ALTERNATIVE MEDIA FOR CHLORELLA VULGARIS CULTIVATION USING PHOSPHORUS RECOVERED FROM BIOMASS FURNACE ASH AND DIFFERENT NITROGEN SOURCES – CIRCULARITY AND PROCESS ECONOMICS

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ABSTRACT

Huge amounts of ashes are generated in industry furnaces, with a significant quantity of phosphorus and other minerals being thus lost or not properly reintegrated into the biogeochemical cycle. In this study, wood-fired furnace ashes were acid-treated to produce an extract rich in phosphorus and other nutrients. With an experimental planning varying solid:liquid proportion (g ash mL^{-1}) and acid concentration (mol H^+ g^{-1} ash), an amount of 1378.1 g L^{-1} of phosphorus were attained at an optimal extraction time of 1 hour. The extract was successfully applied as a source of phosphorus and nutrients for *Chlorella vulgaris* growth with the only extra requirement of nitrogen and carbon sources. As for nitrogen sources, sodium nitrate (the original salt from BG-11) and urea, a cheaper source, were tested. Biomass yield similar to standard BG-11 medium were obtained for both media tested with ash extract (0.78 ± 0.05 g L^{-1} with sodium nitrate and 0.77 ± 0.04 g L^{-1} with urea). An economic analysis of the tested culture media was conducted, showing that to produce 100 m^3 of the proposed microalgae culture media, 65.33 kg of biomass furnace ashes could be recycled and 80% reduction in media cost would be attained by using urea. This demonstrates the potential of obtaining nutrients from an industrial residue and generate high value-added biotechnological products from it.

Keywords: Ash. Culture media. Cost. Microalgae. Phosphorus.

1 INTRODUCTION

Governments and regulatory bodies are increasingly promoting renewable energy, including biomass utilization as fuel, through incentives and policies. Biomass combustion for steam or bioenergy is supported in industries as a greener, cost-effective process that emits less CO_2 and aligns with sustainable development goals[1]. Typically, woody biomasses, industrial wastes, agro-industrial wastes, and urban residues are used as feedstock in biomass boilers. A step further in sustainability is recycling residues from biomass combustion, such as biomass ash and flue gases. Boiler ashes, rich in minerals like phosphorus, are produced in large quantities globally, with about 480 million tonnes generated annually from biomass-fired power plants[2]. Proper management of these residues is crucial to minimize environmental impacts, since they present high alkalinity and eutrophication capacity, and adhere to regulations. Ashes can be repurposed in various applications to support a circular economy[3].

Phosphorus, an essential element for life, is part of a biogeochemical cycle involving soil, plants, animals, and waterways. Human activities, like deforestation, have accelerated phosphorus runoff, contributing to eutrophication. Maintaining phosphorus cycle balance is critical for agriculture and global food security, given the uneven distribution of phosphate rock reserves and rising phosphate prices[4]. A promising option for phosphate recovery is the use of furnace ash, extracting the nutrient and reintegrating it into nature, especially when high value-added products can be generated in the process[5]. Microalgae, such as *Chlorella vulgaris*, can grow rapidly in media containing essential nutrients like phosphorus and nitrogen. *Chlorella* is highly nutritious, with significant health benefits, and has a substantial market value[6]. The direct use of biomass furnace ash in microalgae cultures is challenging due to issues like light obstruction and high alkalinity. This study aims to optimize a process where biomass furnace ashes are treated chemically to create a nutrient-rich solution for *Chlorella vulgaris* cultivation, requiring only additional carbon and nitrogen sources. Economic viability is crucial for the process to be scalable, so a cost analysis comparing standard and ash-based media, including urea as a nitrogen source, was conducted. Few studies have explored phosphorus recovery from ashes for microalgae cultivation. This work is the first to use woody biomass furnace ashes to develop a low-cost culture medium for *Chlorella*, promoting phosphorus reintegration into its natural cycle.

2 MATERIAL & METHODS

The ashes originated from the burning of tree species from the *Pinus* genera were kindly furnished from an industry in Paraná, Brazil. Ashes were macerated to obtain homogenous particles of an average size of 177-350 µm. The ash samples, both crude and post-treated ones, were characterized by ICP-OES (Inductively Coupled Plasma - optical emission spectrometry) to investigate phosphorus content (analytical line of 213.618 nm)[7]. A chemical treatment using acid is proposed to extract phosphorus. The experiments were performed by central composite rotatable design (CCRD) evaluating the proportion between the mass of ashes and the volume of liquid of extraction (0.040 to 0.110 g of ashes mL^{-1}), and the sulfuric acid (H_2SO_4) concentration per mass of ashes (0.014 to 0.056 mol H^+ g^{-1} of ashes). The evaluated response of the designs was the phosphorus concentration (mg L^{-1}). For this, the Erlenmeyer flasks of 250 mL with 70 mL of working volume were kept at 120 rpm for 24 hours. The content of each flask was filtrated to separate the residual solid ash from the liquid. Available phosphorus on the liquid samples was quantified by a colorimetric method (660 nm) based on the formation of a blue-colored phosphorus-molybdic complex[8].

