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Development of a Culture Medium for Microalgae Production Based on Minimal Processing of Oil Palm Biomass Ash

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Abstract: With the increasing participation of biomass in the world energy matrix, large amounts of ash are produced through combustion, resulting in the need to dispose of this waste to minimize the environmental impact. An alternative is to use ashes as phosphorus supplements in microalgae cultures. The present work describes the development and use of a balanced culture medium based on the minimal processing of oil palm biomass ash to cultivate *Arthrospira platensis* Paracas, *Neochloris oleoabundans* UTEX 1185, and *Dunaliella salina* SAG 184. The acid extraction process of phosphorus (P) was defined by evaluating the following parameters: temperature (20 to 70 °C), acid load (0.01 to 0.03 mols/g of ash) of HNO_3, and liquid/solid ratio (50 to 150 mL g^{-1}). The best efficiency of the extraction process was 97%. The use of HNO_3 allowed for the production of an extract containing balanced amounts of N and P sources, the BAX medium (Biomass Ash Extract). This medium was efficient for cultivating the three microorganisms studied, reaching biomass concentrations of 2.03, 0.902, and 0.69 g/L or 84%, 82%, and 99% of the control concentrations for *A. platensis*, *N. Oleoabundans*, and *D. salina*, respectively. In a final scaling-up test, *A. platensis* showed productivity of 0.047 g $L^{-1}d^{-1}$ in a 120 L tank in a greenhouse. BAX can be an alternative nutrient medium for microalgae cultivation, especially in integration with biomass-fueled biorefineries.

Keywords: phosphorus; microalgae; *Dunaliella*; *Arthrospira*; *Neochloris*; nitric acid

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1. Introduction

Bioenergy participation in the world energy matrix has been growing by 8% yearly since 2006 [1]. It is estimated that by 2050, up to 33–50% of the world's primary energy consumption could be provided by biomass [2,3]. Today, plant biomass used directly as an energy source contributes to 10% of the global energy supply. Part of this use is for cooking and heat, generating a scattered residue. However, much of the biomass is used in industries—16 to 18% in the United States of America, India, and Brazil in 2009 [4]. This industrial use generates large and localized volumes of ash, which must be disposed of. Biomass presents significant advantages mainly because of the reduction of CO_2 emissions and reduced dependence on the use of fossil fuels [5].

The increased use of biomass, mainly by the industrial sector, also increases the amount of residual ash generated. Those wishing to apply ash to soil must consider the composition, which is variable and depends on factors such as soil type and composition, crop and cultivation method, season, and combustion temperature [6–9]. A possible use for biomass ash, rich in inorganic compounds, is its reintegration into the soil as a fertilizer. However, the residue is nutritionally unbalanced—virtually all the nitrogen is volatilized upon burning, and high concentrations of silicon, calcium, and aluminum are often present. Proper pretreatment is essential: although less toxic than coal ash, biomass ash can cause environmental problems [9].

On the other side, microalgal cultures—especially those envisaged for large-scale production of lipids for biodiesel production—demand large amounts of nutrients. Biomass ash can provide part of these nutrients, reducing the dependability on fertilizers that are obtained chiefly from mining, except for nitrogen (fixed via the Haber–Bosch process). The most important macroelements for microalgal cultures, besides carbon (as CO_2), are nitrogen (N), sodium (Na), potassium (K), calcium (Ca), phosphorus (P), sulfur (S), magnesium (Mg), and chlorine (Cl) [10,11]. Ash can provide most of these elements but cannot be used directly in liquid culture media because of low nutrient availability (low solubility) and particulate suspended solids' shading effects, which reduce light penetration and thus microalgae culture growth.

Of the nutrients recoverable from ash biomass, phosphorus is the most important for microalgal cultures. It is a non-renewable resource obtained mainly from mining. Researchers have proposed that the growing demand for this element in agronomy will exceed the global exploration capacity of mineral reserves in 50 to 100 years, a concept called "peak phosphorus" [12]. Little of the extracted P is returned to food and feed [13]. A large amount of P is transferred from mined resources to plants and animals through agriculture and the food chain, and most is lost along with its flow, ending up in agricultural leachates, immobilized in landfills, or is lost in industrial and municipal wastewaters. The phosphate content in worldwide freshwater systems is at least 75% higher than pre-industrial levels, and P flows in the oceans have increased from 8 to 22 million metric tons per year during the same time [14]. Therefore, the extraction of P from the ashes of plant biomass is an alternative to supply it for microalgae cultures, and it increase circularity.

The recovery of phosphates from waste streams is intensively researched, with municipal sludge ashes and biomass ashes representing promising nutrient sources. The recovery typically uses low-cost and efficient sulfuric acid [15,16]. Other acids can be used, such as hydrochloric [17], nitric [18], and even organic acids such as oxalic [19]. Among these acids, the only one that can also be used as a nitrogen source is nitric acid. Phosphates from liquid streams such as digestates from municipal wastewater plants can also be recovered as struvite [20–22].

One of the most abundant biomass sources available as fuel, thus generating large quantities of ash, is biomass fractions from oil palm (*Elaeis guineensis*). Palm oil is the most important vegetable oil worldwide, with an annual production of around 70 million tons and growing. Its wastes, such as EFB (Empty Fruit Bunch), palm fiber, palm shells, are generated in large amounts, since only 10% of the whole plant is used for oil production [23,24]. Palm processing industries need large amounts of steam, which is generated in boilers fueled by burning residues, preferentially the fibers from the pressing cake [24]. Searching for potential uses for lignocellulosic materials, many authors evaluated the composition of palm processing residues, including ashes. Significant amounts of potassium and phosphorus are found in the biomass ash (Table 1).

