



Towards resource recovery-oriented solutions in agriculture exploiting structural extracellular polymeric substances (sEPS) extracted from aerobic granular sludge (AGS)

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ARTICLE INFO

Keywords:

Aerobic granular sludge
Structural extracellular polymeric substances
Resource recovery
Hydrogel
Water uptake
Nutrient release

ABSTRACT

In aerobic granular sludge (AGS) for wastewater treatment, microorganisms are embedded in a matrix of extracellular polymeric substances (EPS). A fraction of EPS, the so-called structural EPS (sEPS), can be extracted through physical–chemical methods and form hydrogels. AGS-derived sEPS might be converted into value-added biomaterials, thus contributing to a circular economy-based wastewater sector. This paper aimed to evaluate the feasibility of applying sEPS extracted from AGS and their hydrogels in agriculture. The extraction and hydrogel-forming methods were fine-tuned in terms of chemicals applied and the obtained sEPS and hydrogels were comprehensively characterized for their agronomic properties. It was observed that the chemicals used in the recovery processes influenced the elemental composition of sEPS and derived hydrogels, without significantly modifying the sEPS extraction yield and gelling capacity. Chemical reagents such as K_2CO_3 (thermo-alkaline extraction) and $Ca(NO_3)_2 \cdot 4H_2O$ (ionic cross-linking) promoted a K- and N-enrichment of sEPS and hydrogels, with benefits on their agronomic valorization. sEPS hydrogels can store up to 99.5 wt% of water into their 3D structure, exhibiting properties of interest in agriculture. The 50 °C-dehydrated sEPS hydrogels can swell in both water and KNO_3 aqueous solution, absorbing up to about 12 g H_2O/g $TS_{Hydrogel}$ (TS: Total Solids) and behaving similarly to superabsorbent polymer (SAP)-based hydrogels. After swelling in KNO_3 , they evidenced high K^+ / NO_3^- release capacities (133 mg K^+ and 187 mg NO_3^- per g $TS_{Hydrogel}$). Freeze-dried sEPS hydrogels reversibly absorbed and desorbed water under controlled relative humidity conditions. These properties suggested that AGS-derived sEPS might be converted into valuable biomaterials to improve water-holding capacity and nutrient retention of soils, with advantages for both agricultural and wastewater sectors.

1. Introduction

Over the last two decades, aerobic granular sludge (AGS) process proved to be a feasible alternative to conventional activated sludge systems (CAS) for biological wastewater treatment [1,2]. Granules are dense microbial aggregates having high settling velocity and a multi-layered structure with different redox potential conditions due to e-donors and e-acceptors radial concentration gradients: this results in the establishment of aerobic, anoxic and anaerobic micro-environments in the same biological unit, thus enabling the simultaneous removal of

carbon, nitrogen and phosphorus [2]. In AGS, as in all kinds of biofilms, microorganisms produce a high amount of hydrated extracellular polymeric substances (EPS) forming a polymeric hydrogel matrix in which they are self-immobilized [3,4]. EPS are secreted by bacterial consortia during metabolism and mainly consist of a mixture of proteins, polysaccharides, (phospho) lipids and humic-like substances [3–5]. EPS contribute to the initial aggregation of microbial cells and exert various functions within the bio-aggregates such as maintenance of mechanical and structural stability, protection against detrimental environmental factors and dehydration, increase of microbial tolerance towards toxic

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<https://doi.org/10.1016/j.cej.2024.149819>

Received 30 October 2023; Received in revised form 16 February 2024; Accepted 17 February 2024

Available online 19 February 2024

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compounds, settling properties, and nutrient and organic matter sorption [3–6]. The high diversity and complexity of EPS is strictly dependent on the microbial consortia inhabiting the bio-aggregates and different environmental conditions [5]. A fraction of AGS-derived EPS, the so-called structural EPS (sEPS), has the ability to form hydrogels, contributing to the establishment of the tertiary network structure, and thus to the structural characteristics of granules [4]. Particularly, the mechanical features of AGS are mainly ascribed to the gelling matrix of sEPS, that confers mechanical strength and stiffness to the granule morphology [7]. A gel can be defined as a material made by “a polymer network that is expanded throughout its whole volume by a solvent” [8] (in the case of sEPS hydrogels, by water). The polymer network can be formed through the “physical aggregation of polymer chains (e.g. caused by hydrogen bonds, complexation, etc.), that results in regions of local order acting as the network junction points” [8]. Hydrogels are types of gels that are able to reversibly absorb and exude water or other solvents (i.e. swell and de-swell) [9]: the highly hydrophilic nature of their polymeric chains allows them to associate with large quantities of water without dissolving [10]. sEPS can be extracted from AGS through physical–chemical methods and form hydrogels through cross-linking with divalent metal cations M^{2+} such as calcium [4,11]. The sEPS post-gelling mechanical properties are strictly dependent on the M^{2+} used as ionic cross-linker [12,13].

The recovery of EPS from waste sludge as secondary raw material would allow to reduce the mass of residues to be disposed of, thus improving the environmental and economical sustainability of wastewater treatment [14]. Indeed, excess sludge represents the main waste product in wastewater treatment plants (WWTPs), and its handling/disposal can account for up to 50 % of the total operational costs of WWTPs [15]. Thanks to their peculiar and versatile features (e.g. hydrogel-forming ability, water-holding capacity, adsorption properties), AGS-extracted sEPS can be further processed to be converted into value-added biomaterials of interest in various industrial sectors: flame retardant [16] and water-proof coating [14] in textile and paper industries, metal adsorbents [17,18] and bio-flocculants [19] in wastewater treatment systems as well as additives for cement curing in construction sector [20]. Bearing in mind the above, sEPS may represent a cost-effective and environmentally friendly alternative to synthetic polymer-based materials in various industrial and/or environmental applications, thus contributing to change the critical status of waste sludge in WWTPs and boosting the progressive transition from a petroleum-based to a bio-based and circular economy. Particularly, the high water-holding capacity of AGS-derived sEPS hydrogels would suggest their potential application in agriculture to increase water retention of soils [21]. The valorization of sEPS in agriculture has not yet been comprehensively explored in the scientific literature, but it deserves particular attention given the growing environmental pressures that the agronomic sector is facing (e.g. in terms of prolonged droughts and progressive desertification of soils in many areas). Polymer-based materials like hydrogels feature versatile mechanical and chemical properties that can be engineered in various agriculture-related solutions: superabsorbent polymers (SAPs) for soil conditioning, metal adsorbents for soil and water bioremediation and carriers for controlled-release of nutrient and/or other agrochemicals [22]. Hydrogels currently applied as soil amendments are mainly from the poly(acrylic acid)/polyacrylamide types: these materials are non-renewable, contain potentially toxic monomers (e.g. acrylamide) and are biodegradable only to a limited extent [23]. It was reported that the degradation rate of polyacrylate-based SAPs in agricultural soils varies between 0.21 % and 0.82 % within 12–24 weeks, depending on the polymer cross-linking degree, temperature and soil type [23,24]. Owing to these disadvantages, polyacrylate-based hydrogels are becoming unacceptable from an ecological standpoint, thus driving the research efforts towards more sustainable solutions involving the use of biopolymer-based materials [23]. Taking the above into account, the impact of AGS-derived sEPS in the agronomic sector might be significantly high. The global SAPs

market had a huge growth during the last four decades, which is expected to persist in the coming years too (around 13.34 billion tons of SAPs totally produced by 2020, of which less than 2 % destined to agricultural applications) [25]. This, together with the growing interest towards bio-based and biodegradable polymeric materials, is expected to stimulate the research on sEPS as potential candidates for progressing towards more sustainable and resilient agronomic practices.

As reported in the literature, the EPS composition and characteristics depend on the extraction method applied: distinct extraction and recovery processes might result in differences in terms of both quantity and quality (e.g. extraction yield, biochemical composition, functional groups, etc.) of the extractable EPS [4,6,26]. Among the existing protocols, chemical methods are usually considered more effective, yielding a relatively higher EPS content [6]. The influence of various chemicals on the quality/quantity of the extractable sEPS deserves a dedicated focus. Regarding the sEPS recovery from AGS, higher yield factors (up to about 282 mg VS_{sEPS} extracted per g VS_{AGS} [27], VS: Volatile Solids) are generally obtained by providing a thermo-alkaline solubilization of EPS with Na_2CO_3 , followed by an acidic precipitation of sEPS [4,27]. The recovery yield of the gel-forming EPS could decrease by using a strong base such as sodium hydroxide coupled with high temperatures in the EPS solubilization phase [28]. To the best of our knowledge, the existing methods have never been considered in terms of viability of the extractable biopolymers as biomaterials for specific practical applications [26].

In this perspective, this paper mainly aims to address the agronomic potential of AGS-extracted sEPS and derived hydrogels. The extraction and hydrogel-forming methods were fine-tuned based on agriculture-oriented criteria: to this aim, the influence of distinct chemicals in the recovery/valorization processes on the quality of the resulting sEPS-based biomaterials was evaluated through comparative elemental analyses of both sEPS and derived hydrogels. The swelling, water absorption/desorption capacity and K^+/NO_3^- retention/release ability of sEPS hydrogels were comprehensively addressed to evaluate their potential applicability in agriculture. The methodological approach proposed for the agronomic characterization of AGS-extracted sEPS might be extended to other industrial sectors and/or environmental solutions, thus supporting a broader implementation of resource recovery-oriented strategies in WWTPs.

