Anaerobic Treatment of Municipal Wastewater in a UASB-Digester System

Temperature effect on system performance, hydrolysis and methanogenesis

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To my wife Xin and son HaoYi

Abstract

Abstract

A novel treatment chain for low strength domestic sewage includes low temperature anaerobic treatment as the main process. It can improve the energy efficiency of sewage treatment compared with conventional aerobic sewage treatment. A combination of an Upflow Anaerobic Sludge Blanket reactor and a sludge digester, a UASB-digester system, was proven to be one of the successful anaerobic systems to challenge temperatures as low as 10°C and organic matter concentrations in the range of 382 and 1054 mg chemical oxygen demand (COD)/l. The UASB is operated at low sewage temperature (10°C) and high loading rate. The produced non-stabilised sludge in the UASB is recirculated over the mesophilic digester (35°C) to convert organic solids to methane gas and produce anaerobic biomass fed back into the UASB reactor, where it converts dissolved COD at the low temperature of the waste water.

The effect of sludge recirculation rate and sludge transfer point on the performance of a UASB-digester treating domestic sewage at 15 ˚C was studied in this research. The results show increased total COD removal efficiency when increasing the sludge recirculation rate from 1% to 2.6% of the influent flow rate. Methane gas production increases with the sludge recirculation rate, in the range of 1 to 12.5% of the influent flow rate. A higher sludge transfer point results in an increased suspended COD removal efficiency and a higher VSS concentration of the UASB sludge bed.

Co-digestion was applied for improving soluble COD removal efficiency of a UASB-digester system, operated at low temperatures and treating domestic sewage with a high dissolved/suspended COD ratio. Glucose was chosen as a model co-substrate and added to the sludge digester to produce additional methanogenic biomass, which was continuously recycled to inoculate the UASB reactor. Methane production in the UASB reactor almost doubles and soluble COD removal efficiency equals the biodegradability of the influent dissolved COD, due to a twofold increase in methanogenic capacity, when applying co-digestion 16% of influent organic loading rate. Therefore, co-digestion is a suitable approach to support a UASB-digester for treatment of low temperature domestic sewage.

A pilot scale UASB-digester (130 + 50 L) was studied to treat domestic wastewater at temperatures of 10-20°C at an HRT of 6 h in the UASB reactor and 15 h in the digester. The results show a stable COD removal efficiency of 60 ± 4.6% during the operation at 12.5 to 20°C. COD removal efficiency decreases to 51.5 ± 5.5% at 10°C. The decreased COD removal efficiency is attributed to an increased influent COD load, leading to insufficient methanogenic capacity of the UASB reactor at such low temperature. Suspended COD removal efficiency was 76.0 ± 9.1% at 10-20°C. The effluent COD concentration is 90 ± 23 mg/L at temperatures between 12.5 and 20°C, while soluble COD removal efficiency fluctuates due to variation in the influent COD concentration. 80% of the influent biodegradable COD is recovered as methane gas (including dissolved methane).

Low temperature (10-25°C) hydrolysis after applying a short pre-hydrolysis at 35°C was studied compared with those without the pre-hydrolysis. Batch experiments were executed using cellulose and tributyrin as model substrates for carbohydrates and lipids. Low temperature anaerobic hydrolysis rate constants increase by a factor 1.5 - 10 after applying a short anaerobic pre-hydrolysis at 35°C. The hydrolytic activity of the supernatant collected from the digestate after batch digestion of cellulose and tributyrin at 35°C was higher than that of the supernatants collected from the low temperature (≤ 25°C) digestates. The observed hydrolysis in the UASB of a UASB-digester system, treating domestic sewage at low temperatures (10-20°C) is in line with the elevated hydrolytic activity of mesophilic supernatant.

Effects of temperature and temperature shocks on specific methanogenic activity (SMA), and acetate affinity of the digester sludge were studied. Digester sludge from a UASB (12.5°C)-digester (35°C) system, was fed with acetate at constant temperatures of 10-35°C and at varying temperatures from 35°C to 25, to 15 to 10°C. The results show no lag phase in methane production rate when applying temperature shocks of 35°C to 25, 15, and 10°C. The temperature dependency of the SMA of the digester sludge after the temperature shocks was similar to the one at constant temperatures. Acetate affinity of the digester sludge was high at the applied temperatures (10-35°C). Latter is consistent with the finding of no VFA in the effluent of the UASB-digester, treating low strength, and low temperature (12.5°C) domestic wastewater.

The UASB-digester system to treat low strength, low temperature domestic sewage was provided with a proof-of-principle, and its essential underlying anaerobic processes were sufficiently elucidated to make the technology ready for further scaling up and demonstration in practice.

Keywords: UASB reactor, municipal wastewater treatment, low temperature, digester, pre-hydrolysis, temperature shocks, water scarcity, affinity, UASB-digester, hydrolysis rate constant, half-saturated constant, co-digestion

1 Introduction

List of Abbreviations for introduction:

ABRs: Anaerobic baffled reactors

AF: anaerobic filter

AH: anaerobic hybrid reactor

AMBRs: anaerobic migrating blanket reactors

ANAMMOX: anaerobic ammonia oxidation

AnMBRs: Anaerobic membrane reactors

BOD: biological oxygen demand

CAPEX: capital expenditures

COD: chemical oxygen demand

COD_t: total COD

COD_{ss}: suspended COD

COD_{sol}: soluble COD

CSTRs: Continuous stirred tank reactors

DAMO: denitrification anaerobic methane oxidation

EGSB: Expanded granular sludge bed

GHG: Greenhouse Gas

HRT: hydraulic retention time

HUSB: hydrolytic upflow sludge bed

K_s: half-saturation velocity constant

OPEX: operational expenditures

SAMBR: submerged anaerobic membrane bioreactor

SRT: sludge retention time

TSS: total suspended solids

UASB: upflow anaerobic sludge blanket

1.1 Introduction

The world will increasingly experience water scarcity due to increasing global population, rising water demand, fast urbanization and climate change. In addition to problems in quantity, the quality of fresh water resources is main issue especially in emerging and developing economies (Zinia & Kroeze, 2015). To avoid water resource pollution, municipal wastewater, as one of the main pollution sources, must be treated before discharged into the receiving surface water. However, in developing countries not all the cities have yet adequate wastewater treatment plants, and are generally in need for low-cost and effective solutions. The world-wide numerously applied activated sludge process can provide good effluent quality but consumes high amounts of energy and is characterized by high operational cost (Verstraete et al., 2009). Anaerobic municipal wastewater treatment can be an alternative to reduce energy consumption and operational cost (Siegrist et al., 2008), but is applicable especially at higher temperature climates in tropical countries. Low temperature is still a challenge for anaerobic wastewater treatment of municipal wastewater because of low hydrolysis rate of the influent organic matter and the low methanogenic activity, converting hydrolyzed material into biogas. Many different kinds of anaerobic reactors have been studied to deal with these problems caused by low temperatures. Among these anaerobic reactors, an upflow anaerobic sludge blanket (UASB) reactor-digester is a promising system as it can provide relatively high chemical oxygen demand (COD) removal efficiencies and energy production in the form of methane. Furthermore, it provides stabilized excess sludge compared with other two phase systems like anaerobic filter (AF) - anaerobic hybrid (AH) reactor (AF-AH) or hydrolytic upflow sludge bed (HUSB) - UASB reactor (HUSB-UASB) (or expanded granular sludge bed (EGSB) instead of UASB). In this chapter, an introduction is given into the background and motivation for conducting the research to develop an energy friendly municipal wastewater treatment plant for moderate climate regions, specifically a UASB-digester system.

1.2 Water scarcity

The scarcity of freshwater is increasing due to rising water demands and a changing climate, which is considered as a major risk for the global economy, food security, sanitation and drinking water availability for the society (Garrote et al., 2016). Countries whose renewable water supply cannot sustain 1700 m^3 of renewable water resources per capita per year are considered as water stressed. This demarking amount of renewable water resources is based on estimates of water requirements in households and agricultural, industrial and energy sectors, and the needs of the environment (Shiklomanov, 2000). When availability is lower than 1000 $m^3p^{-1}y^{-1}$, a country experiences water scarcity and lower than 500 $m^3p^{-1}y^{-1}$, absolute scarcity. Countries, particularly Central and West Asia and North Africa, the arid areas of the world, are already close to, or below the 1000 $m^3p^{-1}y^{-1}$ threshold as shown in Fig.1.1. The results of global water scarcity analyses show that up to two thirds of the world population will

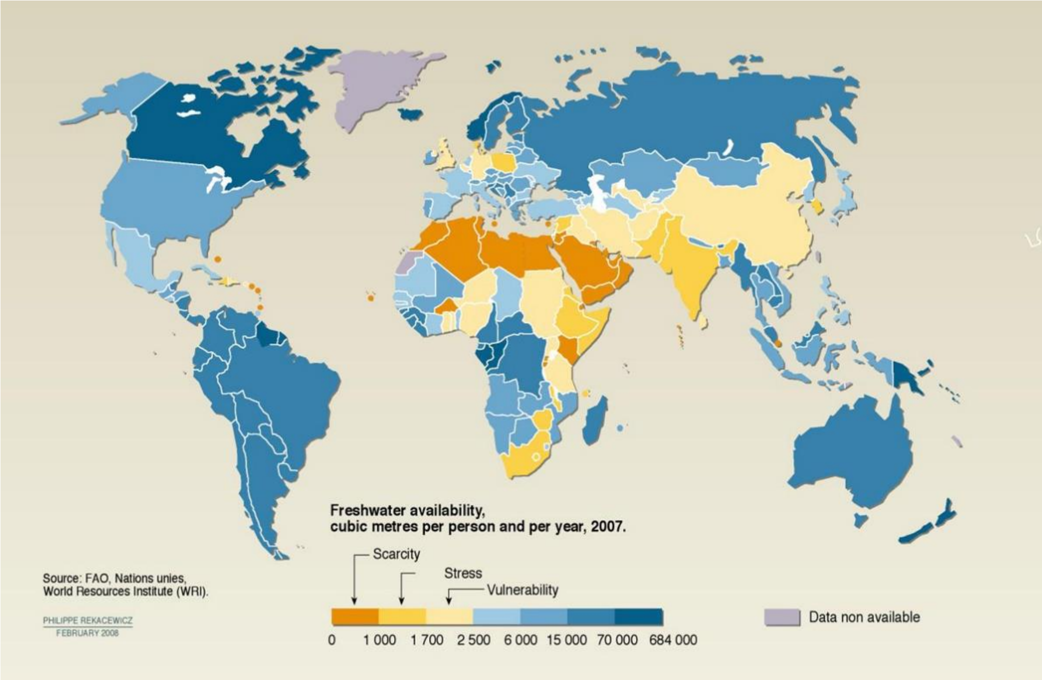

Fig.1.1 Freshwater availability, cubic meters per person and per year, 2007

experience water scarcity over the next decades (Springer & Duchin, 2014).

Wallace (2000) reported that people had less than 1000 $m^3p^{-1}y^{-1}$ in the North-Africa belt (from Morocco to Egypt and including Sudan), and between 1000-2000 $m^3p^{-1}y^{-1}$ in the Middle East and Southern Africa (Rijsberman, 2006). People in Egypt have less than 500-1000 $m^3p^{-1}y^{-1}$ by 2025. Wallace (2000) estimates that the water availability of entire North, East and South Africa, and the Middle East, will drop below 1000 $m^3p^{-1}y^{-1}$ before 2050. West Africa and large parts of South and South-east Asia would range between 1000-2000 $m^3p^{-1}y^{-1}$.

Millions of people are living in water-stressed areas. As an example, farmers near Sana'a in Yemen have deepened their wells by 50 meters over the past 12 years, while the amount of useable water only remains one third of past water extraction yields (Human Development Report 2006). The future of many of the world's water supplies is undoubtedly a story of increasing stress as shown in Fig.1.2. Increased standard of living in developing countries would result in higher per capita water consumption (Ahmed et al., 2014). The world's population is expected to increase to about nine billion by 2050. Most of the three billion additional people will live in the developing or emerging economy countries where water resources are already under stress, including China.

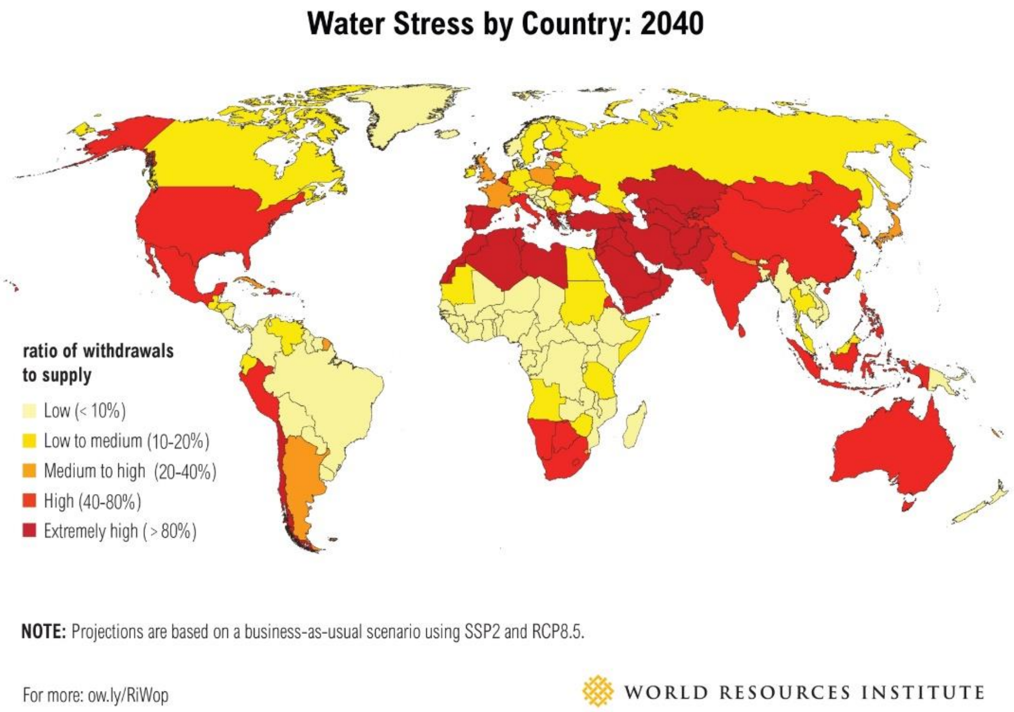

Fig.1.2 Water stress indicator in major basins of the world

China's population is approximately 1.3 billion, which accounts for 20% of the world's total population (Zhang et al., 2016c). Yet China only has 6.5% of the world's total renewable freshwater resources. With its large population, China's water availability is estimated at about 2100 m^3 per capita per year, which is approximately 25% of the world average (Arnell, 2004). China's urban population is more than doubled in less than 25 years and accounted for 43% of the total population in 2005. The large population and rapid urbanization impose heavy pressure on infrastructure development and public services such as drinking water supply and wastewater treatment.

China has been facing increasingly severe water scarcity, particularly in the arid northern part of the country (Zeng et al., 2012). China's water scarcity is characterized by insufficient quantities and poor drinking water quality (Zhou et al., 2014). The problems have negative effects on society and the environment. Rapid economic development, population growth and urbanization trigger a conflict between water supply and demand. Water pollution is a serious problem for water resource protection in China, as well as many other emerging economies and developing countries. Water pollution has extended from point source to non-point source, from fresh water to coastal water, and from surface water to groundwater. Therefore, it is crucial to pay attention to improved wastewater treatment, as an element in mitigating deterioration of water resources quality.

1.3 Conventional municipal wastewater treatment

All over the world, more than one billion people do not have access to safe sanitation and drinking water. 80% of diseases and 30% of deaths are water-related in developing countries as reported in the Human Development Report, United Nations Development Program (De Vries & Lopez, 2013). Industrial and agricultural activities account for a major portion of water pollution, but municipal wastewater, containing urine, feces, kitchen and washing wastes, is the main cause of water related human health problems. Municipal wastewater treatment is therefore a priority to improve human health.

Conventional wastewater treatment consists of the following elements: screening and primary sedimentation followed by an aerobic activated sludge process to remove organic matter and compounds containing inorganic nitrogen and phosphorus. At larger treatment plants, the activated sludge process is often complemented with a sludge digestion reactor where part of the energy in the organic waste material is recovered as biogas. Energy produced in the form of methane in such an anaerobic sludge digester can compensate a quarter to half of the total energy consumption in a conventional wastewater treatment plant (EPA, 2006). The effluent of the wastewater treatment plant is discharged into surface waters when its quality meets local or national standard.

Large fractions of dissolved organic materials are converted to biomass, consuming considerable energy, which still requires further treatment. As a result, the energy consumption of a conventional wastewater treatment plant due to aeration is high, 0.6 kWh per m^3 of wastewater, which accounts for about half of the total energy consumption (McCarty et al., 2011). Electrical energy consumption of wastewater treatment accounts for about 3% of the total electricity load in America, which is similar to other developed countries (EPA, 2006). Due to concerns about climate change, fossil fuel consumption and increasing energy costs, efforts should be made to establish a novel wastewater treatment that is energy efficient and is more sustainable from an energy saving point of view. Therefore, innovations in wastewater treatment have been aimed at reducing costs, saving energy, and lowering the environmental impact.

Municipal wastewater with an COD concentration of 400-500 mg/L contains a potential chemical energy of 1.5-1.9 kWh per m^3 of wastewater (Owen, 1982). If more of the energy potential in wastewater can be recovered and be used for the treatment itself, then a wastewater treatment plant that is a net energy producer rather than a consumer might be achieved. This chapter will provide information to aid in understanding and interpreting anaerobic wastewater treatment, which is much more energy and operational cost friendlier than conventional aerobic wastewater treatment.

1.4 Anaerobic wastewater treatment

1.4.1 Anaerobic conversion steps

During anaerobic conversion of complex substrates such as polysaccharides, proteins and lipids, a complex microbial community consisting of many interacting microbial species is involved. The anaerobic digestion mainly includes 4 steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis (McKeown et al., 2012). Hydrolysis and methanogenesis are considered as rate limiting steps depending on conditions like substrate types, temperature, pH and sludge retention time (SRT) etc.. Therewith, the study focuses only on hydrolysis and methanogenesis.

1.4.1.1 Hydrolysis

Hydrolysis is the first step in the anaerobic treatment of complex wastewater and considered as the rate limiting step (Hendriks & Zeeman, 2008; Lettinga et al., 2001; Pavlostathis & Giraldo-Gomez, 1991b). For instance, the anaerobic hydrolysis rate of cellulose is low due to the insolubility and heterogeneity of cellulose (Hendriks & Zeeman, 2008). Anaerobic hydrolytic bacteria utilize a unique extracellular multi-enzyme complex, called cellulosome for this recalcitrant substrate (Schwarz, 2001). These multi-enzyme complexes make a bridge between the cell envelope and the substrate, which allows the cells to get close to the cellulose. However, many crucial details of cellulose hydrolysis are still unknown.

The hydrolysis of organic solids in anaerobic digestion can be described by first order kinetics (Batstone et al., 2002; Vavilin et al., 1996). Methane will be the main product if hydrolysis is the slowest step compared to acidification, acetogenenesis and methanogenesis (Veeken & Hamelers, 1999). The hydrolysis rate constant can differ due to various experimental conditions such as inoculum source, ratio of biomass and substrate, and available surface of substrate (Sanders et al., 2000; Vavilin et al., 2008).

1.4.1.2 Methanogenesis

Methanogenesis is the last step in anaerobic digestion of organic matter. Acetate is a major product of the fermentation of organic matter and about 70% methanogenesis is through the acetate route under mesophilic conditions, and the rest is through H_2/CO_2 (Aiyuk et al., 2006). Methanogenesis with a high affinity for acetate, is important when treating municipal wastewater with a relatively low COD concentration, at high loading rates. The affinity can be presented by the half-saturation velocity constant (K_s) in the Monod equation (Arnaldos et al., 2015). Varying conditions in K_s quantification experiments are substrate concentration, microbial culture, temperature and experimental set-up (batch or continuous experiment). Generally, the value of K_s of anaerobic sludge increases (i.e. the affinity decreases) when temperature decreases, as shown by Lokshina et al. (2001) and Banik et al. (1998) for treating municipal landfill leachate and synthetic municipal wastewater. K_s and mass transfer limitations may additionally impact methanogenesis and its dependency on temperature (Speece, 2008). A higher K_s and poor mass transfer lead to a higher dependency on temperature.

1.4.2 Effects of low temperature on anaerobic conversion

1.4.2.1 Effects of low temperature on anaerobic hydrolysis

Anaerobic treatment of low temperature municipal wastewater (≤ 15°C) is still a challenge, mainly due to the low hydrolysis rate of organic solids and the related long SRT, and therefore long HRTs (Lettinga et al., 2001). Municipal wastewater has a considerably high COD_{ss} fraction which may account for 50-65% of the COD_t. Non–biodegraded COD_{ss} will accumulate in the sludge bed when the wastewater temperature is low and HRT not long enough. As a result, the SRT, hydrolytic and methanogenic capacity of the sludge will decrease.

The hydrolysis efficiency of COD_{ss} was as low as 12 % during batch digestion for 125 days of cow manure at 5°C (Zeeman, 1991a). When operating a UASB reactor for municipal wastewater treatment at an HRT of 3 h and 17°C, the particulate organic matter was effectively removed by entrapment in the sludge bed, but the hydrolysis efficiency of the entrapped organics was only 0.7 % (Zeeman et al., 1997). Uemura and Harada (2000) showed a drop in the hydrolysis efficiency from 58% at 25°C to 33% at 13°C, when applying a UASB reactor for municipal wastewater treatment at an HRT of 4.7 h. Also the anaerobic treatment of black water in a UASB-septic tank was shown to have a poor performance during the winter period (temperature lower than 14°C); 60% of the influent COD was accumulated as solids in the sludge bed while about 30% was discharged as soluble COD (COD_{sol}) with the effluent (Luostarinen et al., 2007).

1.4.2.2 Effect of fluctuating temperature on anaerobic wastewater treatment

Effects of temperature change on anaerobic processes were investigated in various studies. The difference in biogas production between winter (14-25°C) and summer (24-35°C) in Brazil was studied, when applying a pilot scale tubular continuous anaerobic digester for digestion of cattle manure at an HRT of 60 d (Resende et al., 2015). No difference in average methane yield was found as temperature gradually changed given the long HRT. Biogas production rate under daily down and upward temperature fluctuations was studied when applying anaerobic digestion of cow manure in a continuous stirred tank reactor (CSTR) at 50 and 60°C at an HRT of 20 d (El-Mashad et al., 2004). Biogas production rate at 50°C was higher than at 60°C when a 10°C temperature reduction was applied for 10 h or a 10°C increase for 5 h. Lau and Fang (1997) reported that suddenly applied changes in temperature, from 55 to 37°C, resulted in poor COD removal, granule disintegration and biomass washout when applying a thermophilic granule reactor fed with sucrose and operated at 55°C. Kettunen and Rintala (1997a) reported a 1 d lag-phase when using sludge, collected from a UASB reactor treating leachate at 23°C, for an SMA test at 15°C. Gao et al. (2011) found that a decrease in temperature with 5 and 10°C, starting at 37°C, could be tolerated for a submerged anaerobic membrane bioreactor (SAMBR) operated at an

HRT of 20 h; the same changes, starting at 45°C, led to a significant disturbance of the performance.

1.4.3 Different types of anaerobic reactors

The main anaerobic reactors used for wastewater treatment can be classified as low rate or high rate systems as shown in Fig.1.3. High rate systems are characterized by retention of sludge (SRT>HRT), while most low rate systems have no sludge retention (SRT=HRT)

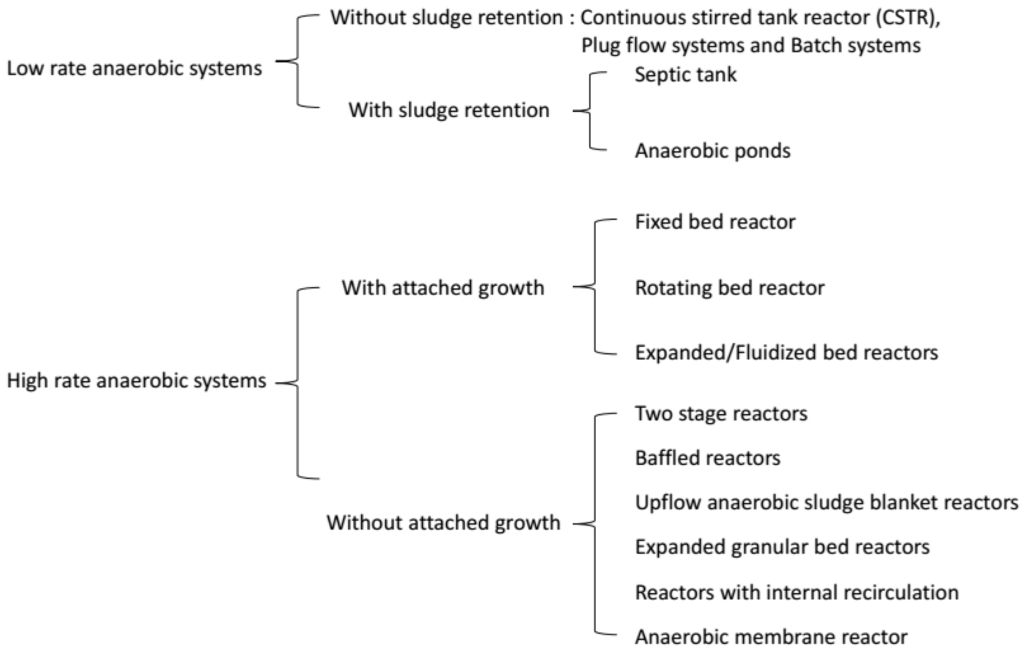

Fig.1.3 Main types of anaerobic reactors used for wastewater treatment (Based on (Sperling & Chernicharo, 2005))

1.4.3.1 Low rate anaerobic systems

<u>Without sludge retention</u>

Anaerobic systems without sludge retention are operated at relatively low volumetric organic loads, long hydraulic retention times (HRTs) and in general fed with highly concentrated waste streams. CSTRs are the most frequently applied systems, and for example used for the stabilization of primary and secondary sludge originating from wastewater treatment plants, for industrial effluents with a high concentration of suspended solids (Li et al., 2011) and animal manure. The applied operational temperature ranges mostly from 25 to 35°C, at HRTs ≥ 20 days, though thermophilic treatment is also more and more applied.

<u>With sludge retention</u>

The septic tank is a unit that has functions of sedimentation and removal of floatable materials. It acts as a low-rate treatment system without mixing and heating possibilities

(Lowe & Siegrist, 2008). Solids are retained in the system as a result of sedimentation. The SRT is therefore much longer than the HRT.

Anaerobic ponds are an alternative for municipal wastewater treatment in warm-climate regions and also often used in the past for wastewater treatment with a high concentration of organic matter (Mara, 1987). Anaerobic ponds can be classified as low volumetric organic loaded reactors due to their large footprint and long HRT. Solids are settled and retained in the system.

1.4.3.2 High rate systems

Anaerobic filters are characterized by the presence of a stationary packing material to which biomass can attach and be maintained within the interstices (Young & McCarty, 1969). The average SRT is above 20 d. A good treatment performance can be achieved because of the longer SRT. The main disadvantage of anaerobic filters is that the accumulation of biomass can lead to blockage or the formation of hydraulic short circuits. Rotating bed anaerobic reactor is also called anaerobic biodisc, in which biomass was attached to submerged discs (Noyola et al., 1988). The SRT is high and blocking should not occur as the rotation of the discs provides shearing forces and remove the excess biomass present between the discs. Expanded bed anaerobic reactors consist of a cylindrical structure, packed with inert support materials like sand, gravel, coal etc. which accounts for about 10% of the total reactor volume (Switzenbaum & Jewell, 1980). In expanded bed anaerobic reactors, the expansion of the bed is maintained between 10-20%; in fluidized bed anaerobic reactors, the expansion varies between 30-100%. The expanded anaerobic reactors have proven to be efficient in treating low strength, pre-treated municipal wastewater at temperatures ≥ 20°C at a short HRT (minimum from 0.5 to 1 h); COD removal efficiencies of 60-70% can be achieved. The fluidized bed anaerobic reactors can achieve a high OLR of 20-30 kg COD/(m^3 d) using soluble wastes and COD removal efficiencies of 70-90% (Garcia-Calderon et al., 1998; Şen & Demirer, 2003). However, van Lier et al. (2015) reported that the fluidized bed anaerobic reactors turned out not to be successful in practice as the biofilm loosened from the support material.

Anaerobic baffled reactors (ABRs) are equipped with vertical baffles that force the liquid to make a sequential downflow and upflow, to enable good contact between the biomass and wastewater (Barber & Stuckey, 1999). OLR of ABRs can reach 36 kg COD/(m^3 d). It can have a smaller depth and be built without a gas separator, which saves construction costs. However, loss of biomass may occur in the case of influent flow variation as the ABRs do not have a gas separator for sludge retention.

An upflow anaerobic sludge bed (UASB) reactor has a gas-solids-liquid separator at the top of the reactor, which separates SRT and HRT (Lettinga, 1995). Biogas produced, provides good mixing of biomass and substrate. The sludge settles after gas separation, which makes the UASB reactor also to work like a clarification tank. The UASB reactor can retain a high concentration of biomass, which is in the form of granules or well-settling flocculent sludge (De Sousa & Foresti, 1996; Torres & Foresti, 2001). The upflow velocity is in such systems in the range of 0.5-2 m/h. A UASB reactor is suitable for treating concentrated and dilute wastewater, with or without suspended solids. Organic loading rates range between 2 and 25 kg COD/m^3/d, depending on type of wastewater and applied temperature (Lier et al., 2008).

An EGSB reactor is a modification of the UASB reactor, which is significantly taller and has a high upflow velocity of 6-15 m/h (Lettinga et al., 1997). Biomass and organic matter can be well mixed due to the high upflow velocity. Slowly settling particulates, present in the influent, do not accumulate in the reactor and are likely washed out with the effluent. Therewith, the EGSB reactor is suitable for low temperature and low strength wastewater, but not suitable for wastewater with a high fraction of low density organic particulates. Internal circulation reactor has a very high upflow velocity, 20-30 m/h (Deng et al., 2006; Pereboom, 1994; Pereboom & Vereijken, 1994). It has 2 three phase separators. One is set in the middle of the reactor, the second set similar to a UASB reactor. Van Lier et al (2015) report the successful full-scale operation of modern EGSB installations, such as the Biobed EGSB and Biopaq IC reactors, applying various wastewaters at loading rates between 25–35 kg COD/(m^3 d).

Anaerobic membrane reactors (AnMBRs) were intensively studied due to their high effluent quality. For AnMBRs of municipal wastewater treatment, the effluent mainly contains macronutrients like nitrogen and phosphorus, while COD, SS and pathogens can be well removed (Liao et al., 2006). Therewith, the effluent can be used in agriculture. Sludge retention provided by the membrane may increase the SMA and biodegradation (Ho & Sung, 2010; Martinez-Sosa et al., 2011). Anaerobic reactors like bench-scale CSTR, UASB, EGSB, UASB-digester coupled with different types of membrane achieved COD removal between 87-92% for municipal wastewater treatment (Chu et al., 2005; Gouveia et al., 2015; Ozgun et al., 2015; Smith et al., 2013).

Most of the studies about AnMBRs are executed in bench scale experiments, and information on cost and energy analysis is limited. The main drawbacks of AnMBRs are the low membrane flux and the related large surface area of the membrane, membrane fouling, high capital and operational costs, which still hinder AnMBRs application (Chernicharo et al., 2015).

1.5 Anaerobic municipal wastewater treatment in tropical areas

The UASB reactor was invented in the 1980s (Lettinga et al. 1980). The first research of a full scale UASB reactor, treating municipal wastewater, was conducted in Colombia (Schellinkhout & Collazos, 1992). Several tropical countries in Latin America and India started to apply anaerobic municipal wastewater treatment technology afterwards. In these countries, climate conditions are favorable for the application of mesophilic anaerobic reactors. In India, full scale UASB reactors have been implemented since 1990 and the UASB reactor is considered as a standard technology for municipal wastewater treatment (Uemura and Harada 2010). As shown

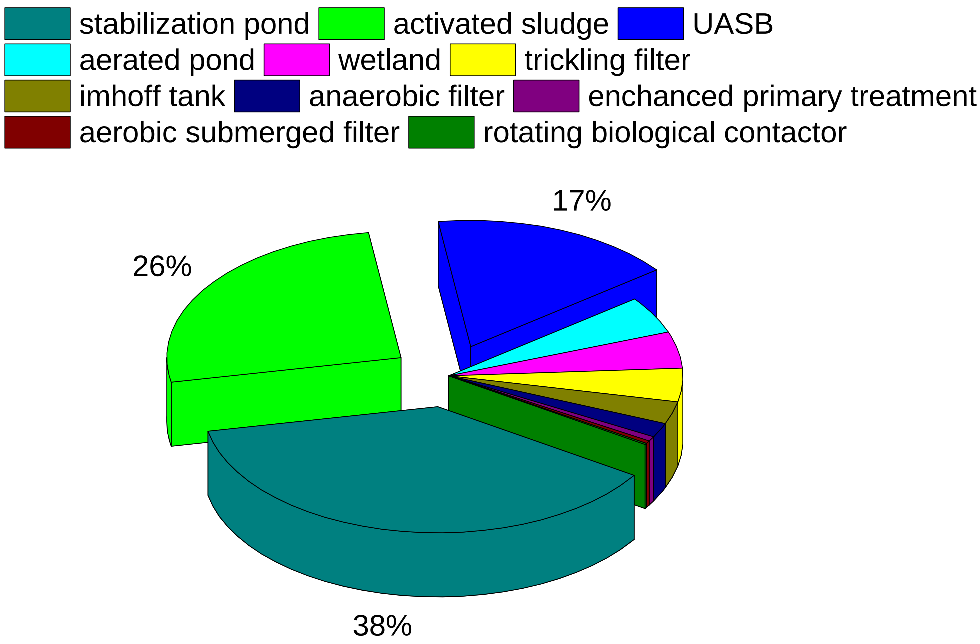

Fig.1.4 Major technologies for municipal wastewater treatment in Latin American region (modified from Noyola et al. (2012))

in Fig.1.4, UASB reactors are the third most applied municipal wastewater treatment technology in Latin American region.

Anaerobic wastewater treatment followed by aerobic post treatment was considered as an alternative to traditional wastewater treatment using an activated sludge process. The costs of a treatment plant with a UASB reactor followed by aerobic biological treatment are usually 20-50% lower for capital expenditures (CAPEX) and 50% for operational expenditures (OPEX) compared with a conventional activated sludge plant (Chernicharo, 2006; Polito Braga et al., 2005). A UASB reactor followed by a stone-filled trickling filter saves 40% CAPEX and 90% OPEX compared with a conventional activated sludge system (Aiyuk et al., 2006). The advantages and disadvantages of anaerobic wastewater treatment are shown in Table 1.1.

The UASB reactor is one of the most frequently applied anaerobic wastewater treatment technologies, being applied in tropical areas (Fang & Chung, 1999; Hulshoff Pol & Lettinga, 1986; Lettinga et al., 1993; Verstraete & Vandevivere, 1999). The performance of full scale UASB reactors applied in Brazil, India, Jordan, Middle East, Colombia and Mexico is shown in Table 1.2. COD, biological oxygen demand (BOD) and total suspended solids (TSS) removal efficiencies varies from 41 to 80%, from 40 to 84% and from 34 to 85% respectively.

1.6 Anaerobic treatment of low temperature wastewater

Temperature is one of the limitations for applying anaerobic municipal wastewater treatment in e.g. the Netherlands. But compared to tropical countries, also reaching discharge limits is a challenge, as they are generally stricter than in tropical countries (in e.g. Brazil there is not (yet) a discharge limit on total nitrogen, but only on ammonium). Removing all BOD in an anaerobic reactor, makes the conventional route for nitrogen removal not possible anymore (unless an external C-source is added); and also phosphate removal is often done via biological phosphorus removal, for which organic carbon is required as well. Ideally nitrogen and phosphorus are recovered after anaerobic treatment, but latter is limited by the low concentrations. Nitrogen can also be removed by autotrophic processes such as anaerobic ammonia oxidation (ANAMMOX) or denitrification anaerobic methane oxidation (DAMO) process (Hendrickx et al., 2012; Kampman et al., 2012). Phosphorus could be removed by iron precipitation (Parsons & Smith, 2008). The present study focuses on anaerobic treatment to recover chemical energy from organic matter in municipal wastewater.

