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Biohydrogen and polyhydroxyalkanoates (PHA) as products of a two-steps bioprocess from deproteinized dairy wastes

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ABSTRACT

In this study a two-steps bioprocess approach aimed at biohydrogen production via dark-fermentation, and polyhydroxyalkanoates-PHA production by mixed microbial cultures, was proposed to valorise two dairy-waste streams coming from cheese whey deproteinization (i.e. *Ricotta* cheese production and ultrafiltration). During the first step, the increase of OLR was tested, resulting in higher daily H_2 volume (3.47 and 5.07 NL H_2 d^{-1} for second cheese whey-SCW and concentrated cheese whey permeate-CCWP) and organic acids production (14.6 and 12.6 g L^{-1} d^{-1} for SCW and CCWP) for both the substrates, keeping good conversion of sugars into H_2 (1.37 and 1.93 mol H_2 mol^{-1} sugars for SCW and CCWP). During the second step, the organic acids were used for PHA production reaching high conversion yields for both the fermented streams (as average 0.74 ± 0.14 mg COD_{PHA} mg^{-1} $COD_{OA\text{-}in}$), with a maximum polymer content of 62 ± 4.5 and 55.1 ± 1.3% (g PHA g^{-1} VSS) for fermented SCW and fermented CCWP respectively. For the results reported, this study could be taken into consideration for larger scale application.

1. Introduction

Cheese whey is produced as by-product from cheese and casein production during milk processing. Currently, the global annual production of cheese whey stands at about 120 million Mg (Nikodinovic-Runic et al., 2013), while in the EU, the total cheese production reached 40 million Mg per year, corresponding to about 66% of the total global production (Nikodinovic-Runic et al., 2013). Italy (as a case study) is among the top five cheese producers in Europe and consequently, large volumes of cheese whey and related wastes are produced. In the Lombardy Region which is the leader region in Northern Italy, about 3.5 billion Mg of cheese whey are annually generated (Colombo et al., 2016). Only about 50% of the produced cheese whey is being further utilised as raw material to produce *Ricotta* cheese, food supplements, such as whey protein concentrate (WPC), and animal feeds (http://www.lattenews.it/il-siero-di-latte-e-una-risorsa-per-diversi-mercati/, visited on January 2019). *Ricotta* cheese production has become the most common approach for utilising cheese whey in the dairy industries due to its relatively low investment cost (Pintado et al., 2001). During *Ricotta* production a liquid by-product "the second cheese whey" (SCW) is generated, mainly composed of lactose and mineral salts (Farkye, 2004), producing for each kg of *Ricotta* cheese produced, about 14–19 L of second cheese whey are generated (Mills, 1986).

Apparently, about 1 million Mg of second cheese whey are produced annually in Italy, however, only a small fraction of second cheese whey is used as animal feed supplement, while a large quantity remains unutilised (Sansonetti et al., 2009).

Besides production of *Ricotta* cheese, the cheese whey ultrafiltration is another process largely employed by dairy industries to treat cheese whey in Italy. Similar to the second cheese whey, the cheese whey permeate (CWP) remains rich in lactose concentration which raises its residual chemical oxygen demand (COD) to about 54 kg m^{-3} and a high content of inorganic salts (Prazeres et al., 2012). Both the high COD and salt content make it unfit for open environment disposal without further pre-treatment. Moreover, due to its high water content the generated CWP is difficult to transport from the source to pre-treatment centres, and therefore, it is common to apply a controlled evaporation process to reduce the water content and cut down the transportable volume. After evaporation the concentrated CWP (CCWP) can have a lactose concentration of up to about 160 g L^{-1} (Pasotti et al., 2017).

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From a waste re-use perspective, the presence of lactose qualifies these two waste streams, i.e. second cheese whey (SCW) and CCWP, as raw materials for potential production of biofuels and other bio-based compounds. For instance, due to their high content of lactose, both the CCWP and SCW have been proposed for the commercial production of attractive biomolcules such as: milk oligosaccharides (Barile et al., 2009), single cell proteins (Schultz et al., 2006), and biofuels including bio-hydrogen (Prazeres et al., 2012), bioethanol (Zoppellari and Bardi, 2013) and biodiesel (Carota et al., 2017). Other emerging researches are focusing on the use of lactose in the cheese whey for the production of organic acids and bioplastics-polyhydroxyalkonates (PHA) (Domingos et al., 2018; Colombo et al., 2016; Duque et al., 2014).

Polyhydroxyalkanoates (PHAs) are polyesters of microbial origin completely renewable and biodegradable (Colombo et al., 2017) able to substitute fossil-derived plastics which production is continuing growing, posing serious problem for their disposal. In this way it has been suggested that the production of biodegradable bioplastics could gradually reduce these problems (Schwarz et al., 2018). Bioplastic started to substitute fossil-fuel-derived plastic in the market, but today they cover only the 1% of the total world plastic production. Anyway its demand is expected to grow to approximately 2.44×10^6 Mg in 2022, representing biopolymers such as polylactic acid (PLA) and above all polyhydroxyalkanoate (PHA) the main biopolymers that will represent this growth.

The main limits that slow down PHA production are the high production costs due to the use of high value substrate such as sugar. In this way the recovery of secondary waste material, such as cheese whey could be interesting to reduce total PHA cost. In addition, it has been reported that volatile organic acids act better than sugar as substrates to produce PHA, since they are the direct metabolic precursors of PHA. Organic acids can be produced starting from wastes by dark fermentation (Villegas Calvo et al., 2018) producing H_2, that add value to the PHA production as H_2 can be used as chemical or to produce energy (Elbeshbishy et al., 2017).

Several studies have been done aiming the utilization of cheese whey for PHAs production by using selected mixed microbial cultures (MMC) (Colombo et al., 2016; Valentino et al., 2015; Duque et al., 2014). On the other hand, several studies have evaluated the feasibility of bio-hydrogen production from non-food biomass and industrial wastes including cheese whey as an alternative to the apparently costly industrial production of H_2 (Romao et al., 2019; Ottaviano et al., 2017). However, to the best of our knowledge, there are no studies done yet to evaluate the potential use of mixed microbial cultures (MMCs) for concomitant production of both H_2 and PHA from deproteinized cheese whey through a cascade concept.

To address the research gap, the present work attempts to integrate H_2 and PHA generation from two dairy waste streams (SCW and CCWP) coming from industrially produced and deproteinized cheese whey. Dark fermentation was suggested to produce hydrogen (H_2) and organic acids (OAs) via using a mixed microbial consortium. The optimal organic loading rates and their influence on the specific (H_2 yield per mol of sugar) and volumetric (H_2 NL d^{-1}) H_2 yields, and on total OAs produced and OAs speciation, was evaluated for the two dairy wastes. In the second step, the OAs produced were used for the production of (PHA) by employing mixed microbial cultures (MMCs).

