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Renewable Mixed Hydrogels Based on Polysaccharide and Protein for Release of Agrochemicals and Soil Conditioning

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Abstract: The study deals with the combination of biopolymers to develop hydrogels intended for agriculture application. The aim is to propose a renewable and eco-compatible solution to enhance agrochemicals and water efficiency and contribute to maintaining soil fertility. We developed a set of hydrogels based on casein and chitosan for water retention and release of agrochemicals, in particular nitrogen fertilizer urea. The weight ratio of biopolymers, from 0.5 to 2, was investigated to understand the influence of their content on the morphology, swelling, swelling-drying cycles, and water retention in soil. The average content of urea in the hydrogels was 30% of the total weight, and up to 80% was released in the soil in 50 days. The biodegradation of the hydrogels in soil has been investigated by the burial method and monitoring the release of CO_2. Results demonstrated that by increasing the content of chitosan, the biodegradation time is prolonged up to 20% in 90 days. The obtained results support the ultimate purpose of the work that the combination of two biopolymers at proper weight ratio could be a valid alternative of the marketed hydrogels with the final goal to promote soil fertility and water retention and prolong biodegradation.

Keywords: renewable hydrogels; casein; chitosan; urea; soil conditioning

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1. Introduction

In agriculture, water and fertilizer distribution represent a key point to maintain soil fertility, improving production yield and harvest quality. However, there are several issues related to the significant amount of fertilizer and water lost through volatilization and leaching when applied in large-scale agriculture. Such losses strongly impact the final cost of agriculture products and lead to long-term environmental pollution [1,2]. Finding innovative technology and approaches able to better manage water usage and enhance the efficiency of fertilizers are research priorities in the agricultural field [3].

Hydrogels are cross-linked polymeric materials with suitable features to absorb, retain, and then slowly release a large amount of water under osmotic pressure. The combination of hydrogels and agrochemicals is a new trend in hydrogels research as it permits the regulation of nutrients and water using a single material [4–7]. Hydrogels are already used in agriculture, but up to now, they have been mainly based on polymers such as poly (acrylic acid) and poly (acrylamide), which have been demonstrated to have long-term impacts on the environment [8]. A promising alternative to currently marketed hydrogels based on polyacrylates could come from natural polymers and biopolymers, which are biodegradable, abundant, renewable, and sometimes available at lower cost [9]. Biodegradable and environmentally friendly hydrogels can be obtained from a large number of biopolymers, including those of food origin like proteins and polysaccharides [10]. The benefits of using food-grade materials include safety, low cost, and wide commercial availability.

Casein (CA), which is found in mammal's milk, is composed of phosphorylated proteins rich in proline with limited secondary and tertiary structures. The main types of casein proteins in milk are α-s1, α-s2, β, and k casein, present at the molar ratios of 11:3:10:4, respectively [11]. Casein-based hydrogels have received increasing attention due to their ability to form networks suitable to hold hydrophilic and hydrophobic compounds. Moreover, networks with different tensile strengths can be obtained by combining with other biomolecules. In addition, casein and casein-based hydrogels are biocompatible, biodegradable, renewable, non-toxic, and the material is easy to obtain at a reduced cost [11]. The casein hydrogels and casein-based hydrogels can be prepared by different approaches, including hot and cold sets, which give different structures. In the development of soil conditioning hydrogels, the water absorption capacity and the release rate over time are two key points linked to the swelling and shrinking behavior. Those parameters are related to the structure in terms of cross-linking density and also by the surrounding environment. To enhance the hydrogel performances by blending with other biopolymers represents a simple, low cost, and environmentally friendly strategy. Among the available biopolymers, polysaccharides are interesting candidates due to their properties in terms of variation in the chemical structure and molecular weight together with biocompatibility, biodegradability, and not toxicity [12].

Chitosan (CS), a positively charged polysaccharide made of D-glucosamine and N-acetyl glucosamine residues, has been largely investigated in various fields due to its biodegradability, biocompatibility, and antibacterial properties [13]. The choice of chitosan is driven by the good results previously obtained in using it for the encapsulation of compounds with various bioactivities [13,14]. Considering the chitosan chemical structure, blending with casein allows the formation of a hydrogel matrix based on hydrophobic, hydrogen-bonding, and ionic interactions together with physical chains entanglements [11]. Such forces result in the formation of different complexes having functional properties that are not found in the polysaccharides or protein alone [15]. Generally, mixed hydrogels have the ability to hold within their structure low molecular weight compounds compared to those made by a single component, as reported in previous work dealing with chitosan-casein complexes [11,16,17]. For the purpose of the present work, the nature and concentration of the components, their weight ratio, and the ionic strength of the surrounding environment have to be considered as they directly affect the mechanical, chemical, and physical properties of the final hydrogel [18]. The aim of this study was to develop an alternative to the already marketed hydrogels based on synthetic polymers as a soil conditioner and carrier for release of agrochemicals with the final goal to maintain and improve the soil quality with a consequent high-quality harvest. Herein, urea was selected as a model fertilizer, as it represents one of the nitrogen-based fertilizers widely used in agriculture. When the urea fertilizers are applied to the soil, microbial enzymes and urease convert urea into ammonium and CO_2. Depending on the temperature, the hydrolysis takes a day to a week. A high soil pH and temperature favor the conversion of ammonium to ammonia which is released into the atmosphere. Losses are highest if the conversion occurs at the soil surface. The ammonium can be converted by soil bacteria into nitrate, through nitrification, with a consequent release of N_2O and NO to the atmosphere. The uptake of ammonium is slower than that of nitrate because ammonium is bound to clay particles in the soil, and roots have to reach it. Most of the ammonium is therefore nitrified before uptake by plants.