1.6.1 Single anaerobic reactors

Temperature of municipal wastewater in large parts of the world is lower than required for anaerobic treatment, at least when a short HRT is applied. Low temperature anaerobic wastewater treatment has recently been intensively studied, and different types of anaerobic reactors have been investigated, as shown in Table 1.3. Generally, these single anaerobic reactors achieved COD removal efficiencies of 37% to 90% at a temperature range of 10 - 25°C. This is achieved by applying anaerobic reactors such as UASB reactors, EGSB reactors, ABRs, anaerobic migrating blanket reactors (AMBRs) and anaerobic membrane bio-reactors (AnMBRs) as shown in Table 1.3. At low temperature, anaerobic treatment with granular sludge and easily biodegradable substrate, methanogenesis is not a limiting factor (Lettinga et al., 1999; Rebac et al., 1999a; Van Lier et al., 1997).

An UASB reactor was investigated for low-strength municipal wastewater treatment at 6 to 32°C, and an HRT range from 25 to 4 h (Singh & Viraraghavan, 2000). The start-up of the UASB reactor was achieved in 60 d at 20°C. COD and BOD removal efficiencies were from 38 to 90% and 47 to 91% respectively. A lab-scale UASB reactor with a height of 1.65 m was studied for treating municipal wastewater at low temperatures in the city of Peru and COD removal efficiencies were achieved between 37 and 62% (Yaya-Beas et al., 2016).

An EGSB reactor was studied under psychrophilic conditions (10-12°C), which was seeded with mesophilic granular sludge and fed with VFA mixture (Rebac et al., 1995; Rebac et al., 1999c; Van der Last & Lettinga, 1992). COD removal efficiencies can exceed 90% with influent COD concentrations from 500 to 800 mg COD/L at an organic loading rate of 12 g COD/ (l d) at HRT of 2.5 and 1.6 h respectively.

Table 1.1 Comparison of anaerobic wastewater treatment of municipal wastewater to activated sludge system

Advantages	Disadvantages
Low operational costs as no energy required for aeration;	UASB requires post treatment step;
Primary sludge sedimentation tank, activated sludge system, secondary clarification and the sludge digester can be replaced by a UASB reactor;	Anaerobic COD removal efficiency is lower than activated sludge process; Dissolved CH_4 is lost in the effluent (especially at low temperature) Potentially higher CH_4 (Greenhouse Gas, GHG) emission due to dissolved CH_4 in effluent anaerobic step;
Energy can be recovered in terms of methane; Small footprint;	Full scale application was not yet commercially developed at moderate to low temperatures.
The sludge production is low, well stabilized and easily dewatered;	
The valuable nutrients (N and P) are conserved which can be reused for agriculture.	

Table 1.2 Performance of Full scale UASB reactors applied in tropical countries

Country	Removal efficiencies (%)			Literatures
	COD	BOD	TSS	
Brazil	58-79	74-84	49-71	Rosa et al. (2012), Oliveira and von Sperling (2011) and Da Silva et al. (2013)
India	41-45	40-60	34-47	Mungray and Patel (2011) and Walia et al. (2011)
Jordan	58	-	62	Halalsheh et al. (2005)
Middle east	71	70	85	Nada et al. (2011)
Colombia	66	78	69	Peña et al. (2000)
Mexico	70-80	-	-	Monroy et al. (2000)

A pilot scale four-chamber ABR achieved a COD removal efficiency of 43% when treating raw municipal wastewater at 12-23°C and an HRT of 12 h for two years (Hahn & Figueroa, 2015). A three-chamber ABR was studied for the treatment of low-strength synthetic wastewater at an influent COD concentration of 300 to 400 mg/L (Manariotis & Grigoropoulos, 2002). COD removal efficiency was 87 and 91% at 26°C and an HRT of 24 and 12 h, respectively. At 16 °C, COD removal efficiency was similar to that before decreasing the temperature. An eight-chamber ABR was studied for treating a dilute wastewater with a COD concentration of 500 mg/L and a COD removal of > 70% was achieved at 10°C and an HRT of 10 h (Langenhoff & Stuckey, 2000).

A compartmentalized AMBR was studied for the treatment of low-strength soluble wastewater at low-temperature (Angenent et al., 2002; Angenent et al., 2001). AMBR was fed nonfat dry milk substrate as a synthetic wastewater at 15 and 20°C in an operating period of 186 days. The influent COD and BOD_5 concentration were constant at 600 and 285 mg/L, respectively. COD_{sol} removal efficiency was 73% at 15°C at an HRT of 4 h, and COD_t removal efficiency was 59%. Biomass was retained effectively and SRT was always greater than 50 d.

The feasibility of an AnMBR for municipal wastewater treatment was investigated and COD removal efficiency of > 89% was achieved at 15°C and an HRT of 6 h (Ozgun et al., 2015).

A pilot scale AnMBR that consisted of a UASB reactor with an external ultrafiltration membrane treating municipal wastewater at 18°C, was evaluated over three years of stable operation (Gouveia et al., 2015). The AnMBR achieved a COD removal efficiency of 87% at an HRT of 7 h, and the effluent COD and BOD_5 concentrations were 100-120 mg/L and 35-50 mg O_2/L, respectively. Specific methane yield varied from 0.18 to 0.23 Nm^3CH_4/kg $COD_{removed}$.

A bench-scale AnMBR equipped with submerged flat-sheet microfiltration membranes was studied using synthetic and actual municipal wastewater (DWW) at 15°C (Smith et al., 2013). The average COD removal efficiency was 92% and provided a good effluent quality of 36 mg COD /L during the operation with simulated wastewater. Dissolved methane in the effluent accounted for a substantial fraction (40-50%) of the total methane production and the effluent was more than saturated according to Henry's law; part of the methane is present as gas microbubbles in the liquid phase. COD removal efficiency averaged 69% during actual DWW operation. The average effluent COD and BOD_5 were 76 mg/L and 24 mg/L, respectively. A microbial analysis on bacterial and archaeal microbial communities in the AnMBR was performed and the results show that a mesophilic inoculum is suited for psychrophilic AnMBRs treating low strength wastewater.

1.6.2 Combined anaerobic reactors

Combinations of AF-AH, an EGSB with an AF, HUSB - UASB and UASB-digester systems were studied in different researches for low temperature anaerobic wastewater treatment as shown in Table 1.3. Each will be discussed in the following paragraph.

1.6.2.1 AF-AH reactor

A two-step anaerobic filter (AF) + anaerobic hybrid (AH) reactor was studied for treatment of municipal wastewater at 13°C. The AH reactor consisted of a granular sludge bed with vertical sheets of reticulated polyurethane foam (RPF) with knobs. The RPF was used for entrapment of solids. This AF+AH system achieved a COD_{ss} removal efficiency of 81% and COD_t removal efficiency of 71% at HRTs of 4 h (AF) and 8 (AH) h (Elmitwalli et al., 2002a; Elmitwalli et al., 2002b). However, the excess sludge that is produced by entrapment of influent COD_{ss} in these systems still needs further treatment. The AF-AH system can achieve a longer SRT in the AH reactor when treating low temperature municipal wastewater containing considerable COD_{ss}. However, excess sludge still needs stabilization.

1.6.2.2 EGSB - AF

An EGSB-AF reactor seeded with mesophilic sludge was studied for the treatment of a medium-strength 5 g COD/L, synthetic, volatile fatty acid-based wastewater for a long-term operation of 625 days at 15°C (Connaughton et al., 2006). COD removal efficiency of > 80% was achieved, and the results were highlighted by a short start-up period of 21 d, a short HRT of 4.9 h, high OLR of 24.6 kg COD/(m^3 d). The contribution of hydrogenotrophic methanogenesis to methane production was increased compared to acetoclastic methanogenesis. The biomass was still mesophilic but can be characterized as strongly active psychro-tolerent. The EGSB-AF system is suitable for low temperature wastewater mainly containing COD_{sol}, but needs pre-treatment for wastewater having a large fraction of COD_{ss}.

1.6.2.3 HUSB - UASB

A two – stage anaerobic treatment pilot plant HUSB-UASB was studied for treatment of raw municipal wastewater at temperatures from 21 to 14°C (Álvarez et al., 2008). The HRT of the HUSB and UASB were from 5.7 to 2.8 h and 13.9 to 6.5 h respectively. COD_t and BOD removal efficiencies were 49-65% and 50 to 77%, respectively. The hydrolysis efficiency of influent suspended solids was 59.7%. Like the AF-AH system, the HUSB-UASB system is able to achieve good COD_{ss} removal at low temperatures, but the sludge produced in the HUSB is not stabilised.

1.6.2.4 UASB-digester

Mahmoud et al. (2004) investigated a UASB-digester system for low temperature municipal wastewater treatment. This system treats wastewater in a UASB reactor at a short HRT. The UASB sludge is recirculated over a heated digester where the wastewater COD_{ss}, captured in the UASB reactor, is converted to methane. The stabilized digester sludge is returned to the UASB reactor where it continues to capture wastewater organic solids and at the same time supplies methanogenic biomass to the UASB reactor for conversion of the COD_{sol} in the wastewater. The UASB-digester

Table 1.3 Anaerobic treatment of low temperature wastewater (influent COD concentration, temperature, HRT and COD removal efficiencies)

Types	Substrate	Influent COD concentration (mg/L)	Temperature (°C)	HRT (h)	COD removal efficiency (%)	Literatures
UASB	Municipal wastewater	621	12.5	4-14	37-62	Yaya-Beas et al. (2016)
	Municipal wastewater	-	11	6	70-90	Singh and Viraraghavan (2000) and Singh and Viraraghavan (2003)
	Wastewater	312	13-25	4.7	70	Uemura and Harada (2000)
EGSB	Mixture of VFA	500-800	10-12	1.6-2.5	> 90	Rebac et al. (1995) and Rebac et al. (1999c)
	Municipal wastewater	-	13	> 3	> 90	Van der Last and Lettinga (1992)
ABR	Municipal wastewater	760	12-23	12	43	Hahn and Figueroa (2015)
	Synthetic low strength wastewater	300-400	26-16	24-12	87-91	Manariotis and Grigoropoulos (2002)
	A dilute wastewater	500	10	10	70	Langenhoff and Stuckey (2000)
AMBR	Non-fat dry milk	600	15	4	59	Angenent et al. (2001)

AnMBR	Milk powder	530	15	6	> 89	Ozgun et al. (2015)
	Municipal wastewater	580-730	18	7	87	Gouveia et al. (2015)
	Municipal wastewater	259	15	16	69	Smith et al. (2013)
	Non-fat dry milk and soluble starch	500	25	6	94	Ho and Sung (2009)
AF-AH	Municipal wastewater	518	13	4 (AF)-8 (AH)	71	Elmitwalli et al. (2002a) and Elmitwalli et al. (2002b)
EGSB-AF	Mixture of VFA	5000	15	4.8	> 80	Connaughton et al. (2006)
HUSB-UASB	Municipal wastewater	118	14	5.7 (HUSB)-11.6(UASB)	53	Álvarez et al. (2008)
UASB-digester	Municipal wastewater	460	15	6	66	Mahmoud et al. (2004)
	Municipal wastewater	330-360	15	6	52	Álvarez et al. (2004)

system includes a sludge digester which enables a low excess sludge production, but the recirculation of the UASB sludge to the digester consumes energy. So far, only limited studies show the feasibility of the UASB-digester system for municipal wastewater treatment at 15°C. However, the temperature of municipal wastewater in moderate climate zones can be as low as 10°C and therefore, the feasibility of the UASB-digester also needs to be assessed at temperatures below 15°C.

1.7 Scope of this thesis

To achieve that anaerobic treatment of low strength municipal waste water can be applied at low temperatures and not only at higher temperature regimes, a pilot-scale UASB-digester is studied in this thesis. The temperature was subsequently decreased, in steps, to 10°C and removal efficiency for COD_t and of its fractions (suspended, soluble and colloidal), methane production of the UASB reactor and the digester and the COD balance were determined. This study addresses the mechanisms behind the successful operation of such a UASB-digester treating municipal wastewater under moderate climate conditions, using real wastewater from an influent of a WWTP in Bennekom the Netherlands, with moderate temperature and COD composition. The research route is shown in Fig. 1.5. The recirculation rate and sludge recirculation point are important control parameters influencing the performance of the system and become part of this research (Chapter 2). The research is carried out with real municipal wastewater with fluctuating COD concentrations and COD fractions. The effect of these fluctuations on reactor performance is studied and mitigation methods for improving performance of the UASB-digester are developed (Chapters 3 and 4). Fundamental aspects are studied in small-scale batch experiments. First order hydrolysis rates and kinetics of methanogenesis are studied after a sudden change in temperature, as taking place in the UASB-digester when transferring sludge between the UASB and the digester and back (Chapter 5 and 6). Results of this study play an important role in understanding the UASB-digester system treating low temperature municipal wastewater and finding the optimal operational conditions.

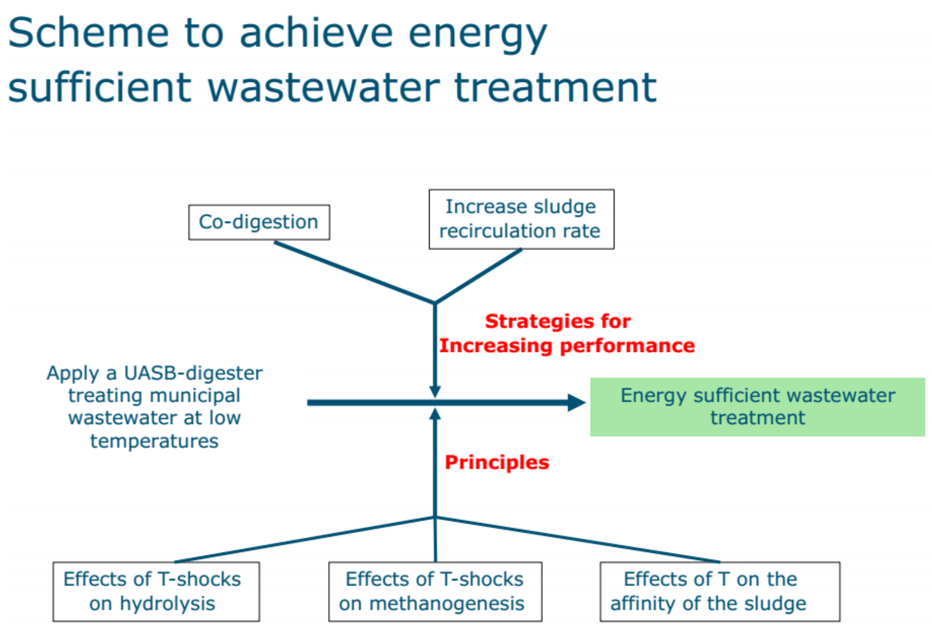

Fig.1.5 The research scheme of this thesis

2 Effects of sludge recirculation rate and Sludge transfer point on a UASB-digester system to treat domestic sewage at 15 ˚C

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Abstract

The anaerobic treatment of low strength domestic sewage at low temperature is an attractive and important topic at present. A UASB-digester system is one of the successful anaerobic systems to challenge low temperature and concentrations. The effect of sludge recirculation rate and height of UABS sludge transfer (HUST) on UASB-digester treating domestic sewage at 15 ˚C was studied in this research. A sludge recirculation rate of 1%, 2.6% and 12.5% of the influent flow rate was investigated respectively. The results showed that the total COD removal efficiency rose with increasing sludge recirculation rate. A sludge recirculation rate of 1% of the influent flow rate leads to organic solids accumulation in the UASB. After the sludge recirculation rate increased from 1% to 2.6%, the stability of the UASB sludge was substantially improved from 0.37 to 0.15 g CH_4-COD/g COD, and the biogas production in the digester went up from 2.9 to 7.4 L/d. The stability of the UASB sludge and biogas production in the digester were not significantly further improved by increasing sludge recirculation rate to 12.5% of the influent flow rate, but the biogas production in the UASB increased from 0.37 L/d to 1.2 L/d. It is recommended to apply a sludge recirculation rate of 2-3% of influent flow rate in a UASB-digester system. Increased HUST resulted in a high VSS concentration of the UASB-digester system.

2.1 Introduction

Given the potential advantages of anaerobic compared to aerobic sewage treatment (e.g. less energy consumption, energy production and a lower sludge production), its application at moderate and low temperatures (≤ 20 ˚C) would be very attractive (Lettinga et al., 2001). High-rate anaerobic reactors, such as Expanded Granular Sludge Bed (EGSB) and Anaerobic Baffled Reactor (ABR), have been reported to successfully treat synthetic wastewater at low temperature (10 ˚C - 20 ˚C) containing mainly soluble chemical oxygen demand (COD) (Langenhoff & Stuckey, 2000; McKeown et al., 2009a). However, at low temperatures (6-15 ˚C) the growth of methanogens is very slow and the hydrolysis of the biodegradable solids in sewage may be the rate limiting step of the process. (LeitÃ£o et al., 2006). As a consequence, suspended organic matter accumulates in the anaerobic reactor when the sludge retention time (SRT) is not sufficiently long (Luostarinen et al., 2007). The accumulated solids in the reactor replace the anaerobic biomass, and the biomass is also lost in the effluent by attachment to washed out solids. As a result, stability, specific methanogenic activity (SMA) and SRT of the sludge in a single Upflow Anaerobic Sludge Bed (UASB) reactor all decrease when the SRT becomes too short due to the organic solids accumulation. As a result, this sludge still requires stabilisation before appropriate reuse or final disposal (Seghezzo et al., 2006), and liquid effluent needs further treatment. The application of long SRT needs long HRT and therefore large reactor volume, which is economically not feasible. The combination of a UASB and a digester (UASB-digester) has been shown to be successful to treat domestic sewage with high concentrations of suspended organic solids at low temperature (Álvarez et al., 2004; Mahmoud et al., 2004 ; Mahmoud et al., 2008).

In this study, municipal sewage was treated in a UASB at 15 ˚C. As shown in Fig. 2.1, sludge recirculation connects a UASB and digester. The un-stabilized suspended sewage COD that is captured by the UASB sludge bed is transferred to the digester, which is operated at 35 ˚C. At the same time, stabilized sludge from the digester is transferred to the UASB, herewith providing additional methanogenic biomass to convert soluble COD. In previous studies, the sludge recirculation rate was determined by control of the sludge bed height (Álvarez et al., 2004; Mahmoud et al., 2004). However, the data about sludge recirculation on the overall process is very limited, and the optimum for the treatment of domestic sewage at low temperature is still not clear. Yet, the amount of sludge that needs to be circulated is crucial to the viability of the UASB-digester, since it determines the required energy input to heat the transferred sludge from 15 ˚C to 35 ˚C.

The height of the UASB sludge transfer (HUST) from which sludge is transferred to the digester is important for the operation of a UASB-digester system and particularly for the dissolved COD removal in the UASB reactor. Previous studies on the UASB-digester system did not elaborate on the effect of HUST. Mahmoud et al (2004) applied sludge transfer from the top of the UASB sludge bed, but recommended doing this from

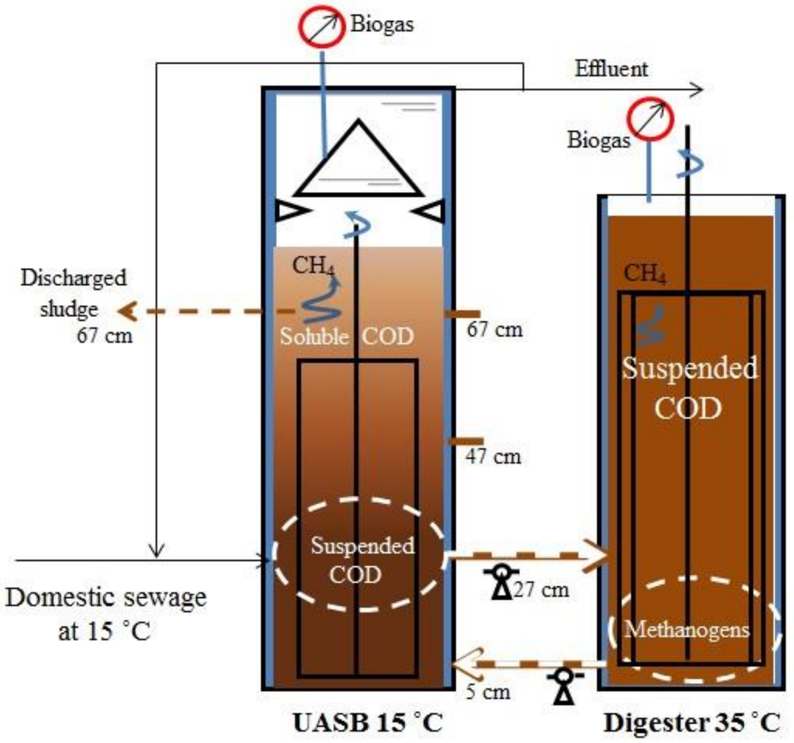

Fig. 2.1 The pilot-scale UASB-digester system in this research.

a lower point since the sludge concentration was higher there. Alvarez et al. (2004) transferred the sludge from 2 different heights, because of available sludge bed height. However, the specific effects of changing the HUST were not shown.

In this work, the effect of the sludge recirculation rate and HUST in UASB-digester system on COD removal efficiency, bio-gas production, the stability and specific methanogenic activity (with acetate) of the UASB-digester sludge, was investigated.

2.2 Method and materials

2.2.1 Inoculum and sewage

The inoculum sludge used in the UASB-digester system was taken from a primary sludge digester operated at 35 ˚C at the wastewater treatment plant (wwtp) of Ede (NL). The screened (<3 mm) sewage came from a collecting system at the wwtp in Bennekom, the Netherlands. It was collected weekly and kept in a closed stirred tank at 5 ˚C.

2.2.2 A UASB-digester system

2.2.2.1 Effects of sludge recirculation test

A pilot scale UASB-digester was operated to treat domestic sewage at 15 ˚C for a period of 372 d. The influent flow rate was about 200 L/d. The following sludge recirculation rates were investigated: 1.8 L/d, 5.2 L/d and 25 L/d for 210 d, 70 d and 92

Table 2.1 The operational and design parameters of UASB-digester in the research.

	UASB	Digester
Total Height (m)	1.15	1
Temperature (˚C)	15	35
Diameter (cm)	23.5	23.5
Volume (l)	50	38
HRT (d)	0.25	21/7.3/1.5
Effluent recirculation (%)	180	-
Up-flow velocity (m/h)	0.5	-
Mixing condition (Rpm)	0.2	84

d respectively. Details of the UASB-digester system are given in Table 2.1. Effluent recirculation over the UASB was applied to increase the up-flow velocity from 0.26 m/h to 0.5 m/h. The sludge bed height in the UASB reactor was manually controlled to be less than 80 cm. The excess sludge was discharged from the height of 67 cm. Sampling points on the UASB reactor were located at 11.5, 27, 47 and 67 cm height.

2.2.2.2 Effects of HUST test

Experiments about effects of HUST was performed after the study of effects of sludge recirculation rate. Sludge recirculation rate was fixed at 5.2 L/d (2.6 % of the 200 L/d influent flow rate). Sludge return point from the digester to the UASB reactor was fixed at 5 cm. The height of the UASB reactor was 100 cm and the height of the sludge bed was controlled at max. 70 cm. A height of UASB sludge transfer (HUST) of 27, 47 and 67 cm was studied in three periods. During period 1 (sludge transfer point at 27 cm), sludge circulation rate was temporarily increased to 25 L/d (days 71-167), thus data were not shown. Effluent circulation over the UASB reactor was applied in period 1 and was stopped from period 2 onwards, which resulted in compaction of the sludge bed. Sludge circulation was temporarily stopped in period 2 (days 30-59) as the height of the UASB sludge bed was below the HUST. COD composition of the sewage is shown in Table 2.2.

Table 2.2 Influent COD concentrations (in mg/L, standard deviation in brackets), n = number of samples

HUST (cm)	n	COD total	COD suspended	COD colloidal	COD dissolved
27	12	605 (133)	282 (868)	82 (32)	241 (50)
47	27	582 (116)	268 (71)	78 (16)	246 (44)
67	6	714 (189)	377 (117)	64 (11)	272 (88)

2.2.3 Batch experiment

Specific methanogenic activity (SMA) of the UASB sludge was determined in duplicate at 15 ˚C. Serum bottles with a volume of 117 ml were used in the test. The substrate was acetate with a starting concentration 1 g COD/L. The volume of UASB sludge was 60 ml. No trace nutrition was added, assuming this was sufficiently present in the sludge samples for the whole test period. The contents and headspace were flushed with nitrogen. The bottles with demi water and without any biomass were used as blanks. The volume of demi water was the same as the volume of the sludge samples. All the samples were incubated at 15 ± 1 ˚C in a shaker with 120 rpm in the dark. The pressure in the bottles was checked twice per day by hand digital pressure meter with a needle.

The stability test of both the UASB and the digester sludge was similar to the SMA test. The test temperature was 35 ˚C, and it was performed without addition of substrate. During the test, the anaerobic degradable compounds were converted to methane. The test was ended when no further methane production was observed (i.e. no further increase in pressure). High value in the results of stability test shows that high anaerobic biodegradable organic compound is in the sludge, which means less stable. The volatile suspended solids (VSS) and total suspended solids (TSS) of the UASB and digester sludge sample in SMA and stability tests are shown in Table 2.3 (in the study of effects of sludge recirculation rate). The UASB sludge samples were taken at 11.5cm height from the bottom of UASB reactor.

For analysis of the gas composition a sample was taken with a 100 µl syringe at the end of all the tests.

2.2.4 Analysis

Concentrations of nitrogen, methane, and carbon dioxide in the headspaces of the activity bottles were measured using a gas chromatograph (Interscience GC 8000 series) equipped with a thermal conductivity detector and Two columns (Molsieve 5A 50 m × 0.53 mm for N_2 and CH_4 and Porabond Q 50 m × 0.53 mm for CO_2). Injector and

Table 2.3 The VSS and TSS concentrations of UASB and digester sludge samples in the SMA and stability test (samples are duplicate and the standard deviation is in the brackets).

Date	VSS concentration		TSS concentration	
(since the operation started)	UASB sludge	Digester sludge	UASB sludge	Digester sludge
(day)	(g/L)	(g/L)	(g/L)	(g/L)
161	7.8 (-)	6.3 (-)	11 (-)	9.5 (-)
277	13.1 (0.13)	7.4 (0.1)	18.6 (0.15)	11.7 (0.04)
307	11.5 (0.04)	6.7 (0.01)	17.5 (0.08)	10.4 (0.06)

detector temperatures were respectively kept at 110 and 99 ˚C, while oven temperature was 50 ˚C.

COD was performed using DrLange tubes (type 514). VSS and TSS of the UASB sludge and the digester sludge were determined according to APHA (2005). The amount of dissolved methane in the UASB effluent was calculated using Henry's law.

2.3 Results and discussion

2.3.1 Effects of sludge recirculation rate

2.3.1.1 COD removal efficiency

Table 2.4 shows the average removal efficiency of total, suspended, colloid and dissolved COD during the three different sludge recirculation rates. The total COD removal efficiency reached the best result with the highest sludge recirculation rate of 25 L/d. Compared to the other two lower sludge recirculation rates of 5.2 L/d and 1.8 L/d, the higher dissolved COD removal efficiency was the main contributor to the improved total COD removal efficiency. Based on the amount of sludge transferred to the digester and the anaerobic biodegradability of the sewage, the improved $COD_{dissolved}$ removal efficiency mainly increased due to the transfer and conversion of dissolved COD in the digester. However, the larger amount of anaerobic biomass provided to the UASB also contributed to the higher dissolved COD removal efficiency. The total COD removal efficiency was lower than expected at all sludge recirculation rates, a possible explanation for this will be discussed later.

Table 2.4 The summary of the suspended, colloid, dissolved and total COD removal efficiency, n is the numbers of samples (the efficiency was the average of all the samples).

Sludge recirculation rate (L/d)	n	COD removal efficiency (%)			
		COD_{total}	$COD_{suspended}$	$COD_{colloid}$	$COD_{dissolved}$
1.8	30	31.8±12.7	61.9±17.7	16.5±23.2	6.3±8.6
5.2	7	32.2±8.1	58.6±16.6	19.1±16.5	5.8±5.8
25	10	37.1±9.8	58.1±21.2	17.9±16.5	17.1±11.5

2.3.1.2 Stability and SMA of UASB-digester sludge

The results of the stability and SMA tests of the UASB and the digester sludge are shown in Table 2.5. The results of stability test with UASB sludge at a recirculation rate of 1.8 L/d shows that this sludge is relatively unstable, i.e. it still contains considerable amounts of biodegradable solids and accumulation of such solids in the sludge bed. Thus, although the total COD removal efficiency was similar compared to the UASB-digester system operation at a sludge recirculation rate of 5.2 L/d as shown in Table 2.4, it was actually attributed to the organic solids accumulation. But the stability of UASB sludge was drastically improved after the sludge recirculation rate had increased from 1.8 L/d to 5.2 L/d. The stability of the UASB sludge only improved 33 percent by further increasing the sludge recirculation rate from 5.2 L/d to 25 L/d. The stability of the digester sludge at recirculation rate 25 L/d remains same to 5.2 L/d. It meant the digester was still stable even at a high sludge recirculation rate 25 L/d. The SMA of the UASB sludge at 15 ˚C became higher at an increasing sludge recirculation rate. This can be attributed to an improved conversion of sewage solids to CH_4 and biomass, and an increased supply of methanogens to the UASB sludge.

2.3.1.3 Methane production

The methane production as a fraction of total COD input and COD removed is shown in Table 2.6. It is clear that both $COD_{methane}/COD_{in}$ and $COD_{methane}/COD_{removed}$ were higher with an increasing sludge recirculation rate. The $COD_{methane}/COD_{removed}$ increased from 0.55 to 0.77 as sludge recirculation rate increased from 1.8 L/d to 5.2 L/d. This confirmed that suspended COD accumulated (as discussed earlier) when operating at low circulation rate of 1.8L/d, since suspended COD removal efficiencies were similar at these two sludge circulation rates (see Table 2.4). The $COD_{methane}/COD_{removed}$ reached 0.92 when the sludge recirculation rate increased to 25 L/d. It indicated a high anaerobic biodegradability of COD removed. Elmitwalli (2001) also reported that the anaerobic bio-degradability of suspended solids in domestic sewage was 78% at 30 ˚C, however, without taking into consideration of dissolved methane. In this research, the $COD_{methane}$ included two parts, which were the collected CH_4 gas and the dissolved CH_4 in the effluent of UASB-digester system. Assuming

Table 2.5 The SMA of UASB sludge at 15˚C and the stability of UASB and digester sludge at 35˚C.

Sludge recirculation rate (L/d)	Stability (g-COD/g-COD)		SMA ($g\text{-}CH_4\text{-}COD\ g^{-1}\ VSS\ d^{-1}$)
	UASB sludge	Digester sludge	UASB sludge
1.8 (0.9%)*	0.37	-	0.040
5.2 (2.6%)*	0.15	0.01	0.048 (0.002)**
25 (12.5%)*	0.10	0.01	0.067 (0.003)**

* the sludge recirculation rate as percentage of the influent flow rate is given between brackets
** standard deviation; three samples were taken at the same time

that the dissolved CH_4 was saturated in the effluent, it was calculated by Henry's law. However, the actual $COD_{methane}/COD_{removed}$ might be lower if CH_4 was not saturated in the effluent.

Table 2.6 also shows the biogas production. A large part of the methane production (5.86 L-CH_4/d according to Henry's law) in the UASB was dissolved in the effluent and combined with a low dissolved COD removal efficiency, the amount of biogas collected in the UASB was very low. It was higher after sludge recirculation rate increased from 5.2 L/d to 25 L/d. This confirmed that, the high dissolved COD removal (in Table 2.4) at sludge recirculation rate 25 L/d was indeed partially due to a large number of methanogens supplied from the digester to the UASB. It enhanced the conversion of dissolved COD to methane in the UASB. The bio-gas production in the digester significantly increased after the sludge recirculation rate had increased from 1.8 L/d to 5.2 L/d. However, it did not rise any further at a sludge recirculation rate of 25 L/d. The reason might be that the bio-gas production of the digester is not only depended on the captured $COD_{suspended}$ from the UASB sludge bed, but also its anaerobic degradability at 35 ˚C.

Assuming that the suspended COD could be efficiently converted to methane, the methane production in the digester could be calculated in the following formula (1):

$$V_{CH4}= COD_{suspended}\times Q_{influent}\times D_{anaerobic\ bio\text{-}degradablity}\times 0.35 \qquad (1)$$

Where VCH_4 is the methane production (L/d); $COD_{suspended}$ is the concentration of suspended COD in the influent (mg/L); $Q_{influent}$ is the influent flow rate of UASB-digester (L/d); D is the anaerobic bio-degradability of suspended solids, which was 0.78 in Elmitwalli's et al. (2001) research, but 0.5 was used in this work on the safe consideration. The methane production in theory should be about 10.5 L/d in this

Table 2.6 Methane production at different sludge recirculation rates (including gaseous and effluent saturated with dissolved methane).

Sludge recirculation rate(L/d)	1.8	5.2	25
$COD_{methane}/COD_{in}$ (g/g)	0.19	0.23	0.3
$COD_{methane}/COD_{removed}$ (g/g)	0.55	0.77	0.92
Bio-gas $*_{digester}$ (L/d)	2.9	7.4	7.5
Bio-gas $**_{UASB}$ (L/d)	0.31	0.37	1.22

* the percentage of methane is 66%

** the percentage of methane is 78%

research. Assuming a 40 kJ/l CH_4 methane heat combustion and an efficiency of 80%, about 336 kJ/d heat could be obtained. It is enough to warm up the transferred sludge from the UASB to the digester from 15 ˚C to 35 ˚C, whose recirculation rate is equivalent with 2-2.5% of the influent flow rate (200 L/d).

The sludge recirculation rates 1.8 L/d, 5.2 L/d and 25 L/d applied in this research represent 0.9%, 2.6% and 12.5% of influent flow rate respectively. Based on the biogas production, COD removal efficiency and the economy of sludge heating, a sludge circulation rate of 2.6% of the influent flow is recommended.

The COD concentrations of influent and effluent are shown in Fig. 2.2 for the different sludge recirculation rates. The dissolved COD concentration contributed from 46% to 53% to total influent COD and this was similar for the suspended COD. The dissolved COD removal efficiency increased about 12% after sludge recirculation rate increased from 5.2 L/d to 25 L/d. However, it only somewhat improved the total COD removal efficiency. Thus, both the $COD_{methane}/COD_{in}$ and total COD removal efficiency were low even with 25 L/d sludge recirculation rate. The dissolved COD was difficult to remove at 15 ˚C in the UASB-digester system and was the main part of the effluent (51-57%). A high contribution of dissolved COD (70%) to total effluent COD was also reported by Álvarez et al. (2004), who also had a high fraction of dissolved COD in the influent (Fig. 2.2). Mahmoud et al. (2004), however, had a low fraction of influent dissolved COD, which resulted in a high total COD removal efficiency. This shows that the influent dissolved to total COD ratio is a key factor in achieving high COD removal efficiency in a UASB-digester system. Elmitwalli et al. (2001) also showed that the maximum conversion of the dissolved COD in domestic sewage was only 62% even at 30 ˚C, this further emphasizes that the removal of dissolved COD is the main challenge in low temperature anaerobic treatment. It highlights that the lack of methanogens leads to a poor dissolved COD removal efficiency. Thus, longer SRT

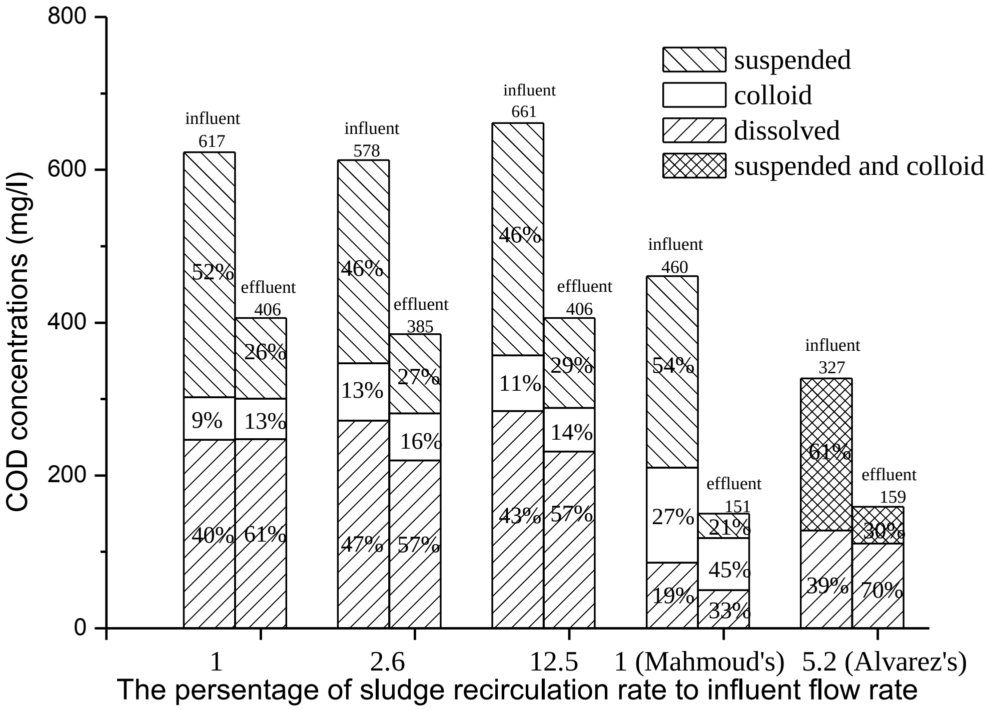

Fig. 2.2 Comparison of COD characteristics in this research and other researchers' during different percentage of sludge recirculation rate to influent flow rate.

