17

Anaerobic Treatment of Milk Processing Wastewater

**Maria Helena G. A. G. Nadais, Maria Isabel A. P. F. Capela,
Luís Manuel G. A. Arroja, and Yung-Tse Hung**

CONTENTS

Abstract Anaerobic processes are widely used for the treatment of milk and dairy effluents. This technology has been subjected to significant development and real-scale application in the last few decades and offers highly favorable perspectives to accomplish a complete biodegradation of the components present in milk processing wastewaters such as sugars, proteins, and fats. Nowadays, anaerobic systems for the treatment of milk wastes can be operated successfully constituting an important contribution for the preservation of environmental quality.

1. INTRODUCTION

The sustainable development of a society requires a reduction of the dependency on fossil energy sources and a decrease in the amount of pollution discharged to the environment (1). Presently, there is a growing interest in alternative energy sources as a result of increased demand for energy coupled with a rise in the cost of available fuel. The needs and priorities of a sustainable society will lead to a situation in which, concerning the treatment of wastes, the possibilities of energy production will be as important as pollution control (1). The rapid industrialization observed in the last century has resulted in the generation of large quantities

From: *Handbook of Environmental Engineering, Volume 11: Environmental Bioengineering*
Edited by: L. K. Wang et al., DOI: 10.1007/978-1-60327-031-1_17

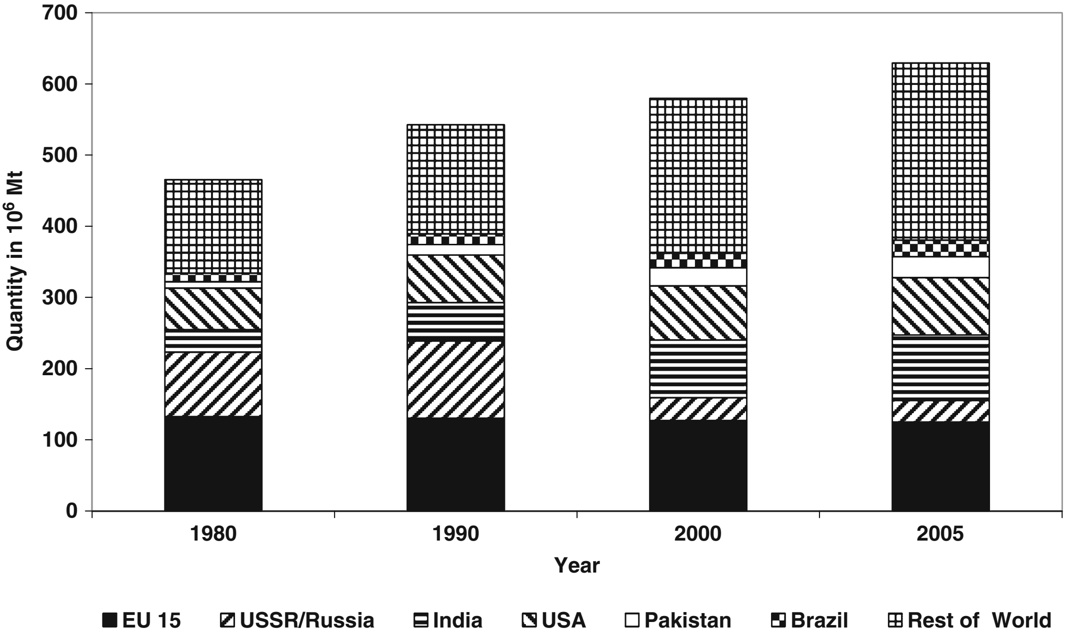


Fig. 17.1. Evolution of milk production in the world (4).

of effluents with high organic contents, which, if treated suitably, can result in a perpetual source of energy (2). Specifically, milk processing effluents have the potential to provide a carbon source in a form that may be converted to methane by anaerobic microorganisms, opening a possibility for a clean energy source together with pollution control (3).

1.1. The Milk Processing Industry

Milk processing industry has grown steadily in most of the countries of the world because of the continuous growth in the demand of milk and milk products. The world milk production has a growing rate around 2.8% per annum (4), see Fig. 17.1. The general tendency in milk processing industry in developed countries is toward the construction of fewer but larger installations and toward higher automation and process efficiency (5). Although there is a negative environmental impact associated with industrialization, this effect may be minimized and energy may be tapped by means of anaerobic treatment of the liquid effluents.

1.2. Major Environmental Problems Caused by Milk Processing Effluents

The environmental impact of industrial milk processing plants can be very severe, especially due to the discharge of large volumes of liquid effluents containing high concentrations of organic matter, nutrients and acid or alkaline products. Although most of these components are biodegradable, some of them such as milk sugars (mainly lactose) are readily consumed in the receiving medium, while some others such as proteins and, especially, fats are quite difficult to degrade (5). The substrates present in milk processing effluents feed algal blooms that deplete dissolved oxygen, damage habitats for fish nurseries, and threaten leisure activities (6). Furthermore, the discharge of the untreated effluent directly onto land or a water body will not use the effluent's potential application as a source of clean energy (methane). The main problems caused by the liquid wastes from milk processing industries are summarized below.

1.2.1. *Direct Discharge into a Water Body*

The decomposition of the organic substrates will cause a severe depletion on the dissolved oxygen of the receiving waters, and it may lead to several important consequences such as anaerobic conditions and bad odor, death of certain branches of a water body, and consequent loss of original biodiversity. The effluents from milk processing industries exert a Chemical Oxygen Demand (COD) in the receiving media that is very high and also very rapid; about 50% of the COD is exerted within 24 h of discharge causing serious problems in the receiving water bodies.

The presence of proteins, phosphorus, and nitrogen based compounds will rise the nutrient level in the receiving water and potentially provide conditions for eutrophication. Quite often, the wastewater temperature is much higher than that of the receiving water medium, and this might cause significant alterations on the life conditions of certain species, not only because of the consequent decrease in oxygen solubility but also because some biological species are sensitive to temperature changes. The pH peaks, typical of milk processing effluents, may also alter the pH of the medium with consequences on the balance of chemical components in the water.

1.2.2. *Direct Discharge onto Land*

The use of milk processing effluents for irrigation is widely spread in underdeveloped countries, but this practice has many environmental disadvantages, e. g., the need for large areas and the effects on water resources due to run-off to water bodies and/or infiltration to groundwater reservations. If the fats content of the rejected effluent is high, then the effects of changing the characteristics of the soil most frequently increasing soil impermeabilization together with excessive organic and nutrient loading also need to be considered. Many small dairy factories dispose of their effluents by irrigation onto lands or pastures. Surface and ground water pollution is therefore a potential threat posed by these practices.

1.2.3. *Treatment in Lagoons*

The treatment of milk processing effluents in lagoons is also widely spread among third world countries. This treatment system requires the use of large areas although not as large as for irrigation. If the bottom and side surfaces of the lagoon are not conveniently impermeabilized, contamination of groundwater by infiltration may occur. On the other hand, if the lagoon is not covered, bad odors may rise, and the methane produced by anaerobic processes may escape to the atmosphere exerting a green house effect.

The effluents from milk processing industries contain predominantly milk and milk products riginated from process losses. Milk losses in an industrial milk processing plant may attain about 0.5–2.5% of the incoming milk, but in some cases, they might reach 3–4% (5). Although the correct action upon the process and the implementation of good management practices may decrease substantially the amount of milk losses to the effluent, there is a lower limit of about 2.5 kg of milk lost per 1,000 kg of processed milk (7). Because of high water consumption, it is estimated that the volume of the discharged effluents is around 2.5 times the volume of the processed milk (3). Taking into consideration that the volume of world milk production in 2005 was about 630×10^6 mton (Fig. 17.1), these data indicate that large amounts of milk are lost and large volumes of milk processing effluents that require adequate treatment are generated.

The conventional methods for the disposal of milk processing effluents include the reutilization of certain fractions present in the effluent, for example, milk whey and lactose (8, 9). Coupled with recovery of some waste constituents or when this is not an economic alternative, several wastewater treatment processes may be used, mainly biological processes. Presently, in milk processing industries, a great percentage of wastewater treatment systems are aerobic, although in the last two decades, there has been a steady growth of anaerobic treatment applications (5).

2. THE EFFLUENTS FROM MILK PROCESSING INDUSTRIES

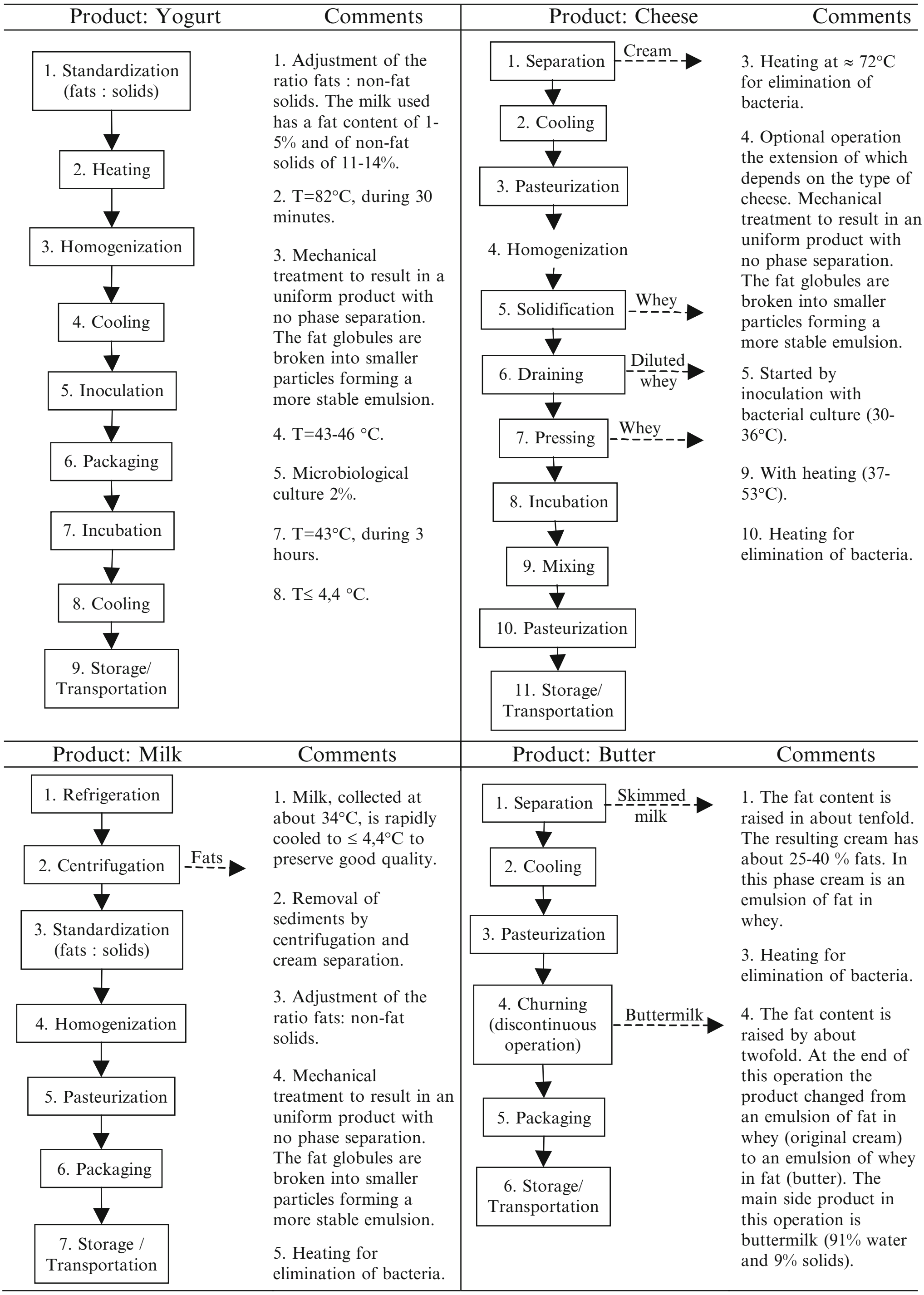
In order to understand the environmental issues of milk processing effluents, it is necessar to consider although briefly the nature of milk and the main characteristics of milk processing industries.

As a consequence of the development of milk preserving techniques, there was a rise in the production of milk products, which was not accompanied by the modernization of production processes and equipment. This caused a higher volume of product losses, spillages, frequent unbalancing in effluent treatment plants, and the surge of a severe problem in effluent treatment. It is not yet quite clear if the degree of modernization of the production processes and installations is related to the volumes of effluent produced (10–13).

2.1. Origins of Liquid Pollution in the Milk Processing Industry

Although the consumption of fresh milk has grown following economic development, a great part of milk utilization occurs after milk has been processed in several operations (heating, transformation in butter, cheese, yogurt, desserts, etc). Figure 17.2 presents the main operations involved in the production of several milk products. Within a milk processing plant, there may coexist the productions of several categories of products, such as, milk, cheese, yogurt, cream, ice-cream, frozen products, food ingredients, whey solids, lactose, etc. Typically, a conventional installation of a milk processing industry is involved simultaneously in the production of several of these products with significant seasonal fluctuations.

As can be seen in Fig. 17.2, the main operations in milk processing are clarification, pasteurization, and homogenization. Pasteurization and clarification do not affect the composition and the characteristics of the effluents that are relevant for effluent treatment processes. On the other hand, through homogenization, the fat globules are reduced from 1–15 μm, as present in raw milk, to 1–2 μm, in homogenized milk, thus forming a stable emulsion. This process is important in terms of effluent characteristics since it implies that a major percentage of milk processing effluents have their fat components in a form that is difficult to separate from the matrix (a stable emulsion) hindering the efficiency of the physical separation systems.



Product: Yogurt – Comments

1. Adjustment of the ratio fats : non-fat solids. The milk used has a fat content of 1-5% and of non-fat solids of 11-14%.

2. T=82°C, during 30 minutes.

3. Mechanical treatment to result in a uniform product with no phase separation. The fat globules are broken into smaller particles forming a more stable emulsion.

4. T=43-46 °C.

5. Microbiological culture 2%.

7. T=43°C, during 3 hours.

8. T≤ 4,4 °C.

Product: Cheese – Comments

3. Heating at ≈ 72°C for elimination of bacteria.

4. Optional operation the extension of which depends on the type of cheese. Mechanical treatment to result in an uniform product with no phase separation. The fat globules are broken into smaller particles forming a more stable emulsion.

5. Started by inoculation with bacterial culture (30-36°C).

9. With heating (37-53°C).

10. Heating for elimination of bacteria.

Product: Milk – Comments

1. Milk, collected at about 34°C, is rapidly cooled to ≤ 4,4°C to preserve good quality.

2. Removal of sediments by centrifugation and cream separation.

3. Adjustment of the ratio fats: non-fat solids.

4. Mechanical treatment to result in an uniform product with no phase separation. The fat globules are broken into smaller particles forming a more stable emulsion.

5. Heating for elimination of bacteria.

Product: Butter – Comments

1. The fat content is raised in about tenfold. The resulting cream has about 25-40 % fats. In this phase cream is an emulsion of fat in whey.

3. Heating for elimination of bacteria.

4. The fat content is raised by about twofold. At the end of this operation the product changed from an emulsion of fat in whey (original cream) to an emulsion of whey in fat (butter). The main side product in this operation is buttermilk (91% water and 9% solids).

Fig. 17.2. Main operations in milk industries (adapted from ref. (13)).

As was referred above, an industrial plant for milk processing is involved in the simultaneous production of various products. The effluent streams originated from the different production lines are discharged in different moments often not coinciding with each other. This results in a final effluent that varies widely both in volume and in composition. Superimposed on these daily variations, there are also some weekly, monthly, and seasonal variations (12).

The effluents from milk processing industries may be divided in five main categories:

- Clean water from heating and cooling operations
- Wastewater with low pollution concentration, originated from the end of cleaning cycles
- Heavily polluted wastewater, contaminated with milk or milk products, originated from the beginning of cleaning cycles or from discharges of raw milk or milk products
- Domestic wastewater
- Rainfall wastewater

Due to the different characteristics of these effluents it is most convenient, for treatment facilitation, to segregate these effluents as some of them can be reutilized or discharged in water bodies, or in a municipal collector, after being subject to a low cost treatment.

The nature of the effluents generated in a milk processing industry is in general very similar reflecting the overwhelming influence of the loss of milk and milk products. Yet, each process generates a wastewater with a specific volume and composition. Table 17.1 presents typical origins of liquid pollution in milk processing industries.

2.2. *Characterization of Effluents from Milk Processing Industry*

The diversity of products and production techniques does not allow a formulation of the characteristics of a typical milk processing effluent. Nevertheless, some general characteristics may be identified (12, 16):

1. Presence of high concentrations of COD, biological oxygen demand (BOD), oils and fats as well as proteins and calcium.
2. Presence of bacterial cultures used in many production processes.
3. Great variability of flow and effluent characteristics as a result of discontinuous production and cleaning in all production processes.
4. Presence of acids, bases, and disinfectants from cleaning process used to inhibit bacterial activity in the production process.
5. Temperature above the normal ambient temperature due to use of hot water for cleaning.
6. Frequent concentration peaks due to the discharge or spillage of raw milk, intermediate or final products and chemicals.
7. High variability in all the above factors.

Table 17.2 presents ranges for some components of milk processing effluents. Milk contains a wide variety of proteins and sugars, casein and lactose being respectively the most important protein and sugar in milk. Fat is present in milk mainly as an emulsion of lipids, which are esters (i.e., triglycerides) of glycerol and long chain fatty acids – LCFA (Fig. 17.3). Long chain fatty acids are carboxylic acids with a hydrophilic acid group in one end and an alkyl hydrophobic group in the other end. The most important LCFAs in milk fat are oleic, myristic, palmytic, and stearic acids (Table 17.3).

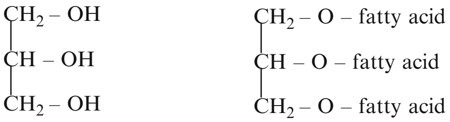
Table 17.1
Typical origins of liquid pollution in milk processing industries (adapted from refs. (14, 15))

Operation	Pollution (kg BOD_5/m^3 processed milk)			
	(a)		(b)	
	Average	Range	Average	Range
Milk reception, churn washing, cleaning	0.26	0.11–0.66	0.31	0.13–0.80
Cooling raw milk, storage, washing tanks and pipelines	0.19	0.07–0.31	0.23	0.08–0.37
Washing tankers	0.25	0.10–0.40	0.30	0.14–0.48
Skimming, storing skimmed milk and cream plus cream pasteurizing	0.66	0.46–1.20	–	–
Churning and washing butter	0.46	0.25–0.80	0.55	0.30–0.96
Evaporating skimmed milk to low total solids	0.23	0.16–0.30	–	–
Evaporating skimmed milk to high total solids and spray drying	0.74	0.14–1.50	–	–
Roller drying	0.53	0.25–1.30	–	–
Pasteurization of milk and storage	0.29	0.10–0.54	0.35	–
Bottling pasteurized milk	0.11	–	–	–
Bottle washing	0.23	0.05–0.37	–	–
Pasteurizing milk, storage, bottling and bottle washing	0.85	0.49–1.70	–	0.12–0.65
Clotted cream	1.20	–	1.44	–
Cream pasteurizing and packing	0.79	–	–	–
Cheese making (hard pressed)	0.89	0.23–2.00	1.07	0.28–2.40
Cottage cheese (washed curd)	15.00	–		
Condensing fresh whey to low total solids	0.25	–	–	–
Condensing stale whey to high total solids	–	–	–	–
Condensate	0.25	–	–	–
Plant washing	0.75	–	–	–
Condensing sweetened separated condensed milk	1.40	–	1.68	1.44–2.04
Full-cream evaporated milk and canning	0.75	1.20–1.70	0.89	0.17–1.80
Ice-cream making and packing (estimated)	1.60	0.50–1.00	–	–

References: (a) (14); (b) (15).

Table 17.2
Characterization of milk processing effluents (adapted from refs. (2, 5))

	Units	Dairy factory	Dairy factory	Cheese factory	Yoghurt and buttermilk factory
pH	–	5.6–8	5–11	7.32	–
COD	mg/L	1,120–3,360	633–4,500	4,430	1,500
BOD	mg/L	320–1,750	241–2,600	3,000	1,000
Suspended solids	mg/L	28–1,900	–	–	–
Total solids	mg/L	–	710–5,100	–	–
VSS	mg/L	–	250–804	–	–
TSS	mg/L	–	240–943	1,100	191
Fats, oil and grease	mg/L	68–240	60–690	754	–



Glycerol Triglyceride

Fig. 17.3. Glycerol and triglycerides.

Table 17.3
Composition of cow milk (data from refs. (17, 18))

Component	% w/w	
Water	85.6–88.1	
Proteins	3.11–3.7	
Lactose	4.48–4.79	
Ash	0.71–0.75	
Non-fat solids	8.43–9.19	
Total solids	11.87–14.34	
Fats	3.44–5.15	
Main LCFAs in milk fats (% of total LCFAs)		
	Range	Average
Oleic (18:1)	25.27–40.31	31.90
Myristic (14:0)	15.56–22.62	19.78
Palmitic (16:0)	5.78–29.0	15.17
Stearic (18:0)	7.80–20.37	14.91

(*n*:*m*)*n* number of carbon atoms in acid chain; *m* number of double bonds.

For a given industrial plant, the concentration of milk and milk products in the effluent depends on the specific process, on the volume of processed milk, on the conditions and characteristics of the equipment, on the loss and waste reduction procedures, on the attitude of management and employees toward environmental problems and on water management practices.

Nowadays, in milk processing industries, the final destiny of liquid wastes is the area in the domain of water management that requires more improvements (12). Although there is in fact a tendency for a shift from many small plants toward fewer and larger installations, there are still many industries located in small rural areas where the access to adequate treatment systems is still a problem (19). In 1979, Brown and Pico (10), made a survey on the characteristics of the milk processing effluents in the USA and concluded that they could be treated in municipal plants. In the last 20 years, this perspective has changed considerably because of the raise in the costs of discharge imposed by the municipal authorities, and presently, the major part of the milk processing industries have on-site treatment installations for total or partial treatment (12).

Besides diluted milk and milk products, dairy processing effluents may also contain variable amounts of cleaning products. The biological oxygen demand exerted by these cleaning chemicals is typically under 200 mg/L, so it is not meaningful as compared to the organic load from milk and milk products in the wastewater. Although not significant in terms of organic load, these cleaning compounds contribute significantly to the refractory COD in the effluent and to the toxicity phenomena and low performance observed in some biological treatment systems. In cleaning procedures (presently CIP – Clean in Place systems are widely applied), there is a need for using disinfection and detergent compounds to inhibit biological growth in the production systems. A large variety of cleaning solutions may be used depending on the equipment, water hardness, and other factors. The most used chemicals for this purpose are nitric acid, phosphoric acid, caustic soda, and sodium hypochlorite, but in some processes, iodide acids and ammonium quaternarium compounds are also used (Table 17.4). Nowadays, due to environmental problems, the trend in the cleaning procedures is toward using more nitric acid and less of the preferable, phosphoric acid. Yet, since from a cleaning process point of view, phosphoric acid is preferable and it is not probable that its utilization will diminish further (12). Notwithstanding its chemical composition, the temperature of the cleaning solution is around 64–82°C, and thus most of the effluents have temperatures higher than normal temperature.

