Bioactive Coating With Low Reactive Sources for Application as Multi-nutrient Fertilizer Granules

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Research Article

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Abstract

Fertilization is essential to provide suitable conditions for plant development and crop productivity, but the environmental cost of fertilizer production is a drawback for achieving a sustainable agriculture. Using unprocessed (raw) nutrient sources such as elemental sulfur ($S^0$) and mineral oxides (ZnO, MnO, CuO) as fertilizers reduces the environmental impact, although they are not readily available to plants. Thus, we developed a polymeric coating material based on gelatinized starch loaded with $S^0$ and oxides, and selected microorganisms – *Aspergillus niger* and *Acidithiobacillus thiooxidans* – aiming at a multi-nutrient fertilizer in which the biological components improve the solubility of the low reactive nutrient sources. The acidifying capacity of both microorganisms led to a synergic release and increased the availability of micronutrients and the elemental sulfur oxidation rate. For instance, the polymeric coating composition enabled a sulfate release of up to 76.4 and 71.3% for *A. niger* and *A. thiooxidans*, respectively. This innovative system can effectively supply nutrients to plants through the use of cheap and low reactivity nutrient sources with the advantage that it can be applied on currently used fertilizer granules, making easier the adoption by producers.

1. Introduction

Fertilizers are defined as sources of macro and micronutrients, which are essential inputs to high crop production. Macronutrients are highly demanded fundamental building blocks of plant structures. On the contrary, micronutrients are required in much smaller quantities by plants, but still limit crop production yields [1–2]. Micronutrients assist on important plant processes and functions, e.g., transport of genetic material, tissue growth, and amino acids synthesis. Moreover, they are constituents of chlorophyll, which is responsible for photosynthesis [2–5]. Micronutrient losses in soil include leaching of soluble salts (e.g., ZnSO$_4$) and/or precipitation of salts (e.g., interaction of Zn$^{2+}$ with phosphate ion, forming zinc phosphate, a poorly soluble salt) [6].

Mineral oxides, such as ZnO, CuO and MnO, display higher elemental contents compared to salts, as well as lower production and environmental costs since they are raw materials. However, these oxides are poorly water-soluble, requiring processes that involve strong inorganic acids and high energy consumption to increase their solubility. This is the typical case of sulfur, a macronutrient often applied as elemental sulfur ($S^0$), but only absorbed by plants as sulfate anions ($SO_4^{2-}$) [7–9]. Moreover, the conversion processes of $S^0$ and micronutrients in soil is limited by the soil microbiological activity, including direct metabolization and indirect effects from secreted organic acids [10–12].

Thus, an adequate soil enrichment with selected microorganisms can potentially increase the availability of these low reactive nutrient sources. Fungi like *Aspergillus niger* (*A. niger*) [13–14] and the bacterium *Acidithiobacillus thiooxidans* (*A. thiooxidans*) [15–16] have demonstrated a significant potential for improving nutrient availability and efficiency [17]. *A. niger* produces organic acids and promotes $S^0$ oxidation through indirect effects related to local pH reduction and enzyme secretion [18–19]. *A. thiooxidans* fixes CO$_2$ and grows chemolithoautotrophically using sulfur as an energy source, thereby
producing sulphuric acid [15]. Despite the recognized potential of these microorganisms in promoting nutrient solubilization, tailoring the fertilizer structure to maximize the benefits from microbial activity still remains an unsolved challenge.

Coating granular fertilizers with adequate materials can provide an advantageous support for microorganism vehiculation, promoting cellular protection during application and nutritional support for its reactivation and growth. We have previously demonstrated that maize starch is suitable to disperse high fertilizer particle contents (mineral oxides and elemental sulfur), and forms continuous, well-dispersed films over the fertilizer granules [13–14]. Maize starch is composed of amylose (30 wt.%) and amylopectin (70 wt.%) macromolecules that can act as an energy source for microbial growth. Starch is also natural, biodegradable, abundant and non-toxic, which are desirable features from an agronomic point of view [13–14].

