-Hi F2P from Varicon aqua readymade media powder (<http://www.variconaquaculture-media.com/products-services/algal-nutrients-and-culture-media/>) [23]. Waste-based media was prepared by diluting the waste stream. Nitrogen as an ammonium salt was supplemented to the waste medium when sourced from aquaculture waste (trout farm waste), as the initial concentration of N was quite low (Table 1). The initial nutrient concentrations, dilution factors and final N:P ratio are shown in Table 1. Microalgae growth occurred for a total experimental duration of 15 days; biological and water chemistry samples were taken during the first three days of the experiment, followed by every other day. pH, temperature and light intensity were measured automatically with an online data collecting system.

Table 1. Nutrient concentrations of selected waste-based media before and after dilution.

Media Formulation	mMol L ⁻¹ N	mMol L ⁻¹ P	N:P Ratio	Dilution Factor mL L ⁻¹	Final N Concentration μMol L ⁻¹	Final P Concentration μMol L ⁻¹	N:P Ratio
Agricultural waste	55.4	4.3	12.7	15.9	880.8	69.3	12.7
Aquaculture waste	8.8	57.4	24.5	1.92	17 (+882) *	110.3	8.15
AD municipal waste	71.4	4.5	15.9	20	1428	89.7	15.9
F/2				1	882	36.2	24.5

* The additional Ammonium salt was added to the aquaculture waste, as the original waste-stream did not have enough N-source.

2.2. Waste-Based Media Preparation and Characterization

2.2.1. Agriculture Waste

The agriculture wastewater derived from spent agricultural digested sludge was composed of mixed waste cattle slurry, vegetable waste and silage. This waste was collected from Farm Renewable Environmental Energy Limited (Fre), Wrexham, United Kingdom (<http://www.fre-energy.co.uk/>). The pretreatment and characterisation of this waste was described in Silkina et al, 2017. The pretreatment of this waste consisted of dilution, mixing, sedimentation and sieving in a 200 L stainless steel vessel. The resulting effluents were then microfiltered using a pilot scale unit equipped with a ceramic membrane (pore size < 0.2 μm) [23]. The resulting particle-free effluents were used as feedstock for microalgae cultivation.

2.2.2. Aquaculture Waste

The aquaculture bio-waste was collected from the Test Valley Trout farm (Romsey, UK), operating in a flow through mode with a settling tank for the collection of solids. The bio-waste was collected from this settling tank and stored at 4 °C to ensure stability. Pretreatment of the bio-waste consisted of 24 hours settling and filtration of the supernatant on a <500 μm pore size mesh. The bio-waste sediment was kept in the settling tank. The filtered bio-waste was characterised by measuring dry mass, total suspended solids, buffering capacity (with HCl and NaOH), particle size distribution and

particle surface charge. Particle size distribution and particle surface charge were determined using a Mastersizer Hydro 2000 MU and a Zetasizer 2000 (Malvern Instruments). The particle surface charge was measured as a relation to the measured electrophoretic mobility. The buffering capacity was determined by acid titration using a 0.5 M HCl solution while continuously monitoring the pH. Dry mass (DM) and total suspended solids (TSS) were determined gravimetrically after drying at 105 °C according to standard methods [26].

2.2.3. Anaerobic Digested (AD) Municipal Waste

This waste source was supplied by Afan AD municipal waste plant, Port Talbot, Wales, UK. Following the Anaerobic Digestion (AD) process, the waste stream was pasteurised and UV treated. Membrane filtration with a pore size of 0.2 µm was also applied to remove any particles and sterilise the waste medium. The physicochemical characteristics of the three nutrient waste streams are presented in Table 2.

Table 2. Physicochemical characteristics of the three waste sources used as media for *N. oceanica* cultivation (the data presented with S.D).

Parameters	Agriculture Waste	Aquaculture Waste	AD Municipal Waste
PH Value	8.5 ± 0.02	8.5 ± 0.2	8.7 ± 0.015
BOD (mg L ⁻¹)	421 ± 16	405 ± 14	433 ± 12
COD (mg L ⁻¹)	2500 ± 256	2786 ± 385	2766 ± 410
Ammonium (mg L ⁻¹)	360 ± 15	26 ± 1.5	890 ± 30
TSS (total suspended solids) (mg L ⁻¹)	210 ± 6.8	472 ± 9	1060 ± 12.1
Phosphorus (mg L ⁻¹)	36.5 ± 0.6	418 ± 2.8	63 ± 0.5
Magnesium (mg L ⁻¹)	5.9 ± 0.8	29 ± 2.6	8.55 ± 1.65
Dissolved iron (mg L ⁻¹)	2.64 ± 0.06	5.93 ± 0.2	2.61 ± 0.03
Conductivity (mS cm ⁻¹)	5.16 ± 0.02	0.858 ± 0.006	4.89 ± 0.2

2.2.4. Membrane Separation Unit

The bio-waste processing unit consisted of a pilot-scale membrane filtration system operating in a cross-flow mode. The main reason was to use this unit to make the three waste streams free from particles and potential biological contaminants, as we would like to use the obtained biomass for future product development. The sludge of all three waste streams was processed by cross-flow filtration and the filtration unit contained two 100 L vessels. One vessel was used for sludge settlement while the other contained the screened suspended sludge and fed the membrane filtration system. A process flow chart of the system is shown in Figure 1. One inch stainless tubing was used to connect, via clamp fittings, two centrifugal pumps, a concentric tube heat exchanger, a pressure control valve, two stainless steel pressure gauges (before and after the filtration module) and a Membralox ceramic MF membrane fitted in stainless steel module. The membrane had a 0.20 µm pore size and an area of 0.22 m². In addition, there were two 1.5 inch clamp butterfly valves in the system. In order to avoid cavitation of the pumps, a minimum holding volume of 5.0 L was established.

