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Dynamic change of pH in acidogenic fermentation of cheese whey towards polyhydroxyalkanoates production: Impact on performance and microbial population

Ana R. Gouveia[a], Elisabete B. Freitas[a], Cláudia F. Galinha[b], Gilda Carvalho[a], Anouk F. Duque[a,*], Maria A.M. Reis[a]

[a] UCIBIO-REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal
[b] LAQV-REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal

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ABSTRACT

Polyhydroxyalkanoates (PHA) are a sustainable alternative to conventional plastics that can be obtained from industrial wastes/by-products using mixed microbial cultures (MMC). MMC PHA production is commonly carried out in a 3-stage process of acidogenesis, PHA culture selection and accumulation. This research focused on the possibility of tailoring PHA by controlling the acidogenic reactor operating conditions, namely pH, using cheese whey as model feedstock. The objective was to investigate the impact that dynamically varying the acidogenic pH, when targeting different PHA monomer profiles, had on the performance and microbial community profile of the anaerobic reactor. To accomplish this, an anaerobic reactor was continuously operated under dynamic pH changes, ranging from pH 4 to 7, turning to pH 6 after each change of pH. At pH 6, lactate and acetate were the dominant products (41–48% gCOD basis and 22–44% gCOD basis, respectively). At low pH, lactate production was higher while at high pH acetate production was favoured. Despite the dynamic change of pH, the fermentation product composition at pH 6 was always similar, showing the resilience of the process, i.e. when the same pH value was imposed, the culture produced the same metabolic products independently of the history of changes occurring in the system. The different fermentation product fractions led to PHAs of different compositions. The microbial community, analysed by high throughput sequencing of bacterial 16 S rRNA gene fragments, was dominated by *Lactobacillus*, but varied markedly when subjected to the highest and lowest pH values of the tested range (4 and 7), with increase in the abundance of *Lactococcus* and a member of the Candidate Division TM7. Different bacterial profiles obtained at pH 6 during this dynamic operation were able to produce a consistent profile of fermentation products (and consequently a constant PHA composition), demonstrating the community's functional redundancy.

Introduction

Polyhydroxyalknoates (PHAs) are a bio-based and biodegradable alternatives to plastic-based polymers. Their high production cost has been limiting for PHAs implementation. Therefore, low cost processes for PHA production, such as the use of mixed microbial cultures (MMCs), have recently emerged [1]. MMCs allow the use of industrial wastes or by-products, since the microbial population can continuously adapt to changes in substrate [1,2]. In fact, several studies have focused on the use of food and agricultural industries' wastes/by-products as substrates for organic acids (HOrgs) [3] and PHA production [1], namely sugar cane molasses [4–7] and cheese whey [8,9]. Cheese whey (CW) is a byproduct resulting from cheese manufacture. The world production of CW is estimated to be around 85 million tons/yr and only 60% of CW is currently valorised in food applications. This by-product is an interesting feedstock for acidogenic fermentation because it is composed mainly of lactose (an easily fermentable sugar), and other compounds in minor amounts, such as proteins, mineral salts and lipids, that can be used as nutrients by microbial cultures. MMC PHA production from complex feedstocks commonly involves a 3-stage process [8,10,11]: (i) acidogenic fermentation, where the organic content of the feedstock is biologically converted into fermentation products

* Corresponding author.
E-mail address: af.duque@fct.unl.pt (A.F. Duque).

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(FP), which are PHA precursors; (ii) culture selection, where an MMC using FP is subjected to a high selective pressure for PHA storage through the use of alternate short presence and long absence of carbon (feast and famine (FF) regime); and (iii) PHA production, where the selected MMC is fed with the FP produced in the acidogenic fermentation aiming to accumulate PHA up to the culture's maximum capacity.

Composition of the FP can be manipulated by tuning the acidogenic reactor operating conditions such as pH [4,9,12–14], retention time [9,15], organic loading rate [16] and temperature [15]. It is expected that different organisms are selected at different pHs and consequently different metabolic pathways are activated/deactivated, leading to different FP compositions [17]. It is known that the FP profiles directly affect the PHA monomer composition [4,8,11,18]. Thus, by changing the acidogenic reactor operating conditions, such as pH, a large range of polymer compositions, with different applications, can be obtained.

PHA producing industries have to comply with market demands in terms of PHA composition, depending on the target application. To be able to respond to a dynamic market demand, several strategies may be proposed when using MMCs to obtain different FP compositions depending on the PHA monomer composition requirements: (i) alternate operation with different feedstocks [8]; (ii) several systems working in parallel at different operating conditions (e.g. pH) producing different FP profiles; or (iii) a single system fed with a single feedstock working continuously by dynamically changing the key operating conditions to obtain several FP compositions. The latter option, while simpler and likely less costly than (ii) and (iii), relies on the resilience of the system to alterations in the operating conditions to comply with the targeted FP profile. Thus far, the effect of dynamic change of pH on acidogenic fermentation is unknown. Therefore, this study has investigated the possibility of manipulating PHA composition by altering the operating conditions (namely pH) of the acidogenic stage, while operated in continuous mode. The impact of dynamic pH changes on the acidogenic fermentation of cheese whey, namely on performance stability, reproducibility and microbial community structure, and on final polymer composition was evaluated.

