ENHANCED WATER USE EFFICIENCY TO SUPPORT THE GREEN ECONOMY IN EU AND INDIA

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Water4Crops - EU

Work Package 1
Valorization, treatment and reuse of agrofood industry wastewaters
Deliverable D1.1
Report on the optimized VFA production from BRWs

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1. Introduction

Work Package 1 focuses on valorization, treatment and reuse of agrofood industry wastewaters. Two types of wastewater were selected for further study: olive mill wastewater (OMW) and biorefinery wastewater (BRW). This report only concerns BRW.

The feasibility of applying anaerobic digestion as a first treatment step to BRWs has been demonstrated in literature. While accumulation of intermediate fermentation products, i.e. volatile fatty acids (VFA) is not desirable in regular anaerobic digestion, it can be exploited for the production of VFA as bulk chemicals. Chang et al. (2010) indeed proposed a VFA-based platform for the bio-based production of fuels and biochemicals, as an alternative for the typical biorefinery platforms based on sugar, syngas, biogas or carbon-rich chains. To the best of our knowledge, this has not been evaluated yet for BRWs.

A VFA-based platform evidently only is viable when the VFA mixtures can be converted in an economical way into biofuels and biochemicals. Therefore, we first described the current state-of-the-art for biorefineries, which will be the benchmark for our own investigations. From there, we selected the appropriate waste stream for VFA production. This was then used to evaluate the impact of selected process parameters on VFA production.

2. State-of-the-art biorefineries

In this part of the work, we address current process schemes and valorization options that have been proposed in literature for BRWs. The production of VFA is one of them.

2.1 Background

The term biorefinery is raising importance in the scientific community and the concept is analogous to today's petroleum refinery, which produces multiple fuels and products from petroleum. Some biorefinery complexes and non-conventional biomass industries are already competitive in the market and many pilot and demo plants are running worldwide, most of them with the purpose to optimize the production of bioethanol and chemicals from lignocellulosic sources (Cherubini & Jungmeier, 2009). The biorefinery produces fuels, solvents, plastics and food for human beings. The key processes in the biorefinery entail ethanol fermentation and lactic acid fermentation (Figure 1). For the biorefinery, different

hybrid techniques were developed from different fields, such as bioengineering, agriculture, polymer chemistry and food science (Ohara, 2003). The main biobased products are obtained from the conversion of biomass to basic products like starch, oil, and cellulose. At present, some fuels are produced from biomass, such as ethanol and biodiesel. In addition, chemicals like lactic acid and amino acids are produced, which are mainly used in the food industry (Kamm & Kamm, 2004). In contrast to this, conventional refineries use petrochemical raw materials to produce a wide variety of fuels, chemicals, and consumer goods. Figure 2 shows the main difference between an oil refinery and a biorefinery.

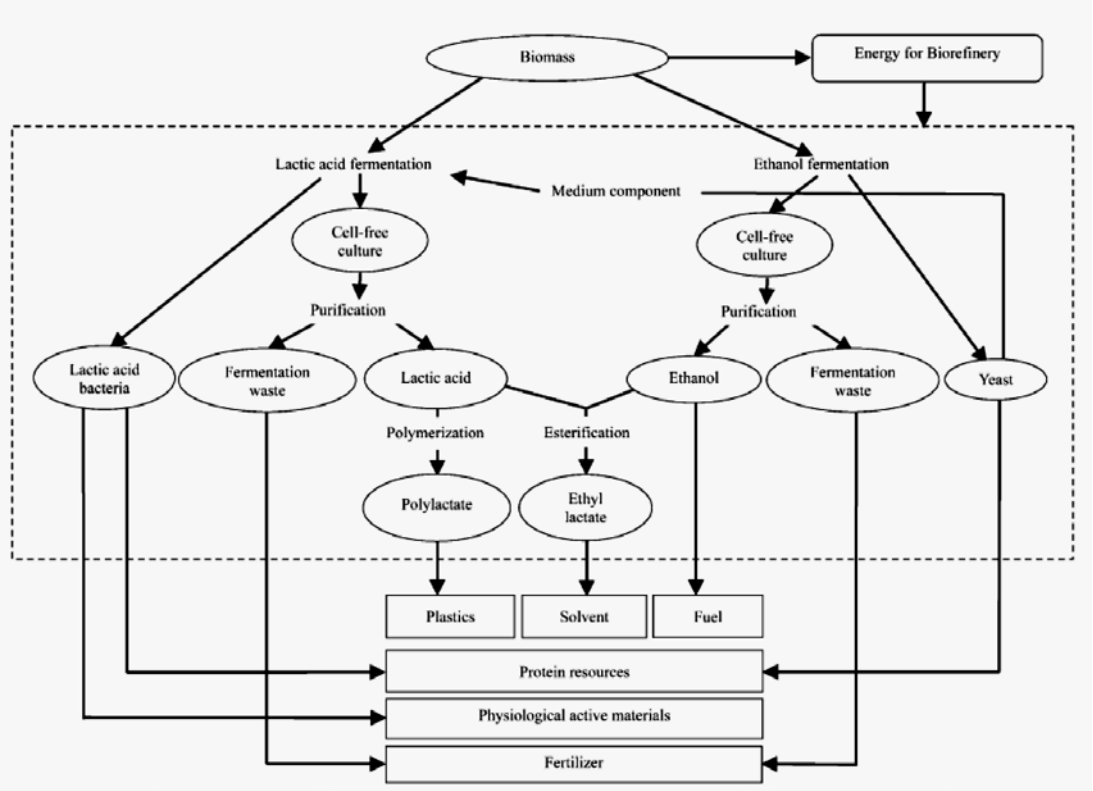

Figure 1 The biorefinery ecosystem, based on lactic acid fermentation and ethanol fermentation (Ohara, 2003)

Presently, concepts of biorefineries are being increasingly discussed. Such biorefineries will produce a multitude of biomass derived products which might replace the petroleum-refinery's products as well as some products which cannot be manufactured in conventional refineries (Kamm & Kamm, 2004 ; Ohara, 2003; Realff & Abbas, 2003). There are three main

biorefinery concepts which are lignocellulose feedstock (LCF) biorefinery, whole-crop biorefinery, and green biorefinery (Kamm & Kamm, 2004). Of these three types, the LCF biorefinery is considered to be the most promising, as the availability of the input material (e.g. straw, grass, waste wood) is relatively high and input material prices are low (Kamm & Kamm, 2004).

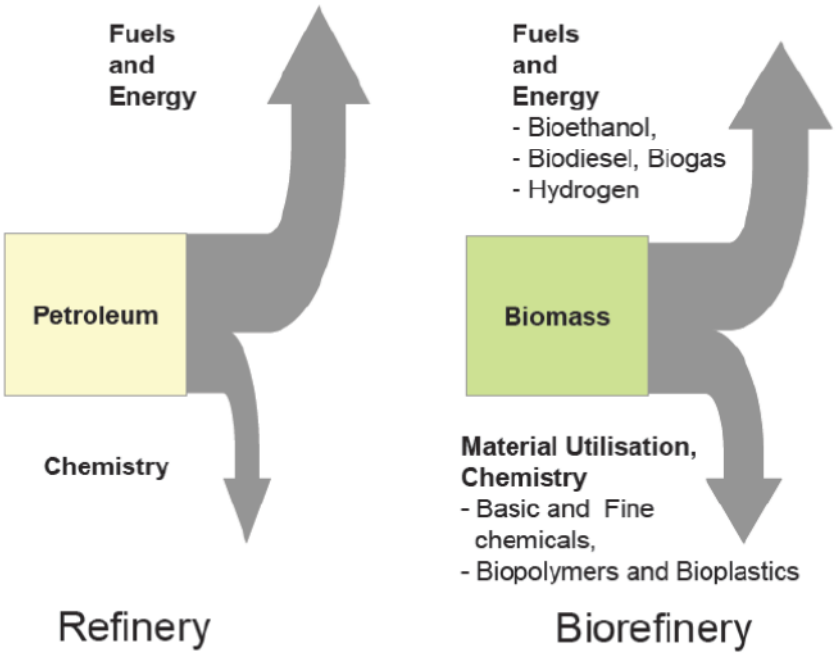

Figure 2 Oil refinery versus biorefinery (Kamm et al., 2006)

2.2 Types of Biorefineries

Biorefineries can be classified into two types: biomass producing-country type and waste-material-utilization type (Ohara, 2000b). In Brazil, the United States, China, Southeast Asia and Australia, biomass such as sugarcane, corn, sugar beet, cassava, sago and potatoes are used in biorefineries. These countries are going to breathe life into a new industry, using agricultural products. In these countries, agricultural policy and industrial policy both relate to the biorefinery. In contrast, Japan and some European countries lack the space for garbage landfill, have insufficient fields for compost and have few agricultural products. In these

countries, the biorefinery serves the dual role of refuse disposal and production of useful products (Ohara, 2000a). In these countries, refuse disposal and environmental pollution policies are connected. Old paper, lumber waste, animal waste and food waste provide the raw material for fermentation and energy resources. In practice however, the bioethanol is made from cereals only.

2.3 Biorefinery industrial processing

Biorefining includes fractionation for separation of primary refinery products. The fractionation refers to the conversion of wood or plant biomass sample into its constituent components (cellulose, hemicelluloses and lignin). Fractionation processes include steam explosion, aqueous separation and hot water systems. Main commercial products of biomass fractionation include levulinic acid, xylitol and alcohols. The main technologies to produce chemicals from biomass are thermochemical, biochemical, chemical, or a combination of them. These will not be covered in this report.

A full realization of the utilization potential of any biomass resource often requires a complex set of operations. Besides the actual chemical transformation steps, a multitude of physical processes are involved in the raw material pre-treatment as well as in the separation of intermediates and products. This is on the production side of the value chain. On the other hand, the wastewater generated as a result of these processes also can be valorised besides being treated in a conventional way. This valorisation component is a core aspect of Water4Crops.

2.4 Products of Biorefinery

Bioethanol is by far the most important biomass based energy product, mainly used in the transportation sector. In recent years biodiesel has also gained in importance as a transportation fuel. Major nations have drawn ambitious plans to improve the share of biofuels in the transport sector. Between 2001 and 2006 alone, the global annual production of biodiesel and ethanol grew by 43% and 23%, respectively. According to the European draft

directive on renewable energy, the target for biofuels share is 10% by the year 2020. The International Energy Agency (IEA) aims for a contribution of 10–20% biofuels in the transportation market in 2030. In the US, the Renewable Fuels Standard (RFS), a provision of the US Energy Policy Act of 2005, expects the supply of renewable energy to increase from 4 billion gallons in 2006 to 7.5 billion gallons by 2012 (Bothast & Schlicher, 2005). Gaseous fuels like biogas and syngas are also derived from biorefineries. Pyrolysis products can be chemically modified to yield dimethyl ether (DME), Fischer–Tropsch diesel, and synthetic natural gas (SNG). Biogas is important from the point of view of decentralized production which can be helpful for sustainable development in rural areas.