The microalgae utilized as a model to evaluate the ash extract capacity as source of nutrients for culture media was *Chlorella vulgaris* LEB-104, obtained from Federal University of Rio Grande (FURG, Brazil). Three cultivation media based on ash extracts were proposed and evaluated. **BG-ASH** is composed of all BG-11 salts excluding dipotassium phosphate, the phosphate source in the standard media (K_2HPO_4 0.040 g L^{-1}), which was replaced by ash extract in a concentration that resulted on the same phosphorus concentration as usual BG-11 with 20% excess (approximately 8.5 mg L^{-1} of elemental phosphorus). To evaluate if the ash extract furnishes, besides phosphorus, enough other elements, the second media was proposed: **N-ASH**. N-ASH media had none of the BG-11 salts except sodium nitrate (the nitrogen source - $NaNO_3$ 1.5 g L^{-1}) and sodium carbonate (carbon source - Na_2CO_3 0.02 g L^{-1}). The absent other salts were replaced by ash extract in the same concentration as BG-ASH. The third tested media is equal to N-ASH but sodium nitrate is replaced by urea ($(NH_2)_2CO$) – the media is named **U-ASH**. Urea was proposed as an alternative nitrogen source to sodium nitrate since it is widely used in the agricultural sector and is a cheap source of nitrogen. Urea concentration on the microalgae cultivation media was calculated to provide the same amount of elemental nitrogen as the regular BG-11 medium. Therefore, 0.53 g L^{-1} of urea was considered equivalent to 1.5 g L^{-1} of sodium nitrate. All culture media pH were adjusted to pH 7.0 with NaOH 1M. Cultivations were performed on Erlenmeyer flasks, with 300 mL of working volume. Initial microalgae concentration was 0.2 g L^{-1}, and the cultures were kept at 120 rpm for 14 days at 25°C. White fluorescent light irradiated the cultures 24h per day at approximately (50 µmol $m^{-2}s^{-2}$. Analysis of biomass concentration (dry mass at 105ºC), culture media pH, and phosphorus concentration was conducted at the end of cultivation. Samples were centrifuged at 3500 rpm for 15 minutes prior to phosphorus analysis.

A cost analysis was conducted to evaluate the economic viability of the proposed culture media using nutrients extracted from woody furnace ashes. A volume of 100 cubic meters of culture media which is reasonable for industrial scale was chosen to evaluate the costs of the process. All the costs to produce this volume of media were accounted, considering three media options: standard BG-11 media, N-ASH and U-ASH (accounting, then, the costs associated with the furnace ashes chemical treatment). The values of salt prices per kilogram were collected from reliable market websites, focusing on industrial grade prices. For each component of the media, prices from three different vendors of bulk chemicals were used to calculate an average price. Each total media cost was calculated by considering the amount of reagent required in the media and the reagents' average costs.

3 RESULTS & DISCUSSION

The results of ICP-OES showed that woody furnace ashes presented 12206.84 ± 433.75 mg/kg of phosphorus on its composition. The solid residue of ashes post chemical treatment presented 180.01 ± 49.76 mg/kg, demonstrating that the chemical treatment caused the release of most part of this element into the liquid extraction solution.

According to the data from the experimental design, the best condition for the process was condition 6 (0.110 g ash per mL and 0.035 mol H^+ per gram of ash), which resulted in 1378.1 g L^{-1} of phosphorus and 100% of recovery. In Figure 1, the kinetics of phosphorus extraction is shown. Concerning phosphorus extraction kinetics, as observed in Figure 1, from 1 hour of process to 8 hours no statistically significant changes on phosphorus concentration were observed (p-value > 0.05), which indicated that the process had already reached an equilibrium. Thus, one hour is proposed as the time to perform the chemical extraction of phosphorus, a suitable process time regarding industrial application. This time of extraction is lower than other studies, such as the study of Tessari et al. (2022)[9], with 8 h, and Xu et al. (2012)[10] with 2 hours as optimal for phosphorus extraction.