2.3. *The Specific Problems of Cheese Whey*

Whey is a liquid waste or a subproduct generated in the cheese making process by the precipitation of casein from milk using acid (resulting in acid whey) or rennet (resulting in sweet whey). Cheese whey represents about 80–90% of the volume of milk used in cheese production with the making of 1 mton of cheese resulting in about 8 mton of liquid whey. Whey contains more than 50% of the milk solids including 20% of the proteins and most of the lactose. The precise composition varies with the different manufacturing methods of casein and cheese products and with milk production season. Because of its very specific characteristics, cheese whey must be regarded on its own in what concerns the wastewater

Table 17.4
Main chemicals used in milk processing industries[a] (adapted from refs. (12, 16))

Compound	Main use	Effects on biological treatment systems
Caustic soda	Alkaline cleaner	FOG emulsification, pH raise, inhibition
Soda ash	Alkaline cleaner	FOG emulsification, pH raise, inhibition
Polyphosphates	Alkaline cleaner	pH raise, inhibition
Sulfated alcohols	Wetting agent, antiseptic, germicide	Inhibition
Alkyl aryl sulfonates	Wetting agent	Inhibition, foaming
Quaternarium ammonium surfactants	Wetting agent, sanitizers, foot washers	Inhibition, foaming
Complex phosphates	Emulsification, protein peptization, dispersion	Raise in P concentration, inhibition
Organic acids (acetic, propionic, citric, lactic, tartaric acids)	High temperature acid cleaning	Inhibition, pH drop
Inorganic acids (phosphoric, nitric, sulfuric acids)	High temperature acid cleaning	Raise in nutrient concentration, inhibition, pH drop
Acid salts	High temperature acid cleaning	Inhibition, pH drop
Sodium hypochlorite	Sanitizer	Inhibition
Iodine compounds	Sanitizers	Inhibition

[a] Other chemicals used in minor doses include: ammonia, trisodium phosphate, hydrochloric acid, hydroxy-acetic acid, sodium metasilicate, hydraulic oils, propylene glycol, emulsifiers, antifoaming agents.

treatment or especially in what concerns its recovery potentialities. In case of small-scale cheese production plants, the problem arises of choosing between treatment in dedicated plant or the investment in modern technologies for recovery of valuable whey components (e.g., recovery of lactose and proteins, or spray-drying, bioconversion of lactose to ethanol or yeast biomass, among others). When analyzing the various options for the destination of cheese whey, it is important to consider that a plant for the recovery of whey or of whey products also generates effluents that require treatment before discharge (20, 21). Although these effluents are much less concentrated than whey, they have an organic content that is significant in comparison with other milk processing effluents (Table 17.5). In the circumstance were no recovery solution may be adopted, it is necessary to find a solution for the final destiny of cheese whey. Whey represents a potential energy source and presents several advantages if it is subject to anaerobic digestion because this solution offers an excellent approach from both energy/resource conservation and pollution control considerations. In general, from an economical point of view, the most convenient treatment solution is anaerobic digestion followed by an aerobic posttreatment in combination with the effluent from the main process. Cheese whey is highly concentrated, highly biodegradable, and has a low bicarbonate alkalinity (Table 17.5). These characteristics make it very difficult to treat whey in high-rate

Table 17.5
Characterization of whey and whey processing effluents (adapted from refs. (20–22, 26, 28))

Parameter	Units	Value (SD)
Concentrated whey		
COD total	mg/L	68,814 (11,518)
COD soluble	mg/L	57,876 (11,272)
TSS	g/kg	1.3 (1.14)
VSS	g/kg	0.94 (0.74)
TKN	mg/L	1,462 (263)
NH_4^+-N	mg/L	64 (31)
P total	mg/L	379 (49)
PO_4-P	mg/L	326 (64)
Effluent from whey processing plant		
T	°C	25.5 (2.5)
pH	–	7.0 (2.0)
BOD_5	mg/L	896 (310)
COD	mg/L	1,624 (556)
TOC	mg/L	546 (167)
TKN	mg/L	109 (80)
NH_4^+-N	mg/L	8.5 (6.3)
TSS	mg/L	261 (180)
VSS	mg/L	188 (149)
Whole whey		
Parameter	Units	Value
COD	g/L	60–70
BOD	g/L	35–45
Deproteinated whey		
COD	g/L	50–60
BOD	g/L	30–40
Acid whey (average composition)		
Humidity	%	94–95
Grease	%	0.3–0.6
Protein	%	0.8–1.0
Lactose	%	3.8–4.2
Minerals	%	0.7–0.8
Lactic acid and other products	%	0.1–0.8
Sweet whey (average composition)		
Humidity	%	93–94
Grease	%	0.3–0.5
Protein	%	0.8–1.0
Lactose	%	4.5–5.0

(Continued)

Table 17.5
(Continued)

Parameter	Units	Value (SD)
Minerals	%	0.5–0.7
Lactic acid and other products	%	0.1–0.4
Raw whey		
COD total	mg/L	57,010–66,040
COD soluble	mg/L	45,800–55,730
SS	mg/L	4,000–6,160
VSS	mg/L	3,840–5,960
NH_4^+-N	mg/L	30–120
PO_4^{3-}-P	mg/L	210–950
Protein	mg/L	4,000–7,000
pH	–	3.0–6.3

biological systems because of the formation of exopolymeric materials that are responsible for low sludge settleability and biomass wash-out (22). It is known that the use of up-flow anaerobic sludge blanket (UASB) reactors for the treatment of milk processing effluents and especially for cheese whey is severely limited by the difficulty in obtaining or keeping a good granulation in the anaerobic sludge (21, 23, 24). It has also been reported that the high level of carbohydrates in whey promotes the growth of acid forming bacteria but is detrimental to methane producing bacteria (25). Because of the rapid acidification of the whey, the treatment in anaerobic systems requires a two-phase process since the addition of extra alkalinity would represent an economical limitation for a one-phase process (26, 27).

Data from anaerobic large-scale installations for whey treatment are sparse, but it appears that loading rates of up to 10 kg COD/m^3-day are applied and COD reductions in long term operation may reach 75–85% (21). Gas yield varies from 35 to 38 m^3 gas with methane content of 60–62% per m^3 of treated whey (21), with an energy value of about 21 MJ/m^3 (29). Most of the reactors are operated at mesophilic temperature although thermophilic operation is also possible.

A number of operational problems have been noted for large-scale whey digesters including pH variations due to deficient equalization or production variation, odor, and detrimental effects of fat and calcium (21). Some strategies to overcome these difficulties were the addition of surfactants or nutrients to improve the performance of the anaerobic digestion (30, 31), the use of a two phase system to improve the stability of the methanogenic phase (26), the combination of thermophilic and mesophilic temperature in different phases of the anaerobic process, and the combination of anaerobic and physical–chemical process (32). Despite the problems, the success of the application of anaerobic technology for methane production from whey or whey processing wastes has been demonstrated (21). Although the major reactor configurations and operation strategies referred above have been evaluated at laboratory, pilot and full-scales, it is still not clear if any of these should be preferred over the others. In any case, the effluent from this anaerobic digestion process is generally not suitable for disposal in natural water bodies, requiring some form of aerobic posttreatment to polish the wastewater before ultimate disposal (29).

2.4. Good Management Practices and Benchmarking

The analysis of the data in Table 17.1 suggests the classification of the discharges from milk processing industries in two main groups:

(i) Intentional discharges not avoidable because they are related with the process itself; and
(ii) Accidental discharges that can be avoided, which occur because of operating errors, and that are not indispensable for good production.

It is important to note that the largest volumes of liquid pollution from milk processing industries originate from intentional discharges, which are cleaning operations of transportation tanks and pipes, and cleaning of equipment whenever there is a halt in production. The large water volumes needed for these cleaning operations result in the volumes of the effluent being higher than the volumes of the processed milk (33). Other effluent sources (accidental discharges) are related to deficiencies in the functioning of the equipment or to operation mistakes that may cause discharges of milk or milk products. The losses from intentional discharges may be minimized with good management practices, while the accidental discharges should be eliminated. As an example of a good practice, the data from de Haast et al. (8) show that increasing the draining time of a 45 L vessel from 3 to 90 s will diminish the volume of milk loss in about 88%.

The progressive adoption of good management practices by milk processing industries will lead to a decrease in the loss of raw materials and to the decrease in water consumption. Consequently, the liquid wastes will become increasingly more concentrated, although with lesser volume, and for this reason even more adequate for anaerobic treatment. According to Bickers and Bhamidimarri (33) the application of good managing practices may decrease the average world value of 0.5–37 m^3 of effluent per m^3 of processed milk to 0.5–2 m^3/m^3. Assuming that all precautions have been taken to minimize the volume of the effluent to the lower possible limit, the load discharged to the receiving medium can only be reduced by an adequate treatment of the effluent (15).

The operators of milk processing industries should strive to reach the benchmarking values for the rejected effluents. Presently, the benchmarking volume for liquid effluent is around 1 m^3/mton of processed milk and a BOD load of under 2.5 kg BOD/mton of processed milk, the optimum being 1–1.5 kg BOD/mton processed milk. In the case of the effluents from cheese or butter production, the BOD concentration in the effluent should be lower than 2 kg BOD/mton of product (34). Table 17.6 presents some values for product losses in a well-run installation.

In order to reach benchmarking values, process control in terms of key parameters will allow identification of opportunities for reducing the product losses and improve process performance. Pollution prevention and control practices for liquid effluents in the milk processing industry include (34):

- Reduction of product losses by means of better production control
- Reutilization of product losses in lower grade products like cattle feed

Table 17.6
Benchmarking values for milk industries (adapted from ref. (34))

Operation	Product losses		
	Milk	Fat	Whey
Butter/transport of skimmed milk	0.17	0.14	n.a.
Butter and skimmed milk powder	0.60	0.20	n.a.
Cheese	0.20	0.10	1.6
Cheese and whey evaporation	0.20	0.10	2.2
Cheese and whey powder	0.20	0.10	2.3
Consumer milk	1.9	0.7	n.a.
Full-cream milk powder	0.64	0.22	n.a.

n.a. not applicable.
Note: data expressed as percentage of the volume of milk, fat or whey processed.

- Optimization of water use and of chemicals for cleaning
- Recirculation of cooling water
- Improvement of water recycling opportunities by segregation of the effluents from sanitary installations, process, cooling operations and condensation, cleaning of transport tanks, and rainwater
- Reuse of condensates for cleaning instead of fresh water
- Use of high-pressure nozzles to minimize water consumption
- Reduction of phosphorus based cleaning products

Additional good pollution prevention practices that can be suggested for milk processing industries are the use of filtration technology and separation of effluent streams (e.g., cheese whey streams).

As with other wastes, the logical way to deal with milk processing effluents is to include the following steps in a waste reduction program (35): (a) prevention, (b) minimization, (c) recycling, and (d) treatment. In the cases were the first three steps have been widely explored and applied, so as to avoid the double cost associated with loss of raw materials and waste treatment, the forth step is the one where more developments are to be expected. Within this framework, anaerobic digestion has a major role.

3. THE ANAEROBIC TREATMENT PROCESS

When considering the pollution of water bodies, there are several wastewater treatment options depending mainly on the type of industry, the effluent characteristics, geographical situation, land availability, and economic factors. In most of the common situations, a complete treatment system for milk processing effluents involves physical and chemical operations as well as biological processes. Biological processes are mostly used for removal of organic matter and since they are based on the maintenance of biological activity, the control of environmental and operating conditions assumes a primordial role. The microorganisms are used to convert the organic matter present in the effluent into several gaseous and dissolved products and new cell material. Since cellular tissues have a specific gravity higher than

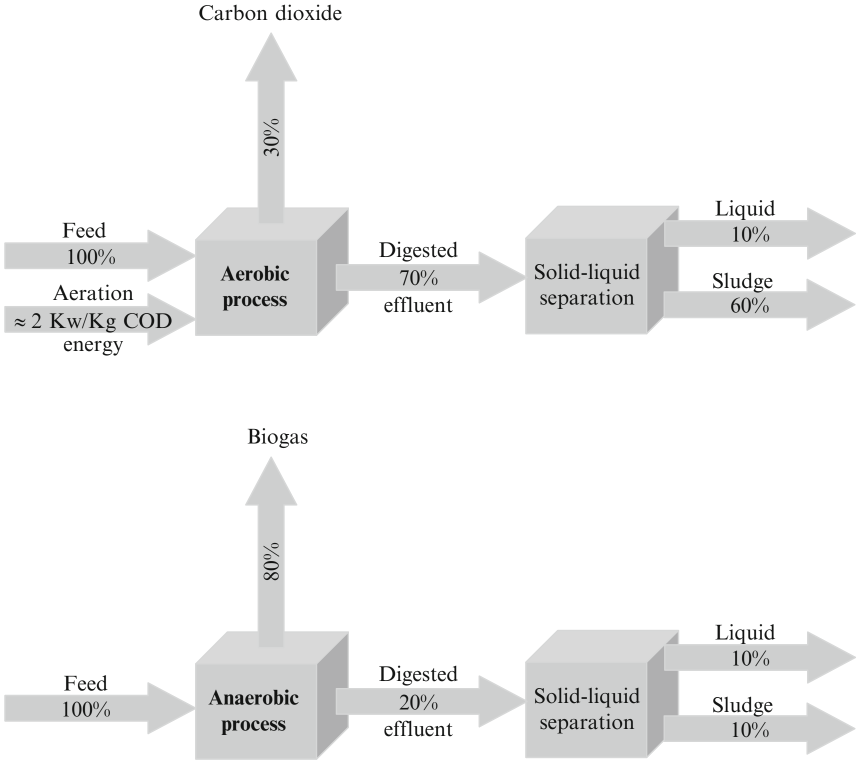


Fig. 17.4. COD mass balances for aerobic and anaerobic processes.

water, the resulting biomass may be removed by gravity settling and organic matter removal is effective only when this separation is achieved.

Biological effluent treatment processes may be classified as aerobic and anaerobic. Aerobic processes use oxygen to digest organic matter, while in anaerobic processes, organic matter is transformed in carbon dioxide and methane in the absence of free oxygen. Figure 17.4 presents the typical mass balances for both processes.

3.1. Description of Anaerobic Process

Anaerobic digestion is the biological degradation of organic or inorganic matter performed by a complex microbiological ecosystem in the absence of a free oxygen source. During the degradation process, the organic matter is converted mainly to methane, carbon dioxide, and biomass.

The compounds involved in anaerobic digestion can be classified as primary substrates present in the wastewater, as intermediate substrates and as final products. For a complex effluent like milk processing wastewater, the primary substrates can be grouped into oils and fats, proteins and hydrocarbons, with each of these substrates being present in the solid, colloidal, or soluble form. Hydrocarbons are easier to degrade than proteins, which in turn are easier than oils and fats (Fig. 17.5).

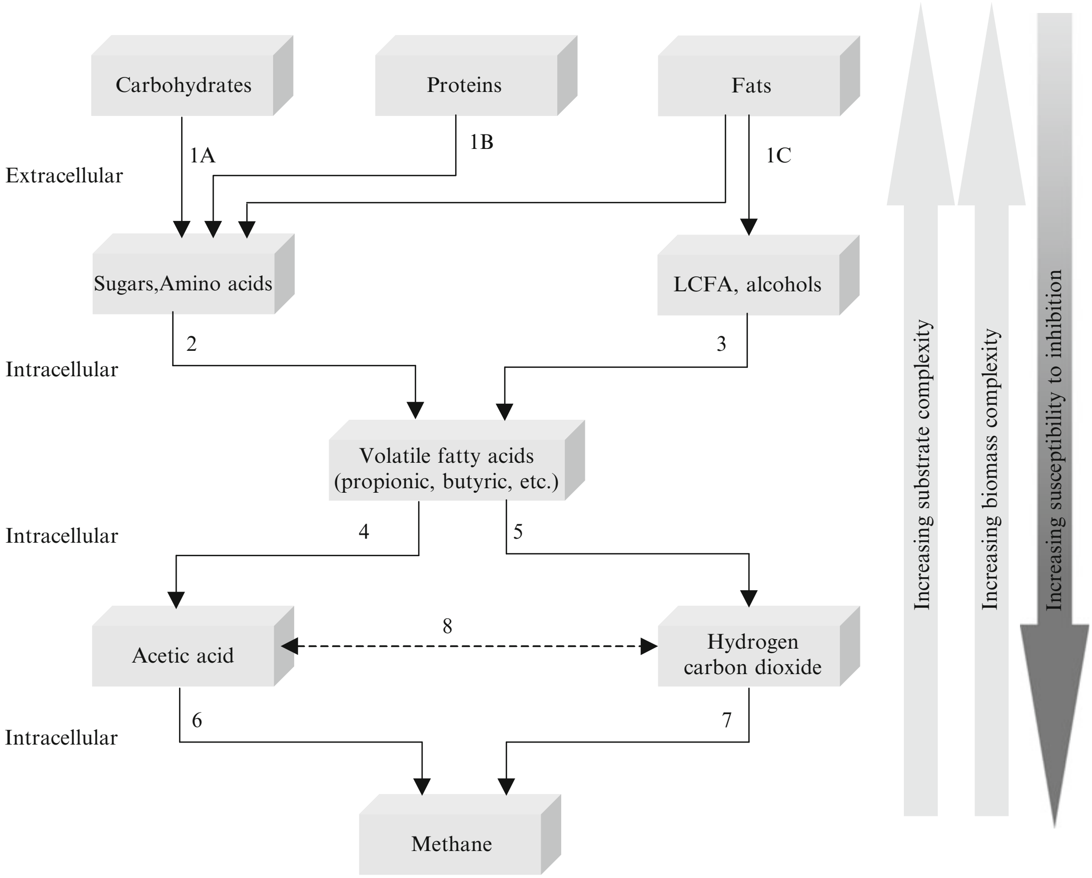


Fig. 17.5. Anaerobic process (see text for meaning of numbers).

The intermediary substrates may be a wide range of gaseous and soluble compounds and the final products are normally gases (methane and carbon dioxide) and bacterial cells.

Anaerobic degradation proceeds by way of a series of parallel and sequential processes performed by a variety of microbial consortia. The process may be divided schematically in four main steps performed by five distinct bacterial populations (Fig. 17.5, Tables 17.7 and 17.8).

The following explanations refer to the reaction steps in Fig. 17.5.

(i) Hydrolysis steps (1A, 1B, 1C): generally the anaerobic digestion of complex substrates starts with the hydrolysis, that is the liquefaction of complex organic compounds (lipids, proteins and polysaccharides) into simpler monomers such as soluble sugars, amino acids, peptides, and long chain fatty acids (LCFA). This is performed by means of extracellular enzymes (exoenzymes) secreted by a complex consortia of hydrolytic and acidogenic bacteria. Hydrolysis is necessary because microorganisms are not able to consume particulate or nonsoluble substrates since these are too large to cross the cell membrane. Therefore, the enzymes are released to the cell environment to break down these insoluble molecules into smaller units that can be processed

Table 17.7
Description of anaerobic process

Step (Fig. 17.5)	Description	Microorganisms	Specific features and environmental conditions
1A	Hydrolysis of carbohydrates	Acidogenic fermentative	Particulate carbohydrates are hydrolyzed to simple sugars
1B	Hydrolysis of proteins	Acidogenic fermentative	Proteins are hydrolyzed to amino acids Need for acclimation of biomass Production of ammonia which may inhibit the process Bacteria (*Clostridium*, *Streptococcus*, *Bacteroides*, *Selenomonas*, *Butyrivibrio*, *Fusobacterium*)
1C	Hydrolysis of fats	Acidogenic fermentative	Lipids are converted to long chain fatty acids (LCFA) Slower hydrolysis step which may avoid VFA accumulation Lipolytic bacteria (*Clostridia* and the *Micrococci*)
2	Fermentation of sugars and amino-acids	Acidogenic fermentative	Simple sugars and amino acids are degraded to volatile fatty acids (VFA) such as acetate, propionate and butyrate
3	β-oxidation of LCFA	Obligate hydrogen producing acetogenic	LCFA are converted to acetate and hydrogen Inhibited by LCFA themselves Need for acclimation of biomass Generally is the rate limiting step (slower than methanogenesis) Hindered by low solubility of LCFA H_2 produced severely inhibits the growth of these strains Only grow in the presence of a hydrogen-consuming partner *Mesophilic bacteria* *Syntrophomonas sapovorans* (*Methanospirillum hungatei* as syntrophic partner) *Syntrophomonas wolfei* (*Desulfovibrio* sp. as syntrophic partner) *Thermophilic bacteria* *Thermosyntropha lipolytica* (*Methanobacterium* sp. as syntrophic partner)

(Continued)

Table 17.7
(Continued)

Step (Fig. 17.5)	Description	Microorganisms	Specific features and environmental conditions
			Short rod thermophile (*Methanobacterium thermoautotrophicum* as syntrophic partner) LCFA is not degraded without the supplement of a exergonic cosubstrate (it is a energy-required process)
4 and 5	OHPA Oxidation of VFA (except acetic) to acetic acid and H_2	Obligate hydrogen producing acetogenic	Higher fatty acids are converted to acetate and hydrogen Only grow in the presence of a hydrogen- or formate-consuming partner *Syntrophobacter wolinii* (propionate decomposer) *Syntrophomonas wolfei* (butyrate decomposer) H_2 produced severely inhibits the growth of these strains Sensitive to pH drop
6	Acetoclastic methanogenesis	Acetoclastic methanogenic	Acetate is converted to CH_4 These bacteria do not adapt to LCFA LCFA inhibition Inhibition by ammonia (hydrolysis product of proteins) *Methanothrix* spp. (now, *Methanosaeta*) and *Methanosarcina* spp. Sensitive to pH drop
7	Hydrogenotrophic methanogenesis	Hydrogenotrophic methanogenic	H_2 and CO_2 are converted to CH_4 Free LCFA inhibition The bacteria play an important role in completion of anaerobic digestion, in accumulating H_2 and in maintaining low levels of H_2 Inhibition by ammonia (hydrolysis product of proteins) *Methanospirillum hungatei* and *Desulfovibrio* sp.
8	Conversion of CO_2 and H_2 into acetic acid and vice-versa	Homoacetogenic	

Table 17.8
Some reactions in anaerobic degradation of milk processing effluents (adapted from refs. (36, 37))

Substrate	Reaction	ΔG^0 (KJ)	Comments
Propionic acid	$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + 3H_2 + CO_2$	+76	Acetogenesis
Butyric acid	$CH_3CH_2CH_2COOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2$	+48	Acetogenesis
Ethanol	$CH_3CH_2OH + H_2O \rightarrow CH_3COOH + 2H_2$	+9.7	Acetogenesis
Palmitic acid	$CH_3(CH_2)_{14}COOH + 14H_2O \rightarrow 8CH_3COOH + 14H_2$	+402.4	Acetogenesis by β-oxidation cycle
$CO_2 + H_2$	$CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O$	−95	Homoacetogenesis, maintenance of low H_2 partial pressure
Acetic acid	$CH_3COOH \rightarrow CH_4 + CO_2$	−31	Acetoclastic methanogenesis
Hydrogen	$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$	−131	Hydrogenotrophic methanogenesis, maintenance of low H_2 partial pressure
Methanol	$4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O$	−312	Methanogenesis

inside the living cell. Hydrolysis is considered the rate limiting step in the degradation of particulate substrates (38), but the hydrolysis of soluble hydrocarbons, globular proteins (as present in milk), and lipids is quite fast.

Several different factors influence the production of the extracellular enzymes responsible for the hydrolysis of lipids, proteins, and sugars. It is known that the production of proteases, the enzymes responsible for protein hydrolysis, may be suppressed when easily degradable substrates are present in the reaction medium (39). The production of lipases, the enzymes responsible for lipid hydrolysis, may be stimulated by the presence of triglycerides or fatty acids (40). Some proteins are known to affect superficial tension and thus inhibit the bonding (adsorption) between lipases and the fat material subject to hydrolysis (41). It has been observed that in the absence of methane production, no lipid hydrolysis occurs (42, 43). At a pH lower than 6, the methanogenic bacteria are inhibited and no methane production takes place, and as a consequence, lipids will not be hydrolyzed. These findings are of special importance when considering the anaerobic treatment of wastewaters containing readily acidified/degradable substrates, such as sugars, together with complex substrates, such as proteins and fats, as it is the case of milk processing effluents.

(ii) Acidogenesis or fermentation steps (2, 3): this is the step where the dissolved compounds resulting from hydrolysis are converted to simple compounds. The substrates are mainly soluble amino acids and sugars, and the products are organic acids and alcohols, among other minor products (lactate, succinate, pyruvate, propionate, butyrate, valerate, acetate, ethanol, ammonia, H_2 and CO_2). In this process, organic compounds serve both as electron donors and acceptors (the process does not need an external electron acceptor), and this is the first step in anaerobic degradation resulting in energy production.

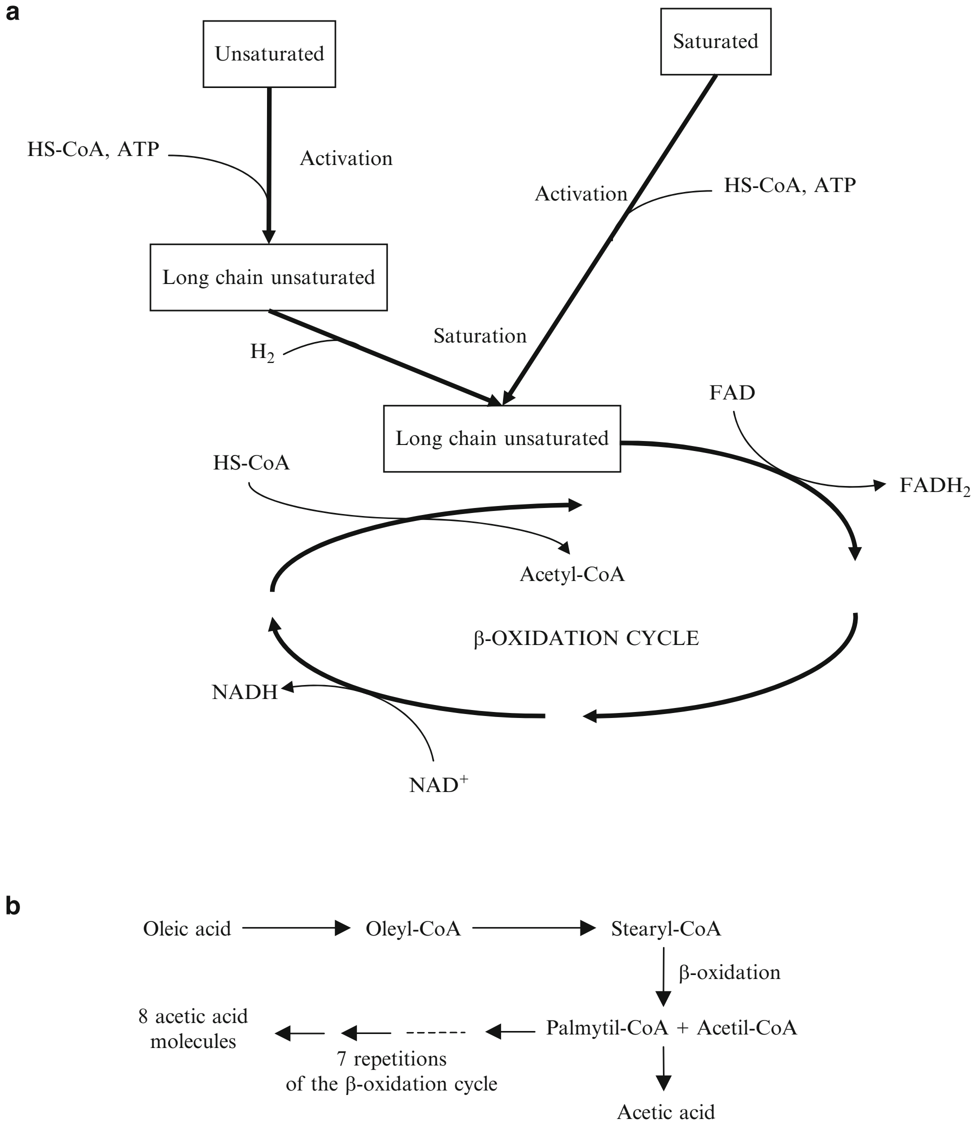


Fig. 17.6. Obligate Hydrogen Producing Acetogenesis (OHPA) (**a**) degradation pathway of LCFA present in milk effluents (**b**) example of β-oxidation cycle for oleic acid (C18:1).

