CHEMICAL ENGINEERING TRANSACTIONS
VOL. 49, 2016
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.aidic.it/cet
Guest Editors: Enrico Bardone, Marco Bravi, Tajalli Keshavarz

ISBN 978-88-95608-40-2; **ISSN** 2283-9216
DOI: 10.3303/CET1649033

Development of a Continuous Biofilm-Based Process for the Bioconversion of Cheese Whey into Volatile Fatty Acids

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The main goal of the present work was to study the continuous production of volatile fatty acids from a cheese whey powder in anaerobic packed bed biofilm reactors (PPBRs). Vukopor S10 ceramic cubes and granular activated carbon were employed as cell immobilization materials. Experiments were done at 37°C, pH 6. Reactors were frequently sampled for metabolites and biogas analyses. Preliminary batch tests, which were carried out in 100-mL anaerobic Pyrex bottles, demonstrated that the process consisted of two sequential phases: (a) the conversion of lactose into lactic acid and (b) the conversion of lactic acid into a mixture of VFAs. The continuous process was settled up in 1-L glass column and two different hydraulic retention times (9 and 6 days) were applied during the continuous process. Almost 80% of lactose bioconversion was achieved in Vukopor reactors, this corresponding to a 10 g/L VFAs concentration.

1. Introduction

Dairy industry is practiced all over the world for the production of milk, cheese and other milk derivate. With fast growth of human population and a respond to the nutrition needing brings to an increase of milk derivate production in the last years. Cheese is the milk derivate most produced around the world and is in Europe where is observed the greatest production of cheese, representing 57% of world production. During cheese production, cheese whey (CW) is a by-product produced in a large amount and is the major and most contaminated waste generated in the cheese production. As rule of thumb it can be said that for production of 1 kg of cheese 10 kg of milk are used and 9 kg of CW are obtained (Prazeres et al., 2012). It has an important lactose content (44-52 g/L) which promotes a high chemical oxygen demand (COD), which can cause severe pollution problems if discharged in the environment (Kosseva et al., 2009).
Anaerobic digestion is a collection of processes by which microorganisms convert biodegradable compounds, as lactose, in the absence of oxygen. First, organic polymers such as carbohydrates are hydrolysed, and then during acidogenesis, acidogenic bacteria convert organic matter as sugars and amino acids into dioxide carbon, alcohol and organic acids (Horiuchi et al., 2002; Itoh et al., 2012). Volatile fatty acids (VFAs) are short chain aliphatic carboxylic acids, which represent the final product of anaerobic acidogenic before methanogeses, last step of anaerobic digestion in which organic acids are convert to methane and carbon dioxide. Since VFAs can be exploited as precursors for the production of several added-value chemicals and materials, the biotechnological conversion of organic agro-industrial wastes into VFAs can be considered a valuable alternative to waste disposal. Since lactose can be bioconverted under anaerobic acidogenic conditions, VFA production from CW can represent a valuable alternative to the waste disposal in agreement with the biorefinery concept. The mentioned process can be carried out by the implementation of a pure or mixed culture. The advantage, of a pure culture, is the high productivity achieved. In other hand, when a mixed culture is employed costs with energy are save due to no sterilization needs. Mixed cultures are composed by many different members of bacteria giving it robustness to support feed variations and synergies effect when extra supplements are needed (Agler et al., 2011). A possible drawback, of production of add-value products from CW, is being a nutrient deficient and so, supplementation is needed to avoid slow microorganisms growth. It was observed that with nutrient supplementation or used of mixed cultures

