Universität für Bodenkultur Wien

Two-Stage Anaerobic Digestion of Industrial Organic Waste – Potentials and Bottlenecks

Investigations of two-stage anaerobic digestion of organic waste from the food and paper industry - olive mill solid waste, sugar beet pressed pulp and paper mill solid waste

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„Wissen ist die einzige Ressource, die sich bei Gebrauch vermehrt.“

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*The key note of this work is the word **peak***

mathematics: A local maximum of a function, e.g. for sine waves, each point at which the value of "y" is at its maximum.

Three peaks in particular are the current troublemaker in the world, causing severe discussions and make the politicians and economists to wrack their brains about it.

The first one is the oil peak,

the second one – the soil peak,

and last but not least – the water peak.

Now we are striving to find out, how to go further with less of these resources, without changing our lifestyle. The following work is meant to contribute to the global topic.

Abstract

Two-stage anaerobic digestion (AD) has been studied as a possibility to overcome the bottlenecks of the fermentation of industrial organic waste. The study was based on three examples: i) substrate containing slow degradable pectic substances, causing foaming and viscosity increase – sugar beet pressed pulp (SBPP); ii) substrate, containing high concentrations of inhibiting substances for the microorganisms accomplishing the AD process – olive mill solid waste (OMSW), and iii) substrate, containing high amount of mineral components and decreased dewatarability caused by residual organic polymers – paper mill sludge (PMS). These three examples cover vast area in the organic waste management in industries, having a substantial environmental impact.

The two-stage AD of SBPP brings several advantages. The faster degradation of the polymers in a separate pre-acidification step allows reduction of the overall hydraulic retention time (HRT), here from 50 to 36 days, and hence lower reactor volumes are required. The pre-acidification occurs the fastest at 55°C and a HRT of four days is enough to obtain the maximum accumulation of volatile fatty acids (VFAs). Also stable operation of a two-stage system was demonstrated. Foaming at higher organic loading rate (OLR), a frequent problem in AD of SBPP, can be avoided. Optimum pH levels can be maintained, both in the first stage and the second stage, without artificial pH adjustment. The tenfold decrease of the viscosity of digestate in the methanogenic stage of the two-stage fermentation decreases the energy input for the reactor stirring about 80%. The observed advantages make the two-stage process economically attractive, despite higher investments for a two reactor system.

Two-stage AD of two-phase olive mill solid waste (OMSW) was applied for reducing the inhibiting factors by optimizing the acidification stage. Single-stage AD and co-fermentation with chicken manure (CM) were conducted coinstantaneous for direct comparison. Degradation of the polyphenols up to 61% was observed during the methanogenic stage. Nevertheless the concentration of phenolic substances was still high; the two-stage fermentation remained stable at OLR 1.5 kgVS/m^3day. The buffer capacity of the two-stage system was twice as high, compared to the one-stage fermentation, without additives. The two-stage AD was a combined process – thermophilic first stage and mesophilic second stage, which pointed out to be the most profitable for AD of OMSW for the reduced HRT from 230 to 150 days, and three times faster than the single-stage and the co-fermentation start-up of the fermentation. The optimal HRT and incubation temperature for the first stage were determined to four days and 55°C. The performance of the two-stage AD concerning the stability of the process was followed by the co-digestion of OMSW with CM as a nitrogen-rich co-substrate, which makes them viable options for waste disposal with concomitant energy recovery.

Pre-treatment of pulp and paper mill sludge (PMS), hydrolytic enzyme pre-treatment and microbiological treatment, both at 30°C, was investigated to enhance the energy recovery in the anaerobic stage of the waste treatment plant. Two approaches were followed. In the first attempt, AD of the whole sludge, no significant improvement of the methane production potential was found. In the second test series only the liquid phase after pre-treatment and solids separation was anaerobically degraded. This option provided up to ten times increased methane production compared to the untreated sample. Sludge mass reduction between 6 and 13 % was achieved after pre-treatment. Moreover this concept can be easily integrated in the established wastewater treatment scheme utilizing an existing upflow anaerobic sludge blanket reactor.

The separation of the biochemical stages of the anaerobic digestion process in different fermenters, and providing the optimal operating condition for the relevant microorganisms led to faster start-up periods, higher organic loading rates, and lower hydraulic retention times. This process stability is achieved with higher investigation costs for the set-up of a pre-acidification fermenter, but saves the costs for keeping the ongoing process stable.

Zusammenfassung

Zweistufige anaerobe Systeme wurden auf ihr Potential, Probleme bei der anaeroben Fermentation von industriellen organischen Reststoffen zu überwinden, untersucht. Als Substrate wurden drei Abfallarten aus Industriezweigen mit einem hohen Umweltbelastungspotential gewählt. Oliventrester enthalten inhibierende Substanzen, Polyphenole, die die Mikroorganismen im anaeroben Reaktor hemmen und die Fermentation zum Stillstand bringen. Demgegenüber stellen Zuckerrübenpressschnitzeln ein Substrat dar, das langsam abbaubare Substanzen, wie Pektine, enthält und dadurch eine Erhöhung der Viskosität und Schaumbildung im Fermenter verursacht. Das dritte Fallbeispiel, Schlamm aus der Papierproduktion, ist ein organischer Abfall mit hohem Anteil an mineralischen Stoffen und eingeschränkter anaerober Abbaubarkeit.

Die zweistufige anaerobe Fermentation von Zuckerrübenpressschnitzeln führt zu schnellerem Abbau von den Pektinen, das die hydraulische Verweilzeit von 50 auf 36 Tage senkt und somit ein geringeres Reaktorvolumen erfordert. Die optimalen Bedingungen für die Vorversäuerung sind 55°C und vier Tage hydraulische Verweilzeit. Die Schaumbildung bei hoher organischer Raumbelastung konnte vermieden werden. Dazu führt die zehnfach niedrigere Viskosität im methanogenen Reaktor zu einer Reduktion des Energieverbrauchs des Rührsystems um 80%. Diese Vorteile machen die Installation eines zweistufigen System plausibel, trotz den hohen Investitionskosten.

In weiteren Untersuchungen wurde das zweistufige System zur Reduktion von inhibierenden Substanzen in Oliventrastern herangezogen. Als Vergleich wurde parallel ein einstufiges System sowie eine Kofermentation mit Hühnermist durchgeführt. Die Polyphenole wurden bis zu 61% in der methanogenen Stufe abgebaut. Trotz ihre hohe Restkonzentration blieb die zweistufige Fermentation auch bei organischer Raumbelastung von 1,5 kg oTS/m^3Tag stabil. Die Pufferkapazität, ein Indikator für Prozessstabilität, des zweistufigen Systems war zweimal höher als im einstufigen Prozess. Thermophile Vorversäuerung und mesophile methanogene Stufe erwiesen sich als optimal für die Fermentation von Oliventrestern. Die optimale hydraulische Verweilzeit ist vier Tage bei 55°C. Auch die Kofermentation mit Hühnermist als stickstoffreicher Substrat, im Verhältnis 30% Oliventrester und 60% Hühnermist, erwies sich als vorteilhaft und trug durch hohe Pufferkapazität zur stabiler Fermentation bei.

Im dritten Fall wurde die enzymatische Vorbehandlung von Schlamm aus der Papierproduktion mit mikrobiologischer Vorbehandlung verglichen. Zwei kommerziell erhältliche hydrolytische Enzymprodukte wurden laut Herstellervorgaben bei 30°C untersucht. Nach der Vorbehandlung wurde die Methanbildungskapazität in zwei unterschiedlichen Ansätzen bestimmt. Im ersten Ansatz wurde das ganze Substrat herangezon. Im zweiten Ansatz wurde das vorbehandelte Substrat abgetrennt, und die Methanbildungskapazität von der flüdssigen Phase bestimmt. Der zweite Ansatz lieferte 10% höhere Methanausbeuten im vergleich zum unbehandelten Substrat. Zusätzlich konnte eine Reduktion des Abfallanfalls zwischen 6 and 13 % erreicht werden. Die genannte Option lässt sich mit relativ einfachen Aufwand in die bestehenden Anlage implementieren.

Generell wurde bewiesen, dass der zweistufige Ansatz in allen drei Fällen entscheidende Vorteile bringt. Die räumliche Trennung einzelner biochemischer Prozesse der anaeroben Fermentation unter jeweils Einhaltung optimaler Bedingungen für die verschiedenen Mikroorganismengruppen führte zu kurzen Anfahrphasen, höherer organischen Raumbelastung und niedrigerer hydraulischen Verweilzeit. Die gewonnene Prozessstabilität erfordert zwar einmalig höhere Investitionen, bringt aber langfristig niedrige Ausgaben für den Betrieb der Fermentation.

Acknowledgement

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Abbreviations

AD	anaerobic digestion
AOX	adsorbable organic halides
BMP	bio-methane potential
CHP	combined heat and power gas engine
CM	chicken manure
CMP	chemo-mechanical pulping
COD	chemical oxygen demand
CTMP	chemo-thermo mechanical pulping
FB	fluidized-bed reactor
FF	fixed-film reactor
FM	fresh mass
GAeq	gallic acid equivalents
LCFA	long chain fatty acids
MP	mechanical pulping
NSSC	neutral sulfite semi-chemical pulping
OMSW	olive mill solid waste
OMWW	olive mill waste water
ORP	oxidation-reduction potential
PMS	paper mill sludge
SRT	solid retention time
SS	suspended solids
TCP	thermo-chemical pulping
TMP	thermo-mechanical pulping
TPOMW	two-phase olive mill waste
TS	total solids
VFA	volatile fatty acids

1 Introduction

1.1 Background

Oil peak, soil peak and water peak - three terms roaring in the media in the last three decades. The first time the term soil peak has been introduced in the 80s (Jackson, 1980). Three decades later also the term water peak has been introduced (Gleick and Palaniappan, 2010). While the term oil peak is more intensively mentioned, the soil and fresh water peaks are even more urgent to handle. These three piles – soil, water and oil are the moving forces of the modern economy and, further, of the modern social system. Oil is possible to be replaced by alternative renewable energy resources. Some of them, solar and wind energy are inexhaustible. Others like fresh water (rivers, lakes, dam lakes) and energy crops, grown for bio-based fuels, are more prone to ethical conflicts in their use. They are not unlimited available and are the living base for humans and animals. An alternative is the energy production from sources which do not exhaust the Earth`s resources. In the same time they should be able even to keep these upright by fitting to the natural circuit of the water and elements and do not disturb the diversity of flora and fauna, or with other words are ecological sustainable. This term is indivisible to the social and economic sustainability and is also an object of active discussions (Morelli, 2011).

On the one hand soil, water and oil availability suffer under the continuous industrial overgrowth. These resources are exhausted in a rate much higher than they can regenerate. In the same time another consequence of the industrial raise is the huge amount of produced waste which needs to be disposed and treated in a proper way. For this reason ***waste management*** has become higher priority since the 90s in the previous century. According to the United Nation Statistic Division waste management "includes (a) collection, transport, treatment and disposal of waste; (b) control, monitoring and regulation of the production, collection, transport, treatment and disposal of waste and (c) prevention of waste production through in-process modifications, reuse and recycling" ("UNSD," 2016).

Implementing the waste disposal into the natural circuit of the Earth by applying of proper tools can accomplish sustainability. The waste should be treated according to its nature, amount, and the socio-economic frames on site (Vaughn, 2009). The industrial organic waste is the kind of waste which incurs steadily and in huge locally concentrated amounts (Haggar, 2010). The co-product recovery is, or at least should be, the first management approach (Waldron, 2009). The landfill is a further waste management tool to deal the organic wastes. The organic municipal or agriculture waste is also composted in order to produce valuable fertilizers.

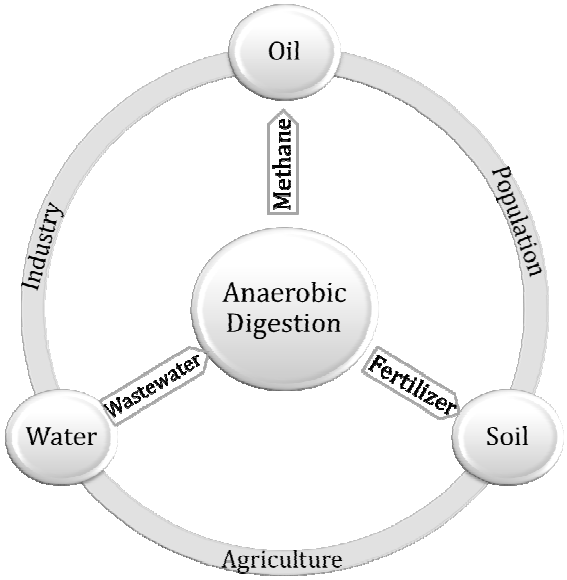


Figure 1: Implementation of the anaerobic digestion into the circuit of the natural resources

An option is the utilization of the organic waste for energy recovery, namely ***anaerobic digestion*** (Figure 1). The implementation of this approach has become popular in the middle of the previous century. Since then it has been optimized and developed continuously. The product – biogas – has a high energy potential used for electricity or as a fuel. The heat from the CHP (combined heat and power) gas engine can also be used. Further, the by-product, digestate, can be applied as a fertilizer and support the soil fertility on site of the plant. By doing so, two of the peak problems are partially solved – the energy from fossile fuels such as petroleum, coal, and natural gas is reduced on the one hand, and the soil necessary for the production of organic crafts is fertilized and instantly recovered.

The anaerobic digestion is a biological process conducted by anaerobic bacteria and Archaea. To provide optimal performance of the microbiologic consortium, specific conditions like temperature, pH, and feed composition should be provided. Considering the industrial organic waste, the bottleneck is the composition of the waste. Inhibiting compounds like fats, nitrogen rich compounds, heavy metals in high concentration can make the process fail and hinder the lively and continuous fermentation process, which is affordable for organic wastes, occurring steadily in huge amounts. The handling of the digestate has to meet the challenge of the further distribution on the fields. The restriction of the nitrogen supply on the field per year prevents from uncontrolled removal of the digestate on the fields. This means that long distances should be considered during the digestate distribution in order to keep the ecological footprint of the process upright. The last but not least factor is the high investigation costs, which are bearable only for developed industries.

Considering these points, this work aims the optimization of the anaerobic fermentation process by investigating the optimal conditions in a ***two-stage anaerobic system***. For this purpose one- and two-stage fermentations in laboratory scale were held out in parallel. Three different organic substrates were treated: olive mill solid waste (OMSW), sugar beet pressed pulp (SBPP) – both residues from the food industry; and pulp and paper mill effluent. Because of the variety in the composition of the organic residues in general, the results should be linked directly to the given substrate, and considered as a guide value for planning and optimizing of fermentations of similar substrates.

This framework summarizes the results published in three peer reviewed articles; two poster contributions to scientific conferences, and one contribution to a book chapter (see Appendix).

1.2 Waste management of food and paper mill industry

This work addresses anaerobic digestion of various industrial organic wastes of different origin. Olive oil and sugar industry are studied as an example of seasonally occurring organic waste concentrated on small area, while paper mill industry waste is an example of continuous occurring organic waste in big amounts and high rate mineral fraction. The topic of the waste management is a critical issue for the sustainability of these industries because of the raising public awareness with its following legislative regulations.

In 2012 about 1.3 billion tonnes of solid waste have been produced in the world cities (1.2 kg per person per day), 46% of which is organic (Figure 2). According to the report of the World Bank this amount is expected to rise up to 2.2 billion by 2025 (Hoornweg and Bhada-Tata, 2012).

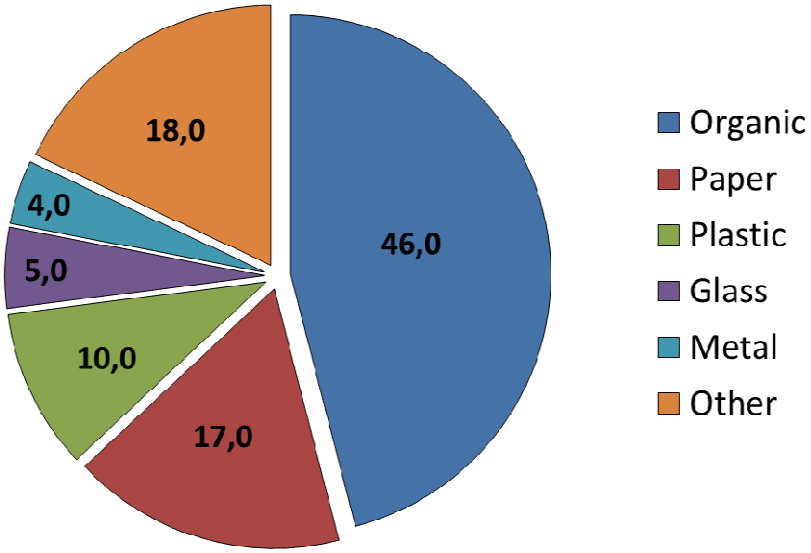


Figure 2: Global solid waste composition (Hoornweg and Bhada-Tata, 2012)

Almost the half of the waste produced in the world is of organic origin, mainly generated already during the production of the food. Therefore analyses of the structure of the given industry branch, as well as the tendencies in its development, are important for the adequate planning of the waste management, and for improving waste flows.

1.2.1 Olive oil industry

The olive oil ***production*** is concentrated in the Mediterranean area, where about 75% of the total production occurs in the EU (Figure 3) mainly in Spain, Italy, Greece and Portugal (FAO, 2015). The olive farms range from 0.5 ha low intensity groves to <500 ha mechanized plantations.

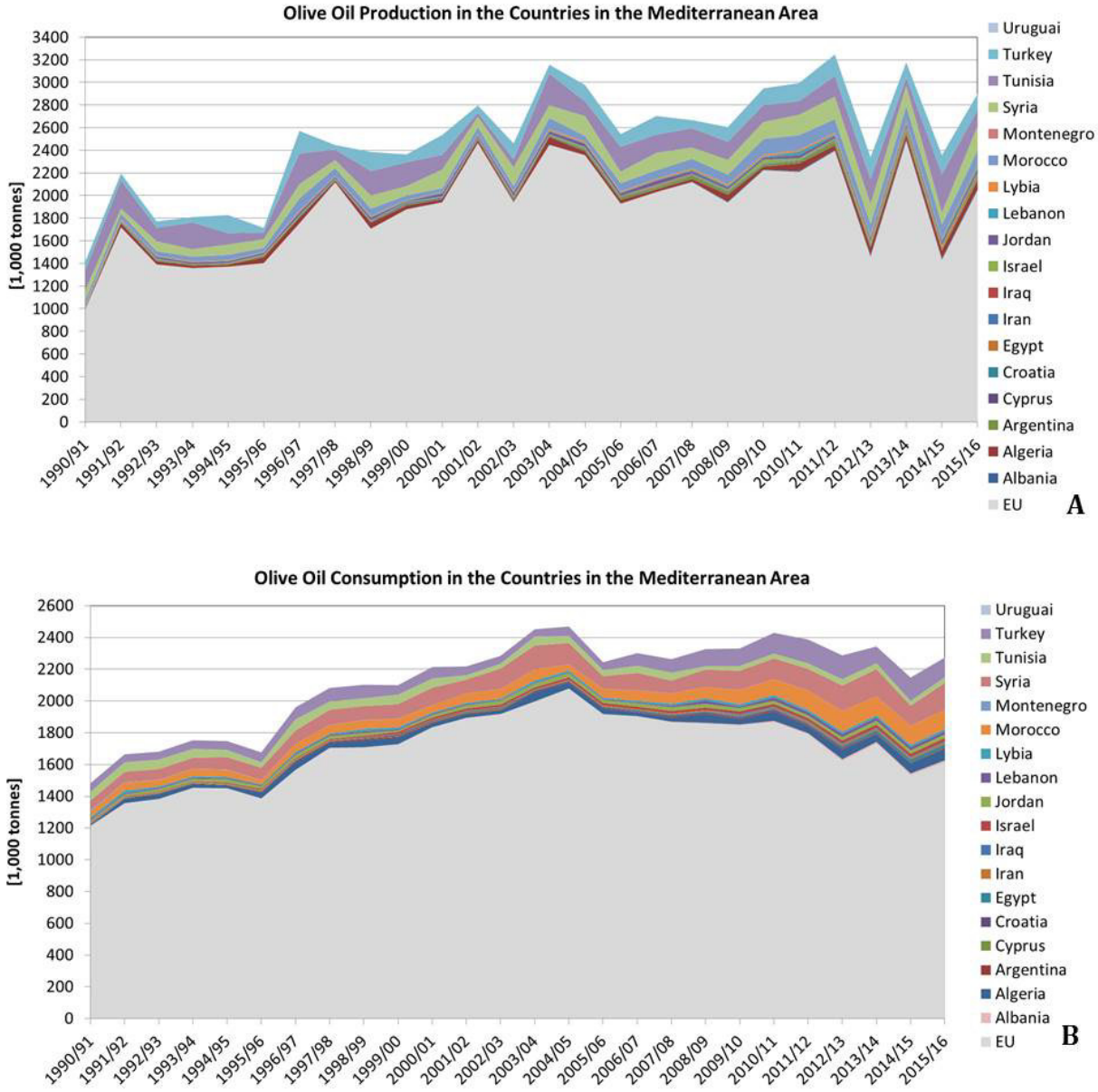
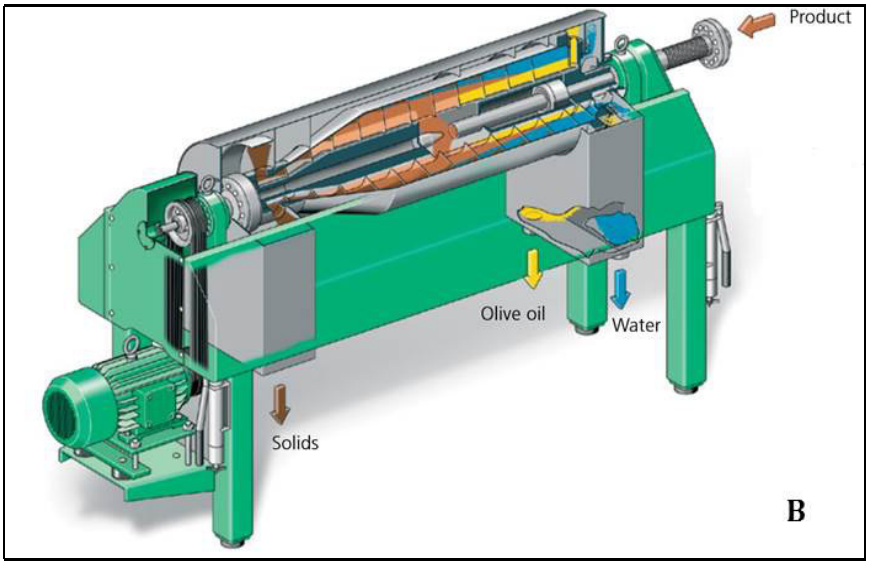


Figure 3: Olive oil consumption and production in the countries in the Mediterranean area (FAO, 2015)

The traditional olive oil production ***process*** consists of pressing of the ground paste between mats (Figure 4A). The outcoming oil-water mixture is separated in two phases into settling ponds. There is no water addition necessary and therefore there is not high amount of waste production. This technique is typical for the small groves. In large scale plantations continuous systems are in use. The older traditional continuous system is the three-phase centrifugation (Figure 4B). Here the olive pulp is added to a horizontal centrifugal machine together with water (one liter water per one kilogram pulp). The outcome is oil, dry pomace (orujo) and vegetable water (OMWW or alpechin). Due to the huge amount of generated waste, since the 90s the three-phase system has been steadily replaced by the two-phase one. In his case the vegetable water is recycled. The output from the two-

phase decanter is oil and wet pomace (alperujo), or two-phase olive mill waste (TPOMW) which is not considered as disposal problem (Figure 4C). Nowadays in Spain and Croatia the two-phase system is already most widely used, since in Italy and Greece three-phase system and traditional pressing are still common and less than 5% of the plants use the two-phase system (Doula et al., 2012). The composition of the wastes from the two- and three-phase systems is compared in Table 1.



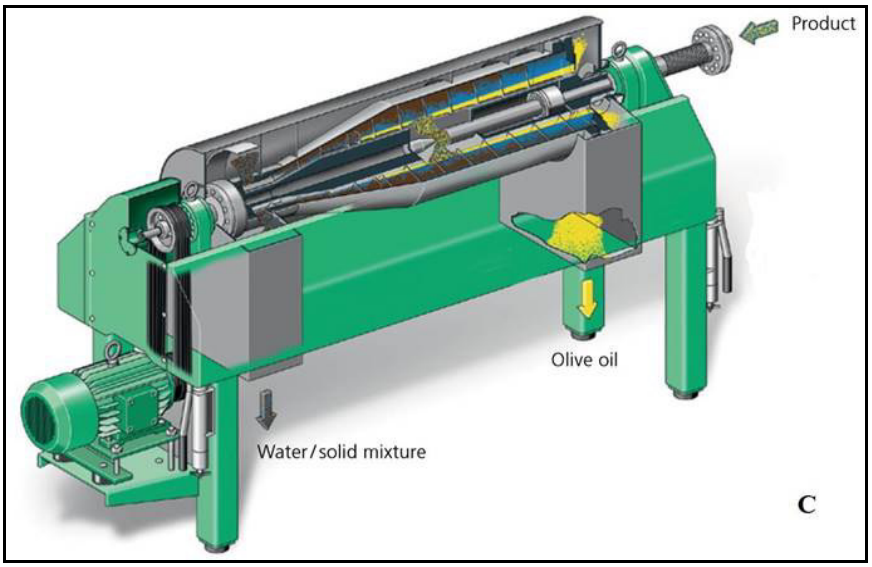


Figure 4: Conventional (A), three- and two-phase centrifugation system (B, C) ("Mungibeddu's," 2016), ("GEA Westfalia Separator Group GmbH," 2016)

Typically the ***olive oil sector*** is a family business, with small plants, which cannot be considered as industrial by the legislative regulations, e.g. the Directive on Industrial Emissions 2010/75/EU (IED), considering the treatment and processing intended for the production of food product from vegetable raw materials with a finished product production capacity greater than 300 tonnes per day, average value on a quarterly basis (European Parliament, Council, 2011). The percentage of large mills in Italy, Greece and Portugal is 9, 1, and 11%, respectively (Doula et al., 2012). Andalucía is a good example to illustrate the impact and the dimensions of the olive oil production. More than 4,500 km^2 is devoted to olive groves containing around 40 million olive trees. During an average year, these trees produce approximately 900,000 tonnes of olives, most of which are turned into some 200,000 tonnes of olive oil. In short, Andalucía produces one-third of Spain's olive oil and a mighty 10% of that used in the entire world, where 98% of the plants are using two-phase system ("Andalucia.com," 2016).

Table 1: Two- and three-phase centrifugation system ("GEA Westfalia Separator Group GmbH," 2016)

Process system	2-phase system	3-phase system
Extraction rate	similar to 3-phase process	similar to 2-phase process
Dilution water	0 – 5 %	20 – 50 %
Moisture in decanter solids	60 – 65 %	50 – 60 %
Amount of solids relative to raw material	approx. 80 %	50 – 60 %
Amount of waste water relative to raw material	max. 5 %	50 – 80 %
Mesh size	5 – 6 mm	6 – 8 mm
COD value of waste water	5 – 10 g/L	40 – 60 g/L

In this study the investigated by-product originates from an olive oil plant in southern Italy using two-phase system, therefore these by-products will be in the center of the discussion. The three-phase system residues will be taken as a comparison.

The ***tendencies*** in the development in this industry branch are expansion of the production in countries being net importers two decades ago, namely the USA, Australia, Chile, and more recently China and India (Figure 5A). Large production and export enterprises are the recent trend. A gap between local small producers and these conglomerates is appearing. One of the results is the mechanized harvesting. Despite of the production fluctuations in the last three years, the tendency in the production and consumption are decrease at about 20% and 5% per year, respectively, combined with raising prices (Figure 5B). The trend in the consumption is increased interest on the extra virgin olive oil which still remains brand of the Mediterranean countries ("Olive Oil Industry Megatrends," 2013).

The ***waste management*** is an inevitable topic in every food industry. The problem with the disposal of the waste from the olive oil production is complex. Both social and ecological aspects play crucial role for the necessity for solving or at least reducing this concern. The waste is generated seasonally, with harvesting period ranging between September and February, which means that large amounts of high pollutant organic waste has to be handled quickly. The characteristics of this waste varies, depending on the centrifugation system, the variety and maturity of olives, region of origin, climatic conditions and associated cultivation/processing methods. The differences in the parameters of the by-products from the olive oil production are summarized in Table 2, based on reviews of Alburquerque et al., (2004); Ouzounidou et al., (2010); and Dermeche et al., (2013).

The parameters listed in Table 2 are witnessing the recalcitrant nature of this waste. Despite the large number of studies and projects on the analyzing of the most proper valorization methods (Paredes et al., (1999); Azbar et al., (2004); Roig et al., (2006); Paraskeva and Diamadopoulos, (2006); Kapellakis et al., (2007); Wiesman, (2009); Al-Khatib et al., (2009); Cardoso Duarte et al., (2011); Nair and Markham, (2012); Doula et al., (2012); Muktadirul Bari Chowdhury et al., (2013); Achinas, (2014)), and for developing new alternatives (Obied et al., (2005); Belaid et al., (2013); Ramos et al., (2013)), still the most common disposal method is the evaporation in storage ponds or the direct spreading on the land. This leads to odor emissions, destruction of soil microbiology in the cases when the waste is not properly pre-treated (Sampedro et al., (2009); Karpouzas et al., (2010); Ouzounidou et al., (2010)), underground seepage, water-bodies pollution, and further comes into conflict with the tourism sector, which is usually not less important income source in the olive oil producing areas. The electrical conductivity, the organic matter, the total nitrogen, total polyphenols, available phosphorus, exchangeable potassium, available iron and the pH have been proposed as indicators for soil quality. Based on them, the disposable amount of olive mill by-products is determined, in order to sustain healthy soil, capable to degrade also polyphenols (Doula et al., 2012).

The olive oil waste consists of a list of ***inhibitors***. Three categories of phenolic compounds were detected in OMWW: cinnamic acid derivatives; benzoic acid derivatives; and compounds related to tyrosol. These are difficult to purify and have inhibitory effect on the bacteria already in low concentrations (Kapellakis et al., 2007). Other compounds which support the recalcitrant nature of these by-products are the lignin, cellulose, hemicellulose, and last but not least, the long chain fatty acids (LCFA). The synergetic impact leads to inhibitions in the municipal treatment plants (Karaouzas et al., 2011). Controlled land application of OMWW is hardly achievable, but it has the potential to enhance soil fertility (Kapellakis et al., 2007).

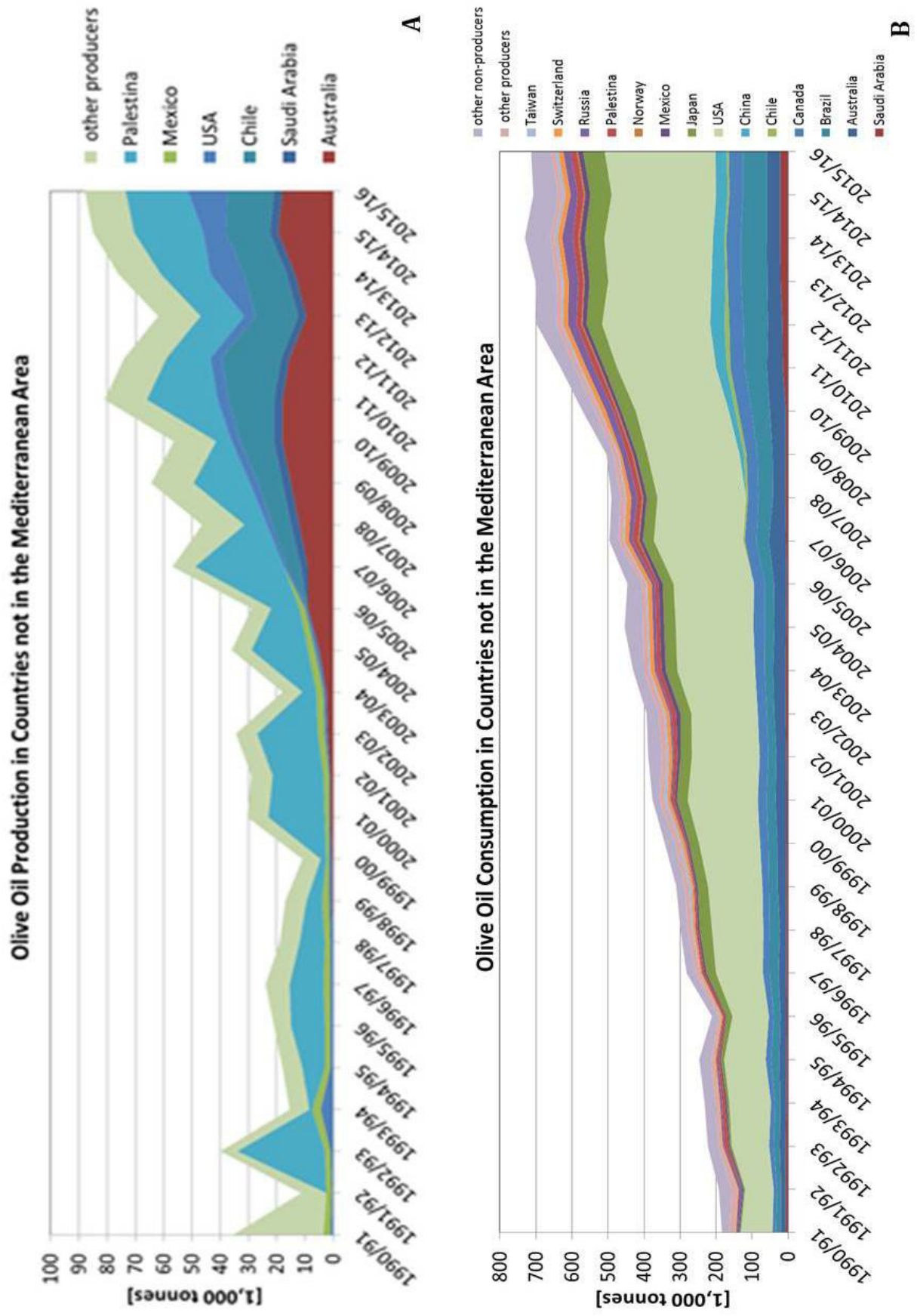


Figure 5: Olive oil consumption and production in the countries not in the Mediterranean area (FAO, 2015)

Table 2: Chemical characteristics of olive oil by-products according to Dermeche et al., (2013); Ouzounidou et al., (2010); Alburquerque et al., (2004)

Parameters	By-products		
	OMWW "alpechin"	Olive cake "orujo"	TPOMW "alperujo"
Pulp [%]		12–35	10–15
Olive-stone [%]		15–45	12–18
Dry matter [%]	6.33–7.19	87.1–94.4	25.5–44.4
Ash [%]	1	1.7–4	1.42–4
pH	2.24–5.9		4.9–6.8
Electrical conductivity [dS/m]	5.5–16		1.78–5.24
Total carbon [%]	2–3.3	29.03–42.9	25.37
Organic matter [%]	57.2–62.1	85	60.3–98.5
Total organic carbon [g/L]	20.19–39.8		
Total suspended solids [g/L]	25–30		
Mineral suspended solids [g/L]	1.5–1.9		
Volatile suspended solids [g/L]	13.5–22.9		
Volatile solids [g/L]	41.9		
Mineral solids [g/L]	6.7		
Volatile acidity [g/L]	0.64		
Inorganic carbon [g/L]	0.2		
Total nitrogen [%]	0.63 (1.25)	0.2–0.3	0.25–1.85
P [%]	0.19 – 1.5	0.03–0.06	0.03–0.14 (0.22)
K [%]	0.44–5.24 (10.8)	0.1–0.2	0.63–2.97
Na [%]	0.15		0.02–0.1
Ca [%]	0.42–1.15		0.23–1.2
Mg [%]	0.11–0.18		0.05–0.17 (0.38)
Fe [%]	0.26 ± 0.03		0.0526–0.26
Cu [%]	0.0021		0.0013–0.0138
Mn [%]	0.0015		0.0013–0.0067
Zn [%]	0.0057		0.0010–0.0027
Lipids [%]	0.03–4.25	3.5–8.72	3.76–18.0 (19.46)
Total phenols [%]	0.63–5.45 (24)	0.2–1.146	0.4–2.43
Total sugars [%]	1.5–12.22	0.99–1.38	0.83–19.3

Total proteins[%]		3.43–7.26	2.87–7.2 (11.5)
Chemical oxygen demand [g/L]	30–320		289.2-321.8
Biological oxygen demand [g/L]	35–132		
Cellulose [%]		17.37–24.14	14.54-24.9
Hemicellulose [%]		7.92–11.00	6.63 (27.3-41.5)
Lignin [%]		0.21–14.18	8.54 (32.3-55.6)

The disposal of waste and by-products in the EU is regulated in the Waste Framework Directive (*WFD*, 2008), according to which waste should be dealt first by prevention, then reuse, recycling, recovery, and finally, disposal. According to Article 5 of this directive, the waste from the olive oil production can be treated as by-product. The legislative regulation is vastly discussed in the report of the EU project PROSODOL, No. LIFE07 ENV/GR/000280 (Doula et al., 2012). The methods for the disposal, treatment or storage of the olive waste differ in the different production countries. The TPOMW is not considered as a waste and is usually air dried to water content less than 50%, a costly process causing greenhouse gases and fumes (Niaounakis and Halvadakis, 2006).

Anaerobic digestion of the residues from the olive oil production has been studied for a long time now (Aveni, (1984); Roig et al., (2006)). Nevertheless, there are scarce examples of anaerobic plants in the practice (Ortner et al., 2013). The small-scale family enterprises cannot afford on-site treatment options. Settling tanks do not provide controlled conditions. The conglomerates, dealing with mechanized harvesting, and need for disposal and energy, have the financial potential for biogas plants.

Another challenge is the seasonal nature of the by-product. The timespan of three months is short for starting up a biogas plant, which should be run continuously. This means that the co-digestion is a viable option for introducing the olive oil production residues into the fermentation process.

1.2.2 Sugar industry

The sugar ***production*** in 2014 was 179.68 Mio. tonnes, produced in 120 countries, 20 % of which from sugar beet (in 40 countries), predominantly in Europe ("SUCDEN," 2016). Sugar beet is among the 20 most produced commodities in the world. The highest share in the production is in Europe - 71.1 % from the 266.830 Mio. tonnes world production in 2014. The top three producers are in Europa: France – 37.630 Mio. tonnes; Russian Federation – 33.513 Mio. tonnes; Germany – 29.748 Mio. tonnes; followed by the USA – 28.472 Mio. tonnes. The tendency is increasing yield and decreasing harvested area (FAO, 2015). Sugar beet is primarily used for the production of sugar. Its production influences directly the sugar production in Europe (Figure 6).

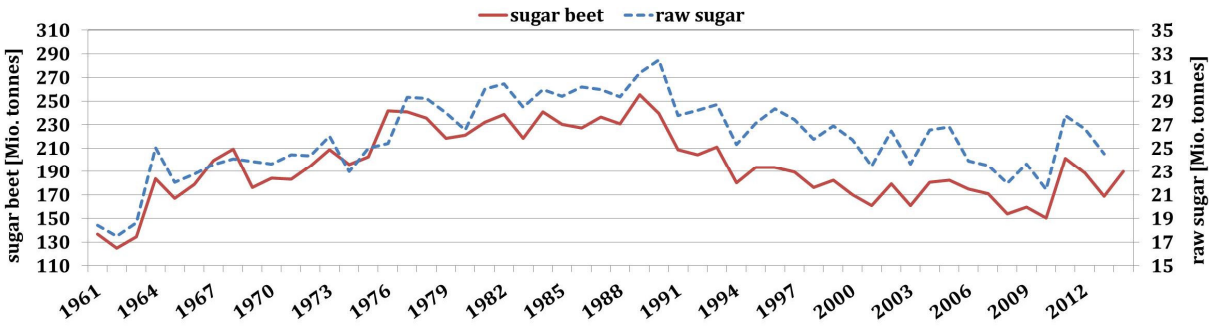


Figure 6: Production of raw sugar and sugar beet in Europe (FAO, 2015)

The sugar beet production in Europe dropped in the beginning of the 90s mainly due to less production in the Russian Federation and Ukraine. The amount produced in the EU remained almost unchanged except of the Mediterranean members Greece, Spain and Italy (Figure 7). In Germany, one of the biggest sugar producers in Europa, 2.890 Mio. tonnes sugar beet pressed pulp were produced in 2014/15. The ***waste management*** in the most of the cases is solved by using of the pressed pulp for animal feed ("WVZ," (2016); Zijlstra and Beltranena, (2013); Teimouri Yansari, (2014)), or as a source for chemicals (Micard et al., 1996), in bio based economy products (Finkenstadt, 2014), and composites, e.g. Curran® ("CelluComp," 2015).

An overview of the production ***process*** and the waste streams is illustrated in Figure 8. Despite of these possibilities, the sugar market in Europa, based on sugar beet, faces the problem with the decreasing sugar prices and needs alternatives for increasing its competitiveness on the world sugar market until it becomes self-sufficient after the end of sugar quotas in 2017, as expected by CEFS (2015).

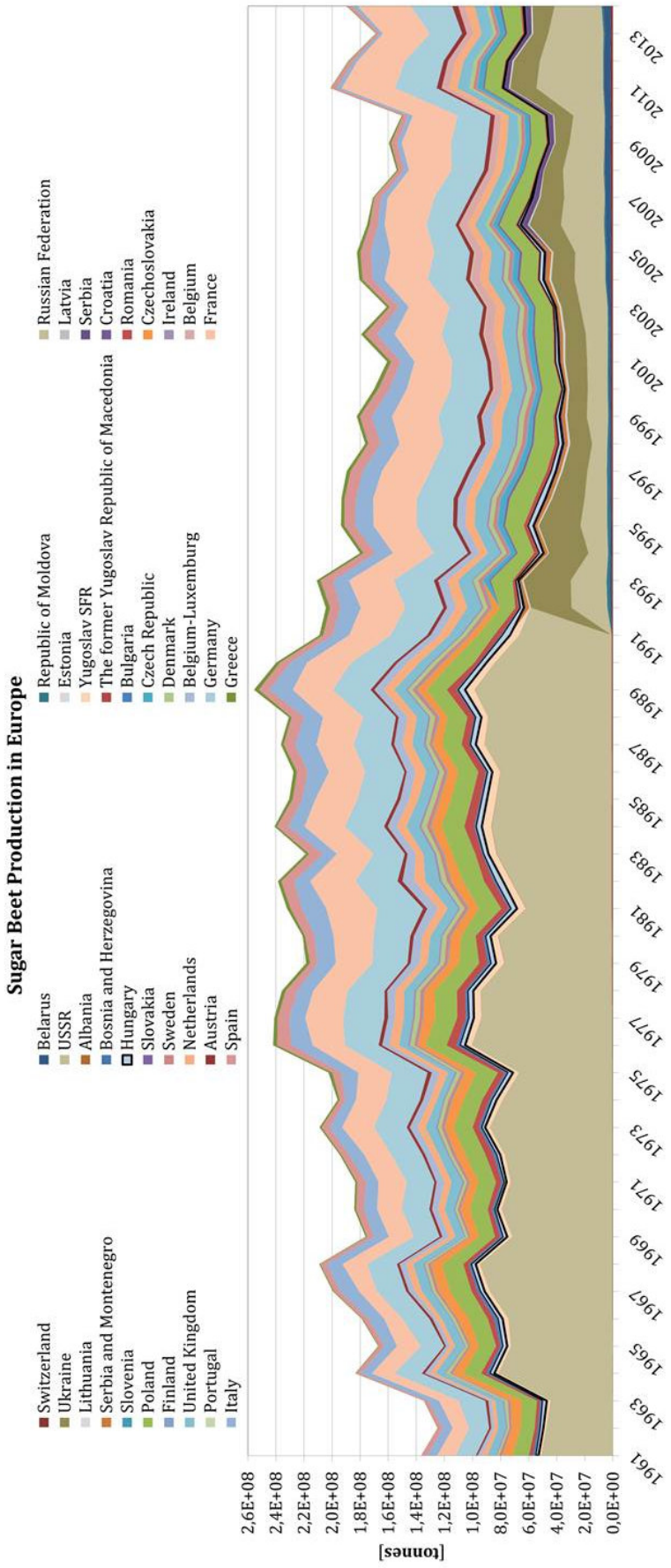


Figure 7: Sugar beet production in Europe(FAO, 2015)

The ***anaerobic digestion*** is regarded as a way to complete the high environmental and social standards of the EU sugar industry by reducing the amount of fossil energy necessary during the production process. Unfortunately, the transport costs of the by-product exceed its value already after 40 km distance. This means, that utilization on-site is important for the sustainable waste management (Csima and Szendefy, 2009). In case that the 13.28 Mio. tonnes exhausted sugar beet pressed pulp, produced in Europa in 2014 would have been converted in electric energy, then 32.9 – 37.9 Mio. kWh would have been produced. The anaerobic digestion of the by-products from the sugar production in Europe is still in development. The first biogas plant on-site of the factory in Europe was built in 2007 in Kaposvár, Hungary. This plant covers 45-50 % of the energy requirement of the factory by operating two 12,000 m^3 fermenters fed with 800-1,000 tonnes pressed pulp daily ("AGRANA," 2016). Further producers of biogas installations for the sugar industry are ("BITECOTM," 2016), Rackwitzer Biogas GmbH ("Suedzucker AG," 2016), or sugar producers supply their by-product to local biogas plants for co-digestion ("Nordzucker AG," 2016a), Suffolk in the UK; Botoš in Serbia; Zórawina, Zalesie in Poland; Donderen in Netherlands ("HoSt," 2016), for the sugar beet campaign takes place only for about four months. In the Netherlands there are two biogas plants built at sugar factories of Suiker Unie: in Dinterloord, operating since 2011, and Vierverlaten, since 2012. The used substrates are sugar beet pulp, sugar beet tails, residues from the potato industry and other agricultural products.

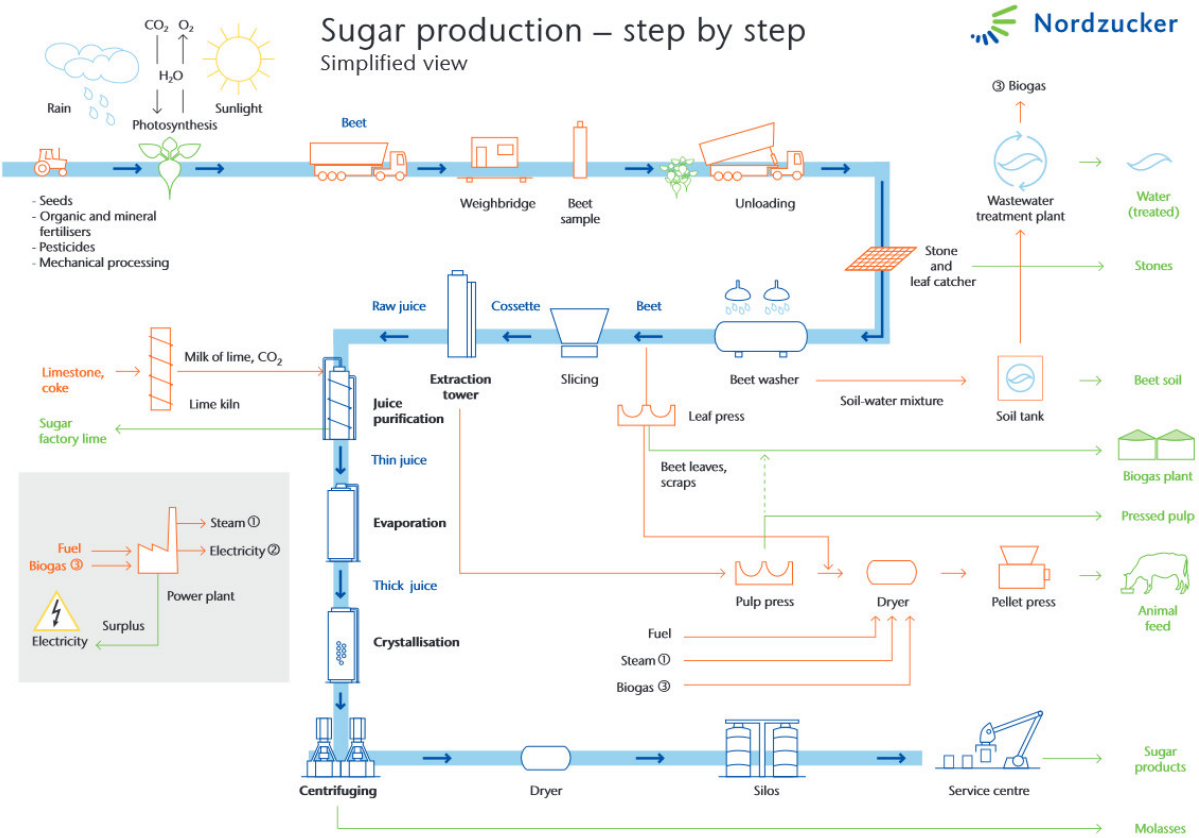


Figure 8: Sugar production process and waste streams ("Nordzucker AG," 2016b)

The exhausted sugar beet pulp has different composition, compared to the sugar beet grown as energy crop with chemical composition offering good possibilities for biological treatment (Table 3). The main components of the sugar beet pulp are sugars – about 74% of the dry matter. The lignocellulosic fraction of the dried pulp contains cellulose 22-30%, hemicellulose 24-32%, pectic substances 24-32%, and lignin 3-4% (Coughlan et al., 1985). The pulp has a cylindrical shape with

6-9 mm diameter and 20-40 mm length, which is suitable for direct input into the fermenter without previous mechanical treatment. The desugared molasses contain a high amount of ions: potassium 160 g/L, sodium 36 g/L and calcium 5 g/L, which can cause ***inhibition*** in the biogas process (Fang et al., 2011).

Table 3: Characterization of the wastes from the sugar production from sugar beet – for comparison also sugar beet silage is shown (Weiland, (1993); Brooks et al., (2008); Demirel and Scherer, (2009); Fang et al., (2011); Alkaya and Demirer, (2011))

Substrate	pH	TS [%]	VS [%]	COD [g/kg]	TKN [g/kg]
Sugar beet pressed pulp	3.9-4.0	15-18	14-17	180-260	1.2-3.1
Sugar beet silage	3.3	20	19	265	3.1
Desugared molasses	n.a.	49.8	32.6	49.8	6.7
Waste water	6.8	6	2.8	6.62	0.01

The high concentration of pectic substances, and not easily degradable hemicellulose and lignin cause difficulties in the reactor operation as foaming and high viscosity at increased loading rates (Brooks et al., 2008).

1.2.3 Paper industry

The paper industry is one of the four biggest industry branches (FAO, 2015). After fluctuations during the last decade, the produced amount of pulp evened out in the last five years (Figure 9). In Europe, 36.3 Mio. tonnes pulp were produced in 2015, or 25.2 % of the world ***production*** (Figure 10), 32 % of which in Sweden and 28.4 % in Finland (CEPI, 2016). Germany is the biggest paper and board producer in Europe, with 5.6 % of the world production after China, the USA and Japan. Sweden is the fifth largest producer of pulp for paper in the world after the USA, Brazil, Canada and China (FAO, 2015). The ***tendency*** in the pulp and paper industry in Europe is increasing the amount of plants producing more than 300,000 tonnes paper per year, and decreasing the pulp production (- 0.8 % for 2015).

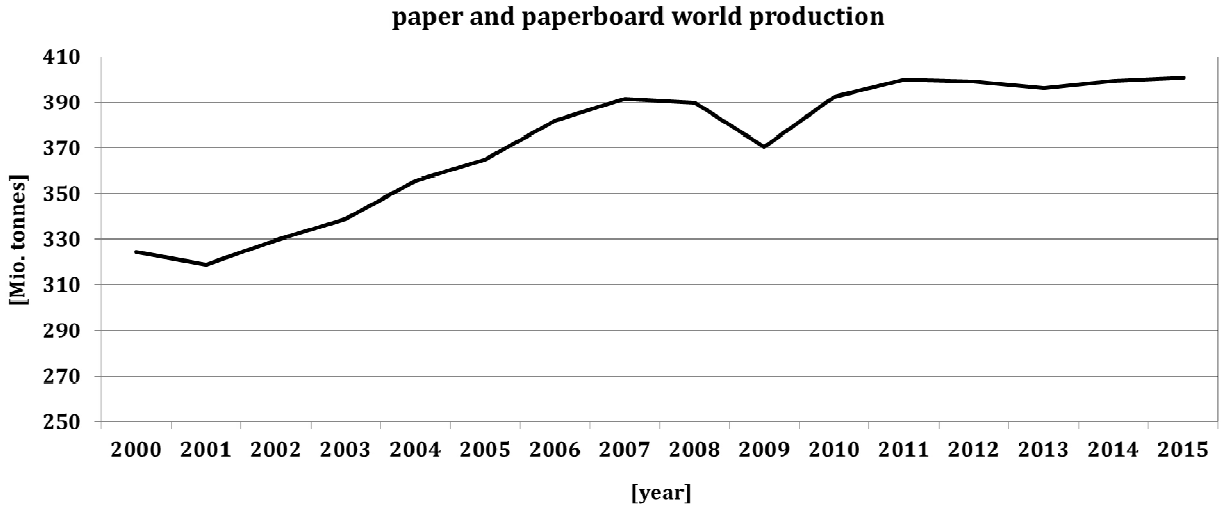


Figure 9: World production of paper and paperboard between 2000 and 2015 (FAO, 2015)

The tendencies in the development of the paper industry structure are important among others also for the proper organization of the ***waste management***. The paper industry was in the 90s the third industry in terms of freshwater withdrawal after the primary metal and the chemical industries in the world (Kallas and Munter, 1994). About 11 Mio. tonnes only in the EU, main part of which is the wastewater treatment sludge, have been produced in 2009 (Monte et al., 2009), when the paper production had a production tall (Figure 9). The environmental impact of the waste has always been pointed out and has been topic of numerous studies (Birge et al., (1989); Owens, (1991); Ali and Sreekrishnan, (2001); Bajpai, (2011)). This topic is of serious concern especially in countries, where the waste management regulations and treatment are not widespread applied yet (Sonnenfeld, (2000); Kostamo et al., (2004); Karrasch et al., (2006)). With the implementation of strict legislative orders (Monte et al., (2009); IPPC, (2015)), the pulp and paper industry has been forced to find effective solution for managing of the waste.

