Optimized Utilization of Quarg Production Residuals

Jan-Christian Mohr

June 2011

A submission presented in partial fulfilment of the requirements of the University of Glamorgan/Prifysgol Morgannwg

for the degree of Doctor of Philosophy

This research programme was carried out in collaboration with the University of Applied Science and Arts Hanover, Germany

BLANK 2

Abstract

Acid whey is a by-product of the quarg production and arises in large volumes in dairies. A considerable disposal problem arises due to the lack of obtainable proceeds from acid whey utilisation. Additionally, sustainable and energy efficient treatment methods for high strength liquid wastes from dairies cleaning operation are needed to reduce the costs of wastewater treatment. Samples of acid whey and spent cleaning solutions from a quarg cheese production plant were collected. The composition and physical properties were analysed and evaluated against waste treatment process requirements. The occurrence of different waste streams, their volumes and frequencies were also investigated.

A laboratory scale membrane nanofiltration plant was designed, and built for investigation of the volume reduction of cleaning process effluents with emphasis to treatment options for the filtration concentrates. The examination of the rheological properties of alkaline CIP wastewaters at different volume reduction ratios clearly shows that these effluents are Newtonian fluids even at high concentrations.

The anaerobic biodegradability of acid whey and mixtures containing portions of alkaline CIP wastewaters at different volume reduction ratios was tested. Characteristic process kinetics for acid whey fermentation in batch mode was observed. The occurrence of a second lag-phase in mixtures containing larger portions of acid whey was identified as phase separation due to rapid acidification of lactose.

Anaerobic digestion (AD) was identified as a suitable treatment option for acid whey and alkaline CIP wastewaters. Four anaerobic digester types were designed with regard to their suitability for high strength waste treatment and were built and operated at laboratory scale. The reactors tested were: a) A Continuous Stirred Tank Reactor (CSTR); b) An Anaerobic Membrane Reactor (AMR); c) An Upflow Anaerobic Sludge Blanket (UASB) reactor; and d) A novel two-stage process design consisting of a combined acidification and crystallization stage and a gaslift driven fluidised bed methanogenic stage.

The operation of the AMR process and also of the UASB process with internal circulation and pH-control using alkaline CIP effluents was evaluated at high loading rates of 7.7 $g \cdot L^{-1} \cdot d^{-1}$ and 10.2 $g \cdot L^{-1} \cdot d^{-1}$ respective. However, in the experiments it was demonstrated that even with perfect biomass retention the operation of one stage anaerobic digestion at high loading rates caused process upsets. Precipitation and accumulation of milk minerals within the sludge was observed in all one stage experiments.

The conclusions drawn from one stage studies led to the design of a novel high-rate digestion system to meet the demands of anaerobic digestion of acid whey and effluents from dairy plant cleaning. The design based on different high-rate industrial reactor designs and incorporate the ideas of staging, crystallisation of calcium salts prior to anaerobic digestion, fluidised bed and internal circulation reactors, and also jet-loop or gaslift reactors. The performance of the novel system when treating acid whey is comparable to the results of well designed, two-stage digesters treating cheese whey which is easier to digest.

Table of Contents

List of Illustrations

List of Tables

List of Abbreviations

AAFEB	Anaerobic Attached Film Expanded Bed (reactor type)
AD	Anaerobic Digestion
ADM1	Anaerobic Digestion Model No. 1
ADP	*Adenosinediphosphate*
AF	Anaerobic Filter (reactor type)
AMR	Anaerobic Membrane Reactor (reactor type)
AOX	Adsorbable Organic Halogens
APHA	American Public Health Association
ASBR	Anaerobic Sequencing Batch Reactor (reactor type)
ATP	*Adenosinetriphoshate*
ATV	Abwassertechnische Vereinigung (German Wastewater Association)
AUBIOS	Automatation Umwelt- und Bioverfahrenstechnischer Prozesse und Systeme
BOD	Biochemical Oxygen Demand
BS	British Standard
BSE	Bovine Spongiform Encephalopathy
CIP	Cleaning In Place
COD	Chemical Oxygen Demand
CSTR	Continuous Stirred Tank Reactor
DAE	Differential Algebraic Equation
DIN	Deutsches Institut für Normung (German Institute for Standardization). The acronym is used for designation of German standards.
DUHR	Downflow-Upflow Hybrid Reactor
EDTA	Ethylenediaminetetraacetic Acid
EGSB	Expanded Bed Granular Sludge (reactor type)
EHS	Enterohaemorrhagic Syndrome
EN	European standard
FAS	Ferrous Ammonium Sulphate
FOS/TAC	Ratio of Volatile Fatty Acids (**F**lüchtige **O**rganische **S**äuren) to **T**otal **A**norganic **C**arbon
GLP	Good Laboratory Practice
HRT	Hydraulic Retention Time
IC	Internal Circulation (reactor type)
IEA	Department of Industrial Electrical Engineering and Automation (at Lund University, Sweden)
Inc.	Incorporated
ISO	International Standard
LCA	Life Cycle Assessment
LCFA	Long Chain Fatty Acids

MARS	Membrane Anaerobic Reactor System (reactor type)
MBR	Membrane Bioreactor (reactor type)
MCAB	Membrane-Coupled Anaerobic Bioreactor (reactor type)
MPAR	Multiplate Anaerobic Reactor (reactor type)
MWCO	Molecular-Weight-Cut-Off
NAD+	Nicotinamide adenine dinucleotide
NADP+	Nicotinamide adenine dinucleotide phosphate
OLR	Organic Loading Rate
ORP	Oxidation-Reduction-Potential
PCP	Pentachlorophenol
PEG400	A Sort of Polyethylene Glycol (with an average molecular weight of approximately 400 Da)
pKa	Acid Dissociation Constant
QAC	Quaternary Ammonium Compounds
SRT	Solid Retention Time
TADU	Thermophilic Anaerobic Digester with Ultrafilter (reactor type)
TBA	True Bicarbonate Alkalinity
TIC	Total Inorganic Carbon
TNT	Trinitrotoluene (explosive material, reagent in chemical synthesis, 2-methyl-1,3,5-trinitrobenzene)
TOC	Total Organic Carbon
UASB	Upflow Anaerobic Sludge Blanket (reactor type)
UF	Ultrafiltration
UFAF	Upflow Anaerobic Filter (reactor type)
USB	Upflow Sludge Blanket (reactor type)
VDM	Verband der Deutschen Milchwirtschaft e. V. (German Dairy Association)
VFA	Volatile Fatty Acids
VRR(t)	Volume Reduction Ratio
WHO	World Health Organization

1. PREFACE

This Ph.D. thesis is based on the results from a research project called AUBIOS carried out at University of Applied Sciences and Arts Hanover in Germany, during the period from June 2001 to May 2007. The experimental work of this thesis was largely performed at the Environmental Process Laboratory at the University of Applied Sciences and Arts Hanover in Germany, with some experimental work conducted in the Wastewater Treatment Laboratory of the Sustainable Environment Research Centre at the University of Glamorgan. Throughout the period, Prof. Alan J. Guwy was the Director of Studies, Prof. Richard M. Dinsdale and Prof. Dennis Hawkes were the supervisors at the University of Glamorgan. Prof. Dr.-Ing. Wilfried Stiller was the supervisor at the University of Applied Sciences and Arts Hanover and Prof. Dr.-Ing. Reimar Schumann was the Director of the AUBIOS Project. Results of the work have been published recently in following papers and oral presentations.

Mohr, J.-C. (2007). Anaerobtechnik: Von der Abwasserreinigung zum Biogasboom. Oral presentation, VDI series of lectures, Hanover, Germany, 24 Oct 2007

Mohr, J.-C.; Stiller, W.; Dinsdale, R.; Guwy, A. (2006a). The Assessment of the Anaerobic Biodegradability of Filtration Residues from the Recovery of Alkaline Dairy Cleaning-In-Place Solutions by Nanofiltration. Proceedings of the 11th European Biosolids and Organic Recources Conference Exibition and Workshop. Wakefield, UK, 13–15 Nov 2006

Mohr, J.-C.; Stiller, W. (2006b). Recycling von Molkerei- Reinigungslaugen und Verwertbarkeit der Filtrationsretentate in Biogasanlagen. Proceedings of the 11th Colloquium Produktionsintegrierte Wasser-/ Abwassertechnik, Bremen, Germany, 13–14 Sept 2006

Mohr, J.-C.; Stiller, W. (2006c). Da Steckt Energie Drin. Behandlung flüssiger Reststoffe aus der Frischkäseproduktion. Pharma+Food, 9/2006 pp. 68–70

Schumann, R., Rößler, J., Hoyer, M., Hülsen, U., Wüst, E., von Ramin, J., Stiller, W., Mohr, J.-C., Gottschlich, M., Stannek, W.; Horn, C. (2005). Angewandter Forschungsschwerpunkt AUBIOS. Automatisierung umwelt- und bioverfahrenstechnischer Prozesse und Systeme. Abschlussbericht 2005. University of Applied Sciences and Arts Hanover, Research unit AUBIOS, Germany

Mohr, J.-C.; Stiller, W. (2005). Biogaserzeugung aus Anfallprodukten. Oral Presentation, Ahlemer Seminar für Führungskräfte und Fachberater in der Milchwirtschaft. Hanover, Germany, 30–31 May 2005

Mohr, J.-C.; Stiller, W. (2003). Verwertung von Sauermolke und Ausschubmedien. Oral presentation, Ahlemer Fachtagung. Hanover, Germany, May 2003

Mohr, J.-C. (2001). Projektierung und Umsetzung einer Laborversuchsanlage zum Membrantrennverfahren Mikrofiltration und zur Untersuchung von Suspensionen und Filtermedien. Diploma-Thesis, University of Applied Sciences and Arts Hanover, Faculty of Mechanical Engineering

See also Appendix, Section 10

2. INTRODUCTION AND BACKGROUND

In Germany, an amount of 28.15 Mio tons of milk had been processed at 198 dairy businesses with 281 operating facilities in the year 2006. In the year 2000 there were 251 businesses with 336 operation facilities (Wohlfarth *et al.,* 2008). However, the amount of utilized milk (27.57 Mio tons in 2000) stagnated (Richards *et al.,* 2005). The decrease of the number of operating facilities is due to a trend in larger facilities in the dairy industry.

Due to the sensitivity of milk and milk products the production and utilization of milk underlies strict regulations and also definition of milk products is regularised in Germany for example by the German Milk Products Ordinance (Bundesrepublik Deutschland, 2010a).

In general the processing of milk involves the following steps:

- Product receiving
- Chilling storage
- Classification and Separation
- Standardisation, Homogenisation, and Pasteurisation
- Product processing
- Packaging and Dispatch

A broad variety of products is produced in dairies. Beside consume milk, butter, cheese, condensed and dried products, cream, yogurt and other fermented products are on the market.

Quarg cheese is very popular in Germany. The output of 781 200 metric tons was the highest compared to the production of other countries in the European Union. Richards *et al.* (2005) reported that the production of quark and fresh cheese in the European Union was 2 530 000 metric tons in 2004.

2.1. Quarg Cheese Production

Quarg is classified as soft, unripened/fresh cheese that can be produced in different varieties from skimmed, partially skimmed, medium fat or full fat by the Codex Alimentarius Standard (WHO, 2006). Additionally a number of specialities with and without spice or fruit preparing, and other flavours are known. Mair-Waldburg (1974) defined quarg as the milk protein precipitated by lactic acid and/or lab, which contains more or fewer different milk constituents such as fat and salts.

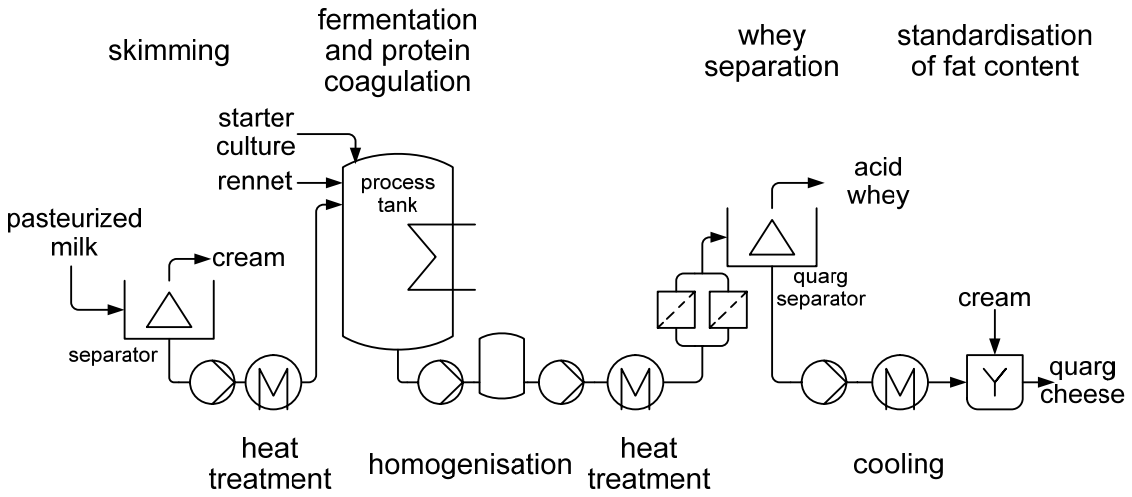

Figure 1 Process scheme of the Thermo-Quarg-Process.

Kessler (1996) gives a detailed description of the most common production method, the thermo-quarg process (Figure 1). The advantage of heat treatments in this process, where the temperature of pasteurized skimmed milk was maintained at (90 to 95) °C for about (5 to 15) min, is a denaturalization of whey proteins. This is intended to make them available to later separation. Without the heat treatment the small whey protein molecules pass the separator nozzles and will be lost with the whey fraction. After cooling down to a temperature range of (28 to 30) °C, the milk is collected in a process tank and (2 to 5) % bulk starter culture and a small amount of rennet is added. The starter cultures are pure or mixed cultures of lactic acid producing bacteria *e.g. Lactococcus lactis*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, and others. The pH decreases during the fermentation due to lactic acid production. A low pH induces the production of curd, a denatured gel coagulation of the milk protein casein. The acidification is stopped at pH 4.6 to pH 4.7 by heating to a range of (60 to 64) °C for about (4 to 6) min. A careful homogenisation gives the curd a preferred structure. The curd is then cooled down to a range of (40 to 44) °C before separation. Centrifugal separators with disc stacks are widely used to separate the acid whey in industrial production. Whey separation by membrane filtration is also possible but less economical due to higher energy demand for pumping action and membrane costs. After separation the quarg is cooled and its fat content is adjusted by adding cream.

Only about one quarter of the thickened milk is coagulated casein and can be achieved in quarg cheese production but approximately three quarter is acid whey and will be removed. From the amount of unripened/fresh cheese, produced in Germany in 2005, a volume of around 2 300 000 m^3 of acid whey can be calculated. A volume of (100 to 200) m^3 of acid whey is produced each day in a standard quarg cheese production plant of a middle sized dairy.

Quarg cheese production plants usually operate in batch mode. Stacks of fermentation tanks and parallel separator configuration allow smooth output rates and work shift wise production.

2.2. Liquid Wastes from the Quarg Production Process

Whey and whey permeates are by-products of cheese production. These milk serums are high strength with a typical chemical oxygen demand (COD) in the range of (50 to 70) $g \cdot L^{-1}$. Large volumes of different whey sorts arise from modern dairies. Since the market for whey based beverages is very limited, dairies try to stimulate their sales volume by creating new products and large efforts in marketing. Fractionation, separation of valuable milk ingredients, dewatering and drying is common practice because whey disposal into sewers without pre-treatment is not permitted in Germany and many other countries. But these utilisation methods require cost and energy intensive treatment. In case of acid whey, a sort of whey arising from the production of some fresh cheeses, the situation is heightened as the use for dairy food production is limited, due to its sour taste. The lactic acid content of acid whey aggravates utilisation procedures established for sweet whey, though they are not generally applicable to acid whey. The utilisation of acid whey as an additive in animal feeds is only possible for small and middle sized dairies which have local farmers as customers. The by-product acid whey, arising from the quarg production process, becomes a liquid waste if no other utilisation options are available. Therefore, while the demand for curd cheeses remains high, the demand for cost effective treatment processes for acid whey is significant.

2.3. Water Consumption at Dairies

Processing of milk is still a water excessive business. The specific water consumption at European dairies is in a wide range of (1.0 to 60) m^3 of water per metric tonne of utilised milk (Garcilaso, 2006). The German Dairy Association reports an average for the consumption of water of 2.06 $L \cdot kg^{-1}$ of milk for the year 1999. The data was based on a survey of 132 dairies (Coldewey *et al.*, 2003). As water is usually not a component of milk products, a large part of the consumed water is discharged as wastewater after use. Most of the water is used for cleaning purpose and smaller volumes are needed for cooling and heating or as steam in physical disinfection. Approximately 90 % of European dairies discharge their effluents into sewers for external treatment in municipal wastewater treatment plants (Abwassertechnische Vereinigung e. V., 1994). Dairy wastewaters contain milk ingredients from production failure and cleaning procedures and chemicals from cleaning and disinfection. Its composition and properties vary not only by the range of products that a specific dairy produces but also varies over time, being influenced by work

schedules and cleaning interval organisation. Heavy loaded wastewater-streams arise from specific operations *e.g.* draining of detergents. These often exceed upper limits for organic load, pH or temperature. Therefore it is common practice to collect all waste streams in tanks or basins for balancing by dilution with less concentrated wastewaters and for neutralisation. Disposal costs can be minimised by use of water saving and recycling. Savings in waste water fees can also be achieved by pre-treatment of high strength waste streams. This would possibly avoid surcharges for exceeding load limits in graded contribution wastewater fees.

2.3.1. Cleaning in Place (CIP)

Cleaning in place (CIP) plants are standard equipment at dairy production plants. These automatic cleaning systems operate without disassembling piping, vessels and other process set-ups. Rinsing water and cleaning agents are transferred into the production plant by mix-proof valves and pumped through the plant in a heated recycling loop. Mix-proof valves or switch panels with monitored tube connectors prevent a mixing or even an infiltration of cleaning agents into the food.

Cleaning in place systems consists of process tanks for centralised storage of cleaning agents and dosage systems for their preparation (Figure 2). Heater and pumps can either be connected to the central process tanks or, located in decentralized cleaning devices which are supplied with water and cleaning agents by various circular CIP-mains. As loss of heat can be reduced and cleaning sequences can be optimized for the connected part of the plant by these so-called "Satellite"- CIP plants, which are more common in larger dairies.

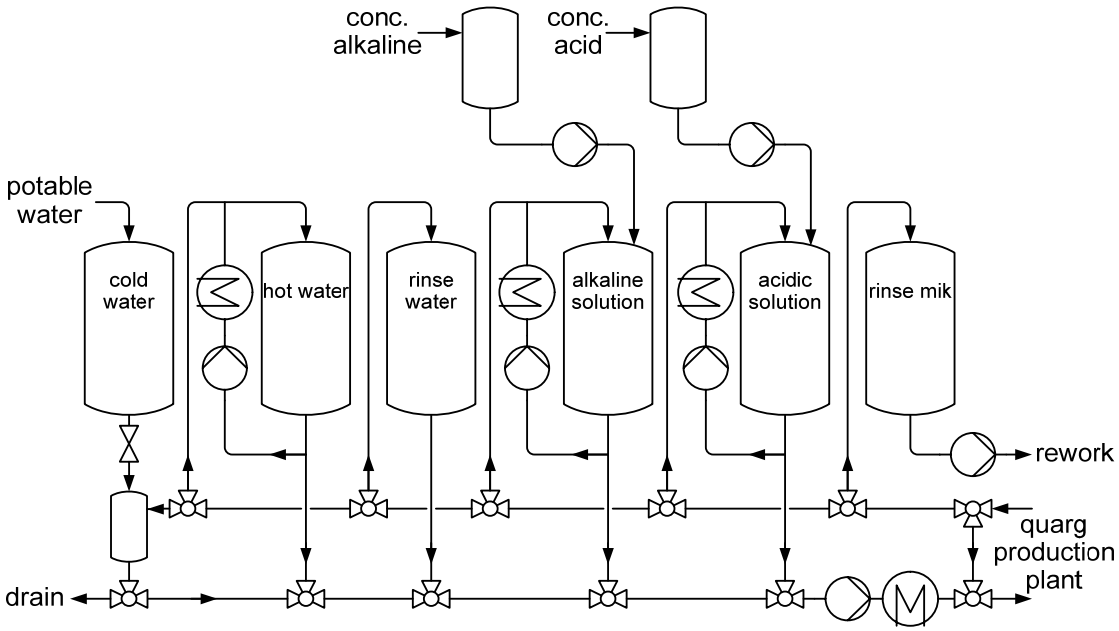

Figure 2 Process scheme of a centralized Cleaning-In-Place (CIP) plant.

CIP plants which are completely independent from a circular CIP-main are available for small solitaire plants like culture fermenter. Some cleaning tasks require specific cleaning agents *e.g.* cleaning of quarg separators. Independent CIP plants can be used parallel to a central CIP plant in this case. Independent CIP plants include all necessary parts: Pumps, process tanks, storage tanks, valves, tubes, and heating equipment.

2.3.2. Detergents

Detergents are not ingredients of the dairy products, though in terms of economics they can be classified as production resources. It can be assumed that all used detergents leave the factory as effluent. The mass of chemicals a dairy consumes varies depending on the product range, on production methods and, not least, on the environmental awareness of the staff and management. The German Institute for Standardisation (DIN) specifies the requirements of detergents in a report about cleaning and sanitising of dairy plants (DIN Deutsches Institut für Normung e.V., 1988) as follows:

Detergents for the dairy industry must:

- be easily and completely solvable
- soak and disintegrate organic deposits
- have wetting power to surfaces
- have the ability to disperse and suspend solid soils
- emulsify fats and oils
- reduce the hardness of water
- have antifoaming effects
- prevent corrosion
- be easily and completely removable from surfaces
- be ecologically harmless and as non-toxic as possible

Detergents can be prepared on site from pure or highly concentrated basic chemicals. If necessary, additives can be added to meet special requirements for a particular cleaning task. Alternatively, preparations that are ready to use after dilution are commercially available for dairy cleaning tasks.

2.3.3. Wastewater from Plant Cleaning

Water and detergents in the process tanks should be used several times. The cleaning efficiency of the solutions decreases with increasing organic load in the process tank. Replacement of solutions depends on the specific CIP operation mode of the dairy CIP system. The spent cleaning solutions were either discarded and renewed batchwise or continuous. The replacement is performed on a basis of time intervals and operators experi-

ence. Gesan-Guiziou *et al.* (2006) stated that replacement is based only on subjective criteria like colour or odour of the solution. Fischer *et al.* (1974) presented the use of conductometry to measure the milk content in alkaline and acidic solutions. Advanced CIP systems use conductometry to detect the current load of the cleaning solution while it cycles through the production plant. However, with conductometry alone it is not possible to detect whether the alkaline cleaning agent is depleted or not. This is due to the complex chemistry of detergents in contact with pollutants. When the detergent loose specific properties during use a replacement is required even when the load, detected by conductometry, is still low.

2.4. Recovery of Water and Cleaning Agents

Nowadays, Cleaning in Place (CIP) is a standard procedure in dairies and large amounts of dairy effluent result from periodic cleaning of such processing plants and can either be extremely acidic or alkaline due to the cleaning products used (Romney, 1990).

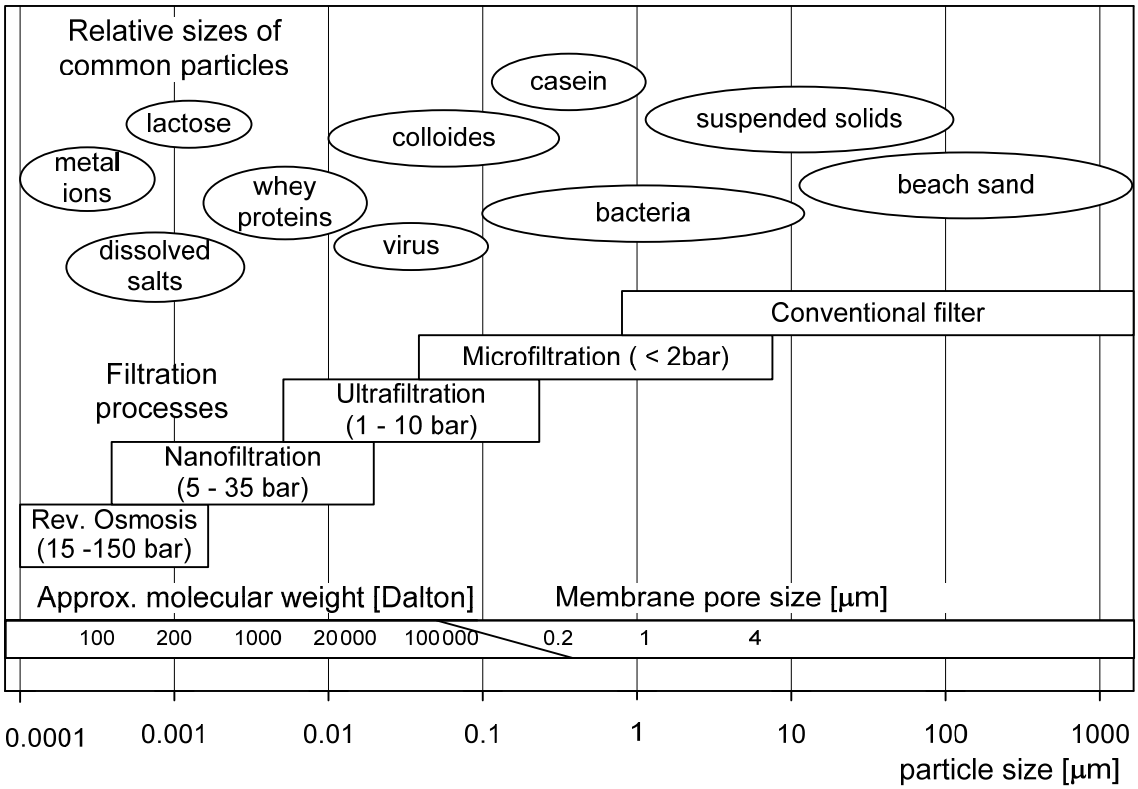

Figure 3 Spectrum of different membrane separation processes.

A simple recycling procedure to extend the operating life of the cleaning solution by removing agglomerates and particles from the cleaning solution is the use of a separator that can be placed in a loop, connected to the process tank. Three or four times a day the sludge will be removed from the separator by lifting the rotor. The sludge is purged out of the separator by approximately 50 L of cleaning solution in this procedure. The lost solution must be replaced and the removal efficiency is poor. The procedure is widely used in

the dairy industry, the frequencies and volumes arising were reported by Mr. Schawe and Mr. Koch-Hartke from the Humana Milchunion dairy (Personal Communication, Meeting of the AUBIOS research group with the Humana Milchunion dairy management and operating staff held on 12th March 2002 in Georgsmarienhütte).

A proper recovery of the caustic cleaning agents means more than just removing particles. An advanced recycling by filtration can be used since caustic and temperature stable membranes are available for cross flow filtration plants. However a significant reduction of the COD of the solution is necessary to preserve the cleaning efficiency of the recovered cleaning solution (Dresch *et al.,* 2001). Suitable processes use micro-, ultra-, or nanofiltration membranes which differ in pore width respectively molecular-weight-cut-off (MWCO) as shown in Figure 3. However, effective COD reduction can only be achieved by using tight membranes with low MWCO *i.e.* a nanofiltration process as shown in Figure 4. A 95 % saving of leach and water is possible when, in addition, a consequent water management is implemented (Otterpohl and Behrendt, 2001).

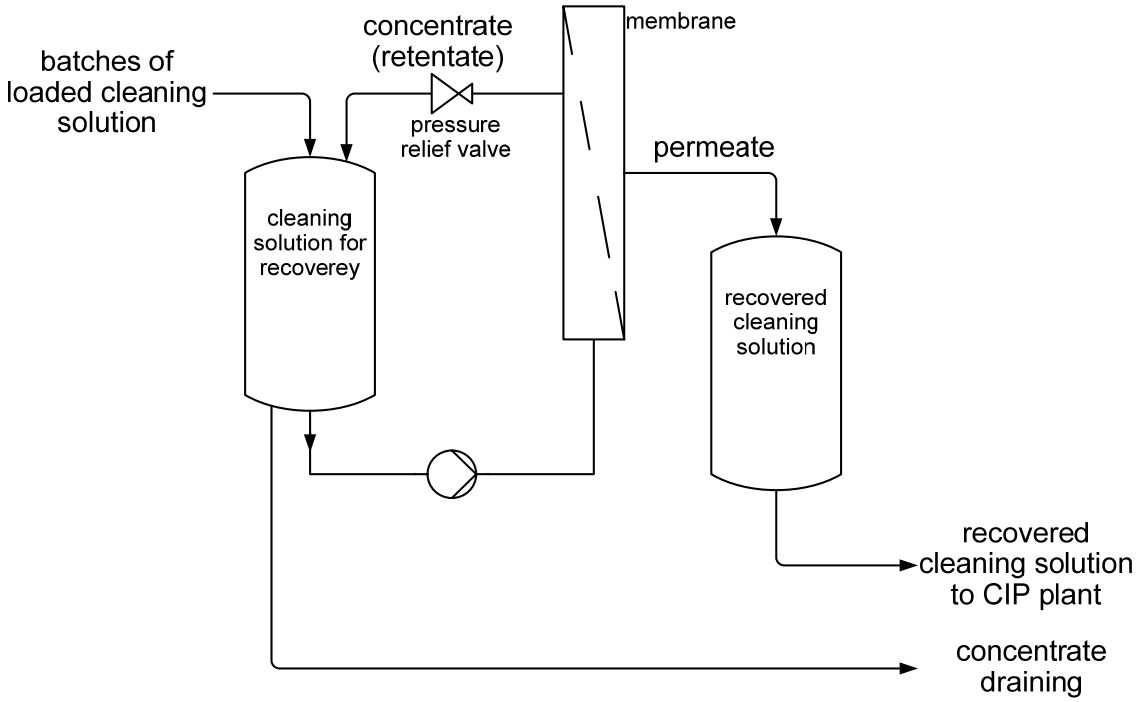

Figure 4 Process scheme of a nanofiltration plant to recycle cleaning agents.

Although CIP is common place, little research has been conducted into the treatment of cleaning solution recovery wastewaters at dairies that use membrane technology. Yacubowicz (1995) reported the development of caustic stable membrane modules for recycling of used cleaning solutions by Membrane Products Kiryat Weizmann Ltd. However, Dresch *et al.* (2001) stated that only a few dairies in the world use this technology at the present time, due to long pay back times and high investment costs. Dresch calculated a pay back time of 14 years. Shorter pay back times of below two years and 7.7 years were

reported by Yacubowicz (1995) and Henk (1993) respectively. A payback time below 1.5 years reached in a beverage producing factory was reported by Bhave *et al.* (2001). This shows clearly that pay back times for CIP fluid recovery can not be stated in general. Wagner (2001) stated that, if more than three solutes are present in a solution it is not possible to predict the performance of nanofiltration. A calculation of payback times is therefore only expressive for the dairy for which it is made, and only if filtration tests have been carried out.

The filtration of spent CIP fluids not only saves water but reduces the amounts of cleaning agents used. A typical residual filtration retentate achieved from filtration of an alkaline CIP cleaning solution (NaOH, 0.1 % to 0.2 %) has a pH range of pH 12.5 to pH 14.0 (Räsänen *et al.,* 2002). A COD of (50 to 250) $g \cdot L^{-1}$ depending on the recirculation index and input COD utilized can be assumed and has got to be reported in the literature. Gèsan-Guiziou *et al.* (2002), Dresch *et al.* (2001) and also Räsänen *et al.* (2002) investigated the recycled cleaning solution as well as membrane and process parameters, but the remaining retentate was described as "drained" or "discarded". Possible reuse, the treatment, and discharge of the concentrate of pressure-driven membrane processes were reviewed by Van der Bruggen *et al.* (2003). However no proper suggestions were made for concentrates from dairy CIP recovery retentates. Henk (1993) considered the disposal of retentates into an anaerobic sludge digester of a municipal wastewater plant and demonstrated the principle applicability of anaerobic digestion for the treatment of retentates from dairy alkaline CIP cleaning solution recovery. However, on-site treatment options had not been investigated.

The review of literature aimed in caustic recycling implies that this technology will play an important role in process water economy of dairies. However, only little information was found that describe utilisation and treatment or effect on biological treatment processes of this high strength wastewater.

2.5. Dairy Wastewater Treatment

Approximately 90 % of the dairies in Germany discharge their effluents to sewers, connected to a municipal wastewater treatment plant (Abwassertechnische Vereinigung e. V. (ATV), 1994). These indirect dischargers are obligated to statutes set by the involved municipal sewage plant and the local public authorities. The remaining 10 % of German dairies operate on-cite wastewater treatment plants and discharge directly to receiving waters governed by the national Water Resources Act and the Waste Water Ordinance (Bundesrepublik Deutschland, 2009). The state of the art dairy wastewater treatment is described by the European Integrated Pollution Prevention and Control Bureau of the

European Commission (Garcilaso, 2006). The German Dairy Association (VDM) publishes recommendations in their guidelines for water and wastewater in dairies (Coldewey *et al.*, 2003).

The composition of dairy wastewaters varies on a daily basis depending on recurrent operations and intermittent releases of wastewater streams. These streams can exceed upper limits of restricted wastewater parameters (indirect discharge) or maximum load of wastewater treatment processes (on-cite wastewater treatment). Mixing and balance tanks are used to intercept highly loaded streams and extreme pH values. A state of the art wastewater treatment plant for dairy wastewater is usually an activated sludge process. In addition to the mixing and balancing tank, an additional tank to be used in the event of losses in the dairy is recommended by Coldewey (2004). Other additional facilities are flotation plants to separate milk fat. To meet the requirements of direct discharge a significant reduction of Nitrogen and Phosphor is necessary. To reduce the nitrogen content, either as nitrate (NO_3^{2-}) or as Ammonium (NH_4-N) an aerobic/anoxic nitrification/denitrification process is still state of the art. A recent development in nutrient removal processes is the use of anoxic partial nitrification and of anaerobic denitrification by ANAMMOX bacteria (Mulder *et al.*, 1995; van der Star *et al.*, 2007). These recently novel processes are characterized by less energy consumption compared to aerobic/anoxic processes due to avoidance of aeration. Additionally a minimal ratio of biological available organic carbon to nitrogen of 1 to 3 in the wastewater is recommended for a conventional nitrification/denitrification process.

Phosphor can only be removed from dairy wastewaters as solid matter either by uptake into the biomass of a biological process or by precipitation using a chemical precipitant. Combined processes are also known (Coldewey *et al.,* 2003).

Anaerobic Digestion is commonly used for sludge stabilisation and to reduce the volume of sludges from activated sludge processes in wastewater treatment plants operated by the dairy for direct discharge. Anaerobic digesters can also be used to pre-treat highly loaded waste streams of dairy wastewaters. The application for digestion of whey and used alkaline wastewaters from plant cleaning operation is the main topic of this work.

2.6. Problem: High Strength Wastes Arising from Quarg Production

There are a number of liquid wastes arising from a common quarg production line with connected CIP cleaning system. These can be subdivided into wastewaters and aqueous by-products *e.g.* acid whey. Wastewaters from a typical quarg production plant are high strength wastes and arise mainly from plant cleaning. If cleaning solution recovery is used, the volume of wastewater from cleaning procedures is drastically reduced. In this case concentrated high strength liquid residues arise from the filtration process. The list below gives an overview of the main liquid waste streams arising from an industrial (thermo-) quarg cheese production:

- Acid whey, a by-product of curd cheese making.
- Rinse water and product loss that cannot be reprocessed.
- Alkaline and acid wastewaters from plant cleaning procedure.
- Wastewaters from chemical disinfection.
- Mixed wastewaters from overflow and floor rinsing.
- Residues from cleaning solution recovery.

These wastes will be discussed further in depth in the following chapters. The review of the literature on anaerobic digestion (Section 4.3) and the results of wastewater volumes arising from the examined dairy (Section 6.1) imply a possible advantage of co-digestion of calcium rich acid whey with spent cleaning solutions which contain a high sodium concentration. To the knowledge of the author, investigations about utilising spent cleaning solutions as alkaline source for pH control or co-digestion with whey have not been reported in the literature.

3. OBJECTIVES OF THE THESIS

The overall thesis aim is to investigate sustainable waste minimisation and effluent treatment systems for the dairy industry in particular quarg cheese production. A novel strategy for dairy CIP reuse concentrate disposal by using biological wastewater treatment processes was developed and investigated. Two high strength waste streams arising from quarg production were chosen as co-substrates of an anaerobic digestion:

- Acid whey, a by-product of fresh cheese production.
- Alkaline wastewater from plant cleaning procedures.

The efficient and environmental friendly waste treatment process helps in meeting the deficiencies of present whey utilization and cleaning agent recovery technology. The research was also focused on the anaerobic digestion of high strength wastes in particular from quarg cheese producing dairies.

The research carried out to meet the objectives can be divided into three sections as follows:

Identify and characterise waste streams and volumes arising

Identification of liquid wastes from a real production plant, as its conditions and volumes are essential data for the investigation of the waste minimisation strategies and waste treatment methods. Frequency and volumes of the waste streams were recorded to evaluate an input-output balance.

Laboratory analysis of acid whey and rinsing waters has been carried out to identify the chemical composition and physical properties of these wastes.

Recycling and reducing volumes of waste

A recycling plant for spent rinsing water and discarded cleaning solutions was developed and evaluated. Nanofiltration membrane technology was employed to utilize and recycle the process water and cleaning solutions. Concentrated residues which result from the filtration of alkaline dairy CIP wastewater were investigated. Treatment of these high strength wastes by anaerobic digestion is novel and should be investigated to improve the sustainability of the process and for its potential as a source of alkalinity and energy.

An alkaline dairy CIP wastewater was characterized and analysed. A synthetic CIP wastewater was then developed and evaluated for later use in laboratory experimentation.

The filtration plant was operated with varying cycle factors to produce retentate samples of different organic concentration. Permeates and retentates from the nanofiltration processes were also analysed.

Investigate anaerobic treatment processes to treat the waste streams

Anaerobic digestion has identified as the most promising technology for treatment of high strength organic liquid wastes. Therefore, it was intended to make the process suitable for treatment of the high strength wastes from fresh cheese production.

Suitability of anaerobic digestion with respect to a pre-treatment of the alkaline wastewater by nanofiltration was investigated to specify a blend of acid whey and alkaline wastewaters optimal for an anaerobic co-digestion. A series of biodegradability tests give information about degradation and also about the kinetics of mixtures from acid whey and alkaline wastewaters.

Appropriate reactor types were selected and operated at laboratory scale. Treatment efficiency, gas production, and process parameters were measured and monitored.

The process was operated and acclimatised with acid whey as its basic substrate. The organic loading rate (OLR) in each experiment was increased stepwise to determine the maximum operational loading rate. Optimized dosage control of CIP alkaline recovery residues was implemented. The effect of this composite wastewater on reactor performance was investigated.

To enhance the process stability of anaerobic acid whey treatment, a novel co-digestion of acid whey and CIP alkaline recycling residuals is proposed. The ability to utilize the whole volume of residues from alkaline CIP cleaning arising from the quarg production process in an anaerobic reactor operated with acid whey was tested. The alkaline CIP residuals can vary in strength (COD concentration) as a result of recovery operations and sodium hydroxide concentration depending on requirements of the plant cleaning. An enhancement of the anaerobic process is postulated by means of additional buffer capacity due to alkaline and reduced sodium toxicity due to the calcium content of the whey. The use of this novel treatment method will allow higher loading rates to be reached in anaerobic whey digestion. The evaluation of co-digestion of acid whey with residues from alkaline cleaning is the main objective of the thesis.

4. LITERATURE REVIEW

4.1. Whey

4.1.1. Survey on Whey Related Problems at Dairies

A survey about whey handling and disposal at dairies was carried out over the project time. In personal communications a set of non official information about the problem to manage large volumes of whey was collected. Mr. Hansen, manager of Nordmilch AG in Zeven, one of Germany's largest dairies, stated in an Interview that, due to the tendency to larger facilities dairies have a high output of whey at a local spot. However, the capacity of classic whey disposal such as animal feeding is limited (personal communication, interview during the Ahlemer Fachtagung conference, Hannover, Germany, 30th - 31st May 2005). Corresponding to this Mrs. Meier, responsible for whey marketing and disposal at Danone GmbH, reported that after expanding the capacity of their facility in Hagenow, Germany the disposal method based on animal feeding, was no longer viable, due to the increased output (personal communication, telephone call, 25th April 2002). Mr. Bergmann, manager at Humana Milchunion explained that the facility in Georgsmarienhütte is still able to sell their whey to local farmers even after expanding their production (personal communication, interview during the meeting of the AUBIOS research group with the Humana Milchunion dairy management and operating staff held on 12th March 2002 in Georgsmarienhütte). Also Mr. Gorzky, manager of Weißenfels, a facility of the Frischli Milchwerke GmbH is able to sell the entire volume of whey produced in his facility. (Personal communication, interview at a meeting of AUBIOS research group members with Mr. Gorzky, manager of Weißenfels/Frischli, Hannover Ahlem, 07th March 2002). However, Georgsmarienhütte is located in a region with a large population of farm animals and Weißenfels located in Hameln is a relatively small facility. Both, Mr. Bergmann and independently also Mr. Gorzky stated that they are concerned about epidemic animal diseases like bovine spongiform encephalopathy (BSE) or aphthous fever (foot and mouth disease) which lead to local emergency mass slaughter and endanger the reliability of this disposal method. Disposal problems can arise, even at smaller dairies. Mr. Kohlhage, manager of the Molkerei Hüttenthal reports that the dairy recently need a certificate of quality if they want to sell their whey to a farmer that uses a quality management system. (Personal communication, e-mail, 20th March 2006)

4.1.2. Definition and Characteristics of Whey

Usually whey is produced during cheese production. It is the serum obtained from milk by separating the casein content, either complete or partly, as defined in the German Milk Products Ordinance (Bundesrepublik Deutschland, 2010a). Whey received from enzymatic

coagulated cheese sorts, is called sweet whey or cheese whey. Acid whey arises when coagulation caused by lactic acid as in the curd cheese process or from heat and acid protein precipitation. Cheese whey and acid whey differ essentially in their pH- values, but also in composition. The main components of whey are lactose/lactic acid, protein from casein or whey protein, and milk fat. The composition of acid whey from curd cheese production varies depending on raw milk characteristics, production method and protein separation efficiency. Composition and properties of cheese and acid whey sorts, compared to whole and skimmed milk, is presented in Table 1, the data is taken from Kessler (1996). Additional information on the mineral components of whey was reported by Sienkiewicz (1990) presented in Table 2.

Table 1 Composition and properties of acid whey compared to cheese whey and other milk products.

	Cheese Whey (cheddar)	**Acid Whey**	**Whole Milk**	**Cream (30 % fat)**
Water [g·L^{-1}]	938	954	875	621
Dry matter [g·L^{-1}]	62	56	125	379
Density [kg·L^{-1}] (15 °C)	1.026	1.024 - 1.025		
Protein [g·L^{-1}]	7.5	7.5	36	28
Total N [g·L^{-1}]	1.2	1.2	6000	4
Fat [g·L^{-1}]	0.5	0.5	35	315
Lactose [g·L^{-1}]	47	40	47	33
Lactic acid [g·L^{-1}]	-	7	-	-
pH	6.10	4.70		
Ash [%]	7	8	7	3

The main content of all whey sorts is, aside from water, lactose. In acid whey, part of the lactose is converted to lactic acid by lactic acid producing bacteria during fermentation as in the following equation:

$$\overset{lactose}{C_{12}H_{22}O_{11}} + \overset{water}{H_2O} \xrightarrow{\text{Fermentation by acidifing bacteria}} 4(\overset{lactic\ acid}{C_3H_6O_3}) \qquad (4\text{-}1)$$

The lactose/lactic acid ratio depends on the fermentation and represents the resulting acidification level. The acidic dissociation value of lactic acid is at pKa = 3.86 and thus it is

partially dissociated. The release of protons cause the lower pH of acid whey compared to cheese whey.

In equation 4-2 the proton release from lactic acid dissociation is presented in half structured form:

$$CH_3CH(OH)COOH \rightarrow CH_3CH(OH)COO^- + H^+ \quad (4\text{-}2)$$

Lactate is a strong anion, and thus lactate salts are derived from available cations, mainly calcium, potassium and sodium. Due to this effect acid whey contains more minerals and salts than cheese whey. Calcium removal by ionic bonding to lactate and drainage with the whey is desirable, since calcium is responsible for bitter taste and is therefore undesired in the quarg cheese.

Table 2 Mineral content of acid whey and cheese whey in $mg \cdot L^{-1}$.

	Cheese Whey (cheddar)	Acid whey
Total nitrogen	1 448	1 223
Phosphorus	412	649
Calcium	466	1 251
Potassium	1 455	1 485
Sodium	505	528
Chlorides (as NaCl)	2 195	2 092

The protein content is considerably higher in cheese whey than in acid whey, due to less complete coagulation in rennet based cheese making. Additionally, in the thermo quarg process a fraction of whey protein is yielded in the quarg.

4.1.3. Whey Utilization

Different kinds of utilization of the whey can be divided by the usage of the products. Thus products of whey can be food or food ingredients, feed for animal husbandry or intermediates for industrial non-food products.

4.1.3.1. Food Products

Acid and cheese whey can be consumed without further treatment. It is known to be healthy and useful as a therapeutic drink, recommended as early as 460 B.C. by Hippocrates (Kar and Misra, 1999). Furthermore whey beverages can contain fruit, vegetable

juices or other flavours, also fermented alcoholic whey beverages are produced. Common whey utilisation methods are based on membrane filtration to separate whey protein, lactose and minerals. Drying of whey or whey fractions prolongs their storage life, otherwise whey is very perishable. Whey permeates, concentrates and powder is used as supplement to various products in the food industry. For example, whey products are used in puddings, bakery products, noodles and other pasta products, in soups and sausages and also in sweets, ice cream, and numerous other products purchased by the food industry (Kessler, 1996). Lactic acid and other organic acids can be produced from whey by fermentation to be a valuable intermediate in food and non food industry. In the food industry lactic acid is used as acidifier, for example in syrups and soft drinks in the beverage industry or for preservation in bakeries and breweries (Sienkiewicz, 1990).

4.1.3.2. Animal Feed

Acid whey is a common supplement in many receipts for feed in cattle and piggery farming and also for pets. Fresh and raw acid whey can be used to soak dry feed. The use of whey as animal feed is a traditional disposal method for small scale dairies with local animal farming. However, modern large scale dairies with high whey output can not find enough customers without long distant transportation (Spreer and Mixa, 1998). Whey as animal feed was discredited by reports of pigs, suddenly dieing shortly after seeming completely healthy (enterohaemorrhagic syndrome EHS). Animal Health Online (2002) reports that incidence of EHS is frequent but not solely located in animal farms using whey as a feed supplement. The disease is also called "whey syndrome". However, Johannsen *et al.* (2000) stated that most of 86 investigated EHS fatalities occurred at farms with liquid feeding, but there was no evidence of an association with a particular feed. See also Section 4.1.1: *Survey on Whey Related Problems at* Dairies

4.1.3.3. Non-Food Products

There are numerous personal hygiene and beauty products based on whey or with whey ingredients. Lactic acid derived from whey fermentation is ingredient of various medicine and cosmetics, and it is used in different operations of the chemical industry *e.g.* for dyeing, printing, tannery procedures, in the utilization of silk, and for lactate based bio plastics, which can be recycled by composting. Examples of fermentation products are also the production of whey based surfactants, de-icers, and tensides. Some yeast cultures which are rich in oil can grow on whey and bio-fuel to be used in diesel engine cars can be produced from this "single-cell-oil". Biogas that is rich in methane and available for heating and power generation can be achieved from mixed culture anaerobic fermentation of whey.

4.1.4. Economic Consideration on Whey Utilization

Present day large scale dairy factories result from condensing of dairy groups wanting fewer business locations with higher milk utilization capacity on economic dues. A large size mixed product dairy produces up to 5 000 m^3 per day of sweet whey, acid whey, and whey permeates which are usually collected and utilized jointly. As dairies have an infra structure for food marketing like packaging, storage, logistics, and trade ways it can be assumed that it was tried to use as much of the milk components as possible for food production. But value adding from whey is hard because of the high energy consumption for separation of whey ingredients and especially for drying into powders. Value adding from acid whey is even harder because of high lactate and lower protein content. The low pH caused by lactate and, the characteristic acidic flavour of acid whey is not favourable in beverage production. Additionally the low pH causes problems when acid whey should be dried. In 2006 the Humana Milchunion in Georgsmarienhütte and also the Weißenfels dairy in Hameln, Germany sold their acid whey to local farmers. According to their managers, both dairies realise a price of 2.5 € per cubic metre of whey. This price can be used to evaluate and to compare treatment and disposal methods.

4.2. Dairy Wastewaters

4.2.1. Dairy Wastewaters from Cleaning Procedures

A cleaning procedure using a CIP system is a sequence of cleaning steps. At first the product has to be removed from the piping and vessels. Remains of the products after draining will be rinsed by a flush of cold potable water or by blowing the product out with compressed air. If possible the product should be collected and reused. Further sequence steps are carried out by alternately circulation of rinse water and cleaning agents through the production plant. The cleaning procedure is different for plants with or without heated parts. A circulation of acid solution can be omitted only when non-heated components have to be cleaned. The sequence step duration, temperatures and flushing velocity as well as concentration and composition of cleaning agents are often optimized to fit the cleaning task to meet the requirements of the connected production unit.

A complete sanitizing sequence, described by Bylund (1995), includes the following steps:

- Product removal.
- Rinsing with warm water (10 min).
- Cleaning by circulation of alkaline detergent solution (0.5 % (m/m) to 1.5 % (m/m), 30 min, 75 °C).
- Intermediate rinsing with warm water (5 min).
- Cleaning and Disinfection:
    - either, for heated parts of the plant:
        - Cleaning by circulation of acid solution (0.5 % (m/m) to 1.0 % (m/m), 20 min, 70 °C).
        - Intermediate rinsing with warm water (5 min).
    - or, for tanks, piping and other non heated equipment:
        - Disinfection with hot water (5 min, 90 °C to 95 °C).
- Post-rinsing and cooling with cold water. (8 min).

Cleaning process intervals are influenced by particular plant configuration. Generally, fermenter vessels have to be cleaned before starting a new fermentation process. A higher frequency of cleaning might be necessary for parts of the plant, *e.g.* the quarg separator needs an intermediate cleaning to recover efficiency if one or more of the separator nozzles, which are approximately 0.5 mm in diameter, are blocked by soil or precipitates. Quarg production lines are divided into sections which are separately connected to the CIP plant. Process tanks, quarg separators and heated equipment *e.g.* plate heat exchangers are common sections. This provides intermediate cleaning procedures that can be performed without emptying the whole plant and enables cleaning steps that are intended to be used only in this particular part of the plant.

A final rinsing will remove remaining cleaning agents. The water must have drinking water quality to avoid recontamination. For pre- and intermediate rinsing the used water do not need to fulfil these high quality requirements. Water from a final rinsing can be stored in process tanks and can then be used for this pre- or intermediate rinsing (Norddeutscher Genossenschaftsverband e. V. Kiel, 1993).

4.2.2. Rinse Water and Product Loss

The thickened milk will be removed from the surface of the acidification tank with cold potable water by rinsing. This dilution does not interrupt the separator or reduce the quarg quality. Therefore only a little of the product will be lost previous to the separator. The removal behind the separator is more difficult due to high viscosity of the quarg. Pumping in drinking water causes a short-circuit flow in the centre of the pipelines. The

quarg firstly adheres to the tube wall and will decay with further rinsing. To avoid a diluted product the fillings have to be stopped before this cleaning step starts. However, the diluted quarg can be collected in a process tank. It is common practice to reprocess these valuable proteins with the next acidified charge. This cost-efficient operation is often called 'rework' of product residues. To avoid high dilution, the removal can be controlled by turbidimetry. Products containing herbs or fruit can not be reworked. These products were discharged into the sewer if diluted with flushing water. The European Commission (Garcilaso, 2006) recommends the usage of 'pigging' in pipelines. These pressure driven solid sliders were used to shove the product from within the pipeline and to separate the product from flushing water. This will reduce the loss of valuable products and reduce water and wastewater costs.

After removing the product, residues of it are still adhering to the surfaces of the plant. A further rinsing needs to be done to improve the usability of following detergents for a longer time. For this purpose recycled water can be used. After several loops, the pre-rinsing water is usually discharged into the sewer.

By cleaning the plant as described above the cleaning solution were enriched with caramelized organic soils, fats, protein as well as bacteria and germs. The organic load will be decomposed to smaller molecules by hydrolization. The colour become dark and the solution started having a bad odour. Alkaline solutions tend to build up foam. The solution must be changed. This can be done by different procedures.

- Complete change

After reaching the maximum load of soil the solution will be removed completely by neutralizing and discharging the process tank. The state of soil can be controlled be conductivity. No recycling of the solution will be done when this method is used.

- Steady renewing

Heavy charged portions from the first rinsing were discarded. The intended loss is then replaced by fresh prepared solution. Daily sharpening of the solution with fresh acid/leach improves the constant cleaning effect of the cleaning agent. The quantity of solution to be added corresponds to the amount that is lost in the cleaning loop by overflow and removal of solids with a separator. At regular intervals settled precipitates must be removed from the process tank

Depending on the income of COD a change of the solution is necessary after 10 to 100 cleaning intervals. It is common praxis to change the solution every 14 days.

This is similar to the time needed to renew an amount corresponding to the volume of the process tank in the steady procedure.

4.2.3. Basic Alkaline Cleaning Chemicals

Basic alkaline cleaning agents like sodium hydroxide and potassium hydroxide, sodium metasilicate, sodium carbonate or sodium phosphate were used to break down protein and other organic deposits. Alkaline detergents should also have an emulsifying effect. Alkaline cleaning procedures use these basic alkaline chemicals in a concentration range of (0.2 to 2.0) % (m/m) (Kessler, 1996). The most common alkaline cleaning chemical in the dairy industry is sodium hydroxide (Romney, 1990; Kessler, 1996; Coldewey, 2004).

4.2.4. Basic Acid Cleaning Agents

Rinsing with acidic solvents is used to remove mineral deposits like scales which were referred to as "milk stone" in dairy plants. These mineral deposits develop preferably on hot surfaces in contact with milk, *i.e.* in heat exchangers and pasteurizing equipment. Cleaning a quarg production plant can mostly be done without any acid cleaning. Only the preheaters operated in the thermo quarg process at high temperature need acidic cleaning. In the dairy industry, inorganic and organic acids are used, most common are nitric acid, phosphoric acid or sulphamic acid. Widely used organic acids are hydroxyl acetic acid, gluconic acid, and citric acid. (Romney, 1990)

4.2.5. Alternative Cleaning Agents

Beside alkaline and acidic formulations, enzymatic cleaners are also known. These cleaners are often mentioned as environment friendly *e.g. in Olsen (2000)*. Eide *et al.* (2003) carried out a life cycle assessment (LCA) to compare new and commonly used cleaning-in-place methods used in dairies including different disinfection strategies. The study aimed to examine the environmental impact of CIP methods. Eide *et al.* (2003) drew the conclusion, that a one phase alkaline cleaning combined with a chemical acidic disinfection has the lowest environmental impact compared to the conventional alkaline/acidic cleaning method using hot water disinfection as described in detail in Section 4.2.1 and also compared it to a procedure using an enzymatic cleaner and chemical disinfection. However, the valuing of the environmental impact of chemicals and their toxicity is not standardized and can therefore be emphasised differently in current life cycle assessment models (Guinée *et al.*, 1996).

4.2.6. Additives

The requirements to make a cleaning agent suitable for the use in an automatic cleaning system can not be fulfilled by only using basic chemicals. Additives enhance specific fea-

tures and properties and therefore are used to create the cleaning agents. Different additives were used to meet the requirements listed above.

Sequestering Agents like sodium polyphosphates or ethylenediaminetetraacetic acid (EDTA) were used to avoid scaling and inorganic deposits by forming a complex with Ca^{2+} and Mg^{2+} ions. The result is a reduction of water hardness. Sequestering agents enhance the dispersing power and help remove soils from surfaces. In the dairy industry EDTA is particularly used for the removal of milk stone, for the cleaning of filtration membranes, and for bottle washing plants (Gekeler, 1999). EDTA can not be removed from wastewater by standard procedures. Due to its ability to remobilize heavy metals from soil and its accumulation in the aquatic eco-system, the use of EDTA should be avoided. A joint statement on the reduction of water pollution by EDTA was agreed in 1991 by the German Industry and the German Federal Ministry for the Environment, Nature Conservation and Nuclear Safety and other German authorities (Bundesrepublik Deutschland, 1991). Until 2001/2002 a significant reduction of approximately 43 % in discharge of EDTA was achieved (Lehmann, 2004).

Surface active agents (short form: surfactants) are a large group of chemicals, characterised by their ability to lower the surface tension of a liquid in which they were dissolved. The essential property of these chemicals is their constitution with a hydrophilic portion on one end and a hydrophobic section on the other, which is often a long alkyl chain. Surface active agents are classified into ionic, anionic, amphoter and non-ionic in order of the charge of their hydrophilic functional groups. A large variety of surface active agents are commercially available and the formulation and functionality of them can vary to specific needs of the application. In the context of cleaning applications they are mainly used to increase the wetting power, to solve hydrophobic matter like fats and oils (emulsifying) or to enhance the dispersion of soils in the solution. Some surfactants have additional effects like disinfective properties (Romney, 1990).

Foaming can cause problems in automatic CIP systems. To avoid this, anti-foaming agents can be added. Acids are often highly corrosive, even to stainless steel. Therefore corrosion inhibitors are needed especially when using acidic cleaning agents. Other usual additives are abrasives, stabilizers, softeners, and oxidisers.