2. Materials and methods

2.1. Extraction and hydrogel-formation

The AGS used in this study as raw material for the recovery of sEPS was the one applied in a previous work [21] coming from a bioreactor treating municipal wastewaters. The extraction of sEPS from AGS was first carried out according to the physico-chemical method reported by Felz et al. [4] with slight modifications. Briefly, the process provided a first step of thermo-alkaline solubilization (200 μ m-sieved AGS placed into a 0.5 % w/v Na_2CO_3 aqueous solution with a concentration of 0.06 g/mL on wet weight basis) under stirred conditions (1 h, 80 °C, 400 rpm) followed by a solid–liquid separation through centrifugation (4000 · g, 4 °C, 20 min). The supernatant (containing the solubilized EPS) was collected and dialyzed against demineralized water (SnakeSkin™ Dialysis Tubing, 3.5 kDa MWCO, 35 mm, Thermo Scientific™; about 24 h of dialysis with one water replacement). The dialyzed EPS aqueous suspension was subjected to acidic precipitation (1 M HCl, pH = 2.20 ± 0.05) and the gelling EPS (sEPS) were then recovered through centrifugation (10000 · g, 4 °C, 5 min) in the form of acidic pellet. Particularly, the sEPS acidic pellet was repeatedly centrifuged (10000 · g, 4 °C, 5 min) to increase its concentration in terms of sEPS total solids (TS) to wet weight (WW) ratio (TS/WW [wt%]). The series of consecutive centrifugations (n° 1–4) was continued until the pellet remained well-defined, i.e. with a “clear” and easily separable supernatant above. Finally, the concentrated pellet of sEPS was re-suspended by adding 0.5 M NaOH

until a pH of 8.5 was reached. The resulting sEPS suspension represented the final product of the extraction/recovery process and therefore the sample used for further experiments and analysis, including the yield factor determination as detailed below.

sEPS hydrogels having cylindrical/disk-like geometries were formed by Ca^{2+} diffusion from an aqueous cross-linker solution (CaCl_2) into the polymeric matrix through a dialysis membrane (SnakeSkin™ Dialysis Tubing, 3.5 kDa MWCO, 35 mm, Thermo Scientific™), according to the method described by Campo et al. [21] and Pagliaccia et al. [12]. The hydrogel-forming experiments were performed varying the Ca^{2+} concentration in the cross-linker solution from 0.010 to 0.225 M and the sEPS concentration of the suspensions at pH = 8.5 (in terms of total solids to wet weight ratios [wt%]) between 0.25 and 3.00 wt%.

The sEPS extraction and hydrogel-forming methods described above (protocols referred to as “Reference” in the manuscript) were initially applied on AGS and sEPS, respectively, and studied as reference protocols. These processes involved the use of chemicals containing sodium and chlorine (Fig. 1) which can be phytotoxic if present in large quantities. For this reason, the described reference methods were then fine-tuned based on agriculture-oriented criteria by means of chemicals potentially able to promote the inclusion of nutrients (particularly, N and K) into the polymeric network (Fig. 1). In the thermo-alkaline extraction, Na_2CO_3 was replaced with K_2CO_3 (protocol named “Agro 1”) or $(\text{NH}_4)_2\text{CO}_3$ (protocol called “Agro 2”) by keeping the same CO_3 molar concentration (about 47 mM); the acidic precipitation was carried out by using HNO_3 instead of HCl , while the re-suspension of the sEPS acidic pellet was performed by adding KOH rather than NaOH . In the hydrogel-formation, two different Ca^{2+} sources were tested as an alternative to CaCl_2 as cross-linking agent: $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (protocols referred to as “Agro 1a” and “Agro 2a”) and $\text{Ca}(\text{C}_2\text{H}_3\text{COO})_2$ (protocols named “Agro 1b” and “Agro 2b”). As described for the Reference protocol, the hydrogel-forming experiments exploiting these agriculture-oriented methods were carried out by testing various Ca^{2+} concentrations in the cross-linker solution between 0.010 and 0.225 M and sEPS concentrations in the range of 0.25 to 3.00 wt%.

2.2. Gravimetric, colorimetric, and elemental analysis

Total Solids (TS, g) and Volatile Solids (VS, g) of AGS, sEPS and derived hydrogels were determined according to standard methods [29]. Total solids to wet weight ratios (TS/WW, g/g) and volatile solids to total solids ratios (VS/TS, mg/g) were hence calculated for each sample. In the case of AGS, WW was determined after sieving at 200 μm . All measurements were performed in triplicates: data are presented as average values \pm standard deviation. For all the extraction methods applied, the sEPS extraction yield was hence calculated on VS basis by gravimetry as the mass ratio of extracted sEPS (i.e. pellets resuspended at pH = 8.5) to initial AGS (i.e. mg VS_{sEPS} /g VS_{AGS}), in agreement with

previous literature reports [4,21].

Comparative elemental analyses of sEPS and derived hydrogels were performed in triplicate by using a CHN-S Analyzer (FLASH 1112 EA/Soil instrument, Thermo®), a Varian 720-ES Inductively Coupled Plasma-Atomic Emission Spectrometer (ICP-AES) equipped with a pneumatic nebulizer and a cyclonic spray chamber and ICS-1000 Thermo-Fisher Dionex conductivity-suppressed ion-chromatographic systems.

Concerning the biochemical composition of sEPS, proteins (PN) were determined through the bicinchoninic acid (BCA) assay [30] using bovine serum albumin (BSA) as standard, while polysaccharides (PS) were evaluated with the anthrone sulfuric acid method [31] using d-glucose as standard. The results were hence expressed as mg PN as BSA equivalent (mg PN_{BSA}) and mg PS as glucose equivalent (mg $\text{PS}_{\text{glucose}}$) per g VS_{sEPS} , as reported in previous studies [21,32].

2.3. Swelling and nutrient loading/release capacities of sEPS hydrogels

The swelling ability of sEPS hydrogels formed from 3 wt% sEPS aqueous dispersions (Ca^{2+} concentration in the cross-linker solution = 0.225 M) was addressed as follows. As suggested in the literature [33], sEPS hydrogels were dehydrated at 50 °C, monitoring the weight loss over time with a Moisture Analyzer (MJ33 Mettler Toledo). The relative percentage of dehydration (RPD, %) was calculated as follows (Eq. (1)):

$$RPD = \frac{WC_0 - WC_{50^\circ\text{C}}}{WC_0} \cdot 100\% \quad (1)$$

where WC_0 [g] and $WC_{50^\circ\text{C}}$ [g] represent the initial and residual water content after dehydration at 50 °C, respectively.

The swelling performance of 50 °C-dehydrated sEPS hydrogels were assessed both in ultrapure water (Milli-Q, 18.2 M Ω /cm) and 0.2 M KNO_3 aqueous solution by gravimetric analysis. To follow the swelling kinetics, the 50 °C-dehydrated sEPS hydrogels were transferred into 150 mL of ultrapure water/ KNO_3 aqueous solution and sampled at scheduled time intervals. The swollen hydrogels were gently pressed with absorbent paper sheets to remove excess water and then weighted on a digital balance. The swelling ratio was therefore calculated over time as g H_2O absorbed per g 50 °C-dehydrated weight (50 °C-DW) of hydrogel (Eq. (2)):

$$\text{Swelling ratio} = \frac{W_s(t) - W_d}{W_d} \quad (2)$$

where W_d [g] is the weight of the 50 °C-dehydrated hydrogel (50 °C-DW) and $W_s(t)$ [g] is the weight of the swollen hydrogel at time t .

In this proof-of-concept study, KNO_3 was hence used as model agrochemical to evaluate the nutrient loading and release capacity by sEPS hydrogels. This choice has been commonly adopted in previous studies in the literature addressing the controlled-release features of hydrogel-like materials [33–35]. Aware of the potential soil and water body pollution due to the nitrate leaching beyond the soil–plant system [36], one of the main reasons for using KNO_3 in this kind of laboratory-scale studies is the inertia of NO_3^- ions in terms of acid/base activity at the applied operative conditions, which allows them not to significantly influence the pH of the aqueous medium in which the nitrate salt is solubilized. pH of the swelling medium consisting of a 0.2 M KNO_3 aqueous solution was monitored over time along the entire swelling experiment, disclosing pH values ranging between 7.44 and 8.00 (Figure S1 in Supplementary Data). The KNO_3 -swollen sEPS hydrogels were then transferred into distilled water (120 mL) to monitor the K^+ / NO_3^- releases over time. At scheduled time intervals (from 0 to 480 min), 10 mL solution aliquots were removed and filtered through 0.45 μm nylon syringe filters. An equivalent volume (10 mL) of distilled water was added to the solution to keep the hydrogels immersed in a volume of 120 mL, thus maintaining a significative driving force for the entire sampling period. The cumulative K^+ / NO_3^- releases over time were determined by analyzing the filtrated bulk samples through ion

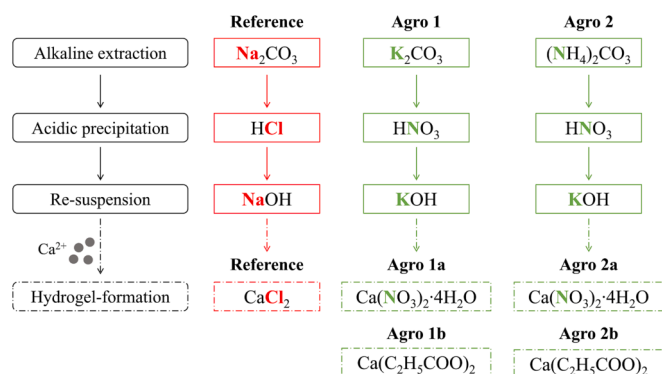


Fig. 1. Schematic representation of the chemicals used in the reference and agriculture-oriented methods for the sEPS extraction and hydrogel-formation.

chromatography. The used method allowed the analysis of anions and cations by two ICS-1000 Thermo-Fisher Dionex conductivity-suppressed ion-chromatographic systems. For the determination of cations, a combination of CG12A (4 x 50 mm) and CS12A (4 x 250 mm) columns was chosen and H₂SO₄ 24.5 mM was used as eluent. For the evaluation of anions, a combination of AG4A SC (4 x 50 mm) and AS4A SC (4 x 250 mm) columns was applied and a Na₂CO₃/NaHCO₃ (1.7 mM/1.7 mM) buffer was used as eluent. On both systems a 25 µL injection loop was used. The experiments of swelling in KNO₃ and subsequent K⁺/NO₃⁻ release in distilled water were only performed with sEPS hydrogels formed from 3 wt% sEPS aqueous dispersions (Ca²⁺ concentration in the cross-linker solution = 0.225 M) according to Agro 1a protocol (Fig. 1): this choice was made based on preliminary results concerning extraction yield and elemental composition, as detailed in the corresponding Results and discussion section.