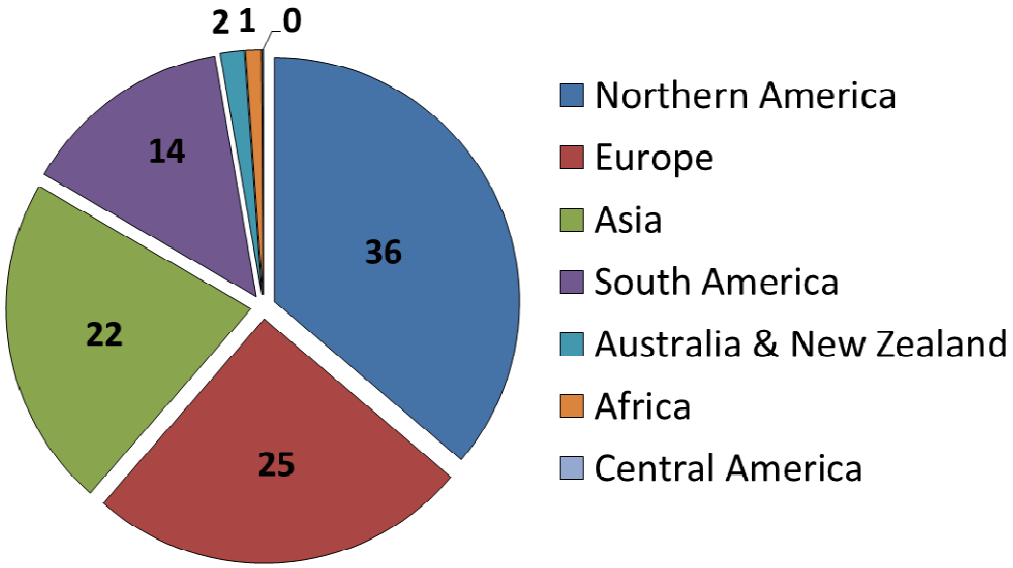


Figure 10: World pulp for paper production in 2015 (FAO, 2015)

The specific water consumption in the biggest pulp and paper producers has been reduced drastically from 250 to 50 (Springer, (2009); CEPI, (2016)) , and even 13 m^3/ton paper in Germany (Holik et al., 2012). Even approaches for closed water circuit have been implemented since the last ten years and the so called Kidney process is in use in some paper plants (CORDIS, 2004), where the process water is in a closed circuit and only the amount evaporated in the dryer and lost with the solid reject is replaced with fresh one. In these cases the investigation costs are justifiable by avoiding waste disposal taxes, which in some areas make up to 60 % of the plant costs (Park et al., 2012). In the USA the effluent water, treated at high level, is returned back to the rivers (Wiegand et al., 2011). Another important achievement is the increase of share of the recycled paper. In Europe 71.5 % of the input in the paper mills comes from recycled paper (CEPI, 2016).

The waste management in the pulp and paper industry depends strongly on the production method and on the available infrastructure. The wastewater is subjected to pre-treatment and afterwards either disposed, or reused in production circuits (Holik et al., 2012). The physicochemical treatment methods as sedimentation/flotation, coagulation and precipitation, adsorption, chemical oxidation, membrane filtration and ozonation are the most explored techniques recently, proper for compounds, resistant to biological treatment, or as tertiary treatment, although they are not wide implemented yet (Pokhrel and Viraraghavan, 2004). The biological treatment, in contrary, is state-of-the-art in the entire existing paper mills, with some exceptions. The tendency in the waste treatment is directed towards internal process changes (Bajpai, 2011).

The solid waste is mainly disposed in landfills, composted or preceded as additive for reuse in other industries: brick, cement, concrete and mortar production or road construction. The important step in this case is the dewatering of the sludge. With the belt filter press and the screw press, used widely in the practice, between 60 and 80 % dry solid content is achievable, 10-20 % of which is organic matter. Considering the high costs for drying systems, the energy recovery starts to play important role in the sufficiency of the production process. Combustion of the dewatered sludge is one of the applied techniques, especially for the waste flows rich in bark and wood residues (Bajpai, 2012).

Table 4: Composition and origin of detrimental substances in waste and process water from the paper industry (Pokhrel and Viraraghavan, (2004); Holik et al., (2012))

Chemical compounds	Origin
Sodium silicate	Peroxide bleaching, deinking, recovered paper
Polyphosphate	Filler dispersing anent
Polyacrylate	Filler dispersing anent
Starch	Coated broke, recovered paper
Humic acids	Fresh water
Lignin derivates, lignosulfonates, hemicelluloses	Chemical and mechanical pulp
Fatty acids	Mechanical pulp, deinking
Tannins, terpenes	Softwood processing
Organochlorine	Bleaching
Organic dyes	Deinking

The technology of the paper production directly influences the waste content and further the treatment options (Figure 11). Similar to the olive oil and the sugar industry, AD is a tool in the waste management system facing both ecological and economical concerns of the pulp and paper industry. The ***anaerobic treatment*** of the effluents from the pulp and paper production has been applied since the early 80s when they started to complete, but not substitute the waste treatment together with the aerobic plants (Habets and Driessen, 2007). Nowadays there are more than 350 anaerobic treatment plants worldwide (Meyer and Edwards, 2014). In general development occurs in the treatment of the wastewater, but not in the sludge processing, where AD is scarce (Hagelqvist, 2013). Since the implementation of the AD, the COD removal capacity increased. The rising share of recycled pulp and paper in the waste streams brings also additional organic and anorganic compounds, like dyes, resin acids, sulphur and organochlorine compounds (Table 5). Already 55.8 % of the paper production is from recovered paper (FAO, 2015). Because of the ***inhibition*** potential of these compounds, often only selected streams are being treated anaerobically, e.g. the paper mill effluents and the evaporator condensates (Meyer and Edwards, 2014). Another concern is also the deviation in the effluent composition (Table 5). The anaerobic reactor systems used in the wastewater treatment in the pulp and paper industry also develop in order to meet the requirements of the changing composition of the waste. At the very beginning CSTR (continuous stirred tank reactor) reactors have been used, soon replaced by UASB systems (upflow anaerobic sludge bed), because of their relatively low loading rates (Habets and Knelissen, 1985). The UASB reactors pointed out with the highly active concentrated granular anaerobic biomass and 2-5 times higher volumetric loading rates. Later, in the 90s, fluidized bed reactors (FB), expanded granular

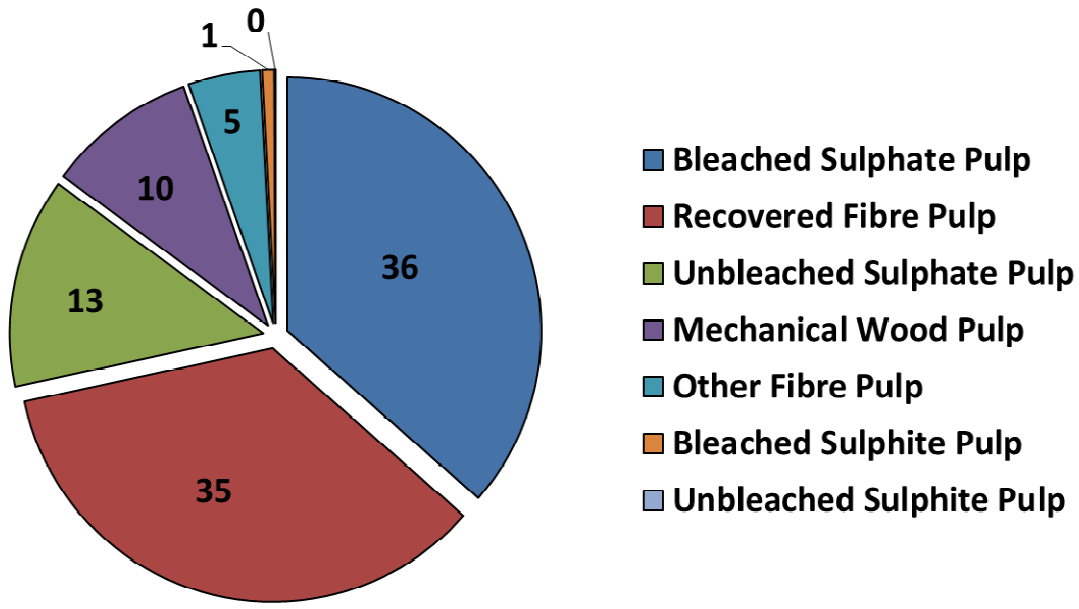


Figure 11: Distribution of the pulping systems in 2015 (FAO, 2015)

sludge bed (EGSB), and internal circulation (IC) reactors were developed with volumetric loading rate 10-20 and 20-30 kg COD/m^3d. In 2007, 75 % of the anaerobic waste treatment occurred in Europa. Almost two third of the reactor systems in use are IC (Habets and Driessen, 2007). Newly upgraded systems on the market are: BIOPAQ®IC ("PAQUES," 2016); Biobed® EGSB ("Veolia Water Technologies," 2016); R2S ("Voith," 2016) - a two-stage high-performance reactor for anaerobic treatment of industrial wastewater with a high calcium content; STP® ECSB ("HydroThane," 2016) - External Circulation Sludge Bed, 2nd generation EGSB; ANAMET ("Purac," 2016) - a two-stage process with a combination of anaerobic and aerobic treatment; an-OPUR (WABAG Group, 2014) - anaerobic contact process with fully mixed reactor (anaerobic activation process).

The advantage of the anaerobic treatment is not only the reduction of the COD load, but also the sludge reduction - 80 % of the biological sludge and 67 % total sludge with increased dewatarability (Habets and Driessen, 2007). As mentioned above the sludge after the primary treatment - sedimentation or clarification - is still containing 80-60 % water and needs enhancing of its dewatarability. Another aspect is the solubilisation of the residual fraction to make it available for anaerobic treatment. The potential of the use of cellulolytic enzymes for achieving this goal is studied in this work. Enzyme treatment in waste management and its benefits has been studied before (Hakulinen, (1988); Duff et al., (1994); Duff et al., (1995); Karam and Nicell, (1997); Ayol, (2005); Parawira, (2012); Mendes et al., (2014)).

Table 5: Chemical composition of pulp and paper mill (Puhakka et al., (1988), Rintala and Puhakka, (1994); Pokhrel and Viraraghavan, (2004); Buzzini et al., (2005); Lin et al., (2011); Bajpai, (2012); Ashrafi et al., (2015); Kamali et al., (2016))

Compound / Process	COD [mg/]	pH [-]	TS [mg/L]	SS [g/L]	AOX [mg/L]	Resins [mg/L]	Volatile acids [mg/L]	N [mg/L]	P [mg/L]	S [mg/L]
MP	91-1,150	6.1-8.1								
TMP	50-7,210	6.8-7.2		127-1,400		30-200	100-560	7-12	2.3	72-700
TCP	1,000-5,600									
CTMP	900-25,000	6.2-12		500-3,200		50-550	1,500-4,550			500-1,500
Kraft bleaching	426-4,112	8.2-13.5	40-8,260	10-74	12.5-22	69-25,000	7,000-10,400	2-350		120-450
Sulfite	4,000-27,100	2.5-5.9		320			2,500			840-4,800
NSSC	5,020-39,800			253-6,095			54-3,200	11-86	0.6-36	97-868
Deinking	78	8.3	450	400						
Debarking	1,275	7		7,150		25-200				
Newsprint mill	925-3,500	7.8	545-3750	250-235	75	16		12.6		490
Paper machine	953-1,116	6.5-7.8	645-1,844	760				11		
Recycled paper mill	3,380-15,000	6.2-7.8	1,900-3,138	300-800						

1.3 Anaerobic Digestion

Anaerobic digestion is primarily a process, occurring spontaneously, when anoxic conditions are achieved, after the bacteria exhaust the oxygen for their metabolism on organic matter. The actors, executing the process are methanogenic Archaea. Methane, the product of the AD of organic matter, is a phenomenon occurring in vast natural habitats: glacier ice, marine and freshwater sediments, marshes, swamps, termites, gastrointestinal tracts of ruminants, municipal solid waste landfills, oil fields, and hydrothermal vents.

The energetic value of methane is 9.968 kWh, and of biogas – 5.0-7.5 kWh, depending on its methane content. The potential of this highly valuable energy provider has been perceived already in the 16th century in Persia. In 1776, Alessandro Volta reported the correlation between the organic matter degradation and the flammable gas. Later, in 1808 Sir Humphry Davy noticed that this gas contains methane (Lusk, 1998). The AD has been studied from the microbiological point of view since the beginning of the 20th century (Buswell and Hatfield, 1936). One of the first biogas plants were reported in India in 1859 (Meynell, 1982), and England, in 1895 (Lusk, 1998). The use of anaerobic treatment for stabilization of sludge started to increase in the 70s of the previous century (Cillie et al., (1969); Pretorius, (1971); Hawkes et al., (1978)). The first anaerobic plants have been implemented in the wastewater treatment systems (EPA USA, (2004); Swedish EPA, (2014)).

The AD has its ***advantages and disadvantages*** compared to the aerobic waste treatment (Speece, 1996). The advantages are:

- uses readily available CO_2 as electron acceptor, and the cost-rich aeration (oxygen supply) is not necessary
- the amount of stabilized sludge is 3-20 times less, since the energy yield from the substrate breakdown is in the final product CH_4
- the final product, biogas, provides valuable gain of energy for electricity or heat production
- the general energy required for the process is reduced
- it is suitable for high-strength organic industrial wastes with high organic loading rates (OLR)
- the activity of the microorganisms remains also after longer periods without feed

The disadvantages are:

- the anaerobic process is slower
- the microorganisms are more sensitive to toxicants
- long start-up periods

Another aspect that should be mentioned is the ***environmental impact*** of the product biogas, in case of poorly technical performance of the plant, is the global warming potential of methane - 21 (compared to CO_2 - 1), which makes its effect on the climate of high consequence (Figure 12).

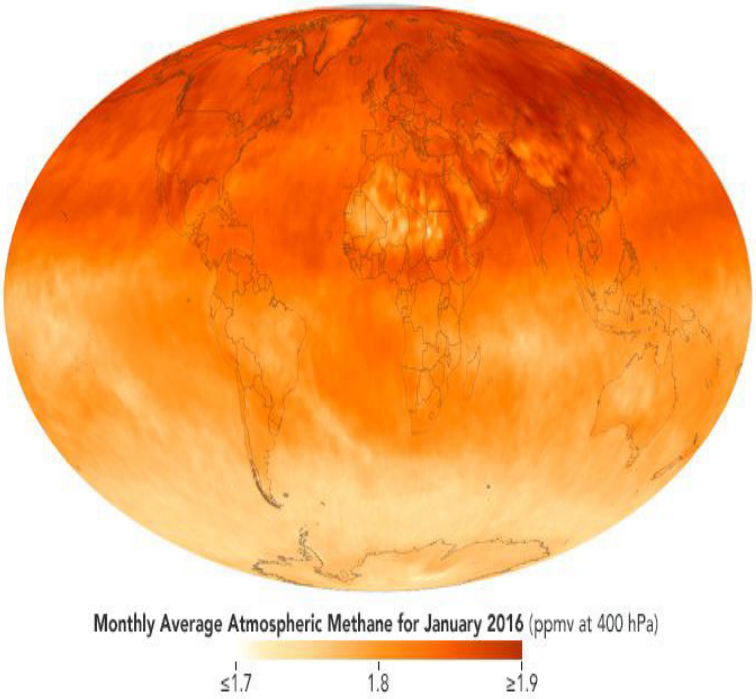


Figure 12: Distribution of atmospheric methane, January 2016: NASA Earth Observatory map by Joshua Stevens, using AIRS data (Voiland, 2016)

The anthropogenic influence plays more and more significant role, by rapidly increasing the uncontrollable methane emissions into the atmosphere (Figure 13).

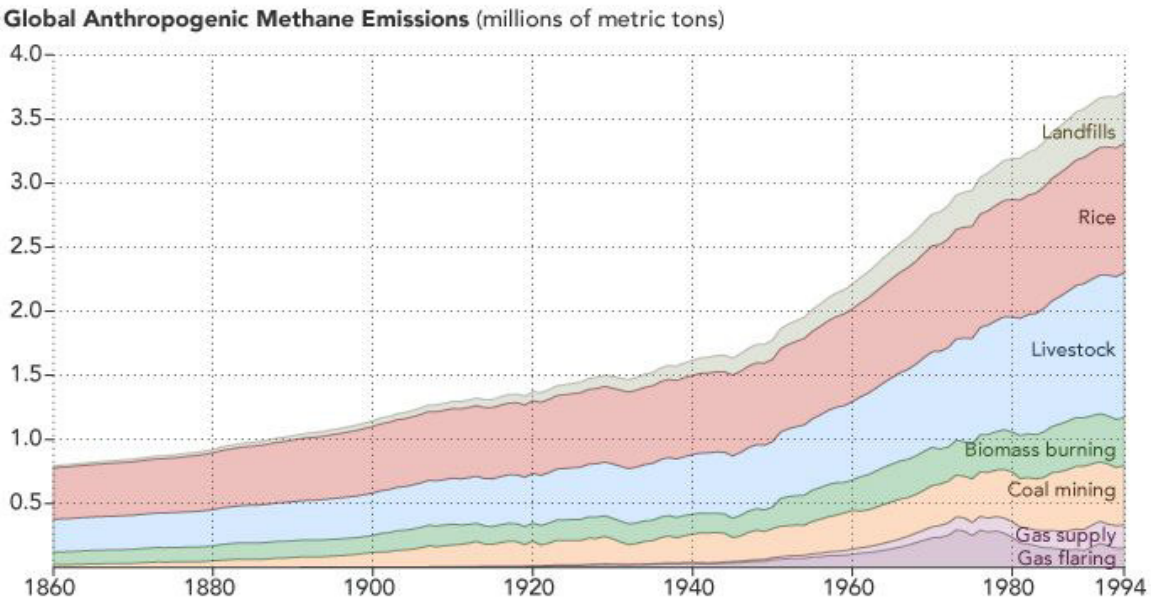


Figure 13: Anthropogenic methane emissions: image by Joshua Stevens, using data from CDIAC (Voiland, 2016)

The tendency is increasing emissions approximately 7 to 10 % within each sector (Figure 14). Methane emissions from wastewater treatment systems are expected to increase by nearly 12 %, and from the oil and gas sector by nearly 35 %. This data proves the necessity of controlled conversion of the organic waste into biogas by modern anaerobic digestion systems and avoiding greenhouse gas emissions.

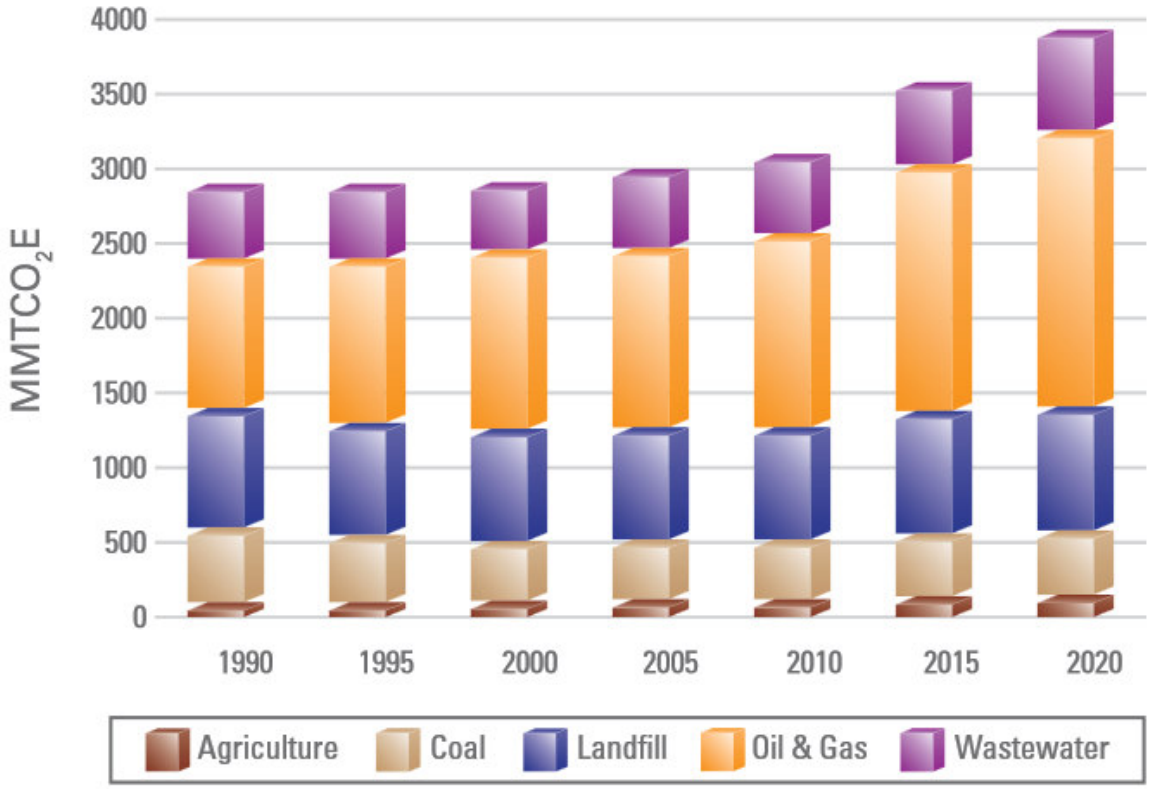


Figure 14: Global methane emissions by sector (Global Methane Emissions and Mitigation Opportunities, 2015)

Nowadays the AD is state of the art industrial scale application for energy recovery from renewable resources and is legislative controlled. The design of the digesters and the technical parameters of their equipment still undergo improvement.

1.3.1 Biochemical processes within AD

The anaerobic fermentation of organic material from its biochemical point of view is a unique process where the co-existence of vast number of microorganisms from two of the three domains - Archaea and Eukaryota leads to cascade of biochemical conversions. The common feature that unites them in this process is the anaerobic or facultative anaerobic metabolism. This process occurs also in the nature where the important regulatory mechanisms temperature, homogenization, and substrate content are uncontrolled and depending on the climatic conditions - in the swampland, the sea and wherever anaerobic conditions develop. In the case of the anaerobic digestion occurring in the four compartment stomach of ruminants, these factors are held constant by the animal body and the substrate is only high content cellulose and lignin plant material. The industrial process anaerobic digestion resembles the conditions in the ruminants by trying to digest substrates different than grass and hay.

Four ***general stages*** are proceeding the anaerobic conversion of organic substrate: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Figure 15) (Braun, 1982).

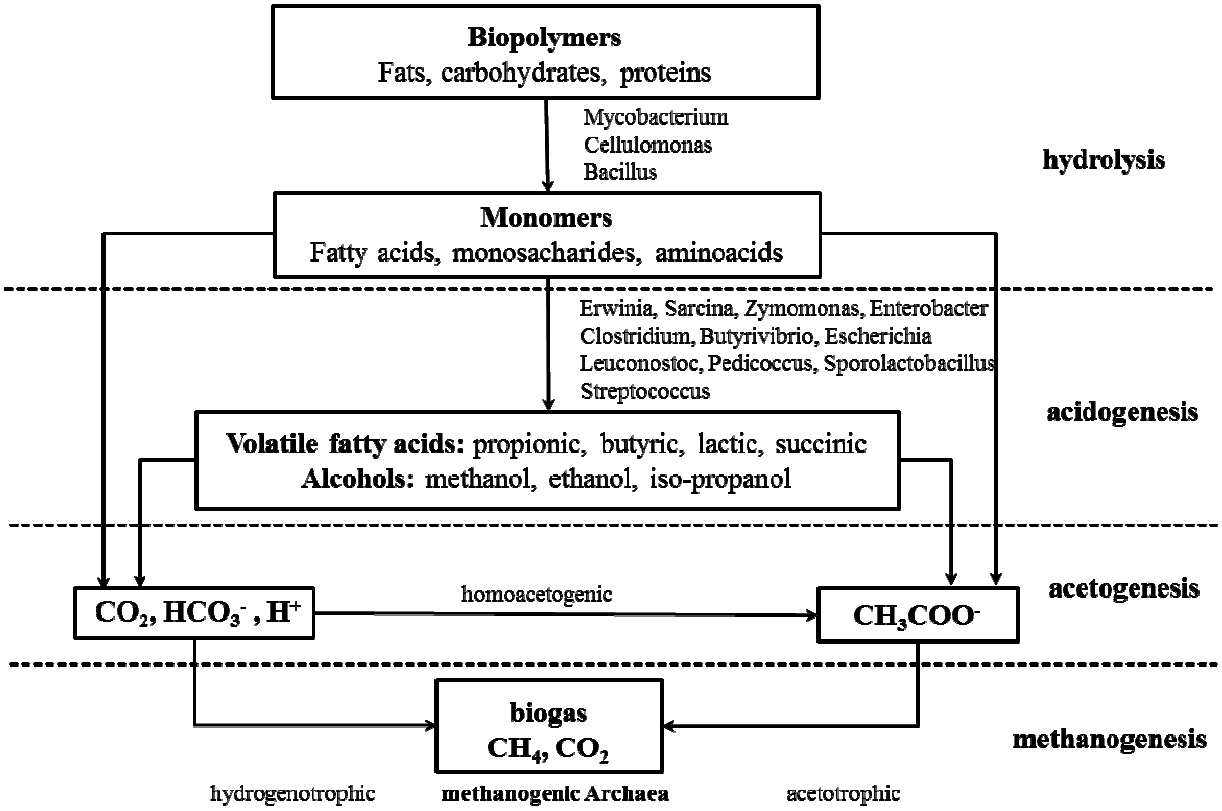


Figure 15: Four stages of the anaerobic digestion

The biochemical reactions during the anaerobic process are summarized in Table 6.

Table 6: Reactions during the anaerobic digestion **(Daniels, (1993); Gerardi, (2003))**

hydrolysis	Complex carbohydrates → simple sugars
	Complex lipids → fatty acids
	Complex proteins → amino acids
acidogenesis	$C_6H_{12}O_6 \rightarrow 2\ CH_3CH_2OH(aq) + 2\ CO_2(g)$
	$C_6H_{12}O_6 \rightarrow CH_3CH_3CH_2COOH(aq)$
	$C_6H_{12}O_6 \rightarrow 2\ CH_3OHCOO^-(aq)$
	$C_6H_{12}O_6 \rightarrow CH_3OHCOO^-(aq) + CH_3CH_2OH(aq) + 2\ CO_2(g)$
	$2\ C_6H_{12}O_6 \rightarrow 2\ CH_3OHCOO^-(aq) + 3\ CH_3COO^-(aq)$
	$3\ C_6H_{12}O_6 \rightarrow 4\ CH_3CH_2COO^-(aq) + 2CH_3COO^-(aq) + 2CO_2(g)$
acetogenesis	$CH_3CH_2OH(aq) + H_2O \rightarrow CH_3COO^-(aq) + H_2(g)$
	$CH_3CH_2COOH(aq) + 2H_2O \rightarrow CH_3COO^-(aq) + CO_2(g) + 3\ H_2(g)$
	$CH_3CH_3CH_2COOH(aq) + 2H_2O \rightarrow 2\ CH_3COO^-(aq) + 2\ H_2$
	$4\ CH_2NCH_2COOH(aq) + 2\ H_2O \rightarrow 4\ NH_4^+ + 2\ CO_2(g) + 3\ CH_3COO^-(aq)$
methanogenesis	$CO_2(g) + 4\ H_2(g) \rightarrow CH_4(g) + 2\ H_2O$
	$HCO_3^- + 4\ H_2(g) + H^+ \rightarrow CH_4(g) + 3\ H_2O$
	$4\ HCOO^-(aq) + 4\ H^+ \rightarrow CH_4(g) + 3\ CO_2(g) + 2\ H_2O$
	$4\ CH_3COCH_3(aq) + CO_2(g) \rightarrow CH_4(g) + 4\ CH_3COCH_3(aq) + 2\ H_2O$
	$2\ CH_3CH_2OH(aq) + CO_2(g) \rightarrow CH_4(g) + 2\ CH_3COO^-(aq) + 2\ H^+$
	$4\ CH_3OH(aq) \rightarrow 3\ CH_4(g) + CO_2(g) + 2\ H_2O$
	$4\ CH_3OH(aq) \rightarrow 3\ CH_4(g) + HCO_3^- + H^+ + H_2O$
	$4\ CH_3NH_3^+(aq) + 2\ H_2O \rightarrow 3\ CH_4(g) + CO_2(g) + 4\ NH_4^+(aq)$
	$CH_3COO^-(aq) + SO_4^{2-} \rightarrow 2\ CO_2(g) + 2\ H_2O + H_2S(g)$
	$H_2(g) + CH_3OH(aq) \rightarrow CH_4(g) + H_2O$
	$CH_3COO^-(aq) + H^+ \rightarrow CH_4(g) + CO_2(g)$
	$CH_3COO^-(aq) + H_2O \rightarrow CH_4(g) + HCO_3^-$

An interaction of environmental circumstances and substrate composition play role for the dynamic constellation of the microorganism consortium, processing this biochemical reaction cascade. And vice versa: the products of the fermentation depend on the diversity of the participating bacteria, which provide these intermediates further to the methanogens. The biochemical pathways of the microorganisms are partially unique for this kind of substrate conversion. Generally all the microorganisms are in tight synergetic relationship despite of the differences in their optimal growth conditions.

Table 7: Bacterial biochemical stages of the anaerobic digestion

Stage	Substrate	Microorganism	Product
Hydrolysis	Polysaccharides	Cellulomonas	Simple sugars
	Proteins	Bacillus	Amino acids
	Lipids	Mycobacterium	Fatty acids
Acidogenesis	Sugars, amino acids, fatty acids	Volatile acid forming bacterium	Propionic, butyric acid, methanol
Acetogenesis	Methanol, propionic acid, butyric acid	*Escherichia coli, Clostridium, Synthrobacter volinii*	Acetate, H_2

Bacteria use exo- and endo-enzymes to degrade the substrates. The particles of the substrates are solubilized by the exo-enzymes in order to allow them to enter the cell, where the endo-enzymes perform the further conversion, e.g. acetate forming bacteria (Table 7). The fermentative pathways from the hexoses are summarized in Table 8.

Table 8: Fermentative pathways during the anaerobic digestion

Fermentative pathway	Product	Microorganism
Lactate fermentation	Lactate, ethanol, CO_2	Bifidobacterium, Lactobacillus, Leuconostoc, Pedicoccus, Sporolactobacillus, Streptococcus
Alcohol fermentation	Ethanol, CO_2	Erwinia, Sarcina, Zymomonas, Enterobacter, Serrata
Butyrate fermentation	Butyrate, butanol, isopropanol, ethanol, CO_2	Clostridium, Butyrivibrio
Butanediol fermentation and mixed acid fermentation	Acetate, formate, lactate, succinate, CO_2, H_2	Enterobacter, Escherichia, Erwinia, Salmonella, Serrata, Shigella
Propionate fermentation	Propionate, succinate	

Acetate forming bacteria produce acetate and H_2. In the same time they are, similarly to the methane-producing microorganisms, sensitive to H_2 pressure higher than 10^{-4} atmospheres. Therefore both microorganism groups are depending on the fast further utilization of H_2. Acetogenic bacteria grow much faster than the methanogens. Sulfate-reducing bacteria as *Desulfovibrio desulfuricans* and *Dsulfotomaculum* are also present in the anaerobic digester, when sulfate is in stock. They utilize acetate and hydrogen similarly to the methanogens. The hydrogen is consumed for the reduction of sulfate to hydrogen sulfide. Under low acetate concentrations the sulfate reducers obtain it more easily and they can outcompete the methanogens under substrate to sulfate ratio <2. At ratios between 2 and 3, the competition is very intensive and at ratio >3 the methane-forming Archaea are favored (Gerardi, 2003). The produced hydrogen sulfide can further inhibit the acetate-forming and the methane-forming microorganisms.

The methanogens are able to degrade substrates at oxidation-reduction potential (ORP) between -200 and -400 mV (Table 9). Approximately 2.5% of the total dry weight mass of the methane-forming Archaea is sulphur, a quite high amount, compared to the rest of the microorganisms. The

digester sludge must contain thiol-group (-SH) compounds, since these produce a reducing environment.

Table 9: ORP and fermentation types in the order of utilization (Gerardi, 2003)

ORP [mV]	Carrier molecule	Condition	Respiration
>+50	O_2	Aerobic	Oxic
+50 to -50	NO_3^- or NO_2^-	Anaerobic	Anoxic
<-50	SO_4^{2-}	Anaerobic	Fermentation, sulfate reduction
<-100	Organic compound	Anaerobic	Mixed acid and alcohol fermentation
<-300	CO_2	Anaerobic	Fermentation, methane production

There are three groups of ***methane-forming Archaea*** according to the used substrates (Figure 16):

- Hydrogenotrophic methanogens, using hydrogen to reduce CO_2 to methane;
- Acetotrophic methanogens, splitting acetate to CO_2 and CH_4 (aceticlastic cleavage). Some of them use CO for methane production. This group reproduces more slowly than the first one and is adversely affected by the accumulation of H_2.
- Methylotrophic methanogens produce CH_4 directly from $-CH_3$ groups from methanol, or methylamine.

These methane forming Archaea belong to four different families (Table 10). Species using these three different substrates appear in each family. Also the optimal temperature range is independent of the substrate. This allows vast species diversity in anaerobic reactor with mixed substrates, which is the case in the most of the agricultural and industrial anaerobic plants.

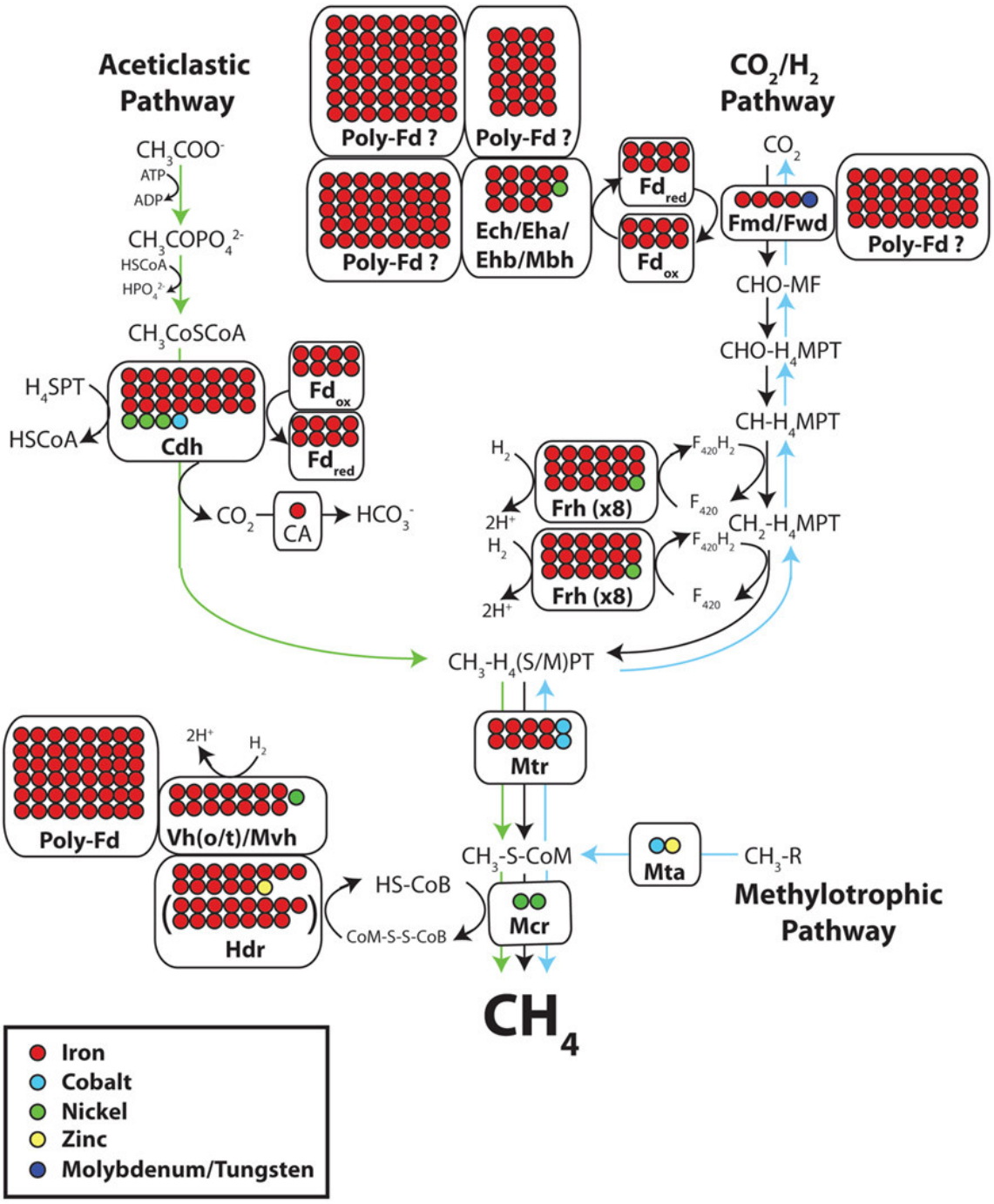


Figure 16: Pathways of methanogenesis (Glass and Orphan, 2012)

The production of methane is unavoidably coupled with several ***coenzymes*** (Figure 17), some of which are typical only for methanogens (Daniels, 1993). The F 420 (5-deazaflavin) is the major electron transferring coenzyme, obligate two electron acceptor. The several existing forms differ only in the number of glutamates. F 420 has been also found in *Streptomyces, Nocardia and Mycobacterium*. Its derivative F 390 is formed when methanogens are briefly exposed to air. A conversion back to F 420 is also possible, when the anaerobic conditions have been recovered. The next coenzyme, methanofuran (MF), has its role in the CO_2 reduction. It has not been described in bacteria or Eucaria yet. The tertahydromethanopterin (H4MPT) is a carbon carrying coenzyme, structurally related to folate. It participates in carbon transfer steps in the methane production from CO_2 and probably in the path of oxidation of methanol for production of CO_2 and electrons.

This coenzyme is found in all methanogens, and in extreme low concentration in *M. stadtmanii*, which produces methane solely from methanol without methanol oxidation. Coenzyme M (CoM), 2-mercaptoethanesulfone acid, is the smallest organic coenzyme found in biological systems. It acts as a methyl carrying coenzyme in the last step of the methanogenic pathway, and is found only in methanogens. Two types of cobamides have also been described in the methyl transfer reactions in the methanogenic pathways, especially from methyl substrates. Factor F 430 is a nickel containing tetrapyrrole, found only in methanogens. Its only role appears to be as a coenzyme in the methyl reductase reaction. The last cofactor is the 7-mercaptoheptanoylthreonine phosphate (HSHTP). The reduced form transfers two electrons to methyl-CoM, producing methane and a heterosulfide of HSHTP and CoM. It may also play a role in the activation of the first, methanofuran-requiring, reaction in methanogenesis from CO_2. It has not been reported in non-methanogens, and has no visible or near UV-absorbance. These coenzymes play role in three main pathways: reduction of CO_2, fermentation of acetate and dismutation of methanol or methylamines (Ferry, 1993).

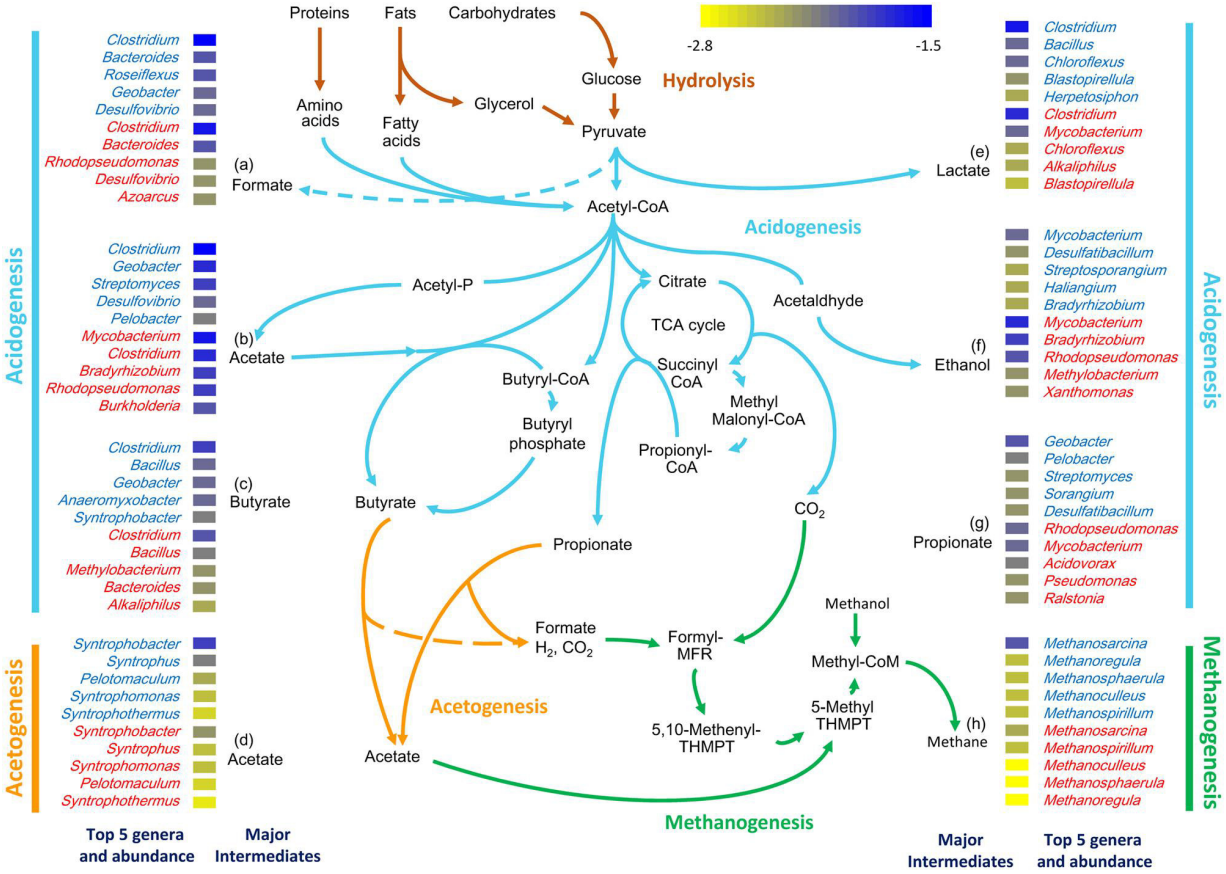


Figure 17: Metabolic pathways during the anaerobic digestion process (Cai et al., 2016)

Table 10: Methanogenic Archaea in the anaerobic digestion process (Balch et al., (1977); Koster, I.W., (1988); Daniels, (1993); Gerardi, (2003))

Order	Family	Genus	Species	Substrate	Optimal Temperature range [°C]
Methanobacteriales	Methanobacteriaceae	Methanobacterium	*M. formicum*	Formate, CO_2, H_2	37-45
			M. thermoautotrophicum	H_2, CO_2, CO, HCOOH	60-65
			M. byranti	H_2	
			M. ruminantium		
			M. alcaliphilum	H_2	
		Methanobrevibacter	*M. arboriphilus*	H_2	37-40
			M. ruminantium	H_2 , HCOOH	
			M. smithii	H_2 , HCOOH	
		Methanosphaera	*M. stadtmanii*	HCOOH, CH_3OH	35-40
	Methanothermaceae	Methanothermus	*M. fervidus*	H_2	83-88
			M. sociabilis		
Methanococcales	Methanococcaceae	Methanococcus	*M. vannielli*	H_2, HCOOH	
			M. frisius	H_2, CH_3OH, $(CH_3)_3N$, CH_3NH_2	35-40; 65-91
			M. voltae	HCOOH, Formate, H_2	30-37
			M. jannaschii	HCOOH, H_2	80-85
			M. deltae	HCOOH, H_2	
			M. maripaludis	HCOOH, H_2	
			M. thermolitotrophicus	HCOOH, Formate	60-65
		Methanothermococcus			
	Methanocaldococcaceae	Methanocaldococcus			
		Methanotorris			
Methanosarcinales	Methanosarcinae	Methanosarcina	*M. bacerii*	Acetate, CO_2, H_2, CH_3OH, $(CH_3)_3N$	30-40; 50-55
			M. mazei	Acetate, CH_3OH, $(CH_3)_3N$	
			M. barceri	Acetate, CH_3OH, HCOOH	37
			M. Gö1	Acetate, CH_3OH, HCOOH	37
			M. thermophila	Acetate, CH_3OH, HCOOH, H_2 , CH_3NH_2 , $(CH_3)_2NH$, $(CH_3)_3N$	50
		Methanolobus	*M. tindarius*	CH_3OH	25; 35-40
			M. bombayensis		
			M. profundi		
			M. taylorii		
			M. vulcani		
			M. oregonensis		
	Methanosaetceae	Methanothrix	*M. soehngenii*	Acetate	35-50
			M. CALS-1	Acetate	60
			M. concilii	Acetate	
	Methermicoccaceae				

Methanomicrobiales	Methanocorpusculaceae	Methanocorpusculum	*M. bavaricum*		30-40
			M. labreanum		
			M. parvum		
			M. sinense		
	Methanomicrobiaceae	Methanoculleus	*M. bourgensis*		35-40
			M. chikugoensis		
			M. marisnigri		
			M. palmolei		
			M. receptaculi		
			M. submarinus		
			M. thermophilus		
		Methanoplanus	*M. limicola*	$HCOOH$, H_2	30-40
			M. endosymbiosus	H_2	
			M. petrolearius		
		Methanohalobus	*M. tindarius*	CH_3NH_2, CH_3OH, $(CH_3)_2NH$, $(CH_3)_3N$	50-55
		Methanohalophius			35-45
		Methanomicrobium	*M. mobile*	$HCOOH$, H_2	
			M. paynteri	H_2	
		Methanogenium	*M. cariaci*	$HCOOH$, H_2	20-40
			M. marisnigri	$HCOOH$, H_2	
			M. tatii	$HCOOH$, H_2	
			M. olentangyi	H_2	
			M. thermophilicum	$HCOOH$, H_2	
			M. bourgense	$HCOOH$, H_2	
			M. aggregans	$HCOOH$, H_2	
		Methanococcoides	*M. methylutens*	CH_3NH_2, CH_3OH	30-35
	Methanospiriliaceae	Methanospirillum	*M. hungatei*	$HCOOH$, H_2	35-40
			M. barkeri	CH_3NH_2, CH_3OH, $(CH_3)_2NH$, $(CH_3)_3N$, Acetate, H_2	

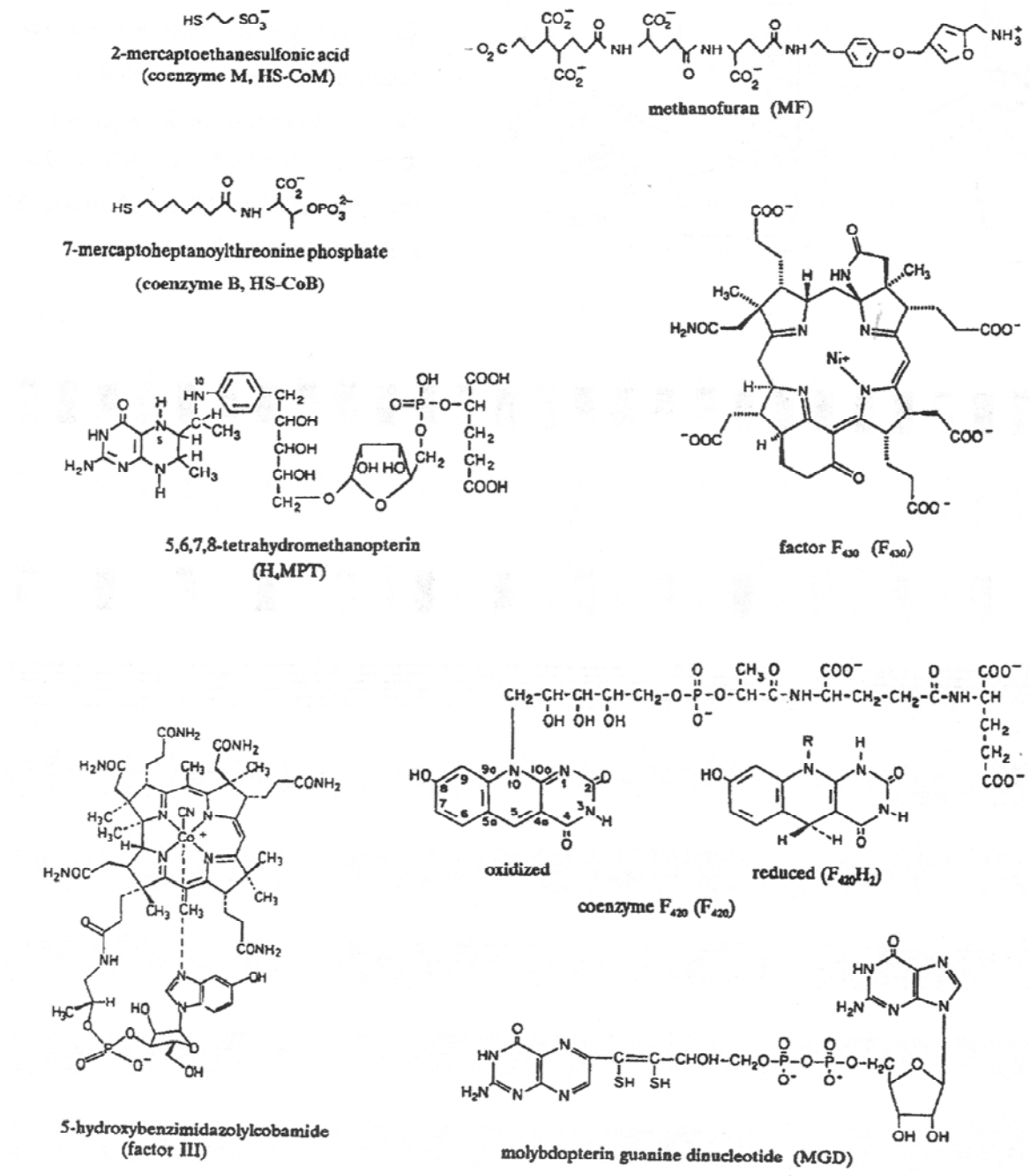


Figure 18: Cofactors in methanogenic pathways (Ferry, 1993)

The microbiological composition in every one of the four stages of the AD process differs and shifts according to the substrate composition and the operational conditions in the fermenter. Figure 17 shows comparison of the microbiological composition in two different wastewater treatment plants (Cai et al., 2016) as one of many examples in the literature.

1.3.2 Biogas production

1.3.2.1 Biogas plants

Anaerobic digestion plants require high ***investment costs***, which is one of their major disadvantages. To enforce their dissemination also of the other renewable energy sources, the EU credits different investigations by laws and programs. Despite these measures, the statistics report negative tendencies in the biogas industrial sector.

The biogas plants are implemented in the agriculture, food and beverage industry and wastewater treatment plants. The development of proper fermentation parameters for a given substrate starts with laboratory experiments. The results achieved on a bench fermenter are often tricky to upscale concerning the volume of the operating costs for keeping the process parameters on the go. Investigation costs, gas utilization, plant operating: stirring, foaming avoidance, fast utilization of the waste, stable process at high HRT are points which should be discussed on the financial level. Carbon footprint of the plant should also be controlled, in order to maintain one of its main goals, namely ecological friendly energy utilization (Fuchs and Drosg, (2010); Wang et al., (2016); (Hijazi et al., (2016); Iordan et al., (2016)).

