BIOPOLYMERIC PARTICLES LOADED WITH SUGARCANE VINASSE REMAINING FROM MICROALGAL TREATMENT AS SOIL FERTILIZERS

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ABSTRACT

Vinasse is the most expressive wastewater from ethanol production and fertigation of sugarcane crops near the refineries has been its main destination. However, when indiscriminately applied, it can cause environmental impacts, justifying the search for alternatives for its use and treatment. The use of vinasse for microalgal growth has gained attention, owing to its fast-growing ability and possibility to accumulate biomolecules of economic and industrial interest. *Phormidium autumnale* has stood out for its viability to grow in agro-industrial effluents indicating the possibility of being integrated into a biorefinery system. After microalgal growth, the remaining vinasse could be employed to produce biodegradable particles intended as agricultural fertilizers. This would increase the viability of the production chain, obtaining a novel product that can expand the ways of vinasse application. This study investigated the production of pectin and alginate particles added with the remaining vinasse from the cultivation of *P. autumnale*. Vinasse addition (raw and remaining from microalgal growth) increased particle size and decreased the moisture content of particles. Raw vinasse enhanced the compression strength of the particles, possibly due to the higher concentration of soluble solids and calcium from the wastewater. The particles could be applied as a fertilizer in several crops, allowing nutrient recycling, and adequate destination of the wastewater.

Keywords: Vinasse, *Phormidium autumnale*, Fertilizers, Biopolymers.

1 INTRODUCTION

The search for alternative technologies that can increase food productivity without causing negative impacts on ecosystems represents a current and urgent challenge considering the world's growing population.[1] Bio-based and biodegradable polymers, such as carbohydrates and proteins, have been considered alternative matrices for the development of systems capable of retarding or controlling the release of fertilizers and other agrochemicals to guarantee more efficient applications. [2] These materials can also act as soil conditioners as they can improve water availability and allow the slow release of fertilizers, ensuring economic and environmental benefits. Alginate and pectin are anionic polysaccharides mainly extracted from brown algae and citrus pomace, respectively. They have been widely used in the food and pharmaceutical industry due to their gelling, thickening, stabilizing, and film-forming properties. In the presence of divalent cations (such as calcium) alginate and pectin can form strong gels by crosslinking between the guluronate and galacturonate residues, in alginate and pectin respectively, and calcium ions.[3]

Vinasse is the main wastewater from the ethanolic fermentation-distillation process and is available in large volumes (10-15 L per liter of ethanol). It has a characteristic smell and dark color, low pH, and high concentrations of organic matter and other nutrients.[4] Brazil is the world's largest sugarcane producer, with an estimated total ethanol production from sugarcane of approximately 27.32 billion liters of ethanol in 2023/24[5], which would generate more than 270 billion liters of vinasse. Fertigation is the main destination for vinasse, enhancing soil fertility, and thus reducing the need for chemical fertilizers. However, when applied in excess it can cause contamination of the soil and groundwater by nutrient leaching, such as potassium. Besides, the high costs of vinasse transportation limit its application to sugarcane-producing areas near the refineries, hindering the correct final destination of the growing volume of this wastewater. So alternatives for its use and treatment have been proposed, such as recycling in fermentation, concentration, microbial growth medium, energy production, and raw material to produce livestock and poultry feed.[6]

The use of sugarcane vinasse as a growth medium for microalgae biomass production has been gaining prominence as they can reach high biomass productivity and accumulate biomolecules of economic and industrial interest. However, the use of vinasse in microalgal cultivation can be limited by the high costs and complexity of autotrophic systems, and the characteristics of the wastewater, which generally exhibit high turbidity. Photosynthesis is the main metabolic path for microalgae, but several species can grow heterotrophically in the dark, consuming soluble organic molecules.[7] The filamentous cyanobacterium *Phormidium autumnale* has been gaining notoriety as studies have shown its potential for bioremediation processes and the production of unicellular lipids, with an average lipid content of 7-15%, depending on the growth conditions.[8] A recent study from our research group investigated the ability of *P. autumnale* to remove organic carbon and nitrogen from vinasse with biomass production, indicating the potential of being integrated into a biorefinery system in the sugar-energy sector.[7] So, using the remaining vinasse from microalgae cultivation to produce biopolymeric particles intended for soil fertilizers will increase the viability of the production chain through an innovative biodegradable product capable of expanding the way vinasse is applied. This work aimed to characterize alginate and pectin-based particles added with the remaining vinasse from *P. autumnale* cultivation as soil fertilizers.