Table 1. Mineral Composition of oil palm biomasses and ash fractions.

	[25]	[26]		[27]	[28]	[29]	[30]	[31]	[32]	[33]
Components	**EFB**	**EFB**	**PS**	**OPA**	**OPA**	**OPA**	**APA**	**POFA**	**POFA**	**POFA**
SiO_2	12.12	27.00	49.70	86.44	35.60	37.00	40.00	64.17	67.72	40.00
CaO	9.65	8.00	10.20	-	12.00	9.20	10.00	5.80	5.57	10.00
K_2O	55.48	44.00	12.20	-	11.00	11.00	12.10	8.25	7.67	12.10
Al_2O_3	0.26	-	7.70	6.49	4.80	14.30	6.10	3.73	3.71	6.10
MgO	1.90	4.80	6.90	1.51	7.20	6.10	6.40	4.87	4.04	6.40
P_2O_5	3.58	3.60	8.40	-	6.80	6.20	8.32	5.18	-	8.20
Fe_2O_3	-	3.00	2.70	4.08	2.00	2.50	-	6.33	4.71	2.50
SO_3	1.66	2.70	-	0.20	-	-	-	0.72	1.07	-
Na_2O	0.09	-	-	0.10	-	0.10	-	0.18	0.16	-
Cl	6.84	5.30	-	-	-	2.90	-	-	-	-

Table 1. *Cont.*

	[25]	[26]		[27]	[28]	[29]	[30]	[31]	[32]	[33]
Components	**EFB**	**EFB**	**PS**	**OPA**	**OPA**	**OPA**	**APA**	**POFA**	**POFA**	**POFA**
C	-	-	-	-	-	-	-	-	-	5.40
TiO_2	-	-	-	-	-	-	-	0.19	-	-
MnO	-	-	-	-	-	-	-	0.18	-	-
MnO_2	-	-	-	0.10	-	-	-	-	-	-
ZnO	-	-	-	-	-	-	-	0.03	-	-
Rb_2O	-	-	-	-	-	-	-	0.06	-	-
Cr_2O_3	-	-	-	0.69	-	-	-	0.03	-	-
Rh_2O_3	-	-	-	0.41	-	-	-	-	-	-
CuO	-	-	-	-	-	-	-	0.08	-	-
RuO_2	-	-	-	0.29	-	-	-	-	-	-
SrO	-	-	-	-	-	-	-	0.02	-	-
ZrO_2	-	-	-	-	-	-	-	< 0.01	-	-
NiO	-	-	-	-	-	-	-	0.02	-	-
Y_2O_3	-	-	-	-	-	-	-	< 0.01	-	-

Note: EFB: empty fruit bunch; PS: palm shell; OPA: oil palm ash; APA: activated palm ash; POFA: palm oil fuel ash.

Recovered, phosphorus can be used as a mineral nutrient for cultivating microalgae and cyanobacteria, since this is one of the essential elements in the cell composition [34,35]. On-site recovery also gives an alternative to value addition and zero-waste development in biorefineries, including a microalgal cultivation step and producing algal biomass rich in proteins, pigments, and even energetic and dietary lipids. Thus, the study's objective is to explore the use of nutrients present in minimally processed palm fiber ash to cultivate the cyanobacteria *Arthospira platensis* and the microalgae *Neochloris oleoabundans* and *Dunaliella salina*, with a focus on phosphorus recovery through treatment with nitric acid, thus developing a nitrogen-to-phosphorus balanced culture media based on minimal processing of biomass ash from the palm oil industry.

2. Materials and Methods

2.1. Oil Palm Ash

Ash from oil palm was obtained from BioPalma S.A., located in Moju, PA, Northern region of Brazil. Oil palm ash was collected and sieved to remove non-calcined materials, such as fibers. The granulometry distribution (Figure 1) was determined using a 6-screens lab sifter (Bertel VP-01, Lazanjeiras, SP, Brazil). Ashes used for extractions were homogenized lightly in a mortar. To remove the moisture eventually adsorbed, the ash was dried in a laboratory oven (Fanem Orion 502, Guarulhos, SP, Brazil) at 85 °C for 15 h.

The residue was analyzed by X-ray fluorescence spectrometry (FRX) (Table 2). The equipment used was an Axios Max (Malvern PANalytical, Almelo, Netherlands). The results were interpreted using the software SuperQ 5l®, from the same manufacturer.

Table 2. Composition of OPA.

Components	wt% of Dry Solids
SiO2	49.40
K2O	11.60
CaO	8.70
P2O5	8.60
MgO	5.80
Fe_2O3	5.60
Al_2O3	1.60
SO3	1.60
Cl	0.70
TiO2	0.20

Table 2. *Cont.*

Components	wt% of Dry Solids
MnO	0.10
SrO	0.10
Na_2O	0.10
ZrO2	<0.10
CuO	<0.10
ZnO	<0.10
LOI.	5.99

Note: LOI.: lost on ignition. Results normalized for 100%.

