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Cheese whey integrated valorisation: Production, concentration and exploitation of carboxylic acids for the production of polyhydroxyalkanoates by a fed-batch culture

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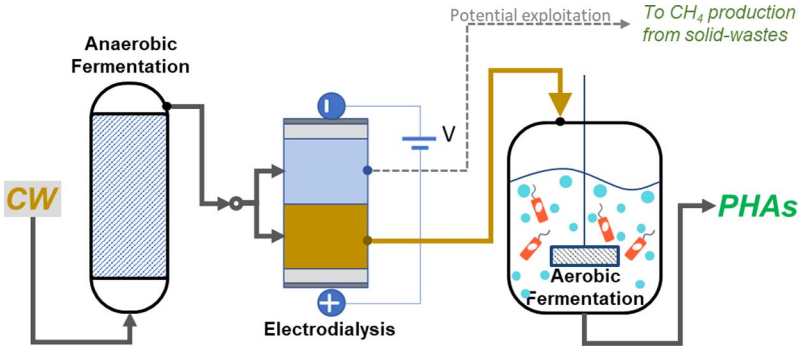
GRAPHICAL ABSTRACT



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ABSTRACT

The present work aimed to verify the feasibility of producing polyhydroxyalkanoates (PHAs) at high concentrations from an alternative and cheap carbon source such as the carboxylic acids (VFAs) obtained by the anaerobic fermentation of cheese whey (CW). An electrodialysis (ED) step was proposed for the obtainment of a concentrated acidic stream, suitable for being employed as feeding solution in the consecutive fed-batch culture system for PHAs production.

Experiments conducted in duplicate shown that a packed bed anaerobic bioreactor resulted a robust and high repeatable culture system for a high performing continuous production of VFAs from CW. The acidogenic effluent contained 12.55 ± 1.10 g L^{-1} of carboxylic acids (*ca.* 90% of the effluent soluble COD), including the hexanoic (4.13 ± 0.56 g L^{-1}) and octanoic (3.12 ± 0.94 g L^{-1}) acids. The application of 7 sequential batch ED processes allowed to obtain a carboxylic acids concentrated stream (*ca.* 63 g L^{-1}); by the achievement of high molar flow. Finally, this stream was utilized as the feeding solution in a fed-batch fermentation aimed to produce PHAs. The attained PHAs yield (*ca.* 0.60 g_{PHAs} g_{VFAs}^{-1}) was comparable to that obtained in a parallel test accomplished with a VFAs-water simulating solution and also to those previously reported from pure VFAs; i.e. no inhibition effects due to the employment of an actual biowaste as the feedstock were detected.

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As a whole, the results allow to conclude that the proposed integrated valorisation scheme fed with CW for the production of PHAs by an ED-concentrated carboxylic acids solution is technically feasible and robust.

1. Introduction

Cheese whey (CW) is the main liquid agro-industrial residue from the dairy industry. Its global generation rate in 2016 was estimated to be *circa* 200 Mt year^{-1} [1], with an annual linear increase of 3% for the last 21 years [2]. CW is a side-product stream with a high chemical oxygen demand (COD, 20–100 gO_2 L^{-1}) and a high biological oxygen demand (BOD, due to the occurrence of hardly biodegradable proteins), which make it necessary to treat CW in dedicated plants before being discharged [3].

A green and valuable alternative to the CW disposal is represented by the possibility of coupling its treatment with the obtainment of added value products, i.e. upgrading the lactose, lipids and proteins occurring in CW into marketable products. In this line, many studies reported the CW valorisation (recently reviewed by *Valta* et al. [4]) through physicochemical processes (e.g., for obtaining proteins [5] or micronutrient fertilizers) or through microbial fermentations that allow obtaining a variety of products [6]. In particular, carboxylic acids (from C2 to C8, from now on mentioned as volatile fatty acids, VFAs) typically accumulated in the effluent of acidogenic fermentation processes mediated by anaerobic mixed microbial cultures. Recently, the production of VFAs by anaerobically fermenting CW in an anaerobic packed bed bioreactor (PBBR) was reported [1]. VFAs are of industrial interest both as ready-for-application products and as precursors in the frame of the carboxylate platform, which includes also their biotechnological transformation into polyhydroxyalkanoates (PHAs) [7].