4.2.7. Disinfection

When required, a disinfection step can follow the cleaning with alkaline and acid cleaning agents. Besides physical disinfection with hot water or steam, chemical disinfectants can be used. Disinfectants loose their effect after a while. Therefore a fresh batch has to be prepared for every disinfection cycle. Schmidt (1999) stated that disinfection by high tem-

perature application is more effective and no wastewater pollution, no residues of chemicals in the plant and no corrosion arises from the use of water or steam. If active disinfectants are discharged into the sewer, they can cause damage to biological wastewater treatment plants (Young, 2001). Often a separate disinfection step can be omitted when the sanitizing effect of the preceding cleaning is sufficient. Disinfection agents can be classified into alkaline, neutral and acidic disinfectants.

Quaternary ammonium compounds (QAC) are kinds of surfactants (see also Section 4.2.6) and are used as neutral disinfectants. However gram-negative bacteria can develop resistance against some QAC. As quaternary ammonium compounds cause heavy foaming, an anti foaming agent must be added. Hydrogen peroxide is also a neutral disinfectant. It is used for its oxidizing effect often in combination with per acetic acid. The advantage of hydrogen peroxide is that no residues arise in wastewaters. An iodophor is a formulation consisting of iodine and a solubilizing agent *i.e.* a strong mineral acid. Iodophor releases free iodine when in solution and has a very strong oxidizing and an excellent disinfection effect. However, iodophors are very corrosive, even against stainless steel. Halogen carbonic acids are compounds of chlorine, bromine or iodine with organic acids and were always used in combination with sulphuric or phosphoric acid (Schmidt, 1999). Active chlorine is used in combination with alkaline and has an additional cleaning effect. The principle of disinfection with chlorine is the oxidizing effect. It is approved for a broad spectrum of microbes and no resistances are known (Schmidt, 1999).

4.2.8. Wastewaters from Disinfection

Disinfection is intended to inhibit microbial life. Chemical disinfection agents are usually discharged after use, due to their limited durability and a loss of effectivity after usage. Nevertheless emissions of active agents released with the wastewater can reach local or municipal wastewater treatment plants and damage biological wastewater treatment processes. Young (2001) examined the effect of different commercial disinfectants on biological wastewater treatment processes and found Quaternary ammonium compounds (compare: Section 4.2.7) and alkyamines to be most toxic. Anaerobic processes and the denitrification stage of advanced nutrient removal processes are most affected.

4.3. Anaerobic Biotechnology

Anaerobic digestion is a microbial decaying process that occurs in nature in manure, in the gastrointestinal tract of cows, in river bed sediments, in marshes and swamps and in other niches in the absence of oxygen but with available organic matter. The principle of biological wastewater treatment by anaerobic digestion is to degrade and mineralize the organic compounds present in the wastewater. It aims to settle out the suspended solids while reducing the organic carbon to methane and carbon dioxide. The advantages of anaerobic digestion in wastewater treatment are often compared to aerobic processes. A strong argument for it's use in treatment of dairy wastewater is the comparison of the net energy balance, in which the costs for aeration in the aerobic processes is juxtaposed to the energy output from anaerobic processes due to methane generation. Nevertheless, effluents from anaerobic digesters usually do not provide the quality to discharge them directly into receiving water courses. Aerobic, anoxic and anaerobic processes are often combined to optimise wastewater treatment. The emerging importance of anaerobic digestion besides treatment of wastewater and power generation from renewable recourses is also due to recent anaerobic biotechnology development. Beside municipal and Industrial wastewater treatment anaerobic biotechnology is currently also used for treatment of solid state organic wastes (Chynoweth *et al.*,1991; Vandevivere *et al.*, 2002, Mohr and Stiller, 2008), for treatment of wastewater containing xenobiotics, for example adsorbable organic halogens (AOX), phenolic compounds, high sulphur concentrations, dechlorination of pentachlorophenol (PCP) to phenol or decontamination of TNT explosive in soils reviewed by Verstraete and Vandevivere (1999). The latest research has broadened the anaerobic biotechnology to fermentative hydrogen gas production (Hussy *et al.*, 2004; Yang *et al.*, 2007). Anaerobic biotechnology is used in advanced development of biological nutrient removal processes reviewed by Ahn (2006). Besides progress in the research of biological technology and process engineering the mathematic modelling of anaerobic processes has entered a new level since the release of the Anaerobic Digestion Model No. 1 (ADM1). The model, designed to create numerical simulation of anaerobic processes, was developed by the IWA Task Group for Mathematic Modelling of Anaerobic Digestion Processes (Batstone *et al.*, 2002)

Anaerobic industrial, agricultural and municipal waste and wastewater treatment is still developing as a topic of current research. Moreover, due to the generation of methane and hydrogen from the process, anaerobic digestion will also play an important role in future energy generation from renewable resources. Since commencement of the German Act on Granting Priority to Renewable Energy Sources in the year 2000 (Bundesrepublik Deutschland, 2010b) with its guarantied recompense of electricity feed in, a strong

upturn in biogas plants, fed with energy crops for the purpose of energy generation, proceed. Ott and Horbelt (2007) gave the number of facilities and the installed electric power of biogas plants in Germany and its development for the last ten years. In a report published by the German Biogas Association they reported that 3 500 biogas facilities were in operation in Germany in 2006. A significant increase in power generation by anaerobic digestion was also shown by Ott and Horbelt (2007).

4.3.1. Historical Background

Johann Baptist van Helmont (1577 - 1644) a physicist and (al)chemist invented the term "gas" and investigated natural gases. Marchaim (1992) reported that van Helmont collected flammable gas from marshland and Möller (2004) stated that he recognized carbon dioxide as a product of fermentation and that he moreover recognized the existence of hydrogen, methane and sulphuric dioxide. Also Alessandro Volta (1745 - 1827) investigated natural gas emissions from marshlands. Volta (1778) concluded that the gas originates from decay of organic matter and that the amount of gas, arise from those sites, is related to the mass of organic matter being digested. Popoff (1875) examined sludge, from river sediment in the estuary area of a sewer, in laboratory batch tests and showed that the production of methane gas from these tests was derived from microbiological activity. Also Hoppe-Seyler (1886) recognised the microbial nature of the methane forming processes. Soon Fermentation experiments were also carried out by Ulysse Gayon (1845 - 1929), where he digested horse manure in a closed fermenter (Gayon, 1884). The large amount of biogas achieved from this experiment inspired his teacher, Louis Pasteur to come up with the idea to use the gas for heating or lightning purposes. Early applications of anaerobic wastewater treatment plants were septic tanks, developed by Cameron (1896), which are covered wastewater pits. The tanks were intended to allow settling of suspended solids. By covering the tanks, an anaerobic milieu develops and the production of gas containing methane sets in. Biogas from septic tanks was used to fuel street lamps in 1895 in Exeter, United Kingdom (Marchaim, 1992). A digester in a leper colony in Bombay, India in 1897 is another early application, reported by Eder and Schulz (2006). The gas, produced in an anaerobic digester, was used for lightning and later to fuel a combustion engine for electric power generation. Microorganisms from a fermentation broth which form methane from hydrogen and carbon dioxide were first isolated and classified by Omeliansky (1902). In the first third of the 20th century, the fundamentals of methanogenic anaerobic microbiology were studied, *e.g.* by Söhngen (1906), Barker (1936). Comprehensive laboratory studies of sludge digestion where carried out by Buswell and Neave (1930). A major step in the understanding of the nature of the microbiology of anaerobic digesters was the discovery of the syntrophic symbiosis of some bac-

terial species in anaerobic digesters. Bryant *et al.* (1967) found that the *Methanobacillus omelianski* culture essentially contain two bacterial species. Further developments in the microbiology of anaerobic digesters will be reviewed in Section 4.3.2.

The industrial usage of anaerobic digestion is closely connected to wastewater treatment. With the rise of industry the emerging need of wastewater treatment was recognised. Cameron (1896) reported the development of septic tanks and wastewater treatment using microorganisms. Karl Imhoff developed an improved version of the septic tank with a settling area and a separate anaerobic section as a lower compartment. The system was first used in Essen- Recklinghausen in 1906 to treat wastewater before releasing it into the river Emscher in Germany's industrial region Ruhrgebiet (Imhoff, 1926). This so called Imhoff tank was widely used in Europe and America (Buswell and Neave, 1930). Understanding the role of the process temperature leads to reactor designs with separate vessels for settling and anaerobic digestion. The anaerobic department was then heated. Subsequent developments involved mixing and reseeding of the digestion unit and aeration of the settling unit. The development of first biomass retention systems in the anaerobic contact process tagged the beginning of modern anaerobic digestion technology.

The fundamentals of industrial anaerobic digestion of organic matter was summarised by McCarty (1964). McCarthy placed emphasis on the future role of anaerobic digestion and anticipated its increased importance for industrial wastewater treatment. This prediction came true, and will likely be true hereafter.

The series by McCarty (1964) was divided into four parts on anaerobic biotechnology and these are still main topics of research in anaerobic digestion. These fundamental topics are reviewed with main focus on treatment of dairy wastewaters and whey digestion in the sections: **Microbiology and Biochemistry** (4.3.2), **Environmental Requirements and Control** (4.3.3), **Toxic Material and their Control** (4.3.4) and **Process Design** (4.3.5). Two further topics in this review represent recent development on **Process Kinetics and Mathematic Modelling** (4.3.6), and **Assessment of Anaerobic Biodegradability** (4.3.7).

4.3.2. Microbiology and Biochemistry

For microbial life it is necessary to acquire energy and material for cell grow. The microbial degradation of a complex molecule to a smaller one can only be done by the microorganisms if there is a yield of energy to make microbial activity possible. Microorganisms use enzymes as biological catalysts to lower the necessary reaction activation energy. Enzymes are proteins with a specific shape and different enzyme groups are involved in the partial steps of the metabolic reaction. In the enzymatic reaction a specific enzyme is coupled to

a substrate molecule and forms a substrate-enzyme-complex. After the reaction the products are released from the complex and the enzyme can couple with the next substrate molecule. Microorganisms obtain their energy from redox reactions. When high energy bonds of a substrate are broken energy is released in the form of electrons. The degradation of a substrate is performed in a series of balanced redox reactions were protons and electrons are transferred by carriers and functional groups. Two carriers, which are used by almost all microorganisms, are *nicotinamide adenine dinucleotide* (NAD^+) and *nicotinamide adenine dinucleotide phosphate* ($NADP^+$). These are able to transport two electrons and one proton by performing reversible oxidation and reduction reactions. This energy can be stored as a high energy bond in a particular molecule, the *adenosinetriphoshate* (ATP). Through the release of a phosphate group, the ATP changes to the low energy form, *adenosinediphosphate* (ADP) and thus the binding energy of 30 KJ can be used by the cell. Free energy from a metabolic degradation of a substrate molecule can again be stored by the ADP molecule by the re-coupling of a phosphate group. For the completion of the metabolic breakdown the cell need a final electron acceptor which will be released from the cell to keep it from becoming charged. The anaerobic fermentation is incomplete respiration. Beside new cells and carbon dioxide, a fermentation product is released, for example mixed acids, alcohol or methane. Aerobic organisms yield a Gibbs-energy of -1 100 $kJ \cdot mol^{-1}$ and synthesise 38 ATP from the consumption of glucose. Utilizing the same substrate, anaerobic fermentation only yields -58 $kJ \cdot mol^{-1}$ corresponding to 2 ATP. The grow rate of a microorganism relays on the energy obtained by its metabolism. Thus, the production of surplus sludge in the (aerobic) activated sludge processes is 10 times higher compared to anaerobic digestion.

The modern view of the microbiology of anaerobic digesters is reviewed by several authors and published in numerous reference books, *e.g.* Stafford *et al.* (1980), Mudrack and Kunst (1991), Marchaim (1992), Gerhardi (2003), Bischhofsberger *et al.* (2005). The authors describe anaerobic digestion as the biological degradation of complex particular organic composites by anaerobic microorganisms. In an anaerobic digester a broad spectrum of syntrophic microorganisms is involved in the degradation process. A community of bacteria generates a metabolic food chain were a group of microorganisms grow on more complex substrates while another group of microorganisms is specialized to the metabolites of the first group.

4.3.2.1. Anaerobic Food Chain

A sequence of physicochemical and bio-chemical reactions causes a breakdown of complex substrates. A methanogenic anaerobic fermentation process is performed by a community of well balanced microorganism groups. The groups are specific to substrates and perform single degradation steps. In the process, the products of degradation steps are substrates to bacteria of another group, following in the chain. The main metabolic pathway of typical methane fermentation is illustrated in Figure 5.

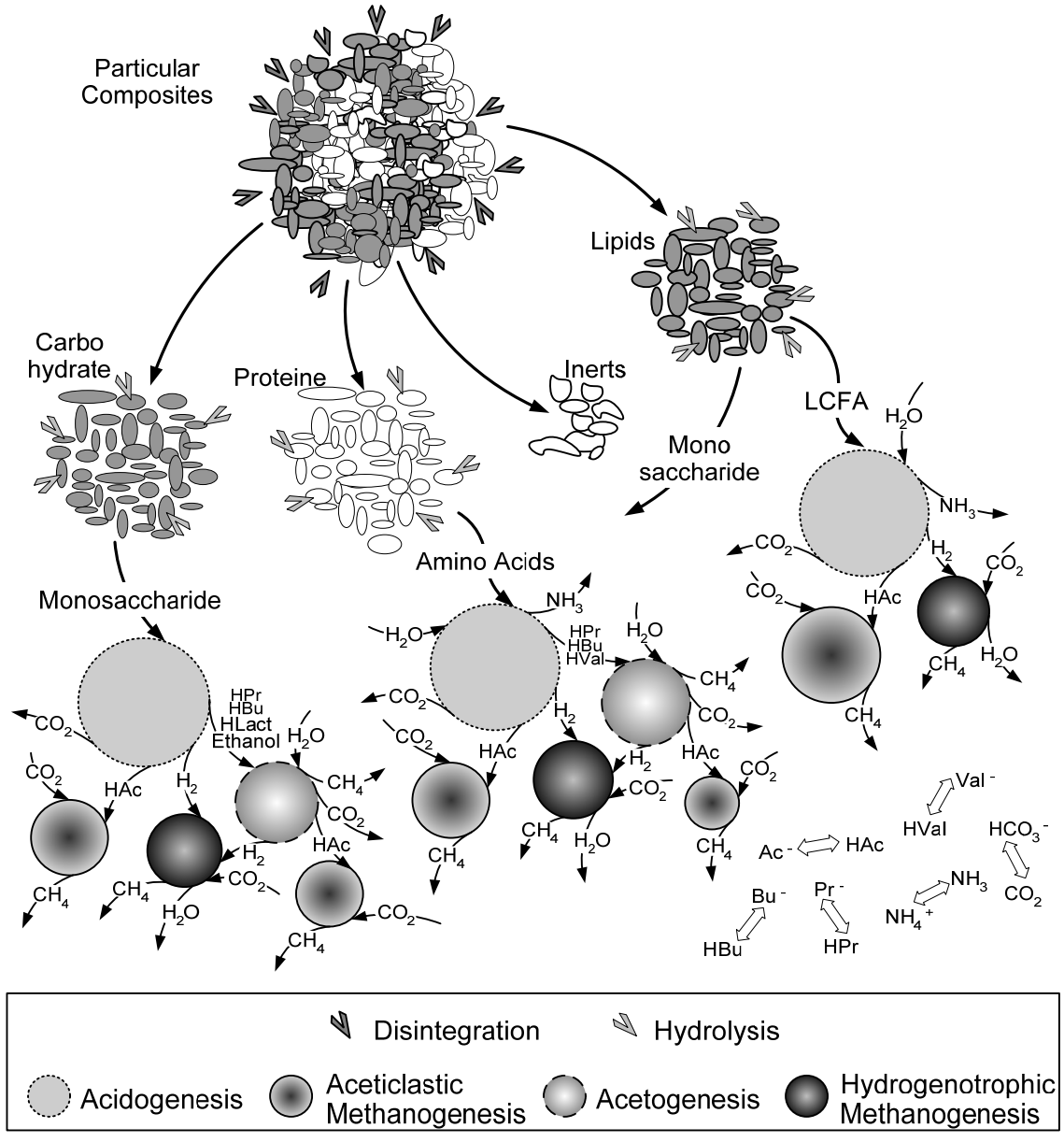

Figure 5 Metabolic pathway of particulate composites. The spotted regions represent a hypothetical composite particular material containing carbohydrate, proteins, and lipids each of 30%. A 10% content of inert material is also assumed. The circles depict biochemical processes in which solved substrate molecules were converted to gaseous end products. The area of the dots represents the COD flow of the processes and is not necessary equal to the mass or volume of the involved microorganisms.

The main metabolic pathway of a particular composite matter containing carbohydrates, proteins and lipids is via intermediate products mainly acetic acid and short chain volatile fatty acids and/or alcohol. Acetic acid can be consumed directly by methanogenic bacteria while other intermediate products must be further degraded before conversation to Methane. Beside acetic acid, hydrogen is also produced on the degradation of short chain fatty acids. A group of methanogens grow in a tight symbiosis with organic acids degrading bacteria. Hydrogenotrophic methanogens produce methane from consumption of hydrogen and carbon dioxide. As the microbial community is very diverse, minor reactions and different pathways can occur, especially when unusual conditions in a digester favour other bacteria than those of methane fermentation. Simplified, the enzymatic and biological activity in the anaerobic food chain can be described in a four step process as follows:

I. Disintegration and Hydrolysis

Bacteria can not consume particulate matter. The bacterial consortia use exoenzymes to initiate and accelerate the disintegration of composites, the solubilisation and the decomposition of organic polymers to make the substrate available to microbial consumption. Substrates are only available when they are soluble and small enough to enter the cell wall. A variety of specific enzymes are produced and released into the environment to break down the substrates to this size. During the disintegration the substrates, complex carbohydrates, protein and lipids will be solubilised. Extra-cellular enzymes are also involved in hydrolysis, a further break down of macro molecules. During the hydrolysis complex carbohydrates were degraded to simple sugars, complex proteins to amino acids and complex lipids to long chain fatty acids (LCFA).

II. Acid forming

A consortium of acidifying bacteria with a high diversity is responsible for the consumption of simple sugars, amino acids and fatty acids derived from their enzymatic activity. A broad spectrum of more or less oxygen tolerant bacteria which can be specialized to single substrates or flexible to consume a variety of substrates are usually present in anaerobic digesters. Products of bacteria involved in this fermentation step are acetic acid, butyrate and other volatile fatty acids (VFA), ethanol, methanol and other alcohols, and succinate, formate or lactate and other components of minor importance. A possible pathway of lactose acidogenesis is the fermentation to lactate which can only be further degraded via butyrate to acetate. Kisaalita *et al.* (1989) used radioactive tracers to reveal the predominant carbon flow routes and found that the direct conversation to butyrates dominate the lactose degradation in an anaerobic digester.

III. Acetogenesis

Only very few products of the acidification step can be consumed directly by methane producing bacteria, *i.e.* acetic acid, formate and hydrogen. Other intermediate products of the acidogenic fermentation *e.g.* propionate, butyrate, alcohols, aromatic compounds require a further fermentation step prior to methanogenesis. However, the conversation of these components is endergonic at standard conditions (pH 7.0, pH_2=0). Thauer *et al.* (1977) described the energy conversation of various reactions in chemotrophic anaerobic bacteria expressed as free Gibbs energy ($\Delta G'_0$). The conversation of the relevant components to acetic acid, hydrogen, and carbon dioxide becomes thermodynamically favourable (negative) if the hydrogen partial pressure is at low levels *e.g.* below 10 Pa for propionic acid oxidation to acetate (Harper and Pohland, 1986). The required low levels can not be maintained when the acetogenic bacteria have to release the hydrogen into an intermediate pool. Acetogenic bacteria depend on a hydrogen scavenging partner to release and transfer the hydrogen directly. This requires a dense contact of the involved species *e.g.* in flocs, biofilms or granules. A symbiotic cooperation of strains by interspecies hydrogen transfer were both partners depend on each other is called syntrophic relationship (Schink, 1997). The hydrogen consuming syntrophic partner of acetogenic bacteria can be hydrogen oxidising methanogens, however in the presence of sulphate in the substrate hydrogen sulphide producing sulphate-reducers compete with the methanogens (Gerardi, 2003).

Acetogenesis is often the rate limiting step in the treatment of easily hydrolysed wastewaters (Yang and Guo, 1991). Additionally, acetogens are relatively sensitive to pH, ORP, and toxic or inhibitory substances

IV. Methane forming

Methanogens are a diverse group in the kingdom of the Archaea. They are of the oldest organisms known. Methanogenic bacteria are strict anaerobes and sensitive to oxygen. Their ability to grow on different substrates is very limited (Mudrack and Kunst, 1991; Bischofsberger *et al.*, 2005; Gerardi, 2003). Acetate, hydrogen, methanol and formate are consumable by methane forming bacteria. Additionally, Methanogens are very sensitive to their environment. Only a narrow band of pH and oxidation-reduction potential is suitable for methanogenic activity. Methane forming is the last step in the degradation of organic matter. Mainly two groups of methane forming bacteria are involved in the typical anaerobic food chain. A group that converts acetic acid to methane and carbon dioxide and another group that converts hydrogen and carbon dioxide to methane. The consumption

of hydrogen lowers the hydrogen partial pressure. This group grows therefore in a tight symbiosis with the hydrogen producing acetogenic bacteria.

Industrial anaerobic bioreactors for wastewater treatment are mixed cultures and they are usually optimised to degrade organic substances and break them down to inorganic matter and methane (Speece, 1996). Favoured conditions are those which allow a balance between acidifying, acetogenic and methane producing bacteria.

4.3.3. Environmental Requirements and Control

Beside the symbiosis of the microorganism groups described in the metabolic pathway the involved species stand in competition for substrates, nutrients and space. The established population the presence and concentration of intermediates and end-products of the process depend on composition and distribution of substrates, and also on environmental conditions that are more or less favourable for single species and syntrophic co-cultures. Industrial anaerobic digesters are designed to provide conditions to enable a process optimal for the desired biocoenosis. The most relevant environmental conditions needed to achieve stable methane fermentation in an anaerobic digester are described as follows:

4.3.3.1. Temperature

Microorganisms can be classified by the temperature range of their highest activity. However the ranges are not standardised, the temperatures given in the literature varies slightly. Microorganisms are classified as being psychrophilic (below 20 °C), mesophilic (20 to 42) °C, or thermophilic (above 50 °C). While the existence of psychrophilic methanogens is in discussion (van Lier *et al.*, 1997), specific methanogens of the mesophilic and thermophilic temperature range are known. Fair and Moore (1937) investigated the activity of a methane fermentation as a function of the temperature. Much later, van Lier *et al.* (1997) found similar results using modern high-rate granular sludge digesters. The findings are widely published in informative and scientific articles and in reference books often without mention of the sources. Thereafter the optimal temperature of a mesophilic digestion is (35 to 37) °C and in a thermophilic fermentation a temperature of 55 °C was found to be most efficient. However, the anaerobic microbial community is very diverse and disintegration, enzymatic hydrolysis and equilibrium reactions are temperature dependent and specific to different substrates. However, the efficiency of an anaerobic digester is strongly affected by adjusting the temperature to optimal range and keeping it constant over the whole volume. Furthermore, an adoption of the microorganisms to a given, constant temperature is possible (Ahn and Forster, 2002). Full scale biogas plants, digesting crops and manure are often operated in the mesophilic range, but at "suboptimal" temperatures in the range of (42 to 44) °C due to self heating as reported

by Lindorfer *et al.* (2008). However, in a large number of monitored biogas plants in Germany, negative effects of operation in this temperature range to the efficiency have not been observed (personal communication, interview with Christoph Martens, MT-Energie GmbH &Co. KG, Rockstedt Germany, 12th. March 2008). It can be assumed that the optimum temperature of a large scale digester depends on multiple factors. Beside the influence of the temperature dependency of the grow rate, operational conditions *e.g.* the distribution and equalisation of heat in the fermenter, fluctuations influenced by season and digester insulation such as substrate specific temperature dependent kinetic rates may influence the reactor performance at a specific temperature.

4.3.3.2. Volatile Fatty Acids, Alkalinity and pH

The activity of hydrogen ions in a digester, expressed as the pH value, is an important operational parameter of the anaerobic methane fermentation. Ranges of pH 6.8 to pH 7.5 usually prevail in stable operated anaerobic digesters (Kapp, 1984). In the anaerobic food chain the acetogenic bacteria and the methanogens are the most sensitive groups regarding the pH range. Acidifying bacteria are more tolerant to low pH values. (Moosbrugger *et al.*, 1993) At values below pH 6.2 the methane production is inhibited and at values below pH 5.0 practically no methane fermentation takes place (Stafford *et al.*, 1980).

The pH of the fermentation broth depends on a dynamic equilibrium of different buffer systems and the concentration of acids and alkaline deriving from the influent and from intermediates produced during the process. Volatile fatty acids (VFA) are the products of acidifying bacteria. The buffering capacity of a digester stabilise the pH at a certain level and enable the methanogenic bacteria to withstand temporarily higher production of VFA. The pH decreases not until the buffer is depleted. Due to this interrelation, the pH of a digester broth alone is not an adequate parameter to estimate the process health.

A buffer system takes effect only in a specific pH range with the highest efficiency (buffer capacity) at a pH value equal to the acid dissociation constant (pK_a) of the involved weak acids. The buffer capacity of an anaerobic digester is predominantly caused by the bicarbonate buffer system. The bicarbonate buffer capacity derived from the production of CO_2 during the process. Only a minor part is from calcium carbonate content of the water. Other relevant weak acid buffer systems commonly found in anaerobic digesters are the volatile fatty acids (VFA) and their dissociated anions *e.g.* acetic acid - acetate, propionic acid - propionate, and butyric acid - butyrate. Alkalinity is also provided by phosphate, sulphate, and ammonia buffer systems. Due to usually low concentrations of phosphate and sulphate in anaerobic digesters these subsystems only play a minor role in the digester alkalinity (Lahav and Morgan, 2004).

A decrease of reactor alkalinity is an indicator of reactor upsets (McCarty, 1964; Hawkes *et al.*, 1994; Guwy *et al.*, 1997). Organic overloads decrease alkalinity due to extensive VFA production. A toxication or inhibition of the relatively sensitive methanogens also leads to accumulation of VFA and to decreasing alkalinity. The response of the alkalinity to such reactor disturbance is fast *e.g.* less than one hour in an organic overload experiment carried out by Hawkes *et al.* (1994). Alkalinity can be determined with simple methods. Thus, the measurement of the digester alkalinity can be considered as a key factor for the process monitoring. Methods and devices for the alkalinity measurement are topic of a number of scientific publications. Several standard methods for the determination of the bicarbonate alkalinity use pH values in the range of pH 4.3 to pH 4.5 as the titration endpoint *e.g.* APHA 2320 (1999) and EN ISO 9963-1 (1995). However, the buffer capacity from fatty acids with pKa values around pH 4.7 influenced the measurement. Jenkins *et al.* (1983) found that the alkalinity determined using an endpoint at pH 5.75 contributes mainly to the bicarbonate alkalinity. They proposed a method to estimate the true bicarbonate alkalinity instead of an uncertain total alkalinity measurement.

The first titration method to determine the volatile fatty acid concentration was introduced by DiLallo and Albertson (1961). The simultaneous measurement of alkalinity and fatty acid concentration was developed by McGhee (1968). The method based on the assumption that the volume of a strong acid needed to titrate a sample from pH 5.0 to pH 4.0 is contributed mainly on VFA alkalinity. The FOS/TAC method published by Nordmann (1977) based on this method is very popular in Germany for the monitoring of biogas plants digesting renewable resources. However, the required filtration step is often left out due to poor filterability of high solids containing substrates from these plants resulting in a poor accuracy. Another disadvantage of this method is an overestimation of fatty acids at higher bicarbonate levels. A further development of the method by Kapp (1984) solved this problem. A 5 point titration method was introduced by Moosbrugger (1991) and later modified by Lahav and Loewenthal (2000). However the measurement of 5 end points requires more time and is susceptible for errors. Additionally the calculation of results is more complicated and requires usually the help of a computer.

A number of problems have been reported in the anaerobic digestion of whey in particular the provision of adequate alkalinity (Speece, 1996). The natural buffering capacity can be enhanced by acclimatisation of the microbial community. To support the buffer capacity, alkaline sources can be added to the reactor. Ghaly *et al.* (2000) have used NaOH and KOH solutions to stabilise the pH at a neutral level by an automatic pH controlled system. Fox *et al.* (1992) used lime for neutralisation but found an eight times higher consumption due to shorter hydraulic retention time (HRT) and a calcium precipitation. In a full scale

study Guiot *et al.* (1995) added $NaHCO_3$ to control the pH of a buffer tank, operated as a pre-acidification stage prior to a granular sludge reactor. Due to large amounts of $NaHCO_3$ needed to reach optimal conditions for acidification, the set point of the control system was adjusted to pH 4.5 instead of pH 6.5.

4.3.3.3. ORP

The environmental Oxidation-Reduction-Potential (ORP) and the ability of the microorganisms to use different electron acceptors are decisive. Aerobic organisms use oxygen as electron carrier at ORP levels above 50mV. A large group of microorganisms, obligate aerobic bacteria, is able to oxidise nitride to nitrate in the absence of oxygen at ORP levels of +50 mV to -50 mV. At ORP levels lower than -50 mV the reduction of sulphate is used by a group of microorganisms and below an ORP of -100 mV the mixed acid and alcohol fermentation may occur. The dominance of facultative anaerobic bacteria relies on an oxygen free environment at ORP levels below -300 mV. These organisms use hydrogen or carbon dioxide as electron acceptors. Approximately, an ORP level below -400 mV is required to achieve a stable methanogenic fermentation process (Pind *et al.*, 2003).

4.3.3.4. Nutrients, Trace Metals and Vitamins

Bacteria require macronutrients such as nitrogen, phosphorus, and sulphur for growth and metabolism activities (Bryant *et al.,* 1971). Additionally, some micronutrients and vitamins are also obligatory (Speece, 1996). Due to lower cell yield, the requirements for nitrogen and phosphor in anaerobic digestion are considerably lower than in aerobic processes (Bischofsberger *et al.*, 2005). The demand for nitrogen and phosphor is often reported in relation to the (bio-available) COD of the substrates. Henze and Harremoes (1983) stated that the requirement depends on the digester loading rate. According to their work, the minimum of nitrogen and phosphor expressed as COD to nitrogen (COD : N) ratio is 1000 : 7 for a low strength waste and up to 400 : 7 for a process operated at high loading rates. Additionally, the nitrogen demand is substrate specific. Carbohydrate rich wastewaters require up to three times more nitrogen compared to proteinous and lipid containing wastewater (Young and Dahab, 1982). Anaerobic bacteria contain significant higher amounts of Sulphur compared to aerobes (Gerardi, 2003). The sulphur concentration present in the wastewater should be equal to the phosphor content. All three, nitrogen, phosphor and sulphur must be in solution to be available to the bacteria. Available forms are nitrogen as ammonium and phosphor as ortho-phosphate. Sulphur must be in the reduced form, *e.g.* as sulphide (Bischhofsberger *et al.*, 2005). Considering the wastewater composition Mudrack and Kunst (1991) recommend a COD : N : P : S ratio in the range of 300 to 800 : 5 : 1 : 1 and accordingly 400 : 7 : 1 : 1 for high-rate digesters.

Unlike to aerobic bacteria anaerobic bacteria in a methane fermentation mixed culture require some trace metals as micronutrients. The presence of iron, nickel, cobalt, molybdenum, tungsten, and selenium is reported to be obligatory (Bischofsberger *et al.*, 2005). In addition some acetogenic species are rely on zinc, copper and manganese. Several publications report beneficial effects of trace metal additions, *e.g.* Hawkes *et al.* (1992) found a significant lower accumulation of volatile fatty acids, particularly propionic acid after shock loads of an UASB reactor fed with ice-cream wastewater compared to a simultaneous reactor set-up without trace metal addition. Jarvis *et al.* (1997) observed an enhanced activity of acetate-utilising methanogens in the digestion of clover-grass silage after addition of cobalt. Gerardi (2003) describes the mechanism of the bacterial trace metal uptake using slime. The slime enables the bacteria to collect and store trace metals beyond needed quantities. Speece (1996) emphasis, that trace-metals must be in solution to be available for the bacteria and he reports that micronutrient deficiencies can cause decreased reactor efficiency and upsets. Both, sweet whey and acid whey contain micronutrients and vitamins in sufficient amounts. However, Kelly and Switzenbaum (1984) observed an increased COD removal efficiency and decreased volatile fatty acid concentration in an anaerobic expanded bed digester treating whey when Nickel, Cobalt, and Iron were added. The findings imply a reduced bioavailability of the natural whey micronutrients.

4.3.4. Toxic Material and their Control

Process failure of whey digestion is due to the tendency of rapid acidification and a resulting accumulation of inhibitory intermediate products Yan *et al.* (1993). The intermediate products are mainly short chain volatile fatty acids (VFA). Acetic-acid can be consumed by methanogens whilst other longer VFA are converted to acetic-acid by acetogenic bacteria. Yang and Guo (1991) found that acetogenesis is the rate limiting step of anaerobic whey permeate digestion. The natural buffer capacity of anaerobic sludge provides a neutral environment in a well balanced reactor but if more VFA are produced than are consumed, a pH drop occurs reported by various authors, *e.g.* Ghaly *et al.* (2000), Yan *et al.* (1993), Mohr and Stiller (2006). Methanogens are very sensitive to low pH levels and rapidly become inactive if the pH remains low resulting failed or sour reactor (Kugelman and Chin, 1971).

McCarty (1964) reported beneficial effect of sodium in a concentration of (100 to 200) $mg \cdot L^{-1}$ for growth of mesophilic anaerobes but strong inhibition above 8 000 $mg \cdot L^{-1}$. Further research was conducted to find optimal sodium concentrations for mesophilic anaerobic bacteria. Kugelman and Chin (1971) reported 230 $mg \cdot L^{-1}$ to be optimal for aceticlastic anaerobes and Patel and Roth (1977) found best conditions at a sodium concentration of 350 $mg \cdot L^{-1}$ for hydrogenotrophic methanogens. Chen *et al.* (2003)

reported increased tolerance of methanogens to high sodium concentration up to 6 000 $mg \cdot L^{-1}$ after adaption over a prolonged period. The sodium toxicity effect can be reduced by adding Calcium to anaerobic reactors. Bashir and Matin (2004) found that a Calcium concentration of 500 $mg \cdot L^{-1}$ is required to antagonise a sodium concentration of 8 200 $mg \cdot L^{-1}$ in a mesophilic anaerobic filter reactor. However, this result was based on trial and error method and additionally, antagonism was formerly reported as soon as 1965 by Kugelman and McCarty (1965). Beside Antagonism effects, also adaption to sodium has been reported. Feijoo *et al.* (1995) stated that sodium concentrations causing a 50 % inhibition range from (3 to 16) $g \cdot L^{-1}$ due to less or more sludge adaptation to sodium.

Surfactants, commonly used in cleaning agents can have toxic effects on the biogas production (Shcherbakova *et al.*, 1999). However, positive effects have been reported (Patel *et al.*, 1996).

Other problems include the requirement for extended acclimatisation periods. Kalyuzhnyi *et al.* (1997) fed their UASB reactor with diluted whey for start-up procedure and increased the loading rate and the waste concentration over a period of 130 days to allow the bacteria to acclimate. The degradation of casein was found to need a period of acclimatisation of the bacteria (Perle *et al.*, 1995). Wildenhauer and Winter (1985) reported an increase of the reactor performance after a short shock load in a fixed film reactor.

4.3.5. Process Design

As anaerobic digestion is not a pure culture process, efforts have to be made to provide environmental conditions to favour the growing of a specific microbial community and to overcome operational problems. In the past decades various reactor designs have been developed to obtain a balanced sequential anaerobic food chain for a wide spectrum of wastewater and other potential substrates at varying strength aimed in high methanogenic activity and/or high COD removal efficiency. The process should provide optimal environmental conditions for anaerobic grow as described in Section 4.3.3. Beside this, the reactor design also focuses on the distribution of the substrates, on supporting an intense contact of the substrates with the biomass, the separation of solid, liquid and gaseous phases and the retention of the biomass. The following section reviews the principles of common reactor types and their application to dairy wastewater and whey treatment. A schematic view of different common reactor types is given in Figure 6. An overview of anaerobic digesters treating whey, found in the literature, their experimental setups and operational data is also presented in Section 9.9, Table 17 on Page 155.

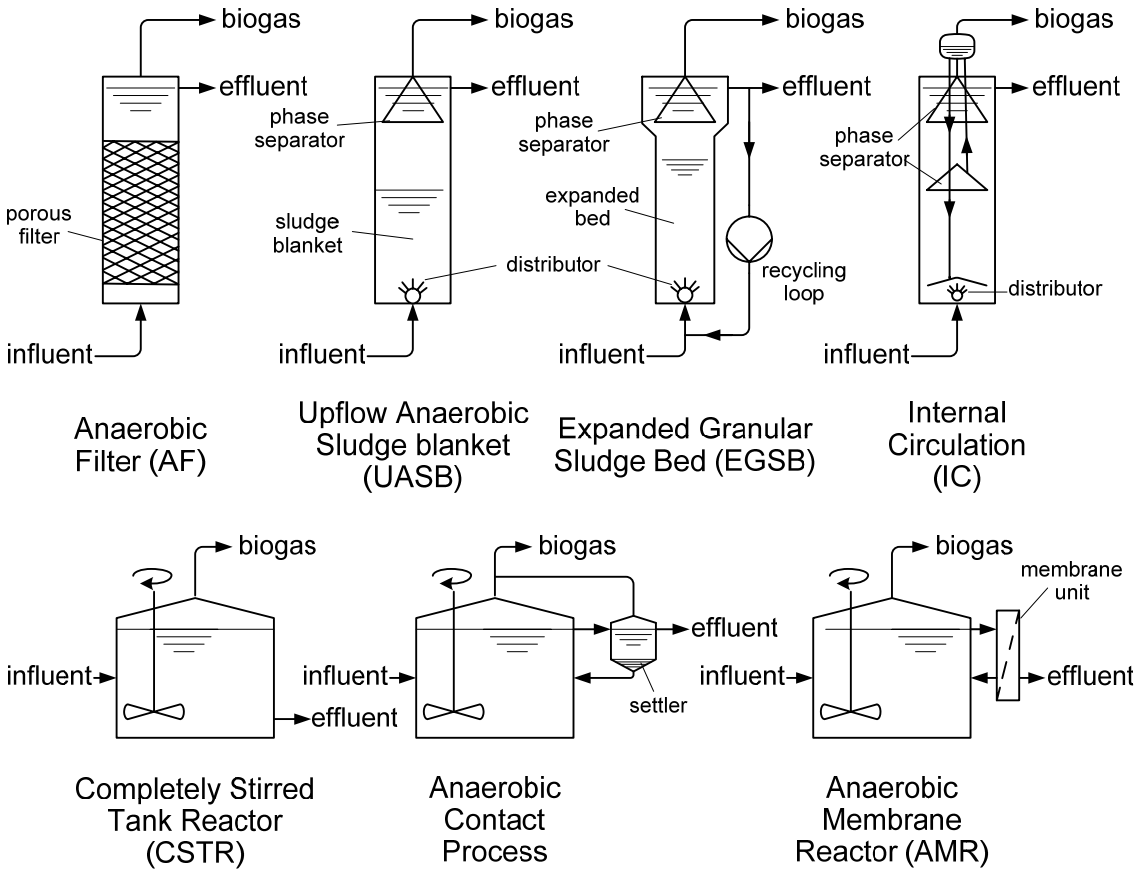

Figure 6 Common types of industrial anaerobic digesters.

4.3.5.1. Attached Grow Digesters

The principle of the anaerobic filter (AF) was presented by Young and McCarty (1969). A support media is installed in the anaerobic filter reactor vessel, providing a surface for attached growth of a biofilm. Small stones, coal, or porous materials like clay can be used as support media, but also rings or otherwise formed plastic pack materials and foams are in use reviewed by Ince *et al.* (2000). Anaerobic filters can be fed from the bottom or like a trickling filter from the top. Several research groups report the usage of anaerobic filter reactors for the treatment of dairy wastewater *e.g.* (Viraraghavan and Kikkeri, 1990; Ince *et al.*, 2000; Omil *et al.*, 2003). Whey treatment in such a system was carried out by Córdoba *et al.* (1995) but only with low strength, diluted whey with less than 9000 $mg \cdot L^{-1}$ COD. A successful whey digestion using an anaerobic filter was reported by Wildenhauer and Winter (1985). The reactor was packed with clay beads and pH controlled by varying the influent flow rate. Wildenhauer and Winter reached a removal efficiency of 95 % at steady state conditions with an organic loading rate of 14 $kg \cdot m^{-3} \cdot d^{-1}$ COD, corresponding to a 5 d hydraulic retention time treating undiluted whey of 79 000 $mg \cdot L^{-1}$ COD. This was one of the highest results for whey digestion found

in the literature. However, the process was only operated for a few months. Some disadvantage of anaerobic filters as reported in Speece (1996) usually occur after long term operation: The difficulties of excess sludge removal and a decreasing efficiency with time due to plugging and channelling of the packing material.

4.3.5.2. Granular Sludge Digesters

Lettinga *et al.* (1980) introduced the first full scale anaerobic digester of the upflow granular sludge blanket (UASB) concept. The 200 m^3 reactor was fed with sugar beet waste and reached an organic capability of 16 $kg \cdot m^{-3} \cdot d^{-1}$ of COD load. The UASB reactor was developed to overcome the lack of proper biomass retention for low strength wastewater. The principle is the formation of sludge aggregates of higher density compared to water. The sludge settles to form a sludge blanket. In a UASB reactor the influent is fed into the reactor bottom and comes into contact with the biomass when passing the sludge blanket. Above the sludge blanket a settling zone allows the biomass to separate and sink back into the sludge bed. In the reactor head a three phase separator is placed where the gas is collected and a further separation of biomass from the supernatant appears. The effluent leaves the reactor via a diversion cut. Lettinga described the formation of granules on experiments with Upflow sludge blanket (USB) reactors resulting in high methanogenic activity and a superior settling capability.

However, the UASB reactor principle lacks efficient mixing resulting in limited organic loading rates (Kato *et al.*, 1994). To overcome this disadvantage the Anaerobic Attached Film Expanded Bed (AAFEB) reactor type was developed and the usage of such a system for whey treatment was presented by Switzenbaum and Danskin (1982) and also by Boening and Larsen (1982). In the AAFEB process a solid packing material of small sized particles is used as the support medium. The particles are coated by a biofilm and the packing builds an expanded bed by the up-flow driven by a recycling pump (Figure 6). Switzenbaum and Danskin (1982) used aluminium oxide particles with an apparent diameter of approximately 0.5 mm, Boening and Larsen (1982) filled their reactor with crushed charcoal with a mean diameter of 0.177 mm. Switzenbaum and Danskin (1982) tested the operation at different organic loading rates up to 30 $kg \cdot m^{-3} \cdot d^{-1}$ COD and constant influent strength of 10 $g \cdot L^{-1}$ COD and in a second run different influent strength varying from 5.0 $g \cdot L^{-1}$ to 20 $g \cdot L^{-1}$ COD while the hydraulic retention time was kept constant at 15.35 h (±10 %). Switzenbaum and Danskin (1982) pointed out that for high strength whey significant longer retention times have to be used indicated by poor COD removal efficiency at higher loading rates. They also stated that the balance between methanogens and acid producing bacteria is easily upset. Furthermore, a loss of biomass via the recycling line was noticed. Also Boening and Larsen (1982) only used low strength whey

(2 000 $mg \cdot L^{-1}$ to 7 000 $mg \cdot L^{-1}$) at similarly short retention times. Their results underline the prediction of Switzenbaum and Danskin (1982) that the treatment of high strength whey requires significantly longer hydraulic retention times. Also in the treatment experiments of Boening and Larsen (1982), low removal efficiencies were a result of higher loading rates.

The development of an expanded granular sludge bed digester was reported by Zoutberg and de Been (1997). The Biobed® EGSB Reactor was developed to overcome the problems of expanded bed reactors and their carrier material. However, the presented system is a granular sludge bed reactor following the UASB principle. By hydraulic optimization of the three phase separator in the reactor head, higher up-flow velocities compared to common UASB systems can be reached. Zoutberg and de Been (1997) stated that no biomass loss was noticed for up-flow velocities up to 15 $m \cdot h^{-1}$. These systems reach stable conditions at high organic loading rates up to 30 $kg \cdot m^{-3} \cdot d^{-1}$ of COD. Also Dinsdale *et al.* (2000) used an EGSB reactor without use of a solid support medium. Dinsdale *et al.* (2000) showed the availability of such a system to treat short chain volatile acids *e.g.* malic, oxalic, and fumaric acids.

A further development, the internal circulation (IC) reactor, was introduced by Vellinga *et al.* (1986). The principle is a granular sludge reactor with two compartments. The compartments are divided by a phase separator where the biogas achieved from the lower compartment is collected and led into a pipe (called a "riser") to a chamber above the reactor head. A second pipe (called a "downer") is connected between this chamber and a distribution system at the bottom of the reactor. The up-flow velocity of the gas drives the liquid flow in the riser to the chamber. In the chamber the gas is separated and the liquid flows back through the downer into the distribution system by gravity and is mixed with influent wastewater. The internal circulation causes a good mixing of the lower compartment and the appearance of an expanded sludge bed. The intensity of the circulation is self regulating by the gas production. The separation of the gas produced in the lower compartment allows a settling of the biomass in the upper compartment. The upper compartment is biological active too, but by lower gas and liquid up-flow velocities this compartment acts more as a UASB reactor. The three phase separator in the reactor head cut of the gas produced in the upper compartment and enhances the settling. The effluent leaves the reactor via a diversion cut. A good example of a full scale IC reactor is given by Habets *et al.* (1997). An application of the IC reactor to dairy effluent was reported by Driessen and Yspeert (1999), but only a very low strength total mix of dairy effluent was treated in a full scale IC reactor. Additionally the wastewater with an average COD of 1550 $mg \cdot L^{-1}$ was pH balanced by addition of hydrochloric acid to a value in the

range of pH 7 to pH 7.5. Driessen and Yspeert (1999) also present an application of the IC system to a high strength (35 000 $mg \cdot L^{-1}$ COD) wastewater from a brewery and show the capability of the system to treat high strength wastewater. However, to the knowledge of the author, no literature is available about the treatment of high strength whey wastewater in a high-rate anaerobic IC reactor system.

4.3.5.3. Membrane Coupled Digesters

The designs of membrane bio reactors (MBR) are similar to each other. Typically a cycle loop with a pump and a membrane is connected to the reactor vessel. In most cases a back pressure valve is used to avoid pressurizing the vessel. A variation is a system with submerged membranes. In these systems the pump is connected to the permeate side of the membrane. Even though membrane coupled anaerobic digester are similar to each other there is no consistent acronym for this type of reactor. Several acronyms can be found in the literature *e.g.* MARS, AMR, MCAB, TADU, MBR, MBS and others. One of the first membrane coupled anaerobic digesters was introduced by Omstead *et al.* (1980). He used a frame and plate membrane system in his lab scale membrane coupled anaerobic bioreactor. The filtration unit was intended as a control mechanism to remove high acid concentrations from the digestion broth. A full scale anaerobic membrane bioreactor system was build in 1984 by Dorr Oliver reported by Li and Corrado (1985) treating dairy wastewater containing whey. However, the organic loading rate of the demonstration plant was low at 8.2 $kg \cdot m^{-3} \cdot d^{-1}$.

The selection of a cross flow membrane suitable for filtration of the anaerobic sludge needs careful consideration of material, pore size and filtration surface. Ceramic membranes offer advantages against those made of polyethylene as they are not hydrophobic and therefore easier to operate. In a long term experiment Harada *et al.* (1994) fed an anaerobic digester coupled to an ultra-filtration UF-membrane with a low strength synthetic effluent for 190 days. Beside a very good COD removal efficiency of 98 % during the experiment, Harada *et al.* (1994) reports decreasing flux permeate flow due to concentration polarisation. In his experiment it was necessary to wash the membrane with pure water every 7 to 10 days to recover the permeate flux. However, Elmaleh and Abdelmoumni (1997) stated that the cake layer resistant has the higher influence. They noticed that above a crossflow velocity of 3 $m \cdot s^{-1}$ no fouling appeared in their experiments. A theoretical approach to predict parameter settings for a membrane system to keep the flux below a critical value was introduced by Choo and Lee (1998). The critical flux method was intended to avoid the deposit of small scale particles on the membrane surface and to prevent irreversible fouling inside the membrane. The pore size for membrane based biomass retention has to be below 0.4 µm to prevent microorganism entering the

membrane wall. Membranes with a pore size less then 0.1 µm need a higher pressure to provide an efficient filtration flux. The demanded filtration area depends on several parameters, mainly pressure, pore size and surface area but it is also affected by the chemical and physical conditions of the filtration medium. Manufacturers give values for filtration flux with pure water at 1.0 bar trans-membrane pressure per square meter filtration area to allow a first assessment of the filtration performance. Yoon *et al.* (1999) compared different membrane materials and found a lower irreversible fouling in ceramic membranes compared to organic membrane materials used for anaerobic digestion. The use of sintered metal membranes at the digestion of dairy manure was tested by Zitomer *et al.* (2004). They reported problems due to the abrasive effect of sand particles which damages the membrane. Similar experience was made by the author (not published) at the filtration of radioactive contaminated wash water with ceramic nanofiltration membranes. The functional layer was worn out by abrasive particles. A damage of the membrane was the result.

In a pilot scale anaerobic digester Pillay *et al.* (1994) tested an alternative microfiltration membrane for the treatment of sludge from a wastewater treatment plant. They used woven fibre membranes with relatively wide pores. In this case the fibre material is not the membrane. Instead of this, the filtration barrier is built up as a fouling layer. Pillay *et al.* (1994) reported that the fouling layer can be removed by mechanical force to the membrane when the flux drops too low. However, the principle is more likely a deep filtration process. Pierkiel and Lanting (2004) connected two different membrane systems to a methanogenic digester treating municipal sewage sludge to compare the flux rates. In their set up, a standard cross flow membrane unit achieves a higher flux than a vibrating membrane disc stack.

Saw *et al.* (1985) identified the reasons for the development of anaerobic membrane reactors in their efficiency in biomass retention. Saw *et al.* (1985) emphasis that (12 to 15) d of solid retention time (SRT) are generally required to balance the wash out of methanogens from a completely mixed anaerobic digester due to low growth rate of the bacteria. Generally, in completely mixed digesters the biomass is always in close contact with the substrate. The advantage of a stirred tank digester with membrane based biomass retention is the decoupling of the solid retention time from the hydraulic retention time. This will led to higher possible loading rates and therefore to smaller reactor vessels.

An aerobic jet loop membrane bioreactor was used by Farizoglu *et al.* (2004) to treat full strength cheese whey at a high organic loading rate of 22.2 $kg \cdot m^{-3} \cdot d^{-1}$ COD at 97 % COD removal efficiency. However the system does not produce methane and the sludge had poor settling capacity and very slimy characteristics.

Laboratory studies of high-rate anaerobic digestion in a small scale membrane coupled bioreactor were carried out by Fuchs *et al.* (2003). The treatment of three wastewaters was tested. Artificial wastewater was digested at an organic loading rate of 20 $g \cdot L^{-1} \cdot d^{-1}$ and the removal efficiency was higher than 90 %. However, the system of Fuchs *et al.* (2003) lacks of an optimal volume flow control strategy. The general problem that the permeate flow varies with the operation time and thus, at a continuous influent flow a change in the digester liquid level can result. To overcome this problem, Fuchs controlled a back pressure valve in the permeate line. However, this had the disadvantage of sub-optimal filtration parameters. Another strategy was followed by Choo and Lee (1998). They operated the system at optimal filtration parameters by pumping surplus permeate back into the digester. As the filtration is a pressure driven process with a high energy consumption this strategy is not very efficient. A novel control strategy was introduced by Mohr and Stiller (2006b). In their system level switches controlled idle times of the pump. With this strategy, surplus permeate was avoided and the filtration unit was operated with optimized parameters. Additionally, shear stress was minimized in this system. Shear stress can cause a physical disruption of the syntrophic association of acetogenic and methanogenic bacteria. This was noticed by several authors *e.g.* McMahon *et al.* (2001), Brockmann and Seyfried (1996)

A membrane coupled anaerobic digester with a separate acidification stage for the treatment of whey at high loading rates was introduced by Saddoud *et al.* (2007). In their experiment the digester was operated at a 24 h hydraulic retention time (HRT) for the acidification stage and 4 d for the membrane coupled section. Organic loading rates in the methanogenic stage up to 19.78 $kg \cdot m^{-3} \cdot d^{-1}$ COD were reached for the overall system. However, only a very short run (45 d) of the system was presented. The system lacks a strategy to avoid augmentation of inorganic whey components in the methanogenic stage. The accumulation of precipitates from milk ingredients in a single stage membrane coupled anaerobic digester treating diluted acid whey at 2 d hydraulic retention time over a period of 120 d was reported by Mohr and Stiller (2006a)

4.3.5.4. Two-Stage Digestion

Partial acidification and methanation in separate vessels of a two-stage system for the digestion of whey have been investigated by several researchers. Under thermophilic conditions, Yang *et al.* (2003) found that the performance of the process could be improved by using a two-stage system compared to a single stage system. However, the two-stage system could require a higher investment and operating costs and two separate interdependent systems could be more difficult to operate. Malaspina *et al.* (1996) combined two-stages in a single vessel by using a partition wall in their downflow-upflow hybrid reactor (DUHR) treating cheese whey. A stable process at a loading rate of 10 $g \cdot L^{-1} \cdot d^{-1}$ COD without pH control treating cheese whey was achieved by careful consideration of the relative volumes of the acidification and methanation reactors of the two-stage design.

Wust (2003) compared single- and two-stage anaerobic sequencing batch reactors (ASBR) for the treatment of high strength cheese wastewater and reported a higher performance for the two-stage system. However both systems were operated with very low organic loading rates and very long hydraulic retention times.

A two-stage system consisting of a CSTR acidification stage and an Upflow Anaerobic Filter (UFAF) as methanogenic stage was used by Ylmazer and Yenigün (1999) to treat cheese whey. They examined different hydraulic retention times for each stage. Optimal parameters are a HRT of 24 h for the acidogenic stage and 4 d for the methanogenic stage. However, the whey they used was diluted with a maximum influent concentration of 20 $g \cdot L^{-1}$ COD.

The effect of reseeding and pH control on the performance of a two- stage mesophilic anaerobic digester operating on acid cheese whey was investigated by Ghaly *et al.* (2000). Ghaly used sodium carbonate ($NaHCO_3$) to maintain the pH of the methanogenic stage to pH 7.0. The design and operational parameters of their two-stage digester was not optimal for whey digestion. The acidification stage was too large resulting in surplus hydraulic retention time of this stage. Nevertheless, the pH of the acidification stage was very low, and thus a poor acidification performance was the result. Beside the poor pH control, the methanogenic stage lacks an effective biomass retention system explaining the need for reseeding.

The separation of the biochemical reactions in a two-stage digester system depends on the conversation rates of the hydrolysis and acidification. In a process treating particulate substrate with low hydrolysis velocity usually methane was produced in both stages. If the substrate can be acidified rapidly and if the hydraulic retention time is short enough, then

the methanogenic bacteria will be washed out off the first stage. In this case acidifying bacteria dominate the culture in the first stage. Nevertheless Lettinga G. (1991) and also Alexiou *et al.* (1994) recommended a partial acidification in the first stage due to a better overall performance compared to a complete acidification of all organics prior to methanation.

The first stage, if operated as an acidification stage, will produce mainly carbon dioxide gas. The COD reduction by conversion of organic compounds to organic acids is very low. Therefore, all of the COD removal must be performed in the methanogenic stage. A pre-acidified wastewater is possibly very available to methanogenic bioconversion. However, it can be very low in pH. Therefore, the suitability of the reactor design must be proved. Additionally high VFA concentrations can be inhibitory to methanogens. Dinsdale *et al.* (2000) examined the availability of an Expanded Granular Sludge Bed (EGSB) Reactor for the digestion of short chain organic acids as they are produced in an acidification stage and found the EGSB to be excellent for this purpose. The dilution of the influent and the good mixing in EGSB reactors may be a reason for the successful operation in the experiments of Dinsdale *et al.* (2000).

Demirel and Yenigun (2004) investigated the anaerobic acidogenesis of dairy wastewater at mesophilic conditions and organic loading rates (OLR) up to 9.3 $kg \cdot m^{-3} \cdot d^{-1}$ of COD. They tested varying hydraulic retention times (HRT) between 12 h and 24 h. At a 12 h HRT the highest degree of acidification (53 %) and an acid production of 3.1 $g \cdot L^{-1} \cdot d^{-1}$ were reached. These findings were reconfirmed by the findings of Fang and Yu (2000).

Optimal pH levels for the acidification of lactose were investigated by Kisaalita *et al.* (1987). They found pH 4.5 and pH 6.0 to be potentially optimal for acidogenic fermentation of lactose. Further research was conducted by Yu and Fang (2001b) to examine the acidogenesis of complex dairy wastewaters at 37 °C and a HRT of 12 h at varying pH levels. A theoretical enzymatic activity was calculated and compared to experimental results. The experimental results fit the theoretical findings in general, but the experimental maximum was found to be pH 5.5 while the calculated theoretical maximum was at pH 5.8. Yu and Fang (2001b) also stated that the pH of the acidification stage influence the distribution of products. From their results it can be concluded that the production of acetic acid increase with increasing pH in the range of pH 4.0 to pH 6.5. Also butyric acid increase slightly with increasing pH while propionate fermentation decreases significantly at lower pH.

4.3.5.5. Pilot and Full Scale Plants for Whey Digestion

In the last decades, several large scale anaerobic digesters treating whey, either as mono-substrate or in combination with other substrates, have been operated. Li and Corrado (1985) report the operation of membrane anaerobic reactor systems (MARS™) developed by Dorr Oliver incorporation, USA. The system is a complete mixed, suspended growth digester with an external membrane filtration unit. A pilot scale plant (198 L) and a full scale demonstration plant (37 850 L) membrane reactor was operated treating whey permeate. Addition of ammonia chloride and an unspecified alkaline for pH control was reported. No information on the volumes consumed was presented. The reported systems were operated treating full strength whey permeate (pilot plant: 62 071 $mg \cdot L^{-1}$ COD, demonstration plant: 59 790 $mg \cdot L^{-1}$ COD) on moderate organic loading rates of 8.5 $g \cdot L^{-1} \cdot d^{-1}$ COD (pilot plant) and 8.2 $g \cdot L^{-1} \cdot d^{-1}$ COD (demonstration plant). The demonstration plant reached a COD removal efficiency of 99.5 % corresponding to an effluent COD of 305 $mg \cdot L^{-1}$. Despite the successful process demonstration, Dorr Oliver Inc. have not built a full scale system beside this demonstration plant, indicating that this system was not economic viable. This may due to high investment and operational costs for the membrane unit.