Also, biorefineries can provide an array of chemicals like adhesives, cleaning compounds, detergents, dielectric fluids, dyes, hydraulic fluids, inks, lubricants, packaging materials, paints and coatings, paper and box board, plastic fillers, polymers, solvents, and sorbents (Cherubini & Ulgiati, 2010). Corn to ethanol biorefineries can produce fiber, germ, and gluten besides Distillers' Dried Grains (DDG) as animal feed (Singh & Eckhoff, 1997). Corn fiber, rich in hemicelluloses, can be further hydrolyzed and fermented to yield additional ethanol. Alternatively, it can be processed into corn fiber oil (Singh & Eckhoff, 1997), characterized by its low cholesterol content (Huang et al., 2008), and corn fiber gum (Singh et al., 1999), which have high commercial value. Gluten rich zein is also a high value product. It has important applications in adhesive, coating, cosmetic, textile, and biodegradable plastics (Fatih Demirbas, 2009; Shukla & Cheryan, 2001). Thus, expanding the corn to ethanol production into a biorefinery framework can provide vital product diversification making the business venture much more economically sustainable. Paper and paperboard are the traditional products obtained from woody biomass from forest. Paper manufacturing, worldwide, has experienced stagnation in the recent past due to competitive products from fossil resources. Off late there has been a rethinking on these operations to expand them as forest biorefineries (FBR). A similar approach can also be applied to agricultural residue based paper production, especially in many developing countries. This can be a much better option than starting a greenfield biorefinery. Such integrated biorefineries can produce ethanol, syngas, DME and electricity among possible energy products and paper/paperboard, fiber reinforced bio-composites, and lignin derivatives as functional biomaterials (Huang et al., 2008). Besides, natural health food components, like phytosterols, folates, and phytates can also be produced (Merchuk et al., 2007). Lignin is the component of the lignocelluloses

which has been traditionally neglected. Lignin is the second most abundant organic substance on earth after cellulose. But currently it is recognized only as a low value biofuel. There has been extensive research worldwide to find better uses for lignin. The largest current application of unaltered lignin is its use as a replacement of phenol in phenol–formaldehyde adhesives or resins (Alonso et al., 2004; Gonçalves & Benar, 2001; Tejado et al., 2007). However, it can be used in many other polymer formulations (Bittencourt et al., 2005; Fernandes et al., 2006; Gregorová et al., 2006), as adsorbents and carbon precursors (Kadla et al., 2002), and raw material for an array of low molecular weight aromatic substances (Embree, 2001; Villar et al., 2001). If used as a raw material for specialty chemicals, lignin can fetch much higher price than when used as a biofuel (Axelsson et al., 2006; Olsson et al., 2006). Use of lignin outside the energy domain can significantly improve the economic and environmental sustainability of lignocelluloses based biorefineries.

2.5 Biorefineries wastewater composition

The issue of energy security, particularly any high dependence on imported oil, as well as a generally increased environmental awareness, has together motivated research into the biorefinery concept for the production of fuel ethanol. The majority of this research has focused on net energy production, feed treatment, separation techniques and micro-organism development, but has often overlooked the downstream impacts of the large volumes of highly polluted stillage produced by the process. The corresponding stillage production, 12–15 L/L of ethanol (Pant & Adholeya, 2007), 6–20 L/L of ethanol (Tewari et al., 2007), is becoming a significant barrier to the further development of this industry. Although the volumes produced by biorefineries fall within the scope of conventional effluent treatment plants, it is the high BOD[1] and bio-recalcitrant COD[2] loads associated with these waste streams (Table 1) that cause the major difficulties in their treatment.

[1] BOD: Biological Oxygen Demand
[2] COD: Chemical Oxygen Demand

Table 1. Characteristics of spentwash or stillage generated from various feedstock (Ref. 1: Pathade, 1999; Ref. 2: Wilkie et al., 2000; Ref. 3: Mahimairaja and Bolan, 2004; Ref. 4: Eskicioglu and Ghorbani, 2011) [Table reproduced and expanded from Satyawali and Balakrishnan, 2008]

Characteristics	Feedstock			
	Cane molasses		Beet molasses	Corn stillage
	Ref. 1	Ref. 3	Ref. 2	Ref. 4
COD (g/l)	65–130	104–134	91	254
BOD (g/l)	30–70	46–96	45	67
COD / BOD ratio	2.5	1.4-2.3	1.95	3.8
Total Solids (g/l)	30–100			124
Total Dissolved Solids (g/l)	80	79–88		
Total Nitrogen (mg/l)	1000–2000	1660–4200	3569	
Total Phosphorus (mg/l)	800–1200	225–3038	163	
Potassium (mg/l)	8000–12000	9600–17 475	10 030	
Sulfur as SO4 (mg/l)	2000–6000	3240–3425	3716	
pH	3–5.4	3.9–4.3	5.35	4.34

2.6 State-of-the-art wastewater treatment

The majority of lignocellulosic ethanol research has focused on microorganisms, pretreatments, hydrolysis and heat integration. Wastewater treatment has been the subject of less research, notable exceptions being NREL3857 (1998) and Torry-Smith et al. (2003). Most of the promising lignocellulosic processes will face wastewater treatment problems that are similar to those of molasses based ethanol production: large wastewater volumes, 12-15 L/L of ethanol (Pant & Adholeya, 2007), 6-20 L/L of ethanol (Tewari et al., 2007), and a high concentration of recalcitrant COD which persists after both anaerobic and aerobic treatments. For lignocellulosic ethanol, these issues are magnified by the necessity to recycle a large

portion of the process water and the fact that the fermentation ethanol concentrations will likely be lower. Anaerobic digestion is an important process in biorefinery context (Gao et al., 2007; Prasad Kaparaju et al., 2010). It can be an important downstream process for generation of biogas from the stillage after the distillation of bioethanol (Buzzini & Pires, 2002; Grover et al., 1999). Anaerobic digestion is also suited to lignin containing wastewaters and process streams (Holm-Nielsen et al., 2009; P. Kaparaju & Rintala, 2011), as well as other waste biomass (Cantrell et al., 2008; Chandel & Chan, 2007).

Regardless of their feedstock, all ethanol-producing biorefineries face the common challenges of treating their effluent efficiently and economically to meet local discharge requirements, and minimising their net water consumption. In terms of process integration, these twin requirements can be addressed by (i) improving existing secondary (i.e. biological) treatment methods to maximise COD reduction, (ii) incorporating a tertiary 'polishing' stage to remove colour, and (iii) using (reverse osmosis) membrane technology to recover process water (Ryan et al., 2009). However, sugar-based production in general presents more wastewater issues than grain-based production.

2.6.1. Biological treatment as core technology

Simple design and low capital/operating costs have made anaerobic digestion a ubiquitous starting point for the treatment of wastewaters with a high organic load. This technology, as applied to distillery effluents, has been comprehensively reviewed in recent years (Pant & Adholeya, 2007; Rajeshwari et al., 2000; Satyawali & Balakrishnan, 2008). The capture of the biogas produced by these systems can provide energy to the plants at a conversion rate of 0.35 Nm^3 gas/kg COD consumed (Tewari et al., 2007). Combined anaerobic/aerobic biological treatments are usually able to reduce stillage BOD to acceptable standards in a cost-effective manner but are unable to decolourise it. Significant concentrations of melanoidins and phenolic/humic compounds contribute both to the heavy colour of stillage and a typical residual COD loading of 4000–10,000 mg/L.

Treatment options vary a lot in their environmental impacts and the extent to which they have been verified on an industrial scale. Cost-effective considerations, such as possible by-product streams and energy charges, must also be taken into account. Disposal options for the post-

biological treatment of biorefineries wastewater include thermal treatment, flocculation, membranes and advanced oxidative processes.

2.6.2. Thermal treatment

After undergoing solids separation, the stillage can be concentrated in multiple effect evaporators to 50–65% solids content. The syrup formed, called 'condensed solubles', has a rich nutritive feed value which has found application as a cattle feed. Alternatively, the concentrate can be spray dried to form a powder with a calorific value which can be used as a boiler feed, often in conjunction with waste biomass (Satyawali & Balakrishnan, 2008). The evaporator condensate can be used as make-up water in the cooking process or as boiler feed. However, the energy cost of evaporation is high, equivalent to 10% of the ethanol produced in a bioethanol biorefinery (Faust et al., 1983), and the viability of this option relies heavily on the value that can be assigned to the resultant products. Additional energy can be recovered by using the vapour formed from boiling in a recompression process.

2.6.3. Coagulation

Coagulation is an effective means of removing TSS[3], COD and color from process wastewater that has undergone full biological treatment (anaerobic and aerobic). Chemical coagulation using ferric chloride or alum is the most common treatment method. The optimum dosage levels for wastewater (COD 4500 mg O_2/L) both fall in the range 15–20 mM metal, yielding better than a 95% color reduction and a 60–80% COD reduction (Migo et al., 1997; Ryan et al., 2009). Above these levels, re-stabilisation is observed, indicating the dominance of an adsorption-charge neutralisation mechanism. The optimum dosage level increases to 60 mM for the treatment of stillage that has not undergone aerobic digestion (Chaudhari et al., 2007; Migo et al., 1997). The optimum dosage for color removal on a metal dosage basis is very similar for aluminium and ferric salts for a wide range of colored organics (Chaudhari et al., 2007; Migo et al., 1997). However, the optimum pH range varies considerably, with ferric salts typically falling in the range 3.7–4.2 in contrast to 5.0–5.5 for aluminium sulphate. This difference can be attributed to a lower pH being required for ferric ions to use organic functional groups as ligands rather than hydroxide ions (Hall & Packham,

[3] TSS: Total suspended solids

1965). Operating costs are here largely based on reactant costs, while the capital component is primarily determined by the sedimentation tanks required for solids separation.

The limitations of coagulation include increased salinity load for the effluent, storage and handling of corrosive chemicals, need for pre- and post-dosing adjustment of pH and sludge handling and disposal. Possible methods for sludge disposal include landfill, agricultural dispersion, wet air oxidation, pyrolysis, and/or refining to create value-added products. It is possible to avoid limitations by using electrocoagulation instead of chemical coagulation. Similar dosage requirements and treatment performance have been reported but without either any salinity increase or the acidifying pH effects of chemical coagulation (Ryan et al., 2009). Flotation of coagulated material by cathodic gases can also assist in reducing the load on downstream solids separation equipment.

2.6.4. Membranes

Utilization of pressurized membrane separation processes, primarily reverse osmosis (RO), is an attractive option mainly due to the ‘guarantee’ of obtaining a specified water quality. Both ultrafiltration (UF) and nanofiltration (NF) have also found application, either as an RO pre-treatment in a cascade process or as stand-alone options in appropriate circumstances. RO processes have the ability to produce high quality water at recoveries approaching 100%. However, the ‘price’ of this level of water recovery is the use of extremely high operating pressures; 70 bar for some distillery wastes (Nataraj et al., 2006). NF membrane systems operate at lower pressures, can maintain a reasonable permeate flux, and have the added capability of being able to selectively fractionate stream solids. This performance is chiefly controlled by the ‘skin layer’ overlaying the basic porous membrane. Microfiltration (MF) and UF are both relatively low pressure options that are reasonably effective in removing suspended solids and larger molecules from a wastewater stream. However, both are quite porous membranes with performance characteristics that are not tight enough to remove sufficient low molecular COD to meet strict effluent discharge standards.

The application of membranes to distillery type wastewaters has utilized all four classes in various combinations. For example, the large organic polymers responsible for the color can be removed by NF, although Mutlu et al. (2002) found that a ‘coarse pre-treatment’ such as MF was essential in preventing fouling and maintaining adequate flux rates in the NF

treatment at 1 bar. At a higher pressure (3 bar), better than 90% removal of both color and COD can be achieved with a permeate flux in excess of 1 m^3m^{-2} h^{-1} (Rai et al., 2008). Similar treatment performance was reported by Nataraj et al. (2006) for an RO pretreatment step using NF (20–50 bar) to remove colloidal color and associated TDS. The condensate arising from evaporation/concentration processes has also been membrane-treated to enable its reuse. Accurate pH control is generally accepted as the most important operational variable to ensure good rejection of target organics (Morin Couallier et al., 2006) and water recovery.

On the downside, fouling is an almost unavoidable result of membrane filtration. Post-biological wastewater streams can have a TDS[4] in excess of 10,000 mg/L and, as noted, require some form of pretreatment prior to using RO. The presence of scale-forming constituents, especially calcium salts/oxides and hydroxides of iron and manganese, can be controlled by pH adjustment and upstream filtration, although periodic cleaning will still be required (Rautenbach et al., 2000).

