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Review

Cheese whey management: A review

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ABSTRACT

Cheese whey is simultaneously an effluent with nutritional value and a strong organic and saline content. Cheese whey management has been focused in the development of biological treatments without valorization; biological treatments with valorization; physicochemical treatments and direct land application. In the first case, aerobic digestion is reported. In the second case, six main processes are described in the literature: anaerobic digestion, lactose hydrolysis, fermentation to ethanol, hydrogen or lactic acid and direct production of electricity through microbial fuel cells. Thermal and isoelectric precipitation, thermocalcic precipitation, coagulation/flocculation, acid precipitation, electrochemical and membrane technologies have been considered as possible and attractive physicochemical processes to valorize or treat cheese whey. The direct land application is a common and longstanding practice, although some precautions are required. In this review, these different solutions are analyzed. The paper describes the main reactors used, the influence of the main operating variables, the microorganisms or reagents employed and the characterizations of the final effluent principally in terms of chemical oxygen demand. In addition, the experimental conditions and the main results reported in the literature are compiled. Finally, the comparison between the different treatment alternatives and the presentation of potential treatment lines are postulated.

1. Introduction

The dairy industry is divided into several sectors, which are associated to the production of contaminated wastewaters. These effluents have different characteristics, according to the product obtained (yogurt, cheese, butter, milk, ice cream, etc.). Moreover, the wastewater management, climate, operating conditions and types of cleaning-in-place also influence the dairy effluents characterization (Pattnaik et al., 2007). Amongst the key parameters characterizing these wastes, the dairy effluents show a relatively high organic load, monitored by BOD (biological oxygen demand) and COD (chemical oxygen demand) in the range of 0.1–100 kg m^{-3} with an index of biodegradability (BOD_5/COD) typically in the range 0.4–0.8. Organic matter content is mainly due to the presence of milk carbohydrates and proteins such as lactose and casein, respectively. Additionally, fat content (0.1–10.6 kg m^{-3}), suspended solids (0.1–22 kg m^{-3}) and nutrients (N and P) also contribute to the contamination levels. The changing nature of dairy effluents makes the treatment a difficult task. Without an appropriate treatment, these effluents pose serious environmental hazards (Rivas et al., 2011). Biological and physicochemical processes are usually suggested to deal with dairy effluents (Kushwaha et al., 2010).

Ice-cream, butter, whey, and cheese production effluents are the most important sources of organic contamination in the dairy industry. Cheese manufacturing is responsible of three main types of effluents; Cheese whey-CW (resulting from cheese production), Second cheese whey-SCW (resulting from cottage cheese production) and cheese whey wastewater-CWW (washing water that contains different fractions of cheese whey and/or second cheese whey). Cheese effluents represent a significant environmental impact in the dairy industry because of their physicochemical characteristics, namely, minerals (0.46–10%), total suspended solids (0.1–22 kg m^{-3}), pH (3.3–9.0), phosphorus (0.006–0.5 kg m^{-3}), Total Kjeldahl Nitrogen (TKN) (0.01–1.7 kg m^{-3}), organic load (0.6–102 kg m^{-3}), etc. The high value of organic matter is caused by the lactose (0.18–60 kg m^{-3}), protein (1.4–33.5 kg m^{-3}) and fats (0.08–10.58 kg m^{-3}) contents. This organic matter is around 99% biodegradable (Ergüder et al., 2001). Accordingly, conventional treatments are based on biological processes. However, when biological processes are not fully controlled, lactose and the casein decomposition generates strong odors, attracts insects, etc. (Rivas

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List of abbreviations

ARBC	Anaerobic rotating biological contact reactor
AS	Activated sludge
ASBR	Anaerobic sequencing batch reactor
ASDFA	Anaerobic Semicontinuous digester with flocculant addition
AUFFLR	Anaerobic upflow fixed film loop reactor
AUFFR	Anaerobic upflow fixed film reactor
CP	Contact process
CPTR	Continuous packed tubular reactor
CSTR	Continuously stirred tank reactors
DF	Diafiltration
DUHR	Downflow–upflow hybrid reactor
JLBR	Jet loop bioreactor
JLMBR	Jet loop membrane bioreactor
MBR	Membrane bioreactor
NRBC	Non-woven rotating biological contactor
PCBR	Packed column bioreactor
TSMAMD	Two-stage mixed anaerobic membrane digester
TSUAD	Two-stage unmixed anaerobic digester
UAF	Up-flow anaerobic filter
UASB	Up-flow anaerobic sludge blanket
UASFF	Up-flow anaerobic sludge fixed film

et al., 2010). Cheese effluent composition can be approached to the following ratio in terms of carbon/nitrogen and phosphorus C/N/P ≈ 200/3.5/1 which, in principle, can be considered as deficient in nitrogen components for biological processes.

Cheese whey is the most contaminated waste generated in the production of cheese (Rajeshwari et al., 2000). Cheese whey characterization depends on the milk quality used (goat, cow, sheep and buffalo), which may vary depending on animal breed, feed, health and lactation stage (De Wit, 2001). Cheese whey can cause an excess of oxygen consumption, impermeabilization, eutrophication, toxicity, etc. in the receiving environments. The volume of effluents produced in the cheese manufacturing industry has increased with the increase in cheese production. Thus, in this context, it can be referred that for the production of 1 kg of cheese, 10 kg of milk are needed, originating 9 kg of cheese whey. Worldwide, 40.7×10^6 tons per year of cheese whey are produced, half of which is produced in USA (Tejayadi and Cheryan, 1995).

2. Cheese whey characterization

Cheese whey is a green-yellowish liquid resulting from the precipitation and removal of milk casein in cheese making processes (Siso, 1996). The yellowish color of whey is caused by the presence of riboflavin (vitamin B2) (De Wit, 2001). The majority of the milk lactose, around 39–60 kg m^{-3}, remains in the cheese whey, constituting the main fraction (90%) of the organic load (Ghaly and Kamal, 2004; Kisaalita et al., 1990). Fat and protein contents are also partially responsible of organic contamination, with values in the range 0.99–10.58 kg m^{-3} and 1.4–8.0 kg m^{-3}, respectively. BOD and COD values range 27–60 kg m^{-3} and 50–102 kg m^{-3}, respectively. The BOD_5/COD ratio is commonly higher than 0.5. Hence, this substrate is suitable to be treated by biological processes. The inorganic contamination of cheese whey is attributable to mineral salts presence (0.46–10%), principally NaCl and KCl (>50%) and calcium salts (primarily phosphates) (Dragone et al., 2009; Venetsaneas et al., 2009). Inorganic contamination is the consequence of NaCl addition during cheese production. High sodium contents can cause problems when operating biological digesters (Backus et al., 1988). Acidic pH (3.8–6.5) and low alkalinity also affect the biological treatment efficiency. In the first case, the filamentous biomass growth is favored (Ghaly, 1996). In the second case, a rapid acidification can be experienced (Castelló et al., 2009). Other inhibiting parameters of the biological processes can be mentioned, such as free ammonia, potassium, volatile fatty acids, etc. (Appels et al., 2008). CW poses a considerable risk of eutrophication attributable to the nitrogen (0.2–1.76 kg m^{-3}) and phosphorus (0.124–0.54 kg m^{-3}) contents.

From the previous statements, it is obvious that cheese whey cannot be directly discharged to the environment without an adequate treatment and/or valorization. From the valorization point of view, cheese whey is a nutrient-rich effluent. Cheese whey contains about 93–94% of water and the following nutrients from the original milk: lactose, soluble proteins, minerals, lactic acid and fats (see Fig. 1). Additionally, significant amounts of other components, such as citric acid, non-protein nitrogen compounds (urea and uric acid), vitamins (B group), etc. are also present in the composition of CW (García Bilbao, 1981; Kosikowski and Wierzbicki, 1973; Kosikowski, 1979; Panesar et al., 2007). β-Lactoglobulin, α-Lactoglobulin, immunoglobulins, serum albumin and lactoferrin have been found in the cheese whey composition (Casal et al., 2006). The nutritional and medical characteristics of the protein concentrates have intensified the interest in cheese whey valorization.

3. Cheese whey treatment

In the past, most of the cheese factories disposed their effluents by land application or direct discharge to receiving waters (rivers, lakes, ocean, etc.) without any pre-treatment. Other less dramatic solutions contemplated the construction of storage tanks/lagoons, the discharge into the municipal sewage system or even animal feeding. Nevertheless, the use of concentrated CW may involve some important drawbacks. Use of dairy effluents in farming practices has incessantly been reduced (Malaspina et al., 1996). The dilution of cheese effluents is an alternative that considers the mixing of CW with less polluted wastewaters like domestic wastewater (Gannoun et al., 2008; Minhalma et al., 2007). However, even diluted effluents may impair the efficiency and stability of microorganisms in biological processes carried out in municipal wastewater treatment plants. Whatever the case, these alternatives are not sufficiently attractive, especially for small-

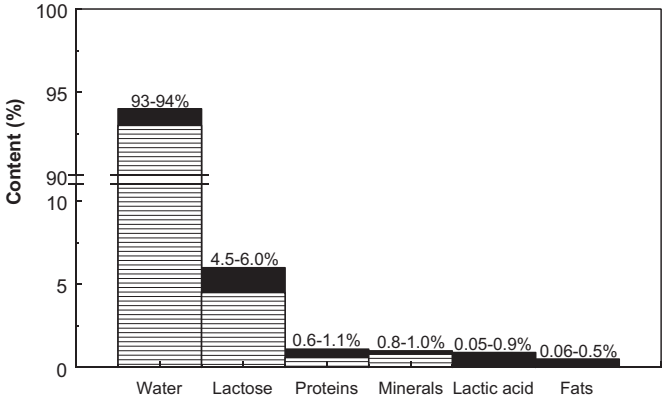

Fig. 1. Cheese whey components (the scalable pattern represents the minimum value and the black solid pattern represents the maximum value).

medium factories, the cheese effluent management becoming an important challenge due to strict legal requirements (Farizoglu et al., 2007; Mawson, 1994).

Three different options in cheese effluent management can be considered. The first one is based on the application of valorization technologies. These technologies are introduced to recover valuable compounds such as proteins and lactose. Each liter of CW contains about 50 g of lactose and 10 g of proteins with a high nutritional and functional value (Domingues et al., 1999). Currently, valorization processes applied to CW constitute the preferential option to treat this by-product, only exceeded by the production of powdered CW. The second option relies on the application of biological treatments. Biological processes can also be used as valorization technologies. For instance, the hydrolysis of lactose and proteins leads to the generation of the lactose monosaccharides (glucose and galactose), peptides and/or amino acids. Controlled fermentation processes are being considered in the production of lactic acid, butyric acid, butanol, acetic acid, glycerol, acetone, ethanol, hydrogen, single cell proteins, etc. The third choice is the application of physicochemical treatments such as coagulation-flocculation, ozonation, Fenton, thermal and isoelectric precipitation, thermocalcic precipitation, acid precipitation, alkaline precipitation, electrochemical oxidation, alkaline subcritical water gasification, etc. These different treatment options will be described further in the text, together with relevant references.

CW management has been focused on the valorization and biological processes, while the CWW control has evolved to the application of biological processes, physicochemical treatments or even the combination of biological and physicochemical technologies (Gannoun et al., 2008).

The costs associated to valorization technologies are not normally tolerable to small and medium factories, so biological and/or physicochemical treatments constitute a viable and the most attractive alternative.

3.1. Biological treatment without valorization

The research on the biological digestion of cheese whey started in the 1970s, with the application of aerobic processes such as activated sludge, trickling filters, lagoon storage, etc. However, these old processes were usually limited by the variability in the inlet properties of effluents and the extremely high pollution load of CW, energy requirements for oxygen supplying, excessive sludge production, difficulties in solids settling and thickening, etc (Blonskaja and Vaalu, 2006; Cordi et al., 2007; Ergüder et al., 2001; Farizoglu et al., 2004; Wildenauer and Winter, 1985). In the middle eighties, the anaerobic digestion achieved a great development facing the difficulties of small and medium factories at the time of implementing aerobic digesters. Thus, in the aerobic process, each kg of degraded COD forms 0.6 kg of sludge while 0.1 kg remains in the final effluent. In contrast, the anaerobic process only generates 0.1 kg of sludge per kg of COD transformed (Blonskaja and Vaalu, 2006). Additionally, the anaerobic process converts the pollutants into gaseous final products, mainly carbon dioxide and methane that can be used as an alternative energy source.