Due to the high loss of urea when applied, the main goal in agriculture is to improve fertilizer efficiency while maintaining crop productivity. Enhanced efficiency fertilizers (EEFs) are products designed to improve nutrient uptake by stabilizing nitrogen products and, consequently, reducing the loss. To date, there are three categories of products being marketed that claim to improve nitrogen use efficiency; (i) nitrification inhibitors, (ii) urease inhibitors, and (iii) controlled-release fertilizer products. These products work by slowing one of the processes within the nitrogen cycle, thereby reducing N loss.

Herein we focused on the third category of EEFs. Loading urea into hydrogel could represent an affordable alternative to the already marketed polymer-coated fertilizers to improve the urea efficiency and reduce the loss due to its additional advantages in improving certain soil's (e.g., sandy) water holding capacity and in water management by reducing irrigation frequency.

We believe that the developed hydrogel could be suitable either in large or small agriculture. They can be applied in the dried state in the soil for water absorption and gradual release of the loaded fertilizers (Figure 1). In addition, hydrogel without fertilizers can be placed in dried form on the soil surface to avoid runoff during heavy rain, thanks to their ability to absorb and retain water.

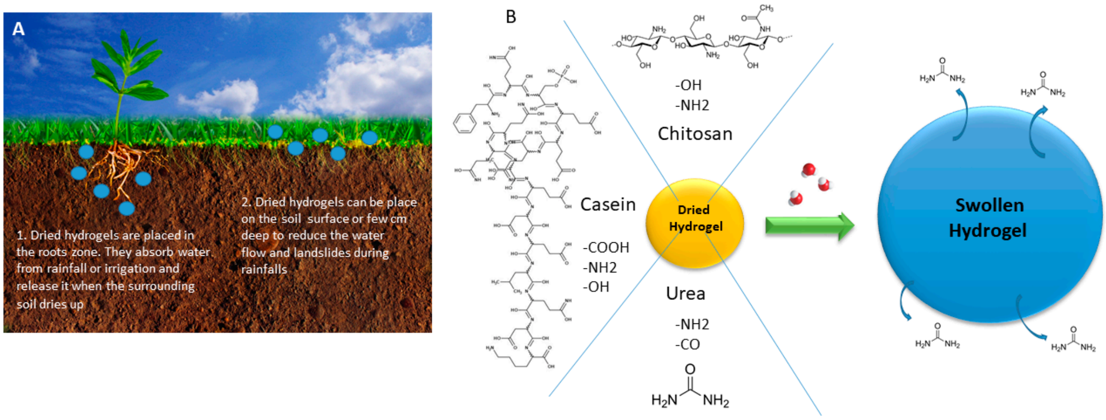

Figure 1. Schematic representation of (**A**) the application in soil of the prepared hydrogels and (**B**) their composition and mechanism of action.

2. Materials and Methods

Medium molecular weight chitosan, casein from bovine milk, urea, 4-dimethylamino benzaldehyde, and sodium tripolyphosphate were purchased from Sigma-Aldrich. Acetic acid and hydrochloric acid were purchased from Chromservis, Prague, Czech Republic.

2.1. *Preparation of Casein-Chitosan-Urea-Hydrogels and Morphological Investigation*

Sets of hydrogels based on chitosan and casein (CS/CA) were prepared by the hot set approach. First, a 12% *w/v* solution of CA was prepared in distilled water and stirred for 24 h. Meanwhile, medium molecular weight chitosan (D.D. >75%) was dissolved in an aqueous solution containing acetic acid (pH 5.5). CA solution was heated to 90 °C and maintained for 15 min under mild stirring to denaturize the proteins, then cooled to 50 °C, and a solution containing CS was added. The pH was adjusted to 4.0 using HCl (0.1 M), and the mixture was stirred for 1 h. Afterward, the temperature was raised to 90 °C for 10 min, and 0.5 mL of tripolyphosphate (TPP) (0.5 mg/mL) was added with a syringe, followed by a quick cooling in the ice bath. Three sets of hydrogels were prepared to have different CS to CA weight ratios reported as CS/CA = 0.5, CS/CA = 1, and CS/CA = 2. The hydrogel containing urea was prepared following the same procedure. Urea (10% *w/v*, V = 5 mL) was added to the prior hydrogel addition of the polysaccharide. The obtained hydrogel was accurately washed with distilled water and stored at 4 °C.

The amount of urea loaded in the hydrogels during preparation was determined by chemical using 4-dimethylamino benzaldehyde (DMAB, 40 mmol/L). The absorbance of the resulting chromogen was measured at 245 nm by UV spectrophotometer (Cary UV 300).

Dried hydrogel tablets were prepared to investigate swelling in media and water retention in soil. The hydrated hydrogels were cut in a cylindrical shape having 10 mm diameter and 2 mm height and dried in the oven at 60 °C until a constant mass was reached (variation 0.005 g). Two groups of four sets of tablets were prepared, one containing urea and the other without.

The influence of CS/CA ratio surface morphology and the inner structure of the dried hydrogels was investigated by scanning electron microscopy (SEM Nova NanoSEM 450 unit set to an operating voltage of 10 kV). For the surface morphology, samples of dimension 0.5 mm × 0.5 mm were placed on the stub using adhesive tape, while for the cross-section, the fresh samples were frozen in liquid nitrogen, cut, and placed on the stub with adeshive tape.

2.2. Rheological Properties

The rheological measurements were performed in triplicates on the swollen hydrogels and the aqueous solution of the single components (chitosan and casein) at the same concentration in which they are found in the hydrogel CS/CA = 1. The measurements were performed on a rotational rheometer (Physica MCR 502, Anton Paar GmbH) set up with a parallel–plate geometry (PP 20/MRD/TI/S, d = 20 mm) at 25 °C. The prepared hydrogels were accurately placed between the plates. The viscoelastic properties of the prepared samples were studied by carrying out oscillation tests (frequency sweep and strain sweep tests). The strain sweep tests were conducted at $\omega = 1.0$ rad/s at the strain amplitude of 0.01% in order to ensure all data fell within the linear viscoelastic region. Frequency sweep tests were performed in the frequency range of 0.1–40 Hz at the above-mentioned strain setting.

