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Waste Biopolymers for Eco-Friendly Agriculture and Safe Food Production

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Abstract: This work addresses environmental problems connected with biowaste management, the chemical industry, and agriculture. These sectors of human activity cause greenhouse gas (GHG) emissions in the air, climate change, leaching of excess mineral fertilizers applied to soil into ground water, and eutrophication. To mitigate this problem in agriculture, controlled release fertilizers (CRFs) are made by coating mineral fertilizers granules with synthetic polymers produced from the fossil-based chemical industry. This strategy aggravates GHG emission. In the present work, six formulations containing sunflower protein concentrate (SPC) and a new biopolymer (BP) obtained from sunflower oil cake and by hydrolysis of municipal biowaste, respectively, and commercial urea were tested as CRFs for spinach cultivation against the control growing substrate Evergreen TS and commercial Osmocote®. The results show large differences in plants' nitrate concentration due to the different treatments, although the same nitrogen amount is added to the substrate in all trials. BP is the key component mitigating nitrate accumulation in plants. The plants grown in the substrates containing BP together with SPC and/or urea, although exhibiting relatively high total N uptake (47–52 g kg^{-1}), have significantly lower nitric to total N ratio (9.6–12.0) than that (15.3–16.5) shown by the plants grown in the substrates containing SPC and/or urea, but no BP. The data confirm that all composites containing BP yield the safest crop coupled with high biomass production. Replication of BP effects for the cultivation of different plants will contribute to the development of a biobased chemical industry exploiting biowastes as feedstock.

Keywords: biopolymers; municipal bio-waste; spinach; agriculture; nitrates

1. Introduction

The augmentation of human population, along with its concentration in cities and increasing consumption habits, is causing several problems connected with biowaste management, the chemical industry, and agriculture. For example, municipal biowaste (MBW) production in Europe is 100 Mt yr^{-1} [1], with more than half still landfilled releasing 25,000 Mm^3 yr^{-1} GHG [2]. To satisfy the food demand, crop production is boosted by applying mineral fertilizer doses higher than those adsorbed by soil and plants. Excess nutrients accumulate in soil, leach into ground water, and cause eutrophication [3]. In this context, for a long time the Universities of Torino and Toulouse focused their research on the valorization of biowaste from urban (MBW) and agro-industrial sources as feedstock

for the production of value added biobased chemical specialties and materials to use in place of commercial products obtained from fossil sources [4].

With specific reference to the agriculture sector, very recently the authors of the present work have reported the manufacturing and thermomechanical properties of a composite material made by twin-screw extrusion followed by injection-molding for use as controlled release fertilizer (CRF) [5]. This material contains urea and two polymers, herein after named sunflower proteins concentrate (SPC) and biopolymer (BP). These are obtained from sunflower oil cake and by hydrolysis of the anaerobic fermentation digestate of municipal unsorted food wastes, respectively. All three components contain nitrogen, have soil fertilizing power, have different solubility in water and, therefore, can release nitrogen in soil. Their fertilizing properties depend on the nitrogen release rate. In addition to the fertilizing property, SPC is well known for its good processability and is suitable to be used as a thermoplastic matrix for manufacturing the CRF composite pellets by twin-screw extrusion followed by injection-molding [5].

To test and assess the performance of the SPC and BP biopolymers, the experimental plant reported in the present work included three key elements, i.e., urea as widely used fertilizer, spinach as a sensitive probe to measure SPC/BP effects, and Osmocote$^®$ as a reference of widely used commercial CRF, representing a typical material made by conventional coating technology. Urea is one of the most important N-fertilizers. Its world consumption is 51 Mt yr^{-1} [6]. Urea has environmental drawbacks deriving from the release of excess nitrogen over the plant uptake rate. Urea is highly soluble in water. In soil, it is hydrolyzed to ammonia [7,8] and then, transformed into nitrates. These are adsorbed by the plant roots and transferred to the leaves. Nitrates in leaves are reduced to ammonia and then converted to proteins. In the presence of excess ammonia, proteins' production is slowed down. Nitrates accumulate in leaves and soil. They may impact the environment negatively, leaching from soil into ground water and causing eutrophication [9]. Nitrates in food plants may also have carcinogenic effects for humans [10]. In the human body, nitrate can be reduced to nitrite, which may cause methemoglobinemia, and the possibility of gastric cancer and other diseases [11,12].