3.1.1. Aerobic digestion

The aerobic digestion is characterized by relatively fast organic matter degradation at room temperature (22–24 °C) requiring short HRT's (hydraulic retention time). However, the high organic load in raw CW makes the aerobic digestion inappropriate. The optimum C/N/P ratio in aerobic processes is roughly 100/5/1 compared to 500/5/1 in anaerobic processes (Janczukowicz et al., 2008). When dealing with highly polluted effluents, limitations in oxygen transfer may occur (Ozmihci and Kargi, 2007b). In general, the high contamination of raw dairy wastewaters might cause the overgrowth of filamentous microorganisms (bulking) and the subsequent difficulties in the sludge settling (Cordi et al., 2007; Donkin, 1997). Similarly to anaerobic processes, proteins and fats may negatively affect the sludge settling properties.

Table 1 summarizes the main parameters in the aerobic digestion processes applied to CW. Non-woven rotating biological contactors (NRBC) can withstand relatively high strength effluents due to its improved oxygen transference. Hence, Ebrahimi et al. (2010), using a three-stage NRBC, treated raw CW with an initial COD around 50 kg m^{-3}. These authors reported a COD removal in the interval 53–78% depending on HRT (8–16 h) with a residual COD of 10.7–24.0 kg m^{-3}. An anaerobic post-treatment was thereafter applied for 16 h to finally achieve an effluent showing a residual COD of 1.6–2.6 kg m^{-3}.

In accordance with the previous statements, the majority of the studies so far reported on aerobic digestion were conducted with diluted CW. Hence, Cordi et al. (2007) studied the application of activated sludge to diluted CW. These authors used two different dilution ratios and HRT's (dilution 1/100 HRT = 6 h and dilution 1/10 HRT = 36 h, respectively) obtaining a COD removal in the range 93.6–95.3%. The residual COD attained when treated the 1/100 CW dilution was below the legal limit value for direct discharge (150 mg L^{-1}). However, when the dilution was 1/10, the treated effluent exhibited a residual COD 1.73 times above the legal limit value.

Amongst the advanced reactor configurations, the development of the so called Jet Loop Bioreactors (JLBRs) has resulted in a high-efficient compact reactor (Petruccioli et al., 2002; Vogelpohl, 2000). These bioreactors are characterized by a high oxygen transfer and mixing, turbulence capacity, small size and reduced costs in terms of installation and energy consumption (Bloor et al., 1995; Dilek et al., 1996). Another efficient configuration is the integrated bioreactor-membrane system (MBR) (Farizoglu and Keskinler, 2006; Farizoglu et al., 2004, 2007) illustrated in Fig. 2. The application of membrane units for solids separation can minimize the principal disadvantages of conventional sedimentation when dealing with high biomass concentrations. Treated wastewater is free of solids and infectious organisms.

Table 1
Bibliographic compilation: aerobic digestion of cheese whey.

Experimental conditions						Results		Residual concentration (kg m^{-3})	Reference
Substrate	Reactor type	COD_i (kg m^{-3})	pH	T (°C)	HRT (day)	Loading (kg m^{-3} d^{-1})	COD removal (%)	COD	
Diluted CW	JLMBR	8.4–36	7.6	24	0.82–2.8	3.5–33.5	84–99	≤5.8	Farizoglu et al. (2004, 2007)
Diluted CW	JLMBR	8.4–36	7.6	24	0.82–2.8	22.2	97	–	Farizoglu and Keskinler (2006)
CW(dilution 1:100)	AS	0.547	7–8	–	0.25	–	93.6	0.04	Cordi et al. (2007)
CW(dilution 1:10)	AS	5.47	7–8	–	1.5	–	95.3	0.26	Cordi et al. (2007)
CW	NRBC	50	6.5	22	0.33–0.67	–	53–78	10.7–24.0	Ebrahimi et al. (2010)
CW	NRBC + UASFF	40–70	6.5	22–36	1.33	–	96–97.4	1.6–2.6	Ebrahimi et al. (2010)

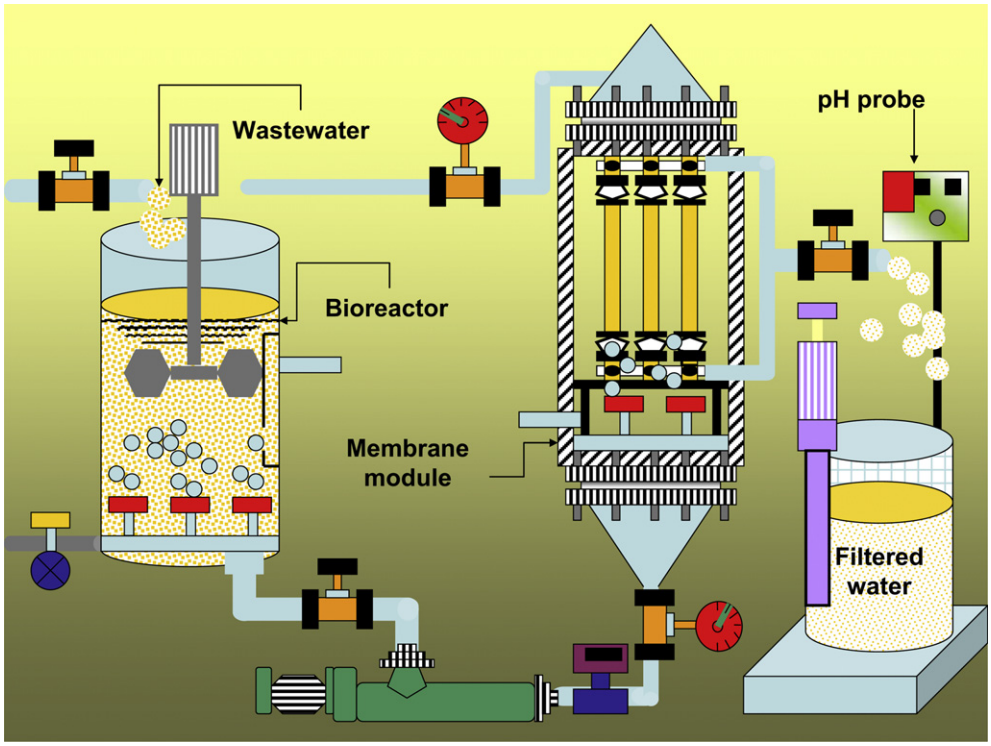

Fig. 2. Bioreactor integrated with membrane system (MBR).

The combination of jet loop and membrane technologies to treat diluted CW (COD up to 36 kg m^{-3}) has also been reported (Farizoglu and Keskinler, 2006; Farizoglu et al., 2004, 2007). Jet loop membrane bioreactors (JLMBR) have demonstrated a high COD reduction efficiency (99%) with residual COD below 5.8 kg m^{-3}. These results are obtained even when high COD loads (range 3.5–33.5 kg m^{-3} d^{-1}) are used. Moreover, this technology is able to tolerate short time changes in the inlet organic load. When raw CW was used the COD removal decreased to values of 81–83%. The JLMBR has also a high efficiency in total nitrogen (99%) and PO_4^{3-} (65–88%) removals (Farizoglu et al., 2007). Amongst the drawbacks, it can be stated that the sludge generated presents some settling problems. Additionally the flux rate through the membranes decreases with usage time.

3.2. Biological treatment with valorization

3.2.1. Anaerobic digestion

As a rule of thumb, cheese whey anaerobic digestion is normally conducted under mesophilic conditions (35–37 °C). The main products formed from the proteins anaerobic biodegradation by proteases include polypeptides, amino acids and ammonia. However, some proteins, such as casein (the main milk protein, 80%), are quite resistant to degradation by microorganisms. Thus, the use of acclimated or specific microorganisms is required. Contrarily, hydrocarbons are more susceptible to biodegradation (Pavlostathis and Giraldo-Gomez, 1991). Hence, lactose can straightforwardly be converted to propionic acid, ethanol and acetate. However, besides of the process easiness, the products obtained in lactose degradation can cause the partial inhibition in the methanogenesis phase (Vidal et al., 2000).

In general, the anaerobic digestion presents high organic removal efficiencies, however the low values of alkalinity (bicarbonate) about 50 meq L^{-1} can lead to failure in anaerobic digesters. Since the degradation rate of generated volatile fatty acids (VFA) by methanogenic bacteria is lower than their production by the acidogenic bacteria, VFA's rapidly accumulate in the medium. The low carbonate concentration avoids its buffering effect and the subsequent acidification of the reaction medium occurs (Janczukowicz et al., 2008; Kalyuzhnyi et al., 1997; Yan et al., 1993). As a result the anaerobic treatment presents great difficulties at the time of maintaining a stable operation (Ergüder et al., 2001; Janczukowicz et al., 2008; Kalyuzhnyi et al., 1997; Malaspina et al., 1996). Additionally to the previous drawbacks, the difficulty in the lipids biodegradation has also been reported (Petruy and Lettinga, 1997). The presence of fats may cause sludge flotation (Perle et al., 1995).

To solve these problems some researches have proposed different alternatives, such as alkalinity supplementation with lime (Gannoun et al., 2008; Patel and Madamwar, 1997; Patel et al., 1995), sodium hydroxide (Cordi et al., 2007; Ebrahimi et al., 2010; Ghaly, 1996); sodium bicarbonate (Ergüder et al., 2001; Mockaitis et al., 2006); sodium bicarbonate + potassium bicarbonate (Frigon et al., 2009).

Also, an increased in the viscosity is observed in anaerobic digestion processes. This viscosity increase may impair the biomass granulation leading to its flotation. This phenomenon occurs mainly when COD concentrations higher than 2.0 kg m^{-3} are used (Mockaitis et al., 2006).

Table 2 illustrates the main parameters of anaerobic digestion processes applied to CW. The UASB (Fig. 3) and UAF reactors are usually used when dealing with CW anaerobic biotransformation. Ergüder et al. (2001) claimed that the UASB is a very efficient reactor when dealing with diluted CW showing COD removals in the proximity of 95–97 %. Moreover, the process led to a high CH_4 yield around 0.424 m^3 kg^{-1} COD by using relatively short HRT in the interval 2.1–2.5 days. The effluent obtained in this work exhibited a residual COD between 1.7 and 2.7 kg m^{-3} (above the limit of direct discharge). Similar results were obtained by Blonskaja and Vaalu (2006). These authors reported 98% COD

Table 2
Bibliographic compilation: anaerobic digestion of cheese whey.

Experimental conditions						Results				Residual concentration (kg m^{-3})		Reference
Substrate	Reactor type	COD_i (kg m^{-3})	pH	T (°C)	HRT (day)	Loading (kg m^{-3} d^{-1})	Gas production (m^3 d^{-1} m^{-3})	% CH_4	CH_4 yield (m^3 kg^{-1} COD)	COD removal (%)	COD	
SCW	AUFFLR	79	6.7	35	5	14	5.6	79	0.4	95	3.9	Wildenauer and Winter (1985)
CW	ASDFA	70	–	–	–	16.1	–	–	–	99	0.7	Barford et al. (1986)
Diluted CW	UASB	4.5–38.1		–	5	–	–	–	–	97	–	Yan et al. (1989)
CW	AUFFR	70	7.0	37	2	35	6.7	72	–	81	13.4	Patel et al. (1995)
CW	DUHR	68.8	6.5–7.5	mesophilic	9	10	10	53	0.33 nL g^{-1} COD	98	1.4	Malaspina et al. (1996)
CW	TSUAD	72.22	7.0	35	20	10 L d^{-1}	12.5.6 L d^{-1}	70.9	0.23 COD	36	33	Ghaly (1996)
Diluted CW	Vertical ARBC	30	7	37	3	–	3.3	73	0.33 m^3 kg^{-1} TS d^{-1}	78	6.6	Patel and Madamwar (1997)
Diluted CW	Horizontal ARBC	30	7	37	3	–	3	73	0.30 m^3 kg^{-1} TS d^{-1}	77	6.9	Patel and Madamwar (1997)
Diluted CW	UASB	55.7–58.4	4–7	35	2.06–2.46	22.6–24.6	23.4 LCH$_4$ L^{-1}	77	0.424	95–97	1.7–2.7	Ergüder et al. (2001)
CW	CP	60.3–66.7	–	36	7	4.3–18.3	5.5–20 L d^{-1}	76	(0.28–0.59) × 10^{-3}	83	4.7	Blonskaja and Vaalu (2006)
CW	UASB		6.5–7.5	36	2.5	0.5–9.0	0.2–18.5 L d^{-1}	78	–	98	4.6	Blonskaja and Vaalu (2006)
CW powder solution	ASBR	0.5–4.0	7.6–84	30	–	0.6–4.8 mg L^{-1} d^{-1}	–	–	–	>90	0.1–0.6	Mockaitis et al. (2006)
CW	TSMAMD	68.6	6.5	37	5	19.78	10	>70	0.3	98.5	1.03	Saddoud et al. (2007)
Pre-treated CW[a]	UAF	5–20	7.2	35	2–5	4	1.3 LCH$_4$ L^{-1} d^{-1}	–	0.28	98	–	Gannoun et al. (2008)
Pre-treated CW[a]	UAF	15	7.2	35	2–5	3	3.2 L d^{-1}	–	0.28–0.38	95	0.75	Gannoun et al. (2008)

[a] Lactic fermentation of diluted CW and lime neutralization.

removal with a HRT of 2.5 days by also using a UASB reactor. The residual COD in this work was 4.6 kg m^{-3}. Gannoun et al. (2008) did use a UAF reactor to process previously pre-treated CW by implementing a fermentation process. These authors reported 95% COD reduction and a high CH_4 yield between 0.28 and 0.38 m^3 kg^{-1}. The effluent had a residual COD of 0.75 kg m^{-3}. This pre-treatment was aimed at eliminating the problems caused by fats and proteins.

