Environmental Technology & Innovation 40 (2025) 104502

Contents lists available at ScienceDirect

Environmental Technology & Innovation

journal homepage: www.elsevier.com/locate/eti

A biorefinery approach for biostimulants and PHAs production from agri-food waste: Improved treatment strategies

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ARTICLE INFO

Keywords:
Hydrolysis
Protein hydrolysates
Enzymatic hydrolysis
Polyhydroxyalkanoates
Biostimulants
Agri-food waste
Biorefinery approach

ABSTRACT

The increase in the global human population means a greater demand for food and plastic goods. Alternative solutions are therefore needed to improve crop production and reduce plastic pollution. This work focused on the conversion of agri-food waste into volatile fatty acids (VFAs, yield 0.3 $kgCOD_{VFA}$/kgCOD) and into bacterial mass rich in polyhydroxyalkanoates (PHAs, yield 0.1 kgPHA/kgCOD), biobased and biodegradable polymers that can be used in place of fossil-based plastic. The PHA-rich biomass was subject to improved hydrolysis, including alkali, acid and enzymatic treatments, for the concomitant extraction of PHAs for the plastic industry and the production of biostimulants for agriculture. While NaOH was most effective in bacterial cell disruption (hydrolysis up to 45.4 %) and nitrogen extraction (63.0 %), the greener and gentler enzymatic treatment still allowed reasonable hydrolysis (33.8 %) and nitrogen extraction (58 %), while preserving the molecular weight of the recovered biopolymer. The seed priming test allowed the selection of the four most efficient protein hydrolysates (NaOH 0.5 M, HCl O.1 M, alcalase and papain without pretreatment) to apply to tomato seedlings via foliar spray and drench, to test for any possible biostimulant effect. The foliar spray treatment resulted in both root and shoot growth promotion (up to 29 %), with no statistical difference between treatments. The drench application was less effective, with only roots showing improved growth (alcalase-treated plants up to 23 %), while shoots were not statistically better than the controls. The results suggest the potential use of enzymatic approaches for the retrieval of PHAs and biostimulants from bacterial biomass.

1. Introduction

In a world suffering from food scarcity (FAO, 2023), with an ever-growing population that has recently broken the threshold of 8 billion (Adam, 2022), crop production must concurrently increase within the framework of sustainability. The contemporary agro-ecosystems could fulfil the increasing food demand by adopting different strategies to boost crop yields, such as genetic improvements of yield-defining traits (Bailey-Serres et al., 2019), improved use of manure and fertilizers (Liu et al., 2024) and irrigation practices (Siebert and Döll, 2010), and the use of plant biostimulants (PBs) (Carillo et al., 2025). PBs are a heterogeneous class of compounds, spanning from humic substances to botanical extracts, which can enhance plant nutrient use efficiency and tolerance to abiotic stress, and boost crop productivity and quality (Du Jardin, 2015). An important category of PBs is that of protein hydrolysates (PHs), which

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https://doi.org/10.1016/j.eti.2025.104502
Received 6 May 2025; Received in revised form 26 August 2025; Accepted 6 September 2025
Available online 8 September 2025

are mixtures of polypeptides, oligopeptides and amino acids produced from protein sources by partial hydrolysis (Schaafsma, 2009). PHs are obtained from protein-rich animal, plant, or microbial-derived biomasses, and the origin of the matrix together with the hydrolysis process applied strongly affects the physicochemical characteristics of the obtained PH (i.e. peptidic content and molecular weight, concentration of free amino acids and micro- and macro-nutrients) (Moreno-Hernández et al., 2022; Pituello et al., 2022). The raw materials employed to produce PHs are often by-wastes of other industries (e.g. collagen from bovine hides of the tanning industry, glutelin and zein from maize gluten of the wet-milling process) while the resulting PBs are high-value products that can bolster crop productivity (Peli et al., 2025; Santi et al., 2017). The mode of application also affects the biostimulation, for instance PHs can be applied by fertigation (directly to the soil or in hydroponic solution), by foliar spray, together with co-formulants in seed treatments or by seed priming (soaking seeds in a solution containing bioactive molecules that can enhance germination and growth favourably modulating the pre-germinative life of the plant) (Colla et al., 2015; Horii et al., 2007). Notwithstanding their heterogeneity, PHs often display similar biostimulant effects on plants, being effective in enhancing plant growth, vegetable and fruit quality, and resistance to abiotic stress (Caruso et al., 2020; Choi et al., 2022; Rouphael et al., 2017). Although their mechanisms of action are still to be elucidated, the observed effects are frequently linked to extensive transcriptional and metabolic reprogramming (Lucini et al., 2020; Peli et al., 2025).

The increase in the world's population not only intensifies the demand for food, but also contributes to a surge in the production and consumption of plastic, which then ends up in the environment (Pilapitiya and Ratnayake, 2024). Since the mid-20th century, plastic production has risen steeply due to its durability, versatility, and low cost (Geyer et al., 2017). However, these very qualities have contributed to a global environmental crisis, affecting ecosystems, wildlife and human health worldwide (Bucci et al., 2020). In response to the plastic pollution crisis, alternative types of plastics of biological origin are being developed, which aim to reduce environmental impact while retaining the beneficial properties of traditional plastics. These bioplastics, made from renewable resources and designed to be biodegradable or compostable, can reduce reliance on finite resources and often have a smaller carbon footprint (Shamsuddin et al., 2017). One type of bioplastics are polyhydroxyalkanoates (PHAs), biodegradable polyesters produced by some microorganisms (Sabapathy et al., 2020) as a form of carbon and energy storage (Raza et al., 2018). They exhibit excellent biocompatibility, flexibility, and mechanical properties similar to those of conventional plastics and have the potential to substitute traditional plastics in various applications (Freches and Lemos, 2017). Their properties can be tailored for specific uses, from packaging to medical applications, although challenges must be addressed to achieve widespread adoption (Guillard et al., 2018; Rosenboom et al., 2022). PHAs face several marketing challenges, in particular higher production costs compared to conventional plastics, which can limit their competitiveness in price-sensitive markets. These costs are linked to the complex and often costly fermentation processes required for their production, the sourcing of raw materials such as renewable biomass, and the relatively lower economies of scale in PHA manufacturing compared to well-established petroleum-based plastics (Yadav et al., 2021). However, the main bottlenecks for production, significantly affecting the overall production costs, are the retrieval of the PHAs from the bacterial biomass and the polymer purification process, as these involve complex, energy-intensive, and environmentally harmful procedures (Chee et al., 2019). Advances in these key areas could enhance the economic viability of PHAs as sustainable alternatives to conventional plastics. One possibility is that of recovering by-products of the PHA extraction process and turning them into high-value products

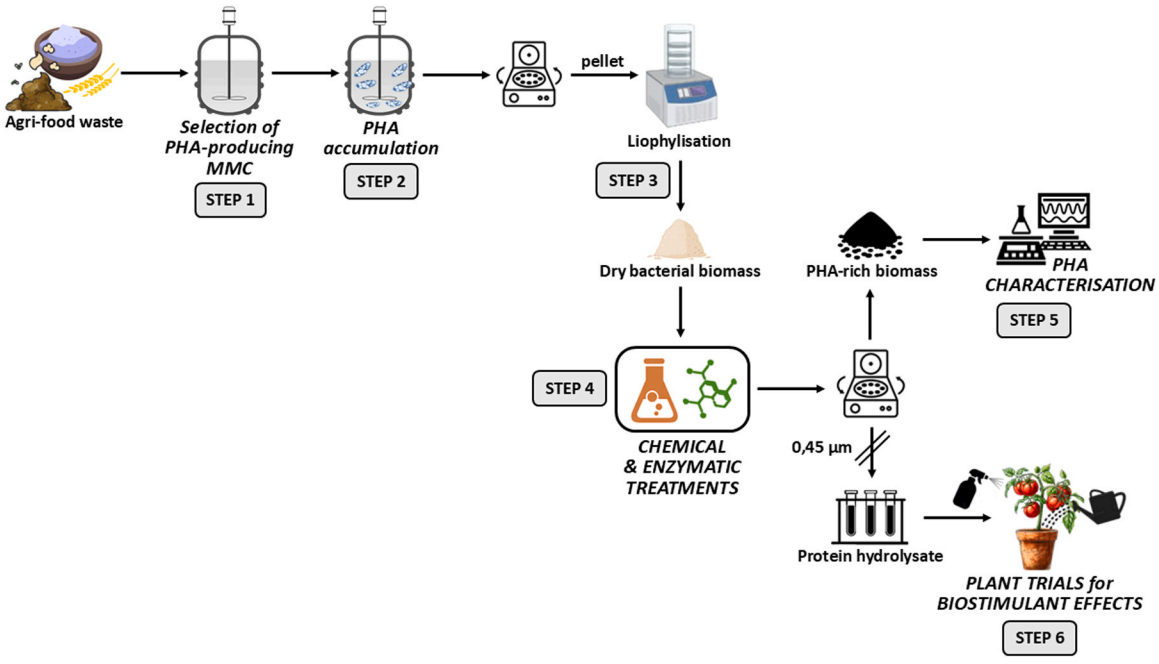


Fig. 1. Schematic representation of the experimental set-up. MMC = mixed microbial cultures.

(such as animal feed, biostimulants, protein hydrolysates, flocculants, adhesives and pharmaceutical), in order to reduce PHAs production costs and maximise profits (Pesante and Frison, 2023; Yadav et al., 2021).