Please cite this article as: Bendada Domingos J.M., Agustin Martinez G., Scoma A., Bertin L., Reis M., Fava F., 2016, Development of a continuous biofilm-based process for the bioconversion of cheese whey into volatile fatty acids, Chemical Engineering Transactions, 49, 193-198 DOI: 10.3303/CET1649033

productivities increased significantly (Prazeres et al., 2012). Bioreactor configuration can also contribute for increasing the productivity, for example the employ of immobilization material. The principal reason is that prevents the culture washout when continuous process is employed due to a higher cell density as well as its robustness formed by the consortium (Keskin et al., 2012, Joshi et al., 2011). With this stronger culture is also possible to work with lower hydraulic retention times (HRT). Another advantage is an easier downstream process since, during the bioconversion the mostly of the cells are separated from the liquid. Immobilization can be performed in several ways. When is performed physically it calls entrapment, otherwise is attachment. Calcium alginate or membranes are the materials usually used for a physically immobilization. Instead, attachment consists cells bound, forming the biofilm. Biofilm is composed by microbial colonies covered a porous matrix or attached to a surface with the polysaccharides segregated by the microorganisms. Because of that, the huge advantage is the fact that is simple to carry out since only depends on microorganisms activity. As it only depends on the comportment of the microorganisms the major disadvantage is related to the fact that binding forces, between the microorganisms and the surface, can be weak (Kosseva et al., 2009). From all of this a several supports can be employed in a packed-bed bioreactor (PBBR), which the most common are porous materials composed by silica, ceramic or activated carbon.

Few experiences about CW anaerobic acidogenic digestion for the production of VFAs with immobilized cells by employing a membrane-based cell recycle (Duque et al., 2014) or fibrous-bed reactors were already described (Kosseva et al., 2009). However, almost no experiments for VFAs production with immobilization in a packed-bed reactor, from CW, have been reported.

One of the most promising perspectives for VFAs employment is their use as substrate for polyhydroxyalkanoates (PHA) production. VFAs mixture obtained during the acidogenic process affects the type of obtained PHA, e.g., acetate and butyrate are converted into hydroxybutyrate (HB) monomers while proprionate is a precursor for hydroxyvalerate (HV) (Bengtsson et al., 2008). Because of that, it is important to control the process conditions in the acidogenic fermenter such as pH, hydraulic retention time (HRT) and temperature in order to achieve a favorable mixture composition. An alternative for a conventional continuous process fermentation is the use of cell immobilizing material. The packed-bed bioreactors (PBBRs) present some advantages such as the achievement of a higher cell density as well microbial robustness. This allows working at lower HRT by avoiding washout problems and conserving microbial consortium.

In the present study, the valorisation of CW by producing VFAs by employing an anaerobic acidogenic packed bed bioreactor was proposed.

2. Materials and Methods

2.1 Materials

Cheese Whey Powder (CWP) was kindly provided by Lactogal, a Portuguese dairy industry. The acidogenic mixed culture inoculum was obtained from a membrane bioreactor operated at Prof. Maria Reis laboratory (Duque et al, 2014), which was dedicated to the production of VFAs from the same CW. The inoculum activation was started by culturing the inoculum sample in a 500 mL-Pyrex bottle, this also allowing to generate enough inoculum volume for all experiments.

The batch experiments were carried out in 100-mL Pyrex bottles (microcosms) and performed in quadruplicate. The microcosms were prepared using an immobilization support to allow development of attached biofilm. In order to be able to perform a comparison between the different supports, the packed and working volume were fixed. The packing volume, that corresponds to the bottle volume occupied by immobilization materials, was set by marking an horizontal line over the glass, corresponding to 45 mL of water, previously added. Then, the bottles were filled up, with water, to 55 mL level, which represents the working volume. This volume will comprise the liquid volume, CWP solution and inoculum, and the immobilization material volume. Then, empty and dried bottles were filled with each support, Vukopor S10 and Granular Activated Carbon, till completing the packing volume. Support accommodation and packing was obtained by giving smalls hits to the bottle. Since both supports have different specific volumes and the working volume was fixed, the liquid amount- comprising the CWP and inoculum necessary to add is different for Vukopor and AC. To determine that, water was added until reaching the 55 mL line. The quantity of added water in each bottle corresponds to the total liquid volume, comprising the inoculum and CWP solution, necessary to add to each bottle. This step was performed with the supports already wet in order to avoid the absorption phenomenon since it was previously observed that AC had a strong absorbent capacity and because during sequential batches the supports will be already wet in the beginning of each. After setting up of all experimental volumes, cultures were started with the corresponding amounts of CWP and inoculum, always bubbling nitrogen to maintain anaerobiose. It were inoculated with 10% (v/v) of acidogenic mixed culture in a CWP solution, which was prepared by diluting 20 g/L of CWP in distilled water, so that its lactose