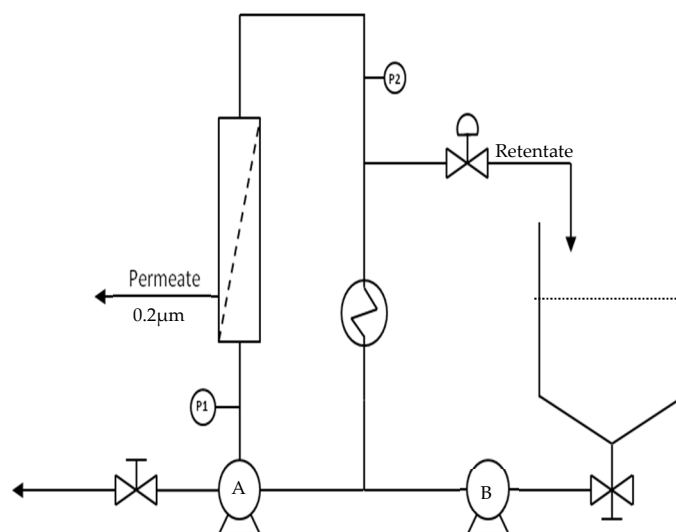


Figure 1. Diagram of microfiltration membrane filtration modified from Gerardo et al, 2015 [27]. P1 and P2—pressure gauges; A and B—centrifugal pumps.

2.3. Strain and Culture Medium

Nannochloropsis oceanica Suda & Miyashita (Eustigmataceae) was supplied by the CSAR culture collection, Swansea University, previously provided by Greece Hellinism Marine institute in 2011 and since cultivated in CSAR culture collection [28].

N. oceanica master culture was maintained in a controlled temperature room (18 °C) with a light intensity of $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ and a light: dark cycle of 16:8 h. The F/2 standard media [29] was used as feedstock for the maintenance of the master culture and inoculum. The inoculum was used during the exponential phase to ensure constant and efficient growth during the experimental phase.

2.4. Cultivation System and Conditions

A vertical tubular photobioreactor (PBR), with a capacity of 1500 L was used. This PBR was based on the Greek Hellinism Marine institute design (Figure 2). The PBR was located in an outdoor greenhouse (51°36'29.1" N 3°58'53.1" W, Swansea, UK), at Swansea University (SU). During the day, the algal culture used natural light; at night, artificial white fluorescent lights were used providing 24 h illumination. Both light phase and dark phase (or dark tank) had a volume of 750 L. Culture mixing was achieved by a pump allowing culture movement from light phase to dark phase, the hydraulic retention time (HDT) in the light phase section was between 7 to 10 min. The temperature and pH were monitored with online probes; temperature ranged from 18 to 24 °C and pH was maintained at 7.5 to 8.5. CO₂ was injected on demand when pH reached 8.1 (pH set at 8 with a hysteresis of 0.1). The CO₂ was injected before the pump, using the pump propeller to allow the formation of microbubble in order to help during the CO₂ uptake of the microalgae cells.