Other advanced reactor configurations like the hybrid reactor (Malaspina et al., 1996) and reactors with flocculant addition (Barford et al., 1986) have also been tested achieving high organic removals from raw CW (98% and 99%, respectively). The hybrid reactor has the advantage of accomplishing the separation phase in the same reactor, reducing, therefore, the operation costs. Chemical flocculation allowed an increase of the biomass concentration. Thereby, the residual COD obtained after the process was reported to be 0.7 and 1.4 kg m^{-3} in reactors with flocculant addition and the hybrid reactor, respectively.

A variety of reactor configurations have also been tested by using a single-stage digestion system (Blonskaja and Vaalu, 2006; Patel et al., 1995; Wildenauer and Winter, 1985) or two-stage digestion system (Ghaly, 1996; Saddoud et al., 2007). The two-stage configuration is more efficient enabling the optimization of operating conditions for both processes: acidogenesis and methanogenesis (Blonskaja and Vaalu, 2006) with different kinetic rates (Saddoud et al., 2007). VFA concentration is reduced even when high organic loads are introduced into the digester and the inhibitor effect observed in the methanogenic phase is reduced. Additionally, the two-stage reactor reduces the costs and increases the biogas production (Ke et al., 2005). Considering the single-stage digestion system, the percentage removal and residual COD present values in the range 81–98% and 4.0–13.4 kg m^{-3} (HRT = 2–7 days), respectively. Saddoud et al. (2007) using a two-stage mixed anaerobic membrane digester (HRT of 5 days) report a COD removal as high as 99% while the residual COD was only 1.03 kg m^{-3}. The membrane system led to an effluent free of suspend solids. However, the flux membrane system was seriously affected by the formation and compaction of a cake layer. Oppositely, Ghaly (1996) reported a poor reduction in organic pollution with a scarce 36% of COD elimination (residual COD = 33 kg m^{-3}, HRT = 20 days) when using a two-stage reactor.

3.2.2. Lactose hydrolysis

The number of commercially available microorganisms capable of metabolizing glucose and galactose are significantly higher than the number of microorganisms able to directly use lactose (Siso, 1996). Berruga et al. (1997) describe the lactose hydrolysis as a low-cost cheese whey pre-treatment. Hydrolysis can be accomplished in two ways. Chemical hydrolysis is characterized by acid conditions (pH < 1.5) and high temperatures (up to 150 °C) (Gekas and López-Leiva, 1985; Guimarães et al., 2010). Chemical hydrolysis can be carried out with acid addition, such as sulfuric acid or using a solid acid, as the acid form of a cationic exchange resin. Chemical hydrolysis has some disadvantages such as protein denaturation, need of pre-demineralization, the appearance of a brown color due to Maillard reactions and the formation of undesirable products (Siso, 1996). As a consequence, the enzymatic hydrolysis is the preferential path for lactose hydrolysis (Kosaric and Asher, 1985). The enzymatic hydrolysis is carried out by means of the lactase enzyme (found in animals, plants, bacteria, fungi and yeasts) that converts the lactose disaccharide into its monosaccharide components, glucose and galactose. The main strains utilized in this process are *Aspergillus* and *Kluyveromyces* (Siso, 1996). Due to the impossibility of lactase reutilization, lactose hydrolysis conducted with the free enzyme

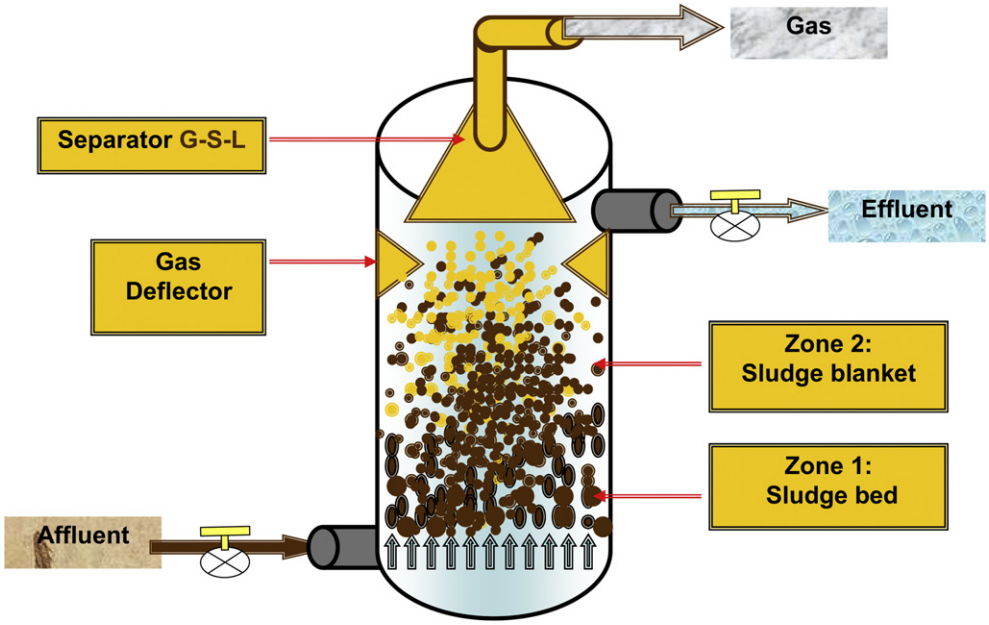

Fig. 3. UASB reactor: fixed bed and upflow reactor.

in solution is not recommended from an economic point of view. Alternatively, heterogeneous processes with the immobilized enzyme on different mediums or insolubilized by polymerization are preferred (Siso, 1996). The use of solid acid or immobilized enzyme leads to economic advantages, namely, the catalyst is maintained in the reactor and reused. Additionally, the post-treatment costs of the product obtained are significantly reduced, since the contamination with catalyst is minimized.

The main disadvantages of lactose enzymatic hydrolysis are: polymerization of lactose or galactose with the formation of oligosaccharides (Gekas and López-Leiva, 1985; Guy and Bingham, 1978) and lactose mass transfer limitations into the cells (Joshi et al., 1987).

3.2.3. *Fermentation to ethanol*

The first studies on alcohol production (ethanol) from CW fermentation date from the 1940s (Browne, 1941; Rogosa et al., 1947). Pollution reduction and lactose conversion to ethanol are achieved simultaneously and remain a common practice at present (see Table 3). Consequently, the treatment of cheese whey and the simultaneous ethanol production has received a wide attention. In this sense several studies have been reported by using raw CW (Ghaly and El-Taweel, 1995, 1997; Kourkoutas et al., 2002; Sansonetti et al., 2009; Zafar and Owais, 2006); CW powder solution (Kargi and Ozmihci, 2006; Ozmihci and Kargi, 2007a, 2007b, 2008); CW permeate from ultrafiltration (Domingues et al., 1999; Dragone et al., 2009; Sansonetti et al., 2009) and even deproteinized CW (Dragone et al., 2011; Izaguirre and Castillo, 1982). This treatment requires a specific group of microorganisms such as *Torula cremoris* (Rogosa et al., 1947); *Kluyveromyces fragilis* (Dragone et al., 2011; Siso, 1996), *Kluyveromyces marxianus* (Dragone et al., 2009; Kargi and Ozmihci, 2006; Kourkoutas et al., 2002; Ozmihci and Kargi, 2007a, 2007b, 2008; Sansonetti et al., 2009; Zafar and Owais, 2006), *Candida pseudotropicalis* (Ghaly and El-Taweel, 1995, 1997; Guimarães et al., 2010; Izaguirre and Castillo, 1982) and *Saccharomyces cerevisiae* (Domingues et al., 1999; Guimarães et al., 2008, 2010), etc. The reaction describing the bio-conversion of lactose to ethanol reveals a theoretical maximum value of 0.538 kg of ethanol kg^{-1} of lactose consumed (Mawson, 1994; Sansonetti et al., 2009).

The alcoholic fermentation of lactose from cheese whey or whey permeates is hardly economically competitive if compared to others substrates such as cane sugar, cornstarch, lignocellulosic biomass, etc (Guimarães et al., 2010). In general, the production of ethanol from non-concentrated cheese whey, with lactose/sugars concentration between 35 and 50 kg m^{-3}, is not economically viable. Although the efficiency of substrate consumption ranges from 70% to approximately 100% with relatively low HRT's (18–50 h), the ethanol concentration is rather low (2.1–20 kg m^{-3}) (Kourkoutas et al., 2002; Ozmihci and Kargi, 2008; Sansonetti et al., 2009; Zafar and Owais, 2006). The obtained effluent shows a considerable residual COD (15 kg m^{-3}) although a better odor is experienced (Kourkoutas et al., 2002).

Alternatively, substrate consumption above 83% and a residual COD in the wide interval 2.6–25.5 kg m^{-3} are obtained when using cheese whey concentrate with lactose/sugar concentration around 125–150 kg m^{-3} (Ghaly and El-Taweel, 1997; Kargi and Ozmihci, 2006; Ozmihci and Kargi, 2007b). The main advantage of dealing with high sugar concentrations is a high conversion efficiency to ethanol and the acceptable values in ethanol concentration in the proximity of 60 kg m^{-3} (Ghaly and El-Taweel, 1997; Ozmihci and Kargi, 2007b).

When lactose fermentation is carried out under anaerobic conditions (Kargi and Ozmihci, 2006; Ozmihci and Kargi, 2007b) the substrate utilization is rather slow, as a consequence, the required HRT notably increases (9–14 days) compared to HRT's needed under aerobic conditions (42 h) (Ghaly and El-Taweel, 1997). The use of extremely high substrate concentrations might affect the ethanol yield and the substrate consumption due to partial inhibition (high osmotic pressure) (Ozmihci and Kargi, 2007a, 2007b). Additionally, an excessive increase in the aeration can lead to a decrease in the ethanol production, since the lactose substrate is preferentially used for biomass growth rather than for ethanol production (Ghaly and El-Taweel, 1995).

Domingues et al. (1999) used a CW permeate (ultrafiltration) under aerobic conditions. These authors experienced a substrate utilization higher than 96% with a residual COD and BOD_5 of 7.22 kg m^{-3} and 2.13 kg m^{-3}, respectively. The effluent had a low biodegradability ($BOD_5/COD \approx 0.30$) and the total nitrogen concentration was 0.710 kg m^{-3}. The final ethanol concentration

Table 3
Bibliographic compilation: fermentation to ethanol of cheese whey.