Herein, we developed a bioactive coating material based on gelatinized starch containing balanced contents of S\(^0\) and oxides as sources of micronutrients (ZnO, MnO and CuO). This system has been used as an encapsulating matrix for the acidifying fungus *Aspergillus niger* or the oxidizing bacteria *Acidithiobacillus thiooxidans* to increase the micronutrient availability of low reactivity sources. The film-forming ability of the composition was very adequate to coat monoammonium phosphate (MAP) granules, which was an ideal vehicle for the targeted composition. Solubilization and soil incubation assays indicated that the supporting fertilizer (MAP) did not affect the sulfur and micronutrients solubilization neither its release rate was affected by the coating. Our results indicate that this composite coating is a simple strategy to deliver multiple nutrients with minimal pre-processing, saving energy and avoiding byproduct generation.

### 2. Materials And Methods

#### 2.1 Materials

Maize starch (St) (Amidex 3001, 30 wt.% amylose and 70 wt.% amyllopectin) was kindly supplied by Ingredion (Brazil). Urea and elemental sulfur (S\(^0\)) (99 by weight – wt.%) were purchased from Synth (Brazil). The mineral oxide sources of zinc oxide (ZnO\(_{\text{Mineral}}\), 74 wt.%), manganese oxide (MnO\(_{\text{Mineral}}\), 65 wt.%), and copper oxide (CuO\(_{\text{Mineral}}\), 28 wt.%) were supplied by Heringer Fertilizers (Brazil). All materials were used as received. Monoammonium phosphate (MAP) granules (diameter ranging between 2.8 and 3.2 mm) were kindly provided by Adubos Vera Cruz, Ltd.

#### 2.2 Microorganisms

*Aspergillus niger* C (BRMCTAA 82), a filamentous fungus, was obtained from Embrapa Food Technology collection (Rio de Janeiro, RJ, Brazil). Spore suspensions of *Aspergillus niger* (*A. niger*) were kept at -18°C in a solution of water with glycerol (20 wt.%) and NaCl (0.9 wt.%). Spores were germinated on Petri dishes containing potato dextrose agar at 30°C. After 96 h, a suspension of grown spores was harvested by adding distilled water. The spore concentration was determined using a Neubauer chamber.
The bacterium *Acidithiobacillus thiooxidans* was obtained from São Paulo State University (Araraquara-SP, Brazil). Suspensions of *Acidithiobacillus thiooxidans* (*A. thiooxidans*) were kept at 4°C in a medium 9K [15]. The bacterium was reactivated on 9K medium in an orbital shaker incubator at 30°C and 150 rpm for 10 days. The final medium was vacuum filtered using Whatman No. 1 filter paper and, subsequently, the cellular concentration was determined using a turbidimetric method.

### 2.3 Coating of fertilizer granules with micronutrients and microorganisms

Biocoating loaded with each microorganism was prepared by maize starch (St) gelatinization. This process followed the methodology described by Klaic et al. (2018), which consisted in the dispersion of starch (8 wt.%) and glycerol (4 wt.%) in distilled water (250 mL) [13–14]. The gelatinization process was carried out by keeping the dispersed starch at about 90 °C for 30 min under stirring until a sticky St paste gel was formed. Elemental sulfur (256 ± 32 nm) and the mineral oxide mix (ZnO, MnO and CuO, average particle sizes of 446 ± 46, 325 ± 55 and 302 ± 51 nm, respectively), both sources of micronutrients, were transferred to the St gel at a proportion of 1:2 between starch (33.6 wt.%) and mix particulate materials (66.4 wt.%), and their dispersion was accomplished by vigorous agitation for 15 min. In addition, ZnO, MnO, CuO and Sº were added at a ratio of 6.4, 7.6, 17.7 and 35.5 wt.%, respectively. As the mineral oxides have impurities, the oxide content added to the matrix was previously calculated to obtain a final micronutrient concentration (Zn²⁺, Mn²⁺ and Cu²⁺) of around 3.75 wt.% and 35 wt.% for Sº. After dispersing the particulate materials in the St gel, the temperature was reduced to 30 °C for incorporating the *A. niger* spores (or *A. thiooxidans* cells). The fungi spore (or bacterial cell) suspensions were prepared as described in Section Microorganisms to achieve a concentration of 1x10⁸ fungi spores (or bacterial cells) per gram of material.