The aim of this work was to offer an alternative and economically feasible pathway to PHA production, by converting agri-food waste into PHA-rich bacterial biomass, which was subjected to hydrolysis treatments capable of releasing the PHAs from the bacterial cells for their use in the bioplastic market; at the same time, the remaining protein-rich liquid hydrolysates were tested on tomato plants for their potential use as PBs. This work stems from the publication of Bastianelli et al. (2023), where chemical and thermal treatments were tested with the same purpose. However, their work involved the application of aggressive hydrolysis treatments with either 6 M hydrochloric acid or 4.2 M sodium hydroxide, and processing temperatures and times up to 110 °C and 22 h. These treatments can be harmful to the environment and expensive, making the process difficult to apply on an industrial scale. For this reason, in the work presented here, the hydrolysis treatments were designed to be more economically and environmentally friendly, reducing processing times and temperatures and using chemicals at lower concentrations. The selection of the processing conditions to be tested was made by consulting recent review works on the most effective methods for PHA recovery from microbial cultures (Kurian and Das, 2021; Pagliano et al., 2021). Enzymatic treatments were added to the experimentation on account of their low environmental impact and gentler cellular lysis, which could preserve PHA integrity and generate peptides with different characteristics compared to the chemical treatments. The efficacy as PBs of the obtained PHs was assessed *via* seed priming. The most efficient PHs were then selected for growth trials in greenhouse conditions with tomato plants, for which the biostimulant effect was tested after application by foliar spray or by drenching in soil.

2. Materials and methods

2.1. Experimental set-up

Fig. 1 illustrates the experimental set-up, whose details for each step are described in the following paragraphs. In summary, agricultural and food waste (hereafter named agri-food waste) was used as feedstock to produce mixed microbial culture biomass able to accumulate PHAs (step 1). A multi-spike feeding strategy was applied to enhance the production of PHAs (step 2). The retrieved biomass was dehydrated (step 3) and subject to chemical or enzymatic treatments (step 4) aimed at extracting PHAs for their possible use in the bioplastic industry, and at using the remaining PHs as PBs. The obtained PHAs were analysed to determine their chemical-physical characteristics (step 5), while the biostimulant properties of PHs were tested in preliminary plant trials on tomato plants (step 6).

2.2. PHA-rich biomass production

PHA-rich microbial biomass was produced at the agricultural cooperative “La Torre” located in Isola della Scala, Verona, Italy, in the frame of the Regione Veneto project EcoDPI (Ecodesign and recycling of personal protective equipment in a circular industrial supply chain) (Salvatori et al., 2025). The feedstock consisted of a carbon source made of pasta by-products (TS = 915.4 kg/tons, TVS = 744.4 kg/tons) recovered from a Barilla spa factory in Parma, Italy, and of digestate as inoculum and source of nutrients (TS = 121.2, TVS = 105.1 kg/m^3), which was obtained from the anaerobic digestion plant located inside the cooperative. These were mixed with water in the proportion of 2.3:5.0:1.0 (water:digestate:pasta by-products) in a 5 m^3 mixing tank equipped with an adjustable stirrer and an internal solution recirculation system. Fermentation of the mixed feedstock to obtain a VFA (volatile fatty acid)-rich fluid for bacterial growth was performed in a 5 m^3 stainless steel bioreactor in mesophilic conditions. The fermenter unit was stirred at 100 rpm and the temperature was kept at 37°C by a hot water double jacket. The fermenter was fed daily with 0.5–1 m^3 of the mixed feedstock, resulting in an organic loading rate (OLR) of 21–33 kgTVS/m^3*d and a hydraulic retention time (HRT) of 4–6 days. The fermented fluid was sieved at 1 mm porosity in a dedicated screw press and then the liquid fraction was ultrafiltered with a Juice Clarification Systems (JU.CLA.S, model MMSL LAB, Italy), equipped with a 0.2 μm ceramic membrane. The VFA-rich filtrated fermented liquid was stored in a 2 m^3 stirred carbon storage unit at the temperature of 25 °C.

The VFA yield, on a COD basis, was determined according to the formula:

$$\text{VFA yield} = \left(\frac{\text{VFA}}{\text{OLR x HRT}}\right)$$

where VFA is the volatile fatty acids concentration in gCOD/L in the fermenter effluent, OLR is the fermenter organic loading rate in gCOD fed per liter of reactor and per day, and HRT is the hydraulic retention time in days.

The selection of the PHA-storing bacteria was performed in 2 m^3 sequential batch reactor (SBR1) equipped with a blower for air diffusion and kept at the temperature of 25 °C, with a HRT of 2 days. A feast and famine (F/F) regime (Reis et al., 2011) was applied to SBR1 to favour the growth of PHA-accumulating bacteria, consisting of four feeding cycles of 12 h (F/F ratio= 0.1), and with a set OLR of 2.3 $gCOD_{VFA}$/(L*d). A 1.2 m^3 SBR kept at the same conditions as SBR1 was used for the PHA accumulation using a multi-spike strategy (Valentino et al., 2019). Each spike consisted of 0.5 $gCOD_{VFA}$/L, and its timing was decided based on dissolved oxygen (DO) values. At the end of the accumulation phase, the biomass was quenched with sulfuric acid (99 %) to reach the pH of 2.0 and then moved to a 1.5 m^3 stirred plastic recovery unit in order to concentrate the bacterial biomass by flocculation (adding the polyelectrolyte SCAEFLOC CL 1908, 0.5 g/L) and overnight decantation. The thickened slurry was then centrifuged and the resulting pellet was retrieved and lyophilised with a Lio 5Pfreeze-drier (5Pascal Srl, Italy).

The microbial culture yield (Y) was determined as the ratio between the produced biomass per batch in grams, and the fed COD along the same batch in gCOD according to the following formula:

$$Y_{batch} = \frac{gMLVSS}{gCOD_{fed}}$$

where gMLVSS is the biomass, expressed as mixed liquor volatile suspended solids in grams produced during the batch process, while $gCOD_{fed}$, is the COD fed into the system in the same time period.

2.3. Chemical and enzymatic treatments

The dehydrated bacterial biomass was analysed to determine total solids (TS), total volatile solids (TVS), total Kjeldahl nitrogen (TKN) and chemical oxygen demand (COD) (see methods in subsection 2.8), and it was then subjected to chemical and enzymatic treatments aimed at the extraction of PHAs and the production of PHs. Each treatment was performed in replicates in 1 L flasks containing 200 mL of solution with a biomass concentration of 4 % (w/v), using a shaking incubator (New Brunswick Innova® 42, Eppendorf) set at a shaking rate of 180 rpm.

The chemical treatment involved the use of either an acid (HCl 36 % w/w, Thermo Fisher Scientific, L13091) or a base (NaOH, Sigma-Aldrich, S5881), which were both tested at the concentrations of 0.1 and 0.5 M. For the acid treatment, the samples were heated at 80 °C for 6 h, while the basic treatment was performed at 30 °C for 3 h. A control sample containing Milli-Q water in place of HCl or NaOH was also processed alongside the real samples.

The enzymatic treatment was performed using either an alcalase from *Bacillus licheniformis* (Novozymes, EC number 3.4.21.62) or a papain from *Carica papaya* (PanReach Applichem, EC number 3.4.22.2). The alcalase enzyme was added at the concentration of 0.3 U for 1 g of biomass, while for the papain 1 g was added for 100 g of biomass. Both enzymes were tested with and without a pre-treatment phase aimed at obtaining a more effective hydrolysis; the pre-treatment consisted of autoclaving the biomass at 121 °C for 1 min. All the hydrolyses (with alcalase or papain, with or without the pre-treatment) were performed at 50 °C for 4 h with a pH of 7.5. The control sample was prepared without the addition of the enzymes.

At the end of both chemical and enzymatic treatments, the samples were centrifuged for 10 min at 20 °C and 3900 rpm. The obtained pellet was lyophilised as above and used to extract, purify and characterise the PHAs (subsection 2.4). The supernatants (hereafter named protein hydrolysates, PHs) were filtered at 0.45 µm, analysed to determine TKN, $N\text{-}NH_3$, N-org, amino acid profile, peptide molecular weights and heavy metals presence, and they were then tested as biostimulants (subsection 2.8).

Each sample was weighed before and after the chemical and enzymatic treatments to determine the degree of hydrolysis, which was calculated as

$$\text{Degree of hydrolysis} = \left(\frac{W0 - W1}{W0}\right) x100$$

where W_0 = weight of sample before the treatment and W_1 = weight after the treatment.

2.4. PHA extraction, purification and characterisation

The lyophilised sample obtained from subSection 2.3 was subject to extraction to determine the percentage of PHAs and the content of the hydroxyvalerate (HV) monomer in the polymer. In detail, 10 mg of finely ground sample were dissolved in 1 mL of chloroform, 2 mL of methanol containing 3 % (v/v) sulfuric acid and 1 mL of a 0.5 % (w/v) benzoic acid solution in chloroform. The sample was heated at 100 °C for 4 h and, after the addition of 1 mL of distilled water, it was mixed for 15 min and centrifuged for 2 min at 2100 rpm. The pellet was used for analysis by gas chromatography following Braunegg and colleagues' protocol (1978). The standards for the calibration curve were prepared with pure poly(3-hydroxybutyric acid-co-3-hydroxyvaleric acid) (Sigma, 403105) alongside the sample and treated following the same protocol. PHA contents in experimental samples were calculated as percentage (w/w) over the sample total volatile solids (TVS). The variation of the PHA content due to the chemical and enzymatic treatments was calculated as

$$\text{PHA variation} = \left(\frac{P0 - P1}{P0}\right) x100$$

where P_0 = weight of PHA in the sample before the treatment and P_1 = weight of PHA after the treatment.