2.4. Water absorption–desorption cycles by freeze-dried sEPS hydrogels under controlled relative humidity conditions

The water absorption and desorption abilities of freeze-dried sEPS hydrogels were studied at different relative humidity (RH) conditions. These experiments were carried out with hydrogels formed from 3 wt% sEPS aqueous dispersions (Ca²⁺ concentration in the cross-linker solution = 0.225 M) according to Agro 1a protocol (Fig. 1). The experimental setup is shown in Figure S2 (Supplementary Data). Known amounts of freeze-dried sEPS hydrogels (0.154 ± 0.003 g for each test) were put into closed glass chambers in which saturated salt solutions were used to control the RH conditions at a temperature of 25.0 ± 0.7 °C [37]. Firstly, the freeze-dried sEPS hydrogels were kept for 96 h at RH = 97.30 ± 0.45 % (thanks to the presence of saturated solutions of K₂SO₄) to promote water absorption. After this conditioning at RH ≈ 97 %, sEPS hydrogels were exposed for 216 h at RH = 52.89 ± 0.22 % and 84.34 ± 0.26 % by using saturated solutions of Mg(NO₃)₂·6H₂O and KCl, respectively, in order to promote water desorption. The residual water content of hydrogels was monitored over time (during both absorption and desorption tests) by gravimetric measurements and expressed as weight percentage referred to the freeze-dried sEPS hydrogel weight (i.e. g H₂O/g_{Freeze-dried hydrogel} · 100 %). A number of experimental setup equal to the pre-selected time intervals of analysis was prepared, so that the RH conditions were not changed due to hydrogel weight measurements.

2.5. Kinetic data treatment

All the observed kinetic profiles were fitted through pseudo-first-order (PFO) and pseudo-second-order (PSO) kinetic models as follows (Eqs. (3) and (4), respectively):

$$q_t = q_e \cdot (1 - e^{-k_1 t}) \quad (3)$$

$$q_t = \frac{k_2 \cdot q_e^2 \cdot t}{1 + k_2 \cdot q_e \cdot t} \quad (4)$$

where q_t [g · g⁻¹, mg · g⁻¹ or %] and q_e [g · g⁻¹, mg · g⁻¹ or %] are the cumulative ratios of water or nutrients absorbed or released at time t and at equilibrium, respectively, while k_1 [min⁻¹] and k_2 [g · g⁻¹ · min⁻¹, g · mg⁻¹ · min⁻¹ or %⁻¹ · min⁻¹] are the pseudo-first-order and pseudo-second-order sorption or release rate constants, respectively [33]. The values of the model parameters were determined minimizing the Sum of Squared Residuals (i.e., sum of the squared differences between q_t measured and q_t predicted from the model) for all the time values. The fitting goodness was expressed with the correlation coefficient R^2 .

3. Results and discussion

3.1. Extraction performance

The influence of distinct chemicals applied in the recovery process on the quantity/quality of the extractable sEPS is highlighted in Table 1. The sEPS extraction yield obtained by applying the Reference protocol (231 ± 14 mg VS_{sEPS}/g VS_{AGS}) agreed with that reported in the literature for an analogous method [21]. The agriculture-oriented methods, i.e. the so-called Agro 1 and Agro 2 protocols, were able to extract an average amount of sEPS (as volatile solids, VS) of about 21 and 19 wt%, respectively, of the organics originally present in the granules, thus showing extraction yields only slightly lower compared to those obtained with the Reference protocol. The minor differences observed among the tested extraction processes can be reasonably ascribed to the different combination of chemicals applied in the various extraction steps (i.e. thermo-alkaline solubilization, acidic precipitation, re-suspension; Fig. 1) that could result in the establishment of slightly different chemical conditions (e.g. initial pH of the alkaline extraction solutions higher for Na₂CO₃ and K₂CO₃, used in Reference and Agro 1 protocols, respectively, than for (NH₄)₂CO₃, applied in Agro 2 protocol). The volatile solids to total solids ratios (VS/TS) of sEPS were comparable among the tested protocols and in agreement with literature data [21]. In all the extracted sEPS, proteins (PN) were dominant compared to polysaccharides (PS): the average PN/PS ratio ranged between 3.47 and 4.58 g/g, depending on the chemicals used, with a higher PS content observed for the sEPS obtained through the agronomy-oriented protocols. Regardless of the extraction method applied, the PN content appeared noticeably high compared to literature data [32]. In this regard, it should be considered that the extracted sEPS likely contained various substances (e.g. humic acids, phenolic compounds, etc.) that could positively interfere with the BCA assay in the evaluation of the target compounds, as highlighted in the literature [32]. As reported in previous reports [21,38], the PN concentrations determined by BCA assay (Table 1) were then compared to those calculated from the Total Nitrogen (TN) values obtained with an analytical technique less prone to interferences (i.e. about 105, 82 and 81 mg TN/g TS_{sEPS} for Reference, Agro 1 and Agro 2 protocols, respectively; Table S1–S3 in Supplementary Data) and average VS/TS ratios listed in Table 1, by using a conversion factor of 6.25 g PN/g TN: the PN concentrations thus estimated would be equal to about 749, 612 and 568 mg PN/g VS_{sEPS} for Reference, Agro 1 and Agro 2 protocols, respectively. Compared to the results emerged from the colorimetric measurements, the PN values calculated from the TN results were still higher but closer to literature data [21]. Despite these analytical uncertainties, it should be considered that the nature of the extractable sEPS macromolecules can be reasonably influenced by the origin of the microbial aggregates (such as microbial consortia, treated wastewater [39], operations, sludge retention time, etc.).

Table 1

Effect of distinct chemicals on the sEPS extraction performance. Volatile solids to total solids ratios (VS/TS) are presented in mg VS_{sEPS}/g TS_{sEPS}. Extraction yields are reported on VS basis (i.e. mg VS_{sEPS}/g VS_{AGS}). The contents of proteins (PN) and polysaccharides (PS) are expressed as BSA equivalent (i.e. mg PN_{BSA}/g VS_{sEPS}) and glucose equivalent (i.e. mg PS_{glucose}/g VS_{sEPS}), respectively: their mass ratios (PN/PS) are given in g PN_{BSA}/g PS_{glucose}.

Protocol	VS/TS [mg/ g]	Extraction yield [mg/g]	PN (as BSA equiv.) [mg/g]	PS (as glucose equiv.) [mg/g]	PN/PS [g/g]
Reference	875 ± 13	231 ± 14	888 ± 22	194 ± 3	4.58 ± 0.18
Agro 1	843 ± 11	207 ± 9	894 ± 2	258 ± 3	3.47 ± 0.03
Agro 2	888 ± 12	190 ± 11	903 ± 26	233 ± 3	3.88 ± 0.06

3.2. Hydrogel-formation and comparative elemental analysis

No differences in terms of gelation threshold (i.e. minimum Ca^{2+} and sEPS concentrations that allow to form hydrogels) were observed varying the chemicals used in the extraction and hydrogel-forming methods. As evidenced in Fig. 2, illustrating a conceptual phase diagram for hydrogels obtained with Agro 1a protocol, hydrogel-formation was observed starting from a sEPS concentration equal to 0.5 wt% (in the presence of 0.225 M Ca^{2+} in the cross-linker solution). For sEPS concentrations lower than 0.5 wt%, an increase in viscosity was qualitatively noted with increasing the calcium concentration, even if the formation of stable hydrogels was not observed. By increasing the sEPS concentration up to 1 wt%, hydrogels were obtained even for lower calcium concentrations ($\text{Ca}^{2+} \geq 0.05$ M). The minimum Ca^{2+} concentration allowing sol-gel transition hence depended on the sEPS concentration. As known in the literature, polymer cross-linking is allowed if binding sites are available and if polymer chains can interact [9]. Being sEPS an heterogeneous class of biopolymers, the binding sites are also expected to be heterogeneous in terms of availability and distribution. Since AGS-derived sEPS are alginate-like polymers in terms of gelation mechanisms [11,21], the concentrations of both Ca^{2+} and available binding sites of the polymer chains reasonably play a primary role in the cross-linking process. Particularly, the formation of a polymer network that is expanded throughout its whole volume by water is only possible at concentrations of both sEPS and Ca^{2+} above a critical threshold. It is worth noting that the gelation threshold in terms of polymer/ionic cross-linker concentrations might be influenced not only by the extraction and gelling methods applied, but also by the origin of the microbial aggregates, thus justifying many differences with literature data [12]. Based on a macroscopical qualitative analysis, and in agreement with literature data experimentally obtained in a previous study carried out with AGS of a different origin [12], increasingly stiff sEPS hydrogels seemed to be obtained by progressively increasing the polymer and cross-linker concentrations above the gelation threshold. The feasibility of tailoring the hydrogel characteristics (e.g.,

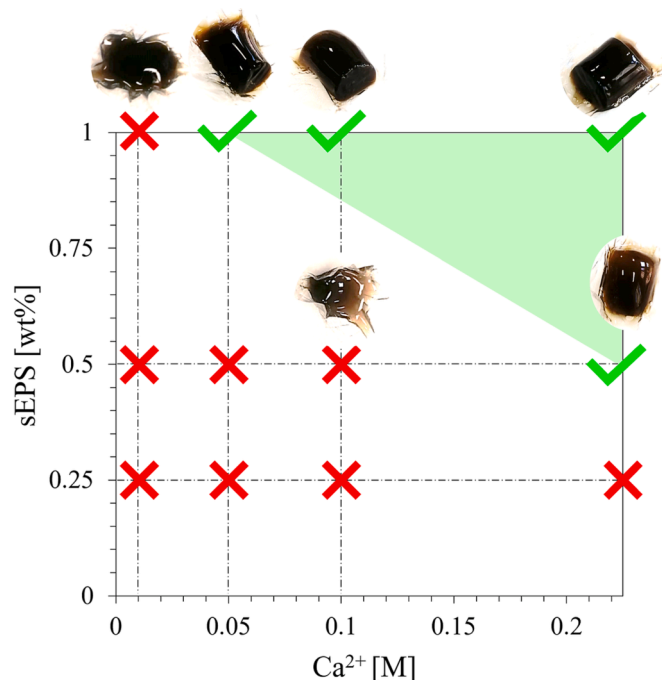


Fig. 2. Conceptual phase diagram of sEPS hydrogels (Agro 1a protocol). The green area represents the conditions that allow the formation of hydrogels, while the red crosses indicate the polymer and Ca^{2+} concentrations for which hydrogels were not obtained. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

physical-chemical and mechanical properties) has important practical implications: depending on the targeted sector, the hydrogel properties might be fine-tuned by playing with the gelling conditions to be applied (i.e. sEPS and Ca^{2+} concentrations) [12]. Particularly, slightly cross-linked hydrogels formed by low concentrated sEPS aqueous suspensions (in the presence of 0.225 mol Ca^{2+} /L) could have a high potential in agricultural applications, thanks to their ability to store and retain large quantities of water (up to 99.5 wt%) into their 3D structure, as detailed in the following sections of the manuscript.

