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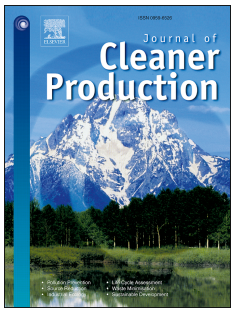

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Antonio Di Martino: Conceptualization, Methodology, Data curration, Analysis and interpretation of data, Writing- Original draft preparation. **Yelena A. Khan:** Methodology, Analysis and interpretation of data, Writing- Original draft preparation. **Silvie Durpekova:** Metodology, Writing- Reviewing and Editing. **Vladimir Sedlarik:** Supervision, Funding acquisition, Writing- Reviewing and Editing**. Ondrej Elich:** Writing- Reviewing and Editing. **Jarmila Cechmankova:** Writing- Reviewing and Editing.

Title page

Ecofriendly renewable hydrogels based on whey protein and for slow release of fertilizers and soil conditioning

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Abstract

Hydrogels show potential in agriculture for overcoming issues associated with conventional fertilizers and irrigation. A combination of hydrogels and fertilizers would limit the loss of fertilizer and curb environmental impact, especially for nitrogen-based compounds, in addition to diminishing the frequency of irrigation. A set of renewable hydrogels, based on a mixture of whey proteins and alginic acid were developed as a soil conditioner and for sustained release of the urea fertilizer. Four separate formulations were prepared from different proteins at a polysaccharide ratio of 1% to 10% w/w (with respect to protein content). The hydrogels were prepared by a heat-set process, applying calcium chloride as the cross-linking agent. The fertilizer was loaded into the hydrogel during the preparation stage to heighten loading efficiency. Investigation was made into the impact of alginic acid content on morphology, swelling behaviour - encompassing repeated swelling-drying cycles, and water retention in soil under different pH conditions. The loading capacity and release of urea from the hydrogels were studied, and the data processed in accordance with a mathematical model to discern any correlation between the structure of the hydrogel, the presence of alginic acid and the release mechanism. The results demonstrate how adding alginic acid promotes possible utilization of the whey protein hydrogel in agriculture.

Keywords: hydrogels, alginate, whey protein concentrate, fertilizers, soil conditioner, swelling

1. Introduction

Recent years have witnessed the development of innovative systems able to control the release of agrochemicals in soil. Such systems are based on various materials, either synthetic, semi-synthetic or natural, and able to yield a large amount of fertilizer while also offering protection against rapid release (*Rabat et al., 2016*). Hydrogels as materials have attracted a great deal of attention due to their capability for absorbing and retaining a large amount of water within their structure (up to hundreds of times their weight) without dissolving (*Calo et al., 2016*). An additional advantage stems from their capacity to release up to 95% of the adsorbed water into the surrounding environment; once dried out, they can be re-hydrated through exposure to water.

Hydrogels are primarily used as soil conditioners in an agricultural context, whereby they control the moisture of the soil and supply water to plants, as well as serving for encapsulation and sustained release

of agrochemicals (*Song et al., 2020*). Although the advantages of employing hydrogels in agriculture have been demonstrated, most commercially available products are based on polyacrylamide (PAAm) and acrylate derivatives (*Lv et al., 2019*). Since these are not fully biodegradable, they are considered potential soil contaminants. Even though PAAm is not actually designated as toxic, commercial formulations contain residual amounts of acrylamide – a neurotoxic and carcinogen, raising concerns over possible contamination of soil and food. Hence, biopolymers have been rising in popularity because they are environmentally friendly and biodegradable, while also usually being less expensive to produce than synthetic materials (*Mishra et al., 2018*; *McClements et al., 2017*).

Of the numerous natural polymers available, the preference is for those with functional groups that make them easy to modify and sensible to variation of the surrounding environment, e.g. pH or temperature (*Tang et al., 2020*; *Thombare et al., 2018*). Hydrogels that are pH-sensitive and based on biopolymers are primarily obtained from polysaccharides, such as chitosan, alginates and cellulose, or from protein which has undergone heat-induced or cold gelation.
Whey proteins make for interesting raw materials in the formulation of hydrogels. They consist of the globular proteins beta-lactoglobulin (ca 65% w/w) and alpha-lactoglobulin (ca 25% w/w), primarily responsible for the capacities of gelling, emulsifying, foaming and hydration (*Gunasekaran et al., 2007*). Gelling is chiefly facilitated through an aggregation process induced by increase in temperature, which unfolds the native protein and exposes polar residues, thereby allowing formation of the gel network (*Kharlamova et al., 2018A; Kharlamova et al., 2018B*). Mixed hydrogels based on a combination of whey proteins and polysaccharides have attracted great interest in recent years, as synergistic interactions between the two components lend additional functional properties to the subsequent hydrogels, compared to those obtained from single components (*Ozel et al., 2017*). The repulsive and/or attractive forces between the two biopolymers give rise to different behaviour, including associative phase separation, while co-solubility instigates soluble/insoluble complexes, which possess functional properties beyond those found in polysaccharides or proteins alone (*Devi et al., 2017, Ates et al., 2020, Chaudary et al., 2020*). Moreover, mixed gels benefit from enhanced structural features alongside the ability to carry active agents. The resultant properties of the gels stem directly from the nature and concentration of the components, the given weight ratio and the ionic strength of the surrounding environment (*Klein et al., 2020*). Herein alginic acid sodium salt has been chosen as component to add to the whey protein concentrate due to its recognised and well-known properties suitable for environmental application (*Sourbh et al., 2018*).

This study set out to develop a set of mixed hydrogels based on a combination of whey protein concentrate and alginic acid sodium salt ,deemed applicable for simultaneous or alternate use as a soil conditioner (by releasing water) and an agent for the controlled release of fertilizers. Soil conditioners aid retention of water in the soil by creating favourable conditions for plant roots to grow; while agents reduce any loss of the agrochemicals they contain and inhibit unwanted conversion - especially for nitrogen-based fertilizers - into derivatives with the potential to harm the environment. In this context, urea was selected as a model to assess the loading and release capabilities of the hydrogels, since it is extensively deployed as a fertilizer in agriculture. The hydrogels were prepared by a heat-set process, after which the effect of alginic acid (1%, 5% and 10% of the weight of the whey protein) was investigated on morphology, swelling, water retention in soil, encapsulation efficiency and urea release kinetics.

2. Materials and methods

2.1 Materials

Alginic acid sodium salt, urea and 4-(Dimethylamino)benzaldehyde were purchased from Sigma Aldrich. Nutri Whey™ 800F (a pure whey protein product, 80% obtained from acid whey) was sourced from

FrieslandCampina Ingredients, NL, while acetic acid and hydrochloric acid came from Chromservis, Prague, Czech Republic.

2.2 Preparation of protein-polysaccharide hydrogels

Sets of hydrogels based on whey protein concentrate (WPC) in combination with alginic acid sodium salt (ALG) were prepared by a heat-set process. A 20% w/w solution of WPC in distilled water was placed under magnetic stirring for 24h for complete dissolution and then filtered to remove any lumps. Sodium azide (0.01% v/v) was added afterwards to prevent bacterial growth. This WPC solution was heated to 80°C and underwent stirring for 20 minutes at that temperature. The WPC solution was subsequently cooled to 50°C and a water solution containing various concentrations of ALG added into it. Four sets of hydrogels were fabricated in total, one solely based on WPC as the control, the other three containing ALG at 1%, 5% or 10% in weight with relation to the amount of WPC, respectively: WPC+1% ALG; WPC+5% ALG; and WPC+10% ALG. The pH was adjusted to 4.0 by HCl (0.1 M) as is close to the pI of the whey protein (pI 4.3). At pH= pI or close to pI the protein undergoes to higher denaturation degree when temperature raise favouring the formation of the hydrogel. In such condition increasing the temperature the protein denaturise faster forming the hydrogel. In addition at pH 4.0 all the carboxylic groups displaced along ALG backbone are in the ionic state. Afterwards, the mixture was stirred for 1h and then the temperature raised to 90°C for 10 minutes. Then, 0.5 ml of $CaCl_2$ (0.5 mg/ml) were added by a syringe, and cooled in an ice bath. The same procedure was adhered to for formulation of the hydrogels with urea, the latter being supplemented after addition of the polysaccharide. The hydrogels obtained were thoroughly washed with distilled water and stored at 4°C.

Figure 1 Schematic representation of hydrogels synthesis procedure. Step 1) addition of sodium azide and increase temperature to 80°C; 2) addition of ALG and urea and cooling to 50°C; 3) raise temperature to 90°C and addition of CaCl2; 4) fast cooling using ice bath to 0°C. ALG, Urea and CaCl2 were added under magnetic stirring.