Material and methods

Reactor setup

A lab-scale continuous stirred tank reactor (CSTR) with a working volume of 1.25 L was operated under anaerobic conditions. The CSTR was inoculated with biomass from an anaerobic wastewater treatment plant (Mutela, Cacilhas) and acclimatised with cheese whey (CW). The CW powder was supplied by a Portuguese dairy industry plant (Lactogal- Produtos Alimentares S. A, Porto) and is mostly composed by lactose (78.4%, w/w), proteins (13.6%, w/w) and fats (1.2%, w/w) (source: Lactogal). The CW powder was diluted with tap water at a concentration of 15 g sugars L^{-1} and was maintained at 4 °C in a refrigerated vessel. As previously reported by Duque et al. [8], no extra nutrients were added to the feeding solution. The average C:N ratio was 100 C-mol:6.3 N-mol.

The CSTR hydraulic retention time (HRT) and, consequently, the sludge retention time (SRT) were controlled by overflow in order to be kept at around 1 day (1.0 ± 0.1 day). The feed flow rate was set up so that the organic loading rate was maintained at about15 g COD L^{-1} d^{-1} (15.9 ± 5.9 gCOD L^{-1} d^{-1}). Flow rates were daily monitored.

The reactor was subjected to a dynamic pH change as described in Table 1. Firstly the CSTR was operated at pH 6 for 46 days (phase I), then shifted to pH 5 for 25 days (phase II), then shifted back to pH 6 for 22 days (phase III), changed to pH 4.5 for 31 days (phase IV), back again to pH 6 for 24 days (phase V), changed to pH 7 for 46 days (phase VI), shifted back to pH 6 for 36 days (phase VII), then at pH 4 for 30 days (phase VIII) and back to pH 6 for 43 days (phase IX). Change in the pH value was performed after a pseudo-steady state was reached, meaning constant FP composition. A decrease in the total fermented products was observed between days 194 and 203 (pH 7). This was due to a temperature drop related to a power failure, therefore the stability at pH 7 was considered between days 177 and 189. Acidogenic biomass was acclimatised at pH 6 (standard condition) and after each pH value shift, the reactor always turned back to pH 6. pH was online controlled by dosing 2 M NaOH. Mixing was provided at 300 rpm and temperature was kept at 30 °C.

Table 1
Performance of the acidogenic reactor operated under dynamic pH change.

Phase	pH	[FP] (gCOD-FP L^{-1})	$Y_{FP/S}$ (gCOD-FP $gCOD^{-1}$)	$Y_{X/S}$ (gCOD-X $gCOD^{-1}$)	r_{FP} (mgCOD-FP L^{-1} h^{-1})	$-r_s$ (mgCOD-CW L^{-1} h^{-1})	q_{FP} (gCOD-FP gCOD-X^{-1} h^{-1})	$-q_s$ (gCOD gCOD-X^{-1} h^{-1})	DF[a] (gCOD-FP $gCOD^{-1}$)	Protein removal (%)
I	6	13.7 (3.0)	0.65 (0.12)	0.15 (0.04)	566.7 (123.4)	842.4 (85.7)	0.18 (0.08)	0.34 (0.08)	0.56 (0.13)	46.4 (8.7)
II	5	14.1 (0.7)	0.58 (0.11)	0.11 (0.03)	568.3 (26.9)	883.7 (62.8)	0.25 (0.05)	0.35 (0.02)	0.50 (0.07)	49.5 (10.1)
III	6	15.1 (0.7)	0.63 (0.12)	0.13 (0.04)	593.9 (67.3)	824.4 (115.4)	0.27 (0.04)	0.43 (0.10)	0.61 (0.08)	40.1 (23.1)
IV	4.5	11.0 (2.0)	0.59 (0.11)	0.12 (0.03)	435.4 (55.3)	730.4 (212.2)	0.22 (0.02)	0.37 (0.08)	0.45 (0.07)	41.8 (21.8)
V	6	11.9 (2.2)	0.55 (0.08)	0.12 (0.03)	504.9 (111.9)	822.1 (185.4)	0.23 (0.06)	0.38 (0.11)	0.42 (0.03)	41.2 (11.5)
VI	7	12.7 (3.6)	0.54 (0.14)	0.13 (0.05)	514.8 (150.4)	985.1 (286.9)	0.22 (0.06)	0.40 (0.11)	0.48 (0.10)	31.4 (15.7)
VII	6	10.7 (1.8)	0.67 (0.06)	0.13 (0.02)	448.5 (86.4)	666.0 (141.5)	0.24 (0.06)	0.35 (0.10)	0.53 (0.09)	49.5 (14.6)
VIII	4	4.3 (0.8)	0.33 (0.04)	0.06 (0.02)	190.6 (42.8)	593.8 (157.7)	0.17 (0.05)	0.52 (0.17)	0.18 (0.03)	59.3 (9.4)
IX	6	11.6 (1.2)	0.68 (0.13)	0.11 (0.02)	513.3 (51.9)	769.9 (155.0)	0.33 (0.06)	0.50 (0.14)	0.57 (0.05)	42.9 (13.8)

The values listed are averages ± standard deviation (values in brackets).
[a] Degree of fermentation.

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