AGS-derived sEPS were previously denoted in the literature as alginate-like exopolysaccharides (ALE) due to their similarities to alginate [11]: indeed, they present similar extraction methods, precipitate at acidic pH values, form ionically cross-linked hydrogels with metal ions and are characterized by the abundance of carboxyl groups [13]. In the light of this, even if sEPS proved to be a more complex polymeric mixture [13], similar industrial valorization strategies might be proposed for both kinds of biopolymers. Considering a global production of alginate of approximately 30 kt per year [40] and the average sEPS extraction yield among the tested protocols (about 19.5 wt% on TS basis), it can be anticipated that about 154 kt TS_{AGS} per year (i.e. around 124 kt VS_{AGS} per year, since $\text{VS}/\text{TS} \approx 0.808$ g/g for the AGS in this study) should be used as feedstock to completely cover the market demand of alginate (and derived hydrogels) through the recovered sEPS. This would hence allow a massive production of sEPS hydrogels to be used in agricultural solutions that already employ alginate-based materials [41]. To give an idea of the high sEPS recovery/production potential, the potential supply of sEPS recovered only from municipal WWTPs located in the Netherlands would exceed global alginate production by a factor of around 2.5 (assuming that all Dutch influents are treated by applying AGS processes), as speculated by Kehrein et al. [40].

Table 2 summarizes the elemental compositions (in terms of molar ratios of each element i , $\text{mmol}_i/\text{C-mol}$) of sEPS and derived hydrogels obtained through the different extraction and gelling methods illustrated in Fig. 1. For more details refer to Tables S1, S2 and S3 (Supplementary Data). It was observed that the potassium/carbon and sodium/carbon molar ratios of the extracted sEPS significantly varied depending on the chemicals used. Particularly, the agriculture-oriented methods provided a higher enrichment in potassium of the extracted sEPS (2.9 and 3.9 wt% as Dry Weight, DW, using Agro 1 and Agro 2 protocols, respectively; Tables S2 and S3) compared to the reference method (about 0.01 wt% as DW, Table S1). Consistently, the sodium concentration of sEPS was significantly reduced from 1.50 to 0.04 wt% as DW by replacing Na_2CO_3 with K_2CO_3 or $(\text{NH}_4)_2\text{CO}_3$ in the thermo-alkaline extraction (Tables S1–S3). The hydrogen/carbon, nitrogen/carbon and sulphur/carbon molar ratios did not vary considerably among sEPS extracted by means of distinct chemicals (except for slightly higher N/C and H/C molar ratios in the case of Reference and Agro 1a protocols, respectively), thus suggesting negligible changes within the organic fraction. The H/C, N/C and S/C molar ratios of sEPS were in line with those reported in the literature [21].

Regardless of the gelling method applied, the calcium/carbon molar ratio significantly increased upon hydrogel-formation, thus indicating the inclusion of Ca^{2+} ions into the sEPS matrix during the cross-linking reaction [21]. Particularly, the calcium content in the hydrogels ranged between 3.0 and 6.4 wt% as DW, depending on the gelling method applied (Tables S1–S3). The nitrogen/carbon molar ratio of sEPS hydrogels formed in the presence of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (Agro 1a and Agro 2a protocols) was significantly higher compared to that of pristine sEPS dispersions. The use of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ hence provided a higher nitrogen enrichment in the resulting hydrogels (11.4 and 9.9 wt% as DW for Agro 1a and Agro 2a protocols, respectively; Tables S2 and S3) compared to the other tested Ca^{2+} sources. This could be likely due to the diffusion of NO_3^- ions into the polymeric matrix during the formation of a cross-linked network. Similarly, the hydrogels formed in the presence of calcium propionate (Agro 1b and Agro 2b protocols) presented larger amounts of carbon (Tables S2 and S3) compared to those

Table 2

Comparative elemental analysis of sEPS and sEPS-based hydrogels obtained by means of distinct chemicals in the extraction and hydrogel-forming methods.

Element		sEPS			sEPS hydrogels				
		Reference	Agro 1	Agro 2	Reference	Agro 1a	Agro 1b	Agro 2a	Agro 2b
C	[mmol/C-mol]	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0
H	[mmol/C-mol]	1619.8	1702.0	1603.7	2261.4	4050.1	3635.2	4118.1	3369.5
N	[mmol/C-mol]	187.9	163.0	159.3	133.5	972.0	101.7	830.7	112.0
S	[mmol/C-mol]	12.6	11.5	12.0	7.7	10.8	11.0	11.4	10.0
P	[mmol/C-mol]	3.7	4.2	3.3	3.6	5.0	4.9	5.4	2.5
Na	[mmol/C-mol]	16.3	0.5	0.5	4.3	0.5	2.1	3.2	0.5
K	[mmol/C-mol]	0.1	20.4	27.8	0.3	3.8	1.5	12.7	4.0
Ca	[mmol/C-mol]	0.4	0.7	0.6	53.8	131.9	110.7	88.9	69.0

observed using $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ as cross-linking agent: this could be attributed to the diffusion of $\text{C}_2\text{H}_5\text{COO}^-$ ions into the polymeric network. For the same reason, the Cl⁻ concentration in the hydrogels decreased from 25.3 to 3.0 – 3.8 wt% as DW by replacing CaCl_2 with $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ or $\text{Ca}(\text{C}_2\text{H}_5\text{COO})_2$ as cross-linking agent (Tables S1–S3). This residual chlorine content might be ascribed to that originally present in the AGS and not removed during the sEPS extraction and gelling processes. These findings suggested that both Ca^{2+} and related counter ions diffused from the aqueous cross-linking solution into the polymeric matrix upon gelation, thus becoming part of the final hydrogel elemental composition. Less significant differences in terms of elemental composition (e.g. H/C and N/C molar ratios) were found among sEPS hydrogels obtained with the same cross-linker but different chemicals in the sEPS extraction process (Table 2 and Tables S1–S3).

It is worth noting that the sodium/carbon and potassium/carbon molar ratios of sEPS hydrogels formed with the reference and agriculture-oriented methods, respectively, were significantly lower compared to those of pristine sEPS (Table 2). This evidence suggested the occurrence of ion exchange mechanisms in binding Ca^{2+} during the cross-linking reaction. In the light of this, the obtained sEPS hydrogels had low concentrations of Na and K (Na = 0.01–0.22 wt% and K = 0.02–0.42 wt% as DW, depending on the chemicals used; Tables S1–S3). The overall decrease in the C + H + N + S concentration observed upon hydrogel-formation (slightly lower for the agro-oriented protocols due to the inclusion of N or C into the polymeric matrix from the $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ or $\text{Ca}(\text{C}_2\text{H}_5\text{COO})_2$ cross-linker solutions, respectively; Tables S1–S3) may suggest the loss of organic matter during the cross-linking reaction (e.g. humic and fulvic acids releases, as indicated by the yellow–brown coloration of the external solution at the end of the gelling process).

It is interesting to observe that sEPS hydrogels presented a higher total solids to wet weight ratio (g TS/g WW) compared to the original sEPS dispersions (Tables S1–S3). Indeed, as previously explained, the hydrogel-formation promoted the inclusion of both Ca^{2+} and related counter ions into the polymeric matrix which can cross-link the polymeric chains (i.e. Ca^{2+}), but also form complexes, precipitates and/or simply dissolve in the free water filling the space within the network porosity. Unfortunately, the elemental compositions here reported are limited to C, H, N, S, Cl and elements detected by ICP-AES, thus not allowing to perform complete mass balances and to understand the exact causes of the observed increases in the TS/WW ratios.

Further studies (e.g. combining chemical imaging, spectroscopic and microscopic techniques) are encouraged to get insights on the differences promoted by the distinct chemicals applied in the extraction process.

3.2.1. Remarks on the compositional properties of sEPS and derived hydrogels in the agricultural sector

The agronomic efficiency of sEPS-based biomaterials is dependent on their content of nutrients and phytotoxic elements (e.g. heavy metals, sodium, chlorine, etc.). As described above, the use of K_2CO_3 or $(\text{NH}_4)_2\text{CO}_3$, HNO_3 and KOH in the extraction process favored an enrichment in potassium of sEPS compared to the Reference method that

could be useful for agronomic purposes. For example, Regulation (EU) 2019/1009 on EU fertilizing products [42] establishes that solid organic fertilizers, designated as Product Function Category PFC 1(A)(I) at Annex I of the aforementioned Regulation, should contain at least 1 wt % N, 1 wt% P_2O_5 and 1 wt% K_2O (if the fertilizer contains more than one declared primary nutrient) [42]: the minimum K concentration was not met for sEPS extracted with the Reference method (Table S1), contrary to what was observed for the agriculture-oriented protocols (Tables S2 and S3). The quality of the extractable sEPS (in terms of elemental composition) might be hence enhanced by playing with the chemicals used in the extraction processes: particularly, the reagents here applied proved to be a feasible option for agronomic applications. On the other side, the nutrient content (especially in terms of K) was significantly reduced upon hydrogel-formation via ionic cross-linking due to the reason described above (N + K + P accounted for 4.4, 11.6, 2.4, 10.5 and 2.8 wt% as DW for hydrogels from Reference, Agro 1a, Agro 1b, Agro 2a and Agro 2b protocols, respectively). Further processes for nutrient enrichment/encapsulation might be therefore encouraged to ensure the hydrogel effectiveness in agriculture-related applications.