content was 15 g/L. Lactose quantity was decided in base of the quantity of lactose content in this CW before lyophilisation. Incubation conditions were 37°C and 150 rpm. Sampling for biogas and metabolites measurements were frequently performed on daily basis. After biogas sampling, the bottles were opened under nitrogen gas sparging to keep anaerobiose and 1 mL of liquid phase was withdrawn for metabolites concentrations analysis. Thereafter, pH was controlled and corrected to 6 by adding NaOH 10 M solution. Two different immobilizing materials were tested, namely, (a) Vukopor S10 (VK) (Vukopor S10 product, Lanik, Boskovice, CZ) whose dimensions, porosity and density were 25 x 25 x 18 mm, 10 ppi and 2.38 g/mL, respectively, and (b) Granular Activated Carbon (AC) (CP4-60 product, Chemviron Carbon, Feluy, Belgium), consisting of cylinders of about 3 mm diameter and 10 mm length, whose density was 1.32 g/mL. Sequential batch tests were performed for inducing biofilm formation.

The continuous process was developed by employing two packed-bed biofilm reactors (PBBRs), which operated in parallel. They were constituted by 1L-hermetically closed glass columns (5 cm of diameter and 40 cm high) wrapped with a silicon tubing serpentine continuously recycling thermostated water, maintaining 37 ± 2°C inside of the bioreactors, and equipped with a down flow recycle line. One PBBR was packed with VK (PBBR-VK), while the other with AC (PBBR-AC). The liquid and the gas effluent were collected in a bottle, hydraulically connected to a 2.5 L “Mariotte” bottle through which the produced biogas volume was determined. PBBR-AC was operated with a HRT of 9 days while PBBR-VK was operated with a HRT of 9 and 6 days. Both had a working volume of 830 mL and were inoculated with 20% of liquid volume of CWP solution, also containing 15 g/L of lactose.

2.2 Analytical methods

HPLC equipped with IR detector and a Varian Hi-Plex H 300 x 7.7 mm column was used for lactose and lactic acid concentrations analyses. A 0.01 N sulfuric acid solution was used as eluent with an elution rate of 0.6 mL/min and a 65°C operating temperature. The samples were centrifuged at 14,000 RPM for 10 minutes and filtered (Spin-X 2 mL centrifuge tube filter 0.45 um cellulose acetate). The VFAs concentrations were determined by gas chromatography using a GC (Agilent Technologies, Milano, Italy) coupled to a Flame Ionization Detector (GC-FID model 7890A) and equipped with a HP-INNOWAX column (30 m x 0.250 mm x 0.25 µm) (Martinez et al., 2015). The samples were centrifuged at 14,000 RPM for 10 minutes; supernatant was diluted oxalic acid solution 60 mM and filtered with 0.45 µm membrane (Spin-X 2 mL centrifuge tube filter 0.45 um cellulose acetate). Biogas volume production in small scale batch experiments was measured using a graduated glass syringe, while a “Mariotte” system was connected to the PBBRs. Biogas composition in terms of H_2, CH_4 and CO_2 was measured by gas-chromatography using a µGC, model 3000 A (Agilent Technologies, Milano, Italy) (Scoma et al., 2011).