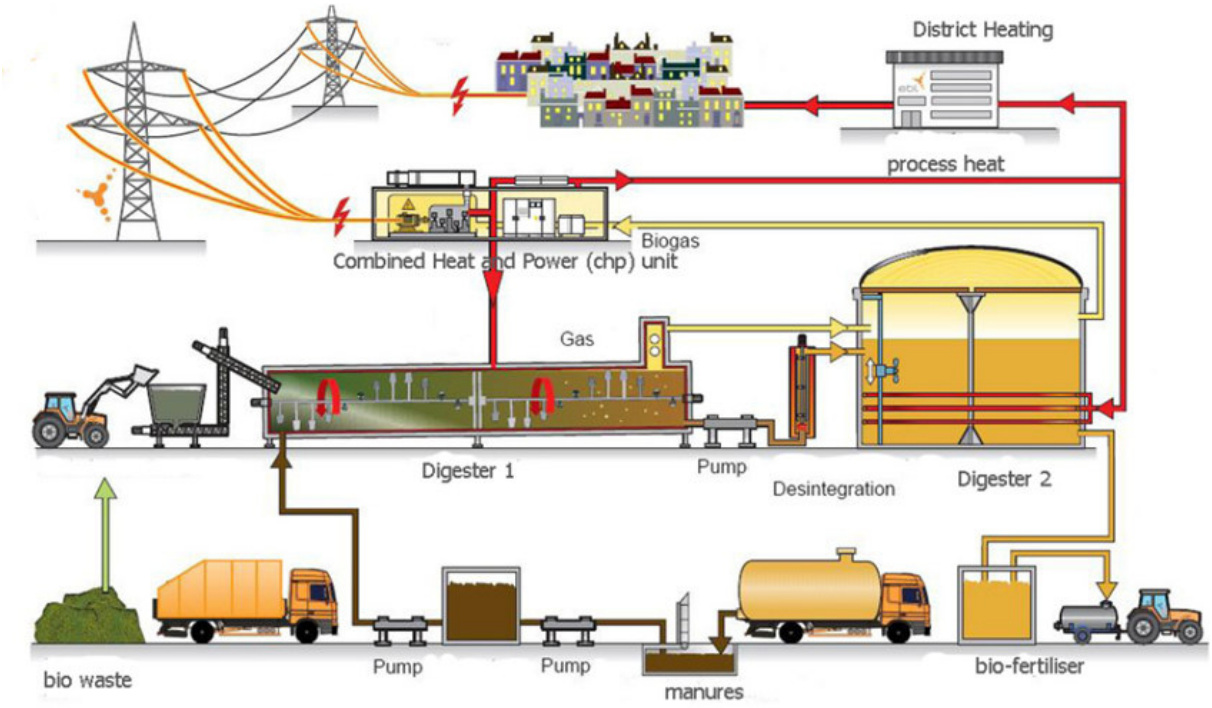


Figure 19: Technological flow process of a biogas plant ("Henan Hi-tech Kingdo Industrial Co., Ltd.," 2016)

In the ***industrial biogas plants*** (Figure 19) the AD is a controlled and well monitored process, which guarantees the optimal biogas yield with profitable methane content. The factors controlling the AD are the temperature, retention time, pH, chemical composition of the treated substrate, and the toxic compounds, which can possibly inhibit the process, as oxygen, ammonia, chlorinated hydrocarbons, benzene ring compounds, formaldehyde, VFAs, LCFA, heavy metals, cyanide, sulfide, salinity, tannins, and last but not least, feedback inhibitors as several intermediates (H_2, VFA).

Table 11: Monitoring parameters for the stable biological process in biogas plants (Döhler et al., 2013)

Parameter	Value
Dry matter [%]	<15
pH [-]	7-8
Ammonium-N [g/L]	<4 at pH=7
Oxygen [mg/L]	<0.4
Sulphur H_2S [mg/L]	<50 at pH=7
VFA [g HA_{eq}/L]	2-6
Alkalinity [g $CaCO_3$/L]	8-15
VFA/Alkalinity [-]	<0.6
Acetic acid [mg/L]	<3,000
Propionic acid [mg/L]	<600
Butyric acid [mg/L]	<50
iso-Butyric acid [mg/L]	<60
Copper [mg/L]	<50
Zink [mg/L]	<150
Chrome [mg/L]	<100

The ***monitoring parameters*** listed in Table 11 are main factors which should be considered during the operation of the plant. Changes in these parameters should be interpreted not only as absolute values, but also referring to their overall progression. Depending on the microbial consortium in every single digester, and the enrichment of different species, adaptation to deviations of the recommended parameter values can be achieved (Chen et al., (2008); Ortner et al., (2014)). Other parameters, like propionate and the butyrate cannot be utilized directly by the methanogenic Archaea, therefore their accumulation is an indicator of stress in the digester. The overall increase of VFA is partly balanced by keeping high buffer capacity in the fermenter. Hydrogen accumulation is inhibiting the acetate-forming bacteria and the acetotrophic methanogens, therefore low hydrogen pressure in the fermenter ($<10^{-4}$ atmospheres) is essential for the undisturbed methane production. Although the energy obtained from the hydrogen utilization is higher than the one from acetate conversion, only 30% of the methane is produced from hydrogen, because of its limited supply.

Another factor that indicates process instability is the ***foam formation***. This can be caused by protein rich substrates, or overloading of the fermenter, with decreased pH and simultaneously discharge of CO_2. On the other hand, foaming can occur when the viscosity in the fermenter broth rises and the digestate cannot be homogenized properly. The consequence is the decreased biogas discharge. Another consequence of low homogenization efficiency is the building of scum layer, which can also cause disturbed biogas discharge and foaming formation. For this reason the increase of the viscosity in the fermenter should be also avoided. In general, the increased viscosity is a consequence of overload of the fermenter, which is coupled to increased total solids contents. There are some compounds in the substrates, which do not increase the solid content, but do increase the viscosity, e.g. pectic substances (Brooks et al., 2008).

The polymers in the anaerobic digester are primarily hydrolyzed by bacteria. The relative bacterial abundance in the anaerobic digester is $>10^{16}$ cells per mL. The three main groups are saccharolytic ($\sim10^{8}$ cells/mL), proteolytic ($\sim10^{6}$ cells/mL), and lipolytic bacteria ($\sim10^{5}$ cells/ mL). The optimal conditions for these species and their endo- and exo-enzymes differ strongly from the ones for the methanogenic Archaea. As long as the biochemical processes should occur in the same anaerobic digester, the requirements of the more sensitive methanogenic microorganisms are taken into account. Not only is the pH optimum shifted, but also the growth rate. The generation times range from 3 days at 35°C to 50 days at 10°C, which requires high retention times in the anaerobic digester, at least 12 days for settling a large population (Gerardi, 2003). Very important is high solid retention time (SRT). These circumstances induce the possible separation of the biochemical phases of the AD in different fermenters (Döhler et al., 2013).

1.3.2.2 Anaerobic wastewater treatment

The anaerobic wastewater treatment is based on the development of high-rate reactions. The solid and the liquid retention time are uncoupled in the reactors resulting in high concentrations of biomass. This is achieved by bacterial growth on inert carrier (FB and FF reactors), or by self-immobilization of the biomass in the form of granules (UASB, EGSB, respectively) (Figure 20). Thick biofilms can induce mass-transfer limitations, resulting in overall limitation of the rector capacity. Some studies report that both internal and external diffusion limitations can influence the substrate utilization (Dolfing, 1985). Other, in contrary, do not detect limitations in the biofilm, but consider that the pH gradient inside the biofilm may cause these (de Beer et al., 1992). Anyway, the general accepted assumption is that the mass transfer is based on diffusion (Pavlostathis and Giraldo-Gomez, (1991); Gonzalez-Gil et al., (2001)).

In the anaerobic treatment of wastewater, the single-stage digester is a large tank, where sludge digestion and settling occur simultaneously. The sludge forms several layers from the bottom to the top of the digester: digested sludge, actively digesting sludge, supernatant, scum layer and gas. The two-stage digestion consists of two following tanks - in the first one the waste is digested by active sludge, mixed and heated continuously, while in the second tank settling and storage prior to the withdrawal and ultimate disposal take place (Bitton, 2005).

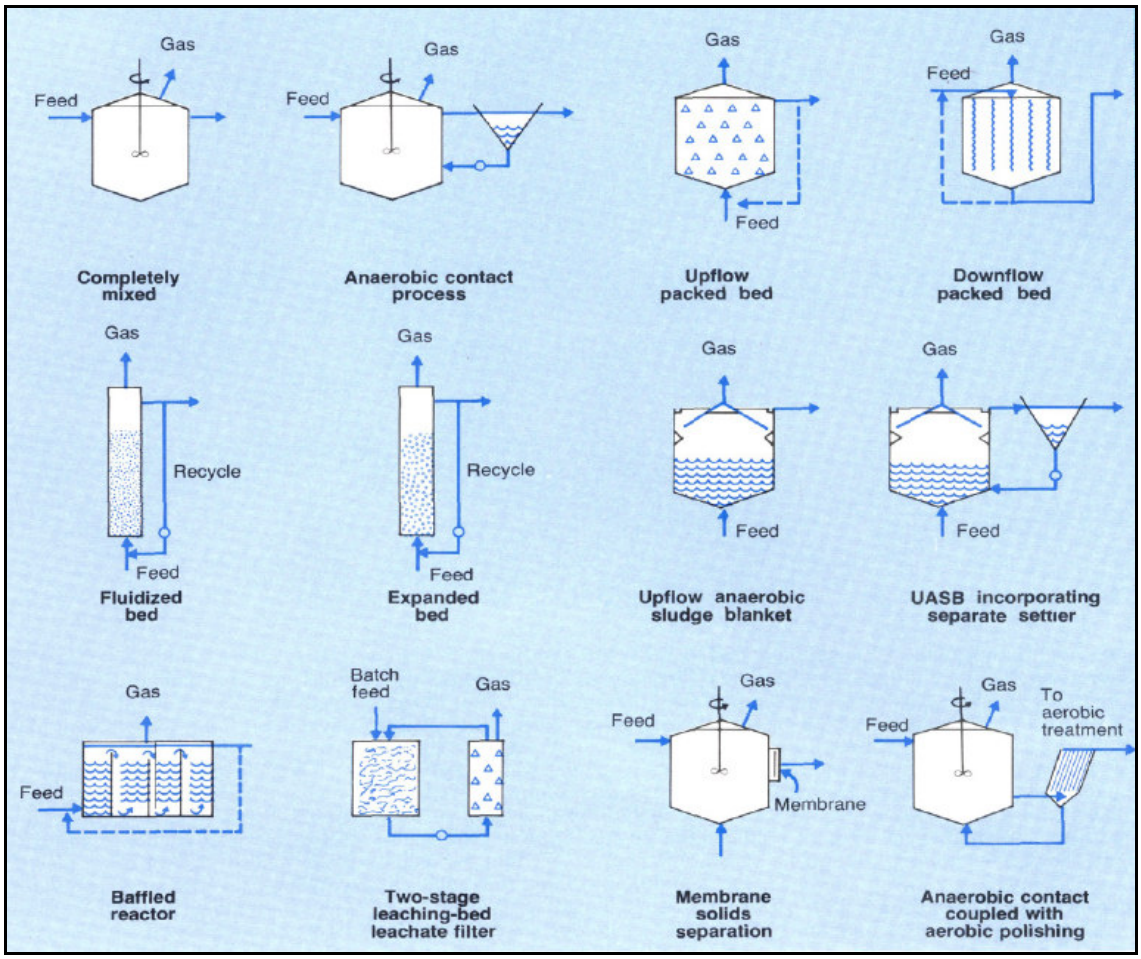


Figure 20: Reactor configuration for AD (Speece, 1983)

The ***bioreactor configurations*** used for the anaerobic treatment of wastewater are septic tank (tank followed by absorption field), UASB, anaerobic filters, anaerobic attached-film expanded-bed, fluidized bed reactors, and anaerobic rotating biological contactor. The septic tank is the oldest system. The produced sludge, septage, is disposed to the land or mixed with municipal wastewater. The floating layer, scum, is preceded to the absorption field and percolates further to the groundwater, leading also to possible contaminations. Later, at the beginning of the 20th century, the UASB reactor has been introduced. This system uses immobilized biomass for increasing the retention time of the sludge. It consists of a bottom layer of packed sludge, a sludge blanket and an upper liquid layer (Lettinga et al., (1980); Lettinga, (1995)). The wastewater flows upwards trough the sludge bed, which is covered with a floating blanket of active bacterial flocks. The sludge flocks are separated from the treated water by settler screens. The so formed compact granular sludge can withstand the sheer force of the upflow of the wastewater. The next developed system is the anaerobic filter (Young and McCarty, 1987). These filters are the anaerobic equivalent of the trickling filter and are packed with support media with a void space >50%. Similar system is the thin film reactor (Berg and Kennedy, 1981). In the anaerobic expanded-bed reactors the wastewater flows upward through a sand bed, where the sludge is attached. The system is suitable for low strength wastewaters with COD < 600 mg/L. The anaerobic rotating biological contactor is similar to the aerobic one. The anaerobic sequencing batch reactor (ASBR) is a batch variation of the UASB reactor. There are also hybrid systems between UASB and anaerobic filter. They have the advantage that the removal of the sludge can be accomplished in situ.

The ***factors for monitoring*** the AD in the wastewater treatment are generally the same as the AD of solid wastes. The difference is in the chemical composition of the wastewater, which usually must be balanced nutritionally (nitrogen, phosphorus, sulfur, trace elements etc.). The optimal C:N:P ratio in the reactors is reported to be 20-30:5:1. The structure of the granules is strongly influenced by the concentration of K, N, P, and Mg ions (Ahring et al., 1993).

1.4 Two-stage anaerobic digestion

The industrial anaerobic digestion can be conducted in single digester, where all of the biochemical processes take place in the same environment, regarding homogenization, retention time, temperature, and pH. This is the so called one-stage AD.

Another, not as often used approach, is the separation of the different phases in the substrate degradation in different fermenter with the possibility to regulate the process parameters according to the requirements of the corresponding microorganisms. This is the two-stage AD (Figure 21).

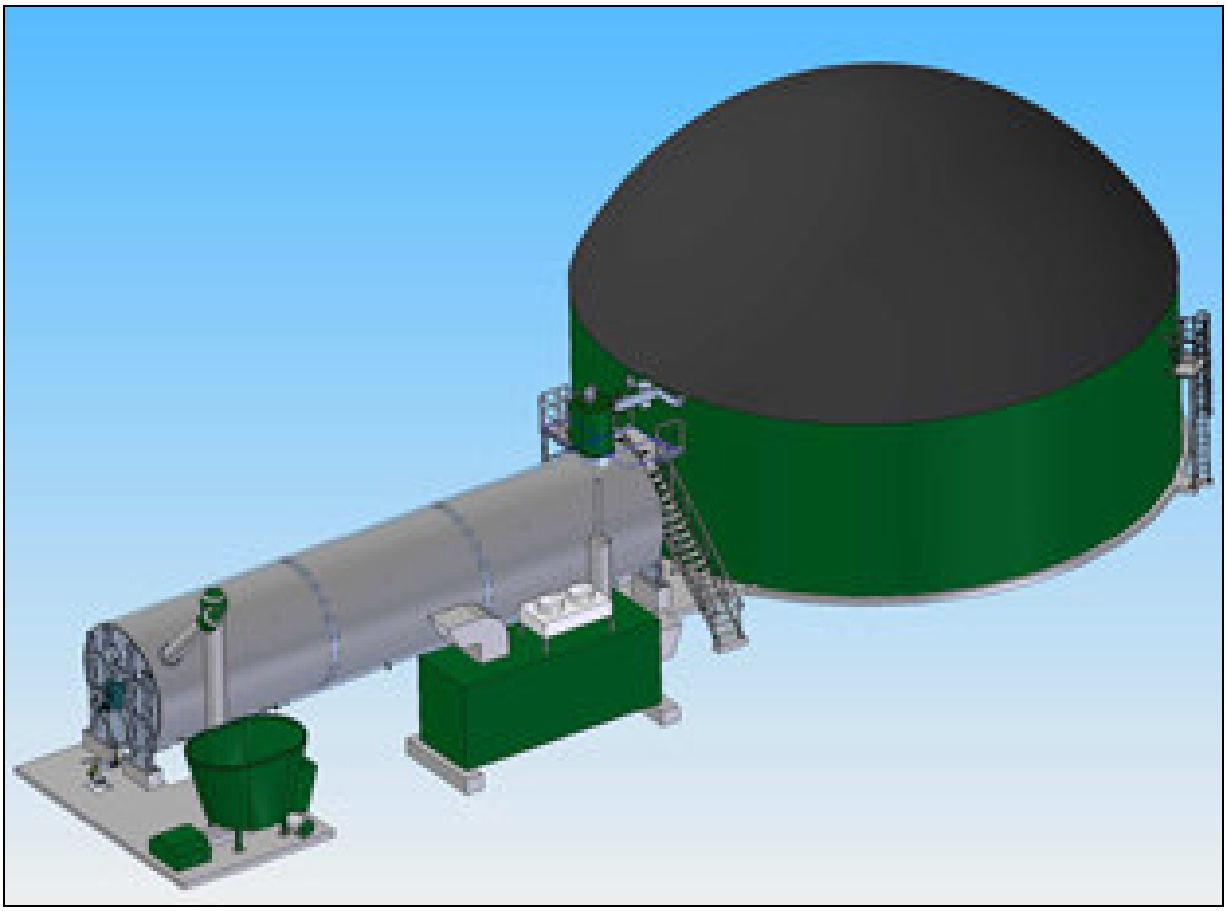


Figure 21: Two-stage biogas plant (ARCHEA New Energy GmbH)

In the first fermenter the hydrolysis and the acidogenesis phase of the anaerobic conversion occur at different operational conditions than the acetogenesis and the methanogenesis in the second fermenter (Ghosh et al., 1987). The effluent from the acid forming digestion phase (pre-acidification) is conveyed to the second methanogenic phase, together with the gas phase from the first fermenter. The advantages of this approach are improvement of:

- process control and stability
- overall process efficiency

- energy productivity and biogas yields
- possibility of processing different biomass species
- substrate conversion
- COD reduction
- avoiding overloading and/or inhibition of the methanogenic population (Schievano et al., 2012)

The high investigation costs is one of the main disadvantages of the two-stage approach, which can be compensated with a stable process with less disturbances and faster start-up in case of substrates containing inhibitory compounds, or which are not fast degradable. The overall biogas yield is not significant higher, compared to the one-stage fermentation for the same substrate (Lindner et al., (2015); Lindner et al., (2016)).

The optimal pH and temperature for the hydrolytic and acidogenic bacteria differs strongly to the one for the methane forming Archaea. Several studies reported optimal pH levels below 6, and temperatures higher than 45°C (Zoetemeyer et al., (1982); Kozuchowska and Evison, (1995); Demirel and Yenigün, (2002)). The gas phase from the acidogenic reactor is CO_2 and hydrogen rich, and considered as a source for hydrogen production (Cooney et al., (2007); Dareioti et al., (2014)). Beside this, also the growth rate of the hydrolytic and acidogenic bacteria is several times higher than the one of the methanogenic Archaea. This physiological feature allows the maintenance of lower HRT in the hydrolytic reactor, leading to lower fermenter volumes.

As already mentioned above, the methanogenic Archaea are more sensitive to the operational conditions than the hydrolytic and acidogenic bacteria. Additionally to this fact, the slower growth rate is hindrance for recovering of the fermentation in case of disturbances. Applying of two-stage AD, conducted in the proper conditions, and namely, high OLR, low pH (4-6) and elevated temperatures (45-50°C) in the first, pre-acidification, stage and low OLR, pH 7-8 and 35-37°C in the second, methanogenic stage provides the optimal environment for all of the species, taking part on the AD. The technical execution should be adapted to the composition of the outcome from the first stage (hydrogen-rich gas and VFA-rich broth) and to ensure their adequate supplementation into the second reactor. All described strains of methanogenic bacteria utilize H_2 as an electron donor for methanogenesis and growth. Formate is an electron donor for approximately one-half of all described strains. The maximum methane formation from formate in *Methanobacterium formicum* at 37° C occurs at pH 8. The H_2 production increased with increasing temperatures (56° C) (Schauer and Ferry, 1980). *Methanobacterium ruminantium* and *Methanospirillum hungatii* possess a formate dehydrogenase is linked to coenzyme F 420 as the first low-molecular weight anionic electron transfer coenzyme. The formate dependent growth of *Methanococcus vaneillii* is stimulated by addition of selenium and tungsten and contains selenocysteine (Jones et al., 1987). Coenzyme F 430, the hydrocorphinoid nickel complex 1, is the prosthetic group of methyl coenzyme M reductase (MCR), the key enzyme in biological methane formation by methanogenic Archaea (Jaun and Thauer, 2007). The supplementation of trace elements into the second-stage of the AD can also lead to their increased availability.

The peculiarity of the two-stage AD was taken as a fundament for the solving of the problems in the AD of several industrial organic wastes.

1.5 Enzymatic pretreatment

Substrate pretreatment is an approach to overcome the bottlenecks on the AD process. This procedure should occur before the anaerobic fermentation, therefore it requires separate tanks. Besides the physical (mechanical, microwave, ultrasound), thermal, and chemical pretreatment methods for enhancing the biogas production from different slowly degradable substrates (Yadvika et al., 2004), the microbiological pretreatment (two-stage fermentation) and enzymatical pretreatment are viable options.

The slow degradation of the lignocelluloses and hemicelluloses in the substrates can turn out to be the limiting step in the AD process. The degradation of polymers by bacteria or fungi occurs by the excreted exo-enzymes. This presumes direct contact of the microorganisms and the polymer, i.e. the ambient environment should contain additionally the optimal nutritional and physiological conditions for the microorganisms. Moreover, well performed homogenization should be provided, depending on the morphology of the hydrolyzing species, e. g. filamentous, clusters, single cells. The production and extraction of hydrolytic enzymes, and their direct application on the substrate presents an option to the microbiological pretreatment. In this case the optimal physiological conditions for the enzyme activity still should be provided, but they can be applied on substrates containing compounds toxic or inhibiting for the microorganisms, e.g. high salt concentrations, heavy metals. Anyway, the problem with the high costs of the commercial enzymes still competes with the efficiency of the results.

The use of cellulolytic enzymes has been studied for a long time yet (Higgins, (1986); Lagerkvist and Chen, (1993); Wawrzynczyk et al., (2003)). The general goals in the studies are increasing the sludge dewatarability (Karam and Nicell, (1997); Roman et al., (2006)), or to enhance the digestability of lignocellulosic-rich biomass (Hendriks and Zeeman, 2009). Namely the effect of enhanced solubilisation of the cellulose fraction after enzymatic pretreatment is the topic of interest in this work. The application of enzymes for saccharification and subsequent increased water extractability can be applied for the whole list of polysaccharides, e.g. arabinoxylans (Severini et al., 2015), starch (Cole et al., 2015), proteins (Torres et al., 2016). The enhanced dewatarability of sludges (Chen et al., 2015) and anaerobically digested biosolids (Dursun et al., (2006); Abu-Orf et al., (2007)) by using enzymes are still object of investigation, together with the microbial flocculants (Tong et al., 1999). The solubilisation of polymers influences several factors influencing the dewatarability of the sludge: suspended solids concentration, exocellular polymers, bound water. Other factors, affecting the dewatarability, which should be mentioned, are the pH and particle size and distribution.

Another consequence from the enhanced dewatarability of the polymers is the decreased viscosity (Hashimoto and Hiraoka, 1990). The viscosity of the fermentation broth is a parameter requiring up to 10% of the self-sustaining energy of a biogas plant (Döhler et al., 2013). This aspect is discussed as a benefit in the two-stage AD of SBPP, where the degradation of pectic substances is leading to low viscosity levels (Stoyanova et al., 2013).

2 Aims

The two-stage AD provides optimal conditions for the microbiological consortium in the AD. The methanogenic Archaea are the more sensitive microorganisms against inhibitors in the overall process, which is supposed to pre-digest the inhibitory compounds for stable process. Carbohydrate polymers are slowly degraded by the hydrolytic bacteria and are therefore the limitation step in the overall process. This limitation is challenge for a controlled technical process, which is supposed to reach optimal performance. The endeavor of this work is the investigation of the applicability of two-stage AD of the three different substrates for achieving stable and technically and economically feasible process. The following three aspects have been investigated in this work:

- Two–stage AD for faster degradation of carbohydrate polymers on the example of sugar beet pressed pulp (publication 1, Appendix) (Stoyanova et al., 2014): Separation of the AD process and the operation of the acidogenic reactor at low pH and high temperature should lead to the efficient degradation of the pectic substances, and further to the reduction of the viscosity and the foaming in the fermentation of pectin-rich organic wastes as an advantage in the technical operation.
- Degradation of inhibiting for the methanogenic Archaea compounds in the first stage of the two-stage AD on the example of olive mill solid waste (publication 2, Appendix) (Stoyanova et al., 2016b): The inhibiting compounds like polyphenols in the OMSW should be degraded in the pre-acidification stage at pH below 6 and 55°C. The desired technical advantage is fast start-up of the fermentation and stable methanogenesis.
- The role of the optimal conditions for the cellulolytic enzymes for the optimal solubilisation of the carbohydrate polymers in wastes with high anorganic content originating from paper mill (publication 3, Appendix) (Stoyanova et al., 2016a): The residual organic fraction in the solid waste fraction after the press belt should be separated before the further disposal of the solid, mostly anorganic, waste, in order to be used for energy recovery, and in the same time the waste amount should be reduced. The water binding capacity of the waste stream should be decreased to achieve better dewatarability in the press belt or centrifuge. Cellulolytic enzymes were applied for achieving these goals.

The optimization of the process parameters is valid for the given substrate, and can be considered as a reference point for further up-scaling, or for the planning of AD of substrates with similar parameters.

3 Results

3.1 General

The experiments with SBPP and OMSW aimed to find out and optimize the fermentation parameters temperature and HRT for two-stage AD within sugar and olive oil industry and to point out its possible advantages. These results should be the basis for industrial scale application. They were carried out in a laboratory CSTR fermenter with total volume of the first stage V=0.001 m^3 and second stage V=0.008 m^3 (Figure 22).

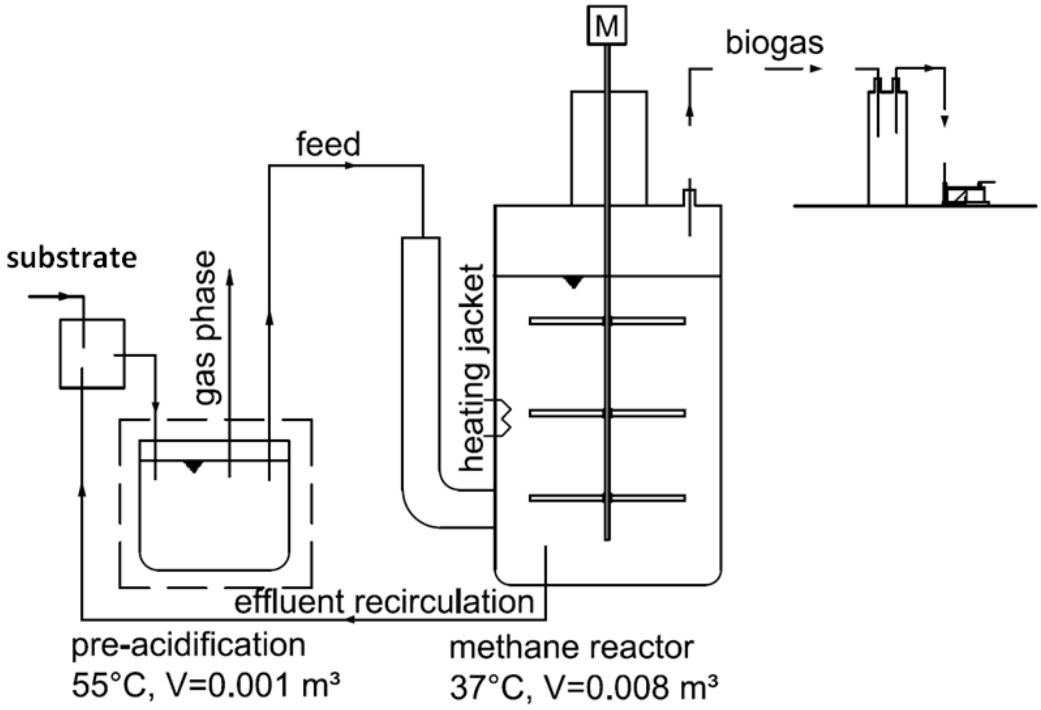


Figure 22: Two-stage process (Stoyanova et al., 2013)

The pretreatment of the PMS was planned in accordance with the parameters of an existing AD plant at a paper mill in Austria. In this case the technical parameters of the plant were considered in the evaluation of the experimental outcomes in terms of practical implementation.

The results obtained within the practical part of the current thesis were published in 3 scientific papers and 2 conference contributions. These publications are attached in the appendix. The following chapters provide an overview of the most important findings.

3.2 Anaerobic digestion of sugar beet pressed pulp

The positive effect of the industrial scale anaerobic digestion of sugar beet pressed pulp can be highlighted with an existing example – the Kaposvár Sugar Factory (Figure 23). The implementation of the biogas plant on the site of the factory in 2007 brought two main advantages for the factory. Firstly, the generated electricity covers over 50% of the demand of the factory and provides independence from shifts of the electricity prices on the long term. Second, the environmental effects makes the plant sustainable and more competitive (Csima and Szendefy, 2009).

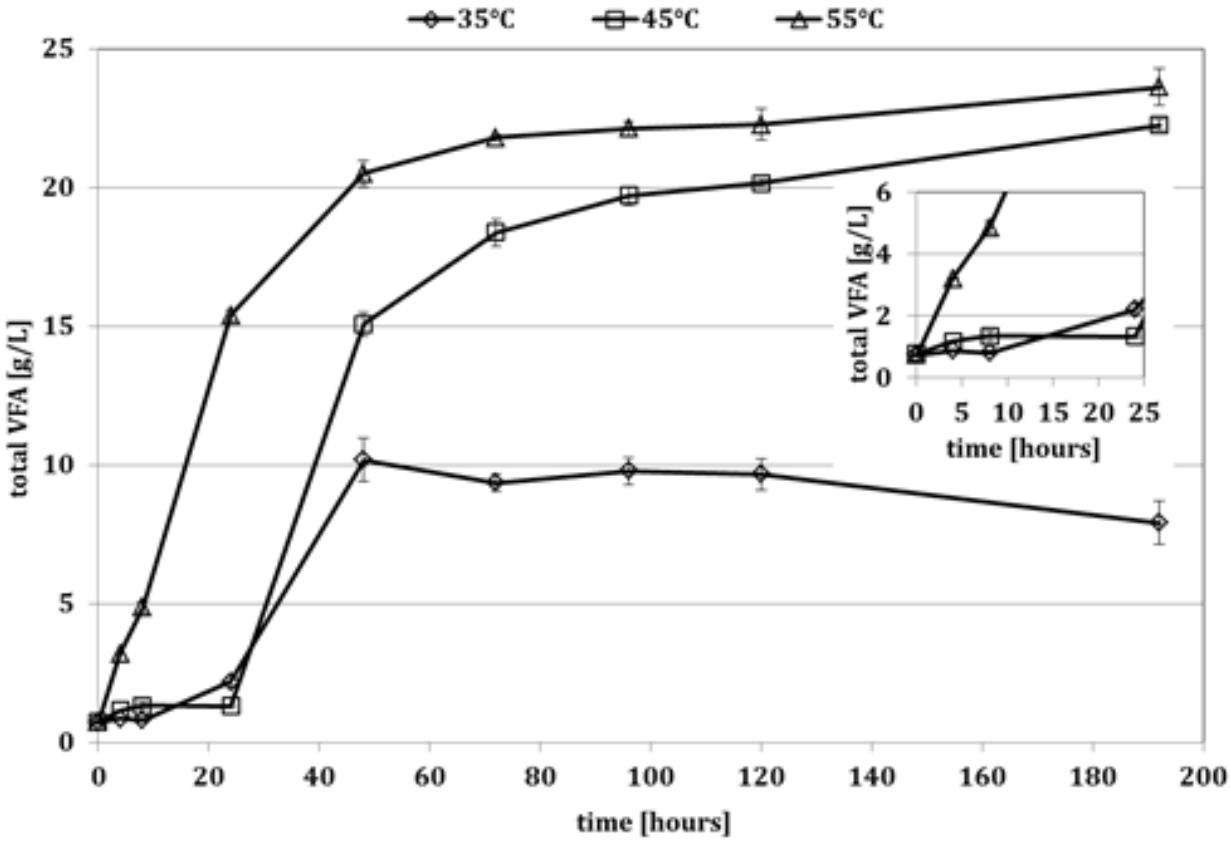
Figure 23: The biogas plant on the site of the Kaposvár Sugar Factory of Agrana in Hungary

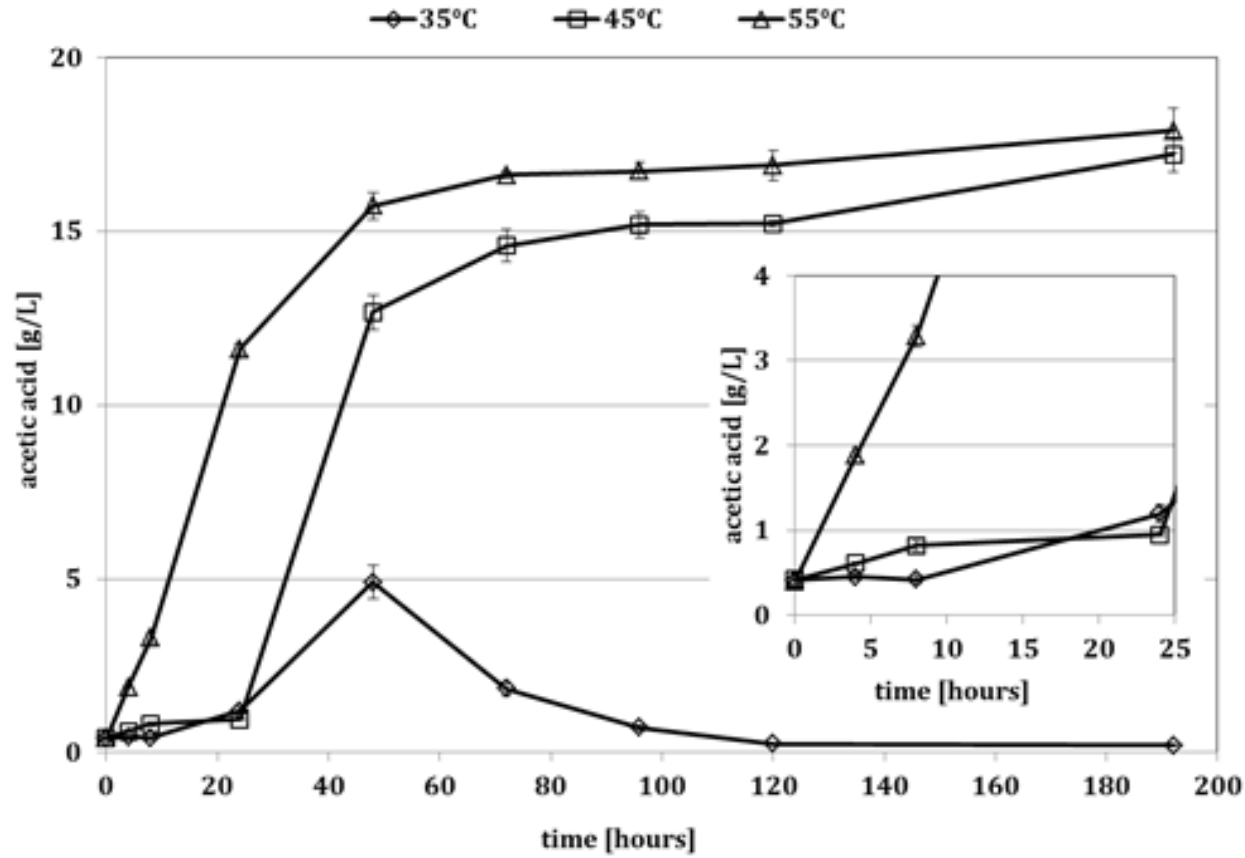
Nevertheless, the anaerobic digestion of sugar beet pressed pulp has its bottlenecks. The main problem is the foam occurring at higher OLR. Factors causing foaming during the AD, are the protein content of the substrate, temperature, mixing, and digester shape. Foaming can be overcome by adding antifoaming agents, or by reducing the OLR. The latter option is not preferable because sugar production is a short term campaign and therefore SBPP has to be utilized as rapidly as possible. This substrate contains also slowly degradable compounds (22-30% cellulose, 24-32% hemicellulose, 24-32% pectin and 3-4% lignin).

The separation of the anaerobic digestion process in two stages in order to influence the degradation kinetics of these compounds promises to turn out to be a reasonable problem solving. In this study the latter option was investigated. First the optimal pre-acidification conditions were determined, and subsequently, one- and two-stage fermentations were carried out in parallel.

3.2.1 Batch experiments for determination of the optimal incubation temperature of the first stage of the two-stage fermentation of SBPP

The focus in this part of the experiment is set on the formation of VFA at three temperature levels: 35, 45, and 55°C. The total VFA concentration should be high, the acetic acid should present the component with the biggest part, and the propionic acid concentration should be the lowest.


35°C
45°C
55°C
total VFA [g/L]
time [hours]
0
5
10
15
20
25
0
20
40
60
80
100
120
140
160
180
200
0
2
4
6
0
5
10
15
20
25


35°C
45°C
55°C
acetic acid [g/L]
time [hours]
0
5
10
15
20
0
20
40
60
80
100
120
140
160
180
200
0
1
2
3
4
0
5
10
15
20
25

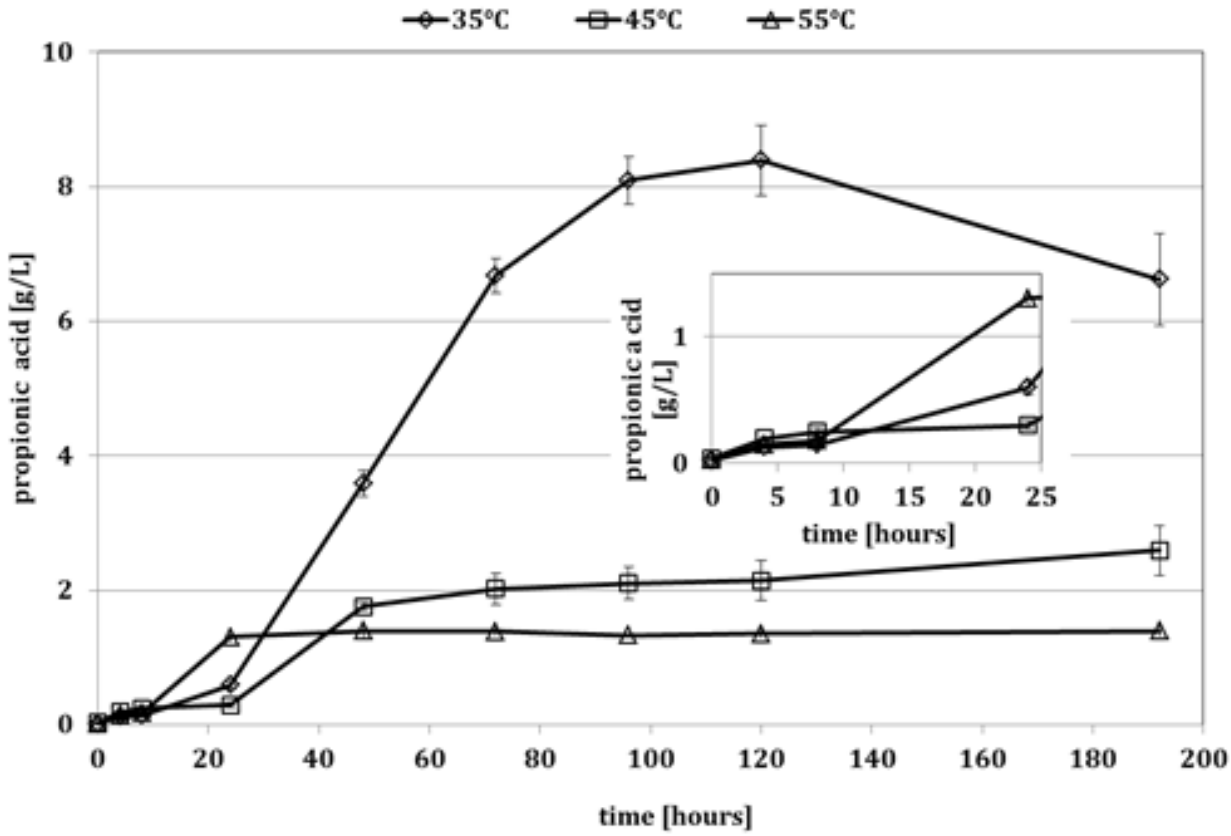


Figure 24: Total volatile fatty acid, acetic acid, and propionic acid concentration in the first stage of two-stage AD of SBPP—batch set-up, small graphs show the zoomed regions with lowest concentration

The results from the HPLC analyses (Figure 24) show that the optimal temperature in aspect of VFA concentration is 55°C, and the optimal HRT – four days.

3.2.2 Comparison of one- and two-stage AD of SBPP

Based on the results above, the first stage of the two-stage AD fermentation was carried out at 55°C and HRT four days (Figure 25). The concentration of the total VFA remained between 12 and 20 g/L, and the pH – between 4.8 and 6.5 without adjusting. It correlated strongly reversed with the concentration of the VFA, which points out the importance of keeping the HRT less than four days. Otherwise shift into methanogenesis is possible.

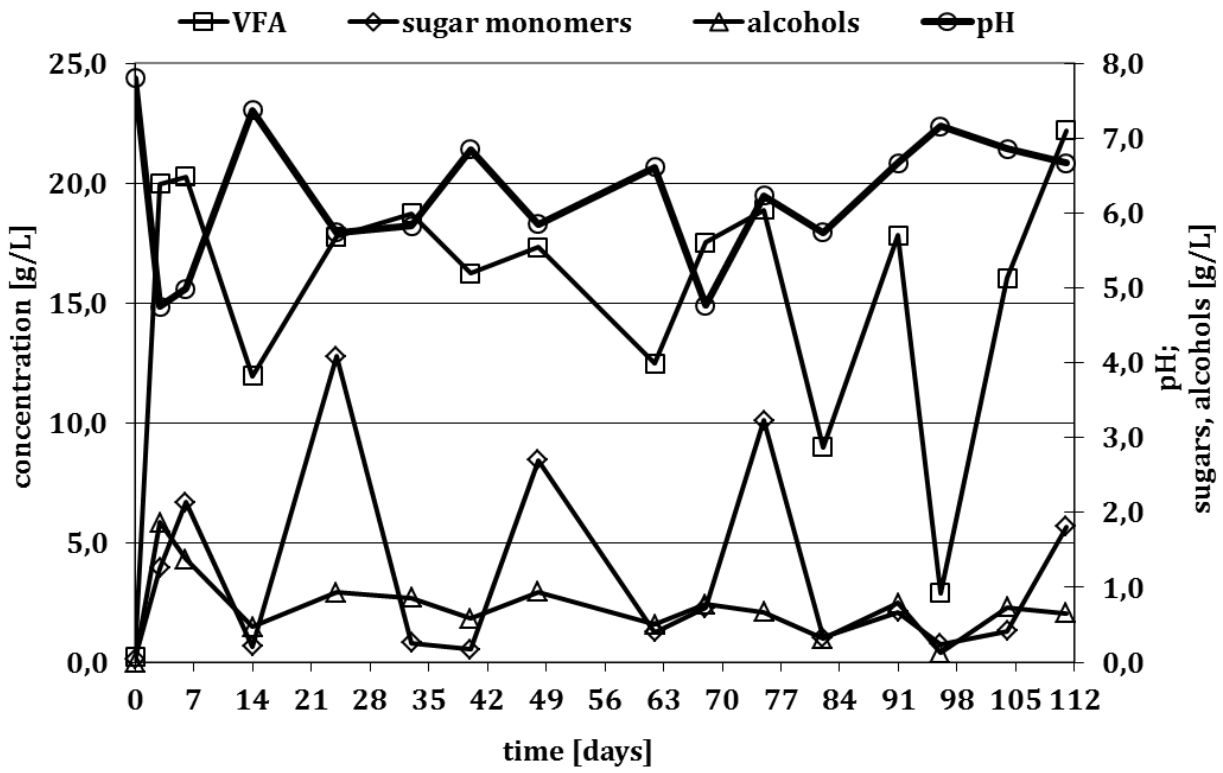


Figure 25: Volatile fatty acids, sugar monomers and alcohols concentration progress during the first stage of the two-stage AD of SBPP , determined weekly, by daily recirculation

The progress of the semi-continuous one- and two-stage fermentations (Figure 26) shows the more stable second stage of the two-stage fermentation, where foaming occurred at twice as lower HRT, and no VFA accumulation occurred during the whole process.

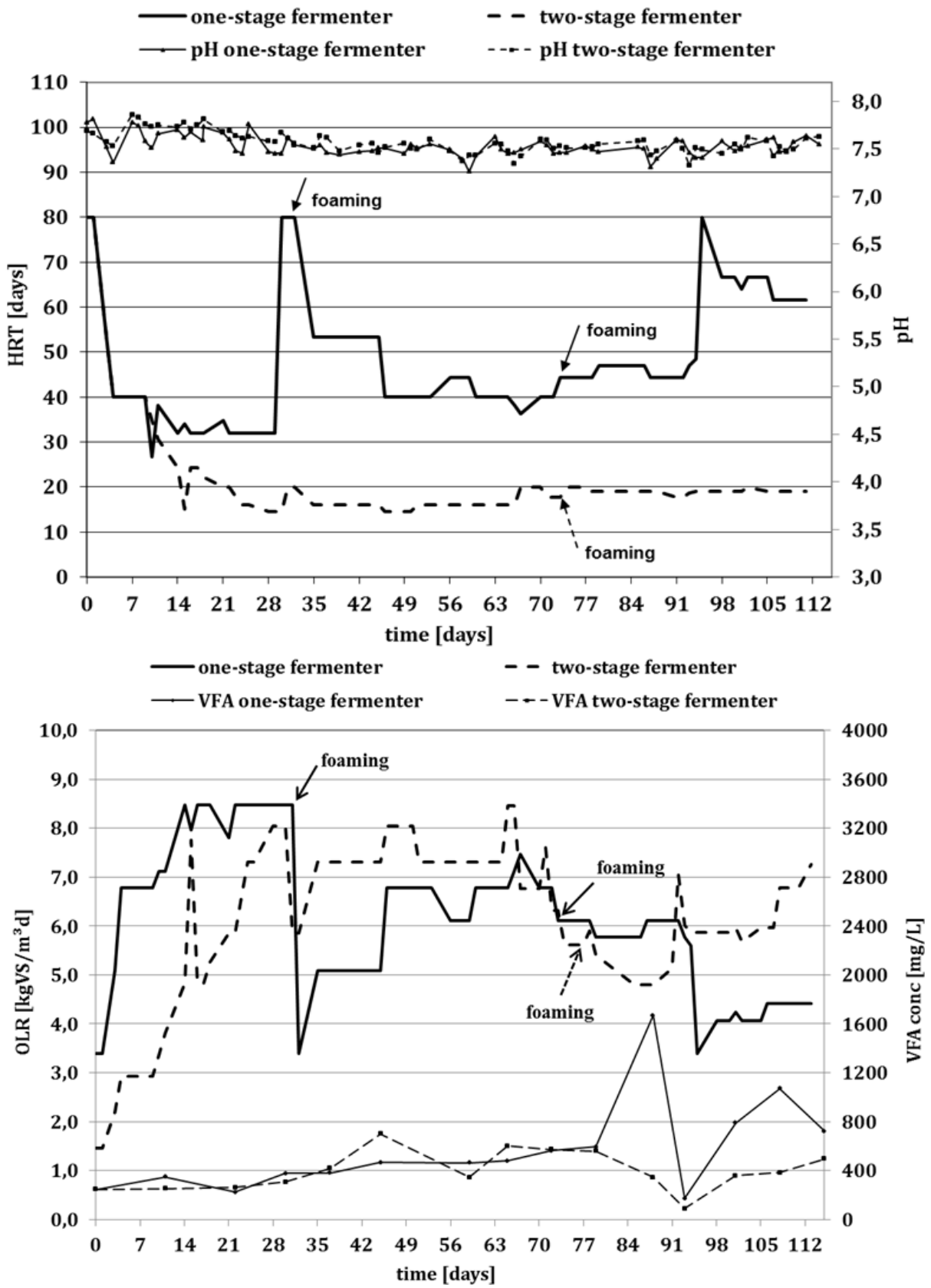


Figure 26: One-and two-stage fermentation process of SBPP: HRT and pH, and OLR and VFA concentration. The arrows show the foaming event during the one-stage fermentation (solid) and two-stage fermentation (dashed)

The viscosity measurements confirmed the effective degradation of the pectic substances, which led to decreasing of the viscosity in the second stage of the two-stage fermenter (Figure 27).

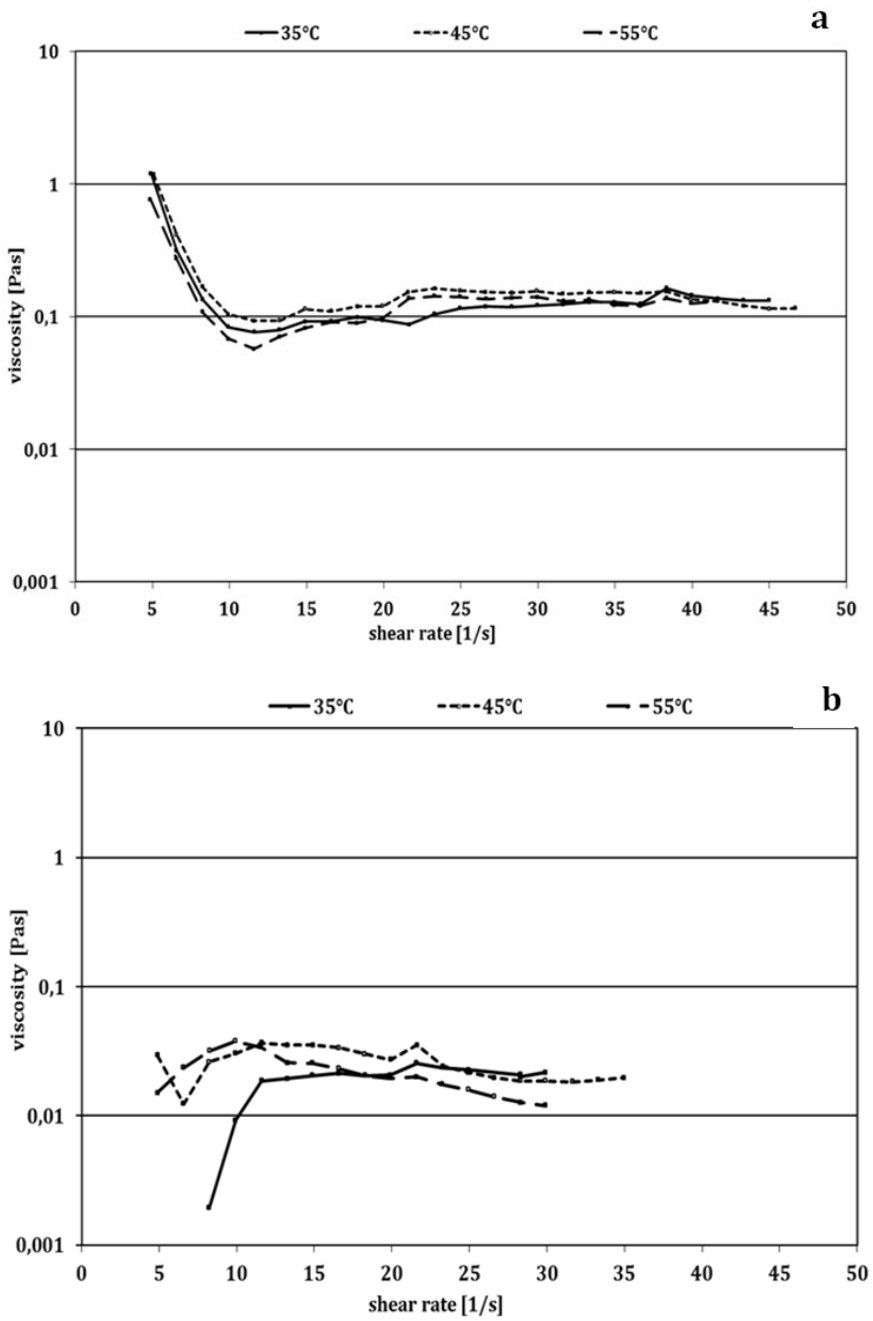


Figure 27: Viscosity of the fermenter content from the one-stage fermenter (VS = 2.64 %) (a), and in the second stage of the two-stage fermenter (VS = 4.06 %) (b)

Rapid degradation of gelling substances (pectines) was confirmed by FTIR analyses. The resulting reduction of the viscosity leads to a five times lower energy demand for reactor stirring. In summary, these advantages make the two-stage process economically attractive, despite higher investments for a two reactor system.

