

CHARACTERIZATION OF THE BIOMETHANIZATION PROCESS IN
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Abstract: The aim of this paper is to show the basic principles of the anaerobic digestion process. All the stages of degradation, such as hydrolysis, acidogenesis, acetogenesis and methanogenesis are characterized. Biodegradable organic matter consists of three main types of substances: carbohydrates, proteins and lipids; the metabolic pathways of their decomposition are described. The last part of the paper presents the co-digestion process, its benefits and technological parameters required to make that process attractive from an economical and environmental point of view.

Keywords: biomethanization, anaerobic digestion pathways, protein degradation, lipids degradation, carbohydrates degradation, co-digestion, anaerobic digestion co-substrates

INTRODUCTION

In environmental issues a widely accepted concept of sustainable development, which implies not only ecological, but also social and economic responsibilities, should be taken into account. A sustainable sewage and solid waste management is a difficult task from economical and technical point of view (Scharff et al., 2006). However, biogas production - one of the accelerating sectors of renewable energy - has become one of the activities which fulfil the above requirements. It has been applied to the degradation of different types of waste such as sewage sludge, the organic fraction from municipal solid waste, agriculture waste (manure), biowaste and industrial organic waste which are usually seen to be of a negative value. Since environmental regulations in the European Union are based on the concept of prevention and control of pollution, therefore waste-sludge management, waste disposal and environmental pollution are strictly linked and the production of biogas from residues seems to be a promising solution. Biogas is formed as a product of the anaerobic digestion process and usually contains about 40-70% of methane. With regard to its high energetic value methane can be considered as a source of energy for producing heat or electricity. It should be noted that the benefits of waste biomethanization depend on the improvement of the process in the aspects of achieving both a higher biogas yield per kilogram of volatile solids (VS), increased methane share in biogas and higher levels of solid degradation. Considering the economical and ecological benefits related to one-source waste anaerobic degradation, co-digestion defined as biomethanization of at least two types of different organic waste seems to be a more favourable solution.

Sewage sludge is characterized by a high content of organic compounds. Its anaerobic stabilization leads to 45-70% degradation and generates biogas containing about 55-70% of methane. Due to the large amount of sewage sludge (SS) produced in wastewater treatment plants

and a high number of operating digesters, the co-fermentation of SS with other types of organic waste can be very attractive. The group of possible co-substrates includes the organic fraction from municipal solid waste (OFMSW), manure and organic waste from different sources. An issue of special significance is the separation of the OFMSW from municipal solid waste and subsequent incorporation of such a co-substrate in biogas production carried out in controlled operating conditions. This approach is consistent with regulations implementing waste management strategies, policies and legal regulations aimed at reducing methane emission from landfills. It has been calculated that methane emission from anaerobic biodegradation of organic substances by bacteria in landfills and in the wastewater treatment plants (WWTP) is respectively about 13% and 10% of the overall annual world methane production (Wuebbles et al., 2002). Moreover, methane is classified among gases causing greenhouse effect and has, on a per molecule basis, a 20 times higher effect than CO₂ (Milich, 1999).

Landfilling characterized by systematic dumping of complex mixture in landfill sites, sometimes even containing toxic residues, has so far been the most popular way of utilizing solid waste. Considering that waste decomposition and sewage digestion are recognized as a significant source of atmospheric methane, a special emphasis should be put on developing technologies and systems allowing to arrest methane and to reduce its uncontrolled emission by using organic-rich waste as a substrate for producing renewable energy. Following this, a tendency is now observed to displace landfilling by anaerobic digestion of the organic fraction from municipal waste. Moreover, anaerobic digestion is an excellent supplement and alternative to composting.

CHARACTERISTIC OF THE BIOMETHANIZATION PROCESS

Organic matter can be degraded anaerobically in nature as well as in technical systems. This process is also called biomethanization or biogasification. Anaerobic digestion is defined as a biomass conversion process without external electron acceptors such as oxygen (indispensable in the aerobic process) and nitrate or sulfate (necessarily present in anoxic processes, i.e. denitrification and sulfate reduction) (Angelidaki and Sanders, 2004, Grady et al., 1999). Degradation of organic compounds due to their complex nature is attributed both to the form (soluble or insoluble) and character of the substrates involved. Insoluble organic materials have to be solubilized and the large soluble organic molecules must be reduced in size to make transport across the cell membranes possible.

The conversion of organic compounds to methane and carbon dioxide requires an activity of various bacterial communities consisting of several groups (producing specific enzymes) which are involved in different metabolic pathways, divided into four main phases. The biomethanization process occurs by a sequence of the following steps:

- hydrolysis,
- acidogenesis,
- acetogenesis,
- methanogenesis.

Methane production is directly related to a decrease of the chemical oxygen demand (COD) in waste during anaerobic degradation, causing the stabilization of the biodegradable organic matter. Thus methane yield can be evaluated from the COD balance in the system, based on the COD removed. In standard conditions methane production is about 0.35 m³ per kg of the COD degraded. Primary sewage sludge in municipal wastewater treatment plants can be characterized by the value of 0.7 m³ methane produced per kg of volatile solids destroyed (Grady et al., 1999).

Particulate organic matter, involving long-chain organic compounds such as proteins, carbohydrates and lipids, is converted into the final products of anaerobic degradation according to the scheme in Figure 1. The scheme shows numerous parallel steps describing conversion of specific substrates (Kashyap et al., 2003). The particular phases, such as hydrolysis, acidogenesis, acetogenesis and methanogenesis, have been characterized individually.

The essential product of the biomethanization process is energy-rich biogas - a mixture of mainly methane and carbon dioxide, while other gases constitute less than 1% of the gas generated. The energetic potential of biogas is high and usually exceeds the level of energy needed in running the digester.

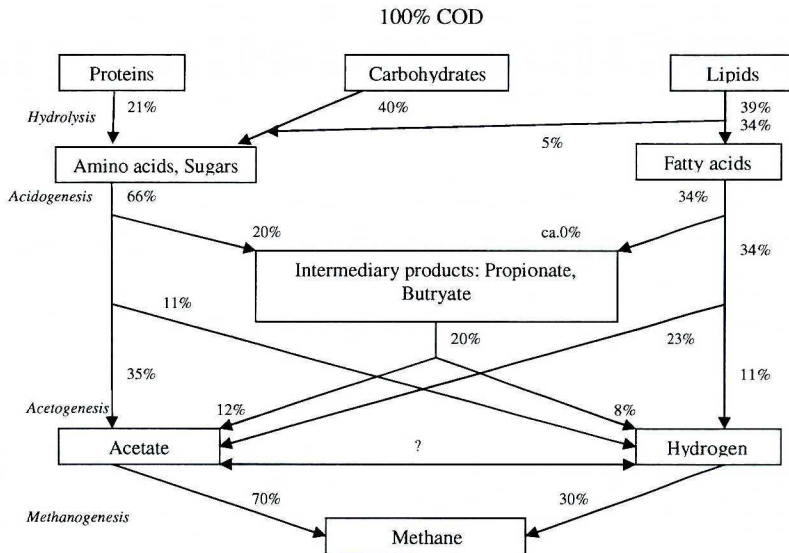


Fig. 1. Scheme of an anaerobic digestion path with COD shares (Siegrist et al., 1993)

Hydrolysis

Hydrolysis of particulates to soluble molecules is the first step in an anaerobic digestion process. Hydrolysis means both the solubilization of insoluble particulate matter and the biological decomposition of organic polymers to monomers and dimers. Such a conversion needs extracellular enzymes produced by hydrolytic bacteria which have an ability to degrade both soