

Gent, May 2010

To my parents.

谨以此博士论文感谢我最敬爱的爸爸妈妈。

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ACADEMIC YEAR 2009-2010

**STRATEGIES TO ENHANCE ANAEROBIC DIGESTION
IN VIEW OF PROCESS STABILITY AND METHANATION**

Jingxing MA

Thesis submitted in fulfillment of the requirements for the degree of
Doctor (PhD) in Applied Biological Sciences

Dutch translation of the title:

Strategieën voor hogere processtabiliteit en methaanvorming bij anaerobe vergisting

The work presented in this thesis was performed at the Laboratory of Microbial Ecology and Technology within the Department of Biochemical and Microbial Technology, and supported by Milieu- en Energietechnologie Innovatieplatform (MIP-Project).

ISBN 978-90-5989-374-0

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Volatile fatty acids

CH_3COOH	Acetic acid
$\text{CH}_3\text{CH}_2\text{COOH}$	Propionic acid
$\text{CH}_3\text{CH}_2\text{CH}_2\text{COOH}$	Butyric acid
HCOOH	Formic acid

Gaseous compounds

CH_4	Methane
CO_2	Carbon dioxide
H_2	Hydrogen
H_2S	Hydrogen sulfide
N_2	Nitrogen

Engineering parameters

COD	Chemical oxygen demand	g COD L^{-1}
COD_S	Soluble chemical oxygen demand	g COD L^{-1}
COD_T	Total chemical oxygen demand	g COD L^{-1}
DM	Dry matter	g DM L^{-1}
DS	Dry solid content	%
E_{HPr}	Propionic acid removal efficiency	%
HRT	Hydraulic retention time	day; hour
OLR	Organic loading rate	$\text{g COD L}^{-1} \text{d}^{-1}$
R_{HPr}	Propionic acid removal rate	$\text{g HPr-COD L}^{-1} \text{d}^{-1}$
SRT	Solid retention time	day; hour
SVI	Sludge volume index	mL L^{-1}
TKN	Total kjeldahl nitrogen	g L^{-1}
TS	Total solid	g TS L^{-1}
TSS	Total suspended solid	g TSSL^{-1}
VFA	Volatile fatty acid	g L^{-1}
VFA_T	Total volatile fatty acids	g L^{-1}
VS	Volatile solid	g VS L^{-1}
VSS	Volatile suspended solid	g VSS L^{-1}

Text abbreviations

CHP	Combined heat and power generator
CODH	Carbon monoxide dehydrogenase
CSTR	Continuous stirred tank reactor
DOC	Dissolved organic carbon
EDTA	Ethylenediaminetetraacetic acid
EPAD	Enhanced propionic acid degradation system
FDH	Formate dehydrogenase
HAc	Acetic acid
HC	High conductivity
HPr	Propionic acid
KW	Kitchen waste
MSW	Municipal solid waste
OFMSW	Organic fraction of municipal solid waste
NTA	Nitrilotriacetic acid
SODM	Superoxide dismutase
POB	Propionate oxidizing bacteria
PS	Primary sludge
SRB	Sulfate reducing bacteria
TOC	Total organic carbon
UASB	Upflow anaerobic sludge bed reactor
WAS	Waste activated sludge
WWTP	Wastewater treatment plant

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GENERAL INTRODUCTION

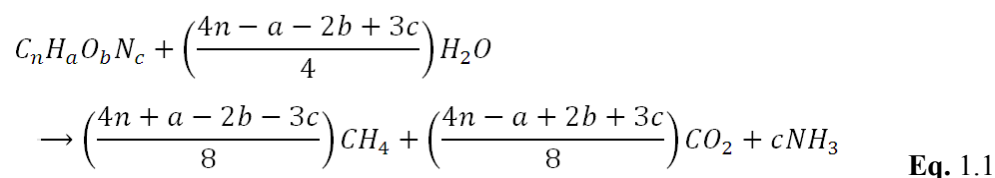
As early as 18th century, a natural process, anaerobic digestion, came into our view. Since then, the research of this process went deeper and deeper together with the growth of science, and today, this exploration is still going on...

1.1 BACKGROUND OF ANAEROBIC DIGESTION

1.1.1 Scientific background

Anaerobic digestion is a fermentation process in the absence of oxygen, in which organic material is degraded by microorganisms. At the same time, biogas, composed of mainly methane (CH₄) and carbon dioxide (CO₂), is produced (Singh and Prerna, 2009). This process is carried out by series of metabolic interactions among a large and varied group of bacteria and archaea, which normally live in a symbiotic relationship (Cardinali-Rezende et al., 2009).

Theoretically, anaerobic digestion obeys to straightforward stoichiometry, which means the degradation of organic matter and the amount of CH₄ production can be calculated based on the Buswell equation, as Eq.1.1 (Ekama, 2009):



However, complex organic materials are not converted in one step to form CH₄ and CO₂. A whole series of microorganisms, each with a limited fermentation capability, gradually break down the molecules in a multi-step process of series and parallel reactions (Figure 1.1).

In general, anaerobic digestion is composed out of three elementary biological processes: hydrolysis, acidogenesis (also known as acidification or fermentation) and methanogenesis, where acetogenesis and methanogenesis happen simultaneously (Gavala et al., 2003).

The digestion processes begin with the enzymatic hydrolysis of the input organic polymers: conversion of the complex, undissolved material (e.g. carbohydrates), into less complex and soluble compounds. This is followed by the acidification where these dissolved compounds are further broken down into a number of simple compounds, such as volatile fatty acids (VFAs). The acetogens then convert these resulting VFAs into acetic acid (HAc), along with additional hydrogen (H₂) and CO₂. Simultaneously, CH₄ is formed by acetotrophic methanogens from HAc and by hydrogenotrophic methanogens from H₂ and CO₂, of which the association is energetic related (Table 1.1) (Pavlostathis and Giraldo-Gomez, 1991).

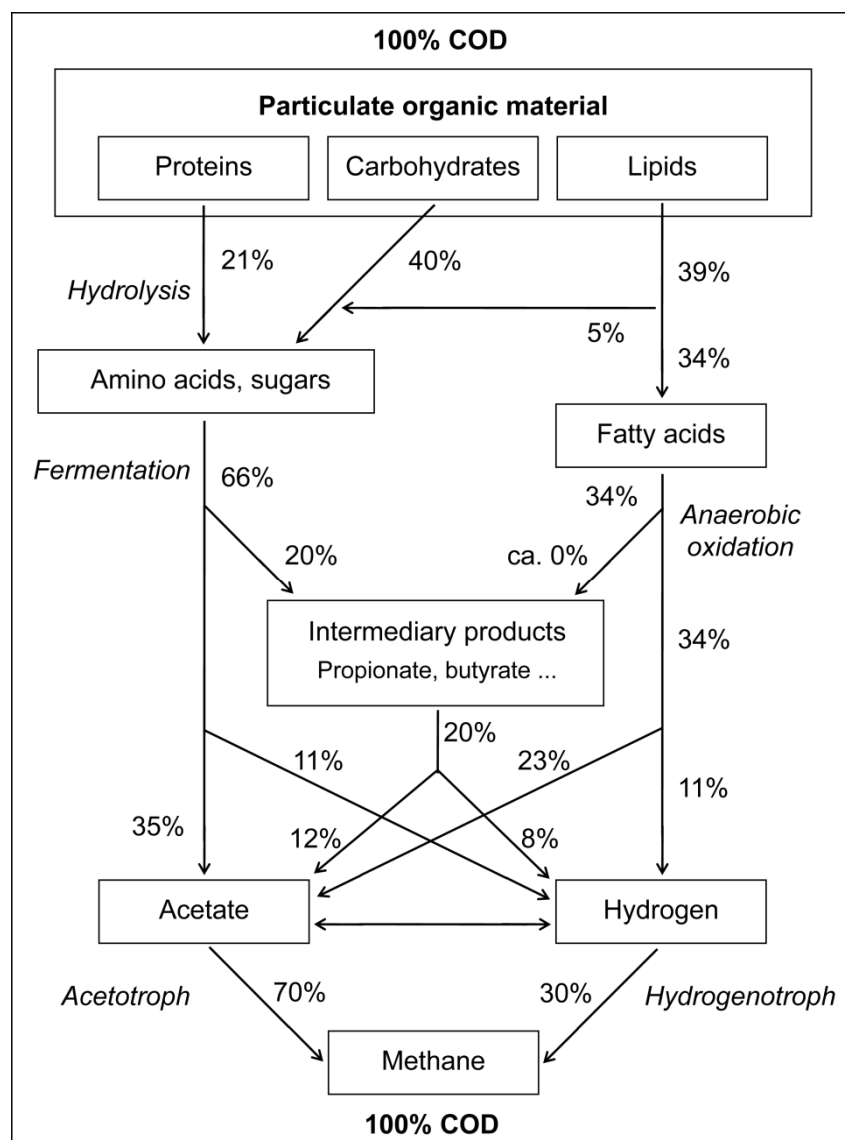


Figure 1.1 Schematic overview of the metabolic steps involved in the complete degradation of complex organic molecules into CH_4 (after Siegrist et al., 1993).

Table 1.1 Reactions of methanogenic pathways and associated free energy per reaction (after Schink, 1997; McInerney, 1999; Winter, 1999; Aiyuk, 2004).

	Reaction	Products	ΔG° (kJ)
	$\text{CO}_2 + 4\text{H}_2$	$\text{CH}_4 + 2\text{H}_2\text{O}$	-135.6
Methanogenesis	4HCOOH	$\text{CH}_4 + 3\text{CO}_2 + 2\text{H}_2\text{O}$	-130.1
	CH_3COOH	$\text{CH}_4 + \text{CO}_2$	-31.0
	<hr/>		
Without H_2 used by methanogens	$\text{CH}_3\text{COO}^- + 4\text{H}_2\text{O}$	$2\text{HCO}_3^- + \text{H}^+ + 4\text{H}_2$	+104.6
	$\text{CH}_3\text{CH}_2\text{COO}^- + 3\text{H}_2\text{O}$	$\text{CH}_3\text{COO}^- + \text{HCO}_3^- + \text{H}^+ + 3\text{H}_2$	+76.1
	$\text{CH}_3\text{CH}_2\text{CH}_2\text{COO}^- + 2\text{H}_2\text{O}$	$2\text{CH}_3\text{COO}^- + \text{H}^+ + 2\text{H}_2$	+48.3
With H_2 used by methanogens	$4\text{CH}_3\text{CH}_2\text{COO}^- + 3\text{H}_2\text{O}$	$4\text{CH}_3\text{COO}^- + \text{HCO}_3^- + \text{H}^+ + 3\text{CH}_4$	-102.4
	$2\text{CH}_3\text{CH}_2\text{CH}_2\text{COO}^- + \text{HCO}_3^- + \text{H}_2\text{O}$	$4\text{CH}_3\text{COO}^- + \text{H}^+ + \text{CH}_4$	-39.4

1.1.2 Anaerobic digestion as a technology

The initial purpose of anaerobic digestion was the treatment of organic wastes, and nowadays the energy recovery is equally important. There are three principal products: digestate, liquor supernatant and biogas, as schematically presented in Figure 1.2. The digestate contains the solid remnants of the original input organic materials, as well the mineralized remains of the dead microorganisms. The liquor supernatant originates both from the moisture content of the original wastes and the water produced during the microbial reactions. Depending on the quality of the liquor supernatant, it can be used as fertilizer or be disposed to the sewer. The very important output for energy recovery is the biogas, which is transported to a combined heat and power generator (CHP), and about 40% and 50% of electricity and useable heat, respectively, can be produced (Karpenstein-Machan, 2001).

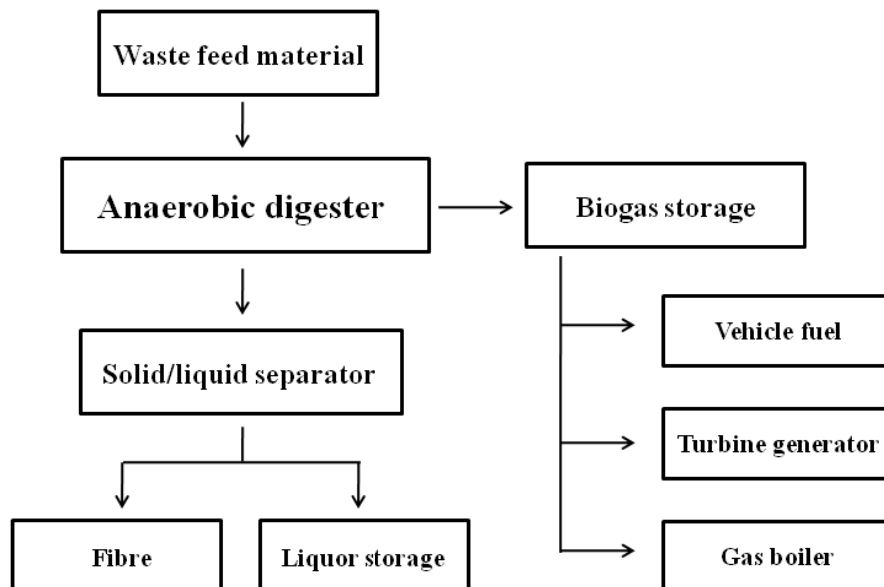


Figure 1.2 A schematic process flow chart of a basic anaerobic digestion products.

From the research laboratory to the field, a variety of waste streams have been shown to be suitable for anaerobic treatment. Feedstock for anaerobic digestion originates mainly from the organic fraction of municipal solid waste (OFMSW), the municipal primary sludge (PS) and waste activated sludge (WAS), and the agriculture as well as the industrial waste streams (Fitzmorris et al., 2009; Sakar et al., 2009).

Anaerobic digestion has evolved into a competitive wastewater treatment technology compared to the aerobic system. The energy requirements and sludge disposal of the conventional wastewater treatment plants (WWTPs) are high, i.e. annual consumption of 20 kWh per IE for a WWTP capacity of 10,000 to 100,000 IE and 100 € per ton of dry solids (DS), respectively. Therefore the operation and maintenance costs of the aerobic system are intensive, i.e. annually 4.1 € per IE for a WWTP capacity of

10,000 to 100,000 IE (Tsagarakis et al., 2003; Nowak, 2006). As a part of an integrated waste management system, the most striking advantages of anaerobic digestion are the lower production of excess sludge based on the chemical oxygen demand (COD) utilization ($0.04 \text{ g COD}_{\text{biomass}} \text{ g}^{-1} \text{ COD}_{\text{removed}}$) (Tomei et al., 2009) and the energy recovery through the biogas production (theoretically around $0.5 \text{ L g}^{-1} \text{ COD}_{\text{removed}}$). From an applied point of view, it has a relevant smaller footprint area ($0.01\text{-}0.05 \text{ m}^2$ per IE), compared to the conventional aerobic system ($0.2\text{-}0.5 \text{ m}^2$ per IE) (Kalogo, 2001). Moreover, as a waste treatment technology, anaerobic digestion decreases the organic waste fraction to be disposed and the diffusive uncontrolled CH_4 emission. Therefore, it can be an alternative for other waste management processes (e.g. landfill).

1.1.3 Biogas: the glamour of anaerobic digestion today

Because of the energy crisis due to the depletion of finite resources of fossil fuels, the advanced waste management strategies set the priority of waste recovery through reuse and recycling, which means that the wastes should be an energy provider and not an energy consumer (Meher et al., 2006; Fytli and Zabaniotou, 2008). Therefore a change of energy source from fossil fuels to biogas, a clean and renewable form of energy, can have important beneficial effects on waste management options.

Up to date, the only technology that has been shown to be capable of extracting the renewable energy from waste streams on a commercial scale is anaerobic digestion, which thus has become a major focus of interests (Pham et al., 2006). The most important biogas components are CH_4 (55-65%), CO_2 (35-45%), sulfur components (H_2S) (0-1%), nitrogen (N_2) (0-3%), and H_2 (0-1%) (Balat and Balat, 2009), though CH_4 percentages vary due to the organic wastes composition, as shown in Figure 1.3.

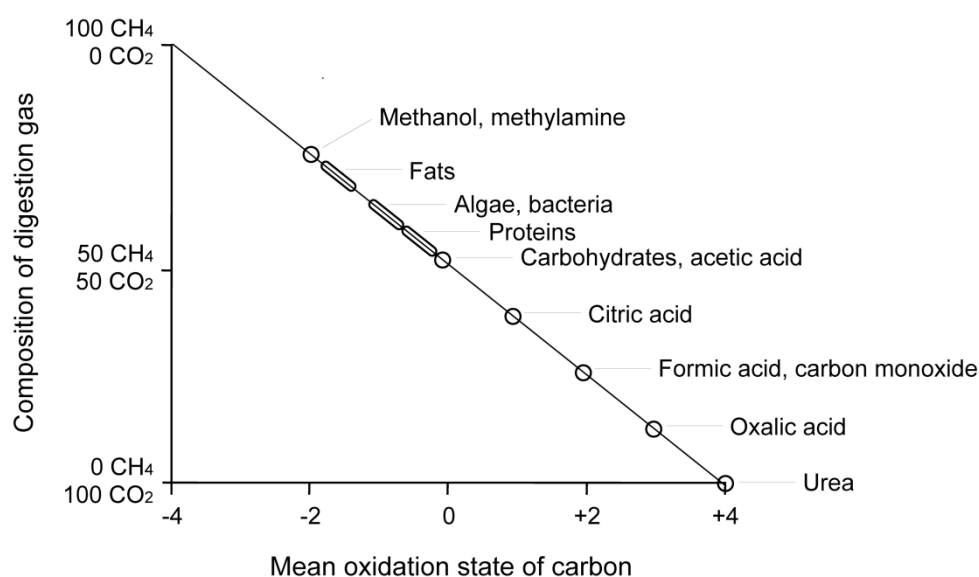


Figure 1.3 Biogas composition depending on the mean oxidation state of the carbon in substrate, assuming total substrate mineralization (after Gujer and Zehnder, 1983).

1.2 LIMITING FACTORS OF ANAEROBIC DIGESTION

1.2.1 Hydrolysis

In solid wastes, the dissolved organic carbon (DOC), which is readily accessible for the acidogens and methanogens, is typically very low (less than 15%) compared to the total organic carbon (TOC) (Kan, 2009). Furthermore, the enzymatic hydrolysis rates of the biopolymers under anaerobic conditions are very slow, as listed in Table 1.2. As a result, the anaerobic digestion of solid wastes is often limited by very long solid retention times (SRT) (20-30 days) and low overall degradation efficiencies (30-50%) (Lin et al., 2009). Therefore researches have been focused on pre-treatment technologies to enhance the solid hydrolysis (see section 1.3.3).

Table 1.2 Average hydrolysis rates (K_H) of different organic substrates at 34-40°C, based on first order kinetics: $C = C_0 \times e^{-K_H t}$ (after Gujer and Zehnder, 1983).

Biopolymer	Hydrolysis products	Enzymes involved	K_H (d ⁻¹)
Lipids	Fatty acid		0.4-0.6
	Glycerol	Lipase	0.1-1.7
	Alcohol		0.08
Proteins	Polypepty		0.02
	Oligopetide	Protease Peptidase	0.03
	Amino acid		
Cellulose	Polysaccharide		0.04
	Oligosaccharide	Cellulase	0.1
	Glucose		
Hemi-cellulose	Polysaccharide		0.54
	Oligosaccharide	Hemi-cellulase	
	Hexose	Xylanase	
	Pentose		

1.2.2 Methanogenesis

For microorganisms growing on soluble substrates, the specific growth rate (μ) and the maximum cell yield (Y^{\max}) are related to the substrate affinity (K_S), the maximum growth rate (μ_{\max}), the substrate concentration (C) and the cellular decay coefficient (K_d) (Denac et al., 1988; Buffiere et al., 1998). In a mature steady-state anaerobic process, a balanced microbial community should be established. Methanogenesis is normally considered as the rate-limiting step in anaerobic digestion of dissolved organics. This is due to the much slower growth rate of methanogens compared to acidogens (Table 1.3) and their higher sensitivity towards the operational conditions (e.g. pH, temperature, etc) (Speece, 1996; Veeken and Hamelers, 2000). Specially, the thermodynamic reactions of

the methanogens are often energetically unfavorable for both the acetotrophic methanogens and the hydrogenotrophic methanogens, of which the associated energy is strongly affected by the hydrogen partial pressure (Table 1.1).

Table 1.3 Growth kinetics parameters of acidogens and methanogens with different substrates (after Denac et al., 1988).

Microorganisms	Substrate	μ_{\max} (h ⁻¹)	Y (g biomass g ⁻¹ COD _{removed})	K _d (d ⁻¹)	K _S (g L ⁻¹)
Acidogens	Monomers	0.05	0.036	0.8	0.14
	Acetate	0.014	0.029	0.02	0.17
Methanogens	Propionate	0.013	0.014	0.01	0.06
	Butyrate	0.015	0.029	0.03	0.5

1.2.3 Propionic acid accumulation

VFAs are important intermediary compounds in the metabolic pathways of methane fermentation. A proper balance between the production and consumption of VFAs is essential for a stable process (Li and Noike, 1992; Wang et al., 1999), since the accumulation of VFAs inhibits not only methanogenesis, but also hydrolysis (Veeken and Hamelers, 2000; Chen et al., 2008).

Up to 30% of all the produced CH₄ potentially originates from the COD fraction of propionic acid (HPr). The acetogenesis of HPr is only possible, if the hydrogen partial pressure is kept between 10⁻⁶-10⁻⁴ bar (De Bok et al., 2004). Obligate H₂ producing acetogens, H₂ consuming methanogens and acetate consuming methanogens are all involved in propionate degradation (Figure 1.4).

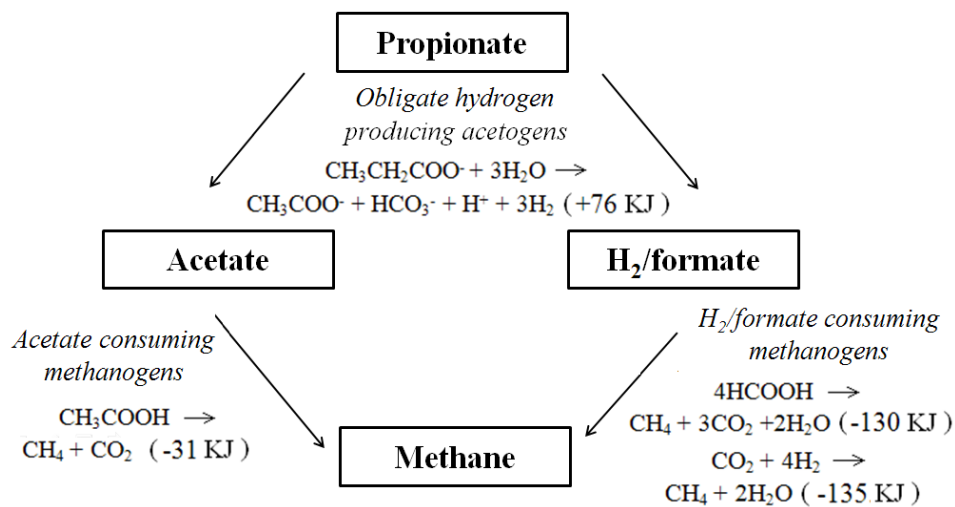


Figure 1.4 Three groups of microorganisms involved in propionate degradation.

Several causes of HPr accumulation have been suggested: the sensitivity of the propionate oxidizing bacteria (POB) against pH below 7.0 (Barredo and Evison, 1991), the nutrients deficiency (Espinosa et al., 1995; Osuna et al., 2004), and the inhibition by the temperature change either in a slow but continuous way or as an immediate response (Dohanyos et al., 1985; Ahn and Forster, 2002; Lindorfer et al., 2008). Since the real cause of the HPr accumulation has not been clarified, the accumulation and degradation of HPr are still the bottlenecks of anaerobic digestion.

1.2.4 Low biogas yield

Currently, more than 4500 biogas plants are under operation in Europe (Balat, 2008). If only aimed at the energy production, the operation is considered to be highly profitable when biogas yields are higher than 30 m³ m⁻³ biomass treated (approximate CH₄ yield of 20 m³ m⁻³ biomass treated) (Angelidaki and Ellegaard, 2003), although it is also dependent on the local green energy policy and price (Europe: by average 0.15 € kWh_{green-electricity}⁻¹) (Karpenstein-Machan, 2001; Walla and Schneeberger, 2008; Munster and Lund, 2009).

Therefore, for the waste streams with biogas potential lower than this value, construction of large centralized biogas plants for combined anaerobic treatment is more desirable due to the lower capital costs (Angelidaki and Ellegaard, 2003). Meanwhile, several studies have also been focusing on the methods to enhance the biogas yields, which might offer the economical possibility of additional income from energy production in case of local community-scale digesters (Karpenstein-Machan, 2001).

1.3 STRATEGIES TO ENHANCE PROCESS PERFORMANCE

1.3.1 Co-digestion

1.3.1.1 Improved process stability

Co-digestion has been applied to a wide range of waste streams. Since the selected co-substrates have their special characteristics, the mixture of two or more organic wastes can balance the feed parameters, such as the C/N ratio, the buffer capacity, the moisture/solid content, etc (Table 1.4). As a consequence, better process stability can be obtained due to the more optimal conditions of the feedstock for the digestion (Demirekler and Anderson, 1998; Rowena et al., 2008; Hejnfelt and Angelidaki, 2009).

For instance, when co-digested with other waste streams, manure usually balances the high organic content, low alkalinity and nutrients deficiency of other co-substrates, while at the same time, the other co-substrates help to lower the ammonia level in

manure, which can be up to 10 g L^{-1} (Creamer et al., 2010). Moreover, the co-digestion of OFMSW is necessary and mainly aimed to balance the solid/moisture content and buffer capacity, since the digestion process alone is instable and problematic due to its complex composition (Bolzonella et al., 2006).

Apart from the mentioned advantages, other interests of co-digestion that have been suggested are: a more successful start-up period, a solution for the possible ammonia inhibition and decreasing of the toxic compounds levels (Mata-Alvarez and Llabres, 2000; Shanmugam and Horan, 2009).

1.3.1.2 Enhanced biogas production

As mentioned in section 1.2.4, anaerobic digestion in the context of energy production by using waste streams with a biogas potential lower than $30 \text{ m}^3 \text{ m}^{-3}$ biomass treated is not economical.

In practice, the conventional anaerobic digestion of manure and municipal sludge are characterized by low biogas yields, ranging from 0.1 to 0.3 L g^{-1} volatile solid (VS) (Bolzonella et al., 2005; Wang et al., 2009). Anaerobic digestion of OFMSW alone can reach the CH_4 yield of around 0.3 L g^{-1} VS, which is approximately $18\text{-}27 \text{ m}^3 \text{ m}^{-3}$ OFMSW treated, if taking its density of around $100\text{-}150 \text{ kg m}^{-3}$ and VS content of 60% into account (Zhang et al., 2008). This value is comparable to the required CH_4 yield value ($20 \text{ m}^3 \text{ m}^{-3}$ biomass treated) in case of economical balance for energy production. However, the co-digestion of OFMSW is considered to improve the process stability as well as biogas production (Elango et al., 2007).

Typically, most types of industrial organic wastes have a CH_4 potential varying from 0.03 to 0.5 L g^{-1} VS, therefore they are considered as very attractive sources of co-substrates for the biogas plants (Angelidaki and Ellegaard, 2003). By selecting easily degradable industrial wastes, a much higher gas yield can be obtained during co-digestion of municipal sludge and industrial wastes, as shown in Table 1.5.

In this way, anaerobic treatment becomes more profitable since the co-digestion benefits not only from the enhanced energy recovery, but also by sharing the capital and operational costs of the biogas plants.

Table 1.4 Literature examples of balanced feed characterization by anaerobic co-digestion.

Main substrate	Co-substrate	Mixture (% VS) (Main:-co-substrate)	T (°C)	Parameter	Balanced value		Reference
					Main substrate	Mixture	
Fruit and vegetable waste	Fish waste	90:10	35		34.2	27.6	Bouallagui et al., 2009
WAS	OFMSW	90:10	38	C/N ratio	8.1	20.6	Zhang et al., 2008
Chicken manure	WAS	40:60	35	Solid content (%)	21.7	10.3	Bujoczek et al., 2000
Ruminal content	Blood	90:10	37	Water content (%)	7.5	5	Lopez et al., 2006
MSW	Industrial sludge	35:65	38	Water content (%)	90	68	Agdag and Sponza, 2005
Municipal sludge	Organic solid wastes	85:15	35	Nutrients	Ca, Mg, K		Edelmann et al., 2000

Table 1.5 Literature examples of CH₄ production enhancement by anaerobic co-digestion of sludge and manure with other waste streams.

Main substrate	Co-substrate	Mix ratio (% VS) (Main:-co-substrate)	T (°C)	CH ₄ yield (mL g ⁻¹ VS)		Reference
				Sole-digestion	Co-digestion	
Sludge	PS, WAS	52:48	35	152	449	Kabouris et al., 2009
	WAS	35:65	52	197	512	
	WAS	50:50	35	174	342	Habiba et al., 2009
Manure	Cow manure	50:50	35	186	321	Heo et al., 2003
	Pig manure	50:50	25	12	78	Alvarez and Liden, 2008
	Swine manure	95:5	37	219	357	Hejmfelt and Angelidaki, 2009
	Cattle manure	50:50	37	267	365	Liu et al., 2009
	Kitchen waste	25:75	35	144	319	Li et al., 2009

1.3.1.3 Possible drawbacks

When wastes are co-digested, there are three possible outcomes on the process stability and biogas production: neutral, synergistic and antagonistic (Zitomer et al., 2008). Normally, the mixture ratio adjustment of the necessary feed parameters can lead to synergistic effects, but co-substrates containing potential toxicants may inhibit biogas production from other co-substrates during co-digestion, and thus antagonistic effects can be observed (Agdag and Sponza, 2005).

Therefore the selection of the co-substrates and their mixture ratio play an important role and also influence the co-digestion performance. In case of one co-substrate containing toxic compounds, the increased portion of other co-substrates in the mixture can lower the overall toxicity level in the feed, which is attributed to the dilution effect.

From an operational view, possible practical inconvenient can rise during the storage and transportation of co-substrates from the production site to the digestion site, especially for the hygiene and odor control due to the potential pre-fermentation. Besides, the more complicated characterizations of the multi-substrates increase the danger of the process instability, which requires more strict parameters control during the process.

1.3.2 Supplementation of micro-nutrients

1.3.2.1 Requirements for micro-nutrients by microorganisms

In addition to the essential requirement of macro-nutrients (average COD/N/P ratio of around 600/7/1), researchers have shown increasing evidences that deficiencies of micro-nutrients (trace metals) can be a severe process limitation and their supplementation may substantially improve digester performance (Table 1.6).

For anaerobic microorganisms, micro-nutrients are crucial constituents in enzymes and enzymatic co-factors and they play an important role in many enzymatic reactions (Hausinger, 1987; Bhattacharya et al., 1995; Thauer, 1998). However, the understanding of the metabolism of trace metals by anaerobic microorganisms is still limited (Lin and Lin, 1997; Bae et al., 2002; Chen et al., 2008).

Table 1.6 Stimulation by metals of anaerobic processes as reported by different literature studies.

Feeding	Experiment	Metal addition	Improvement	Reference
Municipal wastewater	55°C, 35°C, full-scale	Fe, Ni, Co	Increased HPr and HAc utilization rates of 50% and 35%	Zitomer et al., 2008
Municipal wastewater	Batch test, 30°C	Fe, Ni, Co	Stimulation of methanogenic activity	Jansen et al., 2007
Swine wastewater	37°C	Ca	COD removal efficiency enhanced by 33%	Ahn et al., 2006
Potato waste, cattle manure	37°C	Ni, Zn, Cd	25% higher biogas production	Kumar et al., 2006
Canola oil	37°C	Fe	100% removal of VFAs	Li et al., 2006
Methanol	*UASB, 30°C	Co	Increased applicable OLR from 5 to 20 g COD L ⁻¹ d ⁻¹	Zandvoort et al., 2003
VFAs	UASB, 30°C	Ni, Co, Fe, Cu, Mn	Increased applicable OLR from 2 to 10 g COD L ⁻¹ d ⁻¹	Osuna et al., 2004
Sucrose	CSTR, 35°C	Metal salts	Enhanced HAc utilization rate from 0.2 to 0.5 g ⁻¹ VS d ⁻¹	White and Stuckey, 2000
Methanol	Batch test, 30°C	Ni, Co	Increased methane production rate by 18%	Gil and Ostapczuk, 1993
Synthetic medium	UASB	Zn	Improved granulation	Hassan and Wase, 1996
Cane molasses stillage	UASB	Fe, Ni, Co, Mo	15% higher COD removal; 50% higher biogas production	Espinosa et al., 1995
Cheese whey, poultry waste	Batch test, 35°C	Fe, Ni, Co, Cu, Zn	More than 80% increase in biogas production	Patel and Madamwar, 1994

*UASB: upflow anaerobic sludge bed (UASB) reactor.