2 MATERIAL & METHODS

The microalgae *P. autumnale* was maintained in 500 mL Erlenmeyer flasks with BG11 medium. The flasks were kept under stirring at 25°C and 12 h photoperiod (light/dark) until reaching the desired concentration (about 1 g/L). The suspension was centrifugated at 1844 g for 20 min and resuspended in distilled water. Vinasse was collected in a sugar and alcohol facility in Araras/SP, and frozen at -20°C until use. For cultivation, 600 mg of *P.autumnale* biomass was inoculated in 2 L of vinasse previously centrifuged (1281 g /15 min), sterilized, and pH adjusted to 7.6. The flasks were kept in an orbital shaker at 100 rpm, 35°C for 72 h. The medium was centrifuged to separate the biomass. Total organic carbon (TOC) and nitrogen (TN) of raw vinasse and remaining vinasse after microalgal growth were determined in a Shimadzu® TOC/TN analyzer. The concentrations of potassium (K), phosphorous (P), calcium (Ca), and pH were determined by standard methods.[9]

The biopolymeric particles were produced by ionotropic gelation. Alginate (Sigma-Aldrich, USA) and low methoxyl pectin (LM 101, CP Kelco, Brazil) (1:1) were dissolved in distilled water. Then, vinasse (raw vinasse and remaining vinasse) was slowly added to the solution to obtain a final biopolymer concentration of 2% w/v and a water:vinasse ratio of 1:1. The biopolymeric solution was added dropwise in a 5% $CaCl_2.2H_2O$ crosslinking solution with a peristaltic pump. The particles were kept in the crosslinking solution for 30 min and dried at 30°C for 24 h in an air-circulating oven. Particles without vinasse were produced as a control. Samples were designated according to the solvent as control particles (C), raw vinasse particles (Vin), and remaining vinasse particles (R-Vin). Particles were characterized according to the average diameter (Image J®), morphology (scanning electron microscopy, SEM), compression force (TA-XT2, Stable Microsystems)[10], crystallinity (X-ray diffraction), chemical structure (FT-IR), moisture content (W, 105°C/24h), soluble matter in $CaCl_2$ 0.01 mol L^{-1} (S, immersion in water for 24h followed by drying at 105°C/24h), and pH in $CaCl_2$ 0.01 mol L^{-1}. $CaCl_2$ 0.01 mol L^{-1} solution (pH 5.0–5.5) was used to simulate the usual effect of salt concentrations and ionic strength in the soil solution.[11] Analysis of variance (ANOVA) and the Scott-Knott test at a significance level of 5% ($p<0.05$) was performed to detect significant differences using the software R Studio 3.2.4.

3 RESULTS & DISCUSSION

The contents of TOC, TN, K, P, and Ca for raw vinasse and the remaining vinasse used to produce the particles are shown in Table 1. The values found for raw vinasse are within the range reported in the literature.[7,11] As expected, the values of TOC, TN, and nutrients in vinasse decreased after treatment indicating removal by the microalgae.

Table 1 Contents of TOC, TN, K, P, and Ca of raw vinasse and remaining vinasse used to produce the particles.