Nevertheless, the industrial production of PHAs from a VFAs-rich stream by employing a pure culture seems not to represent a winning strategy. Indeed, PHAs production with pure culture requires the achievement of high cell density in order to justify the fermentation costs and thus the fed-batch culture system is supplied with concentrated substrate solutions (e.g. glucose, molasses at 400 g L^{-1}). Unfortunately, acidified effluents contain at most 30 g L^{-1} of VFAs which is one order of magnitude lower than what required for avoiding a cell dilution effect in a fed-batch culture system. The need of managing concentrated VFAs streams is shared also with the other application fields of the mentioned carboxylate platform. This evidence is driving a recent research line dedicated to study the VFAs concentration and separation, as reviewed by *López-Garzón* et al. [8]. Among the proposed strategies, the application of the electrodialysis (ED) approach, where an electrical field applied through ion-exchange membranes works as the driving force to concentrate ions (such as R-COO$^-$) in one stream, has being studied. The main advantages of the ED are the facts that no exogenous compounds addition is required and no harmful residual-stream is generated. The application of ED to recover carboxylic acids such as lactic [9–12], succinic [13–15], fumaric acids or single VFAs [16–23] have been studied for some years ago. However, the VFAs recovery from an acidified effluent (namely, an acidogenic fermented olive mill wastewater) was reported recently by *Scoma* et al. [16] for the first time, in which the feasibility of a conventional ED process was assessed for the VFAs concentration. More recently, an identical integrated approach was reported by *Tao* et al. using an acidified stream arising from thermal hydrolysed waste activated sludge [23]. That study also included a PHAs production test from the VFAs-concentrated stream as suggested previously [16]. However, the actual potentialities and sustainability of such an integrated valorisation scheme should be further assessed, since neither the VFAs concentration achieved by the ED step (< 20 g L^{-1}) nor the biopolymer production (5 g L^{-1} of CDW, with a PHAs content of 30%) allowed speculating about the potential viability of the proposed value chain. Besides, the ED step concentrates also ions potentially responsible for detrimental effects, such as Cl$^-$. This fact should be carefully taken into consideration when concentrating VFAs more than 5 times.

With all this in mind, the aim of the present work was to study an integrated process scheme for the valorisation of CW, which includes the following steps: (a) continuous production of carboxylates in an anaerobic PBBR culture system, followed by (b) the carboxylates concentration in a ED step, this allowing (c) the production of PHAs using the VFAs-concentrated stream as feeding solution in a pure culture fed-batch system. The residual stream from the ED step (the diluate, depleted in VFAs) could be fed to downstream processes such as the anaerobic digestion of solid biowaste, which requires water to dilute the suspended solids (Graphical abstract).

The main parameters such as yield, energy and substrate specific consumptions of each units were analysed in order to carry out preliminary evaluations about the feasibility of the whole integrated biorefinery process here proposed.

2. Material and methods

2.1. Carboxylates production from CW

The acidogenic step was fed with a CW powder as reported previously elsewhere [1]. It was kindly provided by Terra Nostra (Azores, Portugal), and it was mainly composed by (% w/w): lactose (79.3), proteins (9.1) and fats (0.7). The feeding solution was prepared by dissolving the CW powder in distilled water in a concentration equivalent to 25 g L^{-1} of lactose.

The microbial consortium used in the experiments was the same acidogenic microbial culture immobilized on Vukopor® ceramic cubes employed in a previous work [1].

The production of the VFAs-rich stream (CW_{Acid}) was carried out by fermenting the feeding CW solution using the same process configuration applied and described recently by *Domingos* et al. [1]. Very briefly, two parallel anaerobic column biofilm reactors packed with Vukopor® S10 ceramic cubes (PBBR_A and PBBR_B) were operated under continuous acidogenic conditions in order to evaluate the process reproducibility and to generate sufficient amount of VFAs-rich effluent. The PBBRs had a working volume of 0.8 L; process temperature and pH were set up and controlled at 37 ± 2 °C and 6, respectively. The reactors were fed with a HRT of 6 days. Specific bioreactors configuration, control and sampling procedures, as well as the yields calculation formulas, were the same described previously [1].

2.2. Carboxylates concentration using electrodialysis

2.2.1. Experimental approach

The VFAs concentration was carried out by employing an ED step. To this aim, the CW_{Acid} was previously centrifuged (12,000 rpm for 10 min) and filtrated through a cellulose membrane with 1.2 µm pore size.

The ED batch trials were carried out at room temperature, using the same laboratory-scale conventional two-compartments electrodialyser (WORTMANN s.r.l., Brescia - Italy), including the membrane types, described in detail in a previous work [16], where the stack configuration and main operating conditions were also reported. Differently from the operating procedure carried out in the previous work, the experiments were performed at a constant electric potential (10 V) by measuring the electric current decrease (typically ranging from 170 to 18 A/m^2) and using 2 L of electrodes rinse solution (82 g L^{-1} Na_2SO_4). The same protocol for membrane storage and washing procedure was