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Acidogenic Valorisation of High Strength Waste Products from Food Industry

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1. Introduction

Awareness concerning the environment and the use of clean technologies is growing worldwide. As a consequence, research on biodegradability and use of renewable resources for industrial processes has been intensive in the last years. In addition, product and process innovation in food industries is widely regarded as an essential element of competition between food companies, as it will improve their business performance. To face these new challenges, relevant changes in the food manufacturing processes may occur, involving for example the use of different raw materials as an ingredient in new food products. As a result, food processing wastewater will contain complex organic compounds with refractory properties that may compromise the biological treatment processes already existing in the wastewater treatment plants (WWTP) and consequently the fulfilment of legal requirements for wastewater discharge. To overcome this situation, segregation of specific wastewater process streams with refractory characteristics and its individual pre-treatment will be important for a viable and stable treatment in the already existing WWTP.

In this context, acidogenic fermentation is gaining scientific and commercial interest, since it allows improved wastewater treatability as well as additional recovery of bioproducts. Phase separation in anaerobic systems has been studied to enhance treatment efficiencies and/or generate bioproducts from different industrial wastewaters. Several studies contributed to a first technological shift, since optimum growing conditions for acidogenic microorganisms have been developed to treat complex organic substrates that demand for high hydrolytic efforts.

Phase separation in the anaerobic digestion process implies a process configuration employing separate reactors for acidification and methanogenesis connected in series, allowing optimisation of each process separately. Many types of microorganisms and biochemical pathways are involved in the acidogenesis and consequently a large number of bioproducts are usually formed, including carboxylic acids and alcohols.

Currently, acidogenic processes are widely spread among bioreactor engineered technology, and are usually designed for customised applications and for particular high strength waste products. Although methane is usually considered the final product of anaerobic degradation, there are some other valuable by-products which can compete with methane and has a market for itself, such as volatile fatty acids (VFA) that can constitute a valuable resource for biodegradable polymer production (poly-hydroxyalkanoates – PHA) or biological nutrient removal.

The acidogenic microorganisms have specific physiology, nutritional requirements, optimal pH, growth and substrate uptake kinetics. Although global and two-phase anaerobic processes are well studied, little information exists on adequate design and operation of acidogenic digesters. However, higher kinetic rates for acidogenic metabolism may be the most interesting feature on industrial exploitation of this process.

It has been demonstrated that the composition of VFA produced under acidogenic fermentation can be affected by environmental conditions such as pH, retention time, and temperature. In the two-phase anaerobic processes, the physical separation between acid-formers and methanogenic microorganisms leads to the absence of hydrogen uptake by the methanogens, which will lead to hydrogen accumulation and subsequent alteration of the fermentation pathways during the acidogenic phase. So, there will be a shift towards the utilization of a metabolic pathway that does not use proton reduction as a mean of disposing electrons, hence resulting in the production of high amounts of butyrate as an important electron sink product (Fernandes, 1986). Nowadays, this is one of the starting points for the scientific and technological interest on the acidogenic phase in order to understand the nature of metabolic pathways and the energetic content of key-intermediates, which will result in different specific turn-over rates. So, further research is needed in order to establish relations between process conditions during the acidogenic fermentation and VFA production for different potential feedstock.

This chapter aims to contribute to the use of acid-phase anaerobic digestion of high strength waste products as a practical and economically feasible waste management option together with the recovery of valuable organic acids. Hence, the objective of this chapter is to examine acidogenic fermentation of four organic residues (sugar cane molasses, wastewater containing spent coffee grounds, dairy processing fatty slurry and cheese whey), from the point of view of their potential use as feedstock, because VFA produced in the process have a variety of industrial uses. Therefore this chapter intends to highlight acidification as a powerful tool for organic waste streams treatment and management.

1.1 Acidified products as raw materials for PHA production

PHA are polyesters of hydroxyalkanoates stored by more than 100 bacterial genus as sources of carbon/energy or reducing-power. PHA are synthesized and accumulated as intracellular granules usually when there is a limitation in essential component for growth in the presence of excess carbon source. Depending on the substrate provided, microorganisms can include a wide variety of 3-hydroxy fatty acids in the PHA (Braunegg et al., 1998). One of the most widely studied PHA is poly-3-hydroxybutyrate (PHB). PHB is very brittle and crystalline, which makes difficult its utilization in applications that require more flexibility. The incorporation of different monomer units, other than hydroxybutyrate

(HB), in the polymer chain, results in the synthesis of copolymers with improved mechanical properties (Braunegg et al., 1998). PHA are currently produced at the industrial scale using pure microbial cultures and expensive substrates, both contributing to high selling prices. The substrate is one of the main cost factors of PHA production corresponding to 40% of production costs (Reis et al., 2003). The industrial processes developed so far utilize pure cultures or recombinant microorganisms that require very tight sterility conditions and control contributing for high production costs. The use of waste organic carbon and mixed cultures can significantly reduce the price of PHA. The utilization of mixed microbial cultures (MMC) facilitates the use of complex substrates, since microbial population can adapt continuously to changes in substrate. Selection of microorganisms occurs on the basis of its high capacity for PHA storage. Consequently, there is no need for sterile fermentation systems, which contributes to the reduction of the final PHA price (Serafim et al., 2008a).

PHA production in MMC occurs when unbalanced growth conditions are verified, especially those resulting from the periodic absence of an essential nutrient. The most studied processes result from the alternation of oxygen or carbon availability. The former situation, typical in biological nutrient removal systems, usually occurs when the microbial community is submitted to alternating anaerobic (AN) and aerobic (AE) conditions with the carbon substrate supplied at beginning of the AN period. Under AN conditions the microorganisms cannot grow due to the lack of electronic acceptor and some of the bacteria present start to store the external carbon as PHA with simultaneous glycogen degradation. Glycogen, the second internal polymer involved, works as a sink of equivalent reductors. When the AE period begins, stored PHA will be consumed for microbial growth and to replenish the glycogen pool (Serafim et al., 2008a).

Storage of PHA by MMC occurs also when they are subject to transient carbon supply resulting from the alternation of long periods of lack of substrate (famine) with periods with a short time of excess of substrate (feast). During the latter period, substrate uptake is mainly directed to PHA storage and, to a lesser extent, to the biomass growth. After substrate exhaustion, the stored polymer is used as energy and carbon source. This process is known as aerobic dynamic feeding (ADF) or feast and famine. Carbon limitation for a long period causes changes in the macromolecular composition of cells, requiring a physiological adaptation of microorganisms when exposed to high substrate concentration. The dominance of PHA-storing organisms will result from the selective pressure imposed by the operational conditions (Serafim et al., 2008a).

In both situations the carbon substrate should be composed by VFA. In order to obtain a stream rich in these compounds, acidification of the raw material selected for PHA production by MMC is required. The composition of VFA obtained after the acidification step is critical for the development of the process, because not only influences the type of polymer produced but also the microbial composition of the selected population.

By supplying different types of VFA to the culture, PHA with different monomer composition were obtained. Not only in the ADF system using synthetic substrates (Lemos et al., 2006; Serafim et al., 2008b) or a real fermented substrate, like fermented sugar cane molasses (Albuquerque et al., 2007) but also in the AN/AE process with fermented molasses (Pisco et al., 2009). In most of the cases, a feeding stream rich in VFA with even carbon

atoms, such as acetate and butyrate resulted most of the times in the formation of PHB homopolymers. The predominance of VFA with odd carbon atoms composition, such as propionate or valerate led to a PHA copolymer with a higher content in 3-hydroxyvalerate (3HV), which is much more interesting from a commercial point of view (Lemos et al., 2006). Other monomers were also observed, like 2-methyl-3-hydroxyvalerate when synthetic media containing propionate or a mixture of acetate and propionate were fed to a MMC selected under ADF conditions (Lemos et al., 2006). Pisco et al. (2009) reported for the first time the presence of 3-hydroxyhexanoate in PHA produced by a MMC, in this case produced from fermented molasses under AN/AE alternating conditions. Moreover, Albuquerque et al. (2007) found a direct correlation between the VFA profile of the feeding and the polymer final monomer composition. All of these monomers contribute to enhance the polymer properties and it was verified by Serafim et al. (2008b) and Albuquerque et al., (2011) that the properties of PHA produced by MMC follow the same trend as verified for PHA obtained by pure cultures.

Lemos et al. (2008) showed that by supplying different VFA composition were selected MMC with difference microbial composition. Despite the differences in the population, PHA produced by these populations showed no significant differences in their properties, except those that related with the monomer compositions (Serafim et al., 2008b). The stability of the polymer composition is an important aspect of PHA production from real complex wastes by mixed cultures. Because of organic waste streams are often sensitive to seasonal and process variations, acidification processes for their subsequent valorisation into PHA must accomplish manipulation of the operational parameters, in order to achieve a coherent and appropriate VFA profile (Albuquerque et al., 2007, 2011; Serafim et al., 2008a).

2. Methodology

2.1 Waste currents characteristics

In this chapter, acidification processes of four industrial waste products are presented and discussed - sugarcane molasses, spent coffee grounds, dairy processing fatty slurry, and cheese whey. These industrial waste products were selected because they currently constitute environmental problems for their producing industries. Due to their high organic content, these wastes often lead to instability issues inside the conventional bioreactors used for their treatment. Since treatment is mandatory prior to discharge, simultaneous valorisation through acidogenic fermentation was here investigated, from a wastewater treatment point of view with recovery of valuable constituents. The choice of these organic currents covers a large spectrum in the acidification field, since particular composition of each one leads to different biodegradation behaviours.

Organic waste products were collected in different Portuguese food industries. Molasses is a by-product of a sugar refinery industry, and consists mostly of carbohydrates. Spent coffee grounds are a slurry material resulting from instant coffee beverages manufacture, and consist mostly of structural polysaccharides. Dairy processing fatty slurry was collected from a Dissolved Air Flotation system treating dairy industrial effluent and consists mostly of oils and fats. Cheese whey was collected in a medium scale cheese production factory, consisting mostly of proteins. Their organic content is presented in Table 1.