Experimental conditions							Results					Residual concentration (kg m^{-3})				Reference
Substrate	Organism	Reactor type	Lactose$_i$ (kg m^{-3})	pH	T (°C)	HRT (h)	Ethanol production (kg m^{-3})	% ethanol (v/v)	Ethanol yield (kg ethanol kg^{-1} lactose)	Conversion efficiency (%)	Substrate consumption (%)	Lactose	COD	BOD_5	TN	
Pasteurized CW	*Candida pseudotropicalis*	Aerated pilot-scale batch	150	4.9	room	≈70	–	–	0.465	98.3	75	37.5	–	–	–	Ghaly and El-Taweel (1995)
Pasteurized CW	*C. pseudotropicalis*	Without aeration. pilot-scale batch	150	4.9	room	≈ 70	–	–	0.441	89.64	68	48	–	–	–	Ghaly and El-Taweel (1995)
Pasteurized CW	*C. pseudotropicalis*	Aerated Continuous	150	4.1–4.7	29.2–31.6	42	58.0		0.471	99.6	83	≈25.5	–	–	–	Ghaly and El-Taweel (1997)
CW permeate (UF)	*Saccharomyces cerevisiae*	Airlift Continuous	≈50	4.0	30	30	≈ 20	–	0.430	80	>96	< 2	7.22	2.13	0.71	Domingues et al. (1999)
CW	*Kluyveromyces marxianus*	Batch	5[a]	5.5	45	25	7.3	–	–	–	≈90	5.0[b]	–	–	–	Kourkoutas et al. (2002)
CW powder solution	*K. marxianus*	Batch	145[b]	3–7	–	216	–	10.5	0.54[c]	100	98.2	2.61[b]	–	–	–	Kargi and Ozmihci (2006)
CW	*K. marxianus*	Batch	35	4.5	34	24	2.1	–	–	–	≈85.7	5	–	–	–	Zafar and Owais (2006)
CW powder solution	*K. marxianus*	Continuous	100[b]	4.3–4.6	28	54	–	3.7	0.493[c]	91.3	≈58	≈42[b]	–	–	–	Ozmihci and Kargi (2007a)
CW powder solution	*K. marxianus*	Fed-Batch	125[b]	4.0–4.5	26	336	63	7.97	0.54[c]	100	–	–	–	–	–	Ozmihci and Kargi (2007b)
CW powder solution	*K. marxianus*	PCBR Continuous	50[b]	4.2–4.4	25–28	50	19.5	–	0.54[c]	100	70	15.4[b]	–	–	–	Ozmihci and Kargi (2008)
CW	*K. marxianus*	Batch	≈ 43	5	37	18	20	–	0.447	83	≈100	≈0	–	–	–	Sansonetti et al. (2009)
CW permeate (UF)	*K. marxianus*	Batch	≈ 43	5	37	18	≈ 6	–	–	–	≈32.56	≈29	–	–	–	Sansonetti et al. (2009)
CW permeate (UF)	*K. marxianus*	Airlift Continuous	50	4.0	30	–	–	35.4	–	–	–	–	–	–	–	Dragone et al. (2009)
Deproteinized CW powder solution	*Kluyveromyces fragilis*	Batch	200	5.0	35	44	80.95	–	–	–	≈100	≈0	–	–	–	Dragone et al. (2011)

[a] %.
[b] As total sugar.
[c] kg ethanol kg^{-1} sugar.

was comparable to that obtained when using raw CW. When ethanol fermentation of CW permeate was conducted under anaerobic conditions (Sansonetti et al., 2009) the final ethanol concentration and the substrate utilization were reduced in 70% and three times, respectively. Due to the limited substrate utilization, the residual lactose in this process was about 29 kg m^{-3}. Similar results were obtained by Ghaly and El-Taweel (1995) when using concentrated CW under aerobic or anaerobic conditions.

The use of CW powder has some advantages if compared to CW permeate. Thus, the ultrafiltration costs are eliminated and higher lactose/sugar concentration can be fed to the fermentator (Dragone et al., 2011; Ozmihci and Kargi, 2007a, 2007b). Additionally, CW powder has a reduced volume, long-term stability and easier storage and transportation (Kargi and Ozmihci, 2006).

When lactose was concentrated to values in the proximity of 200 kg m^{-3} the final ethanol concentration increased until 10–12%. As a consequence, the distillation costs of ethanol separation significantly decreased (Guimarães et al., 2008; Ozmihci and Kargi, 2007b). It is noteworthy to mention the elevated process efficiency experienced by Dragone et al. (2011) when using a deproteinized CW powder solution with a lactose content of 200 kg m^{-3}. These authors obtained an ethanol concentration in the proximity of 81 kg m^{-3}, that is, about 4 times the maximum values achieved when using raw CW. The authors also report a substrate consumption close to 100%.

Ethanol production is restricted to microorganisms able to directly use lactose (Moulin and Galzy, 1984), namely, *S. cerevisiae* (Domingues et al., 1999). Thus, a potential alternative is the previous enzymatic hydrolysis of lactose by β-galactosidase and subsequent ethanol fermentation (Champagne and Goulet, 1988; Guimarães et al., 2010). The main disadvantages of this pre-treatment are the price of the enzyme β-galactosidase and the slow growth and performance. This process can be developed in two stages or in one step, with a mixture of cultures or with the enzyme and yeast co-immobilized (Axelsson et al., 1991).

Cheese whey ethanol can be used in food, chemical, pharmaceutical and cosmetics industries (Guimarães et al., 2010; Zafar and Owais, 2006) and as an alternative and environmental fuel (Ghaly and El-Taweel, 1997; Staniszewski et al., 2009).

3.2.4. Fermentation to hydrogen

Hydrogen represents a clean energy that does not contribute to the generation of greenhouse gases or acid rain. Due to its low solubility, hydrogen can be easily separated from water and purified (Davila-Vazquez et al., 2009). This gas possesses a high energy yield (Azbar and Dokgoz, 2010; Castelló et al., 2009; Kotay and Das, 2008; Rosales-Colunga et al., 2010; Venetsaneas et al., 2009) of 122 kJ g^{-1}, i.e. 2.75 times the energy content of many hydrocarbon fuels (Kapdan and Kargi, 2006). Moreover, hydrogen can be directly used to produce electricity through fuel cells (Lay et al., 1999).

Use of carbohydrate-rich wastewaters, like cheese whey, is an economically viable option for hydrogen production (Azbar et al., 2009; Yang et al., 2007). Anaerobic fermentation processes from cheese whey (Azbar and Dokgoz, 2010; Venetsaneas et al., 2009), diluted CW (Azbar et al., 2009; Castelló et al., 2009; Ferchichi et al., 2005), CW powder solution (Davila-Vazquez et al., 2008, 2009; Rosales-Colunga et al., 2010) and CW permeate powder (Yang et al., 2007) have been conducted for hydrogen production. This process should lead to a theoretical yield of 8 mol of hydrogen per mol of lactose. The biogas mixture formed in hydrogen production also contains CH_4 and CO_2.

Table 4 illustrates some of the investigations conducted on this particular subject. The anaerobic fermentation is carried out by various microorganisms such as obligatory anaerobic strains of the *Clostridium* species (*Clostridia butyricum*, *Clostridium pasteurianum* and *Clostridium beijerinkii)* (Ferchichi et al., 2005) and facultative anaerobic species like *Enterobacter*, *Citrobacter* sp. and *Escherichia coli* (Rosales-Colunga et al., 2010). Studies have been carried out with mixed microbiological communities under mesophilic conditions (30–38 °C) (Castelló et al., 2009; Davila-Vazquez et al., 2009; Venetsaneas et al., 2009; Yang et al., 2007) or thermophilic conditions (55 °C) (Azbar and Dokgoz, 2010; Azbar et al., 2009).

Hydrogen production can be maximized by controlling some key parameters, like pH (Castelló et al., 2009; Fang and Liu, 2002; Ferchichi et al., 2005; Rosales-Colunga et al., 2010 Yang et al., 2007; Zhang et al., 2003), alkalinity (Castelló et al., 2009; Venetsaneas et al., 2009; Yang et al., 2007), predominant microorganisms (Azbar et al., 2009; Davila-Vazquez et al., 2009; Yang et al., 2007), substrate composition, temperature, humidity, HRT (Castelló et al., 2009; Davila-Vazquez et al., 2009), and supplementation of trace metals, yeast extract and nutrients (Azbar et al., 2009; Ferchichi et al., 2005; Yang et al., 2007), etc. Due to increased yields, hydrogen production is generally carried out in neutral or slightly acidic pH conditions (pH = 4–7.5). Additionally, Davila-Vazquez et al. (2009) experienced a higher volumetric hydrogen production rate (VHPR), an increased hydrogen percentage in the gas and a lower HRT when raising the organic loading rate (OLR) to the digester. Hydrogen production can be reduced due to the presence of methanogenic hydrogen consumer microorganisms (Castelló et al., 2009; Chong et al., 2009; Yang et al., 2007).

The main reactor types used in this process are (by ascending importance order), CSTR (Azbar and Dokgoz, 2010; Azbar et al., 2009; Davila-Vazquez et al., 2009; Venetsaneas et al., 2009; Yang et al., 2007), batch (Ferchichi et al., 2005; Rosales-Colunga et al., 2010; Yang et al., 2007) and UASB (Castelló et al., 2009). The CSTR presents HRT's between 6 and 84 h compared to 12 h for UASB and 24–280 h for batch conditions. As a rule of thumb, batch reactors lead to a higher hydrogen percentage (50–88%) than the rest of reactor configurations (20–60%).

From an environmental point of view, COD reductions around 80–90% and sugar consumption between 86 and 97% have been reported (Azbar and Dokgoz, 2010; Azbar et al., 2009; Ferchichi et al., 2005; Rosales-Colunga et al., 2010; Venetsaneas et al., 2009; Yang et al., 2007). COD in the effluent coming from hydrogen production shows values within the range 4–28 kg m^{-3} when using a CSTR (Azbar and Dokgoz, 2010; Azbar et al., 2009; Venetsaneas et al., 2009) and 0.12–2.0 kg m^{-3} when batch reactors were used (Ferchichi et al., 2005; Rosales-Colunga et al., 2010; Yang et al., 2007). Table 4 suggests that the effluent obtained after hydrogen production cannot be discharged and a post-treatment is required. The residual organic matter remains in the form of volatile organic acids (acetic, propionic, butyric, etc.), alcohols (ethanol) and carbohydrates (unreacted lactose).

Only a few studies have considered the effluent coming from hydrogen production (Azbar and Dokgoz, 2010; Venetsaneas et al., 2009). Venetsaneas et al. (2009) used the hydrogen production effluent for methane generation in a continuous anaerobic bioreactor under mesophilic conditions. These authors report a methane production of 1.0 L CH_4 d^{-1} (yield = 6.7 normal L CH_4 L^{-1}) using a HRT of 20 days. A COD removal of 95.3% was experienced remaining 2.2 kg m^{-3} of COD after the process.

Azbar and Dokgoz (2010) made an attempt to treat the effluent coming from CW hydrogen production by photo-fermentation with *Rhodopseudomonas palustris* (two-stage biological process). The authors claimed the inappropriateness of the process due to the nitrogen and volatile fatty acid content. This effect could be minimized by dilution of the effluent coming from the dark fermentation with a L-malic acid solution, which simultaneously improved hydrogen production. A final yield in the range 2–10 mol H_2 mol^{-1} lactose was also reported. However, the photo-fermentation

Table 4
Bibliographic compilation: fermentation to hydrogen of cheese whey.

Experimental conditions								Results				Residual concentration (kg m^{-3})		Reference
Substrate	Organism	Reactor type	COD_i (kg m^{-3})	$Lactose_i$ (kg m^{-3})	pH	T (°C)	HRT (h)	Volumetric H_2 production	% H_2 (v/v)	H_2 yield (mol H_2 mol^{-1} lactose)	Substrate consumption (%)	Lactose	COD	
Diluted CW	*Clostridium saccharoperbutylacetonicum*	Batch	89.3	41.4	6	30	50–52	47.07 mL h^{-1}	87.5	7.89 mmol g^{-1} lactose	97	1.2	–	Ferchichi et al. (2005)
CW permeate powder solution	Mixed microbial cultures (50 % *Lactobacillus* + 5% *Clostridia*)	CSTR	–	–	4–5	35–38	24	2.8–5.1 L d^{-1}	26–33	1.8–2.3 mM g^{-1} COD	–	–	–	Yang et al. (2007)
CW permeate powder solution	Mixed microbial cultures (50 % *Lactobacillus* + 5% *Clostridia*)	Batch	–	–	5–7	35–38	24	0.39 L h^{-1}	49.6	10.1 mM g^{-1} COD	>95	0.103	–	Yang et al. (2007)
Diluted CW	Mixed microbial communities (99% of *Thermoanaerobacteriaceae bacterium)*	CSTR	40	–	5.5	55	84	2.5 L L^{-1} d^{-1}	42	22 mmol g^{-1} COD	90	–	4	Azbar et al. (2009)
CW powder solution	Anaerobic granular sludge (*Clostridium genus*)	CSTR	–	–	5.9	37	6	23.32–46.61 mmol L^{-1} h^{-1}	49–58	2.1–2.8	–	–	–	Davila-Vazquez et al. (2009)
CW	Indigenous mixed microbial culture	CSTR	60.5	43.8[a]	5.2	35	24	2.9 L L^{-1} d^{-1}	23.8	0.78 mol mol^{-1} glucose	86	6.1[a]	–	Venetsaneas et al. (2009)
CW	Anaerobic mixed microflora	CSTR	–	–	5.5	55	24	2.5–5.0 L L^{-1} d^{-1}	40–60	1.8–5.1	–	–	28	Azbar and Dokgoz (2010)
Diluted CW	Mixed culture (*Megasphaera*, *Anaerotruncus*, *Pectinatus* and *Lactobacillus*)	UASB	10	–	5.0	30	12	122 mL L^{-1} d^{-1}	20–30	–	–	–	–	Castelló et al. (2009)
CW powder solution	*Escherichia coli* (strain constructed)	Batch	–	≈44	7.5	37	280	2.76 mL h^{-1}	–	2.74	91	≈4	–	Rosales-Colunga et al. (2010)

[a] As soluble sugar.

presented some disadvantages such as the energy supplementation and the need of large volume bioreactors.