1.3.2.2 Supplementation of micro-nutrients

In practice, metal supplementation dosages differ from case to case, which lead to huge differences in the range of the reported stimulation levels in literature, as summarized in Table 1.7. These differences are mainly due to the different types of treated waste streams and the variety of the operational conditions among the studies.

Table 1.7 Summary of metal supplementation dosages and chemical formulation in literature studies (compiled from Singh et al., 1999).

Metal	Supplementation	Unit	Chemical formulation
Mg	0.4-17	mg g ⁻¹ COD	MgCl ₂ ·6H ₂ O; MgCl ₂ ·4H ₂ O; MgSO ₄ ·7H ₂ O
Na	2-648	mg g ⁻¹ COD	NaCl; Na ₂ SO ₄
K	10-218	mg g ⁻¹ COD	KCl
Ca	0.2-81	mg g ⁻¹ COD	CaCl ₂ ; CaCl ₂ ·2H ₂ O;
Fe	47-2582	µg g ⁻¹ COD	FeCl ₂ ·4H ₂ O; FeCl ₃ ·3H ₂ O; FeCl ₃ ·6H ₂ O; FeSO ₄ ·7H ₂ O
Al	4-36	µg g ⁻¹ COD	AlCl ₃
Zn	2-600	µg g ⁻¹ COD	ZnCl ₂
Ni	1-3360	µg g ⁻¹ COD	NiCl ₂ ; NiCl ₂ ·6H ₂ O; NiSO ₄ ·7H ₂ O
Co	6-390	µg g ⁻¹ COD	CoCl ₂ ·2H ₂ O; CoCl ₂ ·6H ₂ O;
Mn	12-617	µg g ⁻¹ COD	MnCl ₂ ·4H ₂ O; MnSO ₄ ·H ₂ O;
Cu	1-245	µg g ⁻¹ COD	CuCl ₂ ; CuCl ₂ ·2H ₂ O
B	0.7-31	µg g ⁻¹ COD	Na ₃ BO ₄ ·10H ₂ O; H ₃ BO ₃
Se	2.4-185	µg g ⁻¹ COD	NaSeO ₃ ; Na ₂ SeO ₃ ·5H ₂ O
Mo	4-420	µg g ⁻¹ COD	(NH ₄) ₆ Mo ₇ O ₂₄ ·2H ₂ O; (NH ₄) ₆ Mo ₇ O ₂₄ ·4H ₂ O; NH ₄ Mo ₇ ·4H ₂ O; NH ₄ MoO ₇ ·4H ₂ O

Moreover, since the minimum effective levels of necessary micro-nutrients have not been clarified yet, the supplementation of the micro-nutrients to industrial digesters in practice is preferred in excessive amounts. However, in case of some metal ions which are already present in the influent of anaerobic digesters, such as Na, K, Ca and Mg, further excessive supplementation can lead to their accumulation in digesters. Consequently, a potential metal toxicity effect is possible in case of long-term operation (Bae et al., 2002; Chen et al., 2008).

1.3.2.3 Metal bio-availability

To clearly define the micro-nutrients requirements and their supplementation strategies, the description of the interactions between metals and microbes need to be established. The “active” metal should be capable of binding to enzymes and interfering

with microbial activities. These metals are defined as bio-available to the microorganisms (Aquino and Stuckey, 2007). Although the concept of bio-available metals is important, the measurement of bio-available levels is difficult because it varies depending on the type of microorganism exposed and the environmental factors, such as the total metal concentration, and the kinetics between precipitation, complexation and adsorption (Oleszkiewicz and Sharma, 1990). Therefore in a simplified way, the soluble metal concentration is often used to approximate the bio-available level (Patidar and Tare, 2008). Thus the micro-nutrients supplementation should ensure that their bio-available (soluble) levels reach the minimum requirements which would support a desired digester performance.

During the anaerobic treatment of industrial waste streams, metal deficiencies caused by their limited bio-availability can be due to extensive precipitation, which decreases the soluble metals fraction (Gonzalez-Gil et al., 2003). In these cases, the formation of metal complexes with chelators may be expected to give rise to a certain extent of dissolution of metal compounds, with the concomitant increase of the bio-available metals, which are (directly) available for the uptake by microorganisms (Hu et al., 2008).

Experimental results also support the decisive role of metal speciation in their bio-availability. Gonzalez-Gil et al. (2003) demonstrated that the addition of yeast extract improved the bioavailability of Ni and Co, which was suggested by the formation of dissolved metal complexes from their sulfides. In another study, metal sulfide precipitation could be minimized by additional nitrilotriacetic acid (NTA) at concentration levels of $\mu\text{g L}^{-1}$, which promoted the dissolution of metal ions from precipitates by formation of stable complexes with metals (Hu et al., 2008). These observations imply a possibility to increase the bio-available metal levels by additional chelators, rather than by the direct supplementation of excessive amounts of metals.

1.3.3 Pre-treatments

1.3.3.1 Pre-treatment technologies

Most solid type wastes are by nature heterogeneous in size, composition, structure, and properties. Although sugars, starches, lipids and proteins present in the wastes are among the materials easier to be degraded by the microorganisms, some other fractions such as lignocelluloses and keratin are more difficult to degrade (Romano et al., 2009). For the particulate waste streams with high total solid (TS) content, such as municipal sludge, animal manure, and agricultural residues, pre-treatments prior to enzymatic hydrolysis or digestion are mostly applied to break down the cell walls and release the

soluble organic fraction, and consequently enhance the bio-digestibility and methane production of the waste streams (Taherzadeh and Karimi, 2008). These pre-treatment methods are classified into physical, physico-chemical, chemical and biological (Table 1.8). However, most of them have not been developed enough yet to be effective for full-scale applications.

Not only enzymes, but also bacteria and fungi can be used for biological pre-treatments to improve the digestion as well as the biogas production (Taniguchi et al., 2005; Kurakake et al., 2007). Normally, delignification can be achieved during the process, while the solubilization of the cellulose and hemicellulose can be obtained in part (Eun et al., 2006; Jeganathan et al., 2008). Improved process performance (Table 1.8a) has been observed at lab-scale with food processing wastes, agriculture residues, slaughterhouse wastewater and WAS digestion (Sonakya et al., 2001; Masse et al., 2003; Mendes et al., 2006; Luste et al., 2009).

Low energy requirement, no need of chemicals, and mild environmental conditions are the main advantages of biological pre-treatments. However, the long treatment duration (up to 24 hours) in most biological pre-treatment processes limit their commercial application (Sun and Cheng, 2002).

Physical pre-treatments (e.g. milling) can increase the available surface area by decreasing the particle size of the feedstock, and by decreasing the crystallinity and degrees of polymerization of cellulose (Wen et al., 2004; Jedrczak and Krolik, 2007).

Irradiation is no doubt the most powerful pre-treatment method to disrupt cells with cell disintegration efficiencies of up to 100%, depending on the power input. However, the high power input (up to 2000 kWh m⁻³ biomass treated) is a serious drawback for its industrial applications (Appels et al., 2008; Chen et al., 2008; Park et al., 2010). High-pressure homogenization is one of the most frequently used pre-treatment methods for large scale application (Appels et al., 2008), although it is considered as less effective compared to the irradiation pre-treatments. To obtain significant improvement of digester performance, in practice a combination of pressure pre-treatment with thermal pre-treatment is sometimes applied (Dereix et al., 2006; Phothilangka et al., 2008a).

Chemical pre-treatments involve exposure of wastes to a chemical for a period of time. Acids (e.g. sulfuric acid) are predominantly applied. The major drawback of the chemical pre-treatment methods, particularly at low pH, is the formation of several types of inhibitors such as carboxylic acids, furans and phenolic compounds (Taherzadeh and Karimi, 2008). These chemicals may not affect the enzymatic hydrolysis, but they usually inhibit the microbial growth and fermentative capacity, which results in less biogas production (Taherzadeh and Karimi, 2007). Therefore, the chemical pre-treatments at low

pH should be applied properly by controlling the pH and temperature in order to avoid or at least reduce the formation of these inhibitors.

The chemical and physico-chemical pre-treatments (Table 1.8b) are among the most effective methods and currently they are considered as the most promising processes for industrial applications, mainly due to short treatment duration (within several hours), even though usually very harsh conditions are required (Kim et al., 2000; Mosier et al., 2005).

An effective and economical pre-treatment process should enhance the digestibility of the feedstock and decrease the residues. During the process the possible formation of the inhibitors for hydrolytic enzymes and fermentation microorganisms must be avoided (Bougrier et al., 2008). From a practical view, to ensure the economical feasibility of a selected pre-treatment method, the costs of materials for the construction of the pre-treatment devices and the energy demands should be minimal.

1.3.3.2 Sludge thermal pre-treatment: commercial Cambi process

Thermal pre-treatment of sewage sludge has been shown to increase the sludge biodegradability as well as dewaterability (Phothilangka et al., 2008b). This pre-treatment method is usually under the operational conditions with temperature range of 150-200°C and the adjoining pressures of around 6-25 bar in duration of about 30 minutes. During the process the sludge can be partially solubilized and the cells are disintegrated. With a decreased sludge viscosity by freeing the cell water, it makes the possibility for the later anaerobic digestion to deal with higher sludge concentration (Barlindhaug and Odegaard, 1996).

Contrary to the other lab-scale pre-treatment methods, Cambi is a pre-treatment technology which has been grown to the commercial level. The process design has been developed by the Company Cambi, Norway, based on the thermal pre-treatment process and aiming at minimization of the sludge disposal and maximization of the biogas production. It's full-scale applications have been through the Europe for 15 years (Neyens and Baeyens, 2003). The basic process is by heating the dewatered sludge with DS content of around 15% to around 160-180°C, and then the temperature is decreased to 100°C by depressurizing. Finally the sludge is further cooled down to 40°C and fed into the anaerobic digester.

Table 1.8a Pre-treatment processes reported in literatures: biological pre-treatments and physical pre-treatments.

Category	Process	Method	Remark	Enhancement	Reference
Biological pre-treatments	<i>Enzymatic</i>	Protease and peptidase	Break down proteins into peptides and amino acids	Up to 300% higher solubility;	Sonakya et al., 2001;
		Lipases	Produce glycerol and long chain fatty acids from lipids	Up to 35% increase of biogas production;	Masse et al., 2003; Eun et al., 2006;
		Endoglucanase, cellobiohydrolase and β -glucosidase	Hydrolyze cellulose	Up to 200% increase of COD removal	Mendes et al., 2006; Jeganathan et al., 2008; Luste et al., 2009
	<i>Mechanic</i>	Milling	Decrease of particle size from mm level to μ m level	Up to 10% increase of digestibility	Palmowski and Muller, 2003
		Gamma-ray	50 kGy	15% of TS reduction	Kim et al., 2007
		Electron-beam	5-10 kGy	20% more biogas production	Park et al., 2009
Physical pre-treatments	<i>Irradiation</i>	Microwave	Power of 400-1600 W and temperature of 60-150°C	Up to 10% increase of solubilization	Jin et al., 2009; Park et al., 2010
		Ultrasonic	Temperature of 4000 K and pressure of 40 MPa	65% increase of sludge destruction	Dewil et al., 2006
	<i>Thermal</i>	Hydro-thermal	Temperatures ranging from 60 to 250°C after 30-60 min; optimum temperature of 175 °C	Up to 140% increase of CH ₄ yield; up to 50% increase of solubilization	Gavala et al., 2003; Valo et al., 2004; Lu et al., 2008
		Oven			
	<i>Pressure</i>	Homogenizer	Pressure of 60 MPa	Increase of up to 100% of biodegradability	Harrison, 1991; Appels et al., 2008

Table 1.8b Pre-treatment processes reported in literatures: chemical and physico-chemical pre-treatments.

Category	Process	Method	Remark	Enhancement	Reference
<i>Explosion</i>		Steam	Pressure of 1.5 to 2 MPa for 8 min	Increase of 150% higher solubilization	Yue et al., 2008
		Ammonia	90-100°C for 30 min	30% increase of biomass surface	Chundawat et al., 2007
		Sodium hydroxide	50 meq L ⁻¹ at 20-30°C for 1 h	60% increase of TS reduction	Tanaka and Kamiyama, 2002
<i>Alkaline</i>		Ammonia	Aqueous solution for 10 hours	70% of lignin removal and 60% of solubilization	Kim and Lee, 2006
		Lime	62 meq Ca(OH) ₂ L ⁻¹ for 6 h	Increase in 50% solubilization and 170% biogas yield	Torres and Llorens, 2008
<i>Acid</i>		Sulfuric acid	2-5 hours	Up to 80% of TS destruction	Neyens and Baeyens, 2003
		Hydrochloric acid	6 meq L ⁻¹	6% higher solubilization	Luste et al., 2009
<i>Oxidation</i>		Hydrogen peroxide	50-100 g H ₂ O ₂ kg ⁻¹ TS, 180°C, 5 min	Increase in 10% of CH ₄ yield	Wang et al., 2009
		Wet oxidation	Catalysts required	100% increase of biogas yield	Uellendahl et al., 2008
		Ozone	0.05-0.5 g O ₃ g ⁻¹ TS	10-65% solubilization increase	Chu et al., 2009

Chemical and physico-chemical
pre-treatments

In a Cambi pilot study at HIAS plant, Norway, a solubilization degree of 30% was obtained with the pre-treated sludge at 180°C for 30 minutes (Weemaes and Verstraete, 1998). Another Cambi full-scale study based on 3 years experience showed stable COD solubilization degree of up to 60%, which led to a satisfactory biogas production of 0.49 m³ kg⁻¹ VS treated (70% CH₄). Moreover, the sludge disposal amount was only half of that of the conventional process without Cambi, which was due to the 15% higher DS content in the sludge cake with the Cambi pre-treatment (35%) comparing to the conventional process (20% DS) (Kepp et al., 2000). Moreover, since the total treatment cost of Cambi process is around 50 € ton⁻¹ sludge, the expected pay back of a Cambi application is usually within 2-4 years (Weisz and Solheim, 1999).

1.3.3.3 Application limitations of pre-treatment technologies

Clearly pre-treatment methods are promising techniques to enhance the process performance of anaerobic digestion. The decision to apply these technologies in practice mainly depends on the magnitude of capital investments, energy requirements, operation and maintenance costs. If the energy recovery from the enhanced biogas production can compensate these additional costs, the pre-treatment application becomes economically feasible for the industries. However, in most cases, they may be only considered beneficial for larger WWTP (more than 30,000 IE) due to both the improved biogas production and the decrease of the sludge disposal costs, based on the calculation of the economic efficiency and energy balance (Boehler and Siegrist, 2006). Otherwise, it may be only of interest to plants where severe operational problems exist.

1.4 OBJECTIVES AND OUTLINE OF THIS WORK

Although anaerobic digestion is a well established technology, there is still further potential to expand its efficiency. Therefore the main objective of this doctoral work is to enhance the performance of anaerobic digestion by different approaches, aiming at a better process stability and higher biogas production.

As a first approach for stimulation of the biogas production, co-digestion was investigated. Chapter II describes a study dealing with the biogas enhancement by addition of glycerol to potato processing wastewater. Besides this, the overall process performance was studied, which could be evaluated from the COD and VFAs removal efficiencies. Importantly, the extra energy recovery from the improved biogas production was compared for three different glycerol products.

HPr removal is essential in anaerobic digestion since its accumulation leads to process instability and even process failure. High HPr removal rates and levels have been

reported by macro- and micro-nutrients supplementation in upflow anaerobic sludge bed (UASB) reactors (Wiegant et al., 1986; Fang et al., 1994). In the study described in Chapter III, maximum HPr removal could be achieved under extreme conditions by patient biomass adaptation to the long-term exposure to HPr.

Process failure provoked by HPr accumulation can result in a recovery period of up to 2-3 months. Since the causes of HPr accumulation during anaerobic process have not been unequivocally clarified yet, an external remedy digester, which can be coupled to the main digester to accelerate the recovery, has been developed (Chapter IV). This external digester provides a possibility for the selective HPr removal.

Pre-treatments can improve the substrate digestibility and thus enhance the biogas production. However, different pre-treatment methods have distinct effects on different types of waste streams, and the optimum pre-treatment to be selected must also be based on the economical and practical feasibility, as studied in Chapter V.

In Chapter VI, several aspects related to the approaches evaluated to enhance the anaerobic digestion process are discussed, and finally some major conclusions are summarized and suggestions for future research are recommended.

**ENHANCED BIOGAS PRODUCTION
BY CO-DIGESTION**

APPENDIX I

APPENDIX II

CHAPTER I

CHAPTER II

CHAPTER III

CHAPTER IV

CHAPTER V

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CHAPTER II

ENHANCED BIOGAS PRODUCTION BY CO-DIGESTION

Abstract

The effect of three different types of glycerol on the performance of upflow anaerobic sludge bed (UASB) reactors treating potato processing wastewater was investigated. High chemical oxygen demand (COD) removal efficiencies of around 85% were obtained in both control and supplemented UASB reactors (UASB_C and UASB_T, respectively). By adding 2 ml of glycerol product per liter of raw wastewater, the biogas production could be increased by 0.74 L biogas mL⁻¹ glycerol product, which led to energy values in the range of 810-1270 kWh_{green-electricity} m⁻³ product. Moreover, a better in-reactor biomass yield, based on the volatile solid (VS) enhancement, was observed for the UASB_T reactor (0.012 g VS g⁻¹ COD_{removed}) compared to the UASB_C (0.002 g VS g⁻¹ COD_{removed}), which suggests a positive effect of glycerol on the sludge blanket growth.

Chapter redrafted after: Ma J, Van Wambeke M, Carballa M and Verstraete W 2008 Improvement of the anaerobic treatment of potato processing wastewater in a UASB reactor by co-digestion with glycerol. *Biotechnology Letters* 30 (5) 861-867.

2.1 INTRODUCTION

Since 2001, the total production of crude biogas in the EU countries has increased constantly, corresponding to 2.7 million tons of oil equivalent per year (Demirbas and Balat, 2006). Methane (CH₄) is the main component of biogas, accounting for 60-70%. Its heat value, about 2.5×10^4 kJ m⁻³, is equivalent to 1 kg raw coal or 0.76 kg standard coal (Zeng et al., 2007).

High-rate systems, such as upflow anaerobic sludge bed (UASB) reactors, are widely used for the treatment of several types of industrial wastewaters (Van Lier et al., 1996). Their productivity can be improved by supplementing with readily digestible co-substrates (Van Assche et al., 1983). The aims of using co-substrates can be: i) to maintain a stable pH within the methanogenesis range (Brummeler and Koster, 1990; Kaparaju and Rintala, 2005); ii) to help degradation of low biodegradability substrates (Malpei et al., 1998); iii) to decrease the start-up period (Veiga et al., 1992; Veiga et al., 1994); or, iv) to accelerate the biogas production (Van Lier et al., 2001). In the latter case, different types of materials have been used as co-substrates in anaerobic digestion to enhance the biogas production. However, to our knowledge, there is limited information available on the use of glycerol as a co-substrate.

Glycerol is a sugar alcohol, whose production has increased in the last years since it is a 10% by-product of bio-diesel manufacture. Compared to other co-substrates (food and animal wastes, etc), glycerol has the advantages of being readily digestible and easily storable over a long period. Since the large volumes produced would lead to low prices, glycerol can be an effective co-substrate to facilitate the operation of existing biogas plants.

The objectives of this work were: i) to evaluate technically the use of glycerol as a co-substrate to improve the biogas production during the anaerobic treatment of potato processing wastewater; ii) to compare the effects of different types of commercial glycerol from the market; and, iii) to evaluate economically the advantages of using glycerol as a co-substrate for the anaerobic treatment of industrial wastewaters.

2.2 MATERIALS AND METHODS

2.2.1 Wastewater characteristics

The raw wastewater used as feed was delivered from a full-scale anaerobic digester treating potato processing wastewater (Mydibel, Belgium). Its main characteristics are shown in Table 2.1. It can be observed that the total chemical oxygen demand (COD)

(COD_T) and soluble COD (COD_S) fluctuated from 4.3 to 14.1 g COD L⁻¹ and from 2.7 to 13.5 g COD L⁻¹, respectively, during the experimental period. Due to its low pH, sodium hydroxide (NaOH) was added to the raw wastewater before feeding the UASB reactors to adjust the pH to about 7.5.

Table 2.1 Main characteristics of the potato processing wastewater (n: number of samples; average values with mean error if n<3 or standard deviation if n>3).

Parameter	Unit	Start-up	Phase 1	Phase 2	Phase 3
		n=3	n=4	n=2	n=2
pH		4.8 ± 0.3	5.7 ± 1.2	5.4 ± 0.6	5.5 ± 0.0
COD _T	g COD L ⁻¹	7.3 ± 0.7	4.3 ± 3.2	11.5 ± 2.5	14.1 ± 0.9
COD _S	g COD L ⁻¹	5.8 ± 0.2	2.7 ± 1.0	9.9 ± 3.2	13.5 ± 0.9
VFA _T	g L ⁻¹	2.5 ± 0.2	1.3 ± 1.0	4.0 ± 2.5	7.0 ± 0.3
TKN	mg L ⁻¹	283 ± 42	244 ± 67	n.d.	n.d.
P total	mg L ⁻¹	169 ± 122	83 ± 21	n.d.	n.d.
S-SO ₄ ²⁻	mg L ⁻¹	43 ± 2	41 ± 3	n.d.	n.d.
TSS	g L ⁻¹	2.1 ± 1.1	1.0 ± 0.6	2.4 ± 0.1	3.0 ± 0.3
VSS	g L ⁻¹	1.8 ± 1.0	0.9 ± 0.5	1.6 ± 0.0	2.1 ± 0.2
DM	g L ⁻¹	7.3 ± 0.8	5.2 ± 3.0	11.7 ± 2.5	14.6 ± 0.5
Ash	g L ⁻¹	3.3 ± 0.2	2.4 ± 1.4	7.4 ± 1.0	8.6 ± 0.1
SVI	mL L ⁻¹	79 ± 63	32 ± 36	22 ± 9	70 ± 60
COD _T /N/P		100/3.9/2.3	100/5.7/1.9	n.d.	n.d.

*VFA_T: total volatile fatty acids (VFAs); TKN: total kjeldahl nitrogen; TSS: total suspended solids; VSS: volatile suspended solids; DM: dry matter content; SVI: sludge volume index. n.d.: no data.

2.2.2 Glycerol

Three different types of glycerol products from the market were tested in this study, i.e. the so called pure glycerol, the crude glycerol and the high conductivity (HC) glycerol. Table 2.2 shows the main characteristics of each product.

Table 2.2 Main characteristics of the three different types of glycerol products used.

Parameters	Unit	Theoretical	Pure*	Crude*	HC*
COD _T	g COD L ⁻¹	1540	1200	1120	925
Density	kg L ⁻¹	1.26	1.21	1.22	1.23
S-SO ₄ ²⁻	g L ⁻¹	n.d.	18 (max)	255	254
VFA _T	mg L ⁻¹	n.d.	145	337	394
Conductivity	ms cm ⁻¹	n.d.	n.d.	n.d.	29

n.d.: no data. *n=5, standard deviation < 5%.

2.2.3 Experimental set-up

Two lab-scale UASB reactors, the control reactor (UASB_C) and the supplemented reactor (UASB_T) with a diameter of 5 cm and a volume of 2.3 L were installed and monitored for the three types of glycerol products (Figure 2.1).

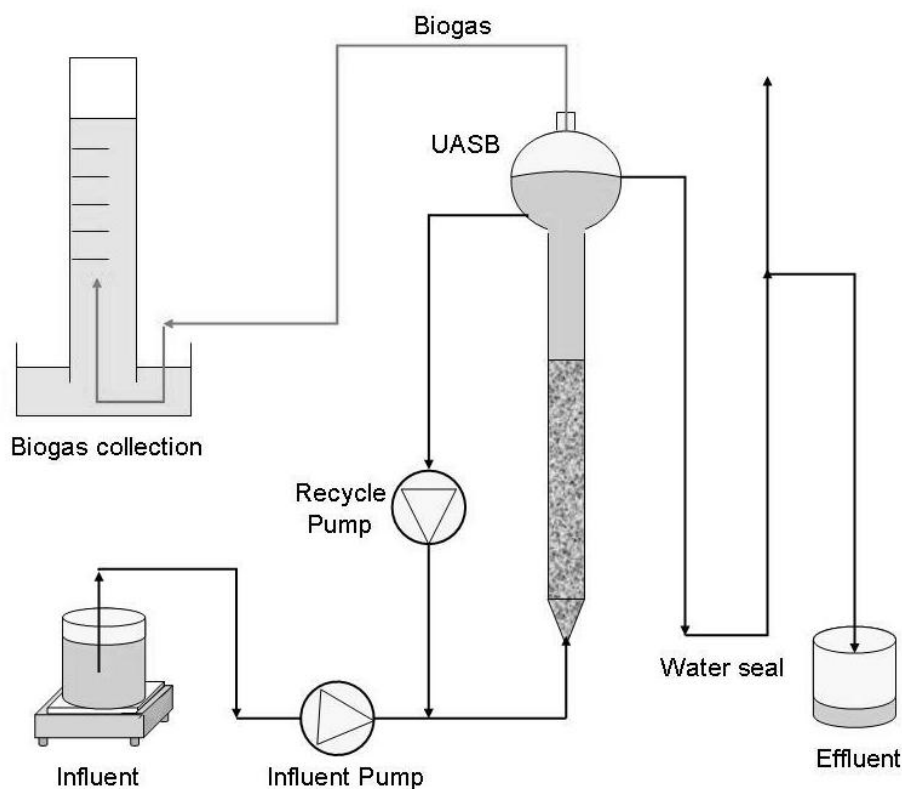


Figure 2.1 Scheme of the lab-scale experimental set-up of the UASB reactor.

Both UASB reactors were inoculated with 500 mL of seed sludge harvested from the full-scale anaerobic digester treating potato processing wastewater (Mydibel, Belgium), which led to an in-reactor volatile solid (VS) concentration of approximate 9 g VS L⁻¹. They were fed semi-continuously (2 min h⁻¹), with a flow-rate of approximately 50 mL min⁻¹ (except in phase 3 of 25 mL min⁻¹), which led to a hydraulic retention time (HRT) of 20 h (except in phase 3 of 40 h). A recirculation with a liquid up-flow velocity of around 1 m h⁻¹ was operated continuously in both reactors. The effluents and the biogas overflowed into the effluent tank and the gas column, respectively, while the sludge was retained by sedimentation in the reactors (Kalogo et al., 2001).

Temperature was maintained at 33 ± 2°C and pH, COD, volatile fatty acids (VFAs) and biogas production were the parameters monitored.

2.2.4 Experimental operation

2.2.4.1 Start-up period

During the start-up period (14 days), the UASB_C reactor was fed with raw wastewater at an average organic loading rate (OLR) of 7.0 ± 0.7 g COD L⁻¹ d⁻¹, while the UASB_T reactor was fed with raw wastewater supplemented with 1 mL of the so-called pure glycerol (Table 2.2) per liter wastewater, which corresponded with an average OLR of 8.2 ± 1.0 g COD L⁻¹ d⁻¹. The aim was to adapt the sludge of the UASB_T reactor to glycerol biodegradation.

2.2.4.2 Experimental period

Three experimental runs (40 days) were carried out in the two UASB reactors. The UASB_C was fed with only raw wastewater throughout the whole experimental period, while the UASB_T was fed with raw wastewater supplemented with the different types of glycerol. Table 2.3 shows the main operational parameters during the start-up and experimental period.

Table 2.3 Operational parameters during the start-up and experimental period of the UASB_C and UASB_T reactors (average values with standard deviations).

Parameters	Unit	Start-up	Phase 1	Phase 2	Phase 3
Duration	day	14	18	12	10
Type of glycerol	-	Pure	Pure	Crude	HC
Glycerol dose	mL L ⁻¹	1	2	2	2
OLR _C	g COD L ⁻¹ d ⁻¹	7.0 ± 0.7	4.9 ± 2.9	8.4 ± 1.3	8.6 ± 0.8
OLR _T	g COD L ⁻¹ d ⁻¹	8.2 ± 1.0	7.6 ± 2.7	11.7 ± 1.9	8.7 ± 1.0

During phase 1 (day 15 to 32), the amount of so-called pure glycerol added to the UASB_T was increased to 2 mL L⁻¹ wastewater to obtain a mature performance of the UASB_T reactor. Subsequently, the OLR of the UASB_T reactor was 7.6 ± 2.7 g COD L⁻¹ d⁻¹ while the UASB_C had a lower OLR of 4.9 ± 2.9 g COD L⁻¹ d⁻¹. During phase 2 (day 33 to 44), the crude glycerol was tested in the UASB_T reactor at the same dose of 2 mL L⁻¹ wastewater. The higher OLR values obtained in both UASB reactors (UASB_C: by average of 8.4 ± 1.3 g COD L⁻¹ d⁻¹ and UASB_T: 11.7 ± 1.9 g COD L⁻¹ d⁻¹, respectively) compared to those of phase 1 were mainly due to the higher COD_T concentrations of the raw wastewater. Finally, during phase 3 (day 31 to 40), the HC glycerol was tested in the UASB_T reactor at the same dose as in the previous phases. Due to the high COD concentrations in the raw wastewater (14.1 ± 1.2 g COD L⁻¹), the HRT was increased to 40 h in both reactors to avoid an organic overload. Similar average OLR values were

obtained for the UASB_C and the UASB_T reactors, i.e. 8.6 ± 0.8 and 8.7 ± 1.0 g COD L⁻¹ d⁻¹, respectively, which are explained by the high COD content of the raw wastewaters and the low COD content of the HC glycerol (only 12% of the total COD fed).

2.2.5 Analytical techniques

Biogas production was followed by liquid displacement and biogas composition was analyzed with an Intersmat IGC 120MB gas chromatograph connected to a Hewlett-Packard 3390A integrator. VFAs were extracted with diethyl ether as described by Holdeman et al. (1977) and measured in a gas chromatograph (Carlo Erba Fractovap 4160) equipped with a flame-ionization detector and a Delsi-Nermag integrator (ENICA-31). pH values were measured with a pH meter (Consort C532) and the other physico-chemical parameters, COD, TSS and VSS, etc, were determined according to the standard methods (Greenberg et al., 1992).

The in-reactor biomass yield (Y) and the particle size distribution were determined in both UASB reactors on day 1 and day 33 (before start-up and at the end of phase 1). The in-reactor biomass yield (Y, in g VS g⁻¹ COD_{removed}) was calculated from the mass balance of VS in the reactor in relation to the amount of COD removed, as:

$$Y = \frac{(VS_{end} - VS_{begin}) \times V_{reactor}}{COD_{removed}} \quad \text{Eq. 2.1}$$

To determine the particle size distribution (wet weight basis), settled sludge from the UASB reactors was sieved through a 0.5 mm pore size.

2.3 RESULTS

2.3.1 Reactors Performance

During the experimental period, the pH in both reactors was rather stable and varied between 7.0 and 7.5 (data not shown). Figure 2.2 illustrates the OLR, the COD_T and VFA_T concentrations in the influent and effluent of both UASB reactors.

Since real industrial wastewaters were used as feeding of the reactors, the COD_T (Figure 2.2B) and VFA_T (Figure 2.2C) concentrations in the influent fluctuated strongly according to the operational conditions of the potato processing industry, which caused important variations on the OLRs applied to the UASB reactors, with values ranging from 2 up to 14 g COD L⁻¹ d⁻¹ (Figure 2.2A). Despite these fluctuations in the influent COD_T levels, the concentrations in the effluent remained constant and low in both reactors, about 100 mg COD L⁻¹ (Figure 2.2B), which led to COD_T removal efficiencies of around 85%. Only during phase 3, lower COD elimination was achieved, i.e. 73% for the UASB_C and

75% for the UASB_T, which was probably due to the increased COD_T concentration in the raw wastewater, around 14.1 g COD L⁻¹.