In order to determine the PHA's average molecular weight (MW), number average molecular weight (Mn) and polydispersity index (PDI = MW/Mn), the extraction of the polymer from the bacterial cell was performed using chloroform ($CHCl_3$) and methanol (MeOH). In detail, the ground biomass from Section 2.3 was digested at 70 °C for 4 h with chloroform (50 mL/g biomass), cooled at room temperature, and filtrated with paper filters. PHA precipitation was obtained by adding methanol (5 mL/mL chloroform), waiting 60 min, filtering as previously and placing the recovered material on a glass petri dish for overnight evaporation of chloroform and methanol. The obtained solid polymer was analysed by gel permeation chromatography equipped with a pump (JASCO PU-4180), a guard column and two columns in series (TSKgel®G6000- HHR TSKGel GMHHR-H), a column oven (JASCO CO-4060) and a refractive index detector (JASCO RI-4030). Chloroform was used as an eluent at a 1 mL/min flow rate. The detector was set at 35 °C, whereas the columns were at 40 °C. Polystyrene standards (0.013–3.05 × 10 6 g/mol) were used to calibrate the system.

2.5. Amino acid profiling by ion chromatography (IC)

The PHs obtained from the chemical and enzymatic treatments were subjected to free amino acid analysis, which was performed by Chelab Srl laboratories - Merieux NutriSciences (Resana, Treviso, Italy, www.merieuxnutrisciences.com/it) following protocols that are not fully disclosable due to copyright. Briefly, the analysis was done in triplicate by ion chromatography with post-column derivatisation with ninhydrin. A total of 25 different amino acids were tested. Methionine was determined as methionine sulfone and then calculated as methionine. Tryptophan was determined according to the AOAC method 2017.03 (Draher and White, 2018). The results are reported as g in 100 g of sample (% w/w).

2.6. Peptide molecular weight analysis by fast liquid chromatography (FPLC)

The molecular weights of the peptides present in the PHs were determined by FPLC using a GE Healthcare AKTA pure chromatography system with a size-exclusion column (Superdex™ 30 Increase 10/300 GL) at room temperature. The supernatants were filtered at 0,45 μm and diluted 1:5 with degassed phosphate-buffered saline (20 mM Na_2HPO_4, 20 mM NaH_2PO_4 e 150 mM NaCl, pH = 7.4). The runs were performed by eluting 1 mL of PBS-diluted sample at a constant flux of 0.5 mL/min. A separate run was dedicated to the co-elution of six molecular weight standards: bovine serum albumin (MW = 66.46 kDa, concentration used 0.05 mg/mL), cytochrome C (MW = 12.40 kDa, 0.20 mg/mL), aprotinin (MW = 6.50 kDa, 0.20 mg/mL), vitamin B12 (MW = 1.35 kDa, 0.07 mg/mL), triglycine (MW = 189.00 Da, 0.20 mg/mL) and glycine (MW = 75.00 Da, 14.00 mg/mL. The absorbance for each sample was monitored at 214 nm (Ambrosini et al., 2022).

2.7. Elemental analysis by Inductively coupled plasma mass spectrometry (ICP-MS)

The presence of heavy metals was tested in the PHs by performing elemental analysis. 22.5 mL of the supernatants were mineralised by digestion with 2.5 mL of ultrapure 65 % HNO_3 at 100 °C for 90 min. The digested samples were filtered at 0.22 μm and diluted to a final HNO_3 concentration of 1 % (v/v) with milli-Q water. The element concentration of the diluted samples was determined by ICP-MS with the machine iCAP™ RQ ICP-MS (Thermo Scientific). Calibration curves were obtained by diluting a custom-made multielement standard (Romil LTD).

2.8. Analytical methods

COD, TS, TVS, TKN, and N-NH_3 were measured according to standard methods (APHA, 1998; IRSA-CNR, 2003), and N-org was obtained by subtracting the values of N-NH_3 from those of TKN. The efficiency of TKN extraction due to the chemical and enzymatic treatments compared to the pre-hydrolysis untreated biomass was calculated as:

$$\text{TKN extraction efficiency} = \quad (TKN1/TKN0)x100$$

where TKN_0 = TKN of the pre-hydrolysys untreated biomass sample and TKN_1 = TKN of sample after the treatment.

2.9. Seed priming tests with Solanum lycopersicum

Seed priming tests, consisting of germination after immersion in the PHs obtained from the chemical and enzymatic treatments, were performed using tomato seeds of the species *Solanum lycopersicum* var. Roma (Blumen Group SpA). The seeds were sterilised by immersion in a 5 % (v/v) NaClO solution for 10 min, then rinsed thrice with Milli-Q sterile water. The PHs were diluted in sterile Milli-Q water to reach a N-org concentration of 1 mg/L, and a control treatment was prepared with only sterile Milli-Q water. The sterilised tomato seeds were immersed in each priming solution and left for 24 h in the dark with gentle shaking, and they were then dried in a sterile environment at room temperature (Wang et al., 2022). For each treatment, four Petri dishes (9 cm diameter) were prepared, each containing 20 dry seeds placed on filter paper previously soaked in 2 mL of sterile Milli-Q water. Germination was obtained by placing the Petri dishes in the dark at 23 °C for 4 days, and root growth analysis was then performed with WinRHIZO™ scanner and automated software (Arsenault et al., 1995).

2.10. Plant trials with Solanum lycopersicum by foliar spray or drench

The possible biostimulant effect of the PHs was tested on tomato plants of the same species used for the seed priming tests (subsection 2.9). The PHs were applied both by foliar spray and by drench, in two different sets of experiments. The tests were run only for the four most effective PHs highlighted with the priming in terms of root growth and replicability of the results. The control was a solution of ammonium dihydrogen phosphate with the same N-org concentration as the tested PHs. The tomato seeds were soaked in water at 4 °C for three days and then sowed into peat (90 %) and perlite (10 %) in trays (4 ×5×5 cm cells) and placed in a greenhouse for 21 days, where artificial light was provided to obtain a 16/8 h light/dark photoperiod, the mean temperature was 23 °C during the day and 18 °C during the night, and relative humidity was 45 %. After 21 days, the seedlings were transferred to bigger trays (5 ×6×6 cm cells, each containing 50 g of soil). On day 27, the PHs or the control solution were applied either by spraying 20 mL on the leaves or by pouring 120 mL in each tray. The PH was diluted prior to application to mirror the composition of commercial

products used in agronomy (*i.e.* Isabion from Syngenta); in particular, the N-org concentration was set at 43.05 mg/L for the spray application, and 7.5 μg for each plant for the drench one. A second application of the PHs or control solution at the same dose was performed on day 33, and the plants were harvested on day 39 for the spray treatment and day 40 for the drench. Fresh plant weights (FW) were determined for roots and shoots after harvest, while dry weights (DW) were recorded after drying at 70 °C for four days using the formula:

$$\text{Weight increase} = \left(\frac{W - WC}{WC}\right)x100$$

where W = medium value of FW or DW of roots or shoots of plants treated with the PH and W_C = medium value for control plants.

Similarly, the root length increase due to the biostimulant effect was calculated as:

$$\text{Root length increase} = \left(\frac{RL - RLC}{RLC}\right)x100$$

where RL = medium value of root length of plants treated with the PH and RT_C = medium value of root length for control plants.

2.11. Statistical analysis

The statistical analysis of the results of the hydrolysis treatments and of the plant trials was performed using the software GraphPad Prism 5 using an ANOVA test followed by a Tukey post-hoc test. An un-paired *t*-test was applied to compare the TKN of PHs obtained from treatments with a solvent concentration of 0.1 M or 0.5 M, or of samples subjected to a pre-treatment *versus* those without a pre-treatment. The results of the analyses are presented in the figures as mean and standard error of the mean, with different letters used to indicate statistically significant data ($p < 0.05$).

3. Results and discussion

3.1. PHA-rich biomass production

The agri-food waste composed of pasta by-products and agricultural digestate was turned into a VFA-rich fluid by acidogenic fermentation. The agricultural digestate was able to buffer the pH drop during the fermentation phase and kept the pH around 6. As described in the materials and methods section, the 5 m^3 anaerobic fermenter operated in mesophilic conditions (around 37° C) with variable HRT, in the range of 4–6 days, and OLR in the range of 21–33 kgVS/m^3 per day. The obtained fermentate showed a TS content of 121.7 kg/m^3, of which TVS were 84.2 % (102.5 kg/m^3). The liquid fraction of fermentate, obtained after screw pressing, was filtered at 0.2 μm in a ceramic filter unit and used as a growing medium for the bacteria during the PHA-producing bacteria selection and PHA-accumulation phases. The characterisation of the filtered fermentation liquid showed a soluble COD of 73.30 kg/m^3, of which VFA constituted 54.0 % (39.57 kg/m^3). The composition of the different VFAs in the filtered fermentate is shown in Table 1: acetic acid was clearly the dominant compound, with concentrations as high as 17 gCOD/L, while propionic and butyric acids reached an average concentration around 10 gCOD/L. Lactic acid showed an average concentration around 15.34 kg/m^3.

