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Alginate Spheres With Urea and Linoleic Acid: A Biological Nitrification Inhibitor Proposal

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Abstract: An alternative to reduce $N\text{-}NO_3^-$ and N_2O losses and improve nitrogen-use efficiency is inhibitors of ammonia monooxygenase and hydroxylamine oxidoreductase enzymes. Linoleic acid is a biological nitrification inhibitor. In this study, alginate spheres were produced to encapsulate urea and linoleic acid. Two procedures were evaluated for the preparation of the spheres: spray needle and gravity. Different (alginate: urea: linoleic acid) ratios were tested: coating (alginate), urea sources (inorganic fertilizer-urea and urine), and inhibitor (linoleic acid). The encapsulation percentage of the actives was evaluated through the percentage of lipids (from linoleic acid) and N (from urea or urine). The release kinetics of the actives in water and soil were also conducted. Spheres with the highest percentage of lipids (10.4%) were obtained by gravity with urine 1:1:0.3. The highest percentage of N (10.8%) corresponded to spheres by gravity with urea 1:3:0.3. Gravity spheres 1:1:0.3 with urea released 93% of N and 100% of lipids after 28 days. Gravity spheres 1:1:0.3 with urine released 90% of N after 28 days and all lipids on the seventh day, so the use of urine in sphere preparation was discarded. The most efficient procedure was gravity 1:1:0.3 for the purpose of using them as nitrification inhibitors.

Key words: agrochemical, biotechnology, coacervation, encapsulation, urine

1. Introduction

Fertilizers are one of the agrochemicals required to produce food and fulfill the needs of an ever-growing population. Between 40% and 70% of applied N is lost through volatilization (N_2O) or leaching ($N\text{-}NO_3^-$), which has a negative impact on the environment [1]. $N\text{-}NO_3^-$ can leach or be converted to N_2O, a greenhouse gas 300 times more potent than CO_2, via denitrification [2]. One proposed method for reducing N loss is the addition of nitrification inhibitors, synthetic molecules that act in the nitrification process. Although there are many on the market, most are synthesized and present some disadvantages [3]. Therefore, biological inhibitors are preferred, a term suggested in 2006 by Subbarao et al. [4], who demonstrated that the root system of Brachiaria humidicola generates compounds capable of inhibiting nitrification. One of these is linoleic acid, which acts on the urease, the ammonia monooxygenase, and the hydroxylamine oxidoreductase enzymes [5]. Several studies have indicated that the encapsulation of synthetic inhibitors can increase their efficiency, but simultaneous encapsulation of nitrogen and a biological nitrification inhibitor has not been reported.

The present study aims to generate spheres of urea and linoleic acid. The release of the active ingredients from the spheres prepared with urea (inorganic fertilizer) and water was also compared with those from urine as a source of urea and solvent.

2. Methodology

2.1 Alginate Spheres Preparation

The coacervation encapsulation method was used.

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Three urea solutions (Yara, USA): 0.5%, 2%, and 6% (w/v) and one conjugated linoleic acid (CLA) solution (Sigma-Aldrich, USA) at 0.6% (w/v) were prepared.

In all the cases, alginate solution (2%, w/v) prepared with acidulated water (pH between 5 and 6) was used as solvent. The alginate:urea:CLA solutions were mixed at different concentration ratios: 1:0.25:0.3, 1:1:0.3, and 1:3:0.3. The mixture was passed through a sieve (mesh 35) and Tween 80 (Meyer, Mexico) was added to reach a final concentration of 3% (w/v).

The spheres prepared as described above were compared with spheres prepared with human urine, which was used as a solvent and an additional source of urea. Urine was collected and stored in the refrigerator for a maximum of three days, after filtered (200-300 μm) and sterilized (120°C, 107 kPa) for 20 minutes. The alginate:urea:CLA ratios evaluated in the spheres with urine were 1:1:0.3 and 1:0:0. The preparation protocol described above was followed.

Coacervate addition (alginate:urea:CLA) was carried out using two procedures: spray needle and gravity. In the first one, a peristaltic pump passed the coacervate through a spray needle with an internal diameter of 0.4 mm (⅛JJCO-SS16 AIR ATOM).

The gravity method consisted of the output of the coacervate through 20 holes of 1-2 mm, arranged at the base of three 1 L plastic bottles. In both cases, the particles fell from a height of 30 cm into a 2% (w/v) $CaCO_3$ equilibrium solution and remained there for 30 minutes to allow reticulation. They were removed and dried at 30°C, overnight.

Lipid extraction was carried out in a Soxhlet apparatus (Lab-Line 5000, Labline Instruments, India): five grams of spheres were added to a 250 mL flat-bottom flask containing 150 mL of n-hexane (Sigma-Aldrich, USA). The excess of solvent was recovered with a rotavapor (RE200, Yamato Scientific, Japan) and the sample was taken to constant weight in a furnace, at 70°C. The weight obtained was considered as the total lipids [6].

N was quantified using a FLASH 2000 elemental analyzer, Thermo Scientific. To do so, the spheres were pulverized, and 60 mg of sample were weighed.

2.2 Release Kinetics of N and Lipids in Water

Ten grams of spheres were placed in 1 L containers with 500 mL of distilled water and incubated at 28±1°C for hours (h) or days (d). After 0.5 h, 3 h, 5 h, 8 h, 24 h, 3 d, 7 d, 14 d, 21 d, and 28 d, the spheres were recovered by filtration and dried in an oven at 30°C. The amount of N in the spheres was quantified using the FLASH 2000 elemental analyzer and lipids by the Soxhlet method.

2.3 Release Kinetics of N and Lipids in Soil

A soil from the municipality of Tepetlaoxtoc de Hidalgo (state of Mexico) was used. The soil had a water pH (1:2) of 7.1 [7], an organic matter content of 1.9% [8], and a sandy loam clay texture [9]. Five grams of capsules were placed in pots containing 250 g of dry soil (< 2 mm) and covered with another 250 g of soil. They were kept at room temperature and at 20% of their field capacity for 0.5 h, 3 h, 5 h, 8 h, 24 h, 3 d, 7 d, 14 d, 21 d and 28 d. At the designated time, the spheres were recovered manually, and the N and lipids were quantified by the aforementioned methods.

3. Results and Discussion

The encapsulation percentage of N was higher in the spheres obtained by gravity with urea in the ratio 1:3:0.3 (10.8%) and 1:1:0.3 (6%). In the case of lipids, the highest encapsulation was obtained in the spheres by gravity with urine in the ratios 1:1:0.3 (10.4%) and 1:0.25:0.3 (8.8%) (Fig. 1).

As the amount of urea 1:1:0.3 was increased to 1:3:0.3, the percentage of lipids decreased. This can be explained by an insufficient alginate mass, insufficient to protect the active ones from dissolution [10]. What was observed in the spheres with urea-fertilizer obtained by gravity 1:0.25:0.3 did not occur in the spheres by gravity 1:1:0.3 with urine. The salts in urine

favoured the formation of mixed micelles, which increased the lipids solubility in the alginate solution [11] and favoured encapsulation.