A recently novel full scale (400 m^3) anaerobic digester treating wastewater and whey was started up in 1992 at the Nutrinor cheese factory in Chambord, Canada reported by Guiot *et al.* (1995). The reactor was a granular sludge type digester with four superimposed compartments (MPAR) initially designed by the École Polytechnique of the University of Montréal. In this system the whey feed was diluted with pre-treated (sieved) municipal wastewater and pre-acidified in a pH controlled buffer tank of 200 m^3 volume prior to methanation in the MPAR. Guiot *et al.* (1995) reported that the system was operated with an organic loading rate of (9 to 14.7) $g \cdot L^{-1} \cdot d^{-1}$ COD, however the buffer tank was not taken into account. The treatment efficiency was high, 92 % of total COD was removed at the highest loading rate. However, high amounts of soda ash were consumed for the pH control of the acidogenic stage, additionally only diluted whey was utilized.

The anaerobic co-digestion of cheese whey from the Lateria Engadina dairy in Bever, Switzerland with activated sludge in an anaerobic digester of the near-by municipal wastewater plant was put in practice in 2005 (Camichel and Wehrle, 2007). The project was based on the economic calculation of Kohle and Nusbaumer (2003). A full scale anaerobic digester treating whey is currently in operation in Austria. At the Landfrisch dairy in Wels, a granular type anaerobic digester of 12 000m^3 Volume is operated at 35°C. Half of the daily influent volume of 360m^3 is demineralised whey the other half is mixed effluent from the dairy (Stutzer, 2006).

4.3.6. Process Kinetics and Mathematic Modelling

Several research groups developed models of anaerobic digestion processes *e.g.* Yang *et al.* (1982); Vavilin and Lokshina (1996). Models for dairy wastewater (Nadais *et al.*, 2001; Hu *et al.*, 2002), lactose (Kisaalita *et al.*, 1989; Yu and Pinder, 1993, Whey and whey permeate (Yang and Guo, 1991; Ghaly and Echiegu (1993) implement the specific parameters of the degradation of dairy ingredients to recently developed standard mathematical models.

The Anaerobic Digestion Model No. 1 (ADM1) developed by the IWA Task Group for mathematical modelling of anaerobic digestion Processes was introduced by Batstone *et al.* (2002) and was intended for general mathematical modelling of anaerobic digestion. As the model use several dynamic state variables, it is recommended to use powerful modern computer based numerical systems for analytics and prediction of anaerobic processes by numerical simulation. Implementations of the model are available as commercial software (Aquasim, Simba). A Scientific Implementation to math software such as The MathWorks™ Matlab®/Simulink® is provided on request by Dept of Industrial Electrical Engineering & Automation, Lund University, Sweden. Volcke *et al.* (2005) introduced a differential algebraic equation (DAE) model implementation that uses differential equations but an external algebraic solution of pH (SH^+). The algebraic solver uses a Newton-Raphson method for calculation of pH and concentration of equilibrium components during dynamic simulation. A Matlab®/Simulink® implementation for plant-wide benchmark simulations of wastewater plants was developed by the Department of Industrial Electrical Engineering and Automation (IEA), Lund University, Lund, Sweden and was presented by Rosen *et al.* (2006b). In the Lund implementation changes to the original model were made to acid-base equations and some initial parameters for better numerical properties and to fix some computational problems. Changes to the original model where also made to avoid unbalances in nitrogen and carbon contents. A DAE model with drastically reduced stiffness was introduced.

4.3.7. Anaerobic Biodegradability Assessment

The broad potential of anaerobic digestion for the degradation of various organic chemicals asserts the need for a screening method to evaluate the biodegradability. Recently developed procedures use small and simple anaerobic methane digesters to test probes of material under specific conditions. The assessment of anaerobic biodegradability can be carried out to gain knowledge about the potential gas production from an organic matter but assessment methods can also be used to evaluate the degradation of pollutants.

A method for the Assessment of methanogenic activity in anaerobic digestion was invited by van den Berg *et al.* (1974). Young and Irwin (1999) compare Bach respirometric tests with continuous cultures and recommended good experimental design and appropriate mathematic techniques. The Standard: BS EN ISO 11734 (1999) can be used for the *"…Evaluation of the "ultimate" anaerobic biodegradability of organic compounds in digested sludge — Method by measurement of the biogas production…."* The use of the OxiTop® Control system for respirometric batch tests was introduced by Süßmuth *et al.* (2001). An overview about the Equipment that can be used for activity tests and the biodegradability of test substances is given by Guwy (2004).

4.3.8. Economic Consideration on Whey Anaerobic Digestion

A possible replacement of 75 % of the liquid fuel oil and propane used in an American dairy by biogas from anaerobic digestion of whey was reported by Wise (1980). However due to the reduced energy consumption of modern dairy processing it is presumably that the rate could be even higher nowadays. The economic potential of whey digestion was also stated by Wildenhauer and Winter (1985).

The calculation based on 100 m^3 daily utilisation of acid whey at a 5 day hydraulic retention time and 0.30 € per m^3 of gas. The result shows that biogas production from whey is very favourably compared to those for marketing as animal feed. However, a dairy producing approximately 100 m^3 of acid whey per day can be classified as small sized dairy nowadays.

Kohle and Nusbaumer (2003) reported about whey disposal at Lataria Engiadinaisa SA, Bever, a Swiss dairy and give a detailed cost estimation for the utilisation of 4 300 m^3 of whey, produced per annum. They calculated total costs of 210 433 franc corresponding to 0.049 franc per litre whey, for the previous utilisation method. The whey was concentrated by reverse osmosis filtration and sold as feed for pig fattening. The calculation involves costs for the operation of the filtration plant and also for cooling, storage, transportation and disposal costs for the permeate discharging. However, sales revenue for the concentrate was not calculated. Kohle and Nusbaumer (2003) suggest the treatment of whey should be by anaerobic digestion and they calculated the economics for two options. The construction of an on-cite plant or alternatively the utilisation of the whey as a co-substrate in the anaerobic digester of the local municipal wastewater treatment plant. For the construction and operation of an on-site plant Kohle and Nusbaumer calculated annual costs of 130 000 franc to 150 000 franc. For whey disposal at the municipal wastewater treatment plant it was calculated, that even accounting investment costs for a new block heat and power plant, the benefit from power generation exceed the costs

from operation of the anaerobic digester and disposal of surplus sludge from the process. Cost savings for the dairy as high as 200 000 franc were also calculated.

An estimate of the investment and operation costs for anaerobic digesters can be calculated from the required reactor type and volume (Böhnke *et al.*, 1993).

For a given amount of substrate with constant strength the reactor volume depends on the organic loading rate (OLR) that can be achieved. Beside lower investment costs, a small reactor saves operating costs due to reduced energy consumption for heating. Therefore the development of a process which could achieve stable operation at higher loading would be of significant environmental and economic benefit.

However, despite these modifications, anaerobic digestion of acid whey has only achieved process stability at relatively low loading rates. Therefore significant problems remain in the alkalinity control of whey anaerobic digestion and greater organic loading rate (OLR) could be achieved with the implementation of control.

5. MATERIAL AND METHODS

5.1. Design of a Membrane Filtration Plant

A laboratory scale, membrane filtration plant was developed and built for waste reduction and cleaning solution recovery, based on state of the art cross flow membrane filtration technology. The filtration plant (photo in Figure 8) is fitted with pumps for pressurization and circulation, process tanks for storage of the filtrate (permeate) and the filtration batch (retentate) which was concentrated by the filtration process as shown in the process scheme in Figure 7.

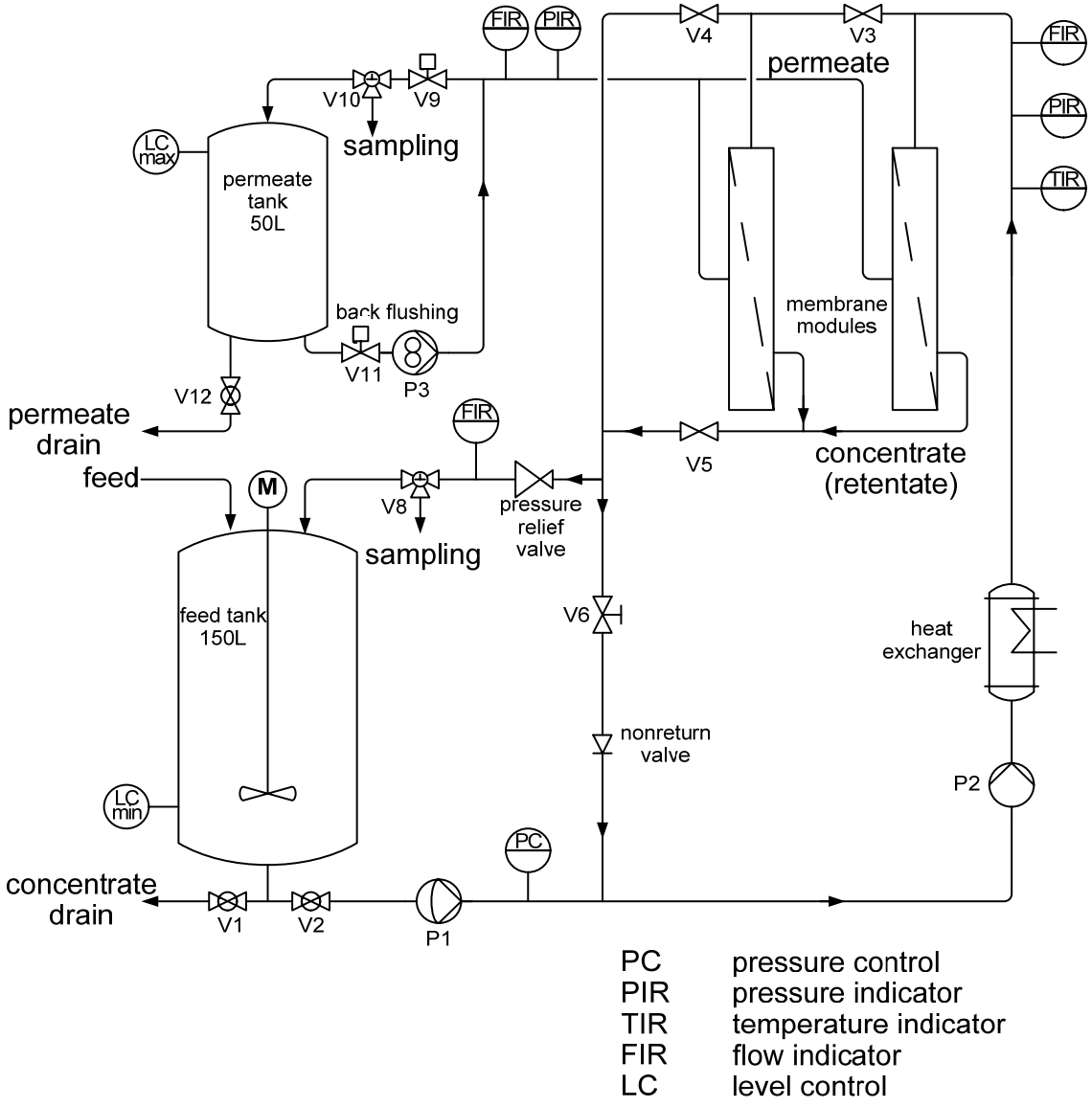

Figure 7 Process scheme of the membrane filtration plant.

In this work, titanium oxide ceramic tubular nanofiltration membranes (INSIDE CéRAM, Tami Industries, Germany) were used. The manufacturer assures caustic resistance in the range of pH 0 to pH 14 for various concentrated acids and alkalines. The nominal molecular weight cut-off (MWCO) is 1.0 kD, the process is therefore stated as nanofiltration. The

membranes with 25 mm outer diameter have a 39 channel design with a hydraulic inner diameter of 2.5 mm of each channel where the filtrate flows from inside (retentate side) outwards (permeate side). Each membrane is placed in a stainless steel housing for collecting and permeate drain off. Compressive seals held the membrane and separate the permeate space from retentate inlet and outlet. The filtration area of each of those membrane modules is 0.25 m².

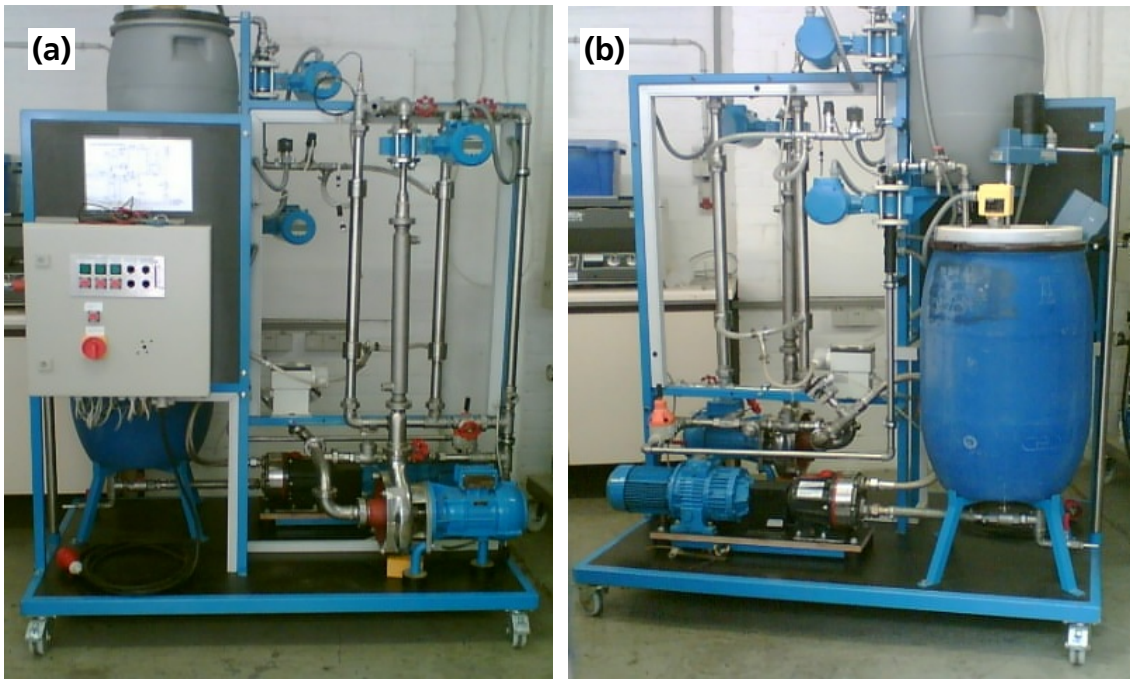

Figure 8 Set-up of the of the membrane filtration plant at the Environmental Process Laboratory at the University of Applied Sciences and Arts Hanover in Germany. (a): Front view with operating panel. (b) Back view with tanks

The difference in pressure between the filtrate side and the permeate side of the membrane decrease over the length of the membrane due to a hydraulic head loss caused by friction and the permeate flow. Pressure sensors are placed near the membrane module inlet and outlet branches and also in the permeate piping to average the transmembrane pressure as in the following equation:

$$\Delta p_t = \frac{p_{in} + p_{out}}{2} - p_f \qquad (5\text{-}1)$$

where Δp_t is the mean transmembrane pressure; p_{in} and p_{out} are the pressures measured at the inlet and outlet branches of the retentate piping and p_f is the pressure measured in the permeate piping (all in bar). Current flow rates of permeate (flux), concentrate, and inner circulation were measured by electromagnetic flowmeters (Altometer K 280, Krohne, Germany). Volumes of permeate achieved were determined using the flowmeters internal sum counter. Operational data of the membrane filtration plant is summarized in Table 3.

Table 3 Operational data of the membrane filtration plant.

Parameter	Values (day 15)
Membrane type	Ceramic (titanium oxide)
Nominal MWCO [Da]	1 000
Filtration area [m^2]	0.5
Max. transmembrane pressure [bar]	7.5
Max. crossflow velocity [$m \cdot s^{-1}$]	1.33
Volume Batch tank	150
Volume Permeate tank	50

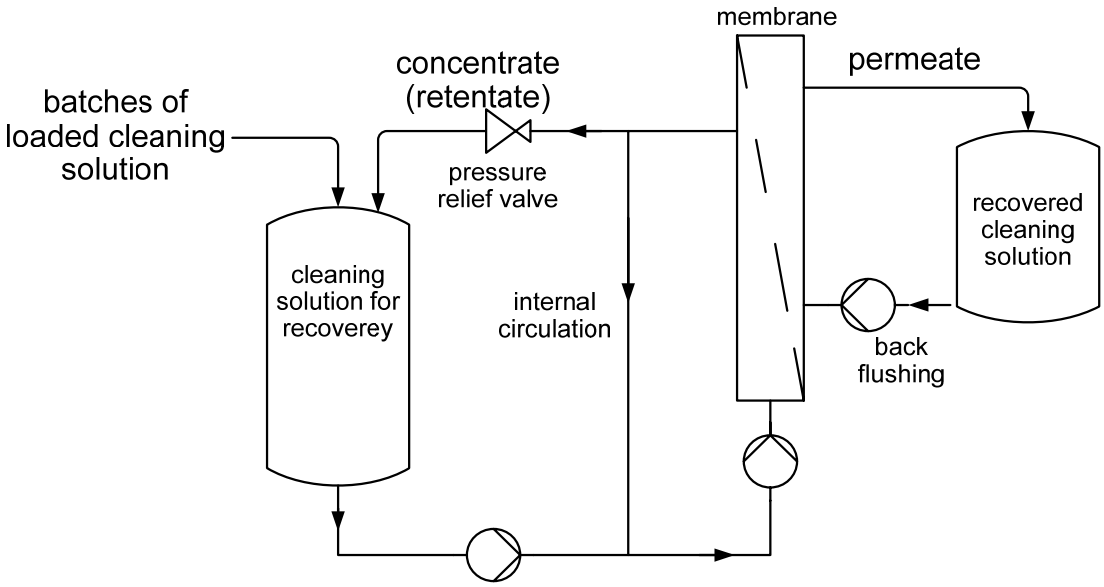

Figure 9 Scheme of the membrane filtration process in batch mode with internal circulation

At batch mode operation of a membrane filtration process the concentrate was recirculated in the plant and its volume was reduced from an initial volume V_0 to a final residue as a result of the volume of Permeate V_P leaving the plant by passing the membrane. The volume reduction ratio $VRR(t)$ of a membrane process can be calculated using following equation:

$$VRR(t) = \frac{V_0}{V_0 - V_P(t)} \qquad (5\text{-}2)$$

If samples of the circulating concentrate were taken during the run the sample volume was regarded in the calculation of volume reduction ratio as follows:

$$VRR(t) = \frac{V_0 - V_S(t)}{(V_0 - V_S(t)) - V_P(t)} \tag{5-3}$$

Where $V_S(t)$ is the sum of all volumes of samples taken since current runtime t.

During filtration the solvent flow through the membrane wall while components with a molecular weight above the nominal cut off can not permeate the membrane and where more or less rejected. The coefficient of rejection of a single component i can be calculated by using the concentration of the component i in the concentrate and in the permeate as in following equation:

$$Rm_i = 1 - \frac{c'_{i,\text{tot}} - c'_i}{c'_i} \tag{5-4}$$

Where Rm_i is the coefficient of rejection of the component i and c'_i is the concentration of component i in the retentate. The concentration of component i for a theoretical total rejection ($Rm_i = 1$) $c'_{i,\text{tot}}$ is calculated by following equation:

$$c'_{i,\text{tot}} = c'_{i,\text{ini}} \cdot VRR(t) \tag{5-5}$$

Where $c'_{i,\text{ ini}}$ is the initial concentration of a component i at process start and $VRR(t)$ is the volume reduction ratio.

5.2. Rheological Measurements

A rotational viscometer was used for the rheometric measurements (Rotovisco RV20, Haake GmbH, Germany). The method is in accordance with German Standard DIN 53019-1 (1980) using a cone and plate measuring system. The rotational speed of the cone was controlled and varied in the range of (0 to 1 000) min^{-1}. The shear rate $\dot{\gamma}$ in s^{-1} resulting from a cone and plate system can be calculated by using following equation:

$$\dot{\gamma} = \frac{2 \cdot \pi \cdot n_{rot}}{\alpha} \cdot \left(1 - \alpha^2 + \frac{\alpha^2}{3}\right) \approx \frac{2 \cdot \pi \cdot n_{rot}}{\alpha} \tag{5-6}$$

Where n_{rot} is the rotational speed (s^{-1}) and α is the cone angle (rad).

A torque indicator calculator unit was used to calculate the shear stress τ in Pa from the torque as follows:

$$\tau = \frac{3 \cdot M}{2 \cdot \pi \cdot r^3} \tag{5-7}$$

Where M is the torque (Nm) and r is the cone radius (m). The current viscosity η in mPa·s at a maintained shear stress was then calculated by using following equation:

$$\eta = 10^3 \cdot \frac{\tau}{\dot{\gamma}} \tag{5-8}$$

Where τ is the shear stress (mPa) at a given rotational speed. Rheological measurements include a set of measurements at different shear rates respective rotational speed settings. The rotational speed was increased stepwise. At each setting the speed was held constant for a minimum of three minutes before recording the torque. The rotational speed was decreased also stepwise, using the same settings, after reaching the maximum shear rate. This was made to detect variations in the viscosity related to shear time.

5.3. Standardized Biodegradability Tests

To evaluate the biodegradability of wastewaters by anaerobic microorganisms a manometric batch tests according to the international standard BS EN ISO 11734 (1999) were performed. The method uses a diluted anaerobic sludge to treat a sample at given concentration. The digested sludge used in the tests was from an anaerobic digester of a municipal wastewater treatment plant. A test medium containing macro nutrients and trace elements according to the standard was used for dilution and wash of the sludge. In sealed test vessels the production of carbon dioxide (CO_2) and methane (CH_4) causes an increase in headspace pressure. The test vessels were sealed and fitted with a pressure measurement device (OXI-Top-C, WTW, Germany), shown in Figure 10.

Figure 10 Test vessels containing inoculated samples, sealed and fitted with OXI-Top-C pressure measurement devices.

Samples and in addition sets of blanks and also sets of reference substances (glucose) were tested in triplicates. Inorganic Carbon of the inoculum sludge and total organic carbon (TOC) of the samples was determined. The ultimate biodegradability was than calculated by balancing the carbon. 1.0 mol of methane and 1.0 mol of carbon dioxide each contain 12 g of carbon. The total mass of gasified carbon in the vessel m_t is the sum of net mass of carbon produced as gas in the headspace from the test compound (m_h) plus the mass of carbon in the liquid of the test vessels (m_l)

$$m_t = m_h + m_l = \frac{12 \cdot 10^3 \cdot (0.1(\Delta p \cdot V_h))}{RT} + \Delta IC_{net} \cdot V_l \qquad (5\text{-}9)$$

Where $\triangle p$ is the mean of the differences between initial and final pressures (mbar) in the test vessels minus those in the blank vessels; V_h is the volume, in litres, of headspace in the vessel; the conversion factor for both, Newton per square metre to millibars and cubic metres to litres is 0.1; $\triangle IC_{net}$ is the mean concentration of inorganic carbon, in milligrams per litre, in the test vessels minus that in the control vessels at the end of the test; V_l is the volume of liquid in the vessel (litres).

Calculate the mass of carbon m_v (mg) in the test vessels, from the test concentration of added carbon, using equation (7):

$$m_V = \Delta c_V \cdot V_l \tag{5-10}$$

Where $\triangle c_v$ is the carbon concentration of the test compound ($mg \cdot L^{-1}$); V_l is the volume of liquid in the vessel in litres.

The total biodegradation D_t (%) is expressed as percentage biodegradability and was calculated using the following equation:

$$D_t = \frac{m_t \cdot 100}{m_v} \tag{5-11}$$

5.4. Digester Design

5.4.1. Design of a Continuous Stirred Tank Reactor (CSTR)

A standard laboratory fermenter (Bioflow110, New Brunswick Scientific, USA) with a working volume of 5.8 L was modified to be used as a continuous stirred tank reactor (CSTR) for anaerobic digestion, see Figure 11. The fermenter comes with a controlling unit for measurement display and automatic or manual control of process sensors and actuators. The fermenter is equipped with a rotational speed controlled stirrer. Temperature was maintained using a heating jacket and a cooling finger. Build in sensors are a resistance thermometer, a pH glass probe, and two level sensors. Four independent peristaltic pumps were controlled either manually or automatic by internal loop controllers. Flow rates were adjusted by digital pulse duration modulation. Two pumps can be used by the internal loop controller for dosage of acid or alkaline solutions to maintain the reactor pH.

For use as CSTR reactor, all fermenter connectors were carefully checked to be gas tight. The biogas produced in the reactor was led through a condenser were most of the water vapour condensed and rinsed back into the reactor. A siphon was used as a water seal to avoid an oxygen contamination from air. A methane sensor and a carbon dioxide sensor and also a gas flowmeter were connected to the gas tubing. These instruments are described further in depth in Section 5.5.11. Influent was dosed in the range of (0 to 26.4) $mL \cdot min^{-1}$ by one of the peristaltic pumps. The effluent was pumped off by another peristaltic pump using the loop controller connected to the level sensors. Alkaline dosage and pH control was implemented and tested but not used in the CSTR experiments.

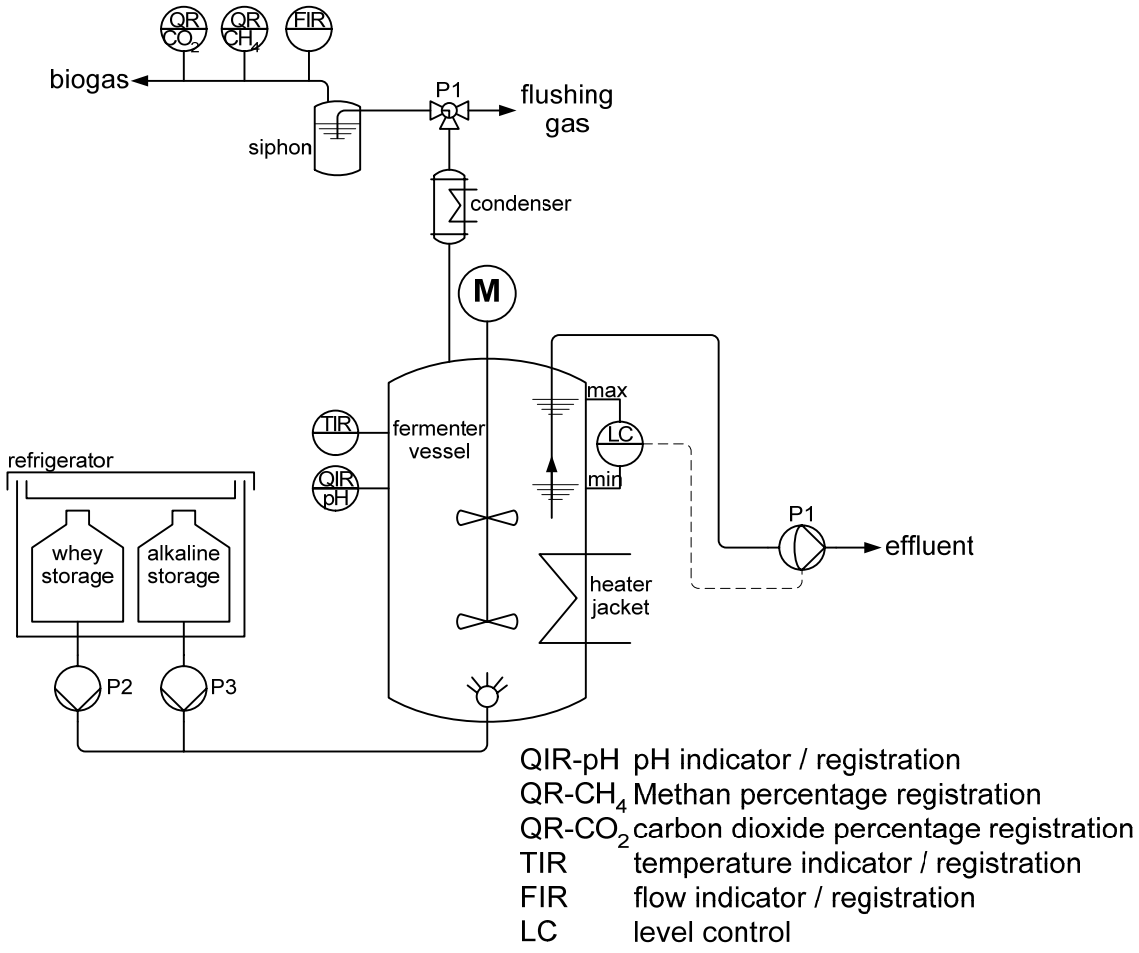

Figure 11 Process scheme of the CSTR Reactor.

5.4.2. Design of an Anaerobic Membrane Reactor (AMR)

The Design of the Anaerobic Membrane Reactor (photo in Figure 13) based on the CSTR described previously. The effluent pump was removed and replaced by a membrane system for biomass retention. Two tubes of 12 mm diameter were immersed into the reactor broth and led through the reactor head plate by a gas tight screwed fitting. The tubes are connected to a cycle loop fitted with a cross flow membrane module. A four piston diaphragm pump (Quattroflow-1000 S, QuattroFlow Fluid Systems, Germany) specially designed for pumping of biomass broth, was placed in the loop. The cross flow velocity can be varied by speed control of the pump. Operation of Influent and cycle loop pumps is controlled by two level sensors in the reactor vessel. The total working volume including the cycle loop was 6.1 L.

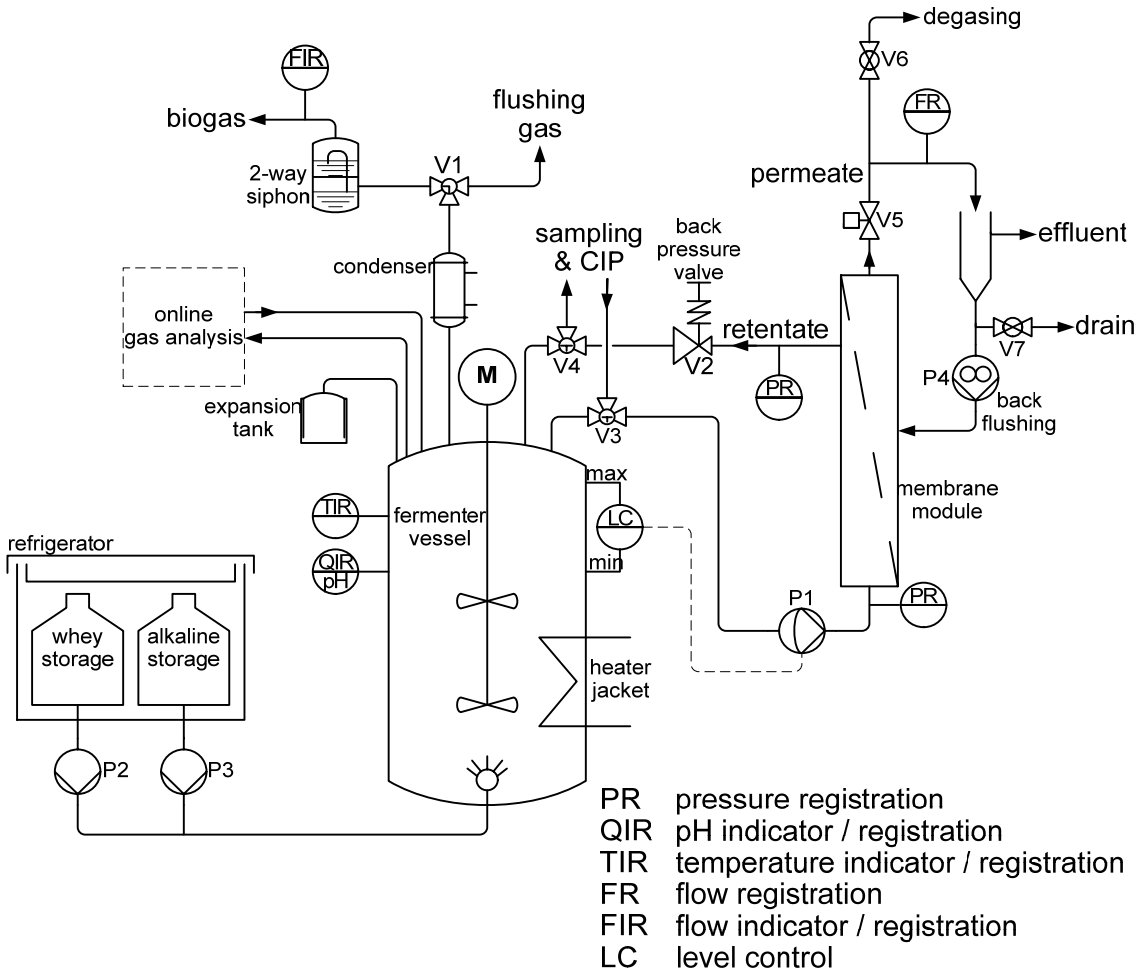

Figure 12 Process scheme of the Anaerobic Membrane Bioreactor (AMR).

A spill valve was placed in the loop to keep a constant system pressure and to prevent the reactor vessel to be pressurised by pumping action. The system pressure was monitored by pressure sensors which are placed near the inlet and outlet branches of the microfiltration module.

A tubular ceramic membrane (*INSIDE* CéRAM, TAMI Industries, Germany) with a pore width of 0.2 µm was used, the process was therefore classified microfiltration. The membrane has 10 mm outer diameter and a 3 channel design with a hydraulic inner diameter of 3.6 mm of each channel. The filtration area was 225 cm². Filtration performance of the plant was monitored by measuring the permeate flow by a low flowmeter (FCH-m-POM-LC, BIO-TECH e.K., Germany) placed in the permeate tube. A process scheme of the reactor set-up with connected microfiltration unit is shown in Figure 12.

Figure 13 Set-up of the of the Anaerobic Membrane Bioreactor (AMR) at the Environmental Process Laboratory at the University of Applied Sciences and Arts Hanover in Germany.

Initial tests were carried out to check the filtration loop and to establish the most suitable working parameters. The filtration area of the filtration unit design was designed to reach a flux capable for a range of HRT at low pressures of (0 to 2.0) bar by using idle times. The average operating system pressure was adjusted to 1.0 bar calculated using two measurements and Equation 5-1, considering Choo and Lee (1998) who recommends a relatively low operating pressure to avoid irreversible membrane fouling. The pumping power of the cross- flow pump was initially set to a delivery rate of 2.0 $L \cdot min^{-1}$ corresponding to a cross flow velocity of 1.0 $m \cdot sec^{-1}$ to avoid high shear rates. Shear stress caused by high cross flow velocities can damage the microorganisms (Brockmann and Seyfried, 1996). This configuration caused problems due to sludge settling in the pump diaphragm and results in a failure of the filtration unit. After stepwise increasing of the velocity to finally 2.8 $m \cdot sec^{-1}$ (5.6 $L \cdot min^{-1}$) the system worked reliably. Negative effects of this higher cross flow velocity were not observed.

The membrane recycling pump was programmed to switch on at indication of a high liquid level in the reactor vessel. After a run of the filtration unit for 10 minutes the system became idle and waited for the next high level event. Thus the reactor volume alternates

for 100 mL corresponding to 1.5 % (v/v) of the reactor liquid volume. This operation mode worked with approximately 60 % idle time and thereby, the hydraulic retention time of the reactor was variable without variation of the filtration area or of the transmembrane pressure and without pumping permeates back into the vessel. This novel control strategy was a process optimization that minimises the operating time and thus the shear stress for the biomass.

5.4.3. Design of an Upflow Anaerobic Sludge Blanket (UASB) Reactor

The laboratory scale UASB digester has a working volume of 5.0 L with a height/diameter ratio of 6:1 and was originally used in the Wastewater Treatment Laboratory at the University of Glamorgan where an initial experiment was carried out (photo in Figure 14a). Further experiments with the reactor vessel equipped with similar peripheral devices were performed at the Environmental Process Laboratory at the University of Applied Sciences and Arts Hanover in Germany (photo in Figure 14b). A process scheme is shown in Figure 15.

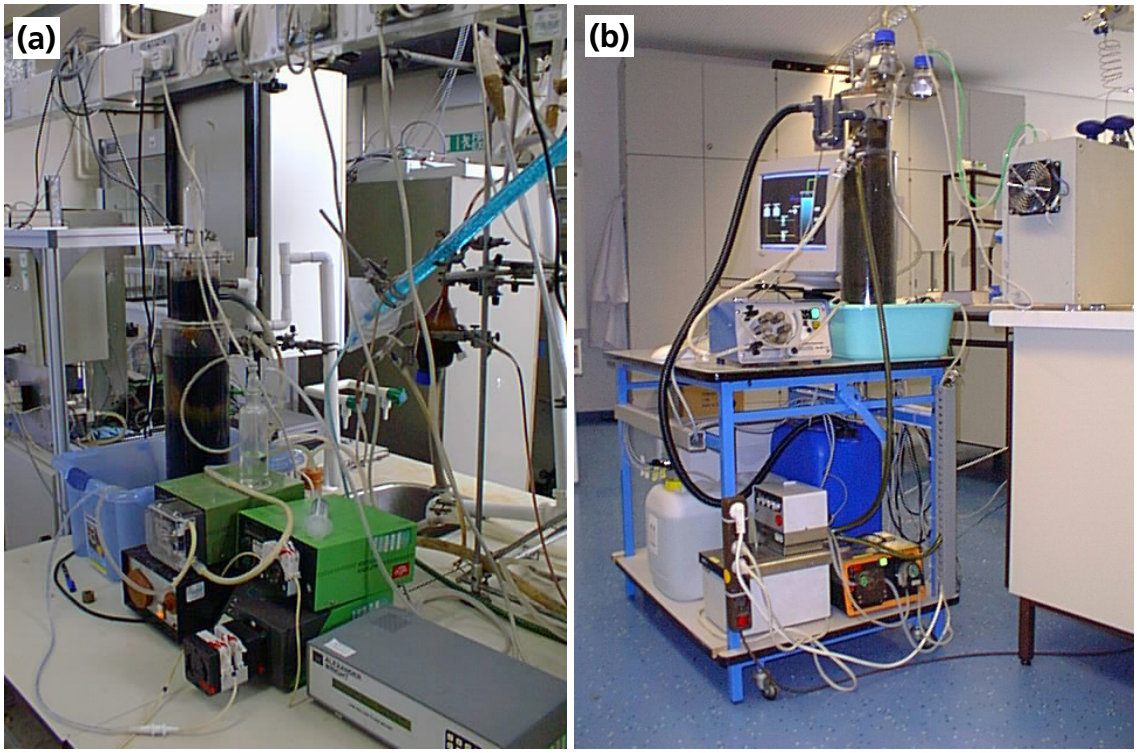

Figure 14 Set-ups of the UASB reactor. (a): In the Wastewater Treatment Laboratory at the University of Glamorgan. (b): In the Environmental Process Laboratory at the University of Applied Sciences and Arts Hanover in Germany

The head of the digester is fitted with a conical phase separator to facilitate good settling. The influent inlet is situated at the base digester and the influent enters the reactor via a horizontal distributing pipe. Peristaltic pumps are used to pump the influent and potable water into the digester. The organic loading rate was adjusted by varying the ratio of the

two pumps. All UASB experiments were performed at a constant hydraulic retention time (HRT) of 48 hours. A recycle loop is employed to improve up-flow velocity during occurrences of low influent flow rates. Effluent leaves the digester over a diversion cut via a manometer so maintaining the headspace pressure and preventing biogas loss. The reactor temperature was controlled by a thermostat, connected to a water jacket and the reactor pH measured by a glass electrode (H8281, Schott, Germany) placed in the reactor head and a digital pH- meter (Model 611, Orion Research Inc., USA).

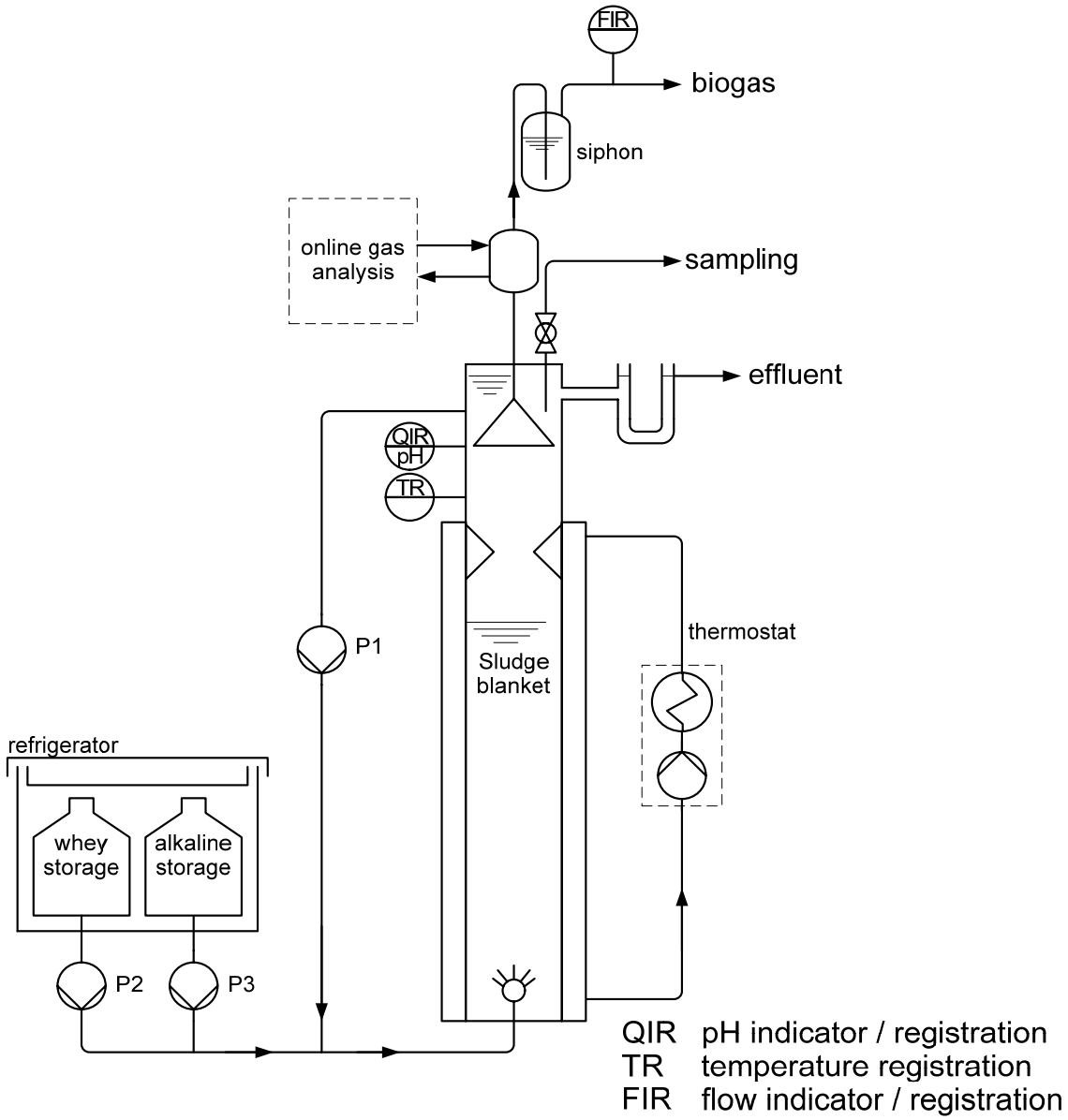

Figure 15 Process scheme of the UASB Reactor.

5.4.4. Design of a Two-Stage High-Rate Digester

The conclusions drawn from one stage studies (Section 9.6) led to the design of a novel high-rate digestion system to meet the demands of anaerobic digestion of acid whey and effluents from plant cleaning. The design based on different high-rate industrial reactor

designs and incorporate the ideas of staging (Section 4.3.5.4), crystallisation of calcium salts prior to anaerobic digestion (van Langerak *et al.* (1997), see also Section 9.5.5), expanded granular sludge bed, fluidised bed, and internal circulation reactors (Section 4.3.5.2), and also jet-loop or gaslift reactors (Beeftink and Staugaard, 1986; Kennard and Janekeh, 1991; Wei *et al.,* 2007).

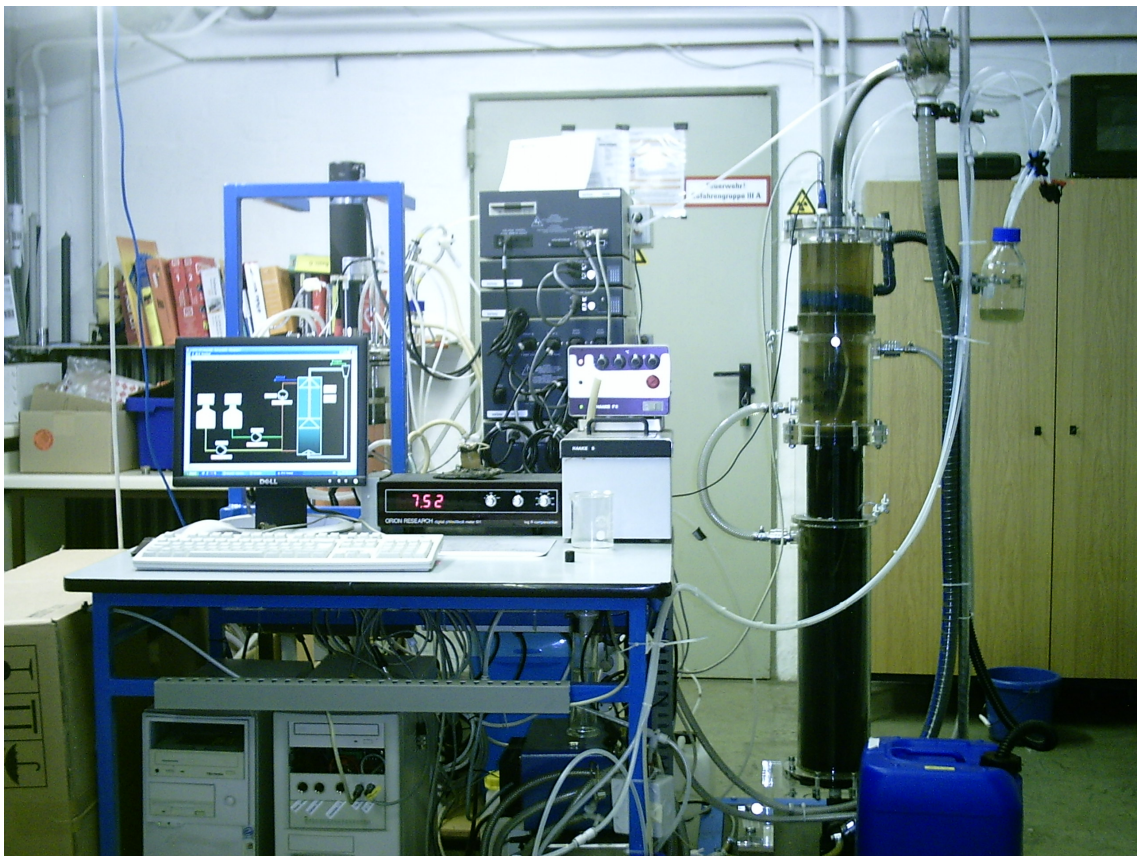

Figure 16 Set-up of the of the two-stage high-rate anaerobic digester at the Environmental Process Laboratory at the University of Applied Sciences and Arts Hanover in Germany.

To overcome biological imbalances due to rapid acidification, a two-stage configuration was chosen. The set-up is shown in Figure 16. A separate acidification stage was intended to convert the high carbohydrate content of whey into acetic and propionic acid and to retain the acetic bacteria in this first stage.

The standard laboratory fermenter (Bioflow110, New Brunswick Scientific, USA) configured as continuous stirred tank reactor (CSTR) described previously was used as acidification stage. The UASB digester, described previously was modified by mounting an additional UASB compartment on the top of the existing one, for the use as methanogenic stage with 10.7 L of working volume. The compartments were fitted with three phase separators. A process scheme is shown in Figure 17.

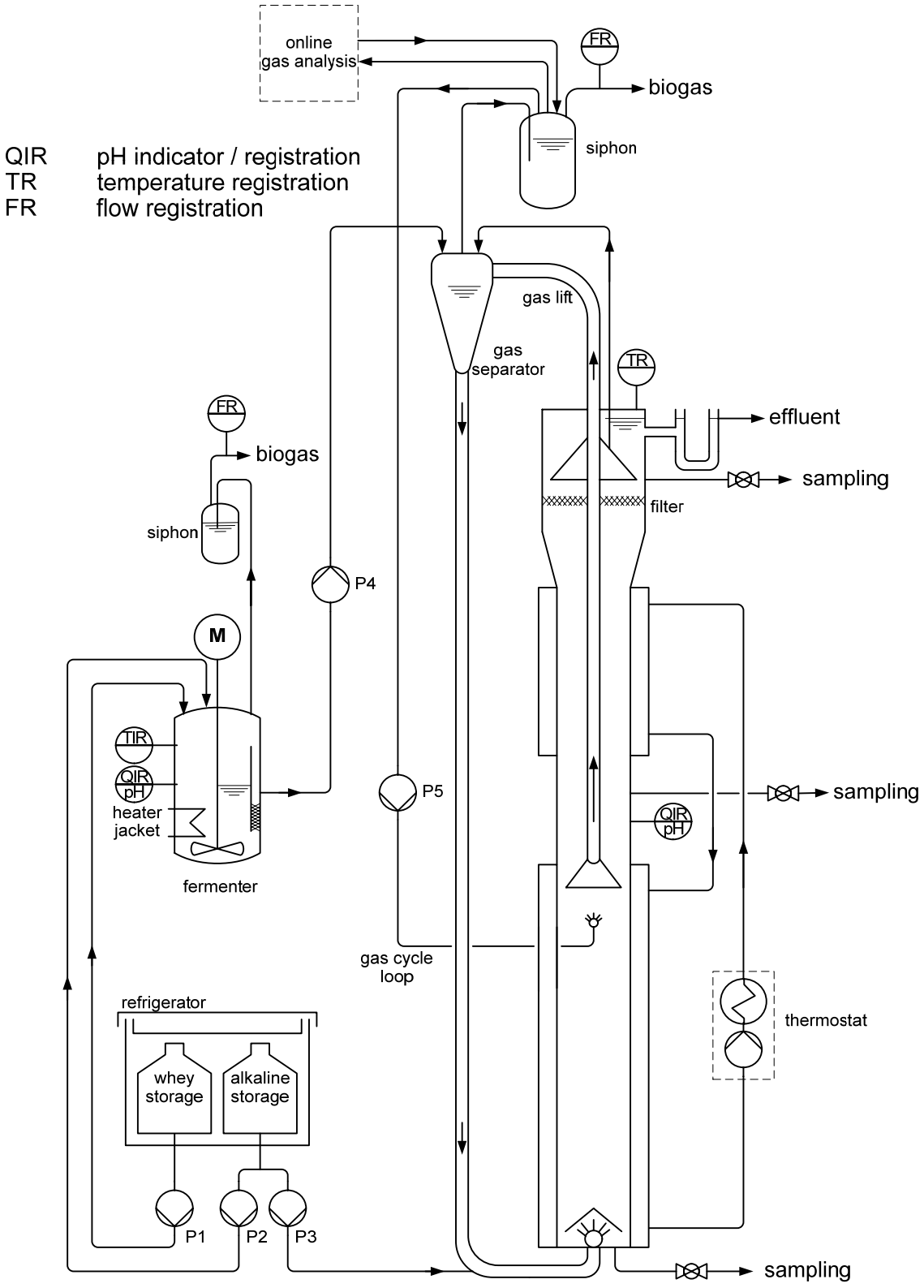

Figure 17 Process scheme of a two-stage high-rate digester with pH control, recirculation loop and gaslift.

A recycling loop was installed to provide a fluidised granular sludge bed in the lower compartment. Rising gas bubbles entrain liquid and biomass from the lower compartment through the riser pipe of the recycling loop into a hydrocyclone, were the biogas was separated and mixed with the off gas of the first stage. The hydrocyclone was mounted approximately 30 cm above the top of the reactor. An adjustable diaphragm pump was used to pump gas from the headspace of the hydrocyclone through a sintered ceramic gas exhauster back into the reactor. This enabled the transport of liquid onto the level of the hydrocyclone by the gaslift principle. The circulation loop was connected to the inlet at the reactor base. Driving force of the liquid recycling was the difference of water levels between reactor and hydrocyclone. The influent from the transferred volume of the first stage was injected into the recycling loop to avoid local organic overloads by intense mixing with the reactor bulk liquid.

The upper compartment acted as a UASB reactor and contained granular sludge. Effluent exited the digester over a diversion cut via a manometer above the upper three phase separator.

The reactor temperature was controlled by a thermostat, connected to a water heating jacket. A flow meter (AWM3300V, Honeywell Inc., USA) was connected to the off gas pipe. The gas was then collected in a gas tight bag with 60 L volume and flared with a standard laboratory gas burner on a regular basis. However, due to a malfunction of the gas flow meter no gas flow was recorded. To estimate the gas production for calculation of performance data, the time to completely fill the gas tight bag was recorded on two occasions.

Simultaneous treatment of alkaline effluents from dairy plant cleaning in place (CIP) processes was considered in the pH control strategy. The pH of the acidification stage was adjusted to pH 6 by automatic dosage of the alkaline CIP wastewater using a peristaltic pump and a control loop with pH glass electrode. The alkaline wastewater originated from the CIP process tank of the quarg production facility at Humana Milchunion and was a 2.0 % (m/m) sodium hydroxide solution loaded with milk ingredients during the cleaning process. The consumption of alkaline CIP wastewater was recorded. Ratio of volumes for acid whey feed and alkaline CIP wastewater varied due to the pH control system.

The organic loading rate (OLR) was calculated as follows:

$$OLR = \frac{c_{influent}}{HRT} = \frac{c_{Whey} \cdot \dot{v}_{Whey} + c_{CIP} \cdot \dot{v}_{CIP}}{\dot{v}_{influent} \cdot HRT} \qquad 5\text{-}12$$

$$\dot{v}_{influent} = \dot{v}_{transfer} = \dot{v}_{Whey} + \dot{v}_{CIP} = const. \qquad 5\text{-}13$$

Where $\dot{v}_{Whey}$ and $\dot{v}_{CIP}$ are the volume flow rates in $L \cdot d^{-1}$ of the acid whey feed and the CIP alkaline solution and c is the concentration of the components in $g \cdot L^{-1}$ COD. HRT is the overall hydraulic retention time in days.

The idea of calcium removal by simultaneous crystallisation within the acidification stage was developed from observations made in initial experiments with the system. Heavy precipitation occurred around the inlet pipe for alkaline CIP wastewater inside of the acidification stage. These precipitate were carried over to the second stage and caused blockings of the inlet distributor. Thus, the acidification stage was modified to allow accumulation and settlement of these precipitates within the acidification reactor by installation of a compartment wall. Additionally a filter sponge was placed in the divided volume to provide a no-mix zone with an immersed outlet pipe for the transfer of the pre-acidified demineralized effluents into the second stage. After this modification the reactor set-up was operated without any upsets.

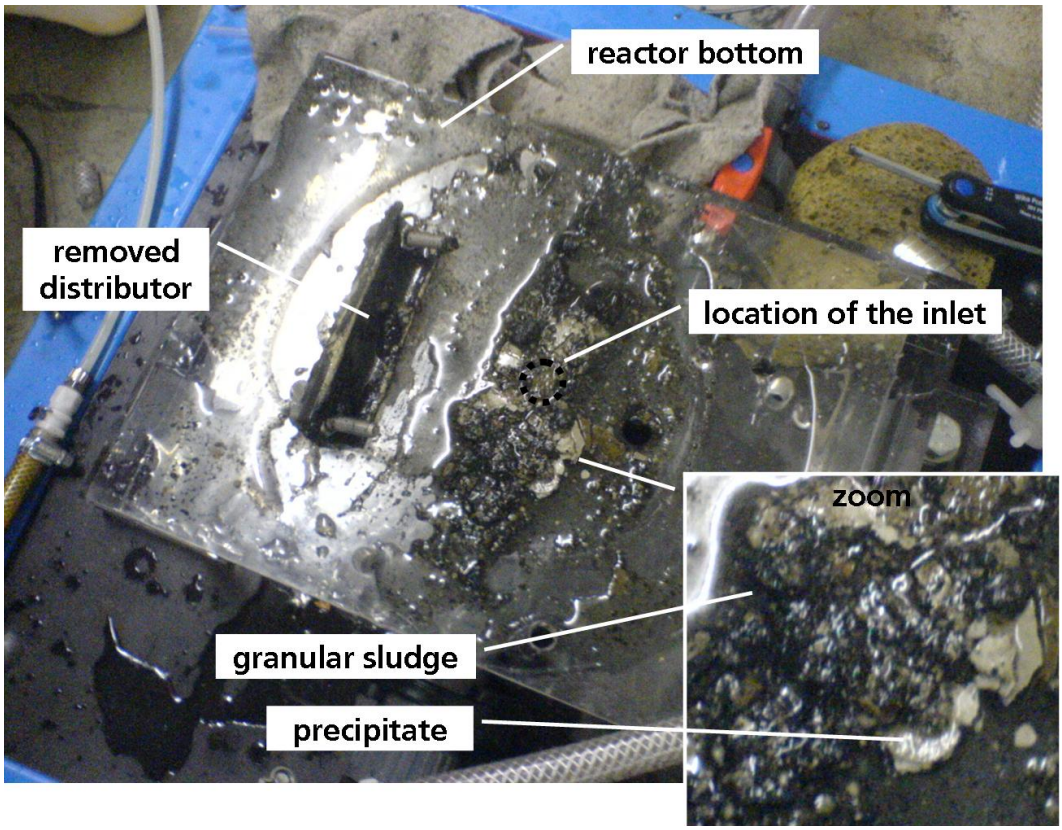

Figure 18 Base of the gaslift/fluidised bed digester stage with dismounted inlet distributor. Aggregates of milk mineral precipitates clogged the inlet distributor.

5.5. Analytical Methods

5.5.1. Sample Preparation and Inocula

The reference dairy was too far away from the laboratory to take samples on a daily basis, so acid whey was collected every few month. The whey was collected directly after its production at approximately 50 °C. It was chilled to room temperature during transportation (2 h). Analytical procedures of whey were performed immediately after arrival in the laboratory. The volume needed for the anaerobic digestion experiments was stored in a freezer at -18 °C. It was defrosted weekly and stored in a refrigerator from which it was directly pumped to the reactor inlet. Chemical Oxygen Demand (COD) was measured and, if necessary, the whey was adjusted to the demanded COD by diluting with potable water.

For the examination of cleaning solutions samples were taken from different sources in the dairy explained further in depth in Chapter 6. Samples of cleaning solutions were taken five times over a 2 year period at this facility. A daily sampling of the sodium hydroxide cleaning solution over a 12 d period was carried out for monitoring of the load evolution in the process tank.

As the load and composition of the initial CIP wastewater can vary widely, a synthetic wastewater with stable conditions was used for waste reduction (filtration) and biodegradability experiments. The synthetic wastewater was made up to match a typical alkaline wastewater from a CIP plant in operation on a quarg cheese production process. The wastewater contained 1.5 % (m/m) sodium hydroxide and an organic load with a COD of 2.5 $g \cdot L^{-1}$. The development, preparation and evaluation of the synthetic wastewater are described in Section 7.1.

Anaerobic Digestion was performed using anaerobic sludge from different large scale plants. The CSTR and membrane reactor experiments were performed using anaerobic sludge from an anaerobic sludge treatment in a municipal wastewater plant. Granular sludge was used as inoculum in the UASB and IC experiments. Except for one initial test run with the UASB described in Section 9.5.1 the sludge was taken from an industrial high-rate anaerobic digester treating wastewaters from bio- alcohol production and distillery (Kraul & Wilkening U. Stelling KG-Gmbh & Co., Hanover, Germany).

5.5.2. Chemical Oxygen Demand (COD)

Oxidisable substances react with sulphuric acid – potassium dichromate solution in the presence of silver sulphate as a catalyst. Chloride is masked by mercury sulphate. The green coloration of Cr^{3+} is evaluated.

COD was measured by open reflux method as described in Standard Methods for the Examination of Water and Wastewater (APHA 5220 B, 1997) instead of the method described in German Standard Methods for the Examination of Water, Wastewater and Sludge (DIN 38414-9, 1986) due to the use of mercury free reagents. If not mentioned otherwise, samples were filtered.

Portions of 5.0 mL of a COD Reagent (Ficodox, Fisher Scientific, Germany) were given into screw cap flasks. Measurements of samples and blanks were done each in triplicates by adding 2.5 mL of the sample respective deionised water to the reagent. The flasks were screwed tight and well shaken before two hours heating at (150 ± 5) °C in a thermostat heater block (LT100, Dr. Lange AG, Germany). After cooling to 20 °C the flasks were emptied into 500 mL beakers and rinsed with deionised water tree times. The samples were then titrated using a 0.0122 mol·L^{-1} ferrous ammonium sulphate (FAS) solution and phenanthroline iron(II) sulphate indicator solution (Ferroin, Fisher Scientific, Germany). Endpoint of titration was the colour change from colourless to orange. Chemical Oxygen Demand (*COD*) was calculated in mg·L^{-1} by using following equation:

$$COD = \frac{(V_1 - V_2) \cdot c_{FAS} \cdot 8000}{V_0} \qquad (5\text{-}14)$$

where V_1 is the mean of volumes of FAS titrated to the samples (mL); V_2 is the mean of volumes of FAS titrated to the blanks (mL); c is the molarity of the FAS (mol·L^{-1}) and the molar mass of ½ O_2 is 8 000 mg·mol^{-1}.

Preparation of FAS (ferrous ammonium sulphate) was done by solving 24.5 g ammonium iron(II) sulphate in 1.0 L deionised water using a 5.0 L volumetric flask. A portion of 50 mL concentrated sulphuric acid was added. After cooling down to 20 °C the FAS was made up to 5.0 L with deionised water. Molarity of the FAS was analysed by titration against a commercial available standard 0.041 7 mole potassium dichromate ($K_2Cr_2O_7$) solution (Fisher scientific, Germany) which contains concentrated sulphuric acid. Ferroin was used as indicator solution. Molarity of the ferrous ammonium sulphate c_{FAS} was then calculated by using following equation:

$$c_{FAS} = \frac{V_P \cdot c_P \cdot 6}{V_{FAS}} \qquad (5\text{-}15)$$

Where V_P is the Volume (mL) and c_P is the molarity (mol·L^{-1}) of the standard potassium dichromate solution; V_{FAS} is the volume of FAS titrated (mL) and 6 is a conversion factor: 6 moles of ammonium iron(II) sulphate are equivalent to 1 mole of dichromate.

5.5.3. Biological Oxygen Demand (BOD)

The 5 d biochemical oxygen demand (BOD5) was determined in accordance with German Standard Methods for the Examination of Water, Wastewater and Sludge: Determination of biochemical oxygen demand after n days (BOD*n*) — Part 1: Dilution and seeding method with allylthiourea addition (DIN EN 1899-1, 1989). Some samples were examined using a photo-spectroscopic cuvette test (LCK555, Hach-Lange, Germany) parallel to the standard method.

5.5.4. Nitrogen

Total-Nitrogen was determined using a standard photo-spectroscopic cuvette test (LCK338, Hach-Lange, Germany). Inorganically and organically bonded nitrogen of a sample was oxidized to nitrate in a screw cap flask using peroxodisulphate. The nitrate ions react under heating (60 min, 100 °C) in a solution of 2.6-dimethylphenol in sulphuric and phosphoric acid to nitrophenol, which was evaluated after chilling, using a spectral photometer (CADAS 50S, Dr. Lange, Germany). This method is equivalent to standard methods Klostermann and Oberdörster (1999) and was used in other scientific works before, *e.g.* Rottiers *et al.* (1999), Rosenberger *et al.* (2002).

5.5.5. Phosphate

In a standard photo-spectroscopic cuvette test (LCK348, Hach-Lange, Germany) phosphate ions react with molybdate and antimony ions in an acidic solution. An antimonyl phosphomolybdate complex was formed, and then reduced by ascorbic acid to phosphomolybdenum blue which can be evaluated using a spectrometric photometer (CADAS 50S, Dr. Lange, Germany). Klostermann and Oberdörster (1999) reported that this method was equivalent to standard methods. The method was also used before, in other scientific works, *e.g.* Rottiers *et al.* (1999), Parawira *et al.* (2004)

5.5.6. Solids

The determination of solids was in accordance with German standard methods for the examination of water, waste water and sludge; parameters characterizing effects and substances (group H); determination of total dry residue, filtrate dry residue and residue on ignition (H1) DIN 38409-1 (1997).

Beakers were prepared by drying in a hot box oven (ULM 500, Memmert, Germany) at 105°C for at least 2 h and cool them down to ambient temperature in a desiccator prior determination of their weigh with a 4 decimal point balance (BP 221 S, Satorius AG, Germany). A known volume of the samples was then added and dried to constant weight at (105 ± 2) °C in a hot box oven. The beaker is cooled in a desiccator, and weigh. After

further 30 min in the oven, the sample is cooled down and weighed again. If the measurements differ less then 2 mg the mass is considered as constant. Otherwise the sample is dried again until mass constancy is reached.

The total dry residue β_{TS} in mg·L^{-1} is calculated by following equations:

$$\beta_{TS} = \frac{m_{DM}}{V_S} \tag{5-16}$$

Where V_S is the Volume of the sample in litres and m_{DM} is the dry mass of the sample in mg calculated as follows:

$$m_{DM} = m_B - m_A \tag{5-17}$$

Where m_B is the mass in mg of the beaker with the residual mass after drying and m_A is the mass in mg of the empty beaker.

After determination of the total dry residue the sample was incinerated at (500 ± 5) °C to constant weight to determine the residue on ignition. The residue on ignition β_{FS} in mg·L^{-1} was calculated as follows:

$$\beta_{FS} = \frac{m_{FS}}{V_S} \tag{5-18}$$

Where m_{FS} is the mass of the incinerated sample calculated as follows:

$$m_{FS} = m_C - m_A \tag{5-19}$$

Where m_C is the mass of the beaker with the residual mass after incinerating in mg and m_A is the mass of the empty beaker in mg as in equation 5-19.

5.5.7. Volatile Fatty Acids (VFA)

Measurement of volatile fatty acids was performed by using a gas chromatograph (Model 5890 Series II, Hewlett Packard, USA) fitted with an capillary column (HP-FFAP 25 m x 0.32 mm x 0.52 µm, Hewlett Packard, USA) and a software based integration and calculation (HP3365 Chemstation, Hewlett Packard, USA). The sample preparation based on the method using ether extraction described by Peck *et al.* (1986). The method was modified to enhance the response. The samples were acidified using phosphoric acid like described by Cruwys *et al.* (2002). Additionally ammonium sulphate solution was added like described by Boe *et al.* (2005). Following ingredients were added to 1 mL of the sample: 1 mL internal standard (2 ethylbutyric acid, 10 mmol), 1.0 mL diethyl ether, 50 µL of phosphoric acid (1.0 mol·L^{-1}), and 100 µL of ammonium sulphate solution (1.0 mol·L^{-1}). The preparation was mixed for 30 s using a small vortex mixer. A centrifugation at 6 000 min^{-1} (EBA20 centrifuge, Hettich GmbH & Co. KG, Germany) for 60 s separates the mixture into

a water layer and an ether layer. 2 µL of the ether layer were injected and analysed. The amount of volatile fatty acids was calculated from the peek area using a reference table derived from calibration with a volatile free acid standard mix (Supelco Inc., USA).

5.5.8. Carbon

The total inorganic carbon (TIC) of the sample is first expelled from the sample with the help of a vented shaker (TOC-X5, Hach-Lange, Germany). The total organic carbon (TOC) is oxidized to carbon dioxide (CO_2). The CO_2 passes through a membrane of a two-stage photo-spectroscopic cuvette test set (LCK386, Hach-Lange, Germany). A change of the sample colour was then evaluated with a photometer. (CADAS 50S, Dr. Lange, Germany) This method was used except in the first biodegradability experiment (Section 8.1) where a TOC Analyser (DC190, Rosemont Dohrman, UK) was used.

5.5.9. pH

Examination of pH, for sample characterisation, titration procedures and for online measurement were done in accordance with German standard methods for examination of water, waste water and sludge *i.e.* DIN 38404-5 (1984).

5.5.10. Bicarbonate Alkalinity

Buffer capacity of the digester liquor was monitored by determining the bicarbonate alkalinity of effluent samples by titration with 0.1 M hydrochloric acid. The endpoint was detected by potentiometry using a pH Probe (Model 766 Calimatic with pH electrode SE101, Knick, Germany). The method based on the work of Jenkins *et al.* (1983) and differs from the standard method for the determination of alkalinity of water according to German standard methods for the examination of water, waste water and sludge (DIN 38409-7, 2005). The Jenkins-method uses a higher pH endpoint (pH 5.75 instead of pH 4.3). The higher endpoint was introduced to avoid interferences of the bicarbonate alkalinity with the buffer capacity from volatile fatty acids. True bicarbonate alkalinity *TBA* expressed as $mg \cdot L^{-1}$ calcium carbonate ($CaCO_3$) was calculated using following equation:

$$TBA = \frac{V_A \cdot c_A \cdot 50000}{V_S} \cdot 1.25 \qquad (5\text{-}20)$$

where V_A is the volume (mL) and c_A is the molarity ($mol \cdot L^{-1}$) of the hydrochloric acid titrant; V_S is the sample volume; 50 000 mg CaCO3 are equivalent to 1.0 M hydrochloric acid and 1.25 is the conversion factor to compensate for the higher pH endpoint.

5.5.11. Gases

Volume percent of methane and carbon dioxide of the biogas was measured by using a measuring system (BacCap-CH_4, BacCap-CO_2, Sentronic, Germany) based on infrared absorption. The system consists of separate methane and carbon dioxide sensor heads with integrated cuvettes, IR-emitter, detector and electronic. The measuring ranges where (0 to 100) % (v/v) methane and (0 to 50) % (v/v) carbon dioxide. The sensors were integrated in the off- gas pipe in the CSTR experiments. The accuracy of first measurements was poor due problems associated with condensing vapour and low gas flow rates. To overcome these problems a gas sample preparation and analysis unit (Figure 19) was connected to the digesters headspace. The unit consists of a gas cooler with condenser, a sample pump, a membrane filter and the gas sensors described above. The sample pump circulates the headspace gas through the unit and back into the reactor headspace. A condensate pump removes condensed water from the condenser and pumps it back into the reactor. The unit was developed and built at the University of Applied Science and Arts Hanover.

The volume of biogas produced in the digesters was measured using two identical low flow meters (ADM2000, Agilent) based on the acoustic displacement principle which is independent from gas composition. The measurement range was (0.1 to 1 000) $mL \cdot min^{-1}$ of gas.

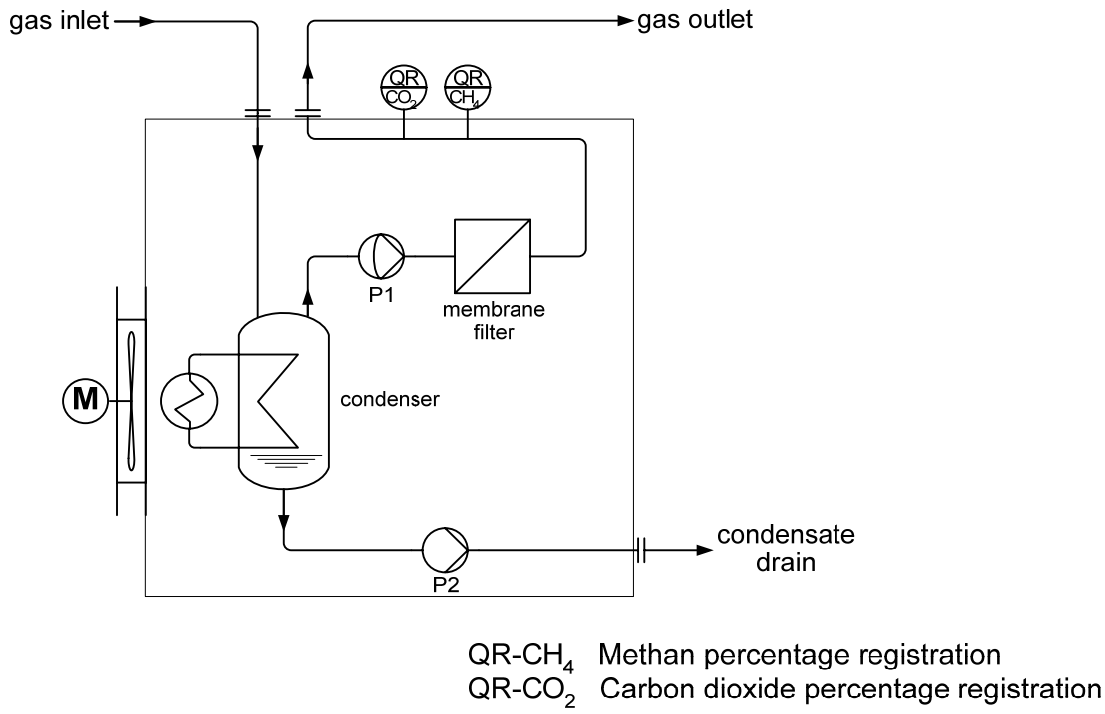

Figure 19 Process scheme of the gas sample preparation and analysis unit.

5.6. Data Storage System

A database software system has been developed and programmed for storage of raw measurement data from online sensors and results of analytical examination. The system is realised by using Microsoft ACCESS and Visual Basic for Applications. Data from measurements and analytical procedures was stored using identifiers and time stamps in database files (backend) which are separate from the graphical user interface file (front-end). Documentation of analytical and online data was printed from automatic generated reports. These reports are intended to fulfil the requirements of the Good Laboratory Practice (GLP) regulations for documentation of analytical examination. All reports generated during the project are listed in the Appendix, Page 169.

6. IDENTIFICATION AND CHARACTERISATION OF THE EFFLUENT STREAMS FROM A QUARG PRODUCING DAIRY

A reference site, an operating facility of the Humana Milchunion in Georgsmarienhütte, Germany was selected as a typical quarg cheese producing dairy for in depth characterisation. The data presented in this section regarding usage of resources was achieved in interviews with the administrative of the Humana Milchunion (personal communication, interview during the meeting of the AUBIOS research group with the Humana Milchunion dairy management and operating staff held on 12th March 2002 in Georgsmarienhütte). Information about the dairy technology was kindly given by the technical personal during regular visits at the production facility of Humana Milchunion, Georgsmarienhütte and via e-mail (personal communication, e-mail, 8th October 2004).

6.1. Volumes and Frequencies of Wastewaters at the Examined Dairy

The Humana Milchunion dairy has two main production plants, one for the production of yoghurt with a milk utilization of 121 000 metric tons in the year 2003 and quarg cheeses with a milk utilization of 156 000 metric tons in the same year. The dairy produces in three work shifts (24 h) and five days a week. The quarg production plant consists of five process tanks for curd thickening and three independent production lines. Approximately 150 metric tons of quarg and 450 metric tons of acid whey were produced each working day from this plant. The consumption of potable water was 260 000 metric tons in the same year. Expressed as specific consumption this implies the use of 0.938 6 kg of water per kg of utilized milk. An overview of the specific and total consumption of water and cleaning agents at the examined dairy is displayed in Table 4.

Table 4 Specific and total consumption of water and cleaning agents at the Georgsmarienhuette dairy in 2003.

Resource	Total Consumption [10^3 kg]	Specific Consumption of Resource per Utilized Milk[1] [g·kg^{-1}]
Milk utilized, total	277 000	-
Milk utilized, quarg production	156 000	-
Potable water	260 000	9 386.0
Sodium hydroxide, liquid 50 % (m/m) NaOH, total	500	1.805
Nitric acid, liquid 53 % (m/m) HNO_3	370	1.3357
Alkaline separator cleaner	20	0.1282

[1] consumption refer to milk utilized for quarg production

A central cleaning in place (CIP) system is used for sanitising of the process tanks, tubes and pumps, and also of filter sieves and heat exchangers, of the quarg and yoghurt production plants at this dairy. Beside the central CIP system, a small independent CIP system is connected to the quarg separators of the quarg production plant. At present the dairy do not operate any membrane based recycling processes for their cleaning in place (CIP) systems, although cleaning solutions were collected in process tanks for reuse and a recycle loop with a connected separator is in use to remove particles from the cleaning agents. The central CIP plant includes dosage stations which are connected to process tanks to prepare the acidic and alkaline cleaning solutions and to re- sharpen the solutions.

Table 5 Effluent streams and their volumes at Humana Milchunion, Georgsmarienhütte, Germany.

	Volume of Waste per Year (2003) [$m^3 \cdot a^{-1}$]	**Frequency**	**Source**
Acid whey	112 000	450 $m^3 \cdot d^{-1}$ 22 $h \cdot d^{-1}$; 5 days per week	quarg production
Sodium hydroxide (NaOH) solution 1.5 % (m/m)	17 000	(10 to 15) $m^3 \cdot d^{-1}$ lost by entrainment, 10 m^3 per month is highly loaded precipitation, discharged at monthly solution replacement	central CIP
Nitric acid (HNO_3) solution 1.5 % (m/m)	9 800	(5 to 8) $m^3 \cdot d^{-1}$ lost by entrainment	central CIP
Cleaning agent solution 1.5 % (m/m)	1 300	(3 to 4) $m^3 \cdot d^{-1}$ lost by entrainment; 2 m^3 per month discharged at solution replacement	local separator CIP

The alkaline cleaning solution is a sodium hydroxide solution in a concentration of (1.5 to 2.0) % (m/m). A small amount of sodium tripolyphosphate was added to enhance the cleaning effect. The additive was dosed into the raw liquid sodium hydroxide of 50 % (m/m) concentration before the alkaline cleaning agent was prepared by dilution with potable water. The acidic cleaning solution was also prepared from raw basic chemicals, *e.g.* from Nitric acid at a concentration of 53 % (m/m) and potable water. The concentration of the ready to use acidic cleaning solution was 1.5 % (m/m). The independent separator CIP plant uses an industrial cleaning agent specially formulated for separator cleaning. It contains potassium hydroxide, sodium hydroxide, sequesterants, phosphonates and antifoam-agents. The cleaning agent was diluted to 1.5 % (v/v) with potable water before use. All cleaning solutions are heated by steam powered heat exchangers

which are placed in a recycling loop connected to the process tanks. The temperature in the process tanks was usually set to 70 °C.

The cleaning procedures take two hours a day total for each production line. A cleaning starts with product removal by rinsing with potable water. The product draining is controlled by turbidimetry and the diluted product is collected and re- processed. After product removal the process tank with the collected product will be disconnected and the first alkaline rinsing starts. The heavy loaded streams from this first alkaline cleaning were drained, controlled by conductimetry. Some loss of cleaning solutions occurs in the automatic self cleaning function of plate separators in which the upper housing of the separator is lifted for a few seconds. This flushes precipitates and agglomerates out of the separator, and in addition, approximately 50 L of cleaning solution is lost. While the product is separated from cleaning agents by no mix valves, the cleaning solutions are not separated that severe. Some carry over of alkaline and the acidic solutions into the rinsing water are tolerated. All these lost volumes have to be replaced by fresh prepared cleaning agents to maintain the volume in the cleaning system. In the central CIP system a small separator is placed in a connected loop to remove agglomerated particles from the cleaning solution. Once a month the CIP process tank is emptied and cleaned, precipitations were removed. The number of effluent streams and their volumes at the factory were identified from the data collected by the chief production manager from the production year 2003. An overview of relevant effluent streams from quarg production is given in Table 5.

6.2. Composition of Acid Whey and CIP Wastewaters

Samples of Acid whey were examined on different occasions. Beside regular measurement of influent strength COD for whey digestion experiments also other relevant parameters were analysed. Results of the whey analysis are given in Table 6. A typical composition of acid whey based on data of Kessler (1996) is shown in Figure 20.

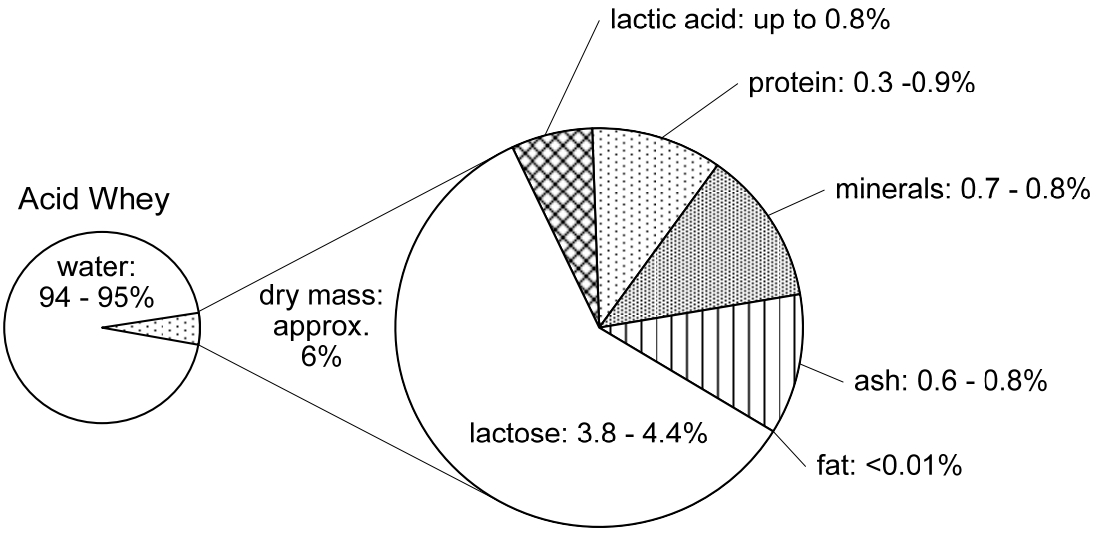

Figure 20 Composition of acid whey.

Samples of the sodium hydroxide cleaning solution from the process tank of the central cleaning in place (CIP) plant were collected from a sampling port in the circular main, samples were taken on two occasions. A single random grab sample and a composite sample prepared by mixing samples taken on a daily basis over a period of 12 days were taken. During the ordinary operation of the CIP-plant a precipitation of organic substances occur in the sodium hydroxide process tank. A sample of these precipitates was collected from the discharged volume during monthly renewal of cleaning solutions. Analysis of samples from the formulated cleaning solution used for the cleaning of quarg separators in an independent de-centralised CIP plant were taken on one occasion.

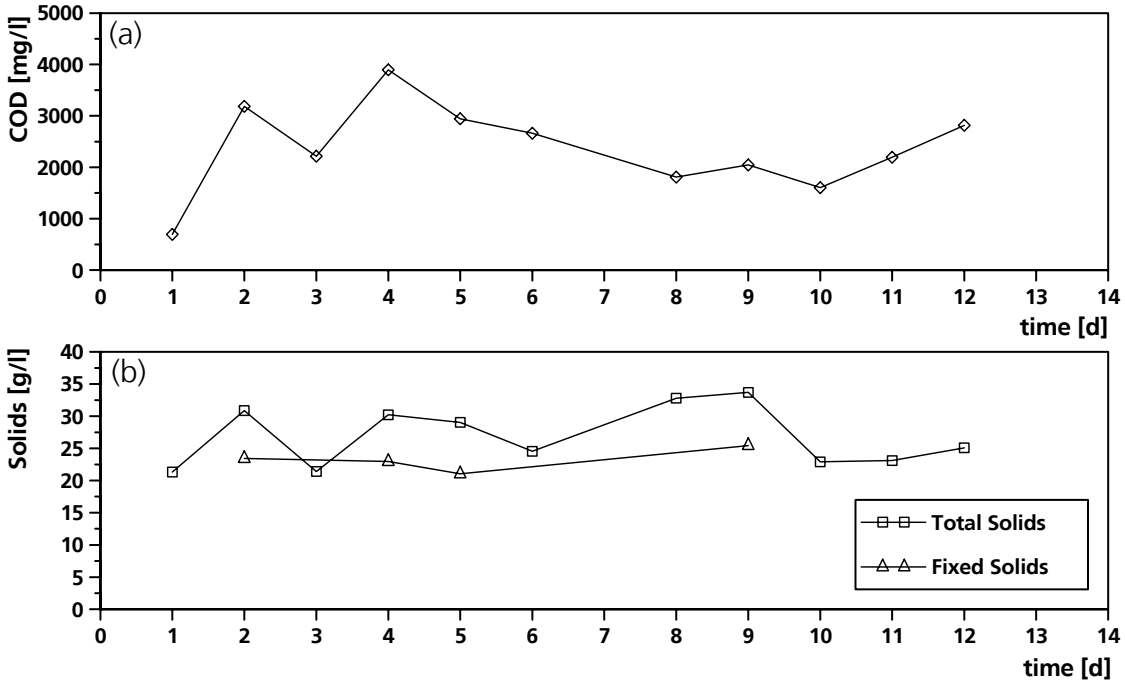

Figure 21 Solids and COD development in a sodium hydroxide CIP solution process tank.

The development of load in the process tank was monitored over a period of 12 days after cleaning of the process tank with complete renewal of the sodium hydroxide solution of the process tank (Figure 21). The load of the cleaning solution increased from initially 695 $mg \cdot L^{-1}$ of COD to 3 185 $mg \cdot L^{-1}$ within one day of operation. The weekly procedure of refreshing on every Wednesday caused temporary decreases in organic load. Thus, at day 3 and day 10 the COD concentrations were measured 2 215 $mg \cdot L^{-1}$ and 1 605 $mg \cdot L^{-1}$ respective. The extensive cleaning procedures on Saturdays (day 6) with larger volumes of discharged spent wash and a corresponding renewal of cleaning solution also decreased the COD level, however to less extend. Also from the total and fixed solids plot the development of the load and refreshing of the solution can be seen. Fixed solids, derived mainly from the sodium hydroxide content, fluctuated only slightly due to dilution by carry over of rinse water and refreshing. The total solids increased from initially 21.32 $g \cdot L^{-1}$ to 30.88 $g \cdot L^{-1}$ at day 2 and decreased to 21.4 $g \cdot L^{-1}$ after the refreshing procedure at day 3. The procedures on day 6 and day 10 caused similar effects. However, the COD measurement of the sample taken at day 8 was unexpected low (1 810 $mg \cdot L^{-1}$). This is not explainable by the process. The measurement is therefore rejected as erratic.

From the measurements of fixed solids it can be concluded that the sodium hydroxide concentration was higher than the value of 1.5 % (m/m) reported by the Humana technical personal. This was proved by titration of the samples to pH 7.0 with hydrochloric acid to determine the OH^- activity. A sodium hydroxide concentration of (1.76 to 1.89) % (m/m) was calculated assuming that the OH^- activity completely derived from this source. Also a random grab sample, taken half a year later, was titrated. In this sample a sodium hydroxide concentration 2.0 % (m/m) was calculated.

Table 6 Chemical and physical properties of acid whey samples.

Parameter	Result	Date of Analysis	Comment
BOD 5 (spect)	(21 665 ± 445) mg·L^{-1}	25.11.2003	triplicate
BOD 5 (rapid)	19 500 mg·L^{-1}	25.11.2003	
TOC (spect)	28 000 mg·L^{-1}	10.04.2006	
COD high (spect)	65.8 g·L^{-1}	25.09.2004	
COD high (spect)	70.8 g·L^{-1}	04.11.2004	
COD high (spect)	65.6 g·L^{-1}	11.11.2004	
COD low (spect)	79 200 mg·L^{-1}	23.03.2005	
COD high (spect)	71.4 g·L^{-1}	27.05.2005	
COD high (spect)	74.6 g·L^{-1}	06.06.2005	
COD high (spect)	74.0 g·L^{-1}	07.06.2005	
COD high (spect)	75.8 g·L^{-1}	11.07.2005	
COD high (spect)	74.0 g·L^{-1}	08.05.2006	Kraft Foods
COD high (spect)	68.8 g·L^{-1}	22.05.2006	Kraft Foods
TS (grav)	(4.861 ± 0.089) g·L^{-1}	30.10.2003	tenfold
FS (grav)	(0.663 ± 0.045) g·L^{-1}	31.10.2003	tenfold
VS (grav)	4.198 g·L^{-1}	31.10.2003	
N total (spect)	652 mg·L^{-1}	04.10.2004	
PO_4 total (spect)	2 280 mg·L^{-1}	07.10.2004	
PO_4 total (spect)	2 495 mg·L^{-1}	07.10.2004	
PO_4 total (spect)	2 740 mg·L^{-1}	17.05.2005	
PO_4 total (spect)	2 465 mg·L^{-1}	25.05.2005	
PO_4 total (spect)	3 030 mg·L^{-1}	25.05.2005	

When not stated otherwise, all samples are from Humana Milchunion. Explanation of method outline: (spect) = spectroscopic, (grav) = gravimetrical; (tit) = by titration; (rapid) = rapid test sensor.

Table 7 Chemical and physical properties of CIP samples.

Sample	Parameter	Result
Central CIP random sample	TS (grav)	30.64 $g \cdot L^{-1}$
	FS (grav)	28.16 $g \cdot L^{-1}$
	VS (grav)	2.48 $g \cdot L^{-1}$
	COD low (spect)	6 995 $mg \cdot L^{-1}$
	COD low (spect)	8 095 $mg \cdot L^{-1}$
	PO4- ortho (spect)	62.9 $mg \cdot L^{-1}$
	PO4 total (spect)	132.5 $mg \cdot L^{-1}$
	OH (tit)	2 % (m/m)
Central CIP 12d composite	COD low (spect)	2 345 $mg \cdot L^{-1}$
	TS (grav)	24.52 $g \cdot L^{-1}$
	FS (grav)	24.2 $g \cdot L^{-1}$
	VS (grav)	0.32 $g \cdot L^{-1}$
	PO_4 ortho (spect)	26.05 $mg \cdot L^{-1}$
	PO4 total (spect)	106 $mg \cdot L^{-1}$
	N total low (spect)	135.5 $mg \cdot L^{-1}$
	OH (tit)	1.84 % (m/m)
Central CIP precipitation	COD high (spect)	(5.843 ± 0.167) $g \cdot L^{-1}$
	N total high (spect)	237 $mg \cdot L^{-1}$
	TS (grav)	38.9 $g \cdot L^{-1}$
	PO4 total (spect)	1 350 $mg \cdot L^{-1}$
Separator CIP random	COD high (spect)	(6.123 ± 0.129) $g \cdot L^{-1}$
	N total high (spect)	281 $mg \cdot L^{-1}$
	PO4 total (spect)	185.5 $mg \cdot L^{-1}$
	TS (grav)	188 $mg \cdot L^{-1}$

All samples are from Humana Milchunion. Explanation of method outline: (spect) = spectroscopic, (grav) = gravimetrical; (tit) = by titration; (rapid) = rapid test sensor.

6.3. Conclusions from Identification and Characterisation of the Effluents

The volumes and frequencies of wastewaters from the Humana Milchunion dairy were identified and analysed. The samples from effluent streams were characterised and the results are discussed above. With the examination of the cleaning procedures and the analytical results of wastewater samples, necessary information for the development of a waste reduction strategy is available. The measurement results of the sodium hydroxide concentration in the CIP solution were given to the Humana technical personal. This leads to a re calibration of the conductivity sensors and thus to a reduced consumption of chemicals. From the evaluation of the results a number of conclusions can be reached:

- The acid whey has a high COD *i.e.* greater than 65 $g \cdot L^{-1}$ COD and a BOD_5 of approximately 20 $g \cdot L^{-1}$. The COD of whey mainly derives from lactose, lactate and proteins. In recent research these components were identified to be very biodegradable (Malaspina *et al.*, 1995)). The high grade of anaerobic biodegradability indicating that anaerobic biological treatment would be appropriate treatment option. Furthermore anaerobic digestion has various advantages compared to aerobic processes as discussed in several publications *e.g.* Speece (1996), Marchaim (1992), Gerardi (2003), Bischofsberger *et al.* (2005) Deublein and Steinhauser (2008). A major advantage is the production of biogas, suitable for the generation of power and heat. But also the investment and operational costs are significant lower in anaerobic processes.

- Alkaline effluents have a medium COD of approximately 3 $g \cdot L^{-1}$ COD and a high concentration of sodium (8.76 $g \cdot L^{-1}$). This high sodium concentration will lead to strong inhibition in an anaerobic process (McCarty, 1964). Therefore would not be appropriate for anaerobic digestion without blending.

- The load of an alkaline cleaning solution used in a steady renewing CIP process was measured as COD and also as total and fixed solids. The load increased rapidly within a day of operation and fluctuated due to usage and refreshing in the range of (1 605 to 3 185) $g \cdot L^{-1}$ COD and (21.4 to 33.72) $g \cdot L^{-1}$ of total solids. During CIP operation a sludge bed of precipitation develops in CIP tank. The precipitation will be removed in regular intervals. The COD of the precipitates varied in a wide range indicating that dilution with supernatant may change. Acidic wastes are arising in low volumes and can be used for balancing the pH of the main effluent stream.

7. STUDIES ON MEMBRANE FILTRATION FOR DAIRY WASTEWATER REDUCTION AND CAUSTIC RECOVERY

7.1. Preparation of a Synthetic Wastewater

Due to the geographical distance of the production facility of the dairy to the laboratory at the university it was decided to use a synthetic wastewater for filtration studies. To ensure conditions similar to the real effluent, four different milk processing products were tested to represent a typical load. Two quarg cheeses, one with 0.2 % fat, one with 40 % fat, protein enriched spray dried whey powder and whole milk were examined. The substances are varying in their composition, mainly milk-protein, lactose and milk fat. Nitrogen and volatile solids per 1 g COD of the synthetic wastewater where compared to a 12 day composite sample from the CIP tank of the reference site. The synthetic wastewaters were prepared by adding 50 $g \cdot L^{-1}$ of the milk products (except whey powder, 20 $g \cdot L^{-1}$) to a 1.5 % (m/m) sodium hydroxide solution. To simulate heating and pumping actions in a CIP plant the synthetic wastewater was homogenized with a vortex mixer, then heat treated at 70 °C for two hours, homogenized and treated further two hours. Samples for analytical measurements were taken directly after two and four hour heating.

A visual examination of the synthetic wastewater preparations after settling and flocculation give information about altering of their components due to reaction with sodium hydroxide, heating and mixing. All preparations were red or brownish, identified as a reaction of lactose with sodium hydroxide. The milk fat of full fat quarg cheese and whole milk caused a strong flotation visible as a layer of flocculated fat, the rest of the solution was clear. In low fat quarg cheese and whey powder preparations the proteins were dispersed, the solution was cloudy. This shows that the flotation of milk fat involve unsolved protein coagulates. The protein of preparations containing milk fat was obviously bound to the floating layer. Visual examination of original CIP wastewater samples show no floating layer and dispersed matter like the preparation with low fat quarg cheese.

Analytical measurements after second heating show a significant increase in COD of full fat quarg cheese. This implies that further heating caused increased fat hydrolysis. This is also confirmed by a decreased flotation layer. The COD to nitrogen ratio of low fat quarg cheese fits better after second heating, but worse for full fat cheese.

The genuine wastewater was higher in total phosphate than explainable by the load with milk components. An additive sequestering agent, containing sodium tripolyphosphate was identified to be the source. In addition the original wastewater had a high relative total solids concentration. As dosage of Sodium hydroxide into the CIP tank is controlled by conductivity measurement, it is presumed that sodium hydroxide concentration is higher than 1.5 % (m/m).

Table 8 Analytical measurements of the synthetic wastewater screening.

	12 day composite sample from dairy CIP tank	Sample prepared with quarg cheese (0.2% fat)	Sample prepared with quarg cheese (40% fat)	Sample prepared with dried whey protein concentrate	Sample prepared with whole milk
pH	13.9	14.0	13.9	14.0	14.0
NaOH conc. [% (m/m)]	1.5 - 2.5	1.5	1.5	1.5	1.5
COD [$g \cdot L^{-1}$]	2.345	72.5	100.5	149.3	68.0
Nitrogen, total [$g \cdot L^{-1}$]	0.1355	4.400	3.175	6.675	1.303
PO_4-P [$g \cdot L^{-1}$]	0.0827	0.418	0.445	1.065	0.323
Total solids [$g \cdot L^{-1}$]	15.9	49.2	62.2	96.2	38.2
Volatile solids [$g \cdot L^{-1}$]	0.61	23.2	44.0	51.92	20.2

Table 9 Ratio of components per sample COD of altered sodium hydroxide solutions containing different milk products.

Ratio of component per COD	12 day composite sample from dairy CIP tank	Sample prepared with quarg cheese (0.2 % fat)	Sample prepared with quarg cheese (40 % fat)	Sample prepared with dried whey protein concentrate	Sample prepared with whole milk
Nitrogen	0.0578	0.0607	0.0316	0.0447	0.0192
PO_4-P	0.0353	0.0058	0.0044	0.0071	0.0048
Total solids	6.7804	0.6786	0.6189	0.6443	0.5618
Volatile solids	0.2602	0.3200	0.4378	0.3476	0.2971

From the results of the visual and analytical examination of the different samples the following conclusions can be reached:

- COD to volatile solids and COD to Nitrogen content ratios were best suited by the preparation with low fat quarg cheese.
- A heating of 4 hours was required to achieve best results for COD to nitrogen ratios.
- High phosphate concentration of the 12 d composite sample is due to use of sequestering agents containing sodium polyphosphates. The exact formulation was not reported by the dairy's operators. The high phosphate content is not represented by the synthetic preparations.
- High total solids ratio of the real wastewater is originated from relatively low COD load compared to the synthetic preparation. This will be equalized when the preparation is diluted with 1.5 % NaOH to its final COD.

Compared to the 12 day composite sample, a preparation with low fat quarg cheese provided the best fit. Therefore it was chosen to simulate a typical used CIP cleaning solution. A heating of 4 hours give the best results for nitrogen content per g COD. The synthetic wastewater was prepared for further examination by dilution to an intended COD with fresh prepared 1.5 % (m/m) NaOH solution.

7.2. Nanofiltration Process

A batch of the synthetic wastewater was prepared as described in Section 7.1. An initial COD of 5.39 $g \cdot L^{-1}$ was measured. The wastewater was then treated using the nanofiltration plant described in Section 5.1. The concentrate was recirculated in the plant to reduce its volume from initial 45.12 L to a final residue of 2.0 L by the nanofiltration process. The transmembrane pressure was controlled and adjusted manually during operation by manipulating the revolving speed of the diaphragm pump. Samples of the circulating concentrate were taken regularly during the run. The COD of the samples was measured on the same day after finishing the filtration process. The permeate flow and the transmembrane pressure were recorded during the run.

7.2.1. Process Parameters of the Filtration

The permeate flow decreased from initial 8.9 $L \cdot h^{-1}$ to 3 $L \cdot h^{-1}$ during the first 200 min of filtration due to an initial layer that typically builds up at the membrane surface when a new cross flow filtration membrane was operated the first time or after intensive cleaning (Figure 22). To remove the membrane surface layer and recover the permeate flow to values above 3 $L \cdot h^{-1}$ a back flushing of permeate was performed. A small pump was used

to force back permeate through the membrane while the retentate pump was switched off. This procedure was manually operated for a 5 min interval after (400, 480, 650 and 680) min of filtration. Each back flushing procedure increases the permeate flow for approximately 1 $L \cdot h^{-1}$. A more rapid decrease of permeate flow was observed after 500 min of filtration. This is supposed to be due to the increased viscosity of the concentrate and a resulting denser layer on the membrane surface which is less permeable. Permeate flow decrease due to cake layer formation and fouling is widely reported (Pellegrino, 2006, Blanpain-Avet *et al.*, 2004); Bilstad,1997). After 710 min of filtration the process was stopped after sudden increase of permeate flow indicating a leak in the seal for separation of permeate and concentrate in the module head. This was confirmed by a dramatically decreased COD retention and a visual detectable permeate turbidity.

The trans-membrane pressure was adjusted to approximately 6.0 bar during the first 300 min of the experiment and was then slightly increased to 7.0 bar and later to 7.5 bar to balance the decreasing permeate flow resulting from higher retentate viscosity as explained above.

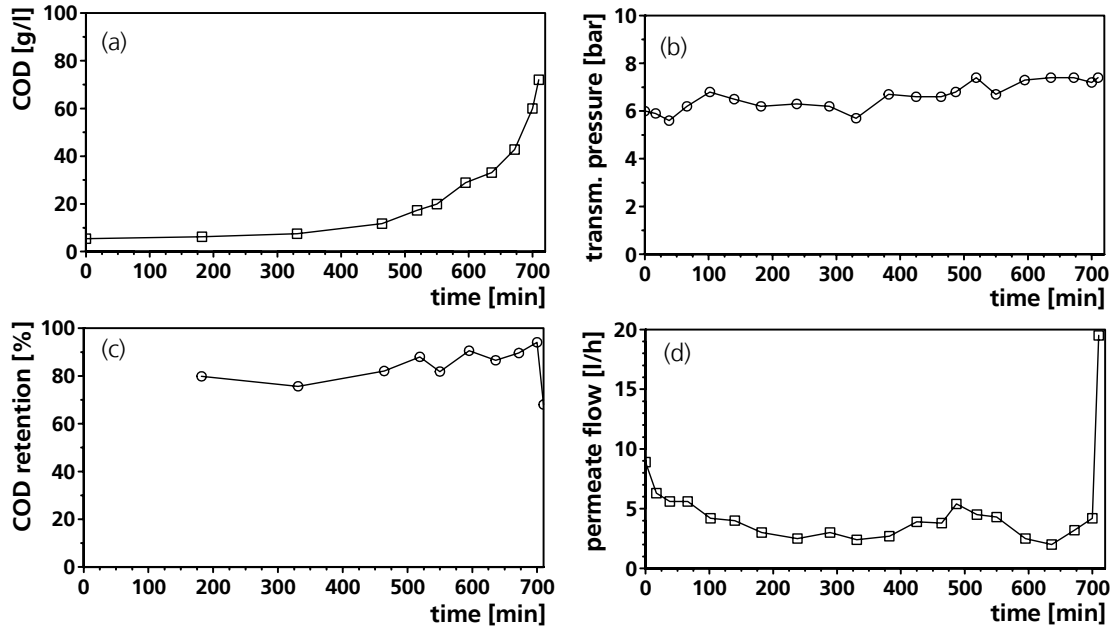

Figure 22 Filtration parameters of a synthetic alkaline cleaning solution. (a): Chemical Oxygen Demand (COD) of samples taken during the run; (b): Transmembrane pressure; (c): Calculated COD retention; (d): Measured permeate flow.

The filtration parameter adjustment can not be stated as optimal conditions for the used membranes. The nominal water flux at 1 bar and 1 $m \cdot s^{-1}$ crossflow velocity at 20 °C, given by the manufacturer, was 30 $L \cdot m^{-2} \cdot h^{-1}$. When treating the retentate at 6 bar, a permeate flow of (10 to 15) $L \cdot m^{-2} \cdot h^{-1}$ had been expected, but only a mean specific permeate flow rate of 8.26 $L \cdot m^{-2} \cdot h^{-1}$ was achieved. The applied cross flow velocity of 1.33 $m \cdot s^{-1}$ was low,

compared to those used in research works of others *i.e.* Henk (1993); Dresch *et al.* (1999); Thus, the low crossflow velocity in combination with a moderate transmembrane pressure of maximum 7.5 bar was identified to cause the unexpected low specific permeate flow.

7.2.2. Calculation of COD Rejection

The volume reduction ratio *VRR*(*t*) was calculated with attention to the volume of the samples removed from the system as in equation 5-3 to investigate the COD rejection further in depth. The *VRR*(*t*) is a polynomial function of second degree and increases with time. It was found that the COD increases with process runtime in a similar manner as shown in Figure 23. In case of the concentration of a single component with a defined rejection the increase of the concentration is linear dependent to the *VRR*(*t*) as shown in equation 5-5 and the rejection can be calculated like in equation 5-4. But, in the case of a multi component parameter like the chemical oxygen demand (COD) the coefficient of rejection can not simply be calculated because of possible drifts during the filtration process. These drifts can occur when single components comprised in the COD have a different permeability. Additionally, if COD is present in particles the rejection coefficient can be influenced when components are solved during the filtration or a breakdown of larger molecules to smaller ones occurs. This can be caused by pumping action and shear stress.

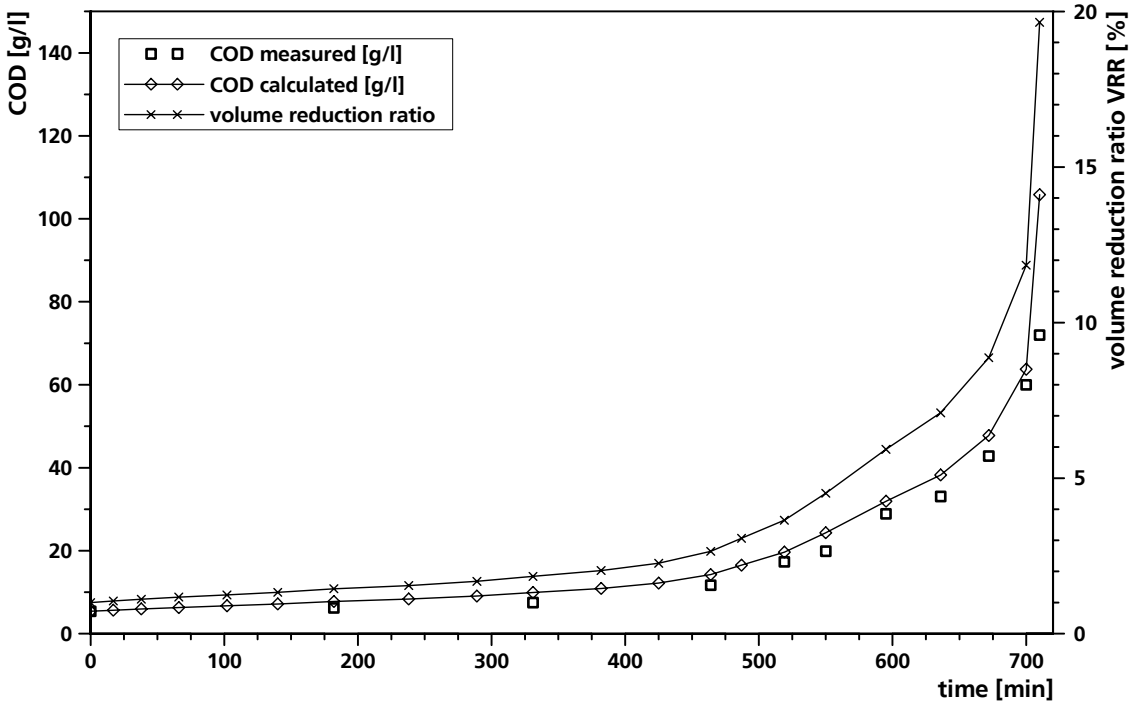

Figure 23 Volume Reduction Ratio of the nanofiltration process. Chemical Oxygen Demand (COD) calculated from initial COD and Volume Reduction Ratio compared to analytical measurements of COD.

If solubilised matter that represents part of the COD passes the membrane the COD can not estimated from the volume reduction ratio. However, in some cases the rejection can

be calculated for the COD and this is a powerful statement about the performance of the used membrane as the COD is a widely accepted parameter for wastewater characterisation. The COD rejection can be used when the measured COD is linear dependent to the volume reduction ratio *VRR*(*t*) over a wide range of volume reduction. The process failure due to a broken seal and the leakage mentioned in Section 7.2.1 was no part of the standard operation. The sample that was taken after this incident was not used for the calculation of the linearity.

The result from this experiment with a regression coefficient of 0.996 4 (Figure 24) indicated a strong linear dependency of the sample COD to the *VRR*(*t*) during intended operation. Therefore the COD rejection can be used to characterise the filtration for a wide range of the volume reduction ratio.

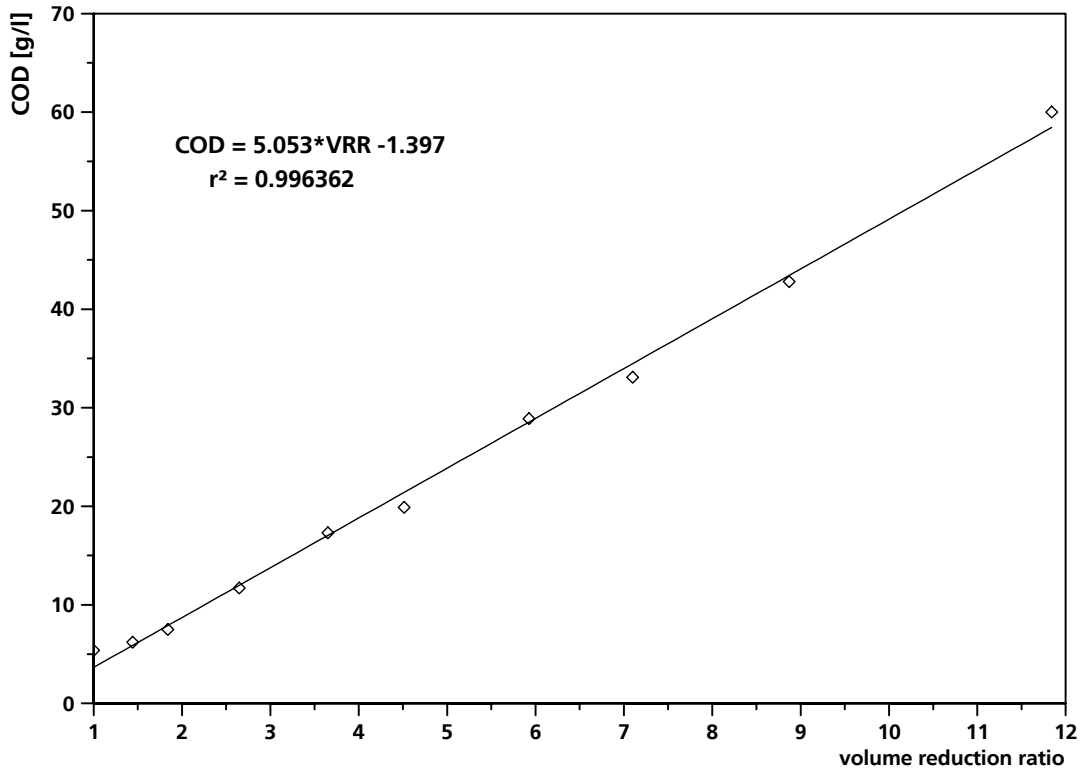

Figure 24 COD of retentate samples vs. Volume Reduction Ratio VRR(t).

7.3. Rheology of Recycling Concentrate

A batch of synthetic wastewaters was prepared like described in Section 7.1. The samples loaded with low fat quarg cheese where altered by mixing and heating. To simulate the samples had an additional mixing with a vortex mixer for 15 min. Samples of different strength where produced by a filtration procedure and their COD was measured. This

concentrated synthetic CIP cleaning solution had a chemical oxygen demand of (70, 104, 155, and 285) $g \cdot L^{-1}$.

Samples were rheological characterised by measurements with the rotational viscometer at 20 °C and a shear rate range of (0 to 500) min^{-1}. Shear stress was measured by increasing the shear rate stepwise to the maximum and also by stepwise decreasing it. Each shear rate was held constant for 3 min while measuring the shear stress. The viscosity η was then calculated from the mean values of measured shear stress τ at the adjusted shear rate using equation 5-8. The results for viscosity at different sample COD and their regression coefficients are given in Table 10

Table 10 Viscosity of concentrated synthetic CIP cleaning solution with different COD.

Sample No.	COD [$g \cdot L^{-1}$]	Viscosity [mPa·s]	Linear Regression Coefficient
CIP1	70	2.56	0.998 07
CIP2	104	10.29	0.999 96
CIP3	155	17.70	0.999 72
Cip4	285	343.75	0.995 06

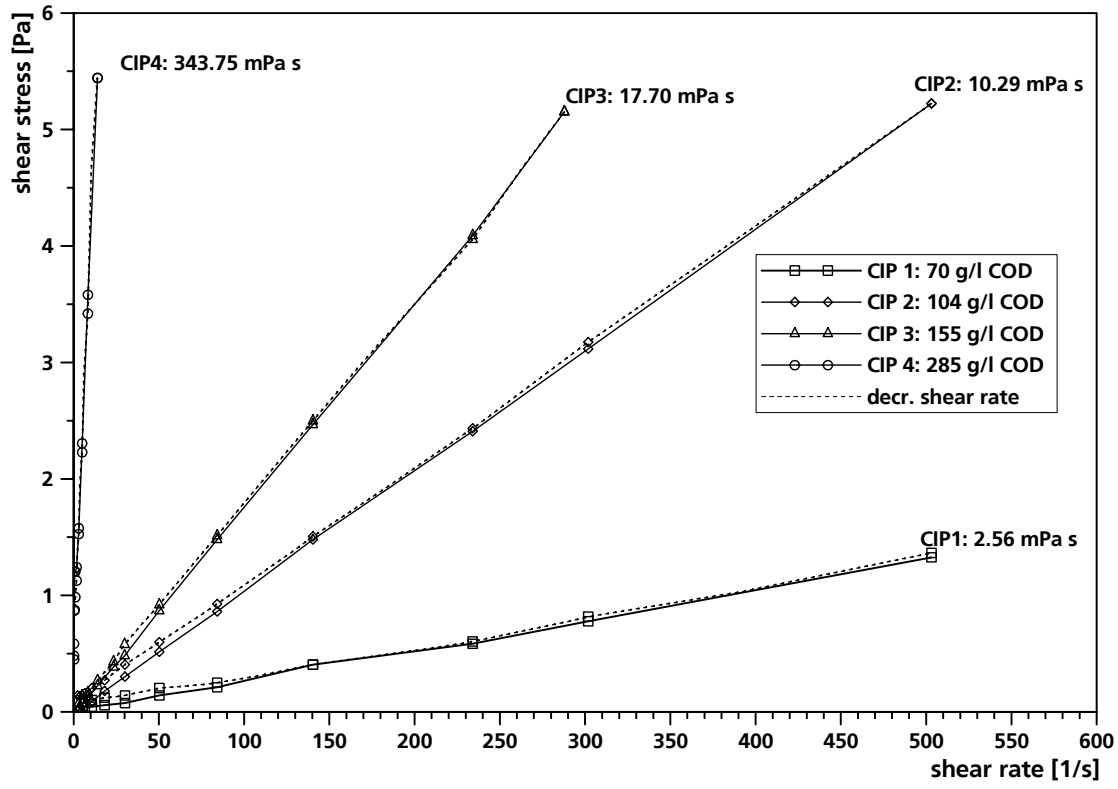

Figure 25 Rheological properties of retentates of different strength.