2.6.5. Advanced oxidative processes

Advanced oxidative processes (AOPs) exploit the aqueous phase oxidation of target (both organic and inorganic) pollutants by hydroxyl free radicals. These radicals promote mineralization by reacting very quickly but non-selectively via addition to a double bond or by abstraction of a hydrogen atom from aliphatic compounds. It has been demonstrated that ozone is efficient at removing more than 90% of the color in wastewater, although parallel COD reductions were modest at 20%. These results are in agreement with Pena et al. (2003). It has been suggested that the decolorisation is due to the cleavage of the chromophoric double bonds between carbon atoms found in melanoidins and other humic substances (Alfafara et al., 2000). However, wastewater also contains high levels of alkalinity (9000 mg/L as $CaCO_3$) due to the presence of bicarbonate ions. These ions increase the ozone dose required and reduce its efficiency because they are strong inhibitors of reactions between hydroxyl radicals and the organic content (Coca et al., 2005). However, ozone generation is expensive and has a short half-life, necessitating on-site generation. Thus many AOPs combine the use of ozone with other agents, such as hydrogen peroxide and UV radiation, to produce amplified concentrations of the reactive hydroxyl radical. The use of

[4] TDS: Total dissolved solids

mobilized/immobilized catalysts (such as $CaTiO_3$ and FeO_3) to enhance radical formation and increase cost effectiveness has also been successfully demonstrated (Carbajo et al., 2007; Sreethawong & Chavadej, 2008). Although ozone is a potential tool for degrading toxic compounds in wastewater, it is not viewed as a cost-effective way to mineralize large COD contents. However, it could have a role to play in stillage treatment as an additional process step in the traditional biological treatment, as ozonation readily breaks down complex organic matter into low molecular weight by-products. The ozonation result will be an increase in the effluent biodegradability by up to 40% (Alfafara et al., 2000), with a consequent increase in aerobic digester performance (Srivastava et al., 2006). The inclusion of an ozonation stage in the treatment of high strength distillery waste is able to double the COD removal of the biological system alone (Beltrán et al., 2000; Sangave et al., 2007), resulting in a final effluent BOD below 20 mg/L, a 70% reduction in COD, and no visible color. This pretreatment option would diminish reactor residence times or permit the use of smaller reactors.

Active varieties that oxidize pollutants at elevated temperatures (125–350 $^{\circ}C$) and pressures are produced by wet air or thermal liquid-phase oxidation (WAO), i.e. hydroxyl radicals (0.5–20 MPa). These oxidation reactions are highly exothermic, and thus once the reactor has reached its operating temperature, the energy requirement is simply the enthalpy difference between the incoming and exit streams. The influent COD needs to be 60,000 mg/L for a wastewater flow to become thermally sustainable in equipment of practical size (Heimbuch & Wilhelmi, 1985; Laughlin et al., 1983). As the reaction heat is liberated within the liquid phase, it can be efficiently and economically recovered.

2.6.6. Treatment costs

Though actual numbers for the treatment costs that are incurred by the biorefineries on the treatment of their wastewater are not available, it can be assumed that these will be substantial considering the enormous volumes of the wastewater that is generated and strict discharge standards in most countries. This is true for industrial wastewater treatment in general. For example, the amount of energy needed for the water infrastructure of USA has been estimated to consume 4 to 5 percent of the electricity produced in this country, with about 1.5 percent of this used for wastewater treatment alone (Logan, 2009). It is estimated that $2 trillion is needed in the US over the next 20 years for building, operating, and maintaining wastewater

and drinking water facilities (WIN, 2001). About $45 billion is needed for wastewater alone, in addition to the current annual expenditure estimated at $25 billion. Recently national water withdrawals by industrial sector were reported based upon United States Geological Survey (USGS) totals, without considering amounts of water returned to the watershed (Blackhurst et al., 2010). This water use also gives a fairly good idea about the costs that might be incurred (Table 2). "Industrial" water use consists of self-supplied water taken from streams or wells and represents 5% of all water withdrawals.

Table 2. Direct and largest indirect water withdrawals for $1 of final demand "Sugar cane mills and refining" and commodity price of 5 Lb of refined white sugar ($0.06/5 lb) (Blackhurst et al., 2010)

Sector name	Water withdrawals (gal./$)	Water wihdrawals (gal./5 lb bag)
Direct use		
Sugar cane mills and refining	0.082	0.026
Indirect, supply chain uses		
Sugar cane and sugar beet farming	270	84
Power generation and supply	11	3.4
Grain farming	2.4	0.75
Pesticide and other agricultural chemical manufacturing	0.39	0.12
Cotton farming	0.28	0.087
Paperboard mills	1.3	0.40

2.7 Wastewater valorization

2.7.1. Water reuse

Some wastewater streams generated from food and beverage plants are clean enough to be used for certain applications onsite without some level of treatment. Other wastewater streams require treatment before they are suitable for reuse. Irrespective of the quality of water

achieved following treatment, water reuse is not used for applications that are in contact with the product. An area of opportunity for water reuse is in utilities, but the reuse water must be of a high quality as the presence of salts or organics causes scaling and corrosion in the boilers and cooling towers (GWI, 2012).

Condensate in the form of vapour, which is generated from steam supply systems and boilers, can be reused at plant sites. Membrane technologies can for instance be used at the industrial sites to recover and reduce the consumption of water. However, membrane technologies can prove to be cost prohibitive, and alternative water sources are likely to be cheaper. In addition, membranes are not suitable for treating wastewater from all waste streams that are to be reused at plant sites.

Reuse can amount to 100% of the water flows. Several process suppliers offer state-of-the-art zero discharge biorefinery plants, in which all waters are reused in the process and small quantities of fresh water are continuously supplemented to compensate for water losses in the process, e.g. due to uptake of water or evaporation. The largest water quantities are recovered from the thin stillage evaporation stage. All waters are typically collected in a buffer tank to balance water production and demand, and are reused for cleaning-in-place, mashing and gas washing for ethanol recovery without extra treatment thanks to the high water quality (mainly condensates). It must be remarked though that cooling water requirements for fermenters and distillation sections are very high and cooling water constitutes the major water flow in biorefineries. These are typically not taken into account when zero discharge concepts are described.

2.7.2. Recovery of valuable products

The starting point for stillage treatment is anaerobic digestion resulting in valuable biogas, which can be used to cover (part of) the energy demand of the overall treatment train. Other valorization routes include incineration to an ash, which can be used as fertilizer, evaporation to an animal feed, or use as a feedstock for production of yeast as an animal feed additive. These are all quite energy consuming. Direct use of stillage (after proper dilution) in irrigation for sugar cane production has been considered as well, because this crop requires large amounts of water and inorganic nutrients. However there are a number of problematic issues, such as the production of stillage when the crops do not require irrigation water, the location

of the plants compared to the land requiring irrigation, the match between the stillage fertilizer content and the crop requirements, the risk of over-application, etc. Intensive land application has indeed shown accumulation of salts in the soil, odor problems, salt and color leaching affecting groundwater and downstream water quality, etc (Willington and Marten, 1982, Satyawali and Balakrishnan, 2008).

The major valuable product obtained from grain-based biorefineries is dried distillers' grains with solubles (DDGS). All nutrients are removed via DDGS while water (mainly condensates) are recycled in the process.

Other valuable products are not present as such, but can be obtained through bioconversion of the waste streams which are highly loaded in organics. We have compiled an exhaustive overview of literature on this topic and refer to ElMekawy et al. (2013) for more details.

2.7.3. Carboxylate platform concept

In the biorefinery concept, the value of each stream must be maximized (similar to oil refineries) (Agler et al., 2011), and such waste treatment creates an opportunity to generate additional fuels or chemicals (i.e. bioproducts), while simultaneously recycling nutrients and water. Processing steps within biorefineries, such as chemical/physical pretreatment, enzyme production, and fermentation and extraction steps, all create large volumes of wastewater that must be treated.

The two best-known biorefinery platforms are

- the sugar platform, in which purified enzymes convert biomass into five- and six-carbon sugars as intermediate feedstock chemicals that are converted further by, for example, fermentation to fuels; and
- the syngas platform, in which thermochemical systems convert biomass into syngas (i.e. synthesis gas, such as CO, H_2, and CO_2) as feedstock chemicals that are converted further by, for example, catalysis to fuels (National Renewal Energy Laboratory: www.nrel.gov/biomass/biorefinery.html).

Recently a third type of platform – the carboxylate platform has been introduced. It envisions to convert organic feedstocks, which are often derived from industrial and agricultural wastes, to short chain carboxylates as intermediate feedstock chemicals, using hydrolysis and

fermentation with undefined mixed cultures in engineered systems under anaerobic conditions (Agler et al., 2011).

The fermentative production of carboxylates particularly from side streams holds the promise of becoming a cost-effective substitute for the present extraction-based and petrochemical sourcing. This approach would not only cope with the scarcity of feedstock, it would even add value to various kinds of fermentable waste streams whilst securing sourcing. For instance in the starch processing industry the majority of side streams go into animal feed manufacturing, whereas another part is used in biorefineries to produce e.g. bioethanol. However, many components present in today's animal feed do not play any functional role for the animals, as is the case for some carbohydrates. Similarly, during fermentation of bioethanol, part of the biomass is not fermented (e.g. cellulose and hemicellulose) and ends up as waste. Hence the non-functional components (as present in animal feed derived from biorefineries) and waste with fermentable sugars should preferably be valorized by fermenting them into e.g. carboxylates that in turn can be supplemented into animal feed to optimize the microbial flora in livestock (substituting the controversial usage of antibiotics). Or they can be further converted in a second (bioprocess) stage into bio-electricity, bioplastics, biosurfactants, etc. This concept will be applied in Water4Crops for the production and recovery of carboxylates, the conversion of carboxylates in the respective alcohols and their recovery.

The feasibility of applying anaerobic digestion as a first treatment step to BRWs has been demonstrated in literature. While process instability and accumulation of intermediate fermentation products, i.e. volatile fatty acids (VFA) is not desirable in regular anaerobic digestion, it can be exploited for the production of VFA as bulk chemicals according to the carboxylate platform approach.

3. Process schemes of selected bioethanol plants

In the Alco Bio Fuel plant, various cereals are used as starting materials, depending on availability and market prices. Wheat, for example, is first ground in a roller mill, and then fed into a mashing system, where it is mixed with water and the enzyme alpha-amylase. It then passes through cookers, where the heat liquefies the starch and the enzymes begin the process of breaking it down into sugars. The mash is then cooled and pumped to a fermentor.

The production of bio-ethanol involves three steps: fermentation, distillation, and dehydration. The industrial fermentation at Alco Bio Fuel is a batch process, producing 150 000-m³ bio-ethanol per year. The fermentation residue is an additional major output of the plant: 130,000 tons of DDGS (Dried Distillers Grains with Solubles), a protein rich animal feed product. Further processing occurs in continuous mode. The distillation process is used to concentrate and purify the alcohol stream. The last phase in the process is dehydration where the remaining water is removed to produce 'anhydrous' alcohol.

The overview scheme is given in Figure 3. The main products of the plant are bioethanol and DDGS. In this zero liquid discharge plant, all waters are reused in the process.

Figure 3 Production and waste(water) treatment concept at Alco Bio Fuel, Belgium (www.alcobiofuel.com).

Another bioethanol plant in Belgium is BioWanze. The facility in Wanze has a production capacity of 300.000 m³ bio-ethanol per year, obtained from 800 000 ton of wheat and 400 000 ton of sugar beet. Nearly all wheat components are used and valorized in the production process. The main products are bio-ethanol, Gluten and ProtiWanze®. The process is schematically represented in Figure 4.

In a first phase, bran is separated from the wheat. For maximal resource utilization, the bran is recovered and combusted in a cogeneration unit, which provides in most energy requirements of the production unit. This makes the site almost self-sufficient for energy and led to minimization of energy costs. This unique concept also allows to reach 70% reduction in CO_2-emissions compared to use of fossil fuel.

BioWanze also has a unique and innovative industrial concept for gluten recovery. After the milling step, the proteins are separated from the starch. The insoluble protein fraction, known as gluten, is extracted. Gluten are separated by mixing the meal with water to form a mash, which is then transported under pressure to obtain the specific elastic structure of gluten. The different components are then separated by centrifugation. The gluten fraction is dried to the desired moisture content and used as human food and animal feed additive or in aquaculture (distributed by BENEO-Orafti as BeneoPro W).

In a next phase, enzymes are added for liquefaction of the starch. Then, bioethanol is produced in a yeast fermentation. The liquid fraction is subjected to distillation and rectification to recover the ethanol, which is then further dehydrated. The Concentrated Distillers' Solubles (CDS), essentially a wheat yeast concentrate of 28% dry matter content, are applied for animal feed (ProtiWanze®), because of their high protein and energy content. CDS production exceeds 200.000 ton per year.