3. Results and Discussion

In order to define the most adequate HRT to be applied in the PBBRs, a preliminary batch experiment was performed with VK and AC filled batch microcosms. Tests with VK demonstrated that the process consisted of two sequential phases (figure 1): (a) the conversion of lactose into lactic acid with a yield (in terms of C-mol) of 90% and (b) the conversion of lactic acid into a mixture of VFAs. After biofilm formation, the whole process of VFAs production lasted 9 days with a yield of 77% (C-molVFAs/C-molLactose). Conversely, the same could not be observed in microcosms with AC, where Lactic acid was never detected. One of the possible reasons is the high affinity of AC for organic compounds as previously reported (da Silva and Miranda, 2013). In this way, it was impossible to distinguish between the produced/consumed and absorbed/desorbed metabolites. Despite AC not revealed positive results, PBBR-AC was settled up in parallel with PBBR-VK to see if under continuous production of VFAs AC could reach an equilibrium between adsorbed and released VFAs.

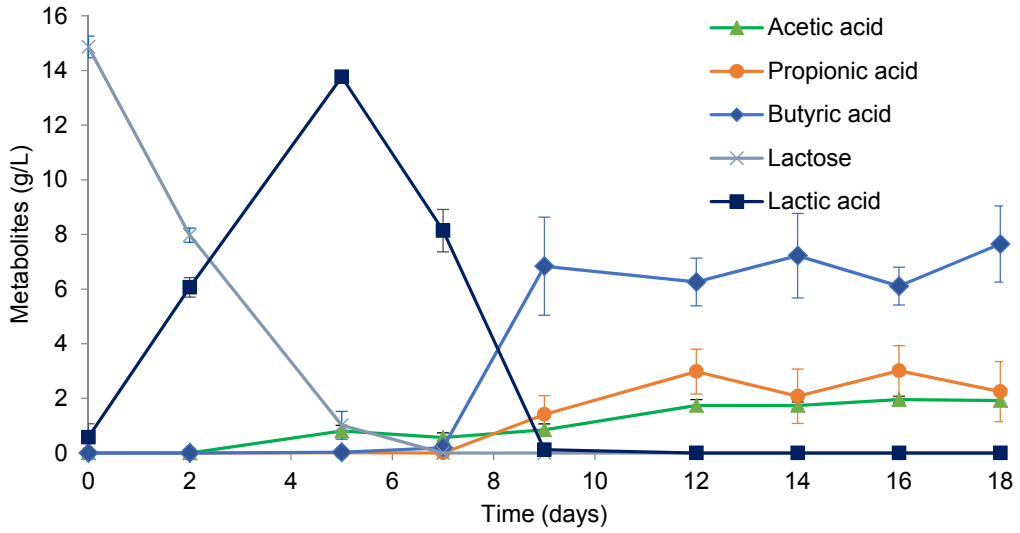

Figure 1: Lactose, lactic acid and VFAs concentrations in microcosms filled with Vukopor S10 during batch experiment.

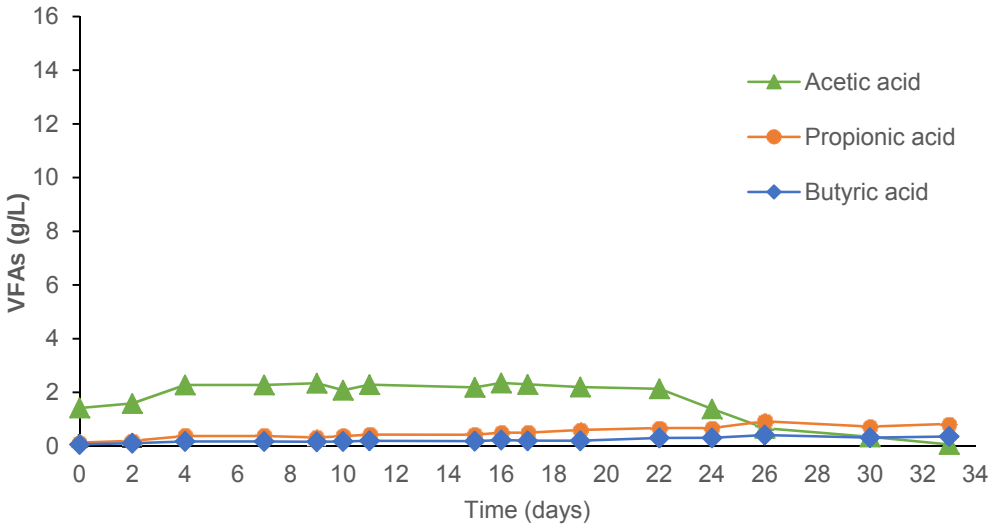

Figure 2: VFAs concentrations in PBBR-AC during continuous operation.