2.3. Swelling, Swelling/Shrinking Behavior and Water Retention in Soil

The hydrogel swelling and swelling-shrinking behaviors are fundamental as they directly affect the release of the loaded compound and describe the displacement of the solvent within the polymeric network [19]. The effect of the pH and the CS/CA weight ratio on the swelling was evaluated by following a reported protocol [20]. 600 mg of dry hydrogel was placed in a teabag and immersed in 500 mL of buffer solution (pH 2.0, 7.0, and 9.0) at room temperature (cc 25 °C). At a determined time (in the interval 0 to 120 min), the sample was removed from the media, surface water was removed by filter paper, and the weight was recorded. The swelling ratio (SR) was calculated as follow:

$$SR = \frac{M_s - M_d}{M_d} \tag{1}$$

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

The swelling-shrinking cycles were performed to evaluate the reusability of the hydrogels. Each cycle was started by placing the dry hydrogel in the media until the swelling equilibrium was reached. After that, the swollen hydrogel was dried at 60 °C and then dipped in the media again. The swelling value at the equilibrium after drying was considered. Each cycle was repeated until the weight did not differ more than 50% of the hydrated gel immediately after preparation. A decrease in the weight of dry hydrogel after each cycle indicates a loss in the mass of the sample, which can be ascribed to structural failure and hydrolysis of the chains.

Water holding capacity or water retention in soil represents an important property to evaluate hydrogel usability for agricultural application as soil conditioners to improve soil quality and enhance fertilizer efficacy [21]. The experiment was performed using commercial soil for gardening containing 30% clay, 40% silt, and 30% sand.

The water retention properties were determined by adding dried hydrogels to the soil and watering with tap water. The hydrogels were dried at 45 °C in an oven until no changes in the weight were reported. A scheme is represented in Figure 2. The parameter evaluated is the effect of the weight ratio between CS and CA on the water retention capacity. Before placing the hydrogel, the soil was dried at 70 °C for 2 days. Afterward, two grams of hydrogel were placed in the container with 500 g of dried soil, irrigated with 300 mL of distilled water, and weighed (Mi). The containers were stored at room temperature and weighed every 2 days up to (for) 30 days (Mt). The water retention in soil was evaluated in terms of water evaporation ratio (WER) calculated by the following equation [16]:

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

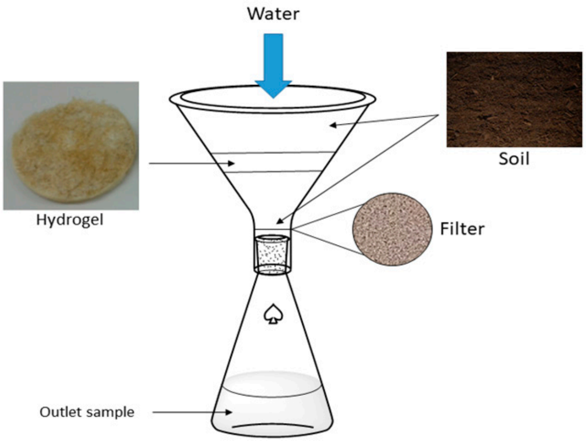

Figure 2. Experimental setup for the water holding capacity and fertilizer release in soil. To perform the experiment, filter paper grade I has been used. The control is the pure soil.

2.4. Urea Release

The release of the urea from the hydrogels generally occurs in 3 stages: media uptake and the dissolution of urea, migration toward the outer surface of the matrix, and then release into the surrounding media [22]. The solute diffusion, swelling, and degradation of the polymeric matrix are considered the driving forces in the transport of the solute from the inner part of the system towards the surrounding environment. The Fick law describes the solute transport from polymeric matrices where the polymer relaxation time is greater than the solvent diffusion time. In the case in which the polymer relaxation time equals the solvent diffusion time, the release is considered anomalous or non-Fickian [23]. The mathematical models permit us to understand the release mechanism of the payload by simplifying the release process.

The in vitro release study represents a key standard for a preliminary assessment of hydrogel properties and to optimize their formulation. The release of urea was evaluated either in soil or in distilled water (pH 7). In the first case, the apparatus settled for water retention capacity was used. A total of 3 g of dried hydrogels were placed in 500 g of soil and watered. The volume of water in the outlet sample was recorded at scheduled time intervals, and the water was treated with 4-dimethylamino benzaldehyde (40 mmol/L).

The absorbance at 245 nm by UV spectrophotometer (Cary UV 300) was measured. For the release in media, 600 mg of dry hydrogel containing urea was placed in 100 mL of media without stirring. The amount of urea released was quantified by treating 3 mL of the release media with 4-dimethylamino benzaldehyde (40 mmol/L), and then the absorbance at 245 nm was measured by UV spectrophotometer (Cary UV 300). The volume of release media withdrawn was replaced with fresh medium to maintain the total constant. In both cases, the urea released (UR) was calculated as follows [20]:

$$UR\ (\%) = \frac{\lambda_\infty - \lambda_t}{\lambda_\infty} \times 100 \tag{3}$$

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

The release is normalized to the weight of the dried sample. The amount of urea is 5% of the total weight of the dried hydrogel.