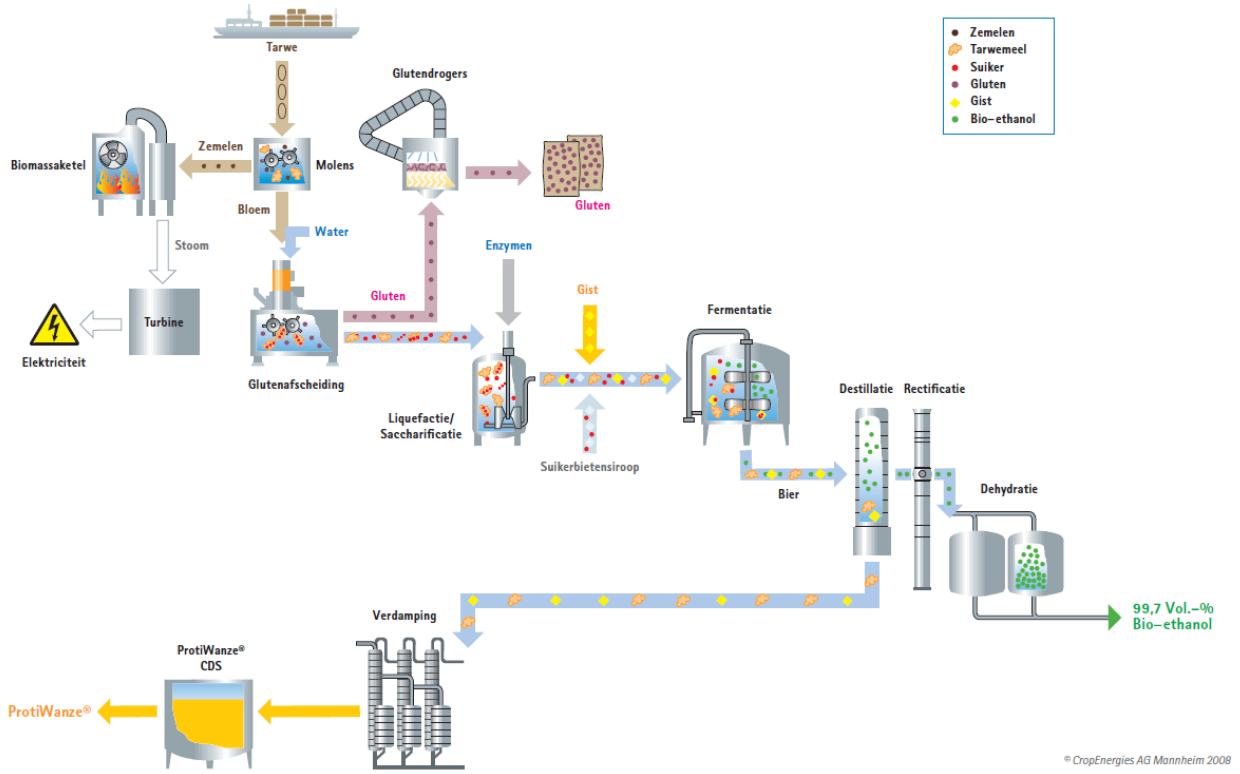

Figure 4 Schematic representation of process scheme at BioWanze, Belgium (www.biowanze.be).

4. Choice of waste stream for VFA production

Wastewater from particularly molasses based bioethanol plants contains compounds which may be inhibitory for an anaerobic treatment step. As failure of anaerobic digestion threatens the criteria for discharge, the wastewater is either diluted prior treatment or an additional treatment step is included. This implies large amounts of water for dilution and expansion of the reactor volume (Pant et al., 2007). In our work, inhibition may be less of an issue because the thin stillage will be taken from a cereals-based bioethanol plant. In any case, we preferentially want to use a concentrated stream without any dilution to ensure maximal VFA concentration levels. In view of separation and recovery of the organic acids from a complex wastewater stream, and their further valorization, the product concentration should indeed be as high as possible. Evaluation of literature data shows that VFA yields on substrate COD are ranging between 0.1 and 0.6. A high substrate COD is thus an important parameter to maximize VFA levels.

In bioethanol plants, wastewater is often treated anaerobically in a first stage. So in principle it is suitable for production of VFA as well. However, the COD levels are often too low to generate VFA concentrations in an appropriate range for product valorization. The thin stillage streams would therefore be more suitable. Thin stillage has a COD of 100 000 mg/l and more. It contains C5 sugars which were not used by the yeast for ethanol production and are a suitable substrate for conversion into organic acids. However, this will reduce the amount of DDGS or CDS and thus has to create sufficient added value compared to the current valorization route.

5. Optimization of VFA production on BRW

The aim of our work is to maximize VFA production from BRWs, by varying a number of operational parameters. In preparation of continuous reactor tests, a batch test was set up to check various inocula for their capacity to produce organic acids and to evaluate the effect of various ratios of substrate to inoculum on the acid production potential.

5.1 Batch test

5.1.1 Experimental set-up

Substrate and inocula were added to 160 ml vials according to the amounts given in Table 3. Volumes were adjusted to 100 ml with distilled water. The pH in the bottles was adjusted to 5. Bottles were closed with rubber caps, flushed with

The substrate was thin stillage. The 3 inocula were the following:

1. granular activated sludge from a potato processing company
2. heat pretreated granular activated sludge from a potato processing company
3. inoculum from an acidogenic fermentor converting vegetable waste as a substrate.

Inoculum 1 was 3 times washed with 20 mM potassium phosphate buffer at pH 5, and sieved with a mesh of 500 µm. Part of the washed inoculum 1 was boiled in water for 15 min and constituted inoculum 2.

5.1.2 Results COD_{total} and $COD_{soluble}$

Conform expectations, increasing substrate levels lead to increasing total and soluble COD levels (Figure 5). For the conditions in which 0.5, 2.5 or 5 g of feed was used, the inoculum made up a major fraction of the total COD. Only at 25 or 50 g levels, the contribution of the substrate was more important. Total COD levels for inoculum 2 (ratio 50:5) and soluble COD levels for inoculum 1 (ratio 50:5) were not in line with the applied quantities, probably as a result of analytical or sampling error.

As a result of substrate hydrolysis, the soluble COD is expected to increase over time. This was apparently only the case for the highest substrate to inoculum ratio of 50:5.

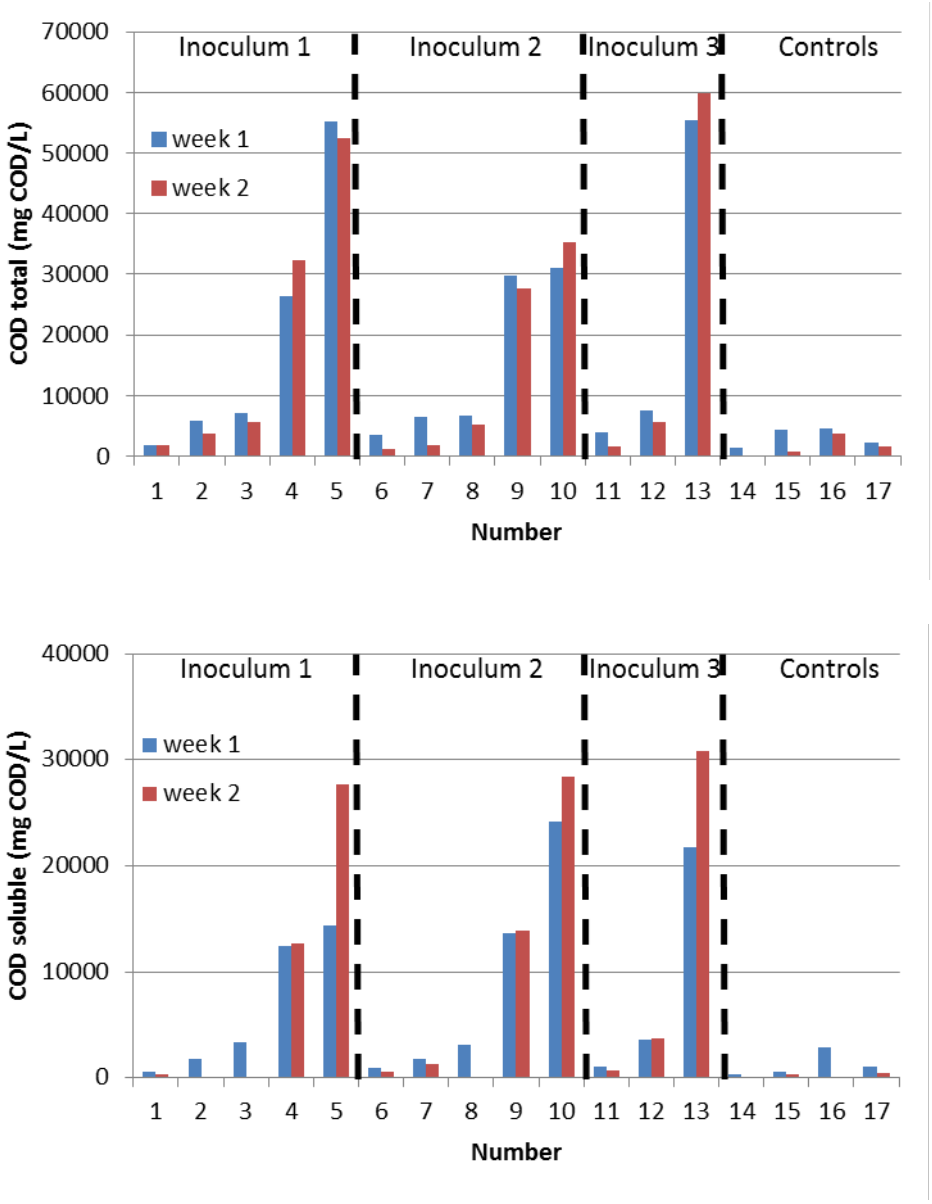

Figure 5 Total and soluble COD after 1 and 2 weeks of incubation.

5.1.3 Results acid production

Figure 6 shows that the total VFA production increased with increasing ratios of substrate to inoculum. For the conditions with a substrate to inoculum ratio higher than 5:5, total VFA production was higher than in the control containing the feed. Furthermore it was noticed that prolonging the incubation time did not significantly enhance the VFA production as the total VFA concentration only slightly increased or even decreased in some cases. VFA production was

negligible in the controls consisting of solely the inoculum. The dominating VFA products for all conditions were acetate and butyrate. Propionate, isobutyrate, isovalerate and caproate were lower than 10%, except for a few cases.

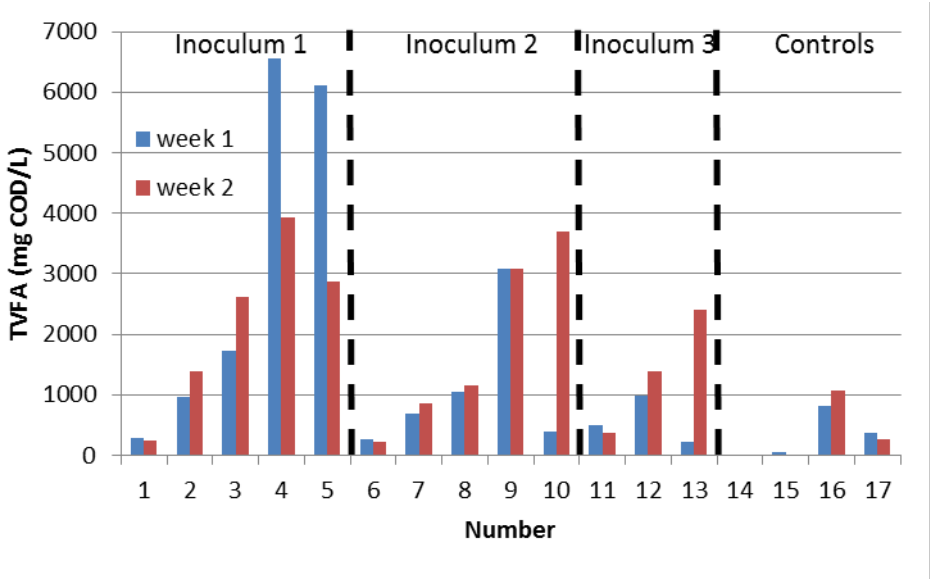

Figure 6 Total VFA production after 1 week and 2 weeks of incubation.

Higher total VFA concentration was obtained when granular activated sludge from a potato processing company was used as inoculum (inoculum 1 and 2). Surprisingly, the heat treatment which is meant to inhibit methanogenesis and stimulate VFA production, did not have this effect. Probably, the initial pH of 5 was low enough to prevent methanogenesis.

Inoculum 3 gave the lowest VFA levels. The latter was obtained from a fermentor that had converted a mixture of vegetables, and was apparently less suitable for the conversion of thin stillage substrate.