2. Materials and methods

2.1. Feedstock collection and pre-treatment

Two industrial dairy waste streams, namely second cheese whey and concentrated cheese whey permeate, were used as substrates. Second cheese whey (SCW), the by-product of *Ricotta* cheese production, was collected from a dairy industry located in Brugherio, in North Italy. Concentrated cheese whey permeate (CCWP), the by-product of cheese whey ultrafiltration, was collected from a cheese whey treatment industry, located at Brescia, North Italy.

Prior to the dark fermentation processes, the two waste streams were exposed to an enzymatic hydrolysis step in order to convert lactose into more fermentable sugars, namely glucose and galactose. Due to the high total suspended solids in the SCW, the material was first centrifuged (8000 g, 15 min, 24 °C) in order to avoid any potential interferences of particulate matter with the enzymatic reaction during the hydrolysis step (Freire dos Santos et al., 2017). Conversely, the CCWP had low content of suspended solids and was thus used in its raw form.

To facilitate the lactose hydrolysis, a 1 mol L^{-1} acetate buffer solution was added to both SCW and CCWP in order to reach pH of 4.5 for optimal enzymatic reactions. The β-galactosidase enzyme from *Aspergillus oryzae* (Sigma-Aldrich, USA) was added in a proportion of 16 mg per g of lactose to effect the hydrolytic process, as determined during preliminary experiments. Then after, the mixed solutions were placed on a shaker and stirred at 150 rpm for 10 h at a temperature of 30 ± 1 °C. At the end of the hydrolysis, the two hydrolysed streams were frozen for future use.

2.2. Biohydrogen and organic acids production via dark fermentation

The dark fermentation processes were carried out in a continuously stirred tank reactor (CSTR) with a total capacity of 4 L with a working volume of 1.5 L. Condition adopted were those to attain faster sugar fermentation (Muri et al., 2016) such as previously described and reported in Villegas Calvo et al. (2018).

Six laboratory scale CSTR reactors were prepared and process performed at thermophilic conditions (55 °C) (Villegas Calvo et al., 2018). Inoculum used was represented by anaerobic digestate taken from a full-scale anaerobic digester fed with corn silage and operated under thermophilic condition (55 °C with a hydraulic retention time –HRT - of 40 days). The inoculum was thermally pre-treated before its use (1 h at 100 °C) to isolate the spore-forming bacteria (Ruggeri et al., 2015).

The six reactors were operated in batch for 3 days using pure glucose as carbon source to acclimatize the hydrogen-producing bacteria; then the reactors were feed with SCW and CCWP. The inoculum vs. feed ratio of 2:1 was used. In particular, 1 L of inoculum was added to 0.5 L of glucose solution adjusted with a mixed nutrients solution (Ren et al., 2006; Ooteghem et al., 2004), keeping the C/N ratio around 30, and pH of 5.5. After filling, the reactors were completely sealed and the headspace (2.5 L) was fluxed with N_2 to ensure anaerobic conditions. During the fermentation process, the pH was maintained in the range of 5.5–5.8 by adding 3 Mol L^{-1} KOH using automatically controlled peristaltic pumps connected to continuous pH measuring probes that were inserted into the fermenting solution. The pH range of 5.5–5.8 was chosen in order to inhibit the growth of methanogenic bacteria that could consume hydrogen during the fermentation process (Ruggeri et al., 2015).

After the batch-mode period, the continuous feeding of the reactors started. In the continuous operation mode, the first two reactors were fed with hydrolyzed second cheese whey (HySCW), another two were fed with the hydrolyzed, and concentrated cheese whey permeate (HyCCWP). The remaining two reactors were fed with pure glucose to serve as control treatments (CTRL). At each feeding interval, a portion of fresh inoculum (50 mL d^{-1}) was added to maintain stable microbial activity (Tenca et al., 2011). The HRT was of 2 days for all the six experimental reactors (Tenca et al., 2011) while, three organic loading rates increased by

38% of sugars content i.e., 8 g sugars L^{-1} d^{-1} (15 g COD L^{-1}) 11 g sugars L^{-1} d^{-1} (20.8 g COD L^{-1}) and 15.2 g sugars L^{-1} d^{-1} (28.7 g COD L^{-1}) corresponding to OLR-I, OLR-II and OLR-III, respectively, were studied to assess their effect on H_2 production. This choice come from Elbeshbishy et al. (2017) that reported in their review on dark biohydrogen fermentation an optimum concentration for H_2 production from cheese whey at 21 g COD L^{-1} and inhibition for >21 g COD L^{-1}.

After the process stability, i.e. constant H_2 production (8th day) was gotten, the OLR testing started for each substrate, by keeping the same OLR for 10 days, thus yielding a combined fermentation period of 41 days for each substrate. The effluent samples (SCW and CCWP), rich in organic acids (OAs) were retained and frozen for subsequent use for the PHA production process.

2.3. Fermented SCW and CCWP pre-treatment and use in PHA production

Prior to their use as substrates for PHA production, streams were centrifuged at 20,000 g for 15 min at room temperature in order to remove all the suspended solids. After the centrifugation, only the supernatants were collected. For the first stage of PHA production, the supernatants were diluted with deionized water and NH_4Cl was added in order to keep the C:N ratio equal to 10 and the organic load equal to about 1,500 mg COD L^{-1}. For the second stage of PHA production, the substrates were prepared by diluting the supernatants until a final organic load equal to 7,500 mg COD L^{-1}. In this case, no ammonia was added since it has been reported that N starvation can determine a greater conversion of carbon into PHA because of cell growth limitation (Serafim et al., 2004).

2.4. PHA production

PHA production by using a mixed microbial culture was performed in a two-stage process. In particular, the first stage was proposed to select PHA storing bacteria, starting from a mixed microbial culture (MMC) while the second stage allowed the production of PHA using the selected microbial culture. Two PHA production processes were performed by feeding the MMC with the fermented SCW (SBR 1) and then using the fermented CCWP as substrate (SBR 2).

The selection of PHA producing bacteria was performed in the first stage by using an inoculum of represented by activated sludge (8 g total suspended solids-TSS L^{-1}) collected at a wastewater treatment plant (Colombo et al., 2017).

The enrichment in PHA producing bacteria was carried out in a Sequencing Batch Reactor (SBR) with a working volume of 1 L, applying an aerobic dynamic feeding (ADF) strategy (Colombo et al., 2017). The selection trend was monitored by determining the duration of the feast phase achievable by measuring the dissolved oxygen (DO) concentration (optical probe FDO 925, WTW, Germany) in the selection media such previously well described (Duque et al., 2014; Valentino et al., 2014, Colombo et al., 2017). During the selection of PHA-accumulating bacteria, 500 mL of activated sludge were used as inoculum fed for each cycle with 500 mL of the fermented stream. The OLR was maintained at 1,500 mg COD L^{-1} d^{-1} and the C:N:P ratio was maintained at 100:9:4 (mmol C: mmol N: mmol P). To evaluate the performance of the selection process, the SBR was monitored once for each SRT.