3.2.5. Fermentation to lactic acid

Cheese whey effluents have been used in fermentation processes to produce lactic acid (Arasaratnam et al., 1996; Mostafa, 1996; Pescuma et al., 2008; Plessas et al., 2008; Roukas and Kotzekidou, 1991, 1998; Silva and Yang, 1995; Tango and Ghaly, 1999). Currently, a high fraction of generated CW is managed by membrane processes, mainly, ultrafiltration. In this situation, permeate has a low protein content and an elevated lactose and mineral salts concentrations. Thus, several works have been carried out aimed at obtaining lactic acid after ultrafiltration of CW (González et al., 2007; Kulozik and Wilde, 1999; Pauli and Fitzpatrick, 2002; Schepers et al., 2006; Vasala et al., 2005). Table 5 highlights some works related to lactic acid production.

Microorganisms used in lactic acid production are *Lactobacillus casei* (Mostafa, 1996; Pauli and Fitzpatrick, 2002; Roukas and Kotzekidou, 1998; Vasala et al., 2005), *Lactobacillus helveticus* (González et al., 2007; Kulozik and Wilde, 1999; Plessas et al., 2008; Schepers et al., 2006; Silva and Yang, 1995; Tango and Ghaly, 1999); *Lactobacillus acidophilus* (Pescuma et al., 2008); *Lactobacillus delbrueckii* (Arasaratnam et al., 1996; Pescuma et al., 2008; Plessas et al., 2008); *Streptococcus thermophilus* (Pescuma et al., 2008) *Lactococcus lactis* (Roukas and Kotzekidou, 1998); L*actobacillus salivarius* (Vasala et al., 2005); *K. marxianus* (Plessas et al., 2008); *Leuconostoc*, *Pediococcus* (Panesar et al., 2007), etc. However, some studies report the use of mixed cultures (Plessas et al., 2008; Roukas and Kotzekidou, 1991, 1998) with synergistic effects.

Although many species have been reported, some researches claimed the extended use of *Streptococcus* (Tango and Ghaly, 1999) and the effectiveness of *L. helveticus* (Plessas et al., 2008; Roy et al., 1986; Tango and Ghaly, 1999). The species *Lactobacillus salivarius ssp. salicinius* and *Bacillus megaterium* can grow in high salinity environments (Vasala et al., 2005).

Lactic acid production from cheese whey or permeate cheese whey obtained without nutrients supplementation (Arasaratnam et al., 1996; Plessas et al., 2008; Tango and Ghaly, 1999; Vasala et al., 2005) is of limited application to industrial scale because of the low productivity (lactic acid production = 3,8–12 kg m^{-3}, HRT = 48–56 h, temperature = 23–37 °C). Nutrients supplementation is a key factor limiting the process efficiency. For instance, manganese constitutes a limiting growth factor for *L. casei*, since this nutrient is a constituent of the lactate dehydrogenase (Krischke et al., 1991). Yeast extract (Aeschlimann and von Stockar, 1990; Arasaratnam et al., 1996; Roukas and Kotzekidou, 1998; Schepers et al., 2006; Vasala et al., 2005), malt combing nuts (Pauli and Fitzpatrick, 2002), peptone (Arasaratnam et al., 1996; Roukas and Kotzekidou, 1998), soya flour (Arasaratnam et al., 1996), whey proteins (Vasala et al., 2005), glucose medium (Mostafa, 1996), $MgSO_4$ (Roukas and Kotzekidou, 1998), $MnSO_4$ (Roukas and Kotzekidou, 1998) and $(NH_4)_2SO_4$ (Arasaratnam et al., 1996) are reported as supplementations used to obtain a good growth and high lactic acid productivity with lactic acid concentration in the range 11–52 kg m^{-3} (substrate consumption 73–100% depending on supplementation type).

A slow microorganism growth has been obtained under non-optimal operating conditions with substrate consumption in the interval 34–85% (unreacted sugar/lactose = 7.5–31.8 kg m^{-3}). However, when increasing the HRT (60–72 h), the temperature (42 °C) or when using mixed cultures, the fermentation performance was notably improved achieving lactic acid concentrations in the range 10.1–20 kg m^{-3}. In the latter case the substrate consumption in the interval 61–83% and a lower remaining sugar/lactose concentration (8–19 kg m^{-3}) were experienced even without nutrients supplementation.

If the supplementation additives (yeast extract, peptone, soya flour, whey proteins, glucose medium, glucose medium, $MgSO_4$, $MnSO_4$, $(NH_4)_2SO_4$) are substituted by proteolytic enzymes or proteolytic microorganisms, the production of lactic acid is doubled up (Vasala et al., 2005).

Immobilized cell systems (biofilm, membrane-based cell recycle reactors, fibrous-bed) present positive characteristics like high lactic acid productivity, easy cell-products separation and easiness of operation at high dilution rates without cell washout (Silva and Yang, 1995). However, immobilized cell systems also have some disadvantages such as low long-term stability due to membrane fouling, cell degeneration, and bed clogging (Silva and Yang, 1995).

Lactic acid is used in food and chemical industries (pharmaceutical products, textiles, leather), primarily as a preservative and as acidulant (Pauli and Fitzpatrick, 2002; Roukas and Kotzekidou, 1991; Tango and Ghaly, 1999). Also it has applications as a biodegradable plastic component (polylactide, polymers, polyhydroxybutryate) (Tango and Ghaly, 1999).

3.2.6. Direct production of electricity through microbial fuel cells

The biological processes mentioned above, to produce methane, hydrogen or ethanol, from cheese whey do not consider the direct production of electricity. Another option for cheese whey valorization is the direct production of electricity through microbial fuel cells (MFCs). MFCs consist of two compartments: one anaerobic and another aerobic, containing an anode and a cathode, respectively. In the anaerobic compartment the microorganisms oxidize the organic matter with formation of carbon dioxide, protons and electrons. Protons migrate to the cathode chamber (aerobic compartment) permeating through the proton exchange membrane (thereafter protons combine with oxygen) while the electrons are transferred to the cathode through of an external circuit, with oxygen reduction to water. This electron flow produces an electrical current that can be measured (Rachinski et al., 2010). MFC technology has been used to produce direct electricity from non-sterilized and diluted cheese whey (Antonopoulou et al., 2010), filter-sterilized and diluted cheese whey (Stamatelatou et al., 2011), raw cheese whey, raw cheese whey inoculated with *Enterobacter cloacae* subspecies *dissolvens* and heat treated cheese whey inoculated with *E. cloacae* (Kassongo and Togo, 2010).

From an environmental point of view, COD reduction close to 100% for a reaction time of 50 h has been reported (Antonopoulou et al., 2010) when non-sterilized and diluted cheese whey were used with an initial COD of 0.73 kg m^{-3} in a two-chamber mediator-less MFC. The obtained maximum power density (18.4 mW m^{-2}) was 6.5% higher comparatively to the pure substrate (lactose). However, the coulombic efficiency was only 1.9%. These authors explained the low coulombic efficiency value due to the presence of indigenous non-electrogenic microbial competition with anodic electrode microorganisms. In this case, COD consumption led to the production of gaseous species, such as methane or carbon dioxide.

The negative effect of indigenous non-electrogenic microorganisms can be reduced by a previous treatment. Thus, Stamatelatou et al. (2011) used cheese whey after centrifugation, filtration and sterilization to eliminate solids and microorganisms. In this work cheese pre-treated whey with 5 different initial concentrations (0.35–6.7 kg m^{-3}) was used. The authors claimed that the reaction time had a linear relationship with the initial cheese whey COD. The maximum power density (38–42 mW m^{-2}) was approximately 2 times higher than the one obtained when no-pretreated and diluted cheese whey was used (Antonopoulou et al., 2010). COD in the effluent coming from microbial fuel cells

Table 5
Bibliographic compilation: fermentation to lactic acid of cheese whey.

Experimental conditions									Results		Residual concentration ($kg\ m^{-3}$)	Reference
Substrate	Cell System/Organism	Reactor type	Supplementation	COD_i ($kg\ m^{-3}$)	$Lactose_i$ ($kg\ m^{-3}$)	pH	T (°C)	HRT (h)	Lactic acid ($kg\ m^{-3}$)	Substrate consumption (%)	Lactose	
Deproteinized Whey	Coimmobilized Ca-alginate gel beds *Lactobacillus casei* and *Lactococcus Lactis*	–	–	–	–	–	–	48	41.3	–	–	Roukas and Kotzekidou (1991)
CW	Static culture *Lactobacillus delbrueckii*	–	–	–	30[a]	6.5	Room	72	20	73.3	8.0[a]	Arasaratnam et al. (1996)
CW	Static culture *L. delbrueckii*	–	–	–	30[a]	6.5	Room	48	12.0	55.0	13.5[a]	Arasaratnam et al. (1996)
CW	Static culture *L. delbrueckii*	–	Yeast extract (10 $kg\ m^{-3}$)	–	30[a]	6.5	Room	72	23.0	90.0	3.0[a]	Arasaratnam et al. (1996)
CW	Static culture *L. delbrueckii*	–	Yeast extract (20 $kg\ m^{-3}$)	–	30[a]	6.5	Room	48	24.5	92.0	2.4[a]	Arasaratnam et al. (1996)
CW	Static culture *L. delbrueckii*	–	Peptone (14.5 $kg\ m^{-3}$)	–	30[a]	6.5	Room	48	22.5	85.7	4.29[a]	Arasaratnam et al. (1996)
CW	Static culture *L. delbrueckii*	–	Soya flour (34.5 $kg\ m^{-3}$)	–	30[a]	6.5	Room	48	23.0	90.0	3.0[a]	Arasaratnam et al. (1996)
CW	Static culture *L. delbrueckii*	–	$(NH_4)_2SO_4$ (10 $kg\ m^{-3}$)	–	30[a]	6.5	Room	60	23.5	100.0	0.0[a]	Arasaratnam et al. (1996)
Deproteinized CW	Agar gel immobilized *L. casei*	CPTR	Glucose medium	–	5.2[b]	7.0	40	4	2.80[b]	49.8	2.6[b]	Mostafa (1996)
Deproteinized CW	Free cells of mixed cultures (*L. casei* and *L. lactis*)	Batch	Yeast extract; peptone; $MgSO_4$; $MnSO_4$	–	50	6.0 ± 0.3	32	24	22.5	93.0	≈3.5	Roukas and Kotzekidou (1998)
Deproteinized CW	Free cells of *L. casei*	Batch	Yeast extract; peptone; $MgSO_4$; $MnSO_4$	–	50	6.0 ± 0.3	32	24	16	80.0	≈10	Roukas and Kotzekidou (1998)
Deproteinized CW	Free cells of *L. lactis*	Batch	Yeast extract; peptone; $MgSO_4$; $MnSO_4$	–	50	6.0 ± 0.3	32	24	11.0	63.0	≈18.5	Roukas and Kotzekidou (1998)
Deproteinized CW	Free cells of mixed cultures (*L. casei* and *L. lactis*)	Fed-batch	Yeast extract; peptone; $MgSO_4$; $MnSO_4$	–	100	6.0	–	24	46	80.0	≈15	Roukas and Kotzekidou (1998)
Deproteinized CW	Coimmobilized cells of mixed cultures (*L. casei* and *L. lactis*) in Ca-alginate beads	Fed-batch	Yeast extract; peptone; $MgSO_4$; $MnSO_4$	–	100	6.0	–	24	47	73.0	≈20	Roukas and Kotzekidou (1998)
Pasteurized CW	*Lactobacillus helveticus*	Batch	–	81.05	48.0	4.4	23	56	3.8	34.0	31.8	Tango and Ghaly (1999)
Pasteurized CW	*L. helveticus*	Batch	–	81.05	48.0	4.4	42	56	10.1	60.6	19.0	Tango and Ghaly (1999)
CW permeate (UF)	*L. casei*	Batch	–	–	50.0	6.0	30	48	>20	–	–	Vasala et al. (2005)
CW permeate (UF)	*Lactobacillus salivarius ssp. salicinius*	Batch	–	–	50.0	6.0	30	48	5–10	–	–	Vasala et al. (2005)
CW permeate (UF) pre-treated with *Acinetobacter* or *Bacillus megaterium*	*L. salivarius ssp. salicinius*	Batch	–	–	50.0	6.0	30	48	>20	–	–	Vasala et al. (2005)
CW permeate (UF)	*L. salivarius ssp. salicinius*	Batch	–	–	50.0	5.5	42	60	≈13	–	–	Vasala et al. (2005)
CW permeate (UF)	*L. salivarius ssp. salicinius*	Batch	Whey proteins + protease mix (Alcalase and Flavourzyme)	–	50.0	5.5	42	60	≈52	–	–	Vasala et al. (2005)
CW permeate (UF)	*L. salivarius ssp. salicinius*	Batch	Whey proteins + Yeast extract	–	50.0	5.5	42	60	≈49	–	–	Vasala et al. (2005)
CW	Mixed cultures (*Kluyveromyces marxianus* and *L. Bulgaricus*)	Static flasks	–	–	50–52	6.6	37	–	16.2 ± 0.2	≈77.8	11.3 ± 0.6	Plessas et al. (2008)