The anaerobic fermenter showed an acidification capacity of 0.3 kg COD_{VFA} per kg COD_{fed}, a value which is in line with typical results obtained in similar studies (Salvatori et al., 2025). With specific reference to nutrient distribution in the filtered fermentate, TKN was 4.25 kg/m^3, of which N-NH_3 represented 59.5 % (2.53 kg/m^3), therefore N-org (obtained by subtracting NH_3 from TKN) was 40.5 % (1.72 kg/m^3) while TP was 0.768 kg/m^3.

As detailed in the materials and methods section, the liquid fraction of the fermentate, rich in short-chain VFAs, was stored in the carbon storage unit and then dosed to the SBR1. The growth of the PHA-accumulating bacteria was stimulated by the application of the F/F regime (Reis et al., 2011) of four feeding cycles of 12 h. The resulting mixed liquor yield for the SBR1 was around 0,4 gVS per gCOD fed to the SBR1, with a final biomass concentration of 2 g/L.

The selected PHA-accumulating bacterial biomass was then transferred to the SBR2, kept at the same conditions as SBR1, and used for the PHA accumulation by means of a multi-spike strategy (Valentino et al., 2019). The feeding spikes, whose duration was decided based on the DO profile, had a concentration of 0.5 $gCOD_{VFA}$/L. The biomass obtained at the end of the accumulation process had an average yield of around 10 kg dry matter per batch cycle. This biomass was quenched to avoid consumption of the stored PHAs and then moved to the recovery unit, where it was recovered and concentrated by flocculation and overnight decantation, and it was then

Table 1
VFA composition of the fermentation fluid obtained from the acidogenic fermentation of the agri-food waste.

Name of VFA	Unit	Value	%
Acetic acid	kg COD_{VFA}/m^3	17.20	43.5
Propionic acid	kg COD_{VFA}/m^3	10.03	25.3
Butyric acid	kg COD_{VFA}/m^3	10.44	26.4
Valeric acid	kg COD_{VFA}/m^3	1.90	4.8
Total VFAs	kg COD_{VFA}/m^3	39.57	100.0

centrifuged and lyophilised before storage.

The dry PHA-rich biomass was subject to characterisation. The final TS content was high due to the biomass being dry (948 g/kg), which was mostly (96.7 %) made up of TVS (917 g/kg). The TKN amount recorded was 63 g/kg over a tCOD of 669 g/kg, representing around 6 % of the sample; this value is in line with those recorded for activated sludge, which normally has a mean TKN content of 5 %, ranging from 2 % to 6 % (Rigby et al., 2016). The amount of PHA in the biomass constituted 37.9 % (w/w) of the TS, which is a satisfactory result considering the use of MMC. Indeed, PHA contents up to 90 % have been recorded only for pure cultures (Sabapathy et al., 2020), while most MMC studies report PHA contents from 15 % to 54 % (Pagliano et al., 2021). The HV monomer content in the PHA polymer (15.4 % w/w) is a consequence of the presence of propionic and valeric acid in the fermentation liquid, since the carbon source type has a great influence on the monomeric composition, with odd-carbon VFAs such as propionic or valeric acid determining higher HV contents (Bengtsson et al., 2008; Duque et al., 2014; Wei et al., 2014).

The final PHA yield of the process was however quite low, with a typical production of less than 100 g PHA per kg of COD of treated agri-food waste. This suggests the necessity to further valorise the residual streams after PHA extraction and purification.

3.2. Chemical and enzymatic treatments

The dry PHA-rich biomass was subject to different chemical and enzymatic treatments, performed with a concentration of hydrochloric acid or sodium hydroxide as low as possible (0.5 and 0.1 M), still allowing effective hydrolysis. Enzymatic treatments were also tested, using proteolytic enzymes among the most efficient in the release of PHAs from the bacterial cells (Pagliano et al., 2021), or in the degradation of plant (Shouket et al., 2020) and bacterial biomass (Kapritchkoff et al., 2006; Kathiraser et al., 2007; Pagliano et al., 2021). The enzymatic treatments were also tested after a heat pre-treatment in autoclave (120 °C for 1 min) to evaluate a possible increase in the hydrolysis efficiency.

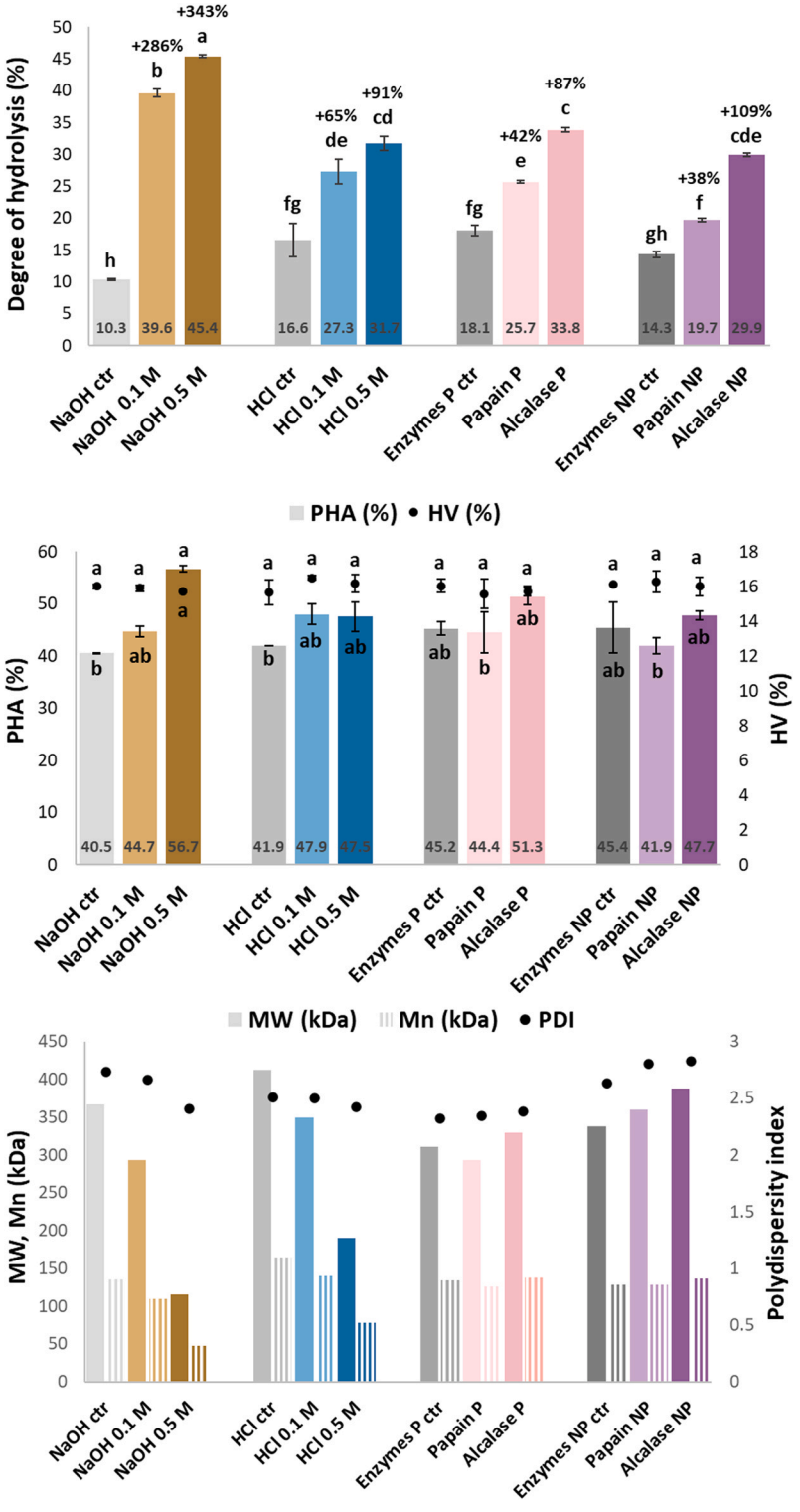
The top picture of Fig. 2 illustrates the degree of hydrolysis obtained after the different treatments. The most effective treatment involved the use of sodium hydroxide 0.5 M, which achieved a degree of hydrolysis of 45.5 %, with an increment of 343 % compared to the NaOH control. Sodium hydroxide at 0.1 M also resulted effective (39.5 % of hydrolysis, +286 % to the control), even if significantly less than the 0.5 M concentration. With the hydrochloric acid treatment, the 0.5 M concentration did not result statistically more efficient than the 0.1 M one (degree of hydrolysis of 27.3 and 31.7 % respectively), but they both showed a significant increment compared to the control (65 and 91 %, respectively), though the hydrolysis was lower compared to when NaOH was used. The enzymatic treatment with the alcalase allowed to obtain similar results to those recorded with HCl 0.5 M, both with (33.8 % of hydrolysis) and without pretreatment (29.9 %). The use of papain resulted the least effective hydrolysis (25.7 and 19.7 % with and without pretreatment), despite being similar to HCl 0.1 when a pre-treatment was applied. Both enzymatic treatments resulted significantly more efficient when the pre-treatment step was included (statistic not shown in Fig. 2), which was to be expected. However, the effectiveness of the enzymatic treatment with alcalase, even in the absence of a pre-treatment (similar to HCl 0.5 M), is a very important result, in view of the development of environmentally friendly processes. It is also worth noting that even the control samples showed evidence of hydrolysis (from 10.3 % to 18.1 %), indicating that factors such as the simple use of a water solvent, a temperature ranging from 30 to 80 °C and shaking at 180 rpm contributed to the disruption of the bacterial biomass.