2.3 Preparation of dry gel tablets

The gel tablets were prepared in accordance with a method reported in the literature (*Kikuchi et al., 1999*). The hydrated gels were cut into tablets with the dimensions of 10 mm in diameter and 2 mm in thickness, and subsequently dried in an oven at 60°C until they had reached a constant mass (variation equalled 0.005 g). Two distinct groups, both comprising four sets of tablets, were assembled in this manner (Table 1). The first group contained hydrogels based on WPC and WPC in combination with ALG at 1% to 10% in weight (of WPC), while the other was given over to hydrogels with the same composition but additionally loaded with urea.

Group I	**Group II**
WPC	WPC + urea
WPC+1% ALG	WPC+1% ALG + urea
WPC+5% ALG	WPC+5% ALG + urea
WPC+10% ALG	WPC+10% ALG + urea

Table 1 Designation of samples - WPC for control purposes, and the remainder with variation in the percentage of ALG.

2.4 Morphology

Scanning electron microscopy (Nova NanoSEM 450) was employed to investigate surface morphology and obtain cross sections of the dried hydrogels so as to understand the impact of ALG content on structure.

2.5 Swelling experiments and swelling/drying cycles

The swelling behaviour of the hydrogels and impact of pH and composition on water uptake were evaluated using an established protocol *(Di Martino et al., 2019)*. In brief, 500 mg of dry hydrogel was placed in a permeable bag and immersed in 300 ml of media at pH 2, 7 and 9 (ionic strength 0.2 M) at room temperature (ca 25°C). At a determined time, the sample was removed from the media, dried with filter paper and accurately weighed. The swelling ratio (SR) was obtained through the following equation:

$$SR = \frac{M_s - M_d}{M_d} \quad [1]$$

where M_s and M_d indicate the weight (mg) of the swollen and dried form, respectively.

The reswelling ability of each hydrogel was gauged by conducting swelling-drying cycles to evaluate the potential of reusing it in relation to the content of WPC and polysaccharide. Each cycle comprised: i. the dry hydrogel being placed in the media and remaining there until swelling equilibrium had been reached; ii. a period of drying at 60°C until constant weight was achieved (no change over time); and iii. the sample being put back in the media again. The value for swelling after each instance of drying in the cycle was recorded. This procedure was repeated until the weight did not differ by more than 50% of the value for the hydrated gel after its initial preparation. Any decrease in the weight of the dry hydrogel after each cycle indicated a loss in the mass of the sample, ascribed to structural failure and hydrolysis of the chains.

2.6 Water retention in soil

The influence of ALG on the capacity of the hydrogel to hold water in soil was evaluated for up to 30 days by carrying out a test for its water evaporation ratio (WER) *(Rabat et al., 2016)*. The soil was dried at 60°C for 2 days. Afterwards, 2g of dry hydrogel were placed in a container with 300g of dried soil, irrigated with 300ml of distilled water and weighed (M_i). The containers were stored at room temperature and weighed every 2 days for the period of 30 days (M_t). The value for W_{ER} was obtained by the following equation:

$$WER \ \ (\%) = \frac{M_i - M_t}{300} \times 100 \quad [2]$$

2.7 Urea release

The release of urea from the hydrogel formulations was investigated in distilled water at room temperature in order to discern the impact exerted by the presence of the polysaccharide. 500mg of the dry hydrogel supplemented with urea was placed in 100ml of media without stirring. The amount of urea released was quantified by treating 3ml of the release media with 4-(Dimethylamino)benzaldehyde

(40mmol/l) and measuring absorbance at 245nm on a UV spectrophotometer (Cary 300 UV-Vis Agilent, UK).

The urea released (UR), expressed in per cent, was calculated as follows *(Tiwari et al., 2019)*:

$$UR(\%) = \frac{\lambda_\infty - \lambda_t}{\lambda_\infty} x100 \quad [3]$$

where λ_∞ and λ_t represent the absorbance of urea released at equilibrium at a certain time (t), respectively. The release studies were performed in triplicate.

3.Results

3.1 Hydrogel Morphology

Figure 2 Morphological evaluation of the hydrogels and the effect of ALG on surface morphology. A, B and C refer to hydrogels based on WPC 20%; D, E and F to WPC+1% w/w ALG. The Figure details G, WPC+5% ALG and WPC+ 10% ALG. The two hydrogels at the top are based on WPC+5% ALG, with those at the bottom on WPC+10% ALG. Therein, H and I represent the surface morphology of WPC+5% ALG, while L and M those of WPC+10% ALG.

Figure 3 Cross-section micrographs of the hydrogels: A) WPC; B) WPC+1% ALG; C) WPC+5% ALG; D) WPC+10% ALG.

Investigation was made of the surface morphology (Figure 2) and cross-sections (Figure 3) of the dried hydrogels without urea by scanning electron microscopy. The micrographs presented in Figure 1 demonstrate how the presence of ALG influenced surface morphology and the surface properties of the material as a consequence. The surface of every sample is nonhomogeneous, with variation in the pattern of the structure. Comparing the two extremes - the surfaces of WPC (Figure 2 B, C) to WPC+10% ALG (Figure 2 L, M) - reveals the first has a visible wave-like structure, while the latter shows particles with dimensions of between 0.5 and 1 μm. It is the ALG in combination with $CaCl_2$ that gave rise to the formation of these particles. The formulation containing only 1% of ALG (Figure 2 E, F) displays no such particles, probably due to its low concentration and the lesser amount of ALG on the surface. The absence of particles in the inner structure of the hydrogel (Figure 3 D) is due to the high viscosity of the WPC+ALG 10% mixture; upon addition of $CaCl_2$, a layer of particles rapidly formed on the surface and a small amount of Ca^{2+} penetrated the inner part of the gels. Differences between the hydrogels are also discernible in their cross-sections. The WPC sample (Figure 3 A) possesses a structure that is porous, wherein pores measure over 100 microns and smaller ones appear in the surface. These pores arose through air bubbles occurring in the heating process of the WPC solution, said process denaturing the protein and facilitating formation of the gel. Adding ALG reduced the extent of porosity. The WPC + ALG 1% sample (Figure 2B) still shows high porosity in its structure, although a thicker layer lays on the surface due to cross-linking occurring between ALG and $CaCl_2$. Increasing the concentration of ALG to 5% and 10% meant that no pores existed in the inner structure due to such cross-linking. The structure of the film, seen here as a cross-section, deeply affected the uptake and displacement of water molecules, and subsequently its swelling properties and the release of urea (Tables 2 and 3 and Figure 4). Supplementation with ALG changed the inner structure of the WPC gel, consequently allowing regulation of its properties in terms of water uptake, retention and swelling–drying cycles, thereby defining the durability of the gel. Release kinetics and loading were additionally affected, along with the degradation process (described in section 3.2).

3.2 In vitro swelling behaviour

Hydrogels designed as a soil conditioner have to adsorb a high amount of water during the swelling process. This stage involves the free adsorption of water, leading to dissociation of the hydrophilic groups and subsequent repulsion between the charged groups with the creation of gaps due to expansion of the polymer chains which are filled with water molecules *(de Kruif et al., 2015)*. A priority is to understand the swelling behaviour and water-holding capacity of the given hydrogel. The amount of water that can be retained in the matrix directly correlates with cross-linking density, the concentration of hydrophilic groups and their orientation within the matrix. Table 2 presents data on increase in SR in the hydrogels following the addition of ALG. This increase occurs in proportion with the content of ALG, i.e. the number of carboxylic groups, and shows a maximum value for the sample with the highest content of ALG (10% of weight of WPC). The swelling process as it happens in the presence of hydrophilic groups essentially comprises four phases: 1) *mobility*, in which the kinetic energy of water-water vibrations is transferred into water-ion vibration (*Horkay et al., 2000.*); 2) *dissociation*, whereby dissociation of COO^- Na^+ into COO^- and Na^+ takes place due to the vibration of water molecules; 3) *hydration*, when negative charges repel against each other and are neutralized by the water molecules, with the concurrent hydration of Na^+ ions; and 4) *swelling*, where changes in hydrogen bonding confine water molecules inside the pores of the hydrogel network structure (*De Loos et al., 2005*).

Figure 4 Effect of pH, percentage and composition on the swelling ratio of the hydrogels. A) pH 2; B) pH 7 and C)pH 9 The data refer to the mean value ± SD (n=3).

The obtained data fully adhere to the phenomenon of rise in water-holding capacity through the act of increasing the concentration of polysaccharide. However, it is necessary to note such increase in capacity and the accompanied increase in swelling varied in the time that both took.