The residual VFA_T concentrations in the effluents of both UASB reactors remained very low, as shown in Figure 2.2C, leading to the VFA_T removal efficiencies higher than 90% throughout the whole experimental period. The major components of the VFAs in the effluents were acetic and propionic acid, accounting for more than 95% of VFA_T as COD; other VFAs were detected at insignificant concentrations (data not shown). The slight increase of residual VFA_T during phase 3 fits with the greater COD_T levels (Figure 2.2B).

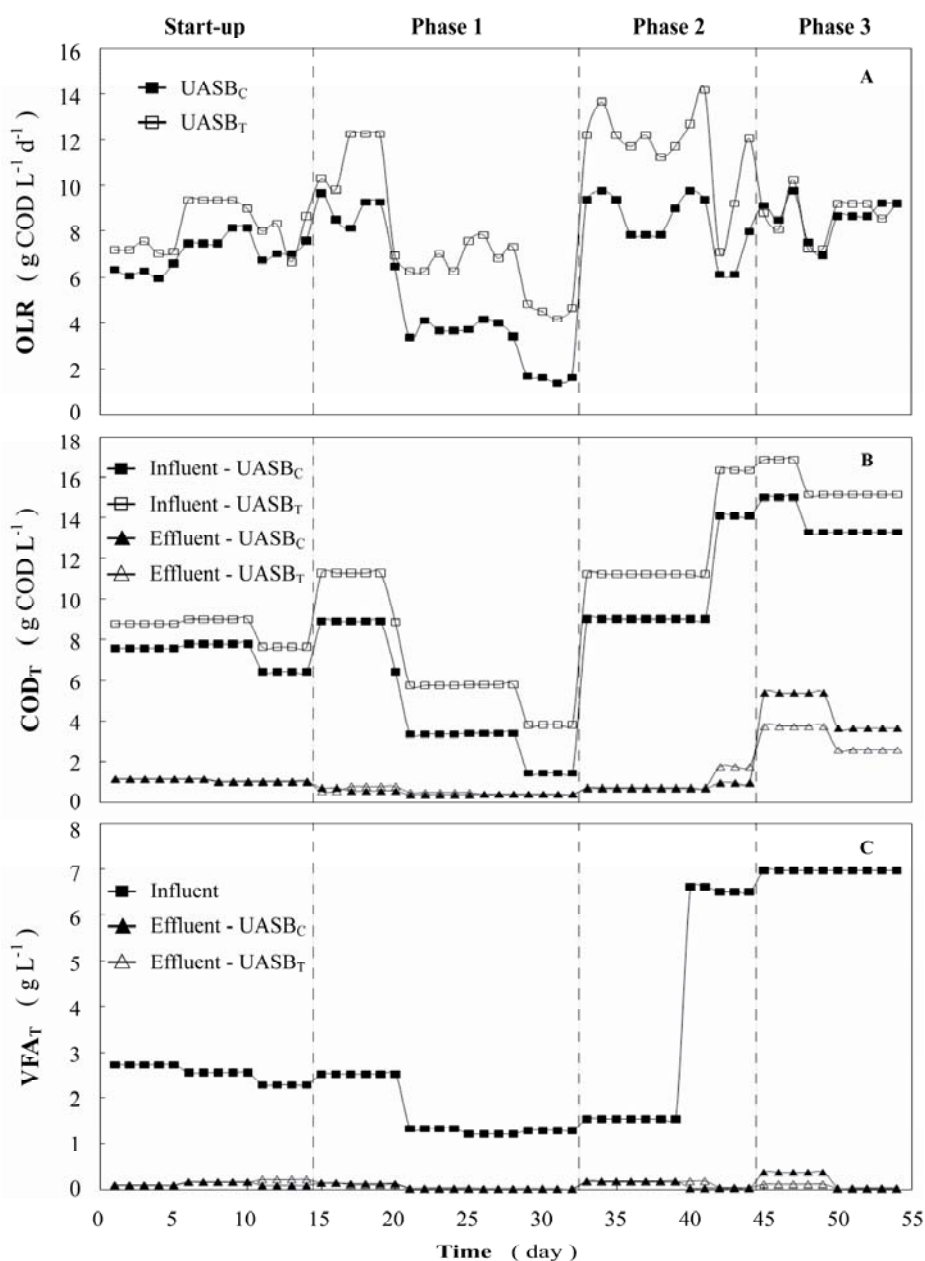
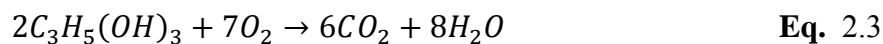
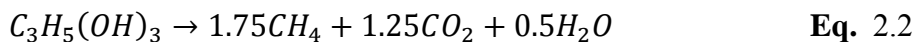


Figure 2.2 (A) OLRs applied to the UASB_C and UASB_T; (B) Influent and effluent COD_T concentrations in the UASB_C and UASB_T; (C) Influent and effluent VFA_T concentrations in the UASB_C and UASB_T.

2.3.2 Effect on biogas production

The theoretical amount of the biogas produced per gram of glycerol product can be calculated from the Buswell formula (Eq. 2.2) and the ideal gas law (Eq. 2.3).



$$PV = nRT \quad \text{Eq. 2.4}$$

where P is the absolute pressure, Pa; V is the volume of gas, m³; n is the moles of gas; R is the gas constant, 8.314 m³ Pa K⁻¹ mol⁻¹; and, T is the temperature, K.

Taking into account the density of the theoretical glycerol (1.26 kg L⁻¹, Table 2.2), it can be calculated that 0.87 L biogas (0.51 L CH₄) can be produced per gram of theoretical glycerol. For the three types of glycerol used in this work, the theoretical amounts of CH₄ which can be obtained are: 0.47 L L⁻¹ so-called pure glycerol, 0.44 L L⁻¹ crude glycerol and 0.37 L L⁻¹ HC glycerol, respectively (Table 2.4).

Table 2.4 Comparison of the extra biogas production from the different glycerol products tested in the UASB_T reactor.

Parameters	Unit	Pure	Crude	HC
COD _T	kg COD L ⁻¹	1.20	1.12	0.93
Biogas production	L L ⁻¹ product	710 ± 200	770 ± 300	740 ± 300
Methane production	L L ⁻¹ product	480 ± 135	440 ± 170	300 ± 120
Theoretical CH ₄ production	L L ⁻¹ product	470	440	370
% theoretical production	%	102	100	80

Figure 2.3 shows the biogas produced experimentally in the UASB_C and UASB_T reactors. Throughout the whole experimental period, higher daily biogas production was achieved in the UASB_T reactor (8.6 ± 2.2 L d⁻¹) than in the UASB_C (5.4 ± 2.5 L d⁻¹), which is explained by the extra glycerol-COD added (79% in phase 1, 23% in phase 2, 13% in phase 3). The biogas produced per liter of wastewater treated was 1.5 times higher in the UASB_T (4.6 ± 2.3 L L⁻¹ wastewater) than in the UASB_C (3.1 ± 2.0 L L⁻¹ wastewater), as illustrated in Figure 2.3B. This means that the addition of glycerol enhanced the biogas production capacity, around 0.74 L biogas (0.41 L CH₄) mL⁻¹ glycerol product added, calculated as the average value from the three glycerol products (Table 2.4).

Comparing the three different types of glycerol products tested (Table 2.4), the so-called pure glycerol gave the highest methane production (0.48 L CH₄ mL⁻¹ product, 0.71 L biogas mL⁻¹ product), followed by the crude glycerol (0.44 L CH₄ mL⁻¹ product, 0.77 L biogas mL⁻¹ product), and finally, the HC glycerol (0.30 L CH₄ mL⁻¹ product, 0.74

L biogas mL⁻¹ product). Hence, as expected, the lower glycerol-COD content in the product, the lower methane production.

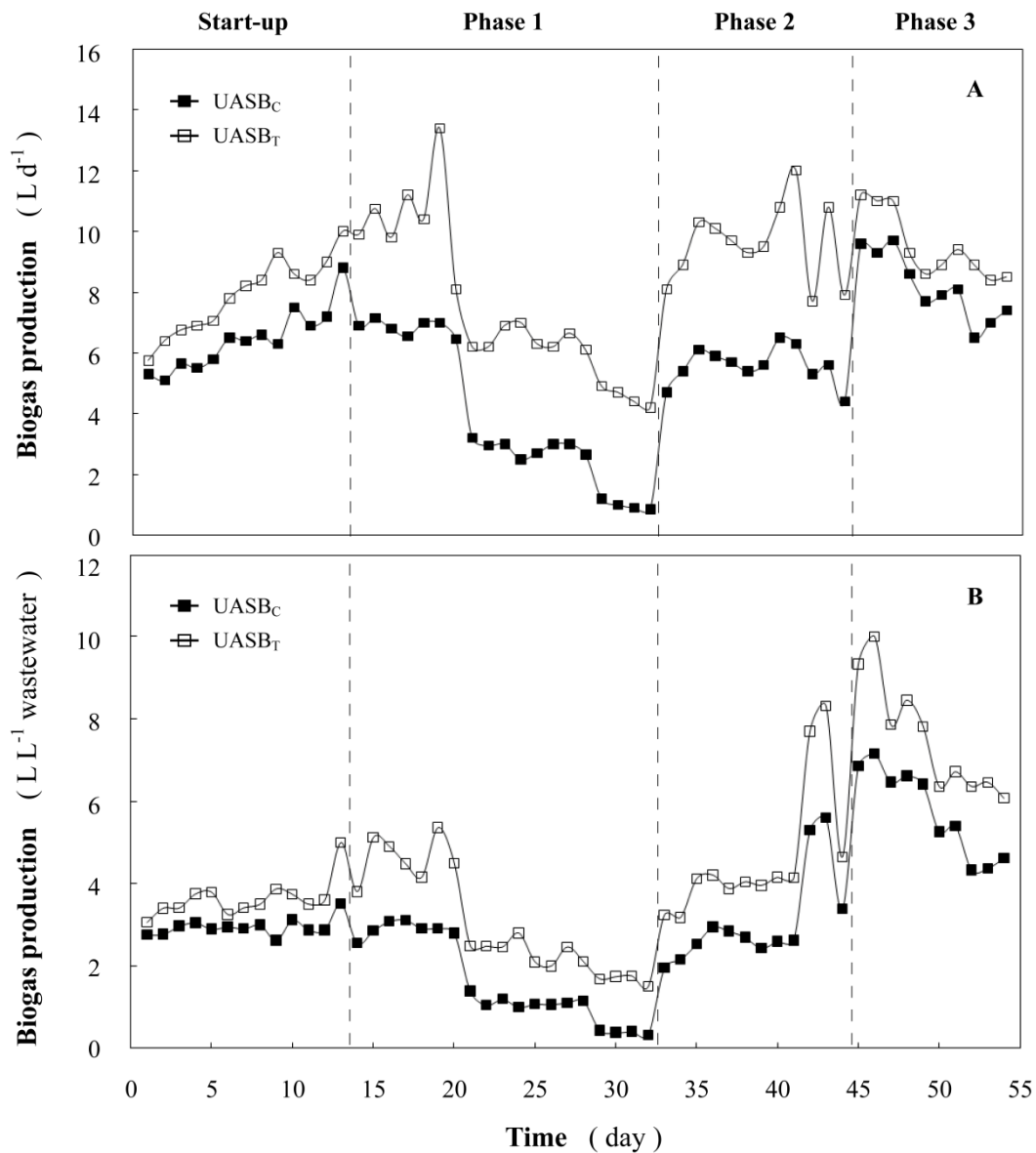


Figure 2.3 (A) Biogas production (L d⁻¹) in the UASB_C and UASB_T; (B) Biogas production (L L⁻¹ wastewater) in the UASB_C and UASB_T.

2.3.3 Effect on biomass granulation

The in-reactor biomass yield (Y) and the particle size distribution were determined in both UASB reactors on day 33 (at the end of phase 1). The results obtained are summarized in Table 2.5. A considerable difference between the two granular sludge beds could be detected visually. The granular sludge bed increased continuously in the UASB_T reactor (from 24 cm to 37 cm), while in the UASB_C reactor, this increase was less significant (from 24 cm to 31 cm). The extra addition of the so-called pure glycerol, and

therefore the supplement of extra organic carbon source, favored the growth of active biomass in the UASB_T reactor (a difference of 3 g VS L⁻¹ was found after 33 days), and subsequently, the in-reactor biomass yield (0.012 g VS g⁻¹ COD_{removed}) was higher compared to that in the UASB_C (0.002 g VS g⁻¹ COD_{removed}).

Table 2.5 In-reactor biomass yield and particle size distribution at the end of phase 1.

Parameters	Unit	UASB _C	UASB _T
In-reactor biomass growth	g VS L ⁻¹	0.39	3.39
COD _{removed}	g COD L ⁻¹	208	283
In-reactor biomass yield	g VS g COD _{removed} ⁻¹	0.002	0.012
< 0.5mm	%	51	59
> 0.5mm	%	49	41

Although no clear difference in sludge structure (granules or fluffy sludge) could be detected visually between both UASB reactors, a larger fraction (on wet weight basis) of small granules (<0.5 mm) was retained in the UASB_T reactor (59%) than in the UASB_C reactor (51%).

Since the duration of phase 2 (with crude glycerol) and phase 3 (with HC glycerol) was quite short for biomass growth (12 and 10 days, respectively), the effect of these glycerol products on biomass granulation was not evaluated.

2.4 DISCUSSION

The co-digestion of potato processing wastewater with three different types of glycerol increased the biogas, and concomitantly the methane production, per liter of wastewater treated by a factor of around 1.5. Among the three glycerol products tested, similar results were obtained with the so-called pure glycerol and the crude glycerol. The HC glycerol gave a slightly lower methane production, related to its lower purity.

Because of the variation in the COD_T amount of the potato wastewater, the percentage of the glycerol-COD added to the wastewater differed strongly (79% in phase 1, 23% in phase 2, 13% in phase 3). Therefore the higher OLRs in the UASB_T, which was mainly resulted from the extra glycerol-COD added, were also observed, i.e. 55% higher in phase 1, 39% higher in phase 2 and 1% higher in phase 3.

Importantly, the OLR differences between the UASB reactors were not only dependent on the COD amounts of the potato wastewater and the extra glycerol added, but also on the influent pump flow-rates. This was the reason that in phase 3 the overall OLRs of both reactors differed only 1%, although the glycerol-COD added was 13% of the

wastewater-COD. By average values, 0.1 L d⁻¹ difference of the influent flow-rate was observed (1.4 and 1.3 L d⁻¹ in each UASB reactor, respectively), and it presented around 1.4 g COD d⁻¹ higher COD input from the wastewater-COD in the UASB_C, which reduced the overall difference of the total COD input from the extra glycerol-COD input in the UASB_T, i.e. 2.4 g glycerol-COD d⁻¹.

Although the OLRs fluctuated strongly according to the characteristics of the raw industrial wastewaters used, no COD or VFAs accumulation occurred in both UASB reactors, which indicates their stable operation. Moreover, no significant difference on COD_T removal efficiency was observed between both reactors (around 85%). Therefore the extra amount of the glycerol-COD added did not stress the process performance of the UASB_T in a long-term of view, and it implied the easily degradability of glycerol.

Despite the presence of sulfur compounds in the glycerol products, the content of H₂S in the biogas produced in the UASB_T reactor was similar to that in the UASB_C reactor (0.2%).

Interestingly, the supplementation of the feeding with glycerol had a positive effect on the in-reactor biomass growth. The in-reactor biomass yield of the UASB_T was 6 times higher than that of the UASB_C, i.e. 0.012 and 0.002 g VS g⁻¹ COD removed, respectively.

The economic evaluation of the three different types of glycerol tested is shown in Table 2.6, which was calculated from the average values of the biogas production given in Table 2.4. It was assumed that 2.7 kWh electrical energy can be produced from 1 m³ methane and 0.15 € as the selling price of green energy per kWh (Karpenstein-Machan, 2001; Walla and Schneeberger, 2008; Munster and Lund, 2009). Although the so-called pure glycerol product represents the highest putative income, it has to be bought at a reasonable cost to become competitive. Therefore, the most profitable glycerol product for co-digestion could be the crude glycerol.

Table 2.6 Economic evaluation of the three glycerol products tested.

Parameter	Unit	Pure	Crude	HC
Biogas	m ³ ton ⁻¹ product	590	630	600
Methane	m ³ ton ⁻¹ product	390	380	240
Energy	kWh _{electricity} ton ⁻¹ product	1050	1017	659
Income	€ ton ⁻¹ product	157	153	99

Overall, it can be concluded that glycerol is a feasible and economically interesting co-substrate to enhance the anaerobic treatment of industrial wastewaters.

APPENDIX I

APPENDIX II

CHAPTER I

CHAPTER II

**PROPIONIC ACID REMOVAL BY MACRO- AND
MICRO-NUTRIENTS SUPPLEMENTATION**

CHAPTER III

CHAPTER IV

CHAPTER V

CHAPTER VI

APPENDIX III

APPENDIX IV

APPENDIX V

APPENDIX VI

CHAPTER III

PROPIONIC ACID REMOVAL BY MACRO- AND MICRO- NUTRIENTS SUPPLEMENTATION

Abstract

The maximum propionic acid (HPr) removal rate (R_{HPr}) was investigated in two lab-scale upflow anaerobic sludge bed (UASB) reactors. Two feeding strategies were applied by modifying the hydraulic retention time (HRT) in the UASB_{HRT} and the influent HPr concentration in the UASB_{HPr}, respectively. The experiment was divided into 3 main phases: phase 1, influent with only HPr; phase 2, HPr with macro-nutrients supplementation; and phase 3, HPr with macro- and micro-nutrients supplementation. During phase 1, the maximum R_{HPr} achieved was less than 3 g HPr-COD L⁻¹ d⁻¹ in both reactors. However, the subsequent supplementation of macro- and micro-nutrients during phases 2 and 3 allowed to increase the R_{HPr} up to 18.1 and 32.8 g HPr-COD L⁻¹ d⁻¹, respectively, corresponding with an HRT of 0.5 h in the UASB_{HRT} and an influent HPr concentration of 10.5 g HPr-COD L⁻¹ in the UASB_{HPr}. Therefore, the high HPr conversion with high throughput and high influent HPr level was demonstrated by macro- and micro-nutrients supplementation.

Chapter redrafted after: Ma J, Mungoni LJ, Carballa M and Verstraete W 2009 Maximum removal rate of propionic acid as sole carbon source in UASB reactors by macro- and micro- nutrients stimulation. *Bioresource Technology* 100 (14) 3477-3482.

3.1 INTRODUCTION

Despite being a conventional and well-established technology, anaerobic digestion stays “fresh” nowadays, since it is not only a part of an integrated waste management system, but also a technology for renewable energy production. However, the high capital costs and low process efficiencies have limited the level of its industrial application. Therefore, the stimulation of this process by different methods attracted much attention in the applied industrial field (Boe et al., 2008; La Motta et al., 2008).

Organic or hydraulic overloading and the presence of toxic compounds are common causes of accumulation of fatty acids (mainly propionate, but also acetate and butyrate), which can give rise to reactor failure (Gallert and Winter, 2005). Contrary to acetate and butyrate, which are gradually consumed, propionic acid (HPr) accumulates easily in the reactor since it is not directly subjected to methanogenesis due to its low acetogenic rate (McMahon et al., 2004). Consequently, the methanogenic phase is inhibited, which can give rise to a low process efficiency (Wang et al., 2006). Although the maximum tolerable level of HPr in anaerobic reactors varies according to the type of waste treated and reactor used, typical values reported in literature vary from 0.8 g HPr L⁻¹ (1.2 g HPr-COD L⁻¹) (Mosche and Jordening, 1998) to 2 g HPr L⁻¹ (3 g HPr-COD L⁻¹) (Barredo and Evison, 1991).

The degradation of HPr decreases when high organic loading rates (OLR) and/or low hydraulic retention time (HRT) are applied (Elefsiniotis and Oldham, 1994; Demirel and Yenigun, 2004). Fang et al. (1994) reported maximum HPr removal rate (R_{HPr}) of 22 g HPr-COD L⁻¹ d⁻¹ in mesophilic upflow anaerobic sludge bed (UASB) reactors operating at a fixed HRT of 12 h and OLR up to 23 g HPr-COD L⁻¹ d⁻¹. The influent was composed of propionate as sole organic substrate (11.5 g HPr-COD L⁻¹) plus trace metals and balanced nutrients. These results are comparable with the maximum R_{HPr} of 25 g HPr-COD L⁻¹ d⁻¹ in a thermophilic UASB reactor, also with an influent composed of propionate as sole organic source and supplemented with macro- and micro-nutrients (Wiegant et al., 1986). Recently, Tatara et al. (2008) obtained similar maximum R_{HPr} of 20.6 g HPr-COD L⁻¹ d⁻¹ in thermophilic UASB reactors at OLR of 66.4 g COD L⁻¹ d⁻¹ (HRT of 4.8 h) with macro- and micro-nutrients supplementation, however, the HPr removal efficiency (E_{HPr}) was very low (around 30%). Nevertheless, in all these studies, the effect of the macro- and micro-nutrients was not investigated since they were supplemented from the beginning of the experiments.

It is widely known that the growth and activity of the methanogenic consortium in anaerobic reactors strongly depend on environmental factors, such as macro- and micro-nutrients availability (Cresson et al., 2006). Table 3.1 shows a summary of

literature values related to the main functions and the stimulatory concentration ranges of different macro- and micro-nutrients commonly used in anaerobic digestion. Already in the 60s, the need of nutrients supplementation for stable anaerobic digestion was brought forward by Speece and McCarty (1962). Macro-nutrients, such as nitrogen, phosphorus, potassium and magnesium, are required for activation or functioning of many micro-organisms. Micro-nutrients, mainly trace metals including nickel, cobalt, and iron, are known for their role as biochemical cofactors for methane production (Speece, 1996). Therefore, it is conceivable that the supplementation of macro- and micro-nutrients can improve the HPr degradation during anaerobic digestion.

The aim of this paper was to evaluate the maximum removal rate of propionic acid in UASB reactors fed with propionic acid as sole carbon source by applying two different strategies to increase the OLR in the systems: increasing the influent flow rate (UASB_{HRT}) and increasing the influent HPr concentration (UASB_{HPr}). In addition, the effect of macro- and micro-nutrients supplementation on the propionic acid degradation was evaluated.

Table 3.1 Main function of macro- and micro-nutrients and their stimulatory ranges of supplement dosage in anaerobic digestion and Rich, 1995; Singh et al., 1998).

Macro-nutrient	Main function	Unit	Supplementation
Nitrogen (N)	Protein synthesis	COD/N ratio	> 100/1.25
Phosphorus (P)	Nucleic acid synthesis	COD/P ratio	> 100/0.25
Potassium (K)	Cell wall permeability	mg g ⁻¹ COD	5–450
Sulfur (S)	Numerous enzymes	mg g ⁻¹ COD	10–2000
Micro-nutrient	Main function	Unit	Supplementation
Cobalt (Co)	Corrinoids, CODH	µg g ⁻¹ COD	100–1000
Copper (Cu)	SODM, hydrogenase	µg g ⁻¹ COD	5–650
Iron (Fe)	CODH, ligand, sulfides	µg g ⁻¹ COD	500–8500
Molybdenum (Mo)	FDH, inhibits sulfur reducers	µg g ⁻¹ COD	65–300
Nickel (Ni)	CODH, synthesis of F430, essential for SRB	µg g ⁻¹ COD	10–13500
Selenium (Se)	Fatty acid metabolism, FDH	µg g ⁻¹ COD	20–600
Zinc (Zn)	FDH, CODH, hydrogenase	µg g ⁻¹ COD	10–1250

CODH: carbon monoxide dehydrogenase; SODM: superoxide dismutase; FDH: formate dehydrogenase; SRB: Sulfate reducing bacteria.

3.2 MATERIALS AND METHODS

3.2.1 Experimental set-up

Due to their high rate performance for the treatment of liquid streams and their flexibility for HRT control, two lab-scale UASB reactors were used in order to achieve a maximum HPr removal rate (Figure 3.1). Each UASB reactor consisted of one cylindrical tube with a diameter of 5 cm and plus a 3-phase separator in the upper part. The total reactor volume was 2.3 L (working volume of 2 L). The feeding was time-controlled, according to the required flow-rates, and the effluent and the biogas were collected in the effluent tank and the gas column, respectively. The reactors were operated under mesophilic conditions ($33 \pm 2^\circ\text{C}$) and the pH was controlled around 7.5 by adjusting the influent pH with NaOH solution (5N). pH, temperature, chemical oxygen demand (COD), volatile fatty acids (VFAs) and biogas production were monitored daily.

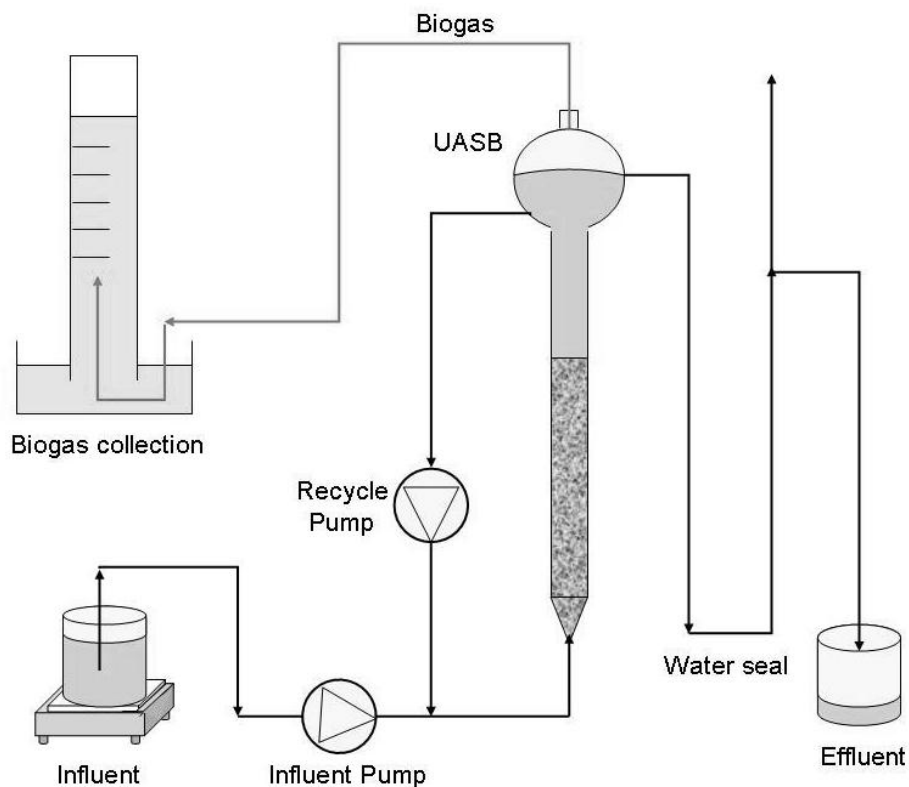


Figure 3.1 Scheme of the lab-scale experimental set-up of the UASB reactor.

3.2.2 Inoculum

Both UASB reactors were inoculated with 500 mL of seed sludge harvested from a full-scale anaerobic digester treating potato processing wastewater (Mydibel, Belgium), which led to an initial in-reactor volatile solid (VS) concentration of around 10 g VS L^{-1} .

3.2.3 Operational strategy

Two different strategies were applied to the UASB reactors to increase the OLR. In the UASB_{HRT}, the HPr concentration in the feeding was kept constant at 0.8 g HPr-COD L⁻¹, and the influent flow-rate was increased progressively, thus decreasing the HRT. In the UASB_{HPr}, a fixed HRT of 12 h was applied and the influent HPr concentration was increased stepwise.

The experiment was divided into three main operational phases according to the feeding composition: phase 1, with only HPr as sole carbon source; phase 2, with HPr supplemented with macro-nutrients; and phase 3, with HPr supplemented with macro- and micro-nutrients. In addition, an extra phase (phase 4) was applied at the end of the experiment to confirm the importance of macro- and micro-nutrients by interrupting stepwise their supplementation.

3.2.4 Chemicals

Concentrated propionic acid (99%, Merck Schuchardt OHG, Germany) was used to prepare the synthetic feeding (HPr solution) and the final pH of the feeding was adjusted to 7.5, approximately, by the addition of NaOH solution (5 N).

The macro-nutrients supplemented are listed in Table 3.2 and they were selected according to Fang and Chui (1993). The supplementation of micro-nutrients was carried out by the commercial product Methanostim Liquid (Avecom, Belgium), which is a solution of technical grade ferric chloride, cobalt chloride hexahydrate, yeast extract and citric acid solution. The applied dosage was 15 µL per liter reactor per day based on industrial use. The individual doses of the supplemented micro-nutrients are (in µg per liter reactor per day): B: 1.26, Co: 0.03, Fe: 2,050, Mn: 6.17, Ni: 0.38 and Zn: 0.30.

Table 3.2 List of macro-nutrients and their dosages.

Chemicals	Dosage (mg g ⁻¹ COD)
Ammonium chloride	260
Magnesium sulfate	128
Potassium monohydrogenphosphate	75
Potassium dihydrogenphosphate	30
Sodium citrate	68
Calcium chloride	52

3.2.5 Analytical techniques

VFAs were extracted with diethyl ether and their quantitative analysis was determined by a capillary gas chromatograph (CE Instruments, Italy) coupled with a flame

ionization detector (FID). pH values were measured with a C532 pH meter (Consort, Belgium) and the other physico-chemical parameters, soluble COD (COD_S), total solid (TS) and VS, were determined according to the standard methods (Greenberg et al., 1992). Biogas production was followed by liquid displacement.

3.3 RESULTS

3.3.1 UASB_{HRT} performance

The pH of the reactor remained constant, around 7.5 ± 0.4 , during the whole experimental period.

Figure 3.2 shows the performance of UASB_{HRT} in terms of applied OLR (Figure 3.2A), effluent COD_S concentration (Figure 3.2B), effluent HPr-COD concentration (Figure 3.2C) and biogas production rate (Figure 3.2D) during the whole experimental period.

During phase 1 (day 0-50), with only propionic acid in the feeding, the OLR was first increased to $1.1 \text{ g HPr-COD L}^{-1} \text{ d}^{-1}$ (HRT of 16 h) from day 0-20, and both the effluent COD_S and HPr concentrations remained low, around $100 \text{ mg COD L}^{-1}$ and $10 \text{ mg HPr-COD L}^{-1}$, respectively. Thus high E_{HPr} and R_{HPr} were obtained, by average 99% and $1.1 \text{ g HPr-COD L}^{-1} \text{ d}^{-1}$, respectively. However, after the further increasing of the OLR to $2.2 \text{ g HPr-COD L}^{-1} \text{ d}^{-1}$ (HRT of 8 h) on day 21, both the effluent COD_S and HPr concentrations rose to around $260 \text{ mg COD L}^{-1}$ and $235 \text{ mg HPr-COD L}^{-1}$, respectively, corresponding with an E_{HPr} of 70% and a R_{HPr} of $1.5 \text{ g HPr-COD L}^{-1} \text{ d}^{-1}$. At the end of phase 1, the OLR was increased to $4.5 \text{ g HPr-COD L}^{-1} \text{ d}^{-1}$ (HRT of 4 h), resulting in effluent COD_S and HPr concentrations around $400 \text{ mg COD L}^{-1}$ and $350 \text{ mg HPr-COD L}^{-1}$, respectively. The biogas production rates remained low during this phase, i.e. $0.4 \text{ L L}^{-1} \text{ d}^{-1}$ ($0.27 \text{ L g}^{-1} \text{ COD}_{\text{removed}}$).