Leaves of spinach are a typical food that can exhibit the nitrates' accumulation effects [13]. Spinach is a high value crop that requires sufficient N fertilizer to ensure optimal growth and to meet high quality criteria [14]. It absorbs NO^{3-} from the soil efficiently but is known to be relatively inefficient in NO^{3-} reduction [15]. The European Commission issued the regulation No. 1258/2011, stating the maximum acceptable concentration of nitrates in spinach [16]. These are 3.5 g kg^{-1} in fresh spinach, 2 g kg^{-1} in preserved, frozen spinach, and 0.2 g kg^{-1} in baby food. Spinach leaves should have high nutritional value from protein content and no toxicity from nitrates.

Commercial Osmocote$^®$ is a typical material belonging to the family of CRFs containing urea granules coated with synthetic polymers [17,18]. These are used to mitigate nitrate accumulation and effects in the environment [19]. The drawbacks of these CRFs are those typical of the fossil-based chemical industry. To substitute synthetic organic materials derived from fossil sources with natural or biobased materials [20], other CRFs have been investigated, based on natural polymers derived from plants [21]. No CRF is known to contain biopolymers derived from biowastes as SPC and BP.

In the previous work [5], the mechanical properties' advantages of the injection-molding technology, compared to conventional coating processes, were discussed. The inclusion of Osmocote$^®$ in the experimental plan of the present work has allowed the vis-à-vis direct comparison of the twin-screw extruded/injection-molded SPC composite and the coated Osmocote$^®$ composite for their performance as CRF for the cultivation of spinach.

2. Materials and Methods

2.1. Materials

The components to fabricate the SPC composites were available from previous work [5]. SPC was obtained by sieving a sunflower oil cake (SOC) using a Ritec (Signes, France) 600 vibrating sieve shaker fitted with a 1 mm grid. The undersize (SPC) was enriched with the smaller particles coming from the kernel of the seed, thus having a high protein content (51%, in proportion to the SPC dry weight). The rest (oversize) was constituted mainly by particles from the seed hull, and it was therefore rich in lignocellulosic fibers and minerals. BP was obtained by hydrolysis at pH 13 and 60 °C of the anaerobic digestate of MBW [4]. The anaerobic digestate was collected from the MBW waste treatment plant owned by the Acea Pinerolese Industriale SpA (ACEA) company in Pinerolo (TO). This plant processes, by fermentation, urban food wastes from separate source collection to produce biogas and solid anaerobic digestate containing the recalcitrant lignocellulosic fraction of the pristine MBW. To prepare the BP, the ACEA anaerobic digestate was taken up in water to yield a slurry at 4 water/digestate (*w/w*) ratio. Potassium hydroxide was added to the slurry until it reached pH 13. The alkaline slurry was heated up to 60 °C for 4 h. Afterwards, the slurry was allowed to settle until the liquid hydrolysate separated from the insoluble phase. The liquid hydrolysate was filtered through a 5 kDa polysulphone membrane to separate the retentate containing BP and the permeate containing the excess unreacted alkali reagent. The retentate was finally dried at 60 °C to yield the solid BP, which was used in the present work. Major components of BP were 45% protein, 13% lignin, and 15% minerals. Urea was a common commercial product.

2.2. Fabrication and Composition of SPC Composites

The SPC composites were fabricated and characterized for their elemental composition (Table 1) in the previous work [5]. Granules containing urea and/or BP dispersed into the SPC matrix were made by twin-screw extrusion. These were converted into dense dark pellets, all having the same (10 mm × 10 mm × 4 mm) dimension, by injection-molding. The tested SPC composites had the following compositions: neat SPC, SPC-U containing 10% urea, SPC-BP containing 10% BP, and SPC-BP-U containing 5% BP and 5% urea. For the neat components and the composite materials, Table 1 reports the C and soil/plant N, P, and K nutrient contents.

Table 1. Total C, N, P, and K concentrations (%, $w\,w^{-1}$) in neat SPC, BP, urea (U), and in the SPC pellets' samples [1].

Formulation	C	N	P_2O_5	K_2O
BP	39.6	6.6	1.1	5.5
U	20.0	46.6	-	-
SPC	38.7 ± 5.2 [a]	6.9 ± 0.9 [a]	2.5 ± 0.1 [a]	1.5 ± 0.1 [a]
SPC-U	37.7 ± 0.2 [a]	10.5 ± 0.1 [c]	2.3 ± 0.1 [a]	1.3 ± 0.1 [a]
SPC-BP	39.5 ± 2.6 [a]	7.0 ± 0.6 [a]	2.6 ± 0.3 [a]	1.8 ± 0.1 [b]
SPC-BP-U	38.6 ± 0.4 [a]	8.5 ± 0.0 [b]	2.1 ± 0.1 [a]	1.5 ± 0.1 [a]

[1] Values in the same column followed by different letters are significantly different ($p < 0.05$).