In the formulation based solely on whey protein concentrate, raising the content of ALG led to swelling equilibrium being reached more rapidly than rise in water-holding capacity. This can be ascribed to the higher number of cross-linking points that slow the movement of water within the hydrogel matrix.
In accordance with the mechanism described above, pH was expected to exert an effect on swelling behaviour. The trends shown in Figure 4 indicate the direct relationship between the pH of surrounding environment and SR – the higher the pH, the higher the value for SR. This is a consequence of the value for pI (the content of protein in WPC) rather than that for the pK_a of the carboxylic groups in ALG.

It is necessary to assess swelling and swelling-drying behaviour when developing soil conditioners and fertilizers as carriers of hydrogels. Repetition of this happens until substantial changes in ability to absorb water are observed (*Leong, et al. 2016).*

Figure 5 Swelling ratio (SR) with reference to equilibrium (maximum swelling); a number of cycles were performed until a decrease of 50% in the mean value for swelling was recorded, compared to the initial value; the data refer to the mean value ± SD (n=3).

Figure 5 presents findings for the hydrogel formulations as they underwent swelling-drying cycles. These relate to changes in water adsorption by up to 50% in a series of cycles, varying in number from 4 to 11 according to the given composition, percentage of ALG and surrounding pH. Out of the 10% formulations, ALG showed the best performance, while the optimal pH was seen to be 7. One such cycle comprised swelling, drying in an oven at 60°C until the content of weight of water equalled less than 10% (of dried weight) and swelling once again. The data gathered pertain to the swelling equilibrium achieved after the drying part of the cycle.

3.3 Water retention in soil

The data plotted in Figure 6 reveal that the water evaporation ratio (WER%) in soil was higher than in soil containing the hydrogel over 30 days of study. The water loss from the soil was caused by evaporation, as regulated by humidity, air temperature and water-holding capacity (*Rabat et al., 2016*). Water retention was seen to be best in the soil with hydrogels and 5% or 10% ALG, said samples showing losses of up to 30% after one month (for 10% ALG) and 36% (after one month), respectively. The WPC hydrogel demonstrated the highest water evaporation loss (over 80%) after one month. The variances in trends and behaviour between WPC and WPC with the ALG hydrogel potentially arose through the different structures of the materials (Figure 2 and 3), since higher porosity leads to less effective retention of water in the tested condition. It also has to be considered that variation in the surrounding environment influenced the water-holding capacity, with any such change reflected in Figure 6.

Figure 6 Water evaporation ratio (WER %) in soil and soil containing the hydrogel formulations; the data refer to the mean value ± SD (n=3).

Water content within the hydrogel is classified as bound, half-bound or free water *(Erol et al., 2019; Barros et al., 2019)*. The latter has the highest mobility and is easily lost. Swollen hydrogels contain an amount of half-bound and bound water that relates to the number of hydrophilic groups per unit volume of the given hydrogels *(Gun'ko et al., 2017; Shah et al., 2018)*. Herein, this pertains to amino and carboxylic groups. Adding ALG increased the concentration of the carboxylic groups per unit volume of hydrogel, with a consequent increase in half-bound and bound water. Interactions between water molecules and biopolymers are based on hydrogen bonds, which are weaker than the break point of the covalent bond. Once water external to the hydrogel evaporates, sufficient thermal energy exists to break the hydrogen bonds between any water stored internally and the structure of the hydrogel *(Casolaro et al., 2016; Sgambato et al., 2016)*.

3.4 Release kinetics

The release kinetics of urea from the hydrogel formulations are illustrated in Figure 7.

Figure 7 Cumulative release of urea from the hydrogel formulations at neutral pH and room temperature: A) overall release patterns; B) release patterns for the interval of 720 min. Data are plotted as ln(time) against $\ln(M_t/M_{eq})$. Graph C) refers to WPC and WPC+1% ALG, while D) shows WPC with 5% and 10% ALG. The data refer to the mean value ± SD (n=3).

The release patterns in Figure 7 A, B reveal that the effect of ALG is significant at the amounts of 5% and 10% WPC. The kinetics referring to the release of urea from WPC and WPC+1% overlap, suggesting the effect of ALG is dependent on concentration. The influence of the polysaccharide on the swelling behaviour of the WPC hydrogel exerted a subsequent impact on its release kinetics and mechanisms. Increasing the concentration of ALG meant a higher number of cross-linking points were present in the structure, and this affected the inner morphology of the hydrogel and the rate of media uptake, the dissolution of the urea and the mechanisms through which was released. Figure 7 (A, B) shows that the presence of ALG at 5% and 10% prolonged the release of urea, with 80% of the total amount of urea being released within one week, while the same amount took nearly 9h for WPC and almost 11h for WPC+1% ALG.

Figure 7A illustrates the general trend for release for the entire period of investigation. A monophasic pattern is depicted for the samples of whey and whey with 1% of ALG hydrogel, demonstrating a steady increase in urea released over time. A double phase of urea release is observed, conversely, in the presence of 5% and 10% ALG. The first is characterized by consistent rise until swelling equilibrium is

reached. Afterwards, a sustained and slow course proceeds, in which around 80% of the loaded urea is released after one week.

Reduction in time scale to the first 12 hours of contact (720 min.) is given in Figure 7B. This describes the urea release patterns for three main phases of the WPC and WPC + 1% of ALG hydrogels, alongside a monophasic one in the presence of 5% and 10% ALG. The WPC and WPC+1% ALG samples exhibited an initial light burst, wherein 5% of urea is released, followed by a lag phase of up to 2 hours and a final stage of heightened increase in the run up to 12 h, by which point almost 80% of urea had been released.

In order to define the mechanisms involved in such release and any relationship with the composition and structure of the hydrogel, the data were processed on the basis of the power of law equation:

$$\frac{M_t}{M_{eq}} = kt^n \quad [4]$$

where M_t and M_{eq} denote the diffusion of urea from the hydrogel at time t and the point of equilibrium, respectively (*Tiwari et al., 2019*). The ratio of M_t to M_{eq} represents the fraction of fertilizer released at the determined time (t), while k is a constant related to the polymer structure and n is the coefficient that describes the type of diffusion. Plotting *ln* (M_t/M_{eq}) vs *ln t* (time) gives n, denoted by the slope of the line. In accordance with the n values obtained, the following mechanisms are reported: n< 0.5 indicates quasi-Fickian diffusion; for n between 0.5 and 1, it is a case I mechanism; and for n> 1, a case II mechanism *(Rizwan et al., 2017; Li et al., 2006).*

Figure 7C and D highlight two distinct trends for WPC and WPC+ALG 1%, pertaining to two related values for n (the 1st and 2nd phases), whereas a single pattern is discernible for WPC + 5% and 10% of alginic acid, with a unique value for n correlating with the entire release process. It suggest that in WPC and WPC containing 1% of ALG at the initial phase of the release either the chain relaxation or the solvent diffusion play a certain role. After a certain time, the major contribution to the release is coming from the solvent diffusion, due to an increase in the chain relaxation time (n value increases). Conversely, in hydrogels having 5 and 10% in weight of ALG the release mechanisms are not varying during all the process.

A summary of the relative equation, with the related values for n and mechanisms involved in urea release, are displayed in Table 2.

Formulation	Equation	n	Mechanism
WPC	*Phase I** y = 0.26x – 3.48 (R^2 = 0.99)	0.26	quasi-Fickian diffusion (urea partially diffuse)
	*Phase II*** y = 1.32x – 8.22 (R^2 =0.99)	1.32	case II
WPC+1% ALG	*Phase I** y = 0.39x – 3.92 (R^2 = 0.92)	0.39	quasi-Fickian diffusion (urea partially diffuse)
	*Phase II*** y = 1.40x – 8.72 (R^2 = 0.99)	1.40	case II
WPC+5% ALG	y = 0.51x – 4.43 (R^2 = 0.95)	0.51	case I
WPC+10% ALG	y = 1.04x – 7.69 (R^2 = 0.98)	1.04	case II

Table 2 Equation for to the trends reported in Figure 6 C and D, with calculated values for *n* and the corresponding mechanisms; * from commencement till 2h; ** over 2h.

The release process includes various phases involving the migration of the loaded from the initial position within the polymer matrix towards the outer surface and then to the surrounding media *(Fu and Kao, 2010)*. The solute diffusion, the swelling ratio, and changes in the structure of the hydrogel matrix are considered influencing factors in the transport of the solute from the hydrogel towards the surrounding environment *(Fu and Kao 2010)*. The equations resumed in the Table 2 and the calculated *n* values, indicate that in all formulations the release is non-Fickian, where the polymer relaxation time is greater than the solvent diffusion time, but quasi Fickian and non-Fickian (case I and case II) where the polymer relaxation time is equal or lower than the solvent diffusion time *(Grassi, 2005)*. The data indicate a direct relationship between the increase in the ALG content and the variation in the release mechanism. As the % of ALG raise the *n* value, indicating a reduction of the chain mobility, due to the increase in the concentration of the crosslinking points. It moves away from the Fickian release trend as the leading force in the release is the solvent diffusion and no chains relaxation.