Waste stream	tCOD (g L^{-1})	sCOD (g L^{-1})	pH	tVFA (mg L^{-1})
Sugarcane molasses	952±130	896±102	6.8±0.4	31±4.9
Spent coffee grounds	61±15	45±16	4.1±0.3	116±10
Dairy fatty slurry	324±71	38±11	5.2±0.1	-
Cheese whey	103±13	98±10	6.2±0.5	22±5.3

Table 1. Main characteristics of the organic waste products from food industry (average ± standard deviation)

2.2 Experimental design and hydraulic modes

Acidogenic fermentation of each waste product was evaluated in order to determine optimum operational conditions for the maximisation of VFA yield. The degree of acidification was generally used to assess acidogenic potential for each current, and it was calculated from the total VFA in COD equivalents relative to the influent soluble COD (Bengtsson et al., 2008). Different approaches and results permitted to investigate their acidogenic potential. Two different types of hydraulic modes of operation and reactors were considered: batch and continuous. While batch experiments permitted a preliminary estimation of the acidogenic potential of each waste product, continuous reactors were operated under long term to ensure and validate continuous production of acidified effluents. Upflow Anaerobic Sludge Blanket (UASB) reactor was operated with dairy fatty slurry and Moving Bed Biofilm Reactors (MBBR) type units were used for molasses, spent coffee grounds and cheese whey. Figure 1 illustrates the main aspects of each bioreactor.

Start-up of the 5 L batch reactors consisted of addition of established volumes of anaerobic biomass and of selected effluents, as well as inorganic nutrients (Capela et al., 1999), and distilled water to make up all the volumes to 5 L. The reactors were purged with a stream of nitrogen for about 1 min, to remove any oxygen content, and then sealed. Stirring was performed with magnetic stirrers and thermostatic condition was ensured with hot water baths. Samples were collected with a syringe at the top of the reactors.

For the test of acidification of dairy processing fatty slurry in continuous mode a UASB reactor made of Perspex with a height of 86.4 cm and a working volume of 6 L was used. The reactor had a gas-solid-liquid separator at the top. The temperature was kept at 35±1 ^{o}C by means of a water jacket. Biogas production was monitored with wet gas meters. The feed was mixed constantly with a mechanical stirrer and pumped to the bottom of the reactor through a peristaltic pump. Samples of the treated waste were collected from the exit in the top settler.

A 2.54 L anaerobic MBBR reactor permitted combination of suspended growth and fixed film processes, taking the advantages of both without being restrained by their disadvantages. Before assemblage, about 40% of the reactor volume was filled with biofilm carrier elements made from polyethylene (Bioflow 9), with a specific surface area of 800 m^2 m^{-3}. Stirring was performed with a mechanical stirrer, and feeding with a peristaltic pump. Thermostatic condition was ensured with hot water baths. Mixed liquor was collected by overflow in a settler with a volume of 0.84 L.

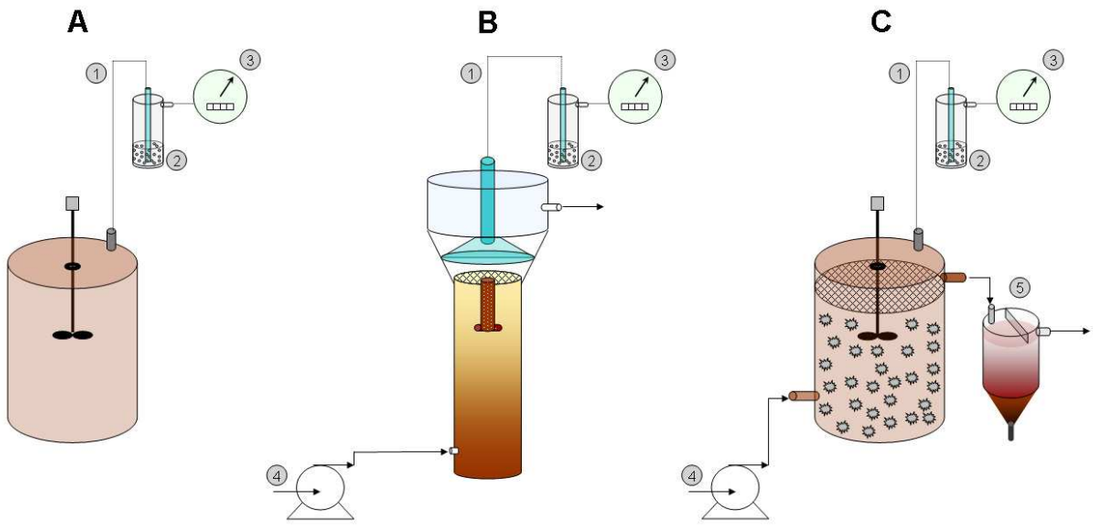


Fig. 1. Anaerobic reactors used in acidification studies: (**A**) Batch anaerobic reactor; (**B**) Upflow anaerobic sludge blanket, UASB; (**C**) Anaerobic moving bed biofilm reactor, MBBR; (1) gas line; (2) gas bubbler; (3) wet gas meter; (4) feedstock; (5) settler

2.3 Analytical methods

All the analyses were performed in accordance with standard analytical procedures (APHA, 2005). Alkalinity and pH were measured according to methods 2320B and 4500-H^+B. Chemical oxygen demand (COD) was measured by colorimetric method (method 5220D) and the solids analyses were performed with glass microfiber filters (Reeve Angel grade 403), analytical balance, drying oven and muffle furnace (methods 2540B, 2540D and 2540E).

Biogas was measured using a wet gas meter and the gas content analysis was done by collecting a gas sample with a gas tight syringe and injection on-column in a gas chromatograph SRI 8610C with thermal conductivity detector set to 75ºC, and a 80/10-2.5 m CRS Hayesep column set to 61ºC. Helium was used as carrier gas. Calibration curves were obtained by injecting standards of methane and carbon dioxide.

The VFA were analysed by gas chromatography by injection of 0.5 µL of filtered sample containing 10% (v/v) of formic acid on-column in a gas chromatograph Chrompack CP9001 with flame ionisation detector set to 220ºC, and a 25 m x 0.25 mm Chrompack CPSIL-5CB column. Helium and nitrogen were used as the carrier and the make-up gases respectively. Calibration curves were obtained by injecting standards of acetic, n-butyric, iso-valeric, n-caproic (even carboxylic chain VFA), propionic, iso-butyric, and n-valeric acids (odd carboxylic chain VFA).

3. Results and discussion

3.1 Sugarcane molasses

Molasses is a by-product of sugarcane processing and it contains a high sugar content ranging from 48 to 50%, mainly sucrose, glucose and fructose, a water content of 17 to 25% and polysaccharides (dextrin, pentosans, polyuronic acids) content of 2 to 5%. Its reduced

polymeric sugars can further react to form fermentable sugar during enzymatic hydrolysis. So far many researchers have mentioned molasses as a carbon source for several biotechnological processes, such as ethanol and citric acid fermentation (Najafpour & Poi Shan, 2003), alcohol and amino acid production, baker's yeast fermentation and improvement of biological denitrification (Quan et al., 2005). However, the use of high loads molasses as an external carbon source for bio-treatment processes has proven to promote VFA build-up containing heavier carboxylic chains (Silva et al., 2009). Espinosa et al. (1995) observed that a molasses OLR = 17 gCOD $L^{-1}d^{-1}$ applied to an UASB led to the accumulation of VFA, in particular propionic acid, being it attributed to the lack of trace nutrients such as Fe, Ni, Mo and Co. Therefore those accumulated VFA can be faced as a valuable recovery product.

Molasses acidification was performed in two MBBR reactors during 160 days at mesophilic temperature (37±2 ^{o}C). In order to study the effect of inoculums type, each reactor was inoculated with distinct anaerobic biomass: AR reactor started operation with acidogenic sludge previously adapted to glucose for 20 days, whereas MR reactor was inoculated with conventional anaerobic biomass from a full-scale municipal digester working on sludge digestion with methane recovery. Hydraulic retention time (HRT) and organic loading rate (OLR) were also investigated to determine optimum operational conditions. Both reactors were submitted to increasing OLR (1 to 70 gCOD $L^{-1}d^{-1}$) and two HRT (6 and 12 h).

In general, output COD varied proportionally to the applied OLR in both reactors. Figure 2A describes soluble COD monitored at the outlet of reactors. Conventional biomass reactor (MR) presented lower COD values than acidogenic biomass reactor (AR), due to the fact that an important fraction of substrates inside reactor followed methanisation pathways. However, when OLR = 33 gCOD $L^{-1}d^{-1}$ was applied, COD values dropped significantly when compared to the previous organic loadings, which is most likely due to HRT change from 12 to 6 h, corresponding to a decrease on COD input. COD removals of 40±9 and 60±7% were achieved under that OLR for AR and MR reactors respectively, and removal in AR reactor is similar to that achieved by Ren et al. (2006) using a bio-hydrogen producing reactor (COD removals in the range 20-40%when varying OLR between 1 and 80 gCOD $L^{-1}d^{-1}$). When OLR changed from 33 gCOD $L^{-1}d^{-1}$ to higher values (52-70 gCOD $L^{-1}d^{-1}$), fermented effluent presenting higher COD content was collected in both reactors, with a significant drop in the COD removal (only 17 and 10% for AR and MR reactors respectively).

As it would be expected, produced methane (Figure 2B) was always higher for MR reactor than for AR during operation at HRT = 12 h, due to the presence of higher amounts of methanogens within the conventional microbial consortium. Moreover, changing of HRT from 12 to 6 h resulted in significant methane production in MR, revealing an effective potential of molasses for methanogenesis and ability of the microbial consortium to deal with moderate OLR (33 gCOD $L^{-1}d^{-1}$), although unsteady-state in methane production was observed at this OLR. Biogas methane content varied between 40 and 55% during longer HRT (12 h) operation, whereas shorter HRT (6 h) at OLR = 33 OLR even resulted in a slight rise on methane content (from 40 to 55%) in AR reactor. An additional increase on OLR from 33 to values above 50 resulted in severe methanogenic inhibition, particularly in MR reactor that exhibited a drop on methane content to values below 5%. Wijekoon et al. (2011) also observed a rising of methane production when the OLR was increased, and it was probably attributed to the ability of the reactor to work in even higher loading rates.

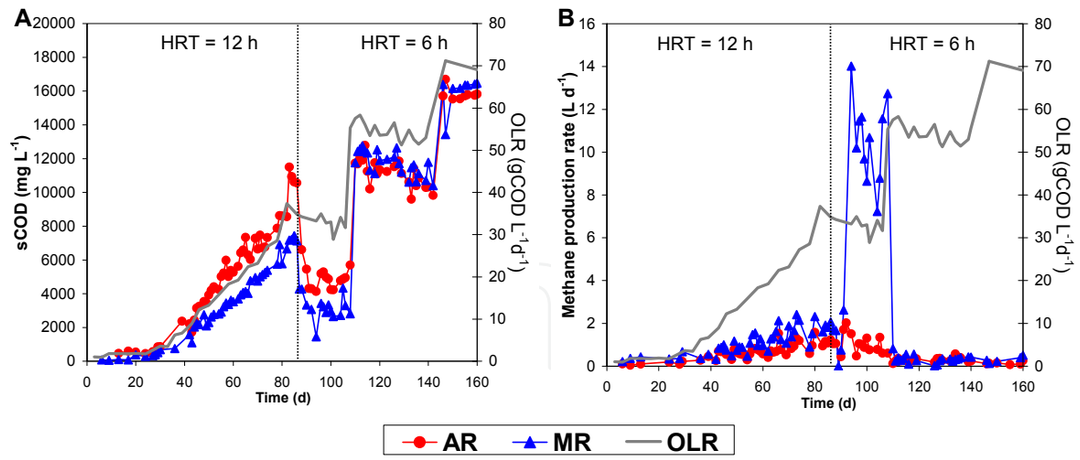


Fig. 2. Performance of MBBR reactors (acidogenic biomass, AR, and conventional biomass, MR) processing sugarcane molasses: A) sCOD in the fermented effluent; B) methane production rate

Figure 3A presents the degree of acidification (DA), which is of fundamental importance when evaluating acidogenic systems. For the tested conditions, it was observed that AR reactor yielded a much higher degree of acidification when compared to the MR reactor inoculated with conventional mixed anaerobic biomass. Values of pH in the acidogenic biomass reactor were also lower than those achieved in the conventional mixed biomass reactor (Figure 3B).

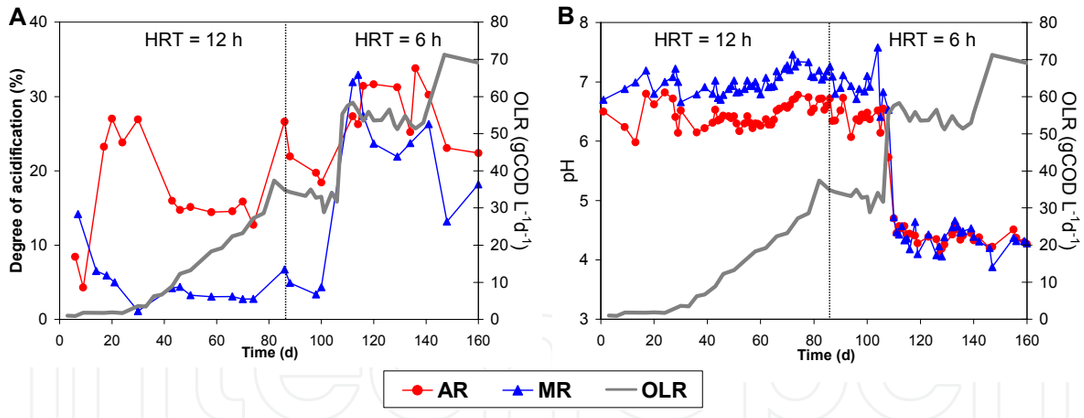


Fig. 3. Performance of MBBR reactors (acidogenic biomass, AR, and conventional biomass, MR) processing sugarcane molasses: A) degree of acidification (VFA/$sCOD_{in}$); B) pH

Higher degrees of acidification achieved in the AR reactor highlight the strategy of using pre-acclimated biomass for VFA production, and it was quite notorious for operation at HRT = 12 h. A DA of 27% was achieved at OLR lower than 8 gCOD $L^{-1}d^{-1}$, while a DA of 16% was achieved at OLR in the range 8-30 gCOD $L^{-1}d^{-1}$. During this period (low OLR), DA for MR reactor was generally 3 to 5 times lower than that for AR reactor. Maximum net production of VFA (30-34% acidification) was achieved under an organic load rate of 52 gCOD $L^{-1}d^{-1}$ for both reactors, with a significant drop in pH (to values around 4 - 4.5), although there had been applied a corresponding increase of alkalinity with increasing OLR

(0.1 g $CaCO_3$ $g^{-1}COD_{in}$). During operation at an OLR in the range 52-55 gCOD $L^{-1}d^{-1}$, some unbalanced conditions in the MR reactor were noticed, as some unsteady condition was observed in the degree of acidification. Conversely, AR reactor kept the acidification degree at 34% and it showed to be well acclimated to those moderate OLR. For higher organic load (70 gCOD $L^{-1}d^{-1}$), it was observed a decrease on the acidification yield in both reactors.

Production of VFA as a function of OLR applied is presented in Figure 4, where the better ability of AR reactor for organic acids production is well emphasised. During operation at HRT = 12 h, VFA production raised with the increasing OLR up to 29 gCOD $L^{-1}d^{-1}$, (from 250 to 2700 mgCOD L^{-1}), while the same OLR increase in the MR reactor yielded an almost constant VFA production (100-400 mgCOD L^{-1}). During that period, acetic (C2), propionic (C3) and iso-butyric (C3) acids were the main forms of VFA. Below OLR = 8 gCOD $L^{-1}d^{-1}$, acetic acid was predominant, thus contributing to low odd-to-even ratios of VFA carboxylic chains (0.16-0.31). Between OLR of 8 and 29 gCOD $L^{-1}d^{-1}$, odd-to-even ratio presented a remarkable increase (1.10-2.85), mainly due to the build-up of propionic and iso-butyric acids that surpassed acetic acid concentration. Similar shift was reported by Ren et al. (2006) when OLR tested was changed from 4 to 13 gCOD $L^{-1}d^{-1}$, with an increasing from 600 to 2200 mg L^{-1} on total VFA. Conversely, Wijekoon et al. (2011) found an increase of n-butyric and a constant propionic acid concentration when the OLR increased, but those loading rates applied (between 5 and 12 gCOD $L^{-1}d^{-1}$) were much lower than in the present study.

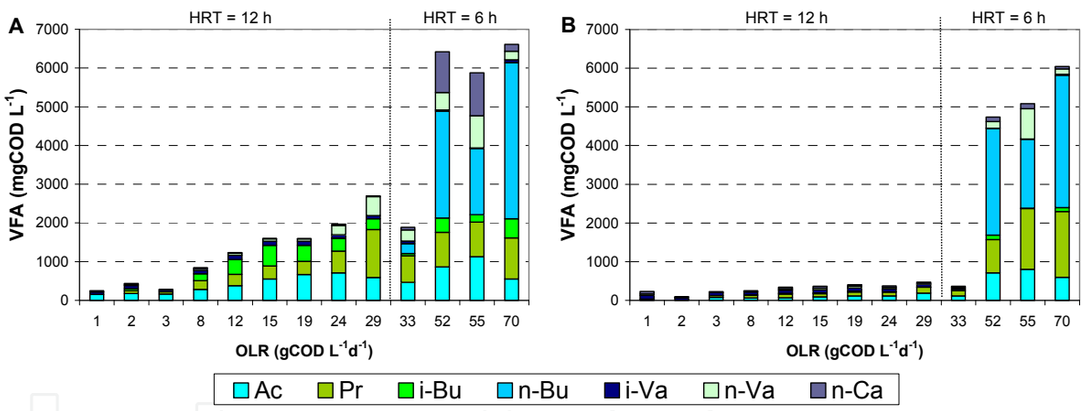


Fig. 4. VFA production as a function of organic loading rate applied: A) Acidogenic biomass reactor, AR; B) conventional mixed biomass reactor, MR

When HRT was set to 6 h, total VFA decreased from 2600 to 1900 mgCOD L^{-1} inside AR reactor, mainly due to decrease on propionic acid concentration (from 1250 to 700 mgCOD L^{-1}). In spite of being suitable for feedstock for microbial PHA production, propionic acid build-up has been pointed as key indicator of methanogenic instability due to its worse biodegradation kinetics (Nielsen et al., 2007; Wijekoon et al., 2011). Shift on OLR from 33 to 52 gCOD $L^{-1}d^{-1}$ led to an increase on DA (Figure 4A) with concomitant build-up of longer carboxylic chain VFA, namely n-butyric (C4) acid that achieved an individual concentration of 2800 mgCOD L^{-1}, despite the biomass tested (AR or MR). That build-up of longer chain VFA matched the severe pH drop observed in both reactors (Figure 4B), which was also referred in other studies. For acidogenic fermentation of molasses at different pH values, Albuquerque et al. (2007) reported that acetate and propionate concentrations decreased

when pH was decreased from 7 to 5, while butyrate and valerate concentrations significantly increased in the same pH shift. For acidogenic fermentation of glucose, Zoetemeyer et al. (1982) referred that lower operating pH favoured the production of longer chain fatty acids, since under these more acidic conditions there are more reducing equivalents available to be incorporated into the fatty acid chains.