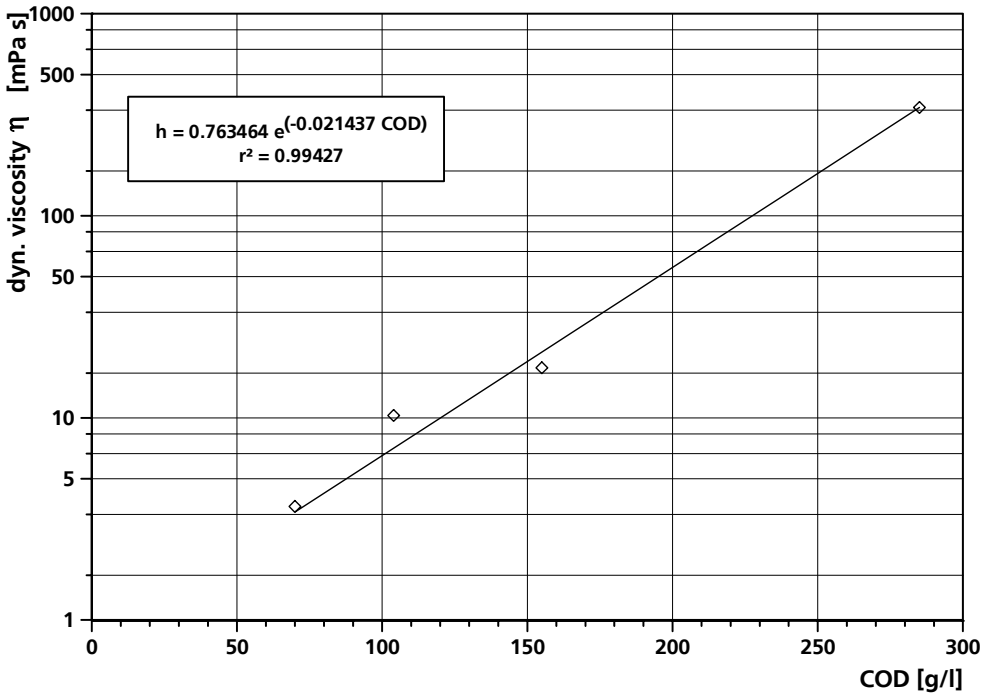

Figure 26 Viscosity vs. COD of retentates from cleaning solution recovery.

The shear stress was found to be linear dependent to the adjusted shear rate as shown in Figure 25 with a high regression coefficient for each sample given in Table 10. The resulting viscosity is constant over the whole shear rate range and there was no threshold shear stress. Viscosity of the samples is not time dependent, as measurement at decreasing shear rate shows the same values than at increasing shear rate steps. Therefore all samples can be assumed as Newtonian fluids. Furthermore a dependency of viscosity on the COD of the nanofiltration concentrates can be assumed (Figure 26).

7.4. Conclusions from Nanofiltration and from Rheology Studies

The sodium hydroxide based alkaline CIP cleaning agent can be recovered from load with milk ingredients. The filtration was performed using a ceramic tubular membrane with a molecular weight cut off (MWCO) of 1 000 Da. The membrane with 3 mm inner hydraulic diameter of the retentate channels was used without pre-treatment. However, the applied transmembrane pressure (max 7.5 bar) and a low cross flow velocity (1.33 $m \cdot s^{-1}$) resulted in a relatively low specific permeate flow of 8.26 $L \cdot m^{-2} \cdot h^{-1}$. A decrease of the permeate flow during the run was caused by reversible fouling. Short back flushing sequences had to be performed to recover the permeate flux.

Samples of the recovered cleaning agent taken during normal operation were low in COD *i.e.* 1 013 $mg \cdot L^{-1}$ and 1 316 $mg \cdot L^{-1}$ indicating a rejection of lactose by the membrane. The COD rejection by a ceramic nanofiltration of a sodium hydroxide solution loaded with milk ingredients was further studied.

The COD rejection during batch operation varied from (78 to 92) % with a mean value of 84 %. The rejection was found to be high for the used membrane compared to results found in the literature. Räsänen *et al.* (2002) archived a COD rejection of 80 % in average for Desal-5 DK spiral wound NF membrane modules with 300 Da MWCO at volume concentration ratios of 16 to 21 treating spent 0.2 % NaOH solution from dairy CIP operation with an initial COD of (8 000 to 10 000) $mg \cdot L^{-1}$. Also Hufemia (1996) reported 80% COD reduction for a MPS-34 NF membrane (Kiryat Weizmann, Ltd.) with 300 Da MWCO treating caustic NaOH wastewater from a bottle washing plant with an initial COD of (5 115 to 10 667) $mg \cdot L^{-1}$. However, this wastewater was pretreated by a microfiltration prior to the Nanofiltration process. Henk (1993) used channelled ceramic membranes in a range from 1.4 µm to 15 kDa, however, even with the densest membranes only a 71 % COD rejection was achieved treating a 2 % sodium hydroxide solution containing whole milk. It can be concludes that the molecular weight cut-off of these micro- and ultrafiltration membranes was too high to achieve lactose rejection. A comparison of the performance of processes for dairy alkaline CIP fluid recovery is given in Table 11. However, when comparing these processes it must be kept in mind that the initial COD load and the composition (carbohydrates, protein, fat) and also the ratio of suspended to solved matter of the investigated effluents can vary widely and influence the results of specific permeate flow (flux) and COD reduction.

Table 11 Performance Characteristics of Alkaline CIP Fluid Recovery Processes

Membrane	MWCO [Da]	transm. pressure [bar]	Flux $[L \cdot m^{-2} \cdot h^{-1}]$	COD rejection [%]	Reference
Tami, INSIDE CéRAM	1 000	6.0 - 7.5	8.3	85	this study
Desal-5 DK	150	9.0	7.1	81	Räsänen *et al.* (2002)
Desal-5 DL	300	8.5	8.9	76	Räsänen *et al.* (2002)
MPS-34	300	14.1	15.0	80	Hufemia (1996)
MPT-34	200	10.0	112	56	Dresch *et al.* (1999)
MPT-34	200	15.0	30 - 70	90	Yacubowicz (1995)
TechSep	15 000	2.0	170	71	Henk (1993)

In the tested application, the COD resulting from filtration can be calculated like a concentration from the volume reduction ratio for the used membranes. The COD is linear dependent to *VRR*(t). It can be concluded that the deterioration of the components caused by the pre-treatment let to stable conditions and further shear stress and elevated tem-

peratures during the filtration had no further influence to the ratio of total COD to soluble COD. This may also apply to aged CIP cleaning solutions. However, it is advised by Dresch *et al.* (2001) to treat the solution as soon as possible to avoid transmission of low molecular mass solutes.

The studies of rheological properties of alkaline CIP wastewaters on different concentrations clearly show that these effluents are Newtonian fluids even at high concentrations. Investigations by Senge (2002) show, that the rheological properties of quarg cheese can be precisely described by the Bingham model. Senge (2002) emphasis, that the dependence of rheology on the temperature of the quarg. Shear stress, temperature and chemical altering of the quarg in an alkaline CIP fluid treated by a nanofiltration process seem to destroy the structure of quarg and affect the rheological properties. Unlike quarg cheese rheology, there is no shear yield rate. In this examination the log of dynamic viscosity is linear dependent to its COD. Thus, the viscosity can be estimated from the retentate COD.

8. STUDIES ON BATCH ANAEROBIC DIGESTION OF ACID WHEY AND CIP WASTEWATER

Standardized respirometric batch tests were used to explore the suitability of anaerobic digestion of whey and alkaline CIP wastewaters with respect to a pre-treatment of the CIP wastewater by nanofiltration. To specify a blend of acid whey and alkaline wastewaters optimal for an anaerobic co-digestion, a series of biodegradability tests were performed. Information about degradation kinetics of pure whey, of alkaline CIP wastewater with and without pre- treatment and also from mixtures of these quarg production residuals was achieved from these studies.

8.1. Biodegradability of Whey and Alkaline CIP Wastewater

To investigate the effect of mixture ratios on the biodegradability of quarg production residuals *i.e.* of whey with alkaline CIP wastewater, respirometric batch tests according to BS EN ISO 11734 (1999) were performed. The synthetic wastewater and four recovery concentrates of different strength were mixed with acid whey at different mixture ratios and tested simultaneously. Pure whey was also tested. Composition and strength of the mixtures is given in Table 12. The examined 14 blends are marked by rhombuses in the parameter matrix, shown in Figure 27. Each blend was tested in triplicate. In addition, triplicate sets of blanks and reference substances (glucose) were tested. Altogether 48 separate bottles, each sealed and fitted with a pressure measurement device (OXI-Top, WTW) were inoculated with digested sludge for 21 d in a temperature controlled incubator at 35 °C. Further tests were conducted to examine a pre-treatment *i.e.* nanofiltration of the alkaline CIP wastewater to different concentrations.

8.1.1. Design of Experiment

The output factor *i.e.* the response of the series was the total biodegradability calculated from the measured pressure, of the OXI-Top system and the analytical measurement of the organic carbon balance like described in the standard method (Section 5.3). The analysis of the dynamic development of the response curves will give additional information about degradation kinetics.

The two input factors that have been chosen to be varied in the anaerobic batch test series are:

- Mixture ratio
- Total COD of the mixture

The mixture ratio of acid whey and synthetic alkaline CIP wastewaters was varied from 100 % (v/v) acid whey to 100 % (v/v) CIP wastewater. The COD of the blends varies due to

the usage of five differently concentrated CIP wastewaters. The concentration of the alkaline CIP wastewater samples ranged from 5.39 g·L^{-1} of a wastewater without pre treatment to a nanofiltration retentate with a maximum concentration of 72.0 g·L^{-1} COD. The used acid whey had a COD of 74.0 g·L^{-1}. The nanofiltration retentate samples used in this experiment originates from the nanofiltration experiment described in Section 7.2. The samples were taken during the filtration operation. COD concentrations of 22.04 g·L^{-1}, 38.69 g·L^{-1}, 55.35 g·L^{-1} and 72.00 g·L^{-1} were measured. The COD of the blends was calculated by adding the COD of each component based on the assumption that mixing alone will not cause reactions affecting the resulting COD. The mixture of whey with the chosen nanofiltration retentate results in an area of possible COD values in the shape of a triangle in the parameter matrix as shown in Figure 27.

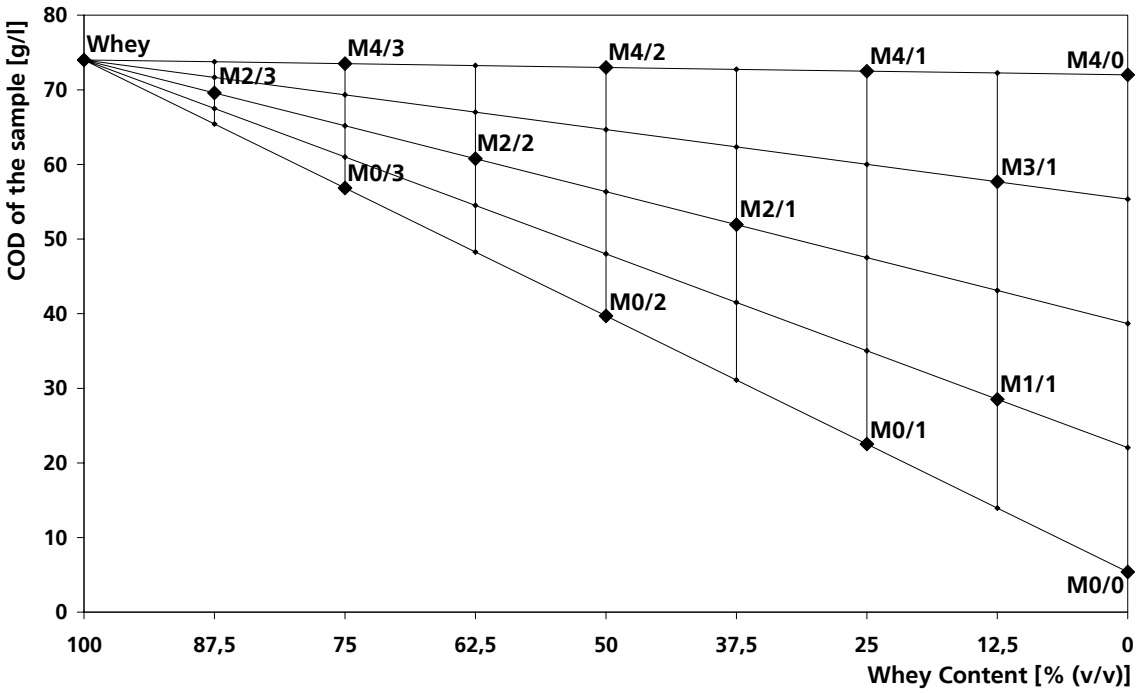

Figure 27 Selection of blends in a design of experiments parameter matrix.

The amount of carbon added to each bottle of diluted sludge was chosen to be 100 mg organic carbon (TOC). From measurements of the TOC for each sample a volume containing 100 mg·L^{-1} of TOC was calculated. Sample Identification numbering, mixture ratios, and the COD strength are given in Table 12.

The sludge for inoculation was washed 3 times to an inorganic carbon content of 3.508 mg·L^{-1} inorganic carbon before use. Total organic carbon (TOC) and COD of each sample was measured. Blend portions of 100 mg TOC were each made up to 50 mL using the test medium specified by the standard method (see Section 5.3), mixed with 100 mL diluted sludge and added to the test bottles.

The prepared medium contained 2.0 ± 1.0 g·L^{-1} total solids and the bottles had a head-space volume of 1 350 mL. The bottles were purged with nitrogen gas before sealed.

Table 12 Preparation of blends: Sample identification, mixture ratios and COD strength.

Description	Sample No.	Volume of Whey in the Blend [% (v/v)]	COD of the Blend [g·L^{-1}]	Volume of Blend (containing 100mg carbon) [mL]
Synth. wastewater (COD = 5.39 g·L^{-1})	M0/0	0.0	5.39	26.172
	M0/1	25.0	22.54	7.178
	M0/2	50.0	39.69	3.666
	M0/3	75.0	56.85	2.617
Concentrate 1 (COD = 22.04 g·L^{-1})	M1/1	12.5	28.53	5.659
Concentrate 2 (COD = 38.69 g·L^{-1})	M2/1	37.5	51.93	3.035
	M2/2	62.5	60.76	2.440
	M2/3	87.5	69.59	2.068
Concentrate 3 (COD = 55.35 g·L^{-1})	M3/1	12.5	57.68	2.552
Concentrate 4 (COD = 72.00 g·L^{-1})	M4/0	0.0	72.00	2.349
	M4/1	25.0	72.50	2.299
	M4/2	50.0	73.00	2.059
	M4/3	75.0	73.50	2.065
Pure whey (COD = 74.00 g·L^{-1})	Whey	100.0	74.00	1.910
Standard (glucose)	STD	0.0	n. a.	-
Blank (pure water)	Blank	0.0	0.00	-

8.1.2. Results of the Batch Test Series

Most of the bottles (> 95 %) were remained correctly sealed and had a viable pressure measurement record. The headspace pressure of the batch digestion bottles was measured in short intervals (84 min) for each of the mixture triplicates. In some cases the measurements of single bottles were rejected since they were identified as leaked and therefore declared as not valid. Additionally some results were rejected due to handling errors during preparation and analytics.

All plots show an increase of pressure due to warming up the headspace gas from laboratory ambient temperature to mesophilic temperature in the incubator. The temperature was maintained to 35 °C by a control loop of the incubator. The pressure increase due to warming up took approximately 4 hours. By using the gas law for ideal gases a pressure increase of 51.2 hPa can be calculated for the increase from initial 20 °C to 35 °C at constant headspace volume. An arithmetic mean plot was calculated from the triplicate sets of the headspace pressure plots of the samples using the plots identified as valid. Some of the raw plots and the resulting means are shown in Figure 28.

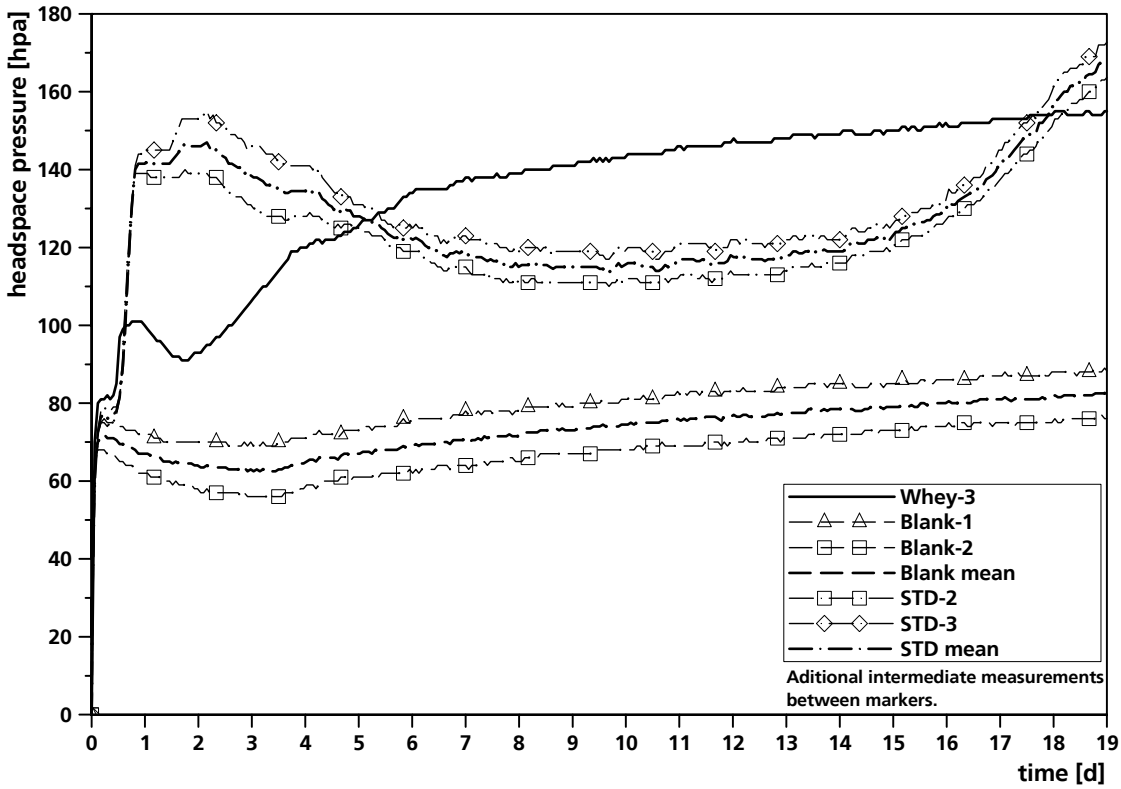

Figure 28 Raw plots of the headspace pressure of pure whey, the glucose standard and the blank sample. Calculated mean plots from blank and standard are displayed in addition.

After warm up the blank samples show a slight decrease in pressure over a period of two days. This can be explained by an oxygen contamination. The red colour of the liquid caused by resazurin indicated a positive redox potential. After consumption of free oxy-

gen an anaerobic respiration took place and the plots of the blank samples show a nearly constant increase of pressure due to decay and degradation of biomass in the solution. A saturation of the pressure of the blank samples could not been observed as the experiment ended after 21 d, though a saturation of the pressure occurs not until the mostly complete breakdown of all organic carbon including organic components and intermediates with low biodegradability or low process rates and also the breakdown of decayed biomass. To observe this decline the experiment had to be extended to approximately 60 d.

The plots of the standard samples show a straight response after a short lag-phase of only a few hours. The standard substrate, glucose is very easy biodegradable and was rapidly consumed by acid producing bacteria. The reaction occurred even faster than the acidification of whey indicated by a higher gradient on pressure increase. However, the reaction started somewhat later due to a slightly longer lag-phase. Unfortunately the standard sample triplicate and one sample triplicate (M2/2) were contaminated with oxygen. The degradation of oxygen consuming bacteria caused a decrease in pressure over a period of approx 7 d for the blank sample triplicates. The gas production restarts extremely delayed after 14 d batch test run time. The pressure plots of blend M2/2 triplicates show the occasional event of a sequencing pressure decrease and increase, indicating a very unstable microorganism population. Sample M2/2 is therefore completely rejected from the interpretation of data.

Normalized plots of the headspace pressures are shown for the comparison of the blends. To normalize the plots their pressure values were subtracted from those of the blank sample. The effect of the temperature increase at digestion start-up was eliminated by this calculation. Also the effects of oxygen contamination with a resulting decrease of pressure due to aerobic respiration was accounted for and eliminated. The slight linear increase of pressure from self digesting sludge under substrate limited condition is also filtered by this procedure. For a better readability the plots are slightly smoothed by creating a floating mean value. The arithmetic mean is formed in this manner for every channel value and one interpolation point to be used to the left and right of the data point.

The normalised mean plots of the pure alkaline CIP wastewater (sample M0/0, COD = 5.39 $g \cdot L^{-1}$), as well as concentrated to 72 $g \cdot L^{-1}$ COD (sample M4/0) and the pure whey sample were compared to each other. The results are shown in Figure 29. Though each bottle was fed with the same amount of organic carbon (TOC) the results are significantly different. The highest headspace pressure was achieved from pure whey. Whey was also the sample with the fastest response and the plot show a high coefficient of the graph indicating a rapid acidification. After a period of 22 h from start-up a local maximum of

33.7 mbar was displayed, followed by a decrease of pressure to a local minimum of 26.5 mbar measured 42 h after start-up. The following increase was quite linear for a while. From the ingredients of whey, mainly lactose, protein, and milk fat, a saturation curve had to be expected. However the whey sample plot shows a reduced rate of pressure increase at day 4 indicating a change in the sequence of degradation steps. The expected saturation curve occurred somewhat later from day 6 on. Saturation was reached after approximately day 18.

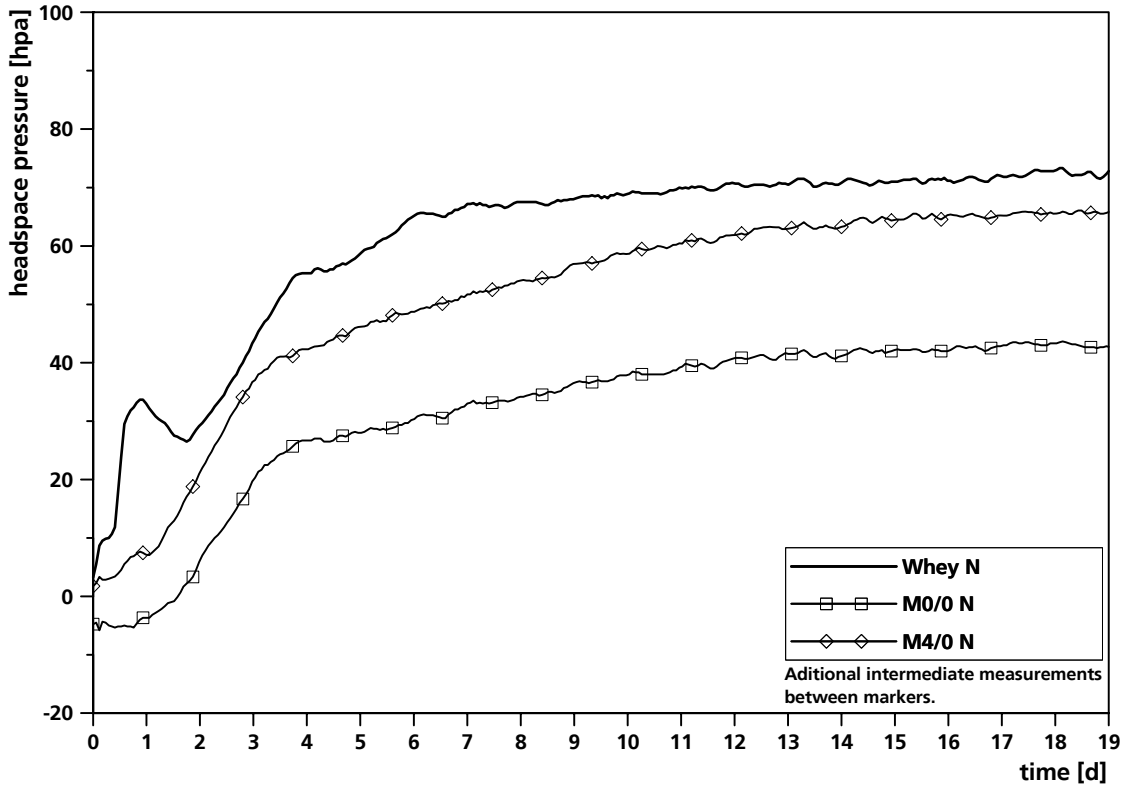

Figure 29 Normalised headspace pressure of alkaline CIP wastewater samples compared to whey samples.

The gas production of the samples containing pure alkaline CIP wastewater without pre treatment started significantly delayed compared to the whey sample indicated by a lower increase in pressure. An extended lag phase can be caused by non-acclimated inoculum sludge (Eskicioglu *et al.*, 2007) however, the CIP cleaning solutions are loaded with quarg containing less lactose compared to whey and thus the acidification rate is expected to be considerably lower than for whey as observed in this experiment. Furthermore the plot of the low strength alkaline CIP wastewater sample (M0/0) showed a decrease in pressure after digestion start. This is a clear indication for an inhibitory reaction (VDI-Richtlinie 4630, 2006). No other of the examined mixtures showed that strong inhibitory effect. An explanation can be given by comparing the blends for the tests as given in Table 12. The sample M0/0 was prepared using 26.172 mL of the alkaline CIP wastewater to achieve a TOC content of 100 mg corresponding to a sodium concentration of 1.5 $g \cdot L^{-1}$ in the bottle

content. An additional sodium content derived from the test medium (approximately 0.16 $g \cdot L^{-1}$) and from the used sludge (unknown concentration). Thus the sodium concentration of sample M0/0 was more than ten times higher compared to other samples and was identified causing the observed moderate inhibition. McCarty (1964) reported a moderately inhibition caused by sodium in a concentration range of the influent of (3.5 - 5.5) $g \cdot L^{-1}$ Na^+ in a continuous anaerobic digestion process. However, in batch test assays Ahring *et al.* (1991) and also Liu. Y. (1991) observed moderate inhibition effects at concentrations as low as 1.6 $g \cdot L^{-1}$ for thermophilic digestion of acetic acid and 0.9 $g \cdot L^{-1}$ for mesophilic digestion of cellulose respectively.

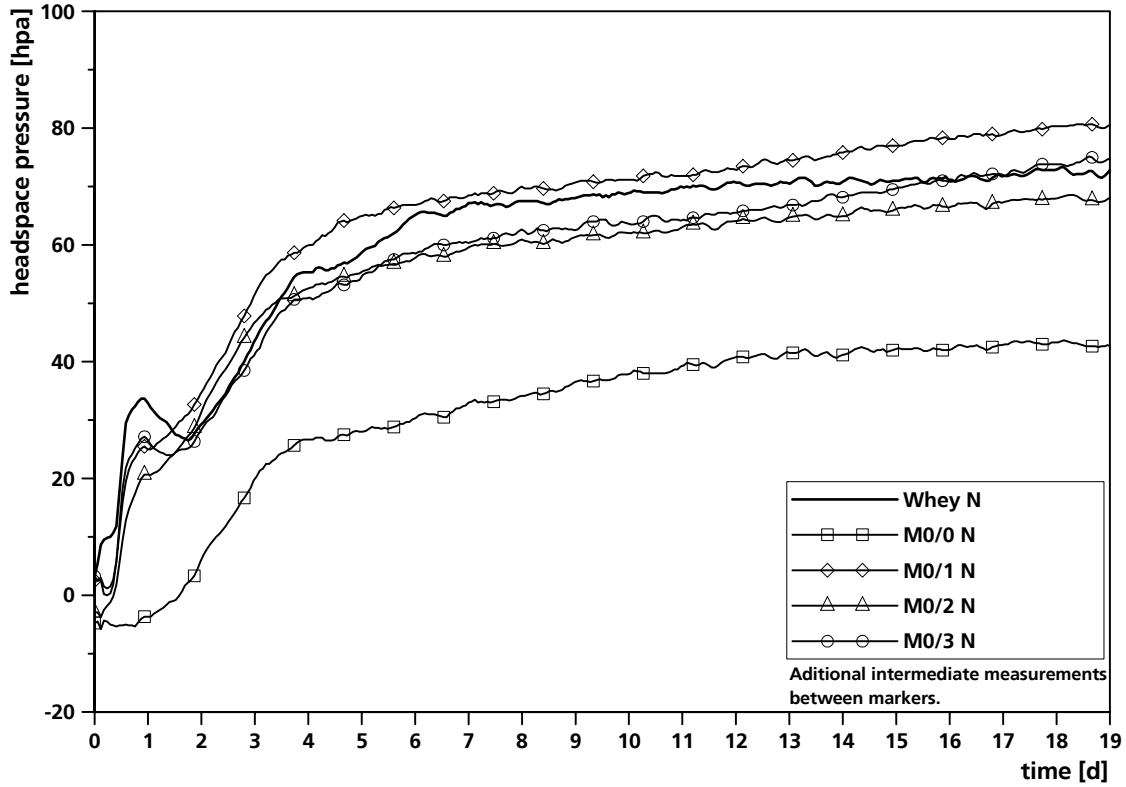

Figure 30 Normalised headspace pressure plots of blends (samples: M0/0 to M0/3) prepared with CIP wastewater (5.39 $g \cdot L^{-1}$ COD) compared to the response of a pure whey sample.

The response of blends of the low strength CIP wastewater (samples M0/0, M0/1, M0/2, and M0/3) are displayed in Figure 30. The responses of blends mixed with whey do not show inhibition effects like that discussed for the pure CIP wastewater sample (M0/0). A short lag-phase of approximately 8 h is followed by a sharp increase in pressure equal to the increase of the whey sample. A local maximum and following decrease of pressure observed for the whey sample in the interval from 22 h to 42 h after start-up was also observed for the blends containing whey. The effect was more or less distinct depending on the mixture ratio. This was also the case on blends with concentrated CIP wastewater, displayed in Figure 31. Thus the effect can clearly assigned to the influence of the whey

degradation kinetics. This requires further examination discussed by means of an additional experiment in Section 8.2.

The distinct change in degradation kinetics observed for whey in the interval from day 4 after start-up can be recognized in the responses of low strength CIP wastewater blends (samples M0/0, M0/1, M0/2, and M0/3) and also for them of the blends with concentrated CIP wastewater (samples M4/0, M4/1, M4/2, and M4/3). However the transition into the saturation curve was more fluent for the blends. The ongoing degradation of the more complex components in the mixtures seemed to mask this effect.

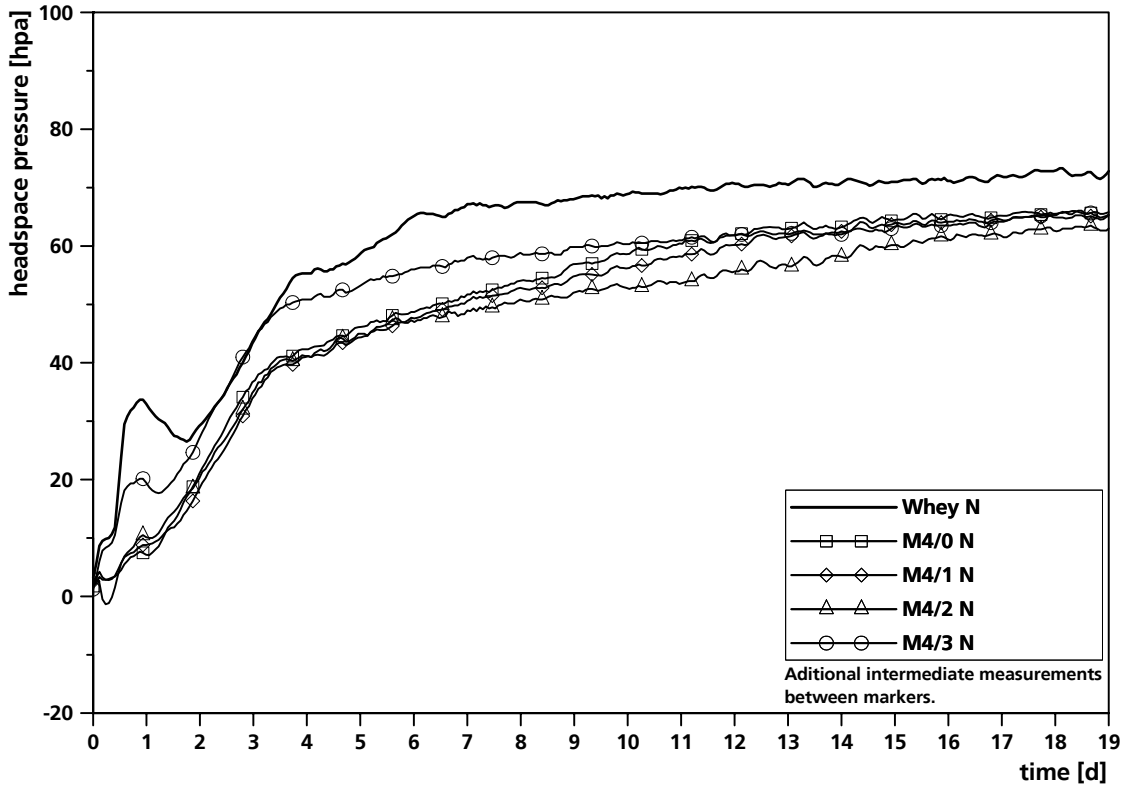

Figure 31 Normalised headspace pressure plots of blends (samples: M4/0 to M4/3) prepared with concentrated CIP wastewater (72.0 g·L^{-1} COD) compared to the response of a pure whey sample.

The results of the ultimate anaerobic biodegradability calculations according to the standard method described in Section 5.3 and the Identification of valid respective rejected measurements are given in Table 13. To compare the results in the context of the design of experiments matrix the results are additionally displayed in Figure 32.

Generally the calculated biodegradability was lower than expected for all samples. This can be estimated on the basis of the result (48.7 %) for the glucose standard sample which was expected to be in the range of 60 % to 90 %. Following possible reason can be assumed: The method required the use of deoxygenized pure water for the preparation of the test medium. The deionised water was poured for several hours with nitrogen

gas using a sintered ceramic gas exhauster placed on the bottom of the flask. Additionally sodium sulphide monohydrate ($Na_2S \cdot 9H_2O$) was used to reduce the oxidation-reduction-potential of the solution. The resazurin indicator was coloured blue after this procedure. However, after preparation of the samples the colour switched to light red indicating the presence of oxygen. The test medium was also used to wash and dilute the inoculum sludge. The presence of oxygen may have weakened the anaerobic biomass and thus reduce the biological activity during incubation.

Table 13 Results of the biodegradability calculation with identification of valid respective rejected measurements

Sample No.	Volume of Whey in the Blend [% (v/v)]	Valid (v) and Rejected (r) Results of the Triplicates (1,2,3)	Ultimate Biodegradability [%]	Standard Deviation $s = \sqrt{\frac{\sum (x - \bar{x})^2}{N-1}}$
M0/0	0.0	(r,v,v)	33.6	5.4
M0/1	25.0	(v,v,r)	42.2	2.0
M0/2	50.0	(v,v,v)	36.4	0.6
M0/3	75.0	(v,v,r)	46.3	3.4
M1/1	12.5	(v,v,v)	38.6	4.8
M2/1	37.5	(v,v,r)	42.2	0.6
M2/2	62.5	(r,r,r)	-	-
M2/3	87.5	(v,r,v)	38.5	3.1
M3/1	12.5	(r,r,v)	45	-
M4/0	0.0	(v,v,v)	39.4	4.6
M4/1	25.0	(v,v,v)	39.7	3.1
M4/2	50.0	(v,r,v)	34.3	3.3
M4/3	75.0	(v,r,v)	39	15
Whey	100.0	(r,r,v)	35	-
STD	0	(r,v,v)	48.7	1.2
Blank	0	(v,v,r)	-	-

The low results for biodegradability do not facilitate a statement about the ultimate biodegradability of the samples as mentioned in the standard. However, all samples were affected in the same way, and thus the results are comparable to each other. The sodium inhibition of the low strength CIP wastewater (sample M0/0) discussed above affects also the result for the biodegradability. The value of 33.6% was the lowest observed in the series. Also the whey sample had a low result (35%). Obviously, blending had a beneficial effect. Blends containing a portion of whey in the range of (75.0 to 87.5) % (v/v) had high results above 38.5 % biodegradability. The highest biodegradability in the series (46.2 %) was observed for a blend with low strength CIP wastewater (sample M0/3) containing 75 % (v/v) whey.

Also blends containing whey in the range of (12.5 to 37.5) % (v/v) reaches relative high results above 38.6 %. However, this region was less favourable due to the expected volumes arising from the process. Additionally, in a continuous anaerobic digestion process, such high rates of alkaline may cause a sodium inhibition due to accumulation that can not be reflected in a batch test.

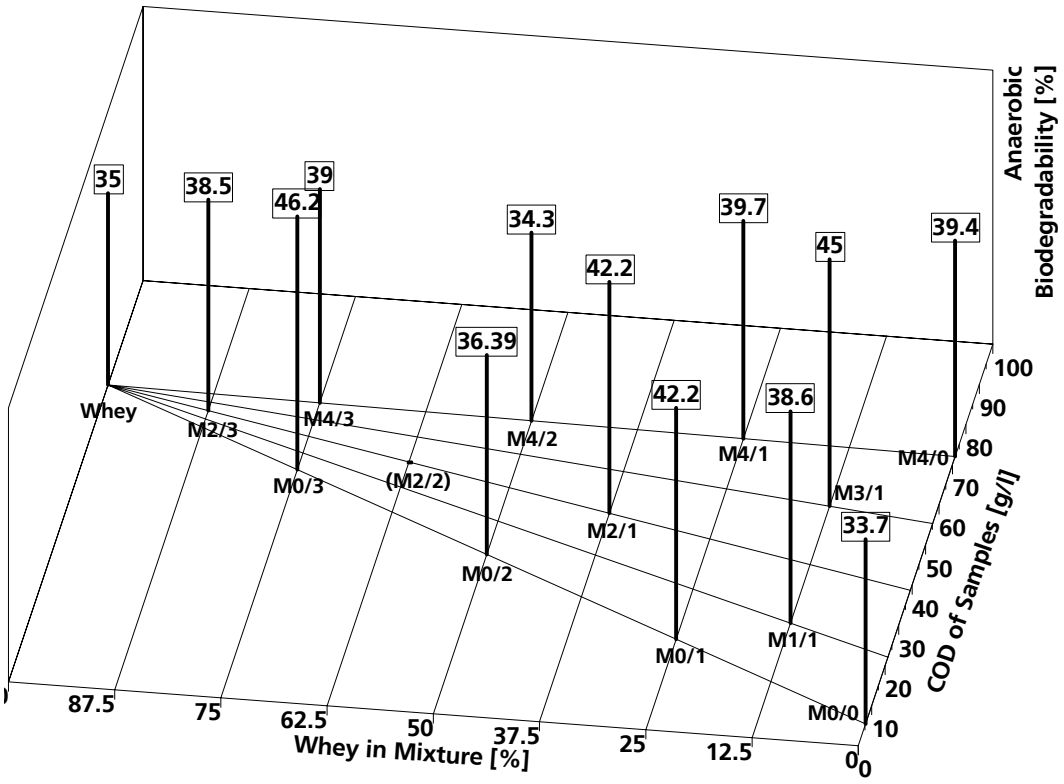

Figure 32 Overview of the biodegradability of whey and mixtures of whey with CIP recovery retentate viewed in the DOE matrix.

8.2. A Kinetic Approach for Acid Whey Batch Fermentation

The observation of temporary pressure decreases in the headspace of the batch tests bottles when treating acid whey samples inspired to a further examination of this phenomenon. In a second experiment, using the OXI-Top Batch digestion system one bottle of a triplicate whey digestion set was modified to measure the development of gases during the run. The plots of the pressure show that the phenomenon was reproducible under the given conditions of the standard method. The results of these tests are more precisely than those of the first experiments. In Figure 33 it can be seen that the digestion of the PEG400 Standard sample compared to the whey sample result in a headspace pressure in the same range. A plot of the headspace pressure in larger scale is displayed in Figure 34a. The development of the first 4 days (96 h) is shown. Additionally the carbon dioxide content in the headspace of a whey sample is given in the same time scale (Figure 34b).

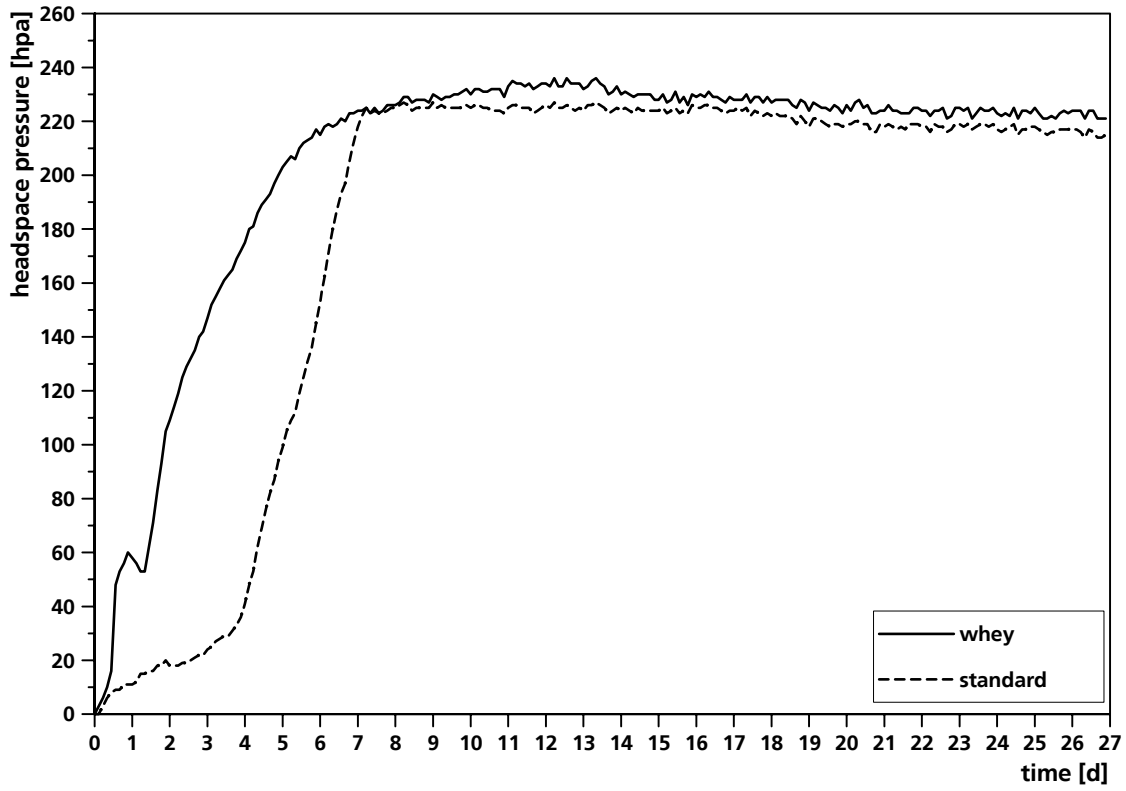

Figure 33 Normalised headspace pressure plots of whey compared to PEG 400 standard.

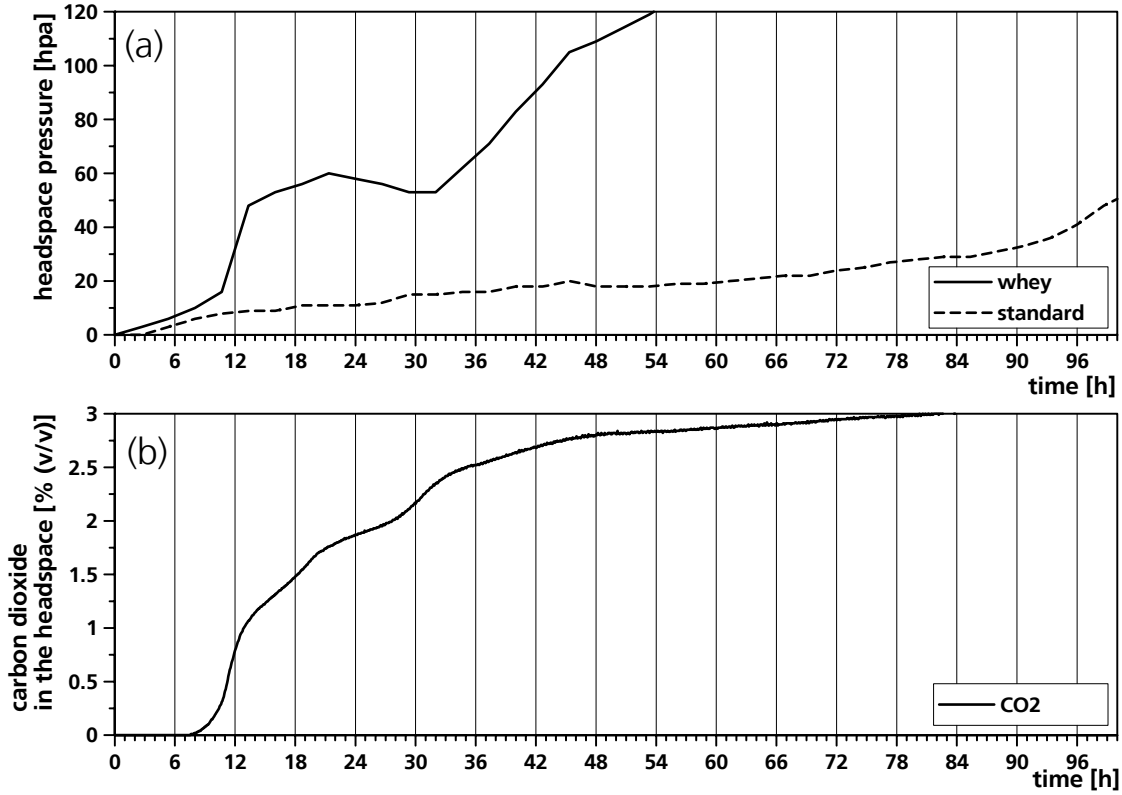

Figure 34 Detail view: Please note that the scale of x-axis is in hours. (a): Normalised headspace pressure of acid whey compared to PEG400. (b): Development of the carbon dioxide content in the headspace of a whey sample digestion.

The development of the headspace pressure implies a phase separation of the fermentation steps. The term 'diauxie', coined by Jacques Monod, is commonly used to describe two separate growth phases in batch studies due to catabolite repression. However, in mixed cultures like anaerobic digestion, distinct kinetic parameters of parallel and sequential processes and various possible metabolic pathways influence the occurrence of phase separation in addition to catabolite repression. In the acid whey batch experiments the phase separation was identified by a delayed pressure development observed 18 h after start-up. Moreover from hour 21 the headspace pressure decreased for 7 hPa during a period of 12 h. Using the ideal gas law the pressure decrease corresponds to a quantity of 0.182 mmol gas. It was concluded that the gas was re-solved into the liquid. Gas-liquid transfer is described by Henry's law expressed as the concentration of a gas component in the liquid due to a gas phase partial pressure. The main gas components in the headspace are nitrogen from initial flushing, produced carbon dioxide, hydrogen, and methane. However the solubility of nitrogen, methane, and hydrogen are very low compared to that of carbon dioxide Sander (1999). Carbon dioxide will mainly be produced by acidogenesis of lactose during the exponential phase during the interval from hour 11 to hour 12. The production rate was reduced by substrate limitation indicated by a reduced slope of the carbon dioxide concentration from hour 13 on (Figure 34b). Carbon dioxide will also be

produced by acetogenesis of lactic acid and volatile fatty acids (compare Figure 5, page 43). However, during the time interval of the observed pressure reduction from hour 21 to hour 31, the consumption of carbon dioxide exceeded the production. Consumption of carbon dioxide is related to aceticlastic and hydrogenotrophic methanogenesis. In addition, hydrogen which is required for hydrogenotrophic methanogenesis is produced during degradation of lactate (Fukuzaki *et al.,* 1991). This led to the conclusion that growth of carbon dioxide consuming bacteria *e.g.* hydrogenotrophic methanogens was favoured while acetogenesis was delayed. Thus, acetogenesis was assumed to be the rate limiting step in anaerobic digestion of whey. This was also confirmed by others *e.g.* Yang and Guo (1991)

Carbon dioxide was identified to be re-solved into the liquid due to delayed acetogenesis while consumption of carbon dioxide was favoured during a period of pressure decrease.

To the authors knowledge the phenomenon of re-solving gas from the headspace of batch tests was not yet reported in any publication. This observation was possible by development of precise respirometric equipment with high measurement frequency and may offer the possibility of advanced kinetic studies using batch tests. This will be a prospect for future research.

8.3. Conclusions from Batch Tests

The biodegradability of mixtures containing simulated alkaline effluents from dairy cleaning procedures concentrated by membrane filtration and acid whey was studied. In general all results were low, due to oxygen intake during preparation of the test bottles. However, comparing the results to each other led to valid conclusions.

An anaerobic biodegradability of 35 % was achieved for the acid whey sample. A significant lower result was achieved from digestion of a sample containing a low strength alkaline CIP wastewater without whey, calculated to 33.7 % degradability. The low result was caused by inhibition observed during the lag-phase. A high sodium concentration of this sample (1.5 $g \cdot L^{-1}$) was identified as inhibitory. Except one sample, all mixtures achieved higher biodegradability than the pure whey sample. The highest value was calculated 46.2 % for the mixture containing 75 % acid whey and 25 % of the low strength alkaline CIP wastewater. Mixture of acid whey with the high concentrated CIP wastewater sample achieved lower biodegradability results when compared with mixtures of whey with less concentrated CIP effluent.

In the mixtures containing a portion of 50 % or more of acid whey the occurrence of a second lag-phase was observed. This was identified as phase separation due to rapid acidification of lactose. The observed phase separation was less distinctive in mixtures

containing less whey. Phase separation was identified to be characteristic for lactose fermentation in batch mode and can be interpreted as a process imbalance.

From these findings, some recommendations for the design of an anaerobic acid whey digestion can be concluded:

- Rapid acidification of lactose and resulting process imbalances at anaerobic digestion of pure acid whey were observed. A two-stage process design is recommended to avoid the accumulation of intermediate volatile fatty acids.
- Co-digestion of acid whey with alkaline effluents from plant cleaning procedures enhances the biodegradability compared to digestion of pure acid whey.
- The high sodium concentration of the examined alkaline effluents from plant cleaning procedures led to inhibitory effects. Therefore, this type of wastewater is unfavourable for single treatment. However, the high alkalinity of these effluents will benefit the anaerobic digestion of acid whey which is low in alkalinity.

9. STUDIES ON WHEY DIGESTION PROCESSES

9.1. CSTR Studies

Several tests and experiments were been carried out with the CSTR reactor using diluted and pure acid whey as feed substrate. Rapid loss of buffer capacity expressed as decreasing bi-carbonate alkalinity and a drop in pH was observed after start-ups, this was especially true in tests were pure acid whey was used.

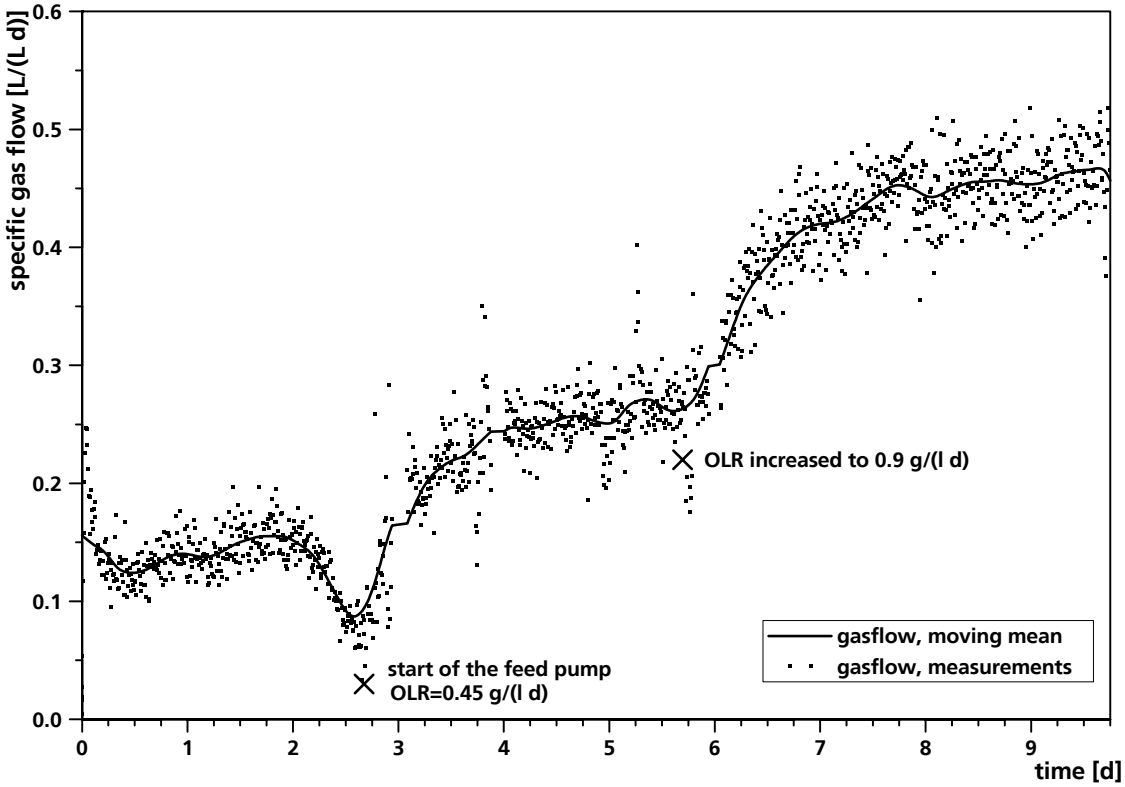

Figure 35 Specific gas flow per litre reactor volume of the CSTR at increasing loading rates (AMR_V001).

One of the experiments, run for a longer period of 45 d in a continuous mode and was performed to verify the dependence of loading rate and biogas production. The plot in Figure 35 shows the gas flow of the CSTR reactor after start up. In this experiment the reactor was filled with 1 L of inoculum sludge and an initial portion of 4.0 L diluted acid whey (3.1 $g \cdot L^{-1}$ COD). The gas production started immediately after the anaerobic digester was fed, and after two days the initial substrate was used up. At the moment the gas production collapsed, the substrate pump was switched on, to pump undiluted acid whey (62.5 $g \cdot L^{-1}$ COD) at continuous loading rate of 0.45 $g \cdot L^{-1} \cdot d^{-1}$ COD into the reactor. The gas production started again and increased in a saturation curve. After reaching a stable gas flow level of 0.26 $L \cdot L^{-1} \cdot d^{-1}$ the pump rate was increased to an organic loading rate of 0.9 $g \cdot L^{-1} \cdot d^{-1}$ COD. As a response the gas production increased to 0.46 $mL \cdot min^{-1}$. This implies that the anaerobic bacteria have been in a saturation phase. The observed reactor

response to the stepwise increased feed ratio at low level is explainable by Monod kinetic with limited substrate supply.

Further operation of the CSTR in this experiment (not displayed) has shown that there was significant biomass wash-out resulted by pumping off the effluent indicated by visual examination of the effluent containing a considerable volume of sludge flocs. Even at a higher loading rate of 3.0 $g \cdot L^{-1} \cdot d^{-1}$ COD the biomass loss had not been compensated by microbial growth. A pH drop to pH 4.0 from initially pH 7.0 occurred after 45 days of operation and also the biogas production dropped to zero, indicate the process failure.

9.2. Conclusions of CSTR Experiments

After initial experiments using acid whey as a substrate for the anaerobic digester, it was clear that the CSTR system suffered from poor biomass retention and therefore poor treatment efficiency. Therefore two different laboratory scale reactor systems were chosen to meet these deficiencies:

- **An Upflow Anaerobic Sludge Blanket (UASB) Reactor**
- **An Anaerobic Membrane Reactor (AMR)**

Both reactor types have good biomass retention and they are suitable for the treatment of high strength wastes as stated by different researchers previously reviewed in Section 4.3.5.

Further work was associated with the design and operation of the chosen reactor types in laboratory scale.

9.3. Anaerobic Membrane Reactor (AMR) Studies

A novel process control strategy was developed to use with pressure driven cross flow membrane filtration based biomass retention systems to minimize shear stress to the separated biomass induced by the membrane cross flow during the filtration as described in Section 5.4.2. A cross flow velocity of 2.8 $m \cdot s^{-1}$ at (1.0 to 1.5) bar working pressure was found to work effectively and using these parameters produced a measured initial flux of 50 $L \cdot h^{-1} \cdot m^{-2}$. The performance decreased to 10 $L \cdot h^{-1} \cdot m^{-2}$ within a week due to organic fouling. However, a regular rinsing with warm (60 °C) sodium hydroxide (0.15 % (m/m) NaOH) and potable water once or twice a week allowed the recovery of the permeate volume flow, indicating the reversible nature of the membrane fouling. The parameters were estimated by increasing the cross flow velocity stepwise during initial experiments until no upsets due to blocking of the membrane system were observed. The permeate flow did not decrease irreversible for the 9 weeks period of the presented experiment indicating that no formation of inorganic precipitants inside the membrane took

place. Moreover the same membrane was used in further operation of the AMR without any upsets or performance loss for several months.

During the experiment (AMR_V007) the organic loading rate (OLR) was increased stepwise to examine the performance of the system. The hydraulic retention time (HRT) was set to 48 hours. An OLR of 2.0 $g \cdot L^{-1}$ d was used during start-up and held for 2 weeks (Figure 36g). During this period the pH of the reactor liquid (Figure 36c) decreased slightly from pH 7.5 to pH 7.0 while the bicarbonate alkalinity (Figure 36d) was measured 6 125 $mg \cdot L^{-1}$ $CaCO_3$ at day 7 indicating good buffer capacity of the anaerobic biomass. The increase to a loading rate of 7.5 $g \cdot L^{-1} \cdot d^{-1}$ COD caused a spontaneous decrease of the pH to pH 6.8 at day 16. The substrate flow was discontinued at day 18, day 24, day 32, day 46, and day 54 to check the ability of the biomass to recover the pH to neutral. The pH only recovered until the disrupted feeding at day 24. No recovery of the pH was detected from day 26 on. The bicarbonate alkalinity decreased to 1 575 $mg \cdot L^{-1}$ $CaCO_3$ at day 36 and dropped within a day from 1 500 $mg \cdot L^{-1}$ to 708 $mg \cdot L^{-1}$ $CaCO_3$ at day 45. Interruption of the substrate flow caused a slight recovery of the alkalinity to 1 275 $mg \cdot L^{-1}$ $CaCO_3$ at day 55 but no change in the pH value and after restart of the feeding alkalinity decreased again to 712 $mg \cdot L^{-1}$ $CaCO_3$ at day 57. This indicated considerable organic loading stress. The buffer capacity was assessed critically low. Therefore, it was decided to add NaOH (Figure 36e) by using the pH controller to enhance the buffer capacity and to attain a neutral environment. The control loop was switched on after increasing the OLR to 10 $g \cdot L^{-1}$ d^{-1} at day 56 It was observed that the pH did not reach neutral conditions, even when the pH control dosed high strength 5N NaOH solution. The bicarbonate alkalinity recovered to 1 300 $mg \cdot L^{-1}$ $CaCO_3$ at day 63. However, pH 6.5 was measured and was not affected by the sodium hydroxide addition.