2.3. Plant Growth Trials

Spinacia oleracea L. "Gigante d'inverno" seedlings, purchased from Beltrame Roberto nursery, strada Sanda, Moncalieri, Italy, were transplanted on the commercial growing substrate Evergreen TS (Turco S.a.s, Moncalieri, Italy) in 2 L pots with 13 cm × 13 cm × 13 cm size (5 plants per pot, and 5 pots per trial). The plants were grown during November and December 2019 in an unheated greenhouse at an average temperature of 18 °C and 90% relative humidity under natural lighting conditions. Surface irrigation was performed manually every three days. After three days, the neat components and the composites were added to the substrate in the following amounts:

- Trial 0: control (growing substrate) containing 25 mg organic N.
- Trial 1: growing substrate + SPC (4.1 g per pot, 283 mg N).
- Trial 2: growing substrate + SPC-U (2.7 g per pot, 284 mg N).
- Trial 3: growing substrate + SPC-BP (4.0 g per pot, 280 mg N).
- Trial 4: growing substrate + SPC-BP-U (3.3 g per pot, 281 mg N).
- Trial 5: growing substrate + urea (0.6 g per pot, 280 mg N).
- Trial 6: growing substrate + BP (4.3 g per pot, 284 mg N).
- Trial 7: growing substrate + urea (0.3 g per pot, 140 mg N) + BP (2.2 g per pot, 145 mg N).
- Trial 8: Osmocote$^{®}$ (1.8 g per pot, 282 mg N).

The trials' plan included the control (trial 0) without added N fertilization, trials 1–7 in the presence of added SPC, BP, and urea products, and trial 8 in the presence of commercial slow-release N, P, K fertilizer Osmocote$^{®}$ [22], which was used as reference material. The amount of SPC materials (trial 1–4) and of urea and BP (trial 5–7) added in each pot was calculated to provide, in each pot, nearly the same total N amount (280–285 mg N) as in trial 8. After 55 days, the shoots and roots were collected, and their respective fresh weights were measured. Two plants per pot were dried at 60 °C for 3 days for the determination of the dry weight of leaves and roots.

2.4. Analyses

Total N was determined by the flash combustion method (i.e., "Dumas method") with thermal conductivity detection using the UNICUBE elementar analyzer (Elementar, Langenselbold, Germany) according to the CSN EN 13654-2 standard method. Nitrate concentrations in leaves and roots at the end of the trial have been analyzed on the fresh material. For each plant, 100 mg of fresh tissue was ground in liquid nitrogen and suspended in 10 mL of deionized water. Suspensions were incubated for 1 h at 45 °C and then centrifuged at 5000 rpm for 15 min. The extract was filtered, and the nitrate N concentration was determined spectrophotometrically by the Griess reaction [23]. The determination of chlorophyll *a* and *b* and of carotenoids was performed on each plant by extraction of 300 mg fresh foliar tissue ground in liquid nitrogen with 10 mL 96% (*v/v*) ethanol. The samples were kept in the dark for 2 days at 4 °C, and the extracts were filtered and then analyzed by spectrophotometry using a Hitachi (Tokyo, Japan) U-2000 spectrophotometer. The absorbance readings were performed at 665 nm for chlorophyll *a*, at 649 nm for chlorophyll *b*, and at 470 nm for total carotene. Chlorophyll *a*, *b*, and total carotenoid concentrations were calculated according to the literature [24].

2.5. Statistical Treatment of Data

The data were evaluated by one-way ANOVA ($p < 0.05$ or 0.01) followed by the Tukey's test for multiple comparison procedures.

3. Results

For the spinach cultivated in the different trials, Table 2 reports the fresh and dry weights of leaves and roots. At the end of the experiment, the plants were healthy and reached the marketable size without apparent differences between the trials, except for the fresh weight of the leaves as higher values were recorded in trials 2 and 3, compared to trial 6. Overall, the experimental values suggest that the control substrate provides a sufficient amount of nutrients to allow a regular and constant growth of the plants.

Table 3 reports the chlorophyll *a*, chlorophyll *b*, and carotenoids concentrations in the fresh spinach leaves. It shows that the chlorophyll *a* content in the plants fertilized with SPC and urea (trials 1 and 5) is significantly higher than that of the control (trial 0). The plants fertilized with SPC and SPC-U (trials 1 and 2) exhibit significantly higher concentrations of chlorophyll *b* than the control (trial 0). The treatments appear not to significantly affect the carotenoid concentration compared to the control (trial 0).

Table 2. Fresh and dry weight of spinach leaves and roots (mean value $\pm$ standard deviation) [1] cultivated in trials 0–8.