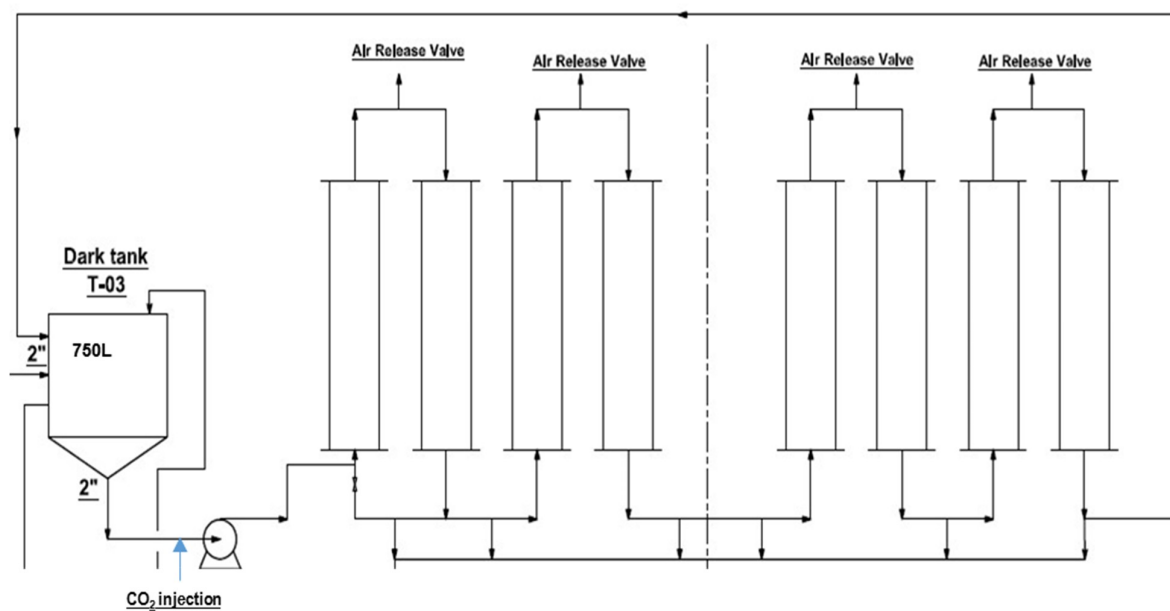


Figure 2. Vertical tubular photobioreactor (PBR, total capacity of 1500 L) used in this experimental study.

2.5. Sampling and Analysis

Every 24 h, cell concentration and bio-volume were measured using a Coulter counter (Multisizer 4, Beckman, USA) Further details are described in [30].

The growth rate was calculated according to Equation (1):

$$\mu = \ln\left(\frac{N_2}{N_1}\right) / (t_1 - t_2) \quad (1)$$

where: $\ln$ = natural logarithm; t_1 = time one (in days/h); t_2 = time two (in days/h); N_1 = Nutrient concentration in the medium at time one; N_2 = Nutrient concentration in the medium at time two.

The doubling time was calculated from Equation (2):

$$\mu = \frac{\ln 2}{T_d} \quad (2)$$

where: μ is the specific growth rate, T_d is the doubling time

Dry weight was measured according to [31], a known volume of algae was pelleted and washed with deionized (DI) water (three times using 25 mL of DI water each time) prior to being filtered onto a preweighed and dried filters (Whatman GF/F 47 mm Ø). The filters with algal biomass were then dried and reweighed until constant weight was reached. Dry weight (g L^{-1}) was then calculated by subtraction of the final filter weight and the prefiltered weight.

Biomass productivity was calculated as the difference in terms of DW between the sample day and the previous day. The results are expressed in $\text{g L}^{-1} \text{d}^{-1}$.

Water samples for nutrient analysis were sample every other day and analysed using an automated segmented flow analyser (AA3, SEAL Analytical, Germany). Ammonium and phosphate were measured. Microalgal biomass samples for biochemical analysis and dry weight were centrifuged (8000 g , 4°C , JA-2, Beckman, Germany), suspended and washed twice with deionised water and frozen at -80°C overnight before freeze-drying for 24 h [32].

The biomass biochemical composition was analysed for lipids using the gravimetric Folch method [33] with modification of [32], for total carbohydrates by DuBois method [34] and for protein content by multiplying the nitrogen content of dried biomass measured using a SerCon GSL elemental analyser (1000°C combustion temperature), by a factor of 6.28 described in [35]. This approach is

analogous to that based on the Kjeldahl digestion [35]. Gross energy content and calorific value (KJ g^{-1} and Kcal g^{-1}) were measured by combustion of freeze dried biomass in a Parr bomb calorimeter using benzoic acid as a standard [36].

All biochemical measurements were carried out in triplicate, except for protein content, which was performed in duplicate.

2.6. Nutrient Uptake Rate Calculation.

To compare the kinetics of nitrogen and phosphorus removal, calculations were made to assess the time necessary to reach 10 mg L^{-1} (ΣN) and 1 mg L^{-1} (P-PO_4^{3-}) (the most restrictive concentrations in European Union Directive 98/15/CE [27] concerning requirements of N and P in the effluents permitted from urban wastewater treatment).

Nutrient uptake rate (%) was calculated based on Equation (3) [37]:

$$R\% = ((R_t - R_0) / R_t) \times 100 \quad (3)$$

where, R is the nutrient removal efficiency and R_t and R_0 are the nutrient concentration at day t and day 0, respectively.

2.7. Statistical Analyses

Statistical analyses were carried out using the R project software on dry weight, nutrient uptake and intracellular nutrient content data. Data normality was tested using a Shapiro test. This test showed that dry weight and nutrient uptake data were not following a normal distribution (dry weight: $p = 0.00105^*$ and $p = 6.955 \times 10^{-6}^{***}$ and $p = 7.472 \times 10^{-5}^{***}$ respectively for ammonium and phosphorus uptake). Intracellular nitrogen and phosphorus content data were following a normal distribution (respectively, $p = 0.07514$ and $p = 0.331$). Non-normal data significance was assessed using GLMs (generalised linear models) furthered by an analysis of variance (ANOVA) on a data set not following a normal distribution. Crossed factors ANOVAS were carried out on normally distributed data. Both statistical methods tested the impact of experimental duration and waste stream (and interaction) for microalgal cultivation on the measured dry weight, nutrient uptake and intracellular nutrient content. When statistical significance was found, post hoc Tukey tests were implemented.