The PHA accumulation was achieved via fed-batch assays carried out in a 500 mL working volume glass reactor, with continuous aeration and stirring. Doing so fermented streams were added to 200 mL of enriched culture (at least 3 SRTs from the beginning of the selection) (Pardelha et al., 2012) adopting a pulse-wise feeding method. The dissolve oxygen (DO) concentration in the media was monitored in continuous, and when DO strongly increased the fermented streams were fed to the reactor (Duque et al., 2014). Total substrate added (calculated as total C dosed) was calculated considering the carbon to the microorganisms ratio had to be the same as that inside the selection reactor, stopping the tests when no DO variation was observed after that the substrate was fed (Colombo et al., 2017).

For the accumulation tests, the operating conditions used were those adopted in the selection reactor, i.e. temperature of 21 ± 1 °C, aeration of 6 L min^{-1} and stirring at 110 rpm. The biomass from the selection process was subjected to accumulation tests using the same substrate as the carbon source and for each SBR two accumulation tests were performed in duplicate.

2.5. Analytical procedures

2.5.1. SCW and CCWP characterization before and after the pre-treatment

Both feedstock materials were characterized in terms of pH, total solids (TS), volatile solids (VS), Chemical Oxygen Demand (COD), total nitrogen (TKN), ammonia (N-NH_4^+) and total hosphorous (P), based on standard measurement procedures (The U.S. Department of Agriculture and The U.S. Composting Council, 2001).

Sugar determination was performed by a high-pressure liquid chromatography binary pump (Binary pump 1525, Waters) equipped with a 300 mm × 7.8 mm Aminex HPX-87H column and refractometer (Refractive Index 2410, Waters). A 0.004 mol L^{-1}. Sulfuric acid mobile phase was used with an operational temperature of 60 °C at a flow rate of 0.6 mL min^{-1} (Villegas Calvo et al., 2018).

The details of the feedstock (i.e., second cheese whey and concentrated cheese whey permeate) characteristics before and after pre-treatment are displayed in Table 1.

2.5.2. Biogas analyses and fermented liquid characterization during the dark fermentation

The daily effluent samples from the dark fermentation processes were analyzed for: pH, TS, COD, TKN, N-NH_4^+, organic acids content and alkalinity based on standard methods as above described. Organic acids (OAs) concentration and speciation were determined by using the same HPLC procedure adopted for sugars determination, but in this case, an UV-Detector (UV detector 2487, Waters) was used. The daily total sugar content in the media was determined by HPLC, as previously reported.

The gas produced was collected in dallying Gas Sampling Bags Supel™ Inert Multi-Layer Foil attached to each reactor's headspace and the volume of collected gas was measured with a Ritter Drum (Gas Meter TG 0.5/5, Germany) by directly connecting the detached gasbag to the gas meter.

The gas composition was analyzed by Gas Chromatograph (Agilent, Micro GC 3000A) equipped with two thermal conductivity detectors (TCD) and two different columns. Hydrogen and methane contents (v/v) were analyzed using a Molsieve/5A Plot column with nitrogen as the carrier gas at a flow rate of 30 mL min^{-1}. The carbon dioxide content was analysed using a different column (Alltech HPPLOT U) with helium as the carrier gas at a flow rate of 30 mL min^{-1}. The operational temperature of the injection port was 100 °C, while that of Molsieve/5A and PLOT U columns was maintained at 100 and 55 °C, respectively.

2.5.3. Substrate and biomass characterization during PHA production

The substrates used for PHA production during the selection and accumulation processes were characterized in terms of pH, TS, VS, COD, organic acids content, N-NH_4^+ and P content (Table S1).

During the selection trials, samples were taken during the cycle once in each SRT; every sample was characterized in terms of total

Table 1
Chemical characterization of second cheese whey and concentrated cheese whey permeate before and after the pre-treatment.

Parameters	SCW[a]	TSCW[b]	HyTSCW[c]	CCWP[d]	HyCCWP[e]
pH	6.05	5.7	4.6	5.7	4.5
TS (%, w/w)	6.8 ± 0.3	6.5 ± 0.2	5.5 ± 0.1	12	10.2
VS (%, w/w)	6.3 ± 0.3	5.9 ± 0.5	5.1 ± 0.5	10.5	8.9
COD (g L^{-1})	86.5 ± 0.7	84.5 ± 2.1	83.26	146.3 ± 3.5	136.2 ± 2.5
Lactose (g L^{-1})	57.7 ± 3	59.7 ± 0.5	3	128	6.05
Glucose (g L^{-1})	–	–	23	1	50.8
Galactose (g L^{-1})	–	–	23	1	45.9
TKN (g L^{-1})	1.43 ± 0	1.35 ± 0	1.1 ± 0.0	0.65 ± 0.01	0.56 ± 0.01
N-NH_4^+ (mg L^{-1})	110 ± 5	70 ± 3	59.8 ± 4.5	61.1 ± 1.9	52.2 ± 1.7
P (mg L^{-1})	503 ± 25	513 ± 16	438 ± 13	1,276.22	1,090.79

[a] SCW: second cheese whey.
[b] TSCW: treated second cheese whey.
[c] HyTSCW: hydrolyzed treated second cheese whey.
[d] CCWP: concentrated cheese whey permeate.
[e] HyCCWP: hydrolyzed concentrated cheese whey permeate.

suspended solids (TSS), volatile suspended solids (VSS), soluble COD, organic acids content, N-NH_4^+ content and PHA content. During accumulation trials, samples were taken continuously in order to measure TSS, VSS, soluble COD, organic acids content and PHA content. Biomass concentration was calculated as VSS according to the standard methods (Duque et al., 2014).

TSS and VSS were determined as reported by Valentino et al. (2015). Organic acids concentrations measured on filtered samples (filter diameter of 0.45 μm) were determined by high performance liquid chromatography (HPLC), as previously reported. The COD and the N-NH_4^+ content (filtered at 0.45 μm) were determined using cuvette test kits (Macherey-Nagel, Germany).

PHA were determined by GC MS using a method adapted from (Serafim et al., 2004) and previously described in Colombo et al. (2017) and Villegas Calvo et al. (2018).

2.5.4. PHA and active biomass growth yield calculation during PHA production

The PHA content in cells was referred to VSS on a mass basis as a percentage [PHA (%) = 100*(g PHA g^{-1} VSS)], taking into consideration that VSS are formed by both active biomass (X) and PHA (Duque et al., 2014). PHA was transformed into COD considering the stoichiometry: 1.67 mg COD mg^{-1} HB monomer and 1.92 mg COD mg^{-1} HV monomer (Valentino et al., 2014). HB precursors were assumed to be acetate, butyrate and lactate; valerate and propionate precursor of HV (Duque et al., 2014). X was calculated on a COD basis considering that 1 g of X contains 1.42 g of COD (Valentino et al., 2014).