Production and profile of VFA from sugarcane molasses is closely correlated with the OLR applied (Ren et al., 2006). It should be mentioned that the massive production of organic acids observed in both reactors during the highest OLR (70 gCOD $L^{-1}d^{-1}$) did not correspond to higher degrees of acidification. Accordingly, high VFA production observed at OLR = 70 gCOD $L^{-1}d^{-1}$ (mainly due to n-butyric acid build-up) did not lead to higher volumetric productivities of VFA, whereas a shift on the HRT from 12 to 6 h combined with a load increase from 33 to 52 gCOD $L^{-1}d^{-1}$ permitted to obtain the maximum amounts of acidified effluent. This was considered the optimum operating condition since it allows for preservation of organic resources (low COD removal) that are demanded for valorisation into VFA.

3.2 Spent coffee grounds

Wastewater from instant coffee substitutes production was collected at a Portuguese food processing industry. Because instant coffee substitutes production implicates hot water solubilization of coffee and substitutes roasted beans, this effluent is a dark coloured liquid with acidic properties and is generated at thermophilic temperatures (>45ºC). This effluent presents high solids content with a significant fraction of organic solids (≈98%). The considerable fraction of insoluble solids (24 to 28%) found in this effluent is consistent with the values for instant coffee processing wastewaters found in literature.

The BOD_5/COD ratio is less than 0.3 which may indicate the presence of low biodegradable compounds at aerobic conditions. In fact, the presence of organic refractory substances like lignin (Dinsdale et al., 1996) tannins and humic acids (Zayas et al., 2007) in coffee and coffee substitutes wastewaters is pointed out as being responsible for the difficulty in treating these wastewaters by biological treatment processes. Nitrogen is deficient in this wastewater: BOD_5:N:P ratio varies between 100:1.5:0.7 - 100:2.8:1.4 which is quite inferior to 100:5:1 considered to be the adequate ratio for aerobic biological treatment.

Acidification of a wastewater containing spent coffee grounds was performed in two MBBR reactors at two temperatures (37 and 55ºC), increasing OLR (2.5-10 gCOD $L^{-1}d^{-1}$) and two values for the HRT (0.5 d and 1 d). The reactors were fed with this effluent previously diluted with tap water. To avoid operational problems in the reactors due to the high solids content of the wastewater in study, TSS level was reduced by settling the effluent for 3 hours and siphoning off the top layer. With this procedure the effluent solids content ranged from 2.2 to 0.8 gTSS L^{-1}. Nitrogen and phosphorus were added to give a COD:N:P ratio of 100:1.75:0.25, according to Dinsdale et al. (1997). Alkalinity (0.1g of $NaHCO_3$ for each g of COD present in feed) was added to provide the system with some buffer capacity. This allowed that reactors pH to vary between 4.5-5.

Figure 5 shows the acidification behaviour of each reactor (A-mesophilic (R1) and B-thermophilic (R2)) during all period of experiment, with respect to the acidified effluent pH,

organic loading rate and hydraulic retention time. The concentration of the sCOD of the acidified effluent is also presented as an indicative parameter to recognize steady state for each operational condition.

In the beginning of the experiment, at the mesophilic temperature (reactor R1), methane was always present, showing methanogenic activity, with pH reaching values higher than 6. It was necessary to cause adverse conditions in the reactor to avoid this situation by a decrease in pH to values lower than 4. This situation leads to an extent of the experimental period at a load of 2.5 gCOD $L^{-1}d^{-1}$. In general, the sCOD out varied proportionally to the applied OLR in both reactors. Mesophilic reactor presented higher amounts of total VFA when compared with the thermophilic reactor for the same organic loads (Figure 5).

For all the experimental conditions, the reactors presented acidification degrees between 20% and 55%, with pH kept at 4.5-5, except for reactor R1 at the beginning of the experiment. HRT had a strong effect on the behaviour of both reactors, mainly for the sCOD, due to the fact that to maintain the some organic load it was necessary to double the flow rate (decrease of HRT from 1 d to 0.5 d) and decrease the input COD in the same proportion.

At mesophilic temperatures (reactor R1), the process yielded an average acidification degree of 40-50%, with values always higher than 30% (exception for the initial time). The equivalent values obtained in all thermophilic assays (Figure 5) were mostly lower, although also varying between 40% and 50%. For a load of 5 gCOD $L^{-1}d^{-1}$, the acidification degree was 41-43% for both HRT (1 d and 0.5 d). At thermophilic operation, the acidification degree decreased to 28% for the highest OLR (10 gCOD $L^{-1}d^{-1}$). These results are in agreement with the results of Dinsdale et al. (1997) where they obtained acidification degrees between 22% and 38% in CSTR reactor treating coffee wastewater at organic loads ranging from 10 to 16 gCOD $L^{-1}d^{-1}$, HRT between 1 and 0.5 d and pH = 5.

In the literature it is agreed that anaerobic acidification process is affected by several operational parameters such as pH, HRT, organic load and temperature. Hence, the effect of HRT, temperature and organic load in the acidification of a wastewater containing spent coffee grounds used in this work is presents in Figure 6.

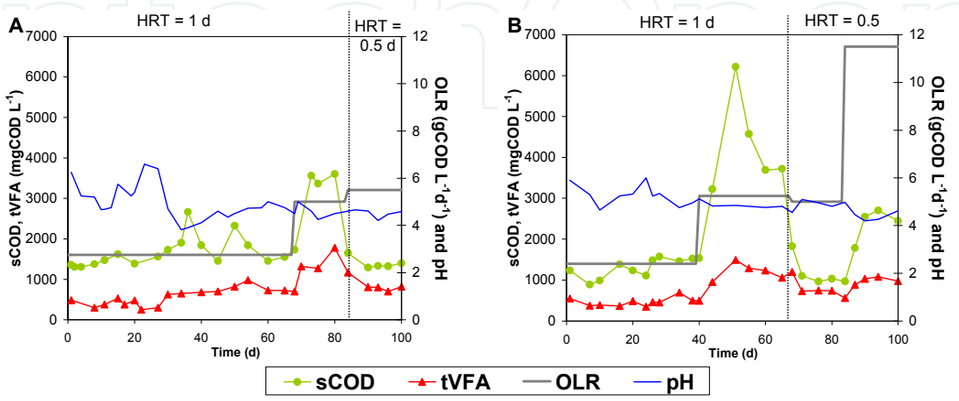


Fig. 5. Performance of MBBR reactors processing a wastewater with spent coffee grounds: A) R1 – mesophilic reactor; B) R2 – thermophilic reactor

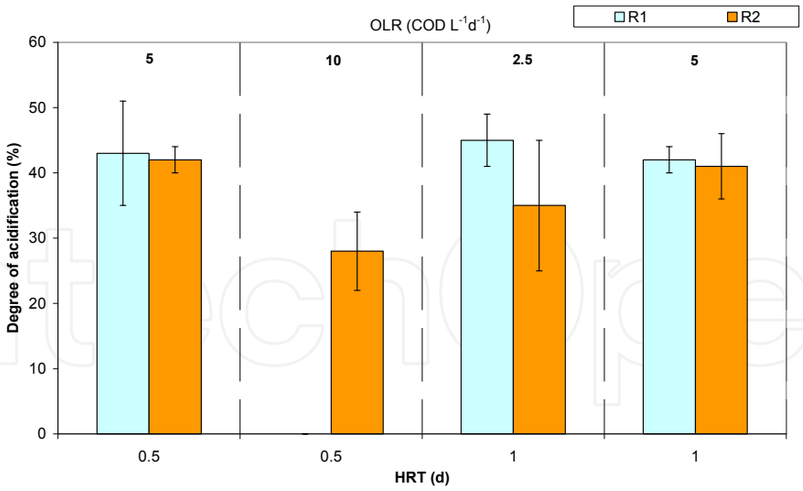


Fig. 6. Acidification degree for the different experimental phases for both reactors (R1 – mesophilic; R2 – thermophilic)

In terms of acidification degree, the operation of reactor R1 at a mesophilic temperature (Figure 6) is not significantly affected by the organic load increase (HRT=1 d and OLR = 2.5→5 gCOD $L^{-1}d^{-1}$), showing a decrease from 44.5% to 42.2%. The decrease of HRT from 1 d to 0.5 d at the same load of 5 gCOD $L^{-1}d^{-1}$, doesn't affect the acidification degree which changed from 42.2% to 42.9%. This behaviour shows that for the operational conditions tested, the HRT of 0.5 d doesn't favour the conversion to VFA.

The thermophilic reactor R2 showed an increase of the acidification degree from 35% to 40% with the increase of the organic load from 2.5 to 5 gCOD $L^{-1}d^{-1}$ (Figure 6, HRT=1 d), which represents an increase of 14%. As already showed for reactor R1, the change in HRT from 1d to 0.5d at the same load of 5 gCOD $L^{-1}d^{-1}$, doesn't affect the acidification process, as the acidification degree changed from 41 to 42%. However, an increase on the OLR from 5 to 10 gCOD $L^{-1}d^{-1}$, affected strongly the acidification degree which changed from 42% to 27%, accounting for a 36% decrease. This value is similar to the one obtained by Dinsdale et al. (1997) for an acidification process of coffee wastewater at an HRT=0.5 d and OLR=16 gCOD $L^{-1}d^{-1}$, without pH control (22% acidification degree).

In conclusion, for the same conditions tested in both reactors R1 and R2, the temperature only affected the first condition (HRT=1 d and OLR=2.5 gCOD $L^{-1}d^{-1}$) with a decrease of 29% when the temperature increased from 36ºC to 55ºC. For the conditions tested the HRT decrease from 1 d to 0.5 d doesn't affect significantly the acidification degree for both temperatures. In the other hand, the increase on the loading rate affected mostly the acidification process at thermophilic temperature, especially for the HRT = 0.5 d.