Regarding the presence of phytotoxic elements, it should be considered that relatively low concentrations of heavy metals were detected in both sEPS and derived hydrogels (together accounting for about 149–244 ppm and 67–181 ppm, respectively, depending on the chemicals used in the extraction and gelling processes; ppm = mg/kg TS). For a complete assessment of the heavy metal concentrations in sEPS and derived hydrogels refer to Tables S1–S3 in Supplementary Data. The presence of heavy metals in the extracted biopolymers might be reasonably ascribed to the pristine AGS (containing a cumulative concentration of heavy metals of about 1160 ppm, Table S4 in Supplementary Data). Indeed, sewage sludge generally retains large part of the metals entering WWTPs: these metals can be partially released during the EPS extraction process or concentrated in the extracted sEPS, theoretically up to 5 times compared to pristine biomass, considering an extraction yield of about 20 wt% on TS basis [43]. The content of heavy metals in sEPS varied depending on the extraction protocol applied. Particularly, the use of Na_2CO_3 for the EPS thermo-alkaline solubilization provided a higher accumulation of Pb, Zn and Cu into the extracted sEPS compared to protocols using K_2CO_3 and $(\text{NH}_4)_2\text{CO}_3$. This evidence hence suggested that the chemicals applied in the recovery process might affect the concentrations of contaminants in the extracted biopolymers. To be noticed that different degrees of lysis and associated release of intracellular metals could be potentially promoted depending on the solvents applied. The heavy metals concentrations of sEPS and derived hydrogels were compared with the concentration limits of Regulation (EU) 2019/1009 [42] for different PFCs of EU fertilizing products (Annex I) to give an idea of their agronomic applicability. Regardless of the chemicals applied, the heavy metal concentrations of sEPS and derived hydrogels respected the maximum limits imposed for organic soil improvers PFC 3(A) and plant biostimulants PFC 6. The values of Cr (which in the present study refer to total Cr measured by ICP-AES) cannot be compared with the Regulation limits expressed in terms of Cr(VI). However, the concentrations of total chromium detected in sEPS and derived hydrogels were themselves below or close to the

maximum Cr(VI) concentration limit established by the fertilizer Regulation (i.e. 2 mg Cr(VI)/kg DW [42]) (Tables S1–S3 in *Supplementary Data*). Moreover, theoretical considerations, supported by literature data, can be made about the expected content of Cr(VI) in the studied polymeric materials. It should be first noticed that Cr(VI) may be relevant in various kinds of industrial wastewaters (such as effluents discharged by steelworks, textile dyeing, leather tanning, electroplating and chemical manufacturing industries) [44–46]: this was not the case of this study, since the AGS used as feedstock originate from municipal wastewater treatment. Concerning the content and speciation of chromium in sewage sludge, Spanos et al. [47] evaluated the concentrations of Cr, Cr(VI) and Cr³⁺ in sewage sludge from different municipal WWTPs, evidencing average Cr(VI) to Cr mass ratios of maximum 2 wt% [47]. To give an approximate figure of the expected Cr(VI) content in sEPS and derived hydrogels, a Cr(VI)/Cr mass ratio equal to 2 wt% was assumed and the Cr(VI) concentrations were hence predicted. Being aware of the speculative nature of these calculations, concentrations ranging between 0.04 and 0.11 mg Cr(VI)/kg TS could be anticipated for sEPS and derived hydrogels. Taking all the above considerations into account, it is reasonable to expect that the concentrations of Cr(VI) of sEPS and derived hydrogels comply with the maximum limit of 2 mg Cr(VI)/kg DW established by the aforementioned Regulation [42]. Nevertheless, it is worth noting that the concentration of heavy metals (and contaminants in general) in sewage sludge and hence in the extracted sEPS is strictly dependent on the origin of microbial aggregates and treated wastewater and therefore it should be addressed on a case-by-case basis.

An aspect to be considered in the evaluation of the elemental composition of AGS and extracted sEPS (in terms of weight concentrations of each element, ppm = mg/kg DW where DW = TS) is the correct estimation of the dry weight (i.e. TS). Based on the results of thermogravimetric (TG) and derivative thermogravimetric (DTG) analyses, Campo et al. [21] observed that AGS-derived sEPS dried at 105 °C are subject to a further weight loss as temperature increases between 200 and 250 °C, which can be ascribed to both residual water evaporation and volatilization of organics such as low thermal resistant proteins [21]. The presence of a portion of water requiring more thermal energy to be removed (i.e. temperatures higher than 105 °C) suggests that sEPS have high water-holding capacity [21]. This feature of sEPS is indeed considered to prevent granules from desiccation [5]. Given that AGS consists of microorganisms embedded in a matrix of sEPS, the same considerations drawn for sEPS (i.e. high water-retention ability resulting in a higher thermal energy necessary to complete dehydration) can be reasonably valid for AGS too [21]. The standard methods [29] applied for conventional activated sludge could therefore lead to an over-estimation of TS in the case of AGS and extracted sEPS [21], and consequently to an underestimation of the weight concentrations of the various elements (such as C, H, N, S, metals, etc.). Bearing in mind the above, there is a potential inaccuracy in the TS determination, since a fraction of water cannot be thermally removed at a temperature below that at which the decomposition of low thermal resistant proteins occurs. To be noticed that, in this work, TS of AGS, sEPS and derived hydrogels were determined according to standard methods (i.e. thermal conditioning at 105 °C for 24 h [29]) to ensure a proper comparison with literature data in terms of extraction yield, elemental analysis, etc. However, the need for a proper drying method allowing complete dehydration without causing organic matter volatilization is highlighted. These aspects were preliminarily addressed in this study by keeping the 105 °C-dried samples at 180 and 215 °C in an oven for 24 h and then weighing them on a digital balance (Figure S3 in *Supplementary Data*). Considering the DW evaluated upon thermal conditioning at 215 °C, the total mass of the determined elements appeared for sEPS higher than 1 g/g DW that is physically impossible (data not shown). This could be because, as previously pointed out, certain organics of sEPS (e.g. low thermal resistant proteins) can volatilize from 200 °C on [21]. It should be noted that, in the case of pristine AGS, Li et al. [48]

reported that organic matter volatilization begins slightly above 200 °C [48]. Furthermore, the short time of thermal conditioning in TGA caused by high temperature increase rates (e.g. 10 °C/min) may not be representative of the drying conditions applied in this study. Indeed, a consistent mass loss, even for compounds whose volatilization is characterized by low kinetics at the applied temperatures, could be promoted by the longer conditioning times used in this work (i.e. 24 h). In summary, the above clearly highlights that a drying temperature of 215 °C cannot be used for an accurate determination of TS, but also suggests that a potential inaccuracy in the measurement of TS by drying at 180 °C for 24 h cannot be ruled out, although the DTG results by Campo et al. [21] indicate that organic matter volatilization begins at temperatures above 180 °C [21]. These considerations deserve a dedicated focus that is strongly recommended in future research, thus enabling the fine-tuning of standard methods for the assessment of the effective TS in the case of hygroscopic complex matrices such as biofilm-based systems and derived EPS.

3.3. Swelling of sEPS hydrogels

Significant differences in terms of 50 °C-dehydration kinetics were not detected among sEPS hydrogels formed through distinct extraction and gelling methods (Fig. 3a): once the maximum dehydration state was achieved, the relative percentage of dehydration (RPD) were 99.7, 99.1, 98.0, 99.5 and 99.4 % for Reference, Agro 1a, Agro 1b, Agro 2a and Agro 2b protocols, respectively.

The 50 °C-dehydrated sEPS hydrogels absorbed large quantities of H₂O upon swelling in Milli-Q water (Fig. 3b). Regardless of the chemicals applied in the extraction phase, the use of Ca(NO₃)₂·4H₂O (Agro 1a and Agro 2a protocols) instead of Ca(C₂H₃COO)₂ (Agro 1b and Agro 2b protocols) as cross-linking agent promoted higher swelling performance, similar to those observed for the Reference method. The extent of swelling in sEPS hydrogels might be influenced by their cross-linking density (also defined as concentration of elastically effective chains, which depends on the hydrogel mechanical properties [9,49]), that inhibits the expansion of the polymer network by osmotic access of water. Hence, weakly cross-linked polymers swell to a larger extent than strongly cross-linked polymers [9]. In the case of polymers forming hydrogels via ionic cross-linking (e.g. by Ca²⁺ addition) such as alginate and AGS-derived sEPS, the density of the connection points (i.e. the cross-linking density) is directly proportional to the strength of the polymeric network (that can be expressed by the Young's modulus E [12] or by the storage modulus G' [9]), which in turn typically increases with increasing the concentration of the ionic cross-linker (e.g. Ca²⁺) [12]. In the light of this, it can be observed that sEPS hydrogels formed by using Agro 1b protocol had the worst swelling performance and the highest Ca²⁺ concentration (6.4 wt% as DW, Table S2). Taking the above into account and being aware of the speculative nature of these considerations, the swelling capacity of sEPS hydrogels formed with Agro 1b protocol could therefore be negatively influenced by their higher Ca²⁺ concentration. More in general, the swelling of hydrogels made from complex raw materials such as sEPS could be affected by multiple factors, concerning, for example, their chemical composition, that in the case of sEPS is highly varied and complex, and not yet fully understood [5]. Given the proof-of-concept nature of this work, further dedicated studies are hence recommended to support and expand the outcome of this research towards a broader and deeper understanding of these complex polymeric materials.

