

Study of the Reuse of Eggshells in the Production of Bioplastics with Soil Enrichment Potential

Giulia Freire Fonseca^{1*}, Cecília Araújo Crisóstomo Oliveira¹, Gisele Gonçalves Chagas¹, Ellen Rayane Vitória Santos Costa Brito¹, Beatriz da Paixão Santos¹, Érica Patrícia Lima Pereira¹

¹SENAI CIMATEC University, Industrial Microbiology, Salvador, Bahia, Brazil

This comprehensive study investigated the significant potential of eggshell, a readily available calcium-rich bio-waste, as an effective and sustainable additive in bioplastics. This innovative approach aims to substantially improve soil fertility during the material's biodegradation, while concurrently mitigating the environmental impacts associated with conventional limestone mining, the traditional source of calcium, as well as addressing the disposal of fossil-derived plastics. The robust methodology involved preparing plant-based raw materials, developing prototypes with systematic variations in composition and concentration, and comprehensively characterizing the formulated biomaterial. Analyses included precise thickness measurements and rigorous soil biodegradation tests through mass loss coupled with calcium carbonate equivalent (CaCO₃) quantification and carbon dioxide (CO₂) emission monitoring. The formulations were systematically studied at concentrations of 0%, 0.25%, 0.5%, and 1% eggshell, with thicknesses ranging from 0.37846 mm to 0.53594 mm. The biomaterials demonstrated promising average soil biodegradation rates of 52.42%, 51.26%, 52.94%, and 57.64% for concentrations of 0%, 0.25%, 0.5%, and 1%, respectively. During the 16 days of biodegradation, the samples released an average of 102.36, 117.55, 128.87, and 138.84 g kg⁻¹ of CaCO₃. These values disregard the baseline CaCO₃ (g kg⁻¹) value found in the soil without the presence of the samples, and it is possible to observe a consistent growing trend in the quantity of this essential nutrient that directly accompanies the increase in eggshell concentration in the formulation. The average aerobic biodegradability rate corresponded to 44.70% within a 14-day interval. Thus, eggshell presented itself as a strong potential additive in bioplastics, enabling formulations that are simultaneously biodegradable and capable of fertilizing the soil.

Keywords: Biodegradation. Bioplastic. Calcium. Eggshell. Fertilizer.

Abbreviations: ADFE, Air-Dried Fine Earth. EF, Eggshell Flour. DABCD, Determination of Aerobic Biodegradability in Soil by Amount of Carbon Dioxide. MLBTS, Mass Loss Biodegradation Test in Soil.

Fossil plastics have revolutionized the industry with the creation of low-cost and multifunctional products. Despite that, since their creation, this polymer has faced contradictions, as highlighted by Conceição and colleagues [1], related to their inadequate disposal in contrast to the dependence of processing companies. However, not only polymers show this growing production and disposal, but also bioproducts such as eggshell.

According to released data by FAOSTAT [2], it is estimated that demand for table eggs will reach 95 million tons. Considering that 10% of the total weight of the egg corresponds to the shell, we will have about 9.5 million tons of a

calcium-rich bio-waste, an essential nutrient for animal and plant metabolism. In this sense, the present study is dedicated to exploring the biotechnological potential of eggshell in the development of a bioplastic with full potential to improve soil conditions after disposal. As objectives, we sought to obtain eggshell flour and English potato starch, both raw materials for the biopolymer. Subsequently, to test different bioplastic formulations and evaluate their potential for soil enrichment with calcium during biodegradation through physical/chemical assays. And finally, to systematize with the literature in the field of botany, the importance of calcium for plant development, given the abundance of this nutrient in eggshells [3].

Theoretical Foundation

The eggshell is calcareous layer in which about 96% of the weight corresponds to calcium

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Address for correspondence: Giulia Freire Fonseca. Av. Orlando Gomes, 1845, Piatã, Salvador, Bahia, Brazil. Zipcode: 41650-010. E-mail: giuliafreire39@gmail.com.