3.3 Anaerobic digestion of olive mill solid waste

Industrial scale plants for anaerobic digestion of OMSW are still scarce not only due to the structure of this agricultural branch, but also due to the severe nature of the substrate. In this case the problems that should be solved are caused by the high concentration of polyphenols in the OMSW, which inhibits the microorganisms and leads to drop in the pH with consequent breakdown of the process. The negative effect of the polyphenols can be avoided by keeping their concentration in the anaerobic digester low. The problem by doing so is the enormous long HRT. This fact makes the installation of anaerobic digester for monofermentation unprofitable. As mentioned above, one of the advantages of the two-stage fermentation is the overcoming of inhibitory effects by separating the biochemical process and allowing the microorganisms to be active at their optimal conditions. Therefore, the two-stage anaerobic digestion was used as approach to reduce the influence of these inhibiting compounds. Additionally, due to the low buffer capacity in the fermenter, co-fermentation with chicken manure (CM), as a substrate with high nitrogen content, was also conducted.

3.3.1 Batch experiments for determination of the optimal incubation temperature of the first stage of the two-stage fermentation of OMSW

The optimal conditions for the pre-acidification of OMSW were, similar to the SBPP, 55°C and HRT four days (Figure 28). The high concentration of sugars in the pre-acidification stage (0.1 – 0.15 g/gVS) witnesses the inhibition of the fermentative bacteria. During the pre-acidification the sugar monomers and dimers are quickly converted into VFA, and their concentration in the pre-acidification of other substrates is significantly lower, e.g. SBPP and PMS in this work.

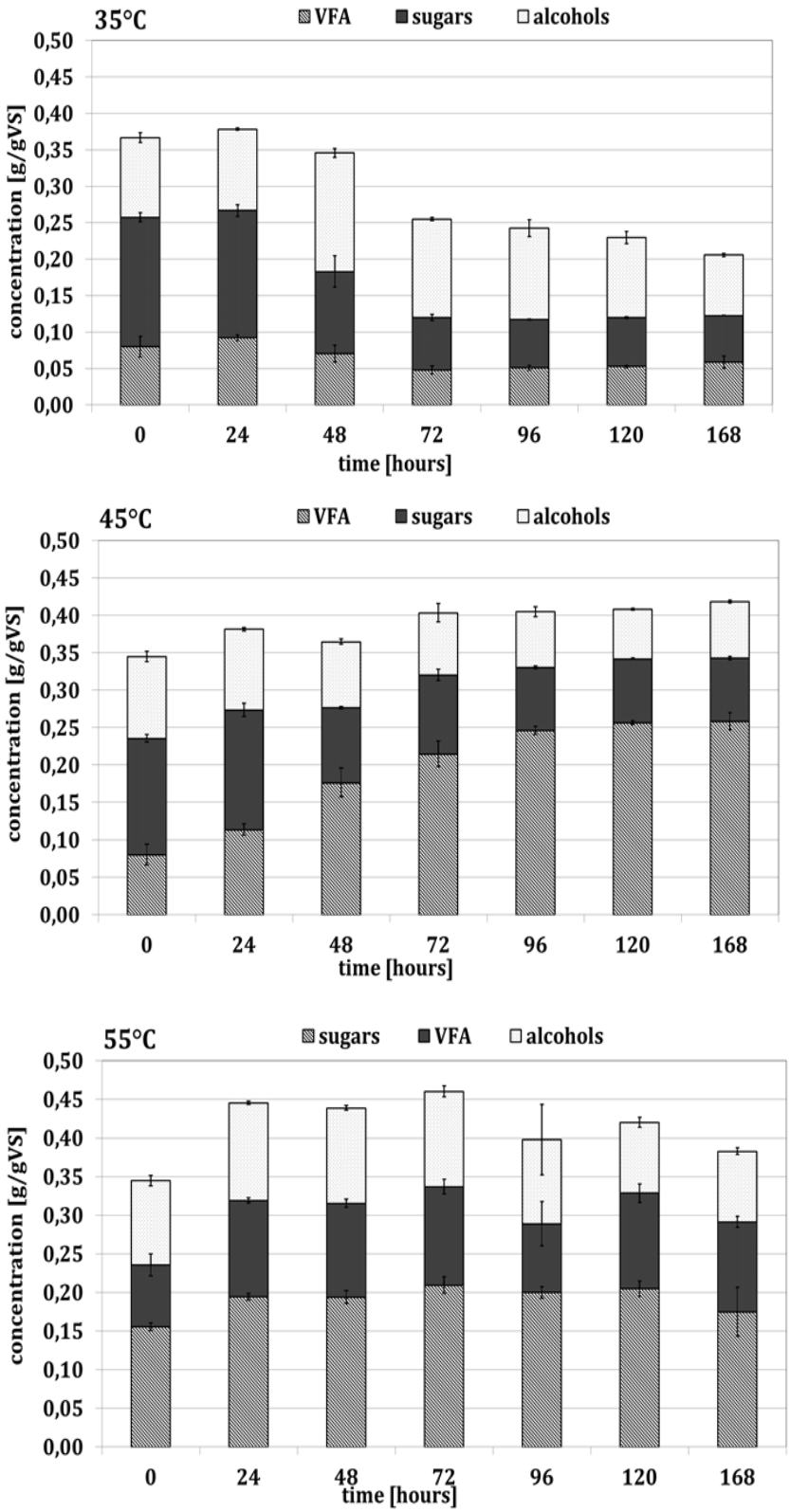


Figure 28: Changes in the concentration of the released monomers sugars, VFAs, and alcohols during the pre-acidification of OMSW at 35°C, 45°C, and 55°C

The microbiology in the anaerobic reactor has been topic of interest for plenty of researches. The composition and the abundance of the species changes during the overall process (Talbot et al., (2008); Kampmann et al., (2012); Levén et al., (2012); Kampmann et al., (2014); Win et al., (2016)).

In this study the shift in the bacterial consortium was illustrated by DGGE analyses of the batch pre-acidification (Figure 29). Within the first 4 days, a strong alteration of bacterial species was observed. Subsequently the composition of the microbial consortium was relatively constant with some prevailing bacterial species. This observation was taken as another indicator, that four days of HRT are adequate to establish an appropriate bacterial community in the pre-acidification step. The pattern of the single-stage includes all bands observed in the course of pre-acidification; however, the bacterial diversity or number of bands was much richer. In contrast, a few dominant species were present in the methanogenic step, which were different from those present during pre-acidification.

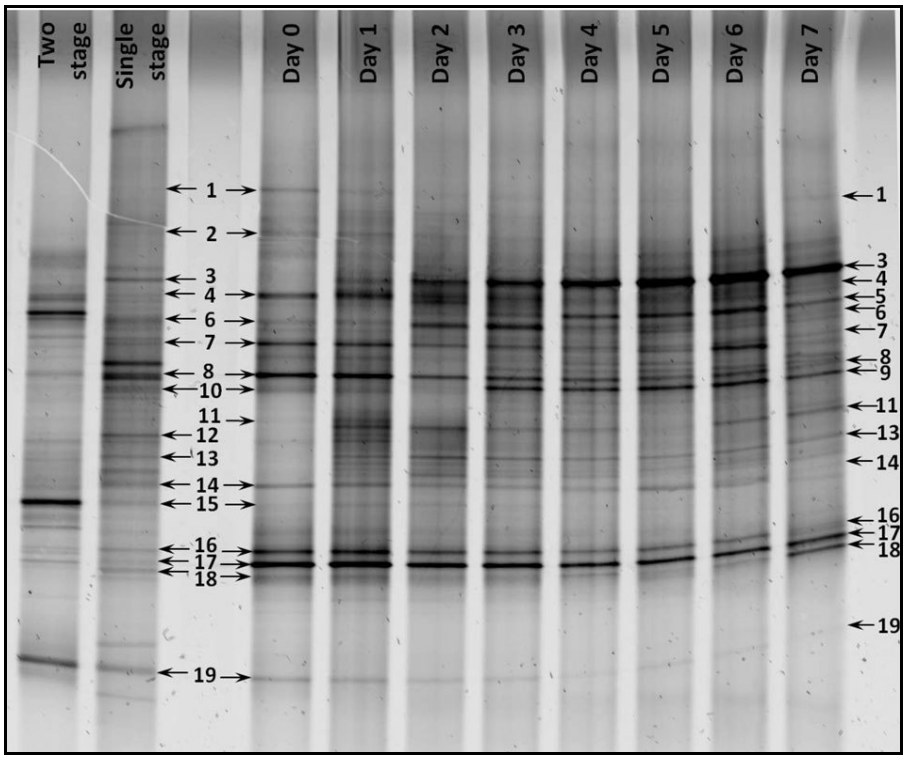


Figure 29: DGGE analyses showing bacterial community shift during pre-acidification of OMSW at 55°C for 7 days. In comparison, bacterial communities in single- and two-stage (second stage) fermentation of OMSW are shown on the left lanes

3.3.2 Comparison of one- and two-stage AD of OMSW and co-fermentation with chicken manure

Further one- and two-stage semi-continuous fermentations, as well as co-fermentation of OMSW with CM were carried out. The pH during the first stage of the two-stage fermentation (Figure 30) remained below 6.0 during the whole experiment. The concentration of the sugars also remained relatively high. It correlated inversely proportional to the concentration of the VFA (days 252-336, Figure 30). The increased VFA concentration led to drop in the pH. Obviously at this time the inhibiting factors regressed, and the fermentation of the sugars and alcohols processed.

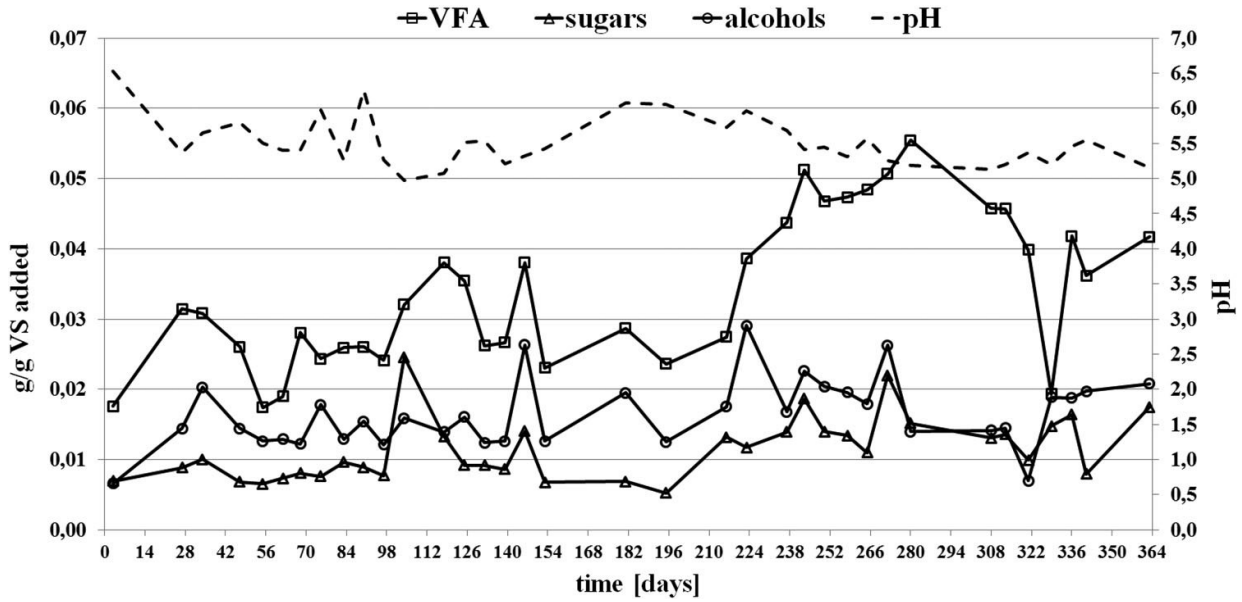


Figure 30: Concentration of the sugar monomers, VFA, alcohols, and pH during the pre-acidification stage (55°C) of the continuous two-stage AD of OMSW

During the fermentation period the pH in the methanogenic stage (Figure 31) remained stable (7.0-7.5). The VFA concentration was below 0.5 g/L, and the buffer capacity was sufficient enough to keep the process stable, despite the increase in the OLR.

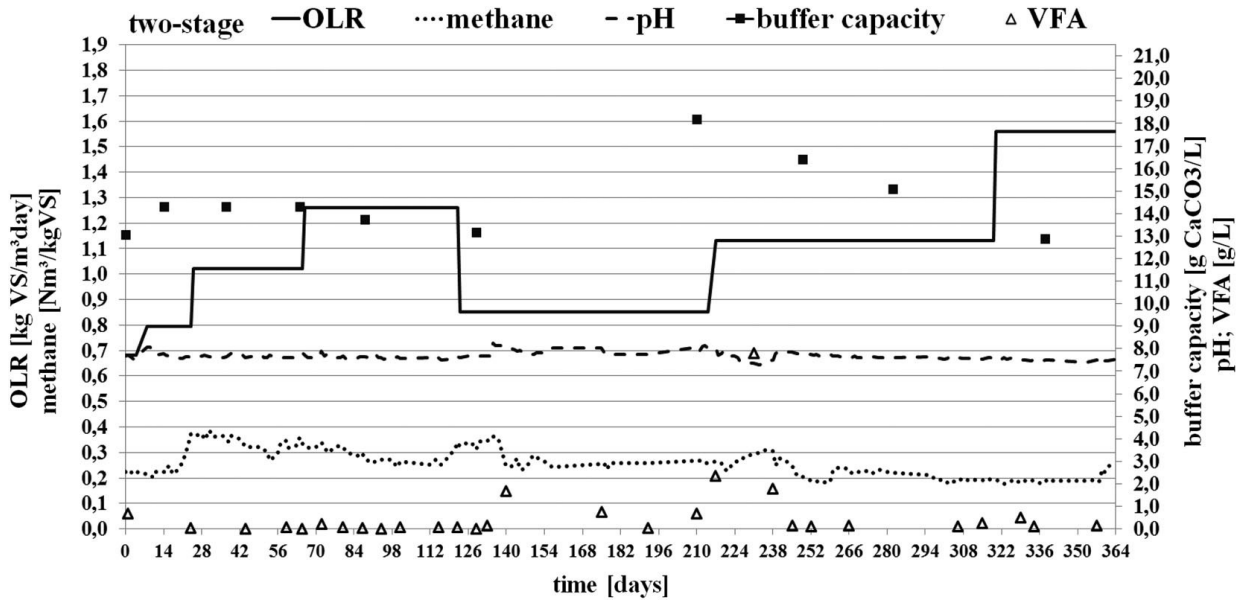


Figure 31: OLR, buffer capacity, and methane production during the mesophilic methanogenic stage of the two-stage semi-continuous AD of OMSW

The same parameters differed in the one-stage fermentation (Figure 32). In this case the buffer capacity was lower and was not sufficient enough to keep the process upright with increasing OLR. This led to break down of the fermentation. The OLR could be increased twice as slowly as in the two-stage fermentation, where the system was even able to recover quickly after new start-up due to technical problem with the heating control (day 120, Figure 31).

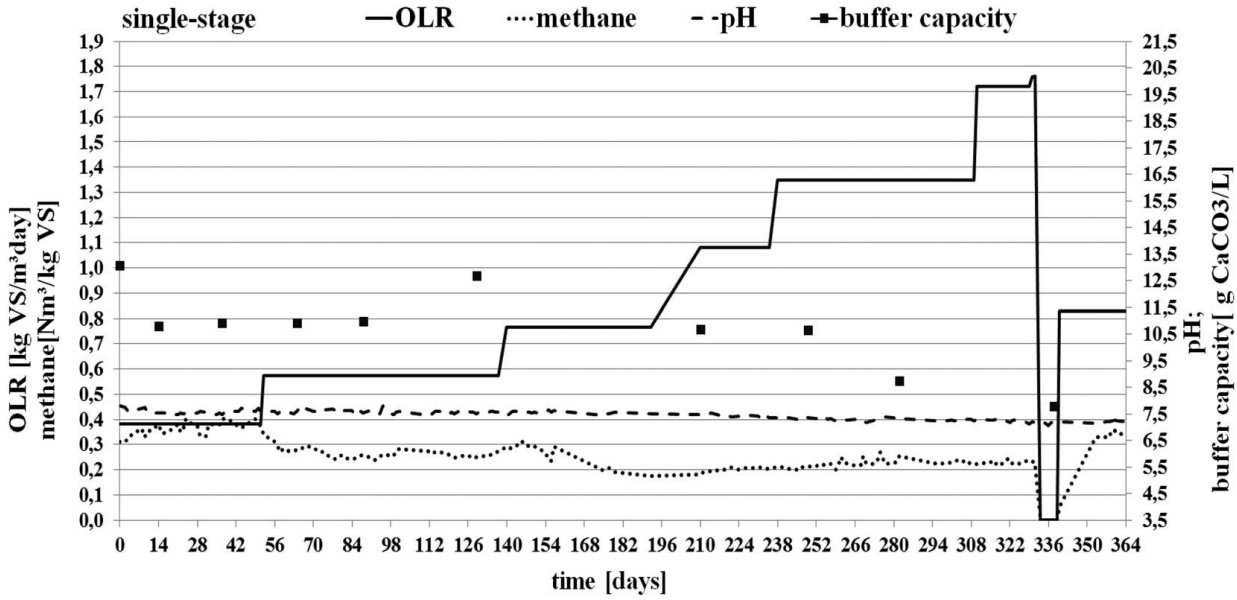


Figure 32: OLR, buffer capacity, and methane production during the single-stage semi-continuous mesophilic AD of OMSW

CM was chosen for co-fermentation as a substrate with high nitrogen content (Figure 33). The NH_4^+-N should increase the buffer capacity in the reactor broth and allow an undisturbed fermentation, as this was the case. The pH remained stable at 7.5. The volatile solids ratio between the two substrates was 30:70 (OMSW:CM). This blending ratio was kept until the end of the experiment.

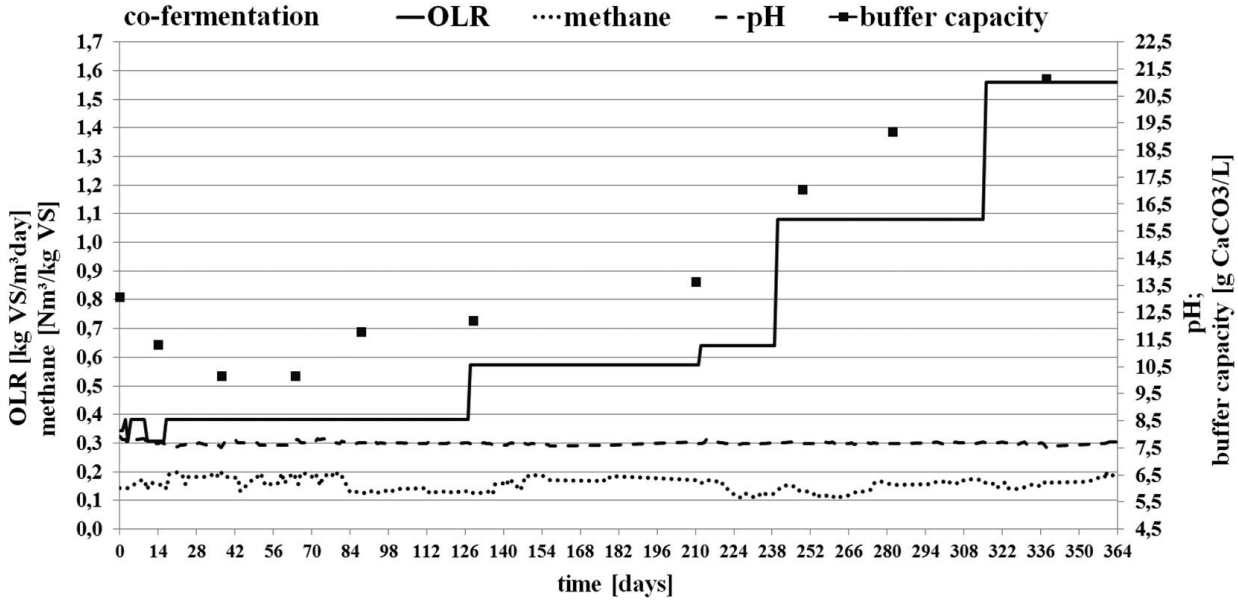


Figure 33: OLR, buffer capacity, and methane production (Nm^3/kgVS) during the semi-continuous mesophilic co-fermentation of OMSW with chicken manure

The concentration of the phenolic substances was determined as they have been reported to be one of the components in OMSW responsible for inhibition. Phenol concentration during the single-stage fermentation was 1.23±0.05 gGAeq/L. In the final phase of the co-fermentation, the concentration of phenolic substances was lower (0.74±0.02 gGAeq/L), obviously due to the reduced share of OMSW in the substrate. It is presumed that this lower phenol concentration contributed to the process

stability even though the OLR was similar to the highest one tested during single-stage fermentation. On the other hand, stable operation was also achieved during two-stage fermentation although it exhibited similar phenol levels as in the single-stage (1.19±0.01 gGAeq/L).

3.4 Anaerobic digestion of paper mill sludge

While the previous tests were carried out on a laboratory scale, the last one was planned to meet the needs of an operating anaerobic digestion plant on the site of the SCA paper mill factory in Pernitz, Lower Austria (Figure 34).

Figure 34: Wastewater treatment at the SCA paper mill factory in Pernitz, Lower Austria ©SCA

The paper mill factory operates since 2011 a two-stage anaerobic plant. This is built additionally to the wastewater treatment plant and achieves 25 % reduction of the primary sludge on the one side. On the other side, additionally 5,000 MWh per year electricity is produced (Werfring, 2012).

Since up to 6 % of the PMS contain organic residues, it is of interest to separate them effectively, before its further disposal to a cement factory. For this reason the solubility of the organic compounds should be increased, and the dewatarability of the sludge boosted. For this purpose additional step in the treatment of the sludge was planned, namely enzymatical pretreatment.

The following tests were designed based on the frame conditions, given by the existing plant equipment (Figure 35).

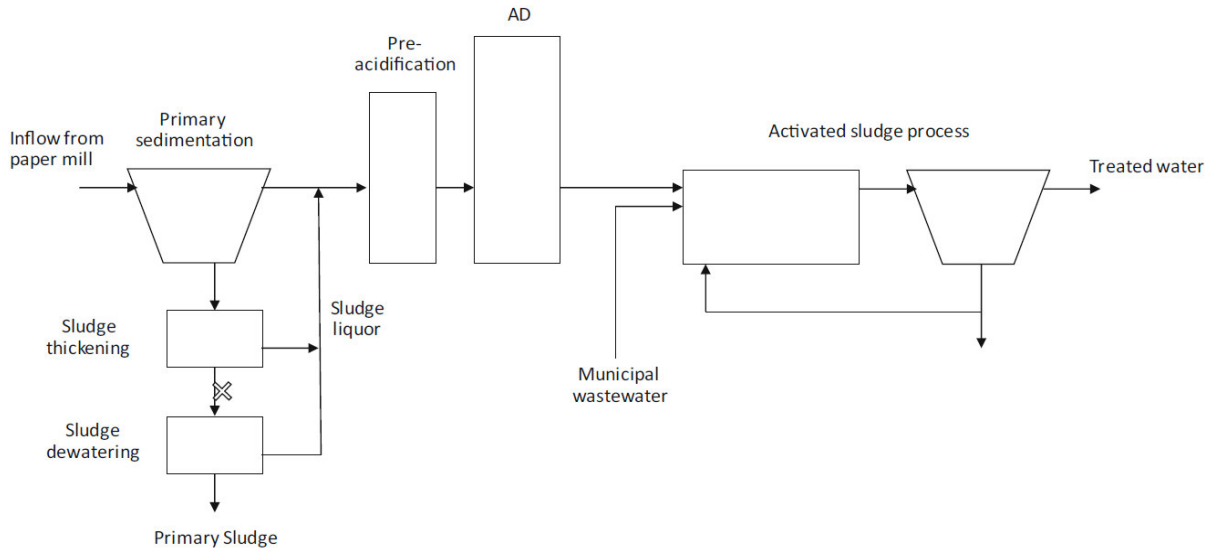


Figure 35: Scheme of the established waste water treatment process (a cross marks the origin of the investigated sludge sample)

The aim was to find out, if enzymatic pre-treatment of the PMS will be sufficient for liquefaction of the residual organic matter, predominantly lignocellulosic material and its improved separation during the filtration. Additionally, the solubilized sugars should contribute to increase in the methane yield in the existing UASB reactor. For this purpose, after enzymatical pre-treatment, the BMP of the PMS was determined: i) directly, as a whole sludge; and ii) after centrifugation, only of the liquid fraction. Two different enzyme products were compared. Microbiological pre-treatment was used as reference to expensive commercial enzyme products.

3.4.1 Enzymatic pre-treatment and BMP of the whole PMS

The enzymatic pre-treatment was carried out at 30°C, according to the manufacturer's instructions. The progression of the VFA during seven days long incubation was monitored, in order to determine the shortest possible incubation period, regarding the high volumes of incubation pond on industrial scale (Figure 36). The VFA concentrations increased during the whole incubation time. In the assays with enzymes 1 and 2 their amount was very similar. In the test with microbiological pre-treatment the concentration starts to increase rapidly only after 48 h, which was obviously the lag phase for the microorganisms to adapt to the substrate.

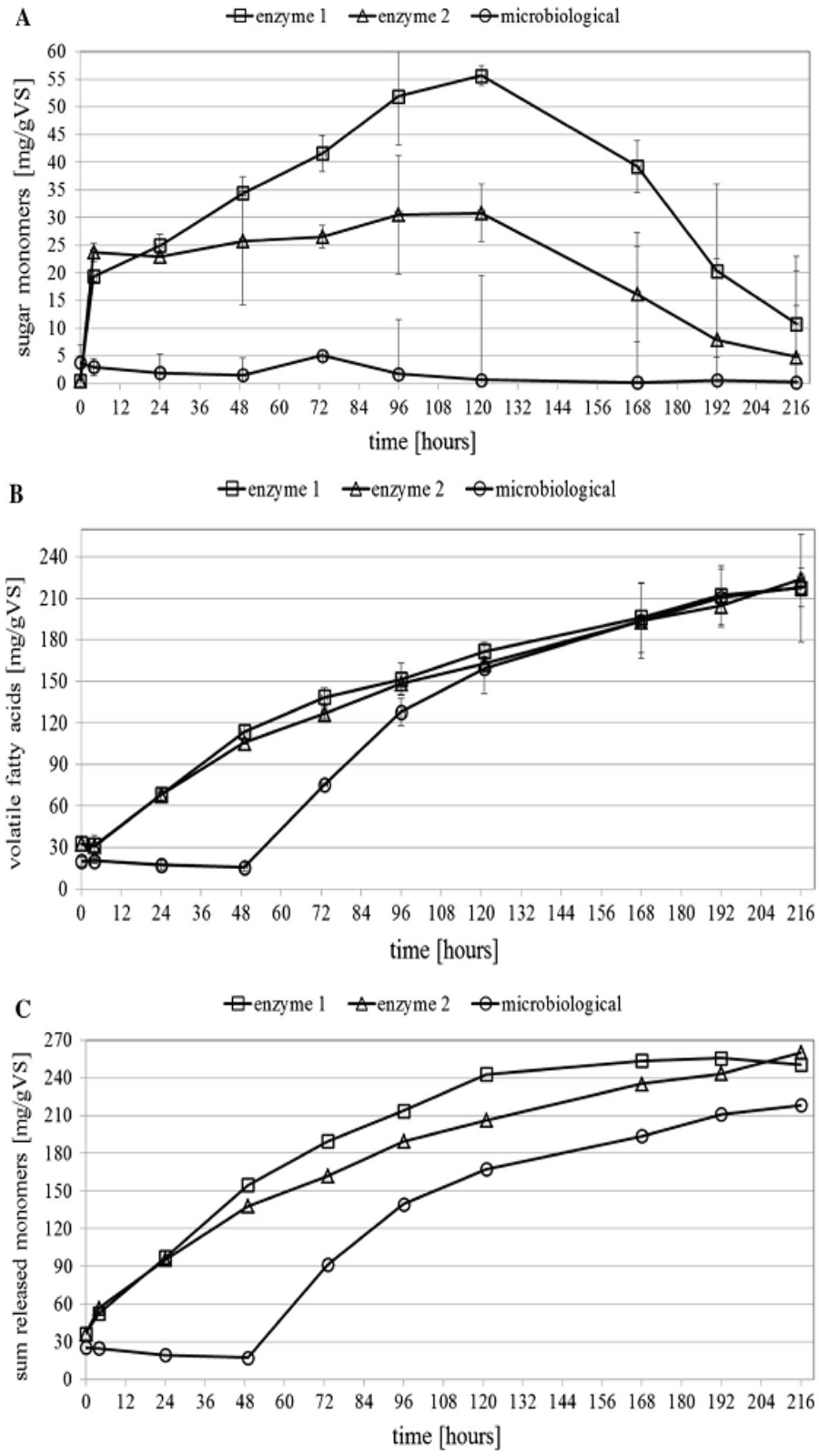


Figure 36: Changes in the concentration of the sugar di-/monomers (a), volatile fatty acids (b) and sum of soluble compounds (c) during the enzymatic and microbiological pre-treatment of paper mill waste at 30°C

The BMP assays of the whole sludge did not influence the methane production (Table 12). Obviously the microbial consortium, present in the AD batch tests is capable to perform the liquefaction of the cellulose and other insoluble carbohydrates by itself.

Table 12: Specific methane production of PMS after enzymatic and microbiological pre-treatment at 30°C for 9 days

	Specific methane production	
	Nm^3/t VS	**Nm^3/t FM**
Pre-treatment with enzyme 1	190.5 ± 29.0	11.2 ± 1.6
Pre-treatment with enzyme 2	187.3 ± 26.9	11.1 ± 1.7
Microbiological pre-treatment	170.8 ± 18.6	10.1 ± 0.6
Not pre-treated	193.3 ± 18.3	11.4 ± 1.1

3.4.2 Liquefaction and BMP tests of the organic fraction of PMS

Based on these results, second test was conducted, in which the BMP only of the soluble fraction, after the enzymatic and microbiological pre-treatment, was determined. This approach followed the idea to implement an intermediary sludge treatment step after the filter belt. In the final dewatering the liquefied compounds are separated from the solids. Subsequently these compounds are returned together with the sludge liquor to the existing treatment process and conversion to methane takes place in the UASB reactor (Figure 35). The incubation periods were 48, 96, and 192 hours (Figure 37).

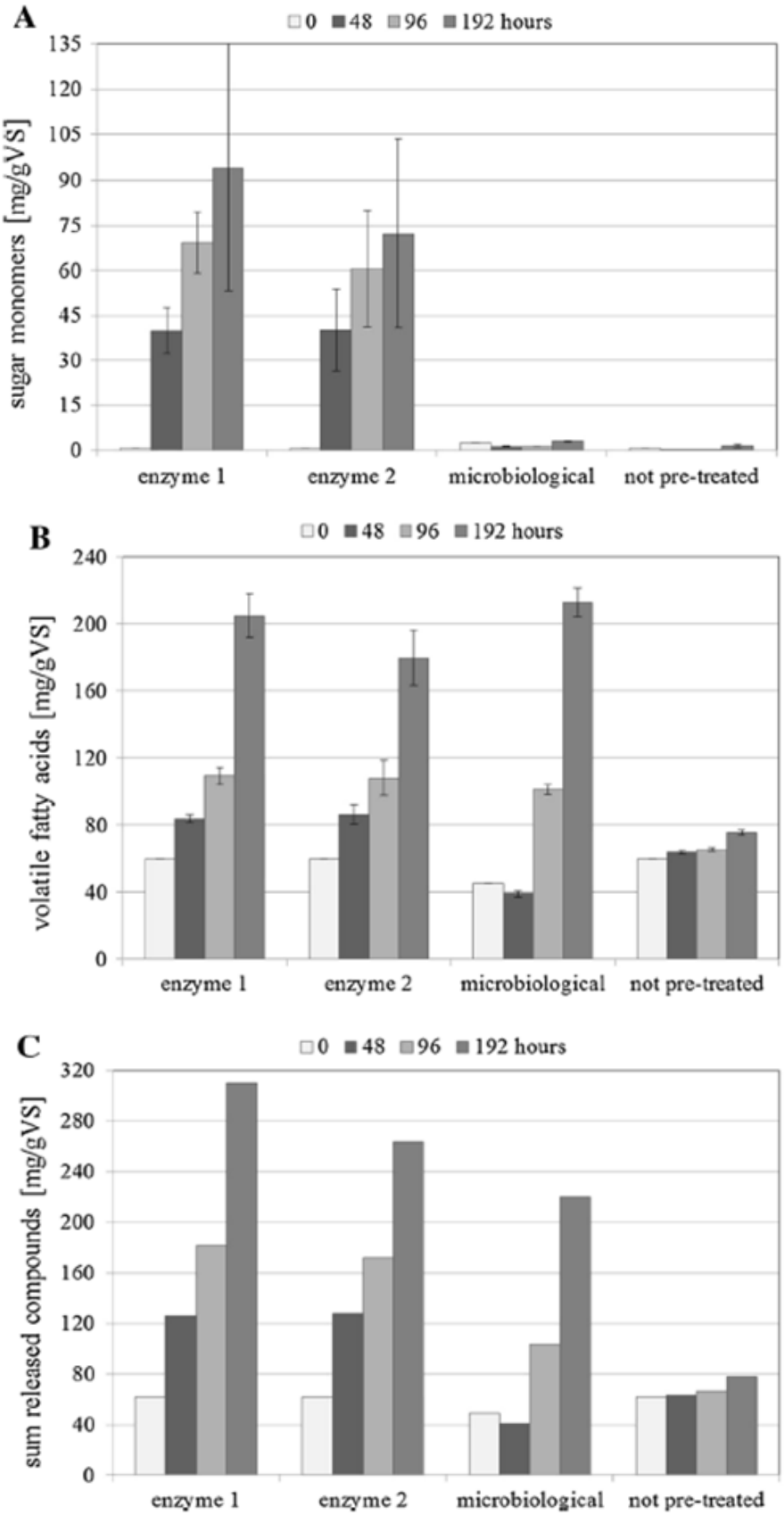


Figure 37: Released sugars (a), VFAs (b) and soluble compounds (c) after 0, 48, 95 and 192 h incubation of paper mill waste at 30°C

After 48 h incubation with enzyme 1 the sum of the concentrations of soluble compounds (Figure 37C) reaches 41 % of the final concentration (192 h) and 59 % after 4 days. In the assay with enzyme 2 the corresponding values were 49 and 65 %, respectively, but the total amount released was lower. In the assay with microbiological pre-treatment, as observed before, no sugar mono-/dimers which are easily convertible were found (Figure 37A). According to the results presented in Figure 37C, within 48 h only 19 % of the soluble compounds obtained at the end of the experiment were released. After a twice as long incubation time (96 h) the concentration increased to 49 %.

Applying the longest pre-treatment period (192 h), in the assay with enzyme 2 the methane production was approximately 12 % lower compared to enzyme 1 (Figure 38). The microbiological pre-treatment yielded the highest methane productivity. This points out that the microbial attack generally helps to weaken the cellulosic structure making it more accessible to subsequent methanization. It should also be noted that the applied analyses of soluble compounds is only an indicator for the degree of solubilisation and that e.g. cellulose oligomers are not measured. As expected, the not treated sample delivered the lowest methane production.

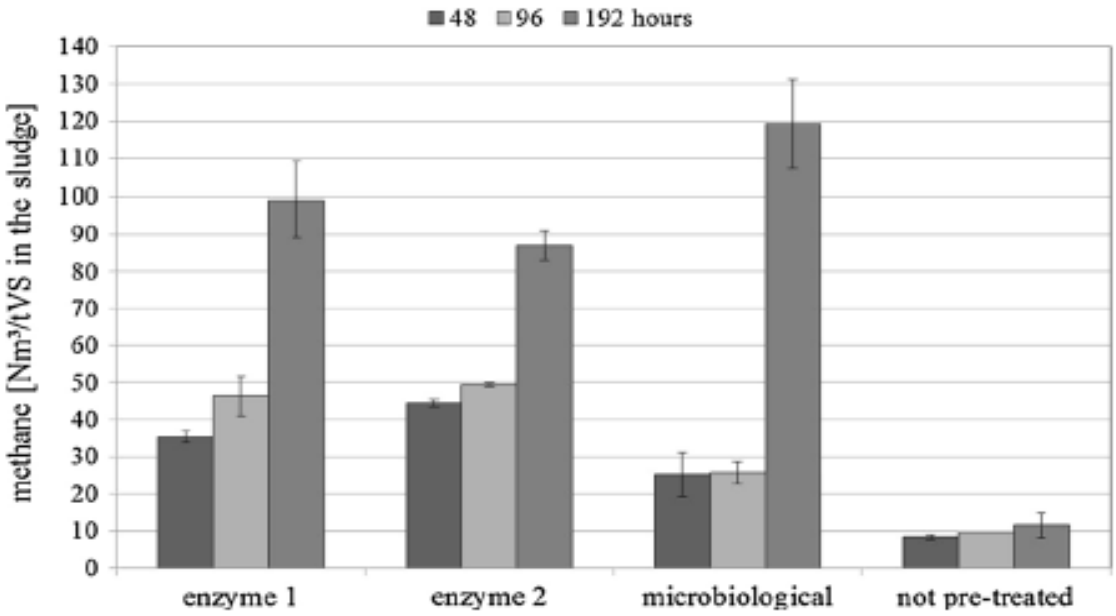


Figure 38: BMP of the liquid phase after pre-treatment of the paper mill sludge for 48, 96 and 192 h, respectively (BMP is expressed as methane yield per VS in the sludge sample)

3.4.3 Options for full scale implementation

Despite the lower methane yield, the second approach is by far easier to accomplish and to implement into the existing wastewater treatment scheme. It requires only an additional tank for incubation and a few pump lines. For solid–liquid separation the existing sludge dewatering step can be used. Therefore, from the economic point of view, it is the much more favorable process. It is worth to look at the development of methane yield with incubation time. The obvious background is that the incubation time determines the size of the necessary additional pre-treatment tank and hence the investment costs (Table 13).

Table 13: Parameters for the on-site implementation of the pre-treatment of paper mill sludge

Pre-treatment duration [d]	2	4	8
Required tank volume [m^3]	1,400	2,800	5,600
Methane yield [m^3/d]			
Enzyme 1	1,261	1,655	3,538
Enzyme 2	1,588	1,763	3,104
Microbiological pre-treatment	896	913	4,253
Volumetric productivity [m^3 methane/m^3d]			
Enzyme 1	0.90	0.59	0.63
Enzyme 2	1.13	0.63	0.55
Microbiological pre-treatment	0.64	0.33	0.76

4 Summary

The two-stage AD of three different substrates – olive mill solid waste, sugar beet pressed pulp and paper mill sludge - was studied to overcome the bottlenecks, observed during their fermentation.

AD of ***sugar beet pressed pulp*** is a promising treatment concept. It produces biogas as a renewable energy source making sugar production more energy efficient and it turns SBPP from a residue into a valuable resource. In this study one- and two-stage mono fermentation at mesophilic conditions in a CSTR were compared. Also the optimal incubation temperature for the pre-acidification stage was studied. The fastest pre-acidification, with a HRT of 4 days, occurred at 55°C. In the methanogenic reactor of the two-stage system stable fermentation at OLR 7 kg VS/m^3d was demonstrated. No artificial pH adjustment was necessary to maintain optimum levels in both the pre-acidification and the methanogenic reactor. The total HRT of the two-stage AD was 36 days which is considerably lower compared to the one-stage AD (50 days). The frequently observed problem of foaming at high loading rates was less severe in the two-stage reactor. Moreover the viscosity of the digestate in the methanogenic stage during the two-stage fermentation was in average tenfold lower than the one-stage fermentation. This leads to decrease of the energy input for the reactor stirring about 80%. The observed advantages make the two-stage process economically attractive, despite higher investments for a two reactor system.

Two-stage fermentation of ***olive mill solid waste*** pointed out to be a straight forward strategy. Pre-acidification and subsequent methanization in a second stage allows overcoming the process disturbances observed during one-stage fermentation. The HRT was reduced 35 %, compared to the single-step fermentation and the increase of the OLR to the highest value of 1.56 kgVS/m^3day could be reached three times faster at stable fermentation. Single-stage mono-digestion of OMSW proved not to be useful due to long adaptation time of the inoculum at start-up and severe process instabilities. Co-digestion with CM was performed as another possible practical way to overcome the limitations. However, co-digestion of substrates derived from different agro-industrial activities, i.e. olive oil production and poultry farming may cause additional logistic problems such as substrate transport and storage. Despite some attempts to identify other potential reasons, it must be generally confirmed that pure solid waste from olive oil production exhibits inhibition of the AD process through its high polyphenol content. Even after long term operation of the processes efficient conversion was only achieved at low OLRs of 1.56 kgVS/m^3day, and high HRT of 150 days, which require proportionally high reactor volumes. For practical implementation further research seems to be necessary to reduce the necessary HRT and to improve process economics.

Two options to yield the inherent energy of ***paper mill sludge*** by means of AD after enzymatic/microbiological pre-treatment were investigated. The first approach, AD of the whole sludge after pre-treatment, was not successful. No significant enhancement of the biomethane production was observed by the applied pre-treatment methods. The measured methane yield of 11 Nm^3/t sludge is only half of the theoretical potential and the necessity to establish an appropriate digester plant makes this approach economically unattractive. The second option, pre-treatment of sludge and supply of the liquid phase to the existing anaerobic reactor (UASB), provided highly promising results. The pretreatment led to 6–13 % mass reduction of the sludge after enzymatic pre-treatment and 17–22 % after microbiological pre-treatment. Such a concept can be easily integrated in the existing wastewater treatment scheme.

According to the data obtained, the implementation of such a process can substantially increase the biogas production.

The essential features of two-stage digestion are:

- faster degradation of the polymers in a separate pre-acidification
- reduction of the overall HRT
- optimal pre-acidification conditions were determined at 55°C and four days HRT
- faster start-up of the fermentation process
- degradation of inhibiting substances for the methanogenic Archaea in the pre-acidification stage
- increase of the OLR by 30-35%
- pre-treatment, in the case of PMS, can lead to 6 – 13% mass reduction of the sludge after enzymatic pre-treatment and 17 – 22% after microbiological pre-treatment

As demonstrated, two-stage AD is a viable option for handling of organic industrial by-products. This approach can effectively enhance process stability as well as reduce operational costs in the long term. It is obvious that in each case such cost advantages must be balanced against increased initial investments and higher complexity of operation for a two stage system - a judgement that has to be made individually case by case. On that note, the outcomes of this study are valuable contribution, without any claim to completeness. They prove the importance of optimization of the process for each distinct waste stream. Nevertheless, the findings clearly demonstrate the high potential of the two stage process for overcoming the bottlenecks of the anaerobic digestion and the benefits of its implementation in the waste management.

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6 Appendix

3 Scientific publications

2 Conference proceedings

1 Book chapter

Scientific publications

Publication 1

Reducing the risk of foaming and decreasing viscosity by two-stage anaerobic digestion of sugar beet pressed pulp

Stoyanova, E., Forsthuber, B., Pohn, S., Schwarz, C., Fuchs, W., Bochmann, G., 2014. Biodegradation 25, 277–289. doi:10.1007/s10532-013-9659-9

Publication 2

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Publication 3

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Stoyanova, E., Bochmann, G., Couperus, A., Fuchs, W., 2016. Waste and Biomass Valorization 1–9. doi:10.1007/s12649-016-9507-3

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ORIGINAL ARTICLE

Reducing the risk of foaming and decreasing viscosity by two-stage anaerobic digestion of sugar beet pressed pulp

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Abstract Anaerobic digestion (AD) of sugar beet pressed pulp (SBPP) is a promising treatment concept. It produces biogas as a renewable energy source making sugar production more energy efficient and it turns SBPP from a residue into a valuable resource. In this study one- and two-stage mono fermentation at mesophilic conditions in a continuous stirred tank reactor were compared. Also the optimal incubation temperature for the pre-acidification stage was studied. The fastest pre-acidification, with a hydraulic retention time (HRT) of 4 days, occurred at a temperature of 55 °C. In the methanogenic reactor of the two-stage system stable fermentation at loading rate of 7 kg VS/m^3 d was demonstrated. No artificial pH adjustment was necessary to maintain optimum levels in both the pre-acidification and the methanogenic reactor. The total HRT of the two-stage AD was 36 days which is considerably lower compared to the one-stage AD (50 days). The frequently observed problem of foaming at high loading rates was less severe in the two-stage reactor. Moreover the viscosity of digestate in the methanogenic stage of the two-stage fermentation was in average tenfold lower than in the one-stage fermentation. This decreases the energy input for the reactor stirring about 80 %. The observed advantages make the two-stage process economically attractive, despite higher investments for a two reactor system.

Keywords Two-stage anaerobic digestion · Sugar beet pressed pulp · Viscosity · Pectin · Foaming

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Introduction

Sugar beet was one of the ten most produced commodities in the world in 2010 (228.45 million tonnes) and the second one in Europe (150.51 million tonnes) (FAO 2010). Processing of one tonne of beets produces about 70 kg of exhausted dried pulp, or about 250 kg of exhausted pressed pulp. The residue is

mainly used as by-product for animal feed. Other utilization possibilities are: (i) producing of protein by using thermophilic microorganisms (Grajek 1988); (ii) as resource for pectin extraction (Phatak et al. 1988); and (iii) obtaining of ferulic acid and oligosaccharides as rhamnose, galacturonic acid, and arabinose after enzymatic saccharification (Micard et al. 1996). However, due to the huge amount of sugar beet pressed pulp (SBPP) processed in the short period of about 4 months, considerable amounts are still disposed which may lead to significant environmental impacts. To reduce these and to increase the sustainability of sugar production, other possibilities for utilization of the residues are necessary. Another problem the sugar industry faces is the sharp fall in sugar prices by 36 % after the reformation of the common market organization for sugar production in the EU in 2006 (CEFS 2011). Converting factory residues into biogas to produce renewable energy and replacing fossil fuels contributes to solving both issues. It decreases costs for sugar production, and it turns SBPP from a potential waste product into a valuable resource.

Anaerobic digestion (AD) is an established and robust technology for converting the energy content of organic wastes into thermal and electrical energy. To make the AD process profitable, it is necessary to optimize the process conditions. Biogas production from SBPP as substitute for fossil fuel in sugar beet factories has been investigated before (Brooks et al. 2008; Hutnan et al. 2000, 2001). An important issue is the availability of the substrate for the methanogenic organisms to achieve a fast and stable fermentation process. The high organic content makes the sugar beets eligible as an energy rich substrate for biogas plants. However, while the sugar components are converted rapidly, a significant part of the organic matter is poorly degradable lignocellulosic material. The lignocellulosic fraction of the dried pulp is composed of 22–30 % cellulose, 24–32 % hemicellulose, 24–32 % pectin and 3–4 % lignin (Coughlan et al. 1985), which are not rapidly degradable. Another, more technical problem, of the AD process for SBPP is foaming in the reactor at high organic loading rates (OLRs), ~10.0 kg COD/m^3d (Brooks et al. 2008). Other factors causing foaming during AD, are the protein content of the substrate, temperature, mixing, and digester shape (Ganidi et al. 2009). Foaming can be overcome by adding antifoaming agents, or by reducing the OLR. The latter option is not preferable because sugar production is a short term campaign and therefore SBPP has to be utilized as rapidly as possible. Otherwise the storage and the ensiling of a substrate with high moisture and low pH will increase the costs for the operation of the biogas plant. However, antifoaming agents are a considerable cost factor too.

An important issue with respect to operational costs for the AD process is the energy consumption of the stirring system. Proper stirring is required to maintain stable temperature conditions and provide optimal homogenization of the fermenter content, and greatly influences the efficiency of the fermentation process. The sensitive microorganisms should not be exposed to high shear stress (Braun 1982). Reactor stirring is influenced by the viscosity of the digestate on the one hand and the amount and composition of solid matter, on the other hand. Poor as well as excessive mixing can cause solid/liquid phase separation and also increase the amount of gas bubbles in the bulk phase, respectively. This leads again to foaming problems (Ganidi et al. 2009). Heating and stirring of the reactor are the highest energy factors during AD. Part of the heating energy is recovered by a combined heat and power (CHP) gas engine heat transfer system. For the reactor stirring, depending on the total solids (TS) and stirrer type, between 12.5 and 23.4 W/m^3 specific electric power is consumed, which is up to 10 % of the electrical energy consumption in the process (Döhler et al. 2009).

The required motor power depends strongly on the viscosity of the stirred medium. In this context, the high content of pectin in SBPP is a factor that may have a high impact on flow behaviour. This cell-wall poly saccharide consists of mainly linear (1 → 4) α-D-galactopyranosyl uronic acid units ("smooth" regions). These are esterified to a various degree with methanol or are partially acetylated (BeMiller et al. 1986). The polymer chain is interrupted with L-rhamnose unites. Side chains from arabinan, galactan or arabinogalactan are linked to the rhamnose ("hairy" regions). One of the most important physical properties of pectin is to form spreadable gels. This results from the interaction of the polymer chains in a three dimensional structure. Hydrogen bonds, divalent cation cross bridges and hydrophobic interactions, as well as some ferulate cross links hold this structure together. In general, the viscosity of the pectin gel

increases with increasing degree of esterification, presence of divalent cations and increasing pH values (BeMiller et al. 1986).

Two-stage AD is one approach for improving the digestion performance (Ghosh and Klass 1978). In order to meet the requirements of different microorganism groups, the process steps take place in two separated reactors. In the first one the hydrolysis and the acidogenesis occur. For the bacteria active at this stage, the optimal pH range is from four to six and the optimal temperature range is 40–60 °C. These conditions conflict the optimal ones for the methanogenic Archaea. The second stage provides the necessary pH between 6.8 and 8.4 to the methanogenic Archaea and a temperature between 37 and 40 °C, for mesophilic fermentation or, alternatively, 50–55 °C for thermophilic. As a result the biochemical conversion of the substrate occurs faster and inhibitory substances can be converted into intermediates (Demirel and Yenigün 2002). The two-stage AD of SBPP can provide an accelerated degradation of lignocellulosic fraction as well as depolymerization of pectic substances due to optimized hydrolysis. This leads to an overall decrease of the hydraulic retention time (HRT) and subsequent reactor volume and more stable process conditions (Alkaya and Demirer 2011).

The current study was conducted to compare the performance of one- and two-stage AD of SBPP. Possible improvement of the reactor efficiency using two-stage AD and decrease of the viscosity in the second stage of the two-stage fermentation has been investigated. The optimal OLR for a stable process, without the need for addition of antifoaming reagents and chemicals for pH adjustment in the first-stage were studied.

Materials and methods

Substrate and inoculum

SBPP is the residue after the extraction of the sugar from the chopped sugar beet. It was obtained from a sugar refinery in Tulln, lower Austria, during the campaign in autumn 2010. The factory processes approximately 3.5 million tonnes sugar beet during a 140 days campaign. The material was stored at −20 °C until use. Table 1 gives an overview of the substrate parameters. The inoculum was a mixture from a mesophilic AD fermenter, digesting thin stillage from bio-ethanol production and a local biogas plant, digesting agricultural waste at mesophilic conditions (Table 1). At the start of the experiment was adapted to mesophillic monodigestion of SBPP, therefore the fast increase of the OLR at the beginning.

Process set-up and operation

Batch experiments for determination of the optimal incubation temperature in the first stage of the two-stage fermentation

The optimal temperature and duration of the first stage of the two-stage fermentation were determined in a batch set-up, by mixing SBPP with inoculum in 2:1 (w/w) ratio in closed reactor vessels. Incubation was conducted for 8 days at 35, 45, and 55 °C, respectively. Daily, the pH value was measured and the formation of sugar monomers, volatile fatty acids and alcohols was determined by HPLC analysis.

Set-up of continuous experiments

The first stage of the two-stage continuous fermentation, the pre-acidification, was carried out by mixing SBPP with mesophilic inoculum from a biogas plant digesting agricultural residues, in 2:1 (w/w) ratio in a gas 1 L tight plastic chamber without continuous stirring. The mixture was incubated at 55 °C in a temperature controlled environment. Daily, feeding was conducted with a mixture of fresh SBPP and fermenter content recirculated from the subsequent methanogenic stage of the two-stage fermenter. The mixture ratio was 2:1 (w/w) SBPP to recirculated sludge. The HRT in the first stage was set to 4 days.

Table 1 Characteristics of sugar beet pressed pulp and inoculum

Characteristics	Sugar beet pressed pulp	Inoculum
pH	5.15	8.36
TS (% fresh weight)	27.13	1.95
VS (% fresh weight)	25.19	1.06
COD (g/kg)	322.03	9.31
TKN (g/kg)	3.64	2.01
NH_4^+–N (g/kg)	0.25	1.19

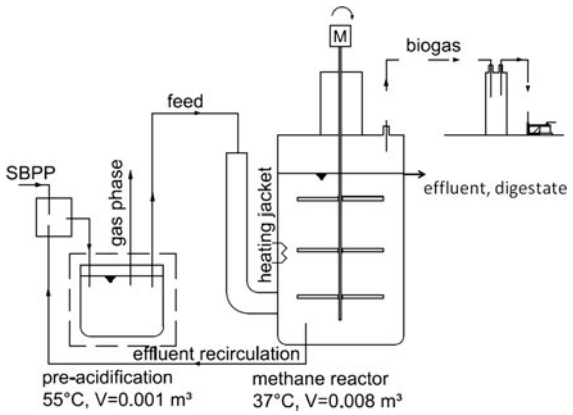

Fig. 1 Two-stage anaerobic digestion of sugar beet pressed pulp. The one-stage reactor is identical to the second stage of the two-stage reactor

The volume increased when increasing the OLR in the second stage by keeping the HRT and the recirculation ratio constant. The removed content of the pre-acidification stage served as the feed for the methanogenic fermenter (Fig. 1).