Conclusions

A set of hydrogels based on natural sources were prepared as a soil conditioner and as carriers for the release of urea. The hydrogels were obtained by a heat-set process, applying whey protein concentrate in combination with alginic acid in addition to $CaCl_2$ as the cross-linking agent. The amount of whey protein was maintained as a constant in the given formulations, while the variable in the experiment constituted the amount of ALG incorporated; this was expressed as the weight ratio between the content of protein and polysaccharide, from a minimum of 1% to a maximum of 10%. The impact of ALG on surface morphology and the inner structure of the hydrogels was evident. The WPC hydrogel showed high porosity with non-homogeneous pores exceeding 100 microns in size. The addition of ALG shifted the structure from porous to non-porous with a more compact inner structure. Porosity affected certain aspects, such as the swelling ratio and water retention in soil. Samples containing a higher amount of ALG demonstrated a different response to pH in terms of SR and time, since a longer period of exposure was required to reach equilibrium. The number of swelling-drying cycles that defined the durability of the hydrogel as a soil conditioner went up in number as the concentration of ALG was raised. The water retention test performed in soil indicated a slower WER for the hydrogels supplemented with 5% and 10% ALG. The results clearly show the advantages of adding ALG into the WPC hydrogel, and its presence also affected the release of urea. Processing the data revealed that the urea was released from WPC and WPC+1% ALG in two main phases under two different mechanisms - quasi-Fickian and case II release. Increasing the amount led to only one mechanism for release in its entirety, this being case I for the hydrogel with 5% ALG and case II for those containing 10% ALG.

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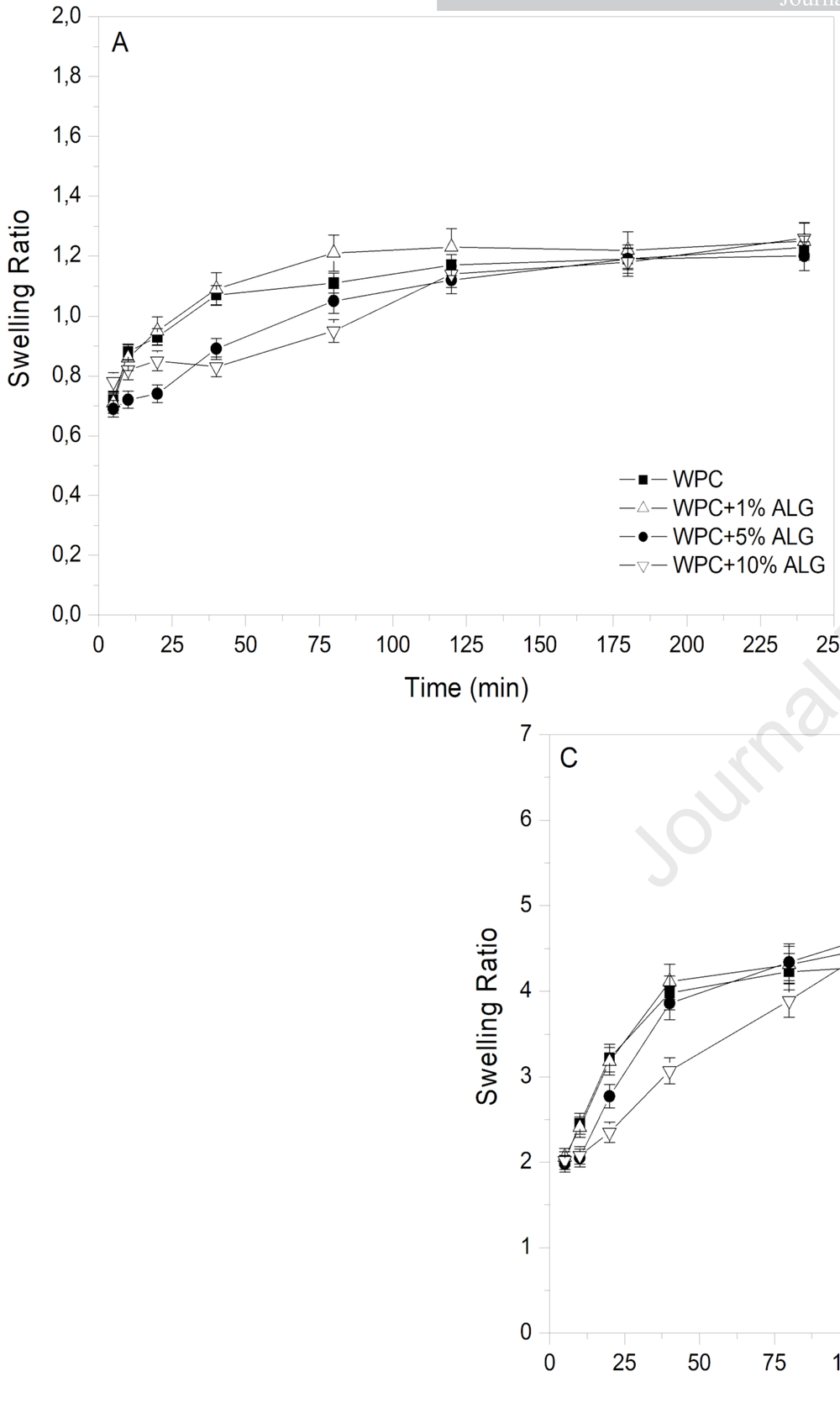