For the SBR, the PHA produced in each cycle (ΔPHA) (%, w/w) was calculated taking into consideration the PHA content at the end of the feast phase and the PHA content immediately upon substrate addition (Oliveira et al., 2017). Parameters considered were calculated as in the following: i. the specific COD consumption rate (-q_{COD}, mg COD mg^{-1} COD_X h^{-1}) as the COD consumed during the feast phase and the time needed to deplete it per unit of active biomass; ii. the specific PHA storage rate (q_{PHA}, mg COD_{PHA} mg^{-1} COD_X h^{-1}) as the amount of PHA stored during the feast phase vs. the time needed to deplete the COD per unit of active biomass; iii. the specific growth rate (q_X, mg COD_X mg^{-1} COD_X h^{-1}) the amount of new active biomass produced during the feast phase vs. the time needed to deplete the COD per unit of active biomass (Valentino et al., 2014).

PHA yield for the SBR was determined considering the PHA stored, expressed as COD, vs. the amount of organic acids depleted (mg COD_{PHA} mg^{-1} $COD_{OA\text{-}cons.}$) and (mg COD_{PHA} mg^{-1} $COD_{cons.}$). In addition PHA yield was also reported on organic acids fed expressed as COD (mg COD_{PHA} mg^{-1} $COD_{OA\text{-}in}$) and on COD fed (mg COD_{PHA} mg^{-1} COD_{in}).

The growth yield was calculated as the ratio between the new biomass produced during the feast phase on COD basis and the amount of COD depleted (mg COD_X mg^{-1} $COD_{cons.}$), as reported Valentino et al. (2014).

In the accumulation batches, the specific rates and yields, except for q_X and the growth yield, that were not considered during the accumulation tests, were calculated as described before, for each pulse. In order to compare different accumulation tests, the average values for the first three pulses and for each parameter were considered.

2.5.5. Statistics

All the results related to H_2 percentage content, H_2 volumetric productivity, H_2 yield and PHA maximum contents were statistically analyzed by one-way ANOVA to compare means with a level of significant difference set at $p < 0.05$; the Tukey test was used as the method to compare means. All statistical analyses were performed by using SPSS software (SPSS Statistics 25.0, IBM, Armonk, NY, USA).

3. Results and discussion

3.1. Dark fermentation

3.1.1. Biohydrogen production

At OLR-I (8 g sugars L^{-1} d^{-1}), the biogas produced during the dark fermentation was composed mainly of H_2 (≈50%, v/v) and CO_2 with no CH_4 production for all the substrates tested (Table 2). The daily H_2 volume (NL H_2 d^{-1}) and the molar H_2 yields (mol H_2 mol^{-1} sugar) were of 2.38 ± 0.47 NL H_2 d^{-1} and 2.87 ± 0.54 NL H_2 d^{-1}, and of 1.70 ± 0.28 mol H_2 mol^{-1} sugar and 1.97 ± 0.38 mol H_2 mol^{-1} sugar for HySCW and HyCCWP, respectively (Table 2). The control experiment (glucose fed reactor) showed a good performance in both terms of daily H_2 volume and specific H_2 yields (Datar et al., 2007), indicating that the dark fermentation test was well designed. Nevertheless the results obtained with the two dairy streams were higher with respect to the control, and moreover, at OLR-I HySCW test showed a conversion yield from sugars to H_2 (mol mol^{-1}) close to the highest value reported in literature for similar substrates, while HyCCWP test obtained the highest value registered (Table 2).

3.1.2. Influence of OLR on biohydrogen production

Increasing the OLR rates from 8 g L^{-1} d^{-1} (OLR-I) to 11 g L^{-1} d^{-1} (OLR-II) resulted into a significant increase in the daily H_2 volume (NL d^{-1}) for all the substrates studied and in a significant growth of

Table 2
Hydrogen gas (H_2) and organic acids (OAs) production for the different organic loading rates (OLR) tested during the dark fermentation processes.

		OLR-I (8 g sugars L^{-1} d^{-1})			OLR-II (11 g sugars L^{-1} d^{-1})			OLR-III (15.2 g sugars L^{-1} d^{-1})		
		Control[a]	HySCW[b]	HyCCWP[c]	Control	HySCW	HyCCWP	Control	HySCW	HyCCWP
Hydrogen										
Daily Gas Volume	NL H_2 d^{-1}	1.94 ± 0.24*a*[*]	2.38 ± 0.47A	2.87 ± 0.54a	3.07 ± 0.48*b*	3.70 ± 0.44B	3.45 ± 0.64b	3.31 ± 0.49*b*	3.47 ± 0.31B	5.07 ± 0.60c
H_2	%, (v/v)	47.3 ± 3.9*a*	45 ± 2.8A	49.11 ± 1.42a	47.4 ± 3.6*a*	44.7 ± 2.8A	49.48 ± 2.0ab	46.8 ± 2.1*a*	43.2 ± 2.1A	50.7 ± 2.5b
H_2 yield	mol H_2 mol^{-1} sugars	1.42 ± 0.18*a*	1.70 ± 0.28B	1.97 ± 0.38a	1.63 ± 0.22*b*	1.93 ± 0.20C	1.87 ± 0.34a	1.33 ± 0.18*a*	1.37 ± 0.09A	1.93 ± 0.29a
Organic acids										
Net Total OAs	g L^{-1} d^{-1}	7.15 ± 0.57	7.83 ± 2.5	6.89 ± 3.4	10.6 ± 1	8.87 ± 2.04	7.76 ± 0.93	12.1 ± 0.5	14.6 ± 3.2	12.6 ± 1.3
Total OAs	mmol L^{-1}	86 ± 26*a*	97 ± 15A	94 ± 7a	131 ± 26*ab*	119 ± 10A	105 ± 17a	184 ± 35*b*	203 ± 30B	169 ± 4b
Formate	% OAs (mmol	2.1 ± 0.6	3.1 ± 0	3.2 ± 1.3	8 ± 2	0	2.8 ± 0.8	7.8 ± 1.1	2.5 ± 0.5	2.4 ± 0.5
Acetate	basis)	17.5 ± 9.5	52.5 ± 6.2	45.4 ± 3.7	16.8 ± 1.5	49.7 ± 1.9	45.3 ± 9.9	24.4 ± 4.9	54.7 ± 8.4	48.4 ± 0.6
Propionate		0	0	0	0	0	0	0	0	0
Isobutyrate		0	0	7.5 ± 0.9	0	0	0	0	0	0
n-butyrate		14.6 ± 7.5	18.6 ± 3	42.9 ± 1	16.1 ± 3.3	34.9 ± 4.3	49.8 ± 4.6	14.9 ± 5	36.3 ± 5.4	49.1 ± 1.4
Isovalerate		0	0	0	0	0	0	0	0	0
Lactate		65.7 ± 12.9	25.8 ± 6.4	1.1 ± 0	59.1 ± 13	15.4 ± 2.5	2 ± 0.7	52.9 ± 22.3	6.5 ± 0.6	0

The statistics are based on a One-Way ANOVA with emphasis on the effect of change in ORLs and the statistical differences were computed using Post hoc Tukey Tests analysis.
[a] Control: glucose fed reactor.
[b] HySCW: hydrolysed second cheese whey.
[c] HyCCWP: hydrolysed concentrated cheese whey permeate.
[*] Averages followed by the same letter are not statistically different for a $p < 0.05$ (italic lower case letters for CTRL; capital letters for HySCW; lowercase letters for HyCCWP).