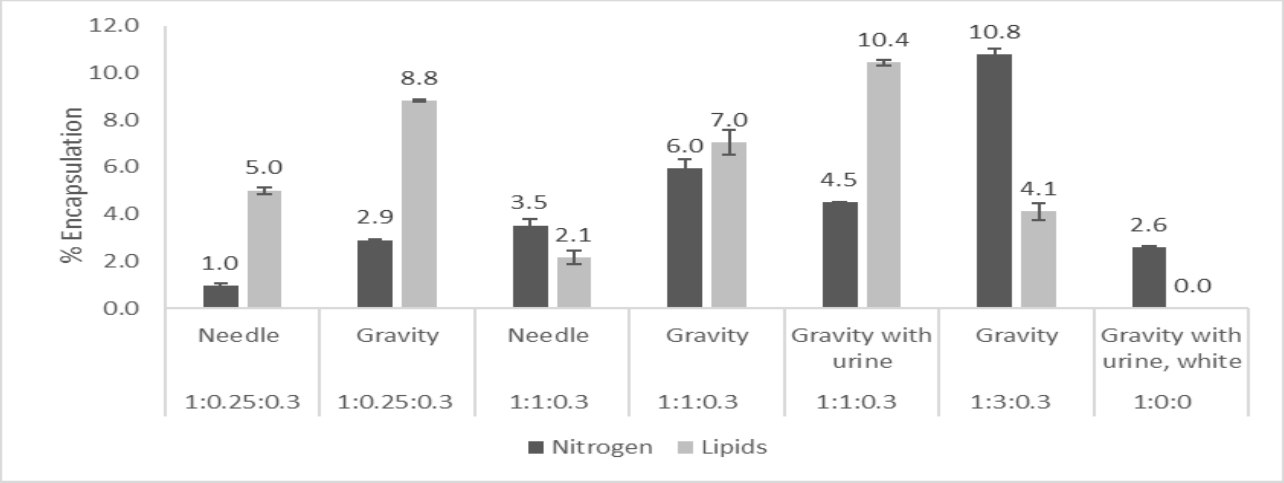

Fig. 1 Nutrient and inhibitor encapsulation with two preparation methods and different ALG:U:CLA ratios. Standard deviation (n = 3).

N encapsulation by gravity with urine was 6% at the ratio 1:1:0.3 and 4.5% at 1:1:0.3. These percentages are lower than those reported in works where urea granules are coated with various materials, in which up to 22% of N has been encapsulated. The higher encapsulation is attributed to the fact that the urea granules are not hydrated before their coating, which prevents N loss during preparation. The amount of N encapsulated with alginate and linoleic acid was greater than that reported in other encapsulations (< 1% N) [12]. To evaluate the use of urine in the preparation of spheres, the spheres prepared by gravity of urea-fertilizer in a 1:1:0.3 ratio and those of gravity with urine in a 1:1:0.3 ratio were selected. The release curves of N and lipids in water and soil were compared (Fig. 2). The release of N in water after five hours was 67% in the spheres without urine and 64% in the spheres that contained it. On the third day, the total amount of the active ingredient was released in both types of spheres. In soil, after five hours, the spheres with urine released 34% of N and the spheres without urine released 32%. Both spheres released around 90% of the active ingredient between days 21 and 28. Because urea is soluble in water (108 g/100 mL) and is absorbed by the polymer, the pore size of the alginate's three-dimensional network is increased, favouring the diffusion of the compound in the polymer [13].

Regarding lipids, the release in water of spheres without urine after five hours was 41%, and for spheres with urine it was 85%. On the third day, spheres without urine released 75% of the active ingredient, while spheres with urine released the total amount. In soil, spheres without urine released 27% of the lipids after five hours, and spheres with urine released 68%. The total amount of the active ingredient in spheres without urine was released on day 28, and in spheres with urine, it was released on the seventh day. The release of lipids from spheres prepared by gravity 1:1:0.3 with urine in soil was similar to their release in water. This is due to the presence of salts in urine that form micelles with fat, favouring their release. Spheres prepared by gravity without urine had a faster release of N and lipids in water than in soil, where ions and particles present are adsorbed by the coating, creating a physical barrier that decreases the swelling degree of the matrix and subsequent dissolution of the active ingredients [13, 14].

In soil, the release of N from gravity spheres without urine was similar to that reported by Wang et al. [13], where carrageenan and alginate were used to coat

urea-fertilizer granules. These coatings allowed for the release of 39%, 72%, and 94% of N at 2, 5, and 25 days, respectively. Longer-lasting N release was reported when preparing alginate capsules with acrylic acid, acrylamide, ammonium persulfate, bisacrylamide, and biochar as a source of N. After 30 days, only 60% of N was released in soil [15]. However, some of these chemical substances are harmful to health, such as acrylamide, which is a carcinogen, and its infiltration into aquifers represents a danger to health, or they are not easily biodegradable, as in the case evaluated in this study.