The methanogenic stage of the two-stage fermenter was conducted in 8 L continuous stirred anaerobic tank reactors (CSTR) at mesophilic conditions (37° ± 1 °C). The vessel was equipped with blade stirrer and a heating jacket was used for temperature control. The fermentation was semi-continuous, the fermenter were fed manually once a day. The single-stage fermentation serving as the reference was conducted in the same type of reactor system. In both cases operation period was 112 days.

Analytical methods

General analytical methods for monitoring the AD process

The stability of the AD process was monitored using the following methods: chemical oxygen demand (COD)—APHA5220B, total Kjeldahl nitrogen (TKN) and ammonium nitrogen (NH_4^+–N)—APHA4500B, TS and volatile solids (VS)—2540B, were determined weekly, according to standard methods (American Public Health Association (APHA) 1999).

The pH value was measured daily with a WTW pH330i electrode SenTix 81.

The gas quality was determined using gas measuring system TRM/816 (Awite Bioenergie). Methane (CH_4) and carbon dioxide (CO_2) were quantified using an infrared sensor (2-beam), and hydrogen sulphide (H_2S), hydrogen (H_2) and oxygen (O_2)—by using an electrochemical detection system. At least 3 L gas is necessary for a single measurement.

Gas quantity was measured using MilliGascounter (Dr.-Ing. Ritter Apparatebau) with a flow rate range 1 mLh^{-1}–1 Lh^{-1}, resolution 3 mL and accuracy ± 3 %.

HPLC measurements were conducted on a Hewlett Packard chromatograph, Series 1100, equipped with Agilent 1100/1200 isocratic pump and refractive index detector with an optical unit temperature of 45 °C. Sugar monomers, volatile fatty acids (VFA) and alcohols, released during the hydrolysis and acidogenesis of the substrate were separated and analyzed by ICSep ICE-ION-300 column (Transgenomic) at 40 °C. The mobile phase consisted of 0.01 N H_2SO_4 solution. The flow rate was 0.325 mL/min with a running time of 120 min. The concentration of the products was quantified using Agilent ChemStation software, Rev. B.01.03 [204] (Agilent Technologies) and external calibration curves.

Viscosity measurement

The differences in the viscosity in both of the reactors, the methanogenic stage of the two-stage fermenter and in the one-stage fermenter, were measured with the macro viscosimeter, designed for identification of the rheological behaviour of slurries in biogas digesters (Pohn et al. 2010). It is based on the so called vane-in-a-large-cup-principle, with a diameter ratio 2.5 ($d_{fermenter}/d_{stirrer} = 2.5$). It allows measurements of the rheological properties of fluids containing particles with diameter up to 15 mm and fibres with a length up to 30 mm. The stirrer motor is ViscoPakt, rheo 57 by HITEC ZANG. The studied temperatures were 35, 45, 55 °C. The rotation speed started from 30 rpm, in steps of 10, and ended when a vortex gap appeared. In this self-constructed measurement system some problems can occur at low torques. Therefore, the measurement error at low rotation speed can be very high. Since the necessary volume for the measurement is 11.5 l, and in order to minimize oxygen stress for the methanogenic microorganisms, two parallel fermentations were carried out, the content of which was used for these experiments.

Pectin extraction

In order to show the decomposition of pectin as a gelling agent causing higher viscosity pectin extraction was carried out. The extraction protocol was optimized based on the method described by (Phatak et al. 1988). The sample was dried at 55 °C until constant weight. The dry sample was mixed with an extraction solvent (0.05 M EDTA, pH 1.5, adjusted with 0.1 M HCl) in ratio 1:50 (w/w). The mixture was incubated for 1 h at 90 °C and filtered through a 20 µm nylon cloth. The filtrate was mixed with 95 % ethanol 1:4 (v/v), and centrifuged for 15 min at 12500 rpm. The pellet was washed three times with 75 % ethanol 1:1 (v/v) and dried at 50 °C.

FTIR spectra analysis

FTIR (fourier transform infrared spectroscopy) spectra of the dried and ground samples for qualifying the pectin extracts were recorded using a Perkin–Elmer Spectrum 100 spectrometer. The absorbance mode was at resolution of 1 cm^{-1} after regular intervals of exposure. The scanning range was 650–4000 cm^{-1}. The background was recorded before every measurement and subtracted by the software. After ATR correction to one, all numerical data were normalized to the intensity by the largest peak in the spectral region from 650 to 1800 cm^{-1}. The reading spectra for each sample were recorded in triplicates.

Results and discussion

Batch experiments for determination of the optimal incubation temperature of the first stage of the two-stage fermentation of SBPP.

In the pre-acidification stage it is important to provide the optimum growth conditions for the enrichment of hydrolytic and acidogenic bacteria, which leads to an accelerated degradation of the polymers (Alkaya and Demirer 2011). The most important parameters are temperature and pH. The optimal temperature for pectinases, polygalacturonases and hemicelluases was reported to be within the range of 30 up to 50 °C (Spagnuolo et al. 1997). Therefore the three temperatures examined in the batch experiments were 35, 45, 55 °C. The pH was measured, but not adjusted. The efficiency of the hydrolysis and acidification was determined by measuring the change of the concentration of the released fatty acids. Since about 70 % of the methane is produced by acetotrophic methanogens (Mah 1977), acetic acid accumulation is preferable in the pre-acidification stage. Also, if accumulation of propionic acid occurs the subsequent methanogenic stage can be inhibited (Lier et al. 1993; Wang et al. 2009). Consequently, its concentration in the pre-acidification stage should be as low as possible. The change in the VFA accumulation is shown in Fig. 2. Recent studies have shown that hydrogenotrophic methanogens can dominate in the anaerobic digester, depending on the substrate (Krakat et al. 2011; Kampmann et al. 2012). As long as hydrogen is released in the gas phase, which was not collected in this batch experiment, its influence could not be studied. The quality and quantity of the gas produced during the first stage have not been measured, because for the available equipment not enough gas volume could be collected between two feed intervals.

The VFA concentration at 55 °C (Fig. 2a) increased already after 4 h up to 3.20 g/L VFA and reached its maximum, 22.12 g/L, after 96 h. The change in the concentration of formic acid, acetic acid, propionic acid, iso-butyric acid, butyric acid, iso-valeric acid, and valeric acid was measured. The concentration of acetic acid (Fig. 2b) started to increase after 8 h at 55 °C and reached 90 % of its maximum value after 72 h. It comprised 76 % of the total released fatty acids. The concentration of propionic acid (Fig. 2c) increased to 1.31 g/L after 24 h and remained stable during the incubation after 198 h. The pH was 5.82 after 24 h and afterwards stable below 5.20.

At 45 °C the VFA formation starts 48 h later, and the total production was 10 % lower. A similar behaviour was found for acetic acid (Fig. 2b). It reached 90 % of its maximum level after 96 h. Nevertheless, it resembled a comparable percentage of the total fatty acids (77 %). In the same time the propionic acid concentration (Fig. 2c) remained relatively stable between 1.94 g/L after 24 h and 2.15 g/L after 198 h. Compared to 55 °C the pH was higher, 5.91 after 48 h going down to 5.67 after 198 h.

The incubation at 35 °C showed the lowest VFA accumulation with high ratio of propionic acid. The pH was considerably elevated, 7.0 and 6.5. This is already in the lower pH range were methanogenic

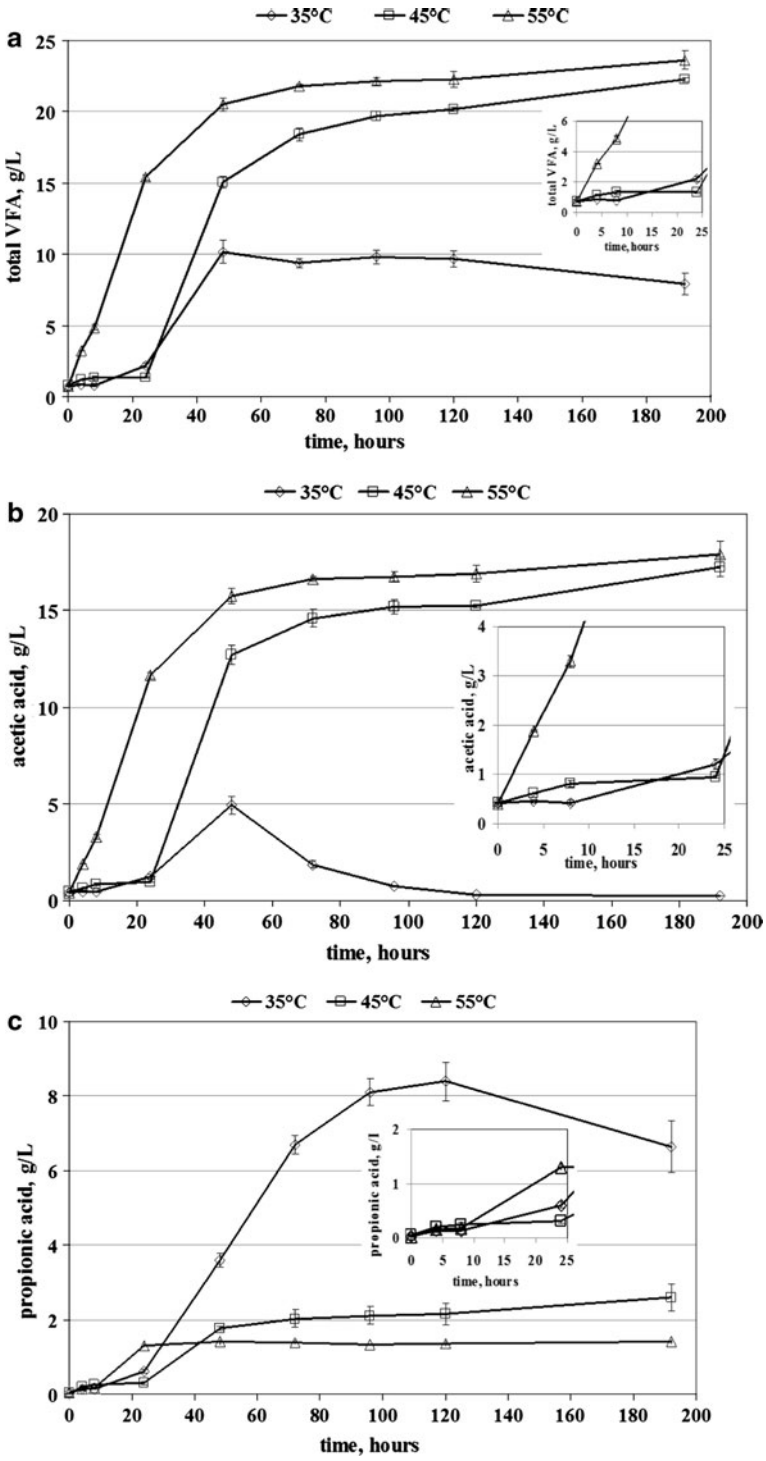

Fig. 2 Total volatile fatty acid (**a**), acetic acid (**b**), propionic acid (**c**) concentration in the first stage of two-stage AD of SBPP—batch set-up, small graphs show the zoomed regions with lowest concentration

activity can occur. This is also the reason why after initial accumulation of acetic acid (4.90 g/L after 48 h), decrease of the concentration was observed (0.71 g/L after 96 h). In contrast, the propionic acid (Fig. 2c) increased steadily and reached its highest value of 8.39 g/L after 120 h. The concentration was 3–4 times higher compared to the experiments at the other temperature levels.

Based on these results the optimal incubation temperature was determined to be 55 °C, due to a stable pH below 5.5, high VFA production and low propionic acid concentration. At the one hand the low pH indicates accumulation of VFA, and on the other hand it boosts the population of hydrolytic bacteria (Demirel and Yenigün 2002). The spontaneous drop in pH under the chosen conditions is enough to suppress methanogenic activity. Hutnan et al. (2001) described the two-stage anaerobic fermentation of sugar beet pulp with artificial adjustment of the pH value between 4.0 and 4.5 in the pre-acidification stage. However, in this case the pH had to be raised again to 7.0 in the methanogenic stage by addition of Na_2CO_3. Hence, and in contrast to these experiments, significant amounts of chemicals are required.

The choice of the HRT in the first stage is also important for the process efficiency. The HRT of 4 days was considered to be optimal, because by that time 90 % of the maximum concentration of VFA is reached.

At industrial scale sufficient thermal energy is usually available from the heat exchange system of the CHP plant, part of which can be used to maintain a temperature of 55 °C in the first stage.

Two-stage AD of SBPP

Based on the results from the batch experiments, the first stage of the two-stage AD was carried out at incubation temperature 55 °C and a HRT of 4 days. The concentration of VFA, sugar monomers and alcohols released during the hydrolysis is shown in Fig. 3. The measured sugar monomers were lactose, glucose, xylose, galactose, rhamnose, arabinose, fructose, and the alcohols were 1,2-propanediol, 1,3-propanediol, ethanol, 2-propanol, 1-propanol. The pH ranged between 7.3 and 5.0 and correlated with the VFA concentration. A decrease of the VFA concentration leads to an increase of the pH value. The average sugar monomers concentration was 1.24 g/L, the alcohols, 0.77 g/L, and the VFA, 15.94 g/L. This demonstrates the fast conversion of the organic matter into VFAs, 60 % of which is acetic acid.

The HRT in the second stage of the AD process was stepwise decreased starting from 80 days. It should be noted that the OLR on the second stage was not constant at a specific HRT due to the variation in substrate composition after the dynamic microbial activity in the first stage. The fast reduction of the HRT at the beginning of the fermentation is due to the fact that the experiment was started with an already adapted inoculum.

The lowest HRT for the second stage was 16 days, corresponding to a maximum OLR of 8.45 kg VS/m^3 d (day 65) (Fig. 4). On day 67, foam production was observed and the HRT was decreased to 20 days. Also the COD degradation decreased from 87 to 77 %. On day 73 an attempt was made to lower HRT slightly, but on the next day severe foaming was observed, nevertheless no increase in the VFA concentration was detected: total VFA concentration on day 45 was 700.5 mg/L. Therefore the HRT was again set to 19–20 days (OLR 6.76 kg VS/m^3 d) and the foaming gradually disappeared. The VFA concentration was below 700 mg/L during the whole fermentation. The maximum concentration of 700.5 mg/L was reached on day 45 (OLR 8.04 kg VS/m^3 d) and afterwards decreased constantly (Fig. 4a). The propionic to acetic acid ratio was below 0.4 during the experiment. In the following period stable operation was obtained with a COD degradation of 84 %. Based on these results, the optimal OLR for the two-stage fermentation in this study was determined to be 7.0 kg VS/m^3 d and HRT of 20 days.

The biogas methane content was 53 % at a HRT 16 days. At a HRT 19–20 days (OLR of 6.76 kg VS/m^3 d) stable methane content of 55 % was measured. On average, gas production was 450 NL/kg VS. The gas from the first stage was not fed into the methanogenic stage, which led to lower total biogas productivity because the hydrogen-rich gas produced in the first stage is used by the hydrogenotrophic methanogenic bacteria to produce methane (Cooney et al. 2007; Ozkan et al. 2011). In practice the gas losses can be overcome by introducing the hydrogen rich gas phase from the first reactor into the second one.

One-stage AD of SBPP and comparison to two-stage AD

In the one-stage fermenter after one and a half week the HRT was reduced to 32 days, corresponding to OLR of 8.47 kg VS/m^3 d, and seemingly stable operation was achieved (Fig. 4). But on the 29th day, foaming was observed although no VFA accumulation occurred. Therefore the OLR was reduced to 3.40 kg VS/m^3 d (HRT 80 days). Again the HRT was

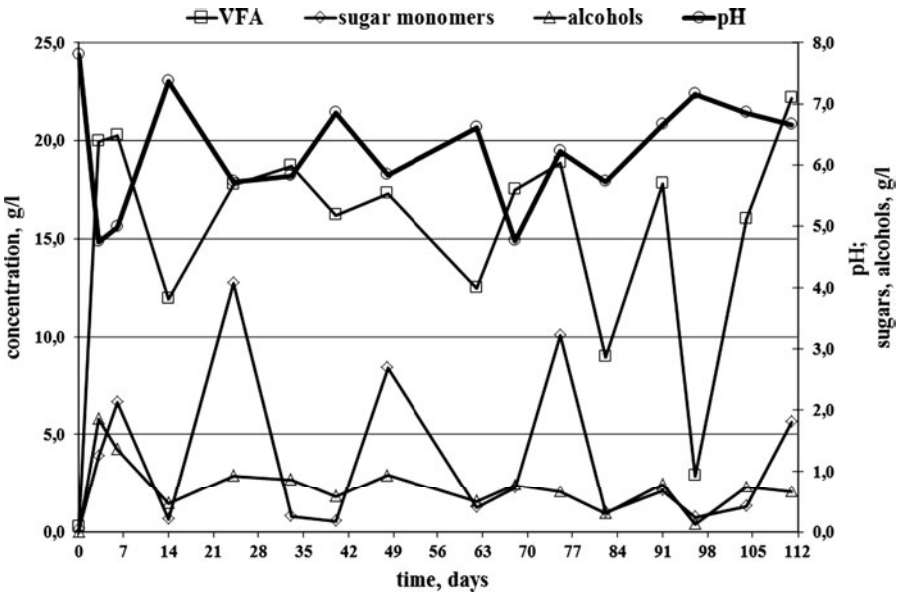

Fig. 3 Volatile fatty acids (VFA), sugar monomers and alcohols concentration progress during the first stage of the two-stage AD of SBPP, determined weekly, by daily recirculation

stepwise decreased to 40 days (OLR 6.78 kg VS/m^3 d). The second time foaming was observed 44 days later (day 73). Therefore the HRT was slightly increased to 44 and foaming significantly receded. At both of the foaming events a decrease of the COD degradation rate was observed: the first time from 88 to 80 %, and the second time—from 85 to 82 %. From day 88 on an increase in the acetic acid concentration was observed. Within 1 week the concentration rose by a factor at about 5 (1.05 g/L). The total VFA concentration was 1.65 g/L with propionic to acetic acid ratio 0.3, both the highest during the experiment. In the following week the HRT was reset to the initial 80 days and then the procedure of stepwise reduction in HRT was once again followed. From these results it is presumed that for one-stage AD in this study without foaming and without COD accumulation a minimum HRT of 50 days (maximum OLR of 6 kg VS/m^3 d) is achievable for this substrate.

The specific biogas production was relatively stable with an average value of 520 NL/kg VS. Nevertheless, a significant correlation was observed between the OLR and the methane content. E.g. in the course of the initial rise of the OLR to 8.47 kg VS/m^3 d (HRT 32 days) the methane content of the biogas dropped from 52 to 45 %. After decreasing the organic load to 5.76 kg VS/m^3 d (HRT 47 days), the methane content increased again to 55 %. The observed values correspond well to data from (Brooks et al. 2008) who reported production of 610 NL biogas/kg VS with a methane content between 50 and 55 %, and 72 % COD removal. They argued that the one-stage fermentation as preferable, because of the lower operational costs and the elaborative and costly handling of the first-stage reactor. Nevertheless, this study showed that the two-stage reactor provides some advantages. It was demonstrated that the first stage can be carried out stably without the need for additives like antifoaming agents and chemicals for pH adjustment. The pH in the one-stage and in the second stage of the two-stage fermenter was similar at 7.5 ± 0.2 during the whole experiment which provides good conditions for methanogenic activity. In both reactor systems the NH_4^+–N concentration of 2.4 g/kg ± 0.5 at pH 7.4 was potentially inhibitory (Braun 1982). The initial NH_4^+–N concentration of the inoculum was already 1.19 g/L, and as long it did not rise progressively, its influence was not considered further. The buffer capacity in the two-stage fermenter did not change significantly during the experiment from 22.1 at the beginning to 21.4 g/L Ca_2CO_{3eq}. In the one-stage fermenter the decrease was higher, from 23.0 to 13.1 g/L Ca_2CO_{3eq}, which could be expected due to the increasing VFA concentration. Nevertheless, it was still enough, not to have influence on the pH value (Fig. 4b).

The OLR for most wet fermentation processes, like energy crops, is between 2 and 4 kg VS/m^3 d (Weiland 2010), but with readily degradable substrates higher values can possibly be obtained. In the one-stage system an OLR of 6 kg VS/m^3 d can be suggested as appropriate to maintain stable operation. Another approach

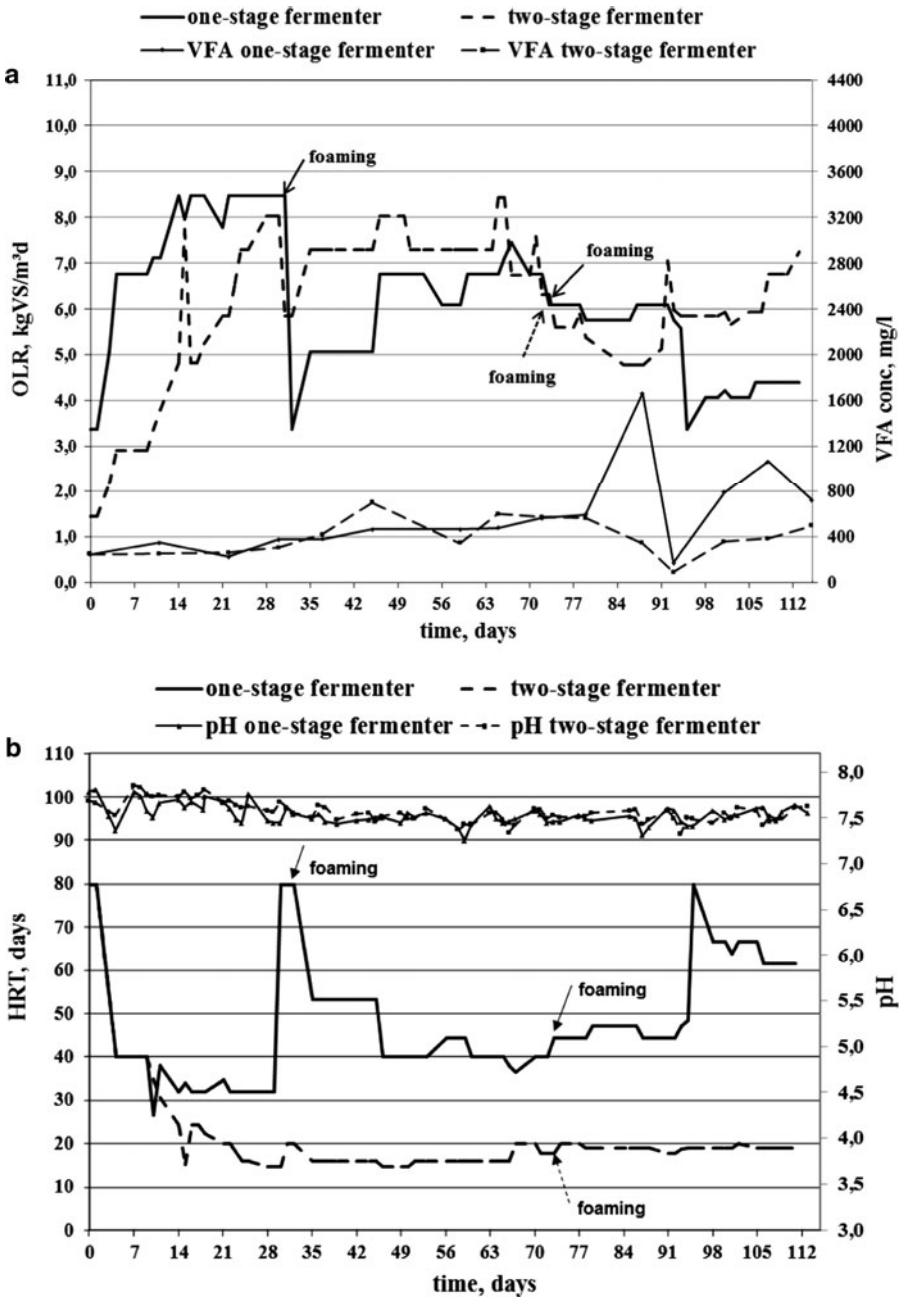

Fig. 4 One-and two-stage fermentation process of SBPP: **a**—organic loading rate (OLR) and VFS concentration, and **b**—hydraulic retention time (HRT) and pH. The *arrows* show the foaming event during the one-stage fermentation (*solid*) and two-stage fermentation (*dashed*)

for carrying out a stable monofermentation of SPPP at higher OLRs is to distribute the OLR by automated feeding of the fermenter several times a day (Scherer et al. 2009). The OLR in the methanogenic stage of the two-stage system was higher, 7 kg VS/m^3 d. The optimum HRT in the single stage system was 50 days. In the two stage system the HRT for the substrate-inoculum mixture was 4 days in the first and 20 days in the second stage, respectively. The overall HRT of the SBPP for the two-stage system was 36 days.

Viscosity measurement

Viscosity was measured in the one-stage fermenter at an OLR of 5.94 kg VS/m^3 d (day 45); and in the second stage of the two-stage fermenter at 8.04 kg VS/m^3 d

(day 46), respectively. The VS contents were 2.64 and 4.06 % for the one- and two-stage fermenter, respectively. In accordance to the method the viscosity was measured until a vortex gap appeared: therefore the maximal shear rate is not the same for all measurements. In the second stage of the two-stage fermenter, where the viscosity was lower, the vortex appeared earlier—at shear rate 30–35 s^{-1} (Fig. 5b). This fermenter had its highest viscosity of 38 mPas at 55 °C at a shear rate of 9.9 s^{-1} (60 rpm). Its lowest viscosity was 2 mPas at 35 °C and shear rate of 8.2 s^{-1} (50 rpm), which corresponds to the operational conditions of the continuous AD of SBPP. For comparison, the viscosity of the one-stage fermenter at 35 °C and shear rate 8.2 s^{-1} is 132 mPas, which is about 60 times higher than the viscosity in the two-stage fermenter at the same conditions. The shear rate at which vortex appeared was also higher: 45 s^{-1} (Fig. 5a).

The digestate in the one-stage fermenter is a non-Newtonian pseudoplastic fluid—its flow properties change according to the shear rate. In the second stage of the two-stage fermenter the fluid behaviour was a Newtonian. At low shear rates the flow behaviour seems not to be Newtonian, resulting from the measurement difficulties at low torques, explained before.

The energy requirement for mixing of the methanogenic reactor was calculated using the standard calculation approach for the specific engine power requirement for stirring (Kraume 2006), $P = (Ne \cdot \rho \cdot n^3 \cdot d^5)/V$, where P is the power, [W/m^3]; Ne—Newton number, [–]; ρ—the density, [g/m^3]; n—rotation speed, [min^{-1}]; d-stirrer diameter, [m], V—volume of the fluid, [m^3], at 35°, and rotation speed 50 rpm. The specific energy required in the two-stage fermenter is about 5 times lower than the one for the one-stage fermenter. Lower viscosity also results in lower stirring rates, necessary to achieve mixing. Viscous fluids prevent small biogas bubbles from escaping from the fermenter broth into the gas phase, which can lead to foaming (Ganidi et al. 2009), and higher stirring rate would be necessary (Zuru et al. 2004). The one-stage fermenter needs a higher stirring rate in order to keep the undigested particles from the sugar beets in the flow. Such particles are not present in the two-stage fermenter. So with the two-stage fermentation a double positive effect on the reactor performance could be observed. The lower reactor stirring costs in the second stage can compensate part

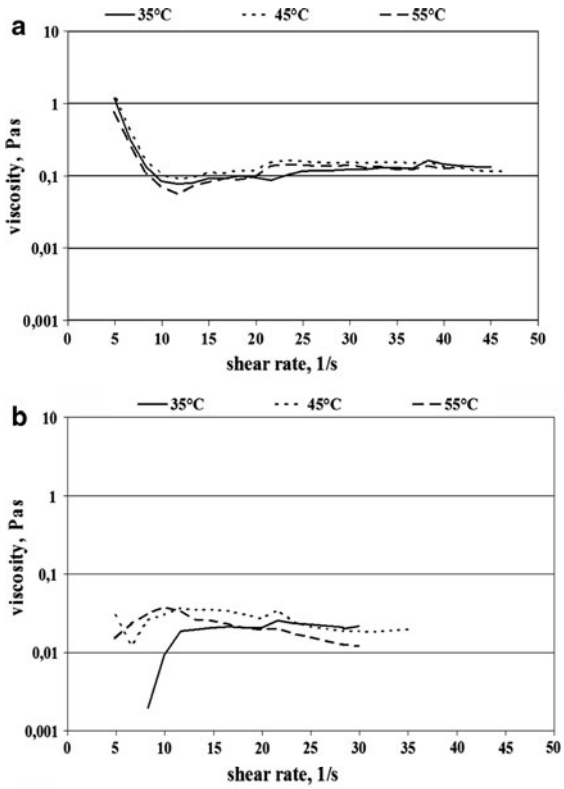

Fig. 5 Viscosity of the fermenter content from the one-stage fermenter (VS = 2.64 %) (**a**), and in the second stage of the two-stage fermenter (VS = 4.06 %) (**b**)

of the additional investment costs for the two-stage fermentation.

The first stage of the two-stage fermenter has high TS 13 %, and sedimentation of the solid fraction does not occur. It only needs mixing after the periodic recirculation once a day, and continuous stirring is not necessary. Energy input for the first was stage considered quite low and therefore viscosity was not measured.

Viscosity decrease in anaerobic reactor as a result of gradual degradation of gel forming polymers during the pre-acidification stage has not previously been reported. However, Toğrul and Arslan (2003) showed decrease in the intrinsic viscosity by decreasing the hydrodynamic volume of the macromolecular chain of cellulose from sugar beet pulp. Hemicellulose and cellulose from sugar beet have been isolated and characterized by Wen et al. (1988). They reported very low viscosities of this fraction of the polymeric compounds—between 1.04 and 2.77 mPas. Therefore,

the pectin fraction was considered to be one of the factors, which could considerably influence the viscosity in AD of SBPP, and was further explored.

Pectin extraction and FTIR structural analysis of the pectin extracts

FTIR analysis of the pectin structure were made to become an insight in the differences of the viscosity in the one-stage fermenter, in order to provide partial explanation (Fig. 6). Commercial apple pectin extract (Fluka) was used as reference. The spectra of the pectin extracted from SBPP and apple pectin are similar. The "fingerprint" region for skeletal C–O and C–C vibration bands of glycosidic bonds and pyranose ring in pectic substances (950–1200 cm^{-1}) has five characteristic bands at 1016–1019, 1052, 1076, 1104 and 1145 cm^{-1} (Kamnev et al. 1998) (Fig. 6a). These bands are present in the spectra of the SBPP and apple pectin extract and are significantly less present in all fermenter extracts. The weak band at 810–838 cm^{-1} represents the presence of α-glycosidic bonds (Mularczyk-Oliwa et al. 2012). It disappears in the spectra from the second stage of the two-stage (TS-AD) and the first stage of the two-stage fermenter (PA-SBPP) extracts, due to the depolymerization of the α-D-galactopyranosyl uronic acid chain.

Beside the fingerprint region, additional information can be taken from FTIR bands. The region between 1200 and 1800 cm^{-1} features the state of the carboxylic groups (Pose et al. 2012). The peak at 1735 cm^{-1} corresponds to the absorbance of ester carbonyl groups (C=O stretching vibration) and in this case shows methylated pectin, or high degree of esterification. This band is strong in the reference extract, weaker in the SBPP extract, and is missing in all other spectra, which indicates full demethylation. Two strong bands at 1600–1650 and 1450 cm^{-1} in the extracts from the one-stage fermenter and the methanogenic reactors correspond to asymmetrical and symmetrical vibration of unesterified carboxylic groups, respectively, confirming deesterification. A strong band at 1670 cm^{-1} appears in the pre-acidification stage extract. This band corresponds to C=O group stretch vibration (1670–1820 cm^{-1}), which indicates a conjugation of side carboxylic groups of pectin molecule chains, if the peak is at the lower wavelength. This indicates again destruction of the polymer pectic molecule (Günzler and Gremlich

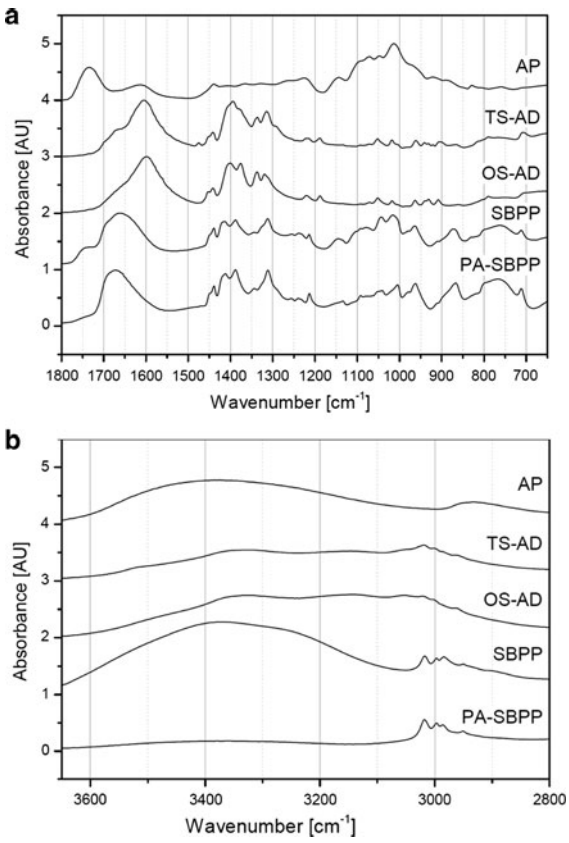

Fig. 6 Fourier transform infrared spectra of pectin extracts from sugar beet pressed pup (SBPP), pre-acidified SBPP (PA-SBPP) and digestate from the methane stage of the one-stage AD (OS-AD) and the two-stage AD (TS-AD) and commercial apple pectin extract (AP) as reference in the range from 650 cm^{-1} to 1800 cm^{-1} (6a) and from 2800 cm^{-1} to 3800 cm^{-1} (6b)

2002). The C–OH stretching in-plane bending peak at 1395–1405 cm^{-1} in the SBPP, PA-SBPP, and OS-AD extracts is disappearing in the TS-AD extract, which indicates also progressing decarboxylation.

Another region in the FTIR spectra is the C–H stretching of CH_2 groups. The band at 1335 cm^{-1} corresponds to in-plan bending of the C–H bonds in the pyranose ring and the peak at 1460 cm^{-1} to in-plan bending of the CH_2 groups. These bands are decreasing in the OS-AD and TS-AD extracts. The bands at 3100 cm^{-1}, characteristic for CH_2 groups are weaker in the OS-AD and TS-AD extracts (Fig. 6b). The same is valid also for the CH_3 bands at 2980 asymmetric vibration. The disappearance of these bands once again indicates the destruction of the pectin subunits (Stuart 2000). However, the symmetric and anti symmetric in-plan bending vibration of CH_3 at 1375

and 1450 cm^{-1} (Sinitsya et al. 2000) remains unchanged in all extracts.

The FTIR structural analyses confirmed rapid deesterification and starting degradation of the pectin fraction during pre-acidification. Ultimately extensive destruction of pectin occurred both in the one and the two-stage fermenter system.

Conclusions

The two-stage AD of SBPP brings several advantages. The faster degradation of the polymers in a separate pre-acidification step can lead to reduction of the overall HRT, in this study from 50 to 36 days and hence lower reactor volumes are required. The pre-acidification occurs the fastest at 55 °C and a HRT of 4 days is enough to obtain the maximum accumulation of VFAs. Also more stable operation of a two-stage system, compared with one-stage AD was demonstrated. The risk of foaming at higher OLR, a frequent problem in AD of SBPP, could be reduced. Optimum pH levels can be maintained, both in the first stage and the second stage, without artificial pH adjustment. Rapid degradation of gelling substances (pectines) was confirmed by FTIR analyses. The reduction of the viscosity in this study led to a five times lower energy demand for reactor stirring. In summary, these advantages may make the two-stage process economically attractive, despite higher investments for a two reactor system.

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Overcoming the bottlenecks of anaerobic digestion of olive mill solid waste by two-stage fermentation

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ABSTRACT

Two-stage anaerobic digestion (AD) of two-phase olive mill solid waste (OMSW) was applied for reducing the inhibiting factors by optimizing the acidification stage. Single-stage AD and co-fermentation with chicken manure were conducted coinstantaneous for direct comparison. Degradation of the polyphenols up to 61% was observed during the methanogenic stage. Nevertheless the concentration of phenolic substances was still high; the two-stage fermentation remained stable at OLR 1.5 kgVS/m^{3}day. The buffer capacity of the system was twice as high, compared to the one-stage fermentation, without additives. The two-stage AD was a combined process – thermophilic first stage and mesophilic second stage, which pointed out to be the most profitable for AD of OMSW for the reduced hydraulic retention time (HRT) from 230 to 150 days, and three times faster than the single-stage and the co-fermentation start-up of the fermentation. The optimal HRT and incubation temperature for the first stage were determined to four days and 55°C. The performance of the two-stage AD concerning the stability of the process was followed by the co-digestion of OMSW with chicken manure as a nitrogen-rich co-substrate, which makes them viable options for waste disposal with concomitant energy recovery.

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Single-stage AD; two-stage AD; co-fermentation; polyphenols; chicken manure

1. Introduction

The production of olive oil is one of the agricultural areas, which produces waste in high amounts on concentrated area. In 2013, 88% of the world production is localized in the Mediterranean area, with 39% of the amount produced in Spain alone.[1] Although the world production decreased from 3,488,907 tons in 2012 [1] to 2,825,730 tons in 2013, the tendency for 2014 was again a rise in the production up to 3,164,000 tons, and being estimated around 2,560,000 tons for 2014/2015.[2] From one ton olives, around 200 kg of olive oil are produced. Since in the 1990s the three-phase extraction system has been replaced by a two-phase one, the amount of wastewater has been reduced from 1.0–1.6 m^3 to 0.2 m^3 per ton processed olives. On the other side, the amount of olive cake has increased from 550 to 800 kg per ton processed olives.[3]

The main characteristic of the two-phase olive cake, also called 'alperujo', are low pH 4.86–6.45, and high C/N ratio (28.2–72.9). It consists of 85–97% organic matter, namely 32–55% lignin, 8–20% fats, and 0.6–3% of the dry weight water-soluble phenols, Na^+ 0.5–1.6 g/kg dry weight.[3] These parameters vary depending on seasonal, geographical, and varietal factors. The low pH, high content of organic matter, long-chain fatty acids (LCFA), and polyphenols do not allow the direct exposure on the soil, because of its phytotoxicity and negative impact on the physicochemical properties of the soil,[4] the microorganisms,[5,6] and the groundwater quality.[7] Moreover, odors and evaporating phenol substances and sulfur oxides cause air pollution during storage.[8] The possibility to extract valuable compounds such as antioxidants,[9] pectins,[10] and fatty acids from the pomace, or to use it as ruminant feed [11] have been studied before. Recently composting of the solid fraction of the olive mill waste is one of the most popular valorization methods, regarding the humidification and the reduction of polyphenol content.[12–14]

Also the feasibility of energy recovery has been considered, for example, by combustion of the cake.[15] However, the total energy recovery in this kind of utilization has been discussed as not sufficient enough.[16] Another option is anaerobic digestion (AD). AD of olive mill wastewater (OMWW) and olive mill solid waste (OMSW) is a technology that already has its application for waste management and energy recovery in the olive oil industry, mostly as a co-substrate.[17] Lot of studies of olive mill waste address wastewater from olive processing due to its huge amount.[18] Promising results were obtained using upflow anaerobic filters

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which provide short start-up time and high process stability.[19] Later the effectiveness of the upflow anaerobic sludge blanket reactor has been recognized exhibiting improved process performance like lower hydraulic retention time (HRT) (around 25 days) and chemical oxygen demand (COD) removal up to 90%.[20] The first approaches on AD of solid waste of olive mills were carried out with olive pulp diluted with water.[21,22] The adjustment of the influent concentration was necessary to overcome the bottlenecks of the olive pomace for AD: high C/N ratio, low alkalinity, residual LCFA, and polyphenols, as well as high amount of potassium.[23] Two-stage AD of OMSW has been studied before as possibility to deal with the possible inhibitions.[24–26] In all of the previous studies the pre-acidification step has been carried out at 35°C, and HRT lower than 20 days, 10–12 optimal has been reported. The co-digestion of OMSW with nitrogen-rich substrates has been in the focus of number of research, as a cost-effective problem solution,[27] with substrates as poultry manure,[28] and cattle excreta [29] in continuous fermentation at mesophilic conditions, laying hen litter in Biochemical methane potential (BMP) tests,[30] and swine manure.[31]

This study aims to point out a reasonable solution of the bottlenecks of the AD of OMSW by applying two-stage AD with direct comparison to single-stage AD and co-fermentation with chicken manure at mesophilic conditions.

2. Materials and methods

2.1. Substrate and inoculum

OMSW was obtained from an olive mill in Sardinia, Italy, as the solid residue from a two-phase extraction system. The co-substrate, chicken manure, was obtained from a local chicken-house in Lower Austria. Both of the substrates were stored at −20°C until use. The inoculum was a mixture from a local biogas plant digesting agricultural crops and an anaerobic digester operated with thin stillage from bioethanol production, both operated at mesophilic conditions. Table 1 gives an overview of the relevant substrate and inoculum parameters.

2.2. Process set-up and operation

2.2.1. Batch experiments for determination of the optimal incubation temperature in the first stage of the two-stage fermentation

The optimal temperature and duration of the first stage of the two-stage fermentation were determined in a batch set-up, by mixing OMSW with inoculum in 2:1 (w/w) ratio in closed reactor vessels. Incubation was conducted for 7 days at 35°C, 45°C, and 55°C, respectively. Daily, the pH value was measured and the formation of sugar monomers, volatile fatty acids (VFA), and alcohols was determined by high performance liquid chromatography (HPLC) analysis.

2.2.2. Set-up of continuous experiments

Three kinds of experiments were conducted: one-stage fermentation with olive waste as sole substrate and single-stage co-fermentation with chicken waste, respectively; and as last experiment a two-stage mono-fermentation of olive waste.

The single-stage fermentation, the co-fermentation as well as the methanogenic stage of the two-stage fermentation were conducted in continuous stirred anaerobic tank reactors (CSTR) with a working volume 6 L at mesophilic (37 ± 1°C) conditions. The vessel was equipped with blade stirrer and a heating jacket was used for temperature control. The fermentation was semi-continuous – the fermenters were fed manually once a day. The operation period of all fermenters was 365 days. The pit rests of the OMSW with size of approximately 1 mm settled on the bottom of the fermenter. Since the fermenter outlet was also on the bottom, they were removed spontaneously with the sampling and recirculation; so they did not disturb the fermentation.

The first stage of the two-stage continuous fermentation, further called pre-acidification, was performed in an extra vessel, a one-liter gas-tight plastic chamber. Mixing was performed manually once per day. The experiment was started by mixing OMSW with mesophilic inoculum, in 2:1 (w/w) ratio without continuous stirring. The mixture was incubated at 55°C in a temperature-controlled environment. Daily, feeding was conducted with a mixture of fresh OMSW and fermenter content recirculated from the subsequent methanogenic stage of the two-stage fermentation. The mixture ratio was 2:1 (w/w) OMSW to recirculated sludge. The HRT in the first stage was set to four days. The removed content of the pre-acidification stage served as the feed for the methanogenic fermenter.

2.3. Analytical methods

2.3.1. General analytical methods for monitoring the AD process

The stability of the AD processes was monitored by the following parameters: COD, total Kjeldahl nitrogen (TKN), ammonium nitrogen (NH_4^+–N), total solids (TS), and volatile solids (VS) which were determined weekly,

Table 1. Characterization of relevant parameters of the substrates and the inoculum.

Parameter		OMSW[a] Charge 1	OMSW[a] Charge 2	Chicken manure	Inoculum
pH	(–)	4.53	4.12		8.36
TS	(% fresh weight)	23.92	33.04	42.12	1.95
VS	(% fresh weight)	22.93	31.68	28.82	1.06
COD	(g/kg)	289.24	321.76	371.80	9.31
TKN	(g/kg)	3.43	4.96	10.28	2.01
NH_4^+–N	(g/kg)	0.26	0.37	4.98	1.19

[a]Charge 1 until day 209; charge 2 from day 210 onward.

according to standard methods.[32] Free ammonia (NH_3–N) concentration in the digestate was calculated from total ammonium nitrogen using Equations (1) and (2).[33]

$$pK_{NH_4} = \frac{2766.16}{T}. \quad (1)$$

$$[NH_3 - N] = \frac{[NH - N]}{10^{pK}NH_4^{-pH} + 1}. \quad (2)$$

Gas quantity was measured using MilliGascounter (Dr.-Ing. Ritter Apparatebau) with a flow rate range 1 mL/h–1 l/h, resolution 3 mL and accuracy ±3%.

The gas quality was determined using gas measuring system TRM/816 (Awite Bioenergie). Methane (CH_4) and carbon dioxide (CO_2) were quantified using an infrared sensor (2-beam), and hydrogen sulfide (H_2S), hydrogen (H_2) and oxygen (O_2) – by using an electrochemical detection system.

Sugar monomers, VFA, and alcohols, released during the hydrolysis and acidogenesis of the substrate were analyzed by HPLC. Before determination the proteins in the samples were precipitated and after centrifugation the supernatant was acidified. The measurement was conducted on a Hewlett Packard chromatograph, Series 1100, equipped with Agilent 1100/1200 isocratic pump and refractive index detector with an optical unit temperature of 45°C. Separation was made on an ICSep ICE-ION-300 column (Transgenomic) at 40°C. The mobile phase consisted of 0.01 NH_2SO_4 solution. The flow rate was 0.325 mL/min with a running time of 120 min. The concentration of the products was quantified using Agilent ChemStation software, Rev. B.01.03 (204) (Agilent Technologies) and external calibration curves.

BMP assays were accomplished in bottles with 500 mL working volume at 35°C, according to VDI 4630, DIN 38 414-S6.[34]

The determination of trace elements was carried out on an inductively coupled plasma – optical emission spectroscope (Jobin Yvon Horiba Ultima) after microwave digestion with nitric acid in a Milestone ultraCLAVE III device at 240°C with a final pressure of 100 bar for 20 min.

Fat content was determined by solvent extraction hexane/2-propanol (3/2 v/v), according to Hara and Radin.[35]

2.3.2. Phenol extraction and determination

The phenol substances were extracted with 80% methanol in water and ultrasound following the protocol provided by Kim and Lee [36] The fermenter broth samples were centrifuged for 5 min at 2500 rpm, because filtration was not possible. The extracts were cleaned from lipids with *n*-hexane. The phenol concentration was determined after the Folin Ciacolteau method [37] as gallic acid equivalents. The absorbance was measured at 750 nm.

2.3.3. DNA extraction and denaturing gradient gel electrophoresis

Total genomic DNA was extracted with the MoBio PowerSoil™ DNA Isolation kit (Cat.No. 12888-100), according to the manufacturer's instruction. 16S rDNA fragments corresponding to nucleotide positions 341–926 of the Escherichia coli 16S rDNA numbering system covering variable V3 and V4 region were amplified with the forward primer 341f–GC [38] and the reverse primer 907r.[39] Hot start [38] touchdown PCR [40] with annealing temperature changing from 63°C to 55°C over 16 cycles was used to avoid nonspecific primer annealing.[41] The PCR was carried out in a PeqSTAR 96 universal gradient thermocycler (Peqlab Biotechnologie GmbH, Erlangen, Germany; Cat. No. 95-95002) and the presence of PCR products was confirmed by analyzing 5 µL of product by electrophoresis in 1% agarose gel with Invitrogen SYBR®Safe DNA gel stain (Life Technologies, Carlsbad, CA, Cat. No. S33102) and UV transilluminator (Bio-Rad Universal Hood II Gel Imager; Bio-Rad Laboratories, Milan, Italy, Serial No. 76S/02161) prior to subsequent denaturing gradient gel electrophoresis (DGGE).

Parallel DGGE was performed with a Dcode System apparatus (Bio-Rad, Hercules, CA) according to the manufacturer's instruction. Amplicons of ca. 560 bp were separated in 6% (w/v) polyacrylamide gels containing a linear 30–70% denaturant gradient (100% denaturant corresponds to 7 M urea and 40% deionized formamide). Electrophoresis was performed in 1× tris-acetate-EDTA (TAE) buffer at a constant voltage of 100 V and a temperature of 60°C for 16 h.[42] The DNA bands were stained by using 0.01% SYBR® Green I nucleic acid gel stain (Sigma-Aldrich Chemie GmbH, Germany; Cat. No. S9430) in 1× TAE buffer (pH 8.0) and were photographed

with a Typhoon TrioTM Variable Mode Imager (Amersham Biosciences, Sunnyvale, CA) at 488 nm wavelength.

3. Results

3.1. BMP tests

Prior to the continuous experiments BMP tests were carried out in order to get first insight about degradation rate, possible inhibitions, and methane potential of the substrates (Table 2).

The observed slower conversion of the fresh OMSW compared to the pre-acidified one indicates the necessity for longer retention times during the single-stage fermentation. The time to achieve 95% of the total methane production was more than twice as long as for the pre-acidified OMSW as well as for the chicken manure. The slightly lower BMP after is due pre-acidification to small methane production during the pretreatment.

3.2. Single-stage fermentation

The single-stage monofermentation (Figure 1) proceeded without disturbances at OLR of 0.76 kg VS/m^{3}day until day 192. Based on previous experience, the chosen increase in the OLR was deliberately low during this period. In earlier experiments, a more rapid increase in the OLR led to a drop of pH to 6.8, however without accumulation of VFAs, and the methane production declined irrecoverably. In the test run the increase in the OLR to 1.10 kg VS/m^{3}day resulted in drop of the biogas production, but no change in the methane content or increased VFA concentration was observed. Successive decrease of the buffer capacity was measured as well. Nevertheless, the OLR was increased again in order to work out the highest possible OLR. The final raise of the OLR to 1.76 kgVS/m^3 day caused more and more severe foaming and on day 333 the feeding had to be stopped. The viscosity of the fermenter broth has not been measured, but it was noticeably higher as at to the beginning of the fermentation. In the course of the test run, the pH dropped from 7.5 at the beginning to 7.0 at the end of the experiment. Methane production decreased steadily with increasing OLR – from 380 to 220 Nm^3/tVS.

3.3. Co-fermentation of OMSW with chicken manure

In order to balance the high C/N ratio, and compensate the low alkalinity of the OMSW, co-fermentation with chicken manure, a substrate with high NH_4^+–N content (Table 1), was conducted. Another reason for choosing CM as co-substrate is its high availability in the region. During the first 183 days the reactor was fed only with OMSW (Figure 2). Subsequently the reactor was fed only with CM for 55 days retaining the OLR. From day 238 on the reactor was fed with a mix of OMSW and CM. The VS ratio between the two substrates was 30:70 (OMSW:CM) and this blending ratio was kept until the end of the experiment. At the same day (238) the OLR was increased from 0.64 to 1.08. With the application of CM as co-substrate the buffer capacity increased significantly, reaching a final value of 21.1 g$CaCO_3$/L at the end of the test run. Throughout the whole fermentation, the pH remained higher than 7.5 and the average overall methane production was around 180 Nm^3/tVS.

3.4. Two-stage monofermentation

3.4.1. Determination of the optimal incubation temperature in the first stage of the two-stage fermentation

In pre-experiments the optimum incubation temperature and period for pre-acidification was investigated. For this purpose the time course of released sugar dimers and monomers, short-chain fatty acids and alcohols were analyzed (Figure 3). At 35°C, instead of the expected increase, a decrease in readily degradable organic compounds was observed (Figure 3(a)). These results are in contrast to the similar experiments conducted with more easily degradable substrates such as pressed sugar-beet pulp.[43] The explanation is that the acidification through formation of VFAs was too slow to suppress methanogenic activity and further conversion of monomers to methane occurred. At 45°C a slight increase in total monomer concentration occurred (Figure 3(b)), whereas at 55°C the monomer concentration raised by 20% (Figure 3(c)). During this experiment the pH dropped from 6.5 on the first day to 5.5 on the second day, and subsequently remained between 4.5 and 5.0. The highest concentration of solubles was measured after 72 h.

Similar concentrations of sugars and alcohols were measured during the whole experimental period.

Table 2. BMP test of fresh OMSW and the pre-acidified OMSW.