After the supplementation of macro-nutrients (from day 51 on), not only the effluent COD_S and HPr concentrations dropped immediately to around $200 \text{ mg COD L}^{-1}$ and $65 \text{ mg HPr-COD L}^{-1}$, respectively, but also the biogas production rate increased to $0.8 \text{ L L}^{-1} \text{ d}^{-1}$ ($0.31 \text{ L g}^{-1} \text{ COD}_{\text{removed}}$). Consequently, both the E_{HPr} and R_{HPr} increased from 48% and $2.1 \text{ g HPr-COD L}^{-1} \text{ d}^{-1}$ (end of phase 1) to 92% and $4.1 \text{ g HPr-COD L}^{-1} \text{ d}^{-1}$ (beginning of phase 2) under the same OLR of $4.5 \text{ g HPr-COD L}^{-1} \text{ d}^{-1}$. In addition, during this phase 2 (day 61-145), the OLR could be further increased up to $18.8 \text{ g HPr-COD L}^{-1} \text{ d}^{-1}$ (HRT of 1 h), resulting in average effluent COD_S and HPr concentrations of $100 \text{ mg COD L}^{-1}$ and $30 \text{ mg HPr-COD L}^{-1}$, respectively, and biogas production rates of $3.5 \text{ L L}^{-1} \text{ d}^{-1}$ ($0.30 \text{ L g}^{-1} \text{ COD}_{\text{removed}}$). Consequently, higher E_{HPr} and R_{HPr} , ca. 96% and $18.1 \text{ g HPr-COD L}^{-1} \text{ d}^{-1}$, were obtained.

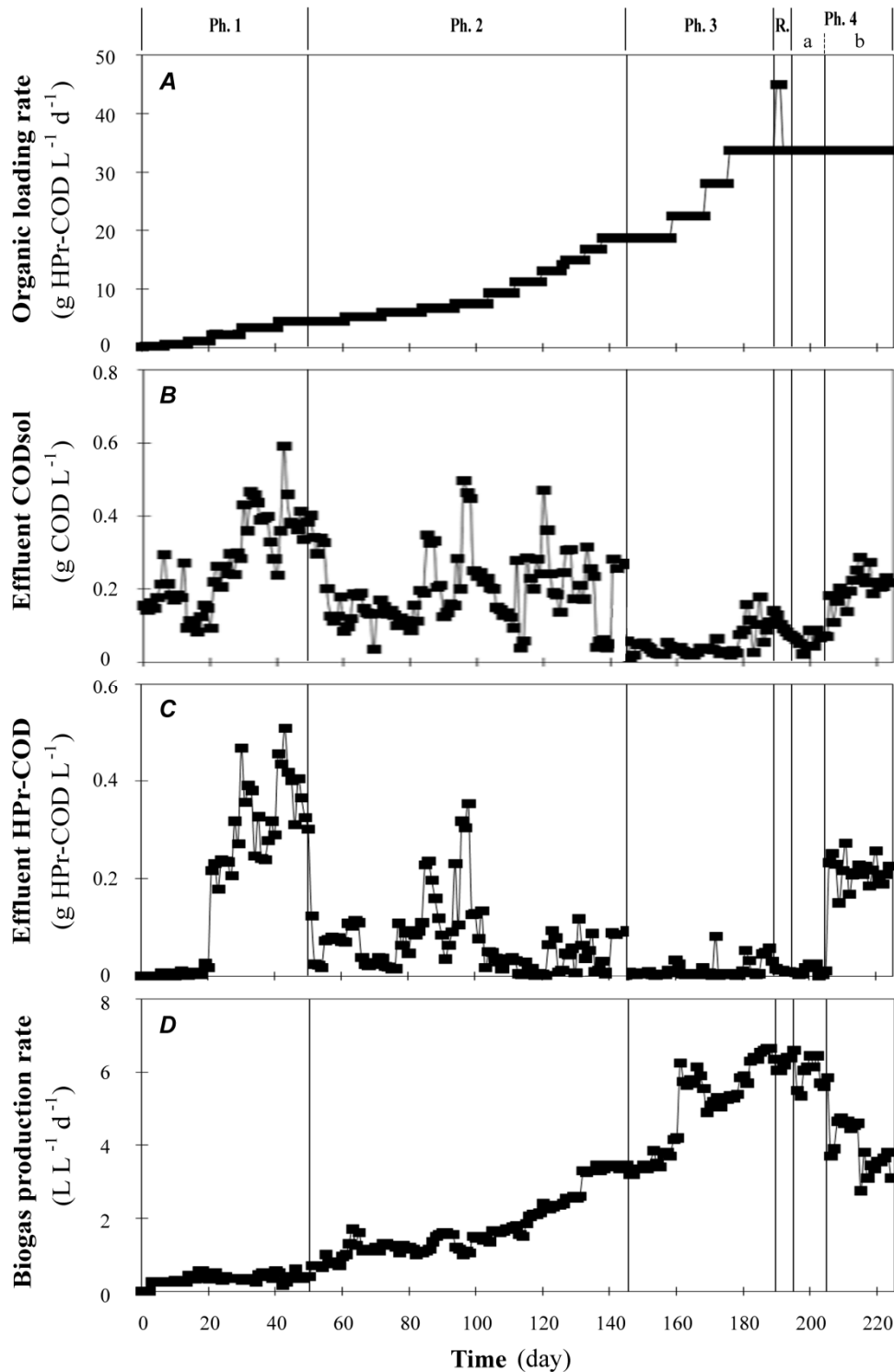


Figure 3.2 Performance of UASB_{HRT} reactor during the whole experimental period. (A) OLR; (B) Effluent COD_S concentration; (C) Effluent HPr-COD concentration; and, (D) Biogas production rate. Ph.1: phase 1, with only HPr in the feed; Ph.2: phase 2, with HPr supplemented with macro-nutrients; Ph.3: phase 3, with HPr supplemented by macro- and micro-nutrients; R: recovery period; Ph.4: phase 4, with no-addition of micro-nutrients (4a) and macro-nutrients (4b).

After the supplementation of micro-nutrients (from day 146 on), the effluent COD_S and HPr concentrations decreased to less than 50 mg COD L⁻¹ and 5 mg HPr-COD L⁻¹, respectively. Besides, an increase of the biogas production rate to 3.8 L L⁻¹ d⁻¹ was observed. During phase 3 (day 146-189), the OLR was further increased to 33.8 g HPr-COD L⁻¹ d⁻¹ (HRT of 0.5 h) and the effluent COD_S and HPr concentrations rebounded to 100 mg COD L⁻¹ and around 20 mg HPr-COD L⁻¹, respectively. Consequently, not only the E_{HPr} and R_{HPr} increased to 97% and 32.8 g HPr-COD L⁻¹ d⁻¹, respectively, but also the biogas production rate was enhanced, i.e. 6.7 L L⁻¹ d⁻¹ (0.34 L g⁻¹ COD_{removed}). At the end of this period (day 90 and 91), the OLR was increased to 45 g HPr-COD L⁻¹ d⁻¹ (HRT of 0.4 h). However, the experiment could not be further advanced under these conditions due to a limitation of the set-up (the reactor tended to overflow at these extremely high influent flow-rates, i.e. 60 L L⁻¹ d⁻¹). Yet, during these 2 days of this very high throughput, the effluent COD_S and HPr concentrations remained at the same level, ca. 110 mg COD L⁻¹ and 15 mg HPr-COD L⁻¹, respectively.

During the recovery period of the influent flow rate at 45 L L⁻¹ d⁻¹ (day 192-195) after the reactor overflow, the effluent COD_S and HPr concentrations, and the biogas production rates reached the same levels as those at the end of phase 3, i.e. around 80 mg COD L⁻¹, 10 mg HPr-COD L⁻¹ and 6.4 L L⁻¹ d⁻¹ (0.36 L g⁻¹ COD_{removed}), respectively.

In order to confirm the positive effect of the macro- and micro-nutrients supplementation during phases 2 and 3, respectively, the inverse effect by interrupting their addition was tested during phase 4 (day 196-225). The no-addition of micro-nutrients (days 196-205) did not affect significantly the average effluent COD_S and HPr concentration values (60 mg COD L⁻¹ and 10 mg HPr-COD L⁻¹, respectively), but the biogas production rate decreased by 8% (5.9 L L⁻¹ d⁻¹). In contrast, the no-addition of macro-nutrients (day 206-225) resulted not only in the decrease of the biogas production rate by 42% (3.4 L L⁻¹ d⁻¹), but also the effluent COD_S and HPr concentrations increased to 233 mg COD L⁻¹ and 211 mg HPr-COD L⁻¹, respectively.

3.3.2 UASB_{HPr} performance

Similarly to UASB_{HRT}, the pH of the UASB_{HPr} remained constant during the whole experimental period, around 7.5 ± 0.4.

Figure 3.3 shows the performance of UASB_{HPr} in terms of applied OLR (Figure 3.3A), effluent COD_S concentration (Figure 3.3B), effluent HPr-COD concentration (Figure 3.3C) and biogas production rate (Figure 3.3D) during the whole experimental period.

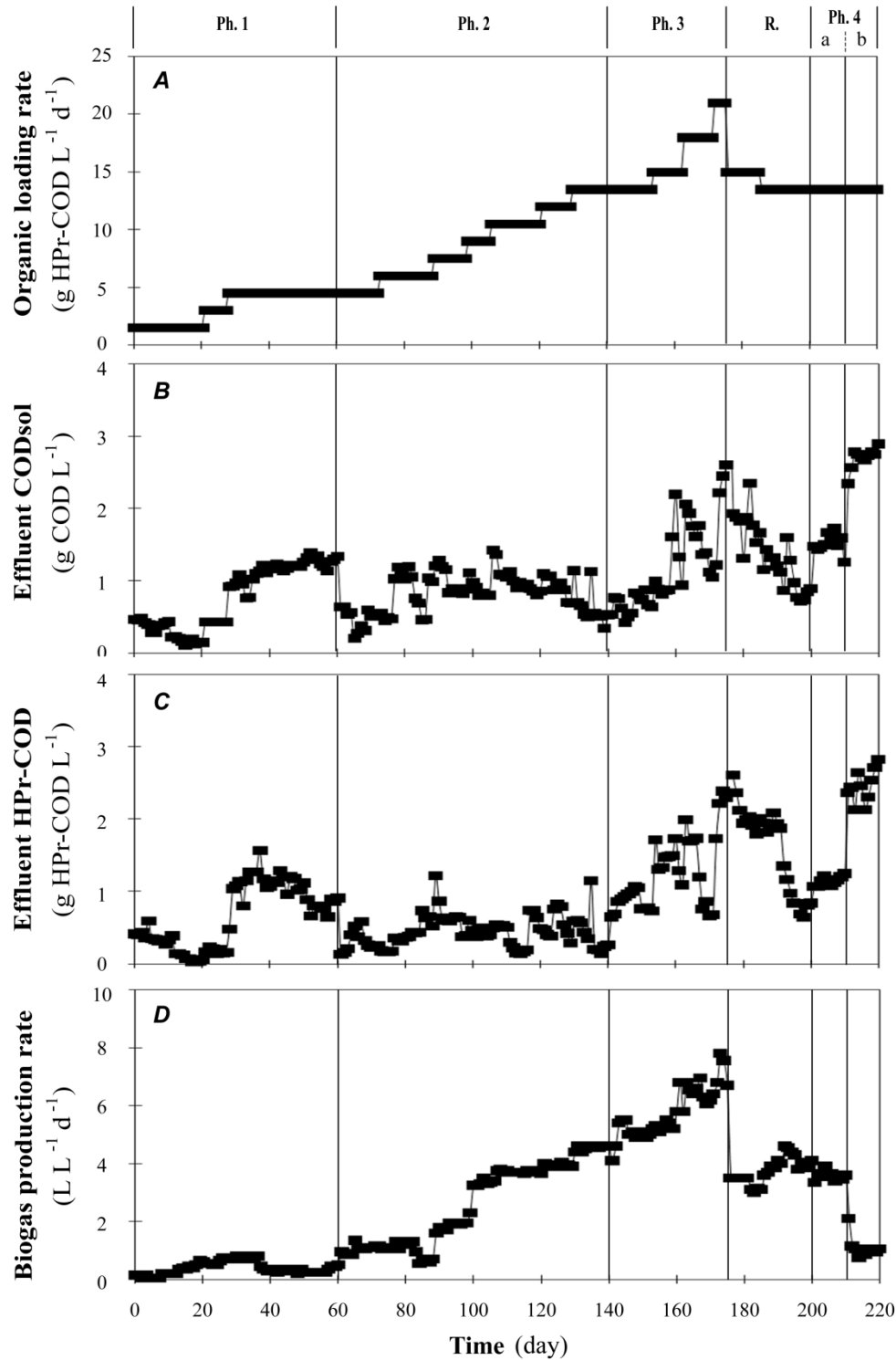


Figure 3.3 Performance of UASB_{HPr} reactor during the whole experimental period.(A) OLR; (B) Effluent COD_S concentration; (C) Effluent HPr-COD concentration; and, (D) Biogas production rate. Ph.1: phase 1, with only HPr in the feed; Ph.2: phase 2, with HPr supplemented with macro-nutrients; Ph.3: phase 3, with HPr supplemented by macro- and micro-nutrients; R: recovery period; Ph.4: phase 4, with no-addition of micro-nutrients (4a) and macro- nutrients (4b).

At the beginning of phase 1 (day 0-27), the OLR was increased from 1.5 to 3 g HPr-COD L⁻¹ d⁻¹ (influent HPr concentration: from 0.8 to 1.5 g HPr-COD L⁻¹). The effluent COD_S and HPr concentrations remained below 0.4 g COD L⁻¹ and 0.2 g HPr-COD L⁻¹, respectively. The E_{HPr} and R_{HPr} were high, around 88% and 2.6 g HPr-COD L⁻¹ d⁻¹, respectively. However, the reactor performance was deteriorated after the further increase of the OLR to 4.5 g HPr-COD L⁻¹ d⁻¹ (influent HPr concentration: 2.3 g HPr-COD L⁻¹) on day 28. Indeed, both the effluent COD_S and HPr concentrations increased to 1.2 g COD L⁻¹ and 1 g HPr-COD L⁻¹, respectively, resulting in low E_{HPr} and R_{HPr}, i.e. 54% and 2.4 g HPr-COD L⁻¹ d⁻¹, respectively. Throughout this phase, the biogas production rate remained lower than 0.5 L L⁻¹ d⁻¹ (< 0.23 L g⁻¹ COD_{removed}).

After the supplementation of macro-nutrients (from day 61 on), the effluent COD_S and HPr concentrations decreased to less than 0.5 g COD L⁻¹ and 0.3 g HPr-COD L⁻¹, respectively, and the biogas production rate increased up to 1.1 L L⁻¹ d⁻¹ (0.32 L g⁻¹ COD_{removed}). Furthermore, during this phase 2, the OLR could be increased to 13.5 g HPr-COD L⁻¹ d⁻¹ (influent HPr concentration: 6.8 g HPr-COD L⁻¹). Under these conditions, high biogas production rate (4.6 L L⁻¹ d⁻¹ or 0.45 L g⁻¹ COD_{removed}), E_{HPr} (97%) and R_{HPr} (13.1 g HPr-COD L⁻¹ d⁻¹) were obtained.

After the supplementation of micro-nutrients (from day 141 on), the biogas production rate increased to 5.1 L L⁻¹ d⁻¹, but also the COD_S and HPr concentrations in the effluent to around 0.8 g COD L⁻¹ and 0.7 g HPr-COD L⁻¹, respectively. During phase 3 (day 141-175), the OLR was further increased to 21 g HPr-COD L⁻¹ d⁻¹ (influent HPr concentration: 10.5 g HPr-COD L⁻¹), which resulted in 25-30% increase of the biogas production rate, i.e. 7.2 L L⁻¹ d⁻¹ (0.49 L g⁻¹ COD_{removed}), as well as the E_{HPr} and the R_{HPr}, up to 77% and 16.4 g HPr-COD L⁻¹ d⁻¹, respectively. However, both COD_S and HPr concentrations in the effluent increased to around 2.4 g COD L⁻¹ and 2.3 g HPr-COD L⁻¹, respectively.

Since the effluent COD_S and HPr concentrations at the end of phase 3 were higher than 2 g COD L⁻¹, a recovery period (day 176-200) was applied, in which the OLR was decreased stepwise to 13.5 g HPr-COD L⁻¹ d⁻¹ (influent HPr concentration: 6.8 g HPr-COD L⁻¹) in order to get effluent COD_S and HPr concentrations below 1 g COD L⁻¹. Consequently, the biogas production rate also decreased and remained constant at 4.2 L L⁻¹ d⁻¹ during this recovery period.

Similarly to UASB_{HRT}, the positive effect of macro- and micro-nutrients was confirmed during phase 4 (day 201-220). The no-addition of micro-nutrients (day 201-210) resulted in an increase of the effluent COD_S and HPr concentrations to 1.5 g COD L⁻¹ and 1.1 g HPr-COD L⁻¹, respectively, and the biogas production rate decreased by 17% (3.5 L

$L^{-1} d^{-1}$). The subsequently interruption of macro-nutrients addition (day 211-220) accentuated this negative effect by increasing the effluent COD_S and HPr concentrations to $2.7 g COD L^{-1}$ and $2.4 g HPr-COD L^{-1}$, respectively, and decreasing the biogas production rates by 60-70% ($1.1 L L^{-1} d^{-1}$ or $0.15 L g^{-1} COD_{removed}$).

3.4 DISCUSSION

Although in most literature studies related to UASB reactors, the OLR is the common used parameter indicating reactor operation conditions, for the start-up phase, the mass OLR is also of interest in order to control the sludge granulation process. In general, it is suggested that it should be around 0.05 to $0.25 g COD g^{-1} VS d^{-1}$ during the start-up and kept below $1 g COD g^{-1} VS d^{-1}$ for a stable performance (Singh et al., 1998). In this study, during the first operational days, the mass OLR of both reactors was below $0.12 g COD g^{-1} VS d^{-1}$. Afterwards, the highest mass OLR achieved in both reactors was lower than $0.44 g COD g^{-1} VS d^{-1}$. Since these values were in the range of the recommended ones and good granulation was observed, the sludge performance was considered as optimal in terms of the mass OLR applied, and this parameter was not considered as a critical for the reactors performance.

Table 3.3 shows a summary of the results obtained in both UASB reactors during the last days of each operational phase.

3.4.1 Without nutrients supplementation

The performance of both UASB reactors without nutrients supplementation was quite poor (Figures 3.2, Figure 3.3 and Table 3.3). The maximum OLR that could be applied in both reactors was $4.5 g HPr-COD L^{-1} d^{-1}$, corresponding to an HRT of 4 h in $UASB_{HRT}$ and an influent HPr concentration of $2.3 g HPr-COD L^{-1}$ in $UASB_{HPr}$. These operational conditions resulted in low E_{HPr} (between 48% and 65%) and R_{HPr} (between 2.1 and 2.9 $g HPr-COD L^{-1} d^{-1}$). This observation was confirmed during phase 4, where the E_{HPr} dropped around 25% in both reactors, upon subsequent interruption of micro- and macro-nutrients supplementation. Although the later E_{HPr} was slightly higher than those obtained in phase 1, it can be explained by the adaptation of the anaerobic microorganisms to HPr degradation after more than 200 days of reactors operation. Concerning the biogas production rate, no more than 20% (maximum $0.5 L L^{-1} d^{-1}$) of the theoretical values were achieved in both reactors during phase 1. During phase 4, although higher biogas production rates were obtained (3.4 and $1.1 L L^{-1} d^{-1}$ in $UASB_{HRT}$ and $UASB_{HPr}$, respectively) as a consequence of the higher OLR applied, the percentages over the theoretical values were similar to those obtained in phase 1, around 33% and 18% in $UASB_{HRT}$ and $UASB_{HPr}$, respectively.

Table 3.3 Summary of the results of the UASB reactors during the last days of each operational phase.

Parameters	Unit	UASB _{HRT}			UASB _{Pr}		
		Phase 1	Phase 2	Phase 3	Phase 1	Phase 2	Phase 3
OLR	g HPPr-COD L ⁻¹ d ⁻¹	4.5	18.8	33.8	4.5	13.5	21
HRT	h	4	1	0.5	4 (constant)		
Influent HPPr concentration	g HPPr-COD L ⁻¹		0.8 (constant)		2.3	6.8	10.5
E _{HPPr}	%	48	96	97	65	97	77
R _{HPPr}	g HPPr-COD L ⁻¹ d ⁻¹	2.1	18.1	32.8	2.9	13.1	16.4
Biogas production rate	L L ⁻¹ d ⁻¹	0.4	3.5	6.7	0.5	4.6	7.2

3.4.2 Effect of macro- and micro-nutrients supplementation

The stimulatory effect of the macro-nutrients was immediately observed after their supplementation (phase 2), since the effluent quality was improved in both reactors by a decrease of the effluent COD_S and HPr concentrations by more than 50%. Consequently, the E_{HPr} increased up to 95%. Moreover, the biogas production rates at the beginning of phase 2, ca. 0.8 and 1.1 L L⁻¹ d⁻¹ (40% of the theoretical value) in the UASB_{HRT} and UASB_{HPr}, respectively, doubled the values obtained at the same OLR (4.5 g HPr-COD L⁻¹ d⁻¹) during phase 1. At the end of phase 2, the maximum OLR applied in the UASB_{HRT} and UASB_{HPr} was 3 and 2 times higher without affecting the E_{HPr} (around 90%), and resulting also in greater biogas production rates (59% and 81% of the theoretical values).

The positive effect of macro-nutrients supplementation was also confirmed during phase 4b, since the performance of both reactors deteriorated (the effluent COD_S concentration increased by 282% and 115%, respectively, and biogas production rate decreased by 42% and 69%, respectively) after stopping the addition of macro-nutrients.

Contrary to the supplementation of macro-nutrients, the stimulatory effects of micro-nutrients addition (phase 3) were less pronounced. The effluent COD_S and HPr concentrations were almost not affected, remaining at the same values or slightly higher than those obtained at the end of phase 2. In contrast, the biogas production rate increased in both reactors by 10%, approximately.

Yet, the supplementation of micro-nutrients allowed the OLR to be further increased to 33.8 and 21.0 g HPr-COD L⁻¹ d⁻¹ in UASB_{HRT} and UASB_{HPr}, respectively, which corresponded to an HRT of 0.5 h and an influent HPr concentration of 10.5 g HPr-COD L⁻¹, respectively. Under these extreme conditions, the R_{HPr} in both reactors could be declared to be very high, i.e. 32.8 and 16.4 g HPr-COD L⁻¹ d⁻¹ (E_{HPr} of 97% and 77%), respectively. The effluent quality in UASB_{HRT} remained good (residual HPr concentrations below 0.1 g HPr-COD L⁻¹), while it was poor in UASB_{HPr}, with residual HPr concentration of 2.3 g HPr-COD L⁻¹. However, it should be considered that an influent HPr concentration of 10.5 g HPr-COD L⁻¹ was used, which is extremely high and less likely to occur in practice.

When the addition of micro-nutrients was interrupted (phase 4a), no significant effect was observed on UASB_{HRT} operation, whereas in UASB_{HPr} the effluent COD_S concentrations slightly increased. However, the biogas production rates decreased in both reactors by 10%.

With the supplementation of the macro- and micro-nutrients, the applied HRT of UASB_{HRT} and the influent HPr concentration of UASB_{HPr} could be stressed to 0.5 h and

10.5 g HPr-COD L⁻¹, respectively, without affecting reactor performance (R_{HPr} of 15-30 g COD L⁻¹ d⁻¹ and E_{HPr} of 75-95%). These conditions surpassed those values widely recommended for UASB configuration by other authors (Barredo and Evison, 1991; Elefsiniotis and Oldham, 1994; Hwu et al., 1997), ca. longer than 12 h and less than 2.5 g HPr-COD L⁻¹.

The positive effect of micro-nutrients was also observed by Climenhaga and Banks (2008), who emphasized the importance of the micro-nutrients for stable digestion at high VFA levels, and Zitomer et al. (2008), who indicated that the propionate utilization rate increased by the enhanced biomass activities benefited from the micro-nutrients.

3.4.3 UASB_{HRT} vs. UASB_{HPr}: practical application

Two different feeding strategies were applied to increase the OLR in the reactors, which consequently affected the performances of the reactors. An extremely short HRT was achieved in UASB_{HRT}, ca. 0.5 h, corresponding to a maximum OLR of 33.8 g HPr-COD L⁻¹ d⁻¹. Interestingly, due to the good biomass granulation, no significant biomass wash-out was observed under these conditions. Moreover, HPr removal was not affected either by these extreme operational conditions, achieving R_{HPr} of 32.8 HPr-COD L⁻¹ d⁻¹ (E_{HPr} of 97%). In the UASB_{HPr}, the maximum influent HPr concentration was 10.5 g HPr-COD L⁻¹, corresponding to a maximum OLR of 21 g HPr-COD L⁻¹ d⁻¹, which is 38% lower than that obtained in the UASB_{HRT}. The R_{HPr} obtained under these conditions, 16.4 g HPr-COD L⁻¹ d⁻¹ (E_{HPr} of 77%), was also 50% lower than the value obtained in the UASB_{HRT}.

In our study, the maximum R_{HPr} achieved in the UASB_{HPr} (21 g HPr-COD L⁻¹ d⁻¹) is in the same range as the values reported by other authors (20-25 g HPr-COD L⁻¹ d⁻¹), whose strategy to increase the OLR was the same as that applied in UASB_{HPr}, i.e. increasing the influent HPr concentration (Wiegant et al., 1986; Fang et al., 1994; Tatara et al., 2008). However, much higher R_{HPr} was obtained in our study in the UASB_{HRT}, in which the OLR was increased by decreasing the HRT. This fact indicates that high throughputs could be advantageous compared to high-concentrated streams. In addition, the recovery of the UASB_{HRT} from the stress conditions at the end of phase 3 took only 2 days while it required 25 days for the UASB_{HPr}, thus indicating the quicker recovery from high hydraulic regimes.

Both strategies are representative of different real situations and the selection will depend on the characteristics of the waste stream to be treated. If low residual HPr concentrations (< 1 g HPr-COD L⁻¹) are expected, the strategy of UASB_{HRT} is suggested, which allows the efficient treatment of high waste volumes (HRT of 0.5 h). If residual HPr

concentrations higher than $2 \text{ g HPr-COD L}^{-1}$ are expected, a longer HRT is required for its efficient removal, and thus the strategy of UASB_{HPr} is more appropriate. In most cases, a combination of these two strategies is recommended.

Although the overall stimulatory effects of the macro- and micro-nutrients supplementation were observed in both reactors, i.e. better effluent quality and higher biogas production rates, the positive outcome of the macro-nutrients was more pronounced on both UASB reactors. The effects of the micro-nutrients supplementation was moderate on the UASB_{HPr} , however, more pronounced on the UASB_{HRT} , which can be explained by the fact that the influent HPr concentration was 8 times lower than that of the UASB_{HPr} .

In general, in practice, nutrients are added into industrial digesters in excessive amounts to ascertain stable performance. But, this excessive supplementation can lead to metals accumulation in the digester, and thus causing toxicity. Therefore, a correct strategy for nutrients supplementation should be defined based on metal uptake by microorganisms and “metal bioavailability”, which also depends on the specific waste characteristics.

3.5 CONCLUSIONS

This work shows that propionic acid is a recalcitrant compound, whose biological degradation is not straightforward. The supplementation of macro- and micro-nutrients stimulates the reactors performance, in terms of higher applied OLR, better effluent quality and greater biogas production rates.

For practical applications, a combination of these two strategies according to the specific characteristics of the waste stream is proposed. A “shock” of the microbial community due to direct exposure to high initial HPr levels should be avoided. On the contrary, by increasing gradually the OLR, it is possible to eliminate HPr at high removal rate. A strategy according to the characteristics of the specific waste stream should be selected, i.e. by lowering the HRT or by increasing the HPr concentration in the influent or a combination of both. Besides, the supplementation of macro- and micro-nutrients to the digesters at levels of mg L^{-1} and $\mu\text{g L}^{-1}$, respectively, can improve the HPr removal.

APPENDIX I

APPENDIX II

CHAPTER I

CHAPTER II

CHAPTER III

**ACCELERATED PROCESS RECOVERY
BY EXTERNAL PROPIONIC ACID DEGRADATION**

CHAPTER IV

CHAPTER V

CHAPTER VI

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CHAPTER IV

ACCELERATED PROCESS RECOVERY BY EXTERNAL PROPIONIC ACID DEGRADATION

Abstract

An enhanced propionic acid degradation (EPAD) system has been conceptually designed and experimentally tested at lab-scale. The system consisted of two components: a liquid/solid separator containing a microfiltration membrane and an upflow anaerobic sludge bed (UASB) reactor specialized in propionic acid (HPr) degradation. Two lab-scale continuous stirred tank reactors (CSTR) were used, i.e. the CSTR_C and the CSTR_T. Firstly, the CSTRs were stressed by organic overloading to obtain high HPr levels. During the recovery period, besides stop feeding, no actions were taken to decrease the residual HPr concentration in the CSTR_C, while the CSTR_T was connected to EPAD system in order to accelerate its recovery. By the end of the experiment, the CSTR_T completely recovered from HPr accumulation, while no significant decrease of the HPr level in the CSTR_C was observed. Based on the experimental results, the up-scaling of EPAD system was evaluated and it would only account for about 2% of the volume of the full-scale digester, thus suggesting that the implementation of a mobile EPAD system in full-scale practice should be feasible.

Chapter redrafted after: Ma J, Carballa M, Van De Caveye P and Verstraete W 2009 Enhanced propionic acid degradation (EPAD) system: experimental validation and practical consideration. *Water Research* 43 (13) 3239-3248.

4.1 INTRODUCTION

Anaerobic digestion is a well established technology for the treatment of high-strength wastes. However, this technology still requires considerable monitoring and optimization due to the frequent process instabilities, which often result in the accumulation of propionic acid (HPr) (Verstraete et al., 2005; Wang et al., 2006).

Two metabolic phases, i.e. acidogenesis and methanogenesis, are involved in anaerobic processes. Consequently, the efficiency and performance of an anaerobic digester strongly depend on the cooperation between the acidogens and the methanogens (Xu et al., 2004). A typical response to this unbalanced cooperation is the accumulation of volatile fatty acids (VFAs). More specifically, HPr levels have been found to rise prior to methane production inhibition (Pullammanappallil et al., 2001). Although the high hydrogen partial pressure or high bio-hydrogen production rate has been suggested by many authors as the main reason causing HPr accumulation in anaerobic digesters (Harper and Pohland, 1986; Mosey and Fernandes, 1989; Fynn and Syafila, 1990), other studies have indicated that HPr accumulation seems to be independent from hydrogen partial pressure (Inanc et al., 1996; Ren et al., 1997). From these contradictory results, it can be concluded that the cause of HPr accumulation during anaerobic processes has not been unequivocally clarified yet.

Moreover, the reported maximum tolerable levels of HPr before inhibiting methane production are not uniform, from 0.8 g L⁻¹ (1.2 g HPr-COD L⁻¹) by Mosche and Jordening (1998) to 5 g L⁻¹ (7.5 g HPr-COD L⁻¹) by Hajarnis and Ranade (1994), although the common values vary between 1 g L⁻¹ (1.5 g HPr-COD L⁻¹) and 2 g L⁻¹ (3 g HPr-COD L⁻¹) (Barredo and Evison, 1991; Dogan et al., 2005). The type of waste treated, the reactor configuration and the operational parameters are the main factors affecting this tolerable level. In practice, it has been generally accepted that HPr concentrations should be kept below 1.5 g L⁻¹ (2.3 g HPr-COD L⁻¹) for a proper process operation.

Solutions to decrease the HPr concentration include reactor configuration modifications, supplementation of proper levels of essential nutrients and bioaugmentation (Kim et al., 2004; Bagi et al., 2007; Tepe et al., 2008). The single-stage continuous stirred tank reactor (CSTR), a commonly used configuration for solid digestion, often results in a poor performance due to the low organic loading rate (OLR) applied and frequent HPr accumulation events (Azbar and Speece, 2001). Therefore, the two-stage anaerobic process, where the acidogenic and methanogenic phases are separated, has been developed to enhance the stability and efficiency of the process. However, the required complex and sophisticated control system limits its application (Demirel and Yenigun, 2004; Bolzonella et al., 2007). Several studies have indicated that the conversion of acetic acid (HAc) and

HPr is stimulated by the addition of macro- and micro-nutrients (Speece, 1996; Cresson et al., 2006) and, in practice, the supplementation of FeSO_4 in excessive amounts to ascertain a stable digester performance has given satisfactory results (Kim et al., 2002; Climenhaga and Banks, 2008; Zitomer et al., 2008). Furthermore, the addition of different hydrogen-oxidizing bacteria has been reported to improve the degradation of HPr (Schmidt and Ahring, 1993; Bagi et al., 2007). However, no mature strategy has been shown so far to be reliable and straightforward in terms of “curing” a full scale “sick” anaerobic digester (i.e. reactor in which HPr is accumulated, ca. $> 1.5 \text{ g L}^{-1}$, resulting in the inhibition of methane production). In practice, most industries simply stop feeding the digesters and passively wait for the decreasing of the HPr level, which can require months before an adequate recovery is obtained.