To define the mechanisms involved in the urea release, in soil and in the media, the release data were processed on the base of the power-law equation, which is a model developed for the release of the compound from a polymeric matrix such as hydrogels. The power-law model is useful when the release mechanism is unknown or when multiple phenomena are involved in the release [24].

$$\frac{M_i}{M_\infty} = Kt^n \tag{4}$$

M_i is the amount of drug released over time t, K is a constant related to geometrical characteristics of the system, M_∞ is the amount of the drug at the equilibrium state, and n is the exponent of the release in the function of time t. Plotting the ln (M_i/M_∞) vs. the lnt (time), the n value is given by the slope of the line. The exponent n indicates the release mechanism from polymeric delivery systems of different geometry (Table 1).

Table 1. Exponent n values and associated drug release mechanism from polymeric systems of different geometry.

Exponent n			
Planar	**Cylinder**	**Sphere**	**Release Mechanism**
≤ 0.5	≤ 0.45	≤ 0.43	Fickian diffusion
$0.5 < n < 1.0$	$0.45 < n < 0.89$	$0.43 < n < 0.85$	Case I transport
$n > 1.0$	$n > 0.89$	$n > 0.85$	Case II transport

2.5. Evaluation of the Hydrogel Biodegradation

A total of 100 mg of dry hydrogel, 5 g of perlite, and 5 g of dry-weight soil were weighed and transferred into biometric flasks sealed with stoppers and incubated at 25 °C. Headspace gas was sampled at appropriate intervals through the septum by a gas-tight syringe and then injected manually into a GC instrument (GC-2010 Plus, Shimadzu), equipped with Porapak Q (1.829 m length, 80/100 MESH) and 5A-molecular-sieve (1.829 m length, 60/80 MESH) packed columns connected in series, as well as a thermal conductivity detector (carrier gas, helium; flow, 53 mL/min; column temperature, 60 °C). The gas-phase (0.1 mL) was taken and analyzed weekly. Concentrations of CO_2 and O_2 were derived from the calibration curve obtained, using a calibration gas mixture with declared composition. The percentage of mineralization pertaining to the carbon content of the initial sample was calculated from the CO_2 concentration found. Endogenous production of CO_2 by soil in blank incubations was always subtracted to obtain values representing net sample mineralization. The blank sample comprised the 5 g soil matter without any hydrogel sample, so the production of CO_2 was related entirely to soil. From the concentration determined, the biodegradation in percentile was calculated as follows:

$$Biodegradation(\%) = \frac{m_{gc}}{m_s w_c} \times 100$$

where m_{gc} (mg) is the mass of carbon evolved as CO_2 and obtained from GC analysis, m_s (mg) is the weight of the polymer sample, and w_c is the percentage (w/w) of carbon in the material investigated. The value of w_c for the given polymer was determined by a total organic carbon (TOC) analyzer (TOC-L, Shimadzu), equipped with a solid sample module (SSM-5000A, Shimadzu). Three parallel flasks were run for each sample, along with four blanks.

2.6. Statistical Analysis

Each experiment was performed in triplicate, and the average values ± standard deviation are reported.

3. Results and Discussion

3.1. Hydrogel Morphology

The SEM micrographs of the unloaded hydrogels (Figure 3B–G) illustrate the impact of the weight ratio between CS and CA on the surface morphology and the inner structure. As can be seen, the surface is not homogeneous with various structural patterns. Looking at the cross-section, all the formulations present cavities in the inner part. The size of the cavities and their distribution seem to be influenced by the weight ratio between the biopolymers; by increasing the polysaccharide content, the cavities became smaller. As the cavities are caused by the removal of the water during the drying process, variation of the component contents is affected, particularly chitosan-casein and chitosan-TPP interactions with consequent changes in the spatial rearrangement of the biopolymers chains, and consequently, the displacement of water molecules within the structure and their diffusion towards the surface during the drying process. Generally, a direct relationship between the size of the cavities and the swelling behavior can be found; bigger cavities promote swelling and diffusion of the media [25]. However, in the present case, samples having an excess of CS showed more swelling despite the presence of smaller cavities. Considering the experimental conditions, in particular the pH, which is higher than the *pKa* value of amino groups in CS, the content of free water is higher than bounded water with a consequent increase in diffusion [26].

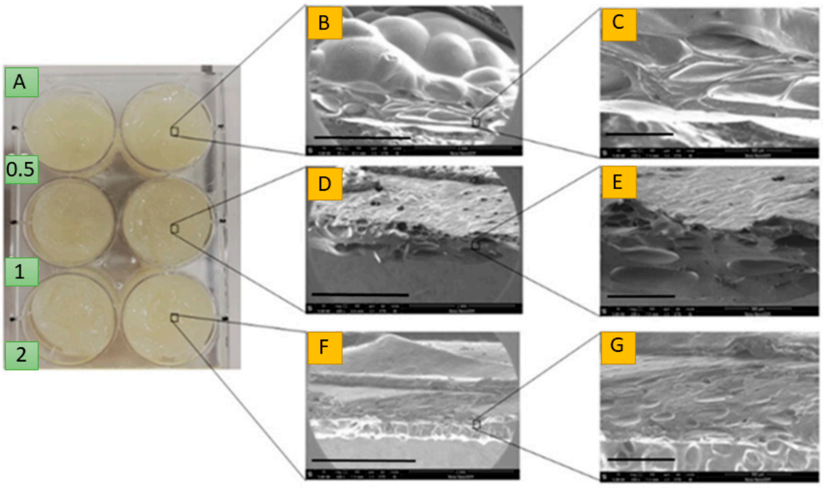

Figure 3. Effect of CS/CA weight ratio on the surface and cross-section morphology. (**A**) Hydrogels photos; the number indicates the weight ratio between CS and CA; (**B**,**D**,**F**) surface and cross-section, scale bar 2 mm; (**C**,**E**,**G**) magnification of the cross-section. Images (**B**,**D**,**F**) scale bar 1 µm (**C**,**E**,**G**) scale bar 500 µm.