Expressing the extent of swelling in terms of equilibrium water content ($EWC = 1 - W_d/W_s$, W_d and W_s : weight of dehydrated and swollen hydrogels, respectively), it can be observed that sEPS hydrogels had $EWC = 0.89$ – 0.92 g/g (Table S5 in *Supplementary Data*) that resulted slightly higher compared to those observed for pristine AGS of 0.88–0.90 (pH < 9.7) [9]. It is well known that the water retention ability of granules is strongly promoted by the gelling extracellular polymeric matrix [5], and therefore it is not surprising that the sEPS-

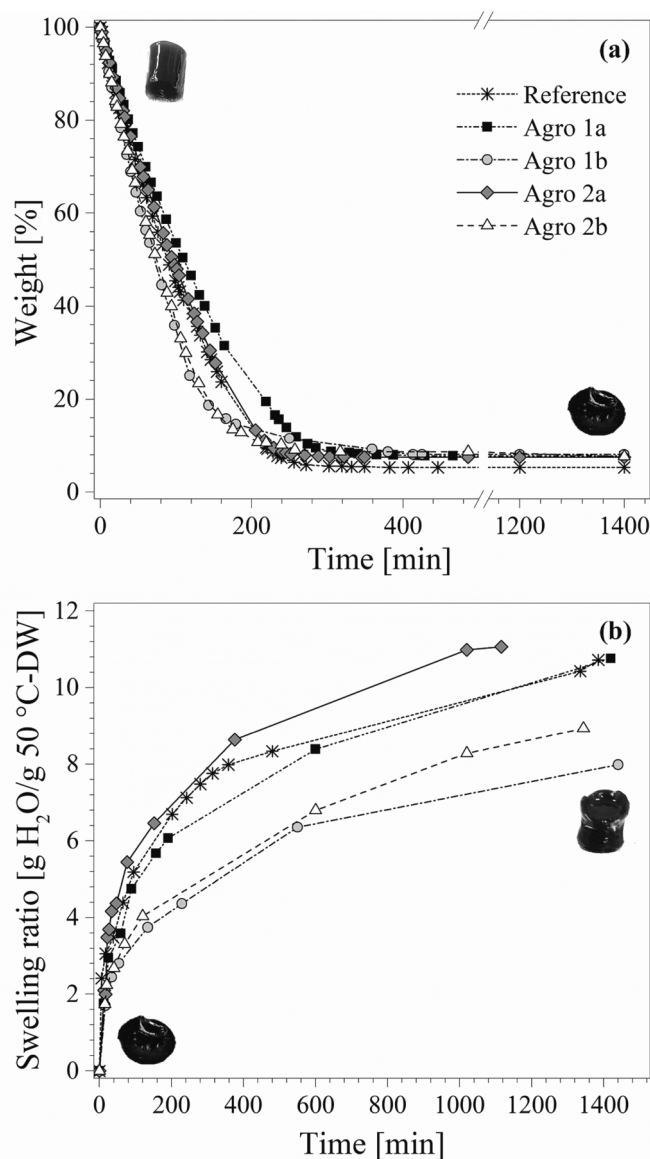


Fig. 3. 50 °C-dehydration kinetics of sEPS hydrogels obtained with different chemicals (a) and increase in their swelling ratio over time upon rehydration in ultrapure water (b). Legend refers to both graphs 3(a) and 3(b).

derived hydrogels show a higher water absorption capacity upon swelling. Overall, the 50 °C-dehydrated sEPS hydrogels derived from Reference, Agro 1a, Agro 1b, Agro 2a, and Agro 2b protocols were able to absorb up to 11.3, 12.1, 10.4, 11.8 and 9.7 g H₂O/g TS_{Hydrogel}, respectively, upon swelling within 24 h of contact time. The increase in swelling ratio was kinetically characterized by two parts: an initial step of fast rehydration within about 60–120 min of contact time followed by a slower absorption process towards equilibrium. Reasonably, sEPS hydrogels are rich in hydrophilic groups able to absorb water molecules, allowing the network to expand. Once swollen, the network behaves like a semi-permeable membrane, regulating the passage of fluid in and out of the network according to osmotic gradients [9]. The larger the osmotic pressure difference between the internal structure and the external solution, the faster the water absorption: for this reason, sEPS hydrogels showed a higher swelling rate in the initial phase of the swelling process. As swelling proceeded, the increase of water molecules diffused into the network caused a progressive decrease in the osmotic pressure gradient with a consequent decrease in the swelling rate [50].

Based on the fitting parameters listed in Table S5 (Supplementary

Data), the increase in swelling ratio over time can be satisfactorily described by a pseudo-second-order (PSO) equation. Regardless of the different swelling performance observed for sEPS hydrogels obtained by means of distinct chemicals, the water uptake hence followed a similar kinetic behaviour. However, no relevant conclusions in terms of water-binding mechanisms can be drawn applying the PSO model. In order to shed light on the water uptake mechanism that occurred in the swelling process, then the experimental data were also fitted using the model proposed by Ritger and Peppas [51] which is based on a modified Fick equation to explain water flow into the hydrogel network [50–52], as illustrated in Eq. (5):

$$\frac{q_t}{q_e} = k \cdot t^n \quad (5)$$

where q_t and q_e [g · g⁻¹] are the mass of water absorbed per g 50 °C-DW of hydrogel at a time t and at equilibrium, respectively, k [min⁻¹] is the kinetic constant (characteristic of the hydrogel), n is the water diffusion exponent and t [min] is the swelling time. For cylinders (as in the case of the studied sEPS hydrogels), when $n < 0.45$, water transport is purely diffusive. On the contrary, when $0.45 < n < 0.89$, water transport is characterized by a complex mechanism, where both diffusion and macromolecular relaxation of the polymeric network control the overall rate of water uptake. Finally, when $n > 0.89$, water transport is defined as Super Case II: the driving factor for the diffusion of water into the polymeric network is the macromolecular relaxation [50–52]. This equation, valid below 60 % of the total equilibrium mass uptake ($q_t/q_e \leq 60$ %) [50–52], was applied to fit the swelling data of sEPS hydrogels. The resulting diffusion parameters are listed in Table S6 in Supplementary Data. Using the q_e values derived from the PSO fitting, this model can simulate with high accuracy the kinetic data ($R^2 = 0.951 - 0.997$) during the initial phase of the swelling process. The diffusion coefficients n ranged between 0.29 and 0.41: being aware of the theoretical nature of these considerations, we can state that the water transport into the hydrogel network seems to be governed by Fick diffusion mechanisms regardless of the chemicals used in the sEPS recovery processes.

The ability of sEPS hydrogels to absorb significant amounts of water by swelling in aqueous systems is typical of superabsorbent polymers (SAPs). SAP hydrogels can be defined as slightly cross-linked 3D polymeric networks, whose properties of interest are the high water-holding capacity and ability to swell in the presence of aqueous media [22,53]. In SAP hydrogels, the density of chain cross-linking results in a significant amount of free volume within the polymeric network, which in turn, combined to the presence of a large number of hydrophilic groups, enables them to absorb and hold large quantities of water, on the order of 10 to 1000 g/g [22,54]. SAPs have a high potential for water management in agricultural applications, where they can be used to maintain the soil humidity for longer periods [22]. In this perspective, sEPS hydrogels could represent a more environmentally friendly alternative to SAPs based on non-renewable and petroleum industry-derived materials that could be potentially pollutant for soils [55] (e.g. synthetic polyacrylamide polymers that have very low degradation rates and contain potentially toxic monomers such as acrylamide [56]).

3.4. Nutrient loading/release capacity of sEPS hydrogels

The swelling kinetics of sEPS hydrogels in 0.2 M KNO₃ aqueous solution are shown in Fig. 4. These experiments were carried out by using Agro 1a protocol for the extraction and hydrogel-formation due to the following reasons: (i) among the chemicals tested in the agriculture-oriented extraction, K₂CO₃/HNO₃/KOH promoted the highest extraction yield (207 ± 9 mg VS_{sEPS}/g VS_{AGS}, Table 1); (ii) the use of Ca (NO₃)₂·4H₂O as cross-linking agent promoted a higher nitrogen enrichment of sEPS hydrogels (up to 11.4 wt% as DW, Table S2 in Supplementary Data) that can be useful for agricultural applications. The extent of swelling in KNO₃ aqueous solution was lower compared to that

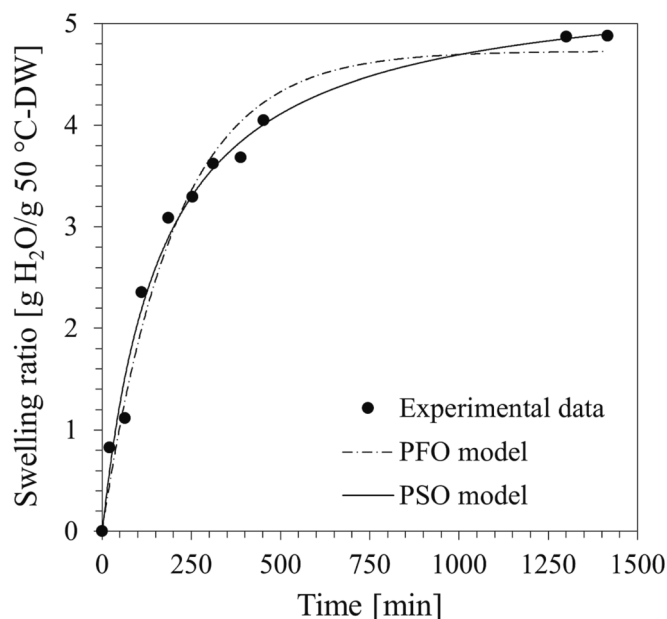


Fig. 4. Experimental swelling kinetics in KNO_3 aqueous solutions of 50 °C-dehydrated sEPS hydrogels and their fitting through pseudo-first-order (PFO) and pseudo-second-order (PSO) kinetic models. The tested hydrogels were formed with Agro 1a protocol.

observed in ultrapure water: the swelling ratio increased up to about 4.9 g $\text{H}_2\text{O}/\text{g}$ 50 °C-DW within 24 h of contact time. Based on the statistical indicators listed in Table S7 (Supplementary Data), the swelling in KNO_3 aqueous solution can be described by a pseudo-second-order kinetic model ($R^2 = 0.989$), such as that in Milli-Q water. The decrease in swelling performance of ionic hydrogels in salt solutions is a phenomenon quite discussed in literature [9,50]: reasonably, the lower extent of swelling observed for sEPS hydrogels in KNO_3 compared to that in Milli-Q water could be mainly ascribed to the higher ionic strength of the surrounding aqueous medium and therefore to a reduction in the osmotic pressure difference between the hydrogel and the external solution [50]. As reported in Table S2 (Supplementary Data), sEPS hydrogels had low K concentrations (likely exchanged with Ca^{2+} during the cross-linking reaction): consequently, large quantities of K^+ diffused from the liquid bulk into the hydrogel matrix upon swelling in KNO_3 (about 52 mg K^+ per g $\text{TS}_{\text{Hydrogel}}$ of the pristine hydrogel). Reasonably, K^+ ions were partially adsorbed on the available binding sites of sEPS and partially diffused as non-bound ions in the free water within the polymeric network. Conversely, being the aqueous medium less concentrated in NO_3^- compared to the pristine hydrogels, part of NO_3^- was released by the hydrogel during the swelling experiment (about 291 mg NO_3^- per g $\text{TS}_{\text{Hydrogel}}$ of the pristine hydrogel). At the end of the process, the sEPS hydrogels presented comparable amount of K^+ and NO_3^- , with a NO_3^-/K^+ ratio equal to 0.90 mEq $\text{NO}_3^-/\text{mEq K}^+$ (to be noticed that a NO_3^-/K^+ ratio of 1.97 mEq $\text{NO}_3^-/\text{mEq K}^+$ is commonly applied in substrates for plant growth [57]). These results thus suggested that the ability of sEPS hydrogels to swell in aqueous phase might be exploited to promote nutrient loading into the 3D polymeric network, by tailoring the ionic composition of the liquid bulk.