The amount of PHA found in the pellet after the treatments was significantly higher than the one recovered from the control sample only when sodium hydroxide 0.5 M was used (Fig. 2, middle picture). In all the other treatments, no differences were found with the control, nor between treatments. The amount of HV in the polymer also remained unchanged before and after the treatments and among the treatments. These data are different from those found by Bastianelli and colleagues (2023), where an evident PHA increase was noticed after the treatments. However, their experiment didn't include a specific control for each treatment, while the results were compared to the biomass prior to hydrolysis, which had a lower PHA content compared to the pre-hydrolysis sample used in our work (27.7 % versus 37.9 %). Other works where NaOH or HCl was used at relatively mild conditions (0.1–0.2 M at 30 °C), similarly to the present work, showed recovery rates up to 90 % for NaOH (Anis et al., 2013; Choi and Lee, 1999; Rodrigues et al., 2022) and 92 % for HCl (Choi and Lee, 1999) with negligible degradation of the PHA, highlighting the efficacy of the methods. The lack of variation in PHA and HV amount in the present work is encouraging in view of the concomitant retrieval of PHAs and biostimulants from the bacterial biomass for any industrial application.

When investigating changes in MW, Mn and PDI after the chemical and enzymatic treatments (Fig. 2, bottom picture), no replicas were possible to obtain due to the low amount of polymer retrieved, therefore no statistical analysis could be performed. However, it appears that MW, Mn and PDI decreased with both chemical treatments (NaOH and HCl), particularly at the concentration of 0.5 M; in contrast, the enzymatic treatment seemed to have no effect on the quality of the PHA, with a slight increase in MW observed for the experiments without pre-treatments. Previous works reported a decrease in MW and PDI when enzymes were used to recover PHAs from bacterial biomass, and the reason for this change was attributed to the presence of SDS or EDTA in the buffer used for the experiment, or of glycerol in the bacteria growth medium (Kachrimanidou et al., 2016; Kathiraser et al., 2007), which are chemicals that were not used in our experiments. Only Kapritchkoff et al. (2006) observed no MW degradation, and indeed the authors report no use of SDS or EDTA. It is worth noticing that the mentioned literature involves the application of enzymatic treatments to pure bacterial cultures, and that the present work is the first – to our knowledge – where enzymes are used for the disruption of bacterial cells of MMC.

3.3. Analysis of protein hydrolysates

The samples obtained after the hydrolysis treatments were centrifuged, and the supernatants (now called PHs) were analysed to



(caption on next page)

Fig. 2. Results of the hydrolysis treatments. Top picture: degree of hydrolysis; the percentages reported on top of the bars represent the increase of each treatment compared to the treatment's control (MilliQ water for NaOH and HCl, phosphate buffer for the enzymes). Middle picture: PHA concentrations in the pellet after the treatments, and HV concentration in the polymer. Bottom picture: average molecular weight (MW), number average molecular weight (Mn) and polydispersity index (PDI) of the PHA extracted from the pellet after the treatments. The data for the three pictures are presented as mean and standard error of the mean, with different letters used to indicate statistically significant data ($p < 0.05$) when comparing each sample against all the others. MW, Mn and PDI were obtained for one replica only, therefore no mean, standard error of the mean nor statistical analysis are reported. Ctr = control, P = with pre-treatment, NP = without pre-treatment.

assess their nitrogen and amino acids content and type, and were then employed as seed priming agents to determine their efficacy in enhancing tomato seedling root growth.

3.3.1. Nitrogen content

The analysis of the nitrogen content of the PHs obtained from the different hydrolysis treatments is shown in Fig. 3. TKN values (top picture) indicate that all the treatments were much more efficient than their controls in the extraction of nitrogen from the bacterial biomass. The most efficient hydrolysis was obtained with NaOH 0.5 M, which extracted 62.7 % of the nitrogen contained in the pre-hydrolysis untreated biomass. However, the alcalase with and without pre-treatment was also effective (58.2 and 51.1 %, respectively), showing results having no statistical difference from those of NaOH 0.5 M. The least efficient treatments were obtained with

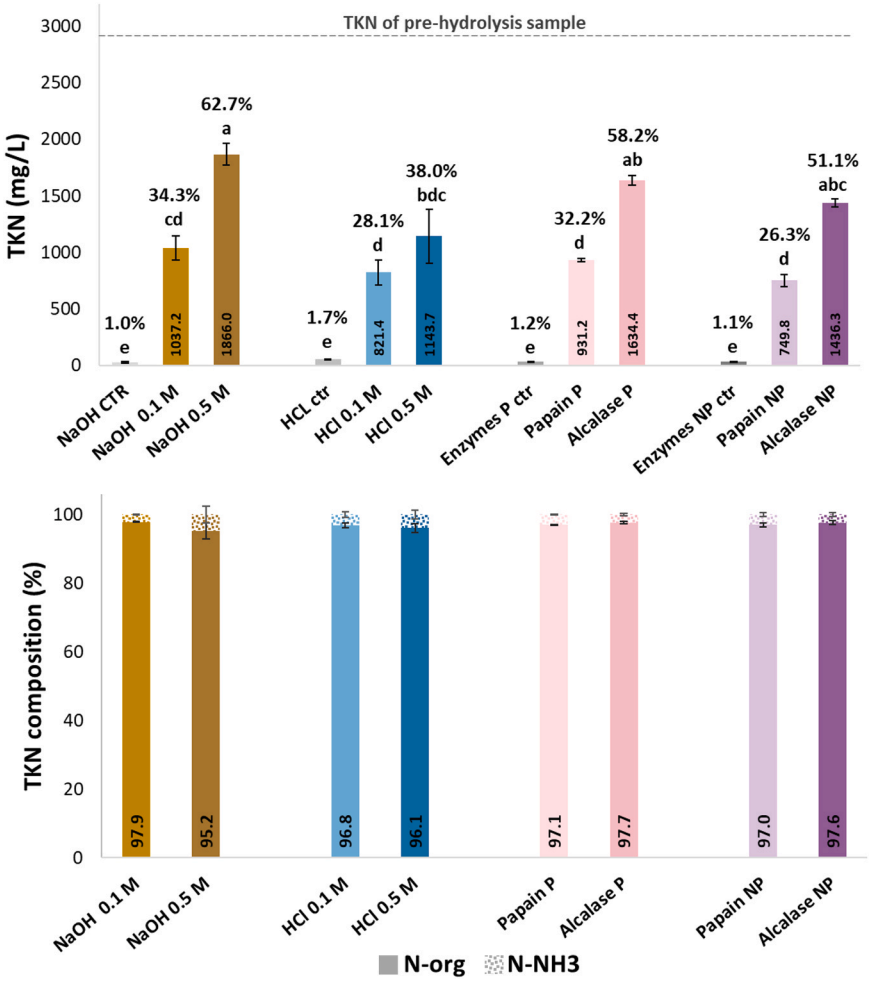


Fig. 3. TKN, N-org and N-NH_3 of the filtered PHs obtained from the different hydrolysis treatments. The top figure shows TKN values for PHs obtained from the different hydrolysis treatments. The percentages indicate the efficiency of TKN extraction compared to the pre-hydrolysis untreated biomass, which is indicated by the dashed horizontal line. The bottom figure shows N-org and NH_3 values, expressed as percentages over the total TKN of each sample. The data of both figures are presented as a mean and standard error of the mean, with different letters used to indicate statistically significant data ($p < 0.05$). obtained with an ANOVA analysis comparing each sample against all the others, controls included. The controls of the single treatments were milliQ water for HCl and NaOH, while phosphate buffer for the enzymatic treatments. P = with pre-treatment, NP = without pre-treatment.

papain (32.2 % and 26.3 % with and without pre-treatment, respectively) and with HCl 0.1 M (28.1 %). With NaOH, the use of the 0.5 M concentration allowed to retrieve more nitrogen compared to 0.1 M, while the difference is not significant for the two concentrations when using HCl. The addition of a pre-treatment to the enzymatic treatments did not cause any significant change for either of the enzymes.

The TKN was mainly made up of N-org (95.2–97.9 %, bottom picture of Fig. 3), with only a small contribution given by N-NH_3 (2.1–4.8 %), whose concentration was highest for NaOH 0.5 M, probably as a consequence of the stronger hydrolysis. The amount of N-org found in the PHs ranged from 749.8 mg/L for papain without pre-treatment to a maximum 1866.0 mg/L for NaOH 0.5 M. This suggests that, despite some treatments being more efficient than others, it is possible to extract a considerable amount of N-org from the bacterial biomass, even with enzymatic treatments. Considering that the PHs normally used as biostimulants in agriculture have a nitrogen content in the order of milligrams to grams per litre, the obtained products are aligned with commercial ones.

3.3.2. *Amino acid profiling*

The free amino acid content of the PHs obtained from the different hydrolysis treatments was investigated. Amino acids and peptides are important components of PHs, since their biostimulant activity has been demonstrated (Colla et al., 2017; Lindsey et al., 2002). Indeed, amino acids have shown a role in signalling, coordinating growth and stress response in plants (Häusler et al., 2014; Heinemann and Hildebrandt, 2021), and their presence in the soil has been linked to the shaping of the root system and the efficiency of nutrient uptake (Canarini et al., 2019). Peptides have an influence on the development of the root apparatus, and can positively contribute to overall root length more than the amino acids they are composed of (Ambrosini et al., 2022).