Low concentrations of volatile fatty acids (Figure 36b) were measured until day 49. Acetic acid, propionic acid, and butyric acid were 6.6 $mg \cdot L^{-1}$, 24.5 $mg \cdot L^{-1}$, and 0 $mg \cdot L^{-1}$ respective. The reactor effluent COD (Figure 36a) was also low until this time, 102 $mg \cdot L^{-1}$ were measured at day 48. After restart of the feeding system at day 57 the concentration of propionic acid increased sharply to 1 938 $mg \cdot L^{-1}$ at day 59 and had a further increase to a maximum value of 2 638 $mg \cdot L^{-1}$ at day 66 indicating strong inhibition of acetogenesis. Also acetic acid and butyric acid concentrations increased, however much slower and to a lesser extent. Maximum concentrations of 1 204 $mg \cdot L^{-1}$ and 1 068 $mg \cdot L^{-1}$, were measured at day 72 for acetic acid and butyric acid respective. The slower accumulation of acetic acid indicated that the acetic acid consuming methanogens are also inhibited by the organic overload and the resulting accumulation of free volatile fatty acids. However they are less sensitive to those unfavourable conditions.

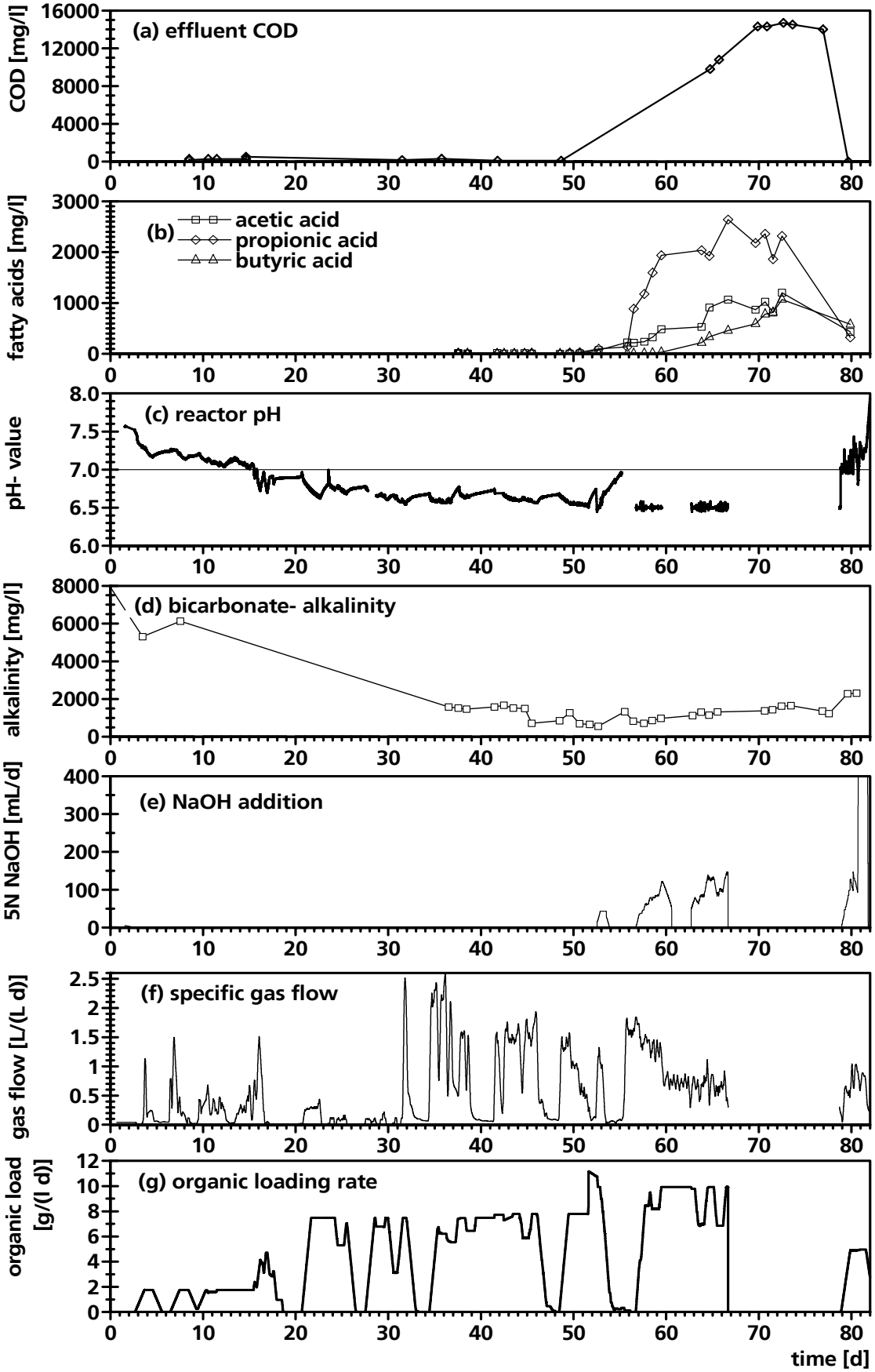

Figure 36 AMR Experiment with and without pH control (AMR_V007). (a): COD concentration of the effluent. (b): Analysis of fatty acids of fermenter liquid samples. (c): Online measurement of the reactor liquid pH value. (d): Bicarbonate alkalinity of fermenter liquid samples. (e): Addition of sodium hydroxide by automatic controlled pump. (f): Gas production of the reactor. (g): Calculated organic loading rate on a COD basis.

The reactor effluent COD (Figure 36a) also increased during overload stress and reached a maximum value of 14 700 mg·L^{-1} at day 72. The approximated toxic dose of sodium-ions according to the works of Feijoo *et al.* (1995) Rinzema *et al.* (1988) Kugelman and McCarty (1965) was not reached by the NaOH dosage, but the biomass, additionally stressed by organic overload, was not able to acclimate to the change of conditions. It was noticed that the colour of the sludge faded from black inoculum to grey at the end of the experiment indicating accumulation of milk ingredients.

Performance data for this experiment (AMR_V007) is presented in Table 14 and was calculated from values at steady state conditions. These were assumed at day 42. The organic loading rate was 7.7 g·L^{-1} d^{-1} corresponding to a COD load of 47.0 g d^{-1}. A COD removal of 46.6 g d^{-1} was calculated, and the gas production was 10.6 L d^{-1} according to a specific gas production of 1.63 L L^{-1} d^{-1}. The methane content was measured 50 % (v/v) at this time.

Table 14 Performance data of the AMR Experiment with and without pH control (AMR_V007).

Parameter	**Values (day 15)**
OLR [g·L^{-1} d^{-1}]	7.7
COD removal efficiency [%]	99.3
Gas yield per COD removed [mL g^{-1}]	227
Methane yield per COD removed [mL g^{-1}]	114

No performance data of a one-stage anaerobic membrane reactor treating whey was found in the literature. The two-stage digester of Saddoud *et al.* (2007) fitted with a membrane coupled methanogenic stage was operated at high loading rates up to 19.78 g·L^{-1} d^{-1}. However, Saddoud *et al.* (2007) treated cheese whey at a considerable higher hydraulic retention time of 1+4 days. Their system reached a COD removal efficiency of 98.5 % and a methane yield per COD removed of 300 mL g^{-1}.

A second experiment (AMR_V011) was performed to investigate the addition of alkalinity for process recovery. The CIP alkaline solution was originally used at Humana Milchunion, Georgsmarienhütte in cleaning procedures. The spent wash was a 2.0 % (m/m) NaOH solution loaded with milk ingredients from the quarg production process.

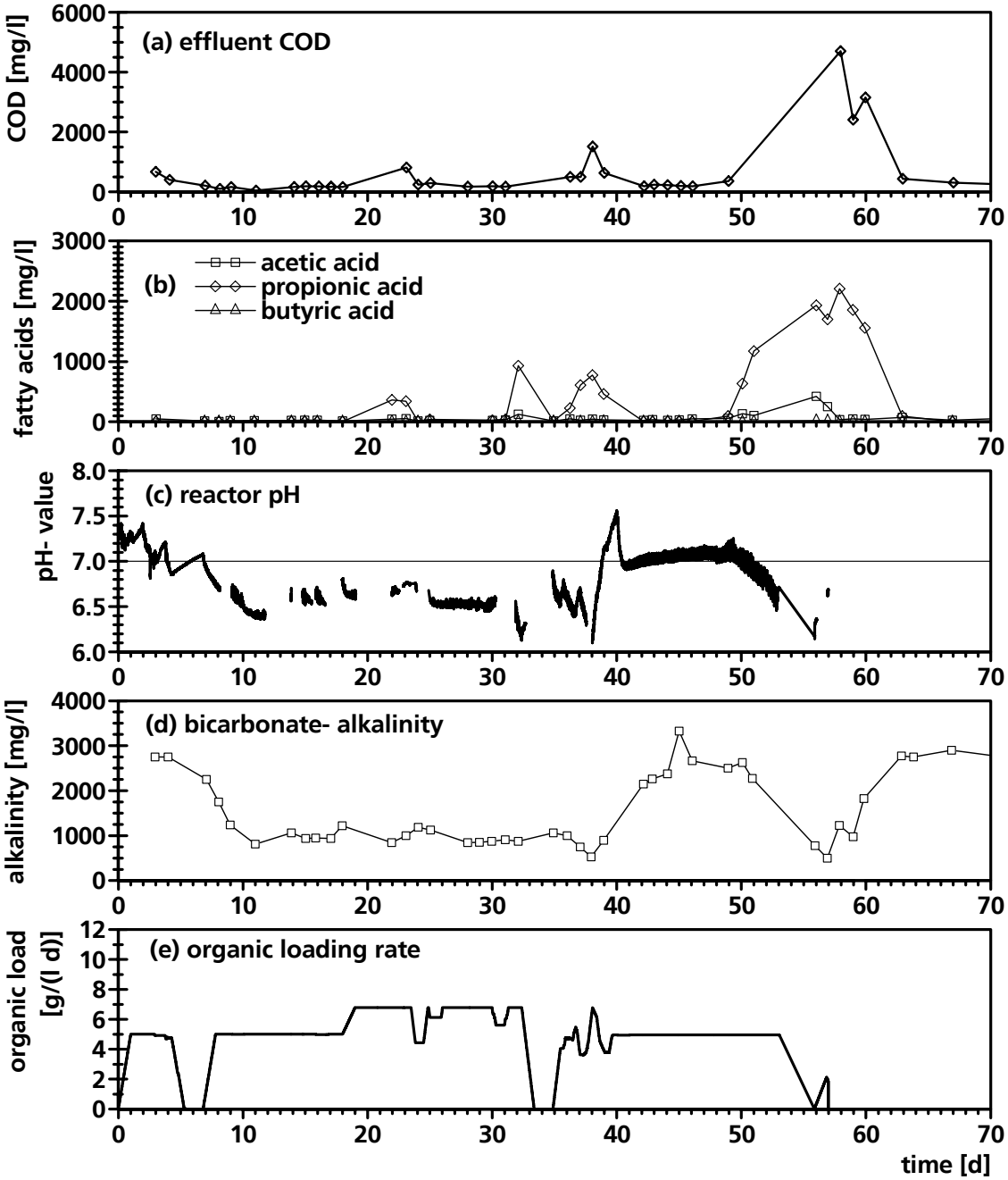

Figure 37 AMR experiment with CIP addition after overload stress (AMR_V011). (a): COD concentration of the effluent. (b): Analysis of fatty acids of fermenter liquid samples. (c): Online measurement of the reactor liquid pH value. (d): Bicarbonate alkalinity of fermenter liquid samples. (e): Calculated organic loading rate on a COD basis.

The digester was started without any pH control. The organic loading rate (Figure 37e) was increased from initially 5 $g \cdot L^{-1}$ d^{-1} to 6.8 $g \cdot L^{-1}$ d^{-1} at day 19. The propionic acid concentration (Figure 37b) increased temporary to 361 $mg \cdot L^{-1}$ at day 22 but recovered to 10 $mg \cdot L^{-1}$ within two days.

Symptoms of overload stress at day 38 were indicated by a decrease of the alkalinity to 525 $mg \cdot L^{-1}$ $CaCO_3$ (Figure 37d), and an increased effluent COD of 1 514 $mg \cdot L^{-1}$ (Figure 37a). Also the propionic acid concentration increased again to 458 $mg \cdot L^{-1}$. The organic

loading rate was reduced to 5 $g \cdot L^{-1}$ d^{-1} the same day and the addition of water for dilution of the whey was stopped and replaced with the CIP alkaline effluent. The pH (Figure 37c) increased from pH 6.1 at day 38 to reach neutral conditions at day 41.

The alkalinity recovered to a range of (2 000 to 3 000) $mg \cdot L^{-1}$ $CaCO_3$. The fatty acid concentration (Figure 37b) and also the effluent COD decreased again to low values. However the process failed after a short period of low-key parameter values at day 57. A first indicator of the upcoming process failure was a rapid increase of the effluent COD followed by the detection of fatty acids. The values of pH and alkalinity decreased with a delay of approximately two days. These parameters are masked by the alkaline addition and are therefore not suitable to predict reactor upsets in a pH controlled anaerobic digester.

Also in this experiment the change of the sludge appearance from a black inoculum sludge to grey sludge at the end of the experiment was noticed. In UASB studies treating cheese whey Kalyuzhnyi *et al.* (1997) observed accumulation of white aggregates and refer to them as "undigested milk ingredients". It was postulated that the high content of calcium present in acid whey cause reactor upsets Clark (1988). Precipitation of calcium carbonate $CaCO_3$ and calcium hydrogen phosphate $CaHPO_4$ from wastewaters with high calcium content occurs in UASB reactors Lettinga and Hulshoff Pol (1991). The phosphate concentration in the dry mass of the sludge was measured a) 22.2 $g \cdot L^{-1}$ PO_4 in the inoculum sludge and b) 45.6 $g \cdot L^{-1}$ PO_4 after 36 days of operation. To exclude solved phosphates the sludge was centrifuged, dried and rewetted with pure water. At day 22 the total phosphate concentration of the whey feed was 562 $mg \cdot l^{-1}$ PO_4 while the effluent concentration was 244 $mg \cdot l^{-1}$ PO_4 corresponding to 56 % reduction of the phosphate. At day 29 the effluent phosphate concentration was 168 $mg \cdot l^{-1}$ PO_4 corresponding to 70 % reduction. It was assumed that the phosphate removal was due to precipitation of Calcium phosphate salts. However, the accumulation of phosphates was calculated only 12.7 % of the phosphate intake indicating a delayed start of the removal process *e.g.* by the requirement for a minimum concentration of calcium-ions.

9.4. Conclusions of Anaerobic Membrane Reactor (AMR) Studies

The principle of an Anaerobic Membrane Reactor provides complete biomass retention. The novel control strategy for the filtration unit has the advantage of avoiding surplus filtrate and thus shear stress on the biomass and energy consumption is minimized. To the knowledge of the author no performance data for a one-stage membrane coupled anaerobic digestion system treating acid whey is present in current literature. This work contributes toward filling this gap. At stable conditions the AMR process was operated with a

high COD removal efficiency of 99.3 % at an organic loading rate of 7.7 $g \cdot L^{-1}$ d^{-1} A gas yield per COD removed of 227 $mL \cdot g^{-1}$ and a methane yield per COD removed of 114 $mL \cdot g^{-1}$ was achieved. However, in the experiments it was demonstrated that even with perfect biomass retention the operation of an anaerobic membrane reactor at high loading rates caused process upsets. Due to cation-toxicity the pH control with alkaline have to be started with careful attention to the process development. A biomass, stressed by organic overload can not be recovered by subsequent pH control. High phosphate removal efficiency, the accumulation of phosphate in the biomass, and the change of the sludge colour indicated the formation of inorganic precipitates within the reactor. However, due to careful parameter estimation for the membrane system, no adverse effects to the filtration performance by irreversible scaling from precipitants were observed.

9.5. UASB Studies

9.5.1. Start-Up of an UASB Reactor Treating Milk

An initial test using the UASB Reactor was performed to evaluate the best start-up procedure and most effective operation. Granular sludge from a previous experiment was used as inoculum. It was stored approximately for 6 month before use. A synthetic wastewater was used as feed for this experiment. It was prepared using low fat milk, with ammonium hydrogen orthophosphate and urea as nutrients. The influent was adjusted to 10 $g \cdot L^{-1}$ COD by diluting the milk with potable water. The HRT was set to 2 days corresponded to an organic loading rate (OLR) of 5 $g \cdot L^{-1} \cdot d^{-1}$ COD. The cycle pump was set to 1 $L \cdot h^{-1}$ corresponding to an up-flow velocity of 1.1 $m \cdot h^{-1}$. The biogas production of the UASB reactor started after 24 h of operation and reached 1.73 $L \cdot d^{-1}$ after 4 days of operation. Daily measurements of bicarbonate alkalinity confirmed rapid acidification as the true bicarbonate alkalinity decreased from initially (2 313 to 1 250) $mg \cdot L^{-1}$ $CaCO_3$ after 4 days of feeding. In the following days the gas production dropped and the effluent became smelly. Thus, the experiment was stopped after 10 days of operation.

Fang and Chui (1994) tested a similar synthetic wastewater in a UASB and in other reactor types. They reached a performance of 95 % COD removal in the UASB reactor at a high OLR of 20 $g \cdot L^{-1} \cdot d^{-1}$ COD and varying HRT of 3 days to 8 days corresponding to different influent COD levels (6.0 $g \cdot L^{-1}$, 4.0 $g \cdot L^{-1}$, 2.6 $g \cdot L^{-1}$). As a result they stated that the removal efficiency was dependent on the organic Loading rate but insensitive to the influent COD. A high reactor performance as in the experiments of Fang and Chui (1994) require an extended acclimatisation period, here 70 days, with stepwise increase of the organic load. The use of a well balanced influent relating to nutrients and trace metals is another reason of their success.

The rapid start-up of the UASB without acclimatisation period and the use of sludge with low biological activity as inoculum are the reasons for the process failure at relatively low organic loading rate. From this experience it was concluded to use a more active inoculum and to stepwise increase the COD loading rates in following experiments.

9.5.2. Influence of Internal Circulation on Whey Digestion

Two UASB experiments have been carried out to investigate the influence of the up-flow velocity. The first set-up was operated using the circulation loop described in Section 5.4.3. The pump of the circulation loop was adjusted to 23.56 $L \cdot min^{-1}$ corresponding to an up-flow velocity of 3.0 $m \cdot min^{-1}$ during the experiment. Results of measurements and analytics of this experiment are displayed in Figure 38a-f. The hydraulic retention time was adjusted to 2 days in all UASB experiments.

9.5.2.1. UASB Experiment with Internal Circulation

The initial organic loading rate was 5 $g \cdot L^{-1} \cdot d^{-1}$ of COD at start-up (Figure 38f). It was kept constant for 29 days with interrupts at day 12 and at day 28 for approximately 24 hours each and a 3 day idle time from day 23 on due to feeding system failures.

One day after start-up, the complete sludge bed lifted, soared up and blocked the three phase separator. It was necessary to pump the biomass manually back to the reactor bottom by using the cycle pump on high number of revolutions for short intervals. A biomass lifting happened another two times at day 5 and day 10. These occurrences were less extensive and affected only parts of the sludge bed. From day 10 on further lifts of the sludge bed had not been observed, however settling of the biomass was not efficient. Frequently, clusters of biomass soared up and build up a layer of scum which was removed regularly by using the cycle pump to convey the solid matter back into the sludge bed.

The reactor pH decreased from initially pH 7.2 to pH 6.8 after start-up and to pH 6.7 after the first biomass lift (Figure 38c). A gently decrease of the pH to a final value of pH 6.3 was observed during the period from start up to day 48. The described biomass lifts at day 5 and day 10 caused temporary increases of the pH value due to contact of the pH probe head with biomass. The biomass provided a higher pH at close range due to its natural buffer capacity. However, the scum layer observed from day 14 on caused fluctuations to temporary low pH values. The pH values recovered after removing of the layer, indicating that the involved biomass was acidic while the supernatant water had a higher pH value. Interrupts in feeding caused temporary increases of the pH value. Particular the three day idle time of the feeding system from day 23 to day 26 allowed a recovery to pH 6.9 which was nearly the initial value.

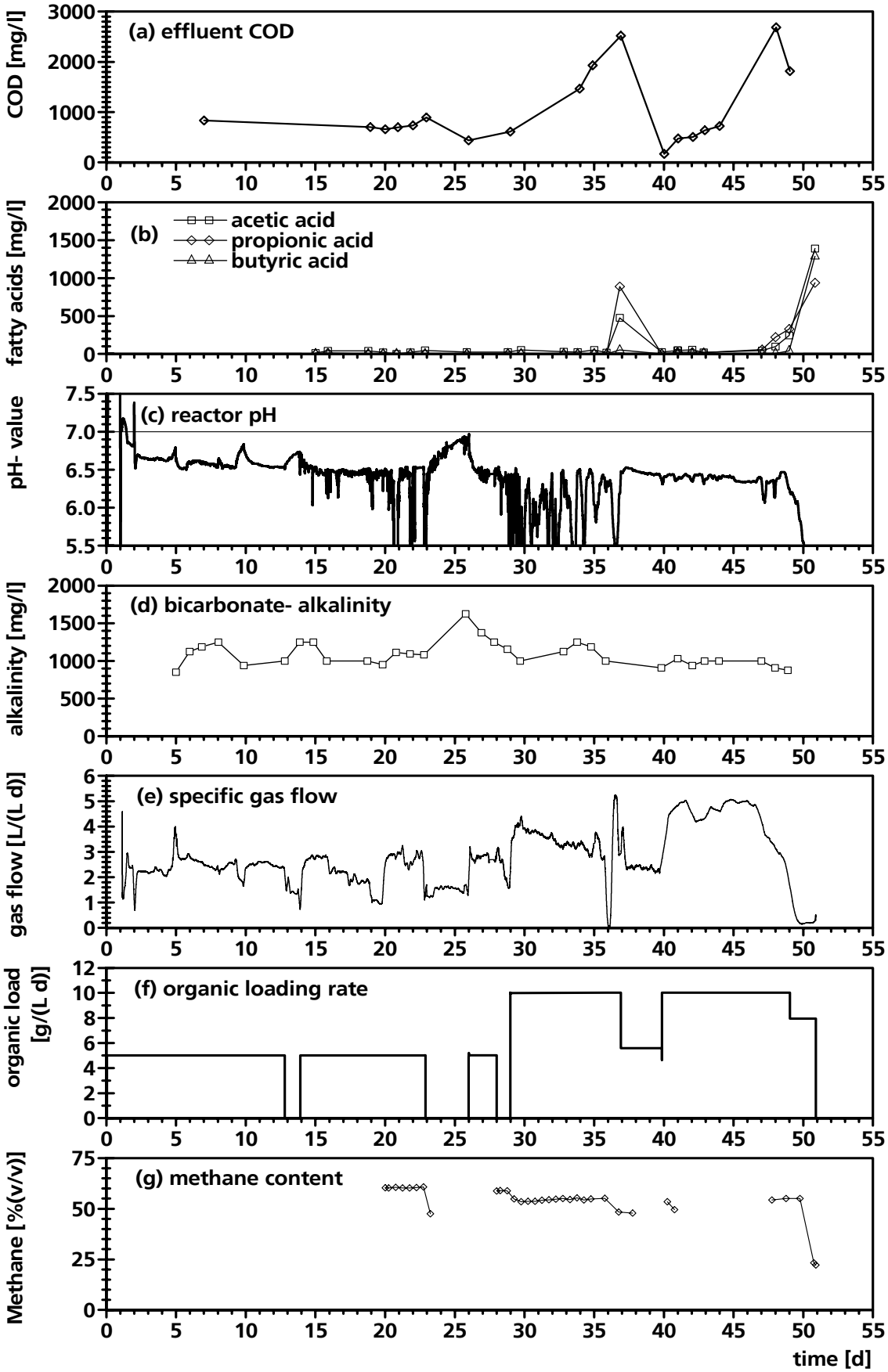

Figure 38 UASB experiment with internal circulation (UASB_IC_V002). (a): COD concentration of the effluent. (b): Analysis of fatty acids of fermenter liquid samples. (c): Online measurement of the reactor liquid pH value. (d): Bicarbonate alkalinity of fermenter liquid samples. (e): Specific gas production per litre reactor volume. (f): Calculated organic loading rate on a COD basis. (g): Methane content of the off gas.

The gas production was stable at approximately 12 $L \cdot d^{-1}$ during the first 28 days with lower gas production during interrupts of the feeding (Figure 38e). The build up of the scum layer from day 15 on was attended by a decrease in the gas production. After removal of the scum layer the gas production recovered. The True Bicarbonate Alkalinity (TBA) was at approximately 1 000 $mg \cdot L^{-1}$ $CaCO_3$ during the whole experiment, except for a temporary increase during day 5 to day 10 and additional increasing due to interrupts of the feeding (Figure 38d). Alkalinity is considered as an indicator for biological imbalances with fast response by several authors as reviewed in Section 4.3.3.2. However the low values measured indicate a weak buffer capacity of the system and thus in this experiment, the pH value was more sensitive to process changes compared to a well buffered system.

The organic loading rate was increased to 10 $g \cdot L^{-1}$ d^{-1} COD from day 29 on. The gas production temporary increased to 20.2 $L \cdot d^{-1}$. However, this higher gas production was not stable. In the following period (day 30 to day 35) the gas production decreased to approximately 9.5 $L \cdot d^{-1}$ at a constant rate. A heavy tendency to build up a scum layer was observed in this period resulting in strong fluctuations in the measured pH values. At day 36, the gas production declined to nearly zero and then fluctuated strongly.

The effluent COD was below 900 $mg \cdot L^{-1}$ during the period of the lower organic loading rate (Figure 38a). After increasing the loading rate to 10 $g \cdot L^{-1}$ d^{-1} the effluent COD increased to a maximum of 2 520 $mg \cdot L^{-1}$ at day 37. The free fatty acid concentration was also low until day 36 (Figure 38b). Acetic acid had not extended values of 51 $mg \cdot L^{-1}$. Propionic and butyric acid concentrations had been below the detection limit. At day 37 the values increased by leaps and bounds. Acetic acid concentration was measured at 475 $mg \cdot L^{-1}$ while propionic and butyric acid concentrations were at 888 $mg \cdot L^{-1}$ and 52 $mg \cdot L^{-1}$ respectively. These high values, the break down of the gas production and the low pH values measured were clear indicators of a biological imbalance due to an organic overload.

To avoid a digester failure the organic loading rate was decreased at day 37 and set to 5 $g \cdot L^{-1}$ d^{-1} COD for a three day period. However, the effluent COD and the fatty acids concentration retrieve to low values. The effluent COD was measured 166.7 $mg \cdot L^{-1}$ at day 40, acetic acid concentration was 23 $mg \cdot L^{-1}$, propionic acid and butyric acid were below the detection limit again. The gas production stabilized to approximately 8.5 $L \cdot d^{-1}$. A reduced tendency to build-up a scum layer was observed and the fluctuation of pH values stopped. However, the reduction of the organic loading rate caused no recovery of the alkalinity values in the reactor.

Nevertheless, the organic loading rate was again set to the higher value (10 $g \cdot L^{-1}$ COD). This caused an increase of the gas production to a maximum of 17.6 $L \cdot d^{-1}$. The gas production remained high until day 47. The first measured sign of the oncoming process failure was a heavy decrease of the gas production from day 47 on. However a change of colour in the biomass was noticed from day 40 on. The dark black inoculum turned to grey. The fatty acids concentration remained low until day 47 but increased to very high values in the period of the following three days. The sludge turned to light grey during this period. Also the effluent COD increased to a maximum value of 2 685 $mg \cdot L^{-1}$ at day 48. The pH value of the process decreased slowly to pH 6.3 at day 46, fluctuated for two days and then decreased rapidly to pH 5.0 within two days indicating the process failure. The process was stopped at day 51.

9.5.2.2. UASB Experiment without Internal Circulation

Without internal cycling the up-flow velocity was 0.1 $m \cdot h^{-1}$ resulting from the influent pumping. The operation was started with 5 $g \cdot L^{-1}$ d^{-1} of COD organic loading rate (Figure 39f). The gas production increased to 2.36 $L \cdot L^{-1} \cdot d^{-1}$ at day 2 (Figure 39e). Within a day the gas production decreased to 1.57 $L \cdot L^{-1} \cdot d^{-1}$. The concentrations of acetic acid, propionic acid, and butyric acid at start-up were 141 $mg \cdot L^{-1}$, 132 $mg \cdot L^{-1}$, and 236 $mg \cdot L^{-1}$ respective (Figure 39b). The concentrations decreased to 15 $mg \cdot L^{-1}$, 0 $mg \cdot L^{-1}$, and 28 $mg \cdot L^{-1}$ at day 3 for acetic acid, propionic acid, and butyric acid respective. The values remained low until day 7.

By increasing the organic loading rate to 7.2 $g \cdot L^{-1}$ d^{-1} at day 3 the gas flow increased to a maximum of 2.3 $L \cdot L^{-1} \cdot d^{-1}$ at day 4 but decreased to 1.28 $L \cdot L^{-1} \cdot d^{-1}$ within two days. At day 6 it was observed that the sludge turned to light grey at the bottom of the fermenter with a sharp transition to the sludge above which remained black (Figure 40a). At day 7 clusters of biomass soared up and caused a scum layer as observed in the previous experiment. The pH was gently decreasing from initially pH 7.11 at start-up to pH 6.3 at day 7 (Figure 39c). After the build up of the scum layer, the pH decreased rapidly to pH 5.3 but recovered fluctuating to values around pH 6.1 for a day. At day 8 the pH dropped to pH 4.3 but only recovered to pH 5.0 with fluctuations caused by the scum layer which had been built up again. The bicarbonate alkalinity (Figure 39d) decreased from initially 1 688 $mg \cdot L^{-1}$ $CaCO_3$ to 1 062 $mg \cdot L^{-1}$ $CaCO_3$ at day 3 and to 688 $mg \cdot L^{-1}$ $CaCO_3$ at day 8.

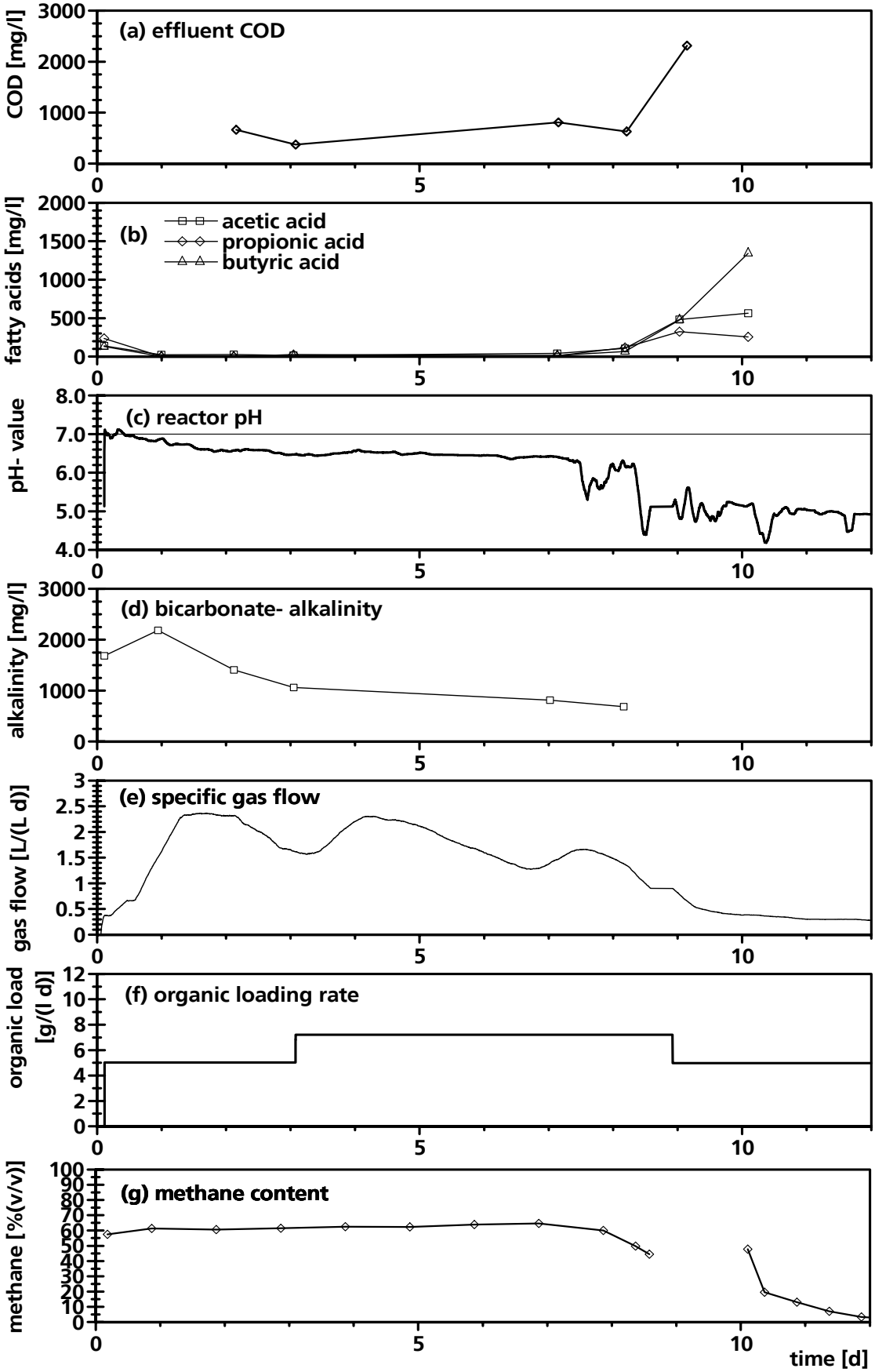

Figure 39 UASB experiment without internal circulation (UASB_IC_V003). (a): COD concentration of the effluent. (b): Analysis of fatty acids of fermenter liquid samples. (c): Online measurement of the reactor liquid pH value. (d): Bicarbonate alkalinity of fermenter liquid samples. (e): Specific gas production per litre reactor volume. (f): Calculated organic loading rate on a COD basis. (g): Methane content of the off gas.

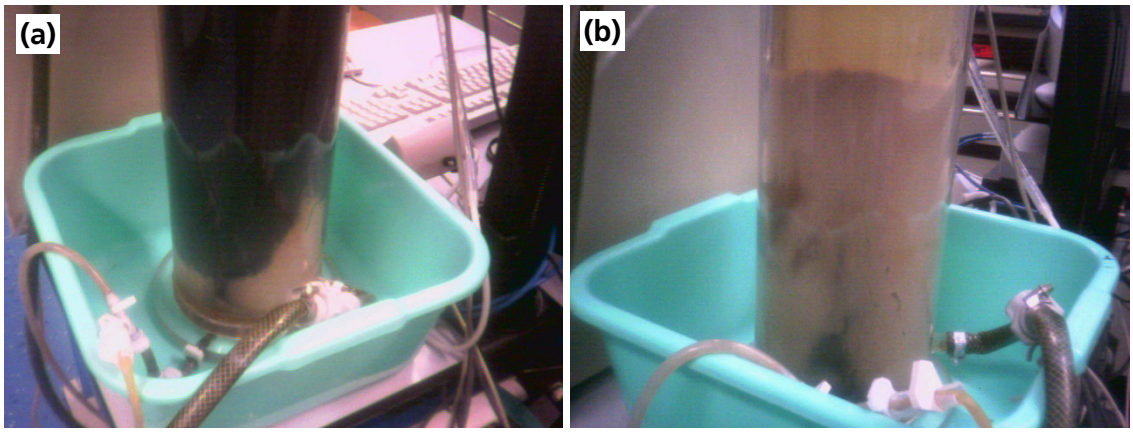

Figure 40 White sludge after UASB operation without recycling loop. (a): Development of light grey sludge on the reactor bottom at day 6. (b): The sludge was completely coloured light grey after process failure at day 12

The fatty acids concentration was on an increased level at day 9. Acetic acid, propionic acid, and butyric acid were 481 $mg \cdot L^{-1}$, 323 $mg \cdot L^{-1}$, and 478 $mg \cdot L^{-1}$ respective. Also the effluent concentration was increased (Figure 39a). It was measured 2 317 $mg \cdot L^{-1}$ COD. During the first 7 days the methane concentration (Figure 39g) was measured at approximately 62 % (v/v) but at day 8 it was decreased to 44 % (v/v). These values indicated a process upset. Thus the organic loading rate was set to a reduced value of 5 $g \cdot L^{-1}$ d^{-1} COD. However at this stadium the whole biomass was observed to be light grey (Figure 40b). The process could not be recovered. The fatty acid concentration had a further increase to 565 $mg \cdot L^{-1}$, 256 $mg \cdot L^{-1}$, and 1 343 $mg \cdot L^{-1}$ for acetic acid, propionic acid, and butyric acid respective. The gas production decreased to values below 1.0 $L \cdot d^{-1}$ with a CO_2 concentration of more than 90 % (v/v). The experiment was stopped at day 12.

9.5.3. UASB Operation with CIP Addition

The UASB operation of whey as a single substrate suffered from adequate alkalinity and thus decreasing pH values in the supernatant reactor liquid occurred. It was intended to overcome this problem by adding CIP alkaline effluents to enhance the alkalinity and to stabilize the pH value during operation. The experiment without internal recycling had shown effects of overloads in the region of the influent inlet. Thus, the experiment with CIP addition was operated using the circulation pump. However, to avoid upsets caused by biomass lifts, the reactor was started without recycling and at a low organic loading rate of 2 $g \cdot L^{-1}$ d^{-1}. Also CIP alkalines were not added during the first 8 days of operation. The results are displayed in Figure 41a-g. Despite these precautions a biomass lift and blocking of the three phase separator occurred at day 2. The biomass was pumped back to the reactor bottom using the circulation pump at day 3. The pH decreased from an initial value of pH 7.2 to pH 6.2 and, caused by the lifted biomass, a further decrease to pH 5.5 at day 4 (Figure 41c). After removing the biomass from the separator the pH re-

covered to values above pH 6.0. Occasionally clusters of the biomass soared up and thus, the circulation was switched on, adjusted to an upflow velocity of 1.36 $m \cdot min^{-1}$. The build up of a scum layer caused a drop to pH 5.5 at day 8. Also the alkalinity was affected by the start up procedure (Figure 41d). The value decreased from initially 1 312 $mg \cdot L^{-1}$ $CaCO_3$ to 667 $mg \cdot L^{-1}$ $CaCO_3$ at day 8.

From day 8 on, CIP alkaline effluent with a COD of 3.5 $g \cdot L^{-1}$ was added. The influent rate was adjusted to 216 $mL \cdot d^{-1}$. At the same time the organic loading rate (Figure 41f) was increased to 5.26 $g \cdot L^{-1}$ d^{-1} COD by increasing the whey concentration while the hydraulic retention time remained constant at 2 days. The addition of the alkaline caused a rapid recovery of the pH. Within two days, pH 7.2 was measured. Due to scum layers the value fluctuated but remained high until day 19. Also the alkalinity recovered to 3 250 $mg \cdot L^{-1}$ $CaCO_3$ at day 15 indicating adequate buffer capability. The increased organic loading rate caused an increase of the gas production from 1.8 $L \cdot L^{-1} \cdot d^{-1}$ at day 5 to 1.6 $L \cdot L^{-1} \cdot d^{-1}$ at day 10 (Figure 41e). Also the methane content increased from 56.5 % (v/v) at day 5 to 71.1 % (v/v) at day 10 (Figure 41g) indicating a higher activity of methanogenic bacteria. The effluent COD was slightly increased from 315 $mg \cdot L^{-1}$ at day 3 to 1 365 $mg \cdot L^{-1}$ at day 15 but remained stable at the adjusted loading rate.

Approximately from day 15 on precipitates in the whey feeding tank caused a malfunction of the feeding system due to blocking of the tubes. The error was noticed and fixed at day 22. The plot in Figure 41f was marked by using a dotted line to identify the period of uncertain organic loading rate. The malfunction caused a deterioration of the relevant process parameters *e.g.* decreased gas production and methane content, decreased alkalinity and pH and the occurrence of acetic and propionic acid (Figure 41b). The process was allowed to recover and restarted at day 24 with an organic loading rate of 5 $g \cdot L^{-1} \cdot d^{-1}$. All monitored process parameters recovered rapidly. The gas flow increased to approximately 6.5 $L \cdot d^{-1}$ and pH 7.0 was measured at day 26. Also the alkalinity and the methane content of the gas recovered. At day 27 the fatty acid concentration was 52 $mg \cdot L^{-1}$ for propionic acid and acetic acid and 1 $mg \cdot L^{-1}$ for butyric acid.

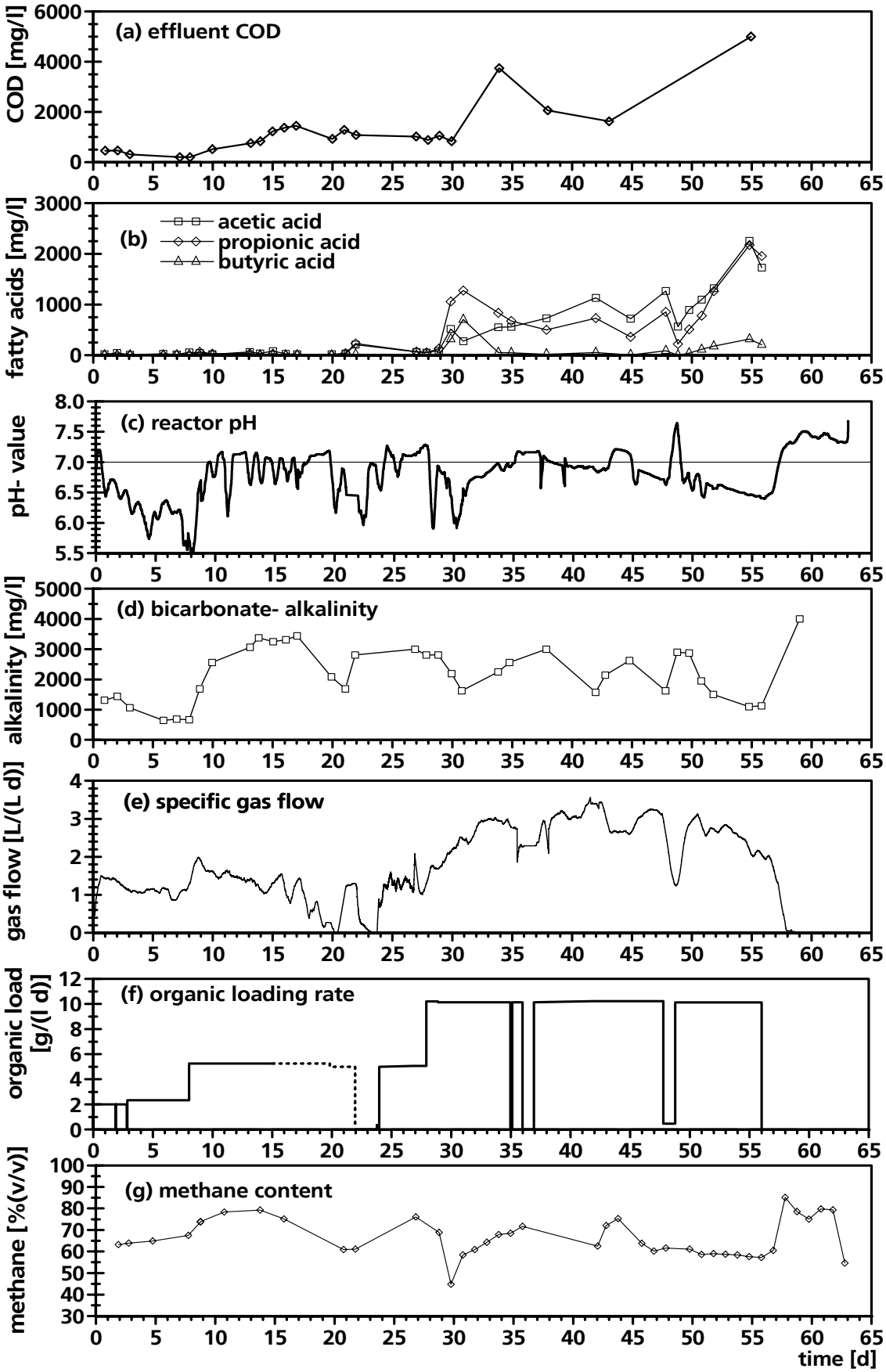

Figure 41 UASB Experiment with CIP addition (UASB_IC_V004). (a): COD concentration of the effluent. (b): Analysis of fatty acids of fermenter liquid samples. (c): Online measurement of the reactor liquid pH value. (d): Bicarbonate alkalinity of fermenter liquid samples. (e): Specific gas production per litre reactor volume. (f): Calculated organic loading rate on a COD basis. (g): Methane content in the off gas.

From day 27 on, an increased organic loading rate of 10.2 $g \cdot L^{-1} \cdot d^{-1}$ was adjusted. Also the internal circulation was increased. The upflow velocity was adjusted to 3.2 $m \cdot min^{-1}$. The gas production increased to 2.9 $L \cdot L^{-1} \cdot d^{-1}$ at day 34. However, the process was significantly stressed from the increased load indicated by deterioration of the relevant process parameters. The methane content of the gas was the fastest indicator. A rapid decrease to 46.5 % (v/v) was measured as soon as day 29 indicating that a rapid acidification took place. At day 31 the alkalinity was decreased to 1 625 $mg \cdot L^{-1}$ $CaCO_3$ and the pH dropped to a temporary minimum of pH 5.9. Caused by this, at day 31 high values of acetic acid (278 $mg \cdot L^{-1}$), propionic acid (1 277 $mg \cdot L^{-1}$), and butyric acid (705 $mg \cdot L^{-1}$) were measured. An increased effluent COD concentration followed (Figure 41a). At day 34 a COD of 3 749 $mg \cdot L^{-1}$ was measured. However, enhanced by an erratic idle time of the feeding system for 24 h at day 36, most parameters recovered within the following days. A recovered methane content of 62.6 % (v/v) was measured at day 35. At day 38, alkalinity was increased to 3 000 $mg \cdot L^{-1}$ $CaCO_3$ and pH 7.0 was measured. The effluent COD was measured 2 064 $mg \cdot L^{-1}$ and the gas production was increased to 3.1 $L \cdot L^{-1} \cdot d^{-1}$. However, butyric acid (21 $mg \cdot L^{-1}$) and propionic acid (502 $mg \cdot L^{-1}$) decreased but were still on high levels. Acetic acid was even increased to 729 $mg \cdot L^{-1}$ indicating that the acetogenesis was still not balanced with the acid production from acidogenesis. The volume of alkaline addition was not sufficient to keep pH and alkalinity in the reactor on an appropriate level at the higher loading rate. The pH decreased linear with fluctuations to pH 6.4 at day 56. Also the alkalinity decreased during the high load period. A low value of 1 100 $mg \cdot L^{-1}$ $CaCO_3$ was measured at day 55. The effluent COD (5 000 $mg \cdot L^{-1}$) and the fatty acid concentration increased to high values at day 55. Acetic acid, propionic acid, and butyric acid were 2 257 $mg \cdot L^{-1}$, 2 172 $mg \cdot L^{-1}$, and 320 $mg \cdot L^{-1}$ respective. From day 46 on, a fading of the sludge colour was noticed indicating the accumulation of precipitates. The feeding was switched off at day 56. At this time the sludge was turned to light grey similar to the observations made in previous experiments.

Performance data was calculated from values at steady state conditions. These were assumed at day 15 and at day 45. At day 15 the organic loading rate was 5.26 $g \cdot L^{-1} \cdot d^{-1}$ corresponding to a COD load of 26.3 $g \cdot d^{-1}$. A COD removal of 23.2 $g \cdot d^{-1}$ was calculated, and the gas production was 7.74 $L \cdot d^{-1}$ according to a specific gas production of 1.55 $L \cdot L^{-1} \cdot d^{-1}$. The methane content was measured 67.9 % (v/v) at this time. At day 45 a loading rate of 10.2 $g \cdot L^{-1} \cdot d^{-1}$ was applied, according to 51.0 $g \cdot d^{-1}$ of COD. The COD removal was 46.9 $g \cdot d^{-1}$, and a gas production of 15.65 $L \cdot d^{-1}$ was measured. This is equal to a specific gas production of 3.13 $L \cdot L^{-1} \cdot d^{-1}$. A methane content of 63.8 % (v/v) was measured. Calculated performance data for this experiment (UASB_IC_V004) is presented in Table 15.

Table 15 Performance data of the UASB experiment with CIP addition (UASB_IC_V004).

Parameter	Low OLR (day 15)	High OLR (day 45)
OLR [$g \cdot L^{-1}\ d^{-1}$]	5.0	10.2
COD removal efficiency [%]	88.4	92
Gas yield per COD removed [$mL\ g^{-1}$]	328	317
Methane yield per COD removed [$mL\ g^{-1}$]	223	202

9.5.4. Discussion of UASB Studies

Anaerobic digestion of whey using UASB type reactors have been reported by several researchers: Clark J.N (1988), digested sweet and acid whey in a 700 L UASB reactor at 14 day hydraulic retention time. Treating acid whey, the loading rates did not exceed 9 $g \cdot L^{-1} \cdot d^{-1}$ and operational problems due to reduced settling ability and biomass washout of the biomass was reported. Clark J.N (1988) made the case that the higher calcium content of acid whey compared to cheese whey may be responsible for this. Also Ghaly *et al.* (2000) reached only low organic loading rates (4.8 $g \cdot L^{-1} \cdot d^{-1}$ at 15 d HRT) when treating acid whey in a two-chamber anaerobic digester. They obtained a gas production of 1.63 $L \cdot L^{-1} \cdot d^{-1}$ and a methane concentration of 52 %. However, due to poor settle ability of the sludge only 58.6 % COD reduction was achieved. Additionally, to avoid rapid acidification and to reach stable conditions, pH control by addition of sodium bicarbonate was necessary. Yan *et al.* (1989) reported a methane yield per COD removed of 340 $mL \cdot g^{-1}$ at an organic loading rate of 4.23 $g \cdot L^{-1} \cdot d^{-1}$. However, also an overload at a high influent concentration of 38.1 $g \cdot L^{-1}$ for their UASB system treating pH-balanced cheese whey with nutrient addition was reported. Yan *et al.* (1989) assumed an imbalance of acidogenic and methanogenic bacteria due to lack of buffer capacity of the whey. At operational parameters similar to the present work, Hwang and Hansen (1990) obtained a COD removal efficiency of 96.9 % and a biogas production of 2.25 $L \cdot L^{-1} \cdot d^{-1}$ corresponding to a methane yield per COD removed of 260 $mL \cdot g^{-1}$. However, they treated diluted, reconstituted cheese whey, pH-balanced and with extra nutrient addition. Yan *et al.* (1993), observed two phases in the sludge bed of an UASB reactor treating cheese whey. An acidification stage near the influent inlet and a methanogenic stage at higher regions of the sludge bed were observed by measuring pH and fatty acid concentration at different heights of the sludge bed. The rapid acidification of the influent and a resulting accumulation of volatile fatty acids were assumed to be responsible for this. Even at low loading rates no dynamical balance was obtained by them. Similar observations were made at the UASB experiments

performed in this study. Kalyuzhnyi *et al.* (1997) digested pre-acidified cheese whey at laboratory and pilot scale UASB reactors at mesophilic (35 °C) and submesophilic (20°C to 30°C) temperatures. They reported the occurrence of some white aggregates inside the sludge bed after 60 days of operation at mesophilic temperature and stated that the presence did not influence the performance of the reactor up to organic loading rates of 28.5 $g \cdot L^{-1} \cdot d^{-1}$. A further increase of the organic loading rate lead to strongly reduced settleability of the sludge. Kalyuzhnyi *et al.* (1997) concluded that extensive inclusion of whey ingredients caused the loss of biomass. However, a discussion about the abrupt change in the sludge characteristic at only slightly higher organic loading rates is missing.

Ergüder *et al.* (2001) reported the digestion of cheese whey in an UASB reactor with addition of alkalinity and trace metal supplements. At hydraulic retention times in the range of (2.06 - 4.95) days and at organic loading rates up to 24.6 $g \cdot L^{-1} \cdot d^{-1}$ the COD removal efficiency was 91.9 % to 97.0 %. A methane yield of 424 $ml \cdot g^{-1}$ of influent COD was reported. No operational upsets were reported. However, Ergüder *et al.* (2001) used an extreme slim (1 : 17.8) reactor with a geometric volume of 2.12 L but calculates with an "effective volume" of only 0.743 L. The very small volume lead to imprecision of the results, the very slim shape of the reactor may cause fringe effects and the calculation of the reactor performance is ambiguous.

El-Mamouni *et al.* (1995) treated whey permeate with addition of calcium hydroxide $Ca(OH)_2$ to enhance the buffer capacity in a multiplate anaerobic reactor and reported that the accumulation of calcium precipitates after three month of operation caused a decrease in COD removal efficiency from 92 % to 31 %.

9.5.5. The Role of Calcium Precipitation and Extracellular Polymeric Substances in Anaerobic Digestion of Whey

A precipitation phenomena with detrimental effects was observed in this study (Figure 40) and also reported by other researchers (Clark, 1988 ; Kalyuzhnyi *et al.,* 1997; Malaspina *et al.,* 1996). The precipitation is presumably attributed to calcium Clark (1988). Acid whey has a considerable high content of calcium compared to rennet whey (Table 2, Page 31). Therefore, the role of calcium with reference to process upsets needs further discussion.

A positive influence to the granulation process was reported for low calcium concentrations (Yu *et al.*, 2001a), but a decrease of biomass activity with increasing calcium concentrations due to mass transfer limitation and toxic effects inside the granules was also reported by them. When high concentrations of Ca^{2+} are present in the wastewater, heavy precipitation of calcium carbonate $CaCO_3$ and calcium hydrogen phosphate $CaHPO_4$ occurs in UASB reactors at reactor internal build-in components and in or on the sludge Lettinga and Hulshoff Pol (1991). A crystallization stage prior to an anaerobic digestion can be used to remove calcium from wastewater (van Langerak *et al.,* 1997).

The addition of phosphate ions in the concentration range of (0.5 – 5.0) $g \cdot L^{-1}$ severely inhibit the extent of calcium carbonate precipitation in an Expanded Granular Sludge Bed (EGSB) reactor (van Langerak *et al.,* 1999). However, also phosphate accumulates in the sludge as observed in the experiments with the anaerobic membrane reactor (Section 9.3).

The location of calcium precipitates influence the stability and performance of anaerobic digestion (van Langerak *et al.*, 2000). A visual examination of sludge granules obtained from the UASB experiment with CIP addition (UASB_IC_V004) pictured in Figure 42 provide an indication of different stages in granulation and the location of precipitates. The bigger granule on the left contain a black core, originated from the inoculum granular sludge, while the smaller one on the right side was grown during the process and was pure white indicating precipitation of milk ingredients within the granule. Both granules are entrapped in a jelly-like, lucent gel structure with dispersed precipitation within it.

The observed gel layer on the granules was developed during the phase of high organic loading rate. Also in the experiment with and without internal circulation jelly-like aggregates were observed. Malaspina *et al.* (1995) reported reduced sludge settleability by excess production of exopolymeric materials. Flemming and Wingender (2001a) describe extracellular polymeric substances (EPS) as polymeric compounds *i.e.* polysaccharides, proteins, nucleic acids, or lipids which form a gel-like, hydrated biofilm matrix in the intercellular space of microbial aggregates. EPS is involved in formation of granules in anaerobic digesters and influence their mechanical properties (Robinson *et al.*, 1984; Dolfing *et al.*,

1985; Forster and Quarmby, 1995; Sallis and Uyanik, 2003). Calcium and Phosphorus were identified to be important constituents of the incinerated extracts from EPS samples Morgan *et al.* (1990). The EPS matrix of algae biofilms benefits the precipitation of calcium carbonate (Heath *et al.*, 1992).

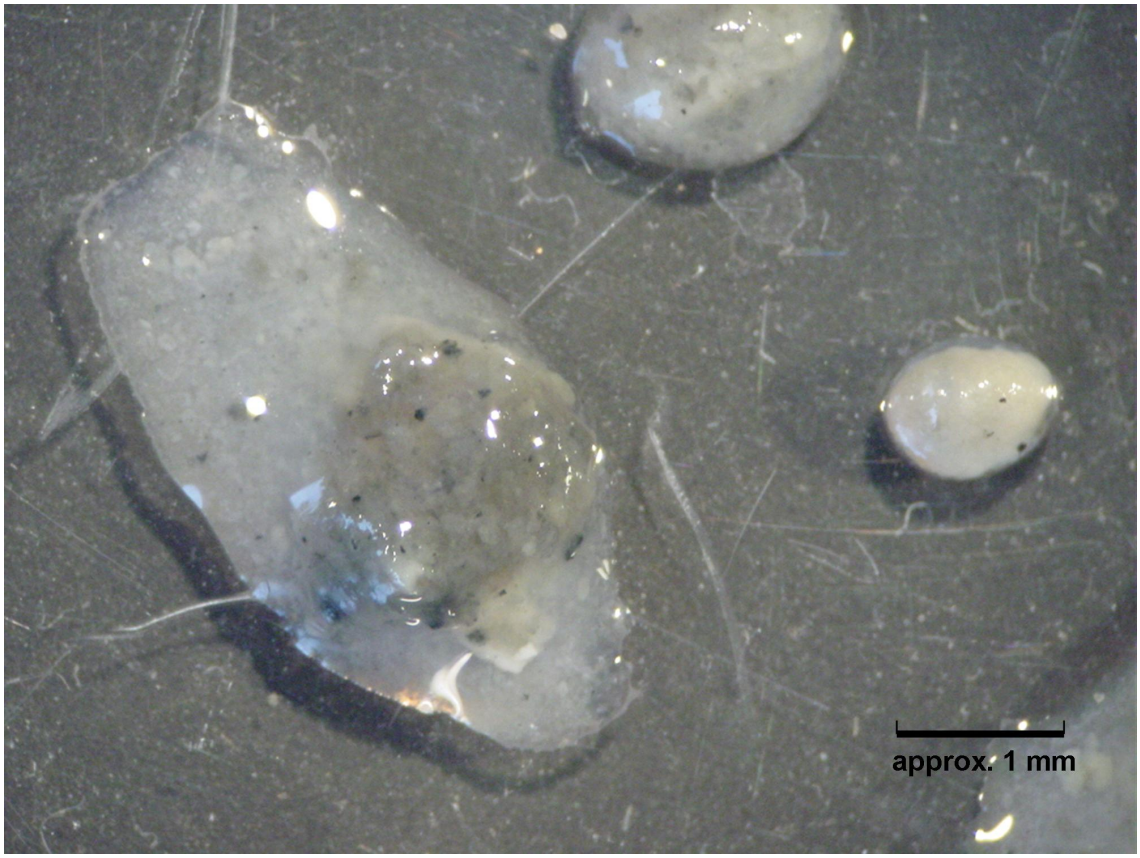

Figure 42 UASB sludge granules from the UASB experiment without internal circulation (UASB_IC_V003). Left: Granule with a black core from inoculum, surrounded by a jelly-like layer grown during organic overload stress. Right: Granule grown during the experiment, coloured white due to precipitated whey minerals.