3.2. Viscoelastic Properties

The complex viscosity defines the ability of the prepared hydrogels to resist deformation in response to stress. Due to the different content of polysaccharides and protein in the hydrogel, it gives information of their contribution to the final properties. From the trends illustrated in Figure 4, there are two main conclusions: (i) the viscosity of the hydrogels is higher than that in the single components, and (ii) the viscosity tends to increase with the chitosan content. The higher viscosity of the hydrogels, compared to the solution containing the components individually, was expected due to the presence of physical cross-linking and interconnection among the polysaccharide chains, which results in a more stable structure with higher resistance to external stress. Variation in the viscosity is also observed by varying the concentration of chitosan; it can be related to the higher number of hydrogen bonds and interactions among the biopolymers and the different special distribution of the biopolymers chains within the matrix with a higher number of cross-linking sites and, consequently, higher viscosity [27].

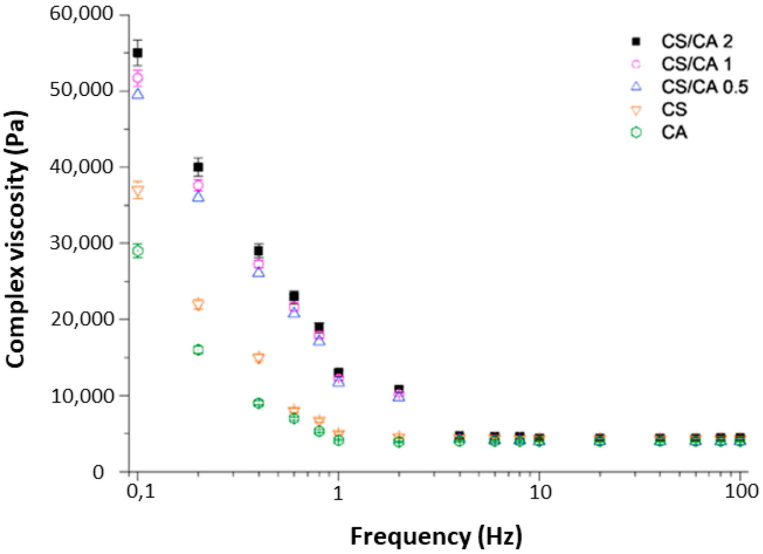

Figure 4. Dependency of complex viscosity on frequency (Hz) for the hydrogel and the components individually.

3.3. Swelling Behavior and in-Soil Water Retention

Hydrogels designed as soil conditioners must adsorb a high amount of water during the swelling process; understanding the swelling behavior and the water holding capacity is a priority. The amount of water that can be held in the matrix is directly related to the density of the cross-linking point, the concentration, and the spatial displacement of the hydrophilic groups within the matrix [28]. In addition, the surrounding environment also plays an important role during the swelling process, as can be seen by the obtained results in vitro and in soil (Figure 5). When the dried hydrogels are hydrated in soil (Figure 5B), the swelling equilibrium occurs in nearly 600 min, while in aqueous media in 60 min at all CS/CA weight ratios tested (Figure 5A).

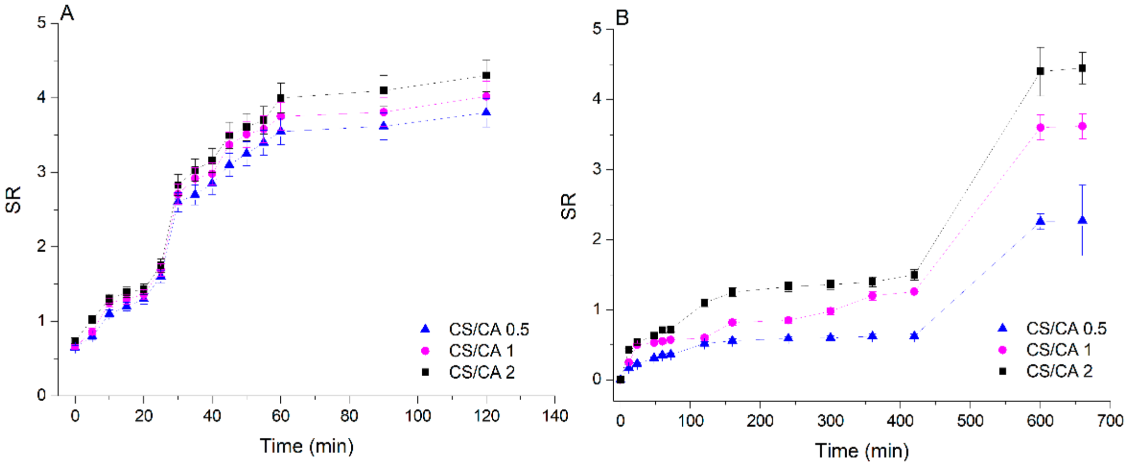

Figure 5. Swelling ratio (SR) of the hydrogels having various chitosan to casein weight ratio (CS/CA) in (**A**) buffer solution pH 7 and (**B**) soil. The SR ratio is referred to as the equilibrium. The data refer to the average value (±SD; n = 3).

The number of swelling/shrinking cycles provides information on the rehydration properties, and consequently, reusability. The bar graph in Figure 6A provides preliminary information on the resistance of the hydrogels to swelling and shrinking cycles and, subsequently, reusability.

