066. CHEESE WHEY VALORIZATION FOR ALTERNATIVE PROTEIN PRODUCTION: TOWARDS A COST-EFFECTIVE AND SUSTAINABLE VALUE CHAIN BASED ON COMBINED ACIDOGENIC FERMENTATION AND PURPLE PHOTOTROPHIC BACTERIA

A. Amini[1*], A. Remelli[1], V. A. Mojarad[1], E. Ficara[1], A. Turolla[1]

1. Department of Civil and Environmental Engineering, Politecnico di Milano, Milan, Italy

* Corresponding author: ali.amini@polimi.it

Keywords: Agri-food biowaste · Resource recovery · Microbial mixed cultures · Volatile fatty acids

Introduction

Purple phototrophic bacteria (PPB) are known for their metabolic versatility and thrive as anoxygenic photoheterotrophs. The application of PPB mixed cultures emerged as a promising sustainable biotechnological solution for resource recovery from wastewater, including several valorization routes of biowaste to valuable materials such as bioplastics, fertilizers, and proteins (Capson-Tojo et al., 2020). In particular, the recovery of single cell proteins (SCP) – particularly focusing on novel high-grade feed ingredients – has highlighted interesting perspectives in terms of nutritional properties and marketability (Alloul et al., 2021). In the view of improving overall process cost-effectiveness and sustainability, the valorization of cheese whey was identified as a valuable alternative, although pre-treatment for the transformation of complex organics into volatile fatty acids (VFA) was deemed necessary to favor carbon uptake in PPB biomass, thus enhancing the process feasibility in the view of its scale-up (Alloul et al., 2018). However, related efforts have been poor so far, indicating a strong need for the application of a robust process engineering approach. In particular, the identification of ideal operating conditions for yield optimization and process stabilization is the next significant step to take. The goal of this study is to assess the valorization of deproteinated cheese whey via a combined process based on acidogenic fermentation (AF) followed by PPB cultivation and harvesting aiming at the development of a cost-effective and sustainable solution for high-quality SCP production. Therefore, both AF and PPB cultivation processes were jointly investigated at the laboratory scale, the former in the view of targeting high production rates of the most desirable VFA mixtures for PPB growth and the latter with the scope of characterizing PPB growth parameters depending on feedstock composition. Estimated parameter values were then used as inputs for a multi-physical modeling framework describing radiation transfer and biokinetics, capable of identifying the optimal conditions for maximizing PPB productivity in a continuous flow photobioreactor (PBR).

Materials and Methods

A 1-L anaerobic sequencing batch reactor (AnSBR) was inoculated with mixed sludge and fed with diluted cheese whey at a concentration of 12 g COD·L-1, supplied by a Grana Padano cheese factory in northern Italy and resulting in 6 g COD·L-1 organic loading rate. The AnSBR was operated in sequential cycles of 12 h, each composed of five stages including feeding (≈ 4 min), reaction (≈ 11 h), sludge discharge (≈ 4 min), settling (50 min), and fermentate withdrawal (≈ 7 min). Hydraulic retention time (HRT) and sludge retention time (SRT) were kept at 2 and 6 days, respectively. VFA composition was analyzed using gas chromatography, and the degree of acidification was calculated as net organic acid production (converted to COD units) divided by the inlet COD value. After removing acidogenic biomass via centrifugation at 7000 rpm, the VFA-rich feedstock was used in batch experiments carried out in flat-plate flasks (≈ 300 mL), in which PPB mixed cultures (enriched from environmental samples) were cultivated under monochromatic (850 nm) light intensity of 40 W·m-2 from one side at controlled conditions of temperature (30°C) and pH (6.8-7.2). PPB samples were then analyzed for the protein content. Figure 1 shows the cheese whey valorization value chain for SCP production. The modelling framework was based on a simplified version of existing Photo-Anaerobic Model reported in the literature (Puyol et al., 2017), including a Haldane-type function to describe the impact of light intensity on growth kinetics.

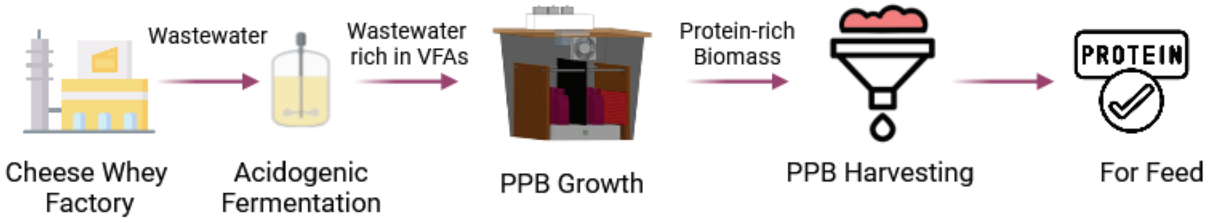

Figure 1: Cheese whey valorization route via combined acidogenic fermentation and purple phototrophic bacteria.