3. Results

3.1. Algal Waste-Based Growth Experiments

N. oceanica showed a high level of acclimation and growth performance on the three waste-based media, comparable to the control conditions which are presented in Figure 3.

After a short lag phase (2–3 days) cultures grew exponentially for 8 to 10 days, and reached a stationary phase after 15 days of cultivation. The average growth rate during the lag phase (3 days) was $0.26\text{--}0.35 \text{ day}^{-1}$ (with 95% Confidence interval, C.I) for all culture conditions. In the exponential phase (8 to 10 days), the growth rate was 0.4 day^{-1} (95% C.I) for the agriculture waste, AD municipal waste and control. *N. oceanica* grown on aquaculture waste had a growth rate of 0.6 day^{-1} (95% C.I). It was 50% faster than the growth under control or the AD municipal waste.

Statistical analysis showed a significant effect of both experimental time and waste stream used for cultivation on the microalgae dry weight (p^{***} for individual factors and interaction, see supplementary material). The post hoc Tukey tests showed that there were no differences in the dry weight from day 0 to day 3 for all waste streams tested, corresponding to a lag phase at the beginning of the experiment. Dry weight was clearly different for tested feedstock for the remaining experiment and microalgae had best performances in terms of growth when cultivated on the aquaculture waste-based media. This was followed by the control medium and municipal waste (Figure 3). Finally, the agriculture waste condition presented less growth over the whole time of experiment (Figure 3).

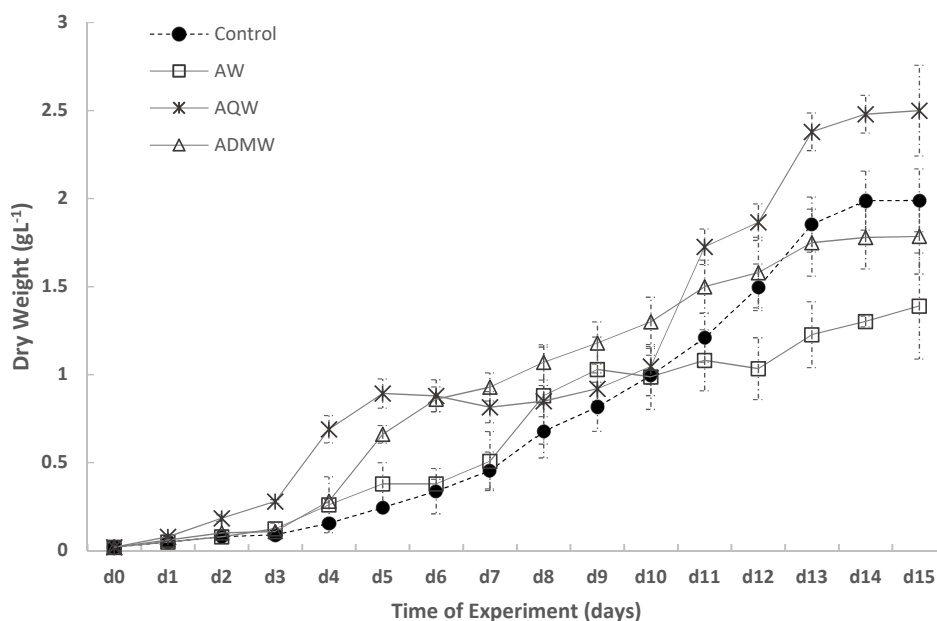


Figure 3. Dry weight of *N. oceanica* (in g L⁻¹) growing on control medium F/2; AW: Agricultural Waste; AQW: Aquaculture Waste; ADMW: Anaerobically Digested Municipal Waste. Error bars represent standard deviation for three biological replicates.

The specific growth rate (μ) and the doubling time during the exponential phase were determined using the Equations (1) and (2) respectively. Table 3 illustrates the calculation of the maximum growth rate during the lag and exponential phases and thus the T_d (doubling time). T_d indicates the possible harvesting frequency of the microalgae culture after it has overcome the lag phase. This harvesting frequency represents an efficiency optimum for the remediation of nutrients by microalgae linking biomass production and water remediation. In that sense, when operating in a continuous mode with frequent harvests, after replenishing water and nutrients, harvesting cycles can take place at 36–40 h intervals for control, agriculture and AD municipal waste-based media conditions. In the case of aquaculture waste-based media every 27 h, the culture could be harvested and the waste-based media and water replenished nearly every day using AQW.

Table 3. Summary of growth and nutrients uptake parameters of *N. oceanica* grown on three waste-based media.

Waste-Based Media	NH ₄ Uptake Rate ($\mu\text{Mol L}^{-1}$)	P Uptake Rate ($\mu\text{Mol L}^{-1}$)	Max Biomass Concentration (g L ⁻¹)	μ at Exp. Phase (day ⁻¹)	Doubling Time	
					(day ⁻¹)	(h)
Agriculture waste	186	8.1	1.3	0.41	1.68	40.39
AD municipal waste	36	0.74	1.78	0.45	1.53	36.80
Aquaculture waste	N/A	2.73	2.5	0.62	1.11	26.71
Control	192	9.6	1.99	0.42	1.64	39.43

3.2. Waste Remediation Efficiency

The experiments revealed that phosphorous is more readily recovered by the microalgal biomass compared to nitrogen. Both forms of nutrients were remediated to levels below that of the water framework legislation after 10 days of culture growth for all the treatment and the control.

