Fractional valorization of purple phototrophic bacteria biomass grown in urban wastewater: comparison with waste activated sludge

Ángel Alejandro Filipigh[1,2], Bárbara Muñoz-Madariaga[1,2], and Silvia Bolado[1,2]

[1]Department of Chemical Engineering and Environmental Technology, School of Industrial Engineering, University of Valladolid, Dr. Mergelina s/n, 47011 Valladolid, Spain
[2]Institute of Sustainable Processes, University of Valladolid, Dr.Mergelina s/n 47011, Valladolid, Spain
Keywords: peptides, monosaccharide, wastewater, purple bacteria.
Presenting author email: angelalejandro.filipigh@uva.es

1. Introduction

Wastewater treatment using purple phototrophic bacteria (PPB) is a sustainable alternative to conventional activated sludge systems. PPB grow under anaerobic conditions using near-infrared light as an energy source, which reduces the energy demand linked to aeration. Due to their high metabolic flexibility, PPB can treat wastewaters (WW) with high organic loads and produce a biomass rich in proteins (≈40-50%) and carbohydrates (20-25%) [1]. Instead of direct use for animal feed as single-cell protein or fertilizer-which requires additional sanitation steps- fractional valorisation of PPB biomass offers higher economic and environmental benefits [2]. The protein fraction is of special interest, as its applications depend on peptide size, amino acid profile, and purity. Biofertilizers and biostimulants usually require low molecular weight peptides or free amino acids to enhance nutrient uptake and plant growth. In contrast, applications such as adhesives, surfactants, or foaming agents demand peptides with defined molecular size and higher purity, since their function depends on chain length and structure [3]. The carbon-rich fraction can be used to produce bioplastics or biofuels [4]. To the authors' knowledge, this is the first study to systematically assess the fractional valorisation of PPB biomass grown in real urban wastewater, using aerobic sludge as a reference. Although both biomasses share a waste origin and similar overall composition, their different cell wall structures may lead to distinct responses under thermal and chemical stress. Three hydrolysis treatments were tested by varying the reagent (NaOH or HCl) and its concentration (0.5 M or 1 M). The aim was to maximize the recovery of peptides and fermentable monosaccharides while limiting chemical degradation. Peptide quality and tecno-functional properties was evaluated. In addition, genomic analysis was performed to link treatment efficiency with microbial composition and cell structure, providing a solid basis for comparing PPB and waste activated sludge (WAS) valorization.

2. Material and Method

The PPB biomass was obtained from an anaerobic photobioreactor (PBR) inoculated with a mixed culture of PSB (purple sulfur bacteria) and PNSB (purple non-sulfur bacteria), and the WAS biomass was obtained from an aerobic digester, both treating municipal wastewater. Biomasses were centrifuged to obtain a total suspended solids (TSS_o) concentration of ≈8% with a volatile suspended solids (VSS_o) fraction of 82.5% and 75% for PPB and WAS, respectively. The PPB biomass composition (based on ash-free dry mass) consisted of 44.9% protein (PR) and 27.7% carbohydrates (CH), whereas the WAS biomass showed a similar protein content (44.4%) but a higher carbohydrate fraction (36.7%). Metagenomic analysis highlighted clear differences in microbial composition and cell structure. PPB biomass was mainly composed of $Gram^-$ bacteria (72%), including phylum as *Proteobacteria* (40.7%), *Bacteroidota* (11.2%), *Chloroflexi* (8.6%), and other minor groups (11.5%). In contrast, WAS was dominated by $Gram^+$ bacteria (75.7%), primarily *Firmicutes* (35.2%) and *Actinobacteriota* (40.5%).