The highest VFA production (6.6 g COD/L) was achieved when granular activated sludge was inoculated at a ratio of substrate to inoculum of 25:5 (number 4). When using heat pretreated activated sludge at the same ratio (number 9), the total VFA concentration was lower (3.1 g COD/L) but the fermentation was directed towards butyrate (Table 4).

Table 4 Concentration of products and fractions (in brackets) after 1 week of incubation.

Products	Substrate:Inoculum 1 25:5	Substrate:Inoculum 2 25:5
Acetate (mg COD/L)	1442 (22%)	296 (10%)
Butyrate (mg COD/L)	3732 (57%)	2795 (90%)
Caproate (mg COD/L)	793 (12%)	0 (0%)
TVFA (mg COD/L)	6550	3091
pH	4.52	4.67
CO_2 (mM)	179 (54%)	110 (33%)
H_2 (mM)	57 (17%)	63 (19%)
CH_4 (mM)	2 (0.6%)	0 (0%)
N_2 (mM)	63 (19%)	139 (42%)

VFAs occurring in fractions lower than 10% are not shown.

Figure 7 illustrates that the pH decreased when VFAs were produced. The pH drop was higher as the total VFA increased. For the conditions where VFA production was negligible (number 1, 6, 11, 14, 15 and 17), pH increased probably due to the release of NH_4^+ which results from the hydrolysis of biomass.

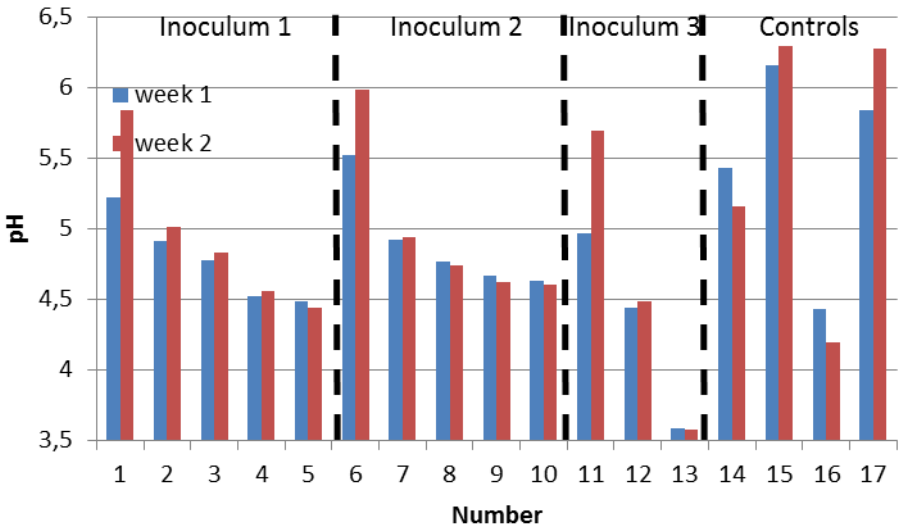

Figure 7 pH change after 1 week and 2 weeks of incubation.

5.1.4 Results gas production

Figure 8 shows that gas production mainly occurred during the first week of incubation. The highest gas production was measured with the granular activated sludge from a potato processing company (inoculum 1 and 2) at a substrate to inoculum ratio higher than 5:5, when also the highest acid production was measured. Under these conditions, mainly CO_2 and H_2 were produced. Conform expectation, small amounts of CH_4 (0.5-2.1%) were only present when using untreated granular activated sludge as inoculum. However, they are negligible.

The headspace composition and gas concentrations for the substrate to inoculum ratio with the highest VFA concentration (number 4) and the most favorable VFA spectrum (number 9) are given in Table 4.

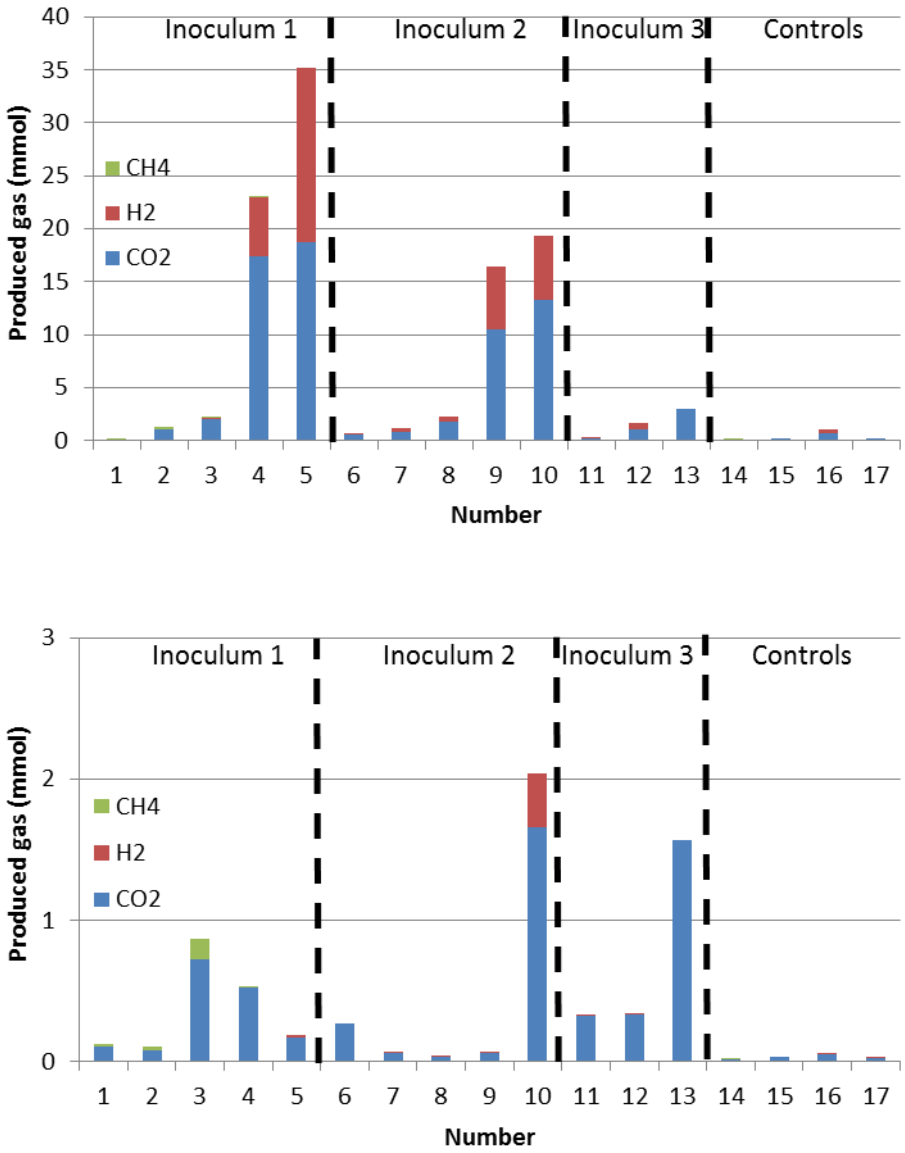

Figure 8 Produced gas in the headspace (mmol) after 1 week (a) and 2 weeks (b) of incubation. Scales are different in each graph.

5.1.5 Conclusions

It can be concluded that

- the results of acid production are in line with those of gas production
- one week of incubation is sufficient for obtaining high VFA concentration
- inoculum 1 is the most suitable one for VFA production on this substrate

- heat pretreatment of the inoculum is not needed to prevent methanogenesis, when pH is low enough. However it leads to a more favorable product spectrum, with a high fraction of a single compound (90% butyrate)
- the optimal substrate to inoculum ratio is 25:5.

Based on the above, inoculum 2 and a substrate to inoculum ratio of 25:5 were used for the continuous reactor test.

5.2 Continuous reactor test

The aim of the continuous test is to vary a series of operational parameters in view of maximal VFA production. Because the feed is highly concentrated, very long hydraulic retention times (HRT) have to be applied not to overload the system. This implies that long time periods are required to reach steady state (typically 3 to 5 HRT) and that it take quite long to evaluate the impact of various parameters on the VFA production and composition. As a result the tests were still running when this report was compiled and the interpretation of results should be considered as a provisional one.

5.2.1 Materials and methods

Inocula and waste stream

Granular activated sludge from a potato processing company was selected as inoculum. It contained 6% TS and 75% VS content, and had almost no (<1%) inorganic carbon content. The granular sludge was washed with 20 mM potassium phosphate buffer (at pH 5) and sieved with a mesh of 500 µm for three times. After the last washing step, the granules were left in the buffer overnight in room temperature. The next day, granular sludge was heat pretreated by boiling in water for 15 min to avoid methanogenesis.

As carbon source, the same thin stillage was used as in the batch test. One single batch was collected and divided into smaller volumes which were stored at -20°C.

Experimental set-up

A 3-L jacketed glass reactor (Applikon, The Netherlands) was filled with 1 L of 20 mM potassium phosphate buffer with pH 5.0. The bioreactor was inoculated with 200 g of pre-treated granular activated sludge and flushed with N_2 during 20 min to remove any residual oxygen. Then the headspace was connected to a volumetric gas counter (Milligas counter, BnC-Ritter) working under atmospheric pressure. The reactor was kept at 30°C by hot water circulation and mixed at 250 rpm. The reactor pH was initially not controlled. When the pH dropped under its set-point value, the pH was adjusted by a pH-stat dosing 2 M NaOH. During the start-up phase, feed was added discontinuous with a flow rate of 0.09 L/d into the bioreactor corresponding to organic loading rate

of 5.0 kg COD/m³.d. The hydraulic retention time (HRT) of the reactor was 22 d. When a working volume of 2 L was reached, the bioreactor was operated in continuous mode. The outflow of the reactor was controlled by a level switch but with 2.5h of time delay compared to influent dosing. The feed tank was kept at 10°C to avoid that fermentation already started inside the tank and was continuously stirred to prevent settling of particles. Every two weeks, the feed was replaced with fresh feed. Gas and liquid samples were taken biweekly for analysis.

Analysis

The biogas was sampled through rubber septa using a gas-tight plastic syringe and analyzed in a gas chromatograph (Trace GC Ultra) equipped with thermal conductivity detectors (TCD). Hydrogen (H_2) content was analyzed with a 2 m stainless steel column packed with molecular sieve 5A (80/100 mesh) using nitrogen as carrier gas at the flow rate of 20 ml/min. Methane (CH_4), carbon dioxide (CO_2), oxygen (O_2) and nitrogen (N_2) in the biogas were separated with 2 m stainless steel HayeSepQ (80/100 mesh) and molecular sieve 5A (80/100 mesh) columns connected in series with a valve. Helium was used as the carrier gas at a flow rate of 15 ml/min.

Liquid samples were centrifuged for 15 min at 7000 rpm, then filtered over 0.45 µm to remove particles before being analyzed.

For analysis of volatile fatty acids (VFAs) samples were acidified with 1:1 (v/v) H_2SO_4 solution and then extracted with diethyl ether. VFAs were analyzed in a GC (CE Instruments-Thermoquest) equipped with a flame ionization detector (FID) and a 15 m AT-1000 filled capillary column (0.53 mm x 1.2 µm). Helium was used as the carrier gas at a constant flow of 6 ml/min. Acetone, ethanol and propanol were analyzed in the headspace of the samples at 60 °C with the same GC fitted with an AT-WAX capillary column (60m x 0.32mm x 1.00µm). The carrier gas was helium and its flow rate was 1.6 ml/min. Lactate was analyzed with a GC (Interscience, CE Instruments-Trace GC) equipped with FID detector, split/splitless injector and AT-1 capillary column (30m x 0.53mm x 5µm column). Samples were treated with $Ce(SO_4)_2$ and warmed up 60°C for 10 min to convert the lactic acid into acetaldehyde which was then analyzed in the headspace.

The Phenol-sulfuric method (Dubois et al., 1956) was used to determine for carbohydrate content, the Total Kjeldahl Nitrogen (TKN) method with conversion factor 6.25 (Pierce and Haenisch, 1948) for protein content. Total and volatile solid contents were analyzed as described in Standard Method

(APHA, 1995) by drying at 105° and 550°C. Total Chemical Oxygen Demand (COD_T), total N and total P were determined with cuvette tests of Hach Lange.

Soluble COD (CODs), PO_4-P and NH_4-N concentrations were analyzed with Hach Lange kits after samples were passed through a 0.45 µm filter.