CW	Mixed cultures (*K. marxianus*, *L. helveticus* and *L. bulgaricus*)	Static flasks	–	–	50–52	6.6	37	–	19.8 ± 0.4	≈82.7	8.8 ± 0.9	Plessas et al. (2008)
CW	*L. helveticus*	Static flasks	–	–	50–52	6.6	37	–	10.1 ± 0.2	≈85.3	7.5 ± 0.6	Plessas et al. (2008)
CW	*L. bulgaricus*	Static flasks	–	–	50–52	6.6	37	–	9.6 ± 0.2	≈62.0	19.4 ± 0.9	Plessas et al. (2008)
CW	*K. marxianus*	Static flasks	–	–	50–52	6.6	37	–	8.8 ± 0.2	≈71.0	14.8 ± 0.7	Plessas et al. (2008)

[a] As total sugar.
[b] %.

presented values in the range 0.018–0.268 kg m^{-3}. In spite of the high COD removal values (95–96%), when high initial COD concentrations were used (initial COD = 6.7 kg m^{-3}) the final effluent still presented an inadmissible COD content requiring a post-treatment.

COD and solid removal obtained (45% and 20%, respectively) with raw cheese whey were higher than those experienced with heat treated cheese whey inoculated with *E. cloacae* (Kassongo and Togo, 2010). In the latter case, COD and solid removal were only 5.0% and 3.4%, respectively. However, the coulombic efficiency in the process with heat treated cheese whey inoculated with *E. cloacae* was 92.5 and 7.4 times higher than the results found when raw cheese whey and raw cheese whey inoculated with *E. cloacae* (subspecies *dissolvens*) were used, respectively (Kassongo and Togo, 2010). Power densities presented values of 16.7, 1.1 and 0.4 W m^{-2} for heat treated cheese whey inoculated with *E. cloacae*, raw cheese whey inoculated with *E. cloacae* and raw cheese whey, respectively. In this study the residual COD presented values in the range 53.4–91.7 kg m^{-3}. These findings imply that the effluent coming from the MFC still constitutes a hazardous waste.

The maximum power density increased approximately 99.7% when sterilized raw cheese whey inoculated with *E. cloacae* (initial COD of 96.5 kg m^{-3}) was used (Kassongo and Togo, 2010), comparatively to sterilized and diluted cheese whey (initial COD of 6.7 kg m^{-3}) (Stamatelatou et al., 2011).

This new alternative for cheese whey valorization presents some other drawbacks for industrial application, specifically, complex biological activity, large operation area, low power production and poor reproducibility (Kassongo and Togo, 2010).

3.3. Physicochemical treatment

The unquestionable importance of proteins in human diet has led in the last years to an increase in research fields focused to find new protein sources. For instance, lactose has found its use as a supplement in baby formulas and as excipient in pharmaceutical products. Lactose can also contribute to the color and taste in bakery and pastry products.

Physicochemical treatments are meant to the reduction of contaminant indicators, such as organic matter, turbidity and suspended solids but also to the recuperation of valuable products present in CW, namely proteins and lactose. Contaminant load reduction can be accomplished by coagulation–flocculation processes with iron salts or electrochemically with iron electrodes. Valorization techniques use chitosan and alginate coagulation/flocculation, electrochemical coagulation, acid precipitation, thermal precipitation or membrane processes to obtain proteins and lactose from cheese whey. In addition, as stated previously, lactose can also be used for the production of glucose and galactose by chemical hydrolysis (Siso, 1996; Souza et al., 2010).

3.3.1. Thermal and isoelectric precipitation

Protein precipitation of cheese whey occurs at moderated temperature with the help of calcium precipitation (thermocalcic precipitation), alternatively at elevated temperatures (thermal precipitation) or by pH decrease until reaching the isoelectric point of micelles (isoelectric precipitation). Thermocalcic precipitation is based on aggregate formation of "lipid insoluble-calcium phosphates" at moderate temperatures (50 °C), neutral pH (7.3–7.5) and the presence of calcium (Mišún et al., 2008; Pereira et al., 2002). Proteins are associated to the aforementioned phosphate aggregates.

The whole treatment consists of two steps: protein thermal precipitation by heating/autoclaving at 90–120 °C or isoelectric precipitation with pH adjustment. The second step consists of protein concentration by centrifugation/filtration. The

inconvenient of the thermal process is the denaturalization of centrifuged proteins.

Table 6 resumes the main supernatant characteristics from the thermal and isoelectric precipitations (Dragone et al., 2011; Lee et al., 2003; Mostafa, 1996; Mukhopadhyay et al., 2003; Roukas and Kotzekidou, 1998; Silva et al., 2010).

Protein isolation from cheese whey by thermal or isoelectric precipitation leads to the formation of a supernatant wastewater with approximately 54 kg m^{-3} of COD. The COD of this supernatant is mainly due to the presence of lactose, which is approximately the same of the raw cheese whey.

Thermocalcic precipitation followed by microfiltration involves the removal of CW non-centrifuged lipids (Fauquant et al., 1985) significantly improving the subsequent ultrafiltration stage (Pereira et al., 2002).

3.3.2. Protein precipitation with coagulant/flocculant agents

Protein precipitation with coagulant agents like sodium polyphosphate, sodium hexametaphosphate, iron salts and polyelectrolytes are effective methods in protein content removal but inefficient in terms of protein recovery due to contamination by the coagulant.

Utilization of a natural chitosan (2-acetamido-2-deoxy-β-D-glucose) polymer is a way to precipitate proteins obtaining pure lactose of high pharmaceutical grade in the supernatant. This coagulant is a linear cationic polymer of high molecular weight obtained by deacetylation of chitin (β (1-4)-N-acetyl-D-glucosoamine) which is manufactured from the outer shell of crustaceans. Lactose prepared from chitosan-treated deproteinized whey was 99.89% pure and meets the standard of pharmacopoeia grade. Chitosan is also efficient in the removal of many metal ions from industrial wastewaters (Su et al., 2003) due to the $-NH_2$ and $-OH$ groups (Su et al., 2005). The high cost of the acid regeneration of the chitosan and the presence of flakes or chitosan powder limit their application in wastewater treatments (Su et al., 2003, 2005). Thus, a method for metal ion imprinted chitosan resin was investigated to reuse several times without loss of adsorption capacity. However, the cost of the template chitosan resin was excessively high (Tan et al., 2001). In the same context, a new surface molecular imprinting adsorbent on the waste biomass from *penicillium* industry is characterized by high mechanical strength, stability in the acid solution and efficient adsorption capacity (Su et al., 2006). Alternatively, Li et al. (2008) developed an adsorbent using molecular imprinting technology and photo degradation through the immobilization of nanometer titanium dioxide on molecular imprinted chitosan matrixes. This adsorbent adsorbs the heavy metal ions and degrades the organic compounds. On the other hand, the chitosan has other properties, namely, can be used as antimicrobial agent. Thus, the work developed by Shi et al. (2008) demonstrated that chitosan/nano-TiO_2 composite emulsion prepared by inverse suspension technology had efficient antibacterial effect against *E. coli*, *Aspergillus niger* and *Candida albicans* after short exposure time.

Table 7 summarizes some of the studies found in bibliography about coagulation/flocculation with chitosan (Bough and Landes, 1976; Casal et al., 2006; Mukhopadhyay et al., 2003; Savant and Torres, 2000), acid precipitation (Sternberg et al., 1975), electrochemical process (Güven et al., 2008; Janson and Lewis, 1994) and alkaline subcritical water gasification (Muangrat et al., 2011) applied to cheese whey effluents. When raw or deproteinized CW were treated with chitosan, turbidity, protein and fats removals of 32–95%; 62–85%; 70–80% were experienced.

Sternberg et al. (1975) compared the performance of two coagulants based on the cheese whey precipitation with

Table 6
Bibliographic compilation: supernatant characteristics from the thermal and isoelectric precipitations.

Process type	pH	Lactose[c]	LA[c]	Protein[c]	Fats[d]	COD[c]	TOC[c,e]	TKN[b]	NH_3[d]	NO_3[d]	PO_4^{3-}[d]	K[d]	Na[d]	Reference
Autoclaved 121 °C/20 min + filtration	–	50	–	–	–	–	–	–	–	–	–	–	–	Mostafa (1996)
Heating 90°C/20 min + centrifugation	–	50	–	–	–	–	–	–	–	–	–	–	–	Roukas and Kotzekidou (1998)
Isoelectric Precipitation + centrifugation + filtration	–	40 ± 0.34	0.4 ± 0.1	0.76 ± 0.03	1.6 ± 0.2	54.1 ± 0.3	18.4 ± 0.7	–	148 ± 4	30 ± 5	635 ± 5	998 ± 5	327 ± 5	Lee et al. (2003)
Heating 95°C/30 min + centrifugation	–	44	–	1.6	2500	–	–	–	–	–	–	–	–	Mukhopadhyay et al. (2003)
Autoclaved + centrifugation	4	3.4[a]	–	–	2.6[b]	–	–	2.2	–	–	–	–	–	Silva et al. (2010)
Heating 115 °C/15 min + centrifugation	–	–	–	–	–	–	–	–	–	–	–	–	–	Dragone et al. (2011)

[a] As free reducing sugars, %.
[b] %.
[c] kg m^{-3}.
[d] mg L^{-1}.
[e] TOC-Total organic carbon.

Table 7
Bibliographic compilation: physicochemical treatment of cheese whey.

Experimental conditions							Removal (%)							Residual concentration (kg m^{-3})	Reference
Effluent type	Treatment	COD_i (kg m^{-3})	Reagent (kg m^{-3})	pH	T (°C)	Time (h)	COD	BOD	Turbidity	Proteins	Lactose	Fat	Ash	COD	
CW and SCW	Precipitation with polyacrylic acid	–	–	3.8–4.2	18	1	–	–	–	62.2–68.4	–	–	–	73.82%[f]	Sternberg et al. (1975)
CW and SCW	Precipitation with trichloroacetic acid	–	12%	–	–	–	–	–	–	85.7–86.7	–	–	–	–	Sternberg et al. (1975)
CW	Chitosan	–	–	6.0	–	–	–	–	90[g]	–	–	–	–	–	Bough and Landes (1976)
CW	Electrochemical coagulation	–	–	–	–	–	–	–	–	73.8	–	–	–	–	Janson and Lewis (1994)
CW	Chitosan	–	0.03	6	20	39	–	–	32	–	–	–	–	–	Savant and Torres (2000)
CW	Complexes Chitosan (alginate; pectin; carrageenan)	–	0.03	6	20	39	–	–	65–72	70	–	–	–	–	Savant and Torres (2000)
CW	Alginate	–	0.03	6	20	39	–	–	52	–	–	–	–	–	Savant and Torres (2000)
Deproteinized and raw CW	Chitosan gel	–	2.5	6.0	30	2	–	87	>90	62–85	4.5–9.1	70–80	50–75	42[f]	Mukhopadhyay et al. (2003)
CW	Chitosan	–	0.25	4.5	–	0.17	–	–	95	–	–	–	–	–	Casal et al. (2006)
Pre-treated CW with Chitosan	Chitosan	–	0.19	6.2	–	–	–	–	–	≈95[c]	–	–	–	–	Casal et al. (2006)
CW powder[b]	Electrochemical[a]	≈27.6	25	5.0	25	8	53.32	–	100	–	–	–	–	≈ 12.9	Güven et al. (2008)
CW powder	Alkaline subcritical water gasification[d] Pressure: 9.5–24.5 MPa	16.8[e]	[NaOH] = 1.67 M; [H_2O_2] = 1.5%	–	300–390	120	–	–	–	–	–	–	–	–	Muangrat et al. (2011)

[a] With iron electrode in the presence of NaCl electrolyte.
[b] Deproteinized and diluted.
[c] As β-lactoglobulin.
[d] H_2 production: 22 g H_2 kg^{-1} of Whey.
[e] As TOC.
[f] As lactose.
[g] As TSS.

polyacrylic and trichloroacetic acids. These authors found a better performance of trichloroacetic acid experiencing a protein recovery of 85.7–86.7%, compared to polyacrylic acid (62.2–68.4%). Protein recovery with polyacrylic acid permits the formation of a white precipitate of protein-polyacrylate at pH 3.8–4.2. A translucent supernatant also was obtained and the remaining solids only represented about 30% of the initial volume.