(Gomec, 2010; Speece, 2008) and plenty of methanogens are required to enhance the removal efficiency of dissolved COD at low temperature.

2.3.2 Effects of HUST

2.3.2.1 Increased UASB biogas production at higher sludge circulation point

Increasing the HUST resulted in a clear increase in biogas production in the UASB reactor (Fig. 2.3). The average biogas productions at the height of 27, 47 and 67 cm were 0.9, 2.8 and 2.8 L/d. The increased biogas production was the result of the increased methanogenic capacity (SMA × VSS) (as discussed later). In addition, it was also explained by a larger amount of dissolved COD originating from partial hydrolysis of the captured suspended COD, due to its longer retention in the UASB reactor. Gas production in the digester decreased with an increase in HUST: in period 1 biogas production was 7.1 L/d in the digester. Increasing the height of UASB sludge transfer (HUST) to 47 and 67 cm (period 2 and 3) resulted in lower digester biogas productions of 3.2 and 3.7 L/d respectively.

2.3.2.2 Improved COD removal

Fig. 2.4 shows that average suspended COD removal efficiencies were 52, 57 and 65 % at the HUST of 27, 47 and 67 cm. The improved efficiencies were probably because the UASB sludge bed was compact and high when transferring the sludge at high position, which enabled good capture of the suspended COD. Overall methane production from the removed COD decreased and was 74, 58 and 44 %, showing that suspended COD accumulated as the HUST increased (as confirmed by the increased

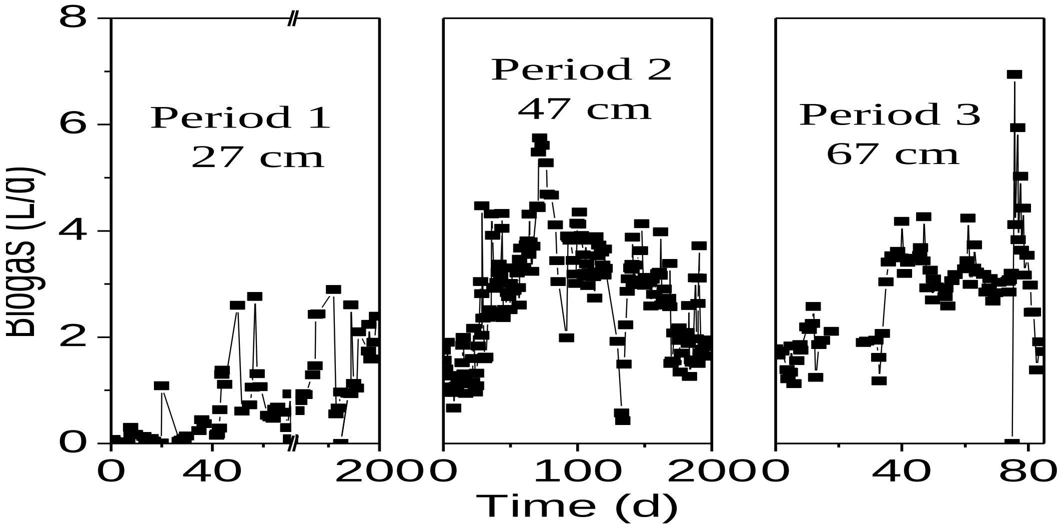

Fig. 2.3 Effects of HUST on biogas production of the UASB reactor in the system

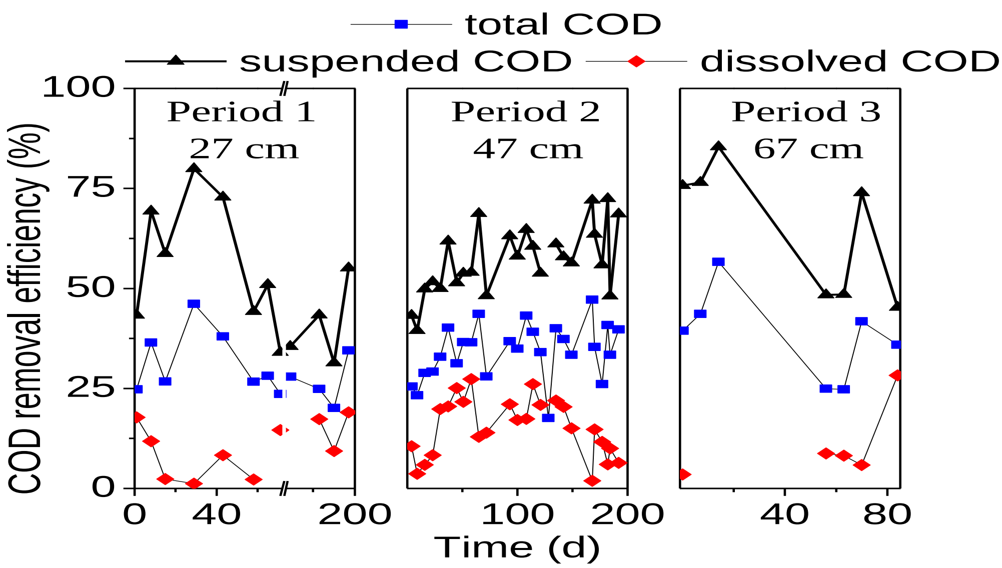

Fig. 2.4 Effects of HUST on COD removal efficiency of the UASB-digester

VSS concentrations, Table 2.7).

As a result, total COD removal efficiency increased to 30, 34 and 38 % at the studied HUSTs. Due to the slow accumulation, the system had not yet reached steady state yet. Longer term experiments will show whether this accumulated COD can be eventually efficiently converted to methane. The low total COD removal efficiencies in this study were due to the relatively low UASB reactor (1 m). Other, higher, reactors have shown higher total COD removal efficiencies of 51-66 % (Mahmoud et al., 2004, Álvarez et al., 2004).

In period 2, dissolved COD removal efficiency initially increased, but later decreased again. This was caused by a net dissolved COD production, due to hydrolysis of the accumulated suspended COD in the UASB sludge bed. The methanogenic capacity of

Table 2.7 Effects of HUST on VSS concentration and SMA$_{15\,^\circ C}$ of the sludge in the UASB-digester (SMA unit: mg CH_4 COD/ (g VSS d), standard deviation was in brackets)

H (cm)	Time	VSS (g/L)					Time	SMA	VSS/
	d	11.5	27	47	67	digester	d	15 ˚C	TSS
27	40	15	11(0.4)	9	8 (0.1)	9 (0.1)	40	33 (1.0)	0.73
47	199	23 (0.2)	16 (0.2)	15 (0.3)	--	12 (0.2)	293	30 (1.0)	0.74
67	63	44 (0.2)	38 (0.2)	21 (0.2)	0.2 (0.1)	7 (0.5)	476	15 (0)	0.72

the sludge bed was still insufficient to convert this additional dissolved COD.

2.3.2.3 Higher solids concentration and improved methanogenic capacity of UASB reactor

Table 2.7 shows that VSS concentrations increased in the UASB reactor. It is hypothesized that suspended COD capture in the UASB reactor improved due to the higher solids concentration in the sludge bed, by allowing more adsorption onto the sludge. Additionally, due to (partial) hydrolysis of this captured suspended COD, the extracellular polymeric substances (EPS) may contribute to a better suspended COD capture. Confirmation of these hypotheses is part of ongoing research.

As shown in Table 2.7, SMA15°C of the UASB sludge decreased with increased HUST. However, total methanogenic capacity of the UASB reactor increased from 9.4 to 11.7 g CH_4-COD/d as the HUST increased from 27 to 47 cm, and was 10.5 g CH_4-COD/d as the HUST further increased to 67 cm.

2.4 Conclusions

Anaerobic treatment of domestic sewage at low temperature is feasible in a UASB-digester system. The removal of dissolved COD was limiting, especially at a high dissolved to total COD ratio in the influent.

Three sludge recirculation rates between UASB (15 ˚C) and digester (35 ˚C) were tested, a higher sludge recirculation rate resulted in:

- Increase in total COD removal efficiency, mainly caused by the transfer of dissolved COD to the digester
- Improved conversion of removed COD to methane
- Improved stability of the sludge in the UASB

Based on the potential energy available in the waste water, a sludge recirculation flow of 2-3 % of the influent flow is recommended.
A higher height of UASB sludge transfer (HUST) has a positive effect on the performance of a UASB-digester system treating sewage at 15°C. It resulted in: 1) Higher biogas production rate in the UASB reactor; 2) Improved suspended COD removal efficiency; 3) Higher solids concentration in the UASB reactor and 4) Increased methanogenic capacity of the UASB sludge bed.

Acknowledgements

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3 Co-digestion to support low temperature anaerobic pretreatment of municipal sewage in a UASB-digester

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Abstract

The aim of this work was to demonstrate that co-digestion improves soluble sewage COD removal efficiency in treatment of low temperature municipal sewage by a UASB-digester system. A pilot scale UASB-digester system was applied to treat real municipal sewage, and glucose was chosen as a model co-substrate. Co-substrate was added in the sludge digester to produce additional methanogenic biomass, which was continuously recycled to inoculate the UASB reactor. Soluble sewage COD removal efficiency increased from 6 to 23%, which was similar to its biological methane potential (BMP). Specific methanogenic activity of the UASB and of the digester sludge at 15°C tripled to a value respectively of 43 and 39 mg CH_4-COD/(g VSS·d). Methane production in the UASB reactor increased by more than 90% due to its doubled methanogenic capacity. Therefore, co-digestion is a suitable approach to support a UASB-digester for pretreatment of low temperature municipal sewage.

3.1 Introduction

Anaerobic biological treatment of municipal sewage has many advantages over aerobic treatment, such as lower operational cost, higher chemical oxygen demand (COD) loading rate, lower excess sludge production and energy recovery in the form of methane (Lettinga et al., 2001; Zeeman & Kujawa-Roeleveld, 2011). So far, full scale anaerobic treatment of municipal sewage has been restricted to tropical areas (Chernicharo et al., 2009; Seghezzo et al., 1998), where the temperature of municipal sewage allows for sufficiently fast hydrolysis of complex organics and suspended solids. Lab scale research has shown the feasibility of low temperature (6-15 °C) application of anaerobic processes with easily biodegradable substrates (such as volatile fatty acids (VFAs), semi skimmed milk or nonfat dry milk) for both high and low strength waste waters (McKeown et al., 2009b; Rebac et al., 1999a). However, low temperature anaerobic treatment of municipal sewage still faces challenges. The main challenge is slow hydrolysis of complex and suspended organic material, and the other is slow growth of methanogens (Álvarez et al., 2008). A UASB-digester system may offer a solution for these challenges (Zhang et al., 2012, Álvarez et al. 2004, Mahmoud et al. 2004).

In this system, only the fraction of the municipal sewage that is transferred from a UASB reactor (15 °C) to a digester (35 °C) needs to be heated. The UASB reactor of this system is operated at cold conditions (8-20 °C), while designed for summer conditions, in order to reduce its hydraulic retention time (HRT). As the loading rate is too high to allow for complete stabilization of entrapped suspended solids in the low temperature UASB reactor, these solids are transferred and stabilized in the sludge digester, which operates at 35 °C. The stabilized sludge from the sludge digester is recycled to the UASB reactor to enhance methanogenic capacity for soluble COD removal at low temperatures. In this manner Mahmoud et al. (2004) achieved an average COD removal efficiency of 66 % at 15 °C with municipal sewage of a low soluble COD fraction (19-24 % of total COD). However, several authors have shown the average COD removal efficiency of the system decreased to only 37-46 % when treating municipal sewage with a considerably higher soluble COD fraction (33-44 %), mainly caused by insufficient methanogenic activity in the UASB reactor (Álvarez et al., 2004; Zhang L. et al., 2011).

An interesting option to improve the performance of UASB-digester system for these types of municipal sewage is to add co-substrate to the sludge digester, which has not been tested yet. This option increases the organic loading on the digester, resulting in a higher methanogen production. This effect of co-digestion is similar to treating municipal sewage containing a high fraction of suspended COD, as it would also lead to a higher organic loading on the digester. As a result of co-digestion, the growth of methanogens will increase and, therefore, also the number of methanogens transferred from the digester to the UASB reactor. In this manner, the methanogenic activity of the

Table 3.1 Operational and design parameters of the UASB-digester in this study

Parameters	UASB	Digester
Total liquid height (m)	1.1	0.8
Diameter (cm)	23.5	23.5
Working volume (L)	50	38
HRT (d)	0.25	15
Up-flow velocity (m/h)	0.2	-
Mixing condition (rpm)	0.2	84
Temperature (°C)	15	35

UASB sludge is expected to increase, as well the soluble sewage COD removal in the UASB reactor.

The aim of this work was to demonstrate that co-digestion improves soluble sewage COD removal of low temperature municipal sewage anaerobic treatment. A pilot scale UASB-digester was studied in this research, and glucose was chosen as a model co-substrate. The applicability of the UASB-digester pretreating low temperature municipal sewage in moderate climates will be discussed, as well as potential substrates for co-digestion.

3.2 Materials and methods

3.2.1 UASB-digester with co-digestion

The operational and design parameters of the UASB-digester system are given in Table 3.1. The sludge bed in the UASB reactor was manually kept below 80 cm by discharging sludge. Very slow mixing of the UASB reactor was performed by a rectangular stainless steel mixer rotating at 0.2 rpm to prevent gas build-up and/or channel formation. The UASB sludge was transferred from a height of 67 cm to the middle of the digester, and the digester sludge was transferred from the bottom to a height of 27 cm of the UASB reactor. This sludge recirculation rate between UASB reactor and digester was 2.5 L/d, which corresponded to 1.25 % of the influent flow rate of 200 L/d. The influent organic loading rate (OLR) of the UASB reactor was about 2.6 kg COD/(m^3·d). Excess sludge was discharged from the digester with an average amount of 1 L/d when the sludge bed in the UASB reactor was higher than 70 cm. At

Table 3.2 Average concentration of influent COD (mg/L) and its fractions in three experimental periods. (period 1: without co-digestion; periods 2 & 3: co-digestion was applied; n: number of the samples)

Period (days)	n	Total COD (CODt)	Suspended COD (CODss)	Colloidal COD (CODcol)	Soluble COD (CODsol)
(1) -105 … 0	10	661 ± 161	351 ± 98	61 ± 10	249 ± 77
(2) 0 … 37	5	727 ± 141	329 ± 112	79 ± 28	319 ± 50
(3) 38 … 189	16	597 ± 109	285 ± 67	71 ± 18	241 ± 45

the start of the experiments, the UASB-digester already had been operated for more than 3 years treating sewage from the same waste water treatment plant (WWTP) (Zhang et al. 2012). The biogas production of the UASB-digester was recorded by gas meters (Ritter, Germany).

The start of co-digestion was defined as day 0, and the time before this as negative days. Experimental work was divided in three periods, and the duration of each period is shown in Table 3.2:

1. without co-digestion;
2. an average co-substrate addition of 8.2 g COD/ d (7 % of the influent COD loading);
3. an average co-substrate addition of 16.6 g COD/d (14 % of the influent COD loading).

Co-substrate was added batch-wise to the digester to avoid imposing a too high COD concentration. This addition was done four times per day, and the COD concentration in the digester after each co-substrate addition was calculated to increase by only 50 and 100 mg COD/L in periods 2 and 3 respectively. All the additional COD was expected to be biodegraded in the digester, and not transferred to the UASB reactor. Therefore, the composition and amount of soluble sewage COD was not influenced by the co-substrate. Glucose was used as a model co-substrate and dosed as a solution of 100 g COD/L.

3.2.2 Sewage

Screened (<3 mm) sewage was collected at the WWTP of Bennekom, the Netherlands. It was collected weekly and kept in a closed stirred tank at 5 °C. Sewage pH was 7.67 ± 0.27 (n = 33). The sewage sample for analysis was taken once a week, one day after the weekly sewage collection. The influent and effluent samples were collected after the influent pump and from the effluent tube respectively. The screened sewage was analysed for total sewage COD (COD_t), 8 μm paper-filtered (Whatman grade 40, Germany) samples for particulate sewage COD (COD_p) and 0.45 μm membrane-filtered (Whatman FP 30/ 0.45 CA, Germany) samples for soluble sewage COD (COD_{sol}). In this study, suspended sewage COD (COD_{ss}) was defined as the fraction larger than 8 μm, whilst colloidal sewage COD was the fraction between 0.45 and 8 μm. Correspondingly, these were calculated as $COD_{ss}=COD_t-COD_p$ and $COD_{col}=COD_p-COD_{sol}$.

3.2.3 Specific methanogenic activity (SMA)

The SMA of the UASB sludge and of the digester sludge were measured at 15 °C. The sludge sample and the substrate (sodium acetate) were added to a serum bottle (120 ml). The initial substrate COD concentration of the mixed solution was 1 g/L. Anaerobic conditions were achieved by flushing the sample with nitrogen gas, and the samples were placed in a shaker at 120 rpm. The duration of an SMA test was 7 days. The increasing pressure in the serum bottle due to the biogas production was recorded by a hand-held pressure meter (GMH 3150, Germany). Calculation of the SMA was done as described by Zhang et al (2012).

One liter of the UASB sludge was sampled each month for an SMA test, after which the sample was disposed of. Sludge samples were collected from the UASB sampling point at a height of 47 cm and from the center of the digester. The methanogenic capacity of the UASB reactor was calculated by SMA of the sludge multiplied by the total amount of volatile suspended solids (VSS).

3.2.4 Biological methane potential (BMP) of municipal sewage

BMP tests were performed with screened sewage, 8 μm filtered and 0.45 μm filtered sewage. Two series of batch experiments were performed for each fraction with serum bottles of 120 mL incubated in shakers (120 rpm) in the dark. In the first series, at 15 °C, digester sludge and UASB sludge were separately used as inoculum. The second series was inoculated only with digester sludge, but at two different temperatures of 15 °C and 35 °C. For each series, about 95 mL of each fraction of wastewater and 5 mL of inoculum sludge were added to each serum bottle. The tests were conducted in duplicate. After adding fractionated sewage samples, the serum bottles were flushed with nitrogen gas. Trace nutrients were assumed to be sufficiently present in the municipal sewage samples. The tests lasted for 60 days, when biogas production stopped. COD and VFAs concentrations were determined for each fraction at the beginning and the end of the test.

3.2.5 Analyses

Total suspended solids (TSS), VSS and pH measurements were performed according to standard methods (APHA, 1998). COD was measured using cuvette tests (Hach Lange). VFAs samples were prepared with formic acid (1.5 % in measured sample) and analyzed by gas chromatography (GC) (HP 5890 GC) equipped with a 2 m x 6 mm x 2 mm glass column packed with Supelco support (100-200 mesh), coated with 10 % Fluorad 431. Oven temperature was 130 °C, the carrier gas was nitrogen saturated with formic acid at a flow of 40 mL/min. Injector temperature was 200 °C and the flame ionization detector was 280 °C. Sample size was 1 µL.

Concentrations of nitrogen, methane, and carbon dioxide in the headspaces of the batch experiments and in the biogas produced by the UASB-digester were measured using a GC (Interscience GC 8000 series) equipped with a thermal conductivity detector and two columns (Molsieve 5A 50 m × 0.53 mm for nitrogen and methane and Porabond Q 50 m x 0.53 mm for CO_2). Temperatures of injector, detector and oven were 110, 99 and 50 °C respectively.

The tested soluble sewage COD concentration did not include dissolved methane COD. Dissolved methane concentration in the effluent of the UASB reactor was determined separately twice per month in triplicate samples. For each sample, about 5.3 g NaCl was added into a 50 mL tube first. The vial was closed with a stopper. Before adding effluent sample into the vial, about 20 mL of air was extracted using a syringe with a needle. About 15 mL of the effluent was slowly injected into the vial. This tube was shaken well to fully mix the salt with the sample. After 30 minutes of settling and reaching equilibrium (transfer of methane to the gas phase), the final pressure was measured by a hand-held digital pressure meter (GMH 3150, Germany) with a needle (the precision was up to 1 mbar). The gas composition was analysed after pressure measurement. The amount of dissolved methane in g CH_4-COD was calculated by the following formula:

$$CH_{4\ \mathrm{dissolved}} = P \cdot C \cdot V \cdot 64 / (R \cdot T)$$

With P the final pressure of headspace in the sample tube (kPa); C the percentage of methane in the biogas; V the volume of the headspace in the tube (L); R = 8.314 J/(mol·K); T = 293 K. 64 is the conversion factor between mole of CH_4 and g CH_4-COD.

3.3 Results and discussion

3.3.1 Characteristics of sewage COD

Total and fractionated COD concentrations (suspended, colloidal and soluble COD) of the sewage are shown in Table 3.2. The average total COD concentration in this study was between 597 and 727 mg COD/L. The COD mainly consisted of suspended (45-53 %) and soluble COD (38-44 %). The fraction of the latter was much higher than 19-24 % reported by Mahmoud et al. (2004) who also investigated a UASB-digester system. The colloidal fraction in the influent was only small (10-12 %) and therefore will not be further included in the results section.

3.3.2 Sewage COD removal efficiency

As shown in Fig. 3.1a, soluble sewage COD removal efficiency of the UASB-digester

Table 3.3 BMP of the sewage fractions: total, suspended and soluble COD. Digester or UASB sludge was used as inoculum. Results are the average of duplicate samples (± standard deviation).

Day of sewage sampling	Inoculum sludge	Temperature (°C)	Total COD (%)	Suspended COD (%)	Soluble COD (%)
79	Digester	15	38 ± 3	52 ± 3	17 ± 1
	UASB		34 ± 2	45 ± 2	17 ± 2
136	Digester	15	47 ± 3	44 ± 8	32 ± 3
		35	56 ± 4	69 ± 1	46 ± 5

increased after applying co-digestion. The average soluble sewage COD removal efficiency was only 6.1 % before co-digestion (period 1), but this increased to 13.2 % after co-digestion started in period 2 and to 23.0 % in period 3, which was in the same range as the BMP of soluble sewage COD at 15 °C (Table 3.3). A similar soluble COD removal efficiency of 30 % in the UASB-digester system was found by Mahmoud et al. (2004), who also treated municipal sewage but with a much lower soluble COD fraction. The observed week to week variation in the soluble COD removal efficiency could be explained by changes in sewage composition and its BMP (17-32 % for soluble COD, see Table 3.3). A similar low BMP of soluble COD of 27.0 % at 15 °C was reported by Elmitwalli et al. (2001). Since not all the soluble sewage COD was anaerobically biodegradable, the effluent contained a high residual soluble COD concentration. Aerobic post treatment will, therefore, be required when implementing this technology at a full scale.

In this study, acetate was the dominant VFAs in both the influent and effluent of the UASB-digester. The average percentages of acetate in the VFAs fraction were 83 % and 86 % respectively for the influent and effluent. The other VFAs consisted of propionate (17 % and 14 %) in the influent and effluent respectively, both corresponding to an average of 11 mg COD/L. The introduction of co-digestion in the digester changed the UASB reactor from a net producer of acetate to a net consumer of acetate (Fig.3.1b). In period 1, without co-digestion, the average acetate concentration

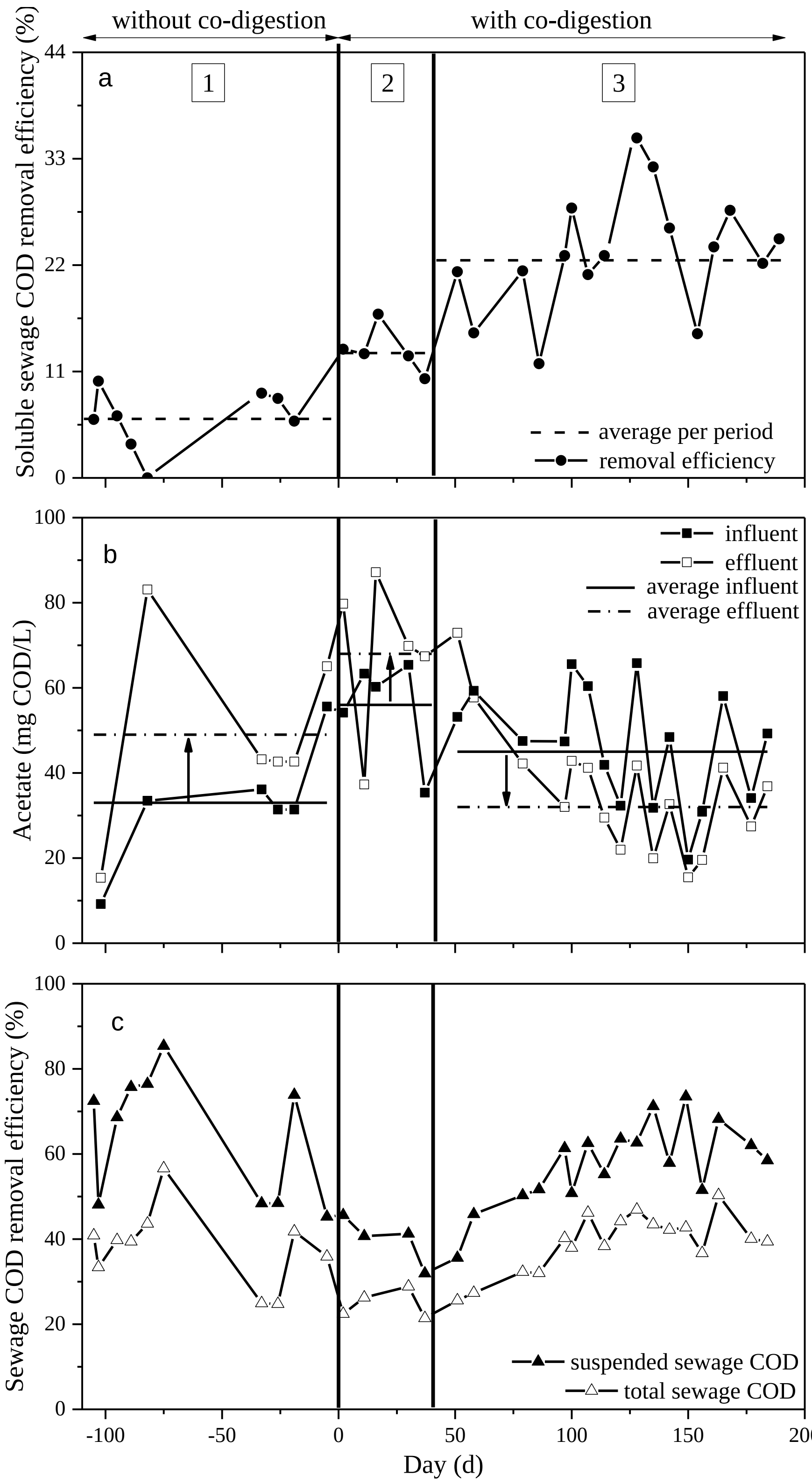

Fig. 3.1 Sewage COD removal efficiency of the UASB digester pretreating municipal sewage in 3 periods. 1 without co-digestion; 2 & 3 with co-digestion, 8.2 and 16.6 g COD/d was added into the sludge digester respectively. (All removal efficiencies excluded glucose-COD. a: sewage soluble COD removal efficiency; b: acetate concentration; c: suspended COD and total sewage COD removal efficiency)

in the effluent increased by 17 mg COD/L compared to the influent. Similarly, in period 2 the average acetate concentration in the effluent still was 12 mg COD/L higher than that in the influent. The net acetate production could be explained by acidification of influent soluble sewage COD, and/or (partial) hydrolysis and acidification of the influent suspended sewage COD. In combination with an insufficient methanogenic capacity of the UASB reactor, this resulted an increased acetate concentration. Glucose or produced VFAs transferred from the digester was considered negligible. This was confirmed by VFAs concentration measurements in the supernatant of the digester sludge in period 3, which were 22 and 2 mg COD/L, respectively 1 and 3 h after a glucose batch addition. In period 3, the average effluent acetate concentration was lower than that in the influent. It remained low with an average of 31 mg COD/L after day 60, with a minimum value of 15.5 mg COD/L. These decreased effluent acetate concentrations could be explained by an increased methanogenic capacity of the UASB reactor as a result of co-digestion, as will also be shown in the next paragraph.

In addition to the improved soluble sewage COD removal efficiency, applying co-digestion also contributed to an increased suspended sewage COD removal efficiency (all the removal efficiencies excluded the glucose-COD). The high suspended sewage COD removal efficiencies in the beginning of period 1 without co-digestion were mainly because of accumulation in the sludge bed (Fig.3.1c). This was confirmed by a low methane production (see Section 3.3.4) in this period. The suspended sewage COD removal efficiencies decreased later in period 1, when the sludge bed could not accumulate more suspended COD. As can be seen from Fig.3.1c, after adding co-substrate, suspended sewage COD removal efficiency maintained stable in period 2 and started to increase at the beginning of period 3. This increase may be explained by a higher extracellular polymer substances (EPS) production in the digester caused by adding glucose (Miqueleto et al., 2010; Miqueleto et al., 2005), though this was not verified in this study. In the UASB reactor, a higher EPS content may contribute to better suspended solids capture in the sludge bed. The suspended sewage COD removal did not yet clearly increase in period 2, as the (hypothesized) EPS production might not have been sufficient. The higher glucose addition in period 3 may also have resulted in a faster establishment of a new equilibrium between EPS production and suspended COD removal efficiency.

Total sewage COD removal efficiency clearly improved in period 3, which increased from 27 % to 50 %. The achieved total and the suspended sewage COD removal efficiencies were 42 % and 62 % respectively, from day 100 onwards. Soluble sewage COD removal contributed for almost a quarter to total sewage COD removal efficiency. The low BMP of soluble sewage COD, as previously discussed and shown in Table 3.3, and its high fraction (40 %) in the sewage explained the low total sewage COD removal efficiency.

3.3.3 Methanogenic capacity UASB

Details of sludge samples taken from the UASB and digester are given in Table 3.4.

Table 3.4 Characteristics of the UASB (sampling point at 47 cm) and of the digester sludge. Results show the average of duplicate samples (± standard deviation).

Day	VSS concentration (g/L)		TSS concentration (g/L)		VSS/TSS		CODt/VSS	
	UASB	Digester	UASB	Digester	UASB	Digester	UASB	Digester
-12	21.3 ± 0.2	6.9 ± 0.5	29.6 ± 0.2	10.1 ± 0.5	0.72	0.68	2.0	1.9
41	14.1 ± 0.3	11.8 ± 0.4	21.5 ± 0.3	18.8 ± 0.5	0.66	0.63	1.9	1.9
119	17.6 ± 0.3	5.0 ± 0.2	26.9 ± 0.2	8.2 ± 0.2	0.66	0.60	1.9	-
147	15.0 ± 0.1	6.0 ± 0.1	22.6 ± 0.1	9.6 ± 0.1	0.66	0.62	2.1	2.0
182	20.5 ± 0.2	6.4 ± 0.4	28.6 ± 0.3	9.2 ± 0.4	0.72	0.70	2.4	2.2

SMA values at 15 °C determined with these values are shown in Table 3.5, as well as calculated methanogenic capacities. The results in Table 3.5 show a clear increase in methanogenic capacity of the UASB reactor after co-digestion was introduced. The capacity almost doubled from 11.3 g CH_4-COD/d in period 1 to 20.0 g CH_4-COD/d in period 3. This increase was mainly caused by the improved SMA of the UASB sludge, which almost tripled from 15 mg CH_4-COD /(g VSS·d) in period 1 to 43 mg CH_4-COD/(g VSS·d) in period 3 (see Table 5). The SMA of the digester sludge also increased to 39 mg CH_4-COD/(g VSS·d) in period 3, almost three times as high as the one in period 1 without co-digestion. The relationship between the amount of glucose addition and methanogenic fraction of the sludge still needs further investigation.

3.3.4 Methane production

The results in Table 3.6 show that the methane production in the UASB reactor increased from 11.1 g CH_4-COD/d in period 1 without co-digestion to 19.3 in period 3 with co-digestion, which was an increase of more than 90 %. This was in agreement with the increased methanogenic capacity of the UASB reactor (11.1 in period 1 and 18.9 g/d, the average in period 3). The measured total methane production (gaseous + soluble) in the UASB reactor closely matched the calculated methanogenic capacity, which indicated that the UASB reactor was operating under non-substrate limiting conditions. This again confirmed that the number of methanogens in the UASB reactor was the limiting factor for low temperature municipal sewage treatment.

Table 3.5 SMA at 15 °C of the sludge of the UASB-digester, VSS in the UASB reactor and methanogneic capacity of the UASB reactor in three periods (tests were performed in duplicate, Methanogenic capacity = SMA (UASB) x VSS.)

Period (day)	SMA ($mg\ CH_4$-COD/ g VSS·d)		Total VSS (g)	Methanogenic Capacity ($g\ CH_4$-COD/ d)
	UASB	Digester	UASB	UASB
(1) -12	15 ± 0[a]	12 ± 0[a]	753	11.3
(2) 41	35 ± 2	23 ± 1	403	14.1
(3) 119	37 ± 3	18 ± 3	522	19.3
147	43 ± 0	39 ± 6	407	17.5
182	36 ± 0.4	-[b]	556	20.0

[a] performed in triplicate

[b] data not available

The dissolved methane concentration in the effluent of the system was found to be 50-60 % of the saturation value calculated with Henry's law. This relatively low percentage may have been caused by a higher ionic strength in the sewage compared to distilled water (Souza et al., 2011).

Based on the soluble sewage COD load and the average BMP of 25 % at 15 °C (Table 3.3), the maximum potential methane production from the influent soluble sewage COD in the UASB reactor would be 12.5, 16.0 and 12.1 g CH_4-COD/d in periods 1, 2 and 3 respectively. The measured methane production in period 3 was much higher than this maximum potential. This indicated that in period 3 an additional 7.2 g (= 19.3 - 12.1) CH_4-COD/d was produced via (partial) hydrolysis of the suspended sewage COD captured in the sludge bed, even at a temperature as low as 15 °C. Most likely this also took place in periods 1 and 2, but this could not be confirmed according to these calculations.

Methanisation of sewage COD in the digester improved from 6.3 g CH_4-COD/d in period 1 to more than 8.8 g/d in period 3 (Table 3.6). This can only be explained by an improved capture and transfer of suspended COD from the UASB reactor to the digester. Average methanisation of influent total sewage COD increased from 12.5 % to mor

Table 3.6 Average methane production in the UASB-digester before and after co-digestion (“based on SMA”= SMA x VSS)

Period	Methane production in the UASB reactor ($g\ CH_4$-COD/d)				Methane production in the digester ($g\ CH_4$-COD/d)			Influent COD load (g COD/d)
	Measured			Calculated	Measured	Calculated		
	Gaseous	Effluent dissolved	Total	Based on SMA	Total	From Glucose	From Sewage COD	
1	5.0	6.1	11.1	11.3	6.3	0	6.3	139.0
2	7.1	7.0	14.1	14.1	14.2	< 8.2 [a]	> 6.0	145.0
3	10.3	9.0	19.3	18.9 [b]	25.4	< 16.60 [a]	> 8.8	119.5

[a] will be lower due to growth

[b] average in period 3

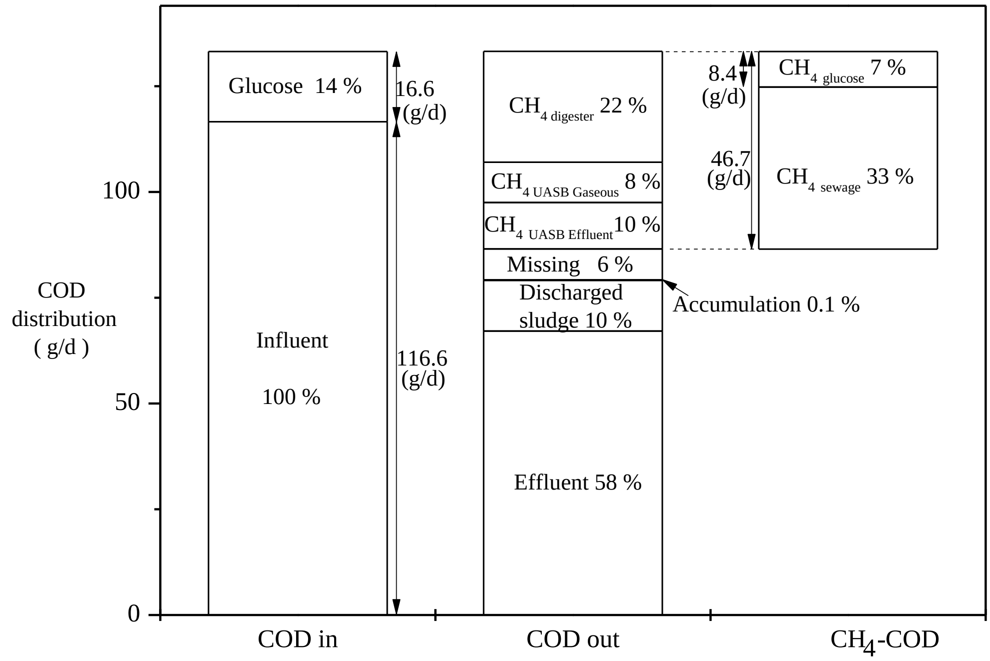

Fig. 3.2 COD balance of the UASB-digester pretreating municipal sewage at 15 °C in period 3 of co-digestion study (day 100-189); COD in has two parts: 1) municipal sewage (100 %); 2) co-substrate (glucose, 14 %). The specified methane production (sewage, glucose) assumes a biomass yield of 0.5 g biomass-COD/g glucose-COD converted. All percentages are relative to the influent sewage COD.

than 13.8 %, and later to more than 24.4 % in periods 1, 2 and 3 respectively (COD loading and methane production from the added glucose were excluded in this calculation).