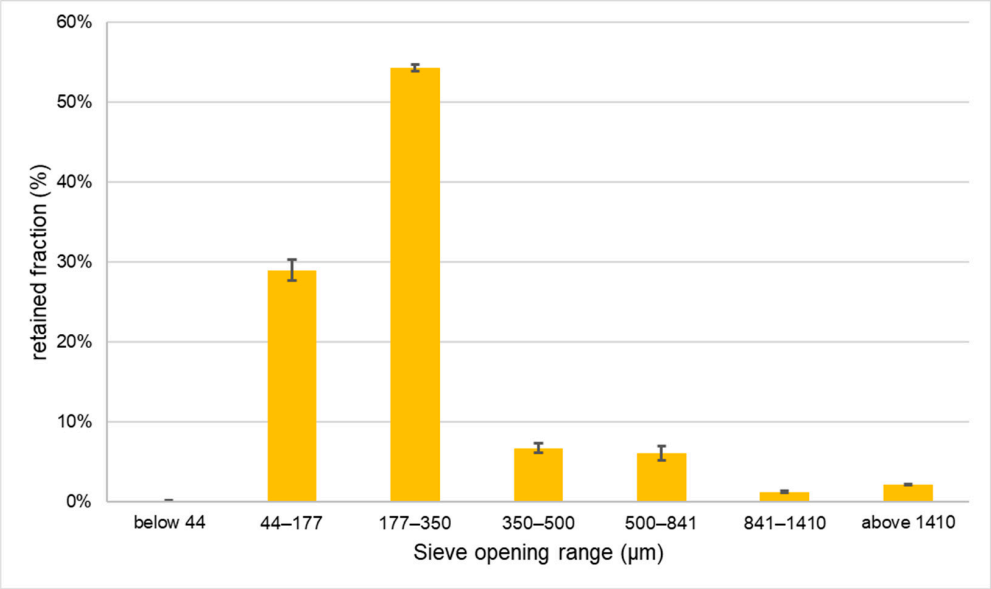

Figure 1. Particle size distribution of oil palm ash.

2.2. Nutrient Extraction from Ash

Ashes were leached to extract phosphate. A 3^3 experimental design was used, based on conditions described in the literature for of acid extraction of phosphate from diverse ash materials [36–42]. The independent variables were temperature, mols of acid (as nitric acid), and Liquid/Solid Ratio (L/S). In all experiments, 1 g of ash was mixed with the acid and an adequate amount of Milli-Q water to reach the final intended volume. The levels of the variables are shown in Table 3.

Table 3. Conditions of the variables in the experiment.

Temperature (°C)	Moles of Acid	L/S (mL g^{-1})
20	0.01	50
45	0.02	100
70	0.03	150

The contact time was defined as 24 h, previously defined as enough time for reaching quasi-equilibrium. After extraction, the mixtures were filtered through nitrocellulose membranes with 0.45 µm pore size to separate the remaining ash from the extract. The extracts were analyzed for the quantification of phosphate.

2.3. Kinetics of Phosphate Extraction

The extraction kinetics of phosphate was evaluated to determine the minimum contact time necessary for extraction. The extraction was performed for 30 h, using the mildest conditions of the initial factorial design (lower temperature and acid concentration, i.e., 20 °C;

0.01 moles of HNO_3; L/S 150 mL g^{-1}, periodic stirring). Samples were periodically collected in 2 mL Eppendorf tubes, centrifuged at 10,000 rpm for 5 min, and the supernatant was evaluated for phosphate concentration.

2.4. Microorganisms and Culture Media

Three microorganisms suitable for cultivation on a large scale were used as models for studying ash extracts as culture media: the cyanobacterium *Arthrospira platensis* strain Paracas and the microalgae *Neochloris oleoabundans* UTEX 1185 and *Dunaliella salina* SAG 184.8.

Inoculum and control culture media: The inoculum of *Arthrospira platensis* was routinely maintained by successive culturing in Zarrouk medium [43], with initial pH 9.5 and at 30 °C. The inoculum of *Neochloris oleoabundans* was routinely maintained by successive cultures in BG11 medium [44], with initial pH 7.5 and at 25 °C. The inoculum of *Dunaliella salina* was routinely maintained by successive culturing in Modified Johnson's Medium (MJM) [45], adjusted to pH 7.1 and at 25 °C. The three cultures were maintained in 3 L Erlenmeyer flasks aerated with 1 vvm of air and irradiation of 25 µmol photons $m^{-2}s^{-1}$. Control cultures for comparison with extracts had the same compositions and conditions, except for aeration—which was by diffusion, with daily manual homogenization.

BAX (Biomass Ash Extract) media: To evaluate the suitability of oil palm ash extracts as a nutrient source for microalgae growth, the extracts were diluted to match the control media concentration. The conditions for extraction were selected among the experiments that showed the highest phosphate solubilization efficiency in the factorial design. Contact time was 24 h. The supernatant (extract) was filtered through a nitrocellulose membrane with 0.45 µm pore size. The extract was separated into three fractions and diluted to match the theoretical usable concentration of P in each Control medium (Zarrouk, MJM, and BG11), respecting the Redfield ratio of 16 mols of nitrogen for each mol of phosphorus. The pH was adjusted with 10 M NaOH to match the initial pH in control cultures. Additionally, 87.7 g L^{-1} NaCl was added for *Dunaliella salina*, specifically to maintain adequate salinity. Culture volume was 500 mL (450 mL culture medium + 50 mL inoculum) in 1 L Erlenmeyer flasks, with aeration by diffusion and gentle manual shaking every 24 h. Initial concentrations were 0.09 g L^{-1} for both *A. platensis* and *N. oleoabundans* and 0.13 g L^{-1} for *D. salina*. Light intensity was 25 µmol photons $m^{-2}s^{-1}$, with continuous light, and constant temperature of 25 °C for *Neochloris oleoabundans*, *Dunaliella salina*, and 30 °C (±5 °C) for *Arthrospira platensis*. Each control and treatment were conducted in triplicate.

2.5. Analytical Methods

Phosphate was determined using a colorimetric assay with Malachite Green (Millipore Sigma, St. Louis, MI, USA) [46]. All tests were performed using genuine triplicates.