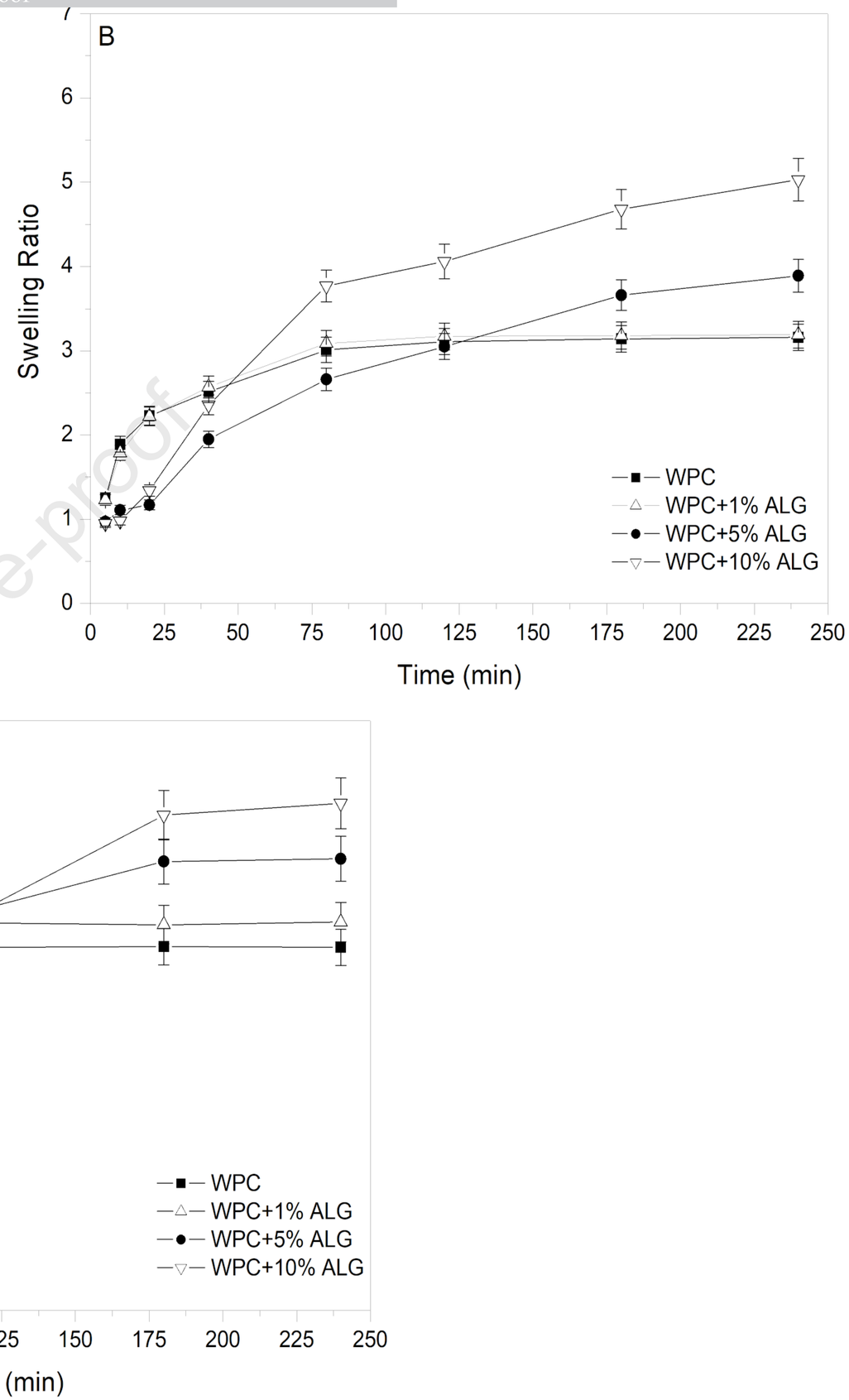

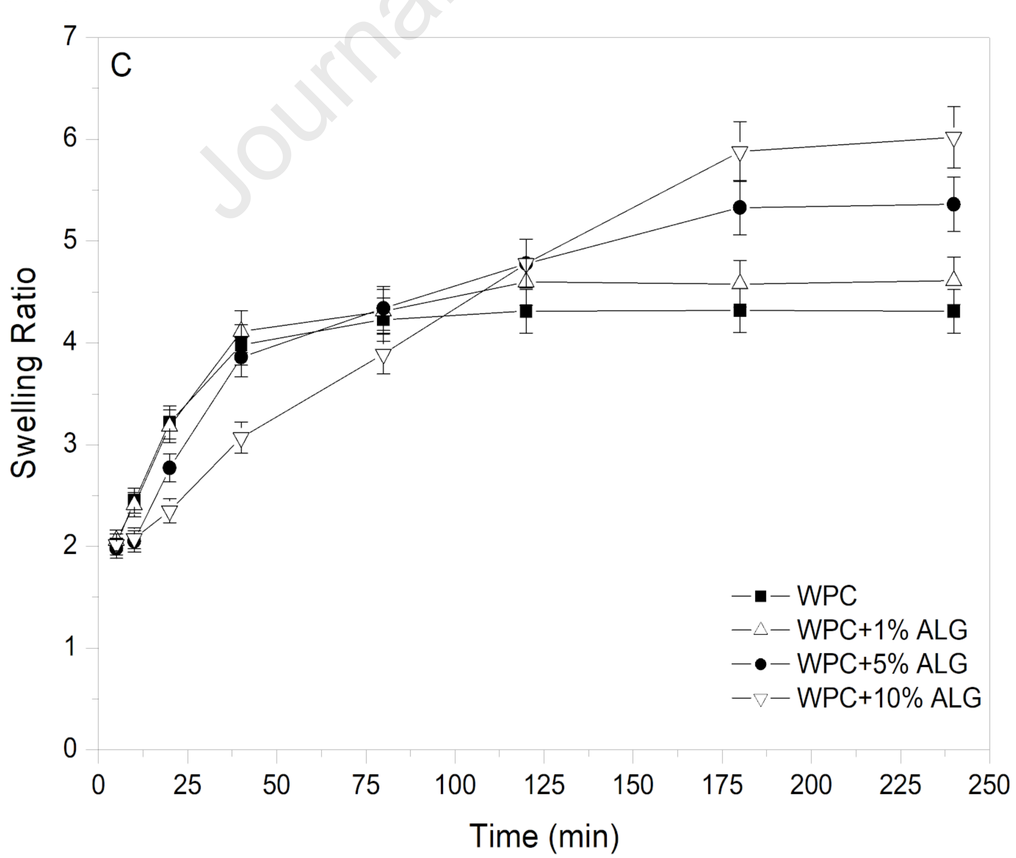

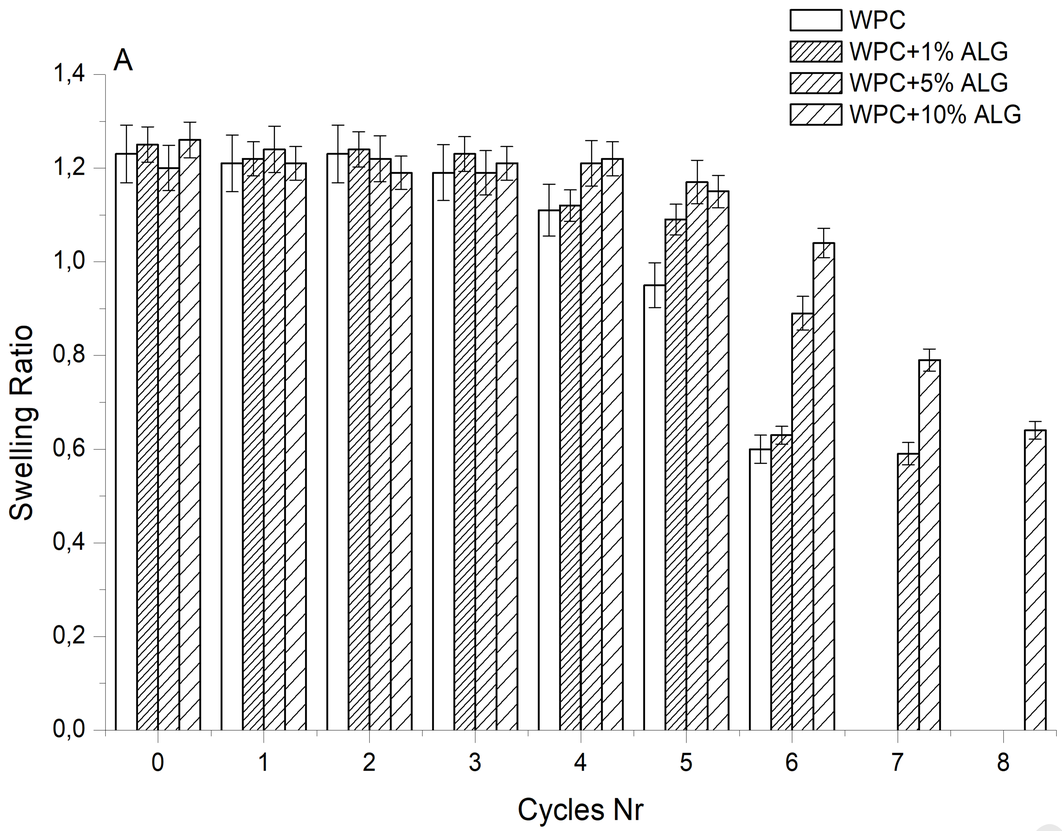

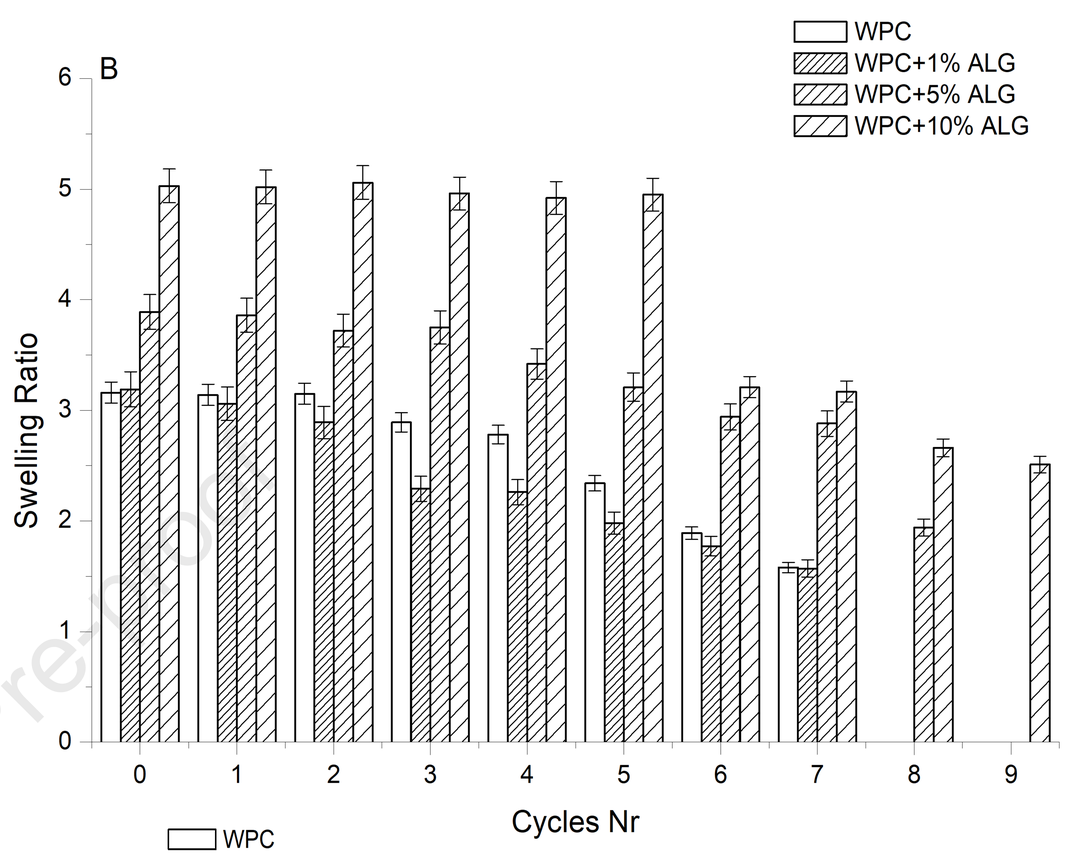

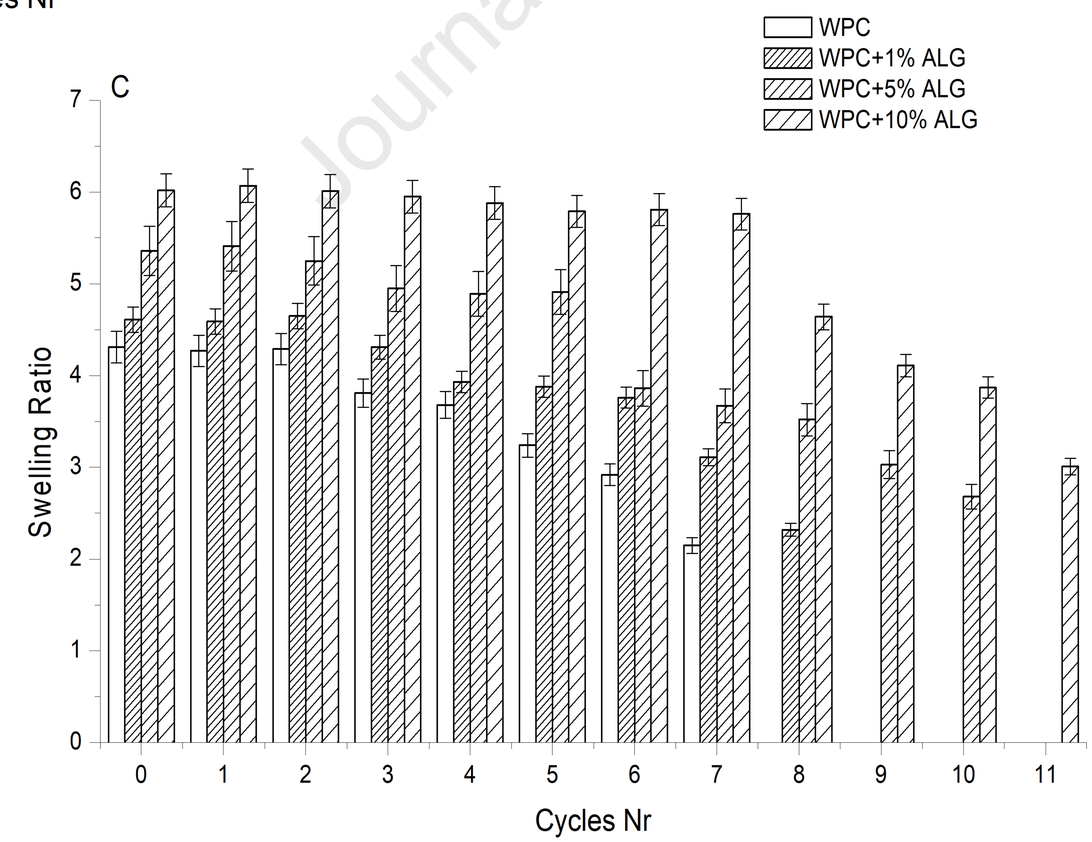

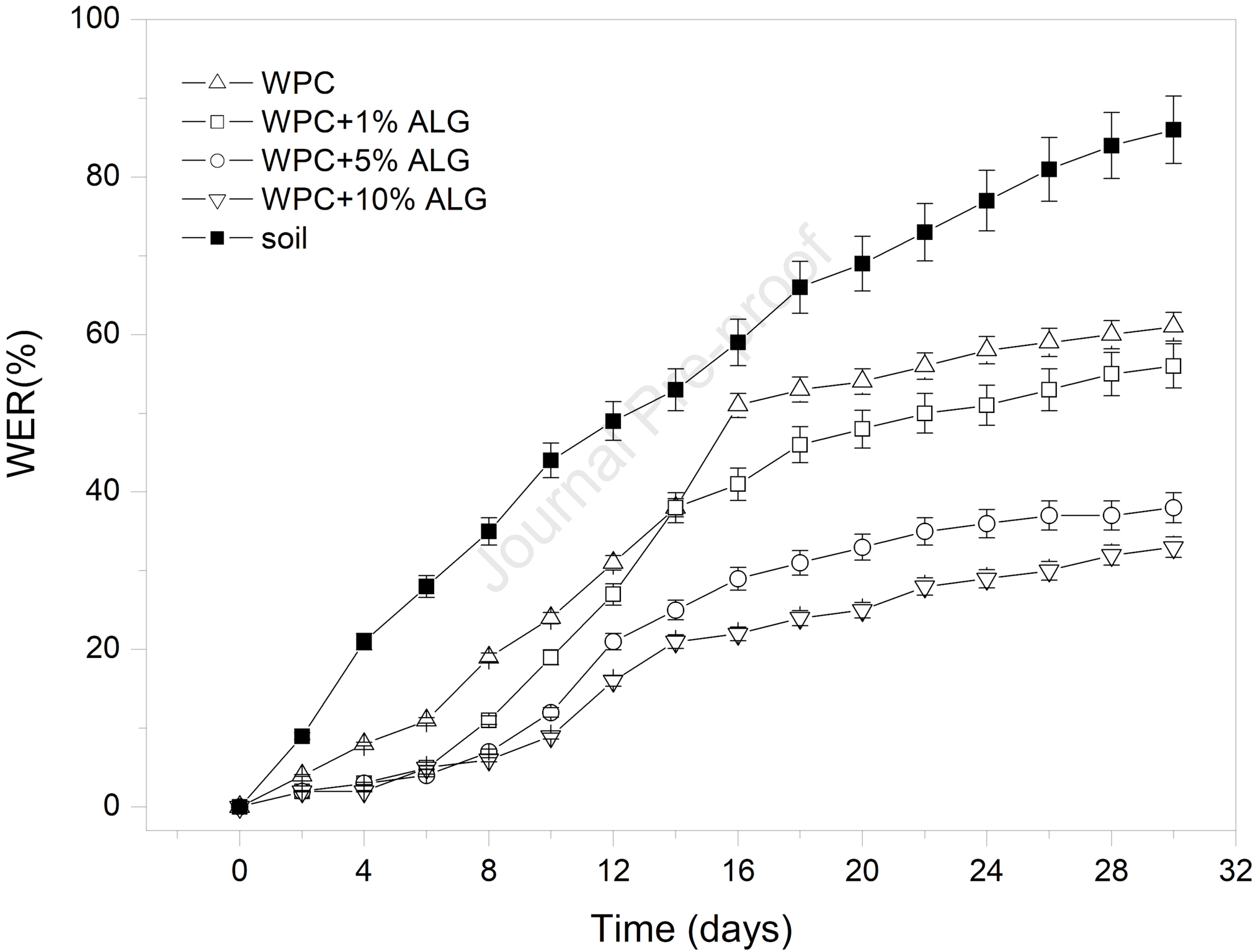

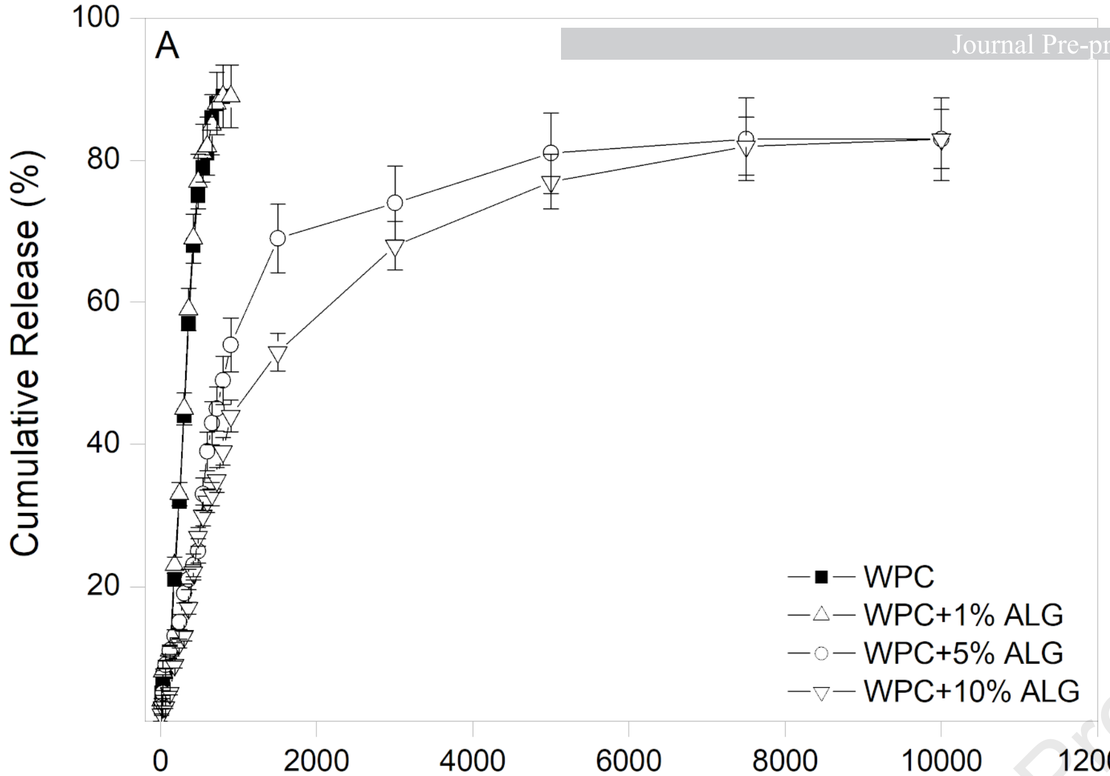

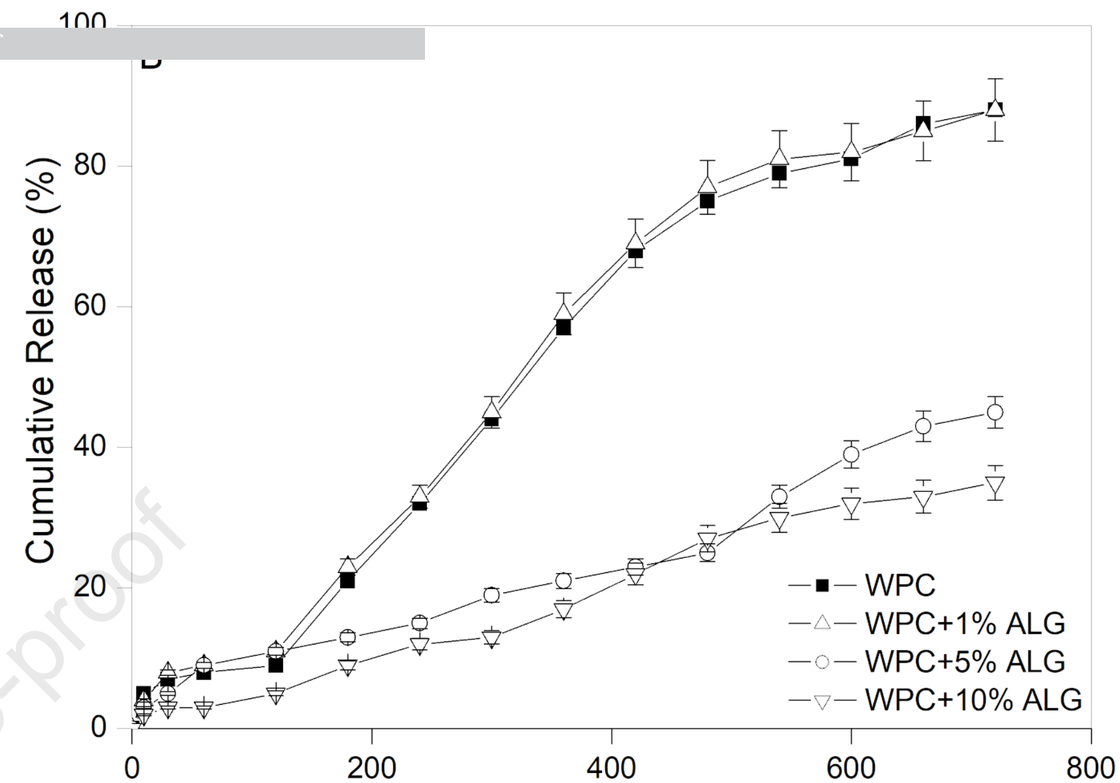

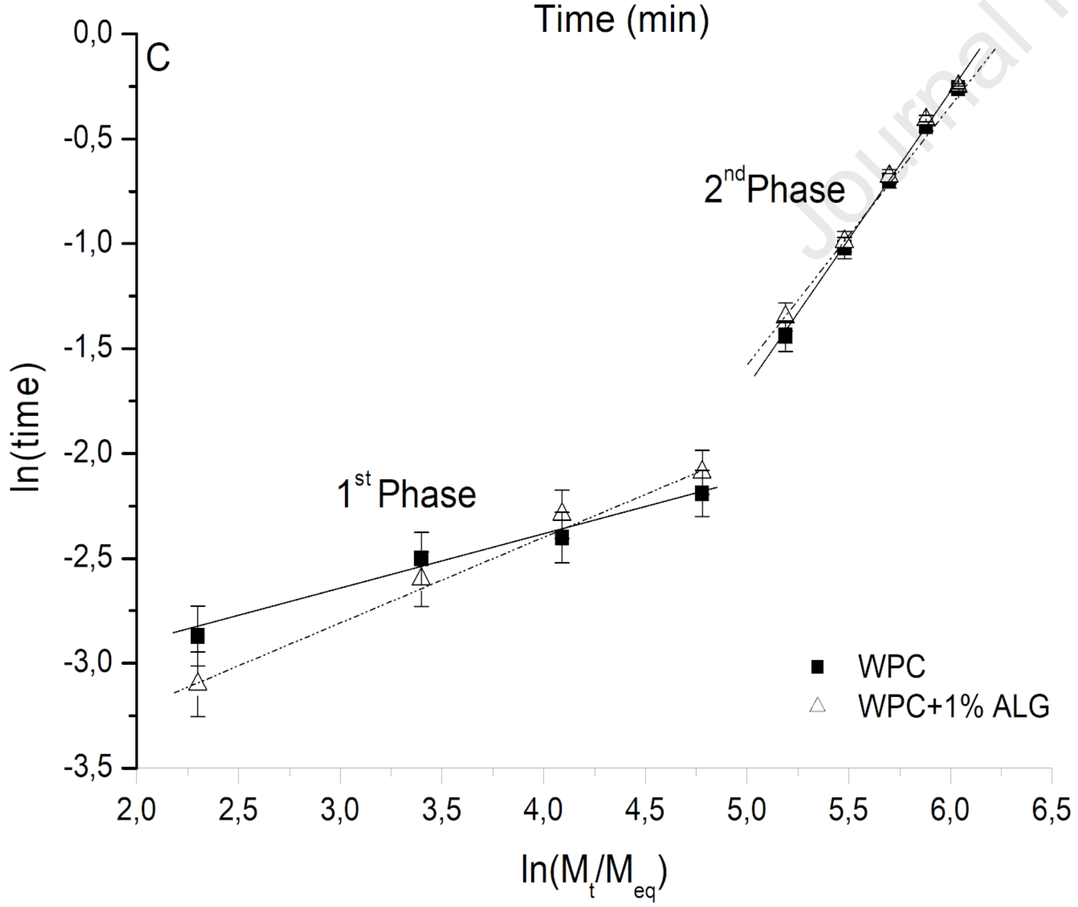

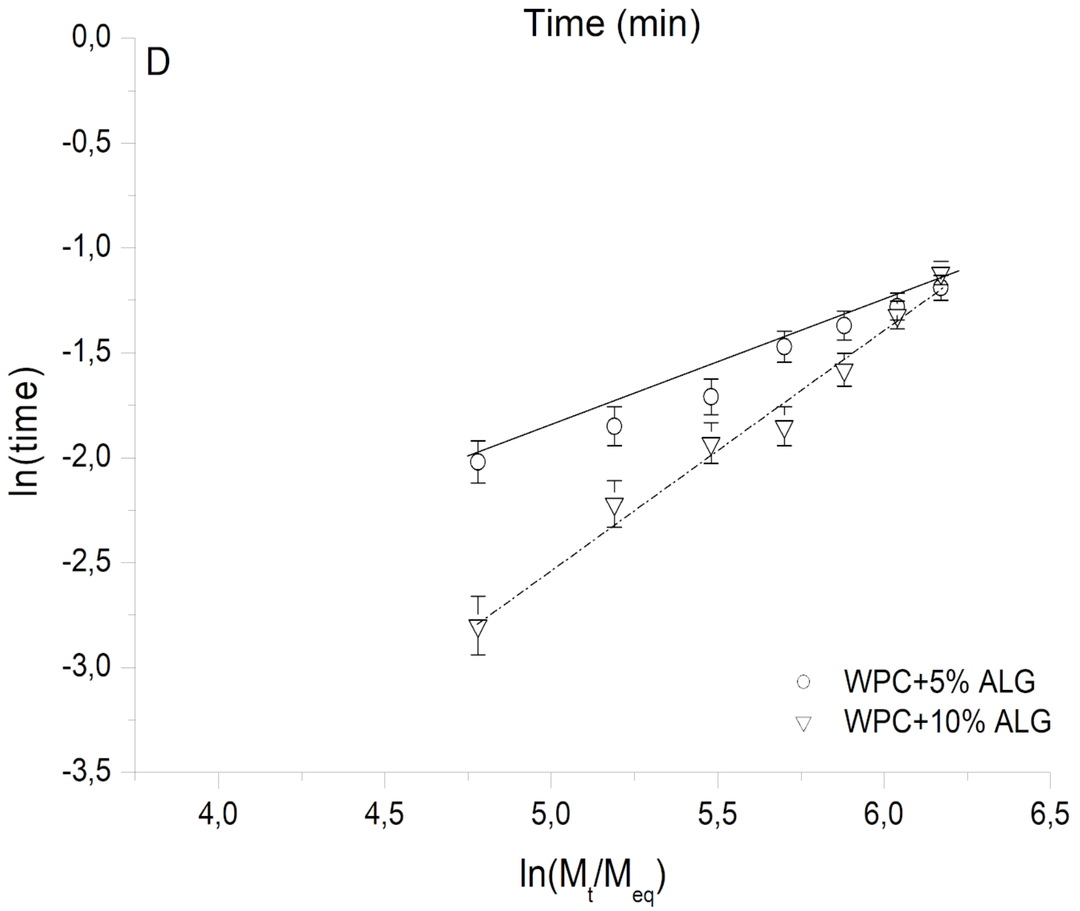

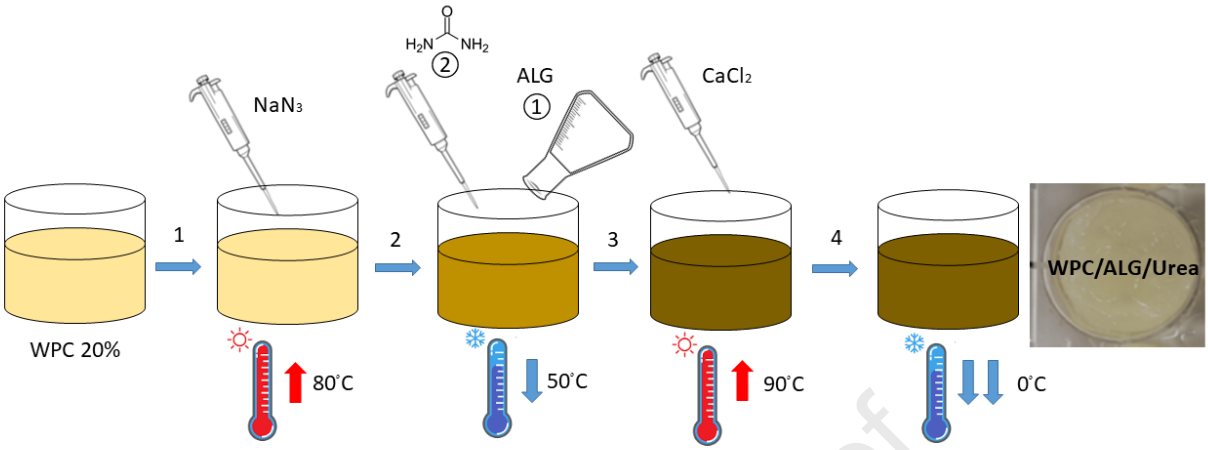

Figure 1

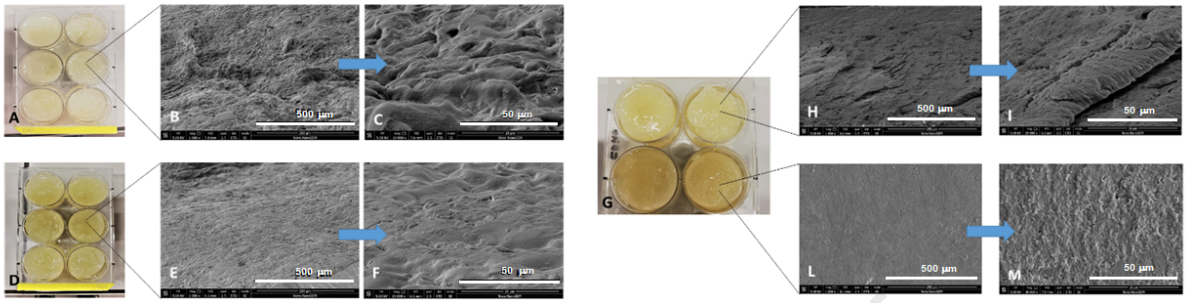

Figure 2

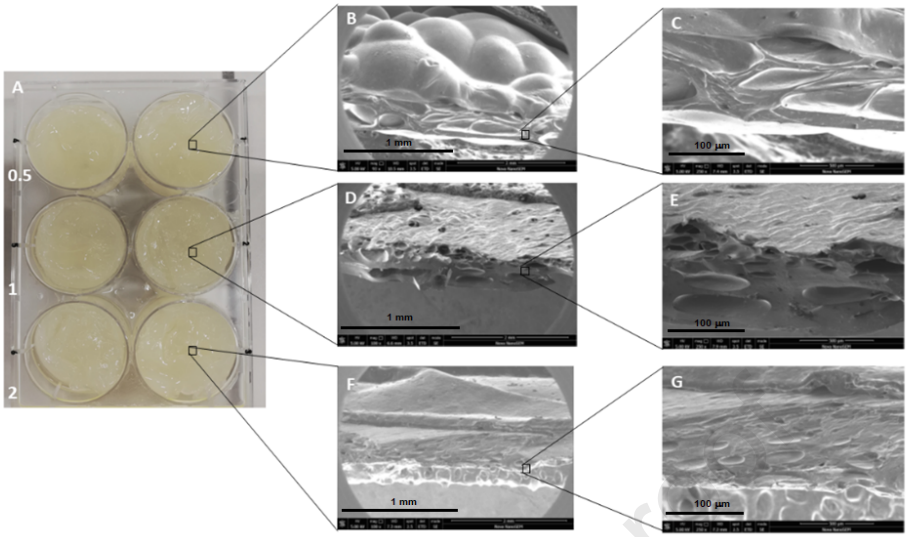

Figure 3

Figure caption

Figure 1. Schematic representation of hydrogels synthesis procedure. Step 1) addition of sodium azide and