The COD mass balance of the UASB-digester system in the stable phase of period 3 (day 100-189) is shown in Fig.3.2. The influent COD loading was used as 100 %, which enabled other COD fractions to be compared with the influent. The average methane production was 46.7 g CH_4-COD/d. This included the methane produced from glucose, which was 8.4 g CH_4-COD/d, assuming a yield of 0.5 g biomass-COD/g glucose-COD converted (Speece, 2008). As the sewage COD load on the system was 116.6 g COD/d, the sewage methanisation was 33 % during day 100-189.

The average BMP of the sewage fed to the UASB-digester system was calculated to be 42-46 %, using the average COD concentrations given in Table 3.2, a biodegradability of suspended sewage COD at 35 °C (69 %), and the average biodegradability of soluble sewage COD at 15 °C (25 %). This implied that the UASB-digester achieved about 75 % of the maximum potential methane production.

3.3.5 General Discussion

Co-digestion successfully improved the performance of the UASB-digester pretreating municipal sewage with a high soluble COD fraction at 15 °C. The amount of added co-substrate-COD was about 14 % of influent COD load. A further improvement could potentially be achieved, but the amount of co-substrate was not yet optimised. At lower temperatures of the municipal sewage, a higher amount of co-substrate may be required, to compensate for a further drop in specific methanogenic activity. However, this still needs to be investigated.

In practice, several resources could be used as substrates, provided they are biodegradable, have a low N/COD ratio and promote EPS production. To avoid additional costs of the transport of co-substrate, excess sludge from aerobic post treatment after the UASB-digester may be used as a local and practical co-substrate. This would amount to about 24 % on influent basis, which is higher than the co-substrate dose used in this study (14 %), but its biodegradability may only be 40-50 %. As other substrates are (much) more complex than glucose, their practical applicability needs to be tested, e.g. for their contribution to the beneficial higher EPS production and the amount of inert material introduced to the UASB-digester system. Also, the number of other co-substrates must be controlled to limit the nitrogen load to the post-treatment.

The UASB-digester effluent does not yet meet discharge standards and requires post treatment to remove residual COD, dissolved methane and nutrients (like nitrogen). Further studies on this system should also focus on a more detailed effluent characterisation. Autotrophic nitrogen removal using Anammox bacteria presents a promising option, as it does not require organic carbon and allows for maximum COD removal and energy recovery in the UASB-digester (Hendrickx et al., 2012). An alternative could be denitrification with dissolved methane (Kampman et al., 2012), which removes both nitrogen and the greenhouse gas methane.

The excess sludge production in the UASB-digester with co-digestion was low with 0.212 g TSS/g $COD_{removed}$, calculated from the results in the stable phase of period 3. This value is similar to the result reported by Mahmoud (2004). The biogas produced by the UASB-digester can be used to generate heat and electricity (e.g. in a combined heat and power unit). The electricity can be used for the aeration in the post-treatment processes. The heat can be locally used for warming up the sludge transferred from the UASB reactor (15 °C) to the digester (35 °C).

3.4 Conclusions

Co-digestion enables wider application of the UASB-digester for low temperature municipal sewage anaerobic treatment. Using glucose as a model co-substrate, we achieved:

- Clear increase in soluble sewage COD removal efficiency from 6.1 to 23.0%, which was similar to its BMP of 17-32 %
- SMA of the UASB and the digester sludge at 15°C tripled to reach 43 and 39 mg CH_4-COD/ (g VSS·d) respectively
- Methane production in the UASB reactor increased by more than 90% because of its doubled methanogenic capacity

- Capture and subsequent methanisation of suspended sewage COD also improved

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4 Anaerobic treatment of domestic wastewater in a UASB-digester demonstrated at a temperature of 10°C

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This chapter is to be submitted

Abstract

Direct anaerobic treatment of domestic wastewater is becoming attractive as it can change a wastewater treatment plant from energy consuming to energy producing. A pilot scale UASB-digester was studied to treat domestic wastewater at temperatures of 10-20°C and an HRT of 6 h. The results show a stable chemical oxygen demand (COD) removal efficiency of 60 ± 4.6% during the operation at 12.5 to 20°C. COD removal efficiency decreased to 51.5 ± 5.5% at 10°C. The decreased COD removal efficiency was attributed to an increased influent COD load, leading to insufficient methanogenic capacity of the UASB reactor. Suspended COD ($COD_{suspended}$) removal was 76.0 ± 9.1% at 10-20°C. Soluble COD removal ($COD_{soluble}$) fluctuated due to variation of the influent COD concentration, but the effluent COD concentration remained 90 ± 23 mg/L at temperatures between 12.5 and 20°C. The methane production (COD_{CH4}) was 39.7 ± 4.4% of the influent COD, which was 80% of influent biological methane potential (BMP). Of the total methane yield, 49% was produced in the UASB reactor operated at a low temperature, and 51% in the digester. Discharged sludge accounted for 8 ± 5% of the influent COD. The specific methanogenic activity (SMA) of the UASB sludge and the digester sludge was 0.26 ± 0.03 and 0.29 ± 0.03 g CH_4 COD/ (g VSS d), respectively. The stability of the UASB sludge and the digester sludge, was 0.25 ± 0.02 and 0.20 ± 0.02 g CH_4 COD/g COD. The methanogenic community analysis revealed an overall dominance of the acetoclastic Methanosaetaceae and the hydrogenotrophic Methanomicrobiales during the operation between 10-20°C.

4.1 Introduction

Anaerobic treatment of domestic wastewater saves energy, generates energy in the form of methane, and only produces a small amount of excess sludge. These advantages of anaerobic treatment result in a reduction of the operational costs compared with conventional domestic wastewater treatment (Speece, 2008). Besides, autotrophic nitrogen removal processes such as anaerobic ammonium oxidation (Anammox) and denitrification coupled to anaerobic methane oxidation (DAMO) are being developed for mainstream nitrogen and methane removal after anaerobic treatment (Hendrickx et al., 2012; Kampman et al., 2012). Latter processes are attractive for combination with anaerobic treatment as organic carbon is not required. This would make it feasible to transform net energy consuming domestic wastewater treatment plants into net energy producing plants.

Anaerobic treatment of domestic wastewater already is applied in tropical countries like Brazil and India, where the temperature of domestic wastewater favors mesophilic anaerobic bacteria (Seghezzo et al., 1998). Lower temperature (< 20°C) anaerobic wastewater treatment however still presents a challenge, mainly because of a low hydrolysis rate of organic solids and low methanogenic growth rates (Lettinga et al., 2001).

Different types of anaerobic reactors have been studied for low temperature wastewater treatment, including expanded granular sludge bed (EGSB) reactors, combinations of an EGSB with an anaerobic filter (AF), up-flow anaerobic sludge blanket (UASB) reactors, anaerobic baffled reactors (ABR), anaerobic migrating blanket reactor (AMBR) and anaerobic membrane bioreactors (Angenent et al., 2001; Ho & Sung, 2009; Langenhoff & Stuckey, 2000; McKeown et al., 2009b; Rebac et al., 1999c; Uemura & Harada, 2000). Generally, these reactors achieved a good chemical oxygen demand (COD) removal efficiency of 70% to 90% at temperatures between 4 and 25°C and for wastewaters that mainly consisted of soluble COD ($COD_{soluble}$). However, domestic wastewaters contain a high fraction of suspended COD ($COD_{suspended}$), typically 55% (Mahmoud et al., 2004). Because of limited hydrolysis of this $COD_{suspended}$ at low temperatures it would result in $COD_{suspended}$ accumulation in the reactor, unlike a very long hydraulic retention time (HRT) is being applied. An upflow anaerobic solids removal reactor (UASR) was studied for $COD_{suspended}$ removal, and it was shown that 65% of the $COD_{suspended}$ could be entrapped in the sludge bed when treating domestic wastewater at 17°C and at an HRT of 3 h (Zeeman et al., 1997). Hydrolysis of $COD_{suspended}$ was shown to be limited, viz. only 0.7 %. A two-step AF + anaerobic hybrid (AH) reactor was studied for treatment of domestic wastewater at 13°C. The AH reactor consisted of a granular sludge bed with vertical sheets of reticulated polyurethane foam (RPF) with knobs. The RPF was used for entrapment of solids. This AF+AH system achieved a $COD_{suspended}$ removal efficiency of 81% and a total COD (COD_t) removal efficiency of 71% at HRTs of 4 h (AF) and 8 (AH) h (Elmitwalli et al., 2002a; Elmitwalli et al., 2002b). However, the excess sludge that is produced by entrapment of influent $COD_{suspended}$ in these systems still needs further treatment.

Mahmoud et al. (2004) investigated a UASB-digester system for low temperature domestic wastewater treatment. This system treats wastewater in a UASB reactor at a short HRT. The UASB sludge is recirculated over a heated digester where the wastewater $COD_{suspended}$, captured in the UASB reactor, is converted to methane. The stabilized digester sludge is returned to the UASB reactor where it continues to capture wastewater organic solids and at the same time supplies methanogenic biomass to the UASB reactor for conversion of the $COD_{soluble}$ in the wastewater. To improve the performance of the UASB-digester system the effect of the sludge recirculation rate between the UASB reactor and the digester and the addition of external co-substrates to the digester were investigated. It was shown that the biogas production of the digester increased from 2.9 to 7.4 L/d, and stability of the UASB sludge was improved from 0.37 to 0.15 g CH_4 COD/ g sludge COD by increasing the sludge recirculation ratio from 0.9 to 2.6% of the wastewater flow rate (Zhang et al., 2012). Further increasing this ratio to 12.5% did not have a significant effect. Co-digestion increases the number of methanogens in the digester and herewith the methanogenic capacity and $COD_{soluble}$ removal in the UASB reactor (Zhang et al., 2013). Glucose as a model substrate was added at an amount of 14% of influent organic loading, and $COD_{soluble}$ removal increased from 6 to 23%, and SMA of the UASB and digester sludge tripled to 43 and 39 mg CH_4 COD/(g VSS d) at 15°C respectively. Therefore, adding co-substrate may be an attractive alternative, especially at very low temperatures and if the $COD_{suspended}$ to $COD_{soluble}$ ratio of the domestic wastewater is low.

Thus, the UASB-digester system was only studied for domestic wastewater treatment at ≥15°C. At this temperature a COD removal of 66 and 52% was achieved at an HRT of 6 h in the UASB reactor as reported by Mahmoud et al. (2004) and by Álvarez et al. (2004), respectively. However, the temperature of domestic wastewater in moderate climate zones can be as low as 10°C and therefore the feasibility of the UASB-digester also needs to be assessed at temperatures below 15°C. For this purpose, a pilot-scale UASB-digester was operated, of which the UASB initially was operated at 20°C. The temperature was subsequently decreased in steps to 10°C and removal efficiency for total COD (COD_t) and of its fractions (suspended, soluble and colloidal), methane production of the UASB reactor and the digester and the COD balance were determined. In addition, the microbial community in the UASB and digester were assessed to provide insight in the effect of temperature on this community and its relation to process performance.

4.2 Materials and method

4.2.1 Experiment set-up

Screened wastewater (< 3 mm) originated from the domestic wastewater treatment plant in Bennekom, the Netherlands. The wastewater was transported to the pilot-scale

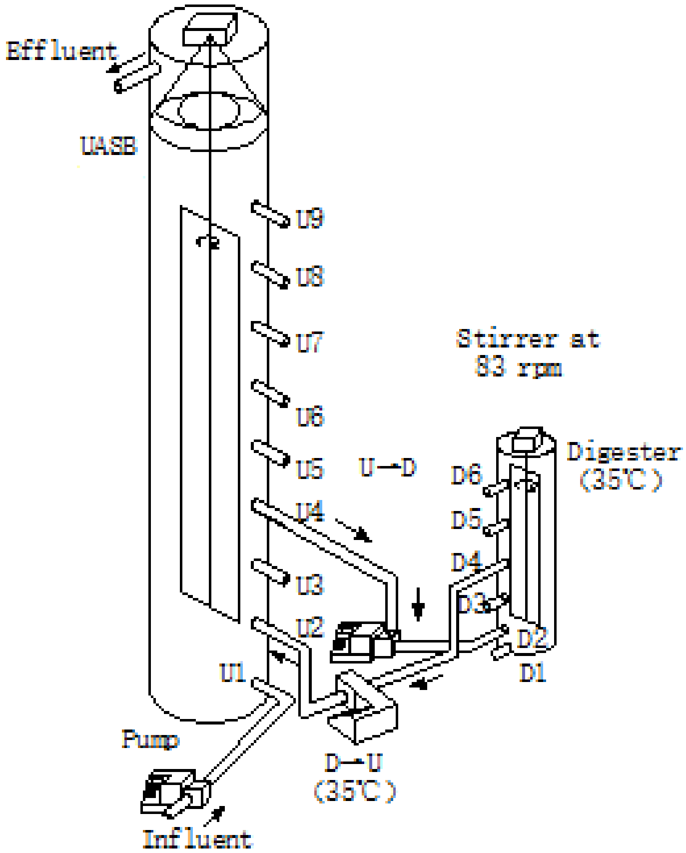


Fig.4.1 A pilot scale UASB-digester used in the study (the UASB reactor was operated at 10-20°C; the digester was operated at 35°C)

UASB-digester. A stirred tank (Mueller, the Netherlands) with a volume of 4 m^3 was used as a buffer to collect (twice per week) and store the sewage before feeding it to the UASB-digester. The temperature of the tank was 4°C to minimize biological conversion processes.

The wastewater passed a double walled metal column (height: 65 cm, diameter of the outside layer and inside layer: 51 and 40 cm). Water at a temperature of 10-20°C provided by a cooler (Julabo FC 1200, Germany) was applied in countercurrent with the wastewater. The water subsequently was guided through a rubber tube surrounding the UASB reactor. Water provided by a water bath (Julabo, Germany) was applied to heat the double walled digester to keep the reactor at 35°C. The UASB reactor and digester were insulated using foam sheets and aluminum.

Along the height of the UASB reactor and digester 9 (distance of 30 cm between them) and 6 (distance of 18 cm between them) sludge sampling and discharge ports were installed (Fig.4.1), respectively. The sludge was recirculated from the UASB sludge port 4 (U4) to the digester sludge port 1 (D1) and recirculated to the UASB reactor from port D4 to port U2. Each two hours the UASB sludge bed was stirred for two minutes at 1 rpm to avoid short circuiting and dead zones. The digester worked as a continuous

Table 4.1 Operational and design parameters of the UASB-digester system

Parameter	UASB	Digester
Temperature (°C)	10-20	35
Diameter (cm)	23.5	23.5
Liquid height (m)	3	1
Working volume (L)	130	43
Up - flow velocity (m/h)	0.5	-
HRT (d)	0.25	0.5
Mixing intensity (rpm)	0.02	83

stirred tank reactor (CSTR), and mechanical mixing was applied at 83 rpm. Weekly 10-15 L of excess sludge was wasted from the UASB reactor from U8 and 0-5 L from the digester from D5.

The design and the operational parameters of the pilot-scale UASB-digester are shown in Table 4.1. The inoculum that was used to start-up the system was 1-year stored sludge from a similar UASB-digester system (Zhang et al., 2013). The UASB-digester in this study had already been operated for a period of 2 years on domestic sewage with a COD concentration of 627 ± 213 mg/L and a temperature of 10-20°C before starting the here presented experiments. A high sludge recirculation rate of 16% of the wastewater flow rate was applied to enable full transfer of fresh influent $COD_{suspended}$ to the digester. To confirm that a sufficiently high recirculation rate was applied, the stability of the UASB sludge was determined in batch experiments (see later). The wastewater temperature was decreased in steps of 2.5°C from 20 to 10°C. Each step lasted for a minimum of 6 weeks, provided that the fluctuation in COD_t removal was less than 10%. The periods of each temperature operation were: 0-46, 47-94, 95-142, 143-212, 213-262 and 263-287 days for 20, 17.5, 15, 12.5, 10 and 11-13°C respectively.

4.2.2 Batch experiments

4.2.2.1 Specific methanogenic activity (SMA) and stability of the sludge

The SMA refers to the maximum rate of methane production per gram of volatile suspended solids (VSS). The stability of sludge presents the fraction of biodegradable

COD that still is present in the sludge and that can be converted into methane. The SMA and stability tests were performed according to the method reported by Zhang et al. (2013). Approximately 30 mL sludge samples were collected from each sludge port of the UASB reactor and mixed for the SMA tests at 10, 15, 25, and 35°C and for stability tests. The digester sludge samples of about 200 ml were collected from port D5.

4.2.2.2 Biological methane potential (BMP) of domestic wastewater

The BMP is the maximum amount of methane, expressed as g CH_4 COD/ g COD, that can be produced from a substrate. The BMP of domestic wastewater was determined at 35°C according to a procedure described by Zhang et al. 2013. Different fractions of the domestic wastewater, viz. raw, paper filtered and membrane filtered domestic wastewater were tested for 30 days because after these 30 days no further methane production was observed. BMP of the influent COD_{total}, $COD_{suspended}$ and $COD_{soluble}$ was calculated. The average BMP in the whole study period was used to calculate the biodegradable fraction of influent OLR.

4.2.2.3 Dissolved methane in the effluent

Dissolved methane concentrations in effluent samples were determined in triplicate by gas chromatography (GC) analyses according to the method described by Zhang et al. (2013).

4.2.3 Microbial community analysis

The samples for microbial community analysis were taken from the influent, the effluent, the mixed sludge from U1-U9 of the UASB reactor, the digester and U4.

45 ml of each sample was collected on 31 d, 88 d, 117 d, 166 d and 249 d for 20, 17.5, 15, 12.5 and 10°C, respectively. Total DNA was extracted from sludge samples that were stored at -20°C, according to (Vilchez-Vargas et al., 2013). A conventional PCR, targeting total bacteria, was performed prior to real-time PCR analysis according to (Boon et al., 2002), using the total bacterial primers P338f and P518r (Muyzer et al., 1993), to verify if no components were present in the DNA extracts that could inhibit PCR. The quality of DNA extracts and PCR products were validated by agarose gel electrophoresis. Real-time PCR analysis was carried out using a StepOnePlus™ Real-Time PCR System (Applied Biosystems, Carlsbad, CA). The methanogens Methanobacteriales, Methanomicrobiales, Methanosarcinaceae, and Methanosaetaceae, as well as total Bacteria were analyzed, as described earlier by Desloover et al. (2015). Each sample was analyzed in triplicate. Real-time PCR quality was evaluated through the different parameters obtained with the StepOnePlus software V2.3 (Table 1, Annex 1). Results were presented as copies per gram of wet sludge.

4.2.4 Analytical methods

The frequency of the measurements in this study is shown in Table 4.2. COD_t,

Table 4.2 Frequency of measurements

	pH	COD concentration	Biogas production rate of the UASB-digester	Methane composition of the UASB-digester	Dissolved methane	Stability and SMA of the UASB-digester sludge	TSS/VSS of the UASB-digester sludge	BMP of influent
Frequency	1-2 times/ week	1-2 times/ week	Daily	1 time / week	1 time / week	1 time/ 2 weeks	1 time /2 weeks	1-2 times / month

Table 4.3 COD concentration, OLR and BMP of domestic wastewater (- : not available)

Temperature (°C)	pH	COD concentration (mg/L)				OLR (g COD/(L d))				BMP (g CH_4 COD/g COD)			
		total	suspended	soluble	colloidal	total	suspended	soluble	colloidal	total	suspended	soluble	colloidal
10-20	7.1-7.8	630 (190)	342 (131)	167 (72)	151 (42)	2.5 (0.8)	1.4 (0.5)	0.67 (0.3)	0.61 (0.2)	0.51 (0.08)	0.55 (0.10)	0.40 (0.12)	-

$COD_{suspended}$, $COD_{colloidal}$, $COD_{soluble}$, UASB sludge COD, digester sludge COD and discharged sludge COD were measured by cuvette tests (Hach Lange, USA). Domestic wastewater, sampled after passing the influent pump, was analyzed for COD_t, distinguishing between 8 μm paper-filtered (Whatman grade 40, Germany) particulate sewage COD (COD_p) and 0.45 μm membrane-filtered (Whatman FP 30/ 0.45 CA, Germany) $COD_{soluble}$. $COD_{suspended}$ and $COD_{colloidal}$ were calculated according to $COD_{suspended} = COD_t - COD_p$ and $COD_{colloidal} = COD_p - COD_{soluble}$, respectively. A mixture of UASB sludge from the ports U1 to U9 was sampled for COD measurement.

Volatile suspended solids (VSS) and total suspended solids (TSS) were measured using standard methods given by American Public Health Association (APHA, 2005). PH was measured using a pH meter (PHM210, Radiometer analytical sas, France). Biogas production was measured by a wet gas meter (Ritter, Germany). Concentrations of nitrogen, methane, and carbon dioxide in the headspaces of the batch experiments and in the biogas produced by the UASB-digester were measured using a gas chromatograph (GC) (Interscience GC 8000 series) (Zhang et al. 2013).

900 μL of influent/effluent sample, filtered by 0.45 μm membrane filter, was mixed with 100 μL of 15% formic acid to prepare VFA samples (1.5% formic acid in the measured sample). GC (HP 5890 GC) was used to determine the VFA concentrations (Zhang et al. 2013).

4.2.5 Calculation

4.2.5.1 Solids retention time (SRT)

The SRT of the UASB digester system was calculated based on VSS concentrations and the number of solids that was wasted from the UASB and from the digester. This will be referred to as the maximum SRT (SRT_{max}). The minimum SRT (SRT_{min}) was calculated in a similar way but included wash-out of VSS with the effluent from the UASB. Effluent VSS concentrations were measured during operation of the UASB reactor at 20°C, and were found to be half of the effluent $COD_{suspended}$ concentration in this effluent (Table 2, Annex 2). No VSS concentrations are determined for the UASB effluent during operation at the other temperatures. Therewith, we took this ratio to calculate the SRT_{min}.

4.2.5.2 Methanogenic capacity

The methanogenic capacity of a reactor is defined as its maximum methane production ability in g CH_4 COD/d, and was calculated by multiplying the SMA of the sludge with the total amount of VSS in the reactor. The methanogenic capacity was compared to the real methane production rate for both the UASB reactor and digester.

4.2.5.3 COD mass balance

For each period of a constant UASB temperature, the amount of COD that was fed to the UASB-digester system was compared with the cumulative distribution of COD to: 1) Methane, 2) Discharged sludge, 3) Effluent and 4) COD that accumulated in the reactors and that was calculated as the difference between the sludge COD in the UASB-digester between the start and the end of each period.

4.2.5.4 Hydrolysis of influent organic solids in the UASB reactor

For each temperature, the hydrolysis yield of the influent $COD_{suspended}$ of the domestic wastewater in the UASB reactor was calculated according to:

Hydrolysis yield = 100× (methane production rate$_{UASB}$ - $COD_{soluble\ removal\ rate}$)/$L_{CODssbiod}$

With hydrolysis yield the fraction of organic solids of the domestic wastewater hydrolysed in the UASB reactor (%), methane production rate$_{UASB}$ the average methane production rate of the UASB reactor (g CH_4 COD/d), $COD_{soluble\ removal\ rate}$, the average $COD_{soluble}$ removal rate, (g COD/d) and $L_{CODssbiod}$ the average loading rate of biodegradable suspended COD (g COD/d).

4.3 Results

4.3.1 COD removal

The UASB-digester system achieved a stable COD removal efficiency (Fig.4.2a) in spite of a decreasing temperature from 20 to 12.5°C. The temperature did not have a significant effect on average COD removal efficiency at 12.5-20°C at P=0.05 level, and the overall average COD removal efficiency was 60.0 ± 4.6 % at 12.5 - 20°C. The mean effluent COD concentration was 242 ± 49 mg/L at an influent COD concentration of 616 ± 140 mg/L. At 10°C the COD removal efficiency decreased to 51.5 ± 5.5%. This was accompanied by a significant increase of the influent COD concentration from 514 ± 110 at 12.5°C to 764 ± 124 mg/L at 10°C. The temperature was subsequently increased from 10 to 11-13°C, in an attempt to recover the performance. As a result, the average effluent COD decreased again to 237 ± 43 mg/L, similar to that achieved at 12.5-20°C.

The results in Fig.4.2b show that the average $COD_{suspended}$ removal efficiencies at temperatures between 10 and 20°C were not significantly different at P= 0.05 level, with an overall efficiency of 76.0 ± 9.1%. The average effluent $COD_{suspended}$ concentration at temperatures of 12.5 to 20°C was 67 ± 28 mg/L at an average influent $COD_{suspended}$ concentration of 306 ± 111 mg/L. The effluent $COD_{suspended}$ concentration increased to 100 ± 23 mg/L as the temperature decreased from 12.5 to 10°C. This increase was probably due to the significant increase of the influent $COD_{suspended}$ concentration from 208 ± 43 to 463 ± 114 mg/L in this period. The high influent COD concentration in this period was therefore mainly due to the increase of the $COD_{suspended}$ concentration. During the last period, operating the UASB at temperatures of 11-13°C, the average effluent $COD_{suspended}$ concentration decreased to 56 ± 11 mg/L, similar to that achieved before at 12.5-20°C.

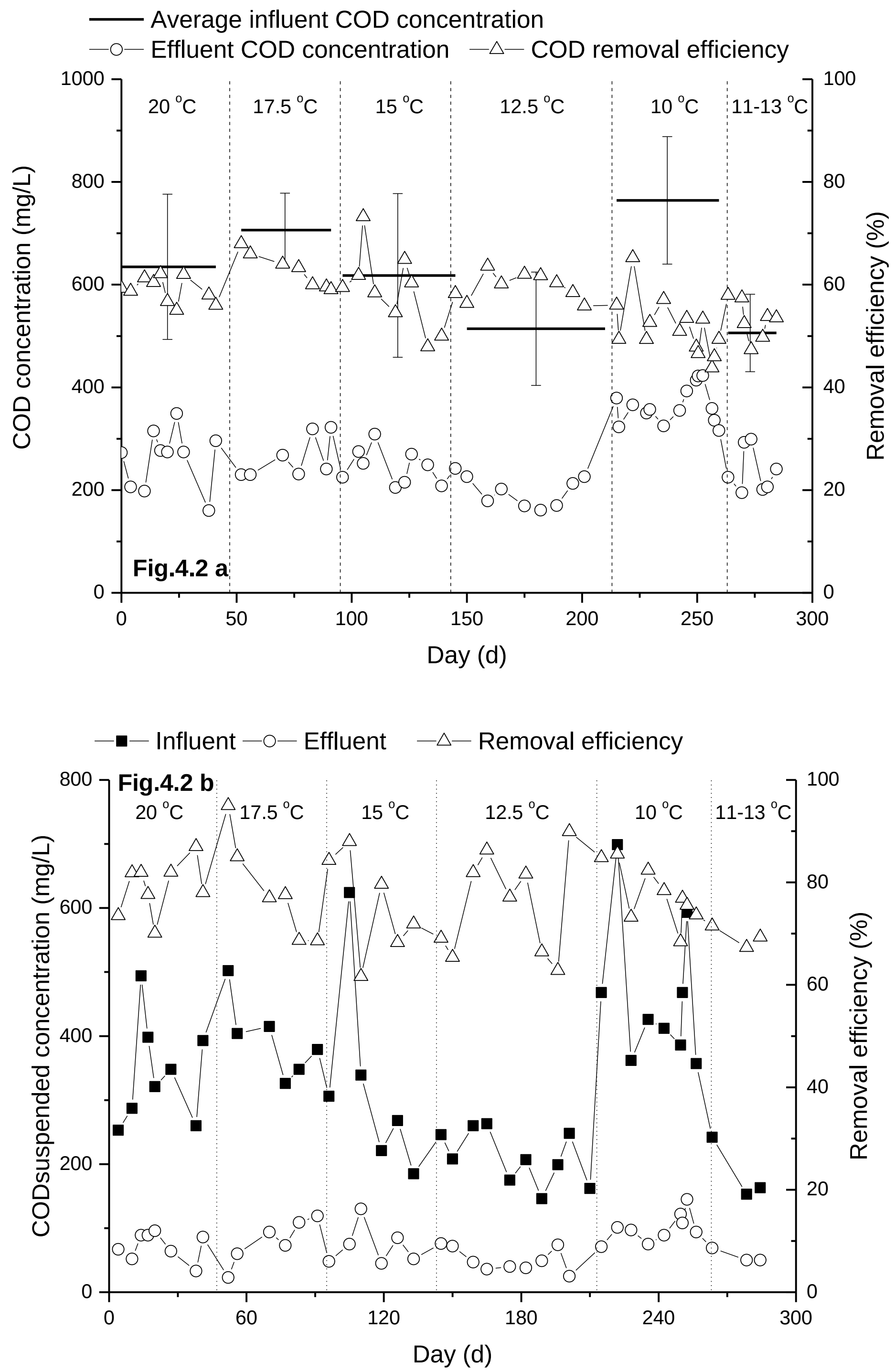
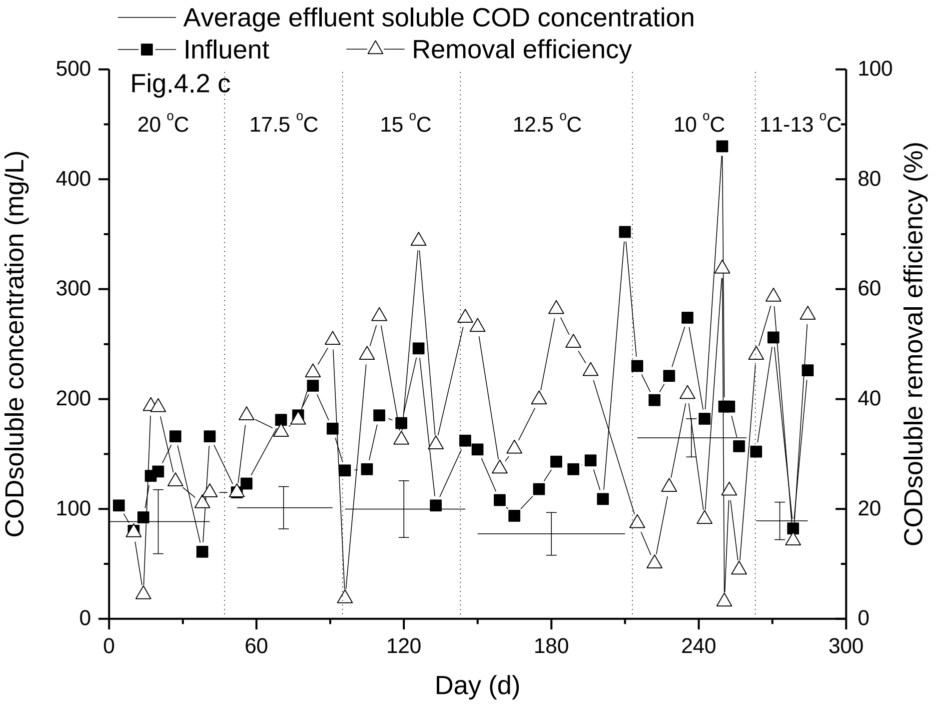


Fig.4.2 COD removal efficiency of the UASB-digester treating municipal wastewater at 10-20°C (a: total COD removal; b: suspended COD removal)



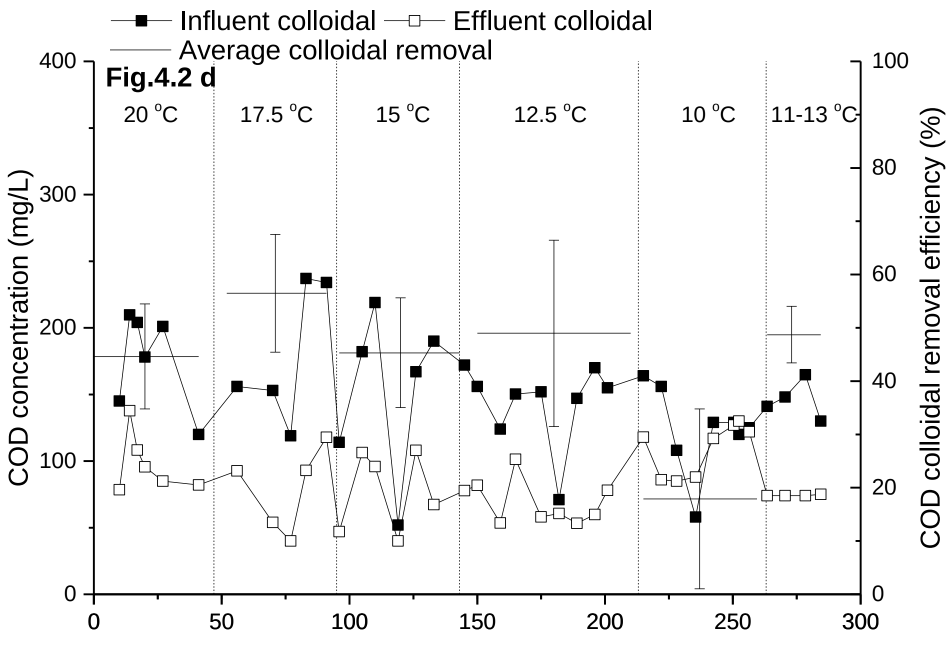


Fig.4.2 COD removal efficiency of the UASB-digester treating municipal wastewater at 10-20°C (c: soluble COD removal; d: colloidal COD removal)

$COD_{soluble}$ removal is shown in Fig.4.2c. The average effluent $COD_{soluble}$ concentration did not significantly change during the period when the temperature was decreased from 20 to 12.5°C at P= 0.05 level, and it was 91 ± 25 mg/L. No VFA could be detected in the effluent (data not shown). The $COD_{soluble}$ decreased was close to the BMP of the influent $COD_{soluble}$ (Table 4.3). At 10°C, the average effluent $COD_{soluble}$ increased to 165 ± 17 mg/L and VFA was detected at a concentration of 36.2 ± 7.9 mg COD/L (about 5%

of the influent COD as shown in Fig.4.5). The latter indicated that the methanogenic capacity of the UASB reactor was insufficient to deal with the increased loading rate. After the temperature was increased to 11-13°C, and the influent COD concentration decreased during the same period, the $COD_{soluble}$ removal efficiency increased to 44.0 ± 20.4% with an average effluent $COD_{soluble}$ concentration of 89 ± 17 mg/L. VFA was no longer detected.

The average $COD_{colloidal}$ removal efficiency was relatively stable at 12.5-20°C (at P=0.05 level): 42.8 ± 17.5% (Fig.4.2d). The average $COD_{colloidal}$ removal efficiency decreased to 17.9 ± 16.9 % at 10°C. The influent $COD_{colloidal}$ concentration of 124 ± 32 mg/L at 10°C was not significantly different from the one (151 ± 47 mg/L) in the whole study period.

4.3.2 Methane production rate

The methane production rate of the digester followed the biodegradable fraction of the influent $COD_{suspended}$ rate during the whole operational period (Fig.4.3). The average methane production rate was not significantly different at P=0.05 level during each period with different influent temperatures. The average methane production was 60 ± 17 g CH_4 COD/d, which was lower than the average influent loading rate of biodegradable $COD_{suspended}$ of 90 ± 36 g CH_4 COD/d. The methane production rate of the digester was 67% of influent loading rate of biodegradable $COD_{suspended}$, which indicated that major part of the influent $COD_{suspended}$ was biodegraded in the digester.

Fig.4 shows the average methane production rate of the UASB reactor at the different temperatures. Methane production included gaseous methane and effluent dissolved methane. The average measured gaseous methane production and load of effluent dissolved methane at 10-20°C were 22 ± 10 and 37 ± 9 g CH_4 COD/d, respectively. The measured load of effluent methane was in agreement with the one calculated with Henry's law using a methane fraction of 65.5 ± 3.1% in the biogas of the UASB reactor.