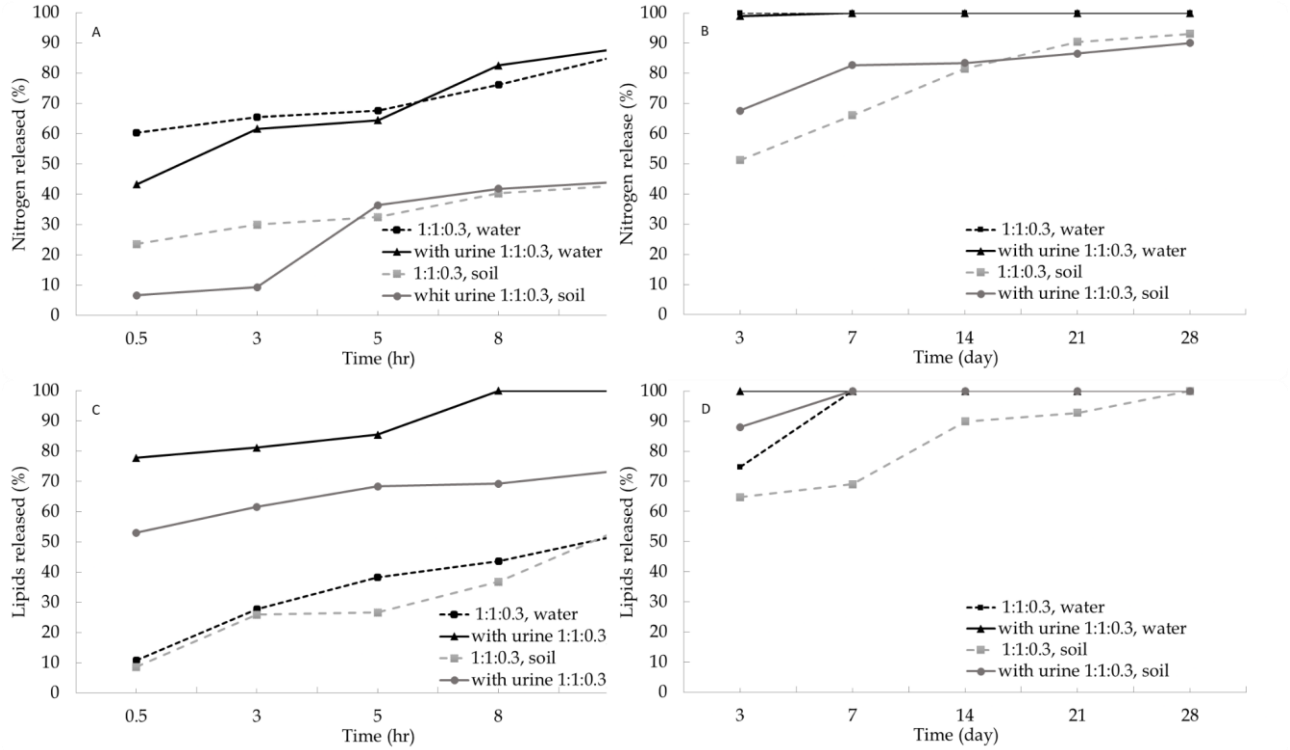

Fig. 2 Release of N in water and soil in hours (A) and days (B), and release of lipids in water and soil in hours (C) and days (D).

4. Conclusions

Alginate-N-lipid spheres were successfully obtained. Of the evaluated methods, the gravity method allows efficient, easy, and fast encapsulation, in addition to not using electric energy to form the spheres. The 1:1:0.3 gravity spheres with urine release all lipids in the soil on the seventh day, so their use in spheres preparation is discarded. Gravity spheres with a 1:1:0.3 ratio with urea-fertilizer and without urine release all N and lipids in the soil after four weeks, so they are preferred for further evaluation as nitrification inhibitors.

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