Since the origin of HPr accumulation has been extensively studied in the past with little success, the objective of this work was not to get insights in the factors causing HPr accumulation, but to provide an adequate remedy to accelerate the anaerobic digester recovery. With this purpose, an enhanced propionic acid degradation (EPAD) system which can be attached to “sick” anaerobic digesters and accelerate their recovery was designed. The performance and efficiency of the EPAD system were validated at lab-scale and its practical feasibility at full-scale was evaluated based on the experimental results. To the best of our knowledge, this is the first study focusing on an add-on remediation technique.

4.2 MATERIALS AND METHODS

4.2.1 EPAD system concept

The EPAD system consists of two components (Figure 4.1): a separator ($\text{EPAD}_{\text{separator}}$) containing a submerged plate membrane aiming to separate the solid and liquid fractions of the mixed liquor from the anaerobic digester, and an upflow anaerobic sludge bed (UASB) reactor ($\text{EPAD}_{\text{UASB}}$) which is aimed to degrade HPr at high rate, relying on a consortium acclimated to HPr degradation (Ma et al., 2009).

The working principle is as follows: the mixed liquor of the full-scale “sick” anaerobic digester is recycled through the $\text{EPAD}_{\text{separator}}$. The resulting permeate is pumped into the $\text{EPAD}_{\text{UASB}}$, where the HPr degradation takes place. The effluent of the $\text{EPAD}_{\text{UASB}}$, with low HPr concentration ($< 0.5 \text{ g HPr-COD L}^{-1}$) is also recycled back to the full-scale anaerobic digester, with the concomitant lowering effect. Therefore, the goal of the EPAD system is to accelerate the decrease of the HPr concentration in the full-scale anaerobic digester, by providing a HPr specialized microbial consortium in a side reactor.

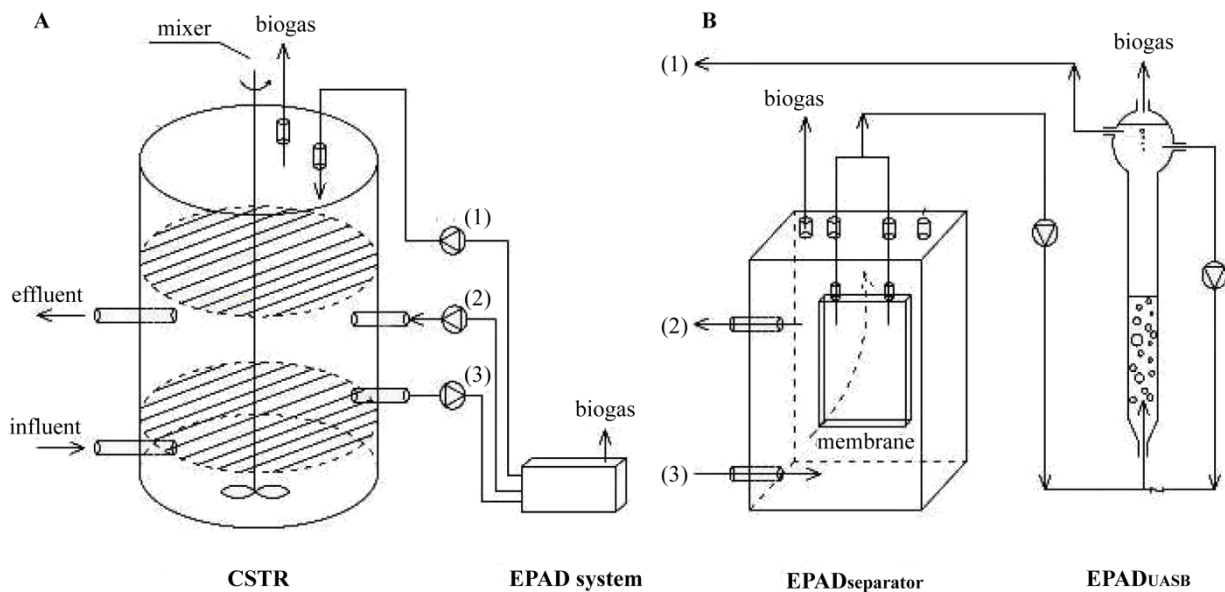


Figure 4.1 (A) EPAD system connected to a full-scale anaerobic digester; (B) Detailed flow sheet of EPAD system.

4.2.2 Experiment set-up

4.2.2.1 The CSTRs

Two lab-scale CSTRs (15 L working volume) were operated to simulate the full-scale anaerobic digesters: the control reactor (CSTR_C), in which no actions rather than the interruption of the feeding were taken to decrease the HPr concentration, and the treatment reactor (CSTR_T), which besides the interruption of the feeding was connected to EPAD system in order to accelerate the reactor recovery.

The CSTRs were inoculated with mesophilic sludge harvested from a domestic wastewater treatment plant (Ossemeersen, Belgium), resulting in an initial in-reactor volatile solid (VS) concentration of around 25 g VS L⁻¹. Kitchen waste (KW) diluted with tap water was used as the daily feeding at a flowrate of 1 L d⁻¹, resulting in a hydraulic retention time (HRT) of 15 d. The composition of the kitchen waste (vegetables, meat, fish, potato, rice, pasta, etc.) varied depending on the deliveries. The average total chemical oxygen demand (COD) (COD_T) and total solid (TS) concentrations were approximately 220 g COD kg⁻¹ KW and 150 g TS kg⁻¹ KW, respectively. Both the raw kitchen waste and the weekly-prepared feeding of the reactors were stored at 4°C.

The experiment was performed in replicate (experiment 1 and 2) and it consisted of two main phases (Table 4.1). In phase 1 (overloading phase), an excessive OLR was applied to both CSTRs to obtain high HPr levels; and, in phase 2 (recovery phase), two different strategies, i.e. stop feeding and EPAD connection, were applied to recover the

CSTRs from HPr accumulation. In experiment 1, the EPAD system was connected to the CSTR_T at the beginning of phase 2 (day 28), while the feeding was continued in both CSTRs. Since the feeding effect surpassed the EPAD system effect, the feeding was stopped from day 34 on in both CSTRs. Consequently, in experiment 2, the feeding of both CSTRs was stopped at the beginning of phase 2 (day 22), without connecting the EPAD system to CSTR_T, in order to evaluate the single effect of interrupting the feeding. From day 36 on, the EPAD system was connected to the CSTR_T. Between both experiments, a self recovery period was applied for the CSTR_C to obtain good effluent quality before starting experiment 2 (data not shown).

Mesophilic conditions ($33 \pm 2^\circ\text{C}$) were applied and the pH of the reactors was controlled at around 7.5 with NaOH solution (5N). pH, soluble COD (COD_S) and VFAs were monitored during the experiments.

4.2.2.2 The EPAD_{separator}

The EPAD_{separator} had a working volume of 4.5 L and it contained a submerged plate membrane (Solis, Holland) made of chlorinated polyethylene (Kubota, Japan). The pore size, the surface membrane area per plate and the specific membrane surface area were 0.4 μm (microfiltration), 0.1 m^2 and 115 $\text{m}^2 \text{m}^{-3}$, respectively (De Gusseme et al., 2009). These plate membranes show good resistance against clogging and they are known for their robustness (Hennebel et al., 2009).

The mixed liquor from the CSTR_T was pumped (15 min h^{-1}) into the bottom of the EPAD_{separator}, where part of the liquid fraction was separated from the solid fraction by the microfiltration membrane. The obtained permeate was pumped into the EPAD_{UASB}. To control the OLR of the EPAD_{UASB} and to abate the membrane fouling, a cycle of 15 min extrusion and 45 min relaxation was applied on the membrane, which resulted in permeate fluxes of 1 $\text{L m}^{-2} \text{h}^{-1}$ (2.5 L d^{-1}) and 0.2 $\text{L m}^{-2} \text{h}^{-1}$ (0.5 L d^{-1}) for experiments 1 and 2, respectively.

4.2.2.3 The EPAD_{UASB}

The EPAD_{UASB} had a working volume of 2 L and it consisted of a cylindrical tube with a diameter of 5 cm and one spheriform 3-phase separator at the upper part. The inoculum was taken from a full-scale mesophilic anaerobic digester treating potato processing wastewater (Mydibel, Belgium) and the initial in-reactor VS concentration was approximately 10 g VS L^{-1} . Before starting the EPAD experiments, this anaerobic biomass was acclimated to HPr methanation (HPr as sole electron donor in the feeding) for 1-2 months (data not shown).

Table 4.1 Summary of the main operational parameters and results of the CSTRs during the whole experiment period.

Experiment 1		CSTR _C					CSTR _T				
Phase	Day	OLR (g COD L ⁻¹ d ⁻¹)	OLR (g COD L ⁻¹ d ⁻¹)	HPPr (g HPPr-COD L ⁻¹)	HAc (g HAc-COD L ⁻¹)	OLR (g COD L ⁻¹ d ⁻¹)	COD _s (g COD L ⁻¹)	HPPr (g HPPr-COD L ⁻¹)	COD _s (g COD L ⁻¹)	HAc (g HAc-COD L ⁻¹)	
1	0	0.7	0.1	0	0	0.7	0.1	0	0.1	0	
	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	
	27	2.8	11.7	4.3	4.7	2.8	11.5	5.0	11.5	4.9	
2	28		12.0	3.9	4.1		11.9	5.0		4.9	
	↓	2.8	↓	↓	↓	2.8	↓	↓	↓	↓	
	34		18.2	5.3	6.6		18.1	5.3		5.9	
2	35		17.8	5.3	6.6		17.4	5.4		5.8	
	↓	--	↓	↓	↓	--	↓	↓	↓	↓	
	65		8.4	3.6	3.5		1.5	0.1		0.3	
Experiment 2		CSTR _C					CSTR _T				
1	0	0.8	0.8	0.1	0.8	0.8	0.8	0.2	0.8	0.2	
	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	
	21	4.2	37.9	13.1	11.8	4.2	30.3	13.4	30.3	10.9	
2	22		30.2	12.4	10.9		32.5	12.3		10.4	
	↓	--	↓	↓	↓	--	↓	↓	↓	↓	
	35		35.4	13.2	11.5		35.7	13.6		12.7	
2	36		34.3	12.8	11.1		28.2	12.3		11.5	
	↓	--	↓	↓	↓	--	↓	↓	↓	↓	
	90		32.9	11.1	11.4		2.8	0.4		0.6	

The reactor content was continuously mixed by recirculation at an upflow velocity of around 1 m h^{-1} and the effluent was recycled back to the CSTR_T. During the first days of the EPAD system connection to the CSTR_T, the OLR of EPAD_{UASB} was controlled by the permeate flow-rate to avoid an organic overloading. Later, a fixed permeate rate (2.5 L d^{-1} in experiment 1 and 0.5 L d^{-1} in experiment 2) was applied and the OLR decreased accordingly with the decrease in the COD_S levels in the permeate. The reactor operated at mesophilic conditions ($33 \pm 2^\circ\text{C}$) and the pH was stable during the whole experimental period, around 7.6 ± 0.2 .

4.2.3 Analytical procedures

Physico-chemical parameters, COD_T and COD_S, TS and VS, were determined according to the standard methods (Greenberg et al., 1992). VFAs were extracted with diethyl ether and the quantitative analysis was carried out in a capillary gas chromatograph (CE Instruments, Italy) which is coupled with a flame ionization detector. The pH values were measured with a pH meter (Consort C532, Germany).

4.3 RESULTS

4.3.1 The CSTRs

The pH in both CSTRs remained constant (7.5 ± 0.2) during the whole experimental period. Table 4.1 and Figure 4.2 show the performance of the CSTRs during the two experiments in terms of the OLR and the residual COD_S, HPr and HAc concentrations.

4.3.1.1 Experiment 1

During phase 1 (day 0-27), the OLR was increased gradually in both CSTRs to $2.8 \text{ g COD L}^{-1} \text{ d}^{-1}$ (Figure 4.2). Consequently the COD_S, HPr and HAc concentrations increased progressively up to around 12 g COD L^{-1} , $4.5 \text{ g HPr-COD L}^{-1}$ and $5.0 \text{ g HAc-COD L}^{-1}$, respectively (Table 4.1). At this moment, both CSTRs were considered “sick” since HPr concentrations were above $1.5 \text{ g HPr-COD L}^{-1}$.

On day 28, the EPAD system was connected to the CSTR_T and the feeding was continued for both CSTRs (OLR of $2.8 \text{ g COD L}^{-1} \text{ d}^{-1}$) during the first days of phase 2 (day 28-34). Since a further increase of the COD_S, HPr and HAc concentrations was observed in both CSTRs (Figure 4.2), up to $18.2 \text{ g COD L}^{-1}$, $5.3 \text{ g HPr-COD L}^{-1}$ and $6.6 \text{ g HAc-COD L}^{-1}$ (Table 4.1), respectively, on day 35, the feeding of the CSTRs was interrupted. From that point, COD_S, HPr and HAc concentrations started to decrease in both CSTRs (Figure 4.2). By the end of phase 2 (day 65), a significant decrease of 91% of the COD_S concentration was observed in the CSTR_T (from 17.4 to 1.5 g COD L^{-1}), while

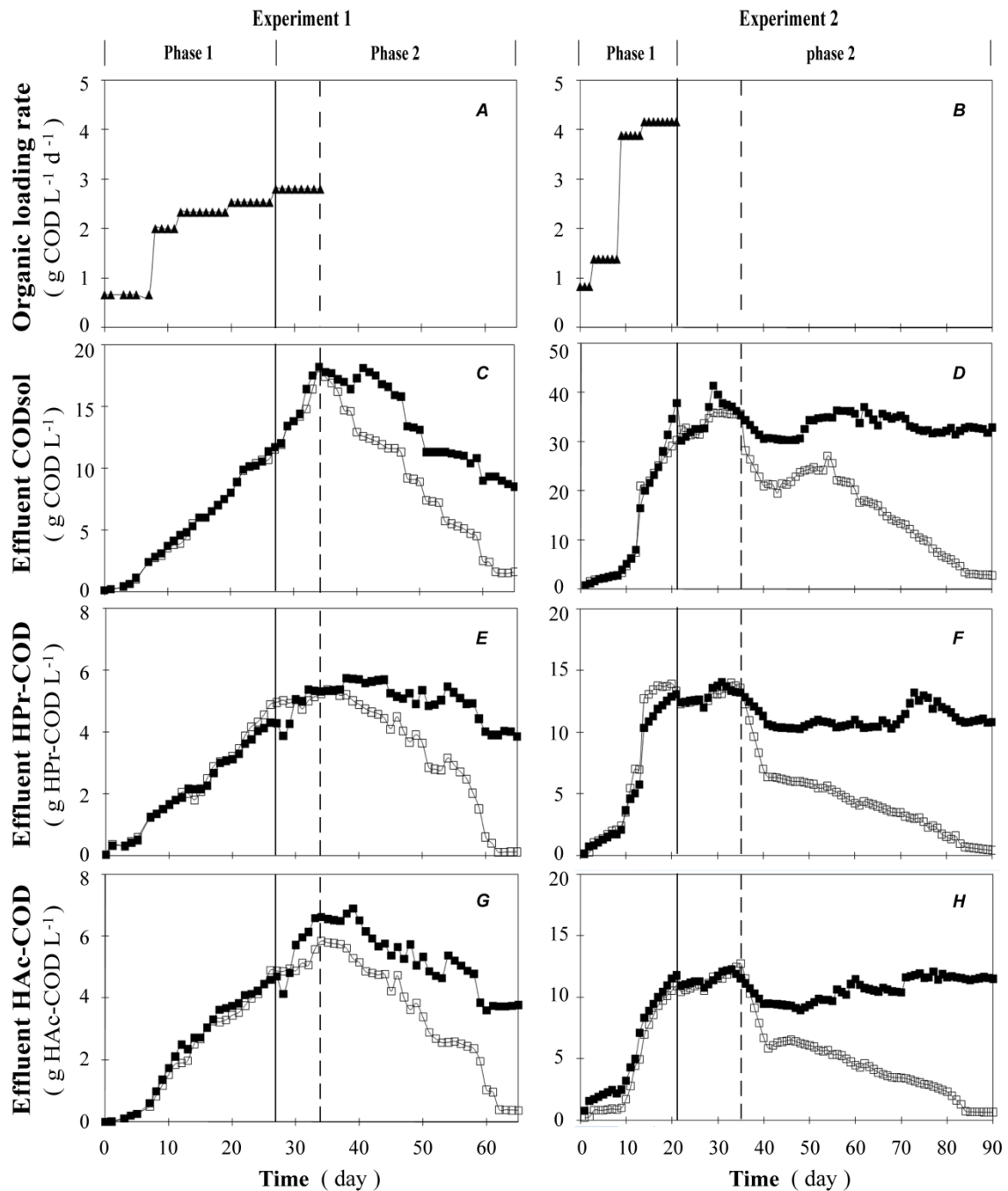


Figure 4.2 Performance of CSTRs during experiment 1 (A, C, E, G) and experiment 2 (B, D, F, H). Phase 1: Overloading phase; Phase 2: recovery phase. (▲) OLR; (■) CSTR_C; (□) CSTR_T. Dashed line indicates when EPAD system effect was evaluated without feeding the reactors.

in the CSTR_C, it was decreased by only 53% (from 17.8 to 8.4 g COD L⁻¹). Similarly, HPr and HAc were almost completely removed in the CSTR_T (from 5.4 to 0.1 g HPr-COD L⁻¹ and from 5.8 to 0.3 g HAc-COD L⁻¹), respectively, whereas a lower elimination was observed in the CSTR_C (from 5.3 to 3.6 g HPr-COD L⁻¹ and from 6.6 to 3.5 g HAc-COD

L^{-1}), respectively. Overall, the accelerated HPr recovery of the $CSTR_T$ by the connection of the EPAD system without feeding took 30 days for experiment 1.

4.3.1.2 Experiment 2

Analogously to experiment 1, both CSTRs became highly dysfunctional during phase 1 when the OLR was increased gradually to $4.2 \text{ g COD L}^{-1} \text{ d}^{-1}$ (Figure 4.2). The COD_S , HPr and HAc concentrations increased sharply to up to $37.9 \text{ g COD L}^{-1}$, $13.9 \text{ g HPr-COD L}^{-1}$ and $11.8 \text{ g HAc-COD L}^{-1}$, respectively (Table 4.1). Considering the observations during experiment 1 that the feeding effect surpassed the EPAD system effect, a different experimental strategy was applied in experiment 2 (Table 4.1). At the beginning of phase 2 (day 22), the feeding of both CSTRs was interrupted without the connection of the EPAD system to the $CSTR_T$. As a consequence, the COD_S , HPr and HAc concentrations did not increase anymore, but they remained more or less constant for 15 days (Figure 4.2). Therefore, on day 36, the EPAD system was connected to the $CSTR_T$, resulting in an immediate decrease of the COD_S , HPr and HAc concentrations with removal efficiencies of 90% (from 28.8 to 2.8 g COD L^{-1}), 97% (from 12.3 to $0.4 \text{ g HPr-COD L}^{-1}$) and 95% (from 11.5 to $0.6 \text{ g HAc-COD L}^{-1}$), respectively, by the end of the experiment (Table 4.1). Meanwhile, the same levels were maintained in the $CSTR_C$ (around 33 g COD L^{-1} , $11 \text{ g HPr-COD L}^{-1}$ and $11 \text{ g HAc-COD L}^{-1}$, respectively). In this case, the accelerated HPr recovery of the $CSTR_T$ by the connection of the EPAD system took 55 days.

4.3.2 The EPAD_{separator}

The ratios between the concentrations of the COD_S and HPr in the permeate and in the bulk liquid ($CSTR$ mixed liquor) were by average 78% and 98%, respectively, during experiment 1 and 88% and 94%, respectively, during experiment 2 (data not shown). However, the HPr permeate flux depended on the permeate flux and the permeate HPr concentration. In experiment 1, the permeate flux was fixed at $1 \text{ L m}^{-2} \text{ h}^{-1}$ (2.5 L d^{-1}) and the initial (maximum) permeate HPr concentration was $5 \text{ g HPr-COD L}^{-1}$, which resulted in the maximum HPr permeate flux of $5 \text{ g HPr-COD m}^{-2} \text{ h}^{-1}$ ($12.5 \text{ g HPr-COD d}^{-1}$). Although the initial (maximum) permeate HPr concentration was higher in experiment 2, i.e. $6.7 \text{ g HPr-COD L}^{-1}$, lower HPr permeate flux was obtained, i.e. $1.3 \text{ g HPr-COD m}^{-2} \text{ h}^{-1}$ ($3.4 \text{ g HPr-COD d}^{-1}$), because the permeate flux was 5-fold lower, $0.2 \text{ L m}^{-2} \text{ h}^{-1}$ (0.5 L d^{-1}).

4.3.3 The EPAD_{UASB}

Figure 4.3 illustrates the performance of the EPAD_{UASB} during the two experiments regarding to the OLR, COD_S , HPr and HAc concentrations.

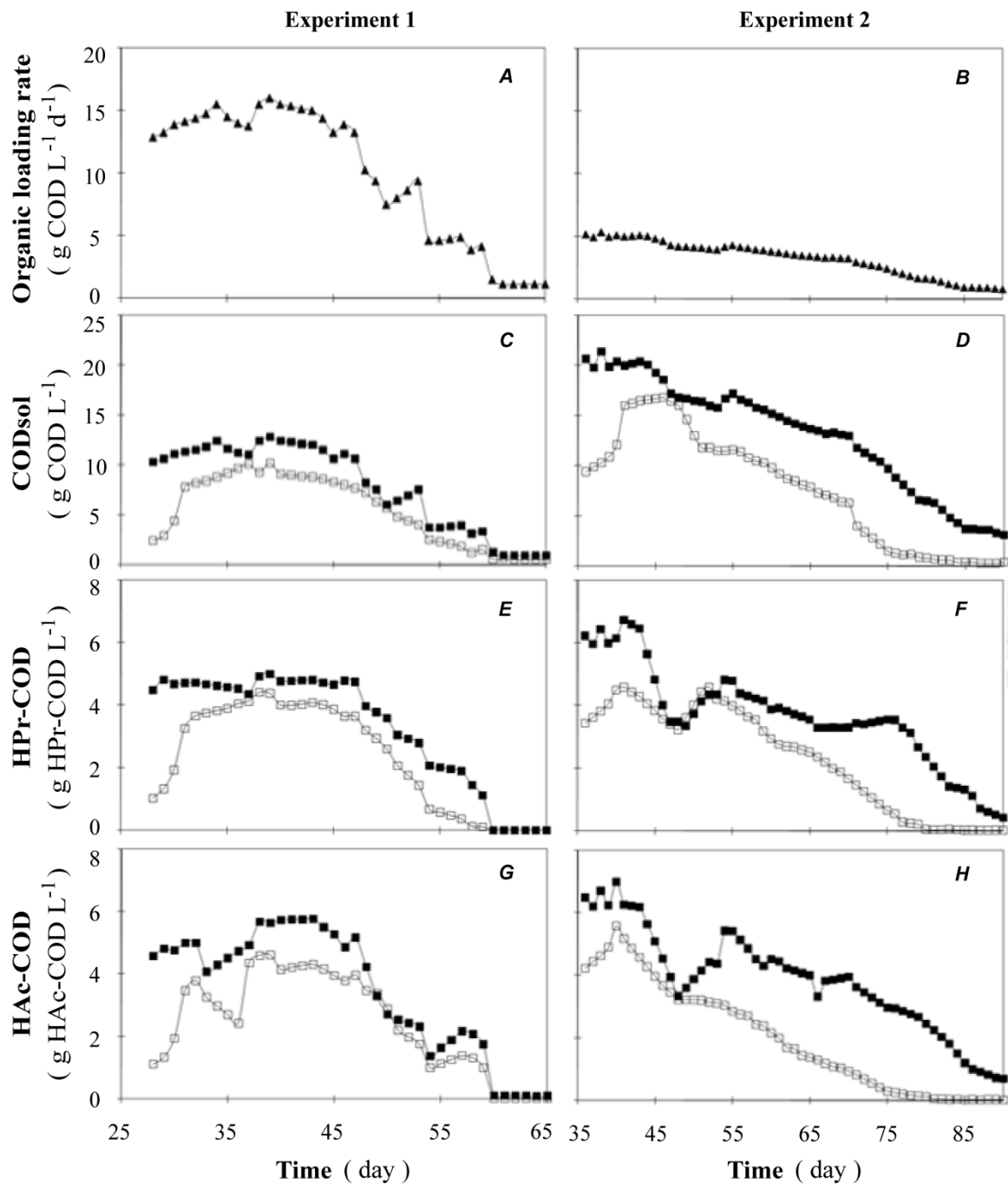


Figure 4.3 Performance of the EPAD_{UASB} during experiment 1 (A, C, E, G) and experiment 2 (B, D, F, H). (▲) OLR; (■) Influent; (□) Effluent.

In experiment 1, the maximum OLR applied to the EPAD_{UASB} during the first days of operation was $16 \text{ g COD L}^{-1} \text{ d}^{-1}$, which corresponded to a maximum permeate HPr concentration of $5 \text{ g HPr-COD L}^{-1}$. The effluent COD_S, HPr and HAc concentrations increased from 2.4 to 8.8 g COD L^{-1} , from 1.0 to $3.8 \text{ g HPr-COD L}^{-1}$ and from 1.1 to $4.0 \text{ g HAc-COD L}^{-1}$, respectively, which resulted in low removal efficiencies ($< 30\%$). From day 35 on, the effluent quality improved gradually and by the end of the experiment, the

COD_S, HPr and HAc concentrations of the effluent decreased to less than 0.5 g COD L⁻¹ (Figure 4.3). The maximum COD_S, HPr and HAc removal rates obtained during experiment 1 were 4.9 g COD L⁻¹ d⁻¹ (COD removal efficiency of 61%), 1.9 g HPr-COD L⁻¹ d⁻¹ (HPr removal efficiency of 92%) and 1.0 g HAc-COD L⁻¹ d⁻¹ (HAc removal efficiency of 75%), respectively.

In experiment 2, since the initial COD_S, HPr and HAc concentrations in the permeate/influent were very high, ca. 20.7 g COD L⁻¹, 6.7 g HPr-COD L⁻¹ and 7 g HAc-COD L⁻¹, respectively, a low influent flow-rate of 0.5 L d⁻¹ was used in order to control the OLR at below 5.5 g COD L⁻¹ d⁻¹. During the first days of phase 2 (day 35 to 46), the effluent COD_S, HPr and HAc concentrations increased up to 16.8 g COD L⁻¹, 4.5 g HPr-COD L⁻¹ and 5.6 g HAc-COD L⁻¹, respectively, resulting in low removal efficiencies (< 10%). From day 47 on, the removal of HPr improved gradually and a maximum HPr removal rate of 0.8 g HPr-COD L⁻¹ d⁻¹ was obtained on day 77, corresponding to an HPr removal efficiency of 92%. Similarly to experiment 1, low effluent COD_S and HAc concentrations (around 0.5 g COD L⁻¹) were achieved at the end of experiment 2 (Figure 4.3).

4.4 DISCUSSION

4.4.1 EPAD system effect: experiment 1 versus experiment 2

In both experiments, the aim of phase 1 was to overload the CSTRs to achieve high HPr levels, and thus giving rise to process failure. The applied OLR could be increased up to 4.2 g COD L⁻¹ d⁻¹ in 21 days in experiment 2 before process failure occurred, which was 1.5 times higher than that of experiment 1 (2.8 g COD L⁻¹ d⁻¹ in 27 days). Consequently, the COD_S, HPr and HAc concentrations at the end of phase 1 were approximately 1.5-2 times higher in experiment 2 compared to those in experiment 1. The latter indicates that, in experiment 2, both CSTRs tolerated higher HPr levels before full process failure occurred, which can be explained by the adaptation of the microorganisms to the HPr accumulation problem since experiment 2 was carried out subsequently after 65 days of experiment 1.

In experiment 1, the feeding of both reactors was continued during the first days of phase 2 (day 28-34), and the CSTR_T was simultaneously connected to the EPAD system. Although the COD_S concentrations evolved similarly in both CSTRs (increase from 12 to 18 g COD L⁻¹), a better evolution was observed in CSTR_T in terms of HPr and HAc concentrations (less increase). These observations implied that the stress imposed by the feeding surpassed the positive effect of the EPAD system. To verify if the interruption of the feeding stress could be sufficient enough for the CSTRs to recover, the feeding was

stopped at the beginning of phase 2 in experiment 2 (day 22-35), without connecting the EPAD system to the CSTR_T. During these 14 days, the COD_S, HPr and HAC concentrations in both reactors slightly increased (around 10-20%), which was due to the hydrolysis and acidification of the residual kitchen waste. These results demonstrated that the simply interruption of the feeding is not sufficient to assure the process recovery, and thus, an active auxiliary help (EPAD system) to restart the failed CSTRs is required.

The acceleration effect of the EPAD system was immediately observed in both experiments. In experiment 1, the HPr concentrations in the CSTR_T decreased to less than 0.1 g HPr-COD L⁻¹ (around 98% removal) after 30 days of connection, while those of the CSTR_C only decreased to 3.6 g HPr-COD L⁻¹ (around 32% removal). Similar results were observed during experiment 2. After 55 days of connection, low HPr levels were obtained in the CSTR_T (0.5 g HPr-COD L⁻¹) while those of the CSTR_C stayed almost constant, at around 12 g HPr-COD L⁻¹. Therefore, it can be concluded that the EPAD system significantly contributed to the recovery of the CSTR_T from the HPr accumulation problem.

Noticeable, the recovery period in experiment 2 was twice longer than in experiment 1. The latter can be explained by the lower HPr removal capacity of the EPAD system in experiment 2, due to the higher influent HPr level of the EPAD_{UASB} (6.7 g HPr-COD L⁻¹) and the lower OLR that could be applied (5.5 g COD L⁻¹ d⁻¹) compared to those of experiment 1, i.e. 5 g HPr-COD L⁻¹ and 16 g COD L⁻¹ d⁻¹, respectively.

4.4.2 HPr removal in the EPAD system

Membrane modules have been applied in anaerobic bioreactors in order to enhance the biomass retention. However, the problems related to cake formation and biofouling limits its acceptance for anaerobic digestion (Choo and Lee, 1998). In general, gas sparging (submerged membrane) and liquid cross-flow superficial velocities (side-stream configuration) are the most common strategies applied to provide surface shear, and subsequently, to control particle deposition (Jeison and Van Lier, 2007). However, both strategies imply an important energy consumption.

In this work, to avoid a complex high-suspended solids content influent entering the EPAD_{UASB}, which would likely disturb its stable performance, a microfiltration membrane was used in the EPAD_{separator} to separate the liquid and the solid fractions of the mixed liquor from the CSTR_T. A maximum permeate flux was not required, but it was tuned to the OLR to be applied in the EPAD_{UASB}. Consequently, low permeate fluxes were applied (1.0 and 0.2 L m⁻² h⁻¹ for experiments 1 and 2, respectively) compared to the common values found in literature, ranging from 5 to 50 L m⁻² h⁻¹ (Fuchs et al., 2003; Dhaouadi and

Marrot, 2008). In addition, a cycle of 15 min extrusion and 45 min relaxation was used to avoid excessive membrane fouling.