The coating process was carried out by dispersing the biopolymer over the MAP granules using a metal turntable coater with 25 cm side shields, rotating at 30 rpm, under air flow heated at 40 °C [20]. Table 1 summarizes the materials and codes of the coated and uncoated granule samples. Coated MAP granules without *A. niger* or *A. thiooxidans* (here denoted as MAP_mix) were also prepared for comparison purposes. Furthermore, physical and chemical characterization, as well as bioactivation experiments in liquid culture medium and soil, were carried out on all materials. The experimental methodologies, including the analytical methods for the determination of micronutrients and solubilized phosphorus, were described in detail in the Supplementary Material.
Table 1
Composition and code of different treatments.

| Composition                                      | Code           |
|-------------------------------------------------|----------------|
| MAP (uncoated)                                  | MAP            |
| Sº/ZnO/MnO/CuO                                  | Mix            |
| MAP + Sº/ZnO/MnO/CuO                           | MAP\textsubscript{Mix} |
| MAP + Sº/ZnO/MnO/CuO + \textit{Aspergillus niger} C | MAP\textsubscript{Mix+A.niger} |
| MAP + Sº/ZnO/MnO/CuO + \textit{Acidithiobacillus thiooxidans} | MAP\textsubscript{Mix+A.thio} |

3. Results And Discussion

3.1 Materials characterization and incubation in liquid culture medium

Figure 1 shows the interface of MAP granules with and without the bioactive coating layer. The SEM image of uncoated MAP exhibits a rough surface (Fig. 1a), while Figs. 1b and 1c show the fertilizer/coating layer interface (highlighted in yellow and green for MAP\textsubscript{Mix+A.niger} and MAP\textsubscript{Mix+A.thio}, respectively). Despite the roughness, Figs. 1b and 1c revealed that the coating layer presented good applicability, uniformity, and adhesion over the MAP granule surface, as well as thicknesses of 101.8 ± 4.2 µm and 105.1 ± 7.3 µm, respectively.

Figure 2 shows the temporal profiles of pH, solubilized P, Zn\textsuperscript{2+}, Mn\textsuperscript{2+} and Cu\textsuperscript{2+}, and of elemental sulfur (Sº) oxidation for the controls and \textit{A. niger}-loaded samples in liquid nutrient medium. All samples evaluated and their nomenclatures were described in Table 1. This experiment provided the minimum conditions required for microorganism germination and fungal growth, evaluating the potential of the bioactive coating in promoting micronutrient solubilization and elemental sulfur oxidation. These results showed that the \textit{A. niger} spores (MAP\textsubscript{Mix+A.niger}) germinated and proliferated throughout the bioactive coating, indicating that the starch coating was efficient to maintain the \textit{A. niger} spores viable after the coating process. Moreover, the starch coating did not delay the phosphorus (P) release, since all granules reached above 80% of P solubilized in 3 days, as shown in Fig. 2a. This result was expected because MAP has a high solubility, and the maize starch coating does not impose a physical barrier to the phosphate solubilization. In addition, it is important to highlight that after the 3th day a slight decreasing in P solubilized was observed for MAP\textsubscript{Mix+A.niger}. This reduction suggests that the microorganisms consumed an amount of P in their metabolic process like cell reproduction and/or production of new molecules/metabolites [21].

Figure 2b presents the temporal profile of pH, and the results showed a lower pH reduction for the Mix (control treatment), remaining virtually the same pH value from the 6th to 12th day. It is possible to note
that MAP (uncoated) and MAP\textsubscript{Mix} (without microorganism) also showed a slight pH decrease until the 3th day of incubation, with pH stabilized at around 5.5. However, the MAP\textsubscript{Mix+A.niger} granules showed a significant pH reduction from 7 to 3, which is associated with the ability of \textit{A. niger} to produce acidity as organic acids [13, 22–23].