Microalgal biomass was evaluated by daily sampling and evaluating the absorbance at 670 nm (UV1601, Shimadzu, Kyoto, Japan) [47–49]. Dry mass analyses were performed every 6 days with 50 mL of sample. Samples were filtered through 3 µm (*A. platensis and N. oleoabundans*) and 1 µm *(D. salina*) nitrocellulose membranes. The biomass was washed with 10 mL ammonium formate (0.5 mol L^{-1}), dried for 24 h at 80 °C, and weighed [50].

2.6. Statistical Analysis

Experimental design planning and analysis were performed using the software Statistica® V. 7.0. Significant effects at the level of 5% confidence were considered through analysis of variance (ANOVA). All figures were prepared using MS-Excel® V. 16.0.

3. Results and Discussion

Preliminary tests using raw ash added with nitrogen sources showed poor microalgal growth, presumably because of self-shading caused by insoluble, suspended particulate material and poor nutrient leaching at culture pH. To evaluate the suitability of culturing

microalgae using nutrients from ash, we first evaluated and optimized the extraction, then tested extracts as the base for culture media, and finally chose conditions for scaling up.

3.1. Phosphate Extraction

It is well known that lower pH promotes more significant dissolution of P from ashes [36,38,41,51,52]. Typically, strong acids such as H_2SO_4 and HCl are used to dissolve phosphorus due to their low cost [36,38,41,52,53]. In this investigation, we used nitric acid: it is a strong acid, forms only soluble salts, and it is suitable as a nitrogen source for microalgal cultures, which would have to be added downstream if another acid were used as extractant. The temperature and liquid/solid ratio used were based on the literature, while the amount of acid was calculated based on the amount of phosphate theoretically extractable to give extracts respecting the Redfield ratio [54,55]. Table 4 shows the experimental conditions and results for a 3^3 experimental design.

Table 4. 3^3 experimental design for phosphate extraction.

Sample	Experiment Conditions			Recovered P_2O_5 (g)	Dissolution of P_2O_5 (%)
	Temperature (°C)	Acid Added, Mols	Liquid/Solid Ratio (mL g^{-1})		
1	70	0.01	50	0.067	78.39
2	70	0.01	100	0.065	75.31
3	70	0.01	150	0.058	67.31
4	70	0.02	150	0.084	97.52
5	70	0.03	150	0.073	85.21
6	70	0.03	100	0.069	79.94
7	70	0.03	50	0.072	83.91
8	70	0.02	50	0.073	85.18
9	70	0.02	100	0.076	88.59
10	45	0.01	50	0.059	68.76
11	45	0.01	100	0.064	74.86
12	45	0.01	150	0.057	66.64
13	45	0.02	150	0.060	69.33
14	45	0.03	150	0.065	76.04
15	45	0.03	100	0.070	81.43
16	45	0.03	50	0.069	79.88
17	45	0.02	50	0.060	69.96
18	45	0.02	100	0.065	75.46
19	45	0.02	100	0.066	76.65
20	45	0.02	100	0.065	75.31
21	20	0.01	50	0.062	72.42
22	20	0.01	100	0.059	68.90
23	20	0.01	150	0.049	57.02
24	20	0.02	150	0.063	73.35
25	20	0.03	150	0.064	74.25
26	20	0.03	100	0.074	86.50
27	20	0.03	50	0.062	72.12
28	20	0.02	50	0.062	72.27
29	20	0.02	100	0.063	72.92

Note: Samples 18, 19, and 20 represent triplicates of the central point in the factorial experiment.

The amount of HNO_3 chosen was defined according to the foreseen formulation of culture media. Absolute amounts from 0.01 to 0.03 mols acid g^{-1} ash were estimated to reach the ideal N:P ratio. The acid concentrations ranged from 0.067 to 0.6 mol L^{-1}. This reasoning made it possible to evaluate the L/S ratio without any conflict between variables simultaneously. An 8% efficiency gain in dissolution between 0.01 and 0.02 mol was observed, but a slight increase of 2% from 0.02 to 0.03 mols of HNO_3. With the L/S studied (50/1, 100/1, and 150/1), 0.02 and 0.03 mols of HNO_3 presented better results. An analysis of the concentration showed that the highest initial acid concentrations did not necessarily give the best dissolution efficiencies: stoichiometry and temperature are more important.

Analyzing temperature, acid addition, and L/S ratio as variables influencing phosphorus extraction, the temperature was the variable that showed the most significant influence

on the dissolution, followed by the number of mols, while L/S was not significant at p = 5%. The increase in temperature from 20 to 45 °C promoted an increase of only 2% in the extraction, which may not justify the energy expenditure of a process where average temperatures can be maintained around 20 °C (Figure 2). However, from 20 to 70 °C, there is an increase of 10% in the dissolution efficiency of P. The increase in temperature and acidity increases solubility of phosphorus from palm biomass ash, similarly to what was is described in the literature for sludge and biomass ashes [36,51].