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carbonate (CaCO_3), while the flour of this byproduct presents 36.9% of bioavailable calcium (Ca) for the organism [4,5]. Thus, it is concluded that eggshell has interesting nutritional values centered on high concentrations of Ca and CaCO_3 .

In view of this, Matteucci and colleagues [6] points out the waste of this resource, which has high utility for soils. This is because this macroelement favors root growth, intensifies microbial activity, and increases the availability of Molybdenum [7].

The presence of calcium in the soil is not limited to performance, as it is also indispensable for the full development of crops, because as stated by Luo [8] it severely interferes with the maintenance of life in the entire ecosystem.

According to Neto [9], the deficiency of adsorbed metallic cations such as Ca is corrected by the liming technique with limestone, as it results in the accelerated formation of nitrates and sulfites and stimulates nitrogen-fixing bacteria. However, according to Neto & Ramalho [10], limestone extraction causes several environmental impacts, such as erosion, irreversible destruction of fossils, among others. Thus, eggshell becomes a potential fertilizer, given its high concentration of a nutrient demanded by agricultural and forest systems. Therefore, it is pertinent for the biotechnology branch to study the reuse of this material for soil improvement, as an alternative to other degrading sources.

Polymeric materials are among the most used tools currently [11]. However, despite their applicability in long-life products, they have been applied as disposable single-use products [12]. In this sense, the use of biodegradable plastics is a viable alternative because they are produced from renewable sources that have a shorter degradation time.

Starch is the most used raw material for plastic application due to its large-scale availability, biodegradability, and low cost [13]. Originally, it is found in granular form, but it is subject to bond breakage at high temperatures with plasticizers such as glycerol with acetic acid, which provide greater flexibility [14]. The work of Furckel [15] obtained

satisfactory results by using agro-industrial residues such as apple flour in starch biopolymers, pointing to the possibility of using other raw materials in starch-based plastic. In this direction, the present work corroborates the concept of circular economy under the conditions of Moreira [12] by conceiving an alternative for a polluting material. The proposal is to use a reused raw material while also attributing a double functionality to it.

Materials and Methods

English Potato Starch Extraction

Following the procedures adopted from Neto & Ramalho [11], the extraction was based on the decantation and drying of the starch from the process of crushing the washed, peeled, and weighed root.

Eggshell Flour (EF)

The chicken eggshells collected by the authors and other collaborators were sanitized, dried, and crushed to form the flour.

Bioplastic Formulation

The bioplastic formulation was grounded on the methodology described by Pereira & Plens [16], with adaptations. Distilled water, starch, acid, glycerin, and eggshell were mixed and heated until reaching a temperature of 90°C . The mixtures were poured into petri dishes and taken to dry in an oven.

Thickness Measurement

Based on Bezerra & Andrade [17], thickness was measured with a micrometer at 5 different points of each polymer to define the overall thickness from a simple average.

Mass Loss Biodegradation Test in Soil (MLBTS)

The experiment was carried out based on the adapted method of Bezerra & Andrade [17]. Each

bioplastic formulation was cut into 3 samples measuring 2cm x 2cm with the aid of a caliper, as shows Figure 1. Subsequently, each one was weighed and buried in different containers with 25g of fertilized topsoil. The mass reduction was monitored by regular weighings and calculated as a percentage.

Quantification of Calcium Carbonate (CaCO₃) Equivalent

The method used was adapted from Teixeira and colleagues [18]. Initially, the soil used in each biodegradation was prepared to obtain Air-Dried Fine Earth (ADFE). Subsequently, these samples were heated with 0.5 N hydrochloric acid (HCl), filtered, and diluted. 50 mL of the resulting solution was taken, 3 drops of 1% phenolphthalein indicator were added, and finally, titrated with 0.25 N sodium hydroxide (NaOH) solution. At the end, the 12 samples percentage of CaCO₃ were calculated, for each triplicate of concentration.