Calculations

Formulas for the different reported parameters are given below.

Table 5 Summary of events during the continuous test.

Day	Date	Event
1	15/05/2013	Start experiment with pH set point 5.3.
8	22/05/2013	Feeding at 2 x 45 ml/day.
16	30/05/2013	Change in pH set point from 5.3 to 5.0. Flushing of the bioreactor.
23	06-11/06/2013	Blockage of the effluent pump on several occasions.
42	25/06/2013	Change of temperature of feed tank from 10°C to 2.5°C.
90	12-14/08/2013	Blockage of the effluent pump on several occasions.
100	22/08/2013	Adjustment headspace pressure to 50 mbar.
101	23/08/2013	Blockage of the effluent pump.
104	26/08/2013	Increase in organic loading rate by decreasing the hydraulic retention time from 22 to 15 d. Feeding at 3 x 45 ml/day.
106	28/08/2013	Adjustment headspace pressure to 200 mbar.
108	30/08/2013	Blockage of the influent pump.
113	04/09/2013	Change of septum for gas sampling. Adjustment headspace pressure to 50 mbar.
114	05/09/2013	Blockage of the influent pump.
119	10-11/09/2013	Several adjustments of headspace pressure to 200 mbar .
128	19-20/09/2013	Blockage of the effluent pump on several occasions.
142	03/10/2013	Bioreactor not operative due to 8h power failure.

5.2.3 Influent

The overall composition of the influent remained fairly constant throughout the experiment. About 53% of the total COD was present in soluble form. Carboxylates were already present in the influent. Although each batch was derived from one master-batch and was kept at 6°C to avoid that fermentation already started inside the tank, the carboxylate concentrations increased. This caused a gradual pH decrease from 5.0 at the beginning of a batch to (on average) 4.0 at the end of a batch.

Lactate constituted a major fraction of the acids. Conform expectation, ethanol was the main alcohol product.

5.2.4 Effluent

TS and VS of the effluent increased with decreasing HRT (Figure 9). The protein and sugar content were rather constant throughout the experiment. As expected, the COD_t increased when the organic loading rate increased. The COD_s remained on the other hand stable during the experiment (Figure 10).

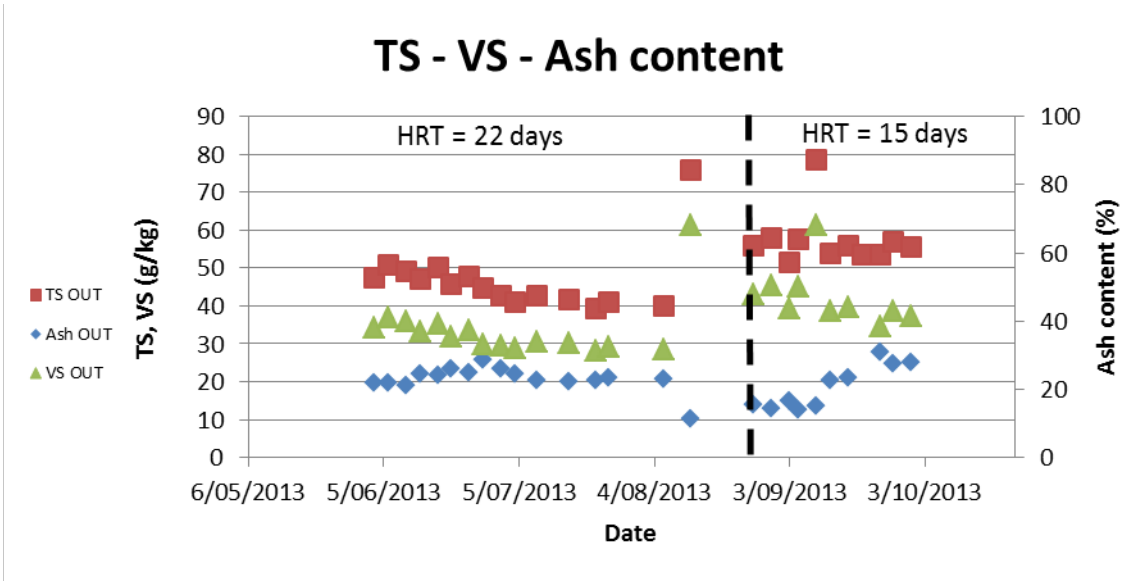

Figure 9 TS, VS and ash content of effluent as a function of time.

The total acid concentration fluctuated during the test (Figure 11). As can be seen from Figure 11 and Figure 12, the VFA concentration stayed constant during the first 34 days (till June 16th). Afterwards, lactate was consumed and VFA concentrations continuously increased. After 65 days (18/07/2013), VFAs were produced up to 25 g COD/L and lactate was removed by 3 g COD/L. Maximal VFA production thus coincides with lactate consumption. Butyrate was the main carboxylate product (Table 6) and its fraction increased in time. It has been described elsewhere that lactate consumption may lead to butyrate production (Arslan et al., 2013).

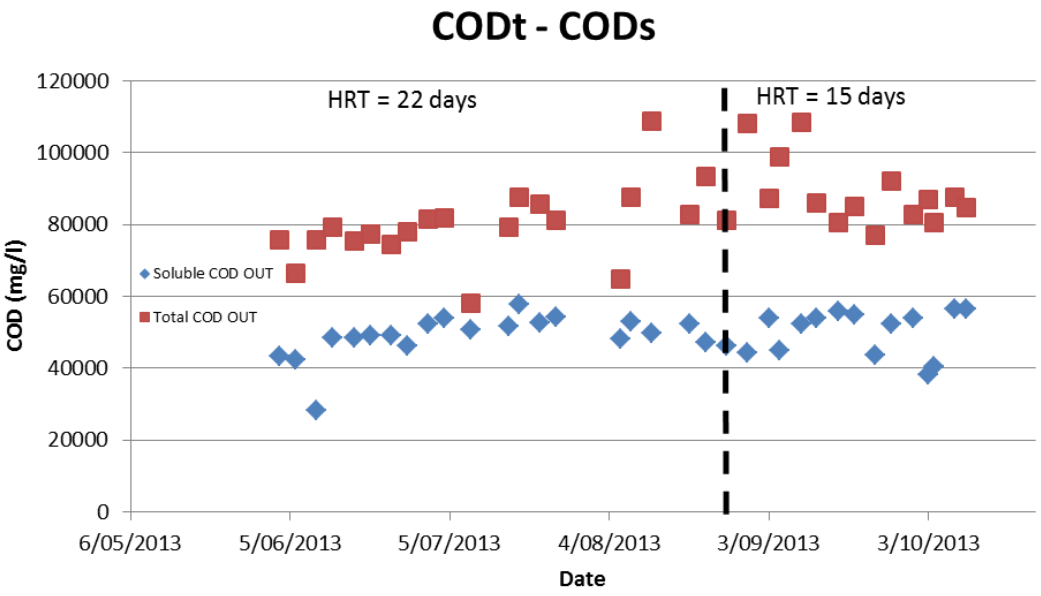

Figure 10 COD_t and COD_s of effluent as a function of time.

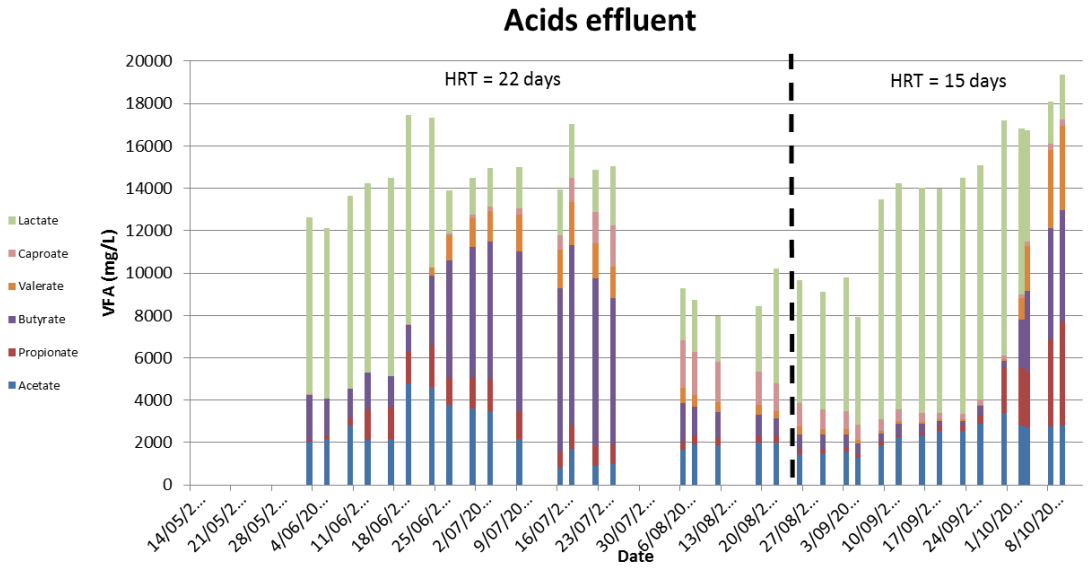

Figure 11 Concentration of acids in the effluent as a function of time.

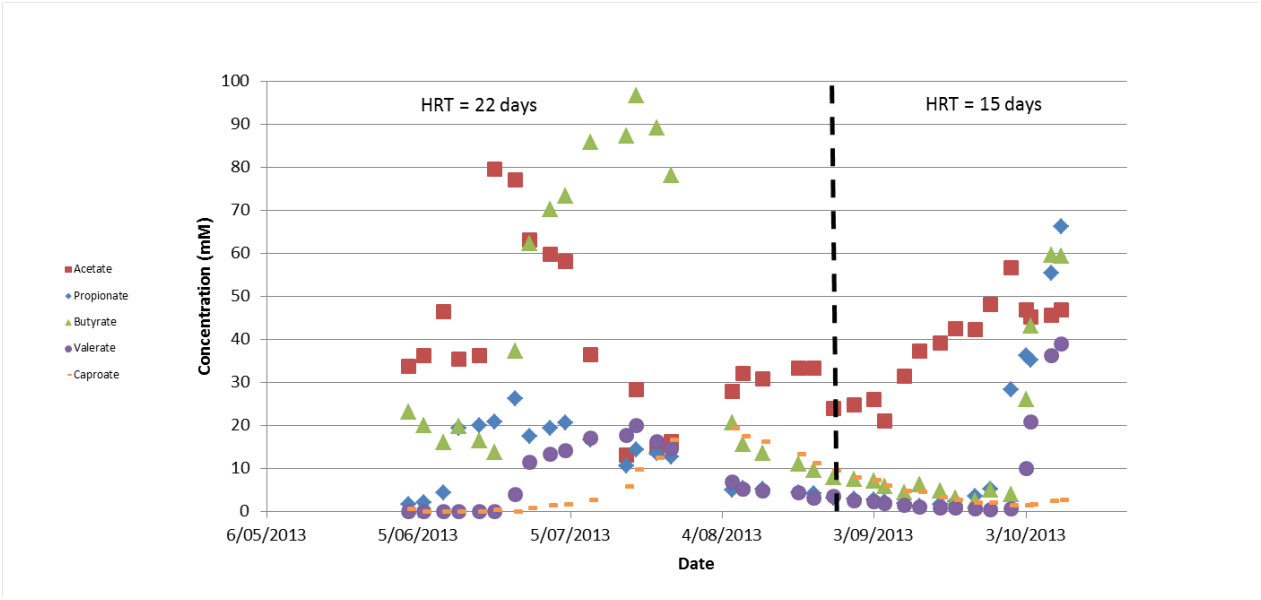

Figure 12 Concentration of acetate, butyrate, valerate and caproate in mM in the effluent as a function of time.