The effect of HRT and organic load on the composition of VFA is presented in Figure 7. At 37ºC (Figure 7A), the VFA present in the acidified effluent are the acetic acid, propionic, n-butyric and n-valeric, being the acetic acid the one who appears in higher concentrations (52 to 61% m/m), followed by the propionic acid with 25 to 32% (m/m) and after the n-butyric acid with 12 to 13% (m/m). These results are not totally in agreement with the results of Alexiou (1998), most probably because in this work the highest organic load tested was 5 gCOD $L^{-1}d^{-1}$ whereas he studied organic loads higher than 18 gCOD $L^{-1}d^{-1}$. At a mesophilic

temperature, the increase of the organic load from 2.5 to 5 gCOD $L^{-1}d^{-1}$, increased all individual volatile acids concentration (total VFA from 615-1335 mg L^{-1} as COD), although in proportion to the total, acetic acid increases in 7% in the acidified effluent in detriment of the propionic acid which is reduced in 13%. The decrease on the HRT decreases the amount of VFA produced (1335-743 mg L^{-1} as COD) and favours the increase on the percentage of acetic acid to the total in 7%, a decrease in the percentage of propionic acid in 10% and the disappearance of the valeric acid which was present before in very low percentages (1.5-2.5%).

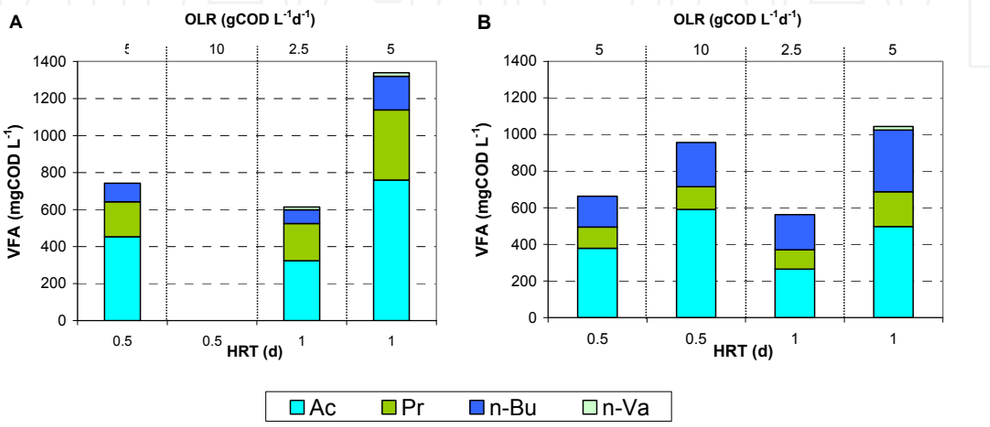


Fig. 7. VFA distribution as a function of HRT and organic load. A) reactor R1 at mesophilic temperature; B) reactor R2 at thermophilic temperature

For the assays at 55ºC, the type of volatile acids present in the acidified effluent is the same that the ones found at mesophilic temperature (acetic, propionic, n-butyric and n-valeric) although with a different distribution. Hence, in terms of VFA composition, it was observed that a temperature shift from 37 to 55ºC enhanced the presence of n-butyric acid rather than propionic acid, but still with the predominance of acetic acid. At thermophilic temperatures (55ºC) Alexiou (1998) found the presence of the same four acids in the acidified coffee effluent at a load of 17.3 gCOD $L^{-1}d^{-1}$, HRT=0.54 d and pH=4.5. The acidified effluent obtained in the experiments of Alexiou (1998) presented 58% acetic, 28% n-butyric, 8% propionic and 2% n-valeric (m/m) which is comparable with the results obtained for reactor R2 from this work during the entire period: 47-62% acetic acid, 34-35% n-butyric, 13-19% propionic acid and 0-1.8% of n-valeric acid (%m/m). A decrease in the HRT stimulates the production of acetic acid (20% increases in the proportion to the total) and inhibits the presence of propionic acid (4% decrease), n-butyric (22% decrease) and the disappearance of n-valeric acid.

In conclusion, the differences found in this work regarding the metabolic pathway for VFA production, i.e. the predominant volatile acids present in the acidified wastewater containing spent coffee grounds are dependent mainly on temperature. It was found that the increase in temperature favours the presence of butyric and acetic acids. Hence, for this type of effluent at thermophilic temperatures doesn't lead to an increase of propionic acid but to an increase of the butyric acid. This behaviour is in accordance with the results by Yu et al. (2002) which studied dairy effluents and concluded that the increase in temperature didn't led to the increase of the ratio of propionic acid to the total. Regarding the effect of

HRT, it was found that the decrease of this parameter favours the increase in the relative percentage of acetic acid and a decrease on the percentage propionic for both temperatures. At thermophilic temperatures the presence of butyric acid is also inhibited. In these conditions, these metabolic pathways are not appropriate for PHA production, but rather for energy production through biogas. So, not only the amount of VFA produced and acidification degree are important but also de distribution of VFA, in order to choose a practical and economically feasible waste management option together with the recovery of valuable organic acids. In this sense, and given the very low biodegradability of this industrial waste, the acidified effluent obtained for each steady state condition was also assessed in terms of biodegradability besides VFA yields, using aerobic BOD tests procedure. For the tested conditions, experimental results indicated that the acidified effluent reached much higher biodegradability degrees with increases as high as 80%.

3.3 Dairy processing fatty slurry

The dairy processing fatty slurry used in this work was collected at a Dissolved Air Flotation (DAF) tank in a dairy factory wastewater treatment plant. Batch assays were performed either at mesophilic (37ºC) or thermophilic (55ºC) temperature ranges in order to investigate the influence of temperature, pH control, added alkalinity and organic load on the acidification of dairy processing fatty slurry. At mesophilic temperature (37ºC) the batch tests were conducted with loads of 5, 10, 15 and 30 gCOD L^{-1} both with and without added alkalinity. Added alkalinity varied in the range of 0-10 $gCaCO_3$ L^{-1}. For the tests at thermophilic temperature (55ºC) two loads were tested (10 and 30 gCOD L^{-1}) with and without added alkalinity and with and without pH control. The tests at 37ºC lasted between 20 and 50 days, the longer tests being those with the higher loads. The tests at 55ºC with no pH control lasted between 110 and 150 days and the tests at 55ºC with pH control lasted 35 days. All the tests were terminated when constancy in the cumulative methane production value was observed for longer than 10 days. The biological anaerobic sludge used in the tests was collected from a wastewater treatment plant in a dairy factory. Due to the characteristics of the fatty slurry the acidification was computed based on feed tCOD.

Cumulative methane production reached at the end of each batch test was a clear function of the applied load (Figure 8A) and mesophilic tests resulted in higher methane production compared to the thermophilic tests. The effect of added alkalinity was not pronounced but pH control (pH4 and pH5) at thermophilic temperature resulted in very low or null methane yield. The factors that most clearly influence the maximum attainable concentration of VFA were the COD load and alkalinity addition (Figure 8B), the VFA concentration peak rising with the rise of any of those two factors. The effect of alkalinity was most pronounced for the higher loads and also the effects of load rise in the rise of the VFA peak was more pronounced in the tests with added alkalinity. For all the tests conducted at loads above 5 gCOD L^{-1} at 37ºC and with no pH control addition of alkalinity caused a rise in the maximum VFA peak of more than 100% compared to the results of similar tests with no added alkalinity.

In all the tests performed at 55ºC the maximum amount of VFA attained was always lower compared to what was observed in the corresponding tests at mesophilic temperature (37ºC). It was observed that for the lowest load tested at 55ºC (10 gCOD L^{-1}) the effect of temperature was not significant whilst for the load of 30 gCOD L^{-1} the negative effect of

temperature rise was only observable in the tests performed with added alkalinity. For the tests conducted at 55ºC it was also noted that the rise in the applied load did not significantly influence the VFA peak and that pH control resulted in slightly lower maximum VFA peaks compared with the equivalent tests conducted with no pH control, even for the test with added alkalinity. Yu and Fang (2001) reported a VFA concentration of approximately 950 mg Ac L^{-1} and around 38% acidification of feed COD for thermophilic acidification of dairy wastewater at pH=5.5 and a load of 4 g COD L^{-1}. Yu and Fang (2002) observed a decrease in VFA production for loads above 8 g COD L^{-1} for batch thermophilic acidification of dairy wastewater. In all the batch tests conducted in the present study with dairy processing fatty slurry in both temperature ranges the highest VFA concentration peak (5356 mg Ac L^{-1}) was reached at 37ºC for a load of 30 g COD L^{-1} with added alkalinity and no pH control. The operating times needed to reach the VFA peaks were 8-15 days at 37ºC and 6-23 days at 55ºC without pH control. Effects of added alkalinity were not clear. In the tests at 55ºC with pH control the times needed to reach the VFA concentration peak were 26-33 days.

The highest acidification (26% of the feed tCOD) was reached in the test conducted with a load of 15 gCOD L^{-1} at 37ºC with added alkalinity (Figure 9). At mesophilic temperature the main VFA produced was acetic acid followed by propionic acid that was detected only for loads above 10 g COD L^{-1}. For thermophilic temperature the highest acidification degree (15% of the feed tCOD) was attained for the lower COD load (10 gCOD L^{-1}) with added alkalinity and no pH control. Acetic and propionic acids were the major VFA produced although butyric and valeric acids were also detected.

COD balances at the end of each batch test (Figure 10) show that in mesophilic temperature the main fraction of the feed tCOD (56%-96%) was converted to methane and that the non-solubilised COD was always below 39% of the feed tCOD. At the end of the tests conducted in thermophilic temperature the main fraction of the feed tCOD remains in non-solubilised form (above 68%) and methanised fraction was always below 23%. Apparently at 55ºC the anaerobic biomass was not sufficiently adapted to the fatty substrate and hydrolysis was the rate limiting step of the acidification process.