The average removal rate of $COD_{soluble}$ at 10-20°C was 26 ± 6 g COD/d (Fig.4). The methane production of the UASB reactor was expected coming from the $COD_{soluble}$ biodegradation. However, the $COD_{soluble}$ removal rate was significantly lower than the average methane production rate of the UASB reactor of 59 ± 10 g COD/d. The difference can be attributed to the hydrolysis of influent organic solids in the UASB reactor, as will be discussed later. This is also in agreement with the results reported by Zhang et al. (2012) and Zhang et al. (2013), and can also explain the lower methane production rate in the digester in comparison with the loading rate of influent $COD_{suspended}$ (Fig.4.3).

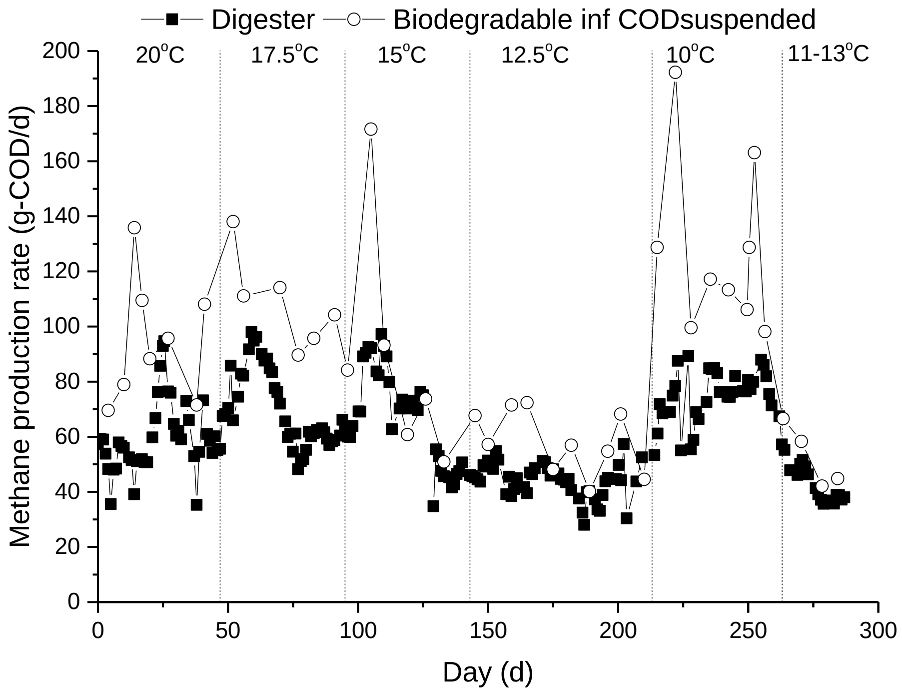


Fig.4.3 Methane production rate (g CH_4 COD/d) of the digester in the UASB-digester treating municipal wastewater at 10-20°C

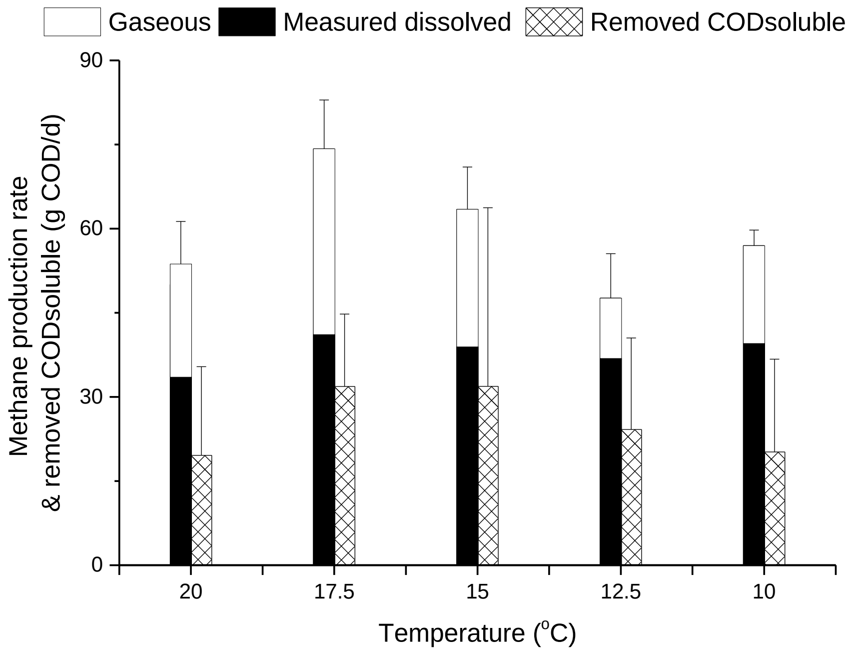


Fig.4.4 Methane production rate (g CH_4 COD/d) and removed $COD_{soluble}$ rate of the UASB reactor in the UASB-digester treating municipal wastewater at 10-20°C

4.3.3 COD mass balance

Fig.4.5 shows the (average) COD balance of the UASB-digester system at 12.5-20°C and at 10°C. Methane production accounted for 40 ± 4% at 10-20°C. Given the influent BMP of 51 ± 8% (Table 4.3), an average 80% of the influent BMP was converted to methane. Methane production in the UASB reactor contributed 49 ± 5% to the total methane production. The gaseous methane and dissolved methane accounted for 18% and 31% of the total methane production, respectively. The average discharged sludge COD at 10-20°C accounted 8 ± 5% of influent COD, which is low compared with traditional wastewater treatment. There was almost no COD (< 3%) accumulation at 10-20°C.

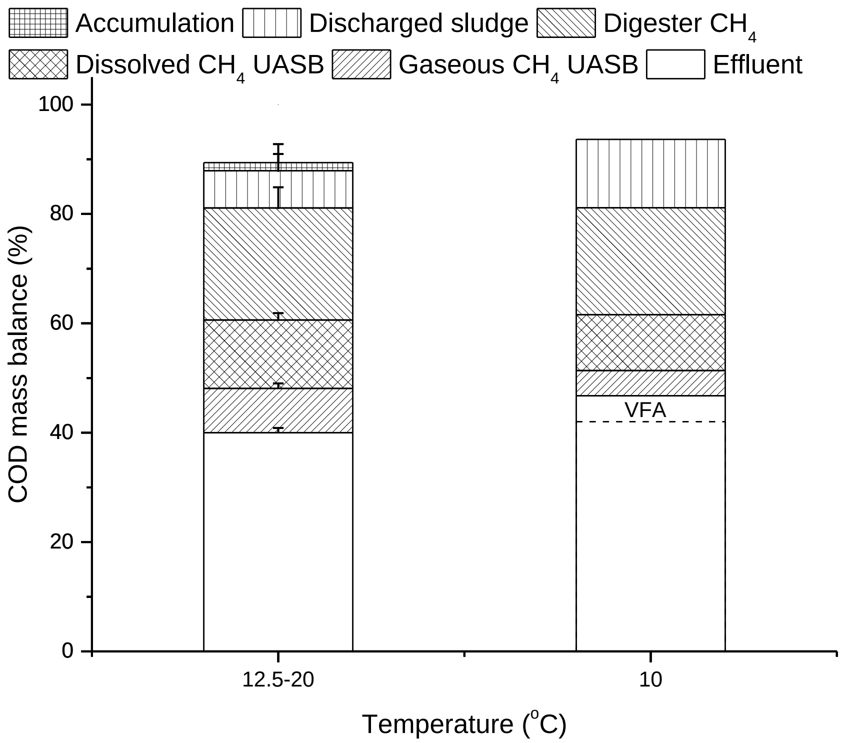


Fig.4.5 COD mass balance of the UASB-digester treating municipal wastewater at subsequently 12.5-20°C and 10°C

4.3.4 Methanogenic capacity

SMA, stability, VSS and SRT of the UASB sludge and the digester sludge were relatively stable throughout the entire operational period (Table 4.4). The average methanogenic capacity of the UASB reactor at 35°C was 264 ± 20 g CH_4 COD/d. The methanogenic capacity of the UASB reactor decreased from 77 ± 4 g CH_4 COD/d at 20°C to 31 ± 4 g CH_4 COD/d at 10°C. The methanogenic capacity of the UASB reactor was sufficient at 12.5-20°C to handle the loading rate of biodegradable influent $COD_{soluble}$. However, the methanogenic capacity at 10°C was insufficient due to the

Table 4.4 SMA, stability, VSS, SRT, and methanogenic capacity of the UASB-digester system treating municipal wastewater at 10-20°C, and BMP of influent $COD_{soluble}$ rate. The standard deviation is in the brackets.

Temperature	SMA at 35°C		Stability		VSS		SRT	Methanogenic capacity			Influent soluble BMP
	UASB	Digester	UASB	Digester	UASB	Digester	UASB-Digester	UASB at 35°C	Digester at 35°C	UASB Low temperatures	
°C	g CH_4 COD /(gVSS d)		g CH_4 COD / g sludge COD		g/L		(d)	g CH_4 COD/d			g CH_4 COD/ d
20	0.27 (0.06)	0.27 (0.05)	0.24 (0.01)	0.20 (0.01)	10.1 (1.4)	8.2 (2.2)	42-139	241 (13)	112 (24)	77 (4)	23 (2)
17.5	0.28 (0.02)	0.27 (0.02)	0.24 (0.03)	0.18 (0.03)	9.0 (1.1)	7.9 (0.6)	39-103	244 (27)	105 (17)	64 (7)	33 (2)
15	0.24 (0.02)	0.29 (0.01)	0.28 (0.03)	0.21 (0.02)	10.7 (0.6)	9.0 (0.8)	75-1670	268 (12)	132 (15)	67 (3)	33 (4)
12.5	0.25 (0.02)	0.27 (0.01)	0.26 (0.01)	0.20 (0.01)	10.9 (0.4)	9.1 (0.11)	59-121	287 (19)	118 (11)	46 (2)	30 (5)
10	0.25 (0.04)	0.29 (0.03)	0.23 (0.02)	0.20 (0.01)	10.3 (0.8)	8.0 (0.70)	42-69	279 (36)	119 (11)	31(4)	46 (4)

significant increase of the $COD_{soluble}$ loading rate during this period. The average stability of the digester sludge and UASB sludge was 0.20 ± 0.01 and 0.25 ± 0.02 CH_4 COD/ g sludge COD, respectively. The average SMA of the digester and UASB sludge was respectively 0.28 ± 0.01 and 0.26 ± 0.02 CH_4 COD/ (g VSS d). SRT of the UASB-digester system was longer than 39 d.

4.3.5 Microbial community analysis

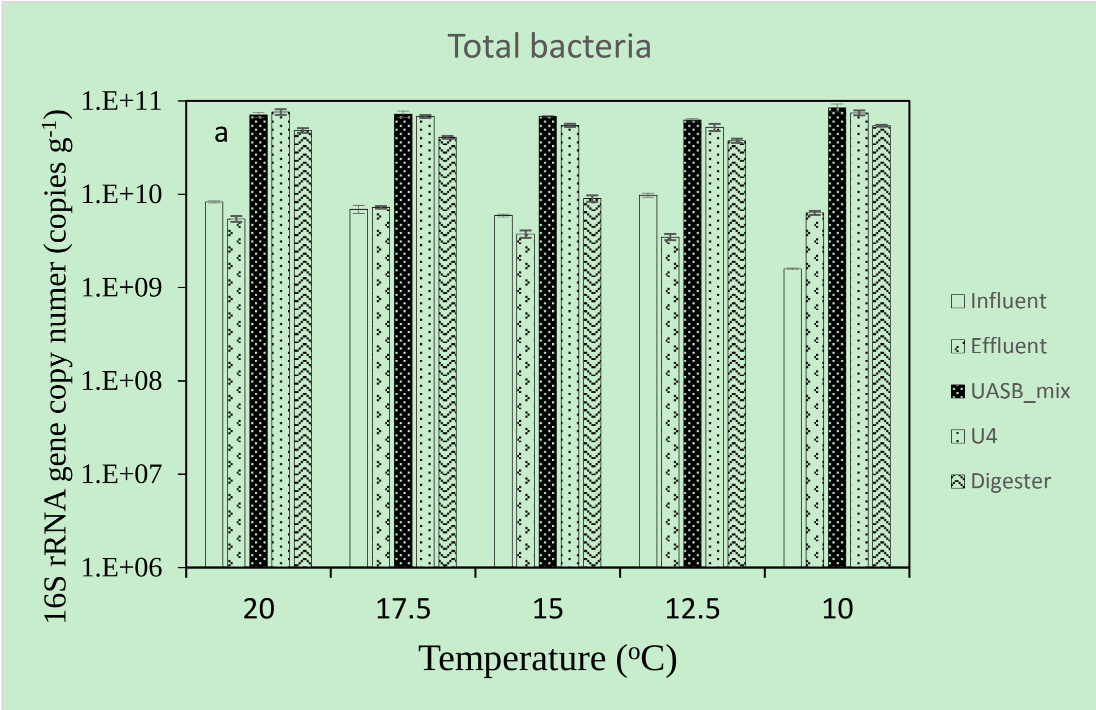
Real-time PCR analysis revealed similar levels of total Bacteria and total Archaea (assumed to be methanogens) in the UASB sludge and in the digester sludge, irrespective of temperature (Fig.4.6a and 4.6b). Total bacteria count in the influent and effluent samples were similar, and on average a factor 10 lower than in the UASB sludge and digester sludge. In contrast, total Archaea were, with the exception of the sample at 20°C, a factor 10 lower in the influent compared to the effluent. Nonetheless, total Archaea were still a factor 20-40 lower in the effluent compared to the UASB and digester samples.

A more detailed view on the methanogenic community revealed an overall dominance of the acetoclastic Methanosaetaceae and the hydrogenotrophic Methanomicrobiales (Fig.4.6c, 4.6d and 4.6e). A lower level of gene copies as found for the different methanogenic groups and total methanogens, could be observed in the influent and effluent samples compared to the UASB sludge and the digester sludge. The methanogenic community was similar in the digester and UASB samples. The number of gene copies of Methanosaetaceae were similar to Methanomicrobiales. However, Methanosaetaceae in the digester sludge showed a slight decrease in abundance at 12.5 and 10°C, compared to the higher temperatures (15-20°C). Methanobacteriales, although being less abundant, showed a clear increase at 10°C. Methanosarcinaceae were not detected in any of the samples.

4.4 Discussion

The present research shows an overall average COD removal efficiency of 60.0 ± 4.6 % at temperatures between 12.5 - 20°C. Latter is somewhat lower as compared to the results of Mahmoud et al. (2004) achieved at a UASB temperature of 15°C, probably as a result of the lower applied $COD_{suspended}$ to $COD_{soluble}$ ratio in the domestic wastewater. A high influent $COD_{suspended}$ fraction can contribute to a high methane production and herewith a high methanogenic biomass production in the digester. Because this biomass is recirculated to the UASB reactor, it helps to enhance the methanogenic capacity of the UASB reactor and thus $COD_{soluble}$ removal.

The present results showed that even at temperatures as low as 12.5°C the methanogenic capacity of the UASB reactor was sufficient to maintain an effluent $COD_{soluble}$ concentration of 91 mg COD/L. However, lower temperatures (10°C), accompanied by higher influent COD loading rate resulted in an overloading of the UASB reactor and higher effluent concentrations. Zhang et al (2013) show that such



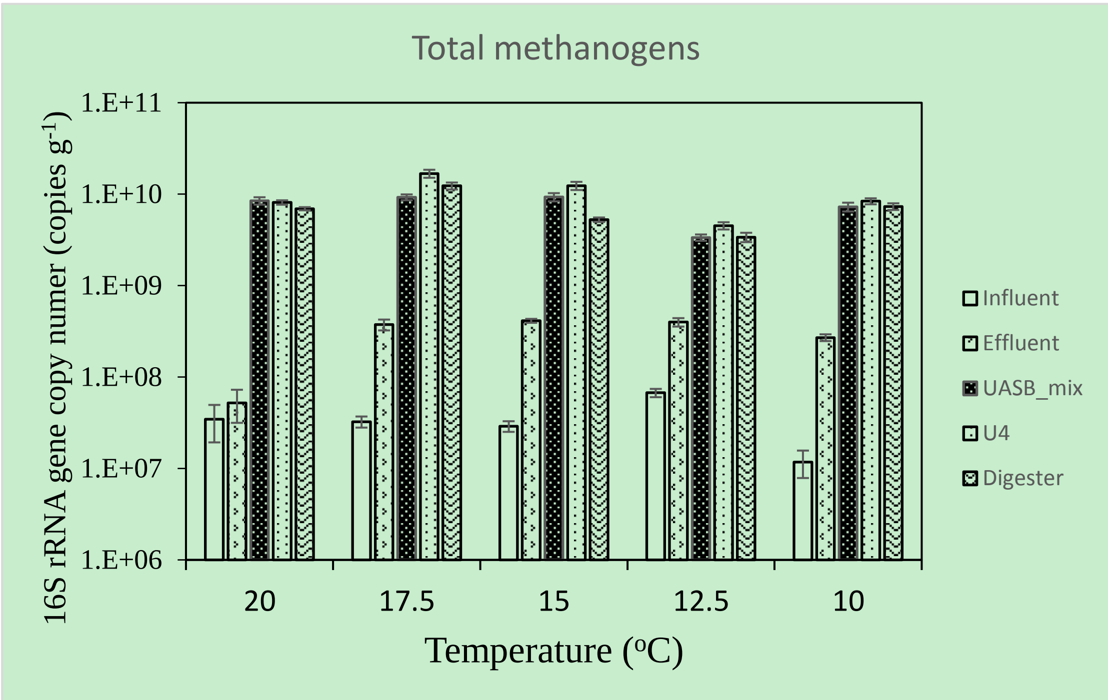
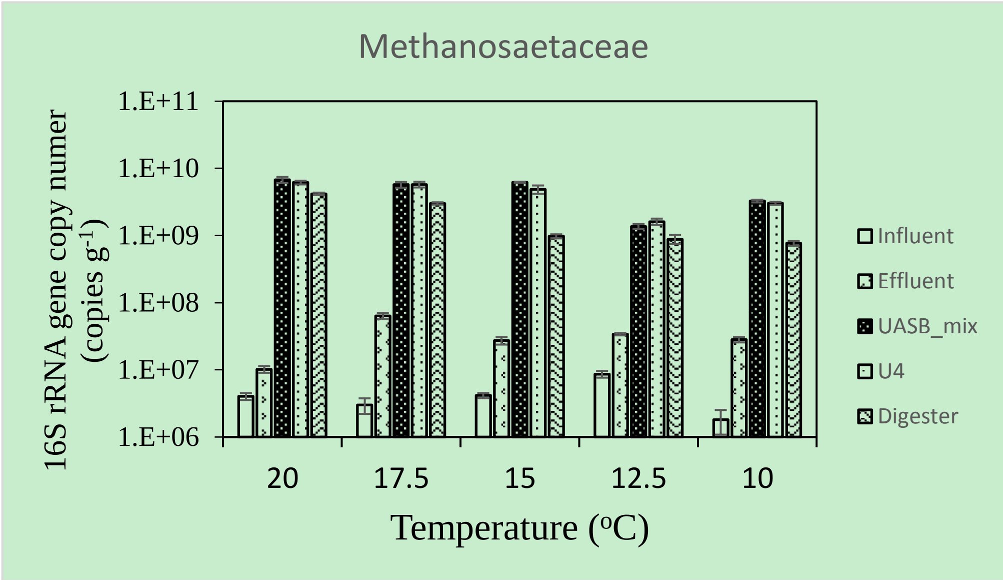
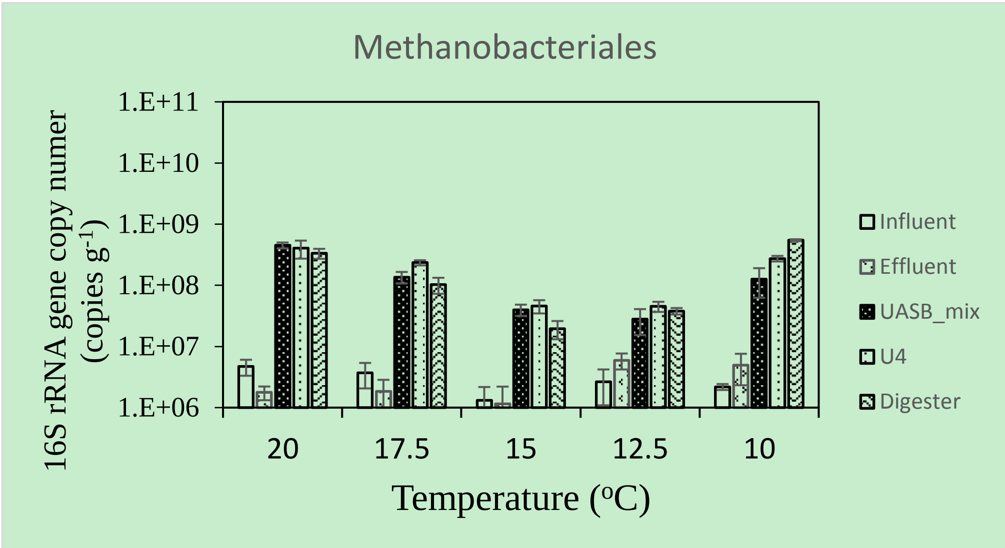
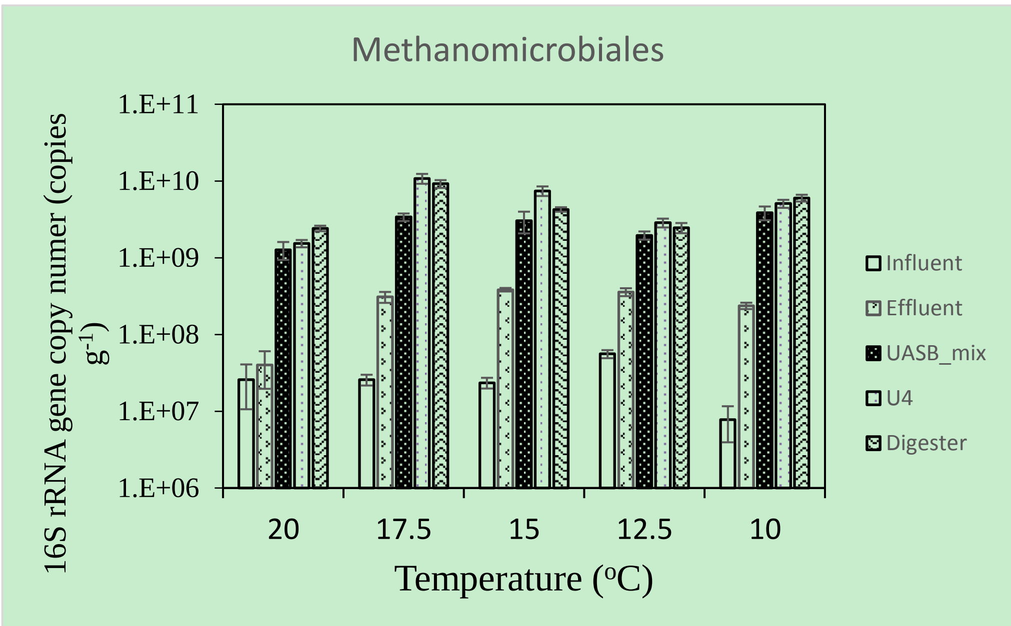


Fig.4.6 Microbial community of the UASB-digester treating municipal wastewater at subsequently 20,17.5,15,12.5 and 10°C. a: total bacteria, b: total methanogens, c: methanosaetaceae, d: methanobacteriales, e: methanomicrobiales


Methanosaetaceae
16S rRNA gene copy numer (copies g-1)
1.E+11
1.E+10
1.E+09
1.E+08
1.E+07
1.E+06
20
17.5
15
12.5
10
Temperature (oC)
Influent
Effluent
UASB_mix
U4
Digester


Methanobacteriales
16S rRNA gene copy numer (copies g-1)
1.E+11
1.E+10
1.E+09
1.E+08
1.E+07
1.E+06
20
17.5
15
12.5
10
Temperature (oC)
Influent
Effluent
UASB_mix
U4
Digester


Methanomicrobiales
16S rRNA gene copy numer (copies g-1)
1.E+11
1.E+10
1.E+09
1.E+08
1.E+07
1.E+06
20
17.5
15
12.5
10
Temperature (oC)
Influent
Effluent
UASB_mix
U4
Digester

lower performance could be mitigated by a (temporary) extra addition of COD to the digester (so called co-digestion) to increase the growth of methanogens and other anaerobic biomass for extra $COD_{soluble}$ conversion in the UASB after the sludge recirculation.

Approximately 38% of the influent biodegradable $COD_{suspended}$ could already be hydrolyzed in the UASB reactor, in spite of the low temperatures. No significant difference was shown for the hydrolysis of influent $COD_{suspended}$ at the different applied temperatures due to the large standard deviation of $COD_{soluble}$ removal. Also other studies towards UASB-digester systems showed a substantial hydrolysis in the UASB reactor at lower temperatures (Álvarez et al., 2004; Mahmoud et al., 2004; Zhang et al., 2013; Zhang et al., 2012). In contrast, the hydrolytic efficiency of organic solids in a separate UASB reactor at 15°C only was 25%, when operated at an HRT of 6 h (Mahmoud et al., 2004). Evidently in the UASB-digester system excess hydrolytic enzymes are recirculated to the UASB reactor and increase hydrolysis at low temperatures (Zhang et al., 2016a). However, it results in an additional $COD_{soluble}$ load and therefore increases the required methanogenic capacity in the UASB reactor. Latter should be taken into account when designing a UASB-digester.

Due to incomplete hydrolysis of wastewater $COD_{suspended,}$ extra $COD_{colloidal}$ may be produced in the UASB reactor. Thus, it was overloading at 10°C. For aerobic reactors it is known that lower temperatures have a negative effect on the flocculation of $COD_{colloidal}$ (van den Brink et al., 2011). Although this has not yet been studied, anaerobic sludge flocculation at low temperatures could be poor as well.

The measured effluent dissolved methane of 74 mg COD /L, corresponding to a production rate of 37 g CH_4 COD/d, did not increase when temperature of the UASB reactor decreased. This is in agreement with Matsuura et al. (2015), who found that the dissolved methane concentration only exhibited small changes between 23.5-28°C and 14.6-24.2°C, i.e. 74 and 72 mg CH_4 COD/L, respectively. Souza et al. (2011) found a similar dissolved methane concentration when applying a UASB reactor treating domestic wastewater at 25°C. However, the dissolved methane was oversaturated at the applied mesophilic conditions. The saturated dissolved methane production based on the solubility of methane at 10 and 20°C should be 84 and 46 g CH_4 COD/d, respectively (Yamamoto et al., 1976). Therewith, methane in the liquid phase in this study was not saturated at 10-20°C. The low dissolved methane concentration was probably due to the low total methane production of the UASB reactor.

Post treatment of the UASB-digester effluent at 242 mg COD/L is required to reach effluent COD standards (e.g. EU standard of 125 mg/L). Downflow hanging sponge (DHS) reactors, rotating biological contactors and trickling filter systems can be alternatives to achieve such an effluent concentration (Beas et al., 2015; Chernicharo et al., 2015; De Almeida et al., 2009; Tawfik et al., 2003). Because methane has a strong global warming potential (25 times the one for carbon dioxide), the dissolved methane in the effluent of 74 mg COD/L should be removed or, preferably, recovered. Hollow-fiber membranes and a poly-di-methyl-siloxane (PDMS) membrane contactor were tested for degasification and to strip the dissolved methane using nitrogen gas, respectively (Cookney et al., 2012; Hatamoto et al., 2010). In this manner 72% and 86% of the dissolved methane were recovered, respectively. Two stages of DHS were applied to subsequently remove the remaining effluent dissolved methane (Matsuura et al., 2015). 58-88% of the dissolved methane was recovered in the first stage, and the residual dissolved methane was almost completely oxidized in the second stage. However, the economic assessment and energy consumption should be considered before applying these technologies.

Methane production of the digester accounted for half of the total methane production, which was higher than the 14% and 33% reported by Mahmoud et al. (2004) and Álvarez et al. (2004), respectively. The higher methane production of the digester in this study was attributed to the higher sludge recirculate rate of 16% of the influent flow rate, resulting in more influent $COD_{suspended}$ transfer to the digester. This also gave a very low $COD_{suspended}$ accumulation in the UASB-digester system and herewith a relatively long SRT (> 39 d). The latter resulted in an improved stability of the UASB sludge (0.25 g CH_4 COD/g COD) compared to stabilities reported by Mahmoud et al. (2004) of 0.36 g CH_4 COD/g COD. Also the SMA of the UASB sludge and digester sludge of 0.26 and 0.28 g CH_4 COD/(g VSS d) were considerably higher than the results reported by Álvarez et al. (2004) of 0.079 and 0.125 g CH_4 COD/(g VSS d) (SMA were measured at 35°C).

Energy cost for heating the sludge recirculated from the UASB reactor to the digester depends on sludge recirculation rate. The sludge recirculation rate in this study was 16% of the influent flow rate. In steady state, no accumulation was found because the high sludge recirculation resulted in a low sludge production. The sludge recirculation rate can be further optimized with respect to energy production, minimizing energy consumption and minimizing the digester volume. Under the applied sludge recirculation rate, methane production can compensate for only 20% of the heating energy at 10°C (see supporting material in Annex 3). In practice, sludge recirculation might be small as the VSS concentration of a full scale UASB reactor treating domestic sewage can be expected to be relative high (about 30 g/L) compared with this study (Florencio et al., 2001). Two alternatives can be used to reduce the heating energy. One is to operate the digester at a lower temperature, e.g. 25-30°C instead of 35°C. A second option would be to concentrate the UASB sludge by sedimentation, thus reducing the amount of water that needs to be recirculated. Furthermore, a heat exchanger could be installed for the recirculated sludge. The energy of the recirculated digester sludge (35°C) can be reused for heating the recirculated UASB sludge (10°C).

Methanomicrobiales and Methanosaetaceae were equally dominant methanogens found in the UASB-digester system during 10-20°C. These methanogens are classified with a high affinity for the substrate. This was identified by the fact that the methane production of the UASB reactor (including dissolved methane) matched well with the methanogenic capacity at 12.5-20°C. Acetoclastic methanogens and hydrogenotrophic methanogens are the two major populations for methane production (Demirel & Scherer, 2008). A similar composition of the microbial community was observed during domestic wastewater treatment in an UASB reactor at 20°C (Saha et al., 2015). This shows that both acetoclastic and hydrogenotrophic pathways are used for methane production at low temperatures. As the biomass was alternatingly exposed to mesophilic and (almost) psychrophilic conditions, it may be that mesophilic selection out competes the psychrophilic selection, or that reaching equilibrium takes an even longer time. However, Bialek et al. (2014) and Bandara et al. (2012) showed that, although both pathways can take place, hydrogenotrophic methanogenesis appears to be the main pathway for methane production at low temperatures, which might explain the apparent increase in abundance of Methanobacteriales at 10°C in the UASB-digester system. In contrast, aceticlastic methanogens were abundant when a bench-scale anaerobic membrane bioreactor (AnMBR) equipped with submerged flat-sheet microfiltration membranes was operated at 15°C treating domestic wastewater (Smith et al., 2013).

The here presented reactor system can become a key technology within a more sustainable treatment scheme for treatment of domestic wastewater as compared to nowadays generally applied conventional activated sludge processes. Coupling this anaerobic system with i.e DAMO technology (Kampman *et al,* 2012) can mitigate the detrimental CH_4 emission and link that with nitrogen removal. An interesting technique for removal and recovery of phosphorus was recently published by Drenkova-Tuhtan et al. (2016), applying nanocomposite magnetic particles for adsorption and desorption of phosphate from wastewater.

Bio-flocculation followed by anaerobic sludge digestion, as applied in the AB process, is referred to as another alternative for activated sludge treatment (Verstraete et al., 2009), and sewage organic matter from which methane can be produced by anaerobic sludge digestion (Faust et al., 2014). Main advantage as compared to direct anaerobic treatment of domestic sewage is the absence of dissolved methane in the liquid anaerobic effluent. However, it needs an energy input of 0.03 kWh/ m^3 (wastewater) for aeration (Khiewwijit et al. 2015).

4.5 Conclusions

A pilot scale UASB-digester treating domestic wastewater at 10-20°C at an HRT of 6 h:

- Achieved a stable COD removal efficiency of 60 ± 4.6%, while temperature decreased from 20-12.5°C at an influent COD concentration of 616 ± 140 mg/L;
- Achieved, at 10°C a COD removal efficiency of 51.5 ± 5.5%; reduction in COD removal efficiency is mainly due to an increased influent COD concentration from 514 ± 110 at 12.5°C to 764 ± 124 mg/L;
- Achieved a high $COD_{suspended}$ removal efficiency of 76.0 ± 9.1%;
- Achieved a varying $COD_{soluble}$ removal due to fluctuations in influent composition; effluent COD concentration maintained 90 ± 23 mg/L at 12.5 to 20°C;

- Achieved a methane yield of 40 ± 4% of the influent COD, which was 80% of the influent BMP. 49% of the total methane production, was produced in the low temperature UASB reactor, and the remainder in the digester. Discharge sludge accounted for 8 ± 5% of influent COD;
- Resulted in a stable SMA of the UASB sludge and the digester sludge of 0.26 ± 0.03 and 0.29 ± 0.03 g CH_4 COD/ (g VSS d); the stability of the UASB sludge and the digester sludge was stable at 0.25 ± 0.02 and 0.20 ± 0.02 g CH_4 COD/g COD;
- Resulted in a microbial population where Acetoclastic Methanosaetaceae and hydrogenotrophic Methanomicrobiales were the dominant methanogens.

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5 Hydrolysis rate constants at 10-25°C can be more than doubled by a short anaerobic pre-hydrolysis at 35°C

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Abstract

Hydrolysis is the first step of the anaerobic digestion of complex wastewater and considered as the rate limiting step especially at low temperature. Low temperature (10-25°C) hydrolysis was investigated with and without application of a short pre-hydrolysis at 35°C. Batch experiments were executed using cellulose and tributyrin as model substrates for carbohydrates and lipids. The results showed that the low temperature anaerobic hydrolysis rate constants increased by a factor of 1.5 to 10, when the short anaerobic pre-hydrolysis at 35°C was applied. After the pre-hydrolysis phase at 35°C and decreasing the temperature, no lag phase was observed in any case. Without the pre-hydrolysis, the lag phase for cellulose hydrolysis at 35-10°C was 4 - 30 days. Tributyrin hydrolysis showed no lag phase at any temperature. The hydrolysis efficiency of cellulose increased from 40 to 62%, and from 9.6 to 40% after 9.1 days at 15 and 10°C, respectively, when the pre-hydrolysis at 35°C was applied. The hydrolysis efficiency of tributyrin at low temperatures with the pre-hydrolysis at 35°C was similar to those without the pre-hydrolysis. The hydrolytic activity of the supernatant collected from the digestate after batch digestion of cellulose and tributyrin at 35°C was higher than that of the supernatants collected from the low temperature (≤ 25° C) digestates.

5.1 Introduction

Anaerobic treatment of municipal wastewater is attractive as it has low operational costs, produces low amounts of excess sludge and recovers energy in the form of methane compared with traditional aerobic wastewater treatment (Chong et al., 2012). Temperature of municipal wastewater in large parts of the world is lower than favourable for anaerobic treatment at least when a short hydraulic retention time (HRT) is applied. Low temperature methanogenesis has recently been intensively studied (McKeown et al., 2012; McKeown et al., 2009a; O'Reilly et al., 2009). Chemical oxygen demand (COD) removal efficiencies of 82 - 92 % were achieved at a temperature range of 4 - 15°C applying anaerobic reactors such as an expanded granular sludge bed (EGSB) reactor, a combined EGSB-anaerobic filter (AF) reactor, and an anaerobic membrane bioreactor (AnMBR) for the treatment of mainly soluble COD (COD_{sol}) (McKeown et al., 2009b; Rebac et al., 1999b; Smith et al., 2013). However, studies on low temperature anaerobic hydrolysis are scarce.

Hydrolysis is the first step of the anaerobic digestion of complex wastewater and considered as the rate limiting step (Hendriks & Zeeman, 2008; Lettinga et al., 2001; Pavlostathis & Giraldo-Gomez, 1991b). Zeeman (1991b) reported on the hydrolysis of suspended COD (COD_{ss}) during the batch digestion of cow manure; hydrolysis efficiency increased from 12 to 27% as temperatures increased from 5 to 25°C during 125 days of batch digestion. When operating an upflow anaerobic sludge blanket (UASB) reactor for domestic sewage treatment at an HRT of 3 h and 17°C, the particulate organic matter was effectively removed by entrapment in the sludge bed, but the hydrolysis efficiency of the entrapped organics was only 0.7 % (Zeeman et al., 1997). Uemura and Harada (2000) showed a drop in the hydrolysis efficiency from 58% at 25°C to 33% at 13°C, when studying sewage treatment applying a UASB reactor at an HRT of 4.7 h and 25 - 13°C. Also the anaerobic treatment of black water in a UASB-septic tank was shown to have a poor performance during the winter period (temperature lower than 14°C): 60% of the influent COD was accumulated as solids in the sludge bed while about 30% was discharged as COD_{sol} with the effluent (Luostarinen et al., 2007).