As most of the removal of HPr was accomplished in the EPAD_{UASB}, a high HPr removal rate in the EPAD_{UASB} is essential for the overall accelerated effect of the EPAD system. In literature, maximum HPr removal rates of 15-20 g HPr-COD L⁻¹ d⁻¹ operating at influent HPr concentrations of up to 17 g HPr-COD L⁻¹ were achieved under mesophilic conditions by increasing gradually the OLR (Fang et al., 1994; Tatara et al., 2008; Ma et al., 2009). In this work, the removal efficiency of the EPAD_{UASB} was low during the first days of the connection to the CSTR_T in both experiments, which indicates a “shock” of the microbial community due to the direct exposure to high initial HPr concentrations (around 4-6 HPr-COD L⁻¹). In this respect, it might be advisable for future implementation of this approach to previously adapt the microbiota of the dedicated EPAD_{UASB} to HPr degradation by gradual exposure to higher HPr levels. Moreover, the supplementation of macro- and micro-nutrients was also shown to be effective to increase the HPr removal rates (Ma et al., 2009).

4.5 UP-SCALING FEASIBILITY

When the full-scale anaerobic digester is connected to the EPAD system, a dynamical process takes place. Figure 4.4 shows the scheme of the calculations performed to estimate the up-scaling feasibility of the EPAD system.

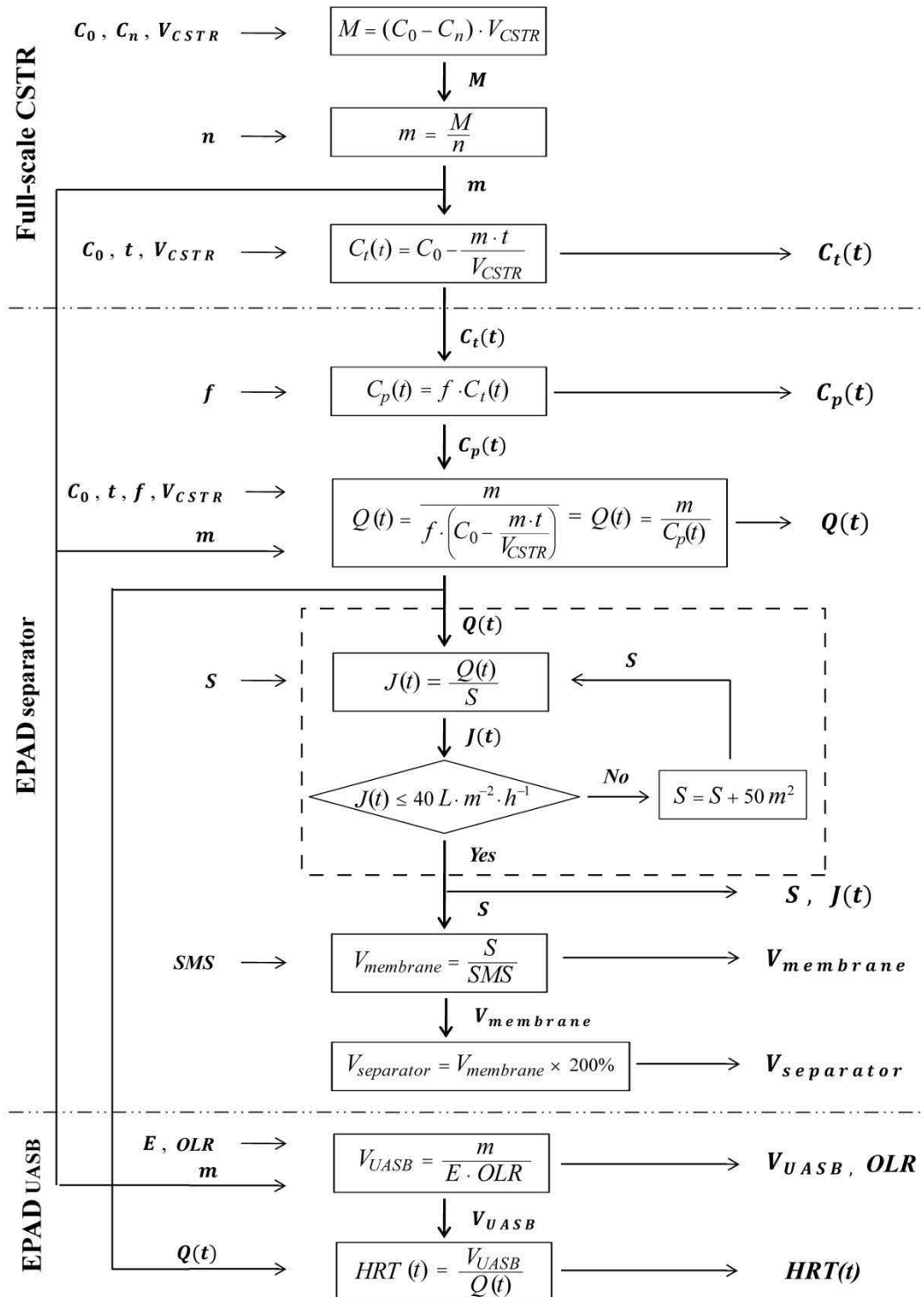


Figure 4.4 Scheme of calculations for the up-scaling feasibility of EPAD system.

In general, full-scale anaerobic digesters can have volumes up to 10,000 m³, although small digesters (< 3000 m³) also exist (Angelidaki et al., 2006). A full-scale anaerobic digester of 3000 m³ (V_{CSTR}) with a HPr accumulation level of 1.5 kg m⁻³ or 2.3 g HPr-COD L⁻¹ (C_0) is assumed. The objective of the EPAD system is to decrease the HPr levels to 0.5 kg m⁻³ or 0.8 g HPr-COD L⁻¹ (C_n) in 10 days (n). Therefore, the total amount of HPr (M) to be removed is 3000 kg HPr (4500 kg HPr-COD) and the daily HPr removal by the EPAD system (m) is 300 kg d⁻¹ (450 kg HPr-COD d⁻¹). The daily HPr concentration ($C(t)$) in the full-scale digester will decrease with time (t), i.e. 2.3, 2.2, ... 1.0, 0.8 g HPr-COD L⁻¹ on days 0, 1, ... 9, 10, respectively.

Based on the experimental results, a constant ratio (f) between the concentrations of HPr in the permeate ($C_p(t)$) and in the bulk liquid ($C(t)$) of 0.9 was considered for the EPAD_{separator}. Since the HPr concentrations in the bulk liquid ($C(t)$) decreases with time, the corresponding $C_p(t)$ also varies with time, i.e. 2.1, 2.0, ... 0.9, 0.7 g HPr-COD L⁻¹ on days 0, 1, ... 9, 10, respectively. In order to maintain a constant OLR in the EPAD_{UASB}, an increasing permeate flowrate ($Q(t)$) is required to compensate the decreasing $C_p(t)$ levels (217, 232, ... 523, 625 m³ d⁻¹ on days 0, 1, ... 9, 10, respectively). According to literature results (Fuchs et al., 2003; Dhaouadi and Marrot, 2008), a permeate flux of around 40 L m⁻² h⁻¹ results in moderate membrane operation. Taking into account that in the EPAD system the permeate flux increases with time, a membrane surface area (S) of 650 m² is selected to ensure that the maximum permeate flux does not surpass 40 L m⁻² h⁻¹.

Considering a specific membrane surface area (SMS) of 115 m² m⁻³ (De Gussemme et al., 2009) and an installation safety factor of 200%, the necessary volume of the EPAD_{separator} is about 12 m³.

Taking into account that 450 kg HPr-COD d⁻¹ must be degraded in EPAD_{UASB} (optimal operational conditions: constant OLR of 15 kg HPr-COD m⁻³ d⁻¹ and stable HPr removal efficiency (E) of 80%), the required volume for the liquid phase of the EPAD_{UASB} (V_{UASB}) is estimated in 30 m³ (total volume 35 m³). Because of the increasing influent flowrate ($Q(t)$), the HRT of the EPAD_{UASB} decreases with time, i.e. 3.1, 2.9, ... 1.3, 1.1 h on days 0, 1, ... 9, 10, respectively. Although an HRT of 1 h is rather low for UASB reactors, Ma et al. (2009) obtained a very high HPr removal rate of 32.8 g HPr-COD L⁻¹ d⁻¹ (influent HPr concentration of 0.8 g HPr-COD L⁻¹) at an extremely low HRT of 0.5 h, which illustrates that low HRT can be applied on UASB reactors by gradually microbial adaptation to the dedicated substrate.

According to the aforementioned calculations, the total volume of the EPAD system would account for 50 m³ (less than 2% of full-scale anaerobic digester volume). The latter gives the possibility to build the EPAD system as a mobile unit, which would be flexible

to be connected to different industrial anaerobic digesters suffering from HPr accumulation problems, thus avoiding the investment by one single industry.

For example, the connection of the previously designed EPAD system optimized to remove HPr at a rate of $12 \text{ kg HPr-COD m}^{-3} \text{ d}^{-1}$ to a full-scale “sick” digester of 5000 m^3 volume and HPr level of $3 \text{ kg HPr-COD m}^{-3}$ will allow the digester to recover (i.e. decrease the HPr level to $0.5 \text{ kg HPr-COD m}^{-3}$) in 1 month.

4.6 CONCLUSIONS

Interrupting the feeding of the overloaded anaerobic digester will not normally suffice to obtain rapid recovery from the HPr accumulation.

The EPAD system significantly contributed to the recovery of the CSTR_T from the HPr accumulation problem, thus allowing for the tested experimental conditions a re-established functionality in 1 to 2 months.

Membrane fouling was successfully avoided in $\text{EPAD}_{\text{separator}}$ by applying cycles of 15 min extrusion and 45 min relaxation, at low VS concentrations and low extrusion rate. The efficiency of the EPAD system will tend to increase with decreasing levels of solids in the mixed liquor.

The performance of the $\text{EPAD}_{\text{UASB}}$ is essential for an optimal functioning of EPAD system. In that way, an anaerobic consortium adapted to HPr degradation should be used in the HPr dedicated $\text{EPAD}_{\text{UASB}}$.

Theoretical calculations estimated a total volume for EPAD system of 50 m^3 , which allows its construction as a mobile unit.

APPENDIX I

APPENDIX II

CHAPTER I

CHAPTER II

CHAPTER III

CHAPTER IV

**IMPROVEMENT OF PROCESS PERFORMANCE
BY PRE-TREATMENTS**

CHAPTER V

CHAPTER VI

APPENDIX III

APPENDIX IV

APPENDIX V

APPENDIX VI

CHAPTER V

IMPROVEMENT OF PROCESS PERFORMANCE BY PRE-TREATMENTS

Abstract

Five different pre-treatment methods, i.e. acid, thermal, thermo-acid, pressure-depressure and freeze-thaw, were investigated to enhance the solubilization and biodegradability of kitchen waste (KW). In the batch solubilization test, the solubilization of different pre-treatments had as hierarchy: thermo-acid > thermo > freeze-thaw > acid = pressure-depressure. However, based on the accumulative biogas production obtained in the batch biodegradability tests, the order observed was: pressure-depressure > freeze-thaw > thermo > control > thermo-acid > acid. In the continuous tests, the performance hierarchy in terms of an acceptable biogas production efficiency of 60% was: pressure-depressure > freeze-thaw > acid > thermo-acid > thermo > control. The highest organic loading rate (OLR) (5 g COD L⁻¹ d⁻¹) was applied in the pressure-depressure and freeze-thaw reactors, almost doubled that of the control reactor (3 g COD L⁻¹ d⁻¹). From the overall analysis, also taking the economical aspects into account, the freeze-thaw pre-treatment was the most feasible process with a net potential profit of around 8.5 € ton⁻¹ KW.

Chapter redrafted after: Ma J, Duong HT, Smits M, Verstraete W and Carballa M 2009 Enhanced biomethanation of kitchen waste by different pretreatments. *Bioresource technology*, *submitted*.

5.1 INTRODUCTION

In Europe, approximately 2.5 billion tons of kitchen waste (KW) are produced yearly, which represents a tremendous potential of utilisable biomass. In the past, the disposal of KW was mostly done by composting or reutilization as animal feed (Kelley and Walker, 1999). However, the latter has been prohibited by the EU since November 2006 (Regulation (EC) No 1774/2002), and since then, most KW has been landfilled with other wastes, resulting in various environmental problems, such as odor emanation, vermin attraction, toxic gases emission, groundwater contamination and waste of valuable land (Shin et al., 2001). Moreover, considering the increasing world population and the significant urbanization in heavily populated countries, the production of KW is likely to increase and, consequently, alternative KW disposal technologies have become a major concern in the last years (Adhikari and Barrington, 2006).

The high biodegradability and water content of KW make it a good candidate for anaerobic digestion with the concomitant benefit of biogas production (Heo et al., 2004). However, due to the high organic particulate matter content, the single-phase anaerobic digestion of KW is not straightforward.

On the one hand, accumulation of intermediary compounds occurs easily, giving rise to an unbalanced fermentation, and consequently, diminishing the stability of the process (Ince, 1998). On the other hand, the soluble organics are converted rapidly to volatile fatty acids (VFAs) at an early stage of the digestion process (Cho et al., 1995), resulting in a drastic pH drop if no sufficient buffering capacity is present (Veeken et al., 2000). In addition, KW has a high protein and fat content which can lead to inhibitory levels of ammonia, sulphide and long chain fatty acids (Braun et al., 2003; Amaral et al., 2004). As a result, anaerobic treatment of KW is often performed at low organic loading rates (OLR) of 2-3 g COD L⁻¹ d⁻¹ to prevent process failure (Hecht and Griehl, 2009), and the biogas yields are usually low, around 50-60% (Banks et al., 2008).

In general, liquefaction (solubilization) and hydrolysis of solids are considered the rate-limiting steps during anaerobic digestion of solid wastes, and their intensification usually leads to a better digestion performance (Shin et al., 2001). By cell disruption, the intracellular and cell wall polymers, including polysaccharides, proteins, lipids, and other macromolecules, are released into the surrounding medium (Bien et al., 2004), becoming more available to microorganisms. Different disintegration methods, such as mechanical (e.g. sonication), chemical (e.g. alkali or acid), osmotic (e.g. NaCl treatment, freezing), oxidative (e.g. ozone), thermal and biological (e.g. enzyme) have been proposed to improve the anaerobic digestion of sewage sludge and the organic fraction of municipal solid waste (OFMSW) (Mata-Alvarez et al., 2000; Kim et al., 2006; Carballa et al., 2009).

However, few studies are available on the effect of these pre-treatment methods on the anaerobic digestion of KW.

The aim of this study was to evaluate the effect of different pre-treatment methods on the anaerobic digestion of KW, in terms of the solubilization efficiency, biodegradability and biomethanation potential. Besides, the practical feasibility of the selected pre-treatment methods was economically evaluated.

5.2 MATERIALS AND METHODS

5.2.1 Kitchen waste and sewage

The KW was provided by a company collecting and treating the organic fraction of industrial kitchens (Trans Vanheede Environment Group, Belgium) and its composition (vegetable, meat, pasta, potato, fish, etc.) varied depending on the deliveries. Table 5.1 shows the characterization of the KW and sewage, such as the total and soluble chemical oxygen demand (COD) (COD_T and COD_S), total solid (TS) and volatile solid (VS), etc. After each delivery, the KW was mixed with a kitchen blender and stored in the fridge (4°C). In order to achieve the required OLR, KW was diluted with sewage coming from a domestic wastewater treatment plant (WWPT) (Ossemeersen, Belgium).

Table 5.1 Main characteristics (average values with standard deviation) of the KW (n=10) and sewage (n=10) used to prepare the feeding of the reactors.

Parameter	KW (n=10) (g kg ⁻¹)	Sewage (n=10) (mg L ⁻¹)
pH	3.8 ± 0.2	6.8 ± 0.2
COD_T	238 ± 40	350 ± 30
COD_S	75 ± 7	80 ± 10
TS	166 ± 14	260 ± 20
VS	155 ± 13	235 ± 10

5.2.2 Pre-treatment methods

All pre-treatment methods were performed just before preparing the feedings for the reactors, which were stored in the fridge at 4°C for maximum 2 weeks during the continuous experiments. All pre-treatments were applied to the raw KW (without dilution with sewage), except the pressure-depressure method, for which dilution was first performed.

5.2.2.1 Acid pre-treatment

Of the raw mixed KW, 1 kg was acidified with HCl (10 N) at room temperature ($18 \pm 2^\circ\text{C}$) until pH 2, checking this value after 24 h of contact time. During the pre-treatment, the KW was continuously mixed for the well distribution of the HCl and the pH was measured at different spots in the container.

5.2.2.2 Thermal pre-treatment

Of the raw mixed KW, 1 kg was autoclaved (Manufacture Belge de Gembloux, Belgium) at 120°C (1 bar) with the following operational cycle: 30 min pre-heating to 120°C + 30 min autoclaving at 120°C + 30 min cooling to room temperature ($18 \pm 2^\circ\text{C}$).

5.2.2.3 Thermo-acid pre-treatment

Of the raw mixed KW, 1 kg was firstly acidified by HCl (10 N) until pH 2 as described in the acid pre-treatment. After 24 h of contact time, the acidified KW was autoclaved at 120°C as described in thermal pre-treatment.

5.2.2.4 Pressure-depressure pre-treatment

A 5 L pressurization reactor (diameter of 16 cm and height of 25 cm) was used. Previous experiments carried out to determine the optimal operational conditions of the pressurization reactor showed that lower initial TS content in the KW and quick depressurization resulted in higher solubilization percentages (data not shown). Consequently, 1 L of mixed KW (previously diluted according to the OLR applied) was pressurized to 10 bar with CO_2 as pressurizing gas. After few minutes of contact time, the depressurization of the reactor to ambient pressure (1 bar) was performed by quickly releasing the CO_2 gas.

5.2.2.5 Freeze-thaw pre-treatment

Of the raw mixed KW, 150 g was frozen to -80°C in an ultra low temperature freezer (New Brunswick Scientific, USA). After 6 h, the frozen KW was thawed in a thermal oven at $55 \pm 2^\circ\text{C}$ (Mettler, Germany) for 30 minutes.

5.2.3 Solubilization efficiency

Samples were taken before and after pre-treatments to evaluate the solubilization effects. The COD_T and COD_S , and TS and VS were the parameters analyzed.

5.2.4 Batch anaerobic biodegradability tests

Seven 1.2 L Erlenmeyers were used as reactors for the anaerobic biodegradability tests, defined according to the different feedings: R_{control} , fed with raw KW; R_{acid} , fed with acidified KW; R_{thermal} , fed with thermally pre-treated KW; $R_{\text{thermo-acid}}$, fed with thermo-acidified KW; $R_{\text{pressure-depressure}}$, fed with pressure-depressure pre-treated KW and $R_{\text{freeze-thaw}}$, fed with freeze-thawed KW.

The reactors were inoculated with 500 mL thermophilic sludge harvested from a pilot-scale thermophilic reactor treating potato wastes ($55.8 \pm 8.4 \text{ g TS L}^{-1}$ and $30.0 \pm 5.6 \text{ g VS L}^{-1}$). The non-pre-treated and pre-treated KWs were added into each reactor according to a $VS_{\text{KW}}/VS_{\text{inoculum}}$ ratio of 1 and the reactors were filled up to 1 L with sewage. 3 g NaHCO_3 was added into each reactor to provide sufficient buffer capacity and the initial pH of the reactors was adjusted to around 7.5 with NaOH (10 N).

The reactors were operated under thermophilic conditions ($55 \pm 2^\circ\text{C}$) in a thermal oven (Memmert, Germany). Biogas production was monitored daily and samples from the mixed liquor were taken 3 times a week for pH, COD_T , COD_S and VFAs analyses.

5.2.5 Continuous anaerobic tests

Continuous tests in thermophilic range ($55 \pm 2^\circ\text{C}$) were performed subsequently after the batch anaerobic biodegradability tests. The pH and the temperature of the feeding were adjusted to 7.5 with NaOH (10 N) and 55°C in the oven, respectively, before feeding the reactors.

The initial OLR applied was $0.5 \text{ g COD L}^{-1} \text{ d}^{-1}$ in the R_{control} , R_{acid} , R_{thermal} and $R_{\text{thermo-acid}}$ and $1.5 \text{ g COD L}^{-1} \text{ d}^{-1}$ in the $R_{\text{pressure-depressure}}$ and $R_{\text{freeze-thaw}}$, and it was stepwisely increased by increasing the concentration of KW in the feed. The initial hydraulic retention time (HRT) was kept constant at 18 days and it was doubled to 36 days when the OLR reached $4 \text{ g COD L}^{-1} \text{ d}^{-1}$.

Biogas production was monitored daily and samples were taken from the supernatant of the reactors (after 30 min of sedimentation to enhance the biomass retention in the reactors) twice a week for pH, COD_S and VFAs determinations. The pH of the reactors was adjusted to around 7.5 with NaOH (10 N) if the pH of below 7.0 was observed.

5.2.6 Analytical techniques

VFAs were extracted with diethyl ether and their quantitative analysis was determined by a capillary gas chromatograph (CE Instruments, Italy) coupled with a flame ionization detector. The pH values were measured with a C532 pH meter (Consort,

Belgium) and the other physico-chemical parameters, COD_T and COD_S, TS and VS, were determined according to the standard methods (Greenberg et al, 1992). Biogas production was followed by liquid displacement.

5.2.7 Cost and benefit analysis

5.2.7.1 Net profits

The net profits (EUR_{net-profit}: € ton⁻¹ KW) were calculated as the difference between the extra benefit from the extra biogas production enhanced by the pre-treatments (EUR_{total-extra-benefit}: € ton⁻¹ KW) and the extra costs associated with the pre-treatments (EUR_{extra-cost}: € ton⁻¹ KW).

5.2.7.2 Extra benefits

The extra benefits from the pre-treatments result from the extra biogas production achieved with the pre-treated KW, compared to the raw KW. The biogas produced from anaerobic digestion is usually converted on-site by a combined heat and power generator (CHP) to produce energy, of which about 40% and 50% of green electricity and useable heat can be obtained. Therefore, the extra benefits can be calculated as:

$$EUR_{total-extra-benefit} = EUR_{benefit-green-electricity} + EUR_{benefit-thermal} \quad \text{Eq. 5.1}$$

where: EUR_{benefit-green-electricity}: extra benefit gained from green electricity production;
EUR_{benefit-thermal}: extra benefit gained from thermal energy production.

$$EUR_{benefit-green-electricity} = E_{biogas} \times V_{biogas} \times f_{green-electricity} \times EUR_{kWh-green-electricity} \quad \text{Eq. 5.2}$$

where: E_{biogas}: energy content of biogas with 65% of CH₄ content: 6.5 kWh_{total} m⁻³ (Verstraete et al., 2009);
V_{biogas}: extra biogas production due to pre-treatment, m³;
f_{green-electricity}: energy conversion yield factor for green electricity: 40% (Walla and Schneeberger, 2008; Pertl et al., 2010);
EUR_{kWh-green-electricity}: green electricity selling price: 0.15 € kWh_{green-electricity}⁻¹ (Karpenstein-Machan, 2001; Walla and Schneeberger, 2008; Munster and Lund, 2009).

$$EUR_{benefit-thermal} = E_{biogas} \times V_{biogas} \times f_{thermal} \times EUR_{kWh-thermal} \quad \text{Eq. 5.3}$$

where: f_{thermal}: energy conversion yield factor for thermal energy: 50% (Walla and Schneeberger, 2008; Pertl et al., 2010);
EUR_{kWh-thermal}: thermal energy selling price: 0.03 € kWh_{thermal}⁻¹ (Europe's energy portal, 2010).

5.2.7.3 Extra costs

Acid pre-treatment

The extra costs of the acid pre-treatment are due to the required HCl for the KW pre-treatment and the NaOH for the neutralization of the pre-treated KW. Considering the technical product prices for HCl and NaOH of 0.1 € L⁻¹ and 0.4 € kg⁻¹ (VWR, Belgium), respectively, and taking into account that their required amounts were 10 L HCl ton⁻¹ KW and 4.8 kg NaOH ton⁻¹ KW (data not shown), the extra costs of acid pre-treatment are approximately 2.9 € ton⁻¹ KW.

Thermal pre-treatment

Assuming no heat loss or water evaporation, the energy requirement of 110 kWh_{thermal} ton⁻¹ KW for the thermal pre-treatment can be calculated as (Daniel, 1998):

$$E_{\text{thermal}} = C_{\text{KW}} \times M_{\text{KW}} \times \Delta T + C_{\text{water}} \times M_{\text{water}} \times \Delta T \quad \text{Eq. 5.4}$$

where: C_{KW} : specific heat capacity of dry KW, 1.92 kJ kg⁻¹ °C⁻¹;
 M_{KW} : dry mass of KW, taking TS value of 166 kg ton⁻¹ KW;
 C_{water} : specific heat capacity of water, 4.18 kJ kg⁻¹ °C⁻¹;
 M_{water} : mass of water in KW, 834 kg ton⁻¹ KW;
 ΔT : temperature increase, 105°C (from 25 to 120°C).

If using gas to provide this thermal energy, the extra costs of the thermal pre-treatment are approximately 6.6 € ton⁻¹ KW (0.03 € kWh_{thermal}⁻¹), taking into account of the utilization efficiency (50%); if considering the electrical energy, the extra cost is about 15.9 € ton⁻¹ KW (industrial electricity price: 0.13 € kWh_{electricity}⁻¹ (Europe's energy portal, 2010)), taking into account of the utilization efficiency (90%). Thus the lower cost from thermal energy is taken for this study, i.e. 6.6 € ton⁻¹ KW.

Thermo-acid pre-treatment

The extra costs of the thermo-acid pre-treatment can be estimated by the sum of those of the acid and thermal pre-treatments, i.e. 9.5 € ton⁻¹ KW.

Pressure-depressure pre-treatment

The extra costs of pressure-depressure pre-treatment are estimated by the energy demand for the compression of certain amount of gas from the initial pressure to the required pressure. According to ideal gas equation,

$$P_A \times V_A = P_B \times V_B \quad \text{Eq. 5.5}$$

where: V_A : initial gas volume, m^3 ;
 V_B : ending gas volume, m^3 ;
 P_A : initial gas pressure, bar;
 P_B : ending gas pressure, bar;

In this study, the pressure was increased from 1 to 10 bar,

$$V_A = 10V_B \quad \text{Eq. 5.6}$$

Assuming a compressor with an active/head space volume ratio of 1:1, the required initial gas volume is $10 \text{ m}^3 \text{ kg}^{-1} \text{ KW}$ (KW density: 1 kg m^{-3}).

Assuming the adiabatic compression (no heat exchange), the energy requirement can be calculated as (Perry et al., 1997):

$$E_{\text{pressure}} = \int_{V_A}^{V_B} \left(P_A + \left(\frac{P_B - P_A}{V_B - V_A} \right) \times (V - V_A) \right) dV \quad \text{Eq. 5.7}$$

Taking the values: V_A of $10 \text{ m}^3 \text{ kg}^{-1} \text{ KW}$ at P_A of 1 bar, V_B of $1 \text{ m}^3 \text{ kg}^{-1} \text{ KW}$ at P_B of 10 bar, the required energy input is $1400 \text{ kWh ton}^{-1} \text{ KW}$. Assuming the compressor efficiency of 80%, the required energy input is around $1750 \text{ kWh ton}^{-1} \text{ KW}$. With the industrial price of $0.13 \text{ € kWh}_{\text{electricity}}^{-1}$, the extra cost of the pressure-depressure pre-treatment is about $227.5 \text{ € ton}^{-1} \text{ KW}$.

Freeze-thaw pre-treatment

The costs for freeze-thaw pre-treatment result from the energy consumption of the freezer, which can be calculated as follows (Daniel, 1998):

$$E_{\text{freezing}} = C_{\text{KW}} \times M_{\text{KW}} \times \Delta T_1 + L_{\text{water}} \times M_{\text{water}} + C_{\text{water}} \times M_{\text{water}} \times \Delta T_2 + C_{\text{ice}} \times M_{\text{ice}} \times \Delta T_3 \quad \text{Eq. 5.8}$$

where: L_{water} : latent heat of fusion of water, 335 kJ kg^{-1} ;
 C_{ice} : specific heat capacity of ice, $2.09 \text{ kJ kg}^{-1} \text{ °C}^{-1}$;
 M_{ice} : mass of ice in KW, $834 \text{ kg ton}^{-1} \text{ KW}$;
 ΔT_1 : temperature decrease of KW, 45 °C (from 25 to -20 °C);
 ΔT_2 : temperature decrease of water, 25 °C (from 25 to 0 °C);
 ΔT_3 : temperature decrease of ice, 20 °C (from 0 to -20 °C).

Taking into account the industrial electricity price of $0.07 \text{ € kWh}_{\text{electricity}}^{-1}$ and the utilization efficiency of the industrial freezer of 90%, the extra costs of freeze-thaw pre-treatment are around $18.8 \text{ € ton}^{-1} \text{ KW}$ (we assume that the energy to thaw the KW comes from the ambient environment).

5.3 RESULTS

5.3.1 Solubilization efficiency

Table 5.2 shows the effect of different pre-treatments on the KW characteristics. After the pre-treatments, the pH of the KW remained at around 4, except for the acid and thermo-acid pre-treatments, after which the pH was about 2.

Table 5.2 Summary of the results obtained during the solubilization tests (n=2).

Pre-treatment	pH	TS (g kg ⁻¹)	VS (g kg ⁻¹)	COD _T (g kg ⁻¹)	COD _S (g kg ⁻¹)	COD _S /COD _T (%)	*Sol. (%)
Control	3.8±0.2	166±14	155±13	238±40	75±7	32±8	--
Acid	2.0±0.3	170±23	155±23	247±43	86±9	35±12	13±7
Thermal	3.8±0.3	175±29	162±25	237±35	93±8	39±9	19±3
Thermo-acid	2.0±0.3	181±25	165±21	246±40	111±8	45±9	32±8
Pressure	3.8±0.2	178±19	162±18	244±20	85±14	35±5	12±7
Freeze-thaw	3.8±0.2	159±27	148±29	228±21	89±4	39±5	16±4

*Sol.: solubilization percentage calculated as the COD_S difference (between the COD_S after pre-treatment and the COD_S before pre-treatment) divided by the COD_S after pre-treatment.

In each pre-treatment, both the COD_S/COD_T ratio and the solubilization percentage increased compared to that of the control (KW without pre-treatment). The highest solubilization was achieved with the thermo-acid pre-treatment (32%), and the lower values (12%) were obtained with the acid and the pressure-depressure pre-treatments.

5.3.2 Batch anaerobic biodegradability tests

Table 5.3 summarizes the results obtained during the batch biodegradability tests and Figure 5.1 illustrates the accumulated biogas production during this test.

Table 5.3 Summary of the results obtained during the batch biodegradability tests.

Reactor	*Days	COD _T (g COD L ⁻¹)			Accumulated biogas production	
		Initial	Final	Removed	(L L ⁻¹)	(L g ⁻¹ COD _T removed)
R _{control}	36	38.2	19.4	18.8	6.5	0.35
R _{acid}	26	38.5	24.4	14.1	2.2	0.16
R _{thermal}	36	42.5	22.5	20.0	7.2	0.36
R _{thermo-acid}	36	44.4	18.4	26.0	7.7	0.30
R _{pressure}	20	39.9	23.0	16.9	8.8	0.52
R _{freeze-thaw}	30	34.1	21.0	13.1	8.3	0.38

*Total duration until 3 subsequent days without additional biogas production.

The highest cumulative biogas production was obtained in the R_{pressure-depressure}, ca. 8.8 L L⁻¹, corresponding to 0.52 L g⁻¹ COD_T removed, while the lowest production, ca. 2.2 L

L^{-1} ($0.16 L g^{-1} COD_T$ removed), was observed in the R_{acid} . Similar biogas yields were obtained in the $R_{control}$, $R_{thermal}$ and $R_{freeze-thaw}$ (around $0.35-0.38 L g^{-1} COD_T$ removed), while a slightly lower value was achieved in the $R_{thermo-acid}$ ($0.30 L g^{-1} COD_T$ removed). The pH of all the reactors during the tests was around 7.5-7.9.

The biogas production in the $R_{control}$, $R_{thermal}$ and $R_{thermo-acid}$ evolved similarly, starting to decrease from day 20 on and reaching the plateau after 35 days (Figure 5.1). In contrast, $R_{pressure-depressure}$ and $R_{freeze-thaw}$ reached the maximum biogas production faster, after approximately 15 and 20 days, respectively. The accumulated biogas production in the R_{acid} was only 34% of that observed in the $R_{control}$, reaching this value after 25 days of experiment.