Trial	Leaves		Roots	
	Fresh Weight (g)	Dry Weight (g)	Fresh Weight (g)	Dry Weight (g)
0	18.7 $\pm$ 3.2 [ab]	1.6 $\pm$ 0.55 [a]	0.86 $\pm$ 0.29 [a]	0.11 $\pm$ 0.04 [a]
1	25.6 $\pm$ 5.3 [ab]	2.3 $\pm$ 0.56 [a]	1.16 $\pm$ 0.22 [a]	0.13 $\pm$ 0.02 [a]
2	27.3 $\pm$ 4.5 [a]	2.4 $\pm$ 0.39 [a]	1.08 $\pm$ 0.23 [a]	0.12 $\pm$ 0.03 [a]
3	27.7 $\pm$ 6.1 [a]	2.3 $\pm$ 0.61 [a]	0.99 $\pm$ 0.26 [a]	0.10 $\pm$ 0.03 [a]
4	23.5 $\pm$ 5.1 [ab]	2.2 $\pm$ 0.54 [a]	0.99 $\pm$ 0.18 [a]	0.11 $\pm$ 0.03 [a]
5	24.5 $\pm$ 5.0 [ab]	2.3 $\pm$ 0.60 [a]	0.98 $\pm$ 0.16 [a]	0.12 $\pm$ 0.03 [a]
6	18.1 $\pm$ 1.9 [b]	1.8 $\pm$ 0.20 [a]	0.89 $\pm$ 0.10 [a]	0.12 $\pm$ 0.02 [a]
7	23.2 $\pm$ 3.4 [ab]	2.3 $\pm$ 0.54 [a]	1.08 $\pm$ 0.14 [a]	0.13 $\pm$ 0.03 [a]
8	22.8 $\pm$ 2.7 [ab]	2.2 $\pm$ 0.27 [a]	1.04 $\pm$ 0.08 [a]	0.13 $\pm$ 0.01 [a]

[1] Within columns, mean values followed by the same letter are not significantly different ($p < 0.01$).

Table 3. Chlorophyll and carotenoids content in spinach leaves expressed as mg kg^{-1} fresh weight (mean value $\pm$ standard deviation) [1] cultivated in trials 0–8.

Trial	Chlorophyll *a*	Chlorophyll *b*	Carotenoids
0	242 $\pm$ 49.0 [a]	123 $\pm$ 28.9 [a]	19 $\pm$ 0.9 [a]
1	330 $\pm$ 32.3 [b]	176 $\pm$ 11.0 [b]	8 $\pm$ 0.3 [a]
2	323 $\pm$ 46.6 [ab]	172 $\pm$ 15.0 [b]	8 $\pm$ 0.4 [a]
3	258 $\pm$ 41.0 [ab]	125 $\pm$ 26.9 [a]	16 $\pm$ 0.8 [a]
4	321 $\pm$ 29.4 [ab]	158 $\pm$ 11.0 [ab]	7 $\pm$ 0.3 [a]
5	341 $\pm$ 29.9 [b]	172 $\pm$ 34.1 [ab]	18 $\pm$ 0.6 [a]
6	241 $\pm$ 55.4 [a]	138 $\pm$ 23.3 [ab]	24 $\pm$ 0.7 [a]
7	309 $\pm$ 22.1 [ab]	158 $\pm$ 19.4 [ab]	11 $\pm$ 0.4 [a]
8	289 $\pm$ 42.2 [ab]	168 $\pm$ 34.5 [ab]	17 $\pm$ 0.6 [a]

[1] Within columns, mean values followed by the same letter are not significantly different ($p < 0.01$).

Table 4 reports the total N concentration in the plants. The experimental data in Table 4 show that the nitrogen uptake per plant is much lower in roots than in leaves. This is expected since most of the nitrate absorbed by the roots is transported in the xylem to the leaves for further reduction to ammonia, which is then used to synthesize the leaf proteins. The results also point out some significant differences between the trials. In trials 1–5, and 7 and 8, the total N concentration in both leaves and roots is higher than that for the control (trial 0). The highest total N concentration values are shown in the leaves of the plants grown on SPC-based fertilizers (trials 1–4) and urea (trial 5).

The data in Table 5 point out large differences in nitrate concentration among the trials, although the same nitrogen amount was added to the substrate in all trials. In all trials, the nitrate concentration in fresh spinach is well below the 3.5 g kg^{-1} safe limit recommended by the European Commission [18]. The highest concentration is found in the spinach fertilized with urea (2816 mg kg^{-1} in trial 5) and in the spinach fertilized with the sunflower proteins-based fertilizer (1890–2559 mg kg^{-1} in trials 1, 2, and 4). The lowest nitrate concentration is found in the plants grown on the control substrate (101 mg kg^{-1} in trial 0) and in the plants fertilized with BP (247 mg kg^{-1} in trial 6). However, these plants exhibit a rather low total nitrogen concentration (Table 4), probably due to the low level of nitrogen mineralization in the substrate. This is confirmed by the low nitric to total N ratio (1.0 and 2.2 in trial 0 and trial 6, respectively), which suggests that most of the absorbed inorganic nitrogen is promptly transformed into amino acids and proteinaceous matter. In all other cases, the nitric to total N ratio ranges from 9.6 to 16.5.