Figure 2c shows the temporal profile of elemental sulfur (S\textsuperscript{o}) oxidation for all treatments. Uncoated MAP and Mix (control treatment) showed elemental sulfur oxidations below 1% over the 12 days of incubation, while MAP\textsubscript{Mix} presented 5% of S\textsuperscript{o} oxidized in the same period. These values indicate that a simple dispersion process does not significantly contribute to the S\textsuperscript{o} oxidation. Moreover, MAP\textsubscript{Mix+A.niger} showed the highest oxidation rates, indicating that the microorganisms in the bioactive coating boosted the S\textsuperscript{o} oxidation to SO\textsubscript{4}\textsuperscript{2−} up to 70% in 12 days. Such a remarkable result can be explained by the fact that \textit{A. niger} acidification is mainly due to the production of organic acids, such as citric acid, oxalic acid and gluconic acid, which present an indirect effect on the elemental sulfur oxidation, since low pH and some enzymes (such as sulfatase) intensify the oxidation of S\textsuperscript{0} [18–19, 24]. The biological oxidation process that converts S\textsuperscript{o} to SO\textsubscript{4}\textsuperscript{2−} is essential for plants, because S\textsuperscript{o} is absorbed only in the sulfate form [25–27].

Figures 2d-2f show the temporal profiles of Zn\textsuperscript{2+}, Mn\textsuperscript{2+} and Cu\textsuperscript{2+} solubilized in liquid nutrient medium, respectively. Similarly to elemental sulfur, the uncoated MAP and Mix showed no significant solubilization rates for those micronutrients, remaining below 1% over 12 days of incubation, while MAP\textsubscript{Mix} showed a maximum solubilization of around 1–7% for the three cations in the same period. This effect was also observed for elemental sulfur, that is, the solubilization was not favored by the dispersion process. On the other hand, for the bioactive coating with \textit{A. niger}, MAP\textsubscript{Mix+A.niger}, the oxide solubilization was significantly enhanced up to 100% for Zn\textsuperscript{2+} and Mn\textsuperscript{2+} and 60% for Cu\textsuperscript{2+} after 12 days.

A similar set of experiments was carried out using the bioactive coating loaded with \textit{A. thiooxidans}. Figure 3 shows the temporal profiles of pH, and solubilized P, Zn\textsuperscript{2+}, Mn\textsuperscript{2+} and Cu\textsuperscript{2+}, as well as of S\textsuperscript{o} oxidation in liquid nutrient medium for the control samples and that containing \textit{A. thiooxidans}. The results showed that the \textit{A. thiooxidans} cells (MAP\textsubscript{Mix+A.thio}) proliferated within the bioactive coating, given that elemental sulfur was oxidized with simultaneous pH reduction. This shows that the starch coating was efficient in keeping the \textit{A. thiooxidans} cells viable after the coating process, as noted for the \textit{A. niger} spores (Fig. 2). As seen for MAP\textsubscript{Mix+A.niger} (Fig. 2a), the \textit{A. thiooxidans}/starch coating was not effective in delaying the P release, as shown in Fig. 3a. In addition, a slight decrease in P solubilized was observed for MAP\textsubscript{Mix+A.thio} after the 3th day of incubation, suggesting that \textit{A. thiooxidans} consumed the solubilized P in its metabolic process at some extent.

Figure 3b displays the temporal profile of pH, and the results showed that MAP\textsubscript{Mix+A.thio} increased the pH of the medium from 2.8 to 4.0. This effect is most likely due to the buffering capacity of MAP and the consequent alkalization of the medium provided by high solubility of MAP, in contrast to that seen in Fig. 2b. It is interesting to emphasize that the acidification mechanisms used by \textit{A. niger} (fungus) to produce acidity is different from that used by \textit{A. thiooxidans} (\textit{A. niger} produces organic acids by a
secondary metabolism, while *A. thiooxidans* produces SO$_4^{2-}$ by consuming S$^0$ as an energy source) [15, 16, 28].