The uptake of ammonium and phosphorus from the growth medium was monitored during the entire cultivation period for the three-waste media and control; data is presented in Figures 4 and 5 and summarised in Table 3. Statistical analysis showed a significant effect of both time of experiment and waste source on ammonium and phosphorus uptake (p^{***} for individual factors and interaction, Table S1). Results of ammonium uptake showed graphically and statistically that ammonium concentration decreased from day 0 to day 8 to reach a minimum concentration from day

8 with no significant differences between waste streams as ammonium was depleted for all tested conditions. In control condition, uptake of all N-source was achieved on day 4 by *N. oceanica*. By that time, cultures grown on aquaculture waste consumed 94% of ammonium. *N. oceanica* had remediated 70% and 50% of the agriculture and AD municipal waste, respectively. After 8 days of cultivation, 99% of ammonium was consumed for all tested treatments in the 1500 L reactor showing an efficient recovery of the ammonium present in all three waste streams.

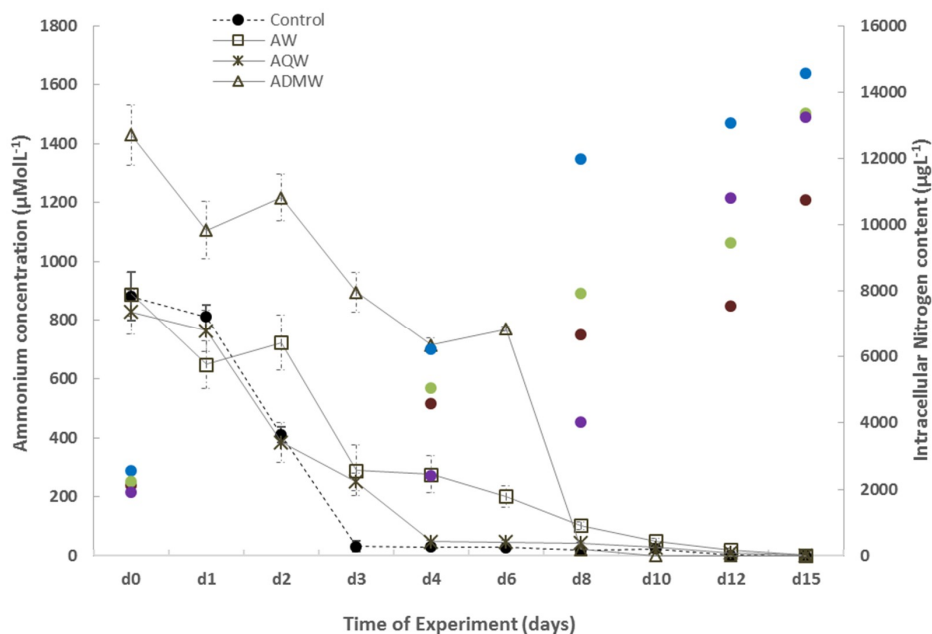


Figure 4. Ammonium uptake (in $\mu\text{Mol L}^{-1}$; lines with markers) and intracellular nitrogen content of *N. oceanica* (in $\mu\text{g L}^{-1}$; scatter) for Control medium F/2; AW: Agricultural Waste; AQW: Aquaculture Waste; ADMW: Anaerobically Digested Municipal Waste. Error bars represent standard deviation for three biological replicates.

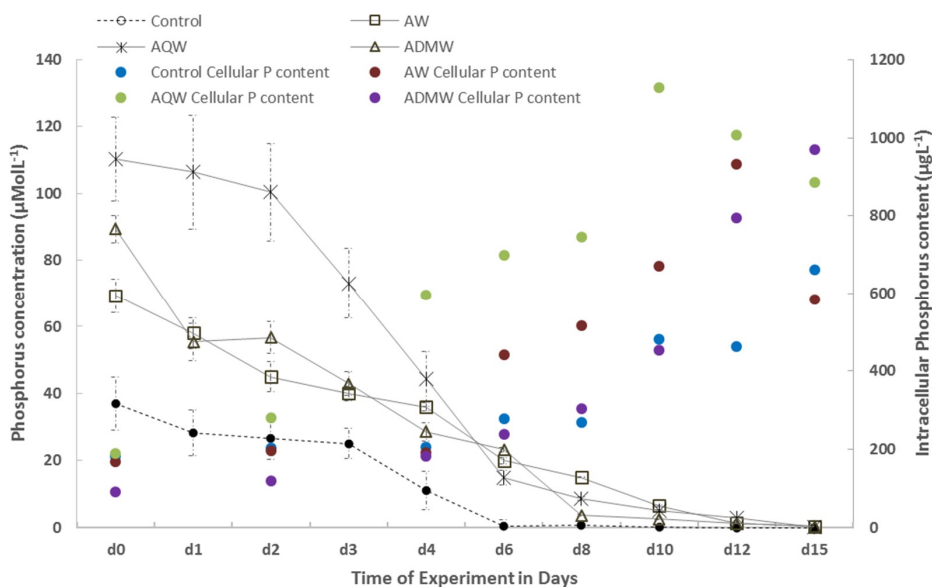


Figure 5. Phosphorus uptake (in $\mu\text{Mol L}^{-1}$; lines with markers) and intracellular phosphorus content of *N. oceanica* (in $\mu\text{g L}^{-1}$; scatter) for Control medium F/2; AW: Agricultural Waste; AQW: Aquaculture Waste; ADMW: Anaerobically Digested Municipal Waste. Error bars represent standard deviation for three biological replicates.