Fig. 4 shows how the amount of free amino acids released by the chemical hydrolyses is much lower compared to the hydrolysis performed by Bastianelli and colleagues (2023), which obtained 63 % of free amino acids over the TKN content for the 6.0 M HCl treatment and 99.9 % for 4.2 M NaOH, while in this work HCl 0.1 M and 0.5 M obtained 1.7 and 20.6 %, respectively, and HCl 0.1 M and 0.5 M 4.1 and 4.6 %, respectively. The comparison with PHs of animal or vegetal origin obtained by enzymatic hydrolysis performed in a commercial establishment (Ertani et al., 2009) also highlights lower values in our experimentation. Clearly, the use of less concentrated chemicals did not allow a thorough hydrolysis of the bacterial biomass; however, the absence of amino acids means that the proteins of the PHs have not been fully degraded, and therefore peptides of different sizes might be present in the PHs. Since peptides and amino acids have been found to exhibit differences in the molecular responses that they induce in plants (Santi et al., 2017), the lower amount of amino acids in the PHs does not directly translate into a diminished biostimulant effect.

The largest content of amino acids was obtained with the NaOH 0.5 M treatment (38.5 mg/100 g of sample), as expected, given its more pronounced degree of hydrolysis; on the other hand, NaOH 0.1 M was not efficient (1.7 mg/100 g), and similar values were obtained for HCl in both concentrations (3.4 and 5.2 mg/100 g for 0.1 and 0.5 M, respectively). The enzymatic treatment with alcalase without pre-treatment was almost as efficient as NaOH 0.5 M (36.1 mg/100 g), showing a wider amino acid distribution, with 13 different types compared to the 7 types produced by NaOH. Papain was the treatment that liberated the smallest amount of amino

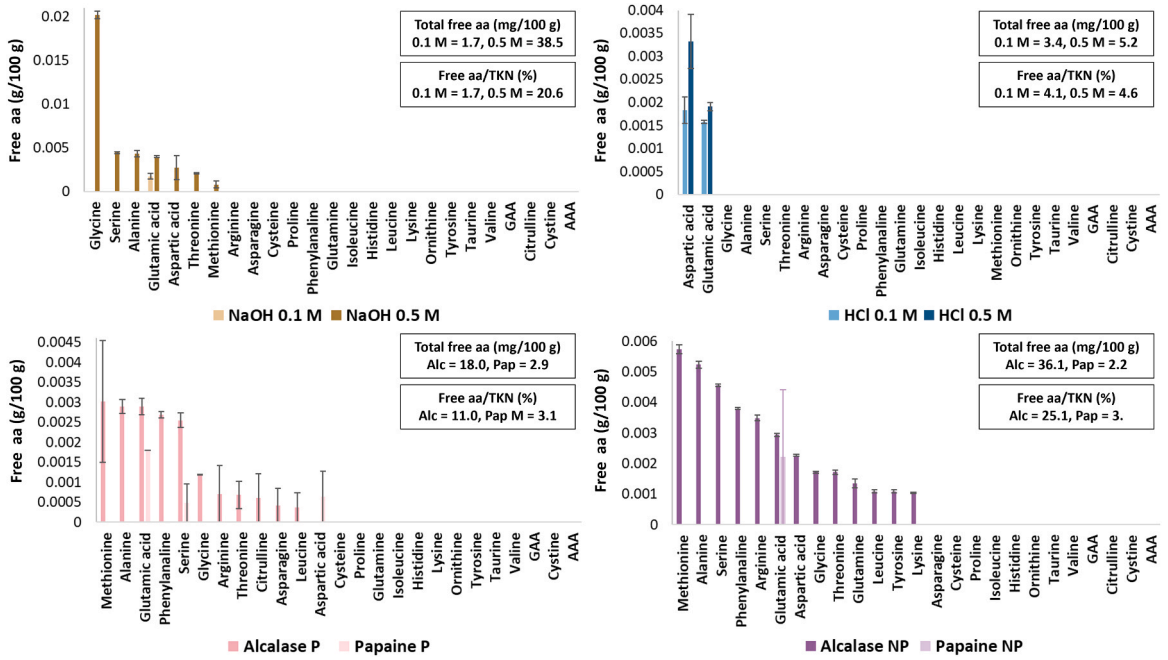


Fig. 4. Amino acid profile of the PHs obtained from the different hydrolysis treatments. The results are given as g over 100 g of the original sample, and presented as mean and error of the mean of three different replicas. Aa = amino acid, AAA = alpha-aminobutyric acid, GAA = gamma-aminobutyric acid, P = with pretreatment, NP = no pretreatment.

acids, with or without pre-treatment (2.9 and 2.2 mg/100 g, respectively), with only serine, glutamic and aspartic acid recorded in the PH, confirming papain as the least efficient hydrolysis treatment. The amino acid profile shows different types of amino acids released by the chemical treatment compared to the enzymatic ones, with mostly aspartic and glutamic acid found in HCl and the addition of glycine in NaOH, while methionine, alanine, serine, phenylalanine, glycine, arginine, glycine and threonine released with the alcalase.

3.3.3. Seed priming tests

The biostimulant effect of the obtained PHs was assayed with a preliminary priming growth test on tomato seedlings. Seed priming is a method of controlled hydration and drying to increase pre-germinative metabolic processes to hasten uniform germination and enhance seedling growth (Paparella et al., 2015). The hydrolysed biomasses showed various degrees of efficacy in promoting root growth (Fig. 5). Two of them, the ones obtained from the treatment with NaOH 0.1 M and HCl 0.5 M, slightly promoted root growth (+10 % and +9 % respectively, compared to control treatments); however, these differences were not significant. The application of all the other PHs as priming agents resulted in significantly longer seedlings' roots. The PHs obtained from the other two chemical hydrolyses, NaOH 0.5 M and HCl 0.1 M, stimulated an increase of 18 % and 30 % respectively. The two enzymatic treatments with pre-treated biomass showed beneficial effects of a similar magnitude, of + 29 % (papain) and + 32 % (alcalase), regardless of the enzyme employed for hydrolysis. Parallelly, the type of enzyme did not affect the efficacy of the products obtained without the pre-treatment as well, which appeared to be the best performing, producing an increase of 37 % (papain) and 38 % (alcalase). Four PHs, one for each treatment, were selected for a thorough characterization, based on the efficiency in promoting root growth and on the reproducibility of the obtained results. From here on, we will refer to these selected PHs, obtained from the hydrolysis with NaOH 0.5 M, HCl 0.1 M, alcalase and papain without pre-treatment, as HCl-PH, NaOH-PH, Alc-PH, and Pap-PH, respectively.

3.4. Analysis of the selected protein hydrolysates

The supernatants of the four selected PHs (HCl-PH, NaOH-PH, Alc-PH and Pap-PH) were analysed prior to the plant trials in order to determine the molecular weight of the peptides found in solution, and the presence of macro- and micro-nutrients and metals.

3.4.1. Analysis of peptides' molecular weight

The four selected PHs were characterized in their peptide molecular mass by a size-exclusion Fast Protein Liquid Chromatography (FPLC). The elution profiles (Fig. 6) showed differences in the degree of hydrolysis and thus specific abundances and ratios between larger and smaller peptides. For a more informative analysis, a mixture of standard proteins, small peptides and amino acids was also eluted, and the relative chromatogram is provided in Fig. 6 alongside the other profiles. Roughly, proteins as big as bovine serum albumin (66,400 Da) eluted with the void volume at 8.5 mL, while the elution range between 9.5 mL to 11.5 mL included proteins spanning from 12,400 to 6500 Da. Peptides composed of less than 15 amino acids were found after 17 mL of elution volume (EV), while tripeptides and free amino acids after 18 mL of EV. The intervals that will be considered for the presentation of the results are the following: EV 7.0–11.5 mL "High MW", EV 11.5–17.0 mL "Medium MW", EV 17.0–18.0 mL "Low MW", EV 18.0–25 mL "Amino acids".

The chromatographic profile of the chemically hydrolysed biomasses (Fig. 6) showed three distinct peaks: the first one around 8.5 mL of EV, followed by a second one at 18.5 mL EV, and a third one at 22 mL. For both profiles, between the first two peaks there is a valley. Table S1 shows that the most abundant fractions in the NaOH-PH are the amino acid one (42 %), followed by the Medium MW one (31 %); the High MW fraction is also well represented (20 %) while the Low MW peptides are much less abundant (7 %). When compared to the profiles of the other PHs, the NaOH hydrolysis clearly produces the largest amount of very small peptides and amino

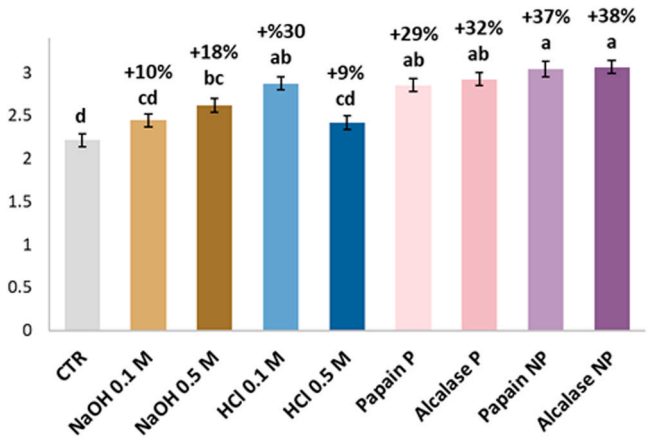


Fig. 5. Effects of seed priming on *S. lycopersicum* seedling growth using the PHs obtained from the different hydrolysis treatments. Percentages indicate an increase compared to the control treatment (sterile Milli-Q water). The graph merges observations from three independent replicates (n = 203). The data are presented as a mean and standard error of the mean, with different letters used to indicate statistically significant data ($p < 0.05$). Ctr = control, P = with pre-treatment, NP = without pre-treatment.