Prolonged HRTs and operating time may lead to the production of longer chain acids. This is also apparent from the gradual appearance of (i-)valeric acid as the second most important VFA product, and at a later stage caproate. Microorganisms can use VFA as electron acceptor and hydrogen or ethanol as electron donor to generate medium chain fatty acids such as valerate and *n*-caproate (Ding et al., 2010; Steinbusch et al., 2011). These reactions are described as:

$acetate^- + 2\ ethanol \rightarrow 2\ n\text{-}caproate^- + 2H_2O$ $\Delta G°'_r = -79$ kJ/mol (R1)

$3\ acetate^- + 2H^+ + 4H_2 \rightarrow n\text{-}caproate^- + 4H_2O$ $\Delta G°'_r = -177$ kJ/mol (R2)

$acetate^- + n\text{-}butyrate^- + H^+ + 2H_2 \rightarrow n\text{-}caproate^- + 2H_2O$ $\Delta G°'_r = -48$ kJ/mol (R3)

$propionate^- + ethanol \rightarrow n\text{-}valerate^- + H_2O$ $\Delta G°'_r = -39$ kJ/mol (R4)

$propionate^- + acetate^- + H^+ + 2H_2 \rightarrow n\text{-}valerate^- + 2H_2O$ $\Delta G°'_r = -48$ kJ/mol (R5)

Figure 12 shows that butyrate and valerate concentrations increased when acetate and propionate levels drop. Ethanol was consumed during this period (not shown). Hence, valerate production may have occurred via the condensation of propionate and acetate (R5) or the conversion of propionate and ethanol (R4). From 18/07/2013 on, caproate production increased and it coincided with butyrate consumption. Because ethanol was not consumed, reaction R1 most probably did not take place. Therefore, it is assumed that caproate was formed from acetate (R2) or acetate/butyrate (R3). Such conversions should be accompanied with hydrogen consumption. As discussed later, this was not the case as hydrogen was actually produced in this stage. This can be explained as follows. The hydrogen increase associated with butyrate production (reaction R6 and R7) probably more than compensated the hydrogen consumption associated with butyrate conversion into caproate.

glucose → *n*-butyrate$^-$ + H^+ + $2H_2$ + $2CO_2$ $\Delta G°'_r$ = -266 kJ/mol (R6)

2 lactate$^-$ + H^+ → *n*-butyrate$^-$ + $2H_2$ + $2CO_2$ $\Delta G°'_r$ = -209 kJ/mol (R7)

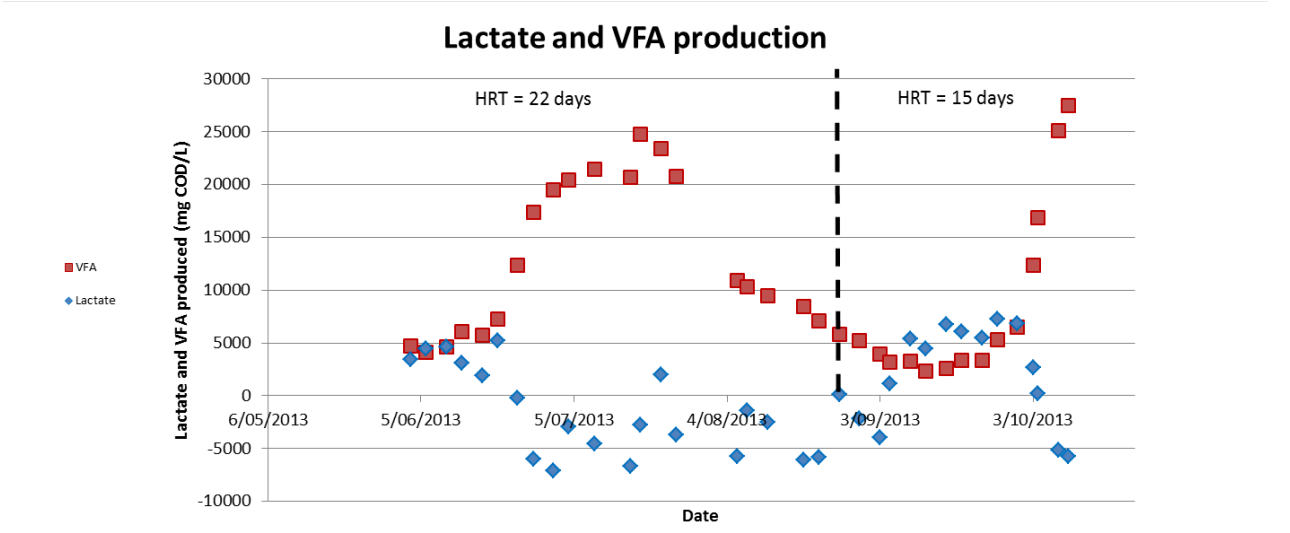

Figure 13 VFA and lactate production as a function of time. Negative values indicate consumption of compounds.

From the end of July onwards, total acid concentration gradually decreased and then remained stable at a lower level of 8 g/L. Decreasing the HRT from 22 to 15 d, and hence increasing the organic loading rate from 5 to 7.5 g COD/L.d on day 104 (26/08/2013), did not have an immediate effect on acid production until day 135 (26/09/2013), when the total acid concentration started to increase again. After day 149 (10/10/2013), VFAs were produced up to 27.5 g COD/L and lactate was removed by 5.7 g/COD L. This time, there was no clear major VFA product. Butyrate, propionate and valerate were present in comparable fractions (Table 6). Although the formation of valerate is coupled with the consumption of propionate (R4 and R5), both acid levels increased (Figure 12). Propionate formation from glucose (R8) probably more than compensated the propionate consumption associated with valerate production (R4 and R5). Acetate levels did not vary much during the whole test period. When a maximum of VFAs were produced, the total acid concentration, expressed in g COD/L, made up about 53% of the soluble COD in the effluent, while this was only about 16% for the influent. The maximum total VFA level reached was higher at the higher organic loading rate.

The pH was adjusted to 5.0 by dosing 2 M NaOH (Figure 15). After 38 and 140 days, a significant pH increase was observed which was probably caused by hydrolysis of proteins, as evidenced by liberation of NH_4 ions (Figure 14). During this period, the concentration of VFAs also increased. A sudden pH increase was also observed after 78 days (31/07/2013) due to an unknown reason. However, it did coincide with reduced NH_4-release and VFA formation.

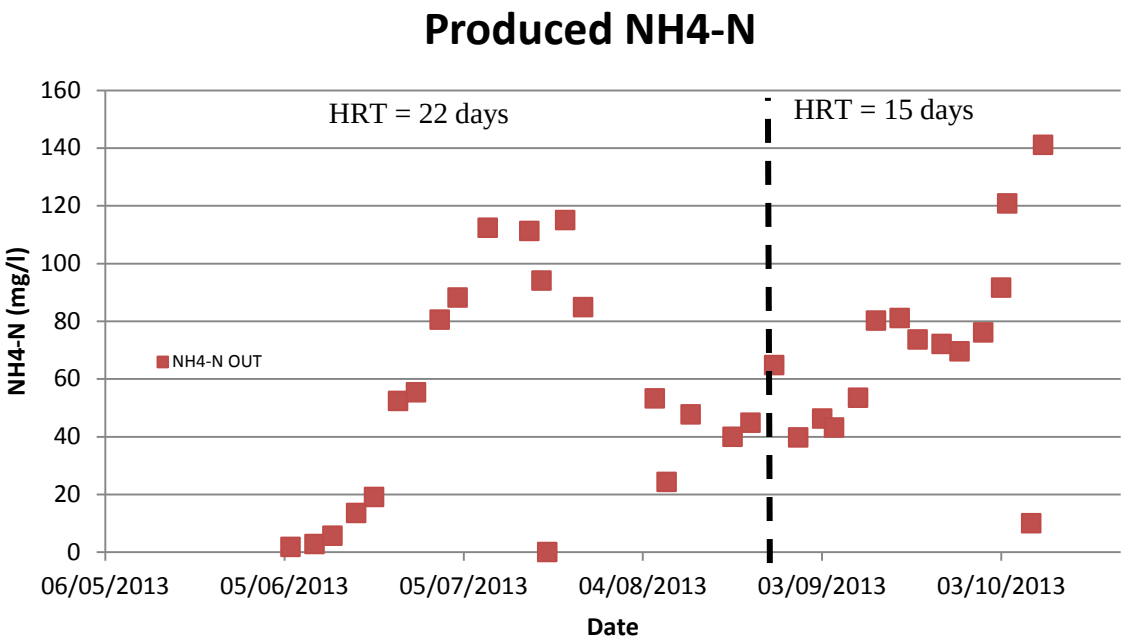

Figure 14 N-NH_4 production as a function of time.

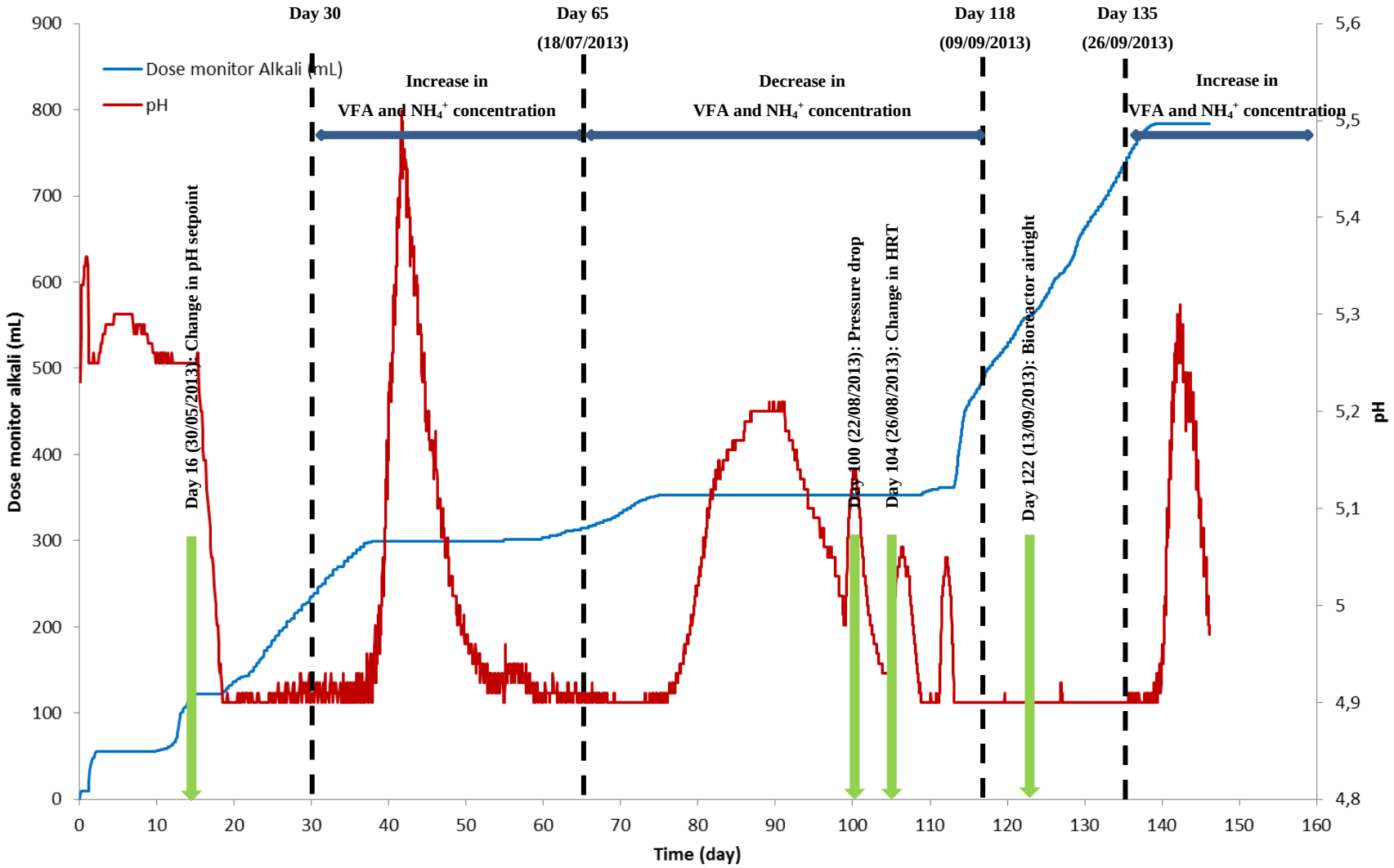

Figure 15 The amount of added alkali (blue line) and pH change (red line) as a function of time.

Table 6 Mean concentrations with standard deviation and fractions of the acids in the effluent when VFA concentration was > 8 g/L.