The phosphorus (P) uptake showed a similar trend with a significant difference in concentrations between waste sources up to day 10 where the phosphorus was almost fully depleted in the tested medium (p^{***} , Table S1). Full P-consumption for all conditions was at day 12. The aquaculture waste experienced the slowest uptake rate while the phosphorus in the control medium was depleted at day 6. Agriculture waste and AD municipal waste were not significantly different and depletion occurred at day 8 for both waste media. The P-uptake was in line with the lag phase in which most of the available P was used up by the cells. In fact, after 6 days of cultivation 60% of the available phosphorus had been consumed for all waste-based conditions. At day 8 and after the available P in the growth medium seemed to be residual with an overall consumption of 99%, P was successfully remediated by *N. oceanica* for all three waste-based media and the control.

The intracellular phosphorus and nitrogen content were also measured during the experiment. Graphically and statistically, both nitrogen and phosphorus cellular content were not significantly different between day 0 and day 4 (Figures 4 and 5). There was a significant increase from day 8 with higher nitrogen content in *N. oceanica* cells in the control followed by the aquaculture waste. A similar trend was observed for the phosphorus intracellular content of *N. oceanica*, with a significant increase from day 8. The intracellular phosphorus content of the microalgae grown on AD municipal waste and agricultural waste was not significantly different as for the nitrogen cell content. The higher phosphorus content was found for the microalgae growing on aquaculture waste and the lowest content was found for the control. The three-fold increase in cellular phosphorus that occurred during the time of experiment was potentially linked to a more frequent cell division and end of the lag phase.

3.3. Biomass Evaluation and Characteristic for Energy and High Value Products Development

The biomass of *N. oceanica* grown on three waste-based media was sampled and analysed for its biochemical composition presented in Table 4.

Table 4. Biochemical composition of *Nannochloropsis oceanica* grown on three waste based formulated media and on F2 (presented per dry matter).

Waste-Based Media	Protein %	Lipids %	Ash %	Carbohydrates %	Energy kcal g ⁻¹
Agriculture waste	28 ± 1.3	21.4 ± 1.5	23.0 ± 0.23	27.6 ± 1.6	5.9 ± 0.1
Aquaculture waste	21.3 ± 1.2	34.8 ± 1.8	23.4 ± 0.17	19.7 ± 1.5	6.4 ± 0.3
Municipal waste	36.6 ± 1.7	17.9 ± 0.4	23.9 ± 0.18	23.5 ± 1.8	4.9 ± 0.2
F2	36.5 ± 1.8	24.5 ± 1.5	21.8 ± 0.16	17.3 ± 1.5	5.5 ± 0.4

The biochemical analysis showed similar content of proteins, carbohydrates and lipids in the biomass grown on waste-based media compared to the F/2 control (Table 4). For example, the AD municipal waste based medium biomass had highest protein content, while the biomass grown on F2 had similar protein content (~36.5%). The biomass grown on the aquaculture and agriculture waste media had protein content 1.3 and 1.7 times lower, respectively, than control. The carbohydrate content ranged from 17.3% to 27.6% with the highest amount found in *N. oceanica* grown on agriculture waste-based medium. Lipid concentration was the lowest for cultures grown on the AD municipal waste-based medium (~17.9%) and the highest in the aquaculture waste-based cultivation conditions (~34.8%). The results of calorific value reveal potential for the use of the biomass as an energy source for feed and other applications. The highest value of 6.43 kcal g⁻¹ was calculated for cultures grown on aquaculture waste, followed by agriculture waste (5.97 kcal g⁻¹). The municipal waste conditions and control had similar calorific results of 4.9 and 5.5 kcal g⁻¹.

4. Discussion

4.1. Cultivation

Data from the algal waste remediation trials performed at a pilot plant of 1500 L at SU showed high levels of remediation capacity and a scalable approach for waste remediation. Scale of algal cultivation is very important for a successful remediation process. It is known that the results obtained in small scale could be extrapolated to the large-scale calculation, only as a modelling prediction. Unfortunately, in a real scale experiment, the same results are not always obtained [38]. For this reason, the bioremediation of three waste streams in the semi-industrial scale of 1500 L PBR was assessed in this study. In the northern latitudes such as Wales, UK, the use of a PBR is inevitable. PBRs are a closed system where algae can be cultivated in controlled conditions. Better results in terms of final production and reliable and consistent production through the year can be obtained by controlling the abiotic parameters. Additionally, the volumetric productivities in PBRs are significantly higher than in open systems and the cultivation processes are not dependent on seasonal variation [39,40]. Originally, the CAPEX cost of a PBR was extremely high and it was impossible to use these systems for waste remediation. Currently, the CAPEX cost of PBRs are reducing due to the use of new materials. With new PBR technology development, the cost has dropped and the implementation of a biorefinery approach with bio-remediation and the use of the obtained biomass for several end users (bio-fertiliser, agroindustry among others) shows that an effective use of these closed systems can be implemented all year around.