Based on previous studies [3], [4], three chemical hydrolysis treatments were selected, all performed with NaOH (R1) or HCl (R2) at 0.5 M (C1) and 1 M (C2) using a 4% (w/w, dry basis) biomass suspension. Treatment A was performed at 50°C for 180 min on a rotary shaker at 150 rpm. For treatments B and C, the biomass was sterilized in an autoclave for 30 min and 60 min, respectively. After each treatment, the hydrolysate was centrifuged. The residual solids were weighted and analyzed to calculate the PR and CH solubilization. The PR and CH recovery as peptides and mono/oligosaccharides were calculated from the analysis of the liquid phase. Total protein in solid fraction was determined using the Total Kjeldahl Nitrogen (TKN) and applying the nitrogen to protein ratio (N:P) of 4.1 and 5.5 for PPB and WAS, respectively, calculated from the amino acid profile, determined by HPLC-UV. In the liquid fractions, monosaccharides and total amino acids recovered were determined by HPLC-IR and HPLC-UV, respectively. Additionally, free α-amino and peptide were quantified using the bicinchoninic acid (BCA) and ninhydrin method. Molecular weight of peptide recovery was determined by SDS-PAGE. Carbohydrates, in the residual solid, were determined using the modified NREL protocol of hydrolysis and HPLC-IR. A two-factor ANOVA was applied to study the effect of type treatment (3 categoric levels: A,B,C) and two continuous factors: type of reagent (2 level: HCl and NaOH) and reagent concentrations (2 level: 0.5 and 1mol/L). Protein solubility was determined by quantifying the protein concentration in the

supernatant after dispersion and centrifugation. EAI, ESI, foaming capacity, and foam stability were measured from the formation and stability of emulsions and foams, while WHC and OHC were determined gravimetrically after mixing with water or oil and subsequent centrifugation. FRAP was assessed spectrophotometrically through the reduction of Fe^{3+} to Fe^{2+}, and chelating capacity was determined from the decrease in absorbance of the Fe^{2+}–1,10-phenanthroline complex.

3. RESULTS AND DISCUSSION

Figure 1 shows protein solubilization (SY) and peptides recovery yields (RY); their difference reflects protein losses associated with treatment severity. Figure 2 shows carbohydrate solubilization and the recovery of fermentable monosaccharides such as glucose and xylose. ANOVA identified treatment type and chemical reagent (HCl/NaOH) as the most significant operational factors. In PPB, protein release ranged from 22 to 40.2% (mean 30.6%), with no significant differences between B and C ($p>0.05$), confirming its higher structural lability, likely related to the typical Gram$^-$ cell envelope architecture. In contrast, WAS showed a much wider range (3.7–35.1% ≈ 33.8%) and significant differences among conditions ($p<0.05$), reflecting its more recalcitrant nature and the need for higher severity to reach comparable yields. Protein losses were moderate (maximum 5.8% in PPB under B-R_2C_2 and 4.1% in WAS under C-R_1C_2), allowing average recoveries of 28.7% in PPB and 21.5% in WAS as peptides and amino acids. Alkaline conditions promoted polypeptide formation, especially in PPB (≈14.6%, from 3.1 to 30.7%) compared to WAS (≈9.8%, from 0.7 to 23.3%), with B-R_2C_2 showing the highest values. This fraction is particularly attractive for industrial applications requiring defined peptide chains, such as adhesives, surfactants, foaming agents, or functional materials. Peptide purity exceeded 50% under mild conditions (A≈B) and decreased to 40–45% as severity increased. In contrast, acidic treatments favored the formation of free amino acids (≈13.3% in PPB and 9.9% in WAS), which are suitable for agricultural formulations such as liquid biofertilizers or biostimulants, where rapid assimilation is essential.

The functional properties of the recovered protein fractions were strongly influenced by peptide molecular-weight distribution and amino acid composition, as confirmed by SDS-PAGE and amino acid profiling. Hydrolysates containing medium-sized peptides (≈55–130 kDa), particularly under B-R1, exhibited the highest emulsifying activity (up to 70.0 m²/g in PPB and 62.8 m²/g in WAS), emulsion stability (82.3 and 81.8 min), foaming capacity (137.1 and 119.8%), and foam stability (90% in both biomasses), indicating excellent interfacial functionality. In contrast, conditions that favored the release of free amino acids and readily soluble low-molecular-weight compounds, such as A-R2 and C-R1, showed higher protein solubility (up to 88.0% in PPB and 77.4% in WAS), enhanced ferric reducing antioxidant power (362–658 µmol TE/g), and strong Fe^{2+} chelating capacity (up to 85.2% and 72.2%, respectively), associated with the increased accessibility of aromatic and polar residues. In the case of A-R2, these properties are likely related to the selective extraction of loosely bound peptides and amino acids under mild alkaline conditions rather than to extensive protein fragmentation, whereas under C-R1 the combination of higher temperature and longer exposure promoted greater release of soluble bioactive compounds. Water- and oil-holding capacities remained within typical ranges for microbial protein hydrolysates (1.7–2.7 g water/g and 1.0–2.3 g oil/g), supporting their use as texture-modifying and moisture-retaining ingredients. Overall, PPB hydrolysates displayed the highest multifunctionality, although WAS achieved comparable values under more severe conditions, particularly under C-R1.