	Period 24/06/2013 to 25/07/2013			Period 25/07/2013 to 10/10/2013		
Composition	Concentration (mg COD/L)	TVFA (%)	Total acids (%)	Concentration (mg COD/L)	TVFA (%)	Total acids (%)
Acetic acid	2697 (± 1600)	13	11	3050 (± 55)	14	11
Propionic acid	1876 (± 528)	9	8	5370 (± 1687)	24	20
i-Butyric acid	71 (± 79)	0	0	239 (± 139)	1	1
Butyric acid	11990 (± 2832)	57	50	7470 (± 2525)	34	28
i-Valeric acid	175 (± 96)	1	1	104 (± 209)	0	0
Valeric acid	2927 (± 946)	14	12	5422 (± 2775)	24	20
i-Caproic acid	< D.L.[a]	0	0	< D.L.	0	0
Caproic acid	1477 (± 1528)	7	6	545 (± 175)	2	2
Lactic acid	2944 (± 1854)		12	5511 (± 3227)		20
TVFA	21213 (± 3097)			22201 (± 7267)		
Total acids	24158 (± 2214)			26917 (± 4280)		

[a]D.L., detection limit.

5.2.5 Gas phase

The headspace of the bioreactor was analyzed for CO_2, N_2, H_2, CH_4 and O_2 (Figure 16). CO_2 was the main gas, followed by H_2. From 04/06/2013 and 03/09/13 onwards, hydrogen concentration in the headspace dropped, potentially due to propionate production, a conversion which is accompanied by H_2 consumption (R8).

glucose + 2 H_2 → 2 propionate$^-$ + 2 H^+ + 2 H_2O $\Delta G°'_r$ = -364 kJ/mol (R8)

After 63 days (16/07/2013), H_2 concentration increased. This could be linked to the conversion of sugars to butyrate (R6) or the conversion of lactate to butyrate (R7) since these reactions are accompanied with H_2 formation. CH_4 was not detected, indicating proper inhibition of methanogenesis.

Over the whole test period, only 173 mL of gas was produced. After 84 days (06/08/2013) no more gas production was observed.

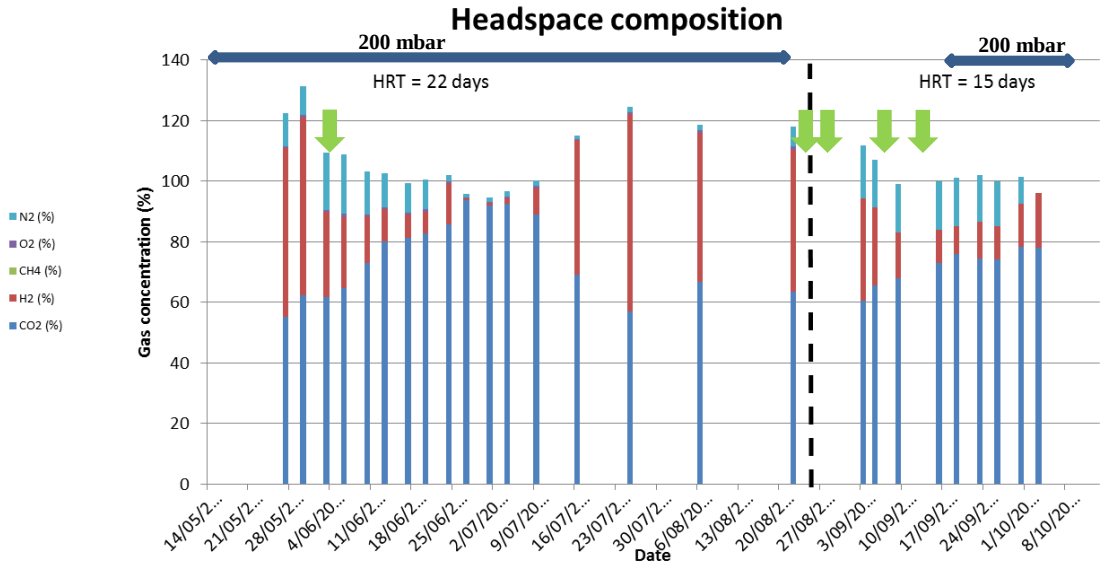

Figure 16 Fraction of gases as a function of time. Green arrow indicate bioreactor headspace pressure adjustment.

5.2.6 Conversions

Overall COD_t removal was 21% and COD_s was converted by 9%. The average degree of hydrolysis was 47%, with levels increasing from 27 to 57%. Sugars were rather well removed (57%). The COD recovery equalled 80%, which is acceptable for organic waste substrates. TS dropped by 23%, probably due to hydrolysis of organic compounds and accumulation in the fermentor and VS was reduced by 31%. As can be seen in Figure 17, these parameters remain rather stable during the experiment and seem to be unaffected by the applied HRT and organic loading rate.

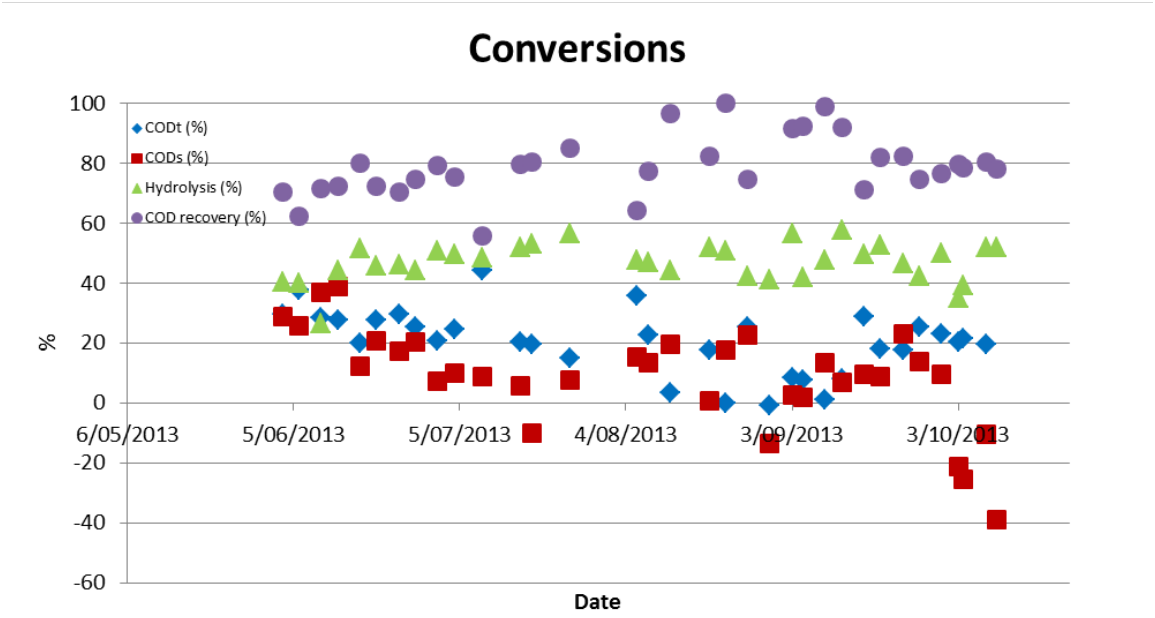

Figure 17 COD_t and COD_s removal, hydrolysis and COD recovery as a function of time.

Maximum total VFA levels are 25 g COD/L. Compared to the incoming COD, this corresponds to a yield of about 25%, which is quite acceptable. Literature review of studies reporting VFA levels obtained from organic waste streams indicate yields between 10 and 50%.

The course of VFA and NH_4^+ production shows a similar pattern at the 2 organic loading rates. After some adaptation period, a sudden pH increase occurred coinciding with ammonia release from proteins. From then onwards, VFA concentrations started to increase with concomitant consumption of lactate. At the lowest organic load, the VFA levels never exceeded 25 g COD/L, and remained at that level for about 1 month. It seems that VFA production stopped at that point

and that VFAs were gradually washed out. In Figure 18 the dilution effect due to the addition of feed on the carboxylate concentration is shown. Propionate and valerate were indeed washed out after maximum VFA levels were reached due to simple dilution. For caproate the maximum level was reached at a later stage than for the total VFA and the dilution effect started later (Figure 18). Acetate and butyrate levels behaved differently (not shown), probably as a result of further production and/or conversion.

Production of VFAs probably ceased due to the inhibition of the produced acids once a certain level was reached. From literature, it indeed appears that VFA levels above 20 g/L are only seldom reported, typically only for systems where lime addition is applied increasing the alkalinity in the system. Because also the ammonia levels peaked and dropped very soon when high VFA levels were obtained in our tests, protein hydrolysis seems to have been inhibited as well.

When a higher organic loading rate was applied, the same pattern was observed as before, except that slightly higher VFA levels were obtained with a quite different composition. Probably, acid levels had to be reduced after the first peak down to sufficiently low levels to remove the inhibitory effect on protein hydrolysis and VFA production and reinitiate hydrolysis and acidification.

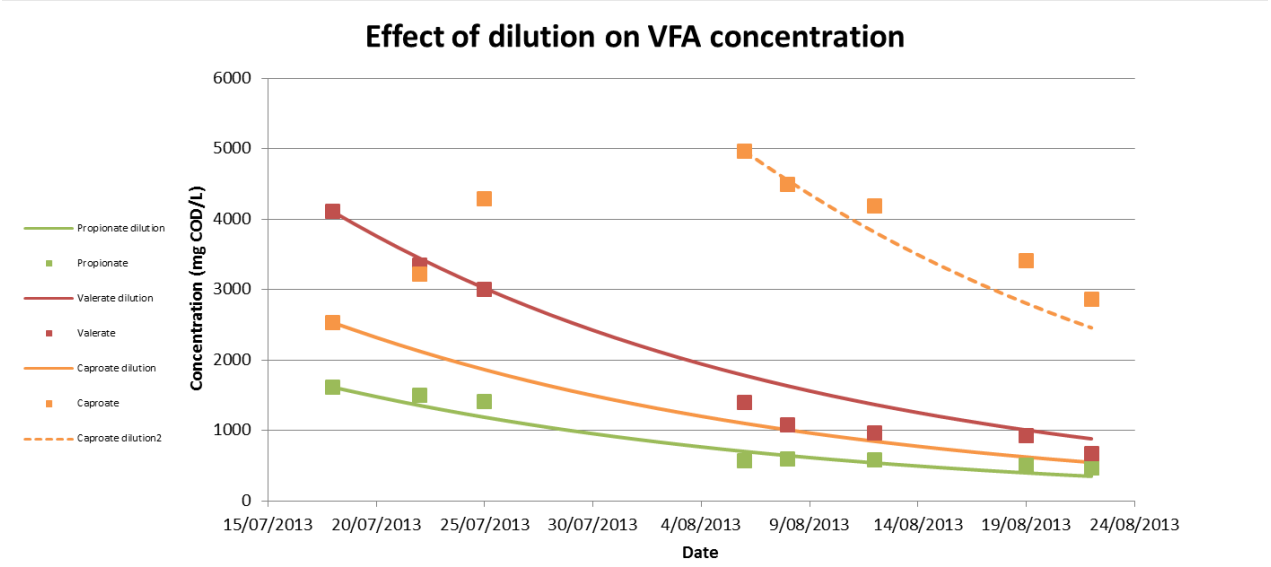

Figure 18 Effect of dilution on VFA concentration and measured VFA concentration in effluent as a function of time.

When the maximum level of acid concentration was reached, the total undissociated acid concentration was 70 to 80 mM (or 6 to 7.2 g/L). Thus about 35% of the acids were present in the undissociated form. A pH set-point of 6.0 would reduce this fraction to 5%, which would significantly lower the inhibitory effect. However, this goes at the expense of higher alkali additions. Alternatively, coupling the fermentation with a separation technique that removes the organic acids could reduce such inhibitory effects and increase the overall yields.

5.3 (provisional) Conclusion

VFA levels of 25-30 g COD/L have been obtained on undiluted thin stillage, but it remains to be confirmed whether such production levels can be maintained in a stable way, as inhibition effects seem to occur. VFA composition varies in time, with gradual production of longer chain fatty acids. A higher organic loading rate led to a more equal distribution of products, which may be undesirable from a product recovery and purification point of view.

The test will be continued with further variations in operational parameters to determine the maximal level of VFAs that can be reached and the stability of the process. Because the feed is highly concentrated, very long HRTs have to be applied not to overload the system. This implies long time periods to reach steady state (typically 3 to 5 HRT) and to evaluate the impact of various parameters on the VFA production and composition.

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