Figure 1. Sample preparation with a caliper



Determination of Aerobic Biodegradability in Soil by Amount of Carbon Dioxide - CO₂ Emitted (DABCD)

The experimental procedure for the biodegradation test was adapted from the ISO 17556 [19] and Tosin & Pischedda [20]. One bioplastic sample of each concentration, sized 2 cm x 2 cm, were introduced into beakers with 200g of soil, whose pH, humidity, and nutrient levels were previously adjusted. The experimental system also included a negative control (soil only) and a positive control (soil with filter paper measuring 2 cm x 2 cm). Each of these beakers was inserted into a CO₂ capture system, composed of a larger beaker containing a 0.5 N potassium hydroxide (KOH) solution. To ensure isolation, the system was hermetically sealed with plastic film, Parafilm®, plastic bag, and adhesive tape. The quantification of released CO₂ was performed every 2-3 days by titrating the KOH solution with 0.3 N hydrochloric acid (HCl). The KOH solution was completely replaced after each titration.

Results and Discussion

English Potato Starch

The yield of the starch extraction process from the potato was approximately 12.98%. This value is considered low when compared to the results by Neto & Ramalho [11], which acquired 50.14% as the average starch yield for sweet potato, 64.82% for mangarito, and 66.03% for cassava. At the same time, Mendonça [21] obtained 7.89% as the average starch yield for mangarito. Such discrepant differences may be due to differences in the growth and harvesting conditions of the tuber used, given that the procedure followed was similar.

Bioplastic Formulation

Different prototypes were made until the expected final appearance was achieved. In the

first, the heterogeneous granulometry of the EF left a shapeless texture, similar to a dough, making demolding impossible due to its brittle texture.

For the second prototyping, adjustments were made to the flour, repeating the grinding step. From this, bioplastics were produced in triplicate for each EF concentration: 5%, 10%, and 15%. The dough-like texture was repeated, as shown in Figure 2, and this result revealed that such an aspect could be associated with the amount of flour added to the formulation.

For the third prototyping, it was necessary to confirm the viability of the formulation without EF. Thus, 2 different acids were tested, acetic and hydrochloric, and for each, two different starch concentrations. In the end, the most satisfactory formulation had 5% acetic acid and 2.5 g of starch as variable components, illustrated in Figure 3.

In addition, the heating temperature was increased, after observing in Santos and colleagues

Figure 2. Second prototyping, in 5%, 10% and 15% concentrations respectively.



Figure 3. Third prototyping with acetic acid.



[14] that the temperature previously used was below the starch breakdown range. Finally, it was possible to demold this prototype with the aid of a spatula. For the last prototyping, the parameters and components defined in the previous one were used, adding EF in lower concentrations than in the previous prototypes, namely: 0%, 0.25%, 0.5%, and 1%. At the end of the process, the expected results for the biopolymer were achieved. Thus, it differs from the best result found by Furckel and colleagues [15], who used 10% of agro-industrial residues in potato starch bioplastic.

Thickness Measurement

The results thickness of each samples used in the MLBTS and DBACD are represented in Tables 1 and 2, respectively.

The average thicknesses of the biopolymers ranged from 0.37846 mm to 0.53594 mm, while the standard deviation values ranged from 0.04568 mm to 0.13042 mm. In a review, Neto & Ramalho [11] obtained average thicknesses between 0.456 mm and 0.6618 mm and standard deviations between 0.032 mm and 0.092 mm. When comparing these results, the bioplastics in this study were less thick, but more shapeless than those in the methodological reference study.

Table 1. Thickness of bioplastics for MLBTS.

Bioplastic	Average Thickness (mm)	Standard Deviation \pm
0.00%	0.40132	0.04568
0.25%	0.53594	0.08387
0.50%	0.52705	0.08754
1.00%	0.40132	0.13042

Table 2. Thickness of bioplastics for DBACD.