the specific H_2 yield (mol H_2 mol^{-1} sugars) for the control and for HySCW (Table 2). However, the H_2 content (%, v/v) in the biogas remained the same for the three substrates tested. A further increase of OLR from 11 g L^{-1} d^{-1} (OLR-II) to 15.2 g L^{-1} d^{-1} (OLR-III) caused a significant decrease of the specific H_2 yield (mol H_2 mol^{-1} sugars) for both control and HySCW, while the daily H_2 volume (NL d^{-1}) and the content of hydrogen (%, v/v) in the biogas did not change significantly for them. On the other hand, increasing the OLR from 11 g L^{-1} to 15.2 g L^{-1} when fermenting HyCCWP resulted into a stable H_2 molar yield (mol H_2 mol^{-1} sugars) and in a significant growth of the daily H_2 volume (5.07 ± 0.6 NL d^{-1}). Moreover, at OLR-III, the highest H_2 content of 50.7 ± 2.5% (v/v) was obtained in HyCCWP experiment (Table 2).

Regarding specific H_2 yield (mol H_2 mol^{-1} sugars) and the daily H_2 volume (NL H_2 d^{-1}), the control test for all the OLRs runs, reported lower values than those obtained by using HySCW and HyCCWP and the fermented liquid had a high content (>50%) of lactate as the main OA. Thus, the lower H_2 molar yield on sugars measured in the control test could be attributed to the presence of lactic acid. In literature, the presence of lactic acid (lactate) as a metabolite during dark fermentation has been associated with lower hydrogen production (Park et al., 2016; Sikora et al., 2013). For instance, during the dark fermentation of galactose (at an OLR of 15 g L^{-1} d^{-1} and HRT of 24 h) in CSTR, Park et al. (2016) observed that when lactic acid increased in the reactor from 26.3 to 266.4 mg COD L^{-1}, the hydrogen yield decreased from 2.2 to 0.8 mol H_2 mol^{-1} galactose (Park et al., 2016). According to Park et al. (2016) lactate accumulation creates a substrate competition where instead of pyruvate which is the ideal substrate for the H_2 production via acetyl-CoA pathway, the lactate is metabolised leading to an almost zero-hydrogen balance pathway. The presence of lactate in the total percentage of OAs could also be the reason to explain the lower molar H_2 yield observed with HySCW at OLR-I with respect to the OLR-II due to the substrate competition effect.

As said before, the specific H_2 yields (mol H_2 mol^{-1} sugars) obtained in this study with HySCW and HyCCWP were in line with the highest results reported in literature for similar substrates (Table 3) or even higher in the case of HyCCWP in OLR-I. Anyway some Authors reported (Table 3) higher volumetric production (NL H_2 d^{-1}) even adopting lower HRT than those used in this work. Anyway for data reported in Table 3, it can be seen that in these cases a lower specific H_2 yield (mol H_2 mol^{-1} sugars) was gotten, except data from Romao et al., (2019) that by 28 h (HRT) and mesophilic condition reported similar H_2 production but using very concentrated sugar solution in 28 h.

The phenomenon could be attributed to the almost total absence of proteins of the dairy waste streams used in this study to perform the dark fermentation process. Proteins have a unique three-dimensional structure whose hydrolysis occurs very slowly during dark fermentation. Thus in typical fermentive biohydrogen reactors treating non-denatured or non-deproteinized cheese whey, the hydrolysis step is a major rate limiting factor and quite often the protein degradation involves some hydrogen consuming reactions (Cabrol et al., 2017). In addition, the high ammonium generated from proteins degradation tends to inhibit the hydrogen producing bacteria (Xiao et al., 2014). In our study the N-NH_4^+ content was in the range of 100–300 mg L^{-1} which is considered non inhibitory (Wang et al., 2018).

Another plausible effect could be that there was most likely no inhibition of H_2 producing bacteria from the accumulated OAs which is commonly reported in literature (Zhang et al., 2012). Moreover, there was a low lactate concentration and absolutely zero propionate in the produced OAs. Previous studies have reported that the propionate-type dark fermentation is a hydrogen-consuming pathway that produces mainly propionate, acetate and some valerate, without significant gas production (Cabrol et al., 2017; Koskinen et al., 2007) meanwhile, as already explained before, the presence of lactic acid as a metabolite during dark fermentation has been associated with lower hydrogen production.

3.1.3. Organic acids production

The main OAs produced from the dark fermentation from both HySCW and HyCCWP were acetate, n-butyrate, and relatively smaller amounts of lactate. The acetate yield obtained from HySCW and HyCCWP were in the range of 49.7–54.7 % of total OAs and 45.3–48.4% of total OAs, respectively, being these values to be compared with the 16.8–24.4% of total OAs obtained from pure glucose (control). The n-butyrate obtained during dark fermentation of HySCW and HyCCWP was in the range 18.6–36.3 % of total OAs and 42.9–49.8% of total OAs, respectively, compared

Table 3
Literature data for H_2 production and yield obtained from cheese whey via dark fermentation and mixed microbial cultures (MMCs).

Reactor type	Inoculum	Substrate	Operating temperature (°C)	pH	HRT (h)	OLR (g sugars L^{-1} d^{-1})	H_2 production (L H_2 L^{-1} day^{-1})	H_2 yield (mol H_2 mol^{-1} hexose)	Ref.
CSTR[a]	Anaerobic granular sludge from full-scale UASB reactor treating confectionery wastewater.	Cheese whey powder	37	5.9	6	92.4–138.6	12.5–25.1	1.05–1.4	Davila-Vazquez et al. (2009)
CSTR	Indigenous microbial consortia cultured from cheese whey wastewater.	Undiluted cheese whey	35	5.2	24	43.8	1.9–2.9	0.61–0.78	Venetsaneas et al. (2009)
CSTR	Anaerobic granular sludge from full-scale UASB reactor treating confectionery wastewater.	Cheese whey powder	37	5.9	6	95–190	17.2–25.8	1.41–1.09	Cota-Navarro et al. (2011)
AFBR[b]	Sludge from UASB reactor treating swine wastewater.	Cheese whey	30	6.0	6	4 (g sugars L^{-1})	2.88	0.55–0.64	Ferreira Rosa et al. (2014)
AFBR	Effluent from UASB reactor sugarcane silage	Cheese whey powder	55	5.0	4–0.5	29.4–235.2	12–98.4	1.84–0.29	Ottaviano et al. (2017)
CSTR	Kitchen waste compost.	Raw cheese whey	30	5.5	24	30.0 (g COD L^{-1} d^{-1})	0.8	0.45	Castello et al. (2018)
CSTR	Not indicated	Ultra filtered cheese whey	36	5.5	12–6	60–120 (g COD L^{-1} d^{-1})	NA	0.67–0.92	Montecchio et al. (2018)
SCTR[d]	Effluent from UASB reactor treating diary wastewater.	Cheese whey permeate	30	5.5	28	20 (g sugars L^{-1})	3.3	1.95	Romao et al. (2019)
CSTR	Anaerobic digestate from full-scale thermophilic digester treating corn silage.	HySCW[e]	55	5.5–5.8	48	8–15.2	2.38–3.47	1.7–1.37	This study
CSTR	Anaerobic digestate from full-scale thermophilic digester treating corn silage.	HyCCWP[f]	55	5.5–5.8	48	8–15.2	2.87–5.07	1.97–1.93	This study

[a] CSTR: Continuously stirred tank reactor.
[b] SCTR: Semi-continuous tank reactor.
[c] NA: Non available (data not given by authors).
[d] AFBR: Anaerobic fluidized bed reactors.
[e] HySCW: Hydrolysed Second Cheese Whey.
[f] HyCCWP: Hydrolysed Concentrated Cheese Whey Permeate.