Sample	TOC (mg L^{-1})	TN (mg L^{-1})	K (mg L^{-1})	P (mg L^{-1})	Ca (mg L^{-1})
Raw vinasse	9521.0 ± 437.0^a	417.3 ± 28.6^a	3263 ± 251^a	70.0 ± 14.5^a	1120 ± 31^a
Remaining vinasse	3756.5 ± 21.9^c	233.8 ± 0.8^c	2268 ± 106^b	39.1 ± 4.5^b	950 ± 36^b

*average ± SD of three measurements. Different letters in the same column indicate significant differences by the Scott-Knott test ($p<0.05$).

The morphology of the alginate and pectin particles without and with vinasse can be observed in Figure 1 (A). All particles exhibited a spherical-like shape. The surface of particles containing vinasse was rough and compact, while the control had a smooth but wrinkled aspect. The cross-section structures of all particles were dense. These differences in the morphology of particles corroborate the other properties (Table 2), which suggested greater stability of the particles with vinasse.

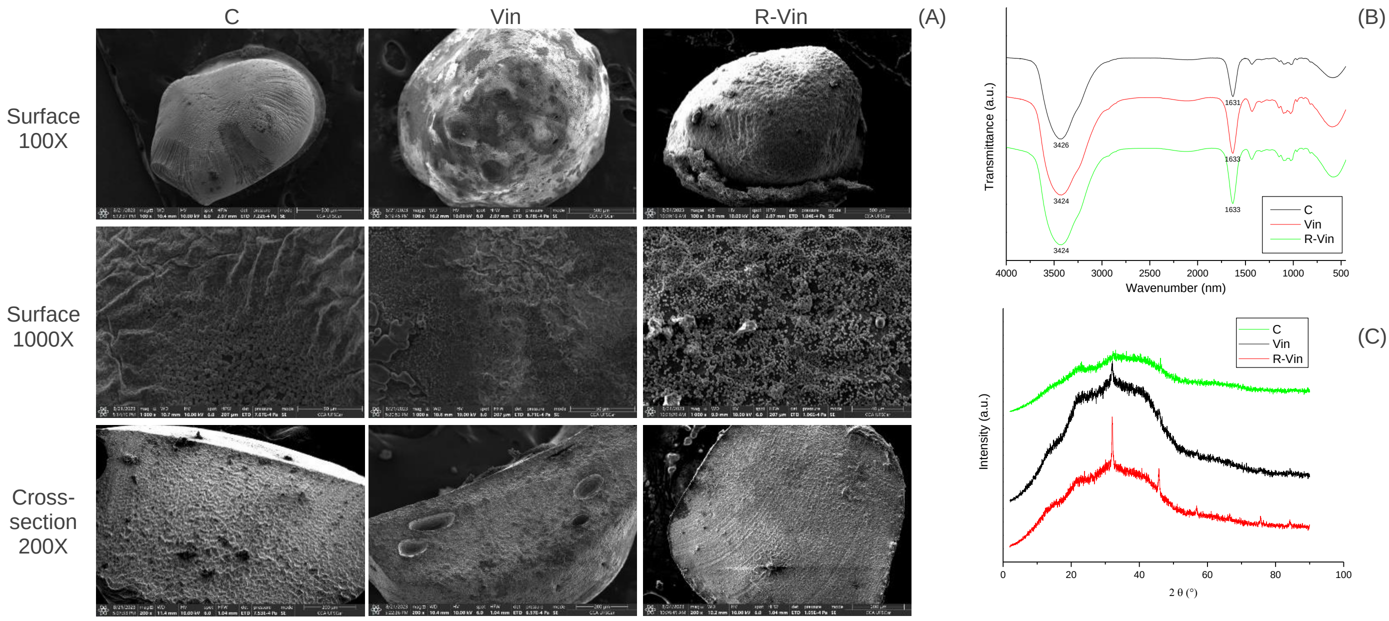

Figure 1 SEM micrographs (A), XRD diffractograms (B), and FT-IR spectra (C) of the biopolymeric particles.