Substrate	Methane (Nm^3/ton VS) after 60 days	Time (days) to achieve a percentage of total BMP		
		50%	75%	95%
OMSW	312.3 ± 50.1	10	27	58
Pre-acidified OMSW	304.8 ± 48.9	10	13	24
Chicken manure	275.0 ± 46.1	5	7	18

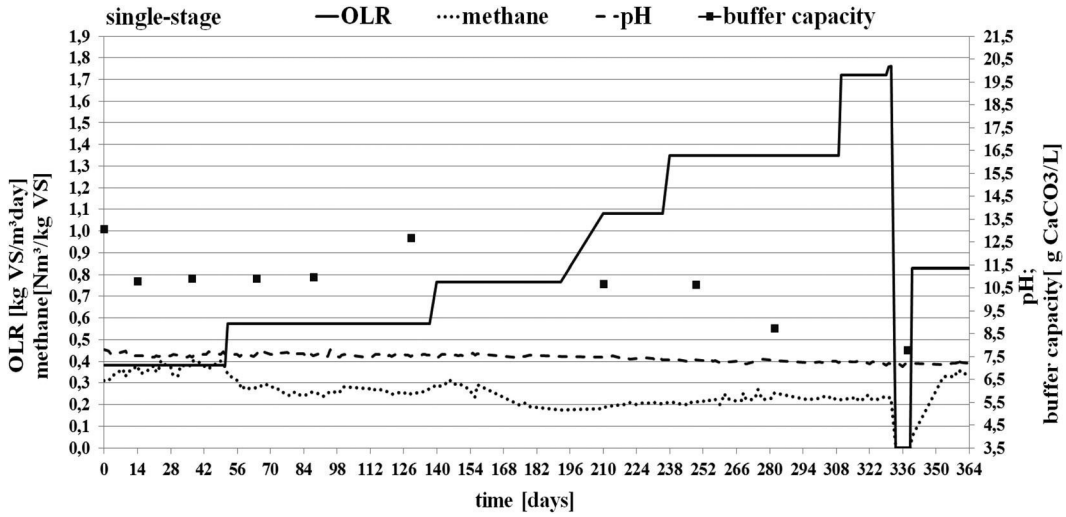

Figure 1. OLR, buffer capacity, and methane production during the single-stage semi-continuous mesophilic AD of OMSW.

According to our own experience,[43] accumulation of sugar dimers and monomers during pre-acidification is unusual because they are rapidly converted to VFAs. Hence, the observation made is an indication for partial inhibition of the acidification step. Based on these findings, a temperature of 55° C was selected for pre-acidification. A HRT of 4 days was chosen to achieve the best possible conversion of organic matter into soluble compounds.

The shift in the bacterial community during pre-acidification was investigated by comparing DGGE banding patterns (Figure 4). Within the first 4 days, a strong alteration of bacterial species was observed. Subsequently the composition of the microbial consortium was relatively constant with some prevailing bacterial species (bands 3, 5, 6–9, 11, 13, 14, 16–19). This observation was taken as another indicator that four days of HRT are adequate to establish an appropriate bacterial community in the pre-acidification step. For comparison Figure 4 shows also samples from single-stage fermentation and the methanogenic step of two-step fermentation which is discussed further down. The pattern of the single-stage includes all bands observed in the course of pre-acidification; however, the bacterial diversity or number of bands was much richer. In contrast, a few dominant species were present in the methanogenic step, which were different from those present during pre-acidification.

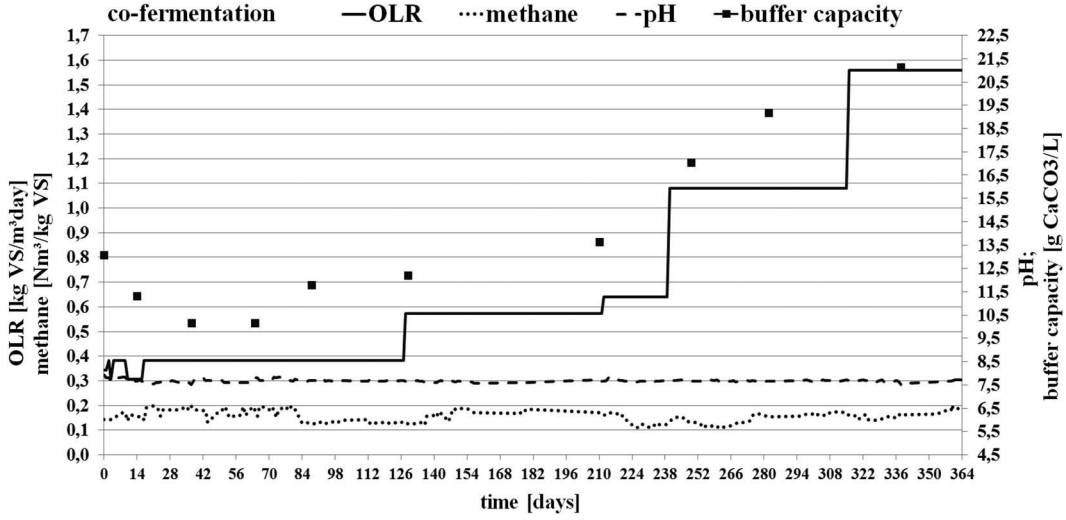

Figure 2. OLR, buffer capacity, and methane production (Nm^3/kgVS) during the semi-continuous mesophilic co-fermentation of OMSW with chicken manure.

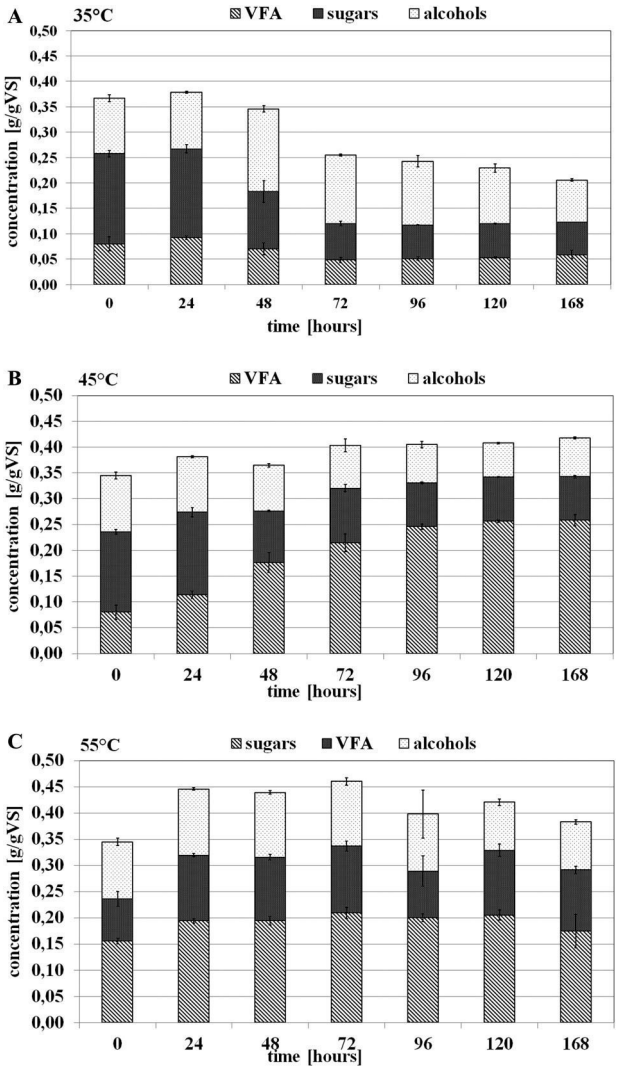

Figure 3. Changes in the concentration of the released monomers sugars, VFAs, and alcohols during the pre-acidification of OMSW at 35°C (a), 45°C (b), and 55°C (c).

3.4.2. Two-stage continuous monofermentation

As explained during two-stage AD of OMSW the pre-acidification stage was carried out at a HRT of four days and at 55°C. The course of the concentration of the monomers is presented in Figure 5. The observed fluctuations in the concentrations of the monomers are mainly due to sludge recycling from the second (methanogenic) reactor. Between days 98 and 122, and later on between days 222 and 322 the concentration of the VFAs increased, which correlated to an increase in the OLR in the methanogenic reactor (Figure 6) and the associated VFA accumulation. The pH in the pre-acidification stage generally followed the fluctuation in VFA concentration.

The results of the second stage are presented in Figure 6. Pre-acidification had a positive impact on the overall process stability of the two-stage fermentation. As a major factor of influence, the buffer capacity was higher as during single-stage fermentation. The OLR was increased up to 1.26 kgVS/m^{3}day within 70 days without drop in the buffer capacity. In comparison in the single-stage monofermentation at an OLR of 1.08 kgVS/m^{3}day the buffer capacity started to decrease (compare Figure 1). On the 120th day the two-stage fermentation was interrupted due to technical problems for 5 days. A restart was conducted at a decreased OLR of 0.85 kg VS/m^{3}day. The OLR was raised again to 1.13 kg VS/m^{3}day on day 217. The final OLR applied was 1.56 kg VS/m^{3}day. In this period the measured buffer capacity was 12.9 g$CaCO_3$/L, which is still considerably high. As a result of increased buffer capacity, the pH remained quite constant around an average of 8.0. Therefore the two-stage fermentation showed high stability during start-up and allowed approximately threefold faster increase in the OLR compared to the single-stage AD. The methane content was approximately 62 ± 3%, and higher compared to the one measured during single-stage fermentation (55%). However, the specific methane yield (Figure 6) was generally the same and again a decrease with increasing loading rates was observed. With the exception of a short period no accumulation of VFAs was observed. Only between the 217th and 238th day the VFA concentration accumulated up to 7.8 g/L, 90% of which is acetic acid. This high amount of VFA resulted in a decrease in the pH from 8.0 to 7.3. However, the reactor recovered quickly within the next eight days.

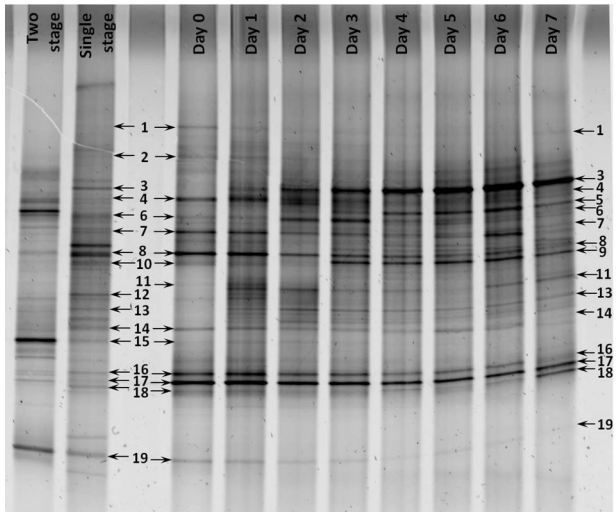

Figure 4. DGGE analyses showing bacterial community shift during pre-acidification of OMSW at 55°C for 7 days. In comparison, bacterial communities in single- and two-stage (second stage) fermentation of OMSW are shown on the left lanes.

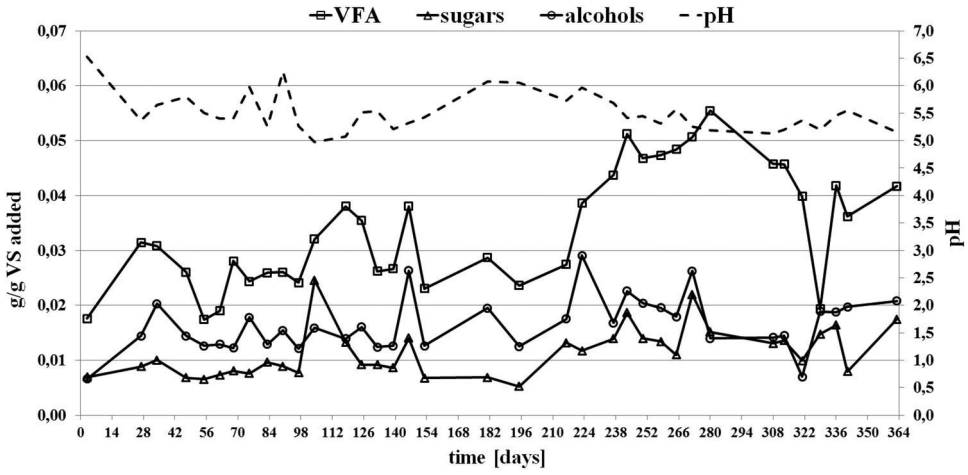

Figure 5. Concentration of the sugar monomers, VFA, alcohols, and pH during the pre-acidification stage (55°C) of the continuous two-stage AD.

3.5. Determination of the possible inhibition factors

The concentration of the phenolic substances was determined as they are reported to be one of the principle components responsible for inhibition. Phenol concentration during single-stage fermentation was 1.23 ± 0.05 gGA_{eq}/L. In the final phase of co-fermentation, the concentration of phenolic substances was lower (0.74 ± 0.02 gGA_{eq}/L) due to the reduced share of OMSW in the substrate. It is presumed that this lower phenol concentration contributed to the process stability even though the OLR was similar to the highest one tested during single fermentation. On the other hand, stable operation was also achieved during two-stage fermentation although it exhibited similar phenol levels as in the single-stage (1.19 ± 0.01 gGA_{eq}/L).

The trace element composition, as parameter related to inhibition of AD processes,[44] was determined in order to identify possible bottlenecks. Their respective concentration at the highest achieved OLR is given in Table 3. However, the measured values did not provide a clear picture. Just in case of co-fermentation elevated NH_4^+–N levels from 1.1 g/L at the beginning to 4.4 g/L at the end of the fermentation, corresponding to 0.10 and 0.26 g/L free NH_3, were observed. Despite of the high fat content in the fresh OMSW, the fat fraction in the anaerobic reactors was completely degraded. The humic acids were considered as a possible factor for the electron shuttling during the biochemical processes in the reactors.[45,46] In this study, the concentration of the humic-like substance correlates with the degradation

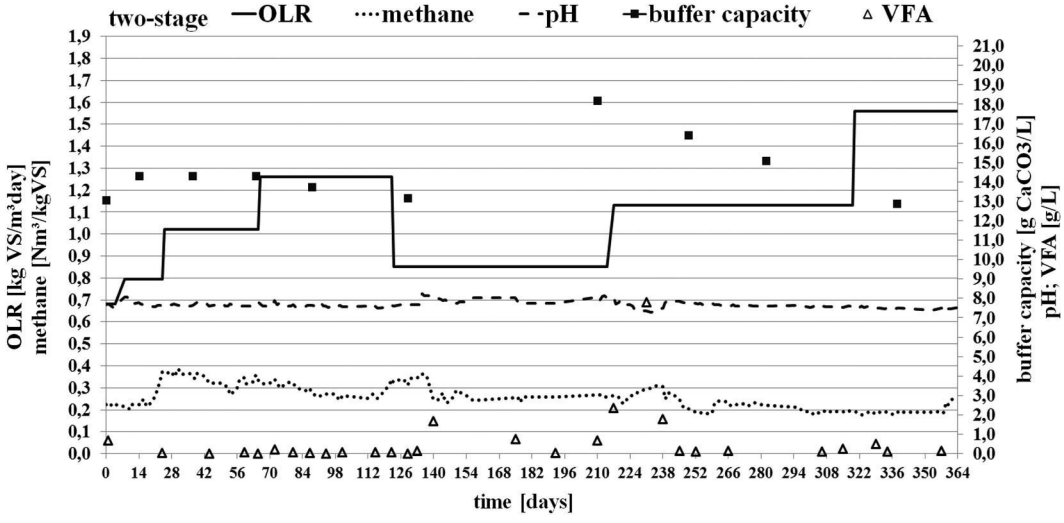

Figure 6. OLR, buffer capacity, and methane production during the methanogenic stage of the two-stage semi-continuous mesophilic AD of OMSW.

Table 3. Relevant parameters for characterization of the AD of OMSW at the end of the experiment.

Parameter		OMSW	Single-stage	Two-stage	Co-fermentation	Pre-acidification
Al	(g/L)	–	0.08	0.22	0.14	–
B	(g/L)	–	0.01	0.01	0.0	–
Mg	(g/L)	0.2	0.25	0.40	0.71	–
Ca	(g/L)	1.02	1.11	1.35	3.33	–
Cu	(g/L)	0.004	0.01	0.01	0.01	–
Fe	(g/L)	0.1	0.28	0.39	0.44	–
Co	(g/L)	–	0.002	0.001	0.004	–
Cr	(g/L)	–	0.02	0.03	0.03	–
Mn	(g/L)	–	0.01	0.01	0.04	–
Mo	(g/L)	–	0.002	0.003	0.002	–
Ni	(g/L)	–	0.02	0.01	0.03	–
Zn	(g/L)	–	0.01	0.01	0.03	–
NH_4^+–N	(g/kgFM)	0.3	0.8	2.3	4.4	0.8
Fat content	(% VS)	11.3	–	–	–	4.5
Phenols	(gGAeq/kgVS)	26.76	17.17	19.39	11.71	13.81
Degradation of polyphenols	(%)	–	73.4	61.2	38.5	–
Humic acids	(g/kg)	22.6	16.8	13.4	8.2	14.8

rate of the polyphenols. However, further investigations are necessary to explain this fact. Also the essential trace elements necessary for the metalloenzymes (Ni, Co, Mo, Se, and Fe) of the methanogenic microorganisms were determined but the measured values do no indicate undersupply.

4. Discussion

Two-stage fermentation of OMSW (a combined process – thermophilic first stage and mesophilic second stage) was investigated to find out an appropriate solution for on-site processing at olive oil mills, and directly compared to one-stage and co-fermentation process. One target was to achieve the highest possible OLR at stable operation conditions. Single-stage AD was characterized by a long start-up period. The highest advisable OLR was 1.36, whereas at an OLR of 1.72 kgVS/m^3day severe foaming and break down of the process occurred. The second option, co-fermentation with CM, provides two advantages: on the one hand, an improvement of the C:N ratio; on the other hand, an increase in the buffer capacity, which helps maintain stable operation conditions. The OLR achieved at the end of the co-fermentation was 1.56 kgVS/m^3day. The approach tested here, co-digestion of the solid fraction of olive mill wastes (OMSW), has not been reported yet, unlike the wastewater fraction. Several studies addressed co-digestion of wastewater from olive mills (OMWW) with nitrogen-rich substrates in order to decrease the C/N ratio. Co-digestion with poultry manure [28] and cattle excreta [29] has been studied in continuous fermentation at mesophilic conditions as well as in BMP tests using laying hen litter [30] and swine manure.[31] Gelegenis et al. [28] reported enhanced biogas production up to a 40% (w/w) share of OMWW to the TS of the feed mixture. They observed that at 50% (w/w) the biogas production decreased considerably. For comparison, in this study the share of OMSW in terms of TS was 23%. While the use of a co-substrate improved process stability as well as the maximum loading rate, this approach also has certain drawbacks. Its practical application is dependent on the availability of such a co-substrate at the specific location. Moreover the increased N-content in the substrate may lead to a NH_4^+ inhibition. The NH_4^+–N concentration determined at the highest loading rate was 4.38 g/kg corresponding to 0.26 g/kg NH_3–N. The concentration of NH_4^+–N (4.38 g/L) at the end of the fermentation is already in the range (1.7 up to 14 g/L NH_4^+–N) were inhibitory effects were observed.[44,47] In fact the critical parameter is the free NH_3 concentration, which is also dependent on pH and temperature. Studies on the effect of free ammonia on the AD process postulated complete inhibition of the activity of the microorganisms at a concentration of 800 mg/L NH_3.[48] Sossa et al. [49] reported 50% inhibition at 365 mg/L, and 100% inhibition at 850 mg/L free NH_3. Here the corresponding free NH_3 was 260 mg/L. However, it has been proved that AD processes can be successfully operated at ammonia levels up to 1060 mg/L, after adequate long-term adaption of the microbial consortium.[50]

The most straightforward approach turned out to be two-stage fermentation incorporating a 4-day pre-acidification step at elevated temperature. Compared to single-stage fermentation, it allowed threefold faster increase in the OLR and a stable process was achieved at the highest OLR of 1.56 kgVS/m^3day at the end of the test run. Rincón et al. [51] investigated bacterial communities in a single-stage reactor treating OMSW. According to their observation the bacterial populations were similar at an OLR of 0.75, 1.5, and 2.25 whereas at 3.0 kgCOD/m^3day, a significant shift in bacterial population occurred, with some species disappearing or

fading away. They concluded that higher OLR causes inhibition of hydrolytic bacteria. These findings are confirmed by the results of this test run. The split of AD into two separated steps allows us to apply tailored conditions for hydrolyses in the pre-acidification stage and hence minimizes inhibitors' effects and improves overall process performance. Other studies have already acknowledged the benefits of pre-acidification, however at 35°C and a HRT of 12 days, respectively.[24,52] Incubation at 55°C in the first step of two-stage fermentation at 55°C has not been tested before for AD of OMSW.

The achieved HRTs of 230, 150, and 193 days in the single-stage, two-stage and co-fermentation, respectively, were still high for an industrial AD plant. Some other studies reported much lower HRTs, however, only after mixing with OMWW,[23] or after dilution with water.[26,53] Generally in all of the fermentation scenarios, the methane production decreased with increasing OLR, indicating a progressive inhibition of the AD process. At the highest OLR, it was 230, 200, and 170 Nm^3/tonVS (226, 197, and 167 Nm^3/tCOD) in the single-stage, two-stage, and co-fermentation, respectively. For comparison, Rincón et al. [26] achieved a methane production of 268 Nm^3/tCOD using the effluent from an OMSW pre-acidification reactor.

Phenolic compounds are considered to be mainly responsible for inhibitory effects during AD of OMSW. They are present in olive fruits and act as a defense against various pathogens.[54] Polyphenolic compounds are a large and complex family of substances, characterized by the presence of large multiples phenol structural units. Several studies have demonstrated that they limit the microbial activity as consequence of biostatic effects.[24,55,56]

The content of phenolic compounds in the fresh OMSW was 8.3 g/kg, which is in the typical range reported by other researchers, 2–11.5 g/kg.[8] Actual phenol levels in the methanogenic reactors were much lower for single, two-stage, and co-fermentation (1.23, 1.19, and 0.74 g/L, respectively). Numerous studies have been conducted on the anaerobic phenol degradation.[24,57–59] The optimal temperature for the AD of polyphenols is considered to be at mesophilic conditions [59] as applied here. Chen et al. [60] underline the important role of methanogens during anaerobic phenol degradation. Nevertheless, Clostridia, the predominant species during pre-acidification, are also known to convert phenol derivatives to benzoate.[61] Despite the low residence time, a significant reduction in phenolic compounds (~ 59%) was observed in first step of two-stage fermentation. Also Rincón et al. [26] described a 40.7% degradation of phenolic compounds in the first stage of a two-stage AD of OMSW. In contrast, Beccari et al. [24] reported degradation of polyphenols only in the methanogenic stage at pH 8.5 and no degradation during the acidogenesis at pH 6.5. Even though substantial removal rates of phenolic compounds were observed in all test runs, the residual concentrations are still considerable and might well explain the lowering of specific methane yield. For instance, Borja et al. [62] studied the impact of the most important phenolic constituents of OMWW on anaerobic methanogenesis. For oleuropein and caffeic, p-hydroxybenzoic and protocatechuic acid inhibition at levels $\geq$1000 mg/L are stated.

Inhibition might not only result from polyphenol accumulation. Several other factors can disturb the AD.[44] One limitation which has become increasingly the focus of attempts to improve AD is adequate supply with trace metals. Metal ions are essential elements of the metalloenzymes in methanogenic microorganisms.[63]

Required concentrations of trace elements are not exactly defined and the suggested values vary in a wide range covering several log steps.[64] However, the measured levels do not indicate a limitation although it should be pointed out that the presence of trace elements does not necessarily confirm their bioavailability.[65] In a recent publication it has been demonstrated that trace metal addition can help to overcome certain other AD process limitations, such as very high ammonia levels.[50] Whether this is the case with OMSW remains subject to further test runs.

5. Conclusions

AD of OMSW is discussed as a viable option for waste treatment with concomitant energy recovery. Two-stage fermentation pointed out to be a straight forward strategy. Pre-acidification and subsequent methanization in a second stage allows overcoming the process disturbances observed during single-stage fermentation. The HRT was 35% reduced, compared to the single-step fermentation and the increase in the OLR to the highest value of 1.56 kgVS/m^3day could be reached three times faster at stable fermentation. Single-stage mono-digestion of OMSW proved not to be useful due to long adaptation time of the inoculum at start-up and severe process instabilities. Co-digestion with chicken manure was performed as another possible practical way to overcome the limitations. However, co-digestion of substrates derived from different agro-industrial activities (i.e. olive oil production and poultry farming) may cause additional logistic problems such as substrate transport and storage. Despite some

attempts to identify potential other reasons, it must be generally confirmed that pure solid waste from olive oil production exhibits inhibition of the AD process through its high polyphenol content. Even after long-term operation of the processes efficient conversion was only achieved at low OLRs of 1.56 kgVS/m^{3}day, and a high HRT of 150 days, which require proportionally high reactor volumes. For practical implementation further research seems to be necessary to reduce the necessary HRT and to improve process economics.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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ORIGINAL PAPER

Enhanced Separation of the Organic Fraction from Paper Mill Effluent for Energy Recovery

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Abstract In this study pre-treatment of pulp and paper mill sludge was investigated to enhance the energy recovery by anaerobic digestion. Two approaches were followed using hydrolytic enzymes or microbiological treatment at 30 °C. In the first attempt, anaerobic digestion of the whole sludge, no significant improvement of the methane production potential was found. In the second test series only the liquid phase after pre-treatment and solids separation was anaerobically degraded. This option provided up to ten times increased methane production compared to the untreated sample. Sludge mass reduction between 6 and 13 % was achieved after pre-treatment. Moreover this concept can be easily integrated in the established wastewater treatment scheme utilizing an existing upflow anaerobic sludge blanket reactor.

Keywords Pulp and paper mill sludge · Enzymatic pre-treatment · Anaerobic digestion · Biogas · Pre-acidification · Sludge dewatarability · Recycled fiber

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Introduction

Pulp and paper production consumes high amounts of energy and water. In Europe 789 mills produce yearly 92.1 Mt paper and board, 27 % of the world production (398.9 Mt), 71.7 % of which is recycled paper [1]. On average the specific energy consumption is 13 MWh/kt product and the wastewater contains 6.2 kg chemical oxygen demand (COD) per ton of product [1]. In the last decades the paper industry has conducted significant effort to reduce their environmental burden. The amount of water necessary for production of 1 ton paper decreased from 500 m^3 in the early years of the paper industry to 50 m^3 and even 15 m^3 in the last years [2]. This progress was driven by tighter legislative regulation such as the EU Directive 2010/75/EU on industrial emissions [3], which addresses paper mill plants producing paper more than 20 tons per day. It stipulates the necessity for biological stabilization of the organic waste fraction taking into account the possible reuse of energy and closed water circuits [4].

Anaerobic digestion (AD), followed by aerobic post-treatment can be considered as state of the art waste treatment technology in the paper mill factories. Recent studies [5] underpin that UASB reactor followed by conventional activated sludge process is the most economically feasible process for these high strength effluents (COD 10,000–5000 mg/L). The advantages of the AD treatment are the low excess sludge production, no need for aeration energy, low nutrient demand, compact installations, and last but not least the production of renewable bioenergy from the biogas. The aerobic post-treatment serves to reduce the concentration of recalcitrant compounds, like resins, which have not been degraded during

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the AD and to meet effluent quality criteria. Until 2007 over 200 of such installations have been reported [4].

Nevertheless one significant problem remains, the high sludge production during the wastewater treatment process. The majority of waste sludge generated from paper production and recycling derives from primary sedimentation. This by-product amounts up to 23 % of the produced paper, the exact quantity depending on paper production process [6]. Primary sludge constitutes a mixture of short cellulosic fibers and inorganic fillers, such as calcium carbonate, china clay, and residual chemicals dissolved in the water [7]. The sludge characteristics vary subject to the milling technology. According to Kyllönen et al. [8], fiber and ash may account between 40 and 90 % and 5–60 % of the solid matter, respectively. Also organic halogens, dyes, phenolic compounds and resins can be contained in the sludge [9].

The dominating sludge management strategies in paper industry are (i) mechanical dewatering followed by composting in order to make material for soil amendment or covering of landfills and (ii) mechanical dewatering and incineration with deposition of the ashes on landfill, or (iii) co-incineration in cement industry or utilization in brickstone production including re-use of the inorganic fraction in the product [7]. Another means of treatment including energy recovery is AD of the sludge. Although this issue has been addressed in several studies [10], sludge processing by AD in paper industry is still a developing technology due to the low methane production potential of the cellulosic material.

The bioconversion of the paper mill sludge has its advantages and deserves deeper research of improving the availability of the organic content for AD. Several studies have proved the increase in methane production of sewage sludge and complex organic matter by using crude and commercial enzymes [11]. These resulted in improved solubilisation, anaerobic digestibility, dewatering and hygenisation. The use of cellulolytic enzymes for treatment of pulp and paper sludges for improved ethanol production have been reported before [12–14]. Therefore the positive effect of cellulolytic enzymes for accelerating the solubilisation of the organic fraction in the paper mill sludge is indisputable. However there are scarce investigations on its potential for improved AD and sludge mass reduction in an existing paper mill plant.

The aim of the study is to explore the options for the pre-treatment, enzymatic or microbiological, to estimate the enhance of the liquefaction of the organic fraction of paper mill sludge for increased anaerobic digestibility and biogas production on the one side, and the reduction of the sludge mass on the other side.

Materials and Methods

Currently Established Process

The study was conducted at a wastewater treatment plant receiving the effluents from a local paper mill treating 160,000 tons recycled paper and producing 130,000 tons paper per year. The generated annual wastewater amount is 3.70 Mio. m^3 ($\sim$10,000 m^3 per day), which is treated as follows: first, the primary sludge is sedimented in a settling tank. The liquid phase is fed to a pre-acidification tank followed by UASB reactor for further anaerobic digestion. Subsequently the effluent is mixed with wastewater from the local municipality and treated in a standard aerobic activated sludge treatment system. The settled primary sludge is thickened and dewatered with a belt filter, and finally in a decanter centrifuge. The sludge water is added to the inflow of the pre-acidification tank. The solid residues with a total solids (TS) content of around 57 % are delivered to a local cement factory. An overview of the wastewater treatment process is illustrated in Fig. 1.

Substrate and Enzymes

The sludge used in this study is the solid fraction after the filter belt press, obtained after paper production from 75 % recycled paper and 25 % fiber from chemical pulping. The material was stored for 4 days (time between delivery and start of the experiment) in tight closed vessels at 4 °C until use. Table 1 gives an overview of the substrate parameters. Two different commercial enzyme products, Petrozym BG-M and Petrozym BG-M1, further termed enzyme 1 and enzyme 2, were used. They contain an enzymatic cocktail derived from *Trichoderma reesei*, an industrially important cellulolytic filamentous fungus. With respect to cellulose degradation the enzyme product comprises a mixture of three major enzyme classes: (i) endoglucanases randomly cutting within the cellulose chain (ii) exoglucanases, in the case of *T. reesei* cellobiohydrolases, which liberate the D-glucose dimer cellobiose consecutively from the ends of the cellulose chain, and (iii) glucosidases which release D-glucose from the soluble oligomeric breakdown products. In enzyme 1 the glucosidase activity is higher whereas in enzyme 2 the focus is on endoglucanase activity. Following the producers suggestions the incubation temperature was 30 °C, optimum pH 6.0, and amount of enzyme product added was 1000 ppm.

For the microbiological treatment and for the BMP tests was used the same inoculum, described in the following section.

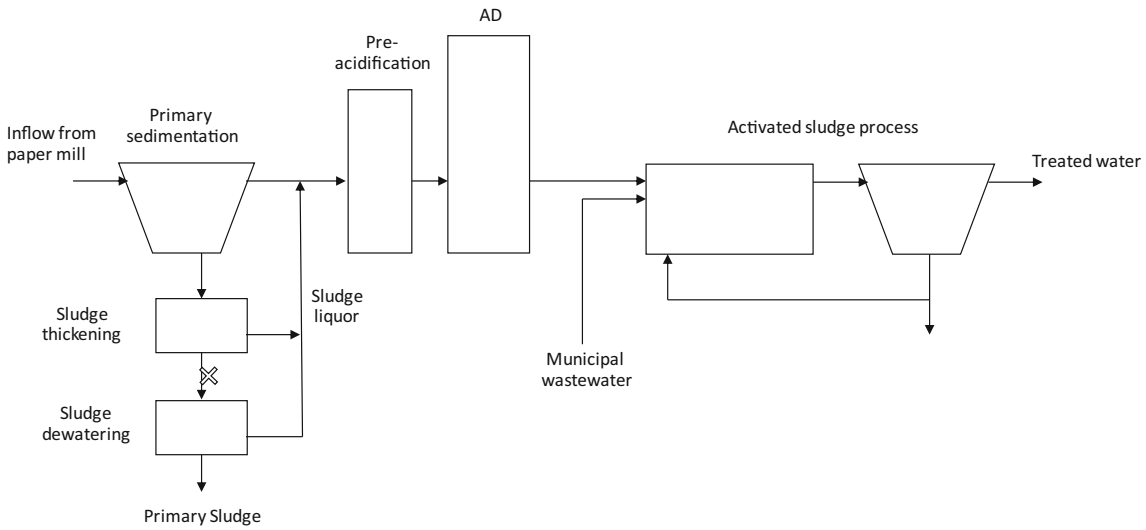

Fig. 1 Scheme of the established waste water treatment process (a *cross marks* the origin of the investigated sludge sample)

Table 1 Characteristics of paper mill sludge and of the inoculum for microbiological pre-treatment

Parameter	Paper mill sludge	Inoculum
pH	6.64	7.6
TS (% fresh weight)	17.10	2.46
VS (% fresh weight)	5.91	1.45
COD (g/kg)	117.15	19.3
TKN (g/kg)	0.95	2.01
NH_4^+–N (g/kg)	0.03	1.19

Test Set-up

The influence of the pre-treatment of paper mill sludge (PMS) with hydrolytic enzymes on the methane production was determined in two consecutive tests. All tests were carried out in triplicates and under non-sterile conditions.

Pre-Treatment and AD of the Whole Sludge

In the first investigations, PMS was incubated with the two different enzyme products (1000 ppm) at 30 °C for 9 days. The experiment was carried out in triplicates in 250 mL tight closed bottles containing 85 g unsterile sludge. A microbiological pre-treatment with inoculum in ratio 1:1 (w/w), simulating the first stage of two-stage anaerobic digestion was carried out in parallel at the same conditions. Daily pH was measured, and 0.2 g samples for determination of sugars, volatile fatty acids (VFA) and alcohols were taken, whereat the measured data were corrected by mass loss. At the end of the incubation the biochemical methane potential (BMP) of the pre-treated PMS was determined. The BMP assays were accomplished at 35 °C, according to VDI 4630, DIN 38 414-S6 [15]. The inoculum was a 1:1 (w/w) mixture from two different sources: mesophilic AD fermenter, digesting thin stillage from bio-ethanol production and local biogas plant, digesting agricultural waste both at mesophilic conditions (Table 1). These both sources were used for inoculum in order to obtain broad range of adapted microorganism consortium. The inoculum was stored at 35 °C at anaerobic conditions for 1 week before the start of the experiment. According to VDI recommendations, the ratio between the VS (volatile solids) of the substrate and the VS of the inoculum was set to ~0.5 (corresponding to a mass ratio of 1:2). BMP tests including not pre-treated PMS (stored at 4 °C) and a blind value for the inoculum were also carried out in triplicate and the values were used for correction of the results of the pre-treatment and the BMP tests, respectively. The provided methane yields are given per ton volatile solids (VS) and fresh mass (FM), respectively, and refer to the initial VS or FM of the PMS at the beginning of the pre-treatment.

Pre-Treatment and AD of Liquid Fraction

In the second test series the sludge was pretreated at similar experimental conditions as during the first test. Based on the previous results from the experiment described in 2.3.1, three different incubation periods were chosen: 2, 4 and 8 days. At the end of the incubation the samples were centrifuged for 15 min at 2000 rpm. The liquid fraction was decanted and weighed to allow calculations of mass balance. In the supernatant the sugars, VFAs, alcohols, and pH of the liquid phase were measured. BMP of the supernatant was determined using the same method as described in Sect. 2.3.1. In this test the mass ratio of the

sample (liquid fraction after centrifugation) to inoculum was 1:1 (w/w).

To allow direct comparison to the data of the first test series, the methane yields were related to the VS present in the original sludge sample taking into account that only the liquid phase after centrifugation is used in the batch test. The used calculation is expressed by the following formula:

$$\text{BMP}\ [\text{ml/gVS}] = \frac{(\text{Methane}[\text{ml}] - \text{BV}[\text{ml}]) * \left(1 - \text{TS}_{\text{sludge}}[\%]\right)}{\text{weight of liquid phase used for BMP test}\ [\text{mg}] * \text{VS}_{\text{sludge}}[\%]}$$

BV Blind value of BMP test, *TS*, $VS_{sludge\ sample}$ TS, VS measured in the original sludge sample.

Analytical Methods

Standard Parameters

General parameters were determined, according to standard methods [16]: chemical oxygen demand (COD)—APHA5220B, total Kjeldahl nitrogen (TKN) and ammonium nitrogen (NH_4^+–N)—APHA4500B, TS and VS—2540B. The pH value was measured with a WTW pH330i electrode SenTix 81.

Analyses of Dissolved Components

HPLC measurements were conducted on a Hewlett Packard chromatograph, Series 1100, equipped with Agilent 1100/1200 isocratic pump and refractive index detector with an optical unit temperature of 45 °C. Sugar di-/monomers: cellobiose, glucose, lactose, xylose, galactose, rhamnose, arabinose, fructose; VFAs: lactic acid, formic acid, acetic acid, propionic acid, iso-butyric acid, butyric acid, iso-valeric acid, and valeric acid, and alcohols: 1,2-propanediol, 1,3-propanediol, ethanol, 2-propanol, 1-propanol, were separated and analyzed by ICSep ICE-ION-300 column (Transgenomic) at 40 °C. The mobile phase was a 0.01 N H_2SO_4 solution. The flow rate was 0.325 ml/minute with a running time of 120 min. The concentration of the products was quantified using Agilent ChemStation software, Rev. B.01.03 [204] (Agilent Technologies) and external calibration curves.

Results and Discussion

Enzymatic Pre-Treatment and BMP of the Whole PMS

The course of the concentrations of the sugar di-/monomers during the enzymatic pre-treatment with the two enzymes, and during the microbiological pre-treatment are shown in Fig. 2. These liquefied sugars represent easy bio-available substrate released from the paper fibers. The concentration of sugars (Fig. 2a) increased rapidly already after 24 h incubation time. For enzyme 1 this was 35 % of the maximum amount of released sugar monomers, in the assay with enzyme 2 it was 77 %, respectively. In both cases the concentration reached its maximum after 120 h, and subsequently the concentration decreased. The assay with enzyme 1 reached around 1.8 times higher concentrations of released sugar di-/monomers, compared to the assay with enzyme 2. It should be mentioned, that during the incubation the pH remained within the optimum range for the enzymes without additional adjustment (pH 5.8–6.6). This strongly simplifies practical implementation of the process and avoids additional costs for chemicals.

In contrast to the observations before, during microbiological pre-treatment the concentration of the sugars remained low throughout the whole incubation period.

The change in VFA concentration completed the picture of the progress of the pre-treatment (Fig. 2b). These compounds result from further microbial conversion of the sugars in the unsterile samples. The VFA concentrations increased during the whole incubation time. In the assays with enzyme 1 and 2 their amount was very similar. In the test with microbiological pre-treatment the concentration starts to increase rapidly only after 48 h, which was obviously the lag phase for the microorganisms to adapt to the substrate. After 120 h incubation time the concentration in all of the three assays matched up to each other.

In Fig. 2c the sum of all soluble compounds identified in the samples are presented. The course of the formation of soluble compounds was relatively similar in the two assays with enzymes whereas the microbiological pre-treatment significantly lacked behind. At the end of the incubation period, after which the BMP assays were conducted, the yield of the released monomers during the microbiological pre-treatment was 15 % lower. This difference is also reflected by a slightly lower methane production (Table 2). However, despite the measured release of soluble components, the general impact on total methane production was low. The specific methane production in the three assays and in the control (untreated sludge) was more or less similar—on average 185 Nm^3/t VS (Table 2). Also the time course of methane production in the BMP tests was relatively similar.

According to these results, AD of the sludge was not improved by the investigated pre-treatment methods and no increase of the methane yield was achieved.

Obviously the microbial consortium, present in the AD batch tests is capable to perform the liquefaction of the cellulose and other insoluble carbohydrates by itself. The BMP results of the untreated sludge fit to data published

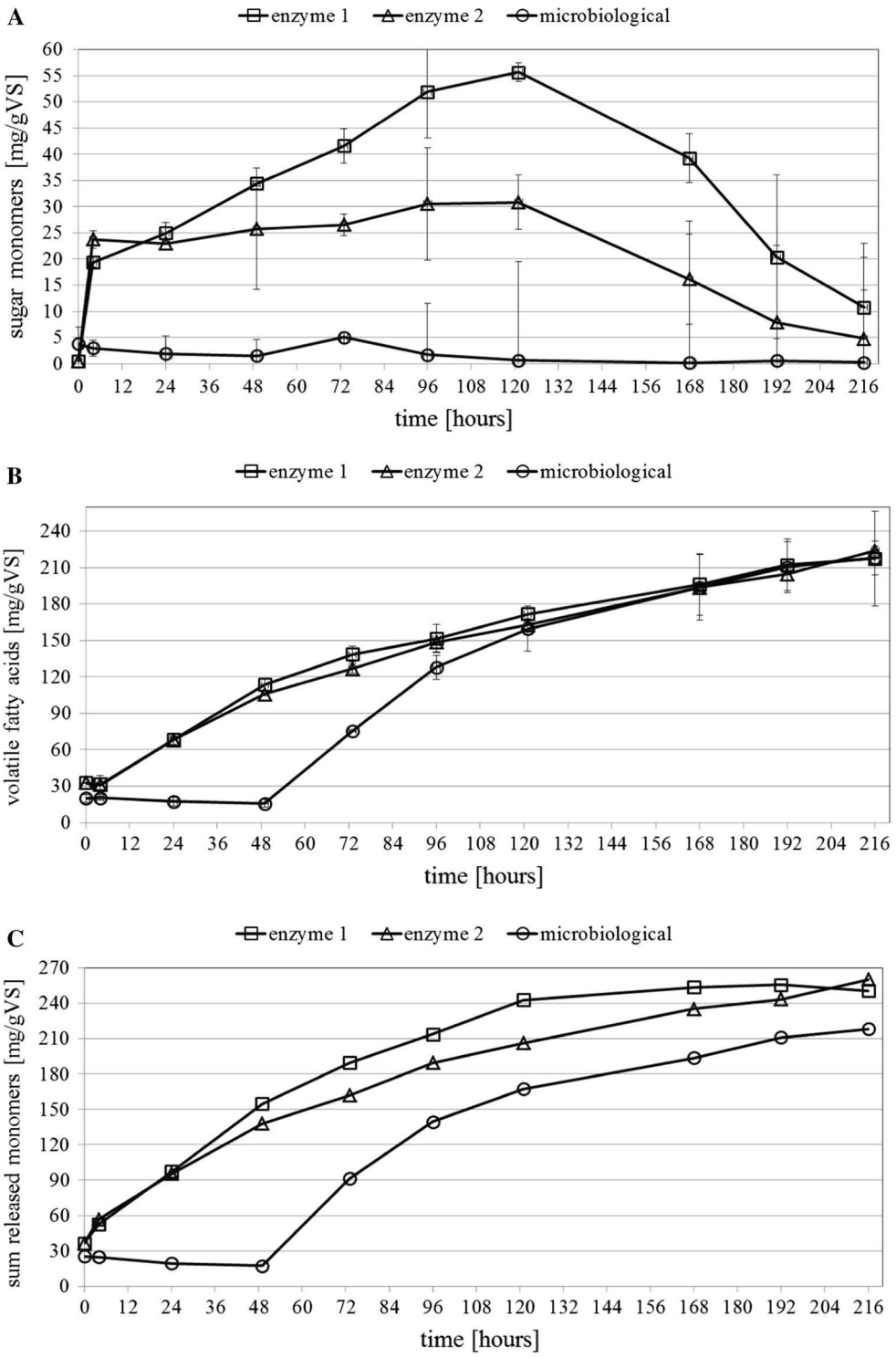

Fig. 2 Changes in the concentration of the sugar di-/monomers (**a**), volatile fatty acids (**b**) and sum of soluble compounds (**c**) during the enzymatic and microbiological pre-treatment of paper mill waste at 30 °C

elsewhere considering that biogas yield from PMS underlies a certain variation depending on the pulping technique. These studies report methane yields from mesophilic AD of whole sludge between 120 and 180 Nm^3/kg VS [17, 18]. Bayr and Rintala [19] determined a little higher methane potential of pulp and paper mill in batch experiments which were 210 and 230 m^3CH_4/t VS at mesophilic and thermophilic conditions, respectively. In continuous AD experiments they achieved methane yields of 190–240 m^3CH_4/t VS_{fed} at organic loading rates (OLR) of 1.0–1.4 kgVS/m^3d. Another study [20] reported a specific biogas production of 380 m^3/t VS at mesophilic conditions

Table 2 Specific methane production of PMS after enzymatic and microbiological pre-treatment at 30 °C for 9 days

	Specific methane production	
	Nm^3/t VS	Nm^3/t FM
Pre-treatment with enzyme 1	190.5 ± 29.0	11.2 ± 1.6
Pre-treatment with enzyme 2	187.3 ± 26.9	11.1 ± 1.7
Microbiological pre-treatment	170.8 ± 18.6	10.1 ± 0.6
Not pre-treated	193.3 ± 18.3	11.4 ± 1.1

and OLR 2.5 kg VS/m^3d, and 130 m^3/t VS at OLR 1.0 kg/m^3d at thermophilic conditions.

With respect to enzymatic pre-treatment of PMS there are scarce literature sources, although enzymes have been applied e.g. to support sludge digestion in municipal wastewater treatment. First discussion about such an approach in pulp and paper industry was published in 1988 [21]. Our findings confirmed recent results of Karlsson et al. [22]. They conclude that pre-treatment by hydrolytic enzymes (a mixture of cellulases, proteases and lipases) to enhance the bioavailability of the organic fraction in PMS did not provide considerable positive effect on biogas production at realistic enzyme concentration levels.

Beside enzymes, other pre-treatment technologies have been suggested to enhance the biodegradability of the recalcitrant organic substances. Microwave, ultrasonic and chemo-mechanical pre-treatment of paper mill waste has been reported [23]. It was demonstrated that microwave pre-treatment at 175 °C increased the methane yield by 90 %, compared to the control in 21 days BMP tests. However, it is also stated that the energy input–output ratio was negative. The chemo-mechanical and the ultrasound pre-treatment were less effective but delivered excess energy of 386 and 1366 kWh/t TS, respectively.

In general, it must be summarized that in our study AD of the whole sludge was not considered a viable option. The observed BMP potential is only around half of the BMP measured for pure cellulose in different degradation test, 345–404 ml/gVS [24], and the investigated pre-treatment methods did not improve methane yields. It also needs to be kept in mind that UASB reactors are not capable to handle sludges with high solid content. Therefore AD of the whole sludge can not be performed in the existing reactor. This implies the construction of a complete new AD process which is economically hardly justified.

Liquefaction and BMP Tests of the Organic Fraction of PMS

The second approach followed the idea to implement an intermediary sludge treatment step after the filter belt. In the final dewatering the liquefied compounds are separated from the solids. Subsequently these compounds are returned together with the sludge liquor to the existing treatment process and conversion to methane takes place in the UASB reactor. Based on the release of soluble compounds during the first experiment, three incubation periods were chosen—48, 96 and 192 h. The reason was the following: on the one hand, the incubation should yield reasonable amounts of liquified compounds; on the other hand, the necessary size of the required extra incubation basin should not be too large.

In this new set of experiments analyses of solubles was only made at the end of the incubation. The results fit well together with the previous experiments. The detailed data are presented in Fig. 3a–c. In summary, after 48 h incubation with enzyme 1 the sum of concentrations of soluble componunds (Fig. 3c) reaches 41 % of the final concentration (192 h) and 59 % after 4 days. In the assay with enzyme 2 the corresponding values were 49 and 65 %, respectively, but the total amount released was lower. In the assay with microbiological pre-treatment, as observed before, no sugar mono-/dimers which are easily convertible were found (Fig. 3a). According to the results presented in Fig. 3c, within 48 h only 19 % of the soluble compounds obtained at the end of the experiment were released. After a twice as long incubation time, 96 h, the concentration increased to 49 %. Despite good progress of solubilization in the last phase of the incubation period, the final concentration was the lowest of the three pre-treatment methods tested.

In accordance to the chosen approach, the BMP was not determined from the complete sample, but from the supernatant after solids removal through centrifugation. As shown in Fig. 4, the specific methane production increased with the increasing incubation time corresponding to the rate of monomers release. Applying the longest pre-treatment period (192 h), in the assay with enzyme 2 the methane production was approximately 12 % lower compared to enzyme 1. Interestingly, the microbiological pre-treatment yielded the highest methane productivity. This points out that the microbial attack generally helps to weaken the cellulosic structure making it more accessible to subsequent methanization. It should also be noted that the applied analyses of soluble compounds is only an indicator for the degree of solubilization and that e.g. cellulose oligomers are not measured. As expected, the not-treated sample delivered the lowest methane production (11 Nm^3/tVS), which is around 10 % of the value obtained for microbiological pre-treatment.

The sludge dewatarability was used as an indicator for the mass loss. It was calculated after the results at the applied centrifugation in this study. The not treated PMSW achieved 42 % mass lost. After 48 h incubation the mass

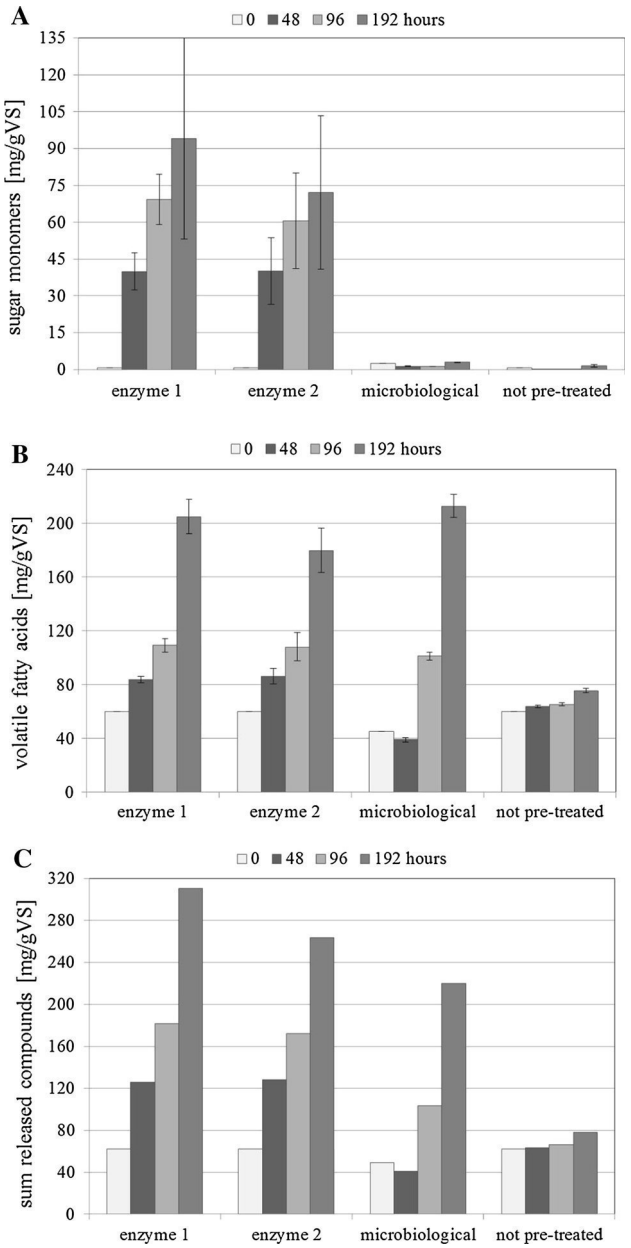

Fig. 3 Released sugars (**a**), VFAs (**b**) and soluble compounds (**c**) after 0, 48, 95 and 192 h incubation of paper mill waste at 30 °C

loss increased up to 48 ± 1 % for the not treated and the enzyme treated sludge (Fig. 5). While the dewatarability of the not treated PMSW remained stable, the mass loos after enzymatic pre-treatment increased further to 55 ± 2 % after 96 h incubation. The dewatarability during the microbiological pre-treatment was the highest. These results fit to the changes in the BMP (Fig. 4), which means that the increased centrifugation efficiency after sludge treatment leads to decreased sludge amount (6–13 % mass reduction of the sludge after enzymatic pre-treatment and 17–22 % after microbiological pre-treatment), and

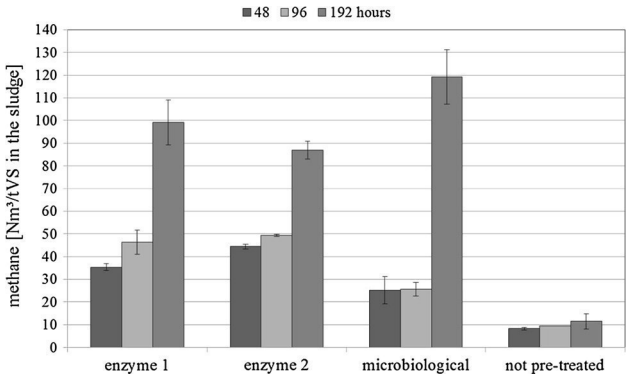

Fig. 4 BMP of the liquid phase after pre-treatment of the paper mill sludge for 48, 96 and 192 h, respectively (BMP is expressed as methane yield per VS in the sludge sample)

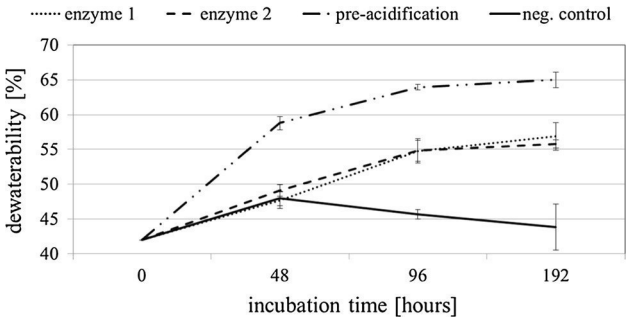

Fig. 5 Dewatarability (weight %) of the PMSW before treatment (0 h) and after incubation for 48, 96 and 192 h, respectively

increased amount of organic substances in the liquid fraction. These results can vary at the centrifugation conditions at industrial scale, but with the same tendency.