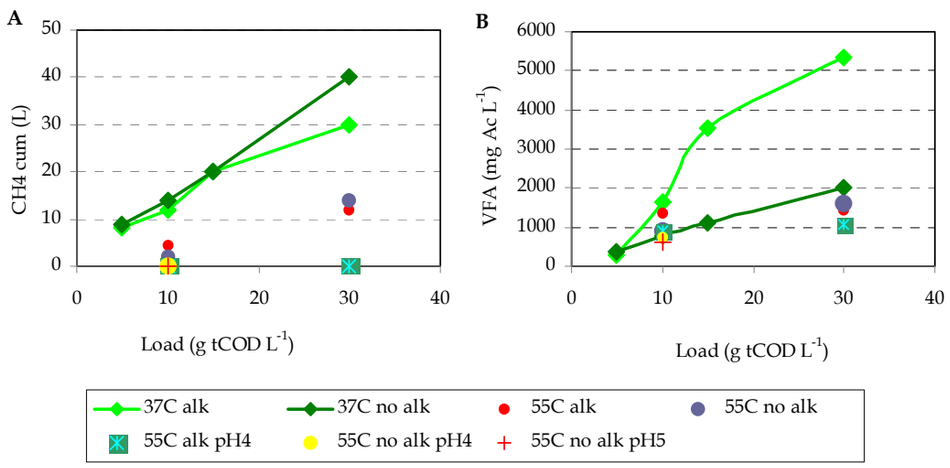


Fig. 8. A) Cumulative methane production in each batch test; B) maximum VFA concentration peak reached in each batch test.

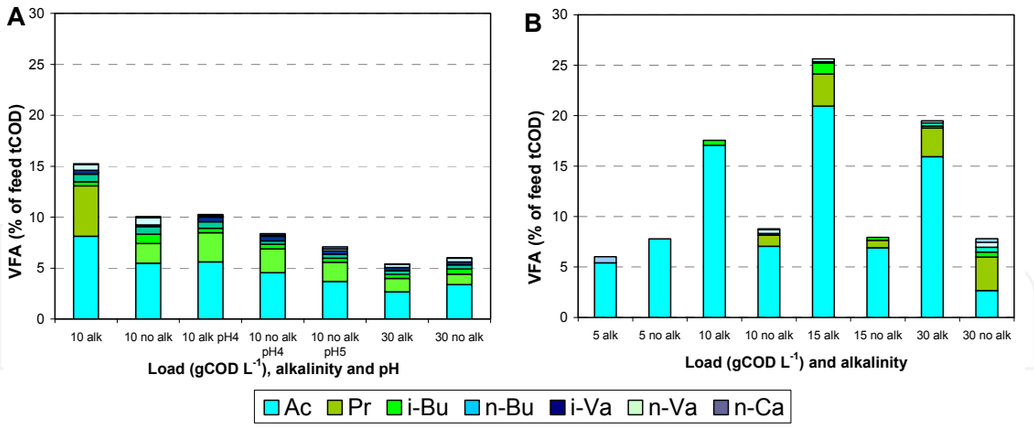


Fig. 9. Maximum degree of acidification reached in the batch tests and corresponding VFA composition. A) mesophilic temperature; B) thermophilic temperature.

A test was also conducted in a mesophilic (35±1)ºC UASB reactor to determine the acidification potential in continuous operation. The reactor was inoculated with anaerobic flocculent sludge from an industrial reactor treating dairy wastewater. The feed was prepared every 4 days diluting the dairy processing fatty slurry and nutrients with tap water; from day 81 onwards alkalinity was supplemented to the feed (1 g $NaHCO_3$ L^{-1} and 1 g $KHCO_3$ L^{-1}). The reactor was operated for a period of 200 days with a constant load of 10 g COD $L^{-1}d^{-1}$ and a hydraulic retention time (HRT) of 12 hours.

The operation of the reactor was divided in four distinct phases (Figure 11) although the COD removal efficiency was similarly low in all phases (average 33%). In the first phase (80 days) no alkalinity was supplemented to the reactor feed and methane production was very low, around 0.4 L d^{-1}, as was the degree of acidification (average 5% of the feed tCOD). In the second phase (days 81 to 125) alkalinity was added to the feed and CH_4 production and acidification improved slightly (3.3 L d^{-1} and 7.6% of the feed tCOD, respectively). From day 126 to 167 the feed to the reactor was suspended whilst the temperature was kept constant (35±1)ºC and the methane production was continuously monitored. This feed interruption was established in order to promote an adaptation of the microbial population present in the biological sludge towards a more complete degradation of the fatty substrates. This adaptation strategy has been previously used in UASB reactors fed with fatty wastewater (Nadais et al., 2011) since it was found that a period without feed improves the biomass capacity to degrade complex fatty substrates. During days 126 to 150 of this feedless period average CH_4 production reached 6.3 L d^{-1} and for the remaining of the feedless period (days 151 to 167) CH_4 production was negligible (Figure 11A). In the fourth phase of operation (days 168 to 200) the average CH_4 production was around 5 L d^{-1} and acidification reached the highest value (33% of the feed tCOD). In all the feeding phases (I, II and IV) the main VFA produced was acetic acid, the concentration of other acids being negligible. The 33% acidification coupled with 22% methanisation of the feed tCOD observed in this work (Table 2) are in accordance with the value of 54% reported by Fang and Yu (2000) for the mesophilic acidification of dairy wastewater at 12 HRT. From Table 2 and Figure 11B it can be seen that hydrolysis improved significantly along the operation period as well as the methanisation of the removed COD. Apparently in the first and second phases acidogenesis was the rate limiting step whilst in the fourth phase acidification improved considerably. This means that the feedless period (phase III) served the adaptation of acidifying microorganisms.

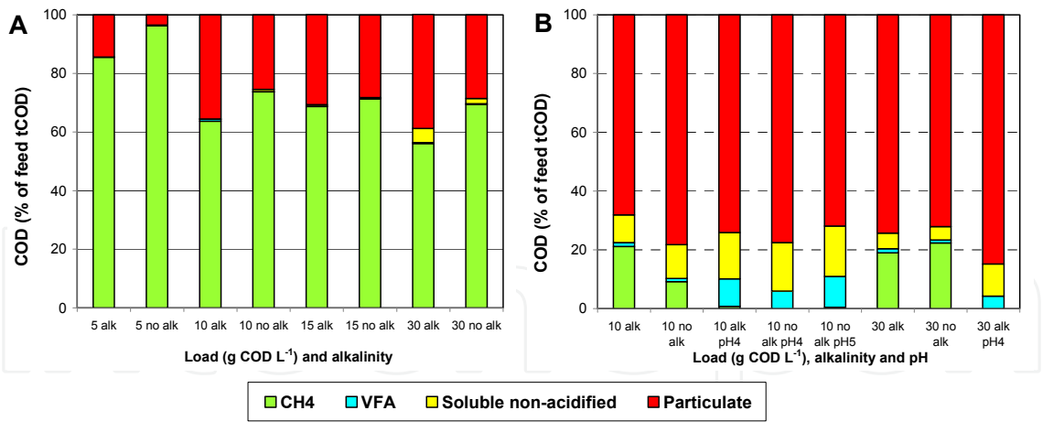


Fig. 10. COD balances at the end of the batch tests. A) mesophilic temperature; B) thermophilic temperature

For continuous operation hydrolysis of the fatty substrate improved compared to the batch experiments and the limiting step was acidification but biomass adaptation towards higher acidification yields was observed. The rate limiting step in batch experiments was hydrolysis, particularly at thermophilic temperature. In batch tests a wider variety of VFA was detected compared to the continuous test. The highest acidification yield (33% of the feed tCOD) was attained in continuous mode although VFA produced consisted only of acetic acid.

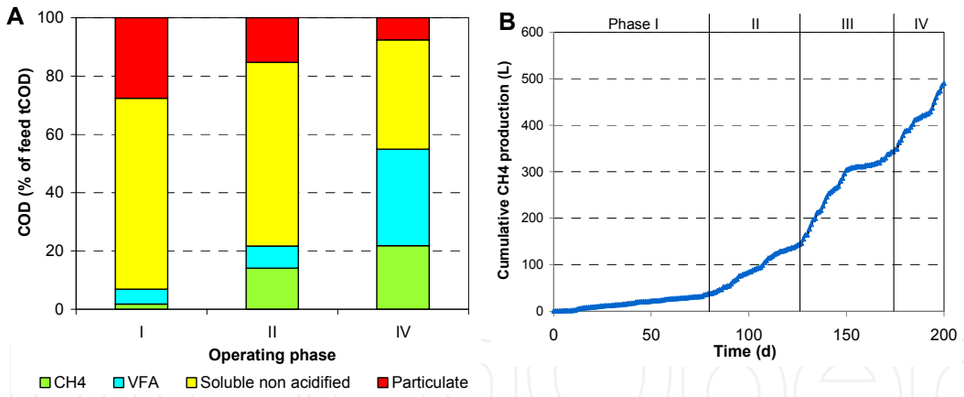


Fig. 11. A) cumulative methane production; B) COD balances for the operation of the UASB reactor.

Operating phase	Hydrolysis of particulate COD (%)	Acidification of feed tCOD (%)	Methanization of feed tCOD (%)	Methanization of removed tCOD (%)
I	50	5	2	6
II	72	8	14	48
IV	86	33	22	72

Table 2. Data from COD balances in UASB reactor acidifying dairy processing fatty slurry.

3.4 Cheese whey

Cheese whey is a lactose-rich by-product of cheese manufacturing. It represents about 85–95% of the milk volume processed and retains 55% of milk nutrients. To use these nutrients, whey is usually processed to separate it into protein, lactose, lipids, and mineral salts. Alternative utilizations for whey include either disposal as waste or use in fertilisers or animal feed (de Wit, 2001). By using whey nutrients as substrates for value-added by-production, both the pollution problem and disposal cost can be simultaneously abated. Even though there have been a number of technological developments in the transformation of whey into other useful products, utilization or disposal of whey is still one of the most significant problems faced by the dairy industry. Whey's high organic content, combined with low buffer capacity, makes anaerobic treatment in high rate reactors difficult, due to tendency to acidify the environment very quickly (Malaspina et al., 1996). Therefore, anaerobic processing of cheese whey is here exploited in order to recover organic acids rather than conventional treatment with methanisation.