Table 4. Total nitrogen concentration and N uptake for spinach plants (mean value ± standard deviation) [1] cultivated in trials 0–8.

Trial	Total N (g kg^{-1} Dry Matter)		N Uptake (mg Plant^{-1})	
	Leaves	**Roots**	**Leaves**	**Roots**
0	34.0 ± 1.82 [a]	20.9 ± 2.15 [a]	54.4 ± 21.6 [a]	2.3 ± 1.01 [a]
1	51.9 ± 1.32 [bcd]	29.8 ± 1.72 [bc]	117.6 ± 32.0 [b]	3.7 ± 0.88 [a]
2	52.7 ± 1.31 [bd]	30.4 ± 2.09 [bc]	128.3 ± 23.6 [bc]	3.6 ± 1.09 [a]
3	48.5 ± 2.04 [bce]	28.2 ± 0.81 [bcd]	113.0 ± 34.3 [ab]	2.9 ± 1.01 [a]
4	52.1 ± 2.99 [bcd]	29.1 ± 1.77 [bc]	114.5 ± 34.5 [b]	3.3 ± 1.12 [a]
5	55.5 ± 1.71 [d]	30.7 ± 2.36 [b]	129.7 ± 37.4 [bc]	3.6 ± 1.20 [a]
6	34.3 ± 3.44 [a]	20.7 ± 3.44 [a]	63.1 ± 13.3 [ab]	2.6 ± 0.87 [a]
7	47.2 ± 2.55 [ce]	26.9 ± 2.14 [bc]	106.2 ± 31.1 [ab]	3.5 ± 0.96 [a]
8	45.1 ± 3.47 [e]	26.5 ± 2.11 [c]	99.8 ± 19.8 [ab]	3.3 ± 0.57 [a]

[1] Within columns, mean values with the same letter are not significantly different ($p < 0.01$).

Table 5. Nitric nitrogen (mean value ± standard deviation) [1] in spinach leaves cultivated in trials 0–8.

Trial	NO^{3-} N (g kg^{-1} Dry Matter)	NO^{3-}/Total N ($w\ w^{-1}$)	NO^{3-} N (mg kg^{-1} Fresh Matter)
0	1.1 ± 0.60 [a]	1.0 ± 0.58 [a]	101 ± 53.7 [a]
1	26.0 ± 4.9 [bcd]	15.3 ± 3.3 [c]	2281 ± 410 [bcd]
2	28.6 ± 2.8 [bc]	16.5 ± 2.0 [c]	2559 ± 297 [bc]
3	15.4 ± 7.5 [e]	9.6 ± 5.1 [b]	1373 ± 575 [e]
4	20.5 ± 7.0 [bde]	12.0 ± 4.8 [bc]	1890 ± 593 [bde]
5	29.8 ± 1.9 [c]	16.3 ± 1.5 [c]	2816 ± 125 [c]
6	2.5 ± 1.4 [a]	2.2 ± 1.5 [a]	247 ± 122 [a]
7	16.4 ± 5.1 [de]	10.5 ± 3.9 [b]	1541 ± 398 [de]
8	14.9 ± 4.0 [e]	10.1 ± 3.5 [b]	1438 ± 323 [e]

[1] Within columns, mean values followed the same letter are not significantly different ($p < 0.01$).

4. Discussion

4.1. Effect of BP on Plant Performances

The data obtained from the present experiment show significant differences only between trials 2, 3, and 6 for the leaf fresh biomass, but not the dry one (Table 2). The average dry weight of the leaves (2.2 g per plant) is consistent with literature data for cultivated spinach under different conditions: 0.6–2.0 g per plant for hydroponic cultures [15,25], 0.2–1.2 g per plant in pot experiments, depending on the amount of added nitrogen [26], and 4.3–5.4 g per plant in trials where different composts were used as fertilizers [27].