Figure 3c shows the temporal profiles of elemental sulfur (S$^0$) oxidation for the controls and *A. thiooxidans*-loaded samples. Uncoated MAP and Mix showed elemental sulfur oxidation below 1% over the 12 days of incubation, while MAP$_{Mix}$ provided a S$^0$ oxidation of 5%. By contrast, the bioactive coating with *A. thiooxidans* MAP$_{Mix+A.thio}$ provided higher oxidation rates, reaching 100% of S$^0$ oxidation. Therefore, *A. thiooxidans* is more efficient than *A. niger* in oxidizing elemental sulfur due to its inherent oxidative metabolism [15–16]. Figures 3d-3f show the temporal profiles of Zn$^{2+}$, Mn$^{2+}$ and Cu$^{2+}$ solubilized in liquid nutrient medium, respectively. MAP$_{Mix+A.thio}$ also enhanced oxide solubilization, reaching 55%, 36% and 12% for Mn$^{2+}$, Cu$^{2+}$ and Zn$^{2+}$, respectively. Although the micronutrient solubilization rates for MAP$_{Mix+A.thio}$ were lower than those for MAP$_{Mix+A.niger}$, their values were higher than those found with the MAP$_{Mix}$, Mix and MAP treatments, especially regarding the Mn$^{2+}$ and Cu$^{2+}$ cations. Although *A. thiobacillus* oxidized all elemental sulfur (Fig. 3c), the buffering effect of MAP on the medium may have impaired the solubilization of oxides [29].

The results showed that, despite *A. thiooxidans* displays the best oxidizing efficiency onto S$^0$, *A. niger* promotes better micronutrient availability from the low reactive mineral sources. This is due to the kind of acidity produced by *A. niger*, e.g., organic acids that dissolve heavy metals by forming soluble metal complexes and chelates [30–31]. Moreover, other compounds that have at least two hydrophilic reactive groups (such as phenol derivatives) may also have been excreted into the culture medium during microbial cultivation and helped solubilize the mineral oxides [30]. Other mechanisms, such as enzymatic reduction of highly oxidized metal compounds may also play a role in the oxide solubilization process, although the enzymatic effect is low in comparison with the acidification due to organic acids [31].

### 3.2 Incubation in soil experiments

Figure 4 shows the temporal profiles of pH, S$^0$ oxidation and Zn$^{2+}$, Mn$^{2+}$ and Cu$^{2+}$ solubilization in the soil incubation experiments, which were performed to evaluate the effects of the bioactive coating under more real conditions. It is possible to notice in Fig. 4a that the control treatment (Mix) had a minimal pH variation remaining at 6.3 after 42 days of incubation. On the other hand, the treatments with the MAP granules showed a similar behavior with increasing pH in 3 days, except for MAP$_{Mix+A.thio}$. MAP solubilization basifies the soil and acts as a buffer, reaching a pH 7.1. For MAP$_{Mix+A.thio}$, pH increased after 7 days of incubation, but significantly reduced after 42 days (Table 2). The increased acidification found with MAP$_{Mix+A.niger}$ and MAP$_{Mix+A.thio}$ was led by oxidation of S$^0$ to SO$_4^{2-}$ and organic acid production, as aforementioned.
Table 2
pH, micronutrients solubilization and elemental sulfur oxidation after 42 day of incubation in soil*.

| Treatment          | pH   | % Oxidized | % Solubilized |
|--------------------|------|------------|---------------|
|                    |      | S          | Zn  | Mn  | Cu  |
| MAP                | 6.49 | ~ 0 a      | ~ 0 | ~ 0 | ~ 0 |
| MIX                | 6.24 | ~ 0 a      | ~ 0 | ~ 0 | ~ 0 |
| MAP<sub>mix</sub>  | 6.30 | 26.71 b    | 3.86| 3.23| 3.24|
| MAP<sub>mix+a.niger</sub> | 6.24 | 76.44 c    | 14.62| 27.67| 10.86|
| MAP<sub>mix+a.thio</sub> | 5.80 | 71.32 c    | 4.89| 11.48| 5.83|

* Differences between mean values were determined by analysis of variance (ANOVA), and differences between treatments were compared using the Fisher’s test (p < 0.05).