Protein gelation is widely used to modify the texture, viscosity and other sensory properties of foods (Kinsella and Whitehead, 1989). Whey proteins may be involved in the development of gel structures within the reactor bulk. These globular proteins remain native during the quarg production process. The gelation mechanism involves two major steps: At first the native protein structure has to be unfolded by a driving force followed by an aggregation to a protein network by association between protein strands. The driving force to denaturize the protein can be induced by heat, pressure, chemical or biochemical processes such as acidification, the use of salts, or enzymatic cross-linking (Totosaus *et al.*, 2002) The acid-induced cold gelation process was investigated by Alting (2003). In this process globular proteins were denatured by heat pre-treatment. In a second step the gelation is induced by lowering the pH or by adding salts. Organic acids such as acetic acid can be used for acid induced gelation (Chawla *et al.*, 1996). Whey proteins can form

gels without heating by addition of calcium salts (Barbut and Foegeding, 1993; Ju and Kilara, 1998). In acid whey, calcium is mainly present as calcium lactate. During acidification and degradation of the lactate, calcium-ions and volatile fatty acids will be released into the bulk liquid. At fermentation of acid whey the denaturing of native whey proteins may be induced by enzymatic reactions and high concentrations of volatile fatty acids. Gel structures can then be formed when a) the concentration of calcium ions and of denatured proteins is high enough, and b) the rates of further protein degradation and of calcium precipitation are lower than the rate of gel forming. Especially at organic overload stress such conditions may arise. Batstone *et al.* (2006) demonstrated the structure of anaerobic granules, consisting of a layer of acidogens and a core of acetogens/methanogens. Fatty acid production and release of calcium-ions from this layer generate favour conditions for an acid-induced cold gelation of whey proteins in immediate proximity of the granule surface. The excessive formation of a gel layer surrounding methanogenic granules was observed after overload stress in the UASB experiments performed in this work (Figure 42). However, such gel layers may also be interpreted as EPS formation. In conformity with this Fukuzaki *et al.* (1991) observed that the content of extracellular polymeric substances developed in methanogenic granular sludge grown on lactic acid was mainly protein rather than carbohydrate. In lower parts of their UASB reactor more EPS was produced. Also the increased production of EPS reported by Malaspina *et al.* (1995) and the abrupt change in the sludge characteristic after increasing the organic loading rates reported by Kalyuzhnyi *et al.* (1997) is explainable by the described mechanism of acid induced gel formation.

9.6. Conclusions of UASB Studies

From the results of this study and from results reported in the literature it can be concluded that UASB type anaerobic digesters are suitable with limitations for digestion of dairy wastes. The rapid acidification of acid whey lactose and the high strength of acid whey led to organic overloads and irreversible deterioration of the sludge bed. The methane production of an UASB without internal circulation, treating diluted acid whey at was completely inhibited within 6 days at a moderate organic loading rate of 7.2 $g \cdot L^{-1} \cdot d^{-1}$. The use of an internal circulation mitigates the effect. However, the resulting increased upflow velocity also increased the loss of biomass due to a reduced settling ability of sludge granules. Precipitation and accumulation of milk minerals within the granules was observed in all UASB experiments. The precipitates were assumed to be mainly calcium carbonate and calcium phosphate salts. The inclusion of whey minerals caused a change of the sludge colour from a black inoculum to granules of pure white colour and was assumed to be related to the reduced settleability of the sludge. Additionally, at overload conditions the

formation of a jelly like layer of extracellular polymeric substances was observed on the surface of granules. A new approach to the formation mechanism of this layer was made assuming the acid-induced cold gelation of whey proteins.

A spent alkaline wastewater from dairy cleaning in place operation which contained 2.0 % (m/m) of sodium hydroxide was used for pH control and this led to a stabilisation of the pH-value towards neutral conditions and an increased alkalinity of the reactor bulk. Thus, co digestion of acid whey with alkaline effluents is an appropriate method to stabilise the process.

The performance of an UASB process with internal circulation and pH-control using alkaline CIP effluents, performed in this study, was reported for two organic loading rates applied to the reactor.

- At an organic loading rate of 5.26 $g \cdot L^{-1} \cdot d^{-1}$ a COD removal efficiency of 88.4 % was achieved. The specific gas production was 1.55 $L \cdot L^{-1} \cdot d^{-1}$ corresponding to a gas yield per COD removed of 328 $mL \cdot g^{-1}$. A methane concentration of 67.9 % (v/v) in the biogas was measured. The resulting methane yield was 223 $mL \cdot g^{-1}$.
- At a higher loading rate of 10.2 $g \cdot L^{-1} \cdot d^{-1}$ applied over a period of 31 days, the COD removal efficiency was 92 %. A specific gas production of 3.13 $L \cdot L^{-1} \cdot d^{-1}$ and a methane concentration of 63.8 % (v/v) were measured. The gas yield and the methane yield per COD removed were 317 $mL \cdot g^{-1}$ and 202 $mL \cdot g^{-1}$ respective.

When comparing the performance of UASB reactors treating whey it is essential to distinguish between acid whey and cheese whey digestion. Stable process conditions for an anaerobic digestion of non-pre-acidified acid whey were not reported beyond organic loading rates higher than 9 $g \cdot L^{-1} \cdot d^{-1}$. Also in this study, the operation at an organic loading rate of 10.2 $g \cdot L^{-1} \cdot d^{-1}$ resulted in process imbalances and finally led to process failure. The results of the UASB reactor experiment, performed in this study, are comparable to those reported from studies of other authors, reviewed in Section 9.5.4. However, the observed imbalances and process failures required revision and optimization of the process design.

9.7. Two-Stage Reactor Studies

The conclusions drawn from the experience of acid whey digestion in one stage digestion processes lead to the development of a novel two-stage process design consisting of a combined acidification and crystallization stage and a gaslift driven fluidised bed methanogenic stage described in detail in Section 5.4.4.

At start-up the acidification stage was seeded using 1 400 mL of granular sludge liquor from an industrial anaerobic digester for wastewater treatment of an alcohol producer (KWST GmbH, Hannover, Germany) as inoculum. The sludge granules were used as inoculum of the gaslift/fluidised bed digester stage. The spent CIP effluent described in Section 5.4.4 was a 2.0 % (m/m) sodium hydroxide solution loaded with a COD of 3.99 $g \cdot L^{-1}$ and was used to maintain the pH of the first stage to a setpoint of pH 6.0 by automatic dosage control. The hydraulic retention time (HRT) of the first stage was maintained to 24 h by adjusting the working volume to 3.58 L, while the hydraulic retention time of the second stage (10.7 L working volume) was set to 3 days by adjusting the transfer pump to 3.58 $L \cdot d^{-1}$. The ratio of influent volumes of whey and spent CIP varied during the experiment due to pH-controlled dosage. The acidification stage was made up to the working volume with diluted whey with a strength of 8.7 $g \cdot L^{-1}$ COD. After start-up and heating to 37 °C the digester was fed continuously. Results of the experiment are presented in Figure 43.

The acidification stage was continuously mixed by a blade agitator at 150 min^{-1} rotational speed. The pH control by dosage of alkaline CIP effluent maintained the pH-value of the acidification stage (Figure 43b) within the range from pH 5.97 and pH 6.03. Also some aggregates formed on the pH-probe resulting in slightly increased fluctuations in the pH value from day 28 on. Thus the probe was cleaned and checked at day 39. At day 36 the pH-control failed due to an empty storage tank of the alkaline spent CIP resulting in rapid acidification to pH 5.38 within 3 h. The pH recovered within 5 min after refilling, however the resulting alkaline shock load led to a fluctuation of the pH-value of the second stage. However, no adverse effects to the process had been observed.

Deposits of fouling matter formed at the reactor interior of the acidification stage and to a great extent around the CIP alkaline inlet pipe by precipitation of milk minerals during the whole experimental period, first observed at day 5. Occasionally clusters of the incrustation flaked off and settled. The precipitate was pure white in colour and fine grained, its solidity were similar to blackboard chalk. Formation of jelly-like gel structures, observed in UASB experiments discussed in Section 9.5.5 had not been observed. A quantitative identification of the mineral composition of the precipitates could not been performed, how-

ever, it can be assumed that large quantities of the precipitated material are calcium minerals rather than potassium- or sodium-salts due to their high solubility. Calcium carbonate in its various polymorphs is presumably the main component of the precipitates. From the findings during the membrane reactor studies (Section 9.3) the forming of calcium phosphate minerals is also assumed. Deposition of milk minerals in and on the granules in the gaslift/fluidised bed reactor stage was not observed. Newly grown granules were of black colour. Thus, the removal of precipitates within the acidification stage benefited the anaerobic digestion process by formation of well settling granular sludge.

After start-up the organic loading rate of the two-stage system was increased stepwise by adjusting the influent concentration from initially 10.0 $g \cdot L^{-1}$ COD (Figure 43e) corresponding to an overall organic loading rate of 2.5 $g \cdot L^{-1} \cdot d^{-1}$ (Figure 43g). After 14 days of operation at this organic loading rate the effluent concentration was measured 369 $g \cdot L^{-1}$ COD (Figure 43a). The consumption of alkaline spent CIP due to pH-control of the acidification stage during this period was 235 $mL \cdot d^{-1}$ corresponding to 6.6 % of the influent volume (Figure 43f). A bicarbonate alkalinity of 4 400 $mg \cdot L^{-1}$ $CaCO_3$ (Figure 43d) and pH 7.63 of the second stage effluent (Figure 43c) indicated a good condition of the process at this time. Thus, the whey feed concentration was increased to 18.9 $g \cdot L^{-1}$ at day 14. This led to an increased consumption of spent CIP of 13.3 % and 16.8 % of the influent volume until day 25 and day 30 respective. The resulting organic loading rate was 4.2 $g \cdot L^{-1} \cdot d^{-1}$ while the effluent COD remained low, measured 352 $g \cdot L^{-1}$ at day 23. The bicarbonate alkalinity of the second stage effluent increased to 6 000 $mg \cdot L^{-1}$ $CaCO_3$ at day 23. Additionally a second sample was taken from the reactor base, measured only slightly lower at 5 600 $mg \cdot L^{-1}$ $CaCO_3$ indicating that no local overload within the lower compartment occurred. The pH-values of the second stage fluctuated in the range of pH 7.4 at day 15 and pH 7.72 at day 21 due to the increased organic load, but recovered to pH 7.63 at day 23.

A slight increase of the whey feed concentration to 23.7 $g \cdot L^{-1}$ COD at day 30 resulted in a further increase of the spent CIP consumption to 23.8 % of the influent volume. However, after acclimatisation to the new whey feed concentration the consumption of spent CIP decreased to 22.1 % at day 36. The organic loading rate was only slightly increased due to dilution by the increased consumption of spent CIP, calculated to 4.86 $g \cdot L^{-1} \cdot d^{-1}$ at day 36.

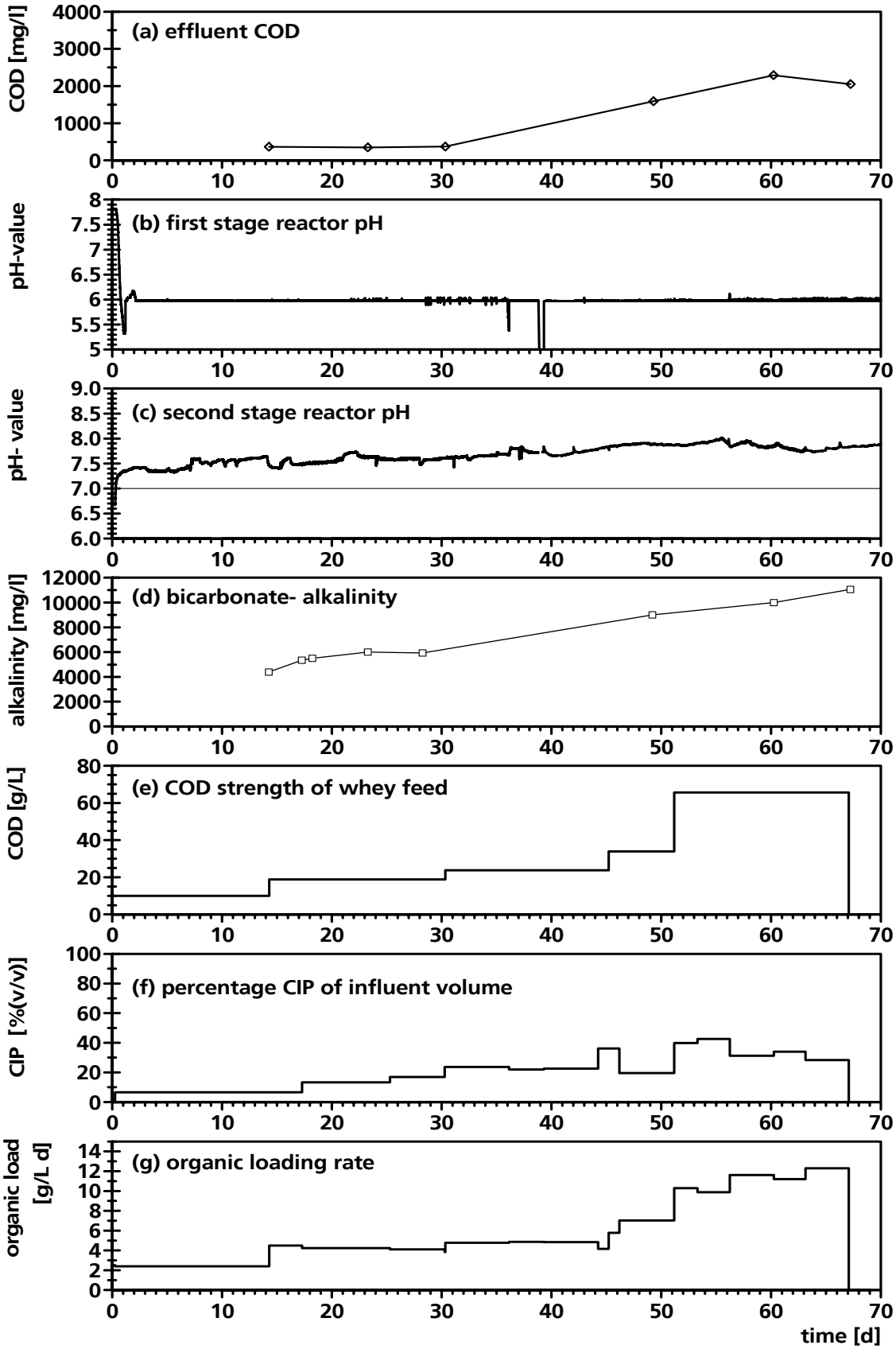

Figure 43 Two-stage high-rate reactor experiment using whey and CIP effluent (UASB_IC_V007). (a): COD concentration of the effluent. (b): Online measurement of the pH value of the acidification stage. (c): Online measurement of the pH value of the methanogenic stage. (d): Bicarbonate alkalinity of the methanogenic stage. (e): COD concentration of the whey feed. (f): Percentage of CIP the influent volume. (g): Overall organic loading rate of the two-stage system.

During the period of low organic loading rate until day 39 occasionally clusters of biomass soared up and accumulated in the three phase separator of the upper compartment. Portions of the poor settable sludge washed out via the diversion cut of the reactor outlet. However the loss of biomass did not reach the excessive wash-out observed in the UASB experiments (Section 9.5.2). To avoid a loss of performance at day 39 the sludge aggregates were removed from the three phase separator of the upper compartment and 1.5 L of inoculum sludge was added to the biomass interior. An effect to the process was not observed indicating that most of the biomass was already well acclimated to the whey based feed. During the application of higher organic loading rates no further biomass wash-out due to poor settable sludge was observed.

After reseeding, two hydraulic retention times were allowed for acclimatisation of the sludge. At day 45 the whey feed concentration was increased to 33.9 $g \cdot L^{-1}$. The consumption of spent CIP increased rapidly to 36.2 % during the following two days but recovered to 19.6 %. The resulting organic loading rate was 7.0 $g \cdot L^{-1} \cdot d^{-1}$ at day 49. The effluent COD was increased to 1 588 $g \cdot L^{-1}$. However, also the bicarbonate alkalinity and the pH-value of the reactor effluent were increased, measured 9 000 $mg \cdot L^{-1}$ $CaCO_3$ and pH 7.87at day 49. The fast recovery to low spent CIP consumption and the high alkalinity indicated that the biomass acclimated very fast to the increase of the feed strength. Therefore undiluted whey with a strength of 65.6 $g \cdot L^{-1}$ COD was used from day 51 on. Once again the consumption of spent CIP increased rapidly, the highest consumption, 42.6 % of the influent volume, was observed in the interval from day 53 to day 56. After acclimatisation the ratio decreased to 34.0 % at day 60 resulting in an organic loading rate of 11.2 $g \cdot L^{-1} \cdot d^{-1}$. At the same time the effluent COD was 2 292 $g \cdot L^{-1}$ and a bicarbonate alkalinity of 10 000 $mg \cdot L^{-1}$ $CaCO_3$ was measured while the pH of the effluent still remained at pH 7.87. The COD strength of the used alkaline CIP solution was increased slightly to 7.3 $g \cdot L^{-1}$ at regular refilling of the storage tank at day 63. No adverse effect of this change was observed. The final measurements at day 67 showed that the effluent COD was slightly lower, measured 2 049 $g \cdot L^{-1}$. Also the consumption of spent CIP decreased to the final value of 28.4 % of the influent volume. The pH-value was stable at pH 7.84 while the bicarbonate alkalinity had a further increase to 11 050 $mg \cdot L^{-1}$ $CaCO_3$ in the effluent.

Based on the current findings, 53.4 % of the whey produced in the examined dairy in the year 2003 could have been digested using the introduced process. However, it can be assumed that the consumption of spent CIP will undergo a decrease with time due to further acclimatisation of the acidifying bacteria. Also an optimization of the acidification/crystallization stage may lead to significant reduced consumption of alkaline CIP effluent. This should be topic of future research work.

Performance data was calculated from values at steady state conditions. Due to failure of the gas flow measurements the gas production was estimated with sufficient accuracy by measuring the time interval between one deflation of the gas tight bag (60 L) to the next, recorded on two occasions. Gas volumes were calculated at day 23 and at day 67. At day 23 the organic loading rate was 4.2 $g \cdot L^{-1} \cdot d^{-1}$ corresponding to a COD load of 60.0 $g \cdot d^{-1}$. A COD removal of 59.2 $g \cdot d^{-1}$ was calculated, a gas production of 60 L in 48 h was observed corresponding to a specific gas production of 2.1 $L \cdot L^{-1} \cdot d^{-1}$. At day 67 a loading rate of 12.3 $g \cdot L^{-1} \cdot d^{-1}$ was applied, according to 175.7 $g \cdot d^{-1}$ of COD load. The COD removal was 168.4 $g \cdot d^{-1}$, and a gas production of 60 L in 20.5 h was observed corresponding to a specific gas production of 4.9 $L \cdot L^{-1} \cdot d^{-1}$. Performance data for this experiment (UASB_IC_V007) is presented in Table 16.

Table 16 Performance data of the two-stage experiment (UASB_IC_V007).

Parameter	**Low OLR (day 23)**	**High OLR (day 67)**
OLR [$g \cdot L^{-1}$ d^{-1}]	4.2	12.3
COD removal efficiency [%]	97.9	95.8
Gas yield per COD removed [mL g^{-1}]	507	416

Data for two-stage anaerobic digestion of acid whey is very rare in the literature. Only Ghaly *et al.* (2000) reported the use of a two-stage system. However, the digester consisted of two vessels, equal in volume. The performance of their digester was poor. Only low organic loading rates of 4.8 $g \cdot L^{-1} \cdot d^{-1}$ at 15 d hydraulic retention time and a COD reduction of 58.6 % was reached, even with addition of sodium bicarbonate and pH-control. Also the gas production was low at 1.63 $L \cdot L^{-1} \cdot d^{-1}$. The downflow-upflow hybrid reactor of Malaspina *et al.* (1996) was operated at 10 $g \cdot L^{-1} \cdot d^{-1}$ organic loading rate feeding raw undiluted cheese whey and reached 98.4 % COD removal efficiency. A high gas yield of 557 $mL \cdot g^{-1}$ of removed COD indicates the proper design of the process. However Malaspina *et al.* (1996) used the anaerobic filter principle with some disadvantages, discussed in Section 4.3.5.1. Another high-rate two-stage anaerobic digestion system treating cheese whey was the membrane coupled CSTR of Saddoud *et al.* (2007) with was operated at an overall organic loading rate of 12 $g \cdot L^{-1} \cdot d^{-1}$. The COD removal efficiency was 98.5 % and a gas yield per COD removed of 300 $mL \cdot g^{-1}$ was achieved. However, the high loading rate was only applied for one hydraulic retention time. There is no evidence about the long term stability of the process.

9.8. Conclusions on Two-Stage Reactor Studies

The design of the novel high-rate anaerobic digester considered the demands of the high strength waste with most of the COD deriving from carbohydrates, its low pH and tendency to rapid acidification during anaerobic digestion. Also considered was the high content of calcium which is characteristic for acid whey. The process was designed to avoid accumulation of precipitates which may otherwise occur in methanogenic granular sludge and also the possible formation of excess extracellular polymeric substances. For this purpose, a novel acidification process with simultaneous precipitation of inorganic milk ingredients was introduced. The concept also involves the idea to use spent alkaline effluents from dairy cleaning in place processes to provide extra alkalinity and to implement pH control. More than half of the whey of the examined dairy can be treated using this source of alkalinity. The final two-stage studies demonstrated enhanced stability of the high-rate anaerobic digestion process on treating full strength acid whey and alkaline dairy wastewaters at high organic loading rates.

Two-stage studies found in the literature mostly used cheese whey as feeding substrate which is easier to digest. To the knowledge of the author no performance data for a two-stage high-rate anaerobic digestion system treating acid whey is present in the literature. This work contributes toward filling this gap. The performance of the novel system used in this study is comparable to the results of well designed, two-stage digesters treating cheese whey. At stable conditions the novel process was operated with a high COD removal efficiency of 95.8 % at a high organic loading rate of 12.3 $g \cdot L^{-1} \cdot d^{-1}$, applied for a period of four hydraulic retention times. A gas yield per COD removed of 416 $mL \cdot g^{-1}$ was achieved.

9.9. Comparison of tested Anaerobic Digestion Processes

When comparing the performance of anaerobic digesters treating whey it is essential to distinguish between acid whey and cheese whey digestion. To the knowledge of the author no performance data for a one-stage membrane coupled anaerobic digestion system treating acid whey is present in current literature. This work contributes toward filling this gap. The results of the one-stage UASB reactor experiment, performed in this study, are comparable to those reported from studies of other authors, reviewed in Section 9.5.4. A novel two-stage high-rate anaerobic digestion system treating acid whey is presented in this work. The performance of the novel system used in this study is comparable to the results of well designed, two-stage digesters treating cheese whey.

Table 17 Overview: Performance data of anaerobic digestion experiments performed and presented in this study.

Parameter	AMR (day 15)	UASB Low OLR (day 15)	UASB High OLR (day 45)	Two-stage Low OLR (day 23)	Two-stage High OLR (day 67)
OLR [g·L^{-1} d^{-1}]	7.7	5.0	10.2	4.2	12.3
COD removal efficiency [%]	99.3	88.4	92.0	97.9	95.8
Gas yield per COD removed [mL g^{-1}]	227	328	317	507	416
Methane yield per COD removed [mL g^{-1}]	114	223	202	-	-
Separation of precipitates	No	No	No	Yes	Yes
Balanced operation	No	No	No	Yes	Yes

When comparing the results of the experiments, performed and presented in this study (see Table 17), it must be kept in mind that in the one-stage experiments with the AMR and also with the UASB system a low alkalinity caused imbalances and process failures. In particular the lack of precipitates removal was identified to cause process upsets. The novel combined acidification and crystallisation stage used in the two-stage studies let to a balanced process with the additional advantage of yielding a valuable product by separation and removal of precipitates from the process. It can be concluded that the AMR and also the UASB reactor, a system tested and proven in thousands of industrial installations, are more successful when operated as the methanogenic stage of a two-stage system with the novel combined acidification and crystallisation process. An overview of anaerobic digesters treating whey, found in the literature, their experimental set-ups and operational data compared to the novel two-stage system used in this study, are presented in Table 18

Table 18 Experimental set-ups for anaerobic digestion of whey found in the literature compared to the two-stage system used in this study.

Reactor Type	Waste Type	Temperature [°C]	Volume [L]	Organic Loading Rate (OLR) [$g \cdot L^{-1} \cdot d^{-1}$ COD]	Hydraulic Retention Time (HRT) [d]	Influent COD Concentration [$g \cdot l^{-1}$]	Treatment Efficiency [% COD removal]	Reference
Two-stage, continuous stirred acidification and crystallisation, Internal circulation, pH control	acid whey, spent alkaline cleaning solutions	37	3.58+10.7	12.3	1+3	65.6 (whey)	95.8	This study
Fixed film loop reactor; packed with porous clay beads.	acid whey	35	2.1	5	14	79	95	Wildenhauer and Winter (1985)
UASB reactor; pH control	diluted cheese whey	33	14.3	0.9-6.0	5	5.0-28.7	97-99	Yan *et al.* (1989)
Downflow- upflow hybrid reactor; two filter sections	cheese whey	mesophilic cond.	51	10	7	68.8	98	Malaspina *et al.* (1996)
UASB reactor	cheese whey, dairy wastewater	20-30	10740	1-6.7	3.3-12.8	16-50	90-95	Kalyuzhnyi *et al.* (1997)
Two phase, continuous stirred acidification, upflow anaerobic filter, pH control	cheese whey powder, nutrients	35	1.5+5.36	-	1+4	5-20	90	Ylmazer and Yenigün (1999)
Two-stage no mix reactor; pH control	acid whey	35	150	4.8	15	74-75	58.6	Ghaly *et al.* (2000)
Two phase continuous stirred tank reactor; pH control	diluted cheese whey	55	1.0+5.0	1.3	7.5	10	96	Yang *et al.* (2003)
Two-stage, Stirred acidification, Stirred tank membrane reactor,	cheese whey		5+15	2.4-12	1+4	10-68.6	98.5	Saddoud *et al.* (2007)

10. CONCLUSIONS

10.1. Summary of Results

In the monitored dairy a volume of 112 000 m^3 per year of acid whey is produced on a 5 days a week basis. The whole factory processed 277 000 metric tonnes milk per year and 0.938 6 kg of water was consumed per kg of utilized milk. Approximately 9 800 m^3 of nitric acid 1.5 % (m/m) and 17 000 m^3 of sodium hydroxide 1.5 % (m/m) are used for cleaning purpose. The characterisation of whey and effluent samples lead to following results:

- The acid whey has a high COD *i.e.* greater than 65 $g \cdot L^{-1}$ COD and a BOD_5 of approximately 20 $g \cdot L^{-1}$. The COD of whey mainly derives from lactose, lactate and proteins. These components were identified to be very biodegradable. The high grade of anaerobic biodegradability indicating that anaerobic biological treatment would be appropriate treatment option.
- Alkaline effluents have a medium COD of approximately 3 $g \cdot L^{-1}$ COD and a high concentration of sodium (8.76 $g \cdot L^{-1}$). This high sodium concentration is inhibitory to the anaerobic process. Therefore would not be appropriate for anaerobic digestion without blending.
- The COD and also total and fixed solids of an alkaline cleaning solution used in a steady renewing CIP process was measured regularly, on a daily basis. The values increased rapidly within a day of operation. Due to usage and refreshing of the cleaning solution the results fluctuated in the ranges of (1 605 to 3 185) $g \cdot L^{-1}$ for COD and (21.4 to 33.72) $g \cdot L^{-1}$ for total solids. Acidic wastes are arising in low volumes and can be used for balancing the pH of the main effluent stream.

The recovery of sodium hydroxide based alkaline CIP cleaning solution loaded with milk ingredients by nanofiltration was investigated. The filtration was performed without pretreatment of the filtration batch, using a ceramic tubular membrane with a molecular weight cut off (MWCO) of 1 000 Da.

- Samples of the recovered cleaning agent taken during normal operation were low in COD *i.e.* 1 013 $mg \cdot L^{-1}$ and 1 316 $mg \cdot L^{-1}$ indicating appropriate rejection of lactose by the membrane.
- The membrane had an effective COD retention of > 80 %. The COD of the permeate was low *i.e.* < 1.5 $g \cdot L^{-1}$.

- The COD of retentates can be calculated using the volume reduction ratio *VRR*(t) in the same way as a concentration. The COD is linear dependent to volume reduction ratio *VRR*(t)
- The examination of the rheological properties of alkaline CIP wastewaters on different concentrations clearly shows that these effluents are Newtonian fluids even at high concentrations. The log of dynamic viscosity is linear dependent to its COD. Therefore a dependency of the viscosity from the retentate COD can be estimated.

The biodegradability of mixtures containing simulated alkaline effluents from dairy cleaning procedures concentrated by membrane filtration and acid whey was studied.

- An anaerobic biodegradability of 35 % was achieved for the acid whey sample.
- Except one sample, all mixtures achieved higher biodegradability than the pure whey sample. A high sodium concentration of this sample (1.5 $g \cdot L^{-1}$) was identified as inhibitory.
- The highest value was calculated 46.2 % for the mixture containing 75 % acid whey and 25 % of the low strength alkaline CIP wastewater.
- Mixture of acid whey with the high concentrated CIP wastewater sample achieved lower biodegradability results when compared with mixtures of whey with less concentrated CIP effluent.
- In the mixtures containing a portion of 50 % or more of acid whey the occurrence of a second lag-phase was observed. This was identified as phase separation due to rapid acidification of lactose. The observed phase separation was less distinctive in mixtures containing less whey. Phase separation was identified to be characteristic for lactose fermentation in batch mode and can be interpreted as a process imbalance. A two-stage process design is recommended to avoid the accumulation of intermediate volatile fatty acids.
- The high sodium concentration of the examined alkaline effluents from plant cleaning procedures led to inhibitory effects. Therefore, this type of wastewater is unfavourable for single treatment. However, the high alkalinity of these effluents will benefit the anaerobic digestion of acid whey which is low in alkalinity.

Four anaerobic digester types were designed with regard to their suitability for high strength waste treatment and were build in laboratory scale. The reactors that had been tested were: a) A Continuous Stirred Tank Reactor (CSTR) without biomass retention; b) A CSTR coupled with a membrane filtration unit for biomass retention; c) An Upflow Anaerobic Sludge Blanket (UASB) reactor; and d) A novel two-stage process design consisting of a combined acidification and crystallization stage and a gaslift driven fluidised bed methanogenic stage.

- The CSTR system suffered from poor biomass retention and therefore poor treatment efficiency.
- An Anaerobic Membrane Reactor provides complete biomass retention. A novel control strategy for the filtration unit avoids surplus filtrate and thus shear stress on the biomass and energy consumption is minimized.
- In the experiments it was demonstrated that even with perfect biomass retention the operation of an anaerobic membrane reactor at high loading rates caused process upsets.
- Due to cation-toxicity the pH control with alkaline have to be started with careful attention to the process development. A biomass, stressed by organic overload can not be recovered by subsequent pH control.
- High phosphate removal efficiency, the accumulation of phosphate in the biomass, and the change of the sludge colour indicated the formation of inorganic precipitates within the reactor. However, due to careful parameter estimation for the membrane system, no adverse effects to the filtration performance by irreversible scaling from precipitants were observed.
- From experiments with UASB type anaerobic digesters it can be concluded that these are suitable with limitations for digestion of dairy wastes. The rapid acidification of acid whey lactose and the high strength of acid whey led to organic overloads and irreversible deterioration of the sludge bed. The methane production of an UASB without internal circulation, treating diluted acid whey at was completely inhibited within 6 days at a moderate organic loading rate of 7.2 $g \cdot L^{-1} \cdot d^{-1}$. The use of an internal circulation mitigates the effect. However, the resulting increased upflow velocity also increased the loss of biomass due to a reduced settling ability of sludge granules.
- Precipitation and accumulation of milk minerals within the granules was observed in all UASB experiments. The precipitates were assumed to be mainly calcium car-

bonate and calcium phosphate salts. The inclusion of whey minerals caused a change of the sludge colour from a black inoculum to granules of pure white colour and was assumed to be related to the reduced settleability of the sludge.

- At overload conditions the formation of a jelly like layer of extracellular polymeric substances was observed on the surface of granules. A new approach to the formation mechanism of this layer was made assuming the acid-induced cold gelation of whey proteins.
- The use of a spent alkaline wastewater from dairy cleaning in place operation containing 2.0 % (m/m) of sodium hydroxide for pH control led to a stabilisation of the pH-value towards neutral conditions and an increased alkalinity of the reactor bulk. Thus, co digestion of acid whey with alkaline effluents is an appropriate method to stabilise the process.
- The design of the novel high-rate anaerobic digester considered the demands of the high strength waste with most of the COD deriving from carbohydrates, its low pH and tendency to rapid acidification during anaerobic digestion. Thus, a two-stage digestion was used.
- The novel process was designed to avoid accumulation of precipitates which may otherwise occur in methanogenic granular sludge and also avoid the possible formation of excess extracellular polymeric substances. For this purpose, a novel acidification process with simultaneous precipitation of inorganic milk ingredients was introduced.
- The final two-stage studies demonstrated enhanced stability of the high-rate anaerobic digestion process on treating full strength acid whey and alkaline dairy wastewaters at high organic loading rates.

10.2. General Discussion and Conclusions

Significant amounts of acid whey arise from quarg cheese production. Additionally, large amounts of water and cleaning agents are consumed in milk processing. From the results of a survey performed by the German dairy association reported by Coldewey *et al.* (2003) the examined dairy can be characterized as middle sized dairy. Compared to other dairies of this size a low specific water consumption per utilized milk of less than 1.0 $kg \cdot kg^{-1}$ was already reached in this facility. This low water consumption or even further savings are only possible by continuous process improvements and regular monitoring. With the examination of the cleaning procedures and the analytical results of wastewater samples,

necessary information for the development of a waste reduction strategy is available. A simple analysis of the fixed solids content of the alkaline detergent in the CIP process tank led to the conclusion that the concentration of sodium hydroxide was 2 % (m/m) instead of the intended value of 1.5 % (m/m). The presentation of the analytical results to the technical personal of the examined dairy led to a re-calibration of the conductivity sensors used for dosage of sodium hydroxide to the process tank and thus to a reduction of sodium hydroxide consumption. Additionally, the concept of using a solitaire CIP plant and a special cleaning agent for the cleaning of the quarg separator was reconsidered and rejected during the period of examination due to high costs for maintenance, water, energy and chemicals. This decision was not made mainly due to the research work. However the attention given to this process during the collection of data may have raised the awareness of the dairy's technical management to this dispensable practice.

Closing loops within the production process by recycling and reuse of resources is a way to overcome the consumption and disposal practice towards sustainability. The recovery of CIP cleaning solutions is one strategy among others to close loops in dairy milk processing. However, the reference document on best available techniques in the food, drink and milk industries of the European Integrated Pollution Prevention and Control Bureau in the European Commission (Garcilaso, 2006) only mention advanced treatment by membrane separation of used CIP cleaning solutions for CIP main cleaning supply as a opportunity for water re-use at dairies and not as best available technique. Thus, the operation of membrane based recovery of cleaning solution is still not very common in dairies. This may due to uncertainty in the case of pay back times (see Section 2.4) but there is still a lack of knowledge about these processes and reports about experiences with alkaline recovery are still rare. In this work the operation and performance of membranes with a molecular weight cut-off of 1kDa was investigated and compared to the results of others with more dense membranes (Räsänen *et al.,* 2002; Dresch *et al.*, 1999; Hufemia, 1996) and with less dense micro- and Ultrafiltration membranes (Henk, 1993) was made. An optimized integration of the recovery unit into the CIP plant was reported by Dresch *et al.* (2001) based on numeric process simulation. Examples for the implementation of membrane based CIP solution recovery processes had been reported for a bottle washing plant (Hufemia, 1996) and for a hard cheese production (Yacubowicz, 1995). However, the current work does not aim in process optimization or implementation into a CIP processes. Instead, the focus was set to the characterisation and treatment of residuals from the recovery, namely the retentates of filtration process. Physical characteristic of retentates were investigated. While the characteristic of permeates, and its capability for re-use was investigated by several authors as reviewed above, no information about rheological prop-

erties of the retentate was found. Samples up to a COD of 285 $g \cdot L^{-1}$ had been tested. The high COD correspond to a volume reduction ratio VRR of 50 to 150 of the recovery plant depending of the initial COD load of the alkaline CIP cleaning solution. The retentates were found to be Newtonian fluids and thus their viscosity was calculated. It can be concluded that a high concentrated retentate (285 $g \cdot L^{-1}$ COD) with a viscosity of 342.75 mPa·s is flowable and pumpable. This is vital information for the design of membrane filtration plants used in dairy CIP cleaning solution recovery.

Only little information about treatment or utilization of residues from caustic recovery in dairies is available in the literature. Henk (1993) considered the disposal of retentates into an anaerobic sludge digester of a municipal wastewater plant and demonstrated the principle applicability of anaerobic digestion for the treatment of retentates from dairy alkaline CIP cleaning solution recovery. However, on-site treatment options had not been investigated. The results of this study contribute towards filling this gap by providing experimental results from tests of the anaerobic biodegradability of retentates and additionally a characterisation of the process kinetics was made. To consider on-site treatment options, not only retentates had been tested. The experimental focus was extended to mixtures of retentates with acid whey. Also alkaline effluents from CIP cleaning without pre-treatment and its mixtures with acid whey had been tested. The analysis of process kinetic characteristics was used for the design of an optimized anaerobic co-digestion process to treat these wastes at high loading rates in a balanced, long term stable process.

A major advantage of anaerobic digestion is the production of biogas, suitable for the generation of power and heat. But also the investment and operational costs are significant lower in anaerobic processes compared to aerobic treatment of wastewater Speece (1996). Thus, anaerobic digestion is an option for economic feasible treatment of liquid wastes, particular streams from cleaning in place operation and surplus by-products from cheese production *e.g.* whey are applicable due to their good anaerobic biodegradability and also due to their high strength in terms of COD. Even though anaerobic digestion of whey was investigated by several researchers, only little work aimed particular in the treatment of acid whey. Additionally, process upsets and imbalances of anaerobic digestion processes of both, sweet whey and, to a greater extend, acid whey had been reported as reviewed in Section 4.3. Balanced anaerobic digestion processes of non-pre-acidified acid whey beyond organic loading rates higher than 9 $g \cdot L^{-1} \cdot d^{-1}$ have not been reported Also in this study, the operation at organic loading rates of 10 $g \cdot L^{-1} \cdot d^{-1}$ in standard one-stage UASB and AMR digestion resulted in process imbalances and finally led to process failure. A major task on the way to a high rate, well balanced anaerobic digestion of acid whey was to identify the causes of process-upsets. In several experiments with

one-stage systems, capable for high rate anaerobic digestion *i.e.* the membrane coupled digester (AMR) and the UASB reactor, rapid acidification of the whey let to insufficient bicarbonate alkalinity and results in process failures. A strategy to balance the process is to add chemical buffers *i.e.* as described by Fox *et al.* (1992). However, adding chemicals is cost intensive and the cations from added salts are burdening the wastewater. To avoid these disadvantages the idea was developed to increase the alkalinity by co-digestion of the acid whey with spent alkaline effluents from cleaning in place processes to provide extra alkalinity and to implement pH control.

In further experiments the co-digestion with automatic dosage of alkaline CIP cleaning solutions using a pH control loop was tested. However, even with stable, neutral pH conditions the processes in UASB and also in the AMR failed when high loading rates had been applied. Local organic overloads in the UASB process have been identified to be responsible for alteration of the granules, resulting in wash-out of biomass. An opaque jelly-like layer surrounding the stressed granules was observed. The mechanism of the development of this layer was discussed in Section 9.5.5. The anaerobic membrane reactor (AMR) system provides perfect biomass retention. However, also this system failed after a longer period of operation. In both, the AMR and also in the UASB process a change of the sludge colour indicated an accumulation of milk ingredients. A 70 % reduction of phosphate was measured indicating a precipitation of phosphates. The high calcium content of acid whey and a comparison of the solubility of salts possibly formed let to the conclusion that the precipitates must be calcium phosphates. The accumulation within the reactor reduced the effective working volume and let to process failures due to organic overloads.

For the design of the optimized process further action was necessary to enhance the alkalinity. Substrates like acid whey tend to acidify rapidly because the lactose is already solved and can directly consume by acidogens. These bacteria grow very fast and compete for space and nutrients with acetogenic and methanogenic microorganisms. Additionally, acidogenic bacteria are less sensitive to low pH values. A two-stage design is intended to relieve the methanogenic stage from extensive grow of acidogens by retaining them in the first stage. Thus, an acidification stage, pH controlled using the alkaline CIP cleaning solutions, was tested. Taking into account the anaerobic process kinetics of acid whey digestion, obtained in the biodegradability tests, a hydraulic retention time of 24 h was adjusted. The design of the methanogenic stage followed the recent development of anaerobic granular sludge processes. Internal circulation type digesters are state-of-the-art high rate digestion systems. However the treatment of whey using this type of digesters was not yet reported in the literature. Other two-stage studies found in the literature

used cheese whey as feeding substrate which is easier to digest. To demonstrate the principle of internal circulation in laboratory scale, the system was equipped with a gas loop used to drive a gaslift. This enhanced the mixing of the lower compartment of the methanogenic stage and by this, local organic overloads were avoided.

Also in the two-stage system precipitation of calcium phosphates occurred. However, accumulation of precipitates was observed solely within the acidification stage. The granules remained black over the whole experimental period. This is a major advantage. The biomass within the acidogenic stage is suspended in the completely mixed fermenter. Due to the fast growing rates of acidogenic bacteria, retention of the biomass is usually not necessary. Thus, precipitates can easily be separated from the bulk liquid by settlement and bottom discharge. The pH level in the acidogenic stage was adjusted to pH 6, however heavy precipitation occurred near the inlet of the alkaline CIP cleaning solution which was a strong base with pH 13 to pH 14. It can be concluded that the precipitates are mainly calcium phosphate $Ca_3(PO_4)_2$ and calcium hydrogen phosphate $CaHPO_4$. The theoretical maximum mass of precipitates from acid whey, containing equal amounts of calcium phosphate and calcium hydrogen phosphate, can be calculated using stoichiometry. Assuming acid whey with 1.25 $g \cdot L^{-1}$ calcium and 2.5 $g \cdot L^{-1}$ total phosphate, a yield of 3.73 $g \cdot L^{-1}$ from acid whey can be estimated. In the two-stage experiment approximately 250 g of precipitates have been achieved from a total volume of 84 L of acid whey. Based on the approximated maximum yield this is 80 % removal efficiency. However, higher yields can be expected from an optimized process.

Calcium phosphate is a highly valuable product and thus, extra profit can be obtained from precipitates, yielded from the novel acid whey treatment process. Besides, a well balanced, high rate anaerobic digestion process was developed and operated. The results are comparable to the performance of well designed, two-stage digesters reported in the literature treating cheese whey which is easier to digest.

10.3. A Large Scale Scenario

An approach for implementation of the novel anaerobic acid whey digestion into industrial scale based on a calculation for the treatment of a volume of 100 m^3 of acid whey (70 $g \cdot L^{-1}$ COD) per day. The design consider a co-digestion with 20 $m^3 \cdot d^{-1}$ of alkaline CIP effluents (5 $g \cdot L^{-1}$ COD). The overall organic loading rate of the design is 12 $g \cdot L^{-1} \cdot d^{-1}$, and thus, a combined acidification/crystallisation stage with 120 m^3 working volume (1 d HRT) and a methanogenic stage with 470 m^3 working volume (3.9 d HRT) can be calculated. The dimensions of the methanogenic stage are approximately 5.5 m in diameter with a height of 28 m including insulation and the degassing chamber at the top of the reactor.

Assuming specific construction costs of 800 €·m^3, result in a price of the plant of 472 000 €. Considering 6 % interest and 2 % repayment, capital costs of 100 €·d^{-1} were estimated.

Table 19 Calculation of the net benefit from anaerobic digestion of acid whey in a large scale installation.

Description	Value
Volume of whey [m^3·d^{-1}]	100
Cost of plant [€]	472 000
Benefit from methane production [€·d^{-1}]	750
Benefit from calcium phosphate [€·d^{-1}]	150
Wastewater fees [€·d^{-1}]	200
Labour [€·d^{-1}]	90
Operational costs [€·d^{-1}]	40
Capital costs [€·d^{-1}]	100
Net benefit [€·d^{-1}]	470

For the calculation of benefits and costs 95 % COD removal efficiency, a gas yield per COD removed of 0.5 m^3·kg^{-1}, and a methane content of 60 % (v/v) was assumed, based on the results of this study. A monetary value of the methane of 0.03 €·kW^{-1}·h which is a typical industrial customers price for natural gas and an energy conversation factor of 9.97 kWh·m^{-3} of methane was used for calculation of the benefit from methane production. With a methane production of 2 025 m^3·d^{-1} from the anaerobic digestion process, the revenue is 750 €·d^{-1}. Additionally a mass of 300 kg·d^{-1} of calcium phosphate can be achieved when 80 % of the calcium will be yielded as calcium phosphates. The benefit from calcium phosphate depends on its purity. For the calculation, a benefit of 0.5 €·kg^{-1} was estimated.

Operational costs regarding pumping, mixing, and heating were considered with 5 % of the benefit from methane production. Maintenance and monitoring of the plant was estimated 2 h·d^{-1} of labour. Additional costs arise from an extra volume of wastewater from the anaerobic digestion *i.e.* by disposal into the sewer and treatment in a municipal wastewater plant. Presuming wastewater fees of 2.00 €·m^3, the additional volume of 100 m^3·d^{-1} from the digestion of acid whey cause costs of 200 €·d^{-1}.

The resulting net benefit from anaerobic digestion of 100 $m^3 \cdot d^{-1}$ acid whey is 470 €·d^{-1} or 171 550 € per annum. The calculation is summarised in Table 19. However the scenario presented here based on many estimations and assumptions. Additionally, energy prices and wastewater fees vary widely and thus a detailed calculation is necessary before starting a large scale project. But also the process itself needs further development and optimisation and not at least an intermediate step *i.e.* the operation of a pilot plant, is recommended before scale-up the process to the final industrial scale.

10.4. Recommendations for future work

Further Experiments should be performed to verify the long term stability of the novel anaerobic digestion process. It can be assumed that the maximum performance was not reached during the experiment presented here. A further increase of the organic loading rate after a prolonged acclimatisation period to the substrates should be tested. Also the ability of the system needs to be verified, to treat retentates from alkaline CIP cleaning solutions recovery.

Future work should also aim in the optimisation of the novel combined acidification and crystallisation stage. A suitable geometric design to allow settlement of precipitates and a device for disposal of the precipitates should be designed for operation over longer periods. Also the chemistry of the precipitation process needs to be investigated further in depth. The composition of the precipitates should be analysed. The pH within the fermentation broth may play an important role on the yield and composition of the precipitates and also on the yield of organic acids. A possible reduction of the pH setpoint will reduce the added volumes of alkaline and thus enable the dairy to treat more of the whey since the available volume of alkaline CIP cleaning solutions is the limiting factor of the current process set-up.

Future work in the field of recycling and reuse of spent cleaning solutions should aim in economics and life cycle assessment (LCA). The feasibility of different membranes for specific wastewater characteristics and particularly examination of membrane lifetime in CIP recovery implementations could help to overcome uncertainty about pay back times. Beside economical considerations, a detailed assessment of the environmental impact of CIP cleaning solution recovery and the comparison with alternative waste reduction strategies is needed. It should be proven that membrane processes for CIP recovery are a step towards more sustainability in dairy cleaning operations.

11. APPENDIX

This appendix is a reference list to electronic content. Most of the content is provided as Adobe Portable Document Format (pdf) files. Additionally there are some Microsoft Excel sheets containing calculations and raw experimental data. Larger datasets are in the form of Microsoft-Access Databases. The Appendix is sorted in folders and the content is directly accessible by hyperlinks. To use the hyperlinks in this document, it is a precondition that the appendix folder is stored in the same directory as this document file.

The folders of the appendix are organized as follows:

Folder "Appendix/ReportsAndRawData":

This folder contains the reports of analytical results and plots of online data from the laboratory experiments. The reports are generated by using the experimental data management software "BiogasDB" based on a Microsoft Access Database system created by the author. See also Section 5.6.

The files are named and titled as follows:

- AMR_V001Report.pdf
  AMR_V001.mdb Digestion of acid whey. Report and database
- AMR_V002Report.pdf
  AMR_V002.mdb Anaerobic digestion of acid whey in Fed Batch Mode. Report and database
- AMR_V003Report.pdf
  AMR_V003.mdb Digestion of acid whey with immersed foam particles. Report and database
- AMR_V004Report.pdf
  AMR_V004.mdb Set-up of the Fermenter with Connected Micro Filtration Module. Report and database
- AMR_V005Report.pdf
  AMR_V005.mdb Digestion of Acid- Whey in an anaerobic membrane reactor. Report and database
- AMR_V006Report.pdf
  AMR_V006.mdb Digestion of Whey: Re-Start. Report and database

- AMR_V007Report.pdf
  AMR_V007.mdb Fermentation of Acid Whey in an Anaerobic Membrane Reactor (AMR). Report and database
- AMR_V008Report.pdf
  AMR_V008.mdb Test of Fermentation Equipment. Report and database
- AMR_V009Report.pdf
  AMR_V009.mdb whey fermentation. Report and database
- AMR_V010Report.pdf
  AMR_V010.mdb Production of synthetic wastewater. Report and database
- AMR_V011Report.pdf
  AMR_V011.mdb acid whey digestion without and with sodium hydroxide CIP fluid addition. Report and database
- AMR_V012Report.pdf
  AMR_V012.mdb Nanofiltration of a synth. Alkaline CIP solution. Report and database
- AMR_V013Report.pdf
  AMR_V013.mdb Anaerobic Biodegradability of acid whey. Report and database
- AMR_V014Report.pdf
  AMR_V014.mdb Evaluation of the ultimate anaerobic biodegradability of acid whey in digested sludge. Report and database
- UASB_IC_V001Report.pdf
  UASBV001.mdb Equipment Test (no experimental data). Report and database
- UASB_IC_V002Report.pdf
  UASBV002.mdb Anaerobic digestion of whey in an UASB reactor with internal circulation. Report and database
- UASB_IC_V003Report.pdf
  UASBV003.mdb UASB reactor treating acid whey without internal circulation. Report and database

- UASB_IC_V004Report.pdf
  UASBV004.mdb UASB Reactor treating acid whey and used alkaline cleaning solution. Report and database
- UASB_IC_V005Report.pdf
  UASBV005.mdb First Start-Up of an Anaerobic Gaslift-Circulation Reactor with pre-acidification (no experimental data). Report and database
- UASB_IC_V006Report.pdf
  UASBV006.mdb Two-stage acid whey digestion in an anaerobic gaslift reactor. Report and database
- UASB_IC_V007Report.pdf
  UASBV007.mdb Anaerobic co-digestion of acid whey and CIP alkaline in a two-stage high-rate digester. Report and database

Folder "Appendix/PersonalCommunication":

This folder contains minutes of personal communication:

- MOH-02c.pdf Minute of a meeting of the AUBIOS research group with the Humana Milchunion dairy management and operating staff held on 12th March 2002 in Georgsmarienhütte, Germany
- MOH-02d.pdf Minute of a meeting of AUBIOS research group members with Mr. Gorzky, manager of Weißenfels/Frischli, Hannover Ahlem, Germany, 07th March 2002
- MOH-02e.pdf Minute of a telephone conversation with Mrs. Meier, responsible for whey marketing and disposal at Danone GmbH, Germany, 25th April 2002
- MOH-04e.pdf Copy of an e-mail communication with Mr. Schawe, technical staff of the Humana Milchunion dairy, Germany, 8th October 2004
- MOH-06f.pdf Copy of an e-mail communication with Mr. Kohlhage, manager of the Molkerei Hüttenthal, 20th March 2006
- Verbatim of an Interview during the Ahlemer Fachtagung conference, Hannover, Germany, 30th - 31st May 2005.
- Verbatim of an Interview with Christoph Martens, MT-Energie GmbH &Co. KG, Rockstedt Germany, 12th March 2008.

Folder "Appendix/Misc":

This folder contains other relevant information *e.g.* Microsoft Excel sheets containing raw data and calculations.

- BiodegradabilityPressurePlots.pdf
  BiodegradabilityExcelCalculation.pdf
  BiodegradabilityExcelCalculations.xls
  The Calculation of the Biodegradability Experiments presented in Section 8.1 is reported in these files. The files include also the calculation for the nanofiltration of the synthetic CIP wastewater, see Section 7.2.
- ProcessKineticStudiesExcelCalculation.xls
- CalculationWheyDigestionLargeScale.xls

Folder "Appendix/Publications":

This folder contains publications, oral presentation sheets and reports published during the project.

- Biosolids2006Conference_ORALPRESENTATION.pdf
  Mohr, J.-C., Stiller, W., Dinsdale, R. and Guwy, A. (2006). T*he Assessment of the Anaerobic Biodegradability of Filtration Residues from the Recovery of Alkaline Dairy Cleaning-In-Place Solutions by Nanofiltration.* Oral Presentation, 11th European Biosolids and Organic Recources Conference Exibition and Workshop, 13th - 15th November 2006, Wakefield, UK
- BremerColloquium2006Conference_ORALPRESENTATION.pdf
  BremerColloquium2006Conference_PROCEEDINGPAPER.pdf
  Mohr, J.-C. and Stiller, W. (2006). *Recycling von Molkerei- Reinigungslaugen und Verwertbarkeit der Filtrationsretentate in Biogasanlagen.* Oral Presentation and Proceedings of 11th Colloquium Produktionsintegrierte Wasser-/Abwassertechnik, 13.- 14. September 2006, Bremen ,Germany
- AhlemerSeminar052005Conference_ORALPRESENTATION.pdf
  Mohr, J.-C. and Stiller, W. (2005). *Biogaserzeugung aus Anfallprodukten.* Oral Presentation, Ahlemer Seminar für Führungskräfte und Fachberater in der Milchwirtschaft, 30.-31. Mai 2005, Hannover, Germany
- AhlemerSeminar052003Conference_ORALPRESENTATION.pdf
  Mohr, J.-C. and Stiller, W. (2003). *Verwertung von Sauermolke und Ausschubmedien.* Ahlemer Fachtagung, Mai 2003, Hannover, Germany

- PharmaAndFood092006_PAPER.pdf
  Mohr, J.-C. and Stiller, W. (2006). *Da Steckt Energie Drin. Behandlung flüssiger Reststoffe aus der Frischkäseproduktion.* Pharma+Food, 9(2006) pp. 68-70

- AUBIOSAbschlussveranstaltung2005_ORALPRESENTATION.pdf
  AUBIOSAbschlussbericht2005_REPORT.pdf
  Schumann, R., Rößler, J., Hoyer, M., Hülsen, U., Wüst, E., Ramin, J. von, Stiller, W., Mohr, J.-C., Gottschlich, M., Stannek, W. and Horn, C. (2005). *Angewandter Forschungsschwerpunkt AUBIOS. Automatisierung umwelt- und bioverfahrenstechnischer Prozesse und Systeme. Abschlussbericht 2005.* University of Applied Sciences and Arts Hanover, Research unit AUBIOS, Germany

- VDIVortrag102007_ORALPRESENTATION.pdf
  Mohr, J.-C. (2007). Anaerobtechnik: Von der Abwasserreinigung zum Biogasboom. VDI series of lectures, oral presentation, Hannover, 24. October 2007.