The chlorophyll content of spinach leaves was also affected by BP application (Table 3). The concentration values reported in Table 3 fall in the range of values reported by other workers for spinach cultivated under various operational conditions. For example, for chlorophyll *a*, the following values are reported: 300–400 mg kg^{-1} in spinach cultivated in field experiments at different N and water applied levels [28], and 860 mg kg^{-1} in spinach cultivated under hydroponic conditions at 105 mg L^{-1} N applied doses [29]. The data for the group of trials 1–7, in which SPC, BP, and/or urea were used, show that trials 3 and 6 exhibit lower contents of chlorophyll and higher contents of carotenoids than the other 5 trials. The statistical analysis of these groups of data does not prove significant differences between most of the trials. Yet, specifically for chlorophyll, the apparent lower chlorophyll concentration recorded in leaves from trials 3 and 6 may be correlated to the lower total N content and uptake (Table 4) measured in leaves. Indeed, it is well known that leaf chlorophyll concentration is linked to leaf N content due to the presence of N atoms in chlorophyll molecules [30]. Some authors observed that BP application to plants enhanced the availability of N requested for chlorophyll formation [31], with positive effects on photosynthetic activity. The apparent higher carotenoid content and lower chlorophyll content in trials 3 and 6 can be related to a trend that plants show in

some growing conditions (particularly under stress), i.e., when chlorophyll values decrease, carotenoids tend to increase, and vice versa [32,33].

The BP application also influenced the total nitrogen content in spinach leaves and roots (Table 4). To be assimilated by plants, nitrogen must be in nitric or ammonia forms. Therefore, nitrogen present in organic forms in soil or substrate must be made available through mineralization by microorganisms. The prevailing form is the nitric one because ammonium ion is promptly oxidized and made less available by adsorption on the soil surfaces. Once absorbed by the roots, nitrates are transferred to the shoots where they are reduced to ammonium and used to first synthesize glutamate and glutamine through the enzymatic nitroreductase and GOGAT systems, and then other amino acids and N-containing compounds. The total N concentration values are consistent with those reported in literature for spinach leaves (22.7–51.5 g kg^{-1}) [15,27]. The low nitrogen concentration found in the plants grown on the BP-added substrate (trial 6) suggests that BP is recalcitrant to the biochemical attack by microorganisms, in accordance with the high lignin-like chemical moieties present in its macromolecular structure [4]. On the other hand, the low nitrogen uptake of the plants grown in trial 6 does not seem to negatively affect the leaf biomass yield as shown in Table 2. This is consistent with previous results reported by other authors, who used BPs in floriculture trials [30,31,34]. These authors agreed that the positive effects exhibited by the investigated biopolymers were due to the biopolymers' chemical structure interacting with the microorganisms and stimulating the plant metabolism, more than to their fertilizing power stemming from their contribution of organic nitrogen as soil fertilizer. On the other hand, although a high N content in the leaves, as shown in Table 4, can be considered a positive result because it is correlated to a high protein concentration, it should not be accompanied with a high nitrate concentration. Accumulation of nitrates in plants generally occurs when the plant uptakes more NO^{3-} than the NO^{3-} assimilable in protein form [35]. Most absorbed nitrate is stored in the vacuole until release for reduction in the cytosol [36]. In the present work, this issue is addressed by the collected data shown in Table 5.

Actually, BP application also affected nitric nitrogen content in spinach leaves (Table 5). Accumulation of nitrates in the vacuoles of the cells occurs when the enzymatic systems leading to the reduction of nitrates and further synthesis of protein matter are inhibited by excessive nitrate uptake. In this scenario, it is noteworthy that the plants grown in trials 3, 4, and 7 substrates, all containing BP together with SPC and/or urea, although exhibiting a high total N uptake (Table 4), have significantly lower nitric to total N ratio (9.6–12.0) than that (15.3−16.5) shown in the other trials (1, 2, and 5) containing SPC and/or urea but no BP. The best fertilizers in terms of high total N content and low nitrates accumulation were the SPC-BP (trial 3) and SPC-BP-U (trial 4) composites, the mix of urea and BP in trial 7, and Osmocote® (trial 8). In all these trials, the nitrate concentration in the spinach leaves is even below the limit of 2 g kg^{-1} recommended for preserved frozen spinach by the European Commission [16]. The data confirm that all composites containing BPs yield the safest crop coupled with high biomass production.