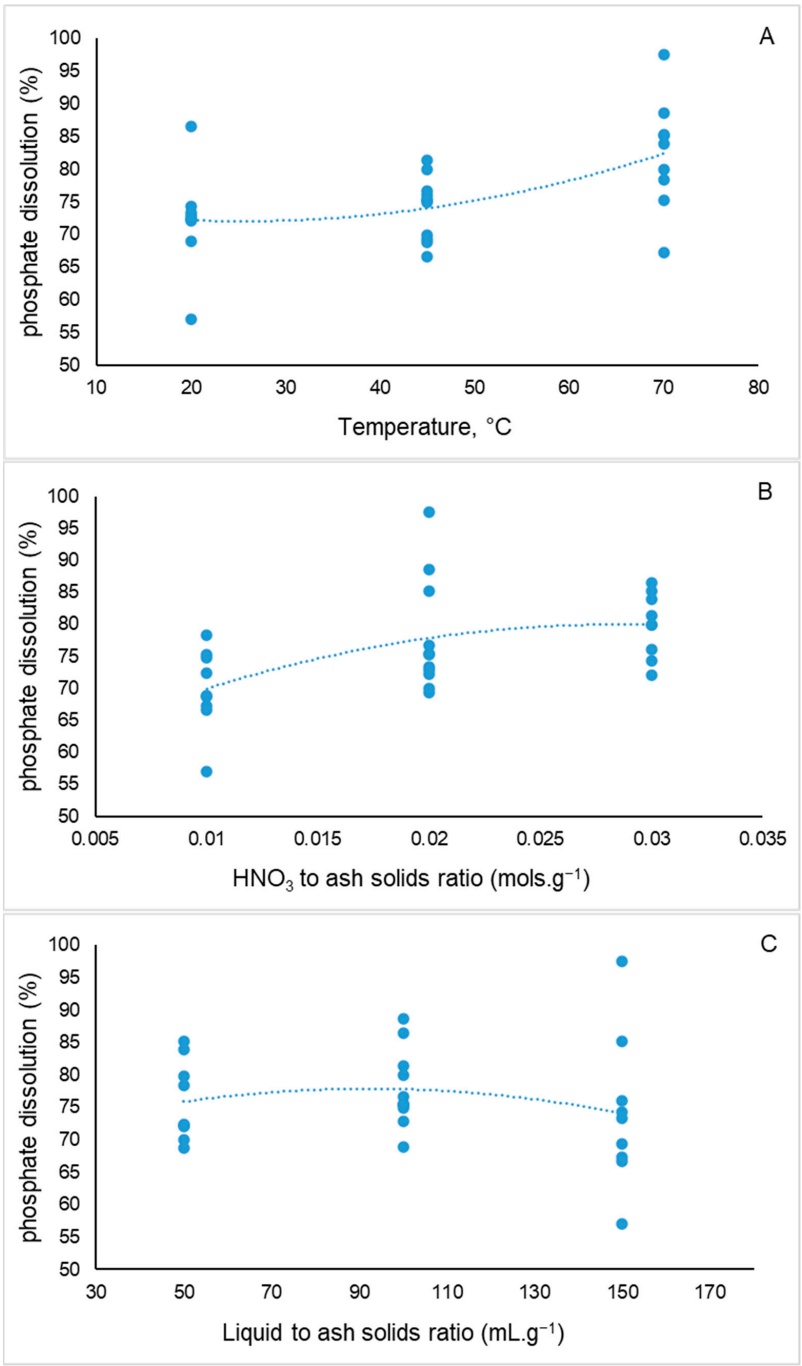

Figure 2. Phosphate dissolution is favored by higher temperatures (**A**) and higher acid content (**B**), but is not significantly affected by concentration (liquid to solids ratio, (**C**) in the range evaluated. The dispersion of each group of points shows the effect of the two complementary variables, e.g., temperature and HNO_3: Ash in Figure 2C.

The dissolution of P_2O_5 varied by 40%. The lowest P dissolution occurred under pH 1.58, while the highest efficiency, 97% (Table 4), was at pH 1.12. Extraction yields near to 100% were reported at pH 1.0 using H_2SO_4 to treat sewage sludge ash [38], with a far lower recovery (40%) using 0.6M HNO_3, however, with an L/S ratio of 5.0, which is 10 to 30 times lower than what was used in this present study. In another study with sewage sludge ash, 0.0075 mol of H_2SO_4 in 150 mL g^{-1} and 0.015 mol of HCl in 150 mL g^{-1} gave satisfactory results, close to 100% phosphate dissolution [36]. A similar result was only obtained in the present study at 70°C with 0.02 mol of HNO_3. This difference may have been due to the intermittent agitation: ash sedimentation may have reduced contact with the liquid. The extraction efficiency also likely varies for different materials because of different compositions [41] and acid neutralization by alkali oxides in the ashes.

The L/S ratio showed no significant influence (at P = 5%) on the dissolution of P at the levels tested, presenting a slightly negative variation of 1.8% from 50 to 150 mL g^{-1}. That could be explained not because the volume does not matter, but because all chemical species formed are soluble at the level tested, and that acid-to-ash stoichiometry and the consequent final pH is critical for this dissolution.

3.2. Kinetics of Extraction

The extraction efficiency was plotted against contact time for 30 h to evaluate the dissolution kinetics. The mildest extraction conditions were chosen: 20 °C; 0.01 mols of HNO_3; 150 mL g^{-1}. P dissolution is highly dependent on the contact time [36,52] and increases with time as an asymptotic curve (Figure 3). Only 15 min was enough to dissolve 45% of the phosphate, but 8 h were needed to reach the equilibrium concentration (an efficiency of 60%). The relatively fast extraction of phosphorus may be due to its presence as soluble salts in the ashes [56] and the small particle size [41].