Electrochemical coagulation with iron electrodes has also been tested to treat a CW powder solution with an initial COD of 27.6 kg m^{-3} (Güven et al., 2008). This process achieved a COD removal of roughly 53%. The clarified supernatant presented a residual COD of around 13 kg m^{-3}. The final turbidity of the supernatant was lower than the corresponding value obtained after chitosan coagulation. Cheese whey protein recovery around 73.8% has also been reported after applying electrochemical coagulation (Janson and Lewis, 1994). Fig. 4 summarizes the application of electrochemical treatments.

3.3.3. *Membrane separation*

Membrane separation processes are extensively utilized to obtain proteins and lactose concentrates from CW and SCW. Membrane processes present some advantages, namely, the reduction of wastewater production with the possibility of reuse and production of a clean effluent (Minhalma et al., 2007). Processes like microfiltration-MF (Pereira et al., 2002; Rektor and Vatai, 2004; Souza et al., 2010), ultrafiltration-UF (Cuartas-Uribe et al., 2006; Domingues et al., 1999; Giacomo et al., 1997; Rektor and Vatai, 2004; Souza et al., 2010; Suárez et al., 2006; Yorgun et al., 2008), nanofiltration-NF (Alkhatim et al., 1998; Cuartas-Uribe et al., 2006, 2009; Minhalma et al., 2007; Suárez et al., 2006; Yorgun et al., 2008), and reverse osmosis-RO (Giacomo et al., 1997; Re et al., 1998; Yorgun et al., 2008) have been widely reported with protein retentions in the ranges: 28–85, 56–81, 87–100, and 94–96%, respectively. Table 8 depicts some membrane processes applied to CW and SCW effluents.

Nanofiltration and reverse osmosis lead to lactose retention values above 89%. Consequently, COD removal efficiencies are in the proximity of 90%. In opposition, microfiltration and ultrafiltration processes present lower lactose retention values below 40%; however these processes are very effective in fat retention ($\approx$100%) (Rektor and Vatai, 2004; Souza et al., 2010).

Microfiltration and ultrafiltration are mainly used to remove fat and proteins, while ion exchange and reverse osmosis are used to purify and concentrate lactose. After concentration, a spray-drying technique is applied to obtain a high purity lactose powder. The obtained protein concentrate is salt free (Kotoupas et al., 2007) with potential applications in pharmaceutical and food industries (Morr and Barrantes, 1998). However, these technologies have limitations from an economic point of view. Due to the need of high pressures, membrane processes are very expensive. Additionally, whey protein concentrates might present a lack of uniformity in the composition (Morr and Ha, 1993).

Few studies have reported the treatment of permeates and the majority of the works apply a fermentation post-treatment to produce ethanol, hydrogen or lactic acid. Thus, effluents coming from the micro and ultrafiltration stages still show a considerable residual COD in the proximity of 54 kg m^{-3} and a phosphate content of 0.800 kg m^{-3}. Permeates coming from nanofiltration or reverse osmosis present lower COD values within the range 1–30 kg m^{-3}, depending on operating conditions (Cuartas-Uribe et al., 2009; Yorgun et al., 2008).

The high COD values of microfiltration and ultrafiltration effluents are mainly due to the lactose content (10%). Additionally, the ultrafiltration permeate has a BOD_5 value of 30–45 kg m^{-3} (Qureshi and Manderson, 1995). The high biodegradability (BOD_5/COD $\approx$ 0.5–0.8) of the ultrafiltration permeate makes viable the application of biological treatments. Additionally, permeates

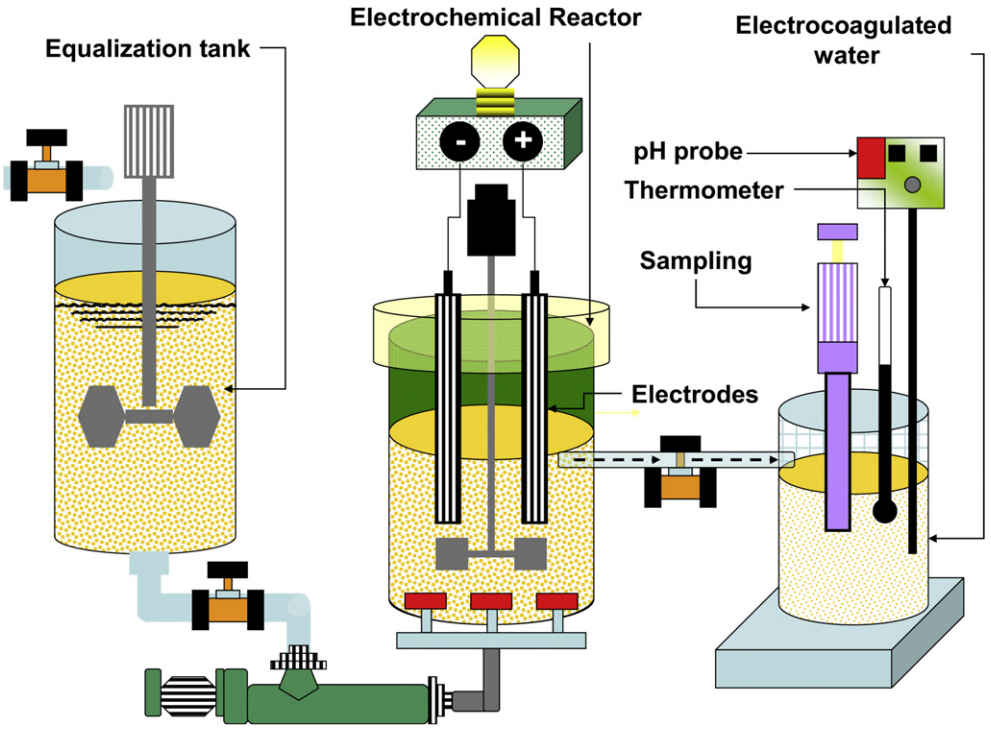

Fig. 4. Electrochemical treatment.

Table 8
Bibliographic compilation: membrane separation of cheese whey.

Experimental conditions		Retention				Permeate						Reference
Process	COD_i (kg m^{-3})	Fat (%)	Lactose (%)	Protein (%)	COD (%)	Lactose (%)	Protein (%)	Fat (%)	Ash (%)	*P*	COD (kg m^{-3})	
MF (centrifuged CW)	–	–	6.5	27.5	–	46.83[a]	6.1[a]	–	–	–	–	Souza et al. (2010)
MF (CW)	–	–	–	84.6	–	–	0.5	0.6[a]	3	–	–	Pereira et al. (2002)
MF(SCW)	–	–	–	75	–	–	0.2	0.08[a]	2	–	–	Pereira et al. (2002)
MF	–	98.7	19.5	67	–	3.14	0.24	0.01	–	–	–	Rektor and Vatai (2004)
UF (centrifuged CW)	–	–	14.5	80.5	–	34.75[a]	2.47[a]	–	–	–	–	Souza et al. (2010)
UF (Centrifugation + UF permeate)	–	–	3.2	56.1	–	33.76[a]	0.93[a]	–	–	–	–	Souza et al. (2010)
UF-DF (Centrifugation + UF permeate)	–	–	4.7	64.5	–	19.75[a]	0.76[a]	–	–	–	–	Souza et al. (2010)
UF (Clarified CW)	–	–	–	–	–	4.6	0.04	0		–	–	Giacomo et al. (1997)
UF	–	–	–	–	–	4.66	0.02	0	0.5	–	–	Domingues et al. (1999)
UF	–	100	40	75	–	2.31	0.18	0.00	–	–	–	Rektor and Vatai (2004)
UF	–	–	–	–	–	45.83–48.12[a]	–	–	0.44–0.47	0.33–0.44[b]	–	Suárez et al. (2006)
UF	–	–	–	–	–	36.6–56.0[a]	2.0–2.2[a]	0.0	–	0.50–0.80[c]	–	Cuartas-Uribe et al. (2006)
UF	100	–	–	78	42.8	–	–	–	–	–	54.3	Yorgun et al. (2008)
NF (UF permeate)	–	–	96	–	–	–	–	–	–	–	–	Cuartas-Uribe et al. (2006)
NF (UF permeate)	–	–	99.5	100	–	–	–	–	–	–	–	Suárez et al. (2006)
NF (UF permeate)	53.25	–	89.06	–	98.1	–	–	–	–	–	1	Cuartas-Uribe et al. (2009)
NF (SCW)	31.2[d]	–	–	–	>98[d]	–	–	–	–	–	–	Minhalma et al. (2007)
NF	–	–	–	–	–	3–10	0.2	–	–	–	–	Alkhatim et al. (1998)
NF	100	–	–	87	74.4	–	–	–	–	–	26.9	Yorgun et al. (2008)
NF	–	–	99.8	100	–	–	–	–	–	–	–	Suárez et al. (2006)
RO (NF permeate)	43.66	–	–	96	94.4	–	–	–	–	–	2.5	Yorgun et al. (2008)
RO (supernatant of heated CW at 100 °C)	68.76	–	–	–	89.8	–	–	–	–	–	7.04	Yorgun et al. (2008)
RO	70.0	–	–	–	92.9	–	–	–	–	–	5	Re et al. (1998)
RO	100.0	–	–	94	92.6	–	–	–	–	–	3.8	Yorgun et al. (2008)
UF + RO (Clarified CW)	–	–	–	–	–	0	0	0	0.02	–	–	Giacomo et al. (1997)

[a] kg m^{-3}.
[b] g kg^{-1}.
[c] As phosphates, kg m^{-3}.
[d] As TOC.

coming from microfiltration and ultrafiltration still contain inorganic salts.

Summarizing, permeates coming from membrane processes still maintain a high value of the main contaminant indicators. As a consequence, these permeates cannot be directly discharged into the receiving environments. Studies about the environmental impact of this type of permeates are scarce (characterization, contamination level, treatment, reutilization).

3.4. Land application

The longstanding practice consists of the disposal of CW on land (Jones et al., 1993; Lehrsch and Robbins, 1996; Lehrsch et al., 2008; Robbins and Lehrsch, 1992, 1998; Robbins et al., 1996; Sharratt et al., 1959, 1962; Watson et al., 1977). This practice is based on the potential fertilizer nature of CW. Thus, this by-product contains salts, organic matter and nutrients such as phosphorus, nitrogen, calcium, sodium, potassium, magnesium, chloride, etc.

Due to the presence of suspended solids and high salinity content, CW disposal might affect the physical and chemical structure of soils (Dragone et al., 2009; Saddoud et al., 2007). In the first case, soil acts as a filter and the solids remain on the soil surface (soil fouling). The solids accumulation might provoke the decrease of soil permeation and gas exchange. However, some researches claim that the majority of solids are sugars and proteins susceptible of biodegradation, improving soil aggregation (Kelling and Peterson, 1981). Salinity content (NaCl) decreases the availability of water for plants, affecting plant growth and fruit production. Additionally, the increase of conductivity destroys soil structure, reducing the aeration extent and water infiltration. Robbins and Lehrsch (1998) suggested that CW must be diluted 1:20 with clean water to obtain an acceptable irrigation water quality (40–60 kg ha^{-1} addition of total salt).