Figures 4b-4d present the results of Zn<sup>2+</sup>, Mn<sup>2+</sup> and Cu<sup>2+</sup> solubilized in soil for the different treatments. Uncoated MAP did not lead to Zn<sup>2+</sup>, Mn<sup>2+</sup> and Cu<sup>2+</sup> solubilization over 42 days of incubation. This occurred due to low amounts of these nutrients in the sample, as observed in the liquid medium experiments (Figs. 2 and 3). Moreover, the treatment MAP<sub>mix</sub> showed slight solubilization for Zn<sup>2+</sup>, Mn<sup>2+</sup> and Cu<sup>2+</sup> of 3.8, 4.1 and 3.4%, respectively. This indicates that the particulate dispersion process was not sufficient to result in a significant oxide solubilization due to the low solubility of oxides in water. This effect points out the role of the microorganisms loaded in the bioactive coatings, creating a synergistic effect that significantly enhanced the oxide solubilization, as well as increased microorganism viability in soil. Noteworthy, these fertilizers are frequently applied in arid soils, which typically display low biological activity.

The MAP<sub>mix+a.niger</sub> sample presented a significant increase in the solubilized micronutrient levels, such as 6.1 and 24.1 for Zn<sup>2+</sup> and Mn<sup>2+</sup> within 3 days of incubation. MAP<sub>mix+a.niger</sub> also presented a linear increase in Zn<sup>2+</sup> solubilization, reaching 11.9% and 14.6% in 14 and 42 days, respectively. Similarly, the solubilized Mn<sup>2+</sup> also increased up to 14 days of incubation, reaching 34.4%, while the amount of Cu<sup>2+</sup> solubilized was 10.9% after 42 days. MAP<sub>mix+a.thio</sub> also had an effect on the solubilization of oxides, with maximum solubilization of 16 and 14% for Mn<sup>2+</sup> and Cu<sup>2+</sup> after 3 and 14 days of incubation. The effects of MAP<sub>mix+a.thio</sub> on the solubilized Zn<sup>2+</sup> and Mn<sup>2+</sup> contents were not as expressive as those obtained with MAP<sub>mix+a.niger</sub>. However, the Cu<sup>2+</sup> solubilization was more significant for MAP<sub>mix+a.thio</sub> (14%) rather than for MAP<sub>mix+a.niger</sub> (10.8%). Noteworthy, the Cu<sup>2+</sup> concentration in solution tends to reduce after 14 days, suggesting a precipitation effect. This can be associated with the increase in pH, leading to the precipitation of metals (especially Cu<sup>2+</sup>) and explains their low solubilization. On the other hand, the organic acids (produced by A. niger) chelate the cations, reducing precipitation and therefore increasing their solubilization, which are defined as acidolysis and complexolysis mechanisms, respectively. To
confirm this hypothesis, Table 3 shows the solubilization of the respective oxides (ZnO, MnO, CuO) in different organic acids (citric and oxalic acid) and inorganic acids (sulfuric and hydrochloric acid). The experiment showed that organic acids (citric and oxalic) were more efficient in solubilizing oxides when compared to sulfuric and hydrochloric acids under similar conditions.

Table 3
Oxide solubilization in different organic and inorganic acids.

| Treatments | Sulfuric acid | Hydrochloric acid | Oxalic acid | Citric acid |
|------------|---------------|------------------|-------------|-------------|
| ZnO        | 13.33 ± 1.39  | 3.93 ± 0.25      | 0.25 ± 0.09 | 24.11 ± 0.97 |
| CuO        | 3.11 ± 0.07   | 0.27 ± 0.01      | 0.22 ± 0.03 | 7.37 ± 0.49  |
| MnO        | 10.00 ± 0.76  | 2.90 ± 0.14      | 10.24 ± 1.49| 16.01 ± 0.96 |

The effects associated with the elemental sulfur dispersion in the biocoating are shown in Fig. 4e. The results showed a low S° oxidation for MAP and Mix, similar to that noted in liquid system (Figs. 2f e 3f). However, for MAPMix, an S° oxidation of around 20% was reached after 12 days, remaining unchanged until the end of the incubation experiment. An elemental sulfur oxidation of about 70% was reached in 42 days of incubation for MAPMix+A.niger and MAPMix+thio. This confirms that the microorganisms have excellent ability to oxidize S°, and that the biocoatings integrated optimal features for the intended application.