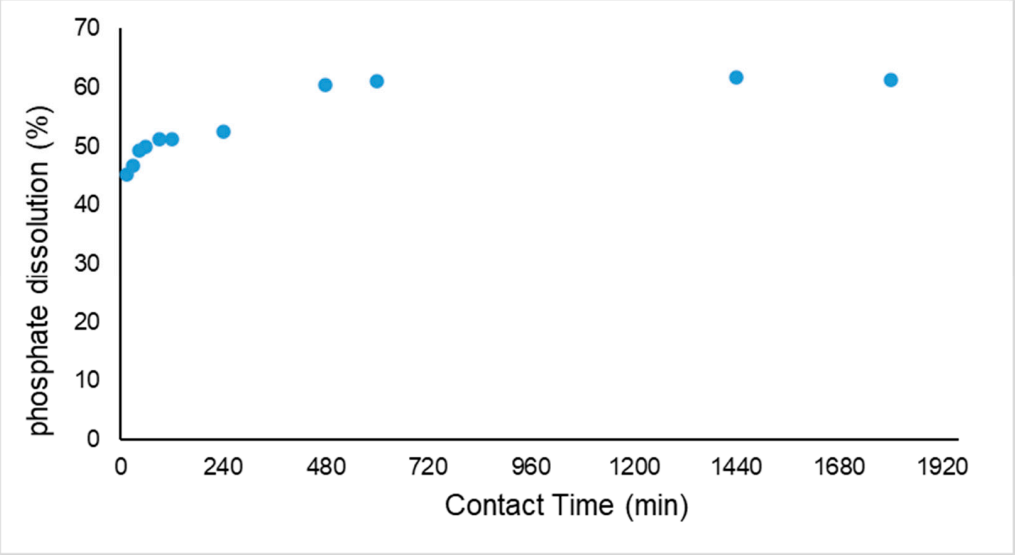

Figure 3. Kinetics of P dissolution.

3.3. Cultivation of A. platensis, N. oleoabundans, and D. salina on BAX Medium

Both nitrogen and phosphorus must be present in microalgae culture media. Although H_2SO_4 and HCl are more common for phosphate dissolution, the use of HNO_3 as extractant acid allowed the supplementation of adequate amounts of nitrogen to cultivate cyanobacteria and microalgae. In balanced growth conditions, microalgae biomass has an average molar proportion of 106:16:1 (C:N:P) on its composition [34], although deviations exist [55,57]. After the extraction of P, it was examined which conditions have the N:P ratio closest to 16 (Figure 4).

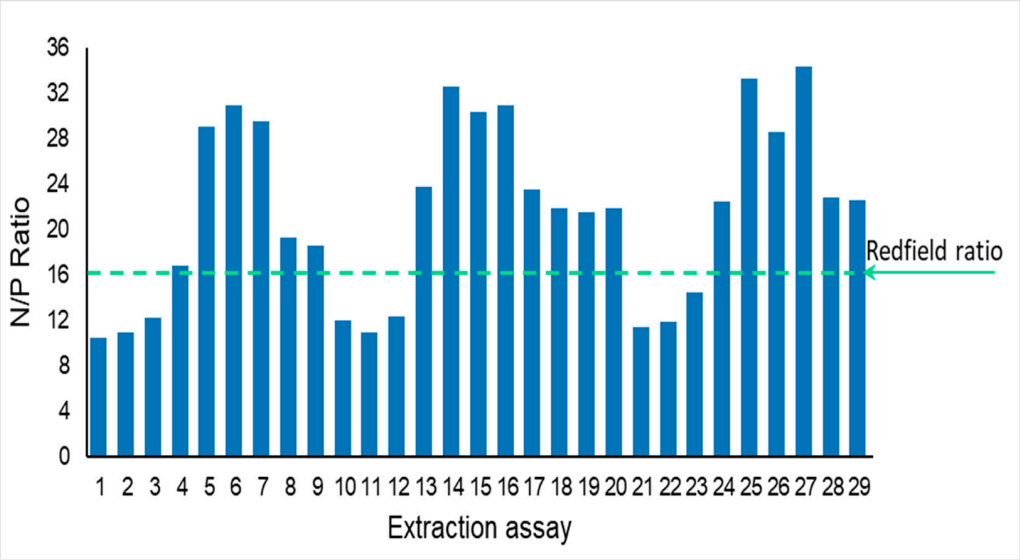

Figure 4. N:P ratio of the palm oil ash extracts.

Values above 16 mean nitrogen excess and below 16 mean phosphorus excess. Besides presenting the best extraction of P, Condition 4 presented the best ratio for cultivation: 16.9 is near the Redfield ratio with a slight excess of nitrogen, recovering 97% of the P extracted from oil palm ash.

After adjusting the pH, BAX was diluted 4.3, 34.3, and 30.6 times to match the theoretical usable concentration of P in each control medium (Zarrouk, BG11, and MJM, respectively), respecting the Redfield ratio. (Figure 5) shows the biomass production of *A. platensis*, *N. oleoabundans*, and *D. salina* in the BAX medium.

A. platensis: At the beginning of the cultivation (Figure 5A), both control (Zarrouk medium) and BAX were similar. The cyanobacteria initially had a low growth rate, probably due to the low initial cell concentration, of 0.09 g L^{-1}. After 18 days, BAX cultivation growth started to slow down. The difference between control and BAX at this point is noticeable. On day 31, BAX cultivation reached 82% of the control biomass final concentration. Growth of *Arthrospira sp*. using POME (palm oil mill effluent) was observed in N:P ratios of 2.7:1 and 2.6:1 [58], while Zarrouk had an N:P ratio of 10:1. It is possible that *A. platensis* does not need an N:P ratio of 16:1, and the excess of nitrate in the BAX medium may have influenced the growth from day 18, promoting a slight reduction in biomass [59]. However, a hypothesis has been raised that the main reason for better growth in the Zarrouk medium is its initially large concentration of CO_2 as sodium bicarbonate.

N. oleoabundans: From day 4 to 8, the BAX culture exhibited a slightly reduced growth compared to the control (BG11 medium), probably due to the process of acclimatizing the cells to the new medium. After day 12, *N. oleoabundans* elevated the growth rate and finished the 31 days of culture with 0.9 g L^{-1}, while the control had 1 g L^{-1} (Figure 5B). Kinetics of cyanobacterial and microalgal growth was examined with poultry litter ash and showed better results when the N source was only ammonium [60]. However, the authors reported that 2.5 g L^{-1} $NaNO_3$ was added to prevent nitrogen limitation, 100 mg L^{-1} Na_2-EDTA was added as a chelating agent, and cultures had aeration of 2 L of air min^{-1} and luminous intensity of 121.5 µmol photons $m^{-2}s^{-1}$, far superior than the diffusion aeration and 27 µmol photons $m^{-2}S^{-1}$ used in the present study.