Most generally, the acidogenesis of amino acids is performed via Strickland reactions, and acidogenesis of soluble sugars is performed via the Embden–Meyerhof pathway. The degradation of LCFA requires an external electron acceptor, and their degradation is closely linked to acetogenesis with obligatory hydrogen production (OHPA), see Fig. 17.6.

An important aspect of the degradation of amino acids is the production of NH_3 that affects the buffer capacity of the media and constitutes an essential nutrient (N). On the other hand,

NH_3 is toxic at high concentrations, but generally the level of proteins in milk processing effluents does not give rise to toxicity by this component. Furthermore, in the acidogenesis step, a sensible drop in buffer capacity may occur. Since many of the methanogenic bacteria, responsible for methane production from hydrogen and carbon dioxide, are very susceptible to low pH values, a drop in pH will result in a decrease in the consumption of hydrogen leading to a shift in the products of acidogenic bacteria. Some of the products (e. g. propionate) will be formed in higher quantities. In these cases, the acidogenic bacterial population may not be able to accommodate the amount of acids produced, and the process will deteriorate; the pH will decrease because of accumulation of volatile fatty acids (VFA), and ultimately methane production will cease. This process is called reactor acidification and should be avoided at all cost namely by the presence of sufficient buffer capacity.

(iii) Syntrophic acetogenesis and hydrogenotrophic methanogenesis steps (4, 5, 7): Syntrophic acetogenesis is the degradation of the fermentation products to acetate using bicarbonate or hydrogen ions as external electron acceptors. Syntrophic acetogenesis is a path for acetate production, in which substrate oxidation is made possible only by the simultaneous reduction of hydrogen ions or by the reduction of bicarbonate to formate. This process is coupled with methanogenesis from hydrogen (hydrogenotrophic methanogenesis) which keeps a low hydrogen concentration as required by the reaction thermodynamics (Table 17.7). The production of hydrogen during the oxidative reactions of acetate production is referred to as acetogenesis with obligate hydrogen production (OHPA) and the hydrogen depletion to produce methane is referred to as hydrogenotrophic methanogenesis. Hydrogenotrophic methanogenesis keeps the hydrogen partial pressure low enough for the thermodynamic conditions need in the acetogenesis to be accomplished (this process is also named interspecies hydrogen transfer).

From the products that result from hydrolysis of milk lipids, that is saturated and unsaturated LCFA and glycerol, it is the LCFA that cause most of the problems in anaerobic digestion. From a biochemical standpoint, glycerol causes no significant problems (44). All the LCFA are degraded via OHPA. The most important mechanism for this degradation is the β-oxidation (42, 45, 46), see Fig. 17.6.

In the β-oxidation mechanism (Fig. 17.6), carbon chain fragments with two carbon atoms are successively removed from the LCFA carbon chain in the form of acetyl-CoA which is then converted to acetate. According to Novak and Carlson (46), depending on the LCFA being saturated or unsaturated, the limiting step of their degradation is the activation by an enzyme molecule or the β-oxidation, respectively (Fig. 17.6). During β-oxidation, the LCFA are degraded by OHPA bacteria to VFA and hydrogen; even numbered LCFA are degraded to acetic acid and hydrogen and odd numbered LCFA are degraded to acetic acid, propionic acid, and hydrogen (45, 47). Since most of the naturally occurring LCFAs are even numbered, the main product of β-oxidation is acetate. Due to the hydrogen production associated with β-oxidation it is clear that the hydrogen consuming bacteria must be sufficiently active in order to keep a low hydrogen partial pressure. This step of the degradation of LCFA through β-oxidation is considered the most problematic in the anaerobic degradation of milk processing effluents. It has been reported that LCFA are not degraded unless this degradation is accompanied by methane formation (42). Thermodynamically, the reactions of the β-oxidation of LCFA are possible only if the hydrogen partial pressure is kept below approximately 10^{-4} atm. Novak and Carlson (46) reported that the hydrogen produced during the degradation of LCFA inhibits this reaction (β-oxidation). The hydrogen depletion for methane production will lower the pH_2 allowing for LCFA degradation. Other authors have also reported that LCFA are inhibitory of their own degradation (48–50).

(iv) Methanogenesis steps (6, 8): acetoclastic methanogenesis is the breaking of acetate into methane and carbon dioxide by highly specialized microorganisms (e. g., *Methanosaeta genus* and *Methanosarcina genus*). About 70% of the methane produced in the anaerobic process results directly from the degradation of acetic acid (45). Some authors (51) have reported that reactors operating in extreme conditions may use an alternative pathway for methane production that is the syntrophic acetate oxidation to hydrogen and carbon dioxide by acetogenic or homoacetogenic bacteria in parallel with hydrogenotrophic methanogenesis. Methanogenic bacteria are the most sensitive bacterial group in what concerns the inhibition by LCFA (50, 52).

4. THE ANAEROBIC TREATMENT OF MILK PROCESSING EFFLUENTS

In the last years, there had been a growing interest on anaerobic systems for the treatment of milk processing effluents due to the known advantages of these processes for treating wastewater with high organic loads.

The adoption of high rate anaerobic technology by the dairy industries has faced several difficulties resulting from the complexity of biological degradation of some compounds present in the wastewater.

Since the main role of anaerobic process is the removal of organic matter and it does not remove significant amounts of nutrients, it is worthy to emphasize that anaerobic treatment is only a pretreatment and must be integrated in a sequence of treatment steps. The anaerobic treatment step is generally followed by an aerobic polishing step before discharge. This may be attained by installing a local aerobic activated sludge unit or in some cases, by the discharge to municipal sewers for later treatment in a municipal plant. In a broader view, anaerobic treatment should be regarded as forming a central part in the concept of Environmental Protection & Resource Conservation, EP&RC (53–55).

4.1. Benefits of Anaerobic Process for Milk Processing Effluents

The advantages and disadvantages of anaerobic vs. aerobic treatment have been widely discussed in literature (54, 56–60), but particularly for the case of milk processing wastewater, there are some specific considerations that deserve to be pointed out:

(a) Large quantities of energy are necessary for aeration (generally the oxygen consumption is over 3.0 kg/kg BOD_5) since milk processing effluents are very concentrated (frequently more than 2,000 mg COD/L, see Table 17.2), and also because they are rejected at high temperature and are readily biodegradable. In the anaerobic process, more than 90% of the substrate energy is retained in the produced biogas being easily recovered onsite and used as a fuel source.

(b) In case of milk processing effluents, which contain appreciable amounts of lipids, the difference in the maximum loads applicable with aerobic and anaerobic systems is of paramount importance. In conventional activated sludge systems (aerobic systems), only low loads can be processed and high biomass concentrations cannot be attained, e. g., about 0.1 kg BOD/kg TSS-day and about 5 $kg\,TSS/m^3$, respectively. In contrast, modern high rate anaerobic systems can accommodate loads ten times as higher allowing for a reduction in reactor size of 60%. This result is a significant benefit considering that the higher costs in a wastewater treatment plant are construction costs.

(c) In aerobic systems, the bulky sludge that is frequently formed with complex fat containing effluents, such as milk processing effluents, is difficult to separate in the final clarifier (6, 14); also, the readily degradable sugars promote the growth of the less dense microorganisms. This leads to biomass loss, clogging of percolating filters (14), and loss of efficiency.

(d) In the case of purely aerobic systems, as is the case of activated sludge systems, it is necessary for the substrate to be in solubilized form so as to be assimilated through the bacterial cell wall. It is known that the anaerobic bacteria have a hydrolytic activity higher than aerobic bacteria. Milk being highly colloidal is not readily degraded by aerobic bacteria as it is by anaerobic microorganisms.

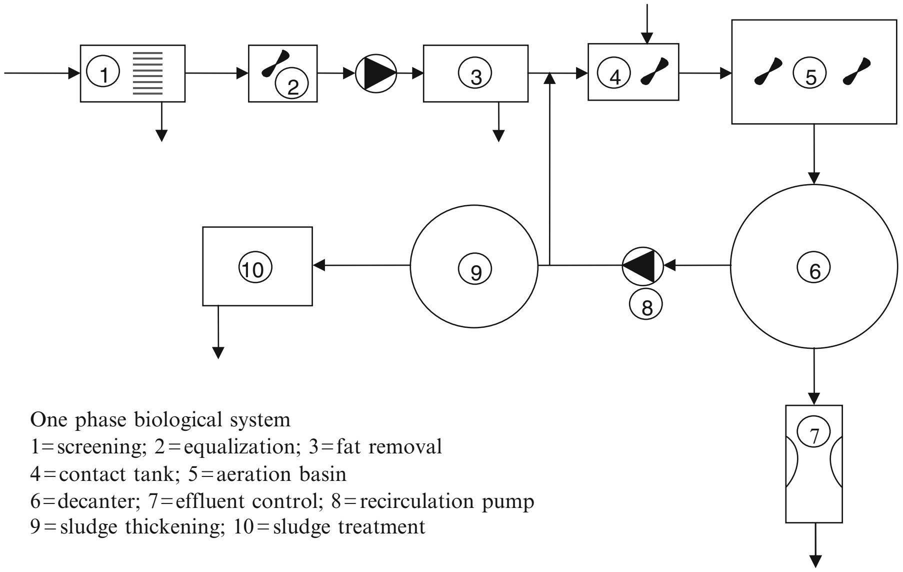
(e) One of the principal advantages pointed to the aerobic treatment systems is the fact that they are able to remove nutrients (phosphorus and nitrogen) coupled to the fact that presently large quantities of phosphoric acid are used as cleaning agents in milk processing industries. In general, the phosphorus content in milk processing effluents is very high and superior to what is necessary for an aerobic process. For this reason, even for aerobic systems, it will be necessary to have an additional polishing step for phosphorus removal, and this need will be more stringent if the legislation for phosphorus discharge will become more restrictive as is the present tendency. These considerations blur a significant difference between anaerobic and aerobic systems which in the past favored heavily the aerobic technology.

4.2. The Role of Anaerobic Systems in a Treatment Plant for Milk Processing Effluents

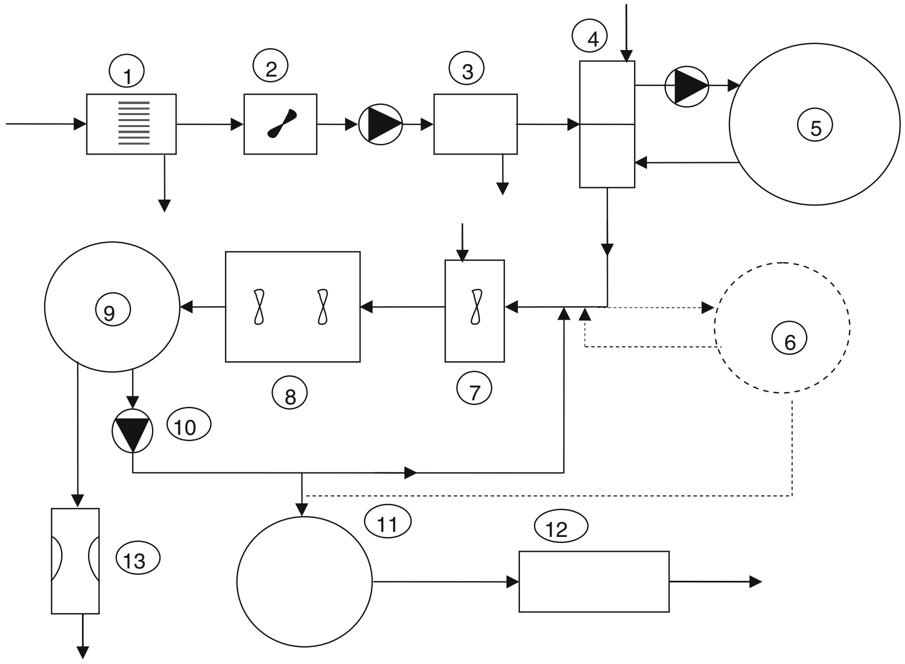
Nowadays in what concerns milk processing industries, the final destination of the liquid effluents is still the area in water management where more improvement is necessary (12). An on-site installation for the treatment of milk processing effluents may be designed to meet the specific demands of a particular wastewater and so provide economic benefits to the industrial plant as well as a reliable protection against organic overload in the municipal treatment plant. Basically, the methods for anaerobic treatment of milk processing effluents are similar to those used for domestic wastewater. Yet, the industrial application of anaerobic systems is more developed than municipal application, since the treatment of industrial effluents is mainly local as opposed to what happens with domestic wastewater. This allowed for the industry to develop and apply especially tailored systems for each case.

Current practice for the treatment of milk processing effluents varies considerably, since each plant rejects a different wastewater depending on the products and processes used. A treatment scheme applicable to a specific case might not be useful for another one and each particular situation calls for a treatability study.

The treatment system used for treating milk processing wastewaters depends on the degree of purification required and on the localization of the discharge point (direct or indirect discharge), but it is generally considered as having three phases: pretreatment, removal of organic matter, and final polishing. The most common configuration for a treatment plant in dairy industries includes gritting for removal of cheese clots and other solids, fat removal, equalization, biological treatment (in one or two phases), and final effluent decanting before discharge (Fig. 17.7). Parallel to this, some sludge handling system must be defined.



One phase biological system
1=screening; 2=equalization; 3=fat removal
4=contact tank; 5=aeration basin
6=decanter; 7=effluent control; 8=recirculation pump
9=sludge thickening; 10=sludge treatment



Two phase biological system
1=screening; 2=equalization; 3=fat removal
4=contact tank; 5=biological filter; 6=decanter 1; 7=contact tank; 8=aeration basin; 9=decanter 2;
10=recirculation pump; 11=sludge thickening; 12=sludge treatment; 13=effluent control

Fig. 17.7. Scheme of typical treatment plants for milk processing effluents (from (13)).

The nature of the preliminary treatment depends on the type of products of the milk processing industry. In general, the pretreatment of milk processing effluents consists of screening, equalization, neutralization and air flotation (for solids and fat removal). An important characteristic of milk solids is that they are not easily settleable, that is solids removal by sedimentation is not very efficient (61). Of the possible air flotation systems, CAF-Coarse Air Flotation or DAF-Dissolved Air Flotation, the DAF system is most convenient because the size of the fat globules after homogenization (around 1–2 μm) makes it difficult to separate the fat from the liquid matrix. In DAF systems, the air bubble has a diameter around 30–50 μm, and this smaller dimension as compared to CAF bubble size is essential for a good efficiency of flotation systems, since the smaller the air bubbles the easier their adherence to fat and/or solid particles. Furthermore, with the addition of coagulants and flocculants, very efficient separation is achievable. For these reasons, DAF has become the standard for fat oil and grease (FOG) removal in the milk processing industry. However, the coagulants and flocculants are fairly expensive and alter the composition of the retained fats and solids, so that reuse as animal feed is sometimes not possible. In what concerns the effects of pretreatment an important remark is that proteins are the only nitrogen source in milk processing effluents. If the pretreatment system employed removes most of the solids (mainly fats and proteins precipitated by acidification of lactose) the remaining wastewater may be nutrient deficient with regards to subsequent biological treatment (16).

Generally, it is more convenient to treat the effluents before they acidify (fresh effluents). Yet, most of the milk processing plants discharge their effluents for short periods of time in each day and in these circumstances, there is probably more advantage in equalizing the effluent, thus damping variations in flow and concentration and diluting harmful substances, than there is disadvantage in treating the acidified effluent. In what concerns cleaning solutions, it is convenient to store them and discharge them throughout the whole period of operation of the treatment plant.

As a general rule, equalization has always to be considered in a system for the treatment of milk processing effluents. The equalization basin smoothes the flow rate, the load, the temperature, and pH variations. If the equalization basin is too small, some fluctuations may occur in the anaerobic reactor. On the other hand, if the hydraulic retention time (HRT) in the equalization basin is too long, the prevailing anaerobic conditions will give rise to odors and also to acidification of some hydrocarbons and precipitation of organic matter resulting in a raise of the solids content fed to the reactor. In practice, the hydraulic retention time varies between 0.5 and 72 h, although the most used range is from 6 to 12 h implying a dimensioning of the basin from 1/4 to 1/2 of the total volume of the daily effluent. The tank should be well mixed and isolated and it should be covered to avoid odors. Generally, the effluent is discharged by means of an overflow or by means of a floating arm take off by gravity or bombing.

Equalization also permits some reaction time for hydrolysis and acidification of the wastewater and for neutralization of residual oxidants from cleaning operations. Through equalization, it is possible to reach a significant reduction in pH allowing economies in terms of chemicals for neutralizing purposes. The need for equalization varies with the type of anaerobic treatment system used. For low load anaerobic systems (e. g., anaerobic lagoons), the need for equalization is minimal, since the high HRT in the treatment systems allows the smoothing of the variations in the characteristics of the wastewater. The average load systems (e. g., contact process) can endure some variations in temperature and pH, but they can suffer a decrease in their performance as a consequence of flow or load variations. The high rate systems (e. g., filters, UASB, EGSB, fluidized beds) are very sensible to variations in the feed, and thus equalization is a must, although some high rate anaerobic systems also use high recirculation ratios (3:1 or higher) in order to minimize the feed variations.

Although the maintenance of a neutral pH as well as the removal of solids before admission to the bioreactor are important, the separation of fats may not be indispensable if a complete biodegradation of these components may be attained inside the system. Anaerobic digestion offers highly favorable perspectives to the accomplishment of this objective. The main problem to be considered is the need of a long residence time for the fat particles so that they may be degraded, and also some care in avoiding the toxicity caused by the LCFA and the accumulation of fats in some parts of the equipment (especially in filters). Milk fat is very difficult to degrade in biological systems and special devices or operating schemes are necessary to favor the contact between biomass and fatty matter in order to attain a complete as possible degradation.

In the great majority of treatment plants, the posttreatment of milk processing effluents includes an aerobic reactor. In case this is an activated sludge system, it should be adapted for nitrification and denitrification of the effluent; that is, it must be adapted to remove nitrogen compounds. The dimensioning of any aerobic posttreatment step receiving the effluent from a first anaerobic reactor must include a nitrification phase and an anoxic zone for denitrification which is generally located at the head of the system.

4.3. *Anaerobic Digestion of Effluent Components*

Due to the effort made in the last decades to gain insight on the microbiological aspects of anaerobic digestion, nowadays sugars and proteins on their own are no longer problematic in the degradation of milk processing effluents. The careful application of the recommendations found in the literature (37) based on research and industrial application in the last years makes it possible to operate reliably any anaerobic reactor treating sugar and/or protein wastewaters. The major problem in anaerobic treatment of milk processing effluents lies in the degradation of the fats and/or their hydrolysis products, i.e., LCFA.

The problems found in the anaerobic treatment of milk processing effluents may be divided in two main classes (52):

1. Loss of biomass due to sludge wash-out; and
2. Inhibition of the microbiological activity of the biomass.

Within this framework, the role of each class of substrates present in milk processing effluents (sugars, proteins and fats) is discussed below.

4.3.1. Sugars

The hydrocarbons present in milk (mainly lactose) are the major source of the high organic load exerted by the effluents from milk processing industries. Lactose is a substrate that is readily available to be degraded by the bacterial populations that are typical of high rate anaerobic reactors (62–66). Special care must be taken in case of very strong effluents, in which the rapid acidification of sugars coupled with eventual lack of alkalinity (e.g., whey or whey processing effluents) may cause reactor instability. In general, the following of the recommendations published in literature about the start-up of high rate reactors will ensure the success of these treatment systems in case of simple soluble substrates like milk sugars.

Although the degradation of milk sugars is relatively rapid and does not present problems (depending obviously of the following of the recommendations mentioned above), the truth is that the presence of sugars will aggravate the problems caused by other components of the effluent (proteins and fats). Due to the wide variations in flow typical of milk processing effluents these streams are directed to equalization basins having hydraulic retention times of 12–24 h. During this retention period in the equalization basin, there is an acidification of the effluent that may cause a decrease in pH below the isoelectronic point of casein (pH about 4.6). This will originate the coagulation and precipitation of the proteins and also the precipitation of fats by entrainment and adsorption to the protein particles. So, due to the conjugation of the high concentration of easily degradable sugars and the presence of proteins and fats, the effluents from milk processing have a high content of solids that may cause problems in their anaerobic degradation because, as it is well known, the lower the degree of substrate solubilization the lower its biological degradation rate. Furthermore, according to some authors (39, 67–71), the production of enzymes capable of degrading complex substrates, e.g., proteinaceous and/or fatty matter, may be hindered by the presence of easily degradable substrates such as glucose, amino-acids, or lactose. Contrary to these verifications, Hwu (48) referred that the degradation of oleic acid (the most common LCFA in milk effluents) was significantly enhanced by the addition of an easily degradable cosubstrate, e.g., butyrate or glucose.

Another problem enhanced, although indirectly, by the existence of high concentrations of sugars in the effluent is sludge flotation, due to the high biogas production coupled with the presence of complex substrates. The tendency of complex substrates, especially fats and LCFA, to adsorb onto the surface of the biomass strongly favors flotation and biomass wash-out by entrainment with gas flow. Petruy (72) reported that in spite of an extensive adsorption of milk fats onto the surface of biomass particles, no significant biomass flotation was observed because of the absence of biogas production.

4.3.2. *Proteins*

When high rate anaerobic systems were applied to the treatment of effluents with significant amount of proteins, the results obtained were not as good as for the case of effluents containing only simple and soluble hydrocarbons (54). Proteins are an important fraction of the polluting load exerted by the wastewater from many milk processing industries, and they may be degraded to VFA and subsequently to methane in anaerobic treatment systems. In these systems and generally in all biological systems, the presence of proteins in the wastewater has been linked with various problems:

- Scum forming leading to accumulation of organic matter (proteins and fats) inside the reactors and to the formation of floating layers on the upper part of the reactors (73–76) that lead to the loss of biomass in the out flow stream. The forming of this scum layer and the accumulation of proteins and fats causes clogging and forces the system to frequent stops for cleaning (77)

- The growth of filamentous organisms that have a tendency to aggregate forming a bulky biomass with very poor sedimentation characteristics also leads to sludge flotation and biomass loss. van Andel and Breure (78) reported that anaerobic biomass fed with a protein rich effluent had a quite viscous appearance and very poor sedimentation behavior. These authors also noted that proteins would easily adsorb onto the biomass particles without being degraded
- Protein mineralization when in high amounts can originate levels of ammonia that are toxic to the anaerobic organisms. Yet in the case of milk processing effluents, the inhibition by ammonia has not been a relevant problem so far (12)
- In what concerns the anaerobic degradation of proteinaceous wastewater a problem of importance is the fact that proteins may eventually not degrade completely and will produce amines that give rise to bad odors. Some suggestions about this problem were given by Lettinga et al. (37) and by Verstraete and Vandevivere (79)
- As was mentioned above, the precipitation of milk proteins (casein) will lead to the formation of aggregates of solid material that are difficult to degrade (80). Protein denaturation (loss of tertiary structure) is a main mechanism of hindering their decomposition
- Several authors have reported that the presence of easily degradable substrates (sugars and hydrocarbons) will hinder the degradation of more complex substrates like proteins and fats (71). This result was observed even with bacterial populations previously adapted to protein degradation

The previous adaptation of the biomass to protein degradation seems a very important parameter for the anaerobic degradation of these substrates. Perle et al. (44) observed that anaerobic non-adapted cultures would not degrade milk protein (casein) but within 3 months of adaptation, the proteolytic activity (mainly extracellular) would rise significantly and protein degradation was very efficient. Apparently, this adaptation is needed only for the hydrolytic step since the nonadapted cultures were able to degrade amino acids resulting from protein hydrolysis (44). In principle, all bacterial groups are able to degrade substrates as simple as sugars and amino acids and hydrolysis is the step requiring a more specialized biomass. Some authors (72, 81) reported that protein degradation by granular biomass presented serious problems due to a large disparity between the removal from the liquid medium and the biological degradation. Even for removal efficiencies of 90%, the protein conversion to methane would not rise above 65% (72). This confirmed that the main initial mechanism of protein (and other complex substrates) removal is mainly from a physical–chemical nature (entrapment and/or adsorption) and not biological (82, 83).

It has been frequently observed that the adsorption of complex substrates onto the granular biomass will lead to the deterioration of granules characteristics (77, 84). The use of granular sludge UASB reactors for the treatment of wastewaters containing milk proteins requires special configurations of GSL separators; even then success is not fully guaranteed.

4.3.3. Fats

Neutral fats, that is, fats before the hydrolytic step, are prejudicial to anaerobic treatment mainly because they originate the flotation of the biological sludge and consequent loss of active biomass (48, 52, 74, 77, 82). It is known that fat has a tendency to ascend to the top of the reactors by flotation and also by entrainment with the liquid flow and/or the released biogas (77). Even in low or medium load reactors and with different configurations of up-flow systems, the accumulation of fat layers in the top of reactors and in the biogas lines has been reported (16, 29, 77).

Since the first investigations reported on the effects of fats in anaerobic digestion (42, 85, 86), it has been clearly established that the LCFA resulting from lipid hydrolysis are responsible for inhibition of various microorganisms even when in millimolar concentrations. These effects have been largely reported in several works on anaerobic treatment applications for complex effluents (48, 52) and specifically for milk fat or milk fat components (44, 72, 83). Unsaturated LCFA are more toxic than saturated LCFA (46). Oleic (C18:1) is the LCFA present in larger quantity (25–40%) in the LCFA mixture resulting from milk fat hydrolysis (14).