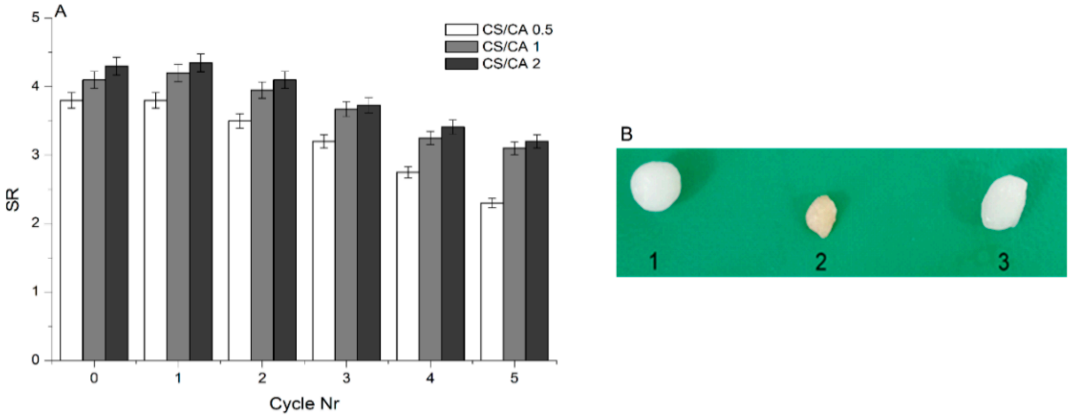

Figure 6. (**A**) Swelling ratio (SR) in buffer media and shrinking (drying in oven) cycles. The SR ratio is referred to as the equilibrium. The data refer to the average value (±SD; *n* = 3). (**B**). Image of CS/CA 2 hydrogel as (1) fresh hydrogel, (2) dried hydrogel, and (3) swollen hydrogel at the 2nd cycle.

The water evaporation ratio in Figure 7 illustrates a major water loss in the soil in the absence of hydrogel during the 30 days of investigation. The water loss is due to water evaporation, which is related to the humidity and the temperature of the air together with the water retention capacity of the soil [29]. The best performance, in terms of less water loss, is observed in the soil in the presence of the hydrogel formulation containing an excess of chitosan. As can be seen, in the first 5 days, the curves overlap, and then differences in the trends arise. Water evaporation is lower in the formulation containing an excess of chitosan with a loss of around 30% in the first 3 weeks and up to 60% after 40 days. Decreasing the polysaccharide content, the loss is from 60% and 70% after 40 days for the formulation containing an equal amount of CS and CA hydrogel and in the range 75–80% in excess of CA. Despite the increase in the value, it is still low compared to the blank (soil), where over 90% of water evaporated during the same time.

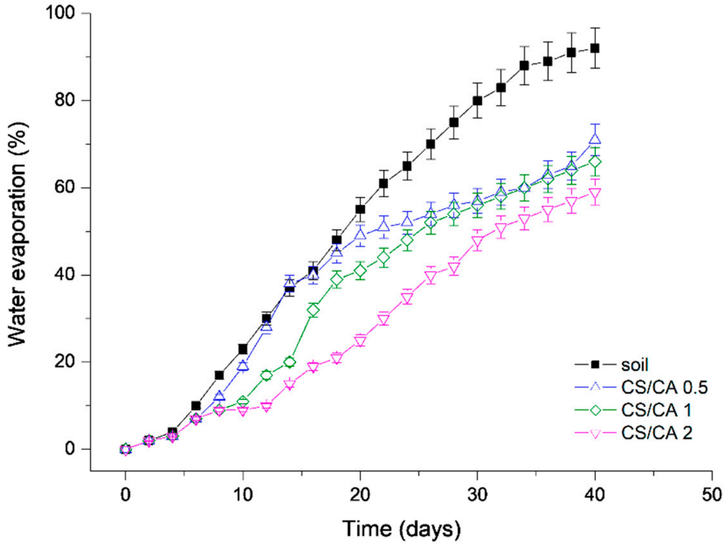

Figure 7. Water evaporation ratio (WER %) in soil and soil containing hydrogels with different chitosan to casein weight ratio (CS/CA). Data refer to the average value ± SD (*n* = 3).

The water content within the hydrogel can be classified as bound, half bound, and free [30]. The free water has the highest mobility and is the first to evaporate. In the swollen hydrogels, the amount of half bonded and bonded water is related to the number of hydrophilic groups per unit volume of hydrogel [25]. In the present case, amino and carboxylic groups are referred to. Adding CS increases the concentration of hydroxyl and amino groups per volume unit of hydrogel with a consequent increase in the half bound and bound water. The interactions between water molecules and the biopolymers are based on hydrogen bonds, which are weaker than the covalent bond and easier to break. Once the water surrounding the hydrogel evaporates, the thermal energy is enough to break the hydrogen bonds between the inner water and the hydrogel structure [31,32].

Figure 6B illustrates the three steps of the swelling-drying cycles which the hydrogels underwent. The figure refers to CS/CA 2 hydrogel, number 1 indicates the fresh hydrogel, number 2 is the dried form, and number 3 represents the rehydrate formulation at the second cycle. The figure provides visual support of the data reported in Figure 6A, in which the SR of the hydrogel at various cycles is reported.

3.4. Urea Content and Release Kinetics

The nitrogen fertilizer was loaded during the preparation step to maximize the loading efficiency. Considering the water solubility of urea and that the whole process was performed in a water solution, over 90% of the initial amount of urea was loaded in all the formulations, indicating that the reported approach is suitable.

The data in Table 2 show the direct correlation between the chitosan content and the ratio between the weight of the hydrated and the dried form of the hydrogel. A higher chitosan content lead to an increase in the weight ratio. This supports the SR values, providing additional confirmation of chitosan in the water uptake and holding capacity. The amount of urea reported per g of dried hydrogel is comparable in all formulations, and it falls in the range of 0.21–0.41 g per g of dried hydrogel, meaning between 20% and 40% of the weight of the dried hydrogel.

Table 2. Evaluation of the ratio between hydrated and dried hydrogel and the content of urea. * referred to the dried hydrogel. W_h = weight of hydrated hydrogel; W_d = weight of dried hydrogel. Data refer to the average value ± SD (*n* = 3).