Bioplastic	Average Thickness (mm)	Standard Deviation \pm
0.00%	0.46609	0.05996
0.25%	0.38100	0.09214
0.50%	0.37846	0.02353
1.00%	0.44958	0.09046

Mass Loss Biodegradation Test in Soil (MLBTS)

The weight variation by triplicates average in grams of the polymers during the test can be observed in Figure 4.

Significant mass reductions can be observed in the initial period of the test. This result contrasts with the mass increase between the first and second weighing observed by Neto & Ramalho [11], who associated this gain with soil adherence to the polymer. In the present study, the magnitude of the mass reduction was sufficient to overcome any initial mass gain resulting from soil adhesion, highlighting the expressiveness of the degradation process.

A significant increase was noted on day 7, which can be attributed to higher soil moisture at the time of weighing, which made it impossible to remove excess soil. The Figure 5, presented below, illustrates the results of the averages of the triplicates regarding the mass reduction, in percentage, for each of the eggshell concentrations used.

Over 16 days of degradation in soil, the bioplastics reduced their mass by an average of

52.68%. This result is similar to that of Neto & Ramalho [11], who obtained averages of 57.46% and 58.76% mass reduction during 24 days of degradation.

As evidenced in Figure 5, the sample without EF addition (0%) exhibited a higher degradation rate than the material with 0.25% EF and similar to the bioplastic with 0.5% EF. This observation can be related to the physical characteristics of the material, specifically its thickness and uniformity. The 0% polymer showed less thickness and greater uniformity. In contrast, the 0.25% was the thickest, while the 0.5% exhibited similar thickness to the 0%, but with greater non-uniformity. Interestingly, the prototypes with 1% eggshell, which had an average thickness equal to 0% and the lowest uniformity among all, showed superior degradation. Such results suggest that the presence of eggshell can accelerate the biodegradation process, while increasing the thickness of the material tends to retard it.

Quantification of Calcium Carbonate Equivalent

The Figure 6 illustrates the amount of CaCO_3 (g kg^{-1}) released by the biopolymers during the 16-day period. It shows the average of the triplicates for the four eggshell concentrations.

The result demonstrates that the eggshell was efficient in fertilizing the soil with calcium, even when present in low concentrations in the biopolymer.

Determination of Aerobic Biodegradability in Soil by Amount of Carbon Dioxide - CO_2 Emitted

The CO_2 emission and degradation percentage are shown in Figures 7 and 8, respectively.

When summing the biodegradation percentages for the entire period, the total aerobic biodegradability rate was 123.75%, 16.34%, 63.65%, 73.69%, and 25.14% for the positive blank and the materials with 0%, 0.25%, 0.5%, and 1% EF, respectively. Although exceeding the theoretical limit, the positive control result

Figure 4. Weight by triplicates average x Biodegradation time.

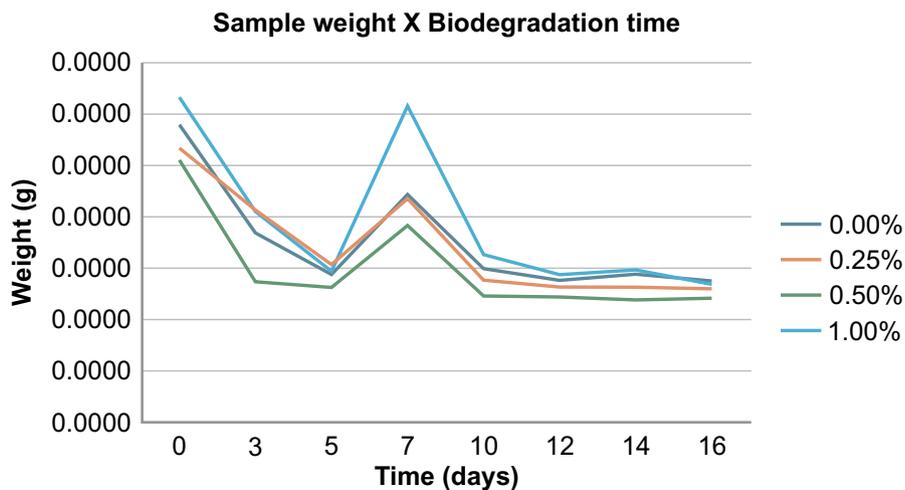


Figure 5. Sample mass reduction by averages of the triplicates.