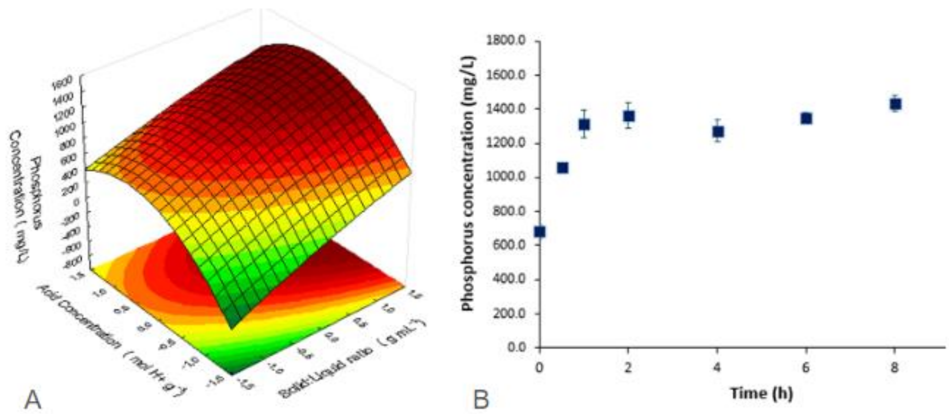

Figure 1 A) Response surface of phosphorus extraction from biomass furnace ashes; B) kinetics of phosphorus extraction.

The results of microalgae cultivation on small-scale flasks on the three tested media are shown in Table 1. The alternative culture media tested not only allowed microalgae growth, but also led to similar biomass concentrations when compared to the standard culture media BG-11. This is important, since the alternative culture media implies a lower cost compared to the standard one, and is aligned to the concept of circular economy, being originated from an industrial residue and reinserting phosphorus into its biogeochemical cycle. The use of urea as nitrogen source also presented positive results, equivalent to the use of sodium nitrate. Urea is economically attractive for large-scale bioprocesses where cost efficiency is important. It is also generally safer to handle and store than sodium nitrate, due to its lower toxicity and higher stability[11]. In the study by Yuniarti et al., different nitrogen sources were tested for *Chlorella* sp. growth, and 0.93 g L^{-1} were attained in the urea-containing medium, which was comparable to the concentration obtained with $NaNO_3$[12]. In the study by Alharbi (2024), 1.35 g L^{-1} *Chlorella* sp. were obtained using urea as nitrogen source (at a concentration of 0.75 g L^{-1})[13]. Besides, for standard BG-11 media the final pH value was 10.3, while for the alternative media the values were 8.8, 10.8 and 9.7, respectively.

Table 1 Results of *Chlorella vulgaris* cultivation across three tested media and control medium, including the total cost for producing 100 m^3 of each medium.

Culture media	Phosphorus concentration (g L^{-1})		Phosphorus consumption (%)	Biomass concentration (g L^{-1})		Cost to produce 100 m^3 of media ($)
	Day 0	*Day 14*		*Day 0*	*Day 14*	
Control BG-11	9.5 ± 1.2	1.5 ± 0.9	85%	0.20	0.78 ± 0.03	125.43
BG-ASH	9.3 ± 1.6	0.5 ± 0.3	94%	0.20	0.71 ± 0.04	Not calculated
N-ASH	8.8 ± 1.0	1.6 ± 0.2	82%	0.20	0.78 ± 0.05	136.59
U-ASH	9.4 ± 0.6	2.4 ± 0.1	74%	0.20	0.77 ± 0.04	25.40

The cost to produce 100m^3 of BG-11 media and N-ASH were similar, which shows that N-ASH would not be advantageous in economic terms, yet it stills present the advantage of reusing a residue from the industry in a circular concept (a considerable quantity of 65.33 kg of ashes would be recycled). The nitrogen source, in this case $NaNO_3$, plays the most significant contribution to the media cost. This is why, on U-ASH, an enormous difference in media cost is observed: changing the nitrogen source from $NaNO_3$ to urea caused 80% reduction on media cost when compared to BG-11 media and N-ASH, with similar microalgae growth observed in experimental data. In the study by Gulhões et al., the use of urea as nitrogen source in *Chlorella* sp. cultivation was seen to reduce culture media costs by 68% compared to BG-11[14]. In another study, by Ribeiro et al., it was found that replacing the nitrogen source with urea reduced 65% of the cost compared to the BG-11 medium[15].

4 CONCLUSION

The present study proposes a low-cost cultivation medium for *Chlorella vulgaris,* inserted in a biorefinery concept in which a residue, biomass furnace ashes, are used to generate a solution rich in phosphorus and other nutrients needed to grow microalgae. The use of biomass furnace ashes extract combined with urea as nitrogen source (U-ASH media) could reduce the cost of *Chlorella* media by almost 80%, with similar microalgae growth performance when compared to standard media (BG-11). Simultaneously, this is an approach to successfully reinserting phosphorus into its biogeochemical cycle. The proposed process and culture media can contribute to the adequate furnace ashes waste management, a highly important issue since the number of combustion facilities of woody biomass for energy generation tends to increase worldwide in the near future, and at the same time generate a biotechnological product, *Chlorella* biomass.

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ACKNOWLEDGEMENTS

This research was supported by the National Council of Technological and Scientific Development (CNPq, Brazil), Coordination for the Improvement of Higher Education Personnel (CAPES, Brazil), and Federal University of Paraná (UFPR, Brazil).