- AUBIOSColloquium042005_ORALPRESENTATION.pdf
  Mohr, J.-C. (2005). Optimierte Fahrweise eines UASB Biogasreaktors zur Abwasserverwertung am Quarkprozess. University of Applied Sciences and Arts Hanover, Forschungsschwerpunkt AUBIOS, Lecture series AUBIOS-Kolloquium

- AUBIOSColloquium122004_ORALPRESENTATION.pdf
  Mohr, J.-C. (2004). Biogaserzeugung aus Sauermolke im Anaerob-Membranreaktor (AMR). University of Applied Sciences and Arts Hanover, Research unit AUBIOS, Lecture series AUBIOS-Kolloquium

- AUBIOSColloquium062004_ORALPRESENTATION.pdf
  Mohr, J.-C. (2004). Membrantrennverfahren für den Biomasserückhalt bei der Biogaserzeugung sowie für das Recycling von Reinigungsmitteln. University of Applied Sciences and Arts Hanover, Research unit AUBIOS, Lecture series AUBIOS-Kolloquium

- AUBIOSColloquium102003_ORALPRESENTATION.pdf
  Mohr, J.-C. (2003). Messungen und Analysen von flüssigen und gasförmigen Proben bei der anaeroben Molkevergärung. University of Applied Sciences and Arts Hanover, Research unit AUBIOS, Lecture series AUBIOS-Kolloquium

- AUBIOSColloquium042003_ORALPRESENTATION.pdf
  Mohr, J.-C. and Sabatin, M. (2003). Datenbankbasierte Versuchsauswertung mit Messdaten aus unterschiedlichen Quellen. University of Applied Sciences and Arts Hanover, Research unit AUBIOS, Lecture series AUBIOS-Kolloquium

- AUBIOSColloquium102002_ORALPRESENTATION.pdf
  Mohr, J.-C. (2002). Biogaserzeugung als Verwertungsmöglichkeit für Sauermolke aus der Produktion von Frischkäse. University of Applied Sciences and Arts Hanover, Research unit AUBIOS, Lecture series AUBIOS-Kolloquium
- UoGTransferReport112004_REPORT.pdf
  Mohr, J.-C. (2004). Optimised Utilization of Quarg Production Residuals, Transfer Report. University of Glamorgan, Wales, not published
- FHHDiplomarbeitMohr2001_DIPLOMATHESIS.pdf
  Mohr, J.-C. (2001). *Projektierung und Umsetzung einer Laborversuchsanlage zum Membrantrennverfahren Mikrofiltration und zur Untersuchung von Suspensionen und Filtermedien.* Diploma Thesis. University of Applied Sciences and Arts Hanover, Faculty of Mechanical Engineering

12. ACKNOWLEDGEMENTS

This Ph.D. thesis was conducted at the research project AUBIOS carried out at the Environmental Process Laboratory at the University of Applied Sciences and Arts Hanover in Germany in co-operation with the Wastewater Treatment Laboratory of the Sustainable Environment Research Centre at the University of Glamorgan. I recognize that this research would not have been possible without the foundation of the research project AUBIOS by the Volkswagen Foundation/ Ministry for Science and Culture of Lower Saxony, Germany, and additionally the financial assistance of the Anglo-German Academic Research Collaboration Programme ARC by the British Council and the German Academic Exchange Service (DAAD). I would like to express my gratitude to those agencies.

I also like to extend my thanks to my supervisors, Prof. Alan J. Guwy, Prof. Richard M. Dinsdale, and Prof. Dennis Hawkes at the University of Glamorgan, and also to Prof. Dr.-Ing. Wilfried Stiller at the University of Applied Sciences and Arts Hanover.

I must also acknowledge the members of the AUBIOS team and my colleagues at the University of Applied Sciences and Arts Hanover. A very special thanks goes out to Prof. Dr.-Ing. Reimar Schumann, the Director of the AUBIOS Project for his help and guidance.

I would also like to thank my family for the support they provided me through my entire life and in particular, I must acknowledge my parents for confidence and support, and especially my wife and best friend, Regina, without whose love, endurance and patience, I would not have finished this thesis.

13. REFERENCES

13.1. Standards

APHA 2320 (1999) *Standard Methods for the Examination of Water and Wastewater: 2320 Alkalinity(1).* American Public Health Association, American Water Works Association, Water Environment Federation.

APHA 5220 B (1997). *Standard Methods for the Examination of Water and Wastewater: 5220 B Chemical Oxygen Demand (COD). Open Reflux Method.* American Public Health Association, American Water Works Association, Water Environment Federation

BS EN ISO 11734 (1999) *Water quality — Evaluation of the "ultimate" anaerobic biodegradability of organic compounds in digested sludge — Method by measurement of the biogas production.* British Standards Institution.

DIN EN 1899-1 (ISO 5815, modifiziert) (1989) *German Standard Methods for the Examination of Water, Wastewater and Sludge. Determination of biochemical oxygen demand after n days (BODn) — Part 1: Dilution and seeding method with allylthiourea addition (H 51).* Normenausschuß Wasserwesen (NAW) im DIN, Deutsches Institut für Normung e.V., DEV-48. Lieferung 2000.

DIN 38404-5 (1984) *German standard methods for examination of water, waste water and sludge; physical and physico-chemical characteristics (group C); determination of pH value (C5).* Normenausschuß Wasserwesen (NAW) im DIN, Deutsches Institut für Normung e.V., DEV-48. Lieferung 2000.

DIN 38409-1 (1997) *German standard methods for the examination of water, waste water and sludge; parameters characterizing effects and substances (group H); determination of total dry residue, filtrate dry residue and residue on ignition (H 1).* Normenausschuß Wasserwesen (NAW) im DIN, Deutsches Institut für Normung e.V., DEV-48. Lieferung 2000.

DIN 38409-7 (2005) *German standard methods for the examination of water, waste water and sludge - Parameters characterizing effects and substances (group H) - Part 7: Determination of acid and base-neutralizing capacities (H 7).* Normenausschuß Wasserwesen (NAW) im DIN, Deutsches Institut für Normung e.V., DEV-48. Lieferung 2000.

DIN 38414-9 (1986) *German standard methods for the examination of water, waste water and sludge; sludge and sediments (group S); determination of the chemical oxygen demand (COD) (S 9).* Normenausschuß Wasserwesen (NAW) im DIN, Deutsches Institut für Normung e.V., DEV-48. Lieferung 2000.

DIN 53019-1 (1980) *Viscometry; Determination of viscosities and flow curves using standard design rotary viscometers with a standard geometry measuring system.* Deutsches Institut für Normung e.V..

EN ISO 9963-1 (1995) *Wasserbeschaffenheit. Bestimmung der Alkalinität. Teil 1: Bestimmung der gesamten und der zusammengesetzten Alkalinität.* European Comitee for Standardization, DEV–35. Lieferung 1996, Dezember 1995 C23.

VDI-Richtlinie 4630 (2006). *Vergärung organischer Stoffe - Substratcharakterisierung, Probenahme, Stoffdatenerhebung, Gärversuche.* Verein Deutscher Ingenieure, VDI-Gesellschaft Energietechnik, Fachausschuss Regenerative Energien, Beuth Verlag, Berlin

13.2. Bibliographic References

Ahn, J-H.; Forster, C. F. (2002) *The effect of temperature variations on the performance of mesophilic and thermophilic anaerobic filters treating a simulated papermill wastewater.* Process Biochemistry, 37(6) pp. 589–594.

Ahn, Y.-H. (2006) *Sustainable nitrogen elimination biotechnologies: A review.* Process Biochemistry, 41(8) pp. 1709–1721.

Ahring, B. K.; Alatriste-Mondragon, F.; Westermann, P.; Mah, R. A. (1991) *Effects of cations on Methanosarcina thermophila TM-l growing on moderate concentrations of acetate: Production of single cells.* Applied Microbiology and Biotechnology, 35(5) pp. 686–689.

Alexiou, I. E.; Anderson, G. K.; Evison, L. M. (1994) *Design of pre acidification reactors for the anaerobic treatment of industrial wastewaters.* Water Science and Technology, 29(9) pp. 199–204.

Alting, A. C. (2003) *Cold gelation of globular proteins.* PhD-Thesis, Wageningen University, The Netherlands.

Animal Health Online (2002) *EHS: Tod ohne Zeugen. Plötzliche Todesfälle bei Mastschweinen und Sauen.* Animal Health Online, [cited 21.12.2003 17:18:00], Available from Internet: http://www.animal-health-online.de.

ATV Abwassertechnische Vereinigung e. V. (1994) *Abwasser bei der Milchverarbeitung. Regelwerk Abwasser-Abfall, Merkblatt M708.* Gesellschaft zur Förderung der Abwassertechnik e. V. (GFA).

Barbut, S.; Foegeding, E. A. (1993) *Ca2+-Induced Gelation of Pre-heated Whey Protein Isolate.* Journal of Food Science, 58(4) pp. 867–871.

Barker, H. A. (1936) *On the biochemistry of the methane fermentation.* Archives of Microbiology, 7(1-5) pp. 404–419.

Bashir, B.; Matin, A. (2004) *Effect of Calcium and Potassium on Sodium Inhibition to Methanogens in Anaerobic Treatment Processes.* Electronic Journal of Environmental, Agricultural and Food Chemistry, 3(6).

Batstone, D.; Keller, J.; Angelidaki, I.; Kalyuzhnyi, S.; Pavlostathis, S.; Rozzi, A.; Sanders, W.; H.; Vavilin, V. (2002) *Anaerobic Digestion Model No.1 (ADM1).* Scientific and Technical Report No. 13, IWA Task Group for Mathematical Modelling of Anaerobic Digestion Processes, IWA Publishing, London.

Batstone, D. J.; Picioreanu, C.; van Loosdrecht, M. C. M. (2006) *Multidimensional modelling to investigate interspecies hydrogen transfer in anaerobic biofilms.* Water Research, 40(16) pp. 3099–3108.

Beeftink, H.; Staugaard, P. (1986) *Structure and Dynamics of Anaerobic Bacterial Aggregates in a Gas-Lift Reactor.* Applied and Environmental Microbiology, 52(5) pp. 1139–1146.

Bhave, R.; Jung, G.; Sondhi, R. (2001) *Potential savings through ceramic membrane caustic reclaim.* Food Processing, 1/2001 pp. 49–52.

Bilstad, T. (1997) *Membrane operations.* Water Science and Technology, 36(2-3) pp. 17-24.

Bischofsberger, W.; Dichtl, N.; Rosenwinkel, K.-H.; Seyfried. C. F.; Böhnke, B. (2005) *Anaerobtechnik.* 2nd ed., Springer: Berlin Heidelberg New York.

Blanpain-Avet, P.; Migdal, J.; Bénézech, T. (2004) *The Effect of Multiple Fouling And Cleaning Cycles on a Tubular Ceramic Microfiltration Membrane Fouled With a Whey Protein Concentrate. Membrane performance and cleaning efficiency.* Food and Bioproducts Processing, 82(C3) pp. 231–243.

Boe, K.; Batstone, D.; Angelidaki, I. (2005) *Online headspace chromatographic method for measuring VFA in biogas reactor.* Water Science and Technology, 52(1-2) pp. 473-478.

Boening, P. H.; Larsen, V. I. (1982) *Anaerobic fluidized bed whey treatment.* Biotechnology and Bioengineering, 24(11) pp. 2539–2556.

Böhnke, B., Bischofsberger, W. and Seyfried, C.F. (1993). *Anaerobtechnik. Handbuch der anaeroben Behandlung von Abwasser und Schlamm*. Springer- Verlag, Berlin

Brockmann, M.; Seyfried, C. F. (1996) *Sludge activity and cross-flow microfiltration - a non-beneficial relationship.* Water Science and Technology, 34(9) pp. 205–213.

Bryant, M. P.; Wolin, E. A.; Wohn, M. J.; Wolfe, R. S. (1967) *Methanobacillus omelianskii, a Symbiotic Association of Two Species of Bacteria.* Archives of Microbiology, 59(1-3) pp. 20–31.

Bryant, M. P., Tzeng, S. F., Robinson, I. M. and Joyner, A. E. (1971). *Nutrient requirements of methanogenic bacteria.* Anaerobic Biological Treatment Processes, F.G. Pohland (ed.). Advances in Chemistry Series, 105, pp. 23–40

Bundesrepublik Deutschland (1991) *Erklärung zur Reduzierung der Gewässerbelastung durch EDTA.* Gemeinsames Ministerialblatt, 42(29) p. 750.

Bundesrepublik Deutschland (2009) *Verordnung über Anforderungen an das Einleiten von Abwasser in Gewässer (Abwasserverordnung - AbwV).* in der Fassung der Bekanntmachung vom 17. Juni 2004 (BGBl. I S. 1108, 2625), die zuletzt durch Artikel 20 des Gesetzes vom 31. Juli 2009 (BGBl. I S. 2585) geändert worden ist.

Bundesrepublik Deutschland (2010a) *Verordnung über Milcherzeugnisse (Milcherzeugnisverordnung - MilchErzV).* vom 15. Juli 1970 (BGBl. I S. 1150), die zuletzt durch Artikel 2 der Verordnung vom 17. Dezember 2010 (BGBl. I S. 2132) geändert worden ist..

Bundesrepublik Deutschland (2010b) *Gesetz für den Vorrang Erneuerbarer Energien (Erneuerbare-Energien-Gesetz - EEG).* vom 25. Oktober 2008 (BGBl. I S. 2074), das zuletzt durch Artikel 1 des Gesetzes vom 31. Juli 2010 (BGBl. I S. 1061) geändert worden ist.

Buswell, A. M.; Neave, S. L. (1930) *Laboratory Studies of Sludge Digestion.* Illinois Division of the State Water Survey, Bulletin No. 30.

Bylund, G. (1995) *Dairy processing handbook.* Tetra Pak Processing Systems AB: Lund, Sweden.

Camichel, R.; Wehrle, F. (2007) *New "Clean Energy St. Moritz/Engadine" – Project (II): Europe's highest dairy turns waste into power.* Bulletin of the Kur- und Verkehrsverein St. Moritz, Available from Internet: http://www.cipra.org/competition/kurvereinstmoritz.

Cameron, D. (1896) *Some Recent Experiments on Sewage Treatment at Exeter.* Engineering, 61 pp. 256–257.

Chawla, S. P.; Venugopal, V.; Nair, P. M. (1996) *Gelation of Proteins from Washed Muscle of Threadfin Bream (nemipterus japonicus) under Mild Acidic Conditions.* Journal of Food Science, 61(2) pp. 362–367.

Chen, W.; Han, S.; Sung, S. (2003) *Sodium Inhibition of Thermophilic Methanogens.* Journal of Environmental Engineering, 129(6) pp. 506–512.

Choo, K.-H.; Lee, C.-H. (1998) *Hydrodynamic Behavior of Anaerobic Biosolids during Crossflow Filtration in the Membrane Anaerobic Bioreactor.* Water Research, 32(11) pp. 3387–3397.

Chynoweth, D. P.; Bosch, G.; Earle, J.; Legrand, R.; Liu, K. (1991) *A Novel Process for Anaerobic Composting of Municipal Solid Waste.* Applied Biochemistry and Biotechnology, 28-29(1) pp. 421–432.

Clark J. N (1988) *Anaerobic digestion of whey in pilot-scale upflow anaerobic sludge blanket digester.* Proceedings of the 5th International Symposium on Anaerobic Digestion, Bologna, Italy, 22–26 May 1988, pp. 489–493.

Coldewey, I.; Hannemann, H.; Bertsch, R. (2003) *Richtlinien für Wasser und Abwasser in Molkereien.* Verband der Deutschen Milchwirtschaft e.V., Köllen-Verlag.

Coldewey, I. (2004) *Umweltverträglichkeit und Nachhaltigkeit in der Milchwirtschaft: Einsatz der online Messtechnik zur Verringerung belasteter Abwasserteilströme bei der Milchverarbeitung.* PhD-Thesis, Technische Universität Dresden, Germany.

Córdoba, P. R.; Francese, A. P.; Siñeriz, F. (1995) *Improved Performance of a Hybrid Design Over an Anaerobic Filter for the Treatment of Dairy Industry Wastewater at Laboratory Scale.* Journal of Fermentation and Bioengineering, 79(3) pp. 270–272.

Cruwys, J. A.; Dinsdale, R. M.; Hawkes, F. R.; Hawkes, D. L. (2002) *Develoment of static headspace gas chromatographic procedure for the routine analysis of volatile fatty acids in wastewaters.* Journal of Chromatography A, 945(1-2) pp. 195–209.

Demirel, B.; Yenigun, O. (2004) *Acid reactor performance assessment in two-phase anaerobic treatment of dairy wastewater.* Proceedings of the 10th World Congress on Anaerobic Digestion, Montreal, Canada, 29 Aug–2 Sept 2004, Vol. 4 pp. 2493-2496.

Deublein, D.; Steinhauser, A. (2008) *Biogas from waste and renewable resources: an introduction.* Wiley-VCH: Weinheim, Germany.

DiLallo, R.; Albertson, O. E. (1961) *Volatile Acids by Direct Titration.* Journal WPCF, Water Pollution Control Federation, 33(4) pp. 356–365.

Dinsdale, R. M.; Hawkes, F. R.; Hawkes D. L. (2000) *Anaerobic Digestion of Short Chain Organic Acids in an Expanded Granular Sludge Bed Reactor.* Water Research, 34(9) pp. 2433–2438.

Dolfing, J.; Griffioen, A.; van Neerven, A. R. W.; Zevenhuizen, L. P. T. M. (1985) *Chemical and bacteriological composition of granular methanogenic sludge.* Canadian Journal of Microbiology, 31(8) pp. 744–750.

Dresch, M., Daufin, G. and Chaufer, B. (1999). Membrane processes for the recovery of dairy cleaning-in-place solutions. Lait, 79(2) pp. 245 – 259

Dresch, M.; Daufin, G.; Chaufer, B. (2001) *Integrated membrane regeneration process for dairy cleaning-in-place.* Separation and Purification Technology, 22-23 pp. 181-191.

Driessen, W.; Yspeert, P. (1999) *Anaerobic treatment of low, medium and high strength effluent in the agro-industry.* Water Science and Technology, 40(8) pp. 221–228.

Eder, B.; Schulz, H. (2006) *Biogas Praxis: Grundlagen, Planung, Anlagenbau, Beispiele, Wirtschaftlichkeit.* 3rd ed., ökobuch Verlag: Staufen/Freiburg, Germany.

Eide, M. H.; Homleid, J. P.; Mattsson (2003) *Life cycle assessment (LCA) of cleaning-in-place processes in dairies.* Lebensmittel-Wissenschaft und-Technologie, 36 pp. 303–314.

Elmaleh, S.; Abdelmoumni, L. (1997) *Cross-flow filtration of an anaerobic methanogenic suspension.* Journal of Membrane Science, 131(1-2) pp. 261–274.

El-Mamouni, R.; Guiot, S. R.; Mercier, P.; Safi, B.; Samson, R. (1995) *Liming impact on granules activity of the multiplate anaerobic reactor (MPAR) treating whey permeate.* Bioprocess Engineering, 12(1-2) pp. 47–53.

Ergüder, T.; Tezel, U.; Güven, E.; Demirer, G. (2001) *Anaerobic Biotransformation and Methane Generation Potential of Cheese Whey in Batch and UASB Reactors.* Waste Management, 21(7) pp. 643–650.

Eskicioglu, C.; Terzian, N.; Kennedy, K. J.; Droste, R. L.; Hamoda, M. (2007) *Athermal microwave effects for enhancing digestibility of waste activated sludge.* Water Research, 41(11) pp. 2457–2466.

Fair, G. M.; Moore, E. W. (1937) *Observations on the Digestion of a Sewage Sludge over a Wide Range of Temperatures.* Sewage Works Journal, 9(1) pp. 3–5.

Fang, H. H. P.; Chui, H. K. (1994) *Comparison of startup performance of four anaerobic reactors for the treatment of high-strength wastewater.* Resources, Conservation and Recycling, 11(1-4) pp. 123–138.

Fang, H. H. P.; Yu, H. Q. (2000) *Effect of HRT on Mesophilic Acidogenesis of Dairy Wastewater.* Journal of Environmental Engineering, 126(12) pp. 1145–1148.

Farizoglu, B.; Keskinler, B.; Yildiz, E.; Nuhoglu, A. (2004) *Cheese whey treatment performance of an aerobic jet loop membrane bioreactor.* Process Biochemistry, 39(12) pp. 2283–2291.

Feijoo, G.; Soto, M.; Méndez, R.; Lema, J. (1995) *Sodium inhibition in the anaerobic digestion process: Antagonism and adaptation phenomena.* Enzyme and Microbial Technology, 17(2) pp. 180–188.

Fischer, J. R.; Brooker, D. B.; Anderson, M. E.; Ruiz, E. L.; Marshall, R. T. (1974) *In-Line Monitoring of the Milk Content of a Detergent Solution by Electrical Conductivity.* Journal of Dairy Science, 57(9) pp. 998–1002.

Flemming, H.-C.; Wingender, J. (2001) *Relevance of microbial extracellular polymeric substances (EPSs) - Part I: Structural and ecological aspects.* Water Science and Technology, 43(6) pp. 1–8.

Forster, C. E.; Quarmby, J. (1995) *The physical characteristics of anaerobic granular sludges in relation to their internal architecture.* Antonie van Leeuwenhoek, 67(1) pp. 103–110.

Fox, E. J.; Clanton, C. J.; Goodrich, P. R.; Backus, B. D.; Morris, H. A. (1992) *Liming an Anaerobic Cheese Whey Digester.* Transactions of the ASAE, 35(1) pp. 269–274.

Fuchs, W.; Binder, H.; Mavrias, G.; Braun, R. (2003) *Anaerobic treatment of wastewater with high organic content using a stirred tank reactor coupled with a membrane filtration unit.* Water Research, 37(4) pp. 902–908.

Fukuzaki, S.; Chang, Y.-J.; Nishio, N.; Nagai, S. (1991) *Characteristics of granular methanogenic sludge grown on lactate in a UASB reactor.* Journal of Fermentation and Bioengineering, 72(6) pp. 465–472.

Garcilaso, I. (2006) *Reference Document on Best Available Techniques in the Food, Drink and Milk Industries.* European Commission, European Integrated Polution Prevention and Control Bureau.

Gayon, U.; Pasteur, M. (1884) *Recherches sur la fermentation du fumier.* Comptes-rendus de l'Acad. des Sciences Paris, 98 pp. 528.

Gekeler, W. (1999) *EDTA in der Milchindustrie.* Deutsche Molkerei Zeitung, 12/1999 pp. 510–514.

Gerardi, M. H. (2003) *The Microbiology of Anaerobic Digesters.* Wastewater Microbiology Series, John Wiley & Sons, Inc., Hoboken, New Jersey, USA.

Gèsan-Guiziou, G.; Boyavalb, E.; Daufin, G. (2002) *Nanofiltration for the recovery of caustic cleaning-in-place solutions: robustness towards large variations of composition.* Desalination, 149(1-3) pp. 127–129.

Gesan-Guiziou, G.; Alvarez, N.; Jacob, D.; Daufin, G. (2006) *Cleaning-in-place coupled with membrane regeneration for re-using caustic soda solutions.* Separation and Purification Technology, 54(3) pp. 329–339.

Ghaly, A. E.; Echiegu, E. A. (1993) *Kinetic of a continous flow no-mix anaerobic reactor.* Energy Sources, Part A, 15(3) pp. 433–449.

Ghaly, A.; Ramkunmar, D.; Sadaks, S.; Rochon, J. (2000) *Effect of reseeding and pH control on the performance of a two- stage mesophilic anaerobic digester operating on acid cheese whey.* Canadian Agricultural Engineering, 42(4) pp. 173–183.

Guinée, J. B.; Heijungs, R.; van Oers, L.; van de Meent, D.; Vermeire, T.; Rikken, M. (1996) *LCA impact assessment of toxic releases. Generic modelling of fate, exposure and effect for ecosystems and human beings with data for about 100 chemicals.* Centre of Environmental Science, Leiden University (CML), Leiden, The Netherlands.

Guiot, S. R.; Safi, B.; Frigon, J. C.; Mercier, P.; Mulligan, C.; Tremblay, R.; Samson, R. (1995) *Performances of a Full-Scale Novel Multiplate Anaerobic Reactor Treating Cheese Whey Effluent.* Biotechnology and Bioengineering, 45(5) pp. 398–405.

Guwy, A. J.; Hawkes, F. R.; Wilcox, S. J.; Hawkes, D. L. (1997) *Neural network and on-off control of bicarbonate alkalinity in a fluidised-bed anaerobic digester.* Water Research, 31(8) pp. 2019–2025.

Guwy, A. J. (2004) *Equipment used for testing anaerobic biodegradability and activity.* Reviews in Environmental Science and Biotechnology, 3(2) pp. 131–139.

Habets, L. H. A.; Engelaar, A. J. H. H.; Groeneveld, N. (1997) *Anaerobic treatment of inuline effluent in an internal circulation reactor.* Water Science and Technology, 35(10) pp. 189–197.

Harada, H.; Momonoi, K.; Yamazaki, S.; Takizawa, S. (1994) *Application of Anaerobic-UF Membrane Reactor for Treatment of a Wastewater Containing High Strength Particulate Organics.* Water Science and Technology, 30(12) pp. 307–319.

Harper, S. R.; Pohland, F. G. (1986) *Recent developments in hydrogen management during anaerobic biological wastewater treatment.* Biotechnology and Bioengineering, 28(4) pp. 585–602.

Hawkes, F. R.; Rozzi, A. G.; Black, K.; Guwy, A. J.; Hawkes, D. L. (1992) *Stability of anaerobic digesters operating on a food-processing wastewater.* Water Science and Technology, 25(7) pp. 73–82.

Hawkes, F. R.; Guwy, A. J.; Hawkes, D. L.; Rozzi, A. G. (1994) *On-Line Monitoring of Anaerobic Digestion: Application of a Device for Continous Measurement of Bicarbonate Alkalinity.* Water Science and Technology, 30(12) pp.1–10.

Heath, C. R.; Callow, M. E.; Leadbeater, B. S. C. (1992) *Deposition of calcium carbonate within algal biofilms on antifouling paints in hard waters.* In: Biofilms - Science and Technology. L. Melo, T.R. Bott, M. Fletcher and B. Capdeville (eds.), NATO ASI Ser. 223, Kluwer Acad. Publ., Dordrecht, pp. 551–556.

Henk, M. (1993) *Recycling of Caustic Cleaning Solutions using Cross-flow Filtration in the Dairy Industry.* PhD-Thesis, Federal Technical University, Zuerich, Switzerland.

Henze, M.; Harremoes, P. (1983) *Anaerobic treatment of wastewater in fixed film reactors: a literature review.* Water Science and Technology, 15(8-9) pp. 1.

Hoppe-Seyler, F. (1886) *Über Gährung der Cellulose mit Bildung von Methan und Kohlensäure.* Zeitschrift für Physiologische Chemie, 10 pp. 201–217.

Hu, W. C.; Thayanithy, K.; Forster, C. F. (2002) *A kinetic study of the anaerobic digestion of ice-cream wastewater.* Process Biochemistry, 37(9) pp. 965–971.

Hufemia, A. M. M. (1996) *Caustic Soda Recovery in a Bottle Washing Plant Using Membrane Technology.* Master-Thesis, Asian Institute of Technology, School of Environment, Resources and Development Bangkok, Thailand.

Hussy, I.; Hawkes, F. R.; Dinsdale, R.; Hawkes, D. L.; Premier, G. C.; Guwy, A. J. (2004) *Fermentative hydrogen production from energy crops and food processing co-products.* Proceedings of the 10th World Congress on Anaerobic Digestion, Montreal, Canada, 29 Aug–2 Sept 2004, Vol. 2 pp. 862–868.

Hwang, S. H.; Hansen, C. L. (1990) *Use of upflow anaerobic sludge blanket (UASB) reactor to treat whey permeate.* Proceedings of the 6th International Symposium on Agricultural and Food Processing Wastes, Chicago, USA, 17–18 Dec 1990, pp. 124-130.

Imhoff, K. (1926) *Fortschritte der Abwasserreinigung.* Carl Heymanns Verlag Berlin.

Ince, O.; Ince, B. K.; Donnelly, T. (2000) *Attachment, strength and performance of porous media in an upflow anaerobic filter treating dairy wastewater.* Water Science and Technology, 41(4-5) pp. 261–270.

Jarvis, A.; Nordberg, A.; Jarlsvik, T.; Mathisen, B.; Svensson, B. (1997) *Improvement of a grass-clover silage-fed biogas process by the addition of cobalt.* Biomass and Bioenergy, 12(6) pp. 453–460.

Jenkins, S. R.; Morgan, J. M.; Sawyer, C. L. (1983) *Measuring anaerobic sludge digestion and growth by a simple alkalimetric titration.* Journal of the Water Pollution Control Federation, 55 pp. 448–453.

Johannsen, U.; Strijkstra, G.; Klarmann, D.; Janthur, I. (2000) *Untersuchungen zum Enterohämorrhagischen Syndrom (EHS) der Schweine.* Praktischer Tierarzt, 81(5) pp. 440–451.

Ju, Z. Y.; Kilara, A. (1998) *Textural Properties of Cold-set Gels Induced from Heat-denatured Whey Protein Isolates.* Journal of Food Science, 63(2) pp. 288–292.

Kalyuzhnyi, S. V.; Perez Martinez, E.; Rodriguez Martinez, J. (1997) *Anaerobic Treatment of High-Strength Cheese-Whey Wastewaters in Laboratory and Pilot UASB-Reactors.* Bioresource Technology, 60(1) pp. 59–65.

Kapp, H. (1984) *Schlammfaulung mit hohem Feststoffgehalt.* Stuttgarter Berichte zur Siedlungswasserwirtschaft, 86. Verlag Oldenbourg, München, Germany.

Kar, T. and Misra, A. K. (1999). *Therapeutic properties of whey used as fermented drink.* Revista de Microbiologia, 30(2) pp. 163-169

Kato, M. T.; Field, J. A.; Versteeg, P.; Lettinga, G (1994) *Feasibility of Expanded Granular Sludge Bed Reactors for the anaerobic treatment of low strength soluble wastewaters.* Biotechnology and Bioengineering, 44(4) pp. 469–479.

Kelly, C. R.; Switzenbaum, M. S. (1984) *Anaerobic treatment: Temperature and nutrient effects.* Agricultural Wastes, 10(2) pp. 135–154.

Kennard, M.; Janekeh, M. (1991) *Two- and Three-phase Mixing in a Concentric Draft Tube Gas-lift Fermentor.* Biotechnology and Bioengineering, 38(11) pp. 1261–1270.

Kessler, H.-G. (1996) *Lebensmittel- und Bioverfahrenstechnik Molkereitechnologie.* Verlag A. Kessler: Muenchen, Germany.

Kinsella, J. E.; Whitehead, D. M. (1989) *Proteins in Whey: Chemical, Physical, and Functional Properties.* Advances in Food and Nutrition Research, 33 pp. 343–438.

Kisaalita, W. S.; Pinder, K. L.; Lo, K. V. (1987) *Acidogenic fermentation of lactose.* Biotechnology and Bioengineering, 30(1) pp. 88–95.

Kisaalita, W. S.; Lo, K. V.; Pinder, K. L. (1989) *Influence of dilution rate on the acidogenic phase products distribution during two-phase lactose anaerobiosis.* Biotechnology and Bioengineering, 34(10) pp. 1235–1250.

Klostermann, S.; Oberdörster, T. J. (1999) *Qualitätsstand photometrischer Betriebsmethoden. Vergleichsuntersuchung Küvetten-Tests und Normverfahren.* GIT Labor-Fachzeitschrift 43/1999 pp. 117–120.

Kohle, O.; Nusbaumer, H. (2003) *Molke-Biogasanlage mit Blockheizkraftwerk für die Molkerei Lataria Engiadinaisa SA, Bever.* Programm Energie und Umwelt im Auftrag des Bundesamts für Energie BFE, Projekt-Nr.: 46'718, Schlussbericht März 2003.

Kugelman, I.; McCarty, P. (1965) *Cation toxicity and stimulation in anaerobic waste treatment.* Journal of the Water Pollution Control Federation, 37 pp. 97–116.

Kugelman, I.; Chin, K. (1971) *Toxicity, synergism, and antagonism in anaerobic waste treatment processes.* Advances in Chemistry Series, 105 pp. 55–90.

Lahav, O.; Loewenthal, R. E. (2000) *Measurement of VFA in anaerobic digestion: The five-point titration method revisited.* Water SA, 26(3) pp. 389–392.

Lahav, O.; Morgan, B. E. (2004) *Titration methodologies for monitoring of anaerobic digestion in developing countries—a review.* Journal of Chemical Technology and Biotechnology, 79(12) pp.1331–1341.

Lehmann, M. (2004) *Verringerung der Gewässerbelastung durch EDTA - Ermittlung der EDTA-Jahresfracht an ausgewählten Messstellen - Bericht 2002/2003.* Proceedings of the Komplexbildner-Fachgespräch, Umweltbundesamt, Berlin, Germany, 18 Nov 2004.

Lettinga, G.; Velsen, A. F. M. v.; Hobma, S. W.; Zeeuw, W. d.; Klapwijk, A. (1980) *Use of the upflow sludge blanket (USB) reactor concept for biological wastewater treatment, especially for anaerobic treatment.* Biotechnology and Bioengineering, 22(4) pp. 699–734.

Lettinga G.; Hulshoff Pol, L. W. (1991) *UASB process design for various types of waste waters.* Water Science and Technology, 24(8) pp. 87–107.

Li, A. Y.; Corrado, J. J. (1985) *Scale-up of the membrane anaerobic reactor system.* Proceedings of the 40th Industrial Waste Conference, Purdue University, West Lafayette, Indiana, USA, 14–16 May 1985, pp. 399–404.

Lindorfer, H.; Waltenberger, R.; Köllner, K.; Braun, R.; Kirchmayr, R. (2008) *New data on temperature optimum and temperature changes in energy crop digesters.* Bioresource Technology, 99(15) pp. 7011–7019.

Liu, Y.; Boone, D. R. (1991) *Effects of salinity on methanogenic decomposition.* Bioresource Technology, 35(3) pp. 271–273.

Mair- Waldburg, H. (1974) *Handbuch der Käse.* Volkswirtschaftlicher Verlag GmbH: Kempten, Germany.

Malaspina, F.; Stante, L.; Cellamare, C. M.; Tilche, A. (1995) *Cheese whey and cheese factory wastewater treatment with a biological anaerobic-aerobic process.* Water Science and Technology, 32(12) pp. 59–72.

Malaspina, F.; Cellamare, C. M.; Sante, L.; Tilche, A. (1996) *Anaerobic Treatment of Cheese Whey with a Downflow-Upflow Hybrid Reactor.* Bioresource Technology, 55(1) pp. 131–139.

Marchaim, U. (1992) *Biogas processes for sustainable development.* Food and Agriculture Organization of the United Nations, Available from Internet: http://www.fao.org/docrep/t0541e/T0541E00.htm.

McCarty, P. L. (1964) *Anaerobic waste treatment fundamentals I - IV.* Public Works, 95 pp.91–94.

McGhee, T. J. (1968) *A Method for Approximation of the Volatile Acid Concentrations in Anaerobic Digesters.* Water & Sewage Works, 115 pp. 162–166.

McMahon, K. D.; Stroot, P. G.; Mackie, R. I.; Raskin, L. (2001) *Anaerobic codigestion of municipal solid waste and biosolids under various mixing conditions—II: microbial population dynamics.* Water Research, 35(7) pp. 1817–1827.

Mohr, J.-C. (2001) *Projektierung und Umsetzung einer Laborversuchsanlage zum Membrantrennverfahren Mikrofiltration und zur Untersuchung von Suspensionen und Filtermedien.* Diploma-Thesis, University of Applied Sciences and Arts Hanover, Faculty of Mechanical Engineering, Available from Internet: http://www.jansnet.com/serioes/index.php.

Mohr, J.-C.; Stiller, W. (2003) *Verwertung von Sauermolke und Ausschubmedien.* Oral presentation, Ahlemer Fachtagung. Hannover, Germany, May 2003.

Mohr, J.-C.; Stiller, W. (2005) *Biogaserzeugung aus Anfallprodukten.* Oral Presentation, Ahlemer Seminar für Führungskräfte und Fachberater in der Milchwirtschaft. Hannover, Germany, 30–31 May 2005.

Mohr, J.-C.; Stiller, W.; Dinsdale, R.; Guwy, A. (2006a) *The Assessment of the Anaerobic Biodegradability of Filtration Residues from the Recovery of Alkaline Dairy Cleaning-In-Place Solutions by Nanofiltration.* Proceedings of the 11th European Biosolids and Organic Recources Conference Exibition and Workshop. Wakefield, UK, 13–15 Nov 2006.

Mohr, J.-C.; Stiller, W. (2006b) *Recycling von Molkerei- Reinigungslaugen und Verwertbarkeit der Filtrationsretentate in Biogasanlagen.* Proceedings of the 11th Colloquium Produktionsintegrierte Wasser-/ Abwassertechnik, Bremen, Germany, 13–14 Sept 2006.

Mohr, J.-C.; Stiller, W. (2006c) *Da Steckt Energie Drin. Behandlung flüssiger Reststoffe aus der Frischkäseproduktion.* Pharma+Food, 9/2006 pp. 68–70.

Mohr, J.-C. (2007) *Anaerobtechnik: Von der Abwasserreinigung zum Biogasboom.* oral presentation, VDI series of lectures. Hannover, Germany, 24 Oct 2007.

Mohr, J.-C.; Stiller, W. (2008) *Untersuchungen zur Feststoff- Fermentation.* forum.new power, 2/2008 pp. 12–15.

Möller, D. (2004) *Historical overview in recognizing the atmosphere: From the antiquity till industrial revolution.* Brandenburg Technical University, Modular Script System: Atmospheric Chemistry and Air Pollution.

Moosbrugger, R. E. (1991) *Determination of Ct and H2CO3* Alkalinity in low alkalinity Solutions using the 5 pH Point Titration method.* PhD-Thesis, University of Cape Town, South Africa.

Moosbrugger, R. E.; Wentzel, M. C.; Ekama, G. A.; Marais, G. v. R. (1993) *A 5 pH Point Titration Method for Determining the Carbonate and SCFA Weak Acid/Bases in Anaerobic Systems.* Water Science and Technology, 28(2) pp. 237–245.

Morgan, J. W.; Forster, C. F.; Evison, L. (1990) *A comparative study of the nature of biopolymers extracted from anaerobic and activated sludges.* Water Research, 24(6) pp. 743–750.

Mudrack, K.; Kunst, S. (1991) *Biologie der Abwasserreinigung.* Gustav Fischer-Verlag: Stuttgart Jena New York.

Mulder, A.; Graaf, A.; Robertson, L.; Kuenen, J. (1995) *Anaerobic ammonium oxidation discovered in a denitrifying fluidized bed reactor.* FEMS Microbiology Ecology, 16(3) pp. 177–184.

Nadais, H.; Capela, I.; Arroja, L.; Duarte, A. (2001) *Kinetic analysis of anaerobic degradation of dairy wastewater.* Proceedings of the 9th World Congress on Anaerobic Digestion, Antwerpen, Belgium, 2–6 Sept 2001, Vol. 1 pp. 203–208.

Norddeutscher Genossenschaftsverband e. V. Kiel (1993). *Richtlinien für Molkereiabwässer.* Norddeutscher Genossenschaftsverband e. V. Kiel (Hersg.); Schmidt & Klauning, Kiel; in Zusammenarbeit mit dem VDM e. V. Bonn; Schriftenreihe Nr. 43

Nordmann, W. (1977) *Die Überwachung der Schlammfaulung. Eine einfache Methode zur Bestimmung der organischen Säuren und der Kalkreserve im Faulwasser.* Korrespondenz Abwasser, Beilage: KA-Informationen fuer das Betriebspersonal von Abwasseranlagen, 3/1977.

Olsen, H. S. (2000) *Cleaning-in-place with a solution containing a protease and a lipase.* United States of America, US Patent 6071356, Available from Internet: http://www.patentstorm.us/patents/6071356-fulltext.html.

Omeliansky, W. (1902) *Ueber die Gärung von Cellulose.* Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten, Zweite Abteilung: Allgemeine, landwirtschaftlich-technologische Bakteriologie, Gährungsphysiologie, Pflanzenpathologie und Pflanzenschutz, 8 pp. 193–201.

Omil, F.; Garrido, J. M.; Arrojo, B.; Méndez, R. (2003) *Anaerobic filter reactor performance for the treatment of complex dairy wastewater at industrial scale.* Water Research, 37(17) pp. 4099–4108.

Omstead, D. R.; Jefferies, T. W.; Naughton, R.; Gregor, H. P. (1980) *Membrane-Controlled Digestion: Anaerobic Production of Methane and Organic Acids.* Proceedings of the 2nd Symposium on Biotechnology in Energy Production and Conservation, Gatlinburg, USA, 3–5 Oct 1979, pp. 247–258.

Ott, M.; Horbelt, A. (2007) *Biogas 2006: Zubau erstmals gleichauf mit Windkraft.* Press release, German Biogas Association, released 01.02.2007.

Otterpohl, R.; Behrendt, J. (2001) *Von der Teilstrombehandlung zur Abwasserfreien Fabrik.* Chapter in: "Von Ökoeffizienz zu nachhaltiger Entwicklung in Unternehmen", von Weizsäcker, E. U.; Stigson, B.; Seiler-Hausmann, J.-D. (eds.), Wuppertaler Institut für Klima, Umwelt, Energie GmbH, pp. 59-75.

Parawira, W.; Murto, M.; Zvauya, R.; Mattiasson, B. (2004) *Anaerobic batch digestion of solid potato waste alone and in combination with sugar beet leaves.* Renewable Energy, 29(11) pp. 1811–1823.

Patel, C.; Sastry, V.; Madamwar, D. (1996) *Tegoprens in anaerobic digestion of a mixture of cheese whey, poultry waste and cattle dung for improved biomethanation.* Applied Biochemistry and Biotechnology, 56(1) pp. 89–94.

Patel, G. B.; Roth, L. A. (1977) *Effect of sodium chloride on growth and methane production of methanogens.* Canadian Journal of Microbiology, 23(7) pp. 893–897.

Peck, M. W.; Skilton, M.; Hawkes, F. R.; Hawkes, D. L. (1986) *Effects of Temperature Shock Treatments on the Stability of Anaerobic Digesters Operated on Seperated Cattle Slurry.* Water Research, 20(4) pp. 453–462.

Pellegrino, J. (2006) *Filtration and Ultrafiltration Equipment and Techniques.* Physical and Chemical Properties Division, National Institute of Standards and Technology Boulder, [cited: 26.12.2006], Available from Internet: http://membranes.nist.gov/ACSchapter/equipment.html.

Perle, M.; Kimchie, S.; Shelef, G. (1995) *Some biochemical aspects of the anaerobic degradation of dairy wastewater.* Water Research, 29(6) pp. 1549–1554.

Pierkiel, A.; Lanting, J. (2004) *Membrane-coupled anaerobic digestion of municipal sewage sludge.* Proceedings of the 10th World Congress on Anaerobic Digestion, Montreal, Canada, 29 Aug–2 Sept 2004, Vol. 2 pp. 738–742.

Pillay, V.; Townsend, B.; Buckley, C. (1994) *Improving the Performance of Anaerobic Digesters at Wastewater Treatment Works: The Coupled Cross- Flow Microfiltration/ Digester Process.* Water Science and Technology, 30(12) pp. 329–337.

Pind, P. F.; Angelidaki, I.; Ahring, B. K.; Stamatelatou, K.; Lyberatos, G. (2003) *Monitoring and Control of Anaerobic Reactors.* Chapter in: "Biomethanation II", Ahring, B. K.; Scheper, T (eds.), Springer: Berlin Heidelberg New York.

Popoff, L. (1875) *Ueber die Sumpfgasgährung.* Pflügers Archiv European Journal of Physiology, 10(1) pp. 114–146.

Räsänen, E.; Nyström, M.; Sahlstein, J.; Tossavainen, O. (2002) *Purification and regeneration of diluted caustic and acidic washing solutions by membrane filtration.* Desalination, 149(1-3) pp. 185–190.

Richards, E.; Wohlfarth, M.; Hellebrand, D.; Wirges, M.; Kapahnke, D. (2005) *ZMP-Marktbilanz Milch 2005, Deutschland - Europäische Union - Weltmarkt.* Verlag ZMP Zentrale Markt- und Preisberichtsstelle GmbH.

Rinzema, A.; van Lier, J.; Lettinga, G. (1988) *Sodium inhibition of acetoclastic methanogens in granular sludge from a UASB reactor.* Enzyme and Microbial Technology, 10(1) pp. 24–32.

Robinson, R. W.; Akin, D. E.; Nordstedt, R. A.; Thomas, M. V.; Aldrich, H. C. (1984) *Light and Electron Microscopic Examinations of Methane-Producing Biofilms from Anaerobic Fixed-Bed Reactors.* Applied and Environmental Microbiology, 48(1) pp. 127–136.

Romney, A. J. D. (1990) *CIP: Cleaning in Place.* Society of Dairy Technology: Cumbria, UK.

Rosen, C.; Vrecko, D.; Gernaey, K.; Pons, M.; Jeppsson, U. (2006b) *Implementing ADM1 for plant-wide benchmark simulations in Matlab/Simulink.* Water Science and Technology, 54(4) pp. 11–19.

Rosenberger, S.; Krüger, U.; Witzig, R.; Manz, W.; Szewzyk, U.; Kraume, M. (2002) *Performance of a bioreactor with submerged membranes for aerobic treatment of municipal waste water.* Water Research, 36(2) pp. 413–420.

Rottiers, A.; Boeije, G.; Corstanje, R.; Decraene, K.; Feijtel, T. C. J.; Matthijs, E.; Schowanek, D. (1999) *Adaptation of the CAS test system and synthetic sewage for biological nutrient removal. Part II: Design and validation of test units.* Chemosphere, 38(4) pp. 711–727.

Saddoud, A.; Hassairi, I.; Sayadi, S. (2007) *Anaerobic membrane reactor with phase separation for the treatment of cheese whey.* Bioresource Technology, 98(11) pp. 2102-2108.

Sallis, P. J.; Uyanik,S. (2003) *Granule development in a split-feed anaerobic baffled reactor.* Bioresource Technology, 89(3) pp. 255–265.

Sander, R. (1999). *Compilation of Henry's Law Constants for Inorganic and Organic Species of Potential Importance in Environmental Chemistry (Version 3)*. Available from Internet: http://www.henrys-law.org

Saw, C. B.; Anderson, G. K.; James, A.; Le, M. S. (1985) *A Membrane Technique for Biomass Retention in Anaerobic Waste Treatment Processes.* Proceedings of the 40th Industrial Waste Conference, Purdue University, West Lafayette, Indiana, USA, 14-16 May 1985, pp. 805–812.

Schink, B. (1997) *Energetics of Syntrophic Cooperation in Methanogenic Degradation.* Microbiology and Molecular Biology Reviews, 61(2) pp. 262–280.

Schmidt, H. J. (1999) *Aspecte der Desinfektion.* Der Doemensianer, Oktober 1999.

Schumann, R.; Rößler, J.; Hoyer, M.; Hülsen, U.; Wüst, E.; von Ramin, J.; Stiller, W.; Mohr, J.-C.; Gottschlich, M., Stannek, W.; Horn, C. (2005) *Angewandter Forschungsschwerpunkt AUBIOS. Automatisierung umwelt- und bioverfahrenstechnischer Prozesse und Systeme. Abschlussbericht 2005.* University of Applied Sciences and Arts Hanover, Research unit AUBIOS, Germany.

Senge, B., Sienkiewicz, T. (2002) *Rheologische Besonderheiten von Frischkäse - Konsequenzen zur Optimierung de Prozesstechnik.* Deutsche Molkerei Zeitung, 14/2002 pp. 26–33.

Shcherbakova, V. A.; Laurinavichius, K. S.; Akimenko, V. K. (1999) *Toxic effect of surfactants and probable products of their biodegradadation on methanogenesis in an anaerobic microbialcommunity.* Chemosphere, 39(11) pp. 1861–1870.

Sienkiewicz, T. (1990) *Whey and Whey Utilization.* Verlag Th. Mann: Gelsenkirchen-Buer, Germany.

Söhngen, N. L. (1906) *Ueber Bakterien, welche Methan als Kohlenstoffnahrung and energiequelle gebrauchen.* Zentralblatt Bakteriologische Parasitenkunde, Abt. II, 15 pp. 513–517.

Speece, R. E. (1996) *Anaerobic Biotechnology for Industrial Wastewaters.* Archae Press: Nashville, Tennessee, USA.

Spreer, E.; Mixa, A. (transl.) (1998) *Milk and Dairy Product Technology.* Marcel Dekker, Inc., New York.

Stafford, D. A.; Hawkes, D. L.; Horton, R. (1980) *Methane Production from waste organic matter.* CRC press: Boca Raton, Florida, USA.

Stutzer, D. (2006) *Abfall zu Geld machen. Abwasserentsorgung in der Getränkeindustrie durch Biogastechnik.* Getränkeindustrie 10/2006 pp. 46–47.

Süßmuth, R.; Doser, C.; Lueders, T. (2001) *Bestimmung der biologischen Abbaubarkeit organischer Stoffe unter anaeroben Bedingungen mit dem Meßsystem OxiTop® Control.* Universität Hohenheim, Applikationsbericht 0600412, Wissenschaftlich-Technische Werkstätten GmbH & Co. KG.

Switzenbaum, M. S.; Danskin, S. C. (1982) *Anaerobic expanded bed treatment of whey.* Agricultural Wastes, 4(6) pp. 411–426.

Thauer, R. K.; Jungermann, K.; Decker, K. (1977) *Energy conservation in chemotrophic anaerobic bacteria.* Bacteriological Reviews, 41(1) pp. 100–180.

Totosaus, A.; Montejano, J. G.; Salazar, J. A.; Guerrero, I. (2002) *A review of physical and chemical protein-gel induction.* International Journal of Food Science & Technology, 37(6) pp. 589–601.

van den Berg, L.; Lentz, C.; Athey, R.; Rooke, E. (1974) *Assessment of methanogenic activity in anaerobic digestion: Apparatus and method.* Biotechnology and Bioengineering, 16(11) pp. 1459–1469.

Van der Bruggen, B.; Lejon, L.; Vandecasteele, C. (2003) *Reuse, Treatment, and Discharge of the Concentrate of Pressure-Driven Membrane Processes.* Environmental Science and Technology, 37(17) pp. 3733–3738.

van der Star, W. R. L.; Abma, W. R.; Blommers, D.; Mulder, J.-W.; Tokutomi, T; Strous, M.; Picioreanu, C.; van Loosdrecht, M. C. M. (2007) *Startup of reactors for anoxic ammonium oxidation: Experiences from the first full-scale anammox reactor in Rotterdam.* Water Research, 41(18) pp. 4149–4163.

van Langerak, E. P. A.; Hamelers, H. V. M.; Lettinga, G. (1997) *Influent calcium removal by crystallization reusing anaerobic effluent alkalinity.* Water Science and Technology, 36(6-7) pp. 341–348.

van Langerak, E. P. A.; Beekmans, M. M. H.; Beun, J. J.; Hamelers, H. V. M.; Lettinga, G. (1999) *Influence of phosphate and iron on the extent of calcium carbonate precipitation during anaerobic digestion.* Journal of Chemical Technology and Biotechnology, 74(11) pp. 1030–1036.

van Langerak, E. P. A.; Ramaekers, H.; Wiechers, J.; Veeken, A. H. M.; Hamelers, H. V. M.; Lettinga, G. (2000) *Impact of Location of CaCO3 Precipitation on the Development of Intact Anaerobic Sludge.* Water Research, 34(2) pp. 437–446.

van Lier, J. B.; Rebac, S.; Lettinga, G. (1997) *High-Rate Anaerobic Wastewater Treatment under Psychrophilic and Thermophilic Conditions.* Water Science and Technology, 35(10) pp. 199–206.

Vandevivere, P.; Baere, L.; Verstraete, W. (2002) *Types of anaerobic digesters for solid wastes.* Chapter in: "Biomethanization of the Organic Fraction of Municipal Solid Wastes", Mata-Alvarez, J. (edt.) pp. 336.

Vavilin, V. A.; Lokshina, L. Ya. (1996) *Modeling of volatile fatty acids degradation kinetics and evaluation of microorganism activity.* Bioresource Technology, 57(1) pp. 69–80.

Vellinga, S. H. J.; Hack, P. J. F. M.; Vellinga, S.; de Vegt, A. (1986) *New type "High Rate" anaerobic reactor.* Proceedings of the NVA-EWPCA Water Treatment Conference, Amsterdam, The Netherlands, 15–19 Sept 1986.

Verstraete, W.; Vandevivere, P. (1999) *New and Broader Applications of Anaerobic Digestion.* Critical Reviews in Environmental Science and Technology, 29(2) pp. 151–173.

Viraraghavan, T.; Kikkeri, S. R. (1990) *Dairy wastewater treatment using anaerobic filters.* Canadian Agricultural Engineering, 33(1) pp. 143–149.

Volcke, E.; van Hulle, S.; Deksissa, T.; Zaher, U.; Vanrolleghem, P. (2005) *Calculation of pH and concentration of equilibrium components during dynamic simulation by means of a charge balance.* Technical report, Ghent University, BIOMATH.

Volta, A. (1778) *Briefe über die natürlich entstehende entzündbare Sumpfluft / von Alexander Volta. Aus dem Italienischen (Lettere sull aria infiammabile nativa delle paludi).* Wintherhur, bey Heinrich Steiner und Compagnie.

Wagner, J. (2001) *Membrane Filtration Handbook: Practical Tips and Hints.* 2nd ed., Revision 2, 2001, Osmonics, Inc.: Minnetonka USA.

Wei, C.-H.; Wang, W.-X.; Deng, Z.-Y.; Wu, C.-F. (2007) *Characteristics of high-sulfate wastewater treatment by two-phase anaerobic digestion process with Jet-loop anaerobic fluidized bed.* Journal of Environmental Sciences, 19(3) pp. 264–270.

WHO (2006). *The Codex Alimentarius, Codex General Standard For Cheese, Codex Standard A-6-1978, Rev.1-1999, Amended 2006.* Food and Agriculture Organization of The United Nations World Health Organization

Wildenhauer, F. X.; Winter, J. (1985) *Anaerobic digestion of high-strength acidic whey in a pH-controlled up-flow fixed film loop reactor.* Applied Microbiology and Biotechnology, 22(5) 367–372.

Wise, D. L. (1980) *Anaerobic fermentation of whey/manure to fuel gas.* Proceedings of the Whey Products Conference, Philadelphia, USA, Oct 1980, pp. 81.

Wohlfarth, M.; Gorn, A.; Hellebrand, D.; Michels, P.; Thielen, M.; Kapahnke, D.; Wirges, M. (2008) *ZMP-Marktbilanz Milch 2008.* Verlag ZMP Zentrale Markt- und Preisberichtstelle GmbH, Bonn, Germany.

Wust, E. (2003) *Single-phase And Two-phase Cheese Wastewater Treatment by Anaerobic SBRs.* PhD-Thesis, Graduate School, Marquette University, Milwaukee, Wisconsin, USA.

Yacubowicz, J. (1995) *AlkaSave process for recovery of caustic and acids in dairies.* Membrane Technology, 1995(67) pp. 7–9.

Yan, J. Q.; Lo, K. V.; Liao, P. H. (1989) *Anaerobic digestion of cheese whey using up-flow anaerobic sludge blancet reactor.* Biological Wastes, 27(4) pp. 289–305.

Yan, J. Q.; Lo, K. V.; Pinder, K. L. (1993) *Instability Caused by High Strength of Cheese Whey in UASB Reactor.* Biotechnology and Bioengineering, 41(7) pp. 700–706.

Yang, K.; Yu, Y.; Hwang, S. (2003) *Selective optimization in thermophilic acidogenesis of cheese-whey wastewater to acetic and butyric acids: partial acidifcation and methanation.* Water Research, 37(10) pp. 2467–2477.

Yang, P.; Zhang, R.; McGarvey, J. A.; Benemann, J. R. (2007) *Biohydrogen production from cheese processing wastewater by anaerobic fermentation using mixed microbial communities.* International Journal of Hydrogen Energy, 32(18) pp. 4761–4771.

Yang, S.-T.; Okos, M. R.; Nye, J. C. (1982) *Kinetics of methane fermentation of whey.* Proceedings of the ASAE Winter Meeting, Chicago, USA, 14–17 Dec 1982, ASAE paper No. 82-3592.

Yang, S.-T.; Guo, M. (1991) *A kinetic Model for Methanogenesis from Whey Permeate in a Packed Bed Immobilized Cell Bioreactor.* Biotechnology and Bioengineering, 37(4) pp. 375–382.

Ylmazer, G.; Yenigün, O. (1999) *Two-phase anaerobic treatment of cheese whey.* Water Science and Technology, 40(1) pp. 289–295.

Yoon, S. H.; Kang, I. J.; Lee, C. H. (1999) *Fouling of inorganic membrane and flux enhancement in membrane-coupled anaerobic bioreactor.* Separation Science and Technology, 34(5) pp. 709–724.

Young, J. C.; McCarty, P. L. (1969) *The anaerobic filter for waste treatment.* Journal of Water Pollution Control Federation, 41(5), Research Supplement to: 41(5) Part II (May, 1969), pp. R160-R173.

Young, J. C.; Dahab, M. F. (1982) *Operational characteristics of anaerobic packed-bed reactors.* Biotechnology and Bioengineering Symposium, 12(4) pp. 303–316; Proceedings of the symposium on biotechnology in energy production and conservation, Gatlinburg, TN, USA, 11 May 1982.

Young, J. C.; Irwin, T. J. (1999) *Treatability Test Assessment.* Industrial Wastewater, 7(1) pp. 37–42.

Young, J. C. (2001) *Impact of cleaning and disinfecting agents on biological treatment processes.* Proceedings of the 7th Annual Industrial Wastes Technical and Regulatory Conference, Charleston, USA, 12–15 Aug 2001.

Yu, H. Q.; Tay, J. H.; Fang, H. H. P. (2001a) *The Roles of Calcium in Sludge Granulation During UASB Reactor Start-up.* Water Research, 35(4) pp. 1052–1060.

Yu, H. Q.; Fang, H. H. P. (2001b) *Acidogenesis of Dairy Wastewater at Various pH Levels.* Proceedings of the 9th World Congress on Anaerobic Digestion, Antwerpen, Belgium, 2–6 Sept 2001, Vol. 1 pp. 519–524.

Yu, J.; Pinder, K. L. (1993) *Intrinsic fermentation kinetics of lactose in acidogenic biofilms.* Biotechnology and Bioengineering, 41(2) pp. 479–488.

Zitomer, D.; Bachmann, T.; Vogel, D. (2004) *Thermophilic anaerobic digester with ultrafilter for solids stabilization.* Proceedings of the 10th World Congress on Anaerobic Digestion, Montreal, Canada, 29 Aug–2 Sept 2004, Vol. 1 pp. 111–116.

Zoutberg, G. R.; de Been, P. (1997) *The Biobed(R) EGSB (expanded granular sludge bed) system covers shortcomings of the upflow anaerobic sludge blanket reactor in the chemical industry.* Water Science and Technology, 35(10) pp. 183–188.