D. salina: (Figure 5C) shows that this microalga had the best growth in the BAX medium compared to the control (MJM medium). The cultures had similar growth since day 0, reaching final concentrations of 0.69 and 0.70 g L^{-1}, respectively.

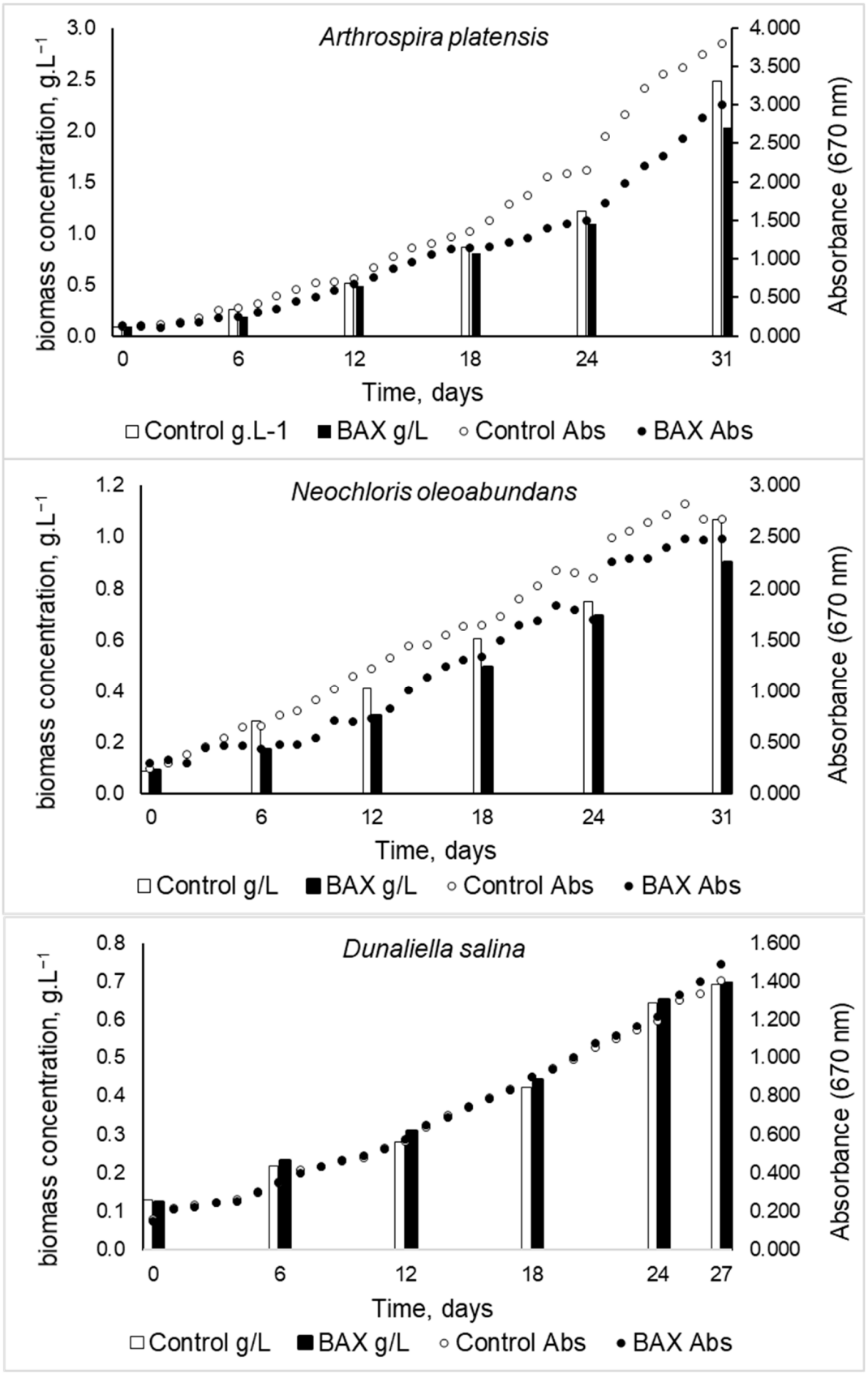

Figure 5. Biomass production of *Arthrospira platensis* (**A**), *Neochloris oleoabundans* (**B**) and *Dunaliella salina* (**C**) on control (synthetic) and BAX medium. All results are average of triplicates.

For all microorganisms, 27 to 31 days of cultivation, without agitation or forced aeration, was necessary to evaluate the behavior of long-term cultures. All considered, the biomass production and growth rate were comparable in BAX and control media, and to the authors' best knowledge, there are no published data of oil palm Ash used to grow cyanobacteria and microalgae. The results were satisfactory and proved that phosphorus in the ashes could be used to grow cyanobacteria and microalgae at rates close to or equivalent to widely popular media.

3.4. Scaling Up

The scaleup (120 L, open tank, greenhouse) was carried out to verify growth replicability in large scale, and also two hypotheses: (1) to verify if the aeration would compensate the limitation of the growth of the microalga cultivated in BAX medium when compared with the Zarrouk medium (limitation observed in the laboratory), and (2) because of the ability of *Arthorspira* to basify the medium to higher values (pH 11), to verify the possibility of reduced growth due to reduced availability of phosphate because of alkaline precipitation as Ca or Mg salts.