Table 2 compiles the final results with statistical analysis of the soil incubation experiment (42 days) in terms of pH, micronutrient solubilization and elemental sulfur oxidation. The statistical analysis indicated a significant difference between treatments without (MAP, Mix and MAPMix) and with (MAPMix+A.niger and MAPMix+thio) microorganisms. The inoculation of microorganisms provided a significant increase in the nutrient availability, where MAPMix+A.niger reached the highest solubilization levels for Zn^{2+}, Mn^{2+} and Cu^{2+}. The difference in solubilization can be associated with the higher potential of organic acids to keep the cations soluble (complexes or chelates) in water. However, the treatments with microorganisms (MAPMix+A.niger and MAPMix+thio) showed no statistical difference in relation to the elemental sulfur oxidation. The starch/sulfur matrix may not have been an ideal system to obtain the maximum oxidizing performance of Acidithiobacillus thiooxidans. On the other hand, Aspergillus niger was versatile for promoting nutrient availability. This indicates a possible synergy between Aspergillus niger and the starch/sulfur matrix, so that this matrix favored the proliferation of A. niger and organic acids production, as described by Guimarães et al. (2018) and Klaic et al. (2018) [13, 24]

Finally, the soil incubation test provides a more adverse environmental condition for microorganisms when compared to the liquid system. It was noted that the bioactive coating was also efficient in keeping the microorganisms viable to act as promoters of micronutrient solubilization and S° oxidation. This shows the potential of the coating strategy developed herein and opens new opportunities for the development of new class of fertilizer coatings using microorganisms.
4. Conclusions

The developed coating formulation, comprising a starch matrix, $S^0$ and oxides (ZnO, MnO, CuO) was effective in encapsulating A. niger or A. thiooxidans cells, and had good adhesion on MAP granules, leading to an ideal vehicle for micronutrients – over other fertilizers. There was a synergy between the nutrients and microorganisms incorporated in the biocoatings, resulting in system acidification and increasing both micronutrient availability and elemental sulfur oxidation. This innovative system can ensure the supply of multiple nutrients to plants by using low reactivity, inexpensive sources through a more environmentally-friendly management. In addition, the biocoating process can also incorporate (or encapsulate) other microorganisms of agronomic interest to be applied on different granular fertilizers.

Declarations

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Declaration on conflict of interest

The authors declare that they have no conflict of interest.

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**Figures**

![Cross-sectional Scanning Electron Microscopy (SEM) images of (a) bare and (b) coated MAP granules with biocoating containing Aspergillus niger (MAPMix+A.niger) and (c) Acidithiobacillus thiooxidans (MAPMix+A.thio). MAP fertilizer and biocoatings MAPMix+A.niger and MAPMix+A.thio are highlighted in](image)

**Figure 1**

Cross-sectional Scanning Electron Microscopy (SEM) images of (a) bare and (b) coated MAP granules with biocoating containing *Aspergillus niger* (MAPMix+A.niger) and (c) *Acidithiobacillus thiooxidans* (MAPMix+A.thio). MAP fertilizer and biocoatings MAPMix+A.niger and MAPMix+A.thio are highlighted in...
blue, yellow and green, respectively. The images were colored using Adobe Photoshop CS5 software. Original images are available in the Supplementary Material – Figure S1.

Figure 2

Temporal profiles of (a) P solubilized, (b) pH, (c) SO₄²⁻ oxidized, (d) Zn²⁺, (e) Mn²⁺ and (f) Cu²⁺ solubilized for control and Aspergillus niger-containing treatments. Mean values ± standard deviations.
Figure 3

Temporal profiles of (a) P solubilized, (b) pH, (c) SO42- oxidized, (d) Zn2+, (e) Mn2+ and (f) Cu2+ solubilized for control and Acidithiobacillus thiooxidans-containing treatments. Mean values ± standard deviations.
Figure 4

Temporal profiles of (a) pH, (b) Zn2+, (c) Mn2+ and (d) Cu2+ solubilized and (d) SO2- recovered in soil incubation experiment for control, Aspergillus niger- and Acidithiobacillus thiooxidans-containing treatments. Mean values ± standard deviations.

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