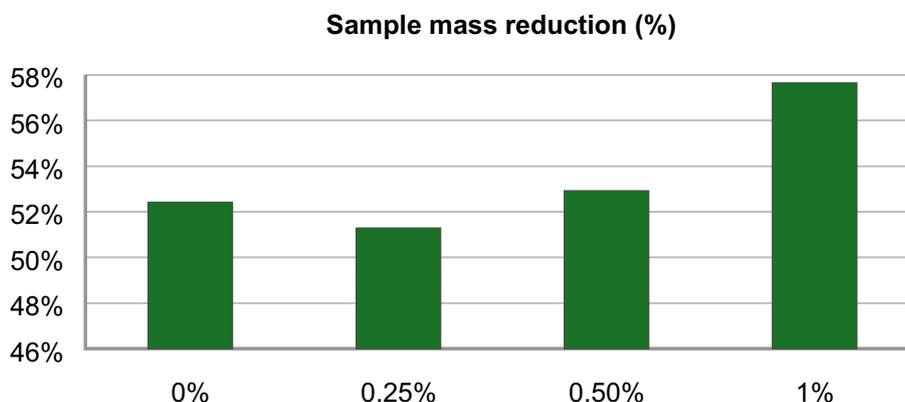


Figure 6. Quantification of CaCO₃ equivalent.