In growing on a larger scale (Figure 6), the growth pattern was similar to that observed in the laboratory, but the initial cell concentration was higher (0.22 g L^{-1}). After 25 days of cultivation, the concentration obtained was 1.40 g L^{-1} and the productivity 0.0472 g $L^{-1}d^{-1}$, both similar to the laboratory scale culture in the same period. At 31 days of culture, the final cellular concentration observed was 1.6 g L^{-1}, corresponding to 80% of the value obtained in the culture on the laboratory scale. The difference of 20% in the cellular concentration between the scales evaluated can be attributed to natural temperature variability from 22 to 36.7 °C, with an average of 36.7 °C, which may have caused a decrease in culture growth—*Arthorspira* requires temperatures between 30 and 38 °C for optimal growth [61]. Another critical factor is that the cultivation on an enlarged scale was conditioned to a natural photoperiod of 13 h/11 h, unlike laboratory cultures where irradiation was constant during the 31 days.

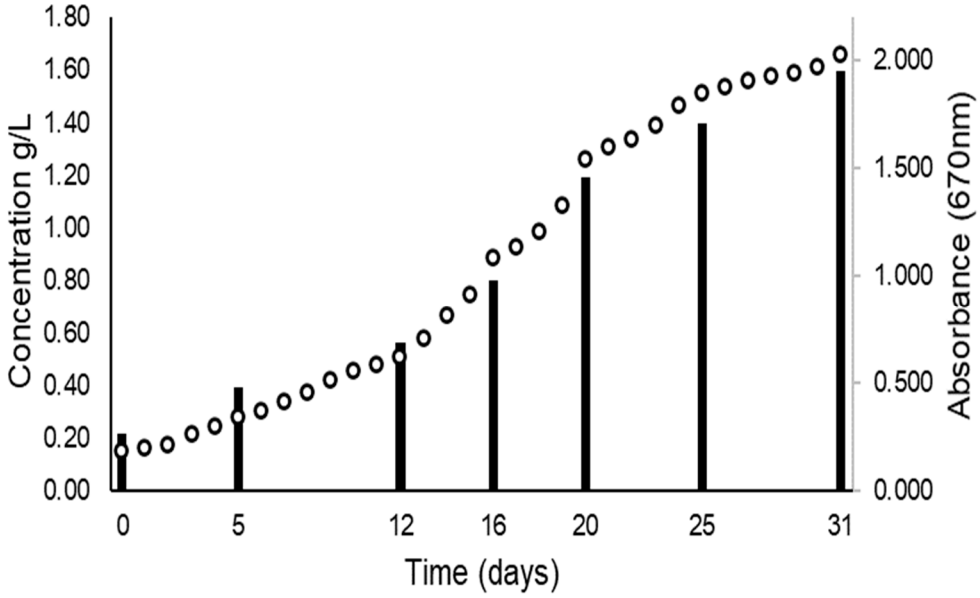

Figure 6. Growth kinetics of scaled-up *A. platensis* cultivation conducted in a greenhouse with a BAX medium.

3.5. Practical Applications and Future Research

Microalgae are intensively researched as potential food, feed and bioenergy sources. However, one of the drawbacks of large-scale cultures of microalgae is the requirement for nutrients, which increases costs and makes only a few biomass products commercially

viable at the moment. The successful cultivation of three important microalgae in ash-extract based culture media (BAX) shows that commercial fertilizers can be substituted as nutrient sources for microalgae, and that the judicious use of nitric acid is adequate to enhance extraction and provide a nitrogen source. BAX could be prepared using other acids, such as sulfuric acid; however, a nitrogen source would have to be added tom complete a culture medium. The use of ash increases the circularity of processes, recovering phosphorus; the high yield shows that a similar process could also be used for recovery of phosphorus for other uses.

The BAX medium is simple to prepare, requiring only pH correction and, for marine microalgae, salt addition. It recycles phosphorus and other mineralized nutrients otherwise lost in biomass ash wastes. However, there are several aspects than can be improved; the three most important are

(i) Scale-up was similar to laboratory cultures, but with modest productivity. It is possible that warmer temperatures lead to better growth. Repeated batches in a long running experiment will also show if some nutrient lacking, e.g., sodium for *Spirulina*, or in excess such as iron (usually present in low concentrations in synthetic media) limit the growth or affect the composition.
(ii) Oil palm ash has a high concentration of potassium, a most needed element in plant fertilizers, but not important enough for microalgae growth. Fractionation of palm ash, e.g., through selective or sequential extraction could make even better use of this residue and maybe integrate it to palm production, reducing the need for fertilizer.
(iii) Large-scale processing of palm oil ash would require reactors, pumps, and filters. Even if the equipment is simple compared to the large-scale equipment used in palm processing, it involves a capital cost that would have to be compensated by the income from biomass products. This requires a process simulation and sensitivity analysis to indicate if microalgae production, although technically feasible, can be profitable in its biorefinery integration with palm processing.

4. Conclusions

This proof-of-concept study proved that minimally processed palm ash could be used in microalgae production. Because liquid media and nitrate as nitrogen source are required, it is sufficient to treat the ash with nitric acid. Phosphorus dissolution by HNO_3 presented satisfactory results at a temperature of 70 °C and an HNO_3 concentration of 0.2 M (a mass proportion of 1.26:1 HNO_3:ash). In these conditions, up to 97% of the phosphorus was dissolved after 24 h. Lower temperatures (20 °C) are enough to extract about 72% of the phosphate, and 8 h was shown to be enough to reach equilibrium in the mildest conditions. The use of the BAX medium was shown to be efficient for the three microorganisms studied, giving biomass production and productivity similar to control cultures performed in synthetic media, albeit with room for improvement. A scaled-up, 120 L cultivation of *A. platensis* conducted in a greenhouse confirmed the feasibility of this residue use approach.

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