Fig. 5 shows the K^+/NO_3^- release kinetics in distilled water by KNO_3 -swollen sEPS hydrogels. The K^+/NO_3^- release mostly occurred during the first half hour (about 75 % of K^+ and 84 % of NO_3^- released in 30 min, corresponding to about 113 mg K^+ and 180 mg NO_3^- released per g $\text{TS}_{\text{Hydrogel}}$ of the pristine hydrogel). This could be mainly ascribed to the high concentration gradient of K^+ and NO_3^- existing between the hydrogel and the surrounding water. Afterwards, as the concentration gradient decreased, part of the residual K^+ and NO_3^- ions were gradually released in water: consequently, the K^+/NO_3^- cumulative release slightly

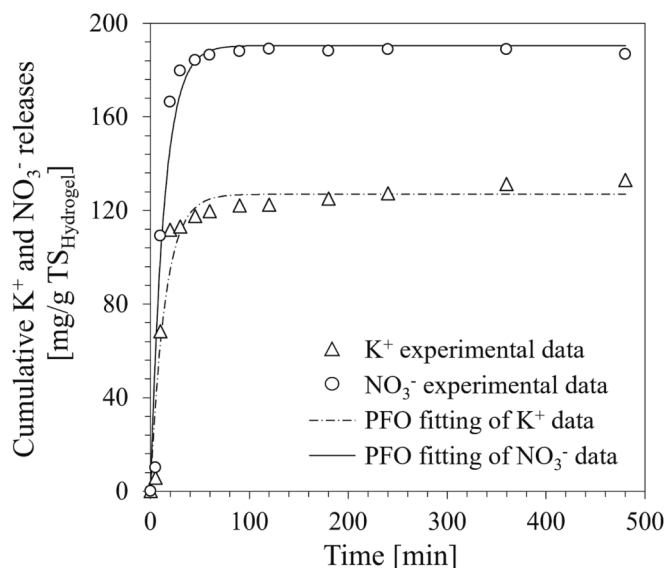


Fig. 5. Experimental K^+/NO_3^- release kinetics in distilled water by KNO_3 -swollen sEPS hydrogels and their fitting through pseudo-first-order (PFO) kinetic model. The tested hydrogels were formed with Agro 1a protocol.

increased up to equilibrium was achieved. Overall, the KNO_3 -swollen sEPS hydrogels were able to release up to 133 mg K^+ and 187 mg NO_3^- per g $\text{TS}_{\text{Hydrogel}}$ in 480 min (about 88 % and 87 % of their K^+ and NO_3^- contents, respectively). According to the R^2 values listed in Table S8 (Supplementary Data), a pseudo-first-order (PFO) equation was the best model for describing the experimental K^+/NO_3^- release kinetics ($R^2 = 0.949 - 0.953$). This suggested that the K^+/NO_3^- release from KNO_3 -swollen sEPS hydrogels was mainly controlled by the diffusive transport of K^+/NO_3^- within the porous network of the hydrogel [33]. The values of the constant k_1 in the PFO model fitting indicated the slower release of K^+ with respect to NO_3^- ($k_1 = 0.068$ versus 0.074 min^{-1}). This interpretation of the experimental profiles agreed with literature reports on alginate gel beads [33]. However, the K^+/NO_3^- releases by sEPS hydrogels appeared faster compared to that observed for alginate gel beads by Wang et al. [33]: this could be influenced by the different gelation methods applied. Compared to this study, Wang et al. [33] carried out hydrogel-formation by dropping the alginate dispersion into a Ca^{2+} solution: this could promote fast and non-controlled gelation kinetics, with the risk to obtain inhomogeneous 3D structures having an inner lightly (or not) cross-linked polymeric core and an external stiff and highly cross-linked polymeric layer (potentially containing most of the alginate molecules in solution) [21] that could act as diffusive barrier slowing down the release of the encapsulated nutrients.

The above results hence suggested that sEPS hydrogels have nutrient retention and release abilities that could be exploited in sustainable agro-practices. However, the currently too high release rates should be optimized, as detailed in the following section 3.6.

3.5. Reversible water uptake and desorption by freeze-dried sEPS hydrogels under controlled relative humidity conditions

The water absorption and desorption kinetics of freeze-dried sEPS hydrogels at different relative humidity (RH) conditions are given in Fig. 6. These experiments were carried out using Agro 1a protocol for the sEPS extraction and hydrogel-formation due to the reasons described above. The lyophilized hydrogels were able to absorb up to 133 % of water (i.e. 1.33 g H_2O per g freeze-dried hydrogel) upon conditioning at RH values around 97 % ($T \approx 25 \text{ °C}$). A similar behaviour was reported for freeze-dried alginate gel beads by Chan et al. [37], who observed a moisture content at equilibrium close to 70 % ($\text{RH} = 80 \text{ %}$, $T = 30 \text{ °C}$) [37]. The water absorption over time by freeze-dried sEPS hydrogels can

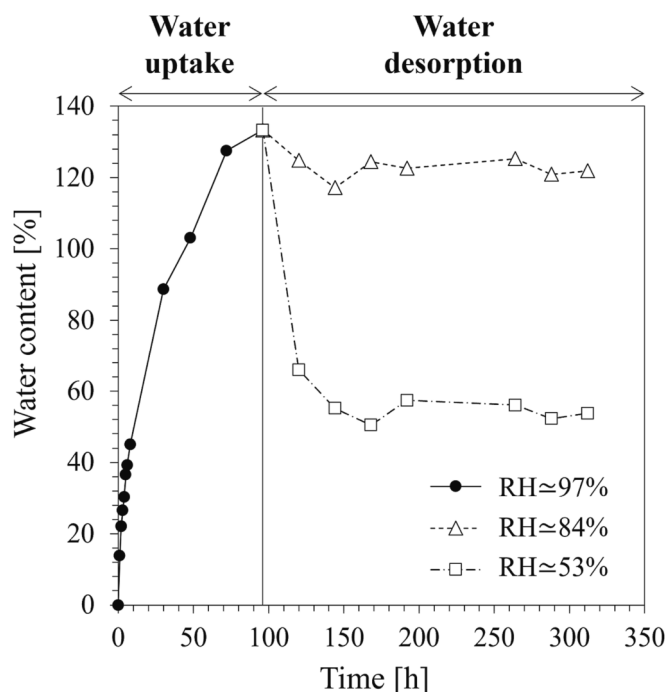


Fig. 6. Residual water content over time of freeze-dried sEPS hydrogels (as weight percentage referred to the freeze-dried sEPS hydrogel weight) during the experiments carried out under controlled relative humidity (RH) conditions: RH \approx 97 % (H₂O uptake), RH \approx 84 % and 53 % (H₂O desorption). The tested hydrogels were formed with Agro 1a protocol.

be described by a pseudo-second-order kinetic model ($R^2 = 0.989$; [Figure S4](#) and [Table S9](#) in [Supplementary Data](#)), similarly to what was inferred for the swelling kinetics in liquid aqueous phase.

Once equilibrated at RH \approx 97 % for 96 h, sEPS hydrogels were conditioned at RH of about 84 % and 53 % for 216 h to monitor the water desorption over time. Desorption mainly occurred during the first 24 h: at RH of about 84 % and 53 %, hydrogels desorbed up to 6 % and 51 % of the H₂O previously absorbed, respectively, in 24 h. At the end of the experiment, the residual water content of sEPS hydrogels conditioned at RH \approx 84 % and 53 % were 122 % and 54 %, respectively (i.e. 1.22 versus 0.54 g H₂O per g freeze-dried hydrogel). Consistently, the hydrogels kept at a lower RH exhibited a higher water loss: about 9 % versus 60 % of the previously absorbed H₂O were desorbed at RH \approx 84 % and 53 %, respectively (i.e. 0.11 versus 0.80 g H₂O desorbed per g freeze-dried hydrogel).

According to these results, sEPS hydrogels are hygroscopic (which means they can absorb moisture from the atmosphere) likely due to the hydrophilic nature of the polymeric matrix enabling a high-water uptake through the expansion of the 3D network during the water transport/diffusion [58]. Hygroscopic hydrogels, which have the ability of capturing and recovering water through absorption – desorption cycles, are emerging as promising materials for high-performance water sorption applications [58,59]: particularly, the reversible water absorption–desorption ability of sEPS hydrogels in moistened environments might be reasonably exploited in agriculture-related solutions.

3.6. Remarks on the applicability of sEPS in agriculture and future perspectives

Thanks to their bio-based and biodegradable nature and promising water and nutrient absorption/release features, sEPS hydrogels could represent a low-impact alternative to petroleum industry-derived SAPs (as highlighted in [Table S10](#) in [Supplementary Data](#)) to progress towards more sustainable and resilient agronomic practices. The ability of sEPS

hydrogels to reversibly absorb and release large quantities of water could be exploited to improve water-retention capacity of soils. sEPS hydrogels could act as “miniaturized water reservoirs” in arid and semi-arid soils, releasing a large part of the absorbed water to plants under moisture stresses or drought conditions through osmotic pressure difference, being the binding forces of water in the hydrogels reasonably lower than the suction force of roots [60].