increase temperature to 80ºC; 2) addition of ALG and urea and cooling to 50ºC; 3) raise temperature to 90 ºC and addition of $CaCl_2$; 4) fast cooling using ice bath to 0ºC. ALG, urea and $CaCl_2$ were added under magnetic stirring.

Figure 2. Morphological evaluation of the hydrogels and the effect of ALG on surface morphology. A, B and C refer to hydrogels based on WPC 20%; D, E and F to WPC+1% w/w ALG. The Figure details G, WPC+5% ALG and WPC+ 10% ALG. The two hydrogels at the top are based on WPC+5% ALG, with those at the bottom on WPC+10% ALG. Therein, H and I represent the surface morphology of WPC+5% ALG, while L and M those of WPC+10% ALG.

Figure 3. Cross-section micrographs of the hydrogels: A) WPC; B) WPC+1% ALG; C) WPC+5% ALG; D) WPC+10% ALG.

Figure 4. Effect of pH, percentage and composition on the swelling ratio of the hydrogels. A) pH 2; B) pH 7 and C) pH 9. The data refer to the mean value ± SD (n=3).

Figure 5. Swelling ratio (SR) with reference to equilibrium (maximum swelling); a number of cycles were performed until a decrease of 50% in the mean value for swelling was recorded, compared to the initial value; the data refer to the mean value ± SD (n=3).

Figure 6. Water evaporation ratio (WER %) in soil and soil containing the hydrogel formulations; the data refer to the mean value ± SD (n=3).

Figure 7. Cumulative release of urea from the hydrogel formulations at neutral pH and room temperature: A) overall release patterns; B) release patterns for the interval of 720 min. Data are plotted as ln(time) against $\ln(M_t/M_{eq})$. Graph C) refers to WPC and WPC+1% ALG, while D) shows WPC with 5% and 10% ALG. The data refer to the mean value ± SD (n=3).

Highlights

- Renewable hydrogels based on alginate and whey protein were prepared
- Correlations between alginic acid content and hydrogel properties were evaluated
- pH responsive swelling behavior
- Slow release of urea and high water holding capacity
- Promising alternative to commercial hydrogels based on synthetic polymers

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

None