Fig 1. Protein solubilization (SY) and peptide recovery yields (RY)

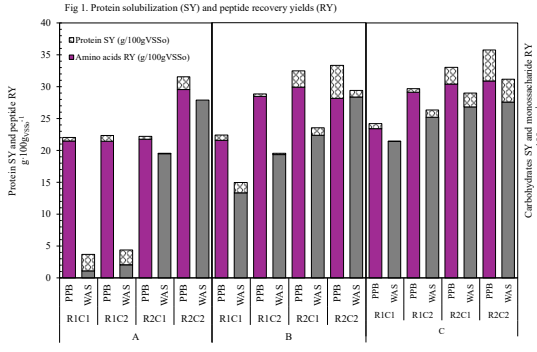
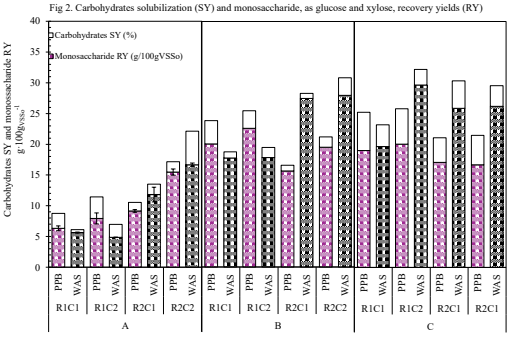


Fig 2. Carbohydrates solubilization (SY) and monosaccharide, as glucose and xylose, recovery yields (RY)



A similar pattern was observed for the carbohydrate fraction (Fig.2). In PPB, carbohydrate release ranged from 8.8 to 25.8% (mean 19%), with B under acidic conditions being particularly effective (25.5%), indicating higher accessibility of structural polysaccharides under moderate severity. In WAS, release reached up to 32.2% (mean 21.8%) but required more severe alkaline conditions, consistent with a more complex extracellular matrix and dense peptidoglycan structure. Carbohydrate losses (≈3.2% in PPB and 2.2% in WAS) were comparable to protein losses. Monosaccharide recovery (mainly glucose and xylose, in free and polymeric forms) averaged 15.8% in PPB and 19.6% in WAS. Free glucose represented ≈6.5% in both biomasses, whereas polymeric glucose

was higher in WAS (5% vs. 2.7%), and xylose showed balanced values. This fermentable sugar fraction opens opportunities for downstream fermentation processes aimed at producing bioethanol, organic acids (e.g., lactic or succinic acid), biopolymers such as PHA depending on the selected microbial platform.

The results confirm that biomass type and chemical reagent determine the efficiency of protein and carbohydrate recovery. PPB exhibited greater structural susceptibility and achieved good yields under moderate conditions, whereas WAS required more severe treatments due to its higher recalcitrance. Alkaline conditions were more suitable for maximizing the production of industrially relevant polypeptides, while acidic treatments favored the generation of free amino acids. Regarding the carbohydrate fraction, the recovery of glucose and xylose highlights its potential as a fermentable stream. Moderate severity was sufficient for PPB, whereas WAS required harsher conditions to enhance sugar release. The recovered monosaccharides can be valorized for bioethanol and organic acid production or used as carbon sources for biopolymer synthesis such as PHA, supporting an integrated biorefinery approach.

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Acknowledgments: This work was supported by Grant PID2023-153356OB-I00, funded by MICIU/AEI/ 10.13039/501100011033 and by ERDF/EU and by Consejería de Educación of the regional government of Castilla y Leon and ERDF/EU (UIC 338 and CLU-2025-2-06). Angel Alejandro Filipigh would like to thank grant PRE021-100176 funded by MICIU/AEI/10.13039/501100011033. Bárbara Muñoz Madariaga would like to thank the University of Valladolid for her PhD scholarships.