Valorization of agricultural residues into microbial proteins and protein hydrolysates by PHA-producing bacteria

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Wastes are a burden for our society, generating costs and producing high levels of CO_2 for their disposal, and being responsible for health and environmental related problems when they are not managed properly (Abdel-Shafy and Mansour, 2018). Nowadays, 2.2 bt of municipal solid waste (MSW) are produced globally each year (Hoornweg and Bhada-Tata, 2012), and by 2050 this number is estimated to grow to 3.4 bt (Hoornweget *et al*, 2013). In view of adopting the circular economy model and of achieving the European Green Deal goal of net zero emission by 2050, these wastes should be turned into valuable resources and value-added compounds for the chemical, agricultural and food industries, rather than being discarded at a cost. Polyhydroxyalkanoates (PHAs), a form of bioplastics synthetized by microorganisms (Raza *et al*, 2018) are one of the valuable end-products that can be obtained, after acidogenic fermentation of the waste and growth of PHA-accumulating bacteria. PHAs are a biodegradable and bio-based form of plastics, however they are limited in their use in place of traditional fossil-based plastics mainly because of the costs for their production, which are mostly related to the extraction procedure from the bacterial cell in a pure form (Chee *et al*, 2019). To avoid extraction costs and therefore obtain economically competitive PHAs, there are two possibilities: either produce other economically valuable compounds from the co-products of PHAs extraction and purification procedures or use directly the cells containing the PHAs as the final product.

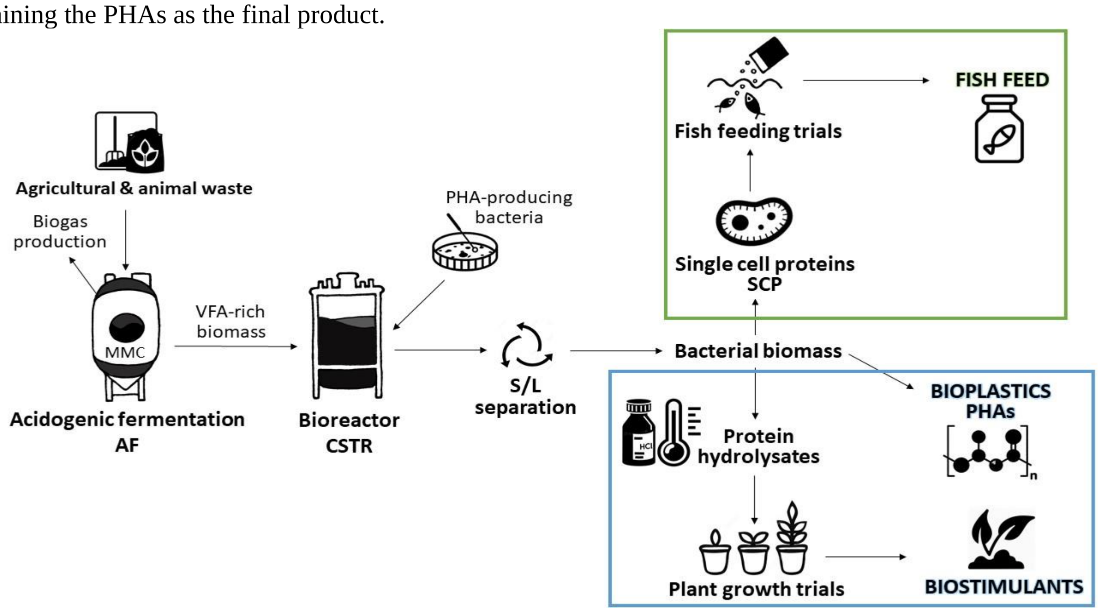

Figure 1. Schematic representation of the experimental set-up. S/L separation = solid-liquid separation.

The aim of this project was to explore both above strategies and obtain as end products fish feed, biostimulants and PHAs (Fig. 1). Agricultural and animal residues coming from the anaerobic digestion plant “La Torre” located in Isola della Scala, Verona, Italy, were used to feed PHA-accumulating bacteria in a continuous stirred tank reactor (CSTR). The bacterial species were *Thauera* sp. Sel9, a proteobacterium often found in wastewater treatment plants and *Rhodopseudomonas palustris*, a metabolically versatile purple non-sulfur bacterium (PNSB), or a mixed microbial consortium (MMC). At the end of the experimental period, the biomass from the CSTR was centrifuged and the solid phase (named single cell protein, SCP) was analysed for its centesimal composition and nutritional value; it was then used to perform feeding trials with Zebrafish larvae (*Danio rerio*) to assess its suitability as fish feed (Fig. 1, green box). As an alternative pathway (Fig. 1, blue box), the bacterial biomass was chemically or thermically hydrolysed in order to disrupt the proteins into free amino acids or small peptides (protein hydrolysates, PHs), while the PHAs were concentrated for their use as bioplastics. The PHs were further tested in growth trials with cucumber and tomato plants (*Cucumis sativus* and *Solanum lycopersicum*) to assess any effect on their growth and productivity.