Sample	W_h (g)	W_d (g)	W_h/W_d	g Urea/g Hydrogel *
CS/CA = 0.5	28.76 ± 2.81	5.85 ± 0.43	4.91 ± 0.11	0.33 ± 0.08
CS/CA = 1	31.18 ± 3.42	6.23 ± 0.59	5.00 ± 0.08	0.28 ± 0.06
CS/CA = 2	29.34 ± 2.67	5.46 ± 0.48	5.37 ± 0.01	0.31 ± 0.10

Release studies were performed in tap water and soil using the set reported in Figure 2. As expected, the release in distilled water was faster than in the soil, and over 80% of the loaded urea was released within 20 h, while the same amount was released in 50 days in soil (Figure 8A,B). The substantial difference is due to the different surrounding environments and, consequently, the forces involved. In the aqueous media, the uptake is faster, and the equilibrium is reached in a short range of time. Conversely, in the soil, its contribution to the adsorption of the water has to be considered, as it interferes with the absorption of the hydrogel. In addition, for the urea to reach the collection vessel, it has to pass through the whole layers of soil, which requires a certain time. As with the timing, the release patterns are also different. In the release in media, three phases are observed: (i) initial burst, (ii) continuous-release, and (iii) stationary release once equilibrium is reached. In the release in soil, only two main phases are observed: the initial lag phase followed by the stationary one. The lag phase is due to the time needed for the water to reach and penetrate the inside of the hydrogel and for it to swell. In fact, looking at the scheme in Figure 1, the hydrogel is placed on the central part of the vessel, meaning that before the hydrogel reaches the swelling equilibrium, the overlying portion of soil has to be saturated. This explains the lag phase.

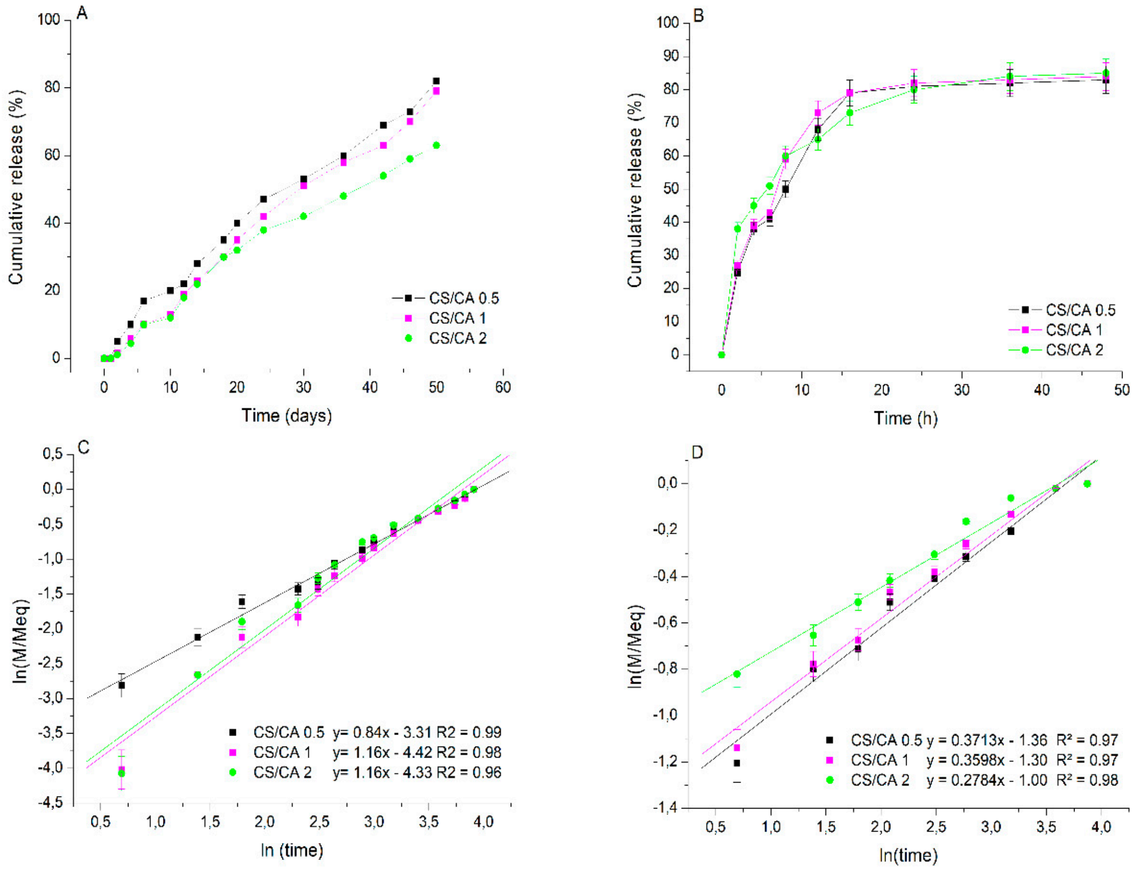

Figure 8. Cumulative release of urea from the hydrogels having different chitosan to casein (CS/CA) weight ratio in soil (**A**,**C**) and in the release media (**B**,**D**) Data refer to the average value ± SD (n = 3).

Looking at the patterns, it is evident that the CS content influences the release rate. The increasing amount of polysaccharides, the intensity of the burst in the release in media, and the overall trend are lower compared to the other two formulations. The faster release is observed in the sample containing an excess of CA. To define the mechanism involved in the release, obtained data were processed by the power of law. The n coefficient value (Table 3) estimates the mechanism.

Table 3. Values of n coefficient and relative release mechanism. Data are processed considering a planar geometry.