For the continuous experiment, a HRT of 9 days was decided for both PBBRs, since it represented the process time according to which satisfactory VFAs yields were observed in VK-batch microcosms.
PBBR-AC was not efficient in VFAs production, since the total VFAs concentration in its effluent was only 2.7 ± 0.5 g/L, corresponding to a yield of about 20%. Some works reported the employment of AC as immobilization material in anaerobic reactors, and also there VFAs concentration was low (Bertin et al., 2010). These unsatisfactory results, in either batch or continuous experiments, were attributed to the high adsorbent capacity of this material. Gao and co-workers (Gao et al., 2011) took advantage of this characteristic and used AC for in situ removal of lactic acid and then its desorption with other chemicals as acetone. Neither adsorption isotherm studies nor analytical or biological analyses of the AC were performed. These procedures could have helped to understand the real effect of AC on this biological system.
Conversely, PBBR-VK revealed versatile and interesting results. During the operation with HRT of 9 days an almost stationary state was reached after 9 days. Under those conditions, the concentrations of butyric and acetic acids were stable (Table 1), but the same did not happen with the other acids; in particular, propionic acid concentration decreased drastically from 6 g/L until 0.8 g/L. A second run was then carried out with a HRT of 6 days. Since the capability of producing propionic acid was almost lost, the latter experiment was

performed with the perspective of getting a higher total VFAs productivity. In fact, a total VFA concentration of 9.0 ± 0.7 g/L was obtained (Table 1), this corresponding to an increased productivity (Table 1). As expected, propionic acid concentration did not achieve even 0.5 g/L. however, the VFA mixture was modified, and a significant increment of caproic acid concentration was observed (Table 1).

Table 1 – Lactose bioconversion yields and concentrations of main VFAs achieved with the PBBR-VK under different HRT conditions.

HRT (days)	Yield (C-molVFAs/C-molLactose)	Total VFAs (g/L)	Productivity (g/L d)	Acetic acid (g/L)	Propionic acid (g/L)	Butyric acid (g/L)	Caproic acid (g/L)
9	78.9±5.7	10.5±0.7	1.2	5.3±0.7	1.0±0.3	2.8±0.3	0.6±0.1
6	79.9±0.1	9.0±0.7	1.5	1.1±0.6	0.4±0.3	4.3±0.3	2.4±0.5

4. Conclusions

Cheese Whey is the waste from dairy industry with more pollution impact for the environment due to its high COD content, in which lactose is the majority responsible. Lactose is a carbon source for some species of microorganisms making the CW a potential substrate for biotechnological processes, like the anaerobic digestion. During this work Vukopor S10 and granular activated carbon were employed as packing materials for the bioconversion of CW into a mixture of VFAs. Batch experiments showed that lactose was firstly converted into lactic acid, which was then converted into VFAs, and that the whole process lasted 9 days. Activated carbon has a huge absorbent capacity, especially for organic compounds. Its characteristic did not allow a complete study. In batch experiments it was achieved a VFAs yield of 30%, while in continuous operation in the bioreactor it was achieved only 20%.Either for batch and continuous experiments, Vukopor S10 showed better performances than granular activated carbon. PPBR-VK allowed developing a versatile process, since it was possible to obtain different VFAs mixtures by modifying the HRT (9 and 6 days). Importantly, the employment of a lower HRT resulted in a higher VFAs productivity, which was obtained by maintaining a high lactose conversion yield (80%).

Acknowledgments

Research funds were received from the European Community's Seventh Framework Programme (FP7/2007-2013) under the Grant Agreement FP7-265669-EcoBioCAP

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