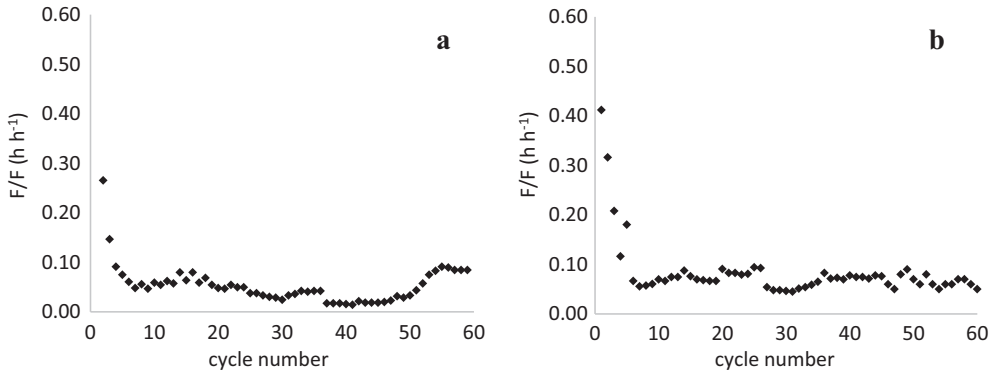

Fig. 1. Selection trend in terms of feast to famine ratio (F/F) for both SBR1 (fermented second cheese whey) (a) and SBR2 (fermented concentrated cheese whey permeate) (b).

to 14.6–16.1% of total OAs obtained from pure glucose (control). It is important to mention that during the fermentation with pure glucose, lactate was the main acid produced, reaching the concentration of 97.4 ± 4.41 mmole L^{-1} in OLR-III (52% of total OAs) (Table 2).

Increasing OLR resulted in an increase in OAs production for all the substrates, as expected, but with a significant increase moving from OLR-II to OLR-III. The maximum values of total OAs were obtained for the highest OLR (OLR-III = 15.2 g sugar L^{-1} d^{-1}). During this period, the total OAs produced (as average) were of 12.1 ± 0.5 g OAs L^{-1} d^{-1}, 14.6 ± 3.2 g OAs L^{-1} d^{-1} and 12.6 ± 1.3 g OAs L^{-1} d^{-1} for glucose (control), HySCW and HyCCWP, respectively. It is important to note that the amount of OAs obtained from HySCW and HyCCWP in OLR-III was in line with those reported in literature for dark fermentation processes of dairy wastes by applying similar OLR (Romao et al., 2019; Ottaviano et al., 2017).

Considering the dark fermentation process for organic acids production, the highest OAs production was obtained at OLR-III for both HySCW and HyCCWP, moreover the composition in organic acids of the two fermented streams remained quite stable for the three OLRs (half butyrate and half acetate for HyCCWP and mainly acetate followed by butyrate and lactate for HySCW), apart for the OLR-I where the fermented HySCW showed a higher presence of lactic acid. Since one of the objectives of this study is related to the subsequent use of the formed OAs in PHAs production, the obtained mix of OAs from OLR-III, due to highest organic acids concentration, was deemed good precursors for PHA producing bacteria according to previous studies by Colombo et al. (2017).

Table 4
Comparison among parameters characterizing MMCs selection performed in this study and in the previous work done (Colombo et al., 2016) with fermented dairy by-products.

Substrate	ΔPHA[a]	Polymer composition[b]	$-q_{COD}$[c]	q_{PHA}[d]	q_X[e]	PHA yield[f]	Growth yield[g]	Ref.
FSCW[h]	11.42 ± 0.93	100:0	508.59 ± 132.7 0.43 ± 0.11	387.48 ± 110.72 0.35 ± 0.10	157.83 ± 54.69 0.16 ± 0.05	0.76 ± 0.01 0.80 ± 0.02	0.31 ± 0.07 0.36 ± 0.08	This study
FCCWP[i]	11.04 ± 2.67	100:0	328.62 ± 17.53 0.28 ± 0.0	229.79 ± 47.81 0.21 ± 0.04	90.89 ± 45.38 0.09 ± 0.05	0.70 ± 0.11 0.73 ± 0.11	0.28 ± 0.15 0.33 ± 0.17	This study
FCW[j]	13.21 ± 1.15	100:0	– 0.4 ± 0.0	– 0.2 ± 0.00	– 0.08 ± 0.01	– 0.7 ± 0.01	– 0.23 ± 0.01	Colombo et al. (2016)

[a] ΔPHA: difference between PHA content (%, w/w) at the end of the feast and immediately upon substrate addition.
[b] Polymer composition expressed as ΔHB/ΔHV in % (w/w).
[c] $-q_{COD}$: specific COD consumption rate. For each cell the first value is expressed as mg COD mg^{-1} COD_X h^{-1}, the second value as mmol C $mmol^{-1}$ C_X h^{-1}.
[d] q_{PHA}: specific PHA storage rate. For each cell the first value is expressed as mg COD_{PHA} mg^{-1} COD_X h^{-1}, the second value as mmol C_{PHA} $mmol^{-1}$ C_X h^{-1}.
[e] q_X: specific growth rate during feast phase. For each cell the first value is expressed as mg COD_X mg^{-1} COD_X h^{-1}, the second value as mmol C_X $mmol^{-1}$ C_X h^{-1}.
[f] PHA storage yield. For each cell the first value is expressed as mg COD_{PHA} mg^{-1} $COD_{cons.}$, the second value as mmol C_{PHA} $mmol^{-1}$ $C_{cons.}$.
[g] Growth yield during feast phase. For each cell the first value is expressed as mg COD_X mg^{-1} $COD_{cons.}$, the second value as mmol C_X $mmol^{-1}$ $C_{cons.}$.
[h] FSCW: fermented second cheese whey.
[i] FCCWP: fermented concentrated cheese whey permeate.
[j] FCW: fermented cheese whey 1 (Colombo et al., 2016).