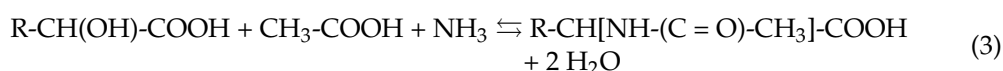*4.2. Plausible Explanation of the BP Effects*

The experimental data point out that BP can mitigate nitrates' accumulation in spinach plants. The fertilizer used in trial 3 was SPC loaded with 10% BP. About 90% of the total nitrogen present in this specimen comes from SPC. Therefore, most of the N uptake of the plants in trial 3 is to be attributed to SPC nitrogen. Indeed, trial 6 has demonstrated that BP nitrogen is relatively less available for the plant to take up. In trial 7, in which urea was the nitrogen source and the same amount of BP was added, high nitrogen uptake is also accompanied by a relatively low nitrate content. The results suggest that BP, although not supplying to the plant as much nitrogen as SPC and urea, strongly affects the pathways responsible for the mineralization of organic nitrogen. A similar effect was observed in the previous work [5] reporting the kinetics of ammonia and organic N release rates of the SPC composites in water. BP was shown to retard the formation of ammonia from urea

hydrolysis and enhance the release of organic nitrogen from SPC. In this case, the effect might be due to a plausible interaction of BP functional groups with urea and SPC.

BP belongs to a family of biopolymers obtained by the same hydrolysis process from fermented lignocellulose biowastes of different sources [4]. All these biopolymers keep the memory of the sourcing lignocellulose materials, are constituted by a mix of macromolecules containing the same carbon types and various acid and basic functional groups capable of interacting with other molecules by protonation and donor-acceptor complexing reactions. The relative ratios of the chemical functionalities in these families of biopolymers depend on the sourcing materials. As a consequence, they have the same multiple properties and performances, although at different levels depending on the sourcing materials. Other BPs, obtained from composted green wastes or composted mixes of green wastes and MBW anaerobic digestates, have been investigated as auxiliaries in the anaerobic fermentation of MBW [37,38] carried out in bioreactors dedicated to the production of biogas. These biopolymers can reduce the ammonia and/or the nitrate level in the process digestate. Other workers have used BPs as animal diet supplements [39]. They have shown that BPs reduce the proteolysis occurring in the caecum intestine of pigs with consequent reduction of ammonia formations. Biagini and coworkers [40] tested the biopolymer obtained from composted mixes of green wastes and MBW anaerobic digestates as a supplement for rabbits' protein diet. They demonstrated that the rabbits fed with the biopolymer-supplemented diet produced manure with significantly lower ammonia and GHG emissions compared to the animals fed with the control diet.
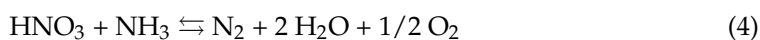
While the confirmation and replicability of the effects of the above biopolymers under different operational conditions and of the BP used in the present work are unquestionable, the role of these biopolymers is not yet clear. So far, it cannot be established definitely whether the biopolymer effects involve pure chemical reactions or biochemical processes with participation of a microorganism. The second hypothesis is the most likely, according to Baglieri and coworkers [41]. These researchers cultivated bean plants using a biopolymer obtained from the hydrolysis of exhausted tomato plants as fertilizers. The biopolymer sourced from the agriculture biowastes bears strong chemical similarities with BP sourced from MBW. The former was found [41] to significantly enhance nitrate reductase, glutamine synthetase, and glutamate synthase activities, and to increase soluble proteins' concentration in shoots and roots, compared to the control. Based on the lack of differences between the concentrations of mineral nitrogen in the control and treated cultivation substrate, as opposed to the significant differences observed for enzymatic activity and soluble proteins' concentration in the plants, Baglieri and coworkers [41] concluded that the biopolymer acts as plant biostimulant with a possible auxin-like effect, more than as soil fertilizer.

On the other hand, a possible action of BP as chemical catalyst must be considered. This is also in view of previous work reporting the property of BPs to catalyze oxidation reactions in the absence of any microorganism [42]. According to the current view of the behavior of urea in soil and the fate of the produced ammonia [7,8,35] for the system investigated in the present work, the following reaction scheme may help clarify the BPs effects:

$$CO(NH_2)_2 + H_2O \leftrightarrows 2\,NH_3 + CO_2 \tag{1}$$

$$NH_3 + 2\,O_2 \leftrightarrows HNO_3 + H_2O \tag{2}$$

$$R\text{-}CH(OH)\text{-}COOH + CH_3\text{-}COOH + NH_3 \leftrightarrows R\text{-}CH[NH\text{-}(C=O)\text{-}CH_3]\text{-}COOH + 2\,H_2O \tag{3}$$

The scheme shows that urea is hydrolyzed to ammonia (reaction (1)) and then, transformed into nitrates (reaction (2) forward). These are adsorbed by the plant roots and transferred to the leaves. Nitrates in leaves are reduced to ammonia (reaction (2) backward) and then converted to proteins (reaction (3)). A recent work [38] investigated the BPs' assisted anaerobic fermentation of MBW in 150 mL shake flasks. It demonstrated that BPs,

in the investigated MBW fermentation system comprising organic, nitrate and ammonia N, catalyze the following chemical redox reaction:

$$HNO_3 + NH_3 \leftrightarrows N_2 + 2\,H_2O + 1/2\,O_2 \tag{4}$$

The calculated Gibbs free energy from literature data [43] for this reaction, i.e., $-360\,kJ/N_2$ mole, shows that reaction (4) is thermodynamically favored. Occurrence of the chemical catalysis by BP in the system investigated in the present work, and the consequent reduction of nitrates by reaction (4), may be a plausible explanation for the lower nitric to total N ratio reported in Table 4 for the composite materials containing BPs (trials 3, 4 and 7), compared to the other materials in trials 1, 2, and 5 that contain SPC and/or urea but no BP.