Many studies have been made with the purpose of analyzing the effects of LCFA in the anaerobic process. One of the first studies was performed by Hanaki and coworkers (85) and lead to the conclusion that LCFA affect the obligate hydrogen producing bacteria and also the acetoclastic and hydrogenotrophic methanogens that are responsible for the conversion of the products from the β-oxydation of LCFA. As a consequence, the LCFA inhibit their own degradation causing a potential serious instability in anaerobic reactors treating fat containing effluents. Hanaki et al. (85) also observed that the LCFA would disappear from solution very rapidly and would accumulate in the solid phase (biomass) without being degraded. These observations have been confirmed by numerous investigations ever since (48, 52, 72, 82, 87, 88). In fact, these inhibition phenomena of the LCFA are closely related to adsorption of the substrate onto the surface of the biological sludge (48). Some authors (89, 90) attributed the LCFA inhibition to the physical interaction between the acids and the cell membrane of the microorganisms. This suggests that the biomass concentration in a reactor has a very important role, since inhibition will be dependent to some extent on the biomass/substrate ratio (91), contrary to what was reported by Koster and Cramer (86) and by Rinzema et al. (49). Some investigations on anaerobic digestion of substrates containing LCFA (52, 86) or containing milk fat (44, 72) have shown that these substrates are extremely toxic to the anaerobic bacteria leading to an immediate decrease in the methanogenic activity of the biomass to which they are added. It has been reported that once their toxic limit (MIC, see Table 17.9 is exceeded, these substrates lead to the death of almost the whole of the acetogenic

Table 17.9
Toxic thresholds for some LCFA present in milk processing effluents (adapted from ref. (86))

LCFA	T (°C)	MIC (mM)	MIC_{50} (mM)
Caprylic $C_{8:0}$	30	6.75	>10
Capric $C_{10:0}$	30	2.6	5.9
Lauric $C_{12:0}$	30	1.6	4.3
Mystiric $C_{14:0}$	30	2.6	4.8
Oleic $C_{18:0}$	30	2.4	4.35

$C_{x:y}$ means carbon chain with x carbon chain length and y double bonds, respectively. MIC_{50} means MIC at which 50% of methanogenic activity remains.

and methanogenic populations (49) and to a significant loss of the physiological activity of the cultures (44). The toxic threshold values reported in Table 17.9 may vary, depending on the operating conditions and especially on the type of sludge and the presence or absence of some metal nutrients, as for example calcium, which has the capacity to precipitate the LCFA, thus lowering their inhibitory action (52). Notwithstanding these variations, it is worthy to refer that any value of MIC or MIC_{50} published in the literature for a specific LCFA will serve merely as a rough indication, since there is a strong synergy effect that turns an LCFA mixture (as present in milk processing wastewaters) much more toxic than the individual acids per se (48, 86). On the other hand, it is also important to note that the bacteria do not respond to the bulk liquid concentration of LCFA, but that they respond to the concentration at the interface between the liquid and the biomass particle (52). The interface concentration is influenced by the mass transfer rate and so also by the biomass concentration in the reactor, and especially by the hydrodynamic characteristics of the system. This relation between the toxicity level of the LCFA and the reactors hydrodynamic conditions means that the response of continuous reactors to a LCFA load may not be estimated from results obtained from batch essays. On the other hand, it also means that the capacities of different reactor configurations to endure LCFA loads may vary substantially.

Since hydrolysis is not the rate limiting step in anaerobic digestion of complex fat containing effluents (42, 44, 85), it would be expected that results obtained for LCFA would apply to emulsified triglycerides as present in milk effluents. However, it is not possible to extrapolate the results obtained for individual LCFA or for LCFA mixtures to the emulsified fats present in milk. The fats, especially in the form of triglyceride emulsions, cause more severe problems than LCFA in the hydraulic functioning of biological reactors, namely sludge flotation and loss of active biomass through wash-out. Laboratory experiments indicated that triglyceride emulsions or milk fats severely impair the stability of anaerobic high-rate sludge bed reactors (52, 72, 92). The main problem detected was the strong wash-out (52) or the flotation of granular sludge (72) or of the flocculent sludge (92). It is noteworthy that sludge flotation and wash-out only occurred after serious overloading during the treatment of LCFA solutions (48, 52) whilst with milk fats or triglyceride emulsions it occurred for very low loads viz. 1–3 $kgCOD/m^3$-day (52, 72, 92). Sludge flotation due to adsorption of fats or LCFA onto the biomass particles is enhanced by the biogas bubbles adhered to the biomass (48, 52). In case of milk processing effluents, it must be stressed that the rapidly acidifying sugars produce high biogas flows in anaerobic systems.

The problem of sludge wash-out so frequently observed and reported for the anaerobic treatment of milk wastes is not adequately solved by the use of a special GSL separator design or by the use of a hybrid configuration like UASB + filter (52, 93) or UASB with several sieve drum separator designs (49, 52, 72). The sieve drum separator requires a brushing device, suffers from severe clogging and does not prevent the loss of small biomass particles. It is known that milk fat tends to cause degranulation of the granular biomass in UASB reactors (48). It has also been reported that the most important bacteria for the LCFA degradation are not amenable to granulation (48), and therefore are easily washed out of the reactor system even with sieve drum separators. The packing layer on the top of hybrid UASB/filter reactors can aggravate the problem of biomass wash-out; great part of the organic matter is retained in the packing leading to the clogging of the filter and accumulation of biogas under it. On the other hand, the occurrence of channeling through the filter medium has been observed, leading to severe biomass wash-out, but the most significant drawback is that the packing layer acts in retaining the biomass on the top of the reactor and impairs its return to the sludge bed (52).

Toxicity by LCFA during anaerobic treatment of milk processing and dairy effluents is commonly a result of inhibition of acetogenic and/or methanogenic bacteria, this two bacterial groups being the slowest growing members of the anaerobic food chain. When treating complex wastewaters like milk processing effluents, inhibition of the extracellular enzymes responsible for the hydrolysis of polysaccharides, proteins and fats may also occur.

4.4. Special Considerations for Anaerobic Treatment of Milk Processing Effluents

The effluents from milk processing industries, like other complex fat containing effluents, form a particular class of wastewaters when considering anaerobic treatment. This is a consequence of the characteristics of the effluents, that is, the simultaneous presence of sugars, proteins, and fats, as discussed above. In view of the particularities of the wastewater and the research developed in the past few years, it is important to discuss the relations between phenomena like biomass adaptation, adsorption of complex substrates onto biomass surface, mass transfer limitations, and inhibition when assessing the application of anaerobic treatment to milk processing effluents.

Adaptation is the acquisition, by a microbial community, of a capacity to degrade substrates that before that adaptation were toxic or inhibitory. In microbiological populations, adaptation to a specific substrate may be a physiological response of existing bacteria, modifying their cells to better cope with the toxic compounds, or more likely it will result from a shift in the microbial population because of the growth of new bacteria that are more tolerant to the toxicity. Obviously in the absence of adaptation, inhibition of the biological process will be observed.

It is generally accepted that biomass gradually exposed to growing concentrations of a toxic or an inhibiting substrate, will develop a resistance to that toxicity or inhibition. An important condition to be met, in order to achieve a good result in the treatment of milk processing wastewater, is that the viable biomass is sufficiently adapted to the substrate. In this way, adaptation is a key factor in the application of anaerobic treatment to milk processing effluents which are potentially toxic effluents (56). Several studies have supported the notion that previous adaptation of the biomass to the substrate is a beneficial or even essential condition for the well succeeded operation of anaerobic treatment systems applied to milk or fat containing effluents (94, 95). Biomass adaptation allows the attainment of a higher treatment capacity (96) and acts as a defense against inhibition effects (97).

In case of milk processing effluents, the potentially inhibitory substrates are proteins, mainly casein, and LCFA, mainly oleic acid (85). Perle et al. (44) reported that the inhibition problems caused by milk lipids and by casein were the underlying reasons for the low performance of anaerobic systems used in the treatment of milk processing effluents. These authors also observed that casein remained undegraded by anaerobic cultures not adapted to this substrate. On the other hand, adapted cultures were very efficient in degrading casein as well as the amino acids formed in this degradation where amino acids had null inhibitory action. Milk fats were considered the most important vehicle for the inhibition of methanogenic activity and general physiological activity (as measured by ATP levels) that has an immediate, but lasting, influence in reducing biogas production in the anaerobic cultures to which they were added. Based on these results, Perle et al. (44) recommended that anaerobic systems should be used in the treatment of milk processing effluents only when the lipid level was under 100 mg/L.

Toxicity or inhibitory effects of a certain substrate is generally discussed in terms of the ratio of inhibitory substrate/biomass (98). If the substrate does not adsorb onto the biomass, then the inhibition effects will be independent of the biomass level and will mainly depend on the substrate liquid concentration. On the other hand, if adsorption is determinant in the inhibition process, then inhibition effects will decrease with decreasing biomass level.

Koster and Cramer (86) reported that although adsorption has an important influence in the inhibition mechanism of LCFA in anaerobic treatment, the inhibitory effect of LCFA in granular sludge was more related to the volumetric concentration of the inhibitor (LCFA) than to the amount of inhibitor per unit of biomass. Rinzema et al. (49) confirmed the importance of the volumetric concentration, as compared to the inhibitor/biomass ratio, and concluded that acetotrophic methanogenic bacteria would not adapt to LCFA when exposed either to toxic or lower than toxic concentrations. The recovering of the activity was only possible through the development of a new population with the capacity of degrading the LCFA by the β-oxidation mechanism. In industrial scale reactors, this means that a "poisoned" reactor will need recovery period of about 1–2 months, and that recovery must be initiated with very low LCFA concentrations. The observations of Rinzema et al. (49) are in accordance with results from Yang and Anderson (75) who after 150 days, observed no adaptation effects in a granular biomass fed with ice-cream effluent. Contrary to this, the works from Hwu (48), Nadais et al. (91), and Alves et al. (99) show that adaptation of anaerobic biomass to milk fats and/or LCFA is possible and highly desirable in the operation of high-rate systems. The works from Nadais et al. (91) show that after a 2 weeks period, significant changes may be detected in the anaerobic flocculent sludge capacity to degrade milk components. Morgan et al. (100) also observed that the modifications in the biological population of a flocculent biomass in high-rate anaerobic reactors fed with ice-cream effluents would go on for several weeks. The authors concluded that the complex nature of this substrate with high protein and fat content was more determinant to the natural selection and to the development of microbial ecology than the type of anaerobic reactor (contact, up-flow filter, UASB, fluidized bed).

The length of the adaptation period is still to be determined and certainly depends on a number of factors like

- Initial biomass characteristics and diversity
- Temperature, at thermophilic temperatures biomass has doubling times much higher than at mesophilic temperatures
- Effluent characteristics

When analyzing the recent works on the anaerobic treatment of complex fat containing effluents, it becomes clear that the main goal in the adaptation of a microbial population to milk processing effluents will be the development of a steady population of syntrophic β-oxidizers capable of overcoming the problems associated with milk fat/LCFA.

Hwu (48) observed that such syntrophic β-oxidizers where not present in granular aggregates and were present in the fine particles washed out in high-rate granular sludge bed systems. For this reason, the use of biomass recirculation was suggested in order to enhance the β-oxydation of LCFA in anaerobic granular sludge systems.

Keeping in view the initial physical–chemical removal mechanism observed in the anaerobic treatment of milk effluents and also the surface mechanism of the inhibition by LCFA, it is obviously important to consider the role of adsorption in anaerobic treatment of milk processing effluents.

The biosorption process is defined as the uptake or accumulation of particles and/or chemical substances by biomass. Acknowledgement of the importance of adsorption phenomena in the anaerobic treatment of complex fat containing substrates has increased parallel to the understanding of the removal mechanisms in wastewater treatment systems. Nowadays, it is well established that adsorption of specific compounds (e.g., proteins, lipids, and long chain fatty acids-LCFA) to bacteria and sludge is a phenomenon that can strongly affect or even completely control the performance of an anaerobic reactor (48, 81, 82).

The adsorption phenomena in the anaerobic treatment of milk processing effluents has been extensively reported in the literature both on flocculent (85, 87, 101, 102) and on granular biomass (48, 81, 82, 87, 103, 104). It was observed that the initial removal of organic matter (about 50% of the initial concentration) from the liquid medium by the biomass was very fast and that pseudo-equilibrium was reached within a short period (1/4 to 24 h) of contact time. An eventual subsequent stabilization reaction would proceed much slower and uniformly. Contrary to what has been reported for other substrates (105, 106), the adsorption of LCFA and milk fats onto anaerobic biomass appears to be nonspecific (89) that is not dependent on bacterial species. Also, in contradiction to what was observed with other substrates (107, 108) the adsorption of fats/LCFA onto anaerobic biomass is influenced by the size of the biomass aggregates being higher for the smaller particles (87, 104). Finally, the adsorption uptakes of fats and LCFA onto anaerobic sludge are higher for higher initial concentration of these complex substrates (48, 101) which was not verified for other substrates (108). So the adsorption of milk fats and/or LCFA onto anaerobic biomass (flocculent or granular) seems to be governed by mechanisms that are somewhat different from those that determine the adsorption of other substrates (mainly non-fatty substances). In a study of anaerobic treatment of milk effluents, Schoepfer and Ziemke (102) found that sludge that had not been fed for several months appeared to loose most of its adsorption capacity. Hwu (48) found a higher adsorption of a mixture of LCFA onto granular sludge adapted to fat containing wastewater in comparison with the adsorption of oleate onto nonadapted granular sludge and ascribed this difference to a synergistic effect of LCFA, but also considered that the sludge adaptation might be of influence. According to Hwu (109), the adsorption of LCFA onto anaerobic sludge is a biologically mediated phenomenon.

This adsorption of milk fats or their hydrolysis products (LCFA) onto the anaerobic granular sludge has been reported to cause disintegration of the granules (23, 72) and to bestow a gelatinous appearance on the sludge (23). Most of the acetogens are hydrophobic (110) and since LCFA act as surfactants in the pH range prevailing in anaerobic reactors, they lower the liquid surface tension thus impairing aggregation of hydrophobic bacteria.

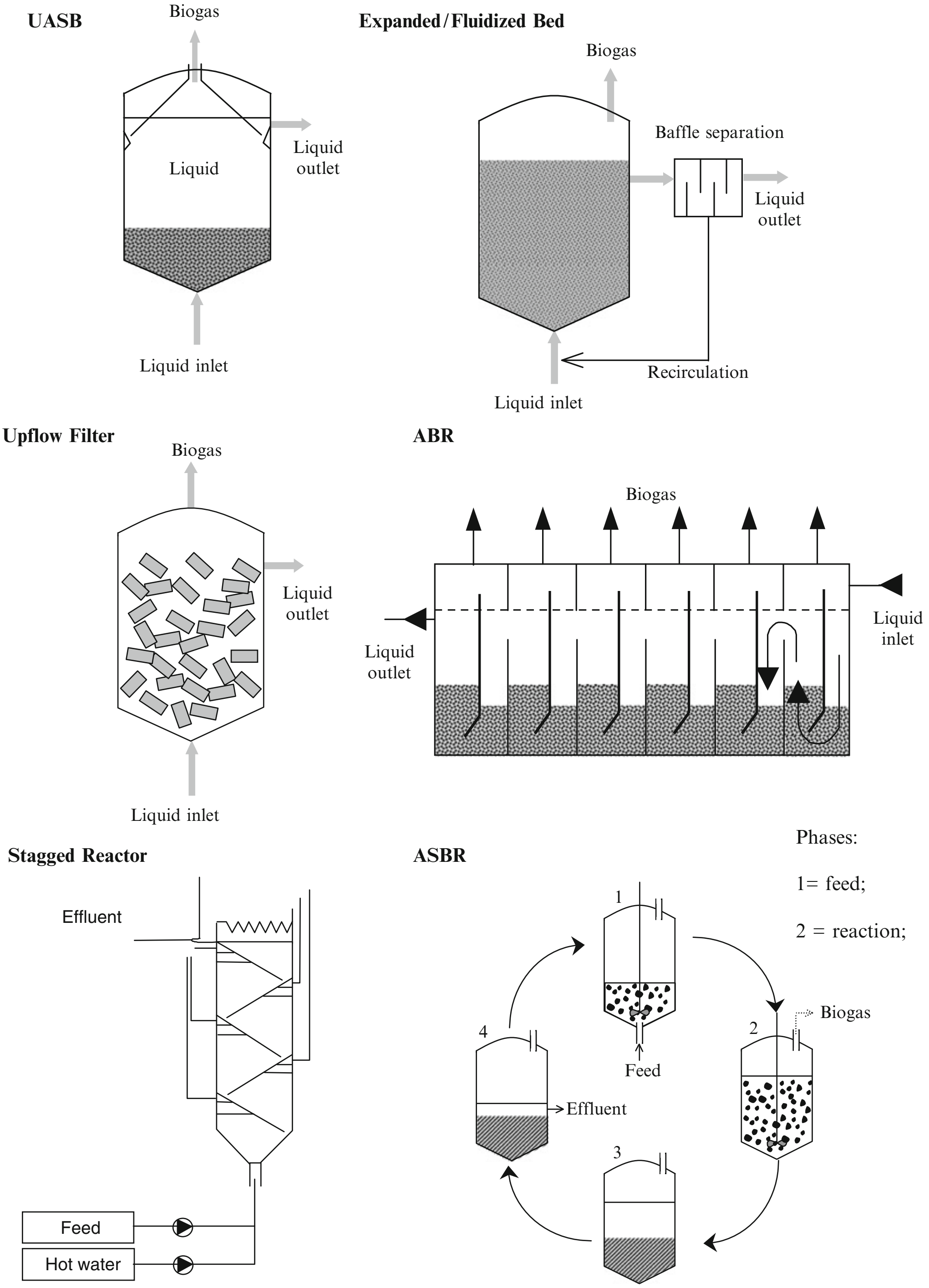
According to Lettinga et al. (37), sludge bed reactors do not necessarily require the use of granular sludge. It is possible to operate these reactors using flocculent sludge provided that it has good settleability. It is known that in the case of milk processing effluents, the limiting step is the hydrolysis of particulate substrates (38) or the degradation of LCFA to short chain acids (85). For these reasons, it is natural that flocculent sludge being predominantly acidogenic (54) will result in a better degradation of complex substrates as compared to granular biomass which is predominantly methanogenic (54). It has been verified (48) that the bacteria that degrade oleic acid in granular sludge bed reactors were found in the fine particles and would not form granules. The reported order in oleate conversion rates for anaerobic biomass was (48): granular < dispersed < washed out. This may be a reason for the poor results published in literature for the anaerobic degradation of milk fats in granular sludge reactors (75, 81). It is also known that UASB reactors inoculated with granular sludge bare lower solids concentration in the feed than flocculent sludge reactors. In view of the high solids content that may be present in acidified milk processing effluents, flocculent biomass appears as the most adequate for the use in anaerobic treatment of these wastewaters.

4.5. Application of Anaerobic Technology to Milk Processing Effluents

As it is well known, an important class of complex wastewater is those effluents containing appreciable amounts of solids and fats/LCFA as it is the case of milk processing effluents. Extensive research has been performed on the anaerobic treatment of these effluents in laboratory, pilot and full-scale installations (48, 52, 72, 82, 92, 111–113). The similarities and differences between the main types of high-rate reactors were extensively covered by Hickey et al. (24) and by Weiland and Rozzi (114). Anaerobic digestion is the most suitable option for the treatment of milk processing effluents. The presence of biodegradable compounds coupled with the advantages of anaerobic process over other treatment methods makes it an attractive option (2).

4.5.1. Types of Anaerobic Systems Used for Milk Processing Effluents

The full-scale application of anaerobic technology to high-strength and high-volume liquid effluents such as milk processing effluents requires the development of reactors, in which the microorganisms converting the waste to methane could be retained in the reactor. Many of the bacteria involved in the process grow very slowly requiring a long solids retention time (SRT) to avoid wash-out. In contrast, the large volumes of liquid wastes to be processed impose a relatively short hydraulic retention time. The most representative anaerobic systems used for the treatment of milk processing effluents are the following: anaerobic lagoons, contact process, anaerobic filter, up-flow anaerobic sludge bed, fluidized/expanded bed, and hybrid configurations. Figure 17.8 shows some of the high-rate anaerobic concepts with actual or predictable application to milk processing effluents. Figure 17.9 illustrates the distribution of real scale high-rate anaerobic systems for treating effluents from dairy and milk processing industries. Table 17.10 presents an overview of world anaerobic full-scale installations in the dairy and milk processing industries.



UASB

Expanded/Fluidized Bed

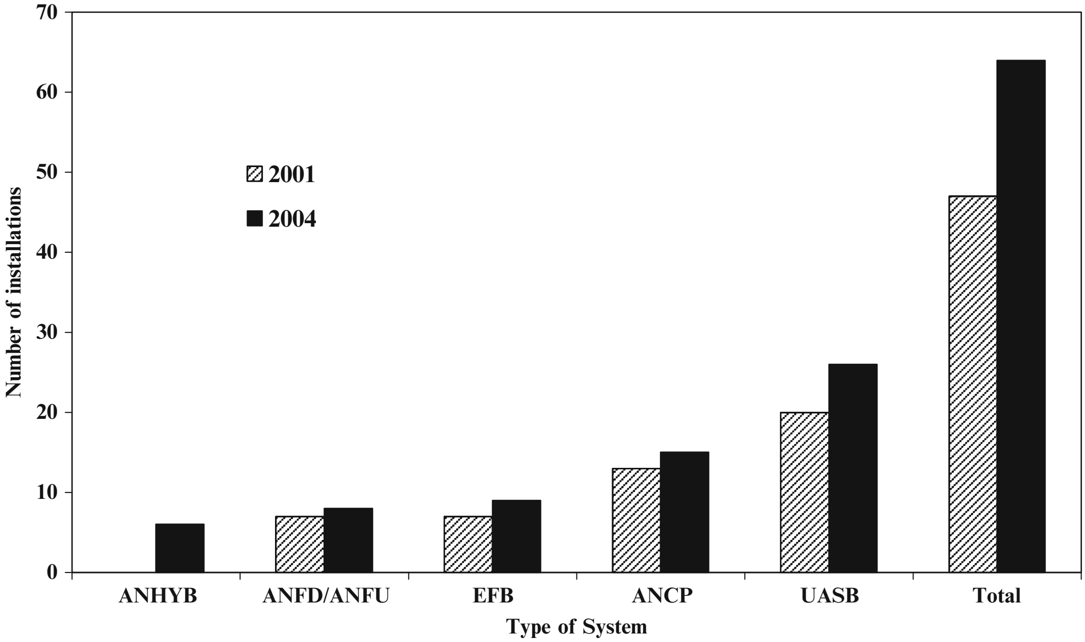
Upflow Filter

ABR

Stagged Reactor

ASBR

Fig. 17.8. High-rate anaerobic systems used for milk processing effluents.



ANYB = Hybrid systems
ANFD = Anaerobic Filter (Downflow)
ANFU = Anaerobic Filter (Upflow)
EFB = Expanded/Fluidized bed
ANCP = Anaerobic Contact Process
UASB = Upflow Anaerobic Sludge Bed

Fig. 17.9. Distribution of real-scale anaerobic treatment systems for milk processing effluents (data from ref. (115)).

4.5.1.1. ANAEROBIC LAGOON

The anaerobic lagoon is the most used system world wide for the treatment of milk processing and other food industry wastes. Their use is common in the third world countries because they need little or no technology and know-how. The construction of anaerobic lagoons is very simple and the process is usually built in one cell, but many combinations can be used both in parallel or sequential arrangement. The high residence times allow for the sedimentation and anaerobic degradation of the organic matter. Occasionally, sludge recirculation and mechanical agitation are used for improvement of contact between substrate and biomass.

In some industries (including milk processing installations), anaerobic lagoons may be present in a natural cover caused by the ascending solids, fats, and oils under quiescent conditions, forming a thick layer. A more modern variation is the synthetic cover to avoid odors and biogas escaping.

Table 17.10
Anaerobic full-scale installations used in the milk processing effluents (data from refs. (16, 115))

Installation	Flow (m^3/h)	Load COD total (kg/day)	Reactor volume (m^3)	Removal COD total (%)	HRT (h)	Load COD total (kg/m^3-day)
Low rate						
Alto Dairy	15	4,100	6,050	85	403	0.68
Arizona Dairies	–	–	–	–	–	–
Luis Farms	–	–	–	–	–	–
M&M Dairies	–	–	–	–	–	–
Bancroft Dairy	38	15,600	10, 400	75	274	1.50
Mid-America Dairy	48	4,560	11,400	85	238	0.40
Tulare, Cidade de	695	59,280	114,000	80	164	0.52
Turkey Hill Dairy	12	2,130	2,840	80	237	0.75
Average rate						
Carbery Milk Products	40	9,600	2,544	95	14	17.65
CCPL – Rio de Janeiro	25	1,980	300	75	12	6.60
Foremost/Leprino	120	25,500	12,500	80	104	2.04
Gold Bond Ice Cream	11	3,300	1,600	85	55	5.50
Haagen Daz Ice Cream	14	4,900	2,300	80	164	2.13
Kerry Ingredients	10	2,343	1,300	85	130	1.80
Lacto-Lima	21	12,640	2,528	87	120	5.00
Lacto-Lusa	21	12,640	2,528	87	120	5.00
Cidade de Madison	–	8,500	–	–	–	–
Mikkeli Dairy	25	1,320	350	75	14	3.77
Millbank Cheese	2	3,245	1,100	85	550	2.95
West Lynn Creamery	47	12, 000	5, 683	85	121	2.11
High rate						
Dunkirk Ice Cream	61	10, 227	1, 350	80	22	7.58
Fermiers Savoyards	5	1, 300	110	90	22	11.82
Kerry Co-op	200	43, 000	4, 150	80	21	10.36
Saint Hubert	42	3, 000	254	80	6	11.81
Agropur	65	6, 500	900	80	14	7.22
Boruclo Whey	146	10, 000	950	75	7	10.53
CCPL	50	3, 000	300	75	6	10.00
Colombo Yogurth	16	1, 818	200	82	13	9.09
Kaserei	67	4, 500	450	80	7	10.00
Kraft	58	3, 274	400	82	7	8.19
So. Caernarvon	5	6, 000	2, 200	85	440	2.73
Sylvester Whey Products	40	6, 100	500	85	13	12.20
Borden/Meadow Gold	32	8, 727	2, 652	80	83	3.29
EDC	6	13, 300	760	75	127	17.50

4.5.1.2. CONTACT PROCESS

The contact process is a perfectly mixed tank (CSTR) in which the biomass in the effluent stream is separated and recycled to the reactor to keep a high concentration of microorganisms in the reactor and a high SRT. The key components for this process are the mixed tank, the effluent degasification unit, and the biomass separation system. Degasification of the effluent is crucial because the biogas entrapped within the biological particles hinders the separation of the biomass from the liquid. Due to the characteristics of the anaerobic flocs, the sedimentation unit should be of parallel plate type. The anaerobic sludge has the same applications as the aerobic sludge (land fertilizer, etc.), but it has the advantage of being more stabilized chemically and producing less odors. This process is still widely used because it is the one that has a higher capacity to degrade solids and fats with no accumulation inside the reactor. But in spite of being used world-wide to the present day, anaerobic CSTRs are increasingly giving way to faster and more efficient higher-rate anaerobic digesters, notably anaerobic filters, UASB reactors, and expanded/fluidized bed reactors.