Microfiltration using membrane technology is being used in microalgae biotechnology for many years, mainly for downstream processing [14], but can also be used for upstream processing by preparing the wastewater to be used during the microalgae growth [12]. Membrane technology is a mature method than has been successfully applied in others sectors such as desalination plants, because the energetic cost of small microfiltration units are low, microfiltration in combination with microalgae cultures can be considered in future WWTP as natural and more environmentally friendly technology than mechanical oxygenation in WWTP.

4.2. Waste Remediation

The bioremediation data from this study was compared with the literature, specifically focused on remediation trials using alternative P-recovery struvite methods, HRAP and ATS technologies (Table 5). Algal remediation trials at SU achieved higher levels of nutrient recovery comparable to the struvite remediation method, ATS and HRAP. Algal bioremediation in the PBR is more efficient in a shorter time in comparison with HRAP and ATS. The struvite method is faster; however, the environmental drawbacks need to be considered [9]. Data collected at SU's 1500 L pilot suggests that using any nutrient source (waste or chemical nutrients) provides efficient growth of microalgae. In addition, within 6 days the ammonium and phosphorous was reduced by more than 80%, which reduced the phosphorous levels to below 1 mg L^{-1} , meaning the water is of dischargeable quality.

In total, 45 g of phosphorous was removed from the bioreactor in batch mode in 4 days, which could be repeated every 4 days. This resulted in 3.7 kg of dried algal biomass being produced in 1500 L PBR in 15 days of cultivation. Forty-five grams of phosphorous is worth less than 5 p (struvite value ~\$765/ton [19], whereas 1 kg of algae can retail for >\$50 as a food supplement and >\$12 as shrimp feed [41]. Additionally, the use of this product as a bio-fertiliser in high concentration requires removal of magnesium as a precipitation agent as it will provide an environmental pollution. This additional removal technology would make this process environmentally unsustainable and increase overall process complexity and costs [13].

Table 5. Bioremediation data (in mMol L^{−1}) from literature [42–49] and from this current study, presenting efficiency of nutrient uptake by algal cultivation, compared with struvite nutrient recovery, HRAP and ATS.

Author	Effluent Type	Parameter	Influent	Effluent	% Recovered	RT (Retention Time)
STRUVITE METHOD						
Yilmazel & Demirer, 2011	Poultry Manure	Ammonia	3.6	Unknown	86%	60 Min
		Phosphate	1.2	Unknown	31%	60 Min
Chong & Thai, 2014	Synthetic WW	Phosphate	0.3	54 ± 2	84%	120 Min
Li et al., 2012	Swine WW	Ammonia	4.9	Unknown	88%	60 Min
HRAP						
Van Den Hende, et al., 2014	Manure	TN	4.3	0.2	43%	4 days
		TP	3.9	1.3	65%	4 days
Gutierrez et al., 2016	Urban WW	N-NH ₄ ⁺	1.5	0.2	95%	1 month
Passos et al., 2015	Municipal WW	N-NH ₄ ⁺	1.05	0.02	95%	1 month
ATS						
Bohutskyi et al., 2016	Agriculture WW	TP	0.004	0.003	22%	1 month
		TN	0.07	0.05	6%	1 month
Craggs et al., 1996	Secondary sewage	TP	2.9	1.4	20%	1 day
		TN	21.13	5.28	40%	1 day
ALGAL PBR						
Current study	Agriculture WW	TON	0.8	0.0006	99.5%	5 days
		Nitrate	0.181	0.0005	99.9%	5 days
		N-NH ₄ ⁺	0.802	0.0003	99.9%	5 days
		T P	0.007	0.0002	99.9%	4 days
	Aquaculture WW	TP	0.11	0.00011	99.9%	4 days
	AD municipal WW	N-NH ₄ ⁺	0.001	0.00012	99.9%	5 days
		TP	0.089	0.00016	99.9%	4 days

Due to the considerable volumes and flow rates involved in the wastewater industry, systems tend to be large-scale. Scalability is one of the major challenges faced by the algal biotechnology industry [50]. The experiments performed in this study took place in a pilot scale (1500 L) bioreactor. This is not a suitable scale for full wastewater treatment, only as a pilot. In the future, the PBR capacity needs to be increased. However, this scale of cultivation is superior to other studies presented in the literature. Nevertheless, direct comparisons with struvite precipitation are difficult. Nutrient recovery via active controlled struvite production is better developed. Phosphate and nitrogen can be removed at a faster rate and, in some cases, largely using struvite systems. This may be the only viable sustainable nutrient recovery option with respect to the large volumes and flow rates in the wastewater industry. In terms of valorisation of the products recovered, microalgae are a more favourable solution. In addition, the running costs of a struvite recovery system are high compared to a microalgal system especially if natural lighting is used and a flue gas source is utilised for CO₂.