Anaerobic batch assays were performed on a factorial basis at 37±2^{o}C, to optimise alkalinity input and OLR (F/M ratio) on cheese whey fermentation. Ranges of the studied factors varied (at four levels) between 1 and 8 $gCaCO_3$ L^{-1} for alkalinity, and 2 and 10 gCOD g^{-1}VSS for food-to-microorganism (F/M) ratio. Sixteen reactors were seeded with an initial concentration of 2 gVSS L^{-1} of anaerobic mixed biomass collected from a full-scale municipal digester working on conventional digestion of waste activated sludge. In order to first investigate the effect of reaction time over VFA production, batch reactors ran for 20 days and VFA profiles for each assay are presented in Figure 12. Response Surface Methodology was applied to predict scenarios for maximisation of VFA production.

Regardless of the alkalinity added or the F/M tested, initial pH values were always recorded in the range of 6.9-7.2 for all experiments and VFA production was clearly achieved in a period of 5 days of incubation. A conservative trend in VFA concentration can be observed, mainly for higher F/M tests (4, 7 and 10 gCOD g^{-1}VSS), whereas the lowest F/M tested (2 gCOD g^{-1}VSS) showed an unexpected disappearance of VFA in all assays after 5 days of reaction. Moreover, maximum VFA plateau increased with F/M ratio. Because of the maximum VFA productions were achieved at 5 days of reaction, response surface methodology (RSM) was applied to results attained in that period (tVFA, degree of acidification and odd-to-even ratio in carboxylic chains). Computation was done using software package StatSoft Statistica. Equation 1 presents the mathematical form of the quadratic model applied (Myers et al., 2009).

$$E(z) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{1,2} x_1 x_2 + \beta_{1,1} {x_1}^2 + \beta_{2,2} {x_2}^2 \quad (1)$$

where E(z) is the response variable, x_1 is the F/M ratio in gCOD g^{-1}VSS, x_2 is the alkalinity added in g L^{-1} as $CaCO_3$, β_0 is the model constant, β_1 and β_2 are linear coefficients (main effects), $\beta_{1,2}$ is a cross-product coefficient (interaction) and $\beta_{1,1}$ and $\beta_{2,2}$ are quadratic coefficients. Table 3 presents the parameters obtained in the quadratic modelling, as well as regression coefficients (r^2) and P-value for each regression. Figure 13 presents the quadratic surface predicted to total VFA production, degree of acidification and odd-to-even ratio of carboxylic chains. Dots represent experimental data.

Parameter	**Response variables**		
	tVFA	**DA**	**Odd-to-even ratio**
β_0	342.8	4.672×10^{-1}	2.277×10^{-1}
β_1	1399	2.020×10^{-2}	-4.937×10^{-2}
β_2	-628.8	6.489×10^{-3}	9.418×10^{-3}
$\beta_{1,2}$	88.59	2.045×10^{-3}	-8.118×10^{-4}
$\beta_{1,1}$	-96.18	-4.588×10^{-3}	3.089×10^{-3}
$\beta_{2,2}$	55.76	1.250×10^{-3}	4.167×10^{-4}
r^2	0.865	0.838	0.861
P-value	8.11×10^{-5}	1.35×10^{-3}	9.10×10^{-5}

Table 3. Summary of the parameters adjusted for response variables

All the P-values were below 0.005, so experimental behaviours are well described by the computed models. Maximum experimental value for tVFA production (10.9 gCOD L^{-1}) was achieved in the assay combining both the highest F/M ratio (10 gCOD g^{-1}VSS) and alkalinity (8 $gCaCO_3$ L^{-1}). Gross production of organic acids increased with the organic load, and the increasing was more marked when alkalinity amount was the highest (increasing from 2450 to 10900 mgCOD L^{-1}). The positive value of the cross-product coefficient ($\beta_{1,2}$ = 88.59) verifies that combined increasing on F/M ratio and alkalinity maximises VFA production. On the other hand, when F/M ratio was the lowest, added alkalinity yielded a much lower raise on the VFA production (from 1300 to 2400 mgCOD L^{-1}).

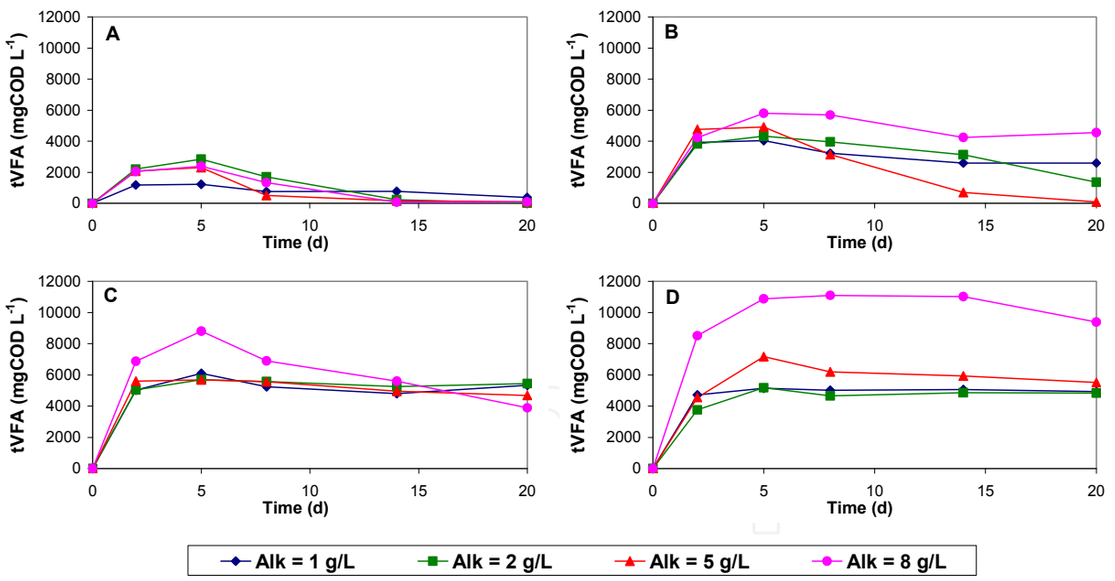


Fig. 12. VFA production from cheese whey as a function of reaction time: A) F/M = 2 gCOD g^{-1}VSS; B) F/M = 4 gCOD g^{-1}VSS; C) F/M = 7 gCOD g^{-1}VSS; D) F/M = 10 gCOD g^{-1}VSS

Calculation of degree of acidification (DA) reflects the net yield of VFA on COD fed (Figure 13B). Experimental DA presented a reverse trend from that obtained for gross VFA production. It is reasonable to argue that higher organic loads yield higher amounts of organic acids. However maximum net yields were observed in small F/M ratios (4 gCOD g^{-1}VSS). For DA, alkalinity presented lower influence than for gross production as well. Prediction for

maximum DA was 0.68±0.25 $gCOD_{VFA}$ $g^{-1}COD_{fed}$ at an F/M = 4.3 and Alk = 8 $gCaCO_3$ L^{-1}. F/M ratio revealed an antagonistic effect, mainly when combined with low alkalinity. Alkalinity present in the system is quickly consumed due to rapid conversion of lactose into VFA, making it necessary to constantly impose pH control to the media (Backus et al., 1988).

Final values for pH were in the range of 6.8-7.9 for lower F/M series (2 and 4 gCOD g^{-1}VSS), while for higher F/M ratios (7 and 10 gCOD g^{-1}VSS) pH was recorded in the range of 4.2-6.0. The lowest pH values in both ranges were observed in the lowest alkalinity-containing assays, in which higher degrees of acidification were achieved. Similar tendency was also observed by Davila-Vazquez et al. (2008), which obtained final values in the range 3.7-3.9 when initial pH was set to the lowest value (4.5) in batch experiments, also corresponding to low gross VFA productions.

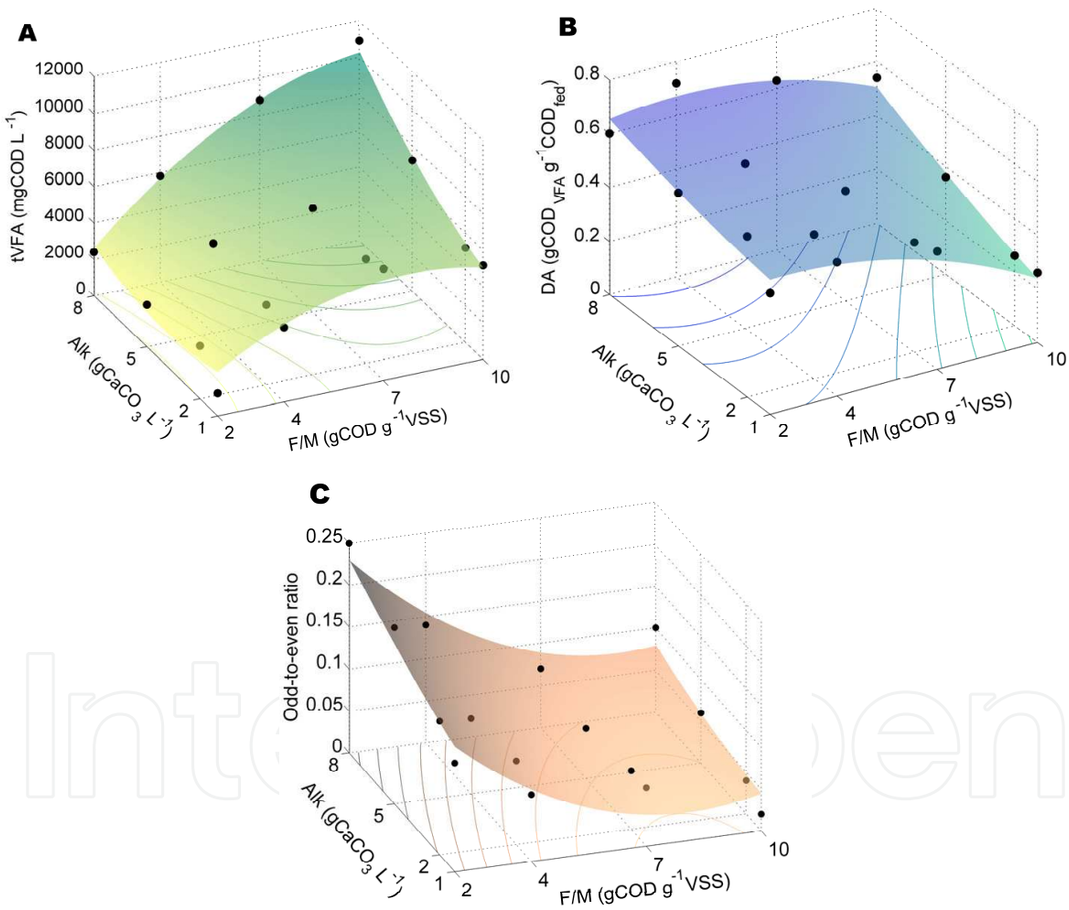


Fig. 13. Response surfaces for whey batch acidification with varying F/M and alkalinity: A) Gross VFA production; B) degree of acidification; C) odd-to-even ratio of carboxylic chains