4.5.1.3. ANAEROBIC FILTER

The anaerobic filter was developed as one of the first anaerobic systems with biomass retention by attachment on a supporting media resulting in high concentrations of biomass and high SRT inside the reactor. The choice of the support media varies from activated carbon, rock, pall rings, PVC supports, and reticulated polystyrene. These systems may be operated in an up-flow or in a down-flow configuration. The wastewater is distributed from above (down-flow configuration) or below (up-flow configuration) the support media. The down-flow systems are much less used than the up-flow systems. In the up-flow systems, wastewater to be treated flows from the reactor bottom upward. The methane forming bacteria stick to the surface of the support medium and also exist in the space between the media in the lower section of the reactor. According to some authors, the biological conversion capacity of the up-flow anaerobic filter reactors is mainly associated with the suspended biomass aggregates in the lower part of the reactor, being the attachment of biofilms to the packing only of marginal importance (116, 117). In what concerns the true fixed film systems, although some modern support materials have been developed with high specific surface areas, the biomass concentration in these reactors is considerably lower than in anaerobic systems with mobile biomass aggregates. Consequently, the maximum conversion rate is lower implying a lower design capacity or a lower safety factor against overloading by the LCFA present in milk wastes. On the other hand, although providing a real safeguard against sludge wash-out, the packing material gives a high risk of clogging and channeling that must be considered in the case of milk processing effluents.

Fixed film reactors offer the advantages of simplicity of construction, elimination of mechanical mixing, better stability at higher organic loads, the capability to withstand large toxic and organic shock loads, and quick recovery after a starvation period (2). The main limitation of this design is that the reactor volume is relatively high compared to other high rate processes due to the large volume occupied by the support media. Another important constraint when considering the treatment of complex substrates like milk processing effluents is clogging of the reactor due to the increase in biofilm thickness and/or to the high suspended solids concentration present in the wastewater (2).

4.5.1.4. UP-FLOW ANAEROBIC SLUDGE BED REACTOR

The development of up-flow anaerobic sludge bed reactors (UASB) was based on the possibility of forming granular biomass aggregates that can be retained inside the reactor without the need of a support medium. In the UASB system, the reactor consists of an up-flow tank with a feed inlet distribution system at the bottom and a three phase separator at the top (Gas–Solids–Liquid separator). The wastewater is evenly distributed over the reactor bottom through inlet pipes and flows upward through a bed of anaerobic sludge at the lower part of the reactor (sludge bed). During the passage through the sludge bed, particulate matter is entrapped and the biodegradable matter is removed from solution by the anaerobic bacteria and converted into biogas and a small fraction of anaerobic biomass. The ascending biogas provides gentle mixing of the sludge bed and is collected at the top of the reactor in a gas collector system from where it is withdrawn. The remaining water–sludge mixture enters a settling compartment, where the sludge can settle and flow back to the sludge bed. The water is collected in effluent gutters and discharged out of the reactor. Effluent recycle (to fluidize the sludge bed) is not required as sufficient contact between the wastewater, and the sludge is guaranteed even at low organic loads because of the influent distribution system. The critical point in UASB systems is the gas–solids–liquid separator. Usually, this is built on a proprietary basis and specific designs have been conceived for specific types of wastes. UASB systems have few mechanical components and so operation and maintenance are easy.

Although initially the granular biomass was considered indispensable for the functioning of the system, some authors suggested the utilization of flocculent sludge when treating complex fat containing wastes (37, 82, 92, 113). The principal reasons for the comparatively large success of the UASB system are its simple and inexpensive construction and its ability to retain very high amounts of high quality biomass and thus accommodate high organic space loads and provide ample safety against shock loads.

A major advantage of UASB reactors is that the technology has comparatively less investment when compared to anaerobic filters or fluidized bed systems. Among notable disadvantages are the somewhat long start-up period, the requirement of a biological sludge with good settling properties, and the need for skilled operation.

UASB systems in particular seem sensitive to pH and load variations, and to high fat and calcium concentrations, all of which were considered to disrupt the settleability of the sludge and sludge granule formation (21).

A very popular technology derived from the initial UASB concept is the Internal Circulation reactor (IC) considered an ultra-high-rate anaerobic system. This system features a two-stage separation/collection of biogas within a tall cylindrical vessel and uses the gas-lift principle to induce internal circulation of treated effluent. The tall cylindrical design of this reactor makes it very suitable for applications where land is at a premium.

4.5.1.5. EXPANDED/FLUIDIZED BED REACTORS

The distinction between expanded and fluidized beds is not clearly defined. In general, it is considered that expanded beds are those subject to an increase in bed volume from 5 to 25%

over the initial (rested state) bed volume. On the other hand, fluidized bed systems have been tested or operated with bed volumes 25–50% higher than initial bed volumes. Contrary to fluidized bed, in expanded bed technology, granular sludge biomass is more used rather than inert support media.

In fluidized reactors (and eventually in expanded bed reactors), the biomass is fixed on small support particles that are retained inside the reactor. The media used are small particle size sand, activated carbon, etc. for fluidized beds and slightly larger particles like sand, gravel, or plastics etc. in expanded beds. Particle bed fluidization occurs beyond a certain up-flow liquid velocity depending on the particle density and other factors such as the pressure loss in the bed. Under fluidized state, each media provides a large surface area for biofilm development. Fluidized bed technology is more effective than anaerobic filter technology as it favors the transport from the bulk to the surface of the aggregates and thus enhances the contact between the microorganisms and the substrate. In relation to the filter process, the fluidized bed system also presents the main advantage of avoiding clogging, yet its capacity for the removal and degradation of suspended solids is almost null. The moving bed systems do not result in higher safety against sludge wash-out. The sludge fluctuation will also occur in these systems when the balance between the liquid up-flow velocity and the biomass/support sedimentation velocity is disturbed because of effects of ascending lipids adsorbed onto the particles. These problems are of special concern in the treatment of milk effluents due to their high content of fats and solids.

In relation to anaerobic filters, these systems have several advantages such as the elimination of bed clogging, lower hydraulic head loss combined with better hydraulic circulation, and a greater surface area per unit of reactor volume and consequent lower capital costs. However, the need for effluent recycling in order to attain bed expansion or fluidization may increase operating costs.

In this system, performance is critically dependent on the efficient distribution of the influent/recycle stream to ensure a rapid, uniform flow through the reactor bed and adequate biomass growth. Significant drawbacks of this configuration range from the relatively high capital and operation/maintenance costs due to the complexity of operation.

The expanded granular sludge bed reactor (EGSB) is a modified form of UASB system, in which a slightly higher superficial liquid velocity is applied (5–10 m/h) as compared to 3 m/h for soluble wastewater and 1–1.25 m/h for partially soluble wastewater in an UASB (37). As a result of bed expansion, the contact between substrate and biomass is very good and the transport of substrate into the sludge aggregates is much better as compared to systems where the mixing is much lower (UASB). Benefits of EGSB reactor over UASB systems are valid especially for low strength VFA containing wastewaters (2).

4.5.1.6. HYBRID CONFIGURATIONS

The hybrid configurations result from a combination of two or more principles of operation of other existing configurations. The most frequent examples are the combination of UASB and filter or UASB and contact process with the objective of raising the sludge inventory in the sludge bed. Such hybrid configurations are designed to take advantage of the beneficial features of several anaerobic processes without realizing the high cost of employing multiple separate processes. On the other hand, the growing interest in these hybrid systems also stems from the fact that none of the simpler initial concepts is fully adequate for some specific effluents.

4.5.1.7. TWO-PHASE SYSTEMS

These processes are based in the assumption that the anaerobic degradation of organic compounds is performed by two main groups of microorganisms that have distinct metabolic characteristics. These systems consist of two reactors in series in which acidogenic and methanogenic phases take place separately. This separation is possible in the circumstances where the acidogenic phase is faster than the methanogenic step, since if the opposite occurs, methanogenic populations will start to grow in the first phase. A major inconvenient pointed to the two-phase processes is the fact that the coordinated activity of the several bacterial groups is essential for the process stability since the phase separation will alter the concentrations of the intermediate species in a way that might turn unfavorable to methanogenic bacterial growth (37).

Specifically for effluents from milk processing and dairy industries, another concept of two-phase systems was presented by Zeeman et al. (118): the up-flow acidifying sludge reactor (UASR), in which the first phase was used to remove proteins and lipids by acidification of easily degradable sugars. The pH drop reaching the isoelectronic pH point of casein (around pH = 4.6) causes precipitation of protein and fats. The acidified effluent from this first phase is treated in an EGSB reactor whilst the protein/fat sludge is treated in a thermophilic reactor.

A recent example of a modification of the conventional UASB reactor is the anaerobic staged reactor developed by van Lier et al. (119) also shown in Fig. 17.8. Basically, in each module of the staged reactor, all of the anaerobic degradation phases occur simultaneously. Consequently, for a nonsoluble and non acidified feed a mainly acidogenic flora will develop in the first stage(s), but also some acetogenic and methanogenic organisms will probably be present. When treating a partially soluble feed, the first stage will serve primarily for hydrolysis and also partially for acidification of the substrate. With the development of the degradation processes in the subsequent stages of the system, a biomass with higher methanogenic activity will develop. The biological sludge will be different in each compartment depending on the prevailing environmental conditions and on the intermediary substrates remaining for degradation. Since the mixture of the whole reactor biomass is avoided, in principle each stage develops a specific type of biomass. In case phase separation occurs in a staged reactor, this would be the consequence of a natural selection. This kind of reactor is especially indicated for thermophilic operation (120) since at thermophilic temperatures, there is a higher inhibition by reaction products or by substrates.

A further example of an anaerobic reactor using the concept of biomass segregation is the anaerobic baffled reactor (ABR) developed by Bachmann et al. (121) from the rotating biological contactor. According to Nachaiyasit and Stuckey (122), the ABR can be considered as a series of UASB reactors, and it was called initially "modified sludge bed reactor" (123) that does not require granular sludge for operation. This reactor concept consists of a series of vertical baffles that force the liquid to flow under or over them from the feed inlet toward the outlet (see Fig. 17.8). The baffles fixed either at the top or at he bottom of the reactor divide the reactor in a number of compartments that cause segregation of biomass and biogas. In this way, the liquid flow and the substrate degradation cause a selection of the trophic groups along the reactor length. Among the new designs of anaerobic high-load reactors, the ABR is quite promising as a new and flexible concept for application to a wide variety of domestic and industrial wastewaters including complex effluents (124, 125). According to Nachaiyasit and Stuckey (123), this reactor design is especially applicable in situations when the wastewater flow to be treated is intermittent and the reactor receives low maintenance and care.

4.5.2. Design Considerations for Anaerobic Systems in Milk Processing Industry

General design considerations common to all anaerobic treatment applications include (115):

- Equalization requirements, volume/time.
- Pretreatment requirements (Total Suspended Solids (TSS)/FOG removal).
- Need for wastewater heating/cooling and type of heat exchanger.
- Nutrient (micro and/or macro) requirements – N, P, S, Fe, Cu, etc.
- Need for pH and/or alkalinity adjustment.
- Odor and corrosion control concerns.
- Handling of biogas, excess sludge and anaerobic effluent.
- Process control requirements – degree of monitoring and control.
- Staffing and training requirements.

Specific design considerations for each particular anaerobic system are presented below (115).

Anaerobic lagoon:

- Availability of space.
- Proximity to subdivisions, commercial areas, and individual residences.
- Hydrogeological and geotechnical constraints (e.g., groundwater level, soil permeability).
- Frequency and magnitude of high winds.
- Duration and intensity of freezing weather.
- Natural cover or synthetic cover.
- Cover resistance to ultraviolet (UV) degradation.
- Sludge recycle, gas collection and reuse, mechanical mixing, and other special features.
- Eventual need to remove settled solids.
- Rainwater/snowmelt removal from cover.

Anaerobic contact process:

- Mesophilic or thermophilic process operation.
- Steel, reinforced concrete, or prestressed concrete reactor construction.
- Side-entering or top-entering mixing.
- Atmospheric versus vacuum degasification.
- Solids removal via sedimentation or gas flotation.
- Lamella or conventional clarifier sedimentation.
- Flow-type versus suction-type conventional clarifier solids removal.
- Special features such as membrane separation and degasifier odor control.

UASB reactor:

- Flow/load equalization.
- Preconditioning (partial acidification) of the wastewater.

- Limitation of influent TSS to 10–20% of influent COD.
- Limitation of FOG levels to $< 100\,\text{mg/L}$.
- Steel or concrete reactor construction.
- Corrosion resistant material selection for cover and proprietary settler.
- Uniform, steady distribution of influent within sludge bed.
- Adequate storage volume for backup sludge supply.
- Specific needs for minimum levels of calcium and micronutrients.

Anaerobic filter system (up-flow or down-flow):

- Flow/load equalization.
- Provisions for wastewater pretreatment to limit TSS and FOG in the feed.
- Preconditioning of the wastewater.
- Steel, reinforced concrete or prestressed concrete reactor construction.
- Internal media material, type and configuration.
- Uniform, steady distribution of influent within sludge bed.
- Provision for removal of solids from the support media.
- Identification of the method for measuring biomass levels in the reactor.

Expanded/fluidized bed reactor system:

- Flow/load equalization.
- Preconditioning (partial acidification) of the wastewater.
- Limitation of influent TSS to 10–20% of influent COD.
- Limitation of FOG levels to $< 100\,\text{mg/L}$.
- Steel or fiberglass reactor construction.
- Corrosion resistant material selection for cover and internals.
- Carrier material selection for systems with a carrier media.
- Carrier cleaning and solids removal system.

4.5.3. Loads and Operating Parameters in Anaerobic Systems for Milk Processing Effluents

Table 17.11 presents some data on the operation of high-rate anaerobic systems used for milk effluents. Information on low and medium rate systems may be found in literature (115, 126).

4.5.4. Summary of Results for Anaerobic Treatment of Milk Processing Effluents

Tables 17.10 and 17.12 present data on industrial-scale, lab-scale, and pilot-scale anaerobic systems used for milk effluents.

4.5.5. Choice of Anaerobic System for Treatment of Milk Processing Wastewater

In general, technologies for wastewater treatment are evaluated based on factors such as sludge management, capital costs, operator requirements, and operating and maintenance costs. A technology is acceptable to an industry if it requires less capital, less land area, and is more reliable when compared to other well-established options. For an anaerobic system, this translates into the process being able to run at high organic and hydraulic loading rates with minimum operating and maintenance requirements.

In the choice of the adequate anaerobic system, the most important factor is the nature of the wastewater to be treated, since not all systems are adequate for some complex substrates. In the

Table 17.11
Loads and operating parameters in anaerobic systems (26, 57, 58, 126)

Anaerobic filter			
Organic load (kg COD/m^3-day)	0.1–30[a]	5–20[b]	2–10[c]
HRT (h)	~ 24[d]		10–15[c]
COD removal (%)	65–75[a]		70–80[c]
Critical solids concentration in feed (mg/L)			450–1,050[c]
UASB			
Organic load (kg COD/m^3-day)		5–15[b]	2–15[c]
HRT (h)	< 24[d]		10–50[c]
COD removal (%)			70–90[c]
Critical solids concentration in feed (mg/L)		10–20% of feed COD[b]	
Expanded bed			
Organic load (kg COD/m^3-day)			2–50[c]
HRT (h)	$\ll 24$[b]		0.5–24[c]
COD removal (%)			70–80[c]
Critical solids concentration in feed (mg/L)	Not critical but solids are not removed[a]		

[a] Ref. (26).
[b] Ref. (126).
[c] Ref. (58).
[d] Ref. (57).

case of milk processing wastewater, the selection of reactor type must take into consideration the main problems discussed above and caused by the simultaneous presence of sugars, proteins, and fats. Nevertheless, milk processing effluents are an application particularly adequate for anaerobic treatment because they have above ambient temperature and high concentrations of organic substrates. In fact, the higher the flow and the concentration of organic matter the higher the economic advantage in the use of anaerobic technology (127).

Apart from the characteristics of the effluent to be treated, the main factors to consider in the selection of anaerobic technology for treatment of milk processing effluents are:

- Lower investment costs (land and technology)
- Higher reliability and flexibility in relation to other well established treatment options
- Lower operation costs
- Absence of environmental emissions, especially odor
- Automated operation
- Maintenance and control costs

A careful analysis of the characteristics of each system must be performed to choose the most adequate technology (Table 17.13).

In order to elect with reliability the most appropriate system, it is necessary to perform a systematic evaluation of the different configurations with the specific wastewater to be treated and if possible with a sample of the biomass that will be available to inoculate the reactor. The choice of the system must be supported by its capacity of being operated at high hydraulic and organic loads with low operation and maintenance costs. Concerning treatability studies, it is

Table 17.12
Summary of the operational conditions for the anaerobic treatment of milk processing effluents (adapted from refs. (2, 5))

Reactor	Volume (L)	T (°C)	HRT (day)	Influent concentration (g COD/L)	COD reduction (%)	OLR (kg CODb/ m^3-day)
AF	2	37	5.9	60.7	98.3	9.4
AF	–	40	1	2.9	93.8	2.9
DSFF	0.7	30	3.3	66	96.0	20
DSFF	0.7	30	0.3	4.1	75.0	15
UASB	1.2	30	0.3	4.1	78.0	15
UASB	4	35	2	29.4	97.5	14.7
UASB	1	35	0.12	2.8	91.1	23.8
UASB	4	35	0.22	2.3	96.0	10.4
UASB	8	30	0.21	1.8	87.0	8.5
FB	0.6	35	0.33	0.34	80.0	1.0
FB	2.5	35	1.33	5.0	92.0	3.8
ASBR	3.5	35	3.2	4.3	96.0	6.25
UASB	4×10^6	35	8	4.4	63.0	0.55
AF	14.2	35	1.9	6	98.0	6.29
SAF	17.7	35	2.05	6	98.0	5.92
TF	40.5	35	0.11	0.333	81.0	4.45
UASB	–	–	2.3–11.6	5–77	95–99	1–28.5
UASB	–	–	5.4–6.8	47–55	90–94	7–9.5
UASB	–	–	3.3–12.8	16–50	90–95	1–6.7
UASB (dairy)	–	–	0.07	2.05	90	31
UASB (cheese whey)	–	–	5	4.5–38.1	–	–
2-stage (cheese whey)	–	–	10–20	72.2	36	–
UFFLR	–	–	5	79	95	14
DSFFR	–	–	5	13	88	2.6
FBR	–	–	0.4	7	90	7.7
FBR	–	–	0.1–0.4	0.8–10	63–87	6–40
AAFEB	–	–	0.6–0.7	5–15	61–92	8.2–22
AnRBC	–	–	5	64	76	10.2
SDFA	–	–	–	69.8	99	16.1
UASB	–	–	1.5	11	94	7.1
UASB	–	–	5	5–28.7	97–99	0.9–6
DUHR	–	–	7	68	97	10
UASB (whey permeate)	–	–	5–0.4	10.4	–	–

Table 17.13
Choice of anaerobic systems for milk processing effluents (adapted from refs. (2, 128, 129))

	CSTR	Contact	Anaerobic filter	Fluidized bed	UASB	EGSB
Start-up	Excellent	Bad	Very good	Good	Acceptable	Acceptable
Start-up period (weeks)	2–4	2–4	3–4	3–4	4–16	4–16
Operation	Acceptable	Acceptable	Excellent	Good	Good	Good
Control	Excellent	Bad	Good	Good	Good	Good
Shock resistance						
Temperature	Good	Bad	Excellent	Excellent	Excellent	Excellent
Toxic	Good	Bad	Excellent	Excellent	Very good	Very good
Organic load	Very good	Bad	Excellent	Excellent	Excellent	Excellent
TSS load	Good	Bad	Good	Acceptable	Acceptable	Acceptable
Channeling effect	Not present	Non-existent	High	Non-existent	Low	Very low
Effluent recycle	Not required	Not required	Not required	Required	Not required	Required
GSL separation	Not required	Not required	Beneficial	Beneficial	Essential	Essential
Carrier packing	Not essential	Not essential	Essential	Essential	Not essential	Not essential
Loading rates (kg COD/ m^3-day)	0.25–3	0.25–4	1–40[a]	1–100[a]	10–30[a]	10–30[a]
HRT (day)	10–60	10–60	0.5–12	0.2–5	0.5–7	0.5–7
Main advantages	Simple technology	Long SRT and relatively short HRT	Simplicity of construction	Resistance to inhibitors	Lower investment relatively to filter and fluidized bed	Excellent contact between biomass and substrate
	Adequate mixing	Good contact between biomass and substrate	No mechanical mixing	Good contact between biomass and substrate	Low loss of solids	
	Good contact between biomass and substrate	Efficiently retained biomass system	Good stability at high loads	No bed clogging	Well settling sludge	
	Eliminates scum and thermal stratification		Good resistance to organic or toxic shocks			
Main disadvantages	Washout of the active biomass	Need for a biomass separation system	Relatively high volume	Need for effluent recycle	Long start-up	Long start-up

(Continued)

Table 17.13 (Continued)

	CSTR	Contact	Anaerobic filter	Fluidized bed	UASB	EGSB
	Long retention times	Need for effective mixing	Clogging of the media	Need for skilled operation	Need for sufficient amount of granular sludge	Need for sufficient amount of granular sludge
	High cost of installation and mixing	Limited tolerance to hydraulic loading and biomass retention	Significant pressure loss		Need for skilled operation	Need for skilled operation
	Biomass growth based system and not a efficiently retained biomass system					
Tolerance to fats and solids in the higher loads	Good	Fair – good	Poor	Poor	Flocculent sludge: fair –good Granular sludge: poor	Poor

[a] The higher loads are achievable only with prior fats removal.

important to remember that the results obtained in laboratory or pilot scale are usually from a short term operation and that in the case of milk processing effluents, the long term behavior of the system may differ significantly from this. In general, when performing laboratory or pilot experiments to access the behavior of a system in the treatment of a certain effluent, the factors that are monitored and used for this evaluation are the higher attainable organic and hydraulic loads, the COD or BOD removal efficiencies and the biogas production. In a preliminary study of the applicability of a certain anaerobic system to milk processing effluents, the optimization of these factors is not enough or, in other words, might lead to the need for high safety factors. This is because the main factor that governs the performance of a reactor treating complex fat containing effluents is the accumulation of the removed organic matter and not the removal efficiency or the production of biogas on their own (113). It is necessary to use these factors in complement with each other so as to calculate the efficiency of methanization of the removed organic material. In this way, a correct evaluation of the reactor performance can be made.

4.5.6. Control of Anaerobic Processes Applied to Milk Processing Effluents

Anaerobic digestion is a process that is significantly affected by the operating conditions. Since the process depends on the formation of several intermediate products that are toxic or inhibitory to the anaerobic biomass, it is important that the rates of reaction are high enough to avoid accumulation of these intermediate compounds inside the system which would result in reactor imbalance and failure.