4.3. Perspectives for a New Biowaste-Based Chemical Industry

The spinach case study reported in the present work shows that, for use in the agriculture sector, materials obtained from urban and agro-industrial biowastes are competitive with materials obtained from fossil sources. Particularly, the BP material obtained from MBW exhibits unique properties that allow modulating the N release rate and fate in soil and in the plant crop. These properties are very important to safeguard the quality of soil, water, and crops. More ambitiously, the present article has relevance also for the sectors of waste management, pollution, and the chemical industry.

Montoneri [4] and Tabasso and coworkers [44] have reviewed the sustainability of the hydrolysis process to produce BPs, as well as the BPs' multipurpose performance and related economic, environmental, and social benefits for several sectors of the chemical industry and agriculture. The engineered composite materials tested in the present work disclose a further benefit offered by the tested BP for developing safe agriculture and food. They prove a new property of BP capable of reducing fertilizer nitrate leaching through soil and eutrophication effects, and at the same time diminish nitrate accumulation in crops. They add further important incentives for valorizing MBW as feedstock for the production of BPs and their use at commercial scale. They also prospect the feasibility of substituting products from fossil sources with products from biowastes. Particularly, because of their origin and special properties, BPs have high potential for developing a sustainable, waste-based industry integrating chemical and biochemical processes.

MBW treatment plants are the ideal settings to this end. At present, they are service providers for citizens, as they perform the collection, disposal, and recycling of urban biowastes. To reduce landfill disposal, the most advanced plants process MBW by anaerobic fermentation yielding biogas and digestate, and by aerobic fermentation producing compost. The value of these products is not enough to cover the plant operational costs [4]. The missing revenue is covered by citizens' taxes. No MBW plant applies chemical processes. Yet, MBWs are a potential source of valuable renewable organic C, which could be recycled in the form of valued-added, biobased products for consumer use.

5. Conclusions

It has been demonstrated that composite controlled release fertilizers made by twin-screw extrusion followed by injection-molding can achieve the same performance in terms of spinach growth, nitrogen uptake, and nitrate accumulation as the commercial Osmocote® controlled release fertilizer, which contains urea coated with synthetic polymers. This result is achieved thanks to the municipal biowaste derived biopolymer. This component can control the nitrogen release rate of urea and from the sunflower protein concentrate, and reduce nitrogen accumulation by the plant while maintaining the same biomass growth and nitrogen uptake of Osmocote®. This finding poses a worthwhile scope for testing the twin-screw extruded/injection-molded biopolymer composites in the cultivation of other plant species in order to contribute to the replacement of current commercial materials coated with synthetic polymers from fossil sources. However, the ambition of the authors of the present work is far beyond developing controlled release fertilizers.

Previous work [4] has demonstrated that the hydrolysis of municipal biowaste from different sources is a cost-effective, feasible process that yields a family of biopolymers for sustainable use in different sectors of agriculture and the chemical industry. The demonstration of the BP biopolymer properties in the present work is a new finding. It widens the fields of application and the potential benefits of BPs. It adds a further argument for developing a waste-based chemical industry, which exploits biowaste as feedstock for the production of value-added chemical specialties and materials rather than plants specifically cultivated for this purpose. This approach, extended to the exploitation of biowastes from urban, agriculture, and agro-industrial sources, would allow not only the replacement of chemicals and materials from fossil sources, but would also dismiss environmentally unfriendly waste disposal practices and keeping agriculture soil for food production rather than for the production of chemicals.

6. Patent

The French patent application submitted 16 October 2020 under number FR2010597 to Institut National de la Propriété Industrielle (INPI) in France and entitled *"Produit pour l'agriculture, et procédé de préparation"* results from both [5] and the work reported in the present manuscript. The inventors of this patent are Philippe Evon, Carlos Vaca-Garcia, Laurent Labonne, and Antoine Rouilly. The owners of this patent are Institut National Polytechnique de Toulouse (INPT) and Institut National pour l'Agriculture, l'Alimentation et l'Environnement (INRAE).

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