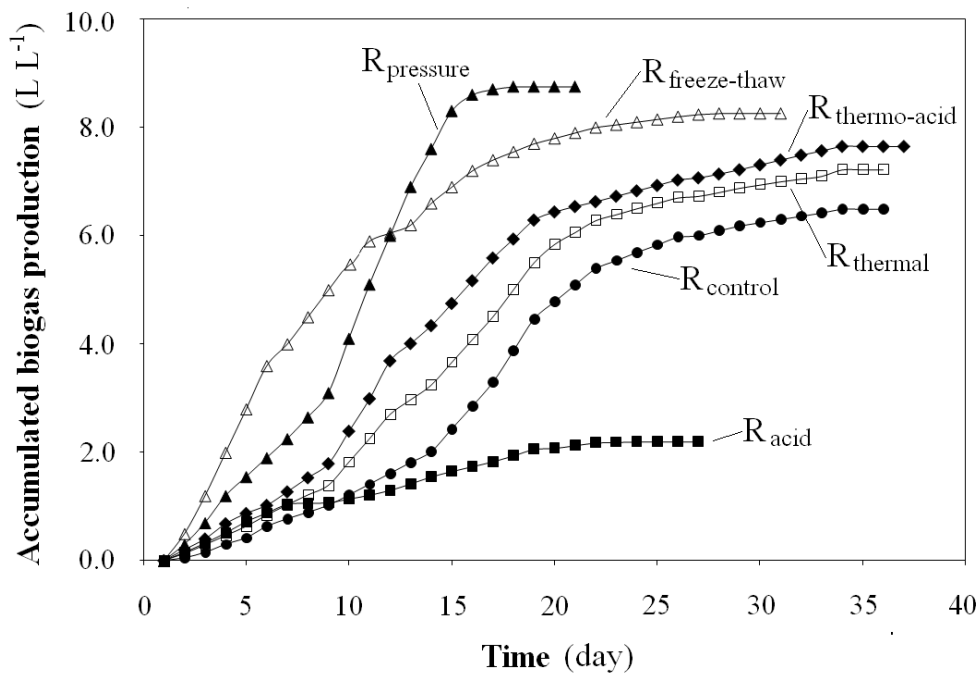


Figure 5.1 Cumulative biogas production during the batch biodegradability tests.

5.3.3 Continuous anaerobic tests

Figure 5.2 presents the performance of each reactor during the continuous anaerobic tests in terms of OLR applied, residual COD_S concentration and biogas production rate.

In the $R_{control}$ (Figure 5.2A), the OLR was gradually increased from 0.5 to $3.0 g COD L^{-1} d^{-1}$ during days 0-79, and consequently the biogas production rate rose to around $0.9 L L^{-1} d^{-1}$, corresponding to an average yield of 55% of the theoretical value. The residual COD_S and propionic acid (HPr) concentrations remained around $3 g COD L^{-1}$ and below $0.5 g HPr-COD L^{-1}$, respectively. During days 80-100, the residual COD_S and HPr concentrations increased sharply to $15.8 g COD L^{-1}$ and $2.3 g HPr-COD L^{-1}$, respectively, and the biogas production rate decreased to $0.4 L L^{-1} d^{-1}$ (26% of theoretical value) as a

response to the further increase of the OLR to $4.0 \text{ g COD L}^{-1} \text{ d}^{-1}$. Therefore, from day 101 on, the HRT was increased to 36 days. However, the residual COD_s and HPr concentrations continued accumulating to $35.7 \text{ g COD L}^{-1}$ and $2.9 \text{ g HPr-COD L}^{-1}$, respectively, for the last 10 days and no biogas production was observed. The reactor was stopped on day 111.

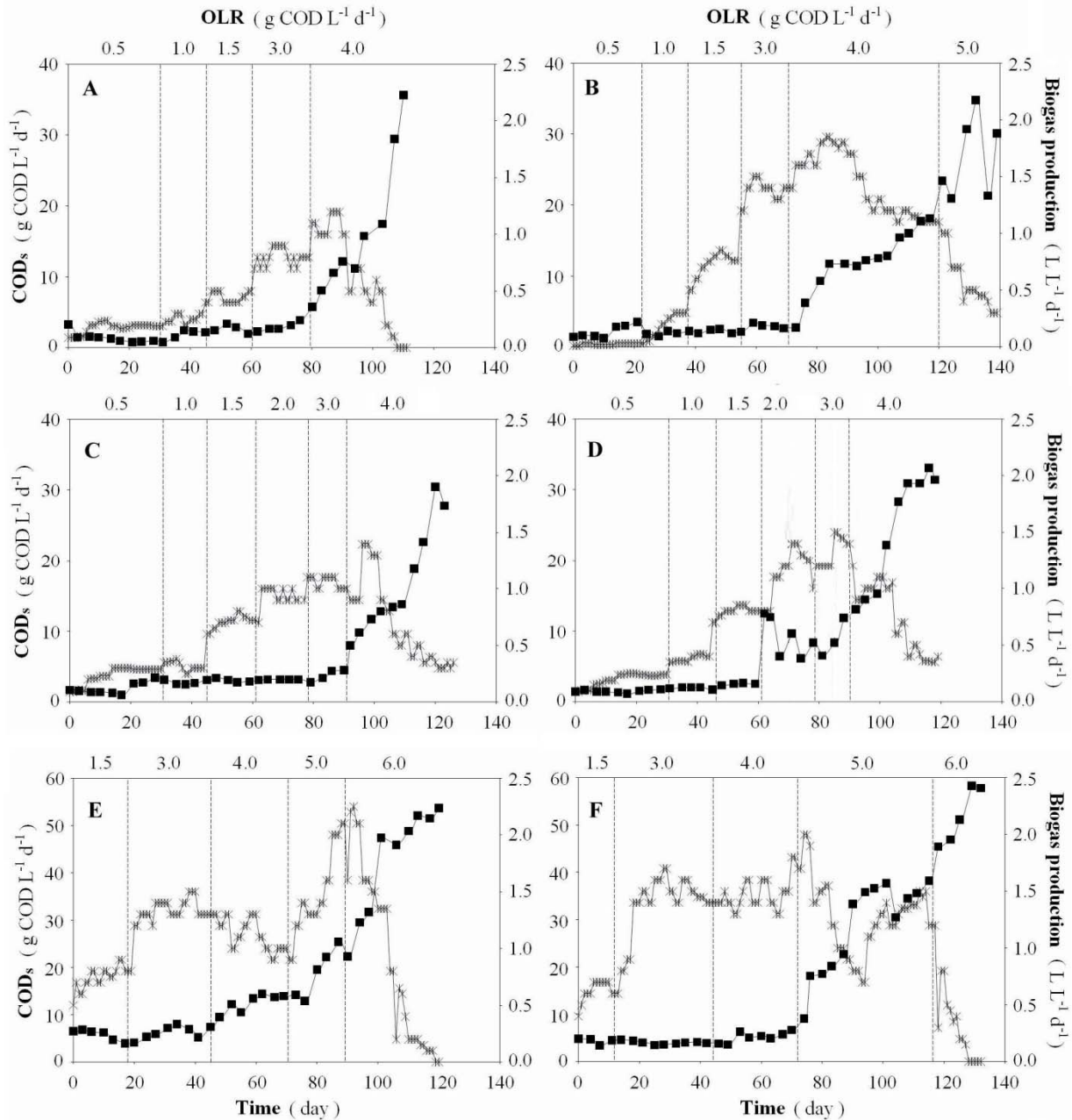


Figure 5.2 Reactors performance during the anaerobic continuous tests in terms of the OLR applied, residual COD_s (■) and biogas production rate (X). (A) R_{control} , the control; (B) R_{acid} , acidification pre-treatment; (C) R_{thermal} , thermal pre-treatment; (D) $R_{\text{thermo-acid}}$, acidification-thermal pre-treatment; (E) R_{pressure} , pressure-depressure pre-treatment and (F) $R_{\text{freeze-thaw}}$, freeze-thaw pre-treatment.

During the first days of the R_{acid} operation (OLR of $0.5 \text{ g COD L}^{-1} \text{ d}^{-1}$), a neglectable biogas production rate (around $0.02 \text{ L L}^{-1} \text{ d}^{-1}$) was observed (Figure 5.2B), corresponding to only 10% of the theoretical value, and the residual COD_S concentration increased to 3.5 g COD L^{-1} . During days 24-72, when the OLR gradually increased up to $3.0 \text{ g COD L}^{-1} \text{ d}^{-1}$, the biogas production rate increased to $1.4 \text{ L L}^{-1} \text{ d}^{-1}$ (95% of theoretical value), and the COD_S and HPr concentrations stabilized at around $2.5\text{-}3.0 \text{ g COD L}^{-1}$ and below $0.5 \text{ g HPr-COD L}^{-1}$, respectively. Therefore, the OLR was further increased to $4.0 \text{ g COD L}^{-1} \text{ d}^{-1}$ on day 73, and as a response, the biogas production rate reached the peak of $1.9 \text{ L L}^{-1} \text{ d}^{-1}$ (89% of theoretical value) on day 84. However, during the next 20 days, the COD_S and HPr concentrations increased sharply to $12.8 \text{ g COD L}^{-1}$ and $2.6 \text{ g HPr-COD L}^{-1}$, respectively, and the biogas production rate decreased to $1.2 \text{ L L}^{-1} \text{ d}^{-1}$ (60% of theoretical value) by day 104. Thereafter, the HRT was increased to 36 days, which resulted in stable COD_S and HPr concentrations and biogas production rate of around $17.5 \text{ g COD L}^{-1}$, $3.8 \text{ g HPr-COD L}^{-1}$ and $1.1 \text{ L L}^{-1} \text{ d}^{-1}$ (58% of theoretical value), respectively. From day 121 on, the OLR was further increased to $5 \text{ g COD L}^{-1} \text{ d}^{-1}$, and the COD_S and HPr concentrations increased again to around $34.5 \text{ g COD L}^{-1}$ and $3.6 \text{ g HPr-COD L}^{-1}$, respectively, and the biogas production rate dropped to $0.3 \text{ L L}^{-1} \text{ d}^{-1}$, which accounted for only 13% of the theoretical value. The reactor was stopped on day 139.

In the $R_{thermal}$ (Figure 5.2C), the biogas production rate increased to around $1.0 \text{ L L}^{-1} \text{ d}^{-1}$ (90% of theoretical value) with the gradual increase of the OLR up to $2 \text{ g COD L}^{-1} \text{ d}^{-1}$, and the COD_S and HPr concentrations increased and stabilized at around 3.2 g COD L^{-1} and $0.4 \text{ g HPr-COD L}^{-1}$, respectively. On day 78, the OLR was increased to $3.0 \text{ g COD L}^{-1} \text{ d}^{-1}$, resulting in higher COD_S and HPr concentrations of 4.5 g COD L^{-1} and $1.1 \text{ g HPr-COD L}^{-1}$, respectively, but the biogas production rate remained at $1.1 \text{ L L}^{-1} \text{ d}^{-1}$ (70% of theoretical value). Although the HRT was increased to 36 days from day 92 on, the increase of the OLR to $4 \text{ g COD L}^{-1} \text{ d}^{-1}$ resulted in higher COD_S and HPr concentrations, ca. $30.5 \text{ g COD L}^{-1}$ and $2.5 \text{ g HPr-COD L}^{-1}$, respectively, and lower biogas production rates of $0.3 \text{ L L}^{-1} \text{ d}^{-1}$ (15% of theoretical value). The reactor was stopped on day 126.

The OLR was gradually increased up to $3.0 \text{ g COD L}^{-1} \text{ d}^{-1}$ in the $R_{thermo-acid}$ during days 0-92 (Figure 5.2D), and the residual COD_S and HPr concentrations and the biogas production rate gradually increased to around 6.4 g COD L^{-1} , $1.2 \text{ g HPr-COD L}^{-1}$ and $1.2 \text{ L L}^{-1} \text{ d}^{-1}$ (80% of theoretical value), respectively. From day 93 on, the OLR was increased to $4.0 \text{ g COD L}^{-1} \text{ d}^{-1}$, which resulted in higher COD_S and HPr concentrations of $33.1 \text{ g COD L}^{-1}$ and $3.4 \text{ g HPr-COD L}^{-1}$, respectively, and lower biogas production rates of $0.4 \text{ L L}^{-1} \text{ d}^{-1}$ (20% of theoretical value). The increase of the HRT to 36 days on day 93 did not have any effect on the reactor performance. The reactor was stopped on day 120.

During the first days (0-44) of operation of the $R_{\text{pressure-depressure}}$ at OLR of 1.5 and 3 g COD L⁻¹ d⁻¹, the COD_S and HPr concentrations remained below 8 g COD L⁻¹ and 1.2 g HPr-COD L⁻¹, respectively, and the biogas production rate increased up to 1.4 L L⁻¹ d⁻¹ (Figure 5.2E). During days 45-72 (OLR of 4 g COD L⁻¹ d⁻¹), the biogas production rate decreased to around 1.0 L L⁻¹ d⁻¹ (50% of theoretical value) and the COD_S concentration increased to around 14 g COD L⁻¹, despite the HRT was increased to 36 days on day 45. The COD_S and HPr concentrations continued accumulating up to 25 g COD L⁻¹ and 3.3 g HPr-COD L⁻¹, respectively, when the OLR was increased to 5 g COD L⁻¹ day⁻¹ on day 73, but the biogas production rate also rose to 2.0 L L⁻¹ d⁻¹ (80% of theoretical value). The highest OLR applied was 6 g COD L⁻¹ d⁻¹, which resulted in a sharp increase of the COD_S and HPr concentrations to 53.7 g COD L⁻¹ and 5.8 g HPr-COD L⁻¹, respectively, by the end of the experiment (day 120), and the biogas production decreased to negligible values.

The operation of the $R_{\text{freeze-thaw}}$ was quite satisfactory up to an OLR of 4 g COD L⁻¹ d⁻¹, since the COD_S and HPr concentrations remained below 7.0 g COD L⁻¹ and 1.0 g HPr-COD L⁻¹, respectively, and a high biogas production rate was reached, ca. 1.7 L L⁻¹ d⁻¹ (85% of theoretical value) (Figure 5.2F). However, the increase of the OLR to 5 g COD L⁻¹ d⁻¹ resulted in higher values of the residual COD_S (around 40 g COD L⁻¹) and lower biogas production rates, ca. 1.4 L L⁻¹ d⁻¹. When the OLR went up to 6 g COD L⁻¹ d⁻¹, the COD_S and HPr concentrations increased sharply to almost 60 g COD L⁻¹ and 8 g HPr-COD L⁻¹, respectively, and the biogas production stopped immediately. The reactor was interrupted on day 133.

5.4 DISCUSSION

The results from the solubilization and batch biodegradability tests showed that there has no direct correlation between the solubilization effect of the pre-treatment and the enhancement of the KW biodegradability. The highest solubilization effect (expressed as solubilization percentage) was observed with the thermo-acid pre-treatment (32%), followed by the thermo, freeze-thaw, acid = pressure-depressure methods. However, the highest cumulative biogas production (expressed by L g⁻¹ COD_T removed) was observed with the pressure-depressure pre-treatment (0.52 L g⁻¹ COD_T removed) followed by freeze-thaw, thermo, control, thermo-acid and acid methods. Therefore, the highest accumulative biogas production was observed in the less effective pre-treatment in terms of solubilization (pressure-depressure) and, conversely, the highest solubilization pre-treatment (thermo-acid) resulted in less biogas production than the control. In both the solubilization and batch biodegradability tests, the acid pre-treatment showed the poorest performance.

This discrepancy between the solubilization effect and the biodegradability is probably explained by the formation of inhibitory or refractory compounds during the pre-treatments, as previously described by Carballa et al. (2006). During acid pre-treatment, the possible formation of several types of inhibitors at low pH, such as carboxylic acids, furans and phenolic compounds, has been reported by Taherzadeh and Karimi (2007). These undesirable by-products inhibit the fermentation process and can invoke less biogas production (Taherzadeh and Karimi, 2008).

The good reactor performance during the continuous anaerobic test was evaluated based on an acceptable biogas production efficiency (60% of theoretical value) and stable in-reactor COD_S and VFA concentrations. In this sense, the optimal OLR applied depended on the type of pre-treatment, i.e. 3 g COD L⁻¹ d⁻¹ in the R_{control}, R_{thermo} and R_{thermo-acid}, 4 g COD L⁻¹ d⁻¹ in the R_{acid} and, 5 g COD L⁻¹ d⁻¹ in the R_{pressure-depressure} and R_{freeze-thaw}. The R_{control} gave the poorest reactor performance among all reactors (0.8 L L⁻¹ d⁻¹; 53%), followed by the R_{thermo} and R_{thermo-acid}, in which higher biogas production rates (around 1.2 L L⁻¹ d⁻¹, 73-80% of theoretical value) were achieved at the same OLR (3 g COD L⁻¹ d⁻¹). These three reactors failed at the OLR of 4 g COD L⁻¹ d⁻¹, with the difference that the biogas production in the R_{control} ceased within 10 days, while those of the R_{thermo} and R_{thermo-acid} gradually decreased to around 0.4 L L⁻¹ d⁻¹, still accounting for 20% of the theoretical value.

The best performance of the R_{acid} was observed at OLR of 4 g COD L⁻¹ d⁻¹ with an average biogas production rate of 1.4 L L⁻¹ d⁻¹ (75% of theoretical value). Deterioration of this reactor was observed at OLR of 5 g COD L⁻¹ d⁻¹, although the biogas production did not cease completely. During the continuous operation of the R_{acid}, no clear inhibition effect from the acid pre-treatment was observed as in the solubilization and batch biodegradability tests. The reason was probably the lower levels of the possible inhibitory by-products present in the feeding. In the batch biodegradability test, the initial COD_T input of the KW (24 g COD_T) was about 50 times higher than the daily feeding during the continuous anaerobic test (from 0.5 to 4 g COD_T). Therefore, assuming that the same percentage of the possible inhibitory by-products was formed during the acid pre-treatment, their levels during the continuous anaerobic test should have been 50 times lower than in the batch biodegradability test, thus not causing inhibition on biogas production.

The R_{pressure-depressure} and R_{freeze-thaw} showed the best performance among all reactors, tolerating OLR of 5 g COD L⁻¹ d⁻¹, with biogas production rates of 2 and 1.4 L L⁻¹ d⁻¹, respectively. Both reactors failed at the OLR of 6 g COD L⁻¹ d⁻¹, but the negative effect of this high OLR was more pronounced in the R_{freeze-thaw}, where the biogas production

sharply ceased, than in the $R_{\text{pressure-depressure}}$, where the biogas production decrease was more gradual. Besides, although the COD_s concentrations were at the same level, around 55 g COD L^{-1} , the HPr concentration in the $R_{\text{freeze-thaw}}$ (7.7 g L^{-1}) was 35% higher than in the $R_{\text{pressure-depressure}}$ (5.7 g L^{-1}). Therefore, it can be concluded that the best reactor performance was the $R_{\text{pressure-depressure}}$.

Pressure pre-treatment has been shown to physically disrupt the cellular material by breaking up the microbial cell walls, and thus intracellular carbon sources as well as nutrients can be released. However, high pressures of more than 200 bar are required (Rai and Rao, 2009). A common machine used for this pre-treatment is the high-pressure homogenizer, which is already commercialized. It is a device consisting of a multistage high-pressure pump and a homogenizer valve (Odegaard, 2004). In this process, disintegration of cells is obtained during the pressuring stage. However, in case of increasing the pressure by injecting gas into a fixed volume of material, the disintegration can happen not only during the pressuring stage, but also during the depressurizing stage, due to the gas explosion effect. Moreover, gas plasticization helps to break down the polymers. In the study of Schimel (2007) with pressure swing pre-treatment (3 pressure-depressure cycles) combined with gas plasticization (biogas) showed 50% higher COD biodegradability under an operation pressure of 1.5 bar. In our study, a single pressure-depressure cycle was applied and CO_2 was used. Although the pressure was 10 bar, the solubilization enhancement was only 12% (Table 5.2).

Although it has been suggested that longer HRT can help the reactor stabilization (Rincon et al., 2008), in our study, the change of HRT from 18 to 36 days at OLR of $4 \text{ g COD L}^{-1} \text{ d}^{-1}$ did not enhance the reactor performance. The slow hydrolysis rates (lipid: $0.1\text{-}0.4 \text{ d}^{-1}$; protein: $0.02\text{-}0.04 \text{ d}^{-1}$ and cellulose: around 0.05 d^{-1}) are the limiting factors of anaerobic digestion of the high TS content wastes (Gujer and Zehnder, 1983). First order kinetics is often considered to describe the hydrolysis of particulate organic matter:

$$\frac{dC}{dt} = -K_H C \quad \text{Eq. 5.7}$$

Assuming a substrate with an initial concentration of C_0 (g L^{-1}) and a hydrolysis rate constant of K_H (d^{-1}), the substrate concentration on day t (C_t) is:

$$C_t = C_0 \times e^{-K_H t} \quad \text{Eq. 5.8}$$

If requiring 60% degradation on day t , C_t should equal to $40\%C_0$, which gives the following equation:

$$t = \frac{0.92}{K_H} \quad \text{Eq. 5.9}$$

Concerning lipids (assuming an average K_H value of 0.2 d^{-1}), 60% degradation will be achieved in 5 days. However, for protein and cellulose with much lower hydrolysis rate (average K_H values of 0.02 and 0.05 d^{-1} , respectively), 60% degradation will be achieved in approximately 46 days and 19 days, respectively.

In our experiment, neither HRT of 18 days or 36 days helped the process performance at OLR of $4 \text{ g COD L}^{-1} \text{ d}^{-1}$. According to the kinetics calculation, more than 45 days are required to obtain 60% degradation of proteins and cellulosic materials. Therefore, the slow degradation rate of KW might be due to its high protein content of up to 30% (dry weight) (Tang et al., 2008). Another possible explanation for the no effect of the HRT could be the already stressful state of the reactors when this modification was performed. Thus, an earlier application of longer HRT might have given a more significant influence.

The maximum applicable OLR for anaerobic digestion of KW varies among studies. Although very high OLR of up to $15 \text{ g COD L}^{-1} \text{ d}^{-1}$ could be achieved in Asian studies (Cho et al., 1995; Shin et al., 2001; Park et al., 2008), other works in Europe could only obtain moderate reactor performance at OLR of around $3 \text{ g COD L}^{-1} \text{ d}^{-1}$ without pre-treatment (Banks et al., 2008; Hecht and Griehl, 2009). In this study with Belgian KW, the best performance in the R_{control} was obtained at the OLR of $3 \text{ g COD L}^{-1} \text{ d}^{-1}$, which was similar to other European studies. Besides the variation in the operational parameters (reactor type, feeding frequency, etc), these discrepancies among several studies can be explained by the variability in the regional food composition. One important factor might be the fat content in the KW, because the anaerobic digestion of fat-rich wastes is problematic due to the formation of long chain fatty acids, which are inhibitory for the methanogens (Carucci et al., 2005).

5.5 ECONOMICAL CONSIDERATIONS

Although all the pre-treatment methods improved the reactor performance, they can be practically feasible only if the economical benefit derived from the extra biogas obtained compensates their application costs. This economical estimation was based on the maximum biogas production rate of each reactor achieved during the good performance period in the continuous tests: 0.8 (R_{control}), 1.4 (R_{acid}), 1.1 (R_{thermo}), 1.2 ($R_{\text{thermo-acid}}$), 2 ($R_{\text{pressure-depressure}}$) and 1.4 ($R_{\text{freeze-thaw}}$) $\text{L L}^{-1} \text{ d}^{-1}$.

Table 5.4 presents the summary of the operational cost and benefit analysis of each pre-treatment method. The electrical requirement of the pretreatment is the main factor affecting the net profits/costs, because the acid pre-treatment without electrical input gave the highest net profit of $20.5 \text{ € ton}^{-1} \text{ KW}$, while the pressure-depressure pre-treatment

with the highest electrical input, resulted in net costs of more than 200 € ton⁻¹ KW. Although the thermal-related pre-treatments have considerably high energy requirements, the biogas enhancement is able to cover the extra costs, thus a net profit of about 5 € (thermal) and 10 € (thermo-acid) ton⁻¹ KW can be obtained. Of course, to obtain a complete estimation, the capital investment of the pre-treatment should also be brought into the balance. Although this aspect warrants further research, the fact that already some KW are frozen at the site of production to optimize hygiene makes the freeze-thaw pre-treatment (net profit of 8.5 € ton⁻¹ KW) of particular interest.

However, it should be noticed that these economical calculations were very roughly estimated. Many issues can affect the profit/cost balance, c.a. local energy price, plant capacity, biogas quality, capital investment, etc.

5.6 CONCLUSIONS

The thermo-acid pre-treatment gave the highest solubilization effect, while the acid and the pressure-depressure methods resulted in low solubilization percentages. On the contrary, the pressure-depressure method gave the highest cumulative biogas production during the batch biodegradability tests, which indicates that the application of such pre-treatments may give rise to the formation of toxic or recalcitrant compounds.

In the continuous tests, all pre-treatments improved the anaerobic digestion of kitchen waste compared to the control in term of higher biogas production rates as well as higher OLR applied. The best performance was achieved with the pressure-depressure and the freeze-thaw methods.

The net profits of the pre-treatments strongly relate to the energy requirements they impose. The highest profits, i.e. 20.5 € ton⁻¹ KW was achieved with the least energy intensive methods, i.e. the acid pre-treatment. Overall, the freeze-thaw treatment (net profit of 8.5 € ton⁻¹ KW) opens interesting perspectives because it does not consume any chemicals, and nevertheless, exhibits a strongly positive effect on the overall biomethanation of KW. Moreover, the odor can be controlled during the kitchen waste storage and transportation.

Table 5.4 Cost and benefit analysis of the pre-treatment methods applied to the KW based on the biogas production obtained during the continuous tests.

Pre-treatment	Unit	Acid	Thermo	Thermo-acid	Pressure -depressure	Freeze-thaw
<i>Thermal energy</i>						
Extra biogas production (65% CH ₄)	m ³ ton ⁻¹ KW	48	24	40	48	56
Green electricity energy obtained	kWh _{electricity} ton ⁻¹ KW	124.8	62.4	104	124.8	145.6
Extra benefit from green electricity energy	€ ton ⁻¹ KW	18.7	9.4	15.6	18.7	21.8
Thermal energy obtained	kWh _{thermal} ton ⁻¹ KW	156	78	130	156	182
Extra benefit from thermal energy	€ ton ⁻¹ KW	4.7	2.3	3.9	4.7	5.5
Total extra benefit	€ ton ⁻¹ KW	23.4	11.7	19.5	23.4	27.3
Pre-treatment cost	€ ton ⁻¹ KW	2.9	6.6	9.5	227.5	18.8
Net profit(+)/cost(-)	€ ton ⁻¹ KW	+20.5	+5.1	+10	-204.1	+8.5

APPENDIX I

APPENDIX II

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CHAPTER VI

GENERAL DISCUSSION

Thanks to the increasing price and taxation of the fossil fuels, the biogas utilization holds its modern importance to economize fossil fuels. Today, the attention for biogas is rising rapidly all over the world ...

6.1 PROCESS ENHANCEMENT BY CO-DIGESTION

6.1.1 Stimulation of biogas production with glycerol

In the past, the main objectives for co-digestion of different wastestreams was to obtain better process stability by the adjustment of the unbalanced C/N ratio, the high solid content, the buffer capacity, etc. Nowadays, due to the requirement of the sustainable energy recovery, another main drive for co-digestion is the stimulation of the methane production. Indeed, since waste treatment plants were recognized as biogas plants, methane as an energy product becomes the major focus.

Because of many newly established anaerobic digesters, the traditional types of organic industrial wastes are foreseen to become scarce. Therefore new types of co-substrates to boost biogas production are sought, and the major requirements are rapid digestion and high methane production.

Under this concept, glycerol qualifies as a satisfactory co-substrate for anaerobic digestion. In Chapter II, different types of glycerol were proven to be easily digestible with 100% biogas production compared to the expected theoretical value. However, the application feasibility depends on the economical balance between the investment due to the glycerol product price and the extra energy income which is obtained from the enhanced biogas production. It has been shown in Chapter II that even by supplementation of a very low quality glycerol product (conductivity of 29 ms cm^{-1}), a stable biogas production of 0.6 L g^{-1} glycerol input could be obtained, which resulted in the income of 66 € ton^{-1} glycerol input. The available crude glycerol price in the market today has dropped to only around $40\text{-}50 \text{ € ton}^{-1}$ (ICIS pricing, 2009), and a further price crush may be possible (Pachauri and He, 2006). Therefore, an overall benefit of more than 20 € ton^{-1} glycerol can be possible.

The additional costs for co-digestion of glycerol are mainly transportation, without any extra operational investments, such as pre-treatment and special storage or dosing equipments. The payback can be directly obtained from the instantaneously increased biogas production. Therefore, with a large quantity produced from the bio-diesel manufacturing (glycerol as by-product) and moderate prices in the market, glycerol can be a good co-substrate to facilitate the existing anaerobic plants to enhance their biogas production.

6.1.2 Enhanced biomass granulation with glycerol

One main difficulty of anaerobic digestion is the low yield of methanogenic biomass, which leads to in-reactor biomass shortage if severe biomass wash-out occurs. Therefore,

enhanced methanogenic biomass growth rate and better granulation to improve sludge settling capacity have been pointed out as the crucial pre-conditions for an efficient anaerobic process (Tiwari et al., 2006). Although the rapid growth and granulation depend on a variety of factors, such as, cell surface characteristics, shear due to upflow and gas production, organic loading rate (OLR), etc (Tiwari et al., 2006), the high energy carbohydrates provide an important contribution to this phenomenon (Thaveesri, 1995).

In Chapter II, enhancements of both the methanogenic biomass growth and granulation by glycerol supplementation were observed in the treatment reactor. Co-digestion with an easily biodegradable substrate (e.g. glycerol) can stimulate the sludge granulation due to the extra chemical oxygen demand (COD) amount received, but other substrates (e.g. protein, cellulose) might deteriorate the sludge characterization (Thaveesri et al, 1994).

6.1.3 Possible drawback of co-digestion with glycerol

A successful co-digestion depends not only on the selection of a suitable co-substrate, but also on the operational parameters applied, specially the mixture ratio, which strongly affects the final characterization of the feedstock. Overdosing of one co-substrate can possibly lead to negative effects, when dealing with substrates containing potential inhibitory degradation products.

Taking glycerol as an example, although glycerol itself has been accepted as a non-inhibitory compound towards anaerobic digestion (Vidal et al., 2000), its degradation products, i.e. volatile fatty acids (VFAs), are important intermediates for the subsequent methane formation (Holm-Nielsen et al., 2008). Therefore, in the view of potential VFA accumulation, the supplementation of glycerol should not surpass specific levels to avoid the possible process failure by the VFA inhibitory action on the methanogens. Moreover, since the fat content of crude glycerol which is produced from bio-diesel manufactory is high, i.e. up to 15% (Thompson and He, 2006), long chain fatty acids (LCFAs) should be expected as one intermediate product during the degradation of glycerol. However, since glycerol is currently underutilized as feedstock for anaerobic digestion or as co-digestion substrate, the effects of LCFAs degraded from glycerol have not been studied.

From different studies, this benchmark level was limited to a supplementation of no more than 100 g glycerol-COD L⁻¹ d⁻¹ for mesophilic conditions at around 32°C (Holm-Nielsen et al., 2008), and no more than 45 g glycerol-COD L⁻¹ d⁻¹ for the thermophilic conditions at around 53°C (Amon et al., 2006; Holm-Nielsen et al., 2008). Further increase of the glycerol supplementation could cause strong process instability,

especially when the glycerol concentration inside the reactor exceeds 7 g L^{-1} , surely due to the increase of the VFAs concentrations (Holm-Nielsen et al., 2008).

6.1.4 Co-digestion for sewage anaerobic treatment

Although anaerobic digestion has been widely applied for the treatment of medium to high strength wastestreams (Foresti, 2002), its application to low strength domestic wastewater treatment has received only limited attention (Kalogo and Verstraete, 2001).