The good performance of an anaerobic reactor for milk processing effluents relies on the equilibrium of the reaction rates and is determined mainly by both the applied load and the influent concentration. Besides the fulfillment of the specific requirements for these particular effluents, other general parameters that are determinant due to the sensitivity of the bacterial populations are the following:

1. Temperature – this is the environmental factor with most influence upon the behavior of the system. The optimum temperature for growth of most of the anaerobic species is around 35°C. The mesophilic range (25–45°C) is most commonly used in anaerobic systems and the number of known thermophilic species (>45°C) is still small. Temperature shocks may negatively affect the performance of any anaerobic reactor. Concerning the rates of biochemical reactions, thermophilic temperatures are favorable as compared to mesophilic. Specifically for LCFA, the higher temperature increases the solubility diminishing negative hydraulic effects (flotation and wash-out). On the other hand, higher LCFA solubilization enhances their inhibitory/toxic action by means of enhanced bioavailability. Thus, thermophilic degradation of LCFA proceeds at higher rates than mesophilic degradation, but the former present higher sensibility to LCFA inhibition/toxicity (48, 130). Nevertheless, reactor recovery after LCFA overload is faster for thermophilic reactors as compared to mesophilic because thermophilic bacteria have higher doubling times. So, the high temperature shocks may be detrimental to anaerobic mesophilic reactors treating milk processing effluents, not only because of direct effect on bacterial populations but also due to the enhanced bioavailability of LCFA caused by higher solubility. The thermophilic anaerobic application at industrial scale is not well documented in the literature.
2. pH – this is also a very important parameter in microbiological metabolism. The major part of the bacterial populations (with the exception of most of the methanogenic species) is capable of growing in a pH range spreading through 3 pH units. Usually, the maximum growth is observed for a pH around 6.5–7.5. One phase systems are operated at a pH of 7.0–7.5 and generally they have a good buffer capacity in pH near neutral values. In two-phase reactors, the first stage (acidogenic) proceeds over a range of pH values but the second step (methanogenic) is most sensible to pH variations, the optimum pH being 6.8–7.2. The most problematic steps of anaerobic digestion in relation to pH changes are the steps in which H^+ is formed (Fig. 17.5). A pH drop may cause a shift in chemical equilibrium and may lead to back reactions and accumulation of toxic intermediate compounds. Milk processing effluents are readily acidifying effluents with generally low alkalinity levels. In the operation of anaerobic systems for these effluents, care must be taken to ensure that enough alkalinity is present or added to avoid sharp pH drops. Special care must be taken upon start-up or overload periods, since in those circumstances, the sequential reactions of anaerobic digestion may shift from balance and lead to accumulation of inhibitory acids.
3. Mixing – in some reactor configurations, mixing is crucial for eliminating gradients in the parameters that exert the most influence upon the kinetics of the process. Some of the ways to improve the mixing characteristics of a reactor are effluent recirculation, biogas recirculation, and mechanical agitation. Presently, the most used form of mixing is the recirculation of part of the produced biogas which also serves as a form of pH control. The mixing in an anaerobic reactor treating milk processing effluents is also important to enhance the contact between biomass and substrate, to decrease mass transfer limitations and to eliminate channeling and

Table 17.14
Monitoring and control of anaerobic reactors for milk processing effluents (adapted from ref. (29))

Parameter	Frequency
Feed	
Flow	Daily
COD, BOD, VFA/alkalinity	Daily
SS, toxic substances	Daily/continuous
Reactor	
Temperature, pH	Daily/continuous
Sludge content	When needed (once per month)
Effluent	
COD, BOD, VFA/alkalinity	Daily
SS	Daily/continuous
N, P	When needed (once per week)
Biogas	
Amount	Continuous
Composition	Daily/continuous
Excess sludge	
Amount	Continuous
Dry matter	When needed

dead volumes, thus avoiding gradients in kinetic rate parameters and minimizing local inhibition effects. Mixing may also have significant benefits in reducing inhibition effects of isolated inputs of toxic substrates.

Table 17.14 presents a summary of other parameters to be monitored and controlled in anaerobic reactors for milk processing effluents.

5. CASE STUDIES

The main operational problems occurring in milk processing wastewater treatment plants are related with process discharges (raw materials and products) or discharges of chemical products. With respect to the process discharges, the most common are the discharges of raw materials like fresh milk and products like whey, which increase sharply the organic load applied to the treatment plants and consequently to the anaerobic reactors. Concerning the use of chemicals, it may occur discharges of sodium hydroxide from CIP units, of soda lime, which is the main neutralizing agent used in the wastewater treatment plants, or cleaning products used for mill disinfection, which have a toxic effect on the anaerobic bacteria, with a consequent potential decrease on their activity, until eventually their complete inactivation.

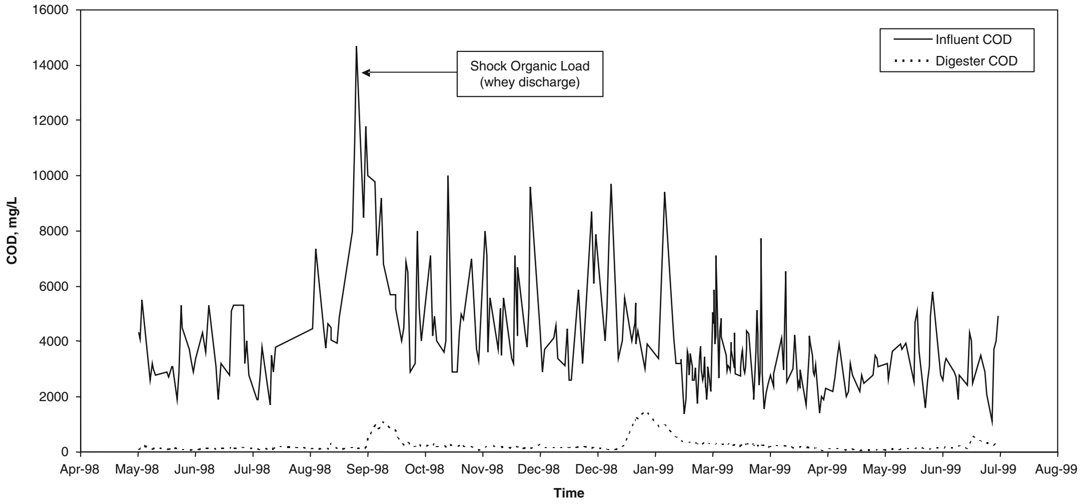


Fig. 17.10. Evolution of COD values (case studies 1 and 2).

In this section, four of these typical situations are described, which had occurred in the treatment plants existing in two milk processing industries producing cheese in Portugal, both using anaerobic contact reactors.

5.1. Case Study 1: Organic Shock Load (Whey Discharge)

Figure 17.10 presents the evolution of the influent COD as well as the COD existing inside the anaerobic contact reactor installed in a wastewater treatment plant treating an effluent from a milk processing mill producing cheese, which also incorporates a whey drying tower. As can be seen from the graph, by mid August 98 occurred a whey discharge because of the malfunctioning of the whey refrigeration system, which resulted in the increase of the organic load applied to the anaerobic reactor to around four times the normal operational value.

The reactor response to the sudden increase in the applied organic load was an increase on the reactor COD content (Figs. 17.10 and 17.12), as well as a sharp increase on the VFA concentration (achieving values higher than 600 mg/L as acetic acid) and a slight decrease in the pH, as can be seen in Fig. 17.11. Simultaneously, it also occurred a very high loss of the total suspended solids (TSS) inside the reactor, which varied from 6,000 mg TSS/L to less than 2,000 mg TSS/L (Fig. 17.12), with the corresponding loss of biomass.

The reactor recovery was attempted by a drastic decrease in the applied organic load. This decrease was obtained by lowering the inlet flow rate to the digester and by-passing the excess flow to the aerobic system, until the achievement inside the reactor of the normal values for the operational and control parameters (VFA, pH and COD concentrations). After this, the inlet flow rate was increased step by step until the total flow rate was achieved. This strategy allowed a gradual recovery of the process although with a slow increase on the TSS concentration inside the reactor, as can be seen in Fig. 17.12. The duration of the recovery time of the process after this accidental discharge was around 5 months. However, in the end of this

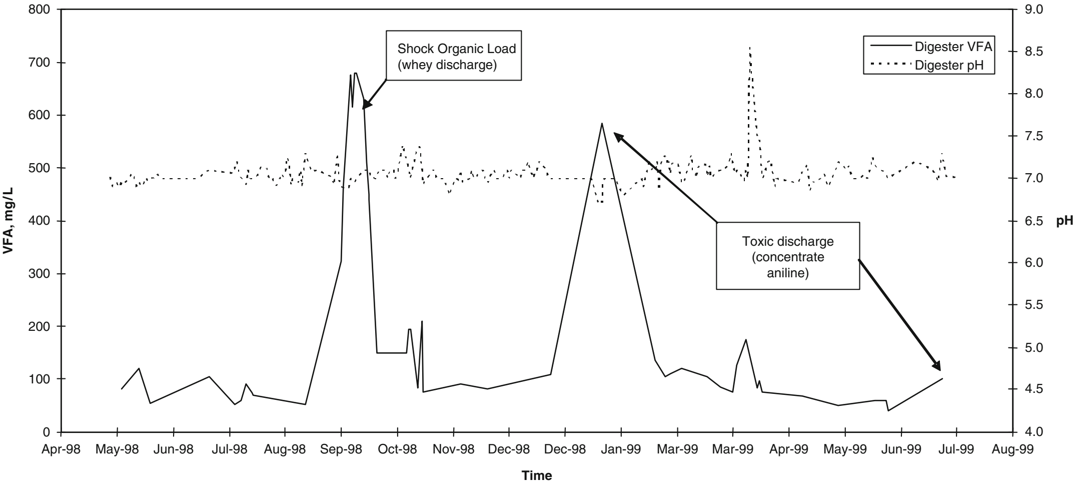


Fig. 17.11. Digester VFA and pH evolution (case studies 1 and 2).

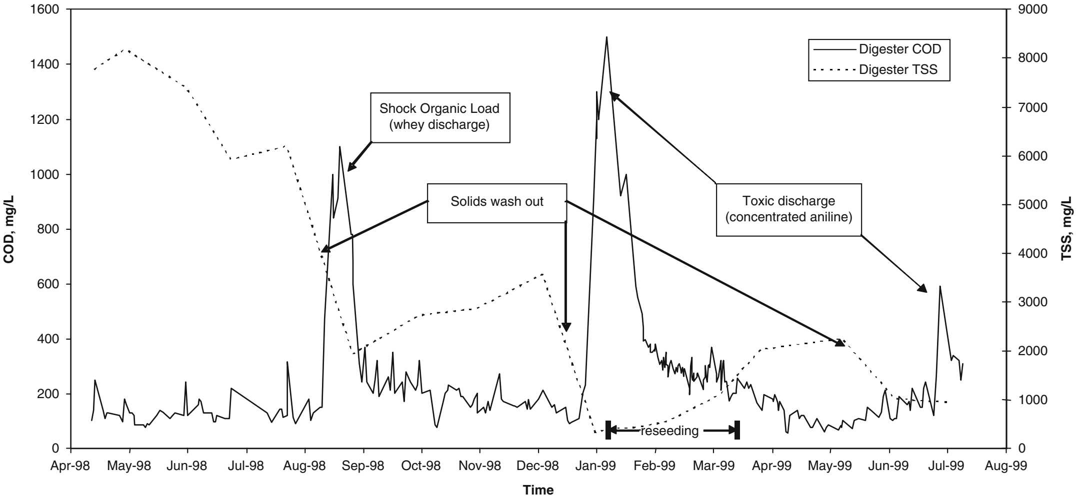


Fig. 17.12. Digester COD and TSS evolution (case studies 1 and 2).

recover period, the TSS inside the reactor was only 3,500 mg TSS/L, lower than the amount before this discharge, which showed that the reactor had not achieved a complete recovery.

5.2. *Case Study 2: Toxic Discharge (Concentrated Aniline)*

In the same milk processing wastewater treatment plant of Case Study 1, and after 5 months trying to recover the reactor after the occurrence of the organic shock load, by the beginning of January 99, an accidental discharge of a toxic product (concentrated aniline used for cheese cover) occurred before the achievement of total recovery of the anaerobic process (reactor

TSS concentration was lower than 4,000 mg TSS/L). This discharge was not reported to the treatment plant manager, so it was not possible to act immediately in the operation of the anaerobic process before the reactor failure. Hence, it was observed biomass inhibition and sludge wash-out. As a consequence, a total loss of the reactor solids content was observed together with a high increase on VFA and COD reactor content concentrations, respectively 600 mg VFA/L and 1400 mg COD/L, as a result of bacteria inhibition and death (Figs. 17.11 and 17.12).

At this moment, due to the very low amount of TSS inside the reactor (less than 500 mg TSS/L), the reactor recovery was only possible through a reactor reseeding and start-up procedure performed from the middle of January 99 until March 99, as can be seen in Fig. 17.12. By May 99, when the reactor was almost recovered and achieving full capacity, 100 mg COD/L, 60 mg VFA/L, and 2,000 mg TSS/L, there was again another accidental discharge of aniline, although in less quantity, which caused again the failure of the reactor. It was necessary to start again the recovery of the reactor through a new reseeding and start-up period (data not presented).

As a conclusion on this type of accidental discharge (toxic compound), it can be stated that the inhibition of the anaerobic bacteria was very severe, causing the failure of the process, and the recovery was possible only through a reseeding and start-up of the anaerobic reactor.

5.3. *Case Study 3: Chemical Discharge (Soda Lime)*

The chemical discharge (soda lime) happened in August 03 in another milk processing mill (also cheese making installation) wastewater treatment plant because of a control valve malfunction in the neutralizing unit. Around 5 m^3 of concentrated soda lime had been added to the anaerobic reactor and pH values higher than 10 had been reached (Fig. 17.13). The COD concentration inside the anaerobic reactor increased almost ten times the normal operational value achieving values higher than 1,200 mg COD/L, as can be seen in Fig. 17.13. To restore rapidly the operational pH values (6.8–7.2), the anaerobic reactor content was neutralized with sulfuric acid. At the same time, and in order to avoid an organic shock load, the inlet flow rate was decreased. After some period, a stepwise increase of the organic loading rate (through the increase of the flow rate) was applied. This procedure had occurred between the middle of August 03 until the end of September 03. However, these actions were not sufficient to prevent biomass wash-out.

From Fig. 17.14, it can be observed that the total suspended solids concentration inside the anaerobic reactor dropped sharply by the end of September 03, as consequence of the biomass wash-out. Hence, a reseeding was planned and was initiated in October 03. Due to the lack of seeding material at this time, the reseed period lasted for 3 months (until December 03) and a new start up procedure was initiated. By the end of January 04, the reactor was working properly at full capacity with a good efficiency, achieving a COD of 150 mg COD/L and a solids content of 12,000 mg TSS/L inside the digester.

As a conclusion on this type of accidental discharge (chemical compound), it can be stated that the inhibition of the anaerobic bacteria was very severe, also causing process failure and the need for a re-seeding and start-up of the anaerobic reactor (recovery time of around 5 months).

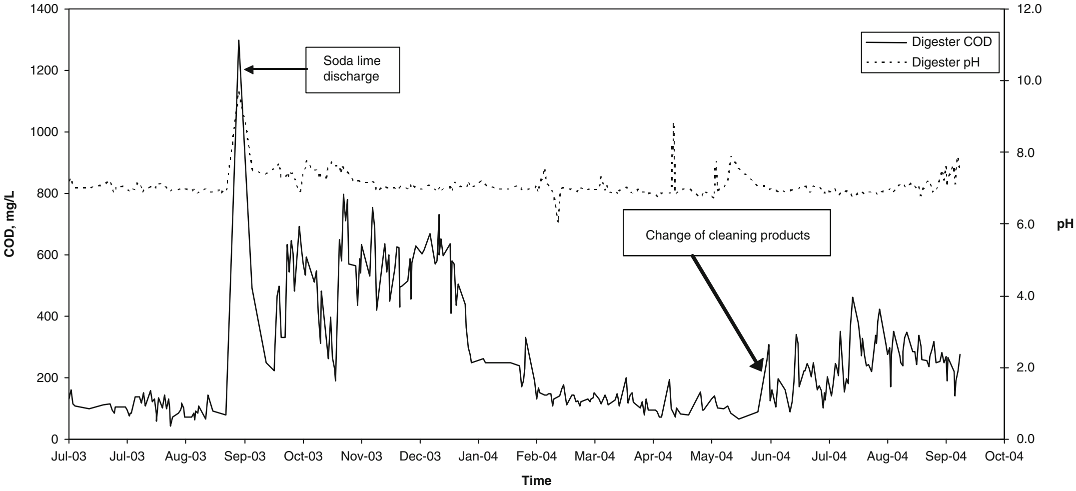


Fig. 17.13. Digester COD and pH evolution (case studies 3 and 4).

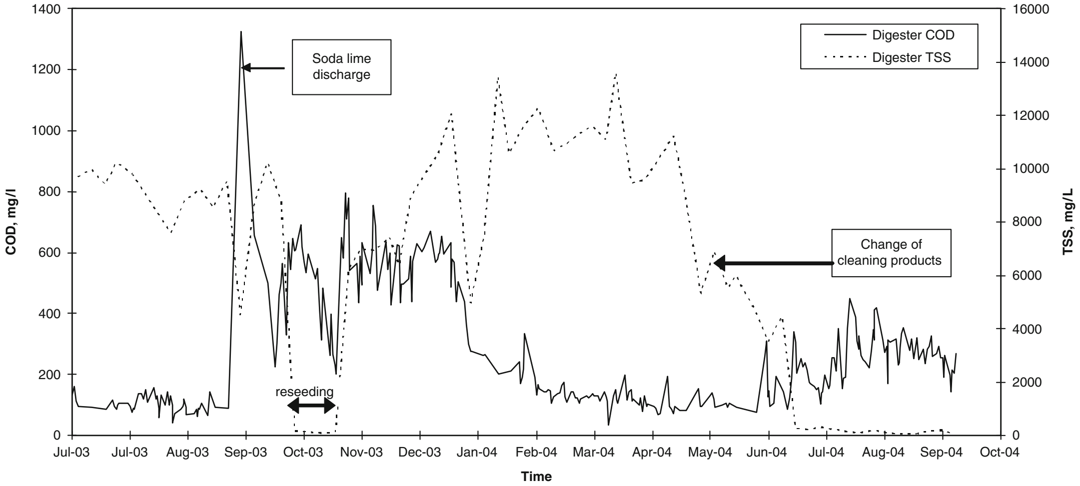


Fig. 17.14. Digester COD and TSS evolution (case studies 3 and 4).

5.4. Case Study 4: Change in Cleaning Products

From Figs. 17.13 and 17.14, it can be observed that, by May 04, something had caused the COD concentration to increase and a very high loss of the total suspended solids inside the anaerobic reactor. After an investigation on the cause of these phenomena, a correlation was established between these facts and a change in the cleaning products (disinfectants) used in the mill during this period. In order to avoid higher organic shock loads, the inlet flow was decreased. By October 04, the anaerobic reactor was working at a low flow rate (25% of the total flow rate) and was still functioning very unstably. Although it was known which cleaning product caused this behavior, the toxic agent was not identified. However, due to process and sanitary reasons, it was not possible to change immediately the cleaning products, so the anaerobic reactor had to work at these unstable conditions during a large period, which caused biomass wash-out. Only after the changing of the cleaning products, it was possible to do a new reseed of the digester and apply the start-up procedure. After this action it was observed a gradual recovery of the reactor (data not shown).

As a conclusion on this type of discharge (change of cleaning products used in the mill), it can be stated that care must be taken wherever there is a need to change the disinfectants used in the process, due to its potential inhibitory effect on the anaerobic bacteria.

6. DESIGN EXAMPLES AND QUESTIONS

In this section, some design examples will be presented for different types of anaerobic reactors treating milk processing effluents from industrial mills. The anaerobic reactors under analysis are: anaerobic contact reactor, anaerobic up-flow filter, and IC reactor (modified UASB reactor).

The anaerobic reactors under study are included in wastewater treatment plants designed to treat industrial effluents from milk processing industries in order to meet legal discharge requirements, with the lowest operation costs.

In general, milk processing wastewater treatment plants using an anaerobic technology have the configuration as presented on the block diagram of Fig. 17.15:

1. Pretreatment consisting of screening, grit removal, flow measurement, oil and grease removal, and equalization.
2. Anaerobic treatment.
3. Aerobic treatment in an activated sludge system designed for the following processes: nitrification/denitrification, aeration and sludge settling and recycle.

6.1. Design Example 1: Anaerobic Contact Reactor (Cheese Mill)

The anaerobic contact reactor is installed in an industrial wastewater treatment plant with the total configuration presented in Fig. 17.15, receiving liquid effluents from a milk processing industry producing cheese. In this mill, there are three different wastewaters to be treated: domestic wastewater, industrial wastewater, and cheese whey (35% of the total daily volume).

The equalization tank was designed for a hydraulic retention time of 15 h, and the tank is provided with a mixing system (propeller type) to avoid solids settling and milk fat flotation. This wastewater component (fat) is separated in a flotation unit (DAF type) to prevent operational problems at the anaerobic reactor, namely sludge bulking and sludge wash-out due to the presence of the milk fat. The flow rate from the equalization tank is constant ($20\,m^3/h$), and the treatment plant operates 24 h/day on a 7 days a week basis.

The anaerobic reactor (Fig. 17.16) consists basically on a cylindrical concrete tank with an inlet distribution system at the reactor bottom. Sludge mixing is provided by sludge recycling at a sufficient flow rate ($150\,m^3/h$) to keep the sludge on the bottom slightly expanded and

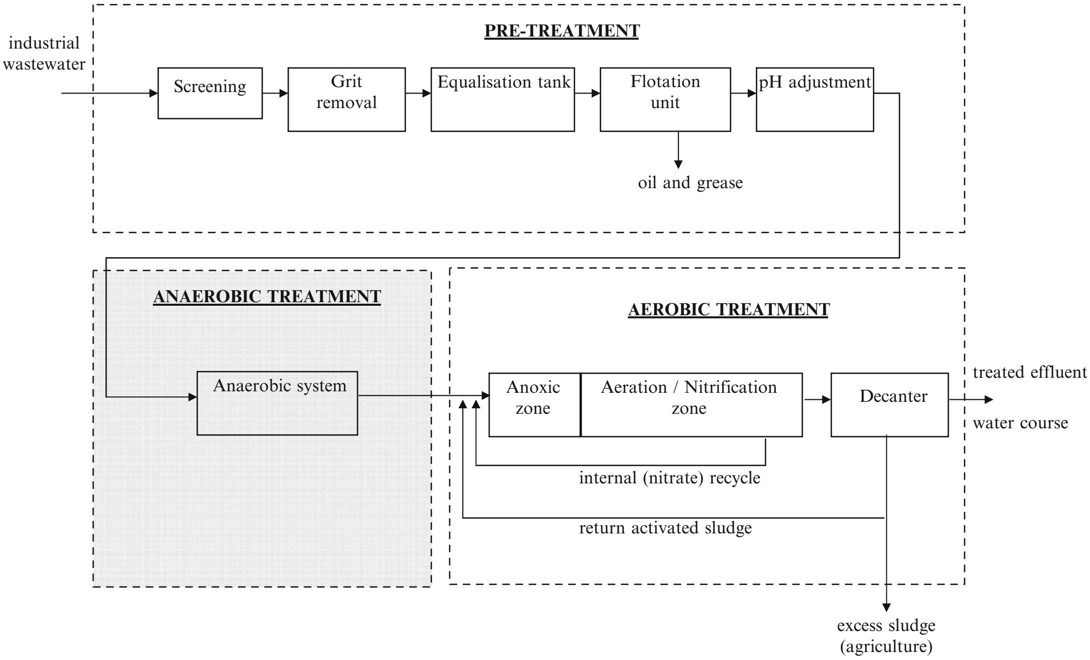
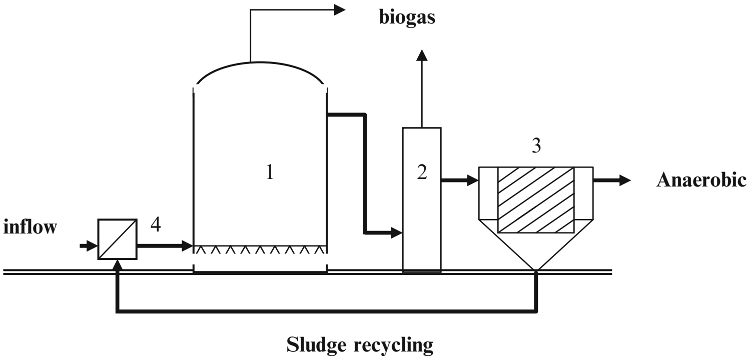


Fig. 17.15. Block diagram for milk wastewater treatment plants.



1 = CSTR anaerobic digester; 2 = degasification tower; 3 = lamella separator; 4 = heat exchanger

Fig. 17.16. Anaerobic reactor (design example 1).

allowing also a good influent distribution. To reach a mesophilic temperature inside the reactor (35°C), the influent is heated by direct steam injection prior to entering the reactor.

The effluent leaving the anaerobic reactor contains a considerable amount of sludge which has to be separated and recycled back to the reactor. Due to the solids settling characteristics,

Table 17.15
Main characteristics of the combined industrial effluent (design example 1)

Parameter	Unit	Value
Flow rate	m^3/day	480
COD	mg/L	25, 800
BOD_5	mg/L	13, 600
TSS	mg/L	1, 400
Total N	mg/L	300
Total P	mg/L	130
Sulphate	mg/L	600
Temperature	°C	24

the separation process is performed in a parallel plate separator (cross flow type) denominated "SEPAFLOC."

The design of the anaerobic contact reactor was based on the main characteristics of the combined industrial wastewater presented in Table 17.15, assuming no COD removal in the pretreatment units.

The total COD load to be treated was 12,640 kg/day, and the maximum design volumetric organic load rate was 5 kgCOD/day-m^3 of reactor volume. The corresponding active volume of the reactor is 2,500 m^3.

The total reactor volume is 2,800 m^3 with a cylindrical shape form. The corresponding reactor diameter is 18.9 m, and the total height is 10.1 m, with a water height of 9.5 m approximately. The reactor is operated at 32–37°C and in a pH range of 6.8–7.5 with an expected control point of 7.2. The estimated biogas production was 234 m^3/day.

The anaerobic contact reactor efficiency was estimated as 87% for COD and as 90% for BOD_5. The assumed effluent characteristics after anaerobic treatment were 4,650 mg/L for total COD, 1,775 mg/L for total BOD_5, and 1,500 mg/L for TSS.

The aerobic activated sludge treatment consists of a tank with two reactors in series: anoxic basin and aeration basin. The anoxic basin, also called denitrification basin, has 330 m^3 of volume and is located in front and adjacent to the aeration basin. This last basin has a volume of 2,250 m^3.

6.2. *Design Example 2: UASB Reactor IC Type (Milk Processing Mill)*

The IC reactor was installed in a wastewater treatment plant designed to treat the liquid effluents from a milk industry producing UHT milk, skimmed and semi-skimmed.