CW application on soils should consider some precautions. Hence, besides the solids and salt contents, the location of water sources should be contemplated to prevent groundwater contamination (Ben-Hassan and Ghaly, 1994; Ghaly and Singh, 1985; Robbins and Lehrsch, 1998).

Some studies have demonstrated that CW application on the recuperation of sodic soils (sodium percentage above 15%) can be efficient by lowering soil pH, SAR (sodium adsorption ratio), ESP (exchangeable sodium percentage) (Jones et al., 1993), and increasing soil flocculation (Robbins and Lehrsch, 1998). Additionally, an increase of crop production can be obtained. In any case, an excessive whey application can lead to a yield decrease (Peterson et al., 1979; Sharratt et al., 1962).

The fertilizing properties of CW have been demonstrated on acid soils in high to moderate rainfall areas and also, on calcareous soils with neutral to alkaline properties (pH = 7.6–8.8) under irrigation in an arid climate (Robbins and Lehrsch, 1998). Lehrsch et al. (2008) obtained an increased aggregation stability between 14 and 25% and a 75% reduction of sediment losses from the degraded calcareous soil in irrigation furrows. These authors stated that CW can improve the structure of eroded or non-sodic soils, with increased aggregate stability (Brown et al., 1998).

Organic matter of the CW is biodegraded to CO_2, organic acids and nitrates. Due to the increased Ca solubility, polysaccharides and other organic extracellular compounds can help to stabilize the aggregates formed (Allison, 1968). Nevertheless, some studies observed crop damage due to the rapid consumption of soil oxygen and rapid drop in the redox potential (−350 mV) (Sharratt et al., 1959).

Table 9
Comparison of the different treatment alternatives: affluent characterization, effectiveness, reactors, recoverable products, by-products and effluent characterization.

Treatment	Cheese whey type	Reactor types or chemical reagents	COD_i (kg m^{-3})	Removal (%)	Recoverable products	By-products	Effluent (kg m^{-3})
Biological							
Aerobic digestion	Raw, diluted	AS, JLMBR, NRBC	0.55–70	COD = 53–99	–	Excess biomass sludge Effluent	COD = 0.04–24.0
Anaerobic digestion	Raw, diluted, powder solution, pre-treated	AUFFLR, ASDFA, UASB, AUFFR, DUHR, TSUAD, ARBC, CP, ASBR, TSMAMD, UAF	0.5–79	COD = 36–99	Gas (53–79% of methane)	Excess biomass sludge Effluent	COD = 0.1–33
Ferm. to ethanol	Raw, pasteurized, UF permeate, powder solution, deproteinized	Aerated and no-aerated pilot-scale batch, batch, fed-batch, aerated or airlift continuous, PCBR, continuous	35–200 as sugar	Sugar = 33–100	Ethanol (2.1–81 kg m^{-3})	Excess biomass sludge Effluent	Sugar ≈ 0–48
Ferm. to hydrogen	Raw, diluted, permeate powder, powder solution	Batch, CSTR, UASB	10–89	Sugar = 86–97	Hydrogen (20–88%)	Excess biomass sludge Effluent	Sugar = 0.1–6.1 COD = 4–28
Ferm. to lactic acid	Raw, deproteinized, pasteurized, UF permeate, pre-treated UF permeate	CPTR, batch, fed-batch, static flasks	30–100 as sugar	Sugar = 34–100	Lactic acid (3.8–52 kg m^{-3})	Excess biomass sludge Effluent	Sugar = 0–32
MFC	No-sterilized and diluted, filter-sterilized and diluted, raw, raw and inoculated, heat treated and inoculated	Two-chamber mediator-less	0.35–96.5	COD = 5–100	Electricity (18.4–16700 mW m^{-2})	Excess biomass sludge Effluent	COD = 0.02–91.7
Physicochemical							
Precipitation	Raw	Polyacrylic acid, trichloroacetic acid	–	Proteins = 62–87	Proteins	Precipitate Supernatant	–
Coagulation–flocculation	Raw, deproteinized, pre-treated	Chitosan, chitosan complexes, alginate, chitosan gel	–	Turbidity = 32–95 Proteins = 62–85 Lactose = 4.5–9.1 Fats = 70–80	Proteins	Precipitate Supernatant	Lactose = 42
Electrochemical	Raw, powder deproteinized and diluted	Iron electrode	27.6	Proteins = 74 COD = 53 Turbidity = 100	Proteins	Precipitate Supernatant	COD = 13
MF	Raw, centrifuged	–	–	Fat = 99 Lactose = 6.5–19.5 Protein = 28–85	Protein concentrate	Concentrate Permeate	Lactose = 31–47 Protein = 2–6 Fats = 0.08–0.6
UF	Raw, centrifuged, clarified, centrifuged and UF permeate	–	100	Fat = 100 Lactose = 3.2–40 Protein = 56–81 COD = 43	Protein and lactose concentrates	Concentrate Permeate	Lactose = 20–56 Protein = 0.2–2.5 Fats = 0 COD = 54
NF	Raw, UF permeate	–	53–100	Lactose = 89–99.8 Protein = 87–100 COD = 74–98	Lactose and protein concentrates	Concentrate Permeate	–
RO	Raw, NF permeate, heated	–	44–100	Protein = 94–96 COD = 90–94	Protein and lactose concentrates	Concentrate Permeate	COD = 2.5–7

Other disadvantages of using CW as a fertilizer source are the cost of transporting a material that is 92–93 percent of water and the limitation of the seasonal application conditions (Robbins and Lehrsch, 1998; Zall, 1980).

4. Discussion and conclusion

The optimal treatment selection, in order to minimize the negative environmental impact of cheese whey becomes very difficult, principally, due to the complexity of the matrix studied. Thus, the optimal treatment line depends on the cheese whey quality and quantity, available technologies, local disposal standards imposed by Environmental Legislations and removal efficiency of the process(es) selected. Additionally, the economic value and costs associated to the post-treatment of the recoverable products, and the requirement of treatment/valorization/elimination of the by-products obtained are other parameters that must be considered in the treatment line. Table 9 summarizes the main treatment alternatives studied, by taking into account cheese whey type, reactors or reagents used, process effectiveness, recoverable products, by-products obtained and effluent characterization.

With the exception of the aerobic digestion, conventional and emerging technologies simultaneously look for the valorization and treatment. The sludge coming from the aerobic digestion, after stabilization, may be contemplated as a viable alternative fertilizer. In this context, it can be noted that all the treatment alternatives lead to by-products which have to be characterized, treated/valorized or ultimately eliminated. Thus, the excess of biomass, chemical precipitates and lactose/protein concentrates are formed in biological processes, precipitation/coagulation–flocculation/electrochemical processes and membrane technologies, respectively. Information on characterization, treatment, valorization or disposal of the chemical precipitates and of the sludge obtained in the biological processes is rather scarce. Nevertheless, several studies include protein and/or lactose concentrates obtained in the membrane technologies. Additionally, the potential valorization of chemical precipitates after coagulation–flocculation processes with iron and aluminum salts in the cheese whey wastewater treatment has also been reported (Rivas et al., 2010).

The main obstacles of biological treatments with valorization are connected to the achievement of optimum conditions to

maximize the production of methane, ethanol, lactic acid, hydrogen or electricity. Differences in organic load, pH, temperature, complexity of biological activity, microbial competition, etc., limit the application and selection of these processes.

Physicochemical treatments (precipitation, electrochemical and coagulation–flocculation principally with chitosan) generally produce clarified supernatants with low fat and protein content. Additionally, protein recovery close to 90% can be achieved. However, these processes partially remove the lactose content (the main responsible of organic matter contamination). Membrane processes have become a viable alternative, specially, nanofiltration and reverse osmosis by two main reasons: firstly, high contamination removal efficiency with COD eliminations between 74 and 98% and secondly, production of protein/lactose concentrates, with protein and lactose recovery within the range 87–100% and 89–100%, respectively. Unfortunately, due to economic reasons, these technologies are not recommended for small-medium cheese factories. The post-treatment of concentrates, membrane fouling and the pollutant permeate production constitute limiting factors.

Concerning the effluent characterization, the majority of processes studied are very efficient in the removal of COD, solids, nutrients, oils and fats and/or protein and lactose recovery. Nevertheless, in most cases, a final effluent that cannot be discharged into the environment is generated. The aforementioned effluent normally exceeds the legal limits imposed by European Environmental Legislations, not only by considering the organic matter load (COD and BOD), but also because of the nutrient content. This drawback is of special concern in sensitive areas, where the N and P can cause the eutrophication of surface waters. Only few study cases presented the effluent with COD values below the limit discharge and in these instances the initial substrate used was diluted to apply:

- aerobic digestion by activated sludge, with initial COD = 0.547 kg m^{-3} (Cordi et al., 2007);
- anaerobic digestion, with initial COD = 0.5–4.0 kg m^{-3} (Mockaitis et al., 2006);
- MFC technology, with initial COD = 0.35–2.7 kg m^{-3} (Stamatelatou et al., 2011).

For these reasons, innovative treatments are unavoidable and imperatives to deal with strength of raw cheese whey and with the effluents obtained that exceed the discharge limit. In this context, there are some studies with post-treatment of pre-treated CW by biological digestion. Ebrahimi et al. (2010) studied the sequence aerobic-anaerobic of raw CW with a total COD reduction above 97%. The effluent obtained presented a residual COD of 1.6–2.6 kg m^{-3}. A notable HRT reduction was achieved in the second step. The physicochemical treatments constitute a viable alternative to cope with the recalcitrant organic matter before or after the biological digestion(s). Other sequences used can be mentioned, namely:

- Fermentation to lactic acid + lime neutralization + anaerobic process (Gannoun et al., 2008);
- Ultrafiltration + fermentation to ethanol (Domingues et al., 1999; Dragone et al., 2009; Sansonetti et al., 2009);
- Membrane process + fermentation to hydrogen (Yang et al., 2007);
- Fermentation to hydrogen + anaerobic digestion (Venetsaneas et al., 2009);
- Fermentation to hydrogen + photo-fermentation (Azbar and Dokgoz, 2010);
- Ultrafiltration + fermentation to lactic acid (Vasala et al., 2005);
- Coagulation–flocculation with chitosan + coagulation–flocculation with chitosan (Casal et al., 2006);
- Ultrafiltration + ultrafiltration (Souza et al., 2010);
- Ultrafiltration + nanofiltration (Cuartas-Uribe et al., 2006, 2009; Suárez et al., 2006);
- Nanofiltration + reverse osmosis (Yorgun et al., 2008);
- Ultrafiltration + reverse osmosis (Giacomo et al., 1997).

Precipitation (with NaOH, lime and H_2SO_4) or coagulation–flocculation (with iron or aluminum salts) as pre-treatment followed by aerobic digestion can constitute a viable alternative to small-medium factories. This treatment line was tested to treat cheese whey wastewater (Rivas et al., 2010, 2011) and presented several advantages:

- pre-treatment cheap and easy to monitor;
- organic matter is partially removed in the pre-treatment;
- the pre-treatment is efficient in the removal of fats, suspended solids, organic nitrogen and phosphorous;
- reduction of the aerobic digestion time;
- elimination of organic matter close to 100% after aerobic digestion;
- sludge obtained with agronomic value.
- recalcitrant organic matter can be reduced by oxidation processes.

On the other hand, coagulation with $FeCl_3$ or precipitation with NaOH appear as an encouraging pre-treatments for fermentation to lactic acid, hydrogen or ethanol. In this case the low removal efficiency of lactose in the pre-treatment produces a rich and biodegradable effluent that can be used as substrate for lactic acid, hydrogen or ethanol productions. Additionally, the effluent coming from coagulation–flocculation (with chitosan or salts of iron or aluminum), precipitation (with NaOH) and electrochemical processes can be potential options for membrane technologies. In this case the fouling problems of membranes are minimized, since the turbidity depletion in the range of 75–100% has been reported in the above processes (Casal et al., 2006; Güven et al., 2008; Mukhopadhyay et al., 2003; Rivas et al., 2010, 2011).

Ultrafiltration (or microfiltration) + fermentation to lactic acid (or hydrogen or alcohol) and Ultrafiltration (or microfiltration) + anaerobic digestion are other promising alternative treatment lines that can be studied. The pre-treatment allows not only the protein recovery in about 80% but also the substrate formation with high lactose content that can be sent for a second valorization by biological process. Thus, different combinations of the technologies must be considered to provide multiple alternatives taking into account the technical and economic potential of each individual cheese factory. In this sense, a bibliographic survey in the subject of post-treatments indicates a lack of studies in this direction, constituting a real challenge in the complete management of cheese whey.

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