Figures 1(B) and 1(C) show the FTIR spectra and XRD diffractograms of the particles, respectively. FTIR spectra exhibit a broad band at 3420 cm^{-1}, attributed to the stretching of the OH bond. In the region of 1800–1500 cm^{-1}, a band at 1631 cm^{-1} (for C) and 1633 cm^{-1} (for Vin and R-Vin) attributed to the asymmetric stretching of the carbonyl group of the carboxylate ion (COO^{-}). This functional group can interact electrostatically with Ca^{+2} described by the "egg-box" model. The displacement of this absorption band has been attributed to this interaction and crosslinking of the alginate chains[12] corroborating the enhancement of the stability of the particles by the presence of the Ca^{+2} in the vinasse. The third region (below 1500 cm^{-1}), known as the "fingerprint" region corresponds to specific bonds of the polysaccharide structure and glycosidic bonds.[12] The XRD diffractograms exhibited predominantly amorphous structures. The control particle showed a broad peak at around 2θ of 20°, a typical pattern for semi-crystalline biopolymers.[13] Vin and R-Vin particles showed sharper peaks probably due to a more organized gel structure induced by vinasse soluble solids.[11]

The properties of the alginate and pectin particles without and with vinasse are reported in Table 2. Vinasse significantly increased the particle size. The compression force also tends to increase with vinasse addition, however, the particles containing the remaining vinasse (R-Vin) were not significantly different from the control. This could be attributed to the presence of solids and calcium that can enhance stability and the crosslinking degree of the polymeric matrix. The mechanical properties are important characteristics since they affect the performance and behavior of a material. A good fertilizer must be strong enough to withstand handling, transport, and storage, without breaking or deforming the granules.[14] The moisture content of the particles varied from 38.95 (C) to 30.19% (Vin). The soluble matter in $CaCl_2$ 0.01 mol L^{-1} was higher for particles containing vinasse, possibly due to the release of soluble solids from the vinasse to the aqueous medium. The same trend was observed for Cerri et al.[11] for chitosan and high methoxyl pectin particles added with raw vinasse. The pH values of the particles in $CaCl_2$ 0.01 mol L^{-1} varied from 5.30 (Vin) to 7.22 (R-Vin). These variations are explained by the characteristic acidity of raw vinasse (~ 4.6) and the pH adjustment for microalgal growth (~ 7.6). The pH values of the particles were within the broad pH range observed for commercial fertilizers, varying from 3.0 to 9.6.[11]

Table 2 Average diameter, compression force, moisture content, soluble matter, and pH of the biopolymeric particles.

Particle	Diameter* (mm)	Compression force* (N)	W** (g/100g)	S** (g /100g)	pH
C	1.81 ± 0.22^{b}	12.79 ± 1.36^{c}	38.95 ± 0.43^{a}	60.63 ± 0.48^{c}	6.73
Vin	2.01 ± 0.16^{a}	18.24 ± 2.82^{a}	30.19 ± 0.20^{b}	62.33 ± 0.68^{b}	5.30
R-Vin	1.98 ± 0.20^{a}	13.53 ± 2.65^{bc}	31.52 ± 0.97^{b}	69.04 ± 0.45^{a}	7.22

*average ± SD of ten measurements. **Average ± SD of three measurements. Different letters in the same column indicate significant differences by the Scott-Knott test ($p<0.05$).

4 CONCLUSION

Stable and uniform alginate and pectin particles added with raw and remaining vinasse from microalgal growth were produced by a simple drip technique into the crosslinking solution. Vinasse addition (raw and remaining from microalgal growth) increased particle size and decreased the moisture content of particles. Raw vinasse significantly enhanced the compression strength of the particles, possibly due to the higher concentration of soluble solids and metal ions from the wastewater. The use of the remaining vinasse from *P. autumnale* growth for producing biopolymeric particles expands the viability of the production chain. This novel biodegradable product can be applied as a slow/controlled release fertilizer in several crops, allowing nutrient recycling, adequate destination of the wastewater, and reduction of the use of conventional fertilizers.

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