The main characteristics of the combined industrial effluent are described in Table 17.16.

The treatment plant also has a configuration similar to the block diagram presented in Fig. 17.15. In this treatment plant, the equalization tank was designed for a hydraulic retention time of 8 h, and the flotation unit (DAF type) is provided with a coagulation/flocculation piping system for chemical precipitation, for the anaerobic reactor protection in case of organic overloading.

Table 17.16
Main characteristics of the combined industrial effluent (design example 2)

Parameter	Unit	Value
Flow rate	m^3/day	3,000
COD	mg/L	2,000
BOD_5	mg/L	1,500
TSS	mg/L	600
Total N	mg/L	100
Total P	mg/L	30
FOG	mg/L	350
Temperature	°C	25–35

The anaerobic reactor, called IC reactor, is a technology based on the UASB process and is essentially an up-flow granular sludge bed system. The IC reactor consists of two UASB compartments on top of each other. The produced biogas is separated in two stages within the reactor, and the biogas collected in the first stage drives a gas lift resulting in an internal circulation of wastewater and sludge.

In the IC system there are four sections:

1. Influent feed and mixing compartment
2. Fluidized bed compartment
3. Recirculation system
4. Polishing compartment

The organic load applied to the IC reactor is 5,400 kg COD/day (equivalent to 4,050 kg BOD_5/day). For this organic load, the designed IC anaerobic reactor has a useful volume of 308 m^3 with a total height of 18.5 m and a diameter of 5 m.

The expected efficiency of the IC reactor is 70–80% for BOD removal, working at a temperature between 25 and 35°C.

6.3. *Design Example 3: UASB Reactor IC Type (Cheese Mill)*

The IC reactor was designed to incorporate a wastewater treatment plant similar to Fig. 17.15, designed to treat the liquid effluents from a milk industry producing cheese, cream, butter, whey, and milk powder.

The equalization tank in this wastewater treatment plant has a hydraulic retention time of 24 h, and the flotation unit is a Plate Water Flotation DAF type.

The liquid effluents from this milk processing industry after passing through the flotation unit have the characteristics described in Table 17.17.

The organic load applied to the IC reactor is 5,600 kg COD/day, and the main dimensions of the reactor are 4 m diameter and 20 m height, with a useful capacity of 250 m^3. The hydraulic retention time is approximately 3.7 h and the average reactor feed flow is 67.5 m^3/h with an average upward velocity of 5.3 m^3/h. Assuming low total suspended solids inlet and

Table 17.17
Main characteristics of the combined industrial effluent (design example 3)

Parameter	Unit	Value
Flow rate	m^3/day	1,600
COD	mg/L	3,500
BOD_5	mg/L	2,800
FOG	mg/L	50–70
Temperature	°C	30

Table 17.18
Main characteristics of the combined industrial effluent (design example 4)

Parameter	Unit	Value
Flow rate	m^3/day	1,100
COD	mg/L	34,900
BOD_5/COD	–	0.53
TSS	mg/L	4,400
Total N	mg/L	500
Total P	mg/L	110
Sulfates	mg/L	460
FOG	mg/L	500
Temperature	°C	30

a temperature above 25°C, the expected removal efficiency is 70–80% for total COD and 80–90% for total BOD with a biogas production of $1{,}800\,m^3/day$.

The effluent from the IC reactor flows to the activated sludge system with an anoxic tank with a volume of $800\,m^3$ and an extended aeration tank with $1{,}800\,m^3$.

6.4. Design Example 4: Anaerobic Filter Reactor (Cheese Mill)

This milk processing mill produces cheese, cream, butter, and milk powder. The wastewater treatment plant designed to treat the industrial wastewater is similar to the standard wastewater treatment plant presented on the block diagram of Fig. 17.15. The characteristics of the raw influent are as follow and reflect the presence of a significant amount of whey (Table 17.18)

The anaerobic reactor (Fig. 17.17) is filled with a carrier material considered an ideal growth medium for the anaerobic biomass and equipped with an influent distribution system over the total bottom surface of the reactor. Due to the growth of the biomass on the carrier material, a very stable performance of the treatment plant is obtained. To ensure a sufficient up-flow velocity inside the anaerobic reactor (1 m/h), there is a recycle back flow from the outlet of the reactor, providing a total influent flow rate of the $700\,m^3/h$.

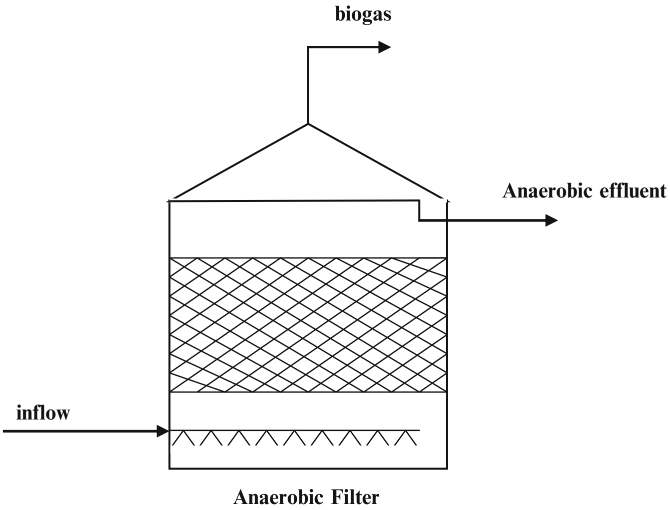


Fig. 17.17. Anaerobic reactor (design example 4).

The anaerobic filter was designed for an organic loading rate of 5 kg COD/m^3-day, and has a hydraulic retention time (HRT) of 7 day (based on the equalized raw influent flow rate) and a useful volume of 7, 680 m^3. The reactor is cylindrical with a total height of 12 m and an internal diameter of 29.8 m.

The COD removal is 80% and the BOD removal efficiency 82%, with a biogas production of 710 m^3/h.

The aerobic treatment is performed in two systems in series, each one comprising an anoxic reactor followed by an aeration tank.

7. TRENDS IN ANAEROBIC TREATMENT OF MILK PROCESSING EFFLUENTS

7.1. Results of Recent Investigations on Anaerobic Treatment of Milk Wastewater

The number and type of anaerobic treatment systems being applied to industrial and agricultural waste streams has grown tremendously since the first technologies were introduced and commercially promoted in the late 1970s and early 1980s. Over the last 30 years, the number of nonlagoon anaerobic installations worldwide has increased by nearly an order-of-magnitude and now probably exceeds 2,200 (115).

Anaerobic treatment technology is being applied more frequently to a variety of unique, high-strength waste streams produced by a wide range of industries and in particular to milk processing wastewaters. Much of the early impetus for such applications was related to complying with discharge regulations. Today, the major impetus for treating such streams is financial, based on the need for a cost-effective, high-performance treatment technology with relatively low operating costs. In addition, the potential economic value of biogas, a by-product of anaerobic treatment, has added a major economic benefit to the picture. Anaerobic digestion is now widely used to treat high-strength industrial wastewaters with COD levels above 2 g/L, especially in case of carbohydrate-rich effluents (131, 132). The most commonly used reactor type is the UASB. More often than not, however, anaerobic digestion of industrial effluents does not proceed optimally because the composition of these effluents is typically time-variable and nutritionally imbalanced. Also, high liquid surface tensions may lead to granule flotation and, as a consequence, poor effluent quality and wash-out of slow-growing bacteria.

The number of full scale applications to wastewater containing lipids or proteins, such as milk processing, is very limited, mainly because problems were encountered with sludge retention (occurrence of sludge flotation and wash-out) and long-chain fatty acids inhibition (long chain fatty acids, LCFA, production as intermediates during lipids degradation), which is especially threatening in systems operated at a low hydraulic retention time. Therefore, control of sludge wash-out and long chain fatty acids inhibition is a prerequisite for increased application of anaerobic treatment to lipid containing wastewaters. This requires a proper choice between the currently existing high rate reactor types: (a) reactors with mobile biomass aggregates, which can accommodate higher biomass concentrations, and (b) reactors with stationary biofilms with better safety against biomass wash-out.

Biomass retention through adequate granulation is of utmost importance in UASB technology, first in order to obtain a good effluent quality and second in order to ensure a minimal cell residence time of 7–12 days, which is required to avoid the wash-out of the slowest-growing anaerobic bacteria (133). Several studies have indicated that the extend of granulation seems to be largely dependent on the feed composition, such as its mineral composition, its sugar/fatty acids ratio, or its surface tension (110, 134). Therefore it appears worthwhile, in order to make UASB technology more reliable, to develop bio-supportive additives able to maintain the granular sludge in a proper state in periods of start-up or low quality input wastewater. Wirtz and Dague (135) succeeded in shortening the period for sludge granulation by adding a cationic polymer, which allowed the increase on the volumetric load of the reactor much more rapidly.

An improvement in the efficiency of an anaerobic digestion, with respect to biomass wash-out, can be brought about by either suitably modifying the existing digester design or by incorporating appropriate advanced operating techniques. Hence, by suitable modifications in the reactor designs and/or by altering the effluent characteristics, the existing high rate digesters can be accommodated for treatment of organic effluents. Based on the characteristics of the different reactors such as efficiency based on loading rate and COD reduction, biomass retention and other factors like cost, operation, and maintenance requirements, UASB and fixed film configuration appear to be the most suitable.

In the last decade, the emphasis has been on the identification of the critical factors affecting performance, so that the reactor efficiency can be improved by maintaining optimal operating conditions. Furthermore, an assessment of the suitability of specific reactors types for different wastewaters has been performed and the possible modifications in the existing process to enhance the system efficiency were discussed. Leal et al. (136) studied the importance of the use of enzymes for hydrolyzing a wastewater from a dairy industry prior to the biological anaerobic treatment. In that study, they propose the use of a hybrid technology – enzymatic treatment associated with anaerobic treatment – to enable the reduction in hydraulic retention time and consequently in reactor volume, since it promotes hydrolysis of fats which cause problems of clogging of the sludge bed in anaerobic reactors of the UASB type.

High rate anaerobic digestion of LCFA requires sufficient mixing of the liquid in the digester and sufficient contact between biomass and substrate, and UASB reactors cannot fulfill these requirements. The gas production rate required to achieve sufficient mixing and contact cannot be achieved if lipids contribute 50% or more to the COD of the wastewater, because at high lipid loading rates exceeding 2–3 kgCQO/m^3-day, UASB reactors failed completely, despite a high initial concentration of highly active, well settling biomass, and total sludge wash-out occurred (112). EGSB reactors do fulfill the requirements of mixing and contact, and the results obtained with these reactors compare very favorably with those published for more conventional digesters. However, a floating layer of undigested fatty acids and minor amounts of biomass was formed in EGSB reactors. Hence, floating layer formation and mixing characteristics in full-scale EGSB reactors require yet further research.

In case of complex wastewater containing significant amounts of fat (e.g., dairy), the continuous operation has proved to cause problems of scum layer and sludge layers on top of the reactors with subsequent biomass wash-out (52, 137). In some recent works (72, 91), it was shown that the continuous operation of UASB reactors treating dairy wastewater resulted in good COD removals but also high COD accumulation in the sludge bed leading to unstable performance of the reactors on the long run. A high degree of organic matter accumulation in anaerobic reactors treating dairy wastes was also detected by Motta Marques et al. (138) and by Guitonas et al. (139). Anderson et al. (140) reported extensive clogging (accumulation) by fatty matter on the support media of an anaerobic filter treating dairy waste. In an investigation on slaughterhouse wastewater treatment in UASB reactors, Sayed (82) suggested that the prevailing mechanism in the removal of soluble and colloidal COD is adsorption to the surface of biomass particles. This adsorption phenomenon will ultimately result in an enclosure of the sludge particles with a film of increasing thickness, and density, which increasingly will hamper the supply of substrate to the bacteria. A feedless or stabilization period would be important to invert this process and stabilize the accumulated (entrapped and adsorbed) organic matter. As a consequence, Sayed (82) suggested that the most adequate form of treating complex and/or fat containing wastewater would be the use of flocculent sludge and discontinuous feeding. This operating mode was successfully tested by Sayed et al. (141) for slaughterhouse wastewater, by Fergala (142) for domestic wastewater and by Nadais et al. (91) for dairy wastewater. The intermittent feeding operating mode was also recommended by Lettinga and Hulshoff Pol (143) for complex wastewater, namely dairy wastewater. Nadais et al. (113) studied the intermittent operation mode and concluded that the stabilization period has a fundamental importance on the operation of the UASB reactors treating complex fat containing wastewater like milk effluents.

Rinzema et al. (112) developed two modifications of the gas–solids separator for the expanded granular sludge bed (EGSB) reactors to prevent excessive sludge wash-out during anaerobic treatment of lipid emulsions: a hybrid reactor with a layer of floating carrier material (reticulated polyurethane foam) above the expanded sludge bed, and a novel EGSB reactor equipped with a sieve-drum separator (EGSB-SDS). The first modification showed to be unreliable in the treatment of emulsified lipids, because the floating support material did not prevent strong sludge wash-out. On the other hand, the EGSB reactor equipped with a sieve-drum separator allowed stable anaerobic digestion of emulsified lipids. However, an incomplete conversion to methane of the organic matter removed from the wastewater was obtained, which should be a point for further investigation. The incomplete mineralization was attributed to the accumulation of a large and rather variable amount of lipids in a thick floating layer, which leads to a further modification of the design of the EGSB-SDS system to solve the floating layer problem. Results obtained with the hybrid reactor design showed that recirculation of the floating lipids to the granular sludge bed enhanced their conversion to methane.

An improvement in the efficiency of an anaerobic digestion, with respect to biomass wash-out, can also be brought about by incorporating appropriate advanced operating techniques. This can be addressed, for instance, by the use of membranes coupled with the anaerobic digester for biomass retention. In a membrane bioreactor (MBR) system, membranes are the main solid–liquid separation devices. Two types of MBR have been used according to the location of the membrane unit, i.e., membranes are submerged in the reactor or positioned external to the reactor. The submerged membrane type has attracted great attention in recent years since it is more compact and energy saving (144–146). It has the drawback that control of membrane fouling is more difficult to achieve than external membrane systems.

Interest in anaerobic digestion is increasing because of the well-known advantages for the treatment of high organic concentration wastewaters. Treatment of dairy wastewaters by means of up-flow anaerobic sludge blanket (UASB) reactors (147–149), hybrid UASB reactors (150), expanded granular sludge bed (EGSB) reactors (81), as well as others based on anaerobic filters (28, 151, 152) have been reported in literature. These papers show that anaerobic treatment can be effectively used for these effluents, in spite of the different operational problems quoted in literature, such as sludge flotation or toxicity/inhibition processes.

Today, there are many processes for the treatment of dairy wastewaters. However, two trends are very clear. They are based either on the recovery of valuable components, mainly proteins and lactose, or on the degradation of all substances that can alter negatively the environmental quality of the water courses.

7.2. Future Expected Developments

The bioprocesses that will be used in future for wastewater treatment will still be chosen as they have been in the past, according to technical feasibility, simplicity, and economics. However, the needs and the priorities of a sustainable society will shift the focus on wastewater treatment from pollution control to resource exploitation. In fact, many bioprocesses can provide bioenergy or valuable chemicals while simultaneously achieving the objective of pollution control. Industrial wastewaters from milk processing are ideal candidates for bioprocessing because they contain high levels of biodegradable organic material, which results in a net positive energy or economic balance. Recovery of energy and valuable materials might reduce the cost of wastewater treatment and somewhat reduce our dependence on fossil fuels (1).

With respect to future developments in the field of anaerobic treatment of milk processing wastewaters, it can be considered:

- Optimization of anaerobic systems through reactor staging, hybridization, thermophilic treatment, accelerated hydrolysis, improved solids retention, and better process control
- Fine-tuning of anaerobic conversions to produce readily disposable effluents
- Utilization of anaerobic treatment processes as a core technology in systems designed to reclaim products from waste streams

Various constructors improved granular sludge bed reactors in recent years aiming at lowering mass transfer resistance and therewith achieving higher organic loading rates. Further improvement might be expected in the field of the treatment of specific wastewaters, so it is foreseen a further development of combination of complementary anaerobic systems, such as hybrid systems. Interesting developments are expected for anaerobic reactors that cannot rely on the development of granular conglomerates or formation of biofilms, for the retention of adequate sludge for successful treatment. This can be achieved by enhanced physical (or physico-chemical) separation of the viable biomass from the treated water. Potential systems are hybrid and/or membrane bioreactors. The major bottle-neck are the relatively high washout of suspended solids and the low rate of hydrolysis in the conventional first generation UASB reactors. Therefore, the improvement of hydrolysis of complex organic matter is of fundamental importance, being the limiting step for the treatment of complex substrates such as the milk processing wastewater. Improved retention of suspended solids in the reactor system will lead to higher sludge retention times, subsequently leading to improved treatment efficiencies. Moreover, a decreased solids load in the effluent will minimize the requirements of the posttreatment step.

Optimization of the reactor configuration can involve staging of the process into separate tanks whereby the conditions for the specific groups of bacteria involved can be optimal. Hydrolysis is greatly improved at high temperatures such as 70°C or more, and a two phase operation scheme whereby the initial treatment occurs at a very high temperature followed by a methanogenic phase at either mesophilic or thermophilic temperatures could be an interesting future development (60).

The breakthroughs dealing with reactor design and operation conditions offer practical solutions to many of the drawbacks that were initially thought to limit the scope of anaerobic digestion, such as instability, temperature requirements, sensitivity to toxicants, shock loads, and feed composition. There remain, however, inherent drawbacks to anaerobic digestion technologies that require further developments in the area of sludge engineering, since sludge adaptation to LCFA may require several weeks to months. Engineered anaerobic consortia therefore are needed to expand the catabolic diversity of sludge and shorten the period of sludge adaptation to toxic substrates. Therefore, it may be advantageous to develop effective and durable anaerobic consortia to inoculate anaerobic reactors treating complex industrial effluents containing lipids and proteins. One option to accelerate the biodegradation of toxic substrates, such as the LCFA, is to inoculate reactors with adequate bacterial strains, so inoculation of reactors with specific degraders can be an effective means to engineer the consortium needed for degradation. Another option is to seed the reactors with sludge granules whose entire microbial association is already adapted to, or engineered for, the degradation of specific compounds. This opens interesting perspectives for the industrial production of these consortia for bioaugmentation of polluted environments or industrial digesters treating complex wastewaters, as the ones containing fat and proteins (79).

Another potential benefit associated with the large-scale availability of specialized microbial consortia is "biochemical rerouting," that is, the induction of desirable biochemical pathways as, for example, the degradation of malodorous primary amines, anaerobic ammonia oxidation, or homoacetogenesis, and the repression of undesirable pathways, such as the formation of malodorous compounds, which will leave the anaerobic digester and give rise to odor problems (79). Hence, attempts should be made to rechannel anaerobic pathways toward other end-products.

A sustainable society requires a reduction on the dependency on fossil fuels as well as a lowering of the amount of pollution that is generated by different activities. Wastewater treatment is an area in which these two goals can be addressed simultaneously, so as a result, there has been a paradigm shift recently, from disposing of waste to using it (1).

The utilization and acceptability of residuals as resources will progressively become the most appropriate, but not the only strategy for coping with environmental pollution, sustainability and survival within the limits of our ecosystem. Hence, prevention and reduction of dairy wastewater pollution can be achieved by means of direct recycling and reutilization of waste components, such as the use of cheese whey for animal feed (44) or by using different wastewater treatments, such as physical–chemical, aerobic and/or anaerobic biological treatment (153). Physical–chemical treatments allow the partial removal of the organic load by protein and fat precipitation with different chemical compounds such as aluminum sulfate, ferric chloride, and ferrous sulfide (154, 155). However, the reagent cost is high and the removal of soluble chemical oxygen demand (COD) is poor. Therefore, biological processes are often used (156).

New treatment processes are being developed that allow recovery of marketable by-products together with anaerobic digestion. For example, membrane reactors seeded with Lactobacillus sp. are being designed to recover lactic acid and other acids from agrochemical wastes, before the latter are treated in conventional anaerobic digesters (157). Wastewater treatment for reuse will emphasize the central role of anaerobic digestion as the most sustainable treatment method for mineralizing organic matter. Hence, anaerobic digestion has the potential to play in future a major role in closing water, raw materials, and nutrient cycles in industrial processes (60).

The combination of anaerobic digestion with other biological or physical–chemical processes will lead to the development of optimized processes for the combined removal of organic matter, sulfur, and nutrients in a milk processing wastewater treatment plant. Hence, advanced methods such as coupling of reactors for suitable pretreatment and posttreatment can result in complete treatment of the effluents within the acceptable limits (158–160).

NOMENCLATURE

AAFEB = Anaerobic attached film expanded bed reactor
ABR = Anaerobic baffled reactor
AF = Anaerobic filter
AFB = Anaerobic fluidized bed
ANCP = Anaerobic contact process
ANFD = Anaerobic filter (downflow)
ANFU = Anaerobic filter (upflow)
AnRBC = Anaerobic rotating biological contact reactor
ANYB = Anaerobic hybrid systems
ASBR = Anaerobic sequencing batch reactor
ATP = Adenosine triphosphate
BOD = Biochemical oxygen demand, mg/L
BOD_5 = BOD after 5 days of incubation, mg/L
CAF = Coarse air flotation
CIP = Clean in place systems
COD = Chemical oxygen demand, mg/L
CSTR = Completely stirred tank reactor
DAF = Dissolved air flotation
DSFF = Down-flow stationary fixed film
DSFFR = Down-flow stationary fixed film reactor
DUHR = Down-flow up-flow hybrid reactor
EFB = Expanded/fluidized bed
EGSB = Expanded granular sludge bed reactor
EGSB/SDS = EGSB reactor equipped with a sieve-drum separator
EP&RC = Environmental protection & resource conservation
FAD = Flavin adenine dinucleotide
FADH = Reduced form of FAD
$FADH_2$ = Reduced form of FAD
FB = Fluidized bed
FBR = Fluidized bed reactor
FOG = Fat, oil and grease, mg/L
HRT = Hydraulic retention time, h
IC = Internal circulator reactor
LCFA = Long chain fatty acids, mg/L
MBR = Membrane bioreactor
MIC = Minimum inhibitory concentration, nM
MIC_{50} = MIC at which 50% of methanogenic activity remains, nM
NAD^+ = Nicotinamide adenine dinucleotide
NADH = Reduced form of NAD^+
NH_3 = Free ammonia, mg/L
NH_4^+ = Ammonium, mg/L

N = Nitrogen, mg/L
OHPA = Obligate hydrogen production acetogenic
OLR = Organic loading rate, kg COD/m^3-day
P = Phosphorous, mg/L
PO_4^{3-} = Phosphate, mg/L
PVC = Polyvinyl chloride
SAF = Staged anaerobic filter
SDFA = Semi-continuous digester with flocculant addition
SRT = Solids retention time, h
SS = Suspended solids, mg/L
T = Temperature, °C
TF = Trickling filter
TKN = Total Kjeldahl nitrogen, mg/L
TOC = Total organic carbon, mg/L
UASB = Up-flow anaerobic sludge blanket reactor
UFFLR = Up-flow fixed film loop reactor
UV = Ultraviolet
VFA = Volatile fatty acids, mg/L
VSS = Volatile suspended solids, mg/L

REFERENCES

1. Angenent L, Karim K, Al-Dahhan MH, Wrenn B, Domiguez-Espinosa R (2004) Production of bioenergy and biochemicals from industrial and agricultural wastewater. Trends Biotechnol 22(9):477–485
2. Rajeshwari K, Balakrishnan M, Kansal A, Lata K, Kishore V (2000) State-of-the-art of anaerobic digestion technology for industrial wastewater treatment. Renewable Sustain Energy Rev 4:135–156
3. Ramasamy E, Gajalakshmi S, Sanjeevi R, Jithesh M, Abbasi S (2004) Feasibility studies on the treatment of dairy wastewaters with upflow anaerobic sludge blanket reactors. Bioresour Technol 93(2):209–212
4. FAOSTAT data base (2005) http://faostat.fao.org/default.aspx?alias=faostat&lang=en
5. Abdulgader M, Yu QJ, Zinatizadeh A, Williams P (2009) Biological treatment of milk processing wastewater in a sequencing batch flexible fibre biofilm reactor. Asia-Pac J Chem Eng 4(5):698–703
6. Carta-Escobar F, Pereda-Martín J, Álvarez-Mateus P, Romero-Guzmán F, Durán-Barrantes M, Barriga-Mateos F (2004) Aerobic purification of dairy wastewater in continuous regime. Part I: analysis of the biodegradation process in two reactor configurations. Biochem Eng J 21:183–191
7. González J, García A, Romero F (1982) Características del vertido de una central lechera. Revista de Agroquímica e Technologia Alimentar 22(4):501–510
8. de Haast J, Britz T, Novello J (1984) The management of waste water in the milk processing industry. S Afr J Dairy Technol 16(2):67–73
9. Mann J (1997) Whey utilization – Part 1. Dairy Ind Int 62(3):17, 18