Options for Full Scale Implementation

Comparing the best values obtained in the two approaches, it is evident that AD of the liquid phase after solids removal provides less methane potential than digestion of the whole sludge, approx. only 60 % at the best conditions. This confirms that during AD of the sludge not only the solubilized compounds are converted, but that cellulosic compounds undergo further degradation by the exoenzymes released by the anaerobic microbial consortium. Nevertheless, and as mentioned before, the second approach is by far easier to accomplish and to implement into the existing wastewater treatment scheme. It requires only an additional tank for incubation and a few pump lines. For solid–liquid separation the existing sludge dewatering step can be used. Therefore, from the economic point of view, it is the much more favorable process. It is worth to look at the development of methane yield with incubation time. The obvious background is that the incubation time determines the

Table 3 Necessary tank volume required for pre-treatment at different incubation periods, predicted biogas gain and methane productivity per tank size installed

Pre-treatment duration (d)	2	4	8
Required tank volume (m^3)	1400	2800	5600
Methane yield (m^3/d)			
Enzyme 1	1261	1655	3538
Enzyme 2	1588	1763	3104
Microbiological pre-treatment	896	913	4253
Volumetric productivity (m^3 methane/m^3d)			
Enzyme 1	0.90	0.59	0.63
Enzyme 2	1.13	0.63	0.55
Microbiological pre-treatment	0.64	0.33	0.76

Data calculated on the basis of the current daily sludge production at the treatment plant (700 m^3/d)

size of the necessary additional pre-treatment tank and hence the investment costs. The current production of wet sludge is approximately 700 m^3 per day (TS 17 %) which finally yields 200 t of dewatered sludge (TS 60 %). Based on the daily production of wet sludge the considerations presented in Table 3 can be made. For the different incubation periods Table 3 presents the tank volume required for pre-treatment, the predicted biogas gain and the productivity expressed as methane production per m^3 tank size installed. For the underlying calculation it was taken into account that the amount of liquid fraction that can be supplied to the UASB reactor depends on the efficiency of solid separation after sludge incubation. It was presumed that separation efficiency is the same as in the currently implemented sludge dewatering step (TS increase from 17 to 60 %).

The highest efficiency per volume is obtained with enzymatic treatment, in particular enzyme 2, applying an incubation time of 2 days. However, on the long run, i.e. at 8 days incubation, the microbiological pre-treatment is gaining better results in terms of total biomethane potential. Both options have certain merits. In the specific case, a short retention time allows the use of an already existing tank of adequate volume. Using enzymatic additives, a highly reasonable level of solubilization can be obtained. On the other hand, a longer retention time allows a more quantitative extraction of biomethanizable compounds, and microbiological pre-treatment can be applied. However, the later choice involves not only a larger incubation tank but it probably needs to be equipped with more installations, e.g. the temperature of the sludge after primary sedimentation is at a convenient level of approximately 35–40 °C, but a proper insulation is necessary to maintain the elevated temperature for an extended period. To mention again, in our experiments the incubation temperature was 30 °C and, as confirmed in other studies, the activity of the cellulases exhibit a strong dependence on temperature [25, 26]. It is also presumed that for longer incubation closed tanks are necessary due to gaseous emissions, caused by the intense formation of odorous organic acids, as well as the production of small amounts of methane.

Currently around 4000 m^3 biogas (80 % methane) per day are derived from the existing UASB reactor. A look at the figures in Table 3 shows that the additional methane potential is considerable and can boost renewable energy production from the waste products generated at the investigated wastewater treatment site. Further pilot investigations are foreseen to work out the optimum settings for full scale implementation.

Conclusions

Two options to yield the inherent energy of paper mill sludge by means of AD after enzymatic/microbiological pre-treatment were investigated. The first approach, AD of the whole sludge after pre-treatment, was not successful. No significant enhancement of the biomethane production was observed by the applied pre-treatment methods. The measured methane yield of $\sim$11 Nm^3/t sludge is only half of the theoretical potential and the necessity to establish an appropriate digester plant makes this approach economically unattractive.

The second option, pre-treatment of sludge and supply of the liquid phase to the existing anaerobic reactor (UASB), provided highly promising results. The pre-treatment led to 6–13 % mass reduction of the sludge after enzymatic pre-treatment and 17–22 % after microbiological pre-treatment. Such a concept can be easily integrated in the existing wastewater treatment scheme. According to the data obtained, the implementation of such a process can substantially increase the biogas production.

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Conference proceedings

Poster 1

Influence of the Pre-acidification on the Anaerobic Digestion of Olive Solid Waste
Stoyanova, E., Vasilieva V., Bochmann G., 2011. International Symposium on Anaerobic Digestion of Solid Waste and Energy Crops, August 28 – September 01, Vienna , Austria, p.133

Poster 2

Two-Stage Anaerobic Digestion of Sugar Beet Pressed Pulp – Optimizing of Reactor Performance
Stoyanova, E., Forsthuber, B., Pohn, S., Schwarz, C., Fuchs, W., Bochmann, G., 2013. IWA-11874, 13th World Congress on Anaerobic Digestion, June 25-28, Santiago de Compostela, Spain, p. 165

Influence of the Pre-acidification on the Anaerobic Digestion of Olive Solid Waste

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Abstract
The biogas production from olive pomace was investigated by comparing one- and two-stage anaerobic digestion process by semi-continuous fermentation in CSTR with 1,8L working volume at maesophilic conditions (37ºC). The pre-acidification step of the two-stage fermentation was carried out at 55 ºC. A mixture from a local biogas plant and a wastewater treatment plant was used as inoculum. The fermentation was started at high HRT – 180 days. The organic loading rate was kept low due to the fast drop in the pH. The COD removal was 90-95% and no VFA accumulation was observed. The adaptation of the microorganisms to the substrate and further to the inhibiting compounds like polyphenols occurred faster in the two-stage process. The OLR could be increased twice after pre-acidification after 50 days compared to the one-stage anaerobic digestion – after 100 days. The biogas production was 384 Nm^3/t oDM at the one-stage digestion and 275 Nm^3/t oDM at the two-stage fermentation wit methane content between 60 – 65%. The specific methane production was determined to be 312 Nm^3/t oDM by means of batch test.
Keywords
Anaerobic digestion, hydrolysis, olive pomace, pre-acidification, polyphenols

INTRODUCTION

The olive oil production in the EU-27 increased from 1,871,000 MT in year 2000 to 2,350,000 MT in year 2011 (United States Department of Agriculture 2011). The emerging olive mill waste contains aqueous and solid phase. The high amount of waste with high chemical oxygen demand (COD) value and low pH causes serious environmental problems and should be pre-treated before disposal on the landfills. The handling of the liquid phase has been studied widely (Roig *et al*. 2006). However, the anaerobic digestion of the olive mill solid waste (pomace) has been poorly investigated yet (Tekin *et al*. 2000). The fermentation can minimize the environmental impact of the solid olive waste and is an appropriate method for sustainable use of the organic waste by producing biogas that can be used for energy production and environmentally friendly fertilizer.

MATERIALS AND METHODS

Substrate

Two and a half phases olive mill solid waste, hereafter called pomace, was obtained from an olive processing plant in Italy and was stored at 4ºC before use. The substrate characteristics are summarised in Table 1.

Table 1. Olive pomace – substrate characterisation

pH (20 ºC)	4.53
dry matter [%]	23.92
organic dry matter [%]	22.93
chemical oxygen demand [g/kg]	289.24
total Kjeldal nitrogen [g/kg]	3.43
NH_4-N [g/kg]	0.26

For the two-stage anaerobic digestion, a pre-acidification step was carried out at 55ºC with a HRT of 2 days, hereafter called hydrolysate. To prepare hydrolysate during the whole fermentation, inoculum from the methane phase of the two-stage reactor was used. The hydrolysate analyses are shown in Table 2.

Table 2. Olive pomace hydrolysate – substrate characterisation

pH (40°C)	4.2 – 4.6
dry matter [%]	14.91
organic dry matter [%]	13.95
chemical oxygen demand [g/kg]	247.31
total Kjeldahl nitrogen [g/kg]	2.80
ammonium nitrogen [g/kg]	0.52

A mixture from a local biogas plant and a wastewater treatment plant was used as inoculum. It was stored at 35°C.

Experimental setup

Two continuous stirred tank reactors (CSTR) with working volume of 1,8 l were incubated at mesophilic conditions (37 ± 1°C) and stirred at 400 rpm with a magnetic stirrer. The fermentation was semi-continuous; a sample for analysis was taken once a week. The pH, temperature and gas production were measured daily, chemical oxygen demand (COD), dry matter (DM), volatile fatty acids (VFA) and gas composition, weekly and total Kjeldahl nitrogen (TKN) and free ammonia, monthly. The reactors were fed once a day.

Methods of analysis

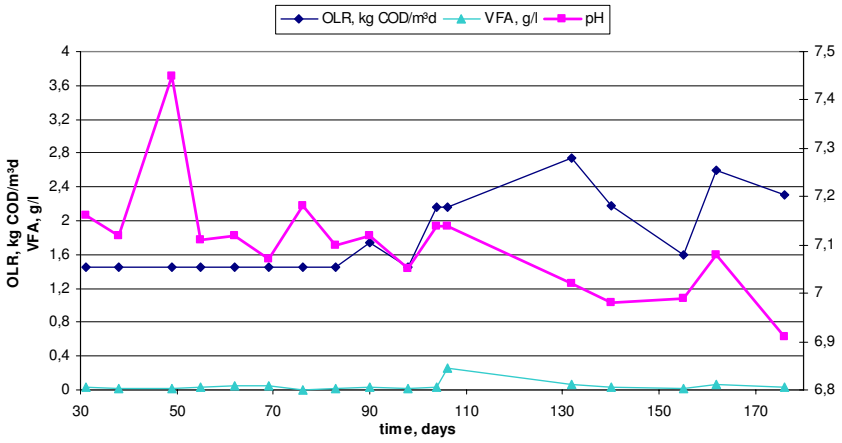
The gas production was measured with a Milligascounter®. The gas composition was determined using an AWITE® TRM816 apparatus. TKN and free ammonia were determined by BÜCHI® apparatus. COD was analysed according to DIN 38414 H41-1 (APHA). VFA were measured by high performance liquid chromatography refractive index detector. For obtaining first insights into the difference in the composition of the hydrolysate and the reactors and to make conclusions for the presence of potential inhibitors, GC²/MS analysis with an Agilent 6890N gas chromatograph (Agilent, Wilmington, US), a 7683D series split/splitless auto-injector, two capillary gas chromatography columns (HP-5MS from Agilent Technologies, Vienna, Austria; 30 m length, 0.25 mm I.D., and 0.25 µm film thickness and SGE BPX50 from SGE GmbH, Germany; 0.8 m length, 0.1 mm I.D., 0.1 µm film thicknesses) connected with a loop jet modulator (Zoex Corporation, Lincoln, NB) and a quadrupole mass spectrometer (Agilent Technologies 5975B inert XL MSD) was carried out.

RESULTS

Operational parameters: VFA, pH and organic loading rate (OLR)

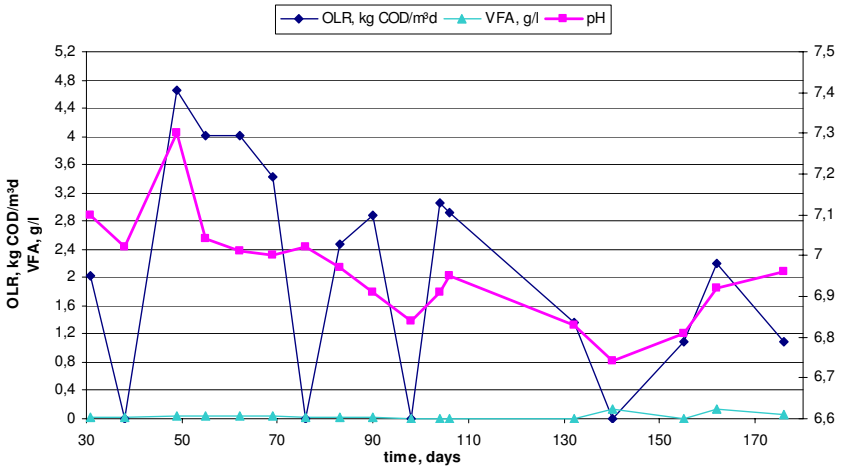
One-stage fermenter. The OLR was low at the beginning of the experiment (1.45 kg COD/m³d) and could be increased twice after 100 days (Figure 1). In the first 30 days the system was very instable despite the HRT of 150 days, and a regular feed was not possible (data not shown). The VFA concentration was below 0.4 g/l and the COD removal was between 90 and 95%. The pH was between 7.0 and 7.2 and dropped fast when the HRT was decreased.

Figure 1. Process parameters VFA, pH and OLR of the one-stage anaerobic digestion fermentation of olive pomace



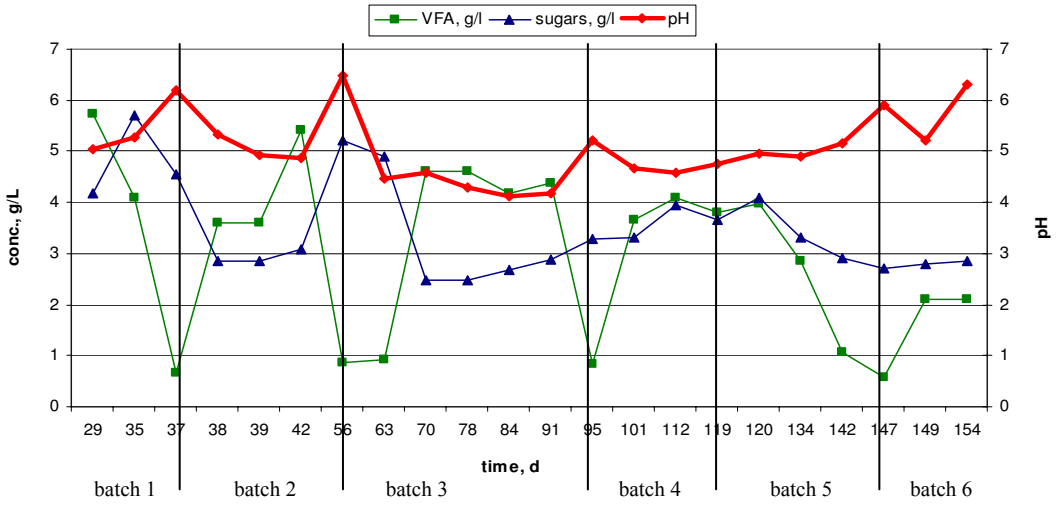
Two-stage fermenter. The OLR was low at the beginning – 2.03 kg COD/m³d and could be doubled after 50 days (Figure 2). The first 30 days the system was not stable and a regular feed was not possible (data not shown). The VFA were degraded entirely, concentration above 0.4 g/l. The pH dropped to 6.8 with decreasing the HRT from 150 days at the beginning at 60 days on the 50th day.

Figure 2. Process parameters VFA, pH and OLR of the two-stage anaerobic digestion fermentation of olive pomace (methane fermenter fed with pre-acidified substrate)



Pre-acidification step. This step was carried out batch-wise at 55°C with a 1:1 ratio of inoculum to fresh olive pulp. The pH was measured daily and the VFA, sugars and alcohols, weekly. Figure 3 shows the progress of these parameters. The pH ranged between 4.2 and 6.4. The values correlated directly with the VFA concentration (2-4 g/l). The variation of the pH correlates with the recirculation times for obtaining hydrolysate. The sugar concentration varies between 2 and 4 g/l.

Figure 3. Pre-acidification of olive pomace at 55°C, pH, sugars and VFA progress



TKN and free ammonia

TKN and free ammonia were measured monthly, to determine if they are present in bacteria inhibiting concentrations (Table 3).

Table 3. TKN and NH_4 – N concentration during the one- and two-stage fermentation of olive pomace

	TKN	NH_4 – N
	(g/kg)	(g/kg)
one-stage fermenter	2.3-2.6	1.2-1.8
two-stage fermenter	0.7-1.2	0.5-0.9

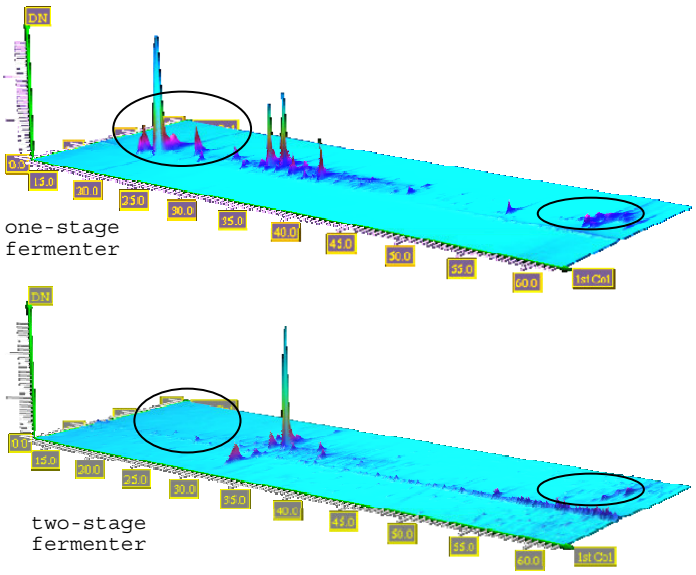
Biogas production and composition

The biogas yield was measured daily. The biogas production of the one-stage fermenter was 384 Nm^3/t oDM and 292 Nm^3/t oDM from the two-stage fermenter. The methane content of the biogas was between 60 and 65%. The specific methane production was determined to be 312 Nm^3/t oDM by means of batch test.

GC²/MS analysis of the fermenter content

Gas chromatographic analyses were made for obtaining first insight into the difference between the fermenter substances (Figure 4).

Figure 4. GC²/MS analysis of the one-stage and two-stage fermenter content from anaerobic digestion of olive pomace



Extraction of polar substances with dichlormethane was made. The peaks observed in Figure 4 were automatically determined by software GC Image™ © 2001–2010 by GC Image, LLC (match above 95%). The peaks, with retention time between 19 and 28 minutes, were determined to be 3-ethylphenol, 3-propylphenol, 2,4-bis-(1,1-dimethylethyl)-phenol. The peaks, with retention time 52 minutes, were referred as squalene and, at 58 minutes retention time, as sigmasterol and sitosterol. In these two areas, differences in the amount of the substances can be observed. The peaks between 30 and 35 minutes retention time were detected as the long chain aldehydes tetradecanal and octadecanal.

DISCUSSION

The first insight into the influence of pre-acidification on the anaerobic digestion of olive pomace showed that the difficulties in the anaerobic digestion which occur due the substrate content can be overcome by applying two-stage fermentation. The separation of the four phases of the anaerobic digestion assures optimal conditions for the hydrolysing and acidifying bacteria on the one hand, and for the methanogenic bacteria on the other hand. The hydrolysate obtained from the pre-acidification step has pH between 4 and 6, which is necessary for the hydrolysing bacteria (Rincón *et al*. 2006). The analysis of the pre-acidification step showed accumulation of sugars (2-4 g/l) during the whole batch incubation and low VFA concentration (2-4 g/l). According to Bochmann *et al*. (2007), the sugars are the first to be degraded in the acidogenesis step, after the hydrolysis of the polycarbohydrates. Therefore, the high concentration of sugars and low concentration of VFA in this case indicates efficient enzymatic hydrolysis and inhibition of acidogenesis (Figure 3). The polyphenols are the major bacteria-inhibiting substances in the olive mill waste (Stasinakis *et al*. 2008). The adaptation of the bacteria to the substrate was examined by Bajaj (2009). In this study, the adaptation of the methanogenic bacteria to the substrate occurred faster in the two-stage fermenter – after 50 days, and after 100 days in the one-stage fermenter (Figure 1 and 2). In the two-stage fermenter, degradation of phenols was observed, while an accumulation occurred in the one-stage fermenter (Figure 4). The data has to be proved by testing different phenol extraction methods (Rios *et al*. 2010). The polyphenols detected in the olives are

glycosylated (oleuropein, verbascoside, rutinoside, lingstroside). Therefore, the high sugar concentration in the hydrolysate may be due to the hydrolysis of the polyphenols. There was difference in the gas composition. The gas production was less after pre-acidification. This is due to the handling of the hydrolysate – during feeding, the volatile substances were lost. For the up-scaling, a proper feed system should be employed. The olive pomace has a low ammonium concentration (Table 3). A buffer capacity can not be developed in the system, which should have a positive influence on the pH drop by increasing the OLR. There could be a possibility for a co-fermentation with a substrate with higher ammonium content (Gannoun *et al*. 2007). Further studies on the effects of the pre-acidification of olive mill solid waste on the anaerobic digestion are under investigation.

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Influence of Pre-acidification on Anaerobic Digestion of Olive Mill Solid Waste

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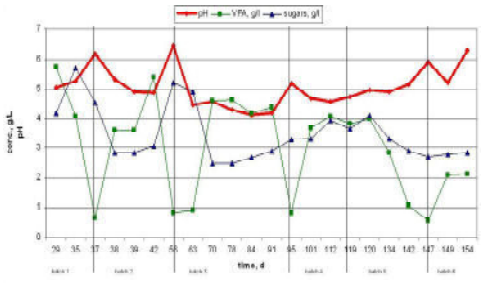
Introduction

The increasing amount of waste from the olive oil industry with high chemical oxygen demand (COD) value and low pH causes serious environmental problems and should be pre-treated before disposal on the landfills. The anaerobic digestion of the olive mill solid waste (pomace) has been poorly investigated yet. The fermentation can minimize the environmental impact of the solid olive waste and is an appropriate method for sustainable use of the organic waste by producing biogas that can be used for energy production and environmentally friendly fertilizer.

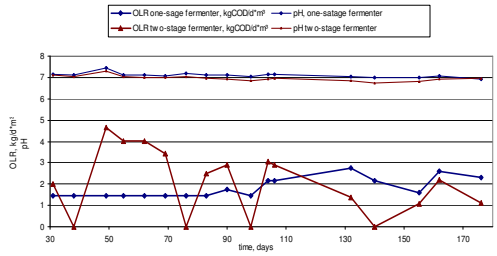
Experimental setup

- semi-continuous fermentation
- mesophilic conditions (37 ± 1 °C)
- one- and two-stage 1.8 l continuous stirred tank reactors



pre-acidification step of the olive pomace at 55 °C - pH, sugars and VFA progress

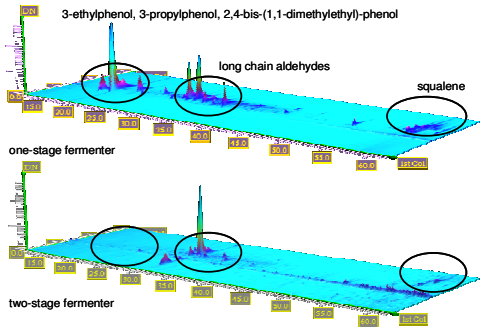
Analysis



OLR and pH changes of the one-stage fermenter with substrate olive mill solid waste (pH – 4.53) and the two-stage fermenter with substrate pre-acidified olive mill solid waste (hydraulic retention time – 2 days, pre-treatment at 55 °C, pH 4.2 – 4,6)

Faster adaptation of the methanogenic bacteria to the substrate in the two-stage fermenter – after 50 days, and after 100 days in the one-stage fermenter could be observed.

The accumulation of sugars (2-4 g/l) and the low VFA concentration (2-4 g/l) indicate either efficient enzymatic hydrolysis and inhibition of acidogenesis, or hydrolysis of the glucosylated polyphenols.



GC²/MS analysis of the one-stage and two-stage fermenter content from anaerobic digestion of olive pomace

Conclusion

The first insights in the two-stage anaerobic mono fermentation of olive solid waste showed the faster acclimatization of the methanogenic bacteria to the substrate, due to the degradation of the polyphenols during the pre-acidification step, as advantage. Further studies on the effects of the pre-acidification of olive mill solid waste on the anaerobic digestion are under investigation.

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Two-Stage Anaerobic Digestion of Sugar Beet Pressed Pulp – optimizing of reactor performance

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Abstract
Anaerobic digestion (AD) of sugar beet pressed pulp (SBPP) has been applied before as method for discharging this waste by obtaining energy and valuable fertilizer. One of the problems occurring during AD of SBPP is foaming. The aim of this study was to investigate the effect of a two-stage AD of SBPP on the reactor performance. One- and two-stage mono fermentation at mesophillic conditions in a continuous stirred tank reactor were compared. Also the optimal incubation temperature for the pre-acidification stage was studied. The effects that have been observed were a stable fermentation at 7 kg VS/m³d, hydraulic retention time of 24 days and reduced foaming in the methanogenic stage of the two-stage reactor. The viscosity of digestate in the methanogenic stage of the two-stage fermentation was in average 10 fold lower than in the one-stage fermentation. The lower viscosity decreases the energy input for the reactor stirring 5 fold.

Keywords
Two-stage anaerobic digestion; sugar beet pressed pulp; viscosity; pectin; foaming

INTRODUCTION

Sugar beet is one of the ten most produced commodities in the world in 2010 (228.45 Mio.tonnes) and the second one in Europe (150.51 Mio. tonnes) with a tendency to increase (FAO, 2010). The processing of one ton of beets produces about 70 kg of exhausted dried pulp, or about 250 kg of exhausted pressed pulp - residue accruing in the short time span of four months and mainly used as animal feed. In order to reduce its environmental impact of and to retain the sugar production sustainable, other utilization possibilities of the waste are necessary. Converting factory residues into biogas to produce renewable energy to replace fossil one decreases costs for sugar production and makes it competitive on the world sugar market. Biogas production from sugar beet pressed pulp (SBPP) as substitute of fossil fuel in sugar beet factories was investigated before (Brooks et al., 2008; Hutnan et al., 2001, 2000). The high organic content makes the sugar beets eligible as substrate for biogas plants. The lignocellulosic fraction of the dried pulp is composed of 22-30% cellulose, 24-32% hemicellulose, 24-32% pectin and 3-4% lignin (Coughlan et al., 1985), which are not fast degradable. The two-stage AD of SBPP accelerates the degradation of carbohydrate polymers and the building of VFA. This leads to decrease of the HRT and subsequent reactor volume (Alkaya and Demirer, 2011). Problem of the AD process of SBPP is foaming in the reactor at high OLRs (10.5 kg COD/m^3d) (Brooks et al., 2008). The current study was conducted to compare the performance of one- and two-stage AD of SBPP. Possible improvement of the reactor performance using two- stage AD and decrease of the viscosity in the second stage of the two-stage fermentation has been studied. The optimal OLR for a stable process, without foaming and without adding of antifoaming reagents and chemicals for pH adjustment in the first-stage were investigated.

MATHERIALS AND METHODS

The continuous experiments were held at mesophillic temperature in CSTR (continuous stirred anaerobic reactors) for 112 days. The first stage of the two stage AD was incubated at 55° C, with HRT four days, and SBPP to inoculum ratio 2:1.The stability of the AD process was monitored according to standard methods (APAH, 1999). Viscosity measurements were carried out in a macro viscosimeter, based on the so called vane-in-a-large-cup-principle, designed for identification of the rheological behaviour of slurries in biogas digesters (Pohn et al., 2010).

RESULTS AND DISCUSSION

First stage of the two-stage AD of SBPP

The release of monomers and the pH changes were measured during the incubation. The pH ranged between 7.3 and 5.0. The VFA concentration correlates with the pH changes – the decrease of the VFA concentration leads to increase of the pH value. The average sugar monomers concentration was 1.24 g/L, the alcohols – 0.77 g/L and the VFA – 15.94 g/L, which shows fast conversion of the sugars into VFAs, where 60% is acetic acid (Figure 1).

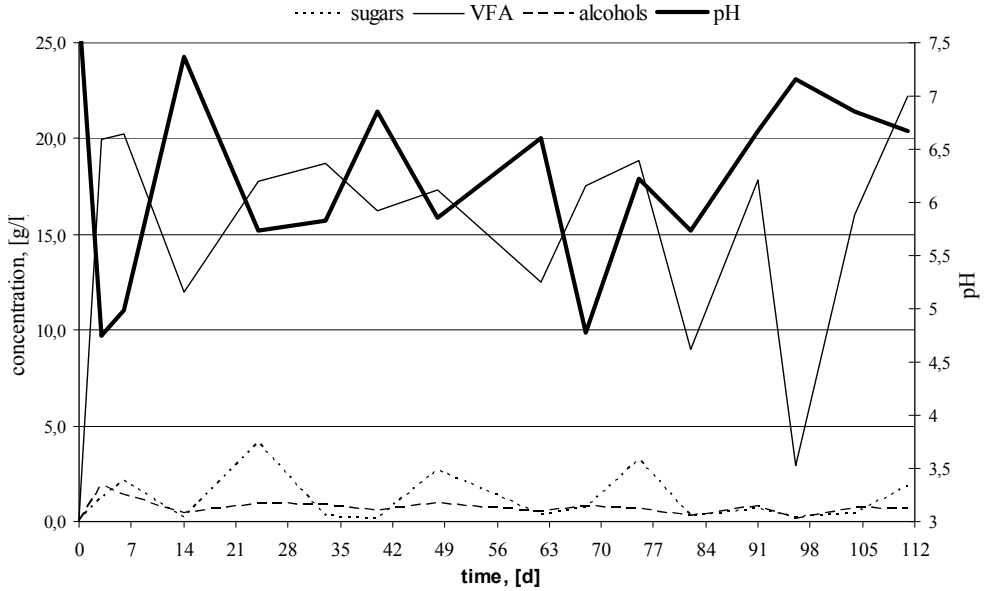


Figure 1 VFA (-), sugars (···) and alcohols (---) concentration progress during the first stage of the two-stage AD of SBPP

Second stage of the two-stage AD and one-stage AD of SBPP

The highest OLR for the two-stage fermenter was 8.45 kg VS/m^{3}d, corresponding to HRT of 16 days (figure 2). At this OLR foaming was observed and the COD degradation decreased from 87 to 77%. Subsequently, the OLR was decreased to 6.76 kg VS/m^{3}d (HRT 20 days). At this OLR the COD degradation reached 84%. The methane content of biogas was at OLR of 8.04 kg VS/m^{3}d (HRT 15 days) 53%. After decreasing the OLR to 7.31 kg VS/m^{3}d (HRT 16 days), the methane content raised up to 57%. At OLR of 5.90 kg VS/m^{3}d (HRT 19 days) the methane content is stable at 55%. Based on these results, the optimal OLR for the two-stage fermentation was determined to be 7.0 kg VS/m^{3}d, HRT of 20 days (HRT 24 for the whole process), without foaming and COD accumulation in the reactor.

The one-stage fermenter was stable for two weeks at OLR 8.47 kg VS/m^{3}d (HRT 32 days) (figure 2). Afterwards, at the 29nd day, foaming was observed without VFA accumulation, and the OLR was reduced to 3.40 kg VS/m^{3}d (HRT 80 days). The second time foaming was observed 44 days later, at OLR 6.78 kg VS/m^{3}d (HRT 44 days). At both of the foaming events a decrease of the COD degradation rate was observed: the first time from 88 to 80%, and the second time – from 85 to 82%. At day 88 an increase in the acetic acid concentration was observed (1.05 g/L). Therefore

the HRT was increased up to 80 days. After 23 days the HRT was reduced to 60 days. Subsequently, an OLR of 6 kg VS/m^{3}d, corresponding to HRT of 50 days, was determined to be optimal for one-stage AD without foaming and without COD accumulation in the digestate.

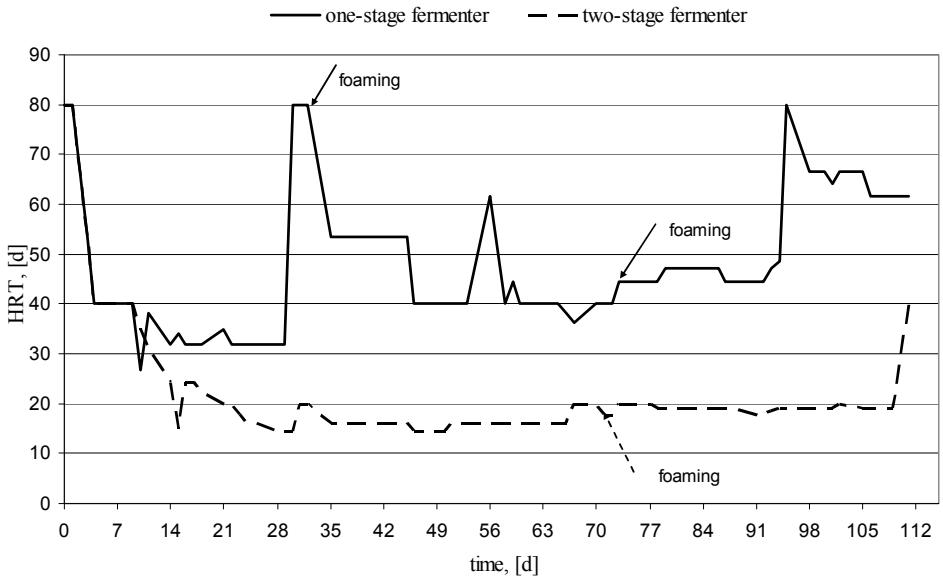
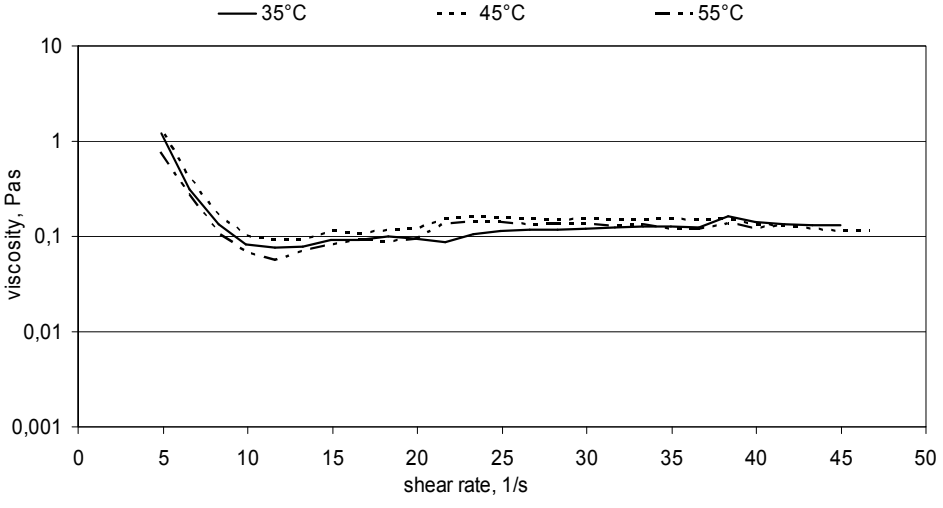


Figure 2 Hydraulic retention times during the one-and two-stage fermentation of SBPP. The arrows show the foaming event during the one- stage fermentation (solid) and two-stage fermentation (dashed).

Viscosity measurements

The viscosity in both, one-stage and the second stage of the two-stage fermenters was measured at an OLR of 5.94 kg VS/m^{3}d and 8.04 kg VS/m^{3}d for the one- stage fermenter and for the two- stage fermenter, respectively. At that time the VS were 2.64 % and 4.06 % for the one- and two- stage fermenter, respectively. The lowest viscosity of the two-stage fermenter was 0.002 Pas at 35°C and shear rate of 8.2 s^{-1}, which corresponds to the operational conditions of the continuous AD of SBPP (figure 3A). For comparison, the viscosity of the one- stage fermenter at 35°C and shear rate 8.2 s^{-1} is 0.132 Pas, which is 66 times higher than the viscosity in the two- stage fermenter at the same conditions. The shear rate at which vortex appeared was also higher – 45 s^{-1}(figure 3B). The digestate in the one-stage fermenter is a non-Newtonian pseudoplastic fluid, and in the two- stage one – a Newtonian fluid. The specific engine power requirement for the stirring in the second stage of the two-stage fermenter is 4.97 times lower than the one in the one-stage fermenter.



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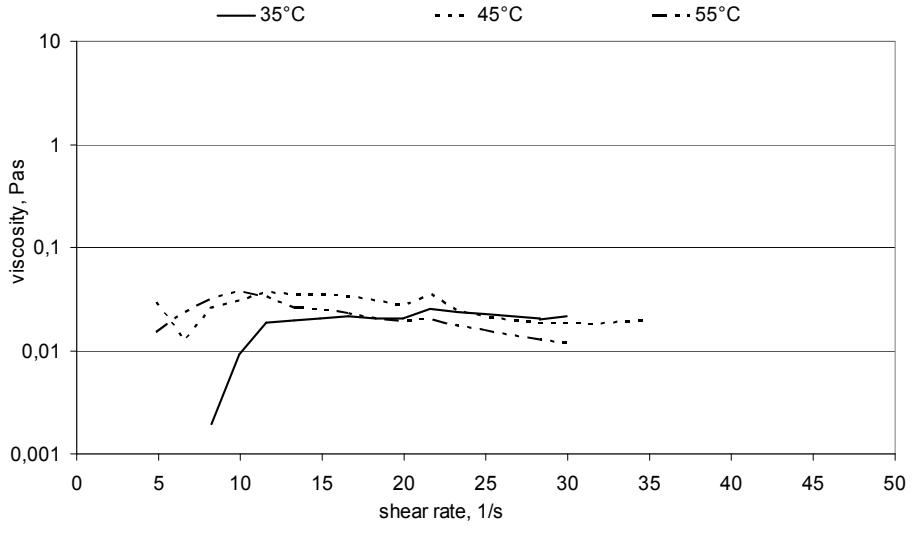

Figure 3 Viscosity of the fermenter content from the one-stage fermenter (VS=2.64%) (A), and in the second stage of the two-stage fermenter (VS=4.06%) (B).

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Two-Stage Anaerobic Digestion of Sugar Beet Pressed Pulp – Optimizing of Reactor Performance

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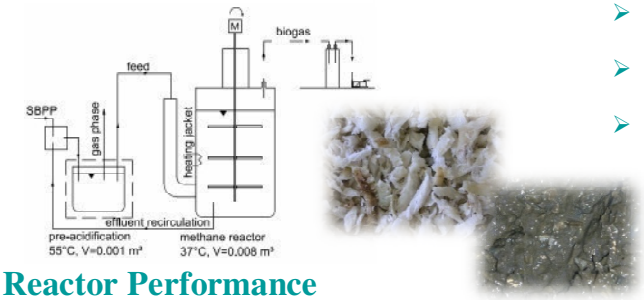
fermentation technologies

Bottlenecks in the AD of Sugar Beet Pressed Pulp

The AD of sugar beet pressed pulp has already been reported as a possibility to replace fossil energy use in the sugar producing industry and making it more competitive on the world sugar market. The problem that occurs during the fermentation is the foaming at higher organic loading rates.

In this study the effect of the two-stage AD on the foaming phenomenon was studied. Beside this another positive effect was observed – reducing of the viscosity. This leads to further reduction of the operating costs and energy savings.

Experimental set-up

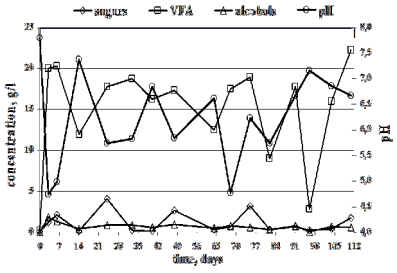


- One- and two-stage anaerobic digestion of sugar beet pressed pulp
- Monodigestion at mesophilic temperature (37°C)
- Reactor working volume of 6L in the one-stage and the second stage of the two-stage fermenter, and 1L in the first stage of the two-stage fermenter

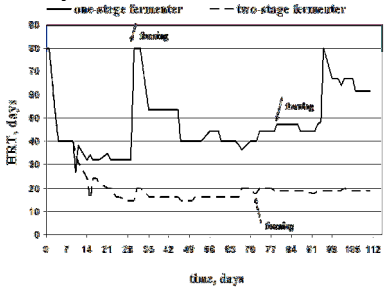
Reactor Performance

The first stage was operated at HRT of four days, without pH adjustment. The incubation temperature was determined to be 55°C (data not shown).

The fermentation in the second stage was stable at HRT 20 days (overall HRT 36 days). In the one-stage fermenter foaming occurred already at HRT of 33 days.



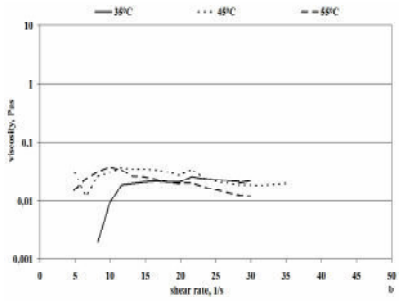
VFA (-), sugars (....) and alcohols (---) concentration progress during the first stage of the two-stage AD of SBPP



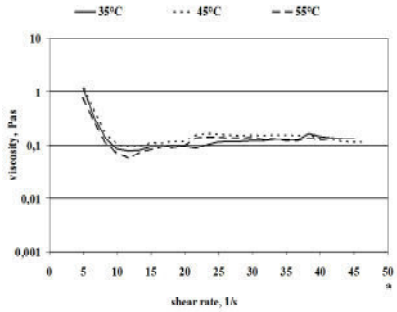
Hydraulic retention times during the one-and two-stage fermentation of SBPP

Viscosity Measurements

The fermenter content in the second stage of the two-stage fermenter was in average 10fold lower than in the one-stage fermenter. At reactor operating conditions, 35°C and shear rate 8.2 s^{-1}, the difference was 60fold. This reduces the energy input for stirring by 20%.



Viscosity of the fermenter content in the second stage of the two-stage fermenter (VS=4.06%, 8.04 kg VS/m^3d), Newtonian fluid



Viscosity of the fermenter content from the one-stage fermenter (VS=2.64%, OLR of 5.94 kg VS/m^3d), non-Newtonian pseudoplastic fluid

Conclusions

Foaming at higher OLR can be avoided in two stage system. Faster degradation of the polymers occurs during the first stage of the two-stage AD. The resulting reduction of the viscosity leads to a five times lower energy demand for reactor stirring. Further reduction of the overall HRT from 50 to 36 days leads to reduction of the required reactor volumes.

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Book chapter

Industrial Residues for Biomethane production
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"Bioenergy Production by Anaerobic Digestion: Using Agricultural Biomass and Organic Waste"

Chapter: Industrial Residues for Biomethane production

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INTRODUCTION

As a result of increasing energy costs and costs for the disposal and treatment of industrial residues, the interest of industrial companies in using renewable energy sources is increasing. An efficient and proper residue management is necessary due to several environmental concerns such as global climate change and diminishing fossil fuel resources.

This is the case, for instance, in the food processing and beverage industries such as dairies, breweries or abattoirs (slaughterhouse). These produce various organic residues with high energy content, and biogas fermentation technology is an attractive option to cope with these residues. Food processing factories, particularly abattoirs or breweries, use a large number of energy intensive processes at different temperatures. The basis for designing and dimensioning such factories is always the peak energy load, which should guarantee a constant energy supply and a secure production. In most cases, only a part of the supplied energy is used, and as a consequence, such factories are neither cost nor energy efficient.

The main running costs in industrial processes are - apart from manpower - the energy supply (natural oil/gas) and the disposal and treatment costs of the residues.

Certain industrial plants such as breweries, bioethanol plants or dairies are in the fortunate situation to gain revenues by selling parts of their residues as animal feed. In general, the revenues are not high but at least it helps keeping the disposal costs down.

Rendering of animal proteins has been an accepted pathway for treatment of slaughterhouse wastes for a long time. Since the appearance of BSE

(bovine spongiform encephalopathy) in Europe, the European Commission banned rendered animal protein from being fed to farmed animals in 2000 (European Commission, 2000, Decision 2000/766/EC). The result was a tremendous increase in disposal fees of slaughterhouse wastes and an additional financial burden on the abattoir industries.

Anaerobic digestion (AD) is an adequate and well-known technology to treat industrial organic residues almost regardless of their consistency. The utilization of industrial organic residues by AD is an appropriate way to improve both the process and the economic efficiency of an industrial factory. Anaerobic digestion produces renewable energy in the form of biogas. Furthermore, it enables a controlled stabilization of the organic material, reduces greenhouse gas emissions and contributes to the closing of nutrient cycles.

The fermentation of process-specific waste materials to biogas yields a highly combustible gas consisting of 55-70 % methane. It can be used in a combined heat and power plant (CHP) to generate heat and electricity, to substitute fossil fuels for heat and steam generation or as vehicle fuel if upgraded to bio-methane.

The implementation of an AD unit onsite has a couple of advantages. The biggest are the reduction of disposal costs, the current national subsidies by the green energy law when generating electricity by CHP and the possibility of waste heat integration (CHP) onsite into the production process. In addition, the effluent of the anaerobic digester, the digestate, represents a high-quality agricultural fertiliser which can be used directly or as a processed concentrate. The reduction of greenhouse gas emissions and the good reputation as green energy technology are additional advantages. On the other hand, there are limitations and technical challenges regarding the composition of the residues. The main issues which have to be considered are: the lack of essential microelements, process instabilities (foaming, low degradation rates and gas yields) caused by the digestion of protein rich material such as slaughterhouse waste and foaming during the pasteurization step when using lipid- and protein rich fractions. Another issue is that certain materials such as brewers' spent grains are rich in components that are difficult to degrade due to their cellulose and/or hemicelluloses content. In this case, a pre-treatment step is necessary to make these components available to anaerobic degradation. Another very important issue in industrial biogas processes is the utilisation of the digestate, the effluent from a biogas plant. Especially if the organic by-products are accumulated in very large amounts (e.g. bioethanol plants) an optimised strategy for the utilisation

of the digestate can become decisive. The integration of the waste heat of the CHP into the production process represents a further challenge.

In the following paragraphs, concrete examples of industries and their potential for anaerobic treatment as well as their main critical issues (bottlenecks) are described in detail. These examples stand for the main challenges in the AD of industrial residues.

Within the selected industrial residues, aspects of high nitrogen impact, need of pre- and post-treatment techniques as well as other inhibiting or limiting factors will be discussed.

I. ANAEROBIC DIGESTION OF ABATTOIR WASTE

Slaughterhouse wastes (SW) are parts of slaughtered animals which are not intended for direct human consumption or animal feed.

It is estimated that humans directly consume only 68% (%w/w) of a chicken, 62% of a pig and 54% of a cow. Large amounts of animal by-products accumulate and have to be disposed of. Certain parts can be recycled and used in human food, cosmetics or pharmaceuticals such as gelatine deriving from bones.
An overview of the annual amounts of animal by-products deriving from pigs, cattle and poultry both in several selected countries and worldwide (total) can be found in Table 1.

Table 1 Overview of estimated amounts of animal by-products (ABP) deriving from slaughter (FAO 2012)

	ABP [t/a]		
	pigs	**cattle**	**poultry**
Austria (2010)	126,500	214,200	28,920
China (2010)	14,835,000	n.a	3,332,000
Egypt (2009)	n.a.	275,400	n.a.
Germany (2010)	1,265,000	1,071,000	273,200
India (2006)	n.a.	746,640	156,400
Italy (2009)	303,600	826,200	n.a.
Poland (2009)	437,000	n.a.	n.a.
Turkey (2009)	n.a.	459,000	288,000
World (2010)	26,162,500	56,640,688	36,763,636

The usual treatment is a rendering process in carcass plants. Although the rendering process is very energy intensive and expensive, the sale of meat and bone meal as an animal feed additive was a valuable source of income for slaughterhouses until new legislation was introduced in 2000 in response to the BSE outbreak. The European Union immediately banned rendered animal proteins from the feeding chain and enacted a law in 2002 for safe and proper disposal of slaughterhouse wastes (Animal by-product (ABP) regulation EC 1774/2002 replaced in 2009 by EC 1069/2009). This situation led to a dilemma. On the one hand a serious protein gap emerged in Europe as meat and bone meal was no longer available as a protein source and on the other hand meat and bone meal turned from a valuable product to a problematic waste. The disposal put an economical burden on agriculture as well as on all other sectors linked to meat production. This act does, however, allow alternative pathways for the treatment of this waste material, such as the utilization in anaerobic digestion systems if approved pre-treatment steps are applied, depending on the by-product category (according to the potential risk to animals, the public or to the environment). The categories described in the following paragraphs are mainly related to slaughterhouse waste and wastewater. It should be mentioned that the animal by-product act regulates the safe disposal of the entire spectrum of animal by-products including also dairy by-products, kitchen and canteen waste and organic waste, which will not be explained here.

RISK CATEGORIES OF ANIMAL BY-PRODUCTS

Cat. I materials (i.e. spinal cord, brain, eyes of cattle) present the highest risk such as TSE (Transmissible Spongiform Encephalopathy) or scrapie and have to be completely disposed of by incineration.

Cat. II includes all materials that do not fit into category I or III and present a risk of contamination with other animal diseases. These may not be used in feed, but can be recycled for other uses (e.g. biogas or composting) after appropriate treatment (sterilisation at 133°C and 3bar for a minimum 20 min, particle size <50mm). Exceptions include intestinal contents, manure or milk that can be used in a biogas plant without any sanitation steps.

Cat. III materials (i.e. by-products derived from healthy animals slaughtered for human consumption, blood) may be used in the production of animal feeds following appropriate treatment in approved processing plants. The treatment comprises pasteurization at 70°C for 60 min minimum with a required particle size ≤12mm).

PATHWAYS OF TREATMENT OF SLAUGHTERHOUSE WASTE

There are different pathways for treating slaughterhouse wastes: either the transformation to electrical and thermal energy or the supply of valuable compounds for biotechnological and chemical transformation to chemical precursors. The last option is still not fully developed and further research is necessary for industrial scale.

The most common way at the moment is the utilisation of rendered meat and bone meal as a secondary fuel in cement plants or waste incineration plants. The meal has similar heat value to lignite and shows very good burning characteristics.

The rendered grease is mainly used in the rendering plant as a substitute for heavy fuel oil for heat generation. An alternative is the transformation to biodiesel.

Slaughterhouse waste is considered to be an excellent substrate for fermentation processes. For instance, the biotechnological production of certain chemical precursors such as polyhydroxyalkanoates (PHA) is an alternative option, as is the formation of bio composites. (Braunegg et al., 2006)

Composting of category III material is also a feasible way to process slaughterhouse waste.

ANAEROBIC DIGESTION OF SLAUGHTERHOUSE WASTE

Last but not least, the anaerobic treatment of slaughterhouse waste in biogas plants is considered to be a challenging and promising alternative. Due to the high protein and lipid content, SW are considered to be a very good substrate for biogas production with expected high amounts of methane. In theory, proteins are able to deliver biogas containing 60% methane and lipids 72% methane. However, in practice a lot of limitations restrict the applicability of SW.

The most significant limitations are the slow hydrolysis rate of certain particulates which are difficult to degrade, as well as foaming and floatation caused by lipid degradation resulting in a biomass wash out and different inhibitory effects caused by several intermediates (i.e. long chain fatty acids (LCFA), hydrogen sulphide (H_2S) or ammonia (NH_3)) formed during the degradation process. (Chen et al. 2008, Salminen et al. 1995, Angelidaki et al. 1993)

For these reasons, it is difficult to digest this material as single substrate. Therefore, slaughterhouse wastes are commonly used as co-substrates in the agro-industrial sector together with canteen waste, manure and/or energy crops.

INTEGRATION OF AD-TECHNOLOGY INTO A ABATTOIR

Few AD plants use SW as a single substrate, but one of these biogas plants is located in St. Martin (Austria). The biogas plant in St. Martin was erected 2003 and is operated only with SW derived from the close-by pig abattoir with an annual capacity of 500,000 heads. By the time of construction this plant was the first abattoir worldwide utilizing wastes in mono-fermentation. The main idea was to reduce its running costs in terms of energy supply and waste disposal by implementing an AD plant onsite (using also some additional rumen content from a cattle abattoir nearby). The overall treatment capacity of the AD plant is 12,000 t organic residues per annum, which covers the waste fractions accumulated during the slaughter process.

The substrate consists of blood, rumen and rumen content, grease separator material, stomach content, colon and wastewater from the slaughtering facilities. Furthermore, rumen content derived from the cattle abattoir is used as well (see Table 2).