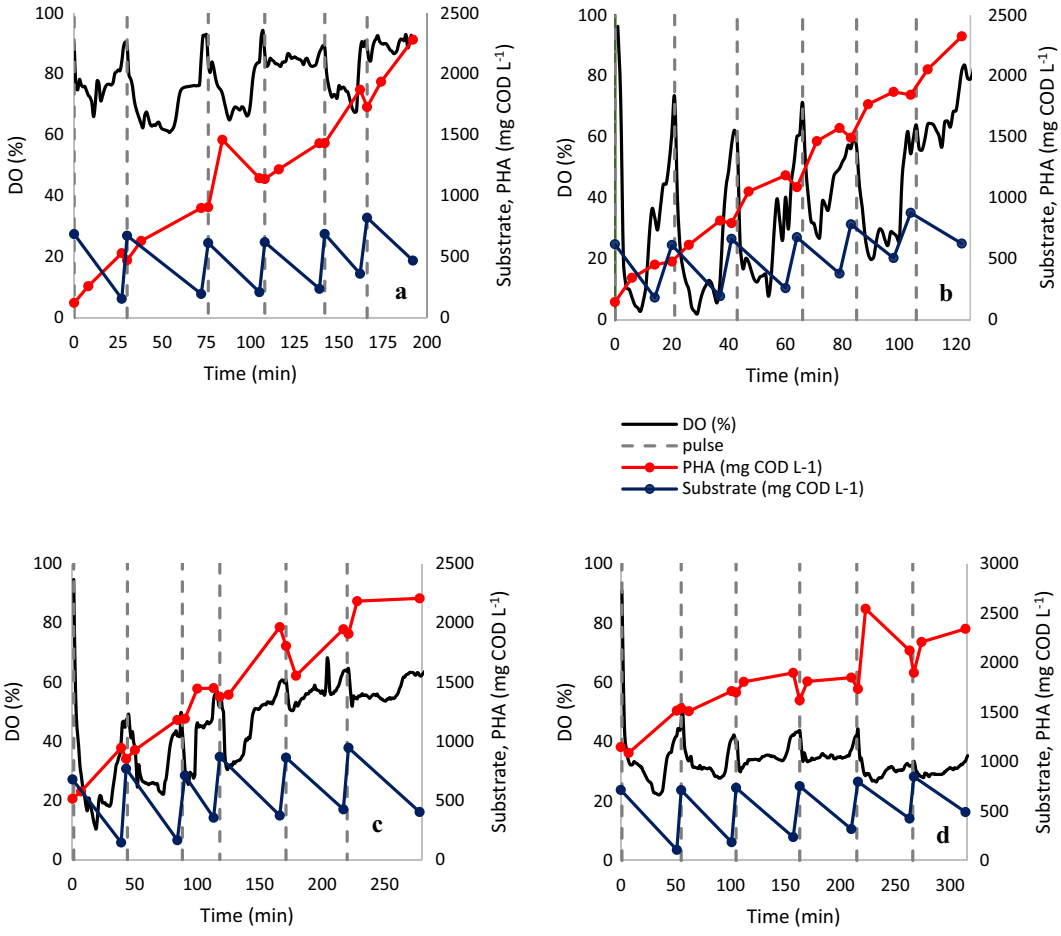

Fig. 2. PHA accumulation processes performed with fermented second cheese whey (a, b) and fermented concentrated cheese whey permeate (c, d).

3.2. PHA production

The two fermented liquid fractions coming from the dark fermentation step (OLR-III), after being appropriately treated (see Table S1), were used as substrates to select PHA storing bacteria starting from an activated sludge by using two sequencing batch reactors (SBR), i.e. SBR1 and SBR2 for fermented SCW and fermented CCWP, respectively. The selection lasted for 30 days for both the reactors.

The feast to famine ratio (F/F) (Fig. 1), that is a rapid indicator of the enrichment trend in PHA storing bacteria, was on average, 0.07 and 0.08 for SBR1 (Fig. 1a) and SBR2 (Fig. 1b), respectively (except for the first cycles where the MMC was adapting to the new growing conditions).

The two F/F average values were clearly lower than 0.33, to be considered as the upper limit for the F/F to maintain good conditions for the selection of PHA storing bacteria in the SBR (Valentino et al., 2014).

During the feast phase for both the SBRs, the specific PHA storage rate was higher than the specific microbial growth rate, indicating a preferred consumption of the carbon source for PHA biosynthesis rather than for bacterial growth (Table 4).

The performances of the two SBRs performed in this work were in line with those previously reported for similar substrates and adopting similar conditions (Colombo et al., 2016). In particular, the two MMCs selected in this study reported comparable ability to convert the consumed substrate into PHA (i.e. PHA storage yield) and similar amount of polymer stored during the feast phase (i.e. as average 11.89 ± 2.19% - w/w) with respect to the MMC selected by Colombo et al. (2016) (Table 4). Moreover, the polymer stored at the end of the feast phase by the two MMCs selected in this work was represented by polyhydroxybutyrate (PHB), as happened in the work performed by Colombo et al. (2016), as logical consequence of the presence of mainly HB precursors (acetate, butyrate and lactate) in the three fermented dairy streams fed to the reactors.

As regards to the PHA accumulation (Fig. 2), even if the tests performed with CCWP presented a higher initial amount of PHA with respect to the tests performed with SCW, both the MMCs selected with fermented SCW and fermented CCWP showed similar performances. In particular, both of them reported a PHA storage yield on organic acids consumed, as average of the four accumulation tests performed, of 0.78 ± 0.2 mg COD_{PHA} mg^{-1} $COD_{OA\text{-}cons.}$, that was about 10–20% higher than the PHA storage yield on total COD consumed (average of 0.72 ± 0.21 mg COD_{PHA} mg^{-1} $COD_{cons.}$). This result indicated the preferred utilization of organic acids as carbon sources for PHA production (Table 5).

Concerning PHA accumulation yield on the substrate fed, the two selected microbial consortia reported analogous results, i.e. as average, 0.74 ± 0.14 mg COD_{PHA} mg^{-1} $COD_{OA\text{-}in}$ and 0.45 ± 0.13 mg COD_{PHA} mg^{-1} COD_{in}, referred to organic acids and COD fed, respectively (Table 5).

The polymer stored during the accumulation trials was completely represented by PHB while the average maximum polymer content on cell dry weight obtained for the two streams tested was 62 ± 4.5% (g PHA g^{-1} VSS) for fermented SCW and 55.1 ± 1.3% (g PHA g^{-1} VSS) for fermented CCWP. Non-significant differences were found between the two substrates ($p < 0.05$; N = 4) (Table 4). The lower amount of polymer stored by using fermented CCWP was probably due to the higher phosphorous content in the substrate with respect to fermented SCW (Table S1). It has been previously reported that high P concentration can negatively interfere with the ability of PHA storing bacteria to accumulate PHA (Venkateswar Reddy and Venkata Mohan, 2012).