The relatively fast nutrient release promoted by sEPS hydrogels would represent a bottleneck in their effective adoption for Controlled Release Formulations (CRFs) of agrochemicals in soil. However, from a mechanistic standpoint, sEPS hydrogels behaved similarly to materials for CRFs classified as physical release formulations of absorption type [61]. In this kind of CRFs, largely using SAPs in the form of hydrogels, the agrochemicals (if soluble) are dissolved in water, loaded in the hydrogels by swelling and then gradually released in the environment with release rates dependent on the concentration gradient between the hydrogel and the surrounding medium [61], as observed for sEPS hydrogels. Taking the above into account, further studies are encouraged to improve the controlled-release characteristics of sEPS hydrogels (e.g. by fine-tuning the cross-linking method) and hence promote their efficient application in soil for nutrient release purposes. For instance, in this kind of ionically cross-linked hydrogels, by increasing the cross-linking density (e.g. by increasing the ionic cross-linker concentration [12]), the mesh size of the polymeric network would reasonably decrease, thus potentially slowing down the release of nutrients due to a decrease in their diffusion coefficient inside the hydrogel network. Alternatively, forming hydrogels with an external highly cross-linked polymeric layer (and/or a chemically modified coating) acting as diffusive barrier could be another strategy to control the release rates.

It is worth noting that the release rate is not only affected by the hydrogel properties, but also by the nature of the surrounding medium (e.g. temperature, pH, ionic strength, etc. [62]). In this study, the nutrient release by sEPS hydrogels was addressed in distilled water. The ion concentration gradient between the hydrogel and the external medium was therefore very high, thus potentially increasing the nutrient release rate compared to that achievable in salt-rich solutions and/or more complex matrices such as real soils. More in general, with increasing ionic strength of the external medium (especially regarding the concentration of multivalent cations), further cross-linking points can be formed, limiting the expansion of the hydrogel network, and hence reducing swelling and release rates [62]. As soil temperature increases, both diffusion coefficient and pore size of the network are expected to increase, thus leading to higher release rates [62,63]. The acidic or alkaline nature of the release medium has also a significant effect on the ion diffusion coefficient, with the highest swelling capacity expected for neutral pH values [62]. In complex environmental matrices, all these factors and many others (e.g. presence of dissolved organic matter) may act simultaneously in regulating the nutrient release kinetics by hydrogel-like materials.

Another aspect to consider in designing this kind of agronomic solutions is that the performance of SAPs depends on their stability in soil which in turn is influenced by their biodegradability. The fast biodegradation of bio-based SAPs may compromise their long-term performance for repeated absorption and release of water and nutrient in the soil [64]. Searching for a balance between long-term functionality and biodegradation rate under the natural conditions of agricultural fields is hence a critical holdup which should be investigated in future research on sEPS-based biomaterials for agronomic uses. A potential strategy might be found in the development of hybrid hydrogels (e.g. by using blends of synthetic and natural polymers [64,65]) exhibiting moderate biodegradation rates and acceptable mechanical strength and stability along their functionality period [64]. It is worth noting that SAPs biodegradation in soils depends on several factors such as soil type, temperature range and cross-linking density [23]. For instance, by increasing the cross-linking density, macromolecules are more confined and potentially less accessible for microbial utilization, thus reasonably

resulting in a slower biodegradation rate and a prolonged structural integrity. In summary, an increase in cross-linking density could result in sEPS hydrogels with both slower release kinetics and prolonged efficacy due to lower diffusion coefficient and biodegradation kinetics, respectively. A preliminary attempt to address the sEPS hydrogel stability was carried in this study by monitoring the Ca^{2+} concentrations over the consecutive dehydration – swelling – K^+/NO_3^- release experiments described in section 3.4 (Figure S5 in Supplementary Data). Indeed, under the simplified experimental conditions tested in this study (e.g. absence of microbial degradation), the loss of the hydrogel structural integrity in the release medium could be ascribed to the loss of cross-linked Ca^{2+} . sEPS hydrogels released most of their Ca^{2+} content (about 98 %) into the liquid bulk upon swelling in KNO_3 , without any significant collapse of their 3D network. Once transferred into distilled water, the KNO_3 -swollen hydrogels released around 67 % of the residual Ca^{2+} ions into the surrounding medium and began to dissolve, likely due to the partial loss of cross-linked Ca^{2+} . In real environmental matrices, especially in the case of Ca-rich soils, the concentration gradient between the hydrogel and the release medium is expected to be lower, thus better preserving the hydrogel structural integrity from cross-linker releases.

Although the above clearly highlights the need for further dedicated studies, the findings of this research in terms of water and nutrient absorption/release characteristics suggested that AGS-extracted sEPS might be integrated into Regulation (EU) 2019/1009 on EU fertilizing products, under Component Material Category (CMC) 9: “Polymers other than nutrient polymers” (Annex II) [42], and hence valorized according to end-of-waste criteria. This Regulation establishes that polymers included into CMC 9 should control the water penetration into nutrient particles and thus the nutrient release and/or increase the water retention capacity or wettability of EU fertilizers [42]. In this regard, biodegradability and phytotoxicity assessments are encouraged in future research to comprehensively verify the compatibility of these waste-derived biopolymers with the aforementioned Regulation [42].

It is worth stressing that the sEPS extraction from waste sludge would not only allow to yield a valuable raw material, but also to potentially increase the sustainability of wastewater treatment [66], especially if integrated chains of resource recovery (including EPS, phosphorus, methane, etc.) are implemented in WWTPs [67]. In this regard, a crucial aspect to be further investigated is related to the influence of the sEPS extraction on the treatment efficiency of the residual (i.e. non-extracted) waste sludge in terms of dewaterability [66] and digestibility [68].

In support of the above, the entire sEPS recovery/valorization chain should be characterized in terms of environmental and economic impacts. Regarding the economic feasibility of extracting sEPS from AGS, it should first be considered that the recovery of sEPS as secondary raw material from waste AGS first causes a significant reduction in the excess sludge mass to be disposed of (by at least 19 – 23 wt% on VS basis). Considering that the excess sludge disposal costs represent one of the greatest incidences of OPEX for wastewater treatment [21], the economic savings from its non-disposal together with the potential market-value of the recoverable biomaterial could make economically sustainable the recovery of sEPS, supporting the CAPEX/OPEX costs of the extraction chain. It is worth pointing out that waste AGS can be concentrated by simple draining up to approximately 11 wt%, thus providing a concentrated feedstock to enter further recovery/valorization processes. This represents a technological and economic advantage with respect to CAS systems, since activated sludge needs to be more intensively processed to achieve considerable concentrations. A more accurate and comprehensive economic assessment is currently hampered by the following aspects. To date, many of the research activities related to sEPS extraction process optimization and derived products development are in the early experimental stages (e.g. Technology Readiness Levels = 3–4), thus complicating the estimation of the economic effort required to scale up the processes and hindering an accurate evaluation of the market-value of the obtainable products. In

fact, to the best of our knowledge, the only relevant data in terms of sEPS large-scale recovery and relative economic benefits are from WWTPs in the Netherlands [69,70]. However, it can be reasonably expected that the growing commercial interest towards bio-based and biodegradable materials in many industrial sectors (including the agricultural field) will stimulate research investments and expand the market potential of sEPS-based products.

4. Conclusions

This paper investigated the agronomic potential of AGS-extracted sEPS and derived hydrogels through a comprehensive assessment of various agriculture-oriented properties. It was found that the chemicals used in the extraction and gelling methods did not strictly affect the quantity of the extractable sEPS and their hydrogel-forming properties, but significantly influenced their elemental composition. Particularly, the comparative elemental analyses revealed that:

- The chemicals used in the extraction process influence the elemental composition of the recovered sEPS;
- The Ca^{2+} source used as cross-linking agent affects the elemental composition of the resulting sEPS hydrogels (due to the Ca^{2+} counter ion diffusion into the polymeric matrix upon gelation);
- The chemicals used in the extraction phase have a less significant effect on the elemental composition of the resulting sEPS hydrogels.

According to these findings, the recovery processes should be critically evaluated based on application-related criteria, considering the potential constraints imposed by the targeted applicative sector.

Looking at the properties of interest in the agricultural framework, the following results are pointed out:

- The 50 °C-dehydrated sEPS hydrogels were able to swell in both water and nutrient-enriched aqueous solutions (KNO_3), absorbing up to about 12 g $\text{H}_2\text{O}/\text{g TS}_{\text{Hydrogel}}$: these high water-binding capacity and swelling ability are characteristic of superabsorbent polymers (SAPs)-based hydrogels, gaining increasing attractiveness in the agronomic sector;
- The KNO_3 -swollen sEPS hydrogels (enriched in potassium compared to the pristine hydrogels) released up to 133 mg K^+ and 187 mg NO_3^- per g $\text{TS}_{\text{Hydrogel}}$ in water within 8 h: sEPS hydrogels might be hence engineered as nutrient loading/release agents upon optimization of the (currently too high) release rates;
- Freeze-dried sEPS hydrogels were able to reversibly absorb and desorb large quantities of water under controlled relative humidity conditions.

All these features suggested the high agronomic potential of AGS-extracted sEPS, thus paving the way towards integrated circular economy pathways in both wastewater treatment and agronomic sector.

CRedit authorship contribution statement

Benedetta Pagliaccia: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Riccardo Campo:** Writing – review & editing, Methodology, Data curation, Conceptualization. **Emiliano Carretti:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Mirko Severi:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Claudio Lubello:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Tommaso Lotti:** Writing – review & editing, Supervision, Project administration, Methodology, Data curation, Conceptualization.

Declaration of competing interest

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.

Data availability

Data will be made available on request.

Acknowledgements

This research was funded by POR FESR Toscana 2014–2020, IDRO. SMART project (CUP: 3647.04032020.157000040). All authors thank the students J. P. Czelnik and G. Pellegrini (University of Florence) for their support in the experimental activity.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cej.2024.149819>.

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