The obtained results showed high remediation capacity and high microalgae biomass production. Additionally, this remediation study highlights the treatment of wastewater to dischargeable levels in scalable, controllable technology. These investigations were limited by the adopted cultivation nature of batch-mode. At this stage, we could predict the dynamic nature of re-supply of nutrients as the culture would be harvested at 2–3.3 day intervals, which would maximise both biomass and the remediation of P and N. The consideration based on 50% of the culture's volume could be harvested at the beginning of the exponential phase every 3.3 days. This represented roughly 1500 L of culture at 2.5 g L^{−1} and a supply of 0.585 mg P L^{−1} of culture. Tables 5 and 6 summarises the potential for microalgae production whilst remediating three bio-waste streams (based on P). Based on a simple mass balance for *N. oceanica* biomass production and remediation of P, 91.3 kg biomass may be produced

annually, which remediated 98.3 g of P using a 1500 L PBR. Seemingly, higher bioreactor volumes will have higher yields of P remediation and biomass production.

4.3. Product Development, Fertilisers and Feed Development.

Nutrient budget calculations with regards to nitrogen and phosphorus for fish farms have been carried out mainly with salmonids in marine and freshwater, but also in the Mediterranean Sea for European Sea Bass and Gilthead Sea Bream to estimate their impact on the eutrophication of the water body [36]. Due to strict governmental regulations on aquaculture, especially in Scandinavia, the trend has been to lower nitrogen and phosphorus levels in the fish feeds and/or to increase their digestibility [51]. Phosphorus and nitrogen are limited nutrients for plants and algae, especially as the dissolved fractions of these nutrients are biologically readily available, whereas the particulate fraction will accumulate on the sediment.

Table 6. Composition of commercially available feed ingredients and selected algal species (per dry matter) (modified from [52]).

Results from [52]	% Crude Protein	% Crude Lipid	% Crude Carbohydrate	% Ash	Gross Energy MJ/kg
Fish meal	63.0	11.0	–	15.8	20.1
Poultry meal	58.0	11.3	–	18.9	19.1
<i>Spirulina</i>	58.0	11.6	10.8	13.4	20.1
<i>Chlorella</i>	52.0	7.5	24.3	8.2	19.3
<i>Tetraselmis</i>	27.2	14.0	45.4	11.5	18.0
<i>Nannochloropsis</i>	42.8	16.6	33.9	6.7	22.6
Results from the current study					
<i>Nannochloropsis oceanica</i>	21–36	17–34	19–27	23	4–6.5

Algal biomass could be used in many ways. Firstly, it is a very good ingredient for feed formulation. Based on the results produced by [52] and [53] (Table 6), our algal biomass presented a good alternative for aquaculture and poultry feed development. *N. oceanica* contains essential omega 3 fatty acids, which are very important components of human and animal diet. Additionally, fish oil supplement production is limited, and fatty acid production from marine algae is considered as an alternative source of this valuable compound. Algae grown using municipal waste can be used as a component of shrimp/fish feed [54]. This represents a significant valorisation of phosphorous recovered as microalgal biomass as opposed to struvite. In addition, the use of microalgae as a waste bio-remediation technology is highly advisable when a circular economy approach is needed [35].

Secondly, the use of algal biomass as a fertilizer could be also considered [55], the biomass contains a high level of nitrogen and phosphorus. It is biodegradable and could be easily used on the land. However, we propose that the produced algal biomass is more valuable in its alternative use as animal feed or extracted compounds for human supplements. Future research needs to be done to evaluate the potential presence of toxic compounds and heavy metals in the algal biomass obtained from bioremediation trials for food and feed consumption. The regulations also need to be complied with for this algal biomass application.

5. Conclusions

In this study, bioremediation of three waste streams at a scale of 1500 L was done using *N. oceanica*. These trials highlighted high level of adaptation of this species to the waste-based media with a high nutrient uptake rate (above 90% at the day 4). The produced biomass was evaluated for product development and based on biochemical composition it would be used as bio-fertiliser and/or potentially suitable for aquaculture feed development, if the biomass heavy metal and biological contaminants analysis comply with regulations. The remediation uptake was compared to the struvite method and algal bioremediation was longer; however, the valorisation of the produced biomass was much higher

and algal biotechnology could be used in circular economy. The results further demonstrate the potential for semicontinuous or continuous cultivation in pilot scale with continuous biomass production.

Supplementary Materials: The following are available online at <http://www.mdpi.com/1996-1073/12/14/2772/s1>, Table S1: Results of GLM+ANOVA test on crossed factors (time of experiment and waste stream) on the variables; dry weight, ammonium and phosphorus uptake and intracellular N and P. Significance is shown by; ***: $p < 0.001$, **: $p < 0.01$ and *: $p < 0.05$. Results of post hoc Tukey tests indicating levels of significance. C: Control; AQW: Aquaculture Waste; ADMW: AD Municipal Waste; AW: Agricultural Waste.

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