VFA profiles varied significantly during the batch experiments and it can be illustrated by the odd-to-even ratio of carboxylic chains (Figure 13C). Although n-butyric and acetic acids were always predominant, lower F/M series assays (2 gCOD g^{-1}VSS) yielded few amounts of odd VFA (propionic and n-valeric acids in the range of 150-500 mgCOD L^{-1}) that

contributed to an increase in the odd-to-even ratio up to 25%. Regarding the shape of the response surface, it is quite visible the antagonistic effect provided by the increase of F/M ratio, which is also verified by the negative value of β_1. In a less extent, a positive value for β_2 highlights a synergistic effect of alkalinity. The lowest experimental odd-to-even ratios (6-7%) were achieved for the F/M = 7 gCOD g^{-1}VSS tests, where n-butyric acid was predominant (54±5% of tVFA).

Butyric acid build-up with an increasing on F/M ratio was also noticed by Yang et al. (2007) and Bengtsson et al. (2008) that reported the predominance of n-butyric and acetic acids in batch experiments with cheese processing wastewater and cheese whey respectively. n-Butyric acid build-up has also been observed in other fermentation studies for cheese whey (Castelló et al., 2009; Davila-Vazquez et al., 2009; Yang et al., 2003). n-Butyric acid occurrence is such magnitude results from an increase on H_2 production, which slows down the overall acid production because of stoichiometric shifts (one mole of butyrate produced instead of two moles of acetate) (Fernandes, 1986). This may be the reason why lower degrees of acidification observed at higher F/M ratios are concomitant with higher n- butyric acid productions achieved at those organic loads, contributing to lower odd-to-even ratios.

Cheese whey was then fed to a MBBR reactor operated for 149 days, for continuous acidification of this organic waste. HRT was maintained at 12 h with OLR varying in the range of 30-100 gCOD $L^{-1}d^{-1}$. Alkalinity added was set to 6 g L^{-1} as $CaCO_3$ and biomass concentration inside the reactor (carrier-attached plus suspended solids) varied between 2 and 24 gVSS L^{-1}. Due to the difficulty for controlling biomass concentration inside high-rate hybrid type reactors (fixed film plus suspended growth) such as MBBR, and consequently the F/M ratio, organic loads and HRT were chosen taking into account not only the previous results for batch acidification tests but also previous studies on cheese whey acidification (Azbar et al., 2009; Castelló et al., 2009; Davila-Vazquez et al., 2009).

Operation at the lowest OLR (30 gCOD $L^{-1}d^{-1}$) produced effluent with high acidification degree (65.6%) (Table 4), in which acetic and n-butyric were the main VFA forms. Moreover during OLR = 30 gCOD $L^{-1}d^{-1}$, almost all of the soluble COD (90.0±7.1%) was present as VFA after fermentation (Figure 14A), which confirms the very high readily fermentable organic content of whey, as also observed by Bengtsson et al. (2008). Similarly to what happened with molasses acidification, the load increase only permitted to get higher amounts of acidified effluent, as acidification degree dropped to values of 36% when OLR was set between 50 and 100 gCOD $L^{-1}d^{-1}$. VFA content in the soluble COD outlet also decreased to values in the range 70-76% at those higher OLR, in spite of gross VFA production being the highest (14.8 gCOD $L^{-1}d^{-1}$) at the highest OLR.

OLR (gCOD $L^{-1}d^{-1}$)	COD removal (%)	DA (%)	pH	Alkalinity (mg L^{-1} as $CaCO_3$)	tVFA (gCOD L^{-1})	Odd-to-even ratio
30	26±7	65.6±6.8	5.07±0.13	1366±203	10.3±0.9	0.74±0.20
50	50±6	36.7±12.8	4.86±0.13	837±328	8.5±2.4	0.62±1.46
100	55±13	36.1±13.8	4.55±0.15	492±397	14.8±2.0	0.37±1.02

Table 4. Monitored variables at different organic loading rates (average ± standard deviation)

VFA productions observed in this study were similar to those reported by Davila-Vazquez et al. (2009), which achieved a total VFA production of 9.7 g L^{-1} with an OLR of 55 g lactose $L^{-1}d^{-1}$ and an HRT of 10 h. On the other hand when those authors reduced HRT to 6 h and increased OLR to 92, only 10.6 g L^{-1} of VFA were collected, which are lower than the value achieved in the present study with an OLR of 100 gCOD $L^{-1}d^{-1}$ (14.8 g L^{-1}). The main reason for this difference may be the higher retention time (12 h) used in this study that permitted to better exploit acidification skills of the reactor.

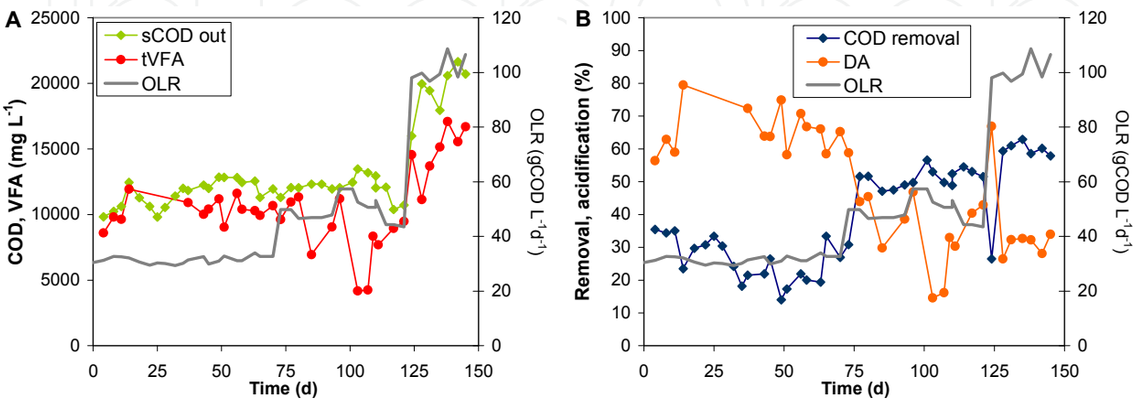


Fig. 14. Performance of MBBR reactors processing cheese whey: A) $sCOD_{out}$ and tVFA; B) COD removal and degree of acidification

Values of pH achieved in all the OLR tested were always higher than 4.5. Bengtsson et al. (2008) reported significant drops in VFA yields for pH values below 4.5, in spite of observing a more stable VFA composition at low values. Those authors also concluded that an increase in pH from 5.25 to 6 produced a shift in VFA profile (odd-to-even ratio ranged from of 25% to 100%), with the predominance of propionic acid. In the present study, a similar trend was detected, since a decrease on the odd-to-even ratio (from 74 to 37%) was observed with the load increase, with the major shifts attributed to a rising of heavier carboxylic chain acids (n-butyric, i-valeric and n-caproic acids) at the higher OLR tested (100 gCOD $L^{-1}d^{-1}$). At last, both degree of acidification and odd-to-even ratio followed trends observed in the batch experiments.

4. Conclusions

The main conclusions are:

High strength waste products from food industries can simultaneously be converted to VFA in acidogenic anaerobic reactor with considerable acidification degree (30-65%) depending on type of waste and reach COD reductions of 40-60%.

Complex and/or slowly biodegradable waste products can be transformed in compounds with higher biodegradability which will improve treatment efficiencies and bioreactor stability.

The optimum operational conditions depend on the waste selected. OLR and pH are two parameters that affect the acidification degree. According to the type of wastes used, the OLR shall be 2.5-20 gCOD $L^{-1}d^{-1}$ for slowly biodegradable wastes (spent coffee grounds and

dairy fatty slurry) and 30-50 gCOD $L^{-1}d^{-1}$ for high strength wastes such as molasses and cheese whey.

Temperature is the main parameter which affects the composition of volatile acids in the acidified wastewater containing spent coffee grounds. Acetic acid was always predominant in all experiments, with propionic acid being the second at mesophilic temperatures and butyric acid at thermophilic temperatures.

Adaptation of biomass is a feasible strategy for treatment efficiency improvement and/or recovery of bioproducts.

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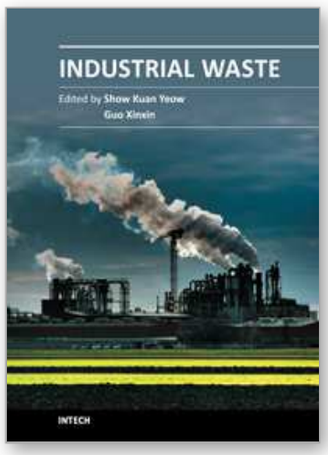
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