Novel anaerobic reactors are being developed to prolong sludge retention time (SRT) to improve hydrolysis efficiency at low temperatures. The hydrolysis of domestic sewage COD_{ss} in an AF or anaerobic hybrid (AH) reactor was respectively 11.8 and 12.3% at 13°C, when operated at an HRT of 4 h (Elmitwalli et al., 2002b), but increased to 36.7 - 42.2% in a combined AF-AH system at an HRT of 2-4 – 4-8 h (Elmitwalli et al., 2002a). A UASB-digester system for low temperature domestic sewage treatment includes a mesophilic digester to stabilize the influent organic solids captured in the UASB sludge (Álvarez et al., 2004; Mahmoud et al., 2004). The hydrolysis efficiency in a UASB-digester system increased from 25 to 44% compared with a single UASB reactor (Mahmoud et al., 2004). In such a UASB-digester system, Zhang et al. (2012) and Zhang et al. (2013) observed an increased hydrolysis activity in the low temperature

UASB reactor treating domestic sewage. The hydrolysis occurring in the UASB-digester system was achieved with sludge that was exposed to an alternating temperature, as the sludge was recirculated between the low temperature UASB reactor and the mesophilic digester. The low temperature hydrolysis in the UASB reactor was initiated with a temporary start-up at 35°C achieved in the digester. The pre-hydrolysis at 35°C could excrete excess hydrolytic enzymes which facilitates hydrolysis. However, the effects of a pre-hydrolysis at 35°C on low temperature anaerobic hydrolysis are not reported in literature.

The hydrolysis of organic solids in anaerobic digestion can be described by first order kinetics (Batstone et al., 2002; Vavilin et al., 1996). Methane can be considered as the main hydrolysis product if hydrolysis is the slowest step compared to acidification and methanogenesis (Veeken & Hamelers, 1999). The hydrolysis rate constant can differ due to various experimental conditions such as inoculum source, ratio of biomass and substrate, and available surface of substrate (Sanders et al., 2000; Vavilin et al., 2008).

The main goal of the present research is to investigate the effect of a pre-hydrolysis at 35°C on low temperature anaerobic hydrolytic activities. Real domestic sewage was purposely not applied as a substrate to rule out potential effects of unknown components present in domestic sewage. Tributyrin and cellulose were used as model compounds for lipids and carbohydrates, of which hydrolysis rates at 35°C have been reported previously in literature (Fernandez et al., 2014 and O'Sullivan et al, 2008). Batch hydrolysis experiments were executed at low temperature (10-25°C) after applying a short start-up at 35°C. The results with a pre-hydrolysis at 35°C were compared with low temperature (10-25°C) hydrolysis without the mesophilic pre-hydrolysis. The supernatant in the hydrolysis tests was collected at 10-35°C, and its hydrolytic activity was tested.

5.2 Material and Methods

5.2.1 Inoculum

Granular sludge originating from a mesophilic anaerobic reactor treating paper-mill wastewater in Eerbeek (NL) was used as inoculum for hydrolysis rate constant tests. The inoculum had been stored at 4°C in gas-tight plastic containers for 2 weeks. The inoculum was incubated at 35°C for 2 weeks without feeding and subsequently washed to remove biodegradable material before conducting hydrolysis experiments.

Digester sludge from a pilot scale UASB (10-12.5°C) digester (35°C) system treating domestic wastewater was used as inoculum for determining the hydrolytic activity, released to the supernatant phase at 10-35°C. Detailed operational parameters of the pilot scale UASB-digester are reported by Zhang et al. (2016b). The digester sludge was, after collection, placed in a cabinet at 35°C for 2-3 days to stabilize and concentrate the sludge.

5.2.2 Determination of hydrolysis rate constants at constant temperatures

First order hydrolysis rate constants of cellulose (Sigmacell® type 50) and tributyrin (Fluka, ≥98%) were determined at different temperatures. Applied conditions for determining the hydrolysis rate constants are shown in Table 5.1 and 5.2. Blanks were executed at the same conditions but without adding substrate and used to correct for hydrolysis of organic materials (including biomass) present in the inoculum. All tests were executed in duplicate. A Bioprocess Control Instrument (AMPTS II, Sweden) was used for determining the methane production for tributyrin hydrolysis. For cellulose using granular sludge as inoculum, serum bottles of 120 ml volume closed with rubber stoppers and aluminium clamps were used. The inoculum was 10 ml at 35 and 25°C, and increased to 20 ml at 15 and 10°C to prevent the accumulation of intermediate products. Volume of inoculums at 25-35°C was lower than 10-15°C to compensate for the higher activity. Methane production of the cellulose tests was monitored by determining the gas composition and the pressure of the headspace. Gas samples size was 0.05 ml. Pressure of the headspace was measured daily using a manual pressure meter (GMH 3150, Germany). For cellulose hydrolysis, using the digester sludge as inoculum, methane production at 35 and 25°C was monitored by Bioprocess Control Instrument (AMPTS II, Sweden).

Dissolved products

0.4 ml liquid samples were collected to determine COD_{sol} concentrations. For cellulose, additionally volatile fatty acids (VFA) and glucose were measured; for tributyrin, additionally VFA and glycerol were measured. The frequency of COD_{sol} measurement depended on the hydrolysis efficiency with time.

5.2.3 Effects of a short pre-hydrolysis at 35°C on low temperature hydrolysis

Next to above described hydrolysis tests, similar tests were executed with a short pre-hydrolysis at 35°C. To make sure that hydrolysis had started with the pre-hydrolysis, temperature was decreased to 25, 15 or 10°C after 3.3, 3.1 and 2.2 days, respectively, for cellulose and after 0.08 days for tributyrin. The difference in pre-hydrolysis time was due to practical considerations. The cooling process was, for each temperature, finished within 15 minutes. A Bioprocess Control Instrument (AMPTS II, Sweden) was applied for all experiments. The serum bottles were transferred to a foam box with ice water and manually mixed for cooling. One sample was added for monitoring the decrease of temperature using a thermometer. Samples were cultivated in coolers (Waeco, Germany) as the temperature reached the targeted value.

Table 5.1 Applied conditions for determining the hydrolysis rate constants of cellulose and tributyrin at different temperatures

	Cellulose	Tributyrin	Cellulose		Tributyrin
Inoculum	Granular sludge		Digester sludge		
Temperature (°C)	35, 25;15,10	35 ;25, 20, 15, 10	10,15	25,35	10-35
Initial COD concentration (g L^{-1})	5	2; 1.25	0.78	0.95	0.95
Inoculum (ml)	10;20	100	87	350	350
Buffer (ml)	0.25;0.5	2.5	-	-	-
Volume of bottles (ml)	120	600	250	600	600
Distilled water (ml)	5;35	150;300	33	50	50
Mixing (rpm)	120	120	120	120	120
Initial pH	7.12	7.12	6.90	6.90	6.90

- Not added

5.2.4 Hydrolytic activity of supernatant phase at 10-35°C

The supernatants of the cellulose and tributyrin hydrolysis tests using the digester sludge from 10-35°C were collected when the hydrolysis efficiency achieved its maximum at the prevailing temperatures. The supernatant phase was collected by centrifuging at 4,500 rpm (Firlabo SW9, France) for 15 mins. The volume of the supernatant was similar as in the hydrolysis tests. Cellulose and tributyrin were used as substrates (in Table 5.1). Hydrolytic activity of the supernatant collected from different temperatures was executed at 35°C, and the procedure was similar as that of the hydrolysis rate constant tests described in paragraph 5.2.2.

Table 5.2 Details of the buffer solution

Material	concentraion	unit
NH_4Cl	4.10	g L^{-1}
KH_2PO_4	0.90	g L^{-1}
$CaCl_2{\cdot}2H_2O$	0.20	g L^{-1}
$MgSO_4{\cdot}7H_2O$	0.22	g L^{-1}
$FeCl_3 \cdot 4H_2O$	4.80	mg L^{-1}
$CoCl_2 \cdot 6H_2O$	4.80	mg L^{-1}
$MnCl_2 \cdot 4H_2O$	1.20	mg L^{-1}
$CuCl_2 \cdot 2H_2O$	0.07	mg L^{-1}
$ZnCl_2$	0.12	mg L^{-1}
HBO_3	0.12	mg L^{-1}
$(NH_4)6Mo_7O_{24} \cdot 4H_2O$	0.22	mg L^{-1}
$Na_2SeO_3 \cdot 5H_2O$	0.24	mg L^{-1}
$NiCl_2 \cdot 6H_2O$	0.12	mg L^{-1}
EDTA	2.40	mg L^{-1}
HCl (36%)	0.002	ml L^{-1}
Resazurin	1.20	mg L^{-1}

5.2.5 Analysis

Samples for measuring dissolved products were prepared by dilution, centrifugation and filtration. The raw sample was diluted 8 times and then centrifuged using a Thermo Electron IEC Micromax centrifuge (with rotor Cat. No. 3590, USA) at 10,000 rpm for 5 minutes. The centrifuged sample was filtered through a 0.45 μm membrane filter (Whatman 10401614, Germany). The filtrate was used for dissolved products analyses.

COD_{sol} concentration was tested by Dr.Lange cuvette (LCK 514, the Netherlands). Biogas composition of the cellulose hydrolysis, and VFA concentration of the cellulose and the tributyrin hydrolysis were tested by gas chromatograph as described by Zhang et al. (2013). Glycerol concentration was determined using a High Performance Liquid Chromatography (HPLC) (Alltech, USA) equipped with a Hi-Plex H column (300 × 6.5 mm) (Varian part nr. 1F70-0830), a Refractive Index (RI-71) detector and a Gynkotek M480 high precision pump. The mobile phase was 5 mM H_2SO_4 at flow rate of 0.6 ml minute^{-1}. Glucose was determined by HPLC equipped with an OA-1000 organic acids column (30 cm ID 6.5 mm) (70°C), a Refractive Index (RI-71) detector and a Gynkotek M480 high precision pump. The mobile phase was 1.25 mmol H_2SO_4 at a flow rate of 0.4 ml minute^{-1}.

5.2.6 Calculation

Methane production during hydrolysis of the cellulose was calculated using equation (1):

$$CH_{4t} = \frac{P_t \cdot V_h \cdot C_t}{100 \cdot R \cdot T \cdot 64 \cdot V_s} \tag{1}$$

Where:

CH_{4t}: Methane production at time t (in mg COD L^{-1});

P_t: Pressure of the headspace at time t (in Kpa);

V_h: Headspace of the serums (in ml);

C_t: Methane composition in the headspace at time t (in %);

R: Gas law constant (in kJ mol^{-1} K^{-1});

T: Absolute temperature (in K);

64: factor converting 1 mole of methane to 64 g COD;

V_s: Volume of the sample solution (in ml).

Hydrolysis efficiency (%) was calculated using equation (2), and hydrolysis products were measured with time during each trial:

$$\text{Hydrolysis }(\%) = \text{net}\sum_t COD_{hydrolysis\ \ products}/(COD_0 \cdot f_b) \cdot 100 \quad (2)$$

Where:

$COD_{hydrolysis\ products}$: for cellulose: COD_{sol} and methane; $COD_{hydrolysis\ products}$ for tributyrin: VFAs, glycerol and methane, expressed as mg COD L^{-1};

COD_0: initial particulate substrate concentration (in mg COD L^{-1});

F_b: the highest biodegradability of substrate achieved in all temperatures (in %).

The net cumulative hydrolysis products of the substrates were obtained after correction for the products of the blank.

In anaerobic digestion model No.1 (ADM1), the disintegration of solids is considered as the first step in anaerobic digestion of composites, such as dead biomass (Yasui et al., 2008). Because model substrates, cellulose and tributyrin, were used in this study, hydrolysis was considered as the first and rate limiting step. First-order hydrolysis model was used for the determination of the hydrolysis rate constant (equation 3).

$$\frac{\mathrm{d}COD_t}{\mathrm{dt}} = -k_h \cdot \ COD_t \quad (3)$$

Where:

k_h: First order hydrolysis rate constant (in d^{-1});

t: Time (in day);

COD_t: Biodegradable particulate substrate concentration at time t (in mg COD/L).

COD_t was calculated (equation 4) :

$$COD_t = COD_0 \cdot f_b - net\sum_t COD_{hydrolysis\ \ products} \quad (4)$$

k_h was estimated by fitting the linear equation (5):

$$\mathrm{Ln}(COD_t) = -k_h \cdot t + Ln(COD_0) \quad (5)$$

The Arrhenius equation was used to analysis the effects of temperature on hydrolysis constant of cellulose and tributyrin (equation 6).

$$k_h = A \cdot \ e^{\frac{-E}{R \cdot T}} \quad (6)$$

Where:

k_h: Hydrolysis rate constant (in d^{-1});

A: Arrhenius constant (in d^{-1});

E: Activation energy (in kJ mol^{-1});

R: Gas law constant (in kJ mol^{-1} K^{-1});

T: Absolute temperature (in K).

E was estimated by fitting the linear equation (7):

$$\mathrm{Ln}(k_h) = -\frac{E}{R} \cdot \frac{1}{T} + LnA \tag{7}$$

5.3 Results

5.3.1 Effects of pre-hydrolysis at 35°C on low temperature hydrolysis of cellulose

The hydrolysis rates of cellulose at 10-25°C clearly increased after the short pre-hydrolysis at 35°C compared with the measured rates at constant low temperatures, as shown in Fig. 5.1 and Table 5.3. Consequently, the hydrolysis rate constants increased as a result of the pre-hydrolysis at 35°C, namely from 0.11 to 0.40 d^{-1} (at 25°C), from 0.03 to 0.11 d^{-1} (at 15°C), and from < 0.01 to 0.10 d^{-1} (at 10°C).

Table 5.3 Hydrolysis rate constant of cellulose and tributyrin with and without the pre-hydrolysis at 35°C (unit: d^{-1}, standard error is in the brackets)

Temperature (°C)	Cellulose		Tributyrin	
	Without pre-hydrolysis	With pre-hydrolysis	Without pre-hydrolysis	With pre-hydrolysis
35	0.48 (0.02)	-	3.9 (0.2)	-
25	0.11	0.40 (0.01)	3.1 (0.2)	2.6 (0.1)
20	-	-	2.2 (0.1)	-
15	0.03	0.11	2.0 (0.1)	2.9 (0.2)
10	< 0.01	0.10	1.0 (0.1)	2.6 (0.1)

- Not measured

When applying pre-hydrolysis at 35°C, no lag phase occurred after decreasing the temperature. For the cellulose hydrolysis without the pre-hydrolysis step, the lag phase was respectively 4 and 6 days at 35 and 25°C; while later it strongly increased to 18 and 30 days at 15 and 10°C, respectively. The reason for the long lag phase at low temperatures might be the long time required for sufficient cellulase excretion and coverage of the cellulose surface with cellulase (Sanders et al 2000).

The hydrolysis efficiency of the cellulose with a short pre-hydrolysis at 35°C increased from 40 to 62%, and 9.6 to 40% at 15 and 10°C, respectively, compared with those without the pre-hydrolysis. A hydrolysis efficiency of 40% was achieved within 9.1 days at 10°C, after applying the pre-hydrolysis at 35°C, while the hydrolysis efficiency at 10°C without the pre-hydrolysis step was extremely low even after 62 days and therewith the calculated hydrolysis constant was lower than 0.01 d^{-1}. Decay of biomass in the sample with substrate was probably lower than that in the blank, and might have led to decrease of the percentage hydrolysis after 9.1 days at 10°C with pre-hydrolysis (Fig.5.1).

The duration of the pre-hydrolysis at 35°C (2.2-3.3 days) prior to low temperature (10-25°C) hydrolysis of cellulose was short compared with the duration of hydrolysis, 44-62 days, at low temperatures (10-25°C) without the pre-hydrolysis. Hydrolysis efficiencies of 22.0, 21.4, and 13.2% were achieved within the pre-hydrolysis at 35°C before decreasing temperature to 25, 15 and 10°C, respectively.

The major hydrolysis product of cellulose was methane, which accounted for 97 - 98% of the hydrolysed COD. The rest was VFA, while the glucose concentration was below the detection limit. The biodegradability of cellulose was 70 ± 2% at a constant temperature of 35°C, and it was 80 ± 4% in the test where pre-hydrolysis at 35°C was applied. The hydrolysis efficiency of cellulose at low temperatures was calculated based on the biodegradability determined at the prevailing temperature.

5.3.2 Effects of pre-hydrolysis at 35°C on low temperature hydrolysis of tributyrin

5.3.2.1 Low temperature hydrolysis (10-15°C) increased after applying a short pre-hydrolysis at 35°C

The hydrolysis rate of tributyrin at 10°C after applying a short pre-hydrolysis step at 35°C clearly increased compared with the one without the pre-hydrolysis, as shown in Fig. 5.2. It also slightly increased for 15°C, but was similar for 25°C. The hydrolysis rate constants at 15 and 10°C with the pre-hydrolysis step at 35°C were 1.5 and 2.6 times higher as those at 15 and 10°C without the pre-hydrolysis (as shown in Table 5.3). The hydrolysis rate after decreasing the temperature from 35 to 25°C was similar to that at a constant temperature of 25°C.

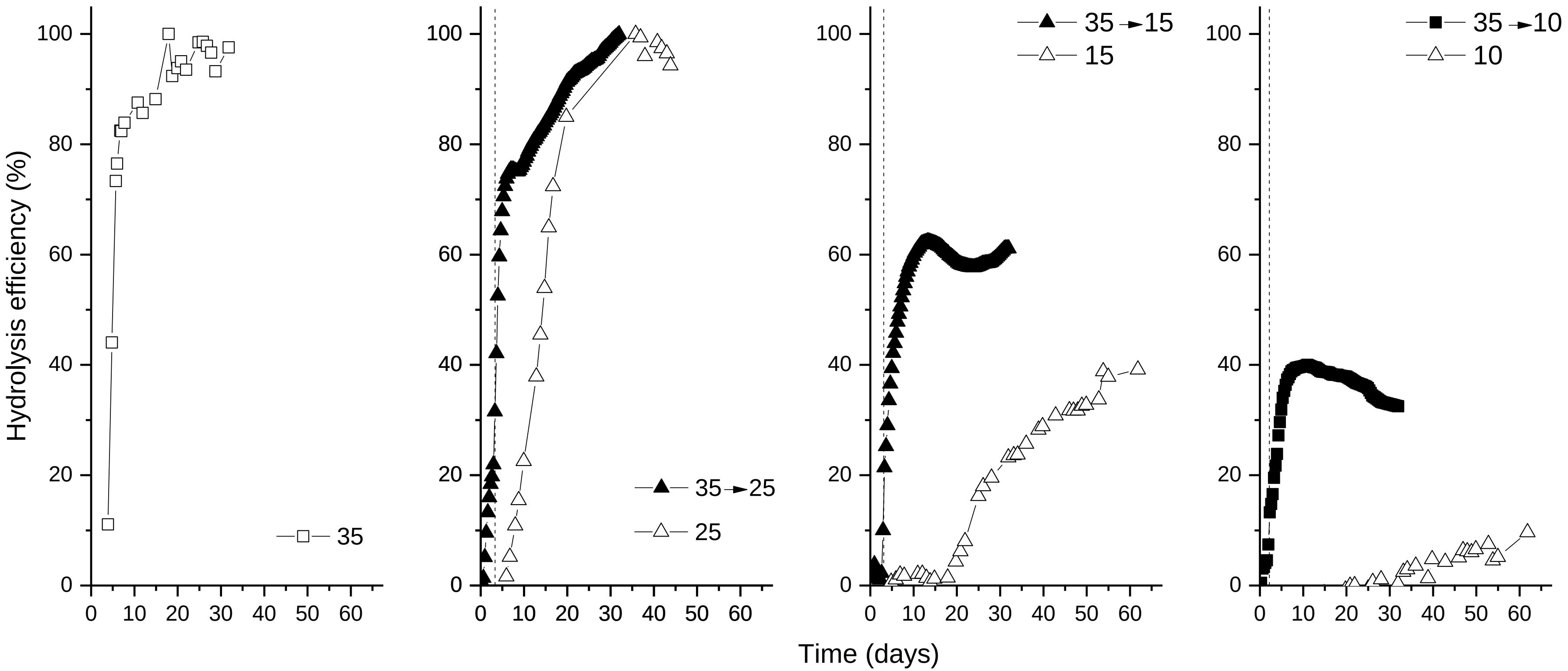

Fig.5.1 Effects of a pre-hydrolysis at 35°C on low temperature anaerobic hydrolysis of cellulose COD;
(cellulose had a biodegradability of 70 ± 2% at 35°C and 80 ± 4% with pre-hydrolysis; cellulose hydrolysis (%) was calculated based on the biodegradability; 35→25, 35→15 and 35→10: temperature was decreased from 35 to 25, 15 and 10°C after 3.3, 3.1 and 2.2 days)

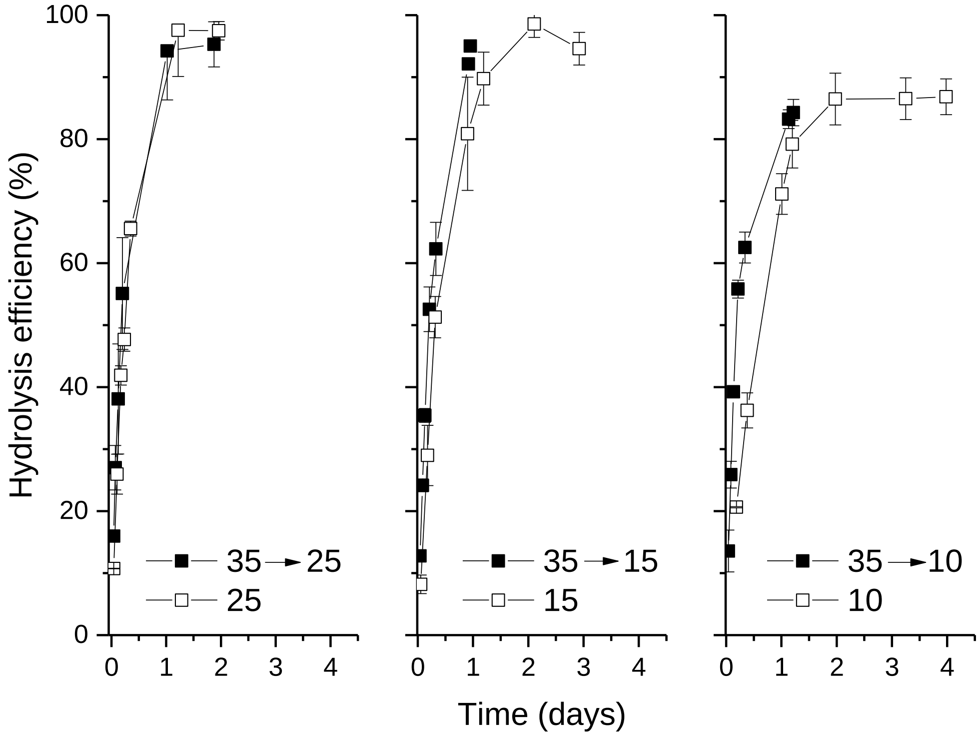

Fig.5.2 Effects of a pre-hydrolysis at 35°C on low temperature anaerobic hydrolysis of tributyrin COD (35→25, 35→15 and 35→10: temperature was decreased from 35 to 25, 15 and 10°C after 0.08 days)

No lag phase was found during the hydrolysis of tributyin at any temperature. The low temperature (10-25°C) hydrolysis efficiency was similar at applying batch digestion with and without pre-hydrolysis at 35°C. The hydrolysis efficiency was nearly 100% at 15-35°C and 87% at 10°C. The duration of the pre-hydrolysis at 35°C (0.08 days) was short compared with the duration of the low temperature hydrolysis (10-25°C) without the pre-hydrolysis step (3.0-4.0 days). A hydrolysis efficiency of 24 - 27% was achieved during the pre-hydrolysis at 35°C before subsequently decreasing the temperature.

5.3.2.2 Hydrolysis of tributyrin at constant low temperatures (10-35°C)

The hydrolysis products of tributyrin in time at constant temperatures are shown in Fig.5.3. The biodegradability of tributyrin varied between 97 and 100% at 15 - 35°C. No lag phase was found in the tributyrin hydrolysis. The hydrolysis products of tributyrin consisted of butyrate, accounting for 56 - 70% at most, and the rest was propionate while no acetate was present (not shown in Fig.5.3). The maximum VFA production accounted for 74 - 82% of the tributyrin COD for all temperatures. The final pH was 6.9 to 7.2 that did not lead to inhibiting hydrolysis and methanogenesis (Veeken and Hamelers, 1999). The accumulation of VFA was likely due to the absence of butyrate consuming bacteria in the inoculum. The total methane production decreased from 40 to 4% as temperature decreased from 35 to 10°C. Glycerol accounted for maximal 7% of the initial triburyrin COD concentration and was fully consumed within 2 - 3 days at 15 - 35°C; at 10°C, 4% was left after 4 days batch digestion.

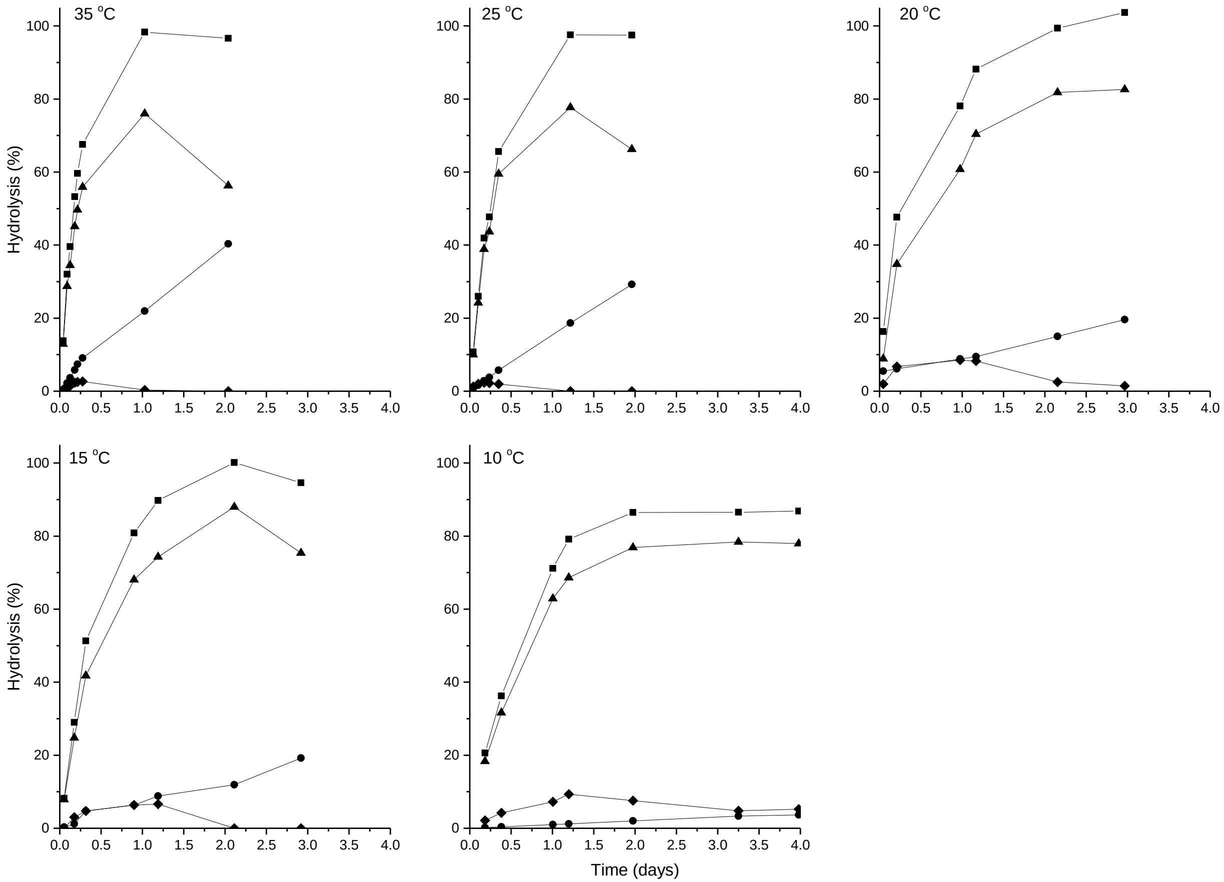

Fig.5.3 Hydrolysis percentage of tributyrin at constant temperatures as a function of time (biodegradability of triburyrin varies between 97 and 100% at 15 – 35°C; hydrolysis percentage of tributyrin (%) was calculated based on the biodegradability of 100%; ■ Total ▲ VFA ● Methane ♦ Glycerol)

5.3.3 Hydrolytic activity of supernatant phase at 10-35°C

Hydrolysis tests at 35°C were also performed using only the supernatant from the digestate after batch digestion of cellulose and tributyrin at 10-35°C. These tests give insight in whether excess enzymes were released to the liquid phase, or active enzyme production by the biomass cause the higher hydrolysis rate when pre-hydrolysis at a higher temperature is applied.

The hydrolytic activity of the supernatant collected from 35°C digestate, after cellulose hydrolysis, was higher than that of the supernatants collected from the 10-25°C “cellulose digestate” (Fig.5.4 and Table 5.4). The hydrolysis rate constant of cellulose determined with the supernatant was similar to that with the digester sludge, while the hydrolytic rate constant for tributyrin in the supernatant was clearly lower than that determined with the digester sludge, as shown in Table 5.4.

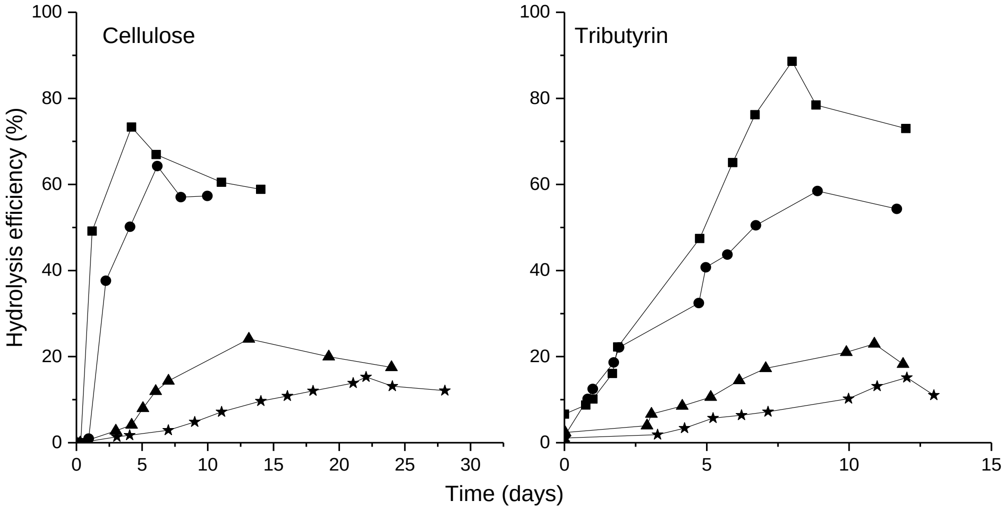

Fig.5.4 Hydrolysis of cellulose and tributyrin using the supernatant at 35°C

(Supernatant was collected from hydrolysis of cellulose and tributyrin at different temperatures, ■: 35●:25▲:15★:10)

The maximum cellulose hydrolysis efficiency, using the supernatants collected from digestate, after cellulose hydrolysis at 35, 25, 15 and 10°C, decreased with temperature from 73 to 51%. No lag phase was found during any of the supernatant hydrolysis tests for cellulose. The maximum tributyrin hydrolysis efficiency also decreased with temperature, viz. from 89 to 42%, when using the supernatants collected from digestate, after tributyrin hydrolysis at 35, 25, 15 and 10°C. No lag phase was found for the supernatant collected at 25 and 35°C, but a lag phase of 2.9 and 3.3 days was found for those collected at 15 and 10°C

5.3.4 Discussion

The hydrolysis rate constants of cellulose and tributyrin, when constant temperature

Table 5.4 Hydrolysis rate constant of cellulose and tributyrin using the digester sludge at 10-35°C and using the supernatant (unit: d^{-1}, standard error is in the brackets, the supernatant experiments were performed at 35°C)

Temperature (°C)	Cellulose		Tributyrin	
	Using the digester sludge	Using the supernatant	Using the digester sludge	Using the supernatant
35	0.46 (0.02)	0.31 (0.02)	4.0 (0.1)	0.21
25	0.20 (0.01)	0.18 (0.01)	2.1 (0.1)	0.10
15	0.03	0.02	1.4 (0.1)	0.02
10	0.01	0.01	1.1 (0.1)	0.01

was applied, decreased with temperature for the range of 35 to 10°C. The same trend was found in other researches (Pavlostathis & Giraldo-Gomez, 1991b). In that study, the hydrolysis rate constants of the six selected components of biowaste decreased as temperature decreased from 40 to 20°C (Veeken & Hamelers, 1999). They also found that the hydrolysis efficiency of cellulose decreased strongly when temperature decreased from 15 to 10°C. Biodegradability of the cellulose at 35°C was used for the calculation of hydrolysis rate constants at low temperatures. Experiments at low temperatures are inaccurate and inconvenient due to the slow rates. Similarly, Cysneiros et al. (2011) reported that the total volatile solids degradation decreased from 53 to 19% as temperature dropped from 37 to 10°C when studying effects of temperature on the trophic stages of perennial rye grass anaerobic digestion. Bohn et al. (2007) found that anaerobic hydrolysis of crop residues decreased from 345 to 46 ml CH_4 g^{-1} VS^{-1} as temperature decreased from 33 to 18°C.

Effects of temperature on the hydrolysis rate constant can be described by the Arrhenius equation for enzyme catalysis when the enzyme concentration is not the limiting factor. The activation energy of tributyrin hydrolysis in the here presented study was calculated to be 36 kJ mol^{-1} (not shown in the results section). The low value is typical for enzyme kinetics reaction (20 - 80 kJ mol^{-1}) (Levenspiel, 2013). As low temperatures led to limiting the cellulose hydrolysis rate, therewith, the activation energy of cellulose hydrolysis was not calculated.

Within the present work low temperature (10-25°C) hydrolysis of cellulose and tributyrin was studied with the pre-hydrolysis at 35°C. The results showed that the hydrolysis rate constants with the pre-hydrolysis at 35°C remained relatively high when decreasing the temperature to 25, 15 and 10°C. Hydrolytic enzyme concentrations, enzyme activity and adherence of anaerobic hydrolytic bacteria to the substrate play an important role in hydrolysis (Azman et al., 2015; Goel et al., 1998). One or several of these factors might limit hydrolysis at low temperatures. It was reported that the cellulase produced by anaerobic bacteria showed a relatively low concentration (filter paper units(FPU) L^{-1} culture broth) and a low productivity (FPU L^{-1} h^{-1}), even under suitable conditions of anaerobic digestion (pH 7 and temperature 37°C) (Adney et al., 1991). Sanders et al (2000) reported an increased lag phase when hydrolysing small starch particles from potatoes in comparison to large starch particles. Latter was partly ascribed to the relatively large substrate surface of the small particles, and partly to the low numbers of hydrolytic enzymes present at the start of the experiment to cover the substrate surface. The increased lag phase at decreased temperature as found in the present research for cellulose hydrolysis indicated a limited cellulase production rate at lower temperatures.

The presented results clearly show an increased hydrolysis rate constant at low temperatures when pre-hydrolysis at 35°C is applied. It is likely that at low temperatures (lower than 20°C), hydrolytic enzyme concentration is low compared with that at 35°C (as shown in Fig.5.5). The results of the present research indicate that for low temperature hydrolysis with the pre-hydrolysis at 35°C, the hydrolytic enzymes produced at the start temperature of 35°C were still active when decreasing the temperature. This relatively high number of enzymes resulted in high hydrolysis rate compared with those starting at low temperatures. Effects of a short pre-hydrolysis at 35°C on low temperature hydrolysis are for the first time studied in the present work. As most literature describes hydrolysis of anaerobic digestion at constant mesophilic or thermophilic conditions (Speece, 2008), no results can be used to compare with low temperature hydrolysis with a pre-hydrolysis at 35°C.