The SCP to be used as fish feed, obtained from MMC, resulted high in its protein content (61.8% on dry biomass) and containing all the essential amino acids for fish; however, it was lacking in lipids, oils and carbohydrates, while PHAs represented 22.5%. The feeding trials showed that animals fed with a combination of commercial feed (CF) and SCP (50:50) performed slightly worse than the control group, while the addition of fish oil or PHAs to the mix improved fish survival (Fig. 2, left) and decreased phenotype alterations (Fig.2, right), resulting in performances even better than the control when oils and PHAs were provided together. This suggests that PHAs producing bacteria could be directly used as SCP without the need of expensive extraction and purification procedures. PHAs could also have further positive effects on the fish by protecting them from pathogenic bacteria (Laranja *et al*, 2017; Suguna *et al*, 2014). The use of PNBS is an added benefit because of their CO_2-capture ability (Alsiyabi et al., 2019).

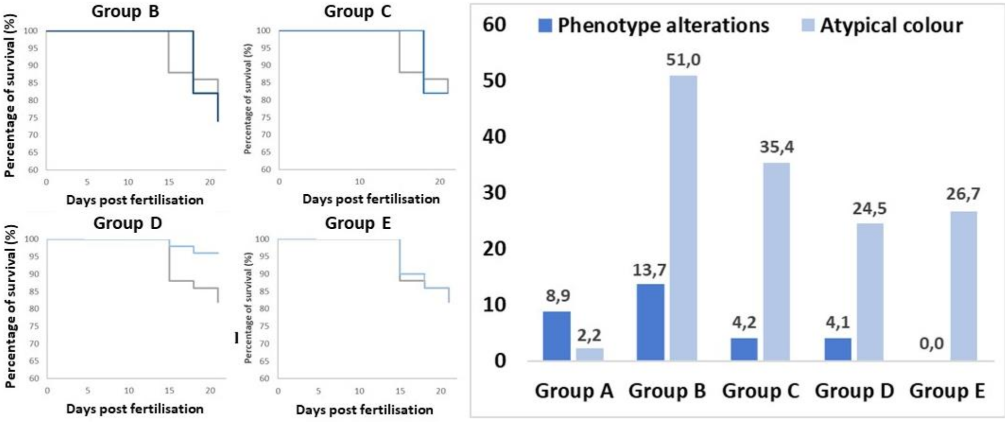

Figure 2. Survival rates (left image), phenotype alteration and atypical colour presence (right) of Zebrafish during the feeding trials. Group A = control, fish were fed with commercial feed only; group B = 50% CF and 50% SCP; group C = 50% CF, 50% SCP plus oils; group D = 50% C, 50% SCP plus oils and PHAs; group E = 50% C, 50% SCP plus PHAs.

Tomato plants grown in pots were treated with the PHs applied as foliar spray and all the three products (acid, base or thermal hydrolysis) showed a biostimulant effect. The results were significative when the root dry weight was assessed (Fig. 3, left), and the aerial growth also seemed improved, however the data could not be validated by the statistical analysis (Fig. 3, middle). When the PHs were applied by drenching at cucumber seedlings grown hydroponically, the treatments were effective in stimulating the aerial fresh weight (Fig. 3, right). PHAs were also recovered during the process, and their concentration was almost doubled when the acid or base hydrolysis was performed, while the basic treatment resulted too aggressive and reduced the PHA content.

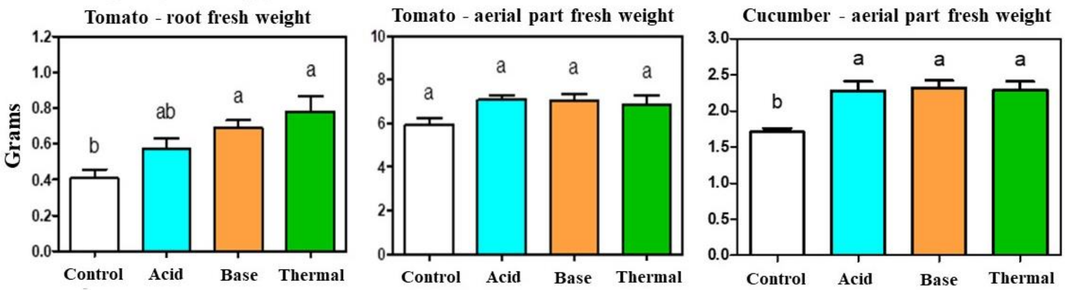

Figure 3. Fresh weight of roots (left) and aerial parts (middle) of tomato plants grown in pot after 12 days of treatment with leaf spray obtained from acid, base or thermal treatment 1mgN-org/L. Fresh weight of aerial parts of cucumber plants after drenching treatment. The statistically significant results ($p<0.05$) are marked by different letters.

These preliminary tests with fish and edible plants suggest that it is possible to biologically convert agricultural residues into products with high commercial value, such as fish feedstuff for pisciculture, biostimulants for agriculture and bioplastics. However, further research is needed in order to ascertain the best treatment for the simultaneous extractions of PHAs and PHs from the bacterial biomass, in other to obtain the highest amount of both end products. Besides, fish trails should be performed on a larger scale and with commercial fish species, and plant trials should be repeated and standardized to allow their comparison with the literature and confirm the results obtained to date.

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