Sample	n_{media}	n_{soil}	$Mechanism_{media}$	$Mechanism_{soil}$
CS/CA = 0.5	0.38	0.87	Non-Fickian	Non-Fickian
CS/CA = 1	0.34	1.01	Non-Fickian	Case II
CS/CA = 2	0.26	1.31	Non-Fickian	Super Case II

In the release in media, data indicate a non-Fickian mechanism where the diffusion and swelling rates are comparable. The polymer chains rearrangement occurs slowly, and the diffusion process simultaneously causes the time-dependent anomalous effect [32]. In soil, the scenario is different, and it depends on the CS/CA weight ratio. In excess of CA, the mechanism is non-Fickian, while an equal ratio of CS and CA is Case II, and in excess of CS is Super Case II. In Case II, the release driving mechanism is the relaxation of the polymeric chains. In this case, the diffusion of the solvent through the system is faster than the chains relaxation process, which marks the inner limit of solvent dispersion at a determined time. A fast increase in the absorption rate of the solvent can be observed at the end of the Case II transport. In this situation, the Case II transport shifts to Super

Case II, which occurs with an excess of chitosan. Super Case II represents an extreme form of transport where during the absorption process, tension and breaking of the polymers occur [33]. The obtained data demonstrate that the impact of the weight ratio between CS and the surrounding environment defines CA and that release studies performed in media can be considered a simulation of what will happen in soil, as different mechanisms are involved.

3.5. Biodegradation under Aerobic Condition

The soil burial test demonstrates the microbial degradation of the hydrogels in the soil, with different rates according to the biopolymers composition. Biological degradation is understood as the degradation of complex organic matter into carbon dioxide, methane, water, minerals, and new biomass by means of a biological metabolic process [33]. This is achieved by microbial enzymes, which first colonize the surface of the material and then secrete a biofilm containing the specific enzymes. The excreted enzymes split the long polymer chains into short-chain fragments, which are transported by means of tunnel proteins in the cell wall into the interior of the microorganism, where they can be metabolized. The degradation behavior depends on numerous factors, including physicochemical conditions of the surrounding environment, material properties, and type of microorganisms [34]. The biodegradation trends, reported in percentage versus the time (day), of three sets of hydrogels having different biopolymers weight ratios, are shown in Figure 9. The results illustrate an inverse correlation between the content of chitosan and the biodegradation rate. On the 90th day of the evaluation, the hydrogel containing casein in excess (CS/CA 0.5) shows a remarkable reduction in the weight, equal to around 60% of biodegradation. Conversely, at a higher amount of chitosan CS/CA 2 at the same time interval, the biodegradation is less than 30%.

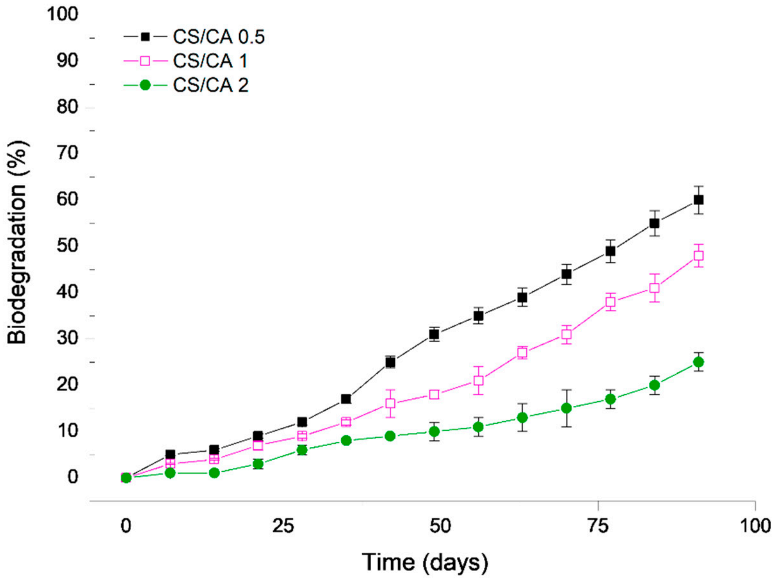

Figure 9. Impact of the chitosan (CS) to casein (CA) weight ratio (CS/CA range from 0.5 to 2) on the biodegradation rate of the hydrogels. Data refer to the average value ± SD (*n* = 3).

4. Conclusions

A set of composite hydrogels based on chitosan and casein were prepared as urea carriers and for soil conditioning. The chitosan and casein content in the hydrogel represents the variable during the preparation, and the impact of the hydrogel's performance and properties have been elucidated. Scanning electron microscopy demonstrates how the surface morphology and, in particular, the inner structure of the hydrogels differs according to the ratio between the biopolymers. Formulations having chitosan in defect compared to casein demonstrate an inner structure characterized by cavities with dimensions that are over 100 μm in those with the lowest amount of the polysaccharide. Conversely, a high content promotes a more compact structure with fewer and smaller cavities. The number of cavities and their dimensions influence several properties like swelling and ability to hold water, the release rate of urea, and biodegradation in soil. The evaluation of the swelling/shrinking cycles, which provide indicative information of the duration of the hydrogel under drying and swelling treatment, indicates that in excess of chitosan, less weight is lost during 5 cycles, which, supported by the microscopic images, indicates a more compact structure. The CS to CA seems not to affect the capacity to hold urea as, in all cases, over 80% of the initial amount has been loaded. Differences were observed in the release patterns, with a faster release in the formulations having an excess of casein. This trend is due to the differences in the inner structure of the hydrogel. Among the release trend in the media and in soil, no correlation can be found indicating that evaluation of the release in media does not reflect the behavior in soil. Processing of the release data, the mechanisms release mechanism differs depending on the polysaccharide content and the surrounding environment. Case I is observable in the formulations having equal content of chitosan and casein (CS/CA = 1) or excess of casein (CS/CA = 0.5) while Case II in those with an excess of chitosan (CS/CA = 2). The obtained results demonstrate that composite hydrogels based on chitosan-casein have proper features for application in soil conditioning and for a more efficient release of agrochemicals and water to promote crop growth.

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