10. Brown, H., Pico, R. (1979) Characterization and treatment of dairy wastes in the municipal treatment systems. In: Proceedings of 34th Purdue industrial waste conference, pp 326–334
11. Royal L (1978) Reduction of milk and milk products wastage. IDF Doc 104:17–27
12. Danalewich J, Papagiannis T, Belyea R (1998) Characterization of dairy waste streams. Current treatment practices and potential for biological nutrient removal. Water Res 32(12):3555–3568
13. Nadais H (2002) Treatment of dairy effluents in UASB reactors with intermittent operation. Ph.D Thesis. University of Aveiro, Portugal (in Portuguese)
14. Wheatland AB (1974) Treatment of waste waters from dairies and dairy – product factories – methods and systems. J Soc Dairy Technol 27(2):71–79
15. de Man G, de Bekker P (1986) New technology in dairy wastewater treatment. Dairy Ind Int 51(5):21–25
16. Totzke D (1992) Anaerobic treatment in the dairy industry. In: Proceedings of food industry environmental conference, pp 3–16
17. Kirk-Othmer (ed) (1995) Encyclopedia of chemical technology, 4th edn, vol 16. Wiley, New York, pp 700–746
18. Hui Y (ed) (1992) Encyclopedia of food science and technology. Wiley, New York
19. Strydom J, Mostert J, Britz T (1995) Anaerobic treatment of a synthetic dairy effluent using a hybrid digester. Water SA 21(2):125–130
20. Fang H (1991) Treatment of wastewater from a whey processing plant using activated sludge and anaerobic processes. J Dairy Sci 74:2015–2019
21. Mawson AJ (1994) Bioconversions for whey utilization and waste abatement. Bioresour Technol 47:195–203
22. Malaspina F, Stante L, Cellamare CM, Tilche A (1995) Cheese whey and cheese factory wastewater treatment with a biological anaerobic-aerobic process. Water Sci Technol 32(12):59–72
23. Sam-Soon P, Loewenthal R, Wentzel M, Marais GVR (1991) A long-chain fatty acid, oleate, as sole substrate in up-flow anaerobic sludge bed (UASB) reactor systems. Water SA 17(1):31–36
24. Hickey R, Wu W-M, Veiga M, Jones R (1991) Start-up, operation, monitoring and control of high rate anaerobic treatment systems. Water Sci Technol 24(8):207–255
25. Hansen C, Hang S (1992) Two-phase anaerobic pilot plant for cheese waste. ASAE paper, 92–6605
26. García PA, Rico JL, Fdz-Polanco F (1991) Anaerobic treatment of cheese whey in a two-phase UASB reactor. Environ Technol 12(4):355–362
27. de Haast J, Britz TJ, Novello J (1996) Effect of different neutralizing treatments on the efficiency of an anaerobic digester fed with deproteinated cheese whey. J Dairy Res 53:467–476
28. Méndez R, Blázquez R, Lorenzo F, Lema JM (1989) Anaerobic treatment of cheese whey: start-up and operation. Water Sci Technol 21:1857–1860
29. Anonymous (1990) Anaerobic treatment of dairy effluents. Bulletin IDF 252:3–23
30. Streicher C, Milande N, Capdeville B, Roques H (1991) Improvement of the anaerobic digestion of diluted whey in a fluidized bed by nutrient additions. Environ Technol 12(4):333–341
31. Desai M, Madamwar D (1994) Surfactants in anaerobic digestion of cheese whey, poultry waste, and cattle dung for improved biomethanation. Trans ASAE 37(3):959–962
32. Cohen A, Thiele JH, Zeikus JG (1994) Pilot-scale anaerobic treatment of cheese whey by the substrate. Water Sci Technol 30(12):433–442
33. Bickers P, Bhamidimarri R (1998) Aerobic treatment of reverse osmosis permeate in the dairy industry for reuse. Water Sci Technol 38(4/5):61–67
34. http://www.cleantechindia.com/eicimage/210602_36/Dairy1.html

35. Anaerobic Digestion of Dairy Factory Effluents. FWR, Report No 455/1/01, available online @ http://www.fwr.org/wrcsa/455101.htm
36. Batstone D (1999) High rate anaerobic treatment of complex wastewater. Ph.D. Thesis. University of Queensland, Brisbane, Australia
37. Lettinga G, Hulshoff Pol L, Zeeman G (1998) Lecture notes on biological wastewater treatment – Part I anaerobic wastewater treatment. Agricultural University of Wageningen, Sub-Department of Environmental Technology, Wageningen, The Netherlands
38. Pavlostathis S, Giraldo-Gomez E (1991) Kinetics of anaerobic treatment. Water Sci Technol 24(8):35–59
39. Ramsay I (1997) Modelling and control of high-rate anaerobic wastewater treatment systems. Department of Chemical Engineering, University of Queensland, Brisbane, Australia
40. Finerty W (1988) β-oxidation of fatty acids. In: Ratledge C, Wilkinson S (eds) Microbial lipids. Academic, London
41. Verger R, Riviere C, Moreau H, Gargouri Y, Rogalska E, Moulin A, Ransac S, Carriere F, Cudrey C, Tretout N (1991) Enzyme kinetics of lypolysis. Lipase inhibition by proteins. In: Alberghina L, Schmid R, Verger R (eds) Lipases: structure, mechanism and genetic engineering, vol 16. GBF Monographs. VCH, Weinheim, pp 105–116
42. Heukelekian H, Mueller P (1958) Transformations of some lipids in anaerobic sludge digestion. Sewage Ind Waste 30:1108–1120
43. Leenders H (1995) Afbraak van vet tijdens de anaërobe slibvergisting, invloed van de concentraties hogere vetzuren en de H2-spanning op de hydrolyse. Doktoraal verslagen serie, nr. 95–134. Vakgroep Milieutechnologie, LU (in Dutch)
44. Perle M, Kimchie S, Shelef G (1995) Some biological aspects of the anaerobic degradation of dairy wastewater. Water Res 29(6):1549–1554
45. Jeris J, McCarty P (1965) The biochemistry of methane fermentation using C_{14} tracers. J Water Pollut Control Fed 37(2):178–192
46. Novak J, Carlson D (1970) The kinetics of anaerobic long chain fatty acid degradation. J Water Pollut Control Fed 42(11):1932–1943
47. McInerney M, Bryant M, Hespell R, Costerton R (1981) *Syntrophomonas Wolfei* gen. nov. sp. sov., an anaerobic, syntrophic, fatty acid oxidising bacterium. Appl Environ Microb 41:1029–1039
48. Hwu C-S (1997) Enhancing anaerobic treatment of wastewaters containing oleic acid. Ph.D Thesis. Agricultural University of Wageningen, Wageningen, the Netherlands
49. Rinzema A, Boone M, Knippenberg K, Lettinga G (1994) Bactericidal effect of long chain fatty acids in anaerobic digestion. Water Environ Res 66(1):40–48
50. Angelidaki I, Ahring BK (1992) Effects of free long-chain fatty acids on thermophilic anaerobic digestion. Appl Microbiol Biotechnol 37:808–812
51. Angenent LT, Sung S, Raskin L (2002) Methanogenic population dynamics during startup of a full-scale anaerobic sequencing batch reactor treating swine waste. Water Res 36:4648–4654
52. Rinzema A (1988) Anaerobic treatment of wastewater with high concentration of lipids or sulphate. Ph.D Thesis. Agricultural University of Wageningen, Wageningen, the Netherlands
53. Hulshoff Pol L, Lettinga G (1986) New technologies in anaerobic wastewater treatment. Water Sci Technol 18(12):41–53
54. Lettinga G (1996) Sustainable integrated biological wastewater treatment. Water Sci Technol 33(3):85–98

55. van Lier J, Lettinga G (1999) Appropriate technologies for effective management of industrial and domestic waste waters: the decentralized approach. Water Sci Technol 40(7):171–183
56. Lettinga G (1995) Anaerobic digestion and wastewater treatment systems. Antonie van Leeuwenhoek 67:3–28
57. IEA Bioenergy (1997) Systems and markets overview of anaerobic digestion. In: Anaerobic digestion activity, Patrick.wheeler@aeat.co.uk
58. Wheatley A, Fisher M, Grobicki A (1997) Application of anaerobic digestion for the treatment of industrial wastewaters in europe. J CIWEM 11:39–46
59. Riggle D (1998) Acceptance improves for large-scale anaerobic digestion. Biocycle 39(6):51–55
60. van Lier JB, Tilche A, Ahring BK, Macarie H, Moletta R, Dohanyos M, Hulshoff Pol LW, Lens P, Verstraete W (2001) New perspectives in anaerobic digestion. Water Sci Technol 43(1):1–18
61. Barnes D, Forster CF, Hrudey SE (1984) Surveys in industrial wastewater treatment, food and allied industries. Pitman Publishing Ltd., London, England
62. Choi E, Burkhead C (1984) Anaerobic treatment of dairy wastes using fixed-film and without media reactors. In: Proceedings of 39th Purdue industrial waste conference, pp 223–233
63. Ghaly A (1989) Biogas production from acid cheese whey using a two-stage digester. Energy Source 11:237–250
64. Kissalita W, Lo K, Pinder K (1989) Influence of dilution rate on the acidogenic phase products distribution during two-phase Lactose anaerobiosis. Biotechnol Bioeng 34:1235–1250
65. Yan J, Lo K, Liao P (1989) Anaerobic digestion of cheese whey using up-flow anaerobic sludge blanket reactor. Biol Waste 27:289–305
66. Yang S-T, Guo M (1990) Kinetics of methanogenesis from whey permeate in packed bed immobilized cells bioreactor. Biotechnol Bioeng 36:427–436
67. Glenn A (1976) Production of extracellular proteins by bacteria. Annu Rev Microbiol 30:41–62
68. Wiersma M, Harder W (1978) A continuous culture study of the regulation of extracellular protease production in vitro. Antonie van Leeuwenhoek 44:141–155
69. Whooley M, O'Callaghan A, McLoughlin A (1983) Effect of substrate on the regulation of exoprotease production by Pseudomonas Aeroginosas. J Gen Microbiol 129:981–988
70. Pausare A, Venugopal V, Lewis N (1985) A note on nutritional influence on extracellular protease synthesis in *Aeromonas hydrophila*. J Appl Bacteriol 58:101–104
71. Breure A, Mooijman K, van Andel J (1986) Protein degradation in anaerobic digestion: influence of VFA and carbohydrates on hydrolysis and acidogenic fermentation of gelatin. Appl Microbiol Biotechnol 24:426–431
72. Petruy R (1999) Anaerobic treatment of protein, lipid and carbohydrate containing wastewaters using the EGSB technology. Ph.D. Thesis. Agricultural University of Wageningen, Wageningen, the Netherlands
73. Shin H, Paik B (1990) Improved performance of UASB reactors by operating alternatives. Biotechnol Lett 22(6):469–474
74. Lettinga G, Hulshoff Pol L (1991) UASB-Process design for various types of wastewaters. Water Sci Technol 24(8):87–107
75. Yang J, Anderson G (1993) Effects of wastewater composition on stability of UASB. J Environ Eng 119(5):958–977
76. Annachhatre A (1996) Anaerobic treatment of industrial wastewaters. Resour Conserv Recycling 16:161–166
77. Samson R, van den Berg B, Peters P, Hade C (1985) Dairy waste treatment using industrial-scale fixed-film and up-flow sludge bed anaerobic digesters: design and start-up experience. In: Proceedings industrial wastewater conference, vol 39, pp 235–241

78. van Andel J, Breure A (1988) Anaerobic purification of proteineous waste water. Poster Paper Book 5th international symposium on anaerobic digestion, Bologna, pp 463–467
79. Verstraete W, Vandevivere P (1999) New and broader applications of anaerobic digestion. Crit Rev Food Sci 28(2):151–173
80. Caine M, Anderson G, Donnelly T (1991) A study into the effect of a series of shocks on a pilot-scale anaerobic filter. In: Proceedings of 45th Purdue industrial waste conference, pp 451–462
81. Petruy R, Lettinga G (1997) Digestion of a milk-fat emulsion. Bioresour Technol 61:141–149
82. Sayed S (1987) Anaerobic treatment of slaughterhouse wastewater using the UASB process. Ph.D thesis. Agricultural University of Wageningen, Wageningen, the Netherlands
83. Nadais H, Capela I, Arroja L, Duarte A (2001) Kinetic analysis of anaerobic degradation of dairy wastewater. In: Proceedings of 9th world congress on anaerobic digestion 2001, Antwerp, Part I, pp 203–208
84. Boari G, Brunetti A, Passino R, Rozzi A (1984) Anaerobic digestion of olive mill wastewaters. Agric Waste 10:161–175
85. Hanaki K, Matsuo T, Nagase M (1981) Mechanisms of inhibition caused by long chain fatty acids in anaerobic digestion process. Biotechnol Bioeng 23:1591–1560
86. Koster I, Cramer A (1987) Inhibition of methanogenesis from acetate in granular sludge by long-chain fatty acids. Appl Environ Microbiol 53(2):403–409
87. Nadais H, Capela I,Arroja, L, Duarte A (2003) Biosorption of milk substrates onto anaerobic flocculent and granular sludge. Biotechnol Prog 19:1053–1055
88. Hwu C-S, Donlon B, Lettinga G (1996) Comparative toxicity of long-chain fatty acids to anaerobic sludges from various origins. Water Sci Technol 34(5/6):351–358
89. Galbraith H, Miller T (1973) Effect of metal cations and pH on the antibacterial activity and uptake of long chain fatty acids. J Appl Bacteriol 36:634–646
90. Galbraith H, Miller T (1973) Physicochemical effects of LCFA on the bacterial cells and their protoplasts. J Appl Bacteriol 36:647–658
91. Nadais H, Capela I, Arroja L, Duarte A (2001) Effects of organic, hydraulic and fat shocks on the performance of UASB reactors with intermittent operation. Water Sci Technol 44(4):45–56
92. Nadais H, Capela I,Arroja, L, Duarte A (2005) Treatment of dairy wastewater in UASB reactors inoculated with flocculent biomass. Water SA 31(4):603–607
93. Fang H, Chui H-K (1994) Comparison of startup performance of four anaerobic reactors for the treatment of high-strength wastewater. Resour Conserv Recycling 11:123–138
94. Hattingh W, Kotzé J, Thiel P, Toerien D, Siebert M (1967) Biological changes during the adaptation of an anaerobic digester to a synthetic substrate. Water Res 1:255–277
95. Sørensen A, Winther-Nielsen M, Ahring B (1991) Kinetics of lactate, acetate and propionate in unadapted and lactate-adapted thermophilic, anaerobic sewage sludge: the influence of sludge adaptation for start-up of thermophilic UASB-reactors. Appl Microbiol Biotechnol 34:3–827
96. Kettunen R, Rintala J (1997) The effect of low temperature (5–29°C) and adaptation on the methanogenic activity of biomass. Appl Microbiol Biotechnol 48:570–576
97. Pereira A, Mota M, Alves M (1999) Efeito da aclimatização do inóculo e da recirculação da biomassa na degradação de ácido oleíco em filtro anaeróbio. In: Proceedings of 6ª Conferência Nacional sobre a Qualidade do Ambiente, vol 3, Lisboa, Portugal, pp 153–162
98. Yang J, Speece R (1985) Effects of engineering controls on methane fermentation toxicity response. J Water Pollut Control Fed 57:1134–1141
99. Alves MM, Vieira JA, Pereira RM, Pereira MA, Mota M (2001) Effect of lipids and oleic acid on biomass development in anaerobic fixed-bed reactors. Part I: biofilm growth and activity. Water Res 35(1):255–263

100. Morgan J, Evison L, Forster C (1991) Changes in the microbial ecology in anaerobic digesters treating ice cream wastewater during start-up. Water Res 25(6):639–653
101. Schroepfer GJ, Ziemke NR (1959) Development of the anaerobic contact process I. Pilot Plant Investigations and Economics. Sewage Ind Waste 31(2):164–190
102. Schroepfer GJ, Ziemke NR (1959) Development of the anaerobic contact process II. Ancillary Investigations and Special Experiments. Sewage Ind Waste 31(6):697–711
103. Sung S, Dague R (1992) Fundamental principles of the anaerobic sequencing batch reactor process. In: Proceedings of 47th Purdue industrial waste conference, pp 393–408
104. Riffat R, Dague R (1995) Laboratory studies on the anaerobic biosorption process. Water Environ Res 67(7):1104–1110
105. Tsetzos M, Bell J (1989) Comparison of the biosorption and desorption of hazardous organic pollutants by live and dead biomass. Water Res 23(5):561–568
106. Bell J, Tsetzos M (1987) Removal of hazardous organic pollutants by biomass adsorption. J Water Pollut Control Fed 59(4):191–198
107. Kennedy K, Pham T (1995) Effect of anaerobic sludge source and condition on biosorption of halogenated aromatics. Water Res 29:2360–2366
108. Ning J, Kennedy K, Fernandes L (1996) Biosorption of 2,4-dichlorophenol by live and chemically inactivated anaerobic granules. Water Res 30(9):2039–2044
109. Hwu C-S, Tseng S-K, Yuan C-Y, Kulik Z, Letinga G (1998) Biosorption of long-chain fatty acids in UASB treatment process. Water Res 32(5):1571–1579
110. Daffonchio D, Thaveesri J, Verstraete W (1995) Contact angle measurement and cell hydrophobicity of granular sludge from up-flow anaerobic sludge bed reactors. Appl Environ Microbiol 61:3676–3680
111. Rinzema A (1993) Anaerobic digestion of long-chain fatty acids in UASB and expanded granular sludge bed reactors. Process Biochem 28:527–537
112. Rinzema A, van Veen H, Lettinga G (1993) Anaerobic digestion of triglyceride emulsions in expanded granular sludge bed reactors with modified sludge separators. Environ Technol 14:423–432
113. Nadais H, Capela I, Arroja L, Duarte A (2005) Optimum cycle time for intermittent UASB reactors treating dairy wastewater. Water Res 39:1511–1518
114. Weiland P, Rozzi A (1991) The start-up, operation and monitoring of high-rate anaerobic treatment systems: a discusser's report. Water Sci Technol 24(8):257–277
115. Totzke D (2004) Personal communication (detotzke@ati-ae.com)
116. Young J, Dahab M (1983) Effect of media design on the performance of fixed-bed anaerobic reactors. Water Sci Technol 15(8/9):369–376
117. Dubourguier H, Prensier G, Albagnac G (1988) Structure and microbial activities of granular anaerobic sludge. In: Lettinga G, Zhender A, Grotenhuis J, Hulshoff Pol L (eds) Proceedings of GASMAT-workshop. Pudoc Wageningen, Lunteren, the Netherlands, pp 18–26
118. Zeeman G, Sanders W, Wang K, Lettinga G (1997) Anaerobic treatment of complex wastewater and waste activated sludge – application of an upflow anaerobic solid removal (UASR) reactor for the removal and pre-hydrolysis of suspended COD. Water Sci Technol 35(10):121–128
119. van Lier J, Boersma F, Debets M, Lettinga G (1994) 'High-rate' thermophilic anaerobic wastewater treatment in compartmentalized up-flow reactors. Water Sci Technol 30(12):251–261
120. van Lier J, Groeneveld N, Lettinga G (1996) Characteristics and development of thermophilic methanogenic sludge in compartmentalized up-flow reactors. Biotechnol Bioeng 50:115–124

121. Bachmann A, Beard V, McCarty P (1985) Performance characteristics of the anaerobic baffled reactor. Water Res 19:99–106
122. Nachaiyasit S, Stuckey D (1997) The effect of shock loads on the performance of an anaerobic baffled reactor (ABR). 2. Step and transient hydraulic shocks at constant feed strength. Water Res 31(11):2747–2754
123. Nachaiyasit S, Stuckey D (1995) Microbial response to environmental changes in an Anaerobic Baffled Reactor (ABR). Antonie van Leeuwenhoek 67:111–123
124. Boopathy R, Larsen V, Senior E (1988) Performance of anaerobic baffled reactor (ABR) in treating distillery wastewater from a Scotch Whisky factory. Biomass 16:133–143
125. Polprasert C, Kemmandamrong P, Tran F (1992) Anaerobic baffle reactor (ABR) process for treating a slaughterhouse wastewater. Environ Technol 13:857–865
126. Totzke DE (2001) Anaerobic treatment technology overview. Internal Report, Applied Technologies, Inc., detotzke@ati-ae.com
127. Page I, Cocci A, Landine R (1998) Low-rate anaerobic pretreatment of high-fat cheese wastewater. In: Proceedings of industrial wastes technical conference, Nashville, TN, pp 173–181
128. Paris JM, Lema JM, Casas C (1983) Criterios de selección de tecnología anaerobia de aguas residuales. Ingenieria Química 175:143–150
129. Burke DA (2001) Dairy waste anaerobic digestion handbook: options for recovering beneficial products from dairy manure. Environmental Energy Company (www.makingenergy.com)
130. Ahring BK (1994) Status on science and application of thermophilic anaerobic digestion. Water Sci Technol 30(12):241–249
131. Pereboom JHF, De Man G, Su IT (1994) Start-up of full-scale UASB reactor for the treatment of terephthalic acid wastewater. In: Proceedings of 7th int. symposium on anaerobic digestion, Cape Town, pp 307–312
132. Hulshoff L, Euler H, Eitner A, Wucke A (1997) GTZ sectoral project 'Promotion of anaerobic technology for the treatment of municipal and industrial sewage and wastes'. In: Anaerobic conversions for environmental protection, sanitation and re-use of residues. REUR Technological series vol 51, pp 96–107, FAO
133. De Zeeuw W (1984) Acclimatization of anaerobic sludge for UASB reactor start-up. Ph.D Thesis. Agricultural University of Wageningen, the Netherlands
134. Thaveesri J, Liessens B, Verstraete W (1995) Granular sludge growth under different reactor liquid surface tensions in lab-scale up-flow anaerobic sludge blanket reactors treating wastewater from sugar-beet processing. Appl Microbiol Biotechnol 43:1122–1127
135. Wirtz RA, Dague RR (1996) Enhancement of granulation and start-up in the anaerobic sequencing batch reactor. Water Environ Res 68:883–892
136. Leal MCMR, Cammarota MC, Freire DMG, Sant'Anna GL (2002) Hydrolytic enzymes as coadjuvants in the anaerobic treatment of dairy wastewaters. Braz J Chem Eng 19(2):175–180
137. Yang YP (1994) Treatment of milk powder wastewater with lipids. Internal Report. Agricultural University of Wageningen, the Netherlands
138. Motta Marques D, Cayless S, Lester J (1990) Process aiders for start-up of anaerobic fluidised bed systems. Environ Technol 11:1093–1105
139. Guitonas A, Paschalidis G, Zouboulis A (1994) Treatment of strong wastewaters by fixed bed anaerobic reactors with organic support. Water Sci Technol 29(9):257–263
140. Anderson G, Kasapgil B, Ince O (1994) Comparison of porous and non-porous media in up-flow anaerobic filters when treating dairy wastewater. Water Res 28(7):1619–1624

141. Sayed S, van der Spoel H, Truijen G (1993) A complete treatment of slaughterhouse wastewater combined with sludge stabilisation using a two stage high rate UASB process. Water Sci Technol 27(9):83–90
142. Fergala M (1995) The anaerobic treatment of complex wastewater. Ph.D Thesis. Department of Water Pollution Control, Van Hall Institute, the Netherlands
143. Lettinga G, Hulshoff Pol LW (1991) UASB-Process design for various types of wastewaters. Water Sci Technol 24(8):87–107
144. Yamamoto K, Hissa M, Mahmood T, Matsuo T (1989) Direct solid-liquid separation using hollow fiber membrane in an activated sludge aeration tank. Water Sci Technol 21:43–54
145. Ueda T, Hata K, Kikuoka Y (1996) Treatment of domestic sewage from rural settlements by a membrane bioreactor. Water Sci Technol 34:189–196
146. Ueda T, Hata K, Kikuoka Y (1997) Effects of aeration on suction pressure in a submerged membrane bioreactor. Water Res 31:489–494
147. Hansen CL, Hwang SH (1990) Use of up-flow anaerobic sludge blanket (UASB) reactor to treat whey permeate. In: Proceedings of the 6th international symposium on agriculture and food processing wastes. Am Soc Agric Eng I11:124
148. Rico JL, García P, Fdz-Polanco F (1991) Anaerobic treatment of cheese production wastewater using a UASB reactor. Bioresour Technol 37:271–276
149. Hawkes F, Donnelly T, Anderson G (1995) Comparative performance of anaerobic digesters operating on ice-cream wastewater. Water Res 29(2):525–533
150. Ozturk I, Ubay G, Demir I (1993) Hybrid up-flow anaerobic sludge blanket reactor treatment of dairy effluent (HUASBR). Water Sci Technol 28(2):77–81
151. Viraraghavan T, Kikkeri S (1990) Effect of temperature on anaerobic filter treatment of dairy wastewater. Water Sci Technol 22(9):191–198
152. Veiga MC, Pan M, Blázquez R, Méndez R, Lema JM (1994) A double-feed anaerobic filter for the treatment of high strength wastewaters. Biotechnol Tech 8(2):77–82
153. Radick K (1992) Dairy wastes. Water Environ Res 64(4):417–418
154. Kárpáti I, Bencze L, Borszérki J (1995) New process for physico-chemical pre-treatment of dairy effluents with agricultural use of sludge produced. Water Sci Technol 22(9):93–100
155. Ruston B (1993) Chemical pre-treatment of dairy wastewater. Water Sci Technol 28(2):67–72
156. Vidal G, Carvalho A, Méndez R, Lema JM (2000) Influence of the content in fats and proteins on the anaerobic biodegradability of dairy wastewaters. Bioresour Technol 74:231–239
157. Braun R, Steffen R (1997) Anaerobic digestion of agroindustrial by-products and wastes. In: Anaerobic conversions for environmental protection, sanitation and re-use of residues. REUR Technological series vol 51, pp 27–41, FAO (ISSN 1024–2368)
158. Wang LK, Hung YT, Lo HH, Yapijakis (2004) Handbook of Industrial and Hazardous Wastes Treatment, CRC Press, NY, 1345 pp
159. Wang LK, Hung YT, Lo HH, Yapijakis (2006) Waste Treatment in the Food Processing Industry. CRC Press, NY, 333 pp
160. Wang LK, Hung YT, Shammas NK (2010) Handbook of Advanced Industrial and Hazardous Wastes Treatment. CRC Press, NY, 1237–1240