The positive effect of pre-hydrolysis at 35°C on low temperature cellulose hydrolysis was greater than that on tributyrin hydrolysis. This difference was probably due to the difference in substrate type; tributyrin is soluble and cellulose is particulate. Sanders (2002) reported an increasing hydrolysis rate constant of dissolved polymer substrates with an increasing biomass concentration, which indicated an infinite surface area for dissolved substrates. The results in this study show a substantial lower hydrolysis rate constant of tributyrin when using digestate supernatant as compared to using digester sludge. The latter could be attributed to the large available substrate surface during hydrolysis of tributyrin when using digester sludge, which resulted in a low number of excess enzymes in the supernatant. The low number of excess enzymes in the supernatant also explained that the positive effect of pre-hydrolysis for tributyrin was only clear for lower temperatures (10-15°C). In contrast, particulate cellulose has a limited surface and, therefore, excess cellulases are excreted to the supernatant during digestion at 35°C. The hydrolytic enzyme activity using the supernatant for cellulose hydrolysis at 35°C was close to the one using digester sludge. These excess cellulases could accelerate the hydrolysis at lower temperatures.

In principle, the effect of a short pre-hydrolysis at 35°C on low temperature anaerobic

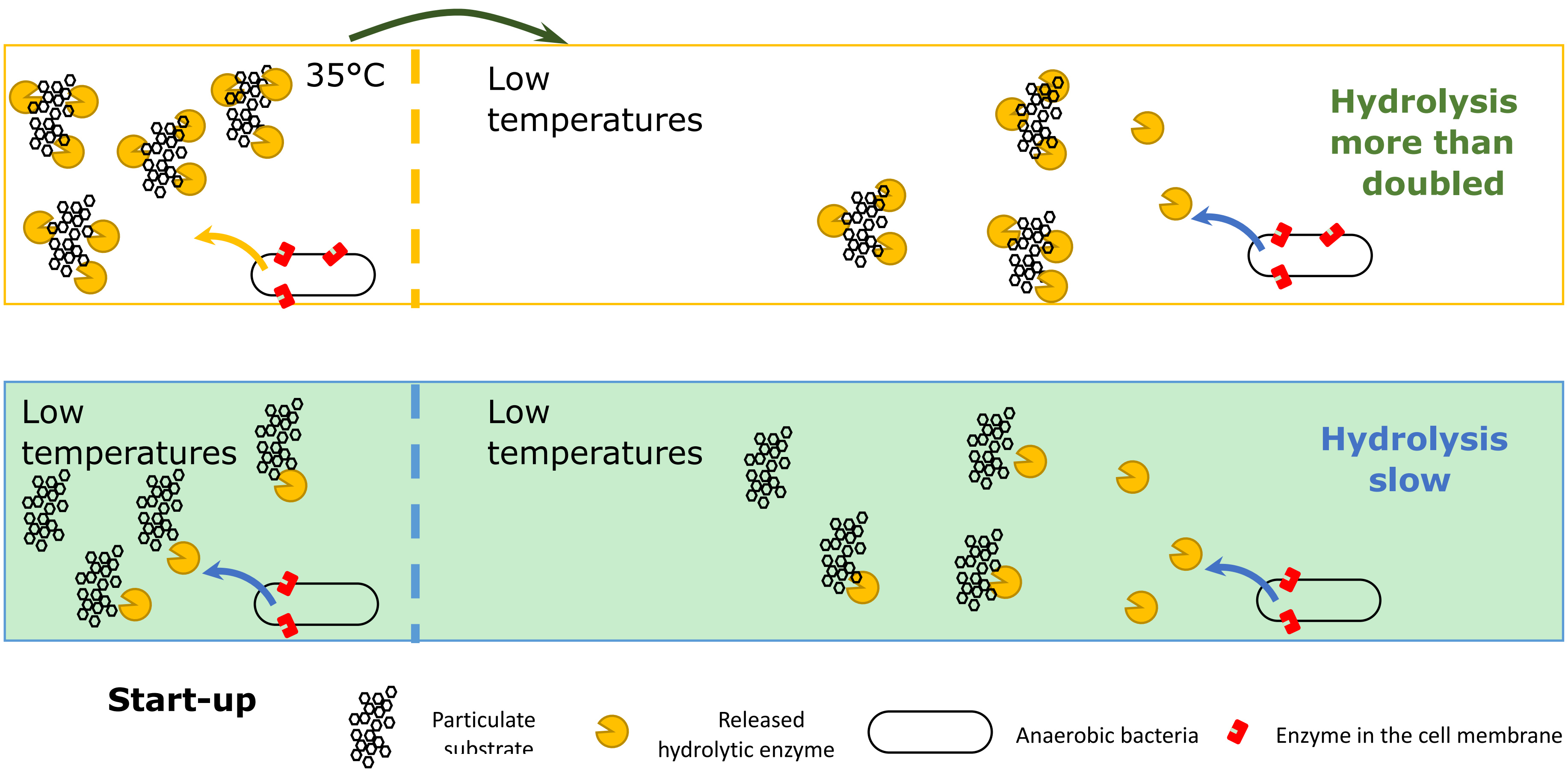

Fig.5.5 Hypothesis on the increased hydrolysis rate constant at low temperatures with a pre-hydrolysis at 35°C

hydrolysis is positive as higher hydrolysis efficiency can be achieved at a certain HRT (SRT). A direct positive effect could be achieved when considering anaerobic digestion of i.e. manure followed by a long term storage at ambient temperature (determined by use of manure as a fertiliser during the crop growing season). The enzymes excreted at high temperature will continue working during the ambient temperature storage. The potential extra conversion could lead to reduced HRT in the digester provided that biogas and digestate storage are integrated to prevent methane emission (Zeeman, 1994).

For a UASB-digester system treating domestic sewage, however, a negative effect is foreseen. In the UASB-digester system, the UASB reactor and the digester is operatedat low temperatures and at 35°C, respectively (Zhang et al. 2012). The recirculated digester sludge of 35°C will elevate the hydrolysis efficiency of organic particulates in the low temperature UASB reactor and therewith the required acidogenic, acetogenic and methanogenic activity. Zhang et al. (2016) showed, despite this increased COD_{sol} production in the UASB, a good performance can be achieved with the UASB-digester at temperatures in the UASB as low as 12.5°C. The energy consumption of a UASB-digester system is limited as only the UASB-sludge, ca. 10-15% of the total wastewater volume, is recirculated over a heated sludge digester (Zhang et al. (2012)) and Zhang et al. (2016). The UASB-digester can therefore be more energy efficient than i.e. an activated sludge system.

5.4 Conclusions

Low temperature (10-25°C) anaerobic hydrolysis rate can be increased by applying a short pre-hydrolysis at 35°C:

- With a pre-hydrolysis at 35°C, the hydrolysis rate constants of cellulose increased from 0.11 to 0.40, from 0.03 to 0.11 and from < 0.01 to 0.10 d^{-1} at 25, 15 and 10°C, respectively; Similarly, the hydrolysis rate constants of tributyrin at 15 and 10°C with the pre-hydrolysis at 35°C were 1.5 and 2.6 times higher as those at 15 and 10°C, respectively, without the pre-hydrolysis.
- No lag phase in hydrolysis was found when decreasing temperature after the pre-hydrolysis at 35°C; while the lag phase without the pre-hydrolysis for the cellulose hydrolysis increased from 4 - 30 days with a temperature decrease from 35 to 10°C. The tributyrin hydrolysis showed no lag phase at any temperature.
- The hydrolysis efficiency of the cellulose with the pre-hydrolysis at 35°C increased from 40 to 62%, and 9.6 to 40% at 15 and 10°C, respectively, compared with those at low temperatures (10-15°C) without the pre-hydrolysis; the hydrolysis efficiency of tributyrin was nearly 100% at 15 - 35°C and 87% at 10°C, which was similar to those with the pre-hydrolysis at 35°C.
- The hydrolytic activity of the supernatant collected after batch digestion of cellulose and tributyrin at 35°C was higher than that collected at low temperatures.

Acknowledgements

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6 Effects of temperature and temperature shocks on specific methanogenic activity and affinity for acetate of sludge exposed to varying temperature conditions (10-35°C)

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This chapter is to be submitted

Abstract

Digester sludge of a UASB (12.5°C)-sludge digester (35°C) was fed with acetate at constant temperatures of 10-35°C and at varying temperatures from 35°C to 25, to 15 to 10°C. Effects of temperature and temperature shocks on specific methanogenic activity (SMA), and affinity of the digester sludge were studied. The results showed that no lag phase in methane production rate occurred when applying the temperature shocks of 35°C to 25, 15, and 10°C. The temperature dependence of the SMA of the digester sludge with the temperature shocks was similar to the one at constant temperatures. The activation energy for the SMA of the digester sludge was 62 kJ/mol. Acetate affinity of the digester sludge was high at the applied temperatures (10-35°C).

6.1 Introduction

Anaerobic treatment of municipal wastewater has three main advantages over treatment using aerobic conventional activated sludge systems: namely the potential low energy consumption due to the absence of aeration, low amounts of excess sludge production reducing costs of sludge processing, and energy recovery from wastewater chemical oxygen demand (COD) in the form of methane (Kassab et al., 2010; Speece, 2008). Generally, anaerobic treatment of municipal wastewater is considered applicable at temperatures between 30-35°C, i.e. in (sub) tropical regions. In moderate climate areas¸ municipal wastewater has temperatures between 10-20°C posing a limitation to anaerobic treatment. Low temperature leads to a low hydrolysis rate of organic wastewater solids and a low specific methanogenic activity of the anaerobic biomass (LeitÃ£o et al., 2006; Lettinga et al., 2001). A combined UASB-digester process has been proposed to resolve these limitations of low temperature and low COD concentration for anaerobic treatment (Álvarez et al., 2004; Mahmoud, 2008; Mahmoud et al., 2004; Zhang et al., 2013).

In this system, the UASB reactor is operated at the low temperature of the wastewater and at a short hydraulic retention time (HRT). The idea is that the UASB sludge blanket captures the suspended COD from the wastewater without biodegradation, and only converts the soluble influent organics to methane. The formed non-stabilized UASB sludge is fed to the digester operated at 30-35°C. Here the suspended COD entrapped in the UASB sludge is hydrolyzed and digested yielding methane and stabilized anaerobic sludge. Returning this sludge from the digester to the UASB reactor provides the UASB reactor with active methanogens for converting soluble COD in the UASB reactor. The biogas production of the digester and the UASB reactor can provide the energy needed for heating the digester (Zhang et al., 2012). The above mentioned combined process can only be successful when the methanogens fed back from the warm digester sludge to the cold UASB reactor maintain adequate methanogenic activity and substrate affinity for converting the soluble wastewater COD in the UASB reactor into methane.

The recirculated digester sludge and the UASB sludge are subjected to sudden changes in temperature upon recirculating the sludge. Temperature of the UASB sludge recirculated to the digester suddenly increases; temperature of the recirculated digester sludge suddenly decreases when entering the UASB reactor. Effects of temperature change on anaerobic processes were investigated in various studies. Biogas production under seasonal temperature change between winter (14-25°C) and summer (24-35°C) in Brazil was studied when applying a pilot scale anaerobic digestion of cattle manure (Resende et al., 2015). No difference in average methane yield was found with the gradually changed temperature. Biogas production rate under daily downward and daily upward temperature fluctuation was studied when applying anaerobic digestion of cow manure at 50 and 60°C at an HRT of 20 ds (El-Mashad et al., 2004). Biogas production rate at 50°C was higher than at 60°C when a temperature change imposed 10°C reduced for 10 h and 10°C increased for 5 h respectively. Lau and Fang (1997) reported that suddenly applied changes in temperature to thermophilic granules from 55 to 37°C resulted in poor COD removal, granule disintegration and biomass washout. Kettunen and Rintala (1997b) reported for mesophilic digester sludge, a sudden decrease in temperature from 35 to 15°C resulted in a one-day lag-phase before acetate consumption recovered. Gao et al. (2011) found that a decreased temperature by 5 and 10°C starting at 37°C could be tolerated for a submerged anaerobic membrane bioreactor (SAMBR); the same changes starting at 45°C led to a significant disturbance of the performance. However, effects of a sudden decrease in temperature on methanogenic activity of sludge from a UASB-digester recirculation system have not been reported before.

High affinity of the sludge of the UASB-digester process for soluble COD and especially acetate, is important when treating municipal wastewater with relatively low COD concentrations at high loading rates. The affinity can be presented by the half-saturation velocity constant (K_s) in the Monod equation (Arnaldos et al., 2015). Monod equation can be expressed in terms of substrate utilization rates (Duran & Tepe, 2004; Pavlostathis & Giraldo-Gomez, 1991a). Varying conditions in K_s quantification experiments are substrate concentration, microbial culture, temperature and experimental set-up (batch or continuous experiment). Generally, the value of Ks of anaerobic sludge increases (i.e. the affinity decreases) when temperature decreases, as shown by Lokshina et al. (2001) and Banik et al. (1998) for treating municipal landfill leachate and synthetic municipal wastewater. Substrate affinity and mass transfer limitations may additionally impact methanogenesis and its dependence on temperature (Speece, 2008). However, the effects of low temperature on the affinity of the sludge in a UASB-digester process have not yet been studied.

In a UASB-digester system, the sludge is continuously exposed to changing temperature, as the sludge is recirculated from low temperature UASB reactor to the warm digester, and then returned again. The sludge recirculation between the reactors at different temperatures may have a positive effect on adaptability of the sludge to a sudden temperature change. In the study presented here, we determined the effects of an immediate temperature drop from 35°C to 25, 15 or 10°C on the methanogenic activity, and the effects of temperature (10-35°C) on affinity constant for acetate using the digester sludge from a UASB-digester process. The results are discussed in relation to the optimization of the UASB-digester for treating low temperature (10-20°C) municipal wastewater.

6.2 Materials and methods

6.2.1 Source of inoculum

The digester sludge used as inoculum in the batch experiments was collected from a digester operated at 35 ± 1°C in a pilot-scale combined UASB-digester process. The influent and internal UASB temperature were kept at 12.5 ± 1°C at the time of sampling. Domestic wastewater, operations and design of the system were similar as described by Zhang et al. (2013), except for the height of the UASB reactor which was increased here to 3.0 m. The influent flow rate and the sludge recirculation rate were 500 L/d and 64-80 L/d, respectively. The UASB-digester process had been operating for municipal wastewater treatment for 3 years, and temperature in the UASB reactor was between 10-20°C.

6.2.2 Affinity of the digester sludge at 10-35°C

The digester sludge was settled for 1 day in a cabinet at 35°C after sampling. The settled sludge of 150 ml and 50 ml supernatant was put in a serum bottle of 300 ml with a sampling port. 0.5 ml of 100 g/L sodium acetate solution was added as substrate in the serum bottle. The initial acetate concentration was 250 mg COD/L. Nitrogen gas was used to flush the solution in the serum bottle to ensure anaerobic condition. Oxi-top (OC 110, Germany) was connected with the serum bottle to automatically read the pressure of the headspace. Each sample was executed in triplicate and placed in the shakers with 120 rpm at 10, 15, 25 and 35°C.

6.2.3 Effects of cold temperature shock on SMA

For cold temperature shock experiments, the digester sludge samples were prepared in the same way as in the affinity experiments, expect for the addition of sodium acetate solution which was 1 ml. The experimental bottles in triplicates were placed in shakers at 120 rpm at 35°C for 1 hour. Then, the temperature of the sludge samples was lowered to 25, 15 or 10°C within 15 minutes by placing the bottles in an ice water bath. Additional samples were used as a control for monitoring temperature using a thermometer. The samples were returned to the shakers at 25, 15 and 10°C after the temperature of the controls dropped to the low temperatures (10-25°C).

6.2.4 Activity monitoring

Gas samples (2 ml) were collected from the sludge sample headspace to measure methane and carbon dioxide composition using gas chromatography (Interscience GC 8000 series) equipped with a thermal conductivity detector and two columns (Molsieve 5A 50 m × 0.53 mm for nitrogen and methane and Porabond Q 50 m × 0.53 mm for CO_2). Temperatures of injector, detector and oven were 110, 99 and 50°C respectively.

Acetate concentration in a liquid sample (1 ml) was measured after centrifuging with a Thermo Electron IEC Micromax centrifuge (with rotor Cat. No. 3590, USA) at 10000 rpm for 5 mins and then by preparing samples with formic acid (1.5% of sample) and analysis was done by GC (HP 5890 GC; glass column of 2 m × 6 mm × 2 mm packed with 10% Fluorad 431 on Supelco-port 100-120 mesh) with an oven temperature of 130 ˚C, and nitrogen saturated with formic acid as the carrier gas applied at a flow of 40 ml/min. The injector temperature was 200 ˚C and the flame ionization detector at 280 ˚C. The sample size was 1 μL.

Pressure of the sludge sample headspace was measured by a hand-held digital pressure meter (GMH 3150, Germany) with a needle (the precision was up to mbar). The volatile suspended solids (VSS) concentration of the digester sludge was measured according to American Standards (APHA 2005).

VFA measurements in the affinity tests were taken one time per 0.5, 1, 2 and 3 h at 35, 25, 15 and 10°C until the substrate was completely consumed. The gas composition was measured every 2 hours.

6.2.5 Data interpretation and calculations

Acetate utilization rate (A) of the digester sludge in the test for determination of affinity constant was calculated by equation (1),

$$A= dC_{acetate}/dt \times 1/VSS \quad (1)$$

Where:

A: acetate utilization rate (g COD/(gVSS d));

$C_{acetate}$: the amount of acetate (g COD);

T: time (d) and VSS is the amount of the digester sludge (g).

SMA (unit: g CH_4 COD/(g VSS d)) of the digester sludge was calculated from the linear part of a curve describing cumulative methane production in time by equation (2). Based on preliminary tests, the applied initial acetate concentration did not influence SMA. Therewith, SMA was calculated in temperature shock experiments and affinity tests.

$$SMA = 64 \times dP/dt \times V \times C_{methane}/(R \times T) \quad (2)$$

Where:

64: a conversion factor for 1 mol methane to g methane COD;

P: pressure of the headspace of the sludge sample (kPa);

V: the volume of the headspace of the sludge sample (L);

C: the percentage of methane in biogas (methane/ (methane + carbon dioxide), %),

R: ideal gas constant (R = 8.314 J/ (mol × K)), and T is room temperature (T= 293 K).

Methane production (CH_4) was calculated by equation (3):

$$CH_4 =64 \times P \times C_{methane} \times V/ (R \times T) \quad (3)$$

The temperature dependence of SMA can be described in Arrhenius equation (Kettunen

& Rintala, 1997a). The temperature dependence of methanogenic activity of the digester sludge (M) is shown in equation (4):

$$SMA = M_0 \times \exp\{-E_a/(R \times T)\} \quad (4)$$

Where:

M_0: a frequency constant in Arrhenius and represents a maximum methanogenic activity of the digester sludge in absence of an activation energy (g CH_4 COD /(g VSS·d))

E_a: the Activation energy (kJ / mol).

E_a was estimated by fitting the linear equation (5):

$$\ln(SMA) = -E/R \times 1/T + \ln(M_0) \quad (5)$$

Acetate concentration (mg COD/L) and acetate utilization rate were used for Monod equation fit by equation (3),

$$A = A_{max} \times S / (K_s + S) \quad (6)$$

Where:

A: acetate utilization rate (g COD/ (gVSS·d));

A_{max}: maximum acetate utilization rate (g COD/ (gVSS·d));

K_s: half-saturation velocity constant of acetate utilization (mg COD/L);

S: acetate concentration (mg COD/L).

6.3 Results

6.3.1 Effects of a sudden temperature drop on SMA

The results in Fig.6.1 show that the methane production of the digester sludge continued without any lag phase, but at a lower rate after an immediate decrease of the temperature from 35 to 25, 15 or 10°C. At each lowered temperature value, the methane production rate of the digester sludge, expressed by slope of the methane production curve, became constant within 12 minutes after changing the temperature.

The temperature dependency of the SMA of the digester sludge was evaluated by applying the Arrhenius model (Fig.6.2). The results show that the temperature dependency of the SMA was similar for the two applied conditions: after applying a temperature shock (Fig.6.1) and at keeping temperature constant (Fig.6.3). The SMA of the digester sludge at 35°C was 0.25 g CH_4 COD/(gVSS d) as shown in Table 6.1. The apparent activation energy for the SMA of the digester sludge using acetate as substrate was 62 kJ/mol (R^2:0.944) (Table 6.1).

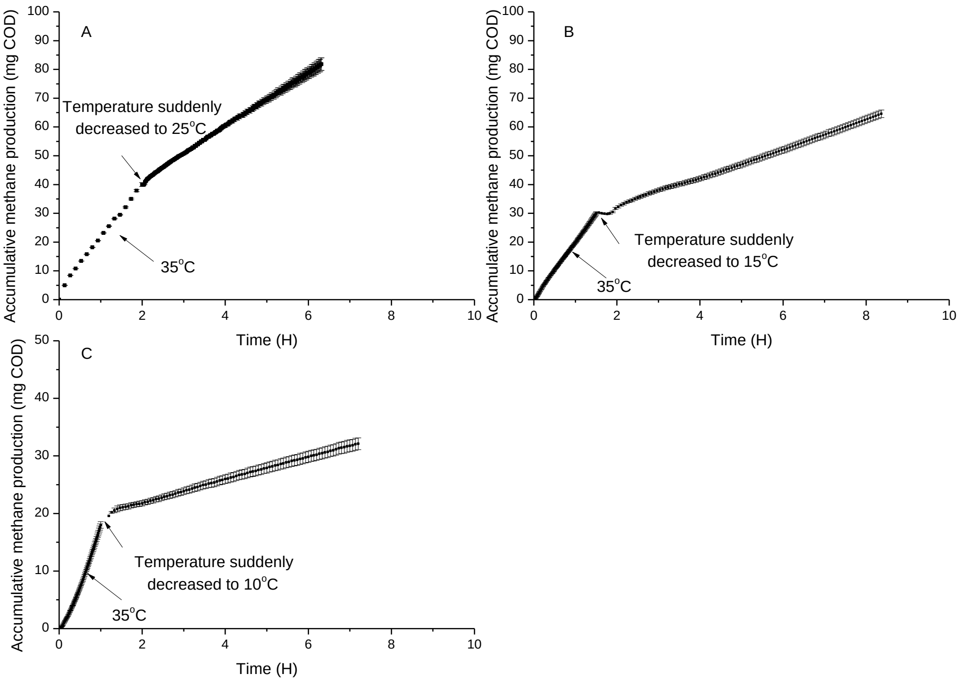

Fig.6.1 Effects of a cold temperature shock on the accumulative methane production during the SMA test of the digester sludge (A, B and C: a sudden temperature decrease from 35 to 25, 15 and 10°C at 2, 1.5 and 1h)

6.3.2 Effects of low temperature on affinity for acetate utilization

As shown in Fig.6.3, methane production rate of the digester sludge maintained constant at each temperature until the substrate acetate was completely consumed to below the detection limit of 1 mg COD/L at 3.2, 5.4, 9.2 and 22 h for 35, 25, 15 and 10°C. The methane production rate decreased to 21% at 35°C afterwards, which was due to the hydrolysis of the digester sludge itself and the same trend occurred at 25°C. The methane production rate at 25°C after the acetate was fully consumed was 45% of the one at 35°C due to the decreased temperature. The methane production rate clearly stopped as acetate was fully consumed at 15 and 10°C.

The results in Fig.6.3 showed that the acetate utilization rate of the digester sludge maintained high at a low acetate concentration for each temperature (35, 25, 15 and 10°C). The lowest acetate concentration before the utilization rate decreased was 3.0, 5.0, 25.1 and 7.9 mg COD/L at 35, 25, 15 and 10°C. The acetate utilization rate was 0.197, 0.118, 0.072 and 0.030 g COD /(gVSS d) at 35, 25, 15 and 10°C. The low acetate concentration combined with the results of the methane production rate discussed before indicate that the digester sludge has a high affinity for acetate. K_s for acetate of the digester sludge at 35°C was 6.5 mg COD/L, while K_s for the other temperatures was Sensing a sudden decrease of the temperature, bacteria can produce cold shock proteins to adapt to a given temperature (Yamanaka, 1999). Cold acclimation proteins were produced to maintain bacterial activity at a 25 to 5°C cold shock and a constant growth at 5°C (Gumley & Inniss, 1996). The EF-2 protein (Mbar_A3686) was considered to

Table 6.1 Lag phase, E_a, K_s and SMA of different types of sludge at different temperatures (unit for temperature: °C)

Authors	Wastewater /Temperature	Reactor	Temperature of SMA tests	Sludge type	Lag phase (days)	E_a (kJ/mol)	SMA[a]/Temperature g CH_4 COD (g VSS d)	$K_{s\ temperature}$ (mg COD/L)
Zhang et al. (this study)	Domestic sewage / 10-12.5	UASB-Digester	10-35	Floc	0	62	0.25/35	6.5_{35}
Kettunen et al. (1997)	Leachate / 23	UASB	5-29	Floc	0.4-3	52	0.24/29	704_{11}-2944_{22}
(Rebac et al. (1999a); Rebac et al. (1995))	VFA mixture/10-12	Expanded granular sludge bed (EGSB)	10-30	Granular	-	68	2.20/30	39^{b}_{10}
Fey and Conrad (2000)	Rice field soil	Batch experiments	10-37	Soil sample	2	61	0.015[c]/37	-
Luostarinen and Rintala (2005)	Black water/10-20	UASB-septic	5-35	Floc	0-10	60	0.08/35	-
McKeown et al. (2009)	Synthetic volatile fatty acid wastewater / 4-16	EGSB - anaerobic filter (EGSB-AF)	4-37	Granular	-	63	1.06/37	-

- Not mention; a: the highest SMA in the tested temperatures; b: elaborated in EGSB; c: methane production rate

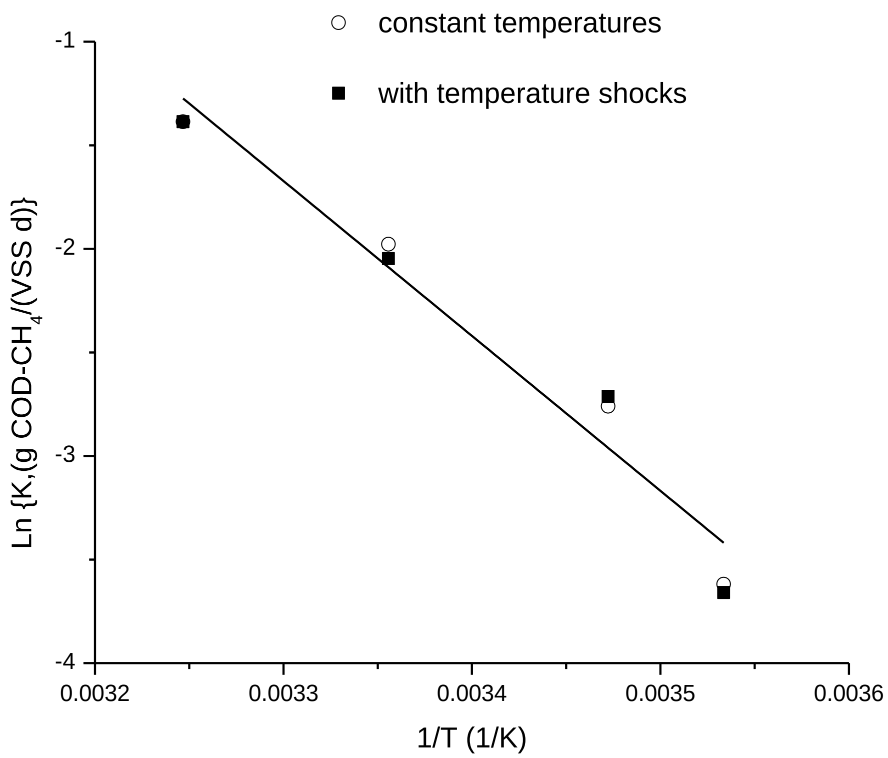

Fig.6.2 Arrhenius model for SMA of the digester sludge using acetate as substrate

play a role in growth of a psychrotolerant methanogens (Gu & Hilser, 2009). This protein was upregulated at 15°C compared with 37°C, not only when applying a temperature shock from 37 to 15°C, but also in the initial phases of growth at 15°C using mesophilic sludge (Gunnigle et al., 2013).

The continuous sludge recirculation of the UASB-digester between low temperature and mesophilic temperature might induce the above mentioned ‘cold shock’ proteins’. almost 0. The SMA of the digester sludge during the affinity tests were 0.260, 0.143, 0.065 and 0.028 g CH_4 COD / (gVSS d) for respectively 35, 25, 15 and 10°C.

6.3.3 Discussion

The effects of a sudden temperature drop from mesophilic conditions (35°C) to low temperatures (10-25°C) on the SMA of the digester sludge showed that the digester sludge is well adapted to a wide range of temperature shocks (10-35°C). After the cold temperature shock, no lag phase was found for methane production, SMA was constant in time at low temperatures, and the temperature dependence of the SMA of the digester sludge after applying the temperature shocks was similar to the ones at constant temperatures (10-25°C). Latter is confirmed by the equal SMA at suddenly decreasing temperature and constant temperature conditions, and non-existence of a lag phase after the suddenly decreasing temperature.

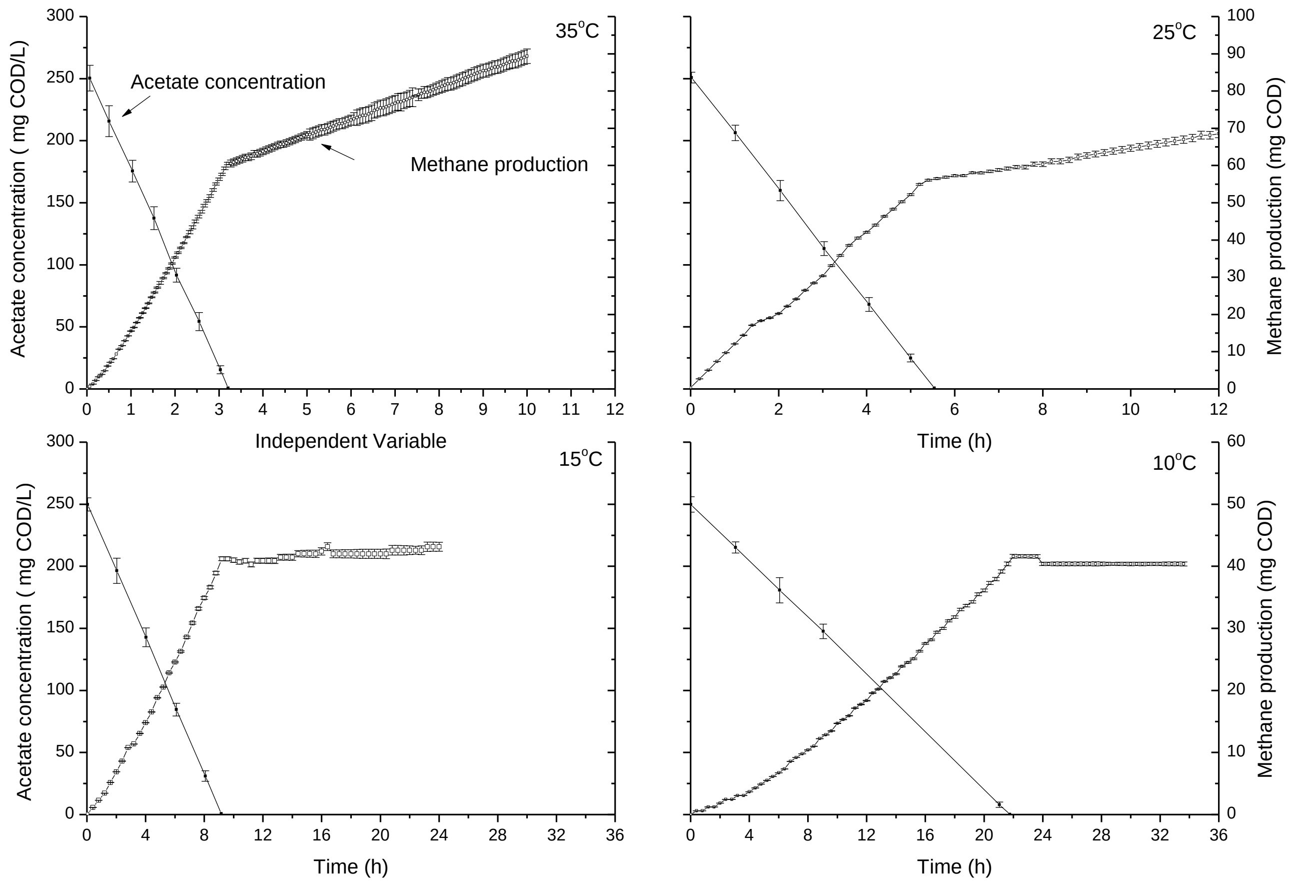

Fig.6.3 Methane production and substrate acetate concentration during the affinity of the digester sludge test at different temperatures 35, 25,15 and 10°C

Lag phase, E_a, K_s and SMA of different types of sludge at different temperatures were shown in Table 6.1. Lag phase was found when performing SMA tests using sludge from low temperature anaerobic reactors (Kettunen & Rintala, 1997a; Luostarinen & Rintala, 2005). The temperature dependence of the SMA can be well fitted by an Arrhenius equation (Fey & Conrad, 2000; Kettunen & Rintala, 1997a; McKeown et al., 2009b; Rebac et al., 1999a; Rebac et al., 1995), and E_a was between 52-68 kJ/mol. SMA of the anaerobic sludge under mesophilic conditions were higher than psychrophilic conditions. Therewith, the anaerobic sludge was still mesophilic after treating low temperature wastewater for a long term operation.

K_s value varied due to different conditions as shown in Table 6.1. Affinity of the digester sludge for acetate in this study was high between 10-35°C. The methanogens with a high affinity for acetate could be enriched as they were mainly grown under a low acetate concentration in the UASB-digester. The methanogenic capacity of the low temperature UASB reactor calculated using SMA and sludge quantity in the UASB reactor matched with the measured methane production (Zhang et al., 2013), and this equality supports that the sludge had a high affinity for acetate. Methanogenic community revealed an overall dominance of the Methanosaeta in the UASB-digester sludge operated between 10-20°C (Zhang et al., 2016b). Methanosaeta was categorized as acetoclastic methanogens and had a minimum threshold concentration of 0.5-5.0 mg COD/L (Jetten et al., 1992) and a high affinity. K_s of the Methanosaeta dominating sludge and Methanosaeta soehngenii was 45 mg COD/L and 30 mg COD/L, respectively (Fukuzaki et al. (1990); Pavlostathis & Giraldo-Gomez, 1991a). At low temperatures, bacteria can change the membrane lipid composition, e.g. increase fatty acid unsaturation and decrease average chain length. The change improves the fluidity of the membrane and compensates the difficult transition of substrate at low temperatures (Nedwell, 1999). Therewith, they can maintain the high affinity as temperature dropped.

6.4 Conclusions

Sludge from an UASB-digester process treating municipal wastewater and continuously exposed to temperature changes from 35 to 12.5°C was shown to have:

- no lag phase in methane production after a sudden temperature drop from 35 to 20, 15 or 10°C
- temperature dependence of SMA of the digester sludge with a sudden temperature drop was similar to that at constant temperatures, and E_a was 62 kJ/mol
- a high affinity for acetate at low temperatures of 35, 25, 15 and 10°C, and K_s was 6.5 mg COD/L for 35°C and almost 0 for the rest temperatures.

The results play an important role in understanding the performance of a UASB-digester progress treating low temperature municipal wastewater, and optimizing its design.

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7 General Discussion

7.1 Introduction

Municipal wastewater must be well treated before being discharged into receiving water thus reducing its impact on the environment. Conventionally, municipal wastewater is treated aerobically, applying the activated sludge process, which consumes a lot of energy for aeration, and produces large amounts of biomass in the form of excess sludge. At the larger treatment plants, this produced sludge is in most cases anaerobically treated for biogas production. The formed biogas generally covers only a fraction of the energy needed for the aeration and other processes in the treatment. Therefore, other more energy efficient, waste water treatment processes are needed. Especially, at higher (tropical) temperatures, anaerobic treatment offers a good and energy effective alternative. However, at moderate temperature conditions, i.e. when in winter time sewage temperatures are as low as 10°C, such an anaerobic treatment method is not yet available. For this reason, within this thesis, a new concept is studied and developed, that allows direct anaerobic treatment to municipal wastewater with temperatures as low as 10°C. Direct anaerobic treatment increases the overall biogas yield and decreases energy cost for the treatment as compared to conventional aerobic treatment of municipal wastewater. The chemical energy of organic matter in the municipal wastewater is recovered in the form of methane. The effluent of the anaerobic treatment contains nitrogen and phosphorus which have to be removed to reach discharge standards. In (sub) tropical countries reuse of the nutrient (nitrogen, potassium and phosphorus) rich effluents can be applied for agricultural purposes, prevailing that pathogens are taken into account (Chernicharo et al., 2015). For low temperature climates reuse in agriculture is in general not attractive due to the relatively short crop seasons and removal of nitrogen and phosphorus from the anaerobic effluent is required. Anaerobic ammonium oxidation processes (Anammox) or denitrifying processes coupled to anaerobic methane oxidation (DAMO) could be applied for autotrophic nitrogen removal following the anaerobic pre-treatment of domestic wastewater (Hendrickx et al., 2012; Kampman et al., 2012).