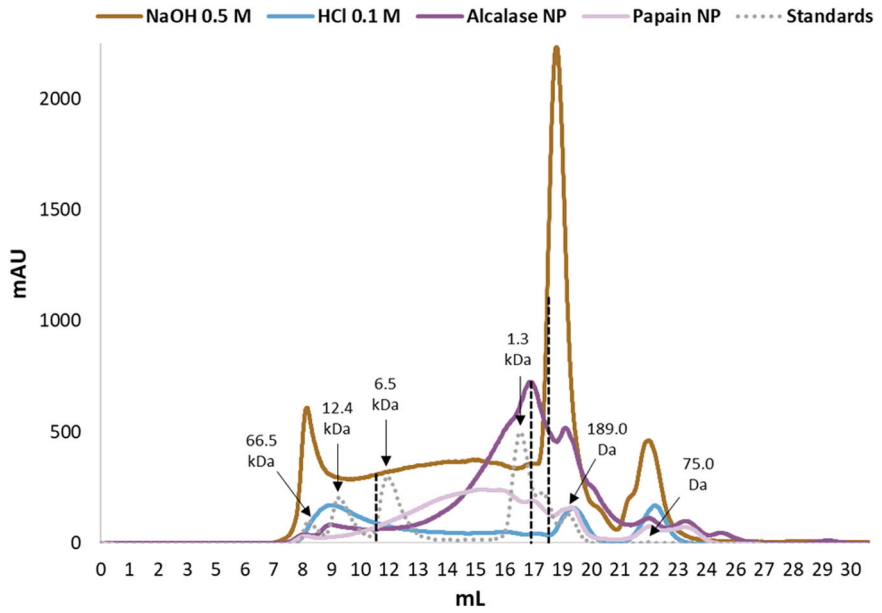


Fig. 6. Chromatogram of the four selected protein hydrolysates. The profiles were obtained with size exclusion chromatography using absorbance at 214 nm. The standards are shown with the grey dashed line and their relative molecular weights are indicated with the arrows. The black dashed lines show the areas of the four intervals considered in the analysis (high MW, medium MW, low MW and amino acids). NP = No pretreatment.

acids. HCl-PH, like NaOH-PH, also presents a small amount of Low MW peptides (5 %) and a similar percentage of Medium MW ones (26 %) and amino acids (35 %). On the other hand, the heaviest fraction is relatively much more abundant for the HCl-PH (35 %). Among the hydrolysis agents, HCl seemed to be the least effective treatment in hydrolysing whole proteins.

The two enzymatic hydrolysis FPLC spectra showed fragmentation profiles that differ from each other. The Pap-PH spectrum had a maximum between 15 and 16 mL of EV, and 4 other local maxima around 17.5, 19, 22 and 23.5 mL. The Alc-PH showed the highest peak at 17 mL, and other three local maxima at the same EV of the Pap-PH, at 19, 22 and 23.5 mL. For both profiles, between the void volume and the maximum there is an increasing slope, much steeper in the case of Alc-PH. Contrary to the chemical treatments, the enzymatic hydrolyses are the most effective in breaking down big proteins, as the High MW fractions consist of only 6 % of the Alc-PH profile, and 9 % of the Pap-PH. While the Alc-PH has a more uniform distribution of peptide sizes among its profile (Medium MW 38 %; Low MW 19 %; Amino acids 37 %) the Pap-PH is overabundant in Medium MW peptides (57 %) compared to all other PHs. Pap-PH Low MW fraction has an intermediate value (9 %) of relative abundance between Alc-PH and the chemical hydrolysis, and, finally, has the lowest one for the aminoacidic fraction (25 %) among all other PHs.

These chromatographic profiles provide a preliminary estimate of the molecular weight and size of the peptides present in the PHs. Currently, there are no studies in the literature explaining the relationship between the size of individual peptides and their activity as biostimulants.

3.4.2. Elemental analysis

The analysis of the content of macro- and micro-nutrients and metals was performed on the selected PHs to evaluate any possible effect on the growth of the tomato plants. The elemental analysis is reported in Table S2 and underlies how the supply of nutrients and metals by the PHs during the plant tests can be neglected, considering the dose of application. A few differences between the PHs were highlighted, such as a higher amount of boron found in the NaOH-PH (around 8 times the amount in the other PHs) and of manganese and zinc in the HCl-PH. The enzymatic PHs were much richer in phosphorus than the chemical ones (probably due to the use of phosphate buffer), while they contained no lead. These differences were likely to be insignificant in the tests with the tomato plants, given the low doses employed for each PH. No heavy metal potentially harmful for plants, such as arsenic and cadmium (Goyal et al., 2020), was detected in the PHs. These results are similar to those reported by Bastianelli and colleagues (2023); however, the concentrations found in our study are smaller compared to their results, with the exceptions of phosphorus and lead, which they found in lower amounts.

3.5. Biostimulant effect assessment

To assess the efficacy as biostimulants of the PHs with other methods of application, tomato plants were grown in a greenhouse and the products were applied by foliar spray or directly drenched in soil.

3.5.1. Foliar spray results

All the foliar spray treatments promoted both root and shoot growth. The root apparatus was the more positively affected, and the application of all the PHs resulted in significant differences in comparison with the control plants; growth of the aerial part was also stimulated in all cases except for the shoot FW of plants treated with the alcalase PH (Fig. 7). The increase in FWs and DWs of the plants treated with the PHs was quite homogeneous and statistically comparable. Root FWs were greater than those of control plants by 16 % (in the case of HCl-PH), 21 % (Alc-PH), 22 % (NaOH-PH) and 29 % (Pap-PH) (Fig. 7 A). In the same ascending order for root DWs an increase of 14 % was observed for the HCl-PH treatment, followed by Alc-PH, and NaOH-PH, both causing an increase of 23 %, and finally Pap-PH with a + 29 % (Fig. 7 B). As mentioned above, the shoot FW of plants treated with Alc-PH is the only case in which a foliar spray treatment did not cause a significant amelioration compared to control plants, causing an increase of only 7 %; the other products effectively stimulated shoot growth by 9 % (NaOH-PH) and 11 % (HCl-PH, Pap-PH) (Fig. 7 C). Lastly, shoot DW values of