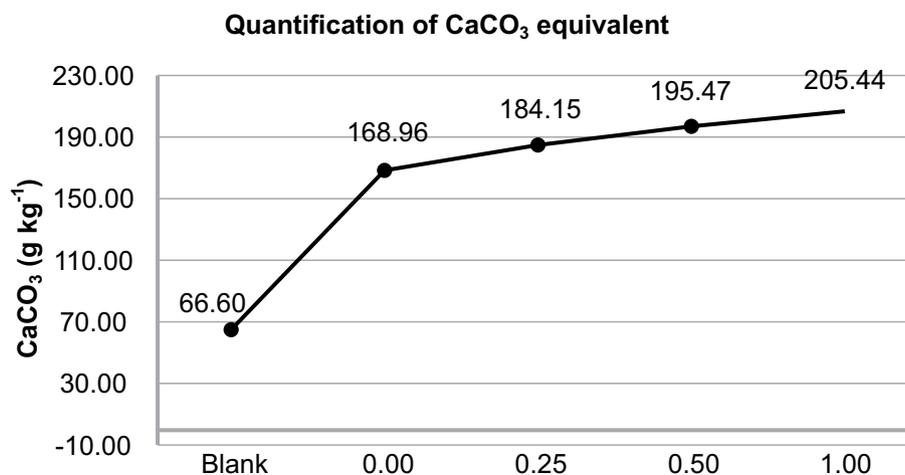
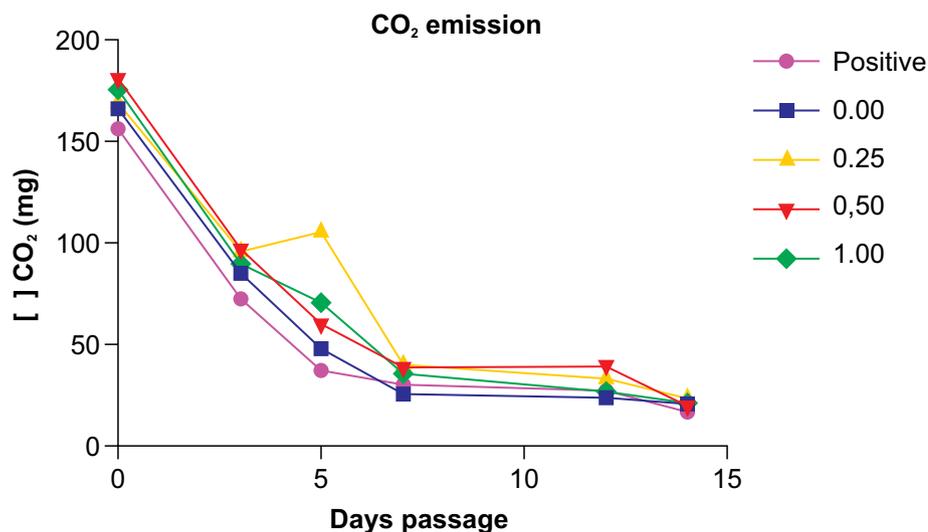
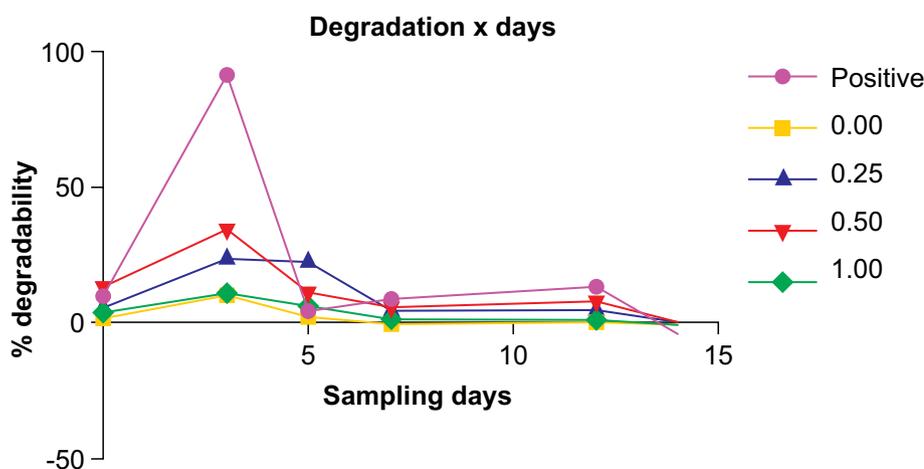


Figure 7. CO₂ emissions in mg.**Figure 8.** Degradation percentage.

unequivocally confirms that the system conditions (temperature, aeration, and humidity) and the microbial inoculum activity were adequate and robust for the mineralization of a biodegradable polymer. Additionally, cellulose is an easily degradable substrate, especially with the low mass used (0.036g), which can lead to significant percentage deviations. Therefore, the assay is considered valid for the evaluation of the tested biopolymers.

Due to the experiment being conducted for 14 days, and the ideal time for this experiment being 6 months, certain inconsistencies can be attributed to this. Furthermore, this specific experiment was not performed in triplicate for each sample, so the

most plausible biodegradation results are from the mass loss assay. Still, the generated data provide an idea of the material's nature, which in future tests can be confirmed by adjusting the parameters mentioned above.

Conclusion

Therefore, the use of eggshell demonstrated success as a functional component in starch-based bioplastics. The results indicate that the additive contributes to soil enrichment by releasing calcium during degradation. This dual functionality represents an innovative solution capable of mitigating the environmental impacts

associated with the disposal of fossil plastics and limestone extraction.

Both biodegradation analyses confirmed the effectiveness of the biopolymer in fertilizing the soil, even at low concentrations of the additive.

Despite the limitations and the short duration of the experiment, especially regarding biodegradation, the data obtained provide a solid basis that validates the concept. Future studies, with adjustments to experimental parameters and longer analysis times, may refine and confirm the potential of this biomaterial as a sustainable and beneficial alternative for agriculture and the environment.

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