In this thesis, anaerobic treatment of municipal wastewater at low temperatures applying a UASB-digester system was studied. The UASB reactor was operated at a short hydraulic retention time (6 hours) and low temperature varying between 10-20°C. The high organic load results in accumulation of non-stabilized suspended solids in the UASB sludge bed. The accumulated sludge is, therefore, recycled over a mesophilic digester, in order to convert the suspended solids to biogas and produce methanogenic and other relevant anaerobic biomass for converting dissolved COD in the low temperature UASB reactor. Sludge recirculation rate, sludge transfer point, co-digestion and operation of the UASB-digester at 10-20°C were investigated. The imposed recirculation of the sludge from the UASB reactor to the digester and back, results in the exposure of the sludge to varying temperature conditions. Therefore, the effects of changing temperatures on hydrolysis and methanogenesis were also studied. This chapter finally discusses the potential application of the new municipal wastewater treatment concept including post-treatment for nitrogen and phosphorus removal.

7.2 Operation of a UASB-digester treating municipal wastewater

7.2.1 Sludge recirculation rate and the sludge transfer point

The sludge recirculation rate between the UASB reactor and the digester influences the performance of the UASB-digester system e.g. COD removal and methane production. The recirculation rate should be sufficiently high for transferring the largest portion of the fresh suspended solids to the digester for production of biogas and anaerobic biomass. However, the recirculation of the UASB sludge to the digester consumes energy for pumping and heating. The balance between energy production (related to the generated methane gas) and consumption (pumping and heating) is a key factor for application.

Increasing sludge recirculation rate improves the COD removal efficiency, mainly as a result of the increased dissolved COD efficiency, of a UASB-digester system (chapter 2). Biogas production of the digester clearly increased from 0.06 to 0.15 m^3 biogas/(m^3wastewater d) as the sludge recirculation rate increased from 0.9 to 2.6% of the influent flow rate. This increase of biogas production was attributed to an increased transfer of fresh influent organic solids to the digester. Increasing sludge recirculation further, from 2.6% to 12.5%, didn't result in a significant rise in methane production by the digester. This indicated that a recirculation rate of 2.6% of the influent flow rate should be sufficient for transferring the influent fresh organic solids from the UASB reactor to the digester (Chapter 2). The stability and specific methanogenic activity (SMA) of the UASB sludge increased with an increased SRR from 0.9 to 2.6% due to less accumulation of influent organic solids in the UASB reactor. COD removal efficiency and methane recovery increased correspondingly.

The sludge recirculation rate needed for adequate functioning of the combined system is strongly depending on the distribution of the solids in the UASB sludge bed and the sludge concentration. When the sludge bed approaches a CSTR, the required sludge recirculation rate will be high, while a plug flow behavior of the sludge bed could allow for a reduced sludge recirculation rate. The UASB reactor in the UASB-digester system described in this thesis behaved like a CSTR reactor as the SMA and stability of the UASB sludge was similar along the sludge bed. However, the sludge concentrations decrease with the height of the sludge bed in the reactor due to gravity. Therefore, the sludge transfer point should be chosen at a sludge bed height where the VSS concentration is adequate.

A high VSS concentration of the transferred sludge would benefit a low sludge recirculation rate and save heating and pumping energy. In chapter 2, it is described that in our approach the transfer point was placed at the bottom of the sludge bed (27 cm from the bottom), while Álvarez et al. (2004) and Mahmoud et al. (2004) applied a sludge transfer point at the top of the sludge bed. The appropriate height of the sludge transfer point for optimal suspended sludge (SS) recirculation in practice should be again determined under full scale conditions. This is because sludge distribution over the height of the reactor can substantially differ between laboratory and full scale applications.

7.2.2 Co-digestion

Co-digestion, adding extra organic matter to the digester of a UASB-digester, clearly improved methanogenic capacity of the low temperature UASB reactor and therewith increased its soluble COD removal efficiency (chapter 3). Co-digestion can be applied for municipal wastewater that has a high ratio in influent soluble COD to suspended COD, to increase the biomass yield in the digester for transfer to the UASB reactor. Glucose was used in this study as a model substrate to investigate the feasibility of co-digestion. The addition of the co-substrate was about 16% of the influent organic loading rate (36% of the biodegradable influent organic load). However, this amount of co-substrate addition was not optimized yet.

When a higher soluble COD load on the aerobic posttreatment and a lower biogas production rate are not desired at times of low waste water temperatures, co-digestion is suggested as a method to mitigate UASB instability when temperature falls below critical levels and/or soluble COD loads increase. This will increase the methanogenic capacity of the UASB reactor at low temperature, which would otherwise not be sufficient for complete conversion of biodegradable dissolved COD. For application in practice, a low nitrogen and phosphorus containing co-substrate is recommended in order to limit the additional nitrogen and phosphorus loading on the post-treatment steps.

7.2.3 Operation at temperatures decreasing from 20 to 10°C

Anaerobic treatment of municipal wastewater using a UASB-digester achieved an average COD removal of 60 ± 4.6% at temperatures of 12.5-20°C (chapter 4). The UASB reactor was operated at an HRT of 6 hours, the digester was operated at an HRT of 15 h, a temperature of 35°C and a sludge recirculation rate of 16% of the influent flow rate was applied. The ratio between the UASB reactor and digester volume is 130/50 (L). A high sludge recirculation rate is applied to show the ‘proof of principle’ and can be further optimized.

COD removal efficiency was lower (51.5 ± 5.5%) at 10°C in comparison to that at a temperature of 12.5-20°C. The decrease in COD removal efficiency at 10°C coincided with an increased influent COD concentration. The increased effluent VFA concentration shows a limited methanogenic capacity of the UASB sludge to cope with the combination of a decreased temperature and an increased loading rate. Yet, co-digestion could be applied under such conditions to enhance the methanogenic capacity of the low temperature UASB reactor.

At temperatures between 10-20°C a mean suspended COD removal of 76.0 ± 9.1% can be achieved in a UASB-digester system treating municipal wastewater. This is similar to the results of Mahmoud et al. (2004) achieved at a UASB temperature of 15°C. The results are similar as reported by Chernicharo (2006) who studied the application of the UASB reactor for municipal wastewater treatment at tropical conditions.

Colloidal COD removal efficiency is limited to 42.8 ± 17.5% at 12.5-20°C. Colloidal COD can be removed by bio flocculation in aerobic reactors (Li et al., 2011). Removal of colloidal COD is in general low when applying anaerobic treatment of municipal sewage at low temperatures (Álvarez et al., 2008).

$COD_{soluble}$ removal efficiency fluctuated probably due to the difference in influent BMP of $COD_{soluble}$, while effluent $COD_{soluble}$ remained stable at 90 ± 23 mg/L and no VFA could be determined in the effluent at temperatures between 12.5-20°C. The effluent $COD_{soluble}$ concentration was similar as reported by (Hülsen et al., 2016). Chernicharo et al. (2015) achieved a lower effluent $COD_{soluble}$ of 30 ± 36 mg/L using a UASB-digester system, which in those cases was mainly due to a low influent $COD_{soluble}$ concentration of 50 ± 10 mg/L.

Methane production accounts for 39.7 ± 4.4% of the influent COD at 10-20°C, which is 80% of influent BMP. In the UASB reactor, 49% of the total methane production is produced of which 63% leaves the UASB dissolved in the effluent and 37% as biogas. This high amount of dissolved methane may offer an opportunity in future to introduce DAMO for further nitrogen removal from the effluent (Kampman et al., 2012). However, although the principle of DAMO has been proven (Hendrickx et al., 2012), the technology needs still extensive developments to reach practical full scale application. The high amounts of methane in effluents pose a problem of greenhouse gas emission to the atmosphere. To make anaerobic wastewater treatments climate change neutral, methane recovery or removal from effluents is an important issue that needs to be addressed in future. Given that 25% of the influent COD can be converted to methane gas, excluding the dissolved methane lost with the effluent, the generated energy is sufficient for the heating of about 3% of the influent flow from 10°C to 35°C. At a recirculation rate of 2.6 % (Chapter 2), the produced energy is sufficient for sludge heating. To improve the energy balance, the following methods can be applied: a) optimize sludge recirculation: b) concentrate the recirculated UASB sludge by sludge sedimentation/filtration prior to transfer to the digester; c) run the digester at 30°C; d) apply a heat exchanger for the recirculated sludge. These items are therefore proposed for further optimization of the UASB-digester system towards full scale application, and briefly elaborated at the end of this chapter.

7.2.4 Effect of temperature fluctuation on methanogenesis

No lag phase was found for methanogenic activity of the digester sludge after suddenly decreasing the temperature from mesophilic conditions (35°C) to temperatures of 10, 15 and 25°C (chapter 5). The temperature dependency of the SMA is the same at constant temperatures and fluctuating temperature conditions. The sludge in a UASB-digester system recirculates between a low temperature (10-20°C) and a high temperature (35°C) environment. Given the high SMA at 35°C, in comparison to the lower temperatures, of the digester and the UASB sludge, the sludge can be characterized as mesophilic.

The affinity of the digester sludge for acetate was high at temperatures of 10-35°C. The dominant methanogens in the digester and the UASB sludge were Methanosaetaceae and Methanomicrobiales methanogens (Chapter 4). These methanogens have a high affinity for acetate and a high methanogenic activity even at minimum substrate concentrations between 0.5-5 mg COD/L at 35°C (Álvarez et al., 2008). The UASB sludge had, as expected, a similar microbial structure as the digester sludge and therefore also a high affinity for acetate. This was confirmed by the fact that the methane production of the UASB reactor (including dissolved methane) matched well with the methanogenic capacity at 12.5-20°C (Chapter 3 and 4).

7.2.5 Short-term pre-hydrolysis at mesophilic condition enhances low temperature hydrolysis

The recirculated sludge was exposed to temperatures, fluctuating between the low temperature in the UASB reactor and the mesophilic temperature in the digester. Therefore, effects of a pre-hydrolysis of organic matter at mesophilic conditions on hydrolysis at low temperature was studied. Hydrolysis tests were executed, applying granular sludge as inoculum from a paper industry in Eerbeek, the Netherlands at low temperatures (10-25°C) with and without a short pre-hydrolysis at mesophilic conditions, using cellulose and tributyrin as substrate (Chapter 6). A short pre-hydrolysis step at 35°C clearly increases the first order hydrolysis rate constant for cellulose at low temperatures (10-25°C). A long lag phase of 40-60 days is occurring when applying cellulose hydrolysis at 10-15°C. The latter indicates that cellulase production is limiting hydrolysis during start-up. No lag phase is occurring at low temperature conditions when pre-hydrolysis at 35°C is applied. This increased hydrolysis rate constant of cellulose at low temperatures is hypothesized to be due to an excess cellulase production during pre-hydrolysis at 35°C.

For cellulose, hydrolysis yield clearly decreases from 100% to 9.6% as temperature decreased from 35 to 10°C. This decrease in yield with temperature decrease can be due to the crystalline structure of cellulose. The yield at 10°C, after a short pre-hydrolysis period at 35°C amounts to 40% in 9.1 days, while that at 10°C without pre-hydrolysis is only 9.6%, achieved in 62 days. This clearly shows that hydrolysis of cellulose, initiated at high temperatures (in our system, the digester) can remain active at low temperatures (in our system, the UASB reactor).

Hydrolysis tests were executed at 35°C, using the supernatant of the digestate after batch digestion of cellulose and tributyrin at 10-35°C to test abovementioned hypothesis. The higher determined hydrolytic activity of the supernatant collected from the digestate at 35°C as compared to that of the supernatants collected at low temperatures (≤ 25°C) digestates confirms the hypothesis that excess cellulases are excreted during pre-digestion at 35°C and can remain active at lower temperatures.

These excess cellulases accelerate the hydrolysis when temperature decreases after a period of high temperature. The increased production of dissolved COD in the UASB reactor as compared to the influent dissolved COD, as observed in the present research (Chapter 4), can be ascribed to the extra hydrolysis as a result of the transferred excess enzymes from the digester to the UASB, in a UASB-digester system. The UASB reactor needs to be designed based on this extra dissolved COD load.

7.3 Outlook

7.3.1 New municipal wastewater treatment

A new concept of municipal wastewater treatment can be achieved using anaerobic treatment as the core biological unit as shown in Fig. 7.1. A UASB-digester is proposed for moderate temperature climate zones, to convert organic material from municipal wastewater into energy, in the form of methane. Bio-flocculation followed by anaerobic sludge digestion, as applied in the AB process, is referred to as another alternative for activated sludge treatment (Faust et al., 2014; Verstraete et al., 2009). Main advantage as compared to direct anaerobic treatment of domestic sewage is the absence of dissolved methane in the liquid anaerobic effluent. However, it needs an energy input of 0.03 kWh/ m^3 (wastewater) for aeration (Khiewwijit et al. 2015). Chemical energy of 1.5-1.9 kWh per m^3 of wastewater can be recovered from municipal wastewater with an COD concentration of 400-500 mg/L (Owen, 1982).

Based on the new concept for upgrading the conventional municipal wastewater treatment plant, the primary sludge sedimentation tank and aeration basins can be replaced by a UASB reactor. Anammox or after further development DAMO processes can be used for autotrophic nitrogen removal (Hendrickx et al., 2012; Kampman et al., 2012).

Phosphorus is a limited resource, which used to be considered as pollutant in conventional wastewater treatment. Iron precipitation is often applied for the removal of phosphorus in conventional wastewater treatment (Parsons & Smith, 2008). The product is however not suitable for reuse in agriculture (De-Bashan & Bashan, 2004), therefore other techniques are required. Recently, (Drenkova-Tuhtan et al., 2016) published on the recovery of phosphate via a sorption/desorption technique, making use of phosphate specific absorbents viz. advanced nanocomposite magnetic particles. The pilot scale experiments results showed a 25-38 times higher phosphate concentration in the desorption as compared to the start solution.

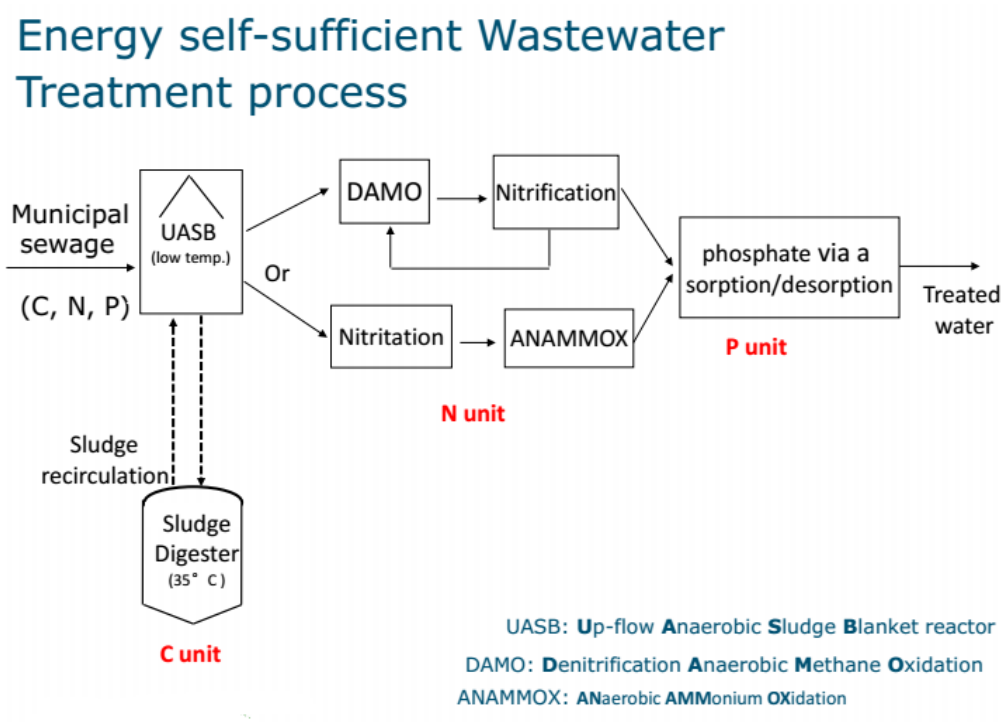

Fig.7.1 New concept of a wastewater treatment plant

The public, government, institutes and companies are involved in or affected by the upgrading of a municipal wastewater treatment plant. It would be fair when representatives of the public, who will pay for the chosen plan, get a chance to give their opinion about these plans. Design of a wastewater treatment plant can be assessed on cost-benefit analysis, safety and function (Guest et al., 2009). The plans should be accepted by the representatives of the public; otherwise new plans should be conceived. However, the interaction between different stakeholders, to decide upon the wastewater treatment process is insufficient or deficient in many countries e.g. China. In China, for a metropolitan area like Beijing, the government did choose the anaerobic anoxic oxic (A2O) process coupled with membrane filtration for municipal wastewater treatment for meeting the stringent standard. The government has to increase the wastewater disposal fee year by year due to the energy and cost consuming wastewater treatment plants, and this will reduce public acceptance. Therefore, a societal drive to reach energy and cost effective waste water treatment can be expected to also arise in China over time, and will create in China and elsewhere in the world a new market for these.

7.3.2 Costs

The operational costs of the new wastewater treatment concept using an anaerobic reactor for organic materials removal is much lower than a conventional wastewater treatment plant. No aeration is needed in the operation of anaerobic reactors, therewith saving electricity consumption. Complete aerobic BOD removal consumes 0.45 kWh, whereas complete anaerobic treatment produces 0.25 kWh (Scherson & Criddle, 2014). The produced heating energy and electricity can be utilized in the wastewater treatment plant itself, for controlling the temperature of the mesophilic sludge digester and for providing electricity for denitrification, aiming at an energy neutral process.

The excess sludge of a UASB-digester in this study was 8 ± 5% of the influent COD, which is much lower than that of a conventional wastewater treatment plant (Chapter 4). The operational cost of excess sludge processing can be significantly reduced using anaerobic reactors due to the low amounts of sludge produced. In further developments, the sludge production due to chemical phosphorus removal and nitrogen removal should also be taken into account. E.g, the operational and maintenance costs are 1.2-1.7 euros /(inhabitant · year) for a Brazilian wastewater treatment plant applying a UASB reactor (Chernicharo et al., 2015), while the average costs for a conventional wastewater treatment plant was 40 euros/p.e/year in 2006 in the Netherlands (UVW, 2006). The absolute difference of the cost between these two case examples can be smaller considering the different sludge disposal and labor costs, and the different effluent discharge standard.

When applying a UASB-digester with a recirculation rate of 16% of the influent flow rate, methane production can compensate for only 20% of the heating energy of the digester, when operating the UASB at 10°C (Chapter 4). Strategies for saving energy are discussed in paragraph 7.2.3. The duration of winter time in countries with moderate climates is generally 3 months, and the time that the wastewater temperature decreases to 10°C is shorter. For different climatic conditions an energy balance over the year is to be established, to make a full feasibility evaluation of our proposed sewage treatment concept.

Another item is the resilience of the UASB-digester system to fluctuations in COD and flow of the sewage. Although in many countries rainwater collection will be uncoupled from sewage infrastructure (Arnaldos et al., 2015), which will be highly beneficial for anaerobic treatment technologies since the COD levels in sewage will significantly increase and fluctuate less, significant daily and seasonal fluctuations can still be expected (Agudelo-Vera et al., 2013). In this study, the UASB-digester approach was developed at constant flow and reduced fluctuation in influent COD concentration. In further optimization towards full application, these flow and COD fluctuations also need to be taken into account.

7.3.3 Recommendations for further research

This research showed that anaerobic treatment of municipal wastewater at 10-20°C using a UASB-digester system can achieve a robust COD removal and methane recovery. In a UASB-digester system, the major operational cost is energy consumption for heating the recirculated sludge. The mitigation methods for saving this energy have been discussed in paragraph 7.2.3 (operation at 20-10°C) and chapter 4.

The efficiency of the UASB-digester system treating municipal wastewater is mainly depending on COD composition ($COD_{suspended}/COD_{soluble}$), sludge retention and sludge recirculation rate. Operational parameters such as sludge recirculation rate and sludge recirculation point, HRT of the UASB reactor, temperature of the digester or co-digestion should be adjusted according to the influent quality.

Separate collection and transport of rainwater and municipal wastewater will decrease hydraulic loading rate, increase COD concentration of the wastewater and reduce its variation. This definitely benefits the application of anaerobic wastewater treatment. There is a large potential for new sewer systems in countries with growing urbanization, where wastewater treatment plants are not present yet. Within so called ‘New Sanitation’ concepts, not only rainwater, but also black water (toilet water) and grey water are separately collected, transported and treated. So far, vacuum collection and transport is applied for the black water to achieve a sufficiently high concentration to allow for energy efficient mesophilic anaerobic treatment in countries with moderate climates. The here presented new treatment system would also allow for the collection and anaerobic treatment of less concentrated black water. The latter could enhance the implementation of ‘New Sanitation’. In these concepts, technology robustness is important, and therefore the resilience of the UASB-digester system to fluctuations in flow, COD, and temperature is an important item for future full scale optimization.

The here proposed nitrogen removal processes, coupled to a UASB-digester system can save energy and do not require organic resources. ANAMMOX and DAMO processes are already studied individually (Kampman et al., 2012; Laureni et al., 2016; Lotti et al., 2015). The volumetric nitrite consumption rate of the DAMO process was shown to be not sufficient yet, and applying a membrane was suggested to increase the biomass retention (Kampman et al., 2012). The performance of ANAMMOX or DAMO, integrated with a UASB-digester system is suggested in further studies.

Pathogen removal should also be concerned for the post-treatment of the UASB-digester system to avoid the spreading of diseases. A downflow hanging sponge reactor can be used to remove pathogens, and guarantees the effluent COD concentration to comply with effluent discharge standards. The UASB-DHS system was tested in a demonstration-scale of 1000 m^3/d capacity in India, where the system has been operated since 2003 (Tandukar et al., 2005).

The fate and the risks of micro-pollutants like pharmaceuticals in the effluent of wastewater treatment plants should be assessed due to a long term consideration of environmental safety. Due to its micro amount, it would be more effective to control from the source rather than the end. E.g. expired pharmaceutical or medicine waste should be collected separately. Still, most pharmaceuticals come from feces and urine (Butkovskyi et al., 2015). Butkovskyi et al. (2015) reported that the removal of pharmaceuticals in a UASB reactor can be better or similar, depending on specific pharmaceuticals, compared to conventional wastewater treatment plants. Poor pharmaceutical removal is expected using a UASB-digester system for sewage treatment, due to the low activity of the UASB sludge at low temperatures. Proper post-treatment, like activated carbon is required (Hernández-Leal et al., 2011), while the energy, costs and removal performance needs to be balanced.

Methane is a greenhouse gas and is emission that should be prevented within the new wastewater treatment concept. The dissolved methane increases when temperature decreases, which is a key issue for application of anaerobic treatment in moderate countries. The DAMO process is suggested as it can remove dissolved methane and nitrogen together. Besides, a membrane technology such as hollow-fiber membranes and a poly-di-methyl-siloxane (PDMS) membrane contactor can be used for degasification and to strip the dissolved methane respectively (Cookney et al., 2012; Hatamoto et al., 2010). Two subsequent stages of DHS were applied to successfully aerobically oxidize the remaining effluent dissolved methane (Matsuura et al., 2015). Besides, vacuum degasification was studied to transfer dissolved gas in the liquid of the UASB reactor inside the membrane, and COD removal efficiency was increased from 83% to 90% (Bandara et al., 2013). However, the economic assessment and energy consumption should be considered before applying these technologies.

Demonstration is the most effective and strongest way to spread the new wastewater treatment concept. Operation experience can be gained through the demonstration which benefits the dissemination of the knowledge of the UASB-digester system. For example, energy recovery from waste-water treatment is considered as one of alternative energy supply technologies in Canada (Cuddihy et al., 2005). The same argument is applicable to China and other parts of the world, where energy friendly wastewater treatment plants are attractive also for rural areas where low operational costs are an important societal boundary condition for effective waste water technology innovation and implementation.

Summary

Summary

A new wastewater treatment concept, applying direct anaerobic treatment of low temperature municipal wastewater is studied within this thesis. The treatment concept results in an increased biogas yield and decreased energy consumption as compared to conventional treatment of municipal wastewater. Chemical energy of organic matter in the municipal wastewater is recovered in the form of methane. The dissolved methane in the effluent could, concurrently with nitrogen, be removed via the denitrifying anaerobic methane oxidation (DAMO) process. Alternatively, the anaerobic ammonium oxidation process (ANAMMOX) could be applied for autotrophic nitrogen removal following the anaerobic treatment of domestic wastewater. Applying such a chain of different biological conversion technologies, can change the treatment of municipal wastewater from energy consuming to energy self-sufficient. In this study, anaerobic treatment of municipal wastewater at low temperatures applying a system of a combination of an upflow anaerobic sludge bed (UASB) reactor and a sludge-digester (UASB-digester) was studied.

Chapter 1 gives a literature review on anaerobic wastewater treatment. Municipal wastewater, as one of the main pollution sources of water systems, must be treated before being discharged into receiving surface waters, to avoid water resource pollution. Anaerobic wastewater treatment can be an alternative to reduce energy consumption and operational costs. However, low temperature is still a challenge for anaerobic wastewater treatment of municipal wastewater due to the low hydrolysis rate of organic solids and the low growth rate of methanogenic biomass needed for biogas production.

Among the anaerobic reactors, designed for low temperature treatment, a UASB-digester is a promising system as, next to removal of dissolved and particulate organics, also biodegradable organic particles are entrapped and converted to methane. Therefore, it provides stabilized excess sludge unlike other anaerobic two phase systems, like the anaerobic filter (AF)- anaerobic hybrid (AH) reactor or hydrolytic upflow sludge bed (HUSB) reactor - UASB (or expanded granular sludge bed EGSB) system, that produce sludge that needs further stabilization. The temperature of domestic wastewater in moderate climate zones can be as low as 10°C and therefore the feasibility of the UASB-digester also needs to be assessed at temperatures below 15°C, which has not been done prior to this study. For this purpose, a pilot-scale UASB-digester was studied, and the temperature was subsequently decreased in steps to 10°C.

A pilot scale UASB-digester system was applied to treat real domestic sewage of Bennekom, the Netherlands. Effects of sludge recirculation rate and height of the UASB sludge transfer point were studied in Chapter 2. A sludge recirculation rate of 1%, 2.6% and 12.5% of the influent flow rate was investigated. The total COD removal efficiency increases with the sludge recirculation rate. A sludge recirculation rate of 1% of the influent flow rate leads to organic solids accumulation in the UASB reactor. The stability of the UASB sludge and the biogas production in the digester substantially improve when increasing the recirculation from 1% to 2.6%, from 0.37 to 0.15 g CH_4-COD/g COD and from 2.9 to 7.4 L/d, respectively. No further improvement is shown

at a recirculation rate of 12.5% of the influent flow rate, but the biogas production in the UASB increases from 0.37 L/d to 1.2 L/d. A sludge recirculation rate of approximately 3% of the influent flow rate is recommended. Additionally, different sludge transfer points were studied. A higher sludge transfer point results in an increased suspended COD removal efficiency and VSS concentration of the UASB sludge bed.

Co-digestion in the digester of the UASB-digester was studied, to enable efficient treatment of municipal wastewater with a high dissolved/suspended COD ratio at low temperatures. (Chapter 3). Glucose was chosen as a model co-substrate. Co-substrate was added in the sludge digester to produce additional methanogenic biomass, which was continuously recycled to inoculate the UASB reactor. Soluble COD removal efficiency increases from 6 to 23%, when applying co-substrate 16% of influent organic loading rate to the digester. The soluble COD removal equals the biodegradability of the influent dissolved COD. Specific methanogenic activity (SMA) of the UASB and of the digester sludge at 15°C triples to a value of respectively 43 and 39 mg CH_4-COD/(g VSS·d). Methane production in the UASB reactor almost doubled due to a twofold increase in methanogenic capacity.

A pilot scale UASB-digester was studied to treat domestic wastewater at temperatures of 10-20°C and an HRT of 6 h in the UASB reactor and an HRT of 15 h in the digester (Chapter 4). The COD removal efficiency remains stable at 60 ± 4.6% when decreasing the temperature from 20 to 12.5°C; it decreases to 51.5 ± 5.5% at 10°C. The decreased COD removal efficiency at 10°C is attributed to an increased influent COD load, leading to insufficient methanogenic capacity of the UASB reactor. Suspended COD (COD_{sus}) removal efficiency is 76.0 ± 9.1% at temperatures of 10-20°C. Soluble COD removal (COD_{sol}) fluctuates due to variation of the influent COD concentration, but the average effluent COD concentration is 90 ± 23 mg/L at temperatures between 12.5 and 20°C. The methane production is 39.7 ± 4.4% of the influent COD, which is 80% of influent biological methane potential (BMP); 49% is produced in the UASB reactor and 51% in the digester; 31% of the produced methane is dissolved in the UASB effluent. Discharged sludge accounts for 8 ± 5% of the influent COD. The methanogenic community is dominated by the acetoclastic Methanosaetaceae and the hydrogenotrophic Methanomicrobiales during the operation of a UASB (10-20°C)-digester (35°C) for domestic sewage treatment.

In Chapter 5 low temperature (10-25°C) hydrolysis was investigated with and without application of a short pre-hydrolysis step at 35°C. Batch experiments were executed using cellulose and tributyrin as model substrates for carbohydrates and lipids. The low temperature anaerobic hydrolysis rate constant increases by a factor of 1.5 to 10, when a short anaerobic pre-hydrolysis step at 35°C is applied. After the pre-hydrolysis step at 35°C, no lag phase occurs at temperatures between 10 and 25°C. Without pre-hydrolysis, the lag phase for cellulose hydrolysis at 35-10°C is 4 - 30 days. Tributyrin hydrolysis shows no lag phase at any temperature. The hydrolysis efficiency of cellulose, after 9.1 days batch digestion at 15 and 10°C, increases from 40 to 62% and from 9.6 to 40%, respectively, when pre-hydrolysis at 35°C is applied. Pre-hydrolysis does not affect the hydrolysis efficiency of tributyrin. The hydrolytic activity of the supernatant, collected from the digestate after batch digestion of cellulose and tributyrin at 35°C, is higher than that of the supernatants collected from low temperature (≤ 25°C) digestates. This effect of pre-hydrolysis at 35°C should be taken into account in the design of a UASB-digester system, as it may increase the soluble COD load on the UASB reactor.

In a UASB-digester system, the sludge is continuously exposed to changing temperatures, as the sludge is recirculated from the low temperature UASB reactor to the warm digester and back. Effects of an immediate temperature drop from 35°C to 25, 15 or 10°C on the methanogenic activity and the effects of temperature (10-35°C) on affinity constant for acetate using the digester sludge from a UASB-digester process, were studied in batch experiments in Chapter 6. Digester sludge of a UASB (12.5°C)-sludge digester (35°C) was fed with acetate at constant temperatures of 10-35°C and at varying temperatures from 35°C to 25, to 15 to 10°C. No lag phase in methane production rate occurs when applying temperature shocks from 35°C to 25, 15, and 10°C. The temperature dependency of the specific methanogenic activity (SMA) of the digester sludge after the temperature shocks is similar to that at constant temperatures. The activation energy for the SMA of the digester sludge is 62 kJ/mol. Acetate affinity of the digester sludge is high at temperatures between 10 and 35°C with apparent affinity constants ≤ 6 mg COD/L.

The results of this research are discussed in Chapter 7. The results of the present research show that anaerobic treatment of municipal wastewater at 10-20°C using a UASB-digester system can achieve a robust COD removal and methane recovery. Especially the good performance at 10°C had not been shown previously. This was achieved when applying a UASB-digester with a recirculation rate of 16% of the influent flow rate. At this recirculation rate, methane production can compensate for only 20% of the heating energy of the digester, when operating the UASB at 10°C (Chapter 4). Strategies for saving energy are a) optimize sludge recirculation: b) concentrate the recirculated UASB sludge by sludge sedimentation prior to the digester; c) run the digester at 30°C; d) apply a heat exchanger for the recirculated sludge.

The integration of nitrogen removal with ANAMMOX or DAMO, with a UASB-digester is suggested for further research. Also, the recovery or conversion of dissolved methane in the effluent is suggested for further research, as its emission to the atmosphere should be avoided, given its high global warming potential. Furthermore, the recovery of phosphate after anaerobic treatment should be considered, as it is a finite resource. Although recovery technologies are available for concentrated phosphate solution, recovery from diluted sewage still requires further research. Demonstration of the UASB-digester system at a larger scale is required for gaining more real operational experience.

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Annex

Annex 1 Quality control of the parameters for real-time PCR analysis

Table 1 gives quality control of the parameters for real-time PCR analysis. These parameters were obtained during analysis with the StepOnePlus V2.3 software. The detection limit was calculated as copies of the target 16S rRNA gene fragment per gram wet sludge, and was determined taking both dilution and extraction efficiency into account.

Table 1 Quality control of the parameters for real-time PCR analysis.

Parameter	Slope	R^2	Efficiency (%)	Detection limit (copies g^{-1})
Methanosaetaceae	-3.7	0.99	85	1.53×10^4
Methanosarcinaceae	-4.6	1.00	64	1.45×10^4
Methanobacteriales	-4.1	1.00	75	1.18×10^4
Methanomicrobiales	-4.1	1.00	76	1.08×10^4
Total bacteria	-3.2	1.00	104	2.76×10^4

Annex 2 Effluent VSS concentration and $COD_{suspended}$ concentration

Table 2 gives effluent VSS concentration and $COD_{suspended}$ concentration, and the ratio of $COD_{suspended}$ concentration to VSS is about 2.

Table 2 Effluent VSS concentration and $COD_{suspended}$ concentration

(sample numbers:8, unit: mg/L)

Effluent VSS concentration	Effluent $COD_{suspended}$ concentration
40 ± 5	82 ± 20

Annex 3 Equations used for assessment of Energy consumption at 10°C

Major energy consumption of the UASB-digester system treating low temperature sewage is heating of the sludge that is transferred from the UASB reactor to the digester. The energy consumption depends on the sludge recirculation rate. So far, applying the proof of principle, a high sludge recirculation rate (16 % of influent flow rate) is applied to test the feasibility of the system for the treatment of sewage at 10°C. The sludge recirculation rate can be decreased after optimization. At present a model is model is developed to predict, making use of the collected data, the optimal sludge recirculation rate.

- Heating consumption

Δ Temperature ×Specific heat capacity of water × Sludge recirculation rate

= 25 × 4.2 × 0.16 × 1000= 16800 (KJ/ m3 treated sewage)

- Energy production of methane

Based on the COD balance, about 40 % of influent COD can be converted to methane. Given the influent COD concentration of 600 mg/L. Heat production of methane is calculated:

Heating production:

Heat value of methane × methane production

= Heat value of methane × (influent COD concentration × 40 % × 0.35)

= 40 × (600 × 40 % × 0.35) = 3360 (KJ/m3 treated sewage)

- Energy balance

Energy balance = energy consumption- energy production = 16800-3360 = 13440 (KJ/ m3 treated sewage)

- Portion of heating energy compensated by methane

$\text{Portion}_{\text{methane}} = 100 \times \text{Heating}_{\text{methane}} / \text{Heating}_{\text{consumption}} = 3360/16800 = 20\%.$

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About the author

Lei Zhang was born on 21st, 1983, in Harbin, China. In September 2005, he graduated from Engineering in Civil Engineering at Harbin Institute of Technology (HIT), China. From 2005 to 2007, he continued the master study. He started the research career as a research assistant at home university. From 2009.09 to 2010.12, he got scholarship from China Scholarship Council to work as a research guest at Sub-department of Environmental Technology in Wageningen University (WUR) in the Netherlands, and continued the research work from 2011.01 to 2011.12 with scholarship from the department. From 2012.01 to 2012.12, he finished research work in HIT. In January 2013, he started the PhD project at Sub-department of Environmental technology in WUR, the Netherlands. From November 2016, he works at School of Mining and Petroleum Engineering, Alberta University, Canada as a postdoc working on the theme of anaerobic wastewater treatment.

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- Member of the Wageningen University PhD Council (WPC) (2013-2015)
- Supervising four MSc theses (2013-2016)

Oral Presentations

- *The effect of sludge recirculation rate on a UASB-digester treating domestic sewage at 15˚C,* X Latin American Workshop and Symposium on Anaerobic Digestion (DAAL), 23-27 October 2011, Minas Gerais, Brazil
- *Sludge transfer point of a UASB-digester system: key to efficient low temperature anaerobic sewage treatment.* 13th World Congress on Anaerobic Digestion: Recovering (bio) Resources for the World, 25-28 June 2013, Santiago de Compostela, Spain

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