Table 2 Chemical characterization of slaughterhouse waste processed in the biogas plant

Substrate	TS [%]	VS [%]	COD [g/kg]	TKN [g/kg]	Relative Amount
Blood (pig)	18.2	16.7	265	28.0	+++
Blood (cattle)	18.5	17.0	260	27.5	+++
Colon (pig)	24.4	22.0	575	10.0	+
Stomach content (pig)	24.4	23.5	408	5.3	++
Rumen content	13.6	13.0	187	3.7	++
Omasum	19.3	18.4	698	15.1	+
Fat scrubber material	6.1	5.4	157	1.3	++

The plant employs conventional two-stage fermentation with CSTRs (continuously stirred tank reactors). As shown in Figure 1, there are two main fermentation tanks, which are loaded in parallel, followed by a third one and a final storage tank for the digestate. According to the European Directive (1069/2009 EC), the material is minced to a maximum particle size of 12 mm and collected in a separate buffer tank followed by pasteurisation at 70°C for 60 min. After passing the recuperator, substrate is pumped into the two main fermenters. The operation temperature is 38°C; higher temperatures are not recommended because the high concentrations of lipids can cause foaming and associated operational problems. The biogas produced in the AD plant is directed to an external biological desulphurisation unit and combusted afterwards in a combined heat and power plant (CHP) with an average monthly output of

approximately 300 MWh electricity and 300 MWh heat. The generated electricity covers about 43% of the abattoir's electricity demand. About 25% of the waste heat generated from the CHP is used for the biogas plant including the sanitation unit and the desulphurisation unit. The major part, about 75%, can be fully used in the abattoir, covering about 90% of the abattoir's heat demand. The overall degree of energetic self-sufficiency of the abattoir is at the moment at about 55%.

The biogas plant at St. Martin is a pioneer project in terms of mono-fermentation of SW and is the result of intensive research activities in this field over the last seven years and the willingness of the facility owner to embrace alternative treatment technologies.

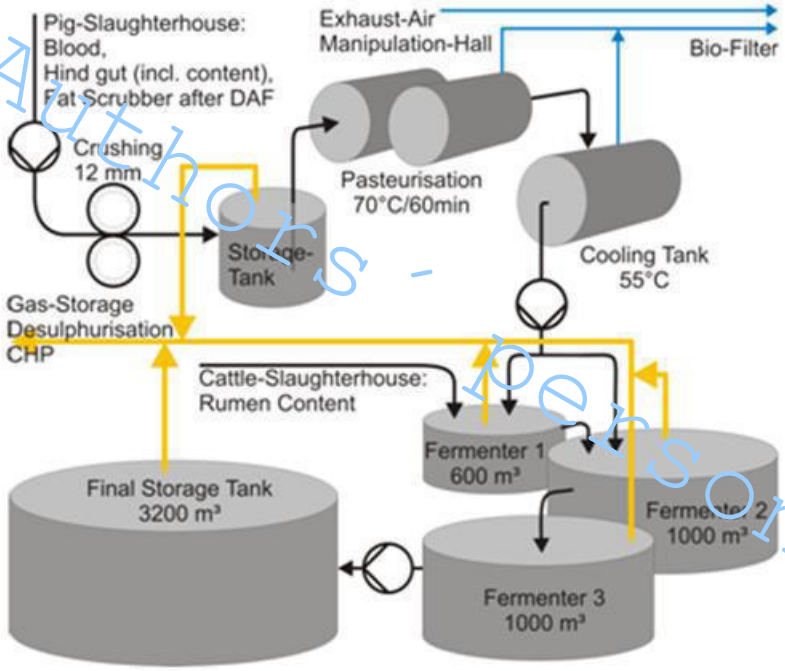

Figure 1 Process scheme of the AD-plant

LIMITATIONS AND BOTTLENECKS

Although there are many advantages there are certain critical issues that have to be considered when using SW in a mono-fermentation.

The high protein content of SW (up to 33 g TKN/kg, mainly from the blood) leads to a **high ammonia concentration** during the degradation process. Free ammonia is well known and described as a potential inhibitor of anaerobic digestion. Among the different groups of microorganisms involved in the degradation, the methanogens are the most sensitive to ammonia inhibition which causes them to slow their cell growth. (Kayhanian et al., 1994).

Methanogen strains isolated from AD sludge such as *Methanospirillum hungatei* show very high sensitivity to ammonia inhibition. Other strains

such as *Methanobacterium thermoautotrophicum*, *Methanobacterium formicicum* or *Methanosarcina barkeri* were found to be less sensitive to higher ammonia (10g/L NH_4) concentrations (Jarrell et al., 1987, Goberna et al. 2010a).

Ammonium (NH_4^+) concentrations have been reported to be starting inhibitory from 1.7 g/l up to 14 g/l (Chen et al. 2008).

The differences are mainly attributed to the various physiochemical conditions such as pH, temperature and different biomass acclimation periods. In terms of free ammonia, it is believed that concentrations above 100 mg/l cause inhibition. High concentrations of ammonium (>5g/l NH_4^+) lead to a shift from aceticlastic to syntrophic acetate degradation pathway. In the syntrophic pathway, acetate is converted to hydrogen and carbon dioxide by syntrophic acetate oxidizing bacteria (SAO), followed by a subsequent utilisation by hydrogenotrophic bacteria. This slows down the degradation process due to the higher doubling time of SAO (28 days) compared to aceticlastic bacteria (about 12 days). (Schnürer et al. 2008)

There are different techniques to counteract ammonia inhibition. Applied and feasible methods include stripping, precipitation, biomass retention, biomass immobilizing on zeolite, addition of ion-exchanger (i.e. zeolite), antagonistic cations such as magnesium or calcium ions or dilution with other liquid wastewater. Dilution is not really recommended as it increases the process cost and the volume of wastewater.

The fermentation of protein-rich material such as SW should take place under **mesophilic conditions** (between 35-40°C). Thermophilic digestion is not recommended as higher levels of free ammonia at this temperature cause higher inhibition. In addition, at higher temperatures foaming and intensified biomass wash out have been observed (Ortner, 2010).

To guarantee full **heat integration** within the facility, heat generated at a constant rate in the CHP should be transferred from periods of low energy demand to periods of high energy demand. That can be achieved by using a hot water storage tank in combination with intelligent recovery networks.

Another critical issue during mono-fermentation is the inefficient supply of essential **micro elements** to the bacterial biomass. This can cause severe constraints in terms of substrate degradation rate which results in reduced biogas production. Due to the high concentrations of hydrogen sulphide, the bioavailability of trace elements such as nickel, cobalt or molybdenum is reduced significantly due to the formation of poorly soluble metal-sulphide precipitates.

The supplementation of trace elements may help to counteract this insufficiency. It is important that addition happens in a well-balanced way; otherwise it can have the opposite effect as overdosing may poison the

microbial community.

ECONOMICS

The costs of industrial AD plants are higher than the costs of a conventional agricultural biogas plant, attributable to the installation of the sanitation unit and comprehensive exhaust air treatment units. In principal, digestate shows good fertilising potential. However, if digestate cannot be used as an organic fertiliser in a direct way, post-treatment units (evaporation, filtration, separation) are required. This may further raise the overall costs.

Generally speaking the economic efficiency of such an AD-plant is strongly dependent on the national legal situation. That means a lot of factors have to be considered, whereby the green energy law and its applicable feed in tariffs, national subsidies and the national disposal costs play the most important role in the decision.

II. INTEGRATION OF AD-TECHNOLOGY INTO BIOETHANOL PRODUCTION

In bioethanol production processes very large amounts of organic by-products are accumulated which are almost all suitable for anaerobic digestion. In grain bioethanol plants, typically all stillage fractions are anaerobically degradable (Drosg et al. 2011, Rosentrater et al. 2006, Cassidy et al., 2008). Integrating anaerobic digestion technology into such grain ethanol plants will be the focus of this chapter.

In sugar cane bioethanol plants, either sugar cane molasses (after the recovery of sugar) can be used for ethanol fermentation or the cane juice directly. Either way, the liquid effluents (vinasse, stillage) are suitable substrates for anaerobic digestion (Nguyen et al. 2009, Harada et al. 1996, Yeoh 1997, Cail and Barford 1985), whereas the solid bagasse is mainly incinerated for energy recovery.

Table 3 Examples of increase in bioethanol production capacities from 2008 to 2010 (Renewable Fuels Association, 2011)

Country	2008 (milllion m³)	2010 (million m³)	Increase (%)
USA	34	50	47
EU	2.8	4.5	61
World	65	95	46

This chapter will focus on the production of bioethanol from grains which is the prevailing process in Europe and the US. In the US - the current world leader in bioethanol production, bioethanol production increased in the last decade almost tenfold (Renewable Fuels Association, 2011). In Table 3 the current increase in bioethanol production capacity of the US, EU and the world are shown. It lies between 46% and 60% in a period of only two years.

Due to such a high quantity of bioethanol produced, also large amounts of by-products are accumulated. The dry-grind bioethanol process from grains produces up to 5.6 t of stillage per m³ of ethanol (Drosg et al., 2008). The state of the art stillage treatment process is drying to animal feed. This consumes a considerable amount of energy, since grain stillage has a water content of about 85-90%. As the bioethanol industry becomes more prominent, there will be a greater need for implementing industrial anaerobic digestion processes. Anaerobic digestion can be a valuable option, depending on the price of animal feed and energy. Since dry-grind grain ethanol production is the prevailing process in the US, it can be estimated that roughly 280 million t/a of stillage are accumulated in US domestic ethanol production. Using anaerobic digestion on the annual stillage produced in the US, roughly 16.3 billion Nm³/a of methane could be recovered. Translated to the European Union about 25 million t/a of stillage are accumulated with a methane potential of approximately 1.5 billion Nm³/a.

STATE-OF-THE-ART STILLAGE TREATMENT

In the dry-grind bioethanol production process, as the prevailing process for grain ethanol production, ethanol, carbon dioxide and animal feed are produced. This process is described in detail by Senn and Pieper (2001) and Bothast and Schlicher (2005). The stillage accumulates as liquid by-product after the distillation of the fermentation broth the so called "beer". In the-state-of-the-art process (see Figure 2) it is separated by centrifuges to thin stillage (liquid phase) and wet cake (solid phase). The liquid phase is concentrated via vacuum evaporation to syrup and mixed with the wet cake. This mixture is finally dried to animal feed called DDGS (Distillers´ Dried Grains with Solubles).

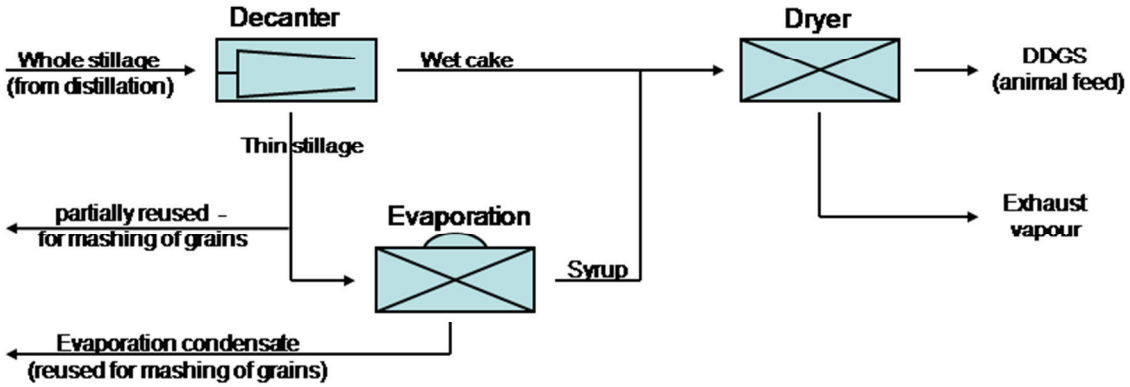

Figure 2 State-of-the-art stillage treatment process in dry-grind bioethanol plants

CHARACTERIZATION OF STILLAGE FRACTIONS

The composition of the stillage fractions in a dry-grind bioethanol plant can vary depending on the input mixture of grains. In table 4 the mean values of the stillage fractions of a large-scale bioethanol plant are shown. In this plant mainly wheat and corn were used as substrates, however, in varying ratios. Due to this fact a high variation of the values occurred. For the estimation of the possible methane production, also BMP (biochemical methane potential) tests were carried out.

Table 4 Mean values of standard parameters of the stillage fractions in a dry-grind bioethanol plant

	pH	TS	VS	COD	TKN	BMP	
Fraction	[-]	[%]	[% of TS]	[g/kg]	[g/kg]	[Nm³ CH_4 /t COD]	[Nm³ CH_4 /t VS]
Whole stillage	4.5	13	91	175	7	290	469
Thin stillage	4.2	8	89	100	4	303	500
Wet Cake	4.4	31	97	458	19	267	425
Syrup	4.3	27	89	348	11	298	470
Condensate	3.1	< 0.01	-	10	< 0.01	292	-

LIMITATIONS AND BOTTLENECKS

Digestate accumulation

The integration of a biogas process into a biofuel production process, multiplies the problem of by-product (digestate) accumulation compared to food production processes. In small and middle-sized biogas plants - especially if they are strongly linked to agriculture - the state-of-the-art utilisation of digestate is land application as fertiliser. The European Nitrate Directive 91/676/CEE limits the application of nitrogen per ha and year, and there is considerable potential of nitrate leaching when digestate is not applied during the time of plant demand (Goberna et al. 2011). As a consequence, the land area which is needed for digestate application increases steadily by increasing biogas plant size and transport costs will increase drastically. In addition, if the utilised raw materials (crops) are not purchased regionally - which is often the case in bioethanol facilities - the willingness of local farmers to utilise the digestate as fertiliser is uncertain, even though digestate is a valuable fertiliser. As a consequence, when integrating biogas technology in such a large-scale process as a bioethanol plant, it is clear that the management of the digestate will be a key issue. In some cases it will be possible to directly apply the digestate as fertiliser in the region, especially if the stillage management at this bioethanol plant has already been land application. At many other bioethanol facilities a detailed digestate treatment strategy will have to be implemented. A variety of technologies are available for digestate treatment (Fuchs and Drosg 2010, Fuchs and Drosg 2011) such as solid-liquid separation by presses and centrifuges, evaporation, membrane purification, etc. The aim of these technologies is to produce process water and a nutrient concentrate. This nutrient concentrate can either be further processed to a marketable bio-fertiliser or the decrease of the water content can allow larger distances for economic land application. At the moment, although there exist already some industrial-scale digestate treatment facilities, the digestate treatment can still not be considered state-of-the-art technology in industrial biogas processes. Apart from that, additional investment and energy will be needed for digestate treatment. However, to which extent depends strongly on the type of technology applied. The selection of a suitable technology is highly dependent on boundary conditions like:

- Availability of waste heat for evaporation
- Flux rates and life time of membranes for membrane purification
- Regionally available agricultural areas for land application
- Market value of the produced fertiliser products

- Quality of process water in the case of a parallel animal feed production

In the case of digestate from bioethanol fractions, one interesting option for digestate treatment can be solid-liquid separation by centrifuges, where the efficiency of the suspended solids removal can be increased by precipitating agents. Another alternative is the evaporation of digestate, if waste heat at the bioethanol plant is available. The produced condensate can be reused - together with evaporated ammonia - as process water and nutrients in yeast fermentation. By adjusting the pH in the digestate the concentration of ammonia in the condensate can be regulated to the amounts needed in yeast fermentation. In the literature (Tiejun and Xiaomei 2010) it is mentioned that the reuse of digestate in the ethanol fermentation is possible, and even beneficial due to its nutrient content. For the recirculation of digestate fractions it is important to know that high volatile fatty acid concentrations can have a negative effect on yeast fermentation. In addition, possible legal restrictions will have to be checked.
The suitable digestate treatment technology depends strongly if parts of the stillage fractions are still processed to animal feed. In this case digestate can influence the smell as well as the colour of the animal feed. In addition, endospores from sporulating bacteria might become a problem. If no animal feed is produced in the process, the influence of recirculating the digestate (or digestate fractions) on animal feed quality has not to be considered. However, a nitrogen sink (e.g. denitrification process, ammonia stripping) will be necessary in the process to reduce the recirculation of nitrogen loads.

Nitrogen Impact

High nitrogen concentrations in biogas feedstocks can have a negative effect on process stability in anaerobic digestion (for details on nitrogen inhibition in industrial residues see the example of abattoirs above). By-products from bioethanol plants show increased nitrogen concentrations since a part of the carbon in the biomass has already been transformed by the yeasts to produce ethanol and CO_2 and removed from the process.

Among the stillage fractions in a bioethanol plant the condensate has the lowest nitrogen concentration. In this fraction practically no nitrogen is present (< 0.01 g/kg) so it will have to be added for anaerobic digestion. Thin stillage has a TKN of about 4 g/kg. Pilot-scale trials (500 l) for almost two years showed that a stable digestion process is possible for thin stillage, if the process is optimised. It can be assumed that also whole stillage can be degraded in a stable process although the nitrogen

concentration is already quite high (7 g/kg). In syrup (11 g/kg) and especially wet cake (19 g/kg) the nitrogen concentration is too high for direct anaerobic digestion. Therefore, these stillage fractions would have to be diluted or a nitrogen sink (denitrification process, membrane extractor process) would have to be integrated.

POTENTIAL FOR ENERGY RECOVERY BY BIOGAS PROCESS INTEGRATION

The potential for energy recovery by anaerobic digestion from each stillage fraction is given in Table 5. There the annual methane production and energy supply of the biogas plant are given. These results are compared to the energy demand of the bioethanol plant which was estimated according to Murphy and Power (2008) and Lurgi GmbH (2006).

Table 5 Potential for energy recovery per stillage fraction in a large-scale bioethanol plant

	Biogas plant – energy recovery		Bioethanol plant	
Stillage Fraction	Methane production	Energy supply[a)]	Energy demand[b)]	Coverage by biogas
	[10^6 Nm³/a]	[GWh_{therm} /a]	[GWh_{therm} /a]	[%]
Whole stillage	66	575	395	146
Thin stillage	25	215	505	42
Wet cake	34	295	495	59
Syrup	24	210	505	41
Condensate	1.7	15	610	2.5

[a)] The biogas production is estimated according to COD load and BMP value. For heat production η_{therm} = 87% was assumed, and the energy demand of the biogas plant was excluded.

[b)] The thermal energy demand of the bioethanol plant is estimated according to the data from Murphy and Power (2008) and Lurgi GmbH (2006). The energy demand varies considerably depending on how much of every stillage fractions is dried to animal feed.

It can be seen clearly that anaerobic digestion has a very high potential for energy recovery in bioethanol production. Table 5 shows that using all the available stillage more than 100% of the energy demand of the bioethanol plant can be provided by biogas. For thin stillage the potential

was 42% of energy coverage. In addition, it is a very promising substrate, especially because it is rapidly and easily degradable and contains no bulky material

Wet cake can provide 59% energy recovery, and syrup 41%. However, both stillage fractions show very high nitrogen concentrations. The reason for the small difference between thin stillage and syrup are the volatile substances that evaporate in the concentration step from thin stillage to syrup. Condensate shows by far the lowest potential (2.5% coverage). Nevertheless, anaerobic condensate treatment can be integrated quite easily into the process, since it contains no particles. In addition, the concentration of potentially inhibitory metabolites for yeasts (e.g. acetic acid) can be reduced by anaerobic digestion of the condensate.

The data in Table 5 show the potential for energy recovery. In real scale processes, the energy recovery will be considerably lower. First of all, the standard energy demand (pumping, stirring) for a biogas fermentation was neglected in Table 5. However, this energy demand is generally not so high in industrial biogas processes (roughly estimated 5-10% of the energy content in the produced biogas). The biggest amount of energy can, however, be needed for an appropriate digestate treatment process, if land application of digestate is not possible in the region Different technologies are available, but no state-of-the-art treatment has evolved up to now. For this reason the energy demand for digestate treatment has not been integrated into this estimation of the potential. It will have to be estimated separately for every case study.

III. ORGANIC RESIDUES FROM BREWERIES

Beer fermentation has been among the first biotechnological processes. Ethanol is the desired fermentation end-product with a final concentration of 4.5 to 6.0 %. Malt is used as the typical source of starch. Beside that, other raw materials such as corn, rice or barley are applied as well. During beer production various organic residues accumulate, mainly non degradable components of malt and yeasts.

In 2009 worldwide 1,809 Mio hectolitres (hl) beer were produced. The biggest producers are China, USA, Brazil, Russia and Germany. (The Barth Report 2010).

CHARACTERIZATION OF ORGANIC RESIDUES

During the brewing process, fermentation and storage approximately 25 kg (FM)/hl of paste-like and solid residues accumulate.

As presented in table 6 the organic wastes consist basically of brewers' spent grains (BSG), break, yeast and wastewater. Due to the consistency of the residues (solid, pasty and liquid) anaerobic digestion seems to be a feasible technology for treatment. The biogas yield of these organic fractions ranges from 60 to 600 Nm^3/t FM.

Table 6: Amount of brewery residues and biogas yield (Pesta et al. 2006)

Residue	Amount [kg/hl]	Biogas yield [Nm^3/t FM]
Malt dust	0.05-0.25	600
Brewers spent grains (BSG)	18.0-20.0	120
Cold break	0.1-0.3	400
Hot break	0.4-2.0	400
Yeast	2.0-2.6	60
Waste water	350-400	0.32

There are two more residues which have to be considered, diatomaceous earth and etiquettes. In most breweries diatomaceous earth is used for filtration. Both materials are not suitable for anaerobic digestion and have to be sorted out. Currently, most of the residues from breweries are used as an animal feed. Beside that there is an increasing amount of BSG, cold and hot break and yeast which is used as a co-substrate for biogas production. In 2010 about 1/3 of the total amount of BSG in Austria were used in biogas plants.

PRE-TREATMENT - OVERVIEW

The four steps of anaerobic digestion are hydrolysis, acidogenesis, acetogenesis and methanogenesis. The time needed for the degradation of biomass to biogas, or macromolecules to mainly methane and carbon dioxide, varies depending on the nature of the chemical bonding of the carbohydrate in the biomass (Noike et al. 1985). The microorganisms in

anaerobic digestion convert simple molecules into biogas. Starch is used by the plants as an energy store and is therefore easy degradable by bacteria, in contrast cellulose is used to maintain the structure of the plant and is for that reason difficult to break down. The breakdown of cellulose is further complicated by the bonds between different cellulose chains, and between cellulose, hemi-celluloses and lignin. Lignin cannot be degraded by anaerobic bacteria.
In recent years different pre-treatment technologies have been developed to increase the degradability of carbon, particularly in ligno-cellulosic material. There are a huge number of pre-treatment technologies, and it is often difficult to decide which of these are suitable.

In general, pre-treatment technologies can be divided into physical, chemical and biological processes. Physical pre-treatment comprises milling, extruding or thermal techniques. An example for a combined physical treatment process is steam-explosion. Chemical pre-treatment technologies include the addition of alkali, acid or organic solvents. Among the biological pre-treatment technologies addition of enzymes as well as a multi-stage digestion including a pre-acidification step have to be mentioned. Generally, a biological pre-treatment process increases the digestion rate, while chemical and physical treatment leads to higher gas yields and higher digestion rate. During both treatments inhibitory substances can be formed. The energy demand of pre-treatment technologies varies strongly (Wellinger et al.,2012).

PRE-TREATMENT TECHNOLOGIES OF BSG

Each residue from brewery process needs different retention times for complete degradation by anaerobic consortia. While yeast, cold and hot break is degraded rapidly, BSG need a higher retention time for a complete degradation. The reason for that is the chemical composition of BSG containing cellulose, hemicellulose and lignin (see Table 7). BSG consist of high amount of holocellulose (Table 7) and request 40 to 60 days for total degradation. Due to this problem different pre-treatment technologies were analysed so far.

Table 7: Composition of brewers' spent grains (Narziß 1995, Kanaucho 2001, Mussatto 2005, Böchelt 2002)

Component	Amount in %
Cellulose	16.2-25.4
Hemicellulose	21.8-28.4
Lignin	11.9-27.8
Proteins	15.2-28.0
Fats	5.5-10.6
Ash	2.4-6.2

The influence of milling of BSG was analysed and evaluated in several research projects. Voigt et al. (2009) reduced the retention time of BSG during anaerobic digestion to 24 - 27 days (OLR 4.9 kg VS/(m^3·d)) in a three stage system. During one stage digestion and retention time of 45 d, a higher OLR (3.4 kg VS/(m^3·d)) and a higher gas yield (+16 %) was realised. The three stage process includes two acidification steps. Voigt presented energy recovery of 25 % of the total energy demand of a brewery. This low amount of recovery can be explained due to the high energy input for the milling process (Voigt et al. 2009).

In 2003 the company von Nordenskjöld presented a milling process in combination with a two step anaerobic digestion system (including an acidification and methanogenesis step). An organic loading rate of approximately 4 kg/m^3·d was realised. Following the anaerobic digestion process by aerobic treatment COD concentration of waste water could be reduced below 100 mg/l (von Nordenkjöld 2003 and 2008). The company ATRES patented a multistage anaerobic digestion process and demonstrated together with the company enbasys the digestion of BSG at Weihenstephaner Brewery in Bavaria/Germany. Hereby the patented hydrolysis process allows a reduction of the hydraulic retention time down to 7 days with the disadvantage of a not fully exploitation of the biogas potential (Pesta 2009).

Bochmann et al. (2007) showed the effect of enzymes on anaerobic digestion of BSG. An increase of volatile fatty acids by about 50% due to a higher hydrolysis rate and thus a higher degradation rate at 40 °C of BSG could be observed. During a continuous digestion process higher gas quantity and quality was measured during a hydraulic retention time of 40

days (Bochmann et al. 2007).
A combined process, pressing by a belt press and subsequent combustion of BSG, was evaluated by the Montan University in Leoben/Austria and the Austrian brewery Gösser. The result showed an increase of total solids (TS) from 20 to 42% by the pressing process. Before the subsequent combustion was carried out, the TS was increased again to 55 % by a drying process. The liquid fraction of the pressing process was digested in the UASB of the brewery. Combustion of the solid fraction supplied partially the thermal energy demand of the brewery (Herfellner et al. 2006).
Through thermo-chemical pre-treatment the biogas yield of BSG could be increased by 28%. A total biogas yield of 155 Nm^3/t FM could be observed (Bochmann et al. 2010). Chemical and mechanical pre-treatment of BSG was analysed by Sezun et al. (2010). Acid and alkali pre-treated BSG showed higher gas production than mechanically pre-treated substrate. In both studies inhibition effects during anaerobic degradation process of the pre-treated BSG have been observed. This resulted in lower degradation rate, and lower gas yield caused by the formation of bacteriostatic compounds, such as furfurals.

ENERGY SUPPLY OF BREWERIES BY AD

Many breweries use anaerobic digestion technology for the treatment of wastewater, but not for solid or paste-like wastes widely applied. In some European countries brewery residues are used as a co-substrate in biogas plants. Currently, in Austria about one third of the total BSG are used in biogas plants. Through anaerobic digestion of the total amount of BSG accumulated in Austria, approximately 21 million Nm^3 CH_4 per year (equal to 210 GWh) can be generated.
The production of beer requires thermal and electrical energy of about 26.8 or 9.9 kWh/hl beer, respectively. Using all residues in a brewery for anaerobic digestion up to 17.9 kWh/hl can be generated. As a consequence approximately 50 % of the energy demand can be covered.

ECONOMICS

An anaerobic digestion unit implemented onsite offers the opportunity of energy recovery by biogas. A large-scale brewery producing 1,000,000 hl annually has an accumulation of 25,000 t/a of solid or paste-like waste 25 kg waste /hl). In this case a total digester volume of 3,000 to 5,000 m^3 is needed; investment costs of 2.5 to 3.0 Mio € are required (Walla et

al. 2008).
Currently, BSG are mainly sold as animal feed. In Austria revenues range between 5 to15 €/t FM. If BSG are used for the production of biogas higher revenues can be expected.
An important point in terms of economics is the accumulation of digestate. After the digestion process 15,000 to 18,000 t of digestate (1 million hl brewery) accumulate with a total solid content of 4-5%. According to different national laws and due to the composition of the digestate it can be used as fertiliser. Another option is digestate treatment, where additional costs of approximately 5-8 €/t have to be considered. Anaerobic digestion of BSG has still to be optimised in economic terms.

IV. ANAEROBIC DIGESTION OF RESIDUES FROM OLIVE OIL PRODUCTION

Another important source for renewable energy generation from industrial residues is waste from olive oil production: olive mill wastewater (OMW) and olive mill solid waste (OMSW). World olive oil production has increased from 2.51 million tons in 2000 to 3.27 million tons in 2010 (FAO 2012).The main olive oil producers are concentrated in the Mediterranean area: Spain 36%, Italy 27%, Greece 15%, Tunisia and Syria 6%, Turkey 4% (Buckland et al. 2010).

EXTRACTION PROCESS

Olive oil extraction can be carried out in a number of ways. In the traditional pressing process, olive emulsion is decanted from the wastewater after pressing and a solid fraction (the olive husk) remains. However, this process is no longer common at industrial scale and centrifuges are being used for olive oil extraction nowadays. Depending on the centrifugation system, two- and three phase systems are common. The three-phase system is popular as it can be completely automated, produces higher quality oil and is a compact process. The huge amount of wastewater – 1 to 1.6 m³ per ton olives - led to the development of the two-phase extraction system, which provides 0.2 m³ wastewater per ton olives and is used by roughly 90% of the olive-mills. In the three-phase system, 550 kg olive cake accumulates per ton olives; in the two-phase system the solid phase amounts to 800 kg olive wet cake per ton olives (Roig et al. 2006).

CHARATERIZATION OF OLIVE MILL WASTE

The characteristics of olive oil wastes (Table 8) vary depending on geographical, seasonal, varietal or methodological factors. Two-phase olive pomasse is an acid effluent consisting of water 60-70%, lignin 13-15%, cellulose and hemicellulose 18-20%, mineral solids 2.5% (Borja et al. 2002). The antimicrobial and the phytotoxic effects, observed when applying the olive-mill wastes directly to the soil are due to the phenolic and long chain fatty acid content and led to the development of other valorisation methods: evaporation, physico-chemical treatment, including coagulation, precipitation, microbiological treatment, composting, extraction of valuable products, solid state fermentation of the solid phase and, last but not least, anaerobic digestion (Roig et al. 2006).

Table 8 Chemical characterization of the waste from olive oil production residues (Alburquierque et al. 2004, Paredes et al., 1999 Gelegenis et al., 2007)

Substrate	pH	TS [%]	COD [g/kg]	Fat [%]	Poly-phenols [% TS]
olive mill wastewater (OMW)	4.80-5.50	4.12-16.38	150	0.55-11.37	1.32-3.99
olive mill solid waste (OMSW)	4.86-6.45	23.92	183-280	2.5-3	0.62-2.39

Beside its low pH and high polyphenol content (Table 8), the OMW has also low alkalinity (3.8 g $CaCO_3$/l) and low amount of ammonium nitrogen 750 mg/l and total Kjeldahl nitrogen (TKN) 1.65 g/l, which contributes to the instability of the anaerobic process (Boubaker et al. 2007).

LIMITATIONS AND BOTTLENECKS

Olive oil production is a seasonal process. The treatment process for safe disposal of such an amount of waste should be flexible in terms of continuality on the one hand, and effective and robust to avoid the necessarity of waste storage over a year, on the other hand. The main advantage of anaerobic digestion of olive mill waste is the high energy potential of the substrate due to its high COD, and the possibility to use the digestate as a fertiliser. The production of biomethane can be up to 25 Nm^3 CH_4 per ton of olives, with a heat-production potential of about 1 GJ per ton olives (Gelegenis et al. 2007) and COD removal up to 90% (Rincón et al. 2006). The anaerobic process should be optimised for fast conversion of the wastes and for phenol and fat degradation.

A large number of laboratory studies over two decades demonstrated the

up-flow anaerobic sludge bed reactor or anaerobic filters as suitable for OMW digestion (Roig et al. 2006). Dilution during the start-up of the reactor is recommended for keeping the concentration of inhibitors low in order to provide adaptation time for the archaea. The upflow anaerobic filter (UAF) operating system offers more process stability and shorter start-up time (Hamdi 1996). Later studies confirmed the effectiveness of the UASB reactor and reported operation parameters like a HRT of up to 25 days, COD removal up to 90%, average organic loading rate (OLR) of 5 kg COD /(m^3·d) and 0.30-0.35 Nm^3 CH_4/kg $COD_{removed}$ (Paraskeva et al. 2006).

The low nitrogen content and buffer capacity, and high content of inhibiting compounds makes the OMW unsuitable for mono-digestion. Co-digestion fermentation with OMSW at thermophilic conditions showed that a HRT of 36 days, OLR of 3.62 kg COD /(m^3·d) are optimal for obtaining 69% soluble COD removal and methane production of 46 Nm^3/m^3 OMW per day (Boubaker et al. 2007). Two-phase anaerobic co-digestion under mesophilic conditions has also been explored (Boubaker et al. 2010). The optimal HRT value for the first stage was determined to be 24 days, and for the second stage 36 days, with 82% COD removal and 70-78% polyphenol removal. Thermophilic co-digestion with abattoir wastewaters in an UAF reduces the main problems encountered during their mono-digestion by optimizing the C/N ratio and decreasing the polyphenol concentration (Gannoun et al. 2007). Poultry and swine manure has also been reported as suitable co-substrates for OMW fermentation. Addition of 70% (v/v) poultry manure with high TKN (4.9 g/l) and alkalinity (20.2 g $CaCO_3$/l) to the OMW resulted in a stable process HRT of 18 days and a biogas yields of 1.53 Nm^3/kg COD per day with a methane content in the biogas of 65% (Gelegenis et al. 2007). Swine manure and OMW optimal mix ratio was reported to be 33 to 67% (v/v). In this case 85-95% COD removal with 0.55 Nm^3 biogas / kg COD per day was reached (Azaizeh et al. 2010).

Pre-treatment methods like addition of soluble calcium salts for precipitating the lipids and pH adjustment with $CaCO_3$ and NH_4^+ led to COD and polyphenol removal of 78-88% and 12%, respectively. Targeted polyphenol removal was tested with coagulation, extraction or oxidation, and lead to 40% COD reduction and up to 13% phenol removal. About 80% of the polyphenols with molecular weight lower than 500 Da were degraded during the methanogenic anaerobic stage. For the phenols with a molecular weight more than 1000 Da, adsorption on betonite was successful. The final aerobic treatment stage achieved up to 96% COD removal (Paraskeva et al. 2006).

Despite its low pH and high organic matter and phenol concentration,

there have been several studies on monodigestion of OMSW. The maximum methane production was found to be 0.244 Nm^3 CH_4 /kg $COD_{removed}$ at standard temperature and pressure conditions (STP) at an OLR of 9.2 kg COD /(m^3·d) and a HRT of 17 days (Rincón et al. 2008). An interesting application of OMSW could be for hydrogen production in two-stage anaerobic fermentation (Koutrouli et al. 2009). The bio-hydrogen potential was estimated at 1.6 mmol H_2 per g total solids in two-stage fermentation (Gavala et al. 2005).

At a large scale, olive mill wastes are applied as a co-substrate in anaerobic digesters (biogas plant in Foggia, Southern Italy). The overview of studies on anaerobic digestion of olive mill residues shows rising interest on its disposal possibilities. The co-digestion of OMW and OMSW or with a nitrogen-rich substrate seems to be appropriate for achieving higher OLR and HRT in order to dispose of the polluting waste as fast as possible in an environmentally friendly way – as digestate - by gaining energy at the same time.

V. WASTES FROM SUGAR BEET PROCESSING FACILITIES

Another organic residue, whose potential for anaerobic digestion should be extended, is the waste from the sugar industry: sugar beet pulp, molasses and sugar beet leaves. These by-products can be used as animal feed, for paper, yeast and amino acid production, for the generation of alcohol including ethanol, and as a soil conditioner (EU 2006). Nevertheless, there are two reasons to implement the anaerobic technology in the sugar producing industry: to reduce the ecological impact and to reduce costs.

World sugar production rose from 166.6 million tons in 2007/08 to 174.1 million tons in 2011/12. In Europe, where sugar is produced from sugar beet, the production increased from 25.7 million tons in 2007/08 to 29.4 million tons in 2011/12 (Licht, 2012). In the European Union and Switzerland, 2.16 million tons molasses and 6.52 million tons fresh pulp (22% dry matter) were accumulated in 2010/11 (CEFS Statistics, 2011). For the processing of one ton sugar beet, excluding drying of sugar beet pulp, about 170 to 330 kWh are needed (Brooks et al. 2008). The Product Carbon Footprint (PCF) of sugar from sugar beet produced and refined in the EU, and from sugar cane imported and refined in the EU has been compared (Klenk et al. 2012). The PCF range for EU refined cane sugar is on average 642-771 kg CO_{2eq}/t sugar, which is similar if not higher than the one for the EU beet sugar – 242-771 kg CO_{2eq}/t sugar. The overseas transport and refining of sugar cane also adds a significant amount of emissions to the PCF. The land use efficiency of beet sugar is higher:

51% more land is required by cane systems to produce an equivalent set of products (sugar and co-products) with equivalent amount green house gas emissions. The impact of the emissions from land use change for sugar cane is also significant, but is rarely taken into account.
Another advantage of converting the energy from the factory residues into biogas to replace energy is the decrease in costs for sugar production to make it competitive on the world sugar market. In 2006 the Common Market Organisation (CMO) for sugar production in the EU was reformed. The quota for sugar production has been reduced by 30% to 13.3 million tons. The prices sank by 36% to 404.4 €/ton in 2009/10 and the minimum sugar price is not guaranteed by intervention mechanisms any more (CEFS Statistics, 2011). This reform and the higher amount of fossil energy needed for the production of sugar beet could give good reason for the use of energy from renewable sources, e.g. biogas from the factory residues.
There are already examples of application of this concept on large scale. The biogas plant in Kaposvár, Hungary (Magyar Cukor ZRT, Agrana) uses since 2008 almost the half of the daily produced sugar beet pulp amount, and produces 110,000 m^3 biogas (55% methane), which covers 40% of the energy need of the factory (Chemie Report 2007). In the Netherlands there are two biogas plants built at sugar factories of Suiker Unie: in Dinterloord since 2011 and Vierverlaten since 2012. The used substrates are sugar beet pulp, sugar beet tails, residues from the potato industry and other agricultural products (Anonymous 2012). The biomethane will be injected in the national grid. Since 2011 a biogas plant has been in operation nearby Parma, Italy with a co-digestion of sugar beet pulp and maize silage (AAT 2012). From summer 2013, a biogas plant on site of the largest European sugar factory, British Sugar, in Wissington, England should start its operation (Pollit 2011).

SUGAR BEET REFINERY PROCESS

The sugar beet is first washed with a dilute CaO solution at pH 10-12 (Figure 3). The liquid is recirculated after sedimentation. The next step is slicing of the sugar beets to pulp. In the subsequent diffusion step, the extraction of the sugar into an aqueous solution at pH 5.6-5.8, 30-60 °dH (German degrees) in countercurrent exchanger, first for 5 minutes at 70-78°C to denature the cells, then for 70-85 minutes at 69-73°C. To avoid microorganism activity, formaldehyde is added. The liquid phase is the so-called raw juice. The solid phase is the pulp with 95% moisture which is then screw pressed to 75% moisture.
Impurities from the raw juice are removed by carbonisation with milk of

lime at 60-70°C, pH 10.8-11.9 for 20 minutes, followed by 30 minutes at 80-85°C. At this stage, impurities such as sulfate, phosphate, citrate and oxalate are precipitated as their calcium salts, and proteins, saponins and pectins also aggregate in the presence of Ca^{2+} ions. By multiple-effect evaporation, usually five stages, the raw juice is converted to thick syrup. The last step is the crystallization. The remaining molasses contain still sugar, and also other impurities (Genie 1982).

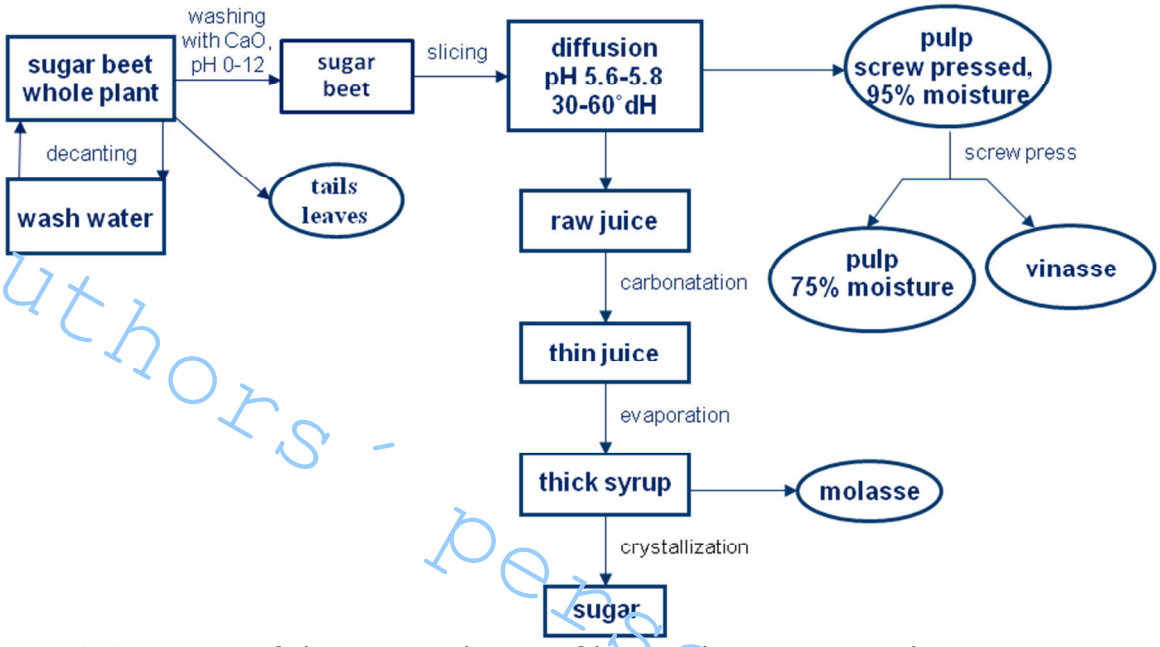

Figure 3 Overview of the accumulation of by-products in sugar beet processing

CHARACTERISATION OF THE RESIDUES FROM SUGAR PRODUCTION

The sugar beet residues most commonly used for biogas production are sugar beet pulp (SBP) and desugared molasses. The characteristics of wastes from the sugar industry are shown in Table 9.

Table 9 Chemical characterization of the wastes from the sugar production from sugar beet – for comparison also sugar beet silage is shown (Weiland 1993, Brooks et al. 2008, Demirel et al. 2009, Fang et al. 2011, Alkaya et al. 2011)

Substrate	pH	TS [%]	VS [%]	COD [g/kg]	TKN [g/kg]
Sugar beet pressed pulp	3.9-4.0	15-18	14-17	180-260	1.2-3.1
Sugar beet silage	3.3	20	19	265	3.1
Desugared molasses	n.a.	49.8	32.6	49.8	6.7
Waste- water	6.8	6	2.8	6.62	0.01

The sugar beet pressed pulp composition offers good possibilities for biological treatment (table 9). The main components of the sugar beet pulp are sugars – about 74% of the dry matter. The lignocellulosic fraction of the dried pulp is: cellulose 22-30%, hemicellulose 24-32%, pectic substances 24-32% and lignin 3-4% (Coughlan et al. 1985). The pulp has a cylindrical shape with 6-9 mm diameter and 20-40 mm length. The desugared molasses contain a high amount of ions: potassium 160 g/l, sodium 36 g/l and calcium 5 g/l, which can inhibit the biogas process (Fang et al. 2011). The interest in the anaerobic digestion of residues from the sugar industry has increased over the last two decades.

Table 10 composition of sugar beet (Micard et al. 1996)

Component	% TS
Rhamnose	2.4
Fucose	0.2
Arabinose	20.9
Xylose	1,7
Mannose	1.1
Galactose	5.1
Glucose	21.1
Galacturonic acid	21.1
Ferulic acid	0.8
Diferulic acid	0.04
Protein (Nx6.25)	11.3

EXPERIENCES IN AD OF SUGAR PRODUCTION RESIDUES

The digestibility of sugar industry residues has been examined at different conditions, concerning the organic loading rate (OLR), temperature, hydraulic retention time (HRT), mono- and co-digestion, and one- and two-stage fermentation. In 1993, Weiland focused on the effect of the C/N ratio on the anaerobic digestion of agro-industrial residues, including SBP, and the one- and two-step fermentation. The C/N ratio of sugar beet pulp was determined to be between 35 and 40, which is optimal for the biogas process. Therefore, Weiland (1993) found no interest in investigating a two-step (also called stage or phase) process. Nevertheless, according to literature other advantages of the two-stage fermentation of sugar beet pulp were shown later. Hutnan et al. (2011)

reported similar biogas production in two-stage as in one-stage anaerobic digestion, but a better COD removal. The optimal HRT in the first stage is two days; the optimal waste to inoculum ratio is 1:1 for sugar beet pulp and waste water at mesophillic temperature (Alkaya et al. 2011). Brooks et al. (2003) achieved a stable biogas production of 530 Nm^3/t COD or 610 Nm^3/t VS at standard temperature and pressure conditions (STP) at an OLR of 10 kg COD /(m^3·d). Methane content was 50-53% and COD removal was 72%. Single-stage, batch, unmixed, leach-bed, laboratory scale thermophilic anaerobic digestion of spent sugar beet pulp resulted in 0.336 Nm^3 CH_4 /kg VS and 95% of the methane yield was achieved after 8 days (Koppar et al. 2008). This result confirms the fast degradability of sugar beet pulp. Biochemical methane potential (BMP) of sugar beet pulp tests resulted in 430 Nm^3 CH_4/ kg VS (Kryvoruchko et al. 2009). Thermophilic co-digestion of sugar beet pulp, desugared molasses and cow manure showed a decrease in the inhibiting potential of the desugared molasses, mainly due to the dilution with manure, which provides a buffer capacity and nutrients (Fang et al., 2011). Sugar beet molasses were digested in an upflow anaerobic fixed bed reactor at mesophillic temperature, with 20 h HRT and influent COD ranging from 7.8 to 9.6 kg COD / (m^3·d). The COD removal ranged from 75 to 93% (Farhadian et al. 2007). Sugar beet leaves improved the methane yield from potato waste up to 62% (0.32 Nm^3 CH_4 /kg $VS_{degraded}$ by optimizing the C/N ratio in batch fermentation process (Parawira et al. 2004). The wastes from the sugar industry have a high energy potential, so this should be used as substitute of fossil energy and for making sugar production more profitable.

VI. CONCLUSION

The residues of five typical food, beverage and biofuel processes with high potential for biomethane production were described in detail. This is by no means a complete list. Information on for example the biomethane potential or chemical characterisation) of other industrial by-products and residues can be found in standard works such as Braun (1982), Speece (1996), Bischofsberger et al. (2005).

Although most sectors of the food, beverage and biofuel industry are able to generate revenue by selling their organic residues as an animal feed, some of them have to pay for their disposal (e.g. abattoirs). The cost of disposal depends on the waste composition and varies from country to country. In Austria, the disposal costs range between 25 and 30 €/t for

blood and between 40 and 45 €/t for other residues coming from the pig slaughter process.
Economic developments in recent years such as constant rising of costs of energy and prices for chemical fertilisers have led to a paradigm shift, especially within the food and beverage industry.
More and more companies are optimising their energy balance in terms of utilising their organic residues to become energetically self-sufficient by using integrated AD technology and combined heat and power plants.
Among the industries presented in this chapter the sugar industry has the strongest interest in integrating AD technology to its production process. There are already a handful of AD plants which are fully integrated into the production process.

Abattoirs also have great interest due to the lack of alternative utilisation. The successful integration of AD technology in the production process of an abattoir in Austria may lead to the construction of further process-integrated biogas plants. AD plants have already been implemented in the bioethanol industry. However, no large-scale mono-fermentation AD plants for breweries or olive mills are known, although there is considerable potential.
The main bottlenecks in the anaerobic digestion of industrial feedstocks are ammonia inhibition and foaming (slaughterhouse waste, bioethanol residues), management of digestate (bioethanol residues), lignocellulose containing compounds (brewer´s spent grains) or other inhibiting substances (e.g. polyphenols in olive oil waste).

The five chosen examples described in this chapter represent these typical bottlenecks and challenges, which can be overcome by use of appropriate technologies and adequate process control.
Moreover there has been much progress especially in the field of efficiency of biomass usage through cascading (bio-refinery concept) of organic residual materials.

To summarise, anaerobic digestion (AD) is a well-known technology to treat industrial organic residues almost regardless of their consistency. The utilisation of industrial organic residues by AD is an appropriate way to improve both the process and the economic efficiency of an industrial factory. Furthermore, it enables a controlled stabilization of the organic material, reduces greenhouse gas emissions and contributes to the closing of nutrient cycles.

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molasses alcohol stillage. Water Science and Technology 36 (6–7) 441-448.

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BERUFSERFAHRUNG

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Projektleitung

Holzforschung Austria, Bereich Holzschutz, Wien

- Projektidee und -umsetzung
- Kostenplanung
- Mitarbeiterführung
- Planung, Organisation, Berichtslegung, und Durchführung von wissenschaftlichen Versuchsreihen
- molekularbiologische Analytik: qPCR
- ELISA und ELASA Methodenentwicklung
- GC-MS Analysen

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Wissenschaftliche Mitarbeiterin

BIOENERGY 2020+ GmbH, Forschungsstätte IFA Tulln

Institut für Umweltbiotechnologie, BOKU

- zweistufige anaerobe Fermentation, reaktorplanung und Prozessoptimierung
- Optimierung von Biogasausbeuten von Reststoffen aus der Lebensmittelindustrie
- Analysen zum Monitoring vom anaeroben Fermentationsprozess: TS, Fettsäurenbesimmung mittels HPLC, CSB, Pufferkapazität

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Projektmitarbeiterin

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- molekularbiologische Analytik: PCR
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Assistentin Event Manager *in Kursalon Hübner*

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Studium der Lebensmittel- und Biotechnologie

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- Umweltbiotechnologie
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SPRACHEN UND IT – KENNTNISSE

Bulgarisch	*Muttersprache*	
Deutsch	*verhandlungssicher*	*C1*
Englisch	*fließend*	*B2*
Russisch	*gut*	*B2*
IT	*MSOffice; Auto CAD; Origin*	

PERSÖNLICHE DATEN

Geburtsdatum /-ort	*4.10.1980 in Dobrich, Bulgarien*
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PUBLIKATIONEN

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Herndl, A., Marzban, G., Kolarich, D., Hahn, R., Boscia, D., Hemmer, W., Maghuly, F., Stoyanova, E., Katinger, H., Laimer, M., 2007. Mapping of Malus domestica allergens by 2-D electrophoresis and IgE-reactivity. Electrophoresis 28, 437–448. doi:10.1002/elps.200600342

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Career objectives Project management; R&D

Key skills and competences

- Research project management, organising tasks effectively
- Business management
- Experience in planning and carrying out experimental research
- Good research skills with creative approach
- To set priorities to the work in response to deadlines
- To resolve problems independently
- Flexible with working hours and travelling

Career history

01/2014 – 09/2016	**junior researcher, project manager** at Holzforschung Austria department Wood protection, molekularbiologcal analysis of wood destroying fungi, qPCR, ELISA, ELASA, GC-MS
07/2010 – 12/2013	**junior researcher,** K1-Centre BIOENERGY 2020+ GmbH, location IFA Tulln, University of Life Sciences, on the topic: Two-stage anaerobic fermentation of organic residues from the food industry; Monitoring of anaerobic fermentation process: HPLC, GC/GC-MS; CSB, buffer capacity; DGGE
10/2009 – 07/2010	**junior researcher** at Holzforschung Austria, preparation of wood samples for further analysis: microwave disintegration, qPCR, potentiometric titration of polymeric betaines in wood preservatives, literature reviews
07/2008 – 08/2008	**traineeship** at Zuckerforschung Tulln, Austria, research on the suitability of hop resistant lactic acid bacteria for ensiling, and optimization for their application in the production process, literature reviews
10/2005 – 10/2007	**junior researcher** at the Plant Biotechnology Unit at the Department of Biotechnology, University of Life Sciences, Vienna on the topic: Serological Investigations of Fruit and Pollen Allergens

Education

01/2017 – 03/2017	**Business management**, Karl-Franzens-University, Graz
03/2002 – 03/2010	studies of **Biotechnology**, University of Life Sciences, Vienna
03/2000 – 02/2002	studies of **Nutritional Sciences**, University of Vienna
10/1998 – 02/2000	studies of **Nutritional Sciences**, University of Food Technology, Plovdiv, Bulgaria

Side-line jobs

03/2000 – 05/2009	Au pair, waitress, shop assistant, customer service

Language skills

Bulgarian	native
German	fluent
English	good
Russian	good

PC MS Office; Auto CAD; Origin

Activities In the evenings and during the weekends I enjoy meeting friends, dancing (Bulgarian folklore dances), reading, gym training, improving my Russian and Italian, enrich also my coin collection. I also enjoy travelling.

References

Stoyanova E, Lundaa T, Bochmann G, Fuchs W (2016): Overcoming the bottlenecks of anaerobic digestion of olive mill solid waste by two-stage fermentation. Environmental Technology 0, 1–25. doi:10.1080/09593330.2016.1196736

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Further references available.