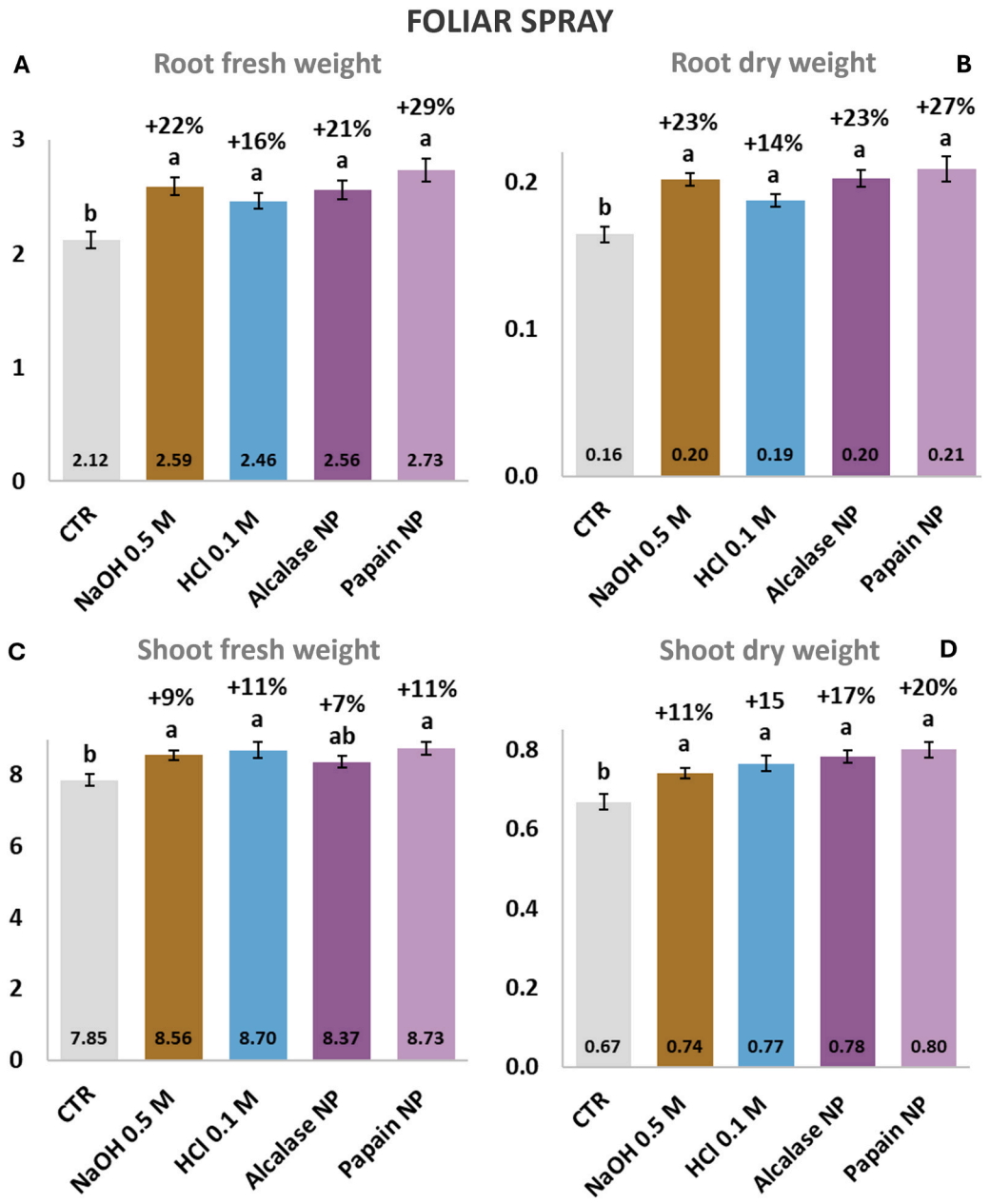


Fig. 7. Effects of the four chosen PHs applied by foliar spray on *S. lycopersicum* in a greenhouse. Percentages indicate an increase compared to the control treatment (H_2O). The graph merges observations from three independent replicates (n = 32). The data are presented as a mean and standard error of the mean, with different letters used to indicate statistically significant data ($p < 0.05$). Ctr = control, P = with pre-treatment, NP = without pre-treatment.

treated plants were also higher than those of the controls of 11 % (NaOH-PH), 15 % (HCl-PH), 17 % (Alc-PH) and 20 % (Pap-PH) (Fig. 7 D).

3.5.2. Drench application results

PH application by drench was less effective than foliar spray: while roots of treated plants were stimulated in their growth, although at different degrees among PHs (Fig. 8 A, B), the aerial parts grew similarly to those of control plants (Fig. 8 C, D). Root FW in treated plants showed a positive trend, however, only the Pap-PH was able to provoke a significant promotion of 16 % (Fig. 8 A). Considering the root DWs, the HCl-PH promoted an increase of 10 %, not enough to be significantly different than control plants, while the other PHs all showed a biostimulatory action, leading to increases of 11 % (NaOH-PH), 20 % (Alc-PH), and 23 % (Pap-PH)(Fig. 8 B). As far as the aerial parts are concerned, none of the PHs applied by drenching were able to ameliorate plant FW nor DW values (Fig. 8 C, D).

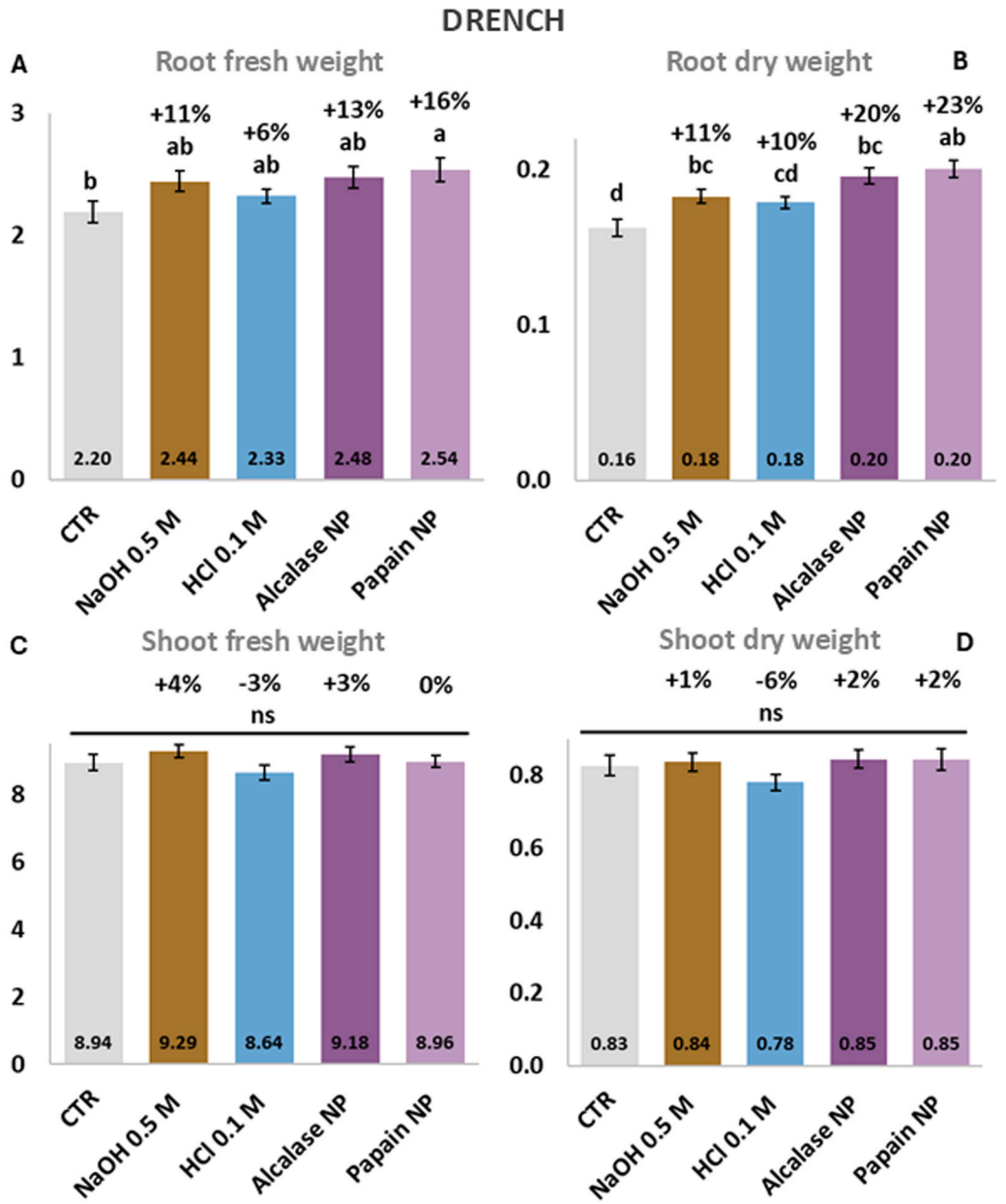


Fig. 8. Effects of the four chosen PHs applied by drench on *S. lycopersicum* in a greenhouse. Percentages indicate an increase compared to the control treatment (H_2O). The graph merges observations from three independent replicates (n = 32). The data are presented as a mean and standard error of the mean, with different letters used to indicate statistically significant data ($p < 0.05$). Ctr = control, P = with pre-treatment, NP = without pre-treatment.

Notably, none of the PHs caused toxic effects in the cultivated plants, no matter which chemistry they were obtained with.

4. Conclusions

Despite the successful production of PHA, the overall yield remained relatively low, with less than 100 g of PHA recovered per kg of COD from processed agri-food waste. This limitation underscores the need to further valorise the residual process streams - particularly through their conversion into high-value products such as biostimulants - in order to improve the economic sustainability of the bioprocess. Biostimulants have been shown to be effective in inducing physiological processes that improve plant growth and their ability to cope with stress, resulting in an increase in crop yield (Martínez-Lorente et al., 2024), and for this reason their use is widely gaining ground in agronomical practices. Bacteria-derived biostimulants are a more recent development (Bastianelli et al., 2023), which allows the recovery and exploitation of underutilised agri-food residues through acidogenic fermentation, therefore reducing waste and generating profits. The work presented here has shown that bacteria-derived biostimulants can be produced with relatively mild chemical treatments, or with eco-friendly enzymatic processes that have a lower impact on the environment. Furthermore, the concomitant extraction of PHA from the bacterial biomass used to produce biostimulants can help reduce production costs through the sale of the obtained bioplastic precursors.

Despite the encouraging preliminary results, further investigations are required to determine the economic impact and the life cycles of both PHAs and biostimulants production through the proposed routes. Indeed, the steps needed to obtain bacterial biomass from the agri-food residues involve additional costs and CO_2 generation compared to the direct hydrolysis of the vegetal biomass, which is the current way of producing waste-derived biostimulants. A detailed life cycle assessment and techno-economic analysis could highlight the involved environmental and economic impacts of the proposed technology and compare it to current practices, to evaluate which one is more sustainable, both environmentally and economically.

CRediT authorship contribution statement

David Bolzonella: Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Giovanna Pesante:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Stefano Ambrosini:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Anita Zamboni:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Mattia Pompermaier:** Writing – review & editing, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declarations

Prof. David Bolzonella is an editor of the journal but was not involved in the review and editorial handling process of this manuscript.

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.

Acknowledgments

We would like to thank Vadim Scerbacov (Innoven Srl, Italy) and the Project EcoDPI (Ecodesign and recycling of PPE in a circular industrial supply chain) for providing the PHA-rich bacterial biomass, Hélène Angellier Coussy (University of Montpellier, France) for the analysis of the molecular weight and Davide Slaghenaufi (University of Verona, Italy) for performing gas chromatography. We are grateful to the company Novozymes for kindly donating the alcalase. Many acknowledgments to Joe Bennett (Biorenewable Development Centre, University of York, UK) for providing the papain and useful advice on its use, as well as Leonardo Gomez (Centre for Novel Agricultural Products, University of York, UK) for his invaluable support, guidance and assistance during the initial phase of this project.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eti.2025.104502.

Data availability

Data will be made available on request.

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