Results and discussion

The fermenter, as reported in Table 1, reached a degree of acidification equal to 70% in line with the findings of Calero et al. (2018), with the SBR strategy effectively working for biomass accumulation. The maximum obtained VFA concentration was 8.15 g COD·VFA L^{-1}, equally divided between acetic and butyric acids.

Table 1: AnSBR experimental output.

Operation Time (d)	VSS at steady state (g VSS·L^{-1})	Maximum total VFA (g COD·VFA L^{-1})	Acetic acid (%)	Butyric acid (%)	Maximum degree of acidification (%)
35	3.7	8.15	49±0.7%	50±0.5%	70

Figure 1a shows that fermented cheese whey is a suitable substrate for PPB growth, completely uptaken in a few days and resulting in high PPB biomass yields (1.3 g COD·g COD^{-1}), competitive with results obtained using synthetic acetate. As reported in Figure 1b, PPB consumed acetic and butyric acid at almost equal pace until they were depleted. No significant variations in substrate consumption and PPB growth were observed for different VFA mixture batches. The amount of protein content reached 57% (g protein·g VSS^{-1}).

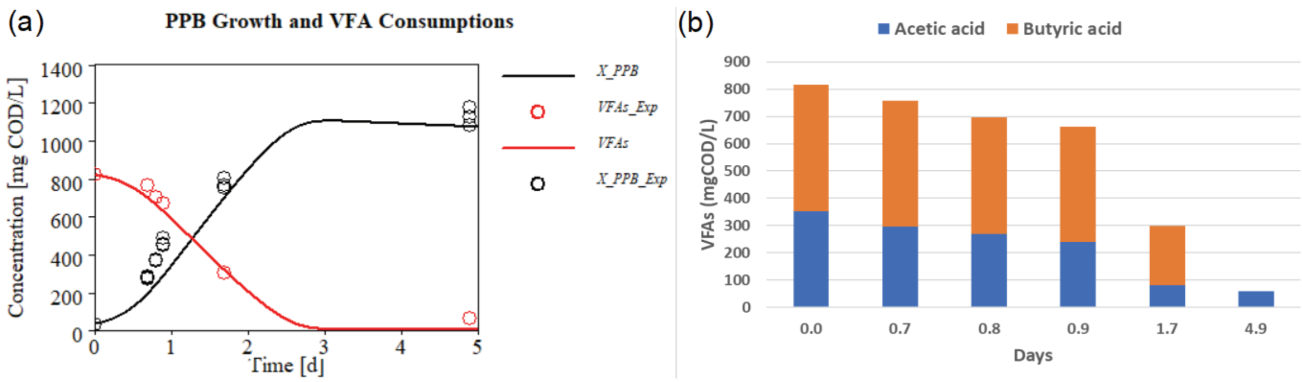

Figure 2: (a) PPB growth and VFA consumption (b) acetic acid and butyric acid consumption over time.

The satisfactory fitting of experimental data by the process model can be observed in Figure 2a, with the estimated parameters compared to those for synthetic acetate in Table 2. No significant differences were observed except for the semi-saturation constant, which was higher for cheese whey as supported by intuition, as well as for the higher yield obtained for cheese whey, being this latter result instead not that much expected.

Table 2: Estimated model parameters.

Parameters	Unit	Cheese whey	Synthetic acetate
Alpha	$s \cdot \mu mol \cdot m^{-2} \cdot d^{-1}$	0.025	0.026
I_{opt}	$\mu mol \cdot m^{-2} \cdot s^{-1} \cdot s^{-1}$	260	260
K_M	$mg\ COD \cdot mg\ COD^{-1} \cdot d^{-1}$	1.80	2
K_S	$mg\ COD \cdot L^{-1}$	69.70	19.24
K_{dec}	d^{-1}	0.01	0.09
K_{hyd}	d^{-1}	0.17	0.07
Y	$mg\ COD \cdot mg\ COD^{-1}$	1.30	0.78

Based on estimated parameters, a model-based optimization procedure was implemented to highlight different combinations of PBR operating conditions leading to the maximization of PPB productivity based on feedstock characteristics and incident light intensity both in case that the PBR was operated with or without HRT and SRT decoupling via a solid-liquid (S/L) membrane separation module downstream of the PBR. Different optima have been identified for PPB concentration, highlighting the need for S/L separation phase to target high productivity.

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