One technical problem is the poor granule build-up (even degranulation) during the sewage treatment (Aiyuk et al., 2006). Both the low amount of readily biodegradable COD (RBCOD) in domestic sewage ($25\text{-}50 \text{ mg L}^{-1}$ on a COD_T around 500 mg L^{-1}) and the adsorption of the suspended solids to the biomass are responsible. These lead to the decrease of the sludge activity (Kim et al., 2003; Aiyuk and Verstraete, 2004). Moreover, the low available COD amount has a direct effect on the low CH_4 production of around $50\text{-}100 \text{ mL g}^{-1}$ COD removed (compared to the expected value of 350 mL g^{-1} $\text{COD}_{\text{removed}}$) during the anaerobic treatment of domestic sewage, as shown in Figure 6.1. This low CH_4 production makes energy recovery infeasible and thus significantly affects the overall economy (Van Haandel and Lettinga, 1994).

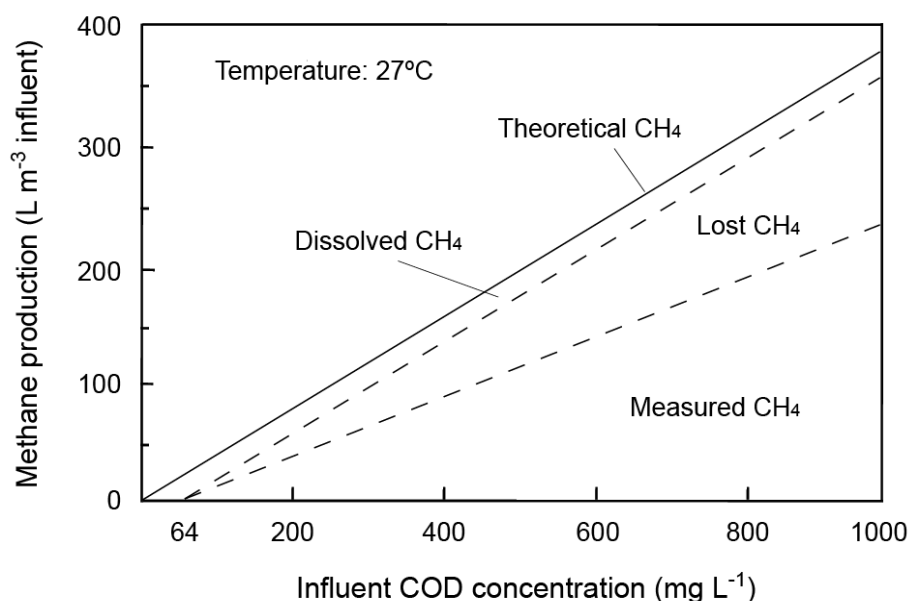


Figure 6.1 The influence of the COD concentration on the CH_4 production at ambient temperature (after Van Haandel and Lettinga, 1994).

To complement the low COD concentrations of the domestic wastewaters, it is evident that the co-digestion process of sewage with other high strength wastestreams can increase the CH_4 production. Moreover, the more balanced feedstock characterization by a diverse mixture has also a positive impact on the process stability (Verstraete et al., 2009).

In Chapter IV, co-digestion of sewage with kitchen waste has been studied. On the one hand, the low COD concentration of sewage was no longer a limiting factor since it was negligible compared to that of the kitchen waste, i.e. around 240 g COD kg⁻¹. The biogas production rate obtained was satisfactory, i.e. higher than 0.3 L g⁻¹ COD removed. On the other hand, anaerobic digestion of kitchen waste has been reported problematic since the intermediary products accumulate easily, e.g. ammonia, VFA, long chain fatty acids (LCFAs), etc, due to the high protein (around 55 g albumin L⁻¹) and lipid content (around 20 g L⁻¹) (Braun et al., 2003; Araya-Kroff et al., 2004; Park et al., 2008). Therefore the mixture with sewage had a dilution effect to the kitchen waste, which potentially offered a buffer to balance such accumulations.

6.2 ENHANCEMENT BY METALS SUPPLEMENTATION

6.2.1 The uncertainty of metal supplementation

Due to the different types of wastestreams used as feedstock and the variety of the operational conditions, the metals supplementation methods and dosages found in literature vary widely. Besides, due to the limited knowledge in the microbial metal uptake mechanisms, the boundary layer between the metal deficiency and metal toxicity in anaerobic digestion is also unclear.

In practice, metals are added into industrial digesters in excessive amounts to ascertain the stable performance, which in the long term can lead to accumulation of metals in the digester and thus causing accumulated toxicity effect (Mueller and Steiner, 1992; Bae et al., 2002; Chen et al., 2008). In addition, the relative toxicities of some metals also depend on pH, type and form of metal ions, and strength and affinity of the binding groups to the surfaces of prevalent microorganisms, which leads to huge differences in the range of both the reported stimulation and toxicity levels in literature.

Consequently, the knowledge of the metals supplementation in anaerobic digestion is limited and thus the supplementation strategies still need to be clarified. However, it is of crucial importance to elucidate this aspect since the costs due to the metal supplementation can be very costly in case of long-term supplementation (techno quality products: FeSO₄ of 8 € kg⁻¹; NiCl₂ of 17 € kg⁻¹; CoSO₄ of 30 € kg⁻¹, etc) (VWR International, 2009).

6.2.2 Metal supplementation methods

6.2.2.1 Supplementation strategies

When supplementing metals to the anaerobic digesters, two strategies are commonly applied: booster supplementation in the beginning and continuous supplementation throughout the operational period.

Research aimed at cobalt supplementation for methanol conversion by Gonzalez-Gil et al. (1999) showed that the continuous supplementation strategy was more effective than the booster supplementation in overcoming cobalt limitations by 60% higher accumulated CH₄ production, while 10 times lower amount of cobalt in total was required in the continuous strategy. However, a 10 hour delay of the exponential increase of CH₄ yield in the continuous supplementation (30 h after the start-up) was observed, comparing to that of the booster supplementation (20 h after the start-up). This delay was probably due to the lower supplementation amount of the continuous strategy. Thus, a method combining these two strategies is suggested: the booster supplementation in the beginning for an effective recovery of the metal deficiency and the continuous supplementation to compensate the metals losses from the precipitation in the digester and the washout in the effluent.

Moreover, a pulse supplementation occasionally during the process with a moderate dosage (5 times of the continuous supplementation dosage) can be necessary to overcome an acute metal limitation. Yet, the metal losses from the wash-out in this strategy can be up to 30 times higher than that of the continuous supplementation (Fermoso et al., 2008).

6.2.2.2 Supplementation unit

Often, the nutrients supplementation medium is prepared based on the reactor volume (per m³ reactor) without taking into account of the particular process conditions such as the OLR, the feedstock characterization and the amounts of metals already present in the feeding. In this way, the medium preparation may not match the actually required metals for the specific process, but simply ensures the metal variety with excessive dosages.

An alternative method is by preparing the supplementation medium per g COD of the feedstock (Fang et al., 1994). This strategy seems more reasonable since the requirement of the metals varies with the strength of the applied OLR. The argument can be that the major requirements of the metals in anaerobic digestion are for the growth and metabolism of microorganism. Thus another strategy based on the in-reactor biomass amount (per g volatile solid (VS) in reactor) might be also appropriate (Osuna et al., 2004). For instance, the supplementation of cobalt can be achieved by pre-incubating the inoculum in a CoCl₂

solution (0.13 g L^{-1}) for 24 hours. However, after 77 days of operation, about 55% of the cobalt was washed out with the reactor effluent (Zandvoort et al., 2004).

6.2.2.3 Importance of feed pre-characterization

Different types of industrial wastestreams already contain a wide range of metals with different soluble concentrations. Therefore, supplementation of all metals is actually not necessary. Instead, it should be focused on the absent or insufficient metals. Moreover, accumulated toxicity effects derived from the continuous metals supplementation with excessive amounts can be observed, if no sufficient sulfate is available in the bulk for the later metal precipitation, especially in the lab-scale studies (section 1.3.2.2).

From the economical view, since the metal supplementation is normally in a long-term continuous way, the pre-characterization of the wastestreams is important to find out the types of metals that are already available in the feedstock. Thus the costs can be minimized by only supplementation of the metals in deficiency.

Considering the supplementation of one metal with a rather low dosage of $10 \mu\text{g g}^{-1}$ COD (Table 1.7) to a 5000 m^3 industrial anaerobic digester with OLR of $10 \text{ g COD L}^{-1} \text{ d}^{-1}$, the total metal supplementation amount is 0.5 kg d^{-1} . Taking an average price of a metal techno production of 20 € kg^{-1} (section 6.2.1), the daily supplementation cost is about 10 € d^{-1} . In case of the presence of sulfate, multiple metals supplementation or booster supplementation, this daily cost can go easily 10 times higher. Currently the analysis cost of one waste sample in laboratory is in the range of $200\text{-}400 \text{ €}$ (estimated as half day engineering work). Hence, the saving from a more dedicated metal supplementation recipe compiled after the pre-characterization can be cost-effective.

6.2.3 Metal bio-availability

Supplementation of metals can increase the metal bio-available (soluble) levels, but it can be economical undesirable due to the chemical costs. Another possibility is to supplement chelators to the digester, which may dissolve metal compounds by complexation from their precipitation, and thus the increased soluble metal levels can give rise to the metal uptake by microorganisms (Hu et al., 2008).

Common chelators to increase the metal soluble concentrations are citrate, yeast extract, nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), amino acids, etc. However, microorganisms can also produce chelators to uptake metal ions and diffuse them through cell membrane (Clemens, 2001). If strong chelators, such as EDTA, are supplemented to the digester, stable metal complex are formed in the solution. In this case, although the soluble metal levels are increased, the metal ions are actually not

bio-available for the microorganisms since the chelators produced by microorganisms are not strong enough to re-complex and transfer the metal ions from their stable complex forms to the cell. Therefore, the supplemented chelators should be a “bridge” between the metal precipitation and the uptake by microorganisms. This can be achieved by the weak chelators (e.g. citrate, NTA), rather than by the strong ones (e.g. EDTA), which can cause a metal “sink” in solution. As one example, in a batch experiment of Aquino and Stuckey (2007) comparing the chelation effects of EDTA and NTA on methanogenesis, it was observed that EDTA (1 mM) decreased the CH₄ production rate while the NTA (1 mM) solubilized almost all of the available metals. The inhibitory effect of EDTA addition was explained by the unavailability of metals caused by the metals complexation with EDTA.

An important concern of the supplementation of chelators is the chemical cost. Taking yeast extract as example, trace doses of yeast extract may be effective in keeping essential metals in solution (Gonzalez-Gil et al., 2003). However, the addition of yeast extract to full-scale anaerobic reactors may not be economically feasible due to its high market price (techno quality product of 40 € kg⁻¹) (VWR International, 2009). On the other hand, using the cheaper chelation agents, such as citrate (techno quality product of 5 € kg⁻¹) (VWR International, 2009), the operational costs can be minimized by lowering the metal supplementation dosage.

6.3 ABATEMENT OF PROPIONIC ACID ACCUMULATION

6.3.1 Propionic acid accumulation in anaerobic digestion

HPr accumulation in anaerobic digesters has long been considered as a problem for process stability. The HPr level can be used as an indicator of potential process instability (Nielsen et al., 2007), since it is a sensitive parameter against the change of the operational conditions, such as pH, temperature or OLR (Barredo and Evison, 1991; Lindorfer et al., 2008).

The reported maximum HPr concentrations vary from 1.2 to 7.5 g HPr-COD L⁻¹, depending on different wastestreams and operational conditions (Hajarnis and Ranade, 1994; Mosche and Jordening, 1998). Moreover, it is also important to keep the in-reactor propionate to acetate ratio below 1 (Pullammanappallil et al., 2001).

The gradual build-up of the HPr levels can happen during reactor start-up and operational shock (e.g. overloading), which results in the delay of full sludge activity (Gallert and Winter, 2008). It can take weeks to months before the reactor recovers (Lindorfer et al., 2008). However, there is not yet a promising and effective recovery method for HPr accumulation.

6.3.2 Microbial adaptation tolerance

Although propionate oxidizing bacteria (POB) are sensitive to pH, temperature, toxins, etc, high HPr utilization rate (Chapter III: up to 33 g HPr-COD L⁻¹ d⁻¹) can be achieved by a slow and patient adaptation of POB, by means of the gradual increase of the OLR (Wiegant et al., 1986; Fang et al., 1994; Tatara et al., 2008).

Moreover, the HPr tolerable level of the microorganisms can be improved as well. In Chapter IV, the CSTR failed in the first experiment at an OLR of 2.8 g COD L⁻¹ d⁻¹ and an HPr concentration of 5 g HPr-COD L⁻¹. The failure of this reactor in the second experiment happened at an OLR of 5 g COD L⁻¹ d⁻¹ and an HPr concentration of 13 g HPr-COD L⁻¹, indicating that the tolerance level of the CSTR doubled after a successful HPr recovery.

6.3.3 Supplementation of nutrients

Trace metals supplementation, especially Fe, Ni and Co, can accelerate the HPr degradation rate (Espinosa et al., 1995; Osuna et al., 2004). In Chapter III, the maximum HPr utilization rates without and with nutrients supplementation were 3 and 33 g HPr-COD L⁻¹ d⁻¹, respectively, which indicated a 10 fold enhancement. Yet, the application to the industrial wastewaters is not clear, since very dynamic process kinetics are involved.

For example, in some cases of process failure due to the HPr accumulation, supplementation of FeSO₄ was often found to be helpful, but in others not (Jansen et al., 2007). Actually, trace metals appear to be present in many industrial effluents (Speece, 1988; Espinosa et al., 1995), but their soluble levels are not always sufficient to the microorganisms, due to the metal precipitation by sulfate compounds. Consequently, a successful supplementation of the trace metals strongly depends on the degree of the further precipitation in the digester, as discussed in session 6.2.3.

6.3.4 Recovery by means of a side reactor

The increase of the HPr level in the anaerobic digesters is always a potential danger to process failure. Although it is possible to obtain high HPr degradation rates by slow adaptation of the microorganisms, the adaptation process can take very long time (Chapter III: more than 200 days). Therefore it is often not practical feasible to apply this approach to industrial digesters.

Since the HPr recovery is not straightforward, an external remedy technology is necessary to accelerate the HPr degradation in the main digester. In Chapter IV, an extra system was designed and specialized for the HPr degradation, which mainly relied on the

HPr degradation capacity in a upflow anaerobic sludge bed (UASB) reactor with a consortium acclimated to HPr degradation. The advantage is that the time-consuming adaptation process is applied to a separated system without interfering with the main digester process. Moreover, after the HPr abatement by the connection of such a system, the recovered main digesters can be expected to be able to tolerate more stressful HPr conditions, as discussed in 6.3.2.

According to the calculated system for a volume of 50 m³ (Chapter IV: less than 2% of a full-scale 3000 m³ digester), the roughly estimated capital investment of such a system will be around 10,000-15,000 € (Gettier and Moser, 1998; EPA, 2002; Nelson and Lamb, 2002), which is quite costly for the occasional HPr accumulation events. However, its mobility/flexibility due to the small system volume gives the possibility to be connected to different industrial digesters. Thus the required investment can be shared by several partners.

6.4 PRE-TREATMENTS APPLICATION

6.4.1 Focus of the pre-treatments

The aim of the pre-treatments has always been to obtain enhanced hydrolysis. Mechanical pre-treatments were the first methods used to decrease the solid particle size prior to anaerobic fermentation process and the changes in the particle surface area were expected to improve the biological degradation. The obvious link is that the smaller particles with increased surface area can be better reached by microorganisms and exoenzymes. However, the improvement of the degradation was not significant because of the slow biological hydrolysis rates (Table 1.2). Irradiation pre-treatments are able to decrease the particle size of the substrates, produce no microbial inhibitors and usually lead to up to 60% higher solid reduction. However, these methods have high energy demands (up to 2000 kWh m⁻³ biomass treated) and are not economically attractive (Hendriks and Zeeman, 2009).

On the other hand, the dissolution of the cell content from the cell wall rupture makes these organic components readily available for degradation. Thus pre-treatments aiming at enhancing solubilization have been studied intensely. However, the degree of the solubilization improvement does not ensure the same degree of the digestibility improvement (see Chapter V). During the pre-treatment processes, especially through chemical methods, toxic compounds might be formed. During thermal pre-treatment at temperatures higher than 200°C, sugars and amino acids react to melanoidines, which are difficult to degrade, and other toxic compounds, such as phenols and furfural, can be formed (Hendriks and Zeeman, 2009).

Therefore digestibility becomes a more relevant description for the pre-treatment efficiency than solubilization. A successful pre-treatment process relies on the combined enhancement of the COD removal, VS conversion and biogas production in the subsequent batch digestion as well as in the long-term stability of the anaerobic digestion process. Thus, research of pre-treatments should always be based on a more sophisticated experimental setup, including solubilization test, batch CH₄ production test and continuous feeding test.

6.4.2 From the research laboratory to the field

In recent years, more and more pre-treatment researches are focused on the irradiation technology. When providing extreme conditions such as temperatures of more than 3500°C, pressures of 50 MPa, complete cell wall destruction can be achieved (Jin et al., 2009; Park et al., 2010). However, the costs associated with these kinds of pre-treatment methods are electrical input related and, although the latter has been always mentioned in literature, the average numbers have not been calculated (Ahn et al., 2009; Naddeo et al., 2009).

At laboratory scale, a satisfactory pre-treatment method is defined by the improved digestibility and increased biogas production, even if little enhancement is observed (less than 10% improvement) (Kidak et al., 2009). However, at industrial scale, the application of a pre-treatment method mainly depends on the increase of the biogas production, and it will be only feasible if the benefit from the extra biogas production can compensate the pre-treatment investment. Often the payback time is expected to be within 2-3 years. Today there is clearly still a huge gap between the laboratory and the industrial practice, since the pre-treatment method in research has been moved on to the electronic technology and the industrial full-scale pre-treatments implemented are still limited to simple physical, chemical or mechanical methods.

The question to the researchers can be formulated as follows: Is it more relevant to explore the high treatment efficiencies based on high energy consuming technologies, which is still economically infeasible? Or would it not be better to search for the methods with slightly reduced treatment efficiencies but which are economically directly related to practice?

Therefore, in Chapter V the investigated methods chosen were thermal, thermo-acid, pressure-depressure and freeze-thaw pre-treatments, which are possibly economically feasible. Although the acid pre-treatment gave the highest net profit of approximately 20.5 € ton⁻¹ kitchen waste, the lag phase during the beginning gave uncertainty to this method, since the chemical pre-treatments have the possibility of release or formation of toxic

compounds during the process. In this case, the physical pre-treatments are more favorable. With respect to the freeze-thaw pre-treatments, although the net profit was lower, around 8.5 € ton⁻¹ kitchen waste, the extra benefit was the odor control during the kitchen waste storage and transportation. Therefore, after an overall consideration, freeze-thaw would be the best pre-treatment method for anaerobic digestion of kitchen waste.

6.5 GENERAL CONCLUSIONS AND FUTURE R&D

In terms of the commercial biogas production, research is exploring new types of feedstock to be co-digested for the existing anaerobic digesters. Crude glycerol from bio-diesel manufactory was proved a suitable co-substrate for this purpose. The process performance was not disturbed by the additional glycerol-COD and higher biogas production rate was achieved. An overall benefit of more than 20 € ton⁻¹ glycerol can be possible, although depending on the glycerol quality and the price. Further researches should also focus on the potential inhibitions from the intermediate products during glycerol degradation, such as LCFAs. Moreover, reliable resources are important both in terms of adequate amounts and the long-term supply. In order to create a feedstock market with low prices, researches are encouraged to give priority to the waste streams which are involved with high disposal costs or increasing production amounts, such as the industrial by-products, the organic fraction from the landfill, etc.

Although both the macro- and micro-nutrients supplementation enhanced the process performance of the UASB reactors with satisfactory HPr removal rate under extreme conditions, more pronounced results was observed from the macro-nutrients supplementation in both reactors. Therefore the further researches on the role of the micro-nutrients to microorganisms are required. The understanding of metal uptake by microorganisms is complex since a dynamic system is involved with several individual metal systems and various processes, such as chemical speciation, precipitation, biological uptake, etc. The difficulty on dosage quantification is that the interaction between the metal and biomass from the microbial aspect is still unclear. Therefore, in order to develop a more rational method for the metals supplementation, studies on process kinetics of the metals in the bulk (precipitation and dissolution) and the kinetics of metal uptake by the microorganisms are important. Besides, more studies on chelation effects on the metal bioavailability are necessary.

Although the reactor recovery from HPr accumulation could be stimulated by an external reactor which was dedicated to the HPr removal with high capacity, it is still important to explore the process mechanisms related to the HPr accumulation topic. The key point of HPr accumulation is the H₂ partial pressure. Besides that, the interactions

with HAc and formate are also crucial. Therefore, a combined mathematic model may help to understand better the evolution of these compounds during the process. Moreover, it is important to prevent the HPr accumulation, therefore reliable process indicators and evaluation strategies should be developed for a better control of the HPr levels inside the reactor.

The researches on the pre-treatments present a variety of technologies. Often the pre-treatment could increase the solubilization of the feedstock, but it must be taken into consideration that pre-treatments (such as thermal or acid methods) may give rise to the formation of toxic or recalcitrant compounds. For long-term continuous operation, a stable operation should be evaluated from the process evolution based on the COD, VFA and biogas production. However, the applicability of these methods is dependent on the extra biogas production achieved, which is actually the income resource to cover the extra cost due to the operation of the pre-treatments. Nevertheless, the link between the laboratory and the industry is weak, especially lack of information related to the pre-treatment cost. Experiments should be completed by several steps, including the solubilization test, batch CH₄ production test and continuous feeding test. Moreover, the economical aspects should be considered, which can give more confidence in relation to practical feasibility of the investigated technologies.

APPENDIX I

APPENDIX II

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APPENDIX I

APPENDIX II

CHAPTER I

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APPENDIX IV

ABSTRACT - SAMENVATTING

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ABSTRACT

A variety of wastestreams have been shown to be suitable for anaerobic treatment, both in laboratory studies and industrial applications. Nowadays, the energy recovery from biogas production and the treatment of organic wastes are equally important when evaluating anaerobic digestion. Although the latter has been recognized as a well-established technology, solids hydrolysis, slow growth of methanogens, propionate accumulation and limited biogas yields are still the major drawbacks of this technology. Therefore, further studies aiming at expanding the treatment efficiencies of anaerobic digestion are necessary.

The objective of this doctoral thesis is to enhance the performance of anaerobic digestion by different approaches, aiming at a better process stability and a higher biogas production.

As a first approach, co-digestion for intensification of the biogas production was investigated in Chapter II by supplementing three different types of glycerol to potato processing wastewater. The addition of 2 mL of glycerol product per liter of raw wastewater could increase the biogas production by 0.74 L biogas mL⁻¹ glycerol product without affecting the COD removal efficiency (85%). In energetic terms this resulted in 810-1270 kWh_{electricity} per m⁻³ glycerol product, depending on the glycerol quality. Besides, a 5-fold higher biomass yield was observed in the reactor supplemented with glycerol compared to the control reactor, which suggests a positive effect of glycerol on the sludge blanket growth.

Propionic acid (HPr) removal is essential during anaerobic digestion since its accumulation leads to process instability and even process failure. It has been reported in the literature that high HPr removal rates could be achieved by macro- and micro-nutrients supplementation. In the study described in Chapter III, two feeding strategies, by modifying the hydraulic retention time (HRT) and the influent HPr concentration, respectively, were applied to obtain maximum HPr removal in up-flow anaerobic sludge bed (UASB) reactors. After a patient biomass adaptation to the long-term exposure to HPr together with nutrients supplementation, a maximum HPr removal rate of up to 32.8 g HPr-COD L⁻¹ d⁻¹ could be achieved under extreme conditions with high HPr throughput (HRT of 0.5 h) and high influent HPr levels (10.5 g HPr-COD L⁻¹). Therefore, the presence of macro- and micro-nutrients is clearly essential for stable and high HPr removal in anaerobic digestion.

Since the HPr accumulation events occur occasionally during anaerobic digestion, an enhanced propionic acid degradation (EPAD) system, an external remedy digester, has

been conceptually designed and experimentally tested at lab-scale (Chapter IV). The system consisted of two components: a liquid/solid separator containing a microfiltration membrane and an UASB reactor specialized in HPr degradation. The experimental results demonstrated that the interruption of the feeding was not sufficient for the digester to recover from HPr accumulation. However, a complete recovery could be obtained by connecting the anaerobic digester to the EPAD system. Moreover, the up-scaling of the EPAD system was economically estimated and it would only account for about 2% of the volume of the full-scale digester, thus suggesting that the implementation of a mobile EPAD system in full-scale practice should be feasible.

In Chapter V, five pre-treatment methods, i.e. acid, thermal, thermo-acid, pressure-depressure and freeze-thaw, were investigated to enhance the solubilization, biodegradability and biomethanation of kitchen waste (KW). The highest solubilization percentage obtained in the batch solubilization tests did not correspond with the best biodegradability efficiency achieved in the digestibility tests, which was probably due to the formation of inhibitory compounds during the pre-treatments. In the continuous tests, the performance hierarchy in terms of the maximum organic loading rate (OLR) applied that allowed an acceptable biogas production efficiency of 60% was: pressure-depressure ($5 \text{ g COD L}^{-1} \text{ d}^{-1}$) > freeze-thaw > acid > thermo-acid > thermo > control ($3 \text{ g COD L}^{-1} \text{ d}^{-1}$). From the overall analysis, also taking the economical aspects into account, the freeze-thaw pre-treatment was the most profitable process with a net potential profit of around $8.5 \text{ € ton}^{-1} \text{ KW}$, as well as the positive hygienic and odour control aspects.

In conclusion, new types of feedstock for co-digestion are important, in terms of stimulation of biogas production. The requirements relate to both adequate amounts and the long-term supply. Considering the process stability, metal bio-availability is crucial for metal supplementation, however, the knowledge is limited. A variety of technologies are available for pre-treatments, nevertheless, the link between the laboratory and the industry is weak, mainly due to the economical aspects.

SAMENVATTING

Anaerobe vergisting is geschikt voor de behandeling van diverse afvalstromen, zowel op laboschaal als voor industriële toepassingen. De energierecuperatie uit de biogasproductie en de behandeling van organisch afval zijn beiden belangrijke aspecten bij het evalueren van de anaerobe vergisting. Alhoewel dit proces beschouwd wordt als een ingeburgerde technologie, zijn er nog steeds enkele obstakels zoals een moeilijke hydrolyse van vaste stoffen, trage groei van methanogenen, accumulatie van propionzuur en een beperkte biogasopbrengst. Dit benadrukt de noodzaak van verder onderzoek om de behandelings efficiëntie van anaerobe vergisting te verbeteren.

Dit doctoraatsonderzoek is toegespitst op de verbetering van anaerobe vergisters met als ultieme doel om een betere processtabiliteit en een hogere biogasproductie te bekomen.

In Hoofdstuk II werd co-vergisting onderzocht ter verbetering van de biogasproductie door het toevoegen van drie verschillende soorten glycerolproducten aan afvalwater uit de aardappelindustrie. Het toevoegen van 2 mL glycerolproduct per liter afvalwater kon de biogasproductie verhogen met 0.74 L biogas mL⁻¹ glycerolproduct zonder de COD verwijderings efficiëntie (85%) te beïnvloeden. In energetische termen resulteerde dit in 810-1270 kWh_{elektriciteit} per m⁻³ glycerolproduct, afhankelijk van de glycerol kwaliteit. Bovendien werd ten opzichte van de controlereactor een vijfmaal hogere biomassa-opbrengst waargenomen in de reactor behandeld met glycerol. Deze waarneming wijst op een positief effect van glycerol op de groei van het slibbed.

De verwijdering van propionzuur (HPr) is onontbeerlijk tijdens anaerobe vergisting aangezien accumulatie leidt tot een onstabiel proces of zelfs falen van het proces. In de literatuur wordt vermeld dat hoge HPr verwijderingssnelheden kunnen bekomen worden door toevoegen van macro- en micro-nutriënten. Om een maximale HPr verwijdering te bekomen in opwaartse-stroom anaerobe slib bed reactoren (UASB), werden twee voedingsstrategieën toegepast in Hoofdstuk III door het aanpassen van respectievelijk de hydraulische verblijftijd (HRT) en de HPr concentratie in het influent. Na een geleidelijke aanpassing van de biomassa aan de langdurige blootstelling aan HPr in combinatie met nutriëntentoevoeging, werd een maximale HPr verwijderingssnelheid bereikt van 32.8 g HPr-COD L⁻¹ d⁻¹. Dit gebeurde onder extreme omstandigheden met een snelle HPr verwerkingscapaciteit (HRT van 0.5 u) en hoge HPr influent concentraties (10.5 g HPr-COD L⁻¹). De aanwezigheid van macro- en micro-nutriënten bleek uit dit onderzoek essentieel om een stabiele en hoge HPr verwijdering te garanderen bij anaerobe vergisting.

Aangezien HPr accumulatie regelmatig wordt waargenomen tijdens anaerobe vergisting, werd een enhanced propionic acid degradation (EPAD) systeem conceptueel

ontworpen en experimenteel getest op laboschaal (Hoofdstuk IV). Deze externe hulpvergister bestaat uit twee componenten: een vloeistof/vaste stof afscheidingstoestel dat een microfiltratie membraan bevat en een UASB reactor gespecialiseerd in HPr afbraak. De experimentele resultaten toonden aan dat de onderbreking van de voeding niet voldoende is om een vergister te laten herstellen van HPr accumulatie. Volledig herstel kon daarentegen wel bekomen worden door de anaerobe vergister te verbinden met het EPAD systeem. De opschaling van het EPAD systeem werd economisch berekend en zou slechts 2% van het totale volume vereisen, wat aangeeft dat de implementatie van een mobiel EPAD systeem een haalbare oplossing is in praktijk.

In Hoofdstuk V werden vijf voorbehandelingsmethoden, namelijk zuur, thermisch, thermisch-zuur, compressie-decompressie en vriezen-dooien, onderzocht voor de bevordering van het oplosbaar maken, de biodegradeerbaarheid en de biogasomzetting van keukenafval. Het hoogste percentage van oplosbaar maken van het keukenafval in batchtesten, kwam niet overeen met de hoogste biodegradeerbaarheid efficiëntie tijdens vergistingstesten. Dit is waarschijnlijk te wijten aan vorming van inhiberende componenten tijdens de voorbehandelingen. Tijdens continue testen werd de hiërarchie bepaald in termen van maximale organische volumetrische belasting (OLR) met een aanvaardbare biogasproductie efficiëntie van 60%: compressie-decompressie (5 g COD L⁻¹ d⁻¹) > vriezen-dooien > zuur > thermisch-zuur > thermisch > controle (3 g COD L⁻¹ d⁻¹). In een globale evaluatie die ook economische aspecten in rekening bracht, was de vriezen-dooien voorbehandeling het meest rendabele proces met een netto potentiële winst van ongeveer 8.5 € ton⁻¹ keukenafval en bijkomende voordelen in de vorm van hygiënische aspecten en beperkte geurhinder.

Als besluit kan gesteld worden dat nieuwe grondstoffen voor co-vergisting belangrijk zijn voor de stimulatie van de biogas productie. Een grondstof is geschikt als ze in voldoende mate beschikbaar is en op lange termijn een gegarandeerde aanvoer heeft. Met het oog op processtabiliteit is inzicht in de biobeschikbaarheid van metalen cruciaal om de benodigde metaal toevoeging te bepalen. De kennis hierover is tot dusver beperkt, wat aangeeft dat verder onderzoek noodzakelijk is. Tot slot is er een breed gamma aan voorbehandelingstechnologieën beschikbaar, maar de link tussen labo-onderzoek en industrie is nog beperkt, voornamelijk door economische aspecten.

APPENDIX I

APPENDIX II

CHAPTER I

CHAPTER II

CHAPTER III

CHAPTER IV

CHAPTER V

CHAPTER VI

APPENDIX III

APPENDIX IV

APPENDIX V

CURRICULUM VITAE

APPENDIX VI

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Gent, May 2010

*I would like to thank my promoter and supervisor, all my friends and colleagues,
for your supports through all these years !*

I will always think of you no matter where I am !

Echo 