Comparing the accumulation tests performed with fermented SCW and fermented CCWP with other studies related to PHA production from MMCs by using fermented dairy by-products (Table 5) it was possible to see that in this study very high yields of PHA storage, referred to organic acids consumed, were obtained. These data were in line with the highest yield reported in literature by using dairy wastes as substrate (0.86 ± 0.07 mmol C_{PHA} $mmol^{-1}$ $C_{OA\text{-}cons.}$), which was obtained by Oliveira et al. (2017). The conversion yield of the consumed substrate into PHA calculated by Oliveira et al. (2017) considered also the presence of a small amount of ethanol other than organic acids in the substrate; if they had calculated the yield without considering ethanol (that is

Table 5
Comparison among parameters characterizing PHA accumulation tests performed in this study and in other studies carried out with fermented dairy by-products.

Substrate	PHA content end test[a]	Polymer composition[b]	$-q_{COD}$[c]	q_{PHA}[d]	PHA Yield[e]	PHA Yield[f]	PHA Yield[g]	PHA Yield[h]	PHA Yield[i]	PHA Yield[j]	Ref.
FSCW[k]	62 ± 4.5	100:0	644.04 ± 109.7 40.55 ± 0.09	471.60 ± 92.1 90.42 ± 0.08	0.84 ± 0.28 0.88 ± 0.30	0.77 ± 0.14	0.75 ± 0.17	0.46 ± 0.10	682 ± 130	274 ± 60	This study
FCCWP[l]	55.1 ± 1.3	100:0	437.80 ± 78.83 0.37 ± 0.07	350.00 ± 69.3 90.31 ± 0.06	0.82 ± 0.13 0.87 ± 0.14	0.72 ± 0.14	0.69 ± 0.26	0.45 ± 0.16	567 ± 210	268 ± 100	This study
FCW[m]	~32	87:13	– 0.29 ± 0.02	– 0.25 ± 0.01	– 0.86 ± 0.07 OA contain also ethanol (13% C mol)	–	–	–	–	–	Oliveira et al. (2017)
FCW1	65.9 ± 4.6	100:0	– 0.3 ± 0.0	– 0.2 ± 0.0	0.6 ± 0.0 –	–	–	–	–	–	Colombo et al. (2016)
FCW2	81.4 ± 5.7	60:40	– 0.5 ± 0.1	– 0.4 ± 0.0	0.7 ± 0.1 –	–	–	–	–	–	Colombo et al. (2016)
FCW	–	85 ± 2:15 ± 2	227 –	106 –	0.4 –	–	–	–	–	–	(Valentino et al. (2015)
FCW	65	81:19	– 0.45 ± 0.07	– 0.3 ± 0.06	0.67 ± 0.13 –	–	–	–	–	–	Duque et al. (2014)

[a] PHA content at the end of the test expressed as % (g PHA g^{-1} VSS).
[b] Polymer composition expressed as ΔHB/ΔHV in % (w/w).
[c] $-q_{COD}$: specific COD consumption rate. For each cell the first value is expressed as mg COD mg^{-1} COD_X h^{-1}, the second value as mmol C $mmol^{-1}$ C_X h^{-1}.
[d] q_{PHA}: specific PHA storage rate. For each cell the first value is expressed as mg COD_{PHA} mg^{-1} COD_X h^{-1}, the second value as mmol C_{PHA} $mmol^{-1}$ C_X h^{-1}.
[e] PHA storage yield on organic acids consumed. For each cell the first value is expressed as mg COD_{PHA} mg^{-1} $COD_{OA\text{-}cons.}$, the second value as mmol C_{PHA} $mmol^{-1}$ $C_{OA\text{-}cons.}$.
[f] PHA storage yield expressed as mg COD_{PHA} mg^{-1} $COD_{OA\text{-}in}$.
[g] PHA storage yield expressed as mg COD_{PHA} mg^{-1} $COD_{cons.}$.
[h] PHA storage yield expressed as mg COD_{PHA} mg^{-1} COD_{in}.
[i] PHA produced expressed as g PHA kg OA_{in}.
[j] PHA produced expressed as g PHA kg COD_{in}.
[k] FSCW: fermented second cheese whey.
[l] FCCWP: fermented concentrated cheese whey permeate.
[m] FCW: fermented cheese whey.

another possible precursor of PHA), the final value would have been higher with respect to what reported.

Regarding the maximum PHAs amount stored by the cultures during the accumulation tests, the result of this study was in a similar range of average values calculated considering the maximum PHA content for the four accumulation tests performed i.e. 61.1 ± 20.8% (g PHA g^{-1} VSS), reported in the literature when using fermented dairy by-products as substrates.

The accumulation tests performed by using fermented SCW as substrate gave a total PHA production yields of 274 ± 60 g PHA kg^{-1} COD_{in}, i.e. 682 ± 130 g PHA kg^{-1} OA_{in} and of 268 ± 100 g PHA kg^{-1} COD_{in}, i.e. 567 ± 210 g PHA kg^{-1} OA_{in}, when fermented CCWP was used as carbon source. In both processes the overall PHA production yield on organic acids fed was similar and for fermented SCW it was higher than that reported by Domingos et al. (2018) by using fermented cheese whey to feed a pure culture of *Cupriavidus necator* DSMZ 545 (600 g PHA kg^{-1} OA).

Despite the differences in terms of maximum amount of PHA reached, the very similar performance reported by the cultures selected with fermented SCW and fermented CCWP represents a good result in terms of reproducibility of PHA production process by using two different fermented dairy streams.

Taking into consideration the COD yield after dark fermentation calculated for OLR III of 0.76 g COD g^{-1} COD_{sugar} and 0.66 g COD g^{-1} COD_{sugar} for HySCW and HyCCWP, and PHA yield of 0.46 g COD_{PHA} g COD_{in} and 0.45 g COD_{PHA} g COD_{in} for HySCW and HyCCWP, the overall calculated yield were as in the following: 0.35 g COD_{PHA} g COD_{sugar} and 0.30 g COD_{PHA} g COD_{sugar}.

Now taking into consideration cheese whey waste costs equal to zero because they represent a waste to be safely disposed, the cost for PHA production equal to 1,000 € Mg^{-1} (Villegas et al., 2018) and the PHA value of 3,410 € Mg^{-1} (Villegas et al., 2018), and considering PHA yields (calculated from data before reported) of 14 kg PHA m^3 HySCW and 24,7 kg PHA m^3 HyCCWP, the net revenue can be calculated in: 33.6 € m^3 HySCW and 59.3 € HyCCWP, i.e. 610 € Mg_{TS}^{-1} HySCW and 581 € Mg_{TS}^{-1} HyCCWP.

4. Conclusions

The two bioprocess approach from HySCW and HyCCWP allowed to get very good yields in both the steps performed. During the dark fermentation step, the increase of OLR resulted in higher volumetric H_2 yield and OAs production for both the substrates, keeping good performance of sugars conversion into H_2. The quite constant OAs composition of the fermented streams allowed their use as substrates in PHA production step, where OAs conversion into PHA was high and similar for both the fermented dairy streams, underlining the reproducibility of the process. For the results obtained, this study could be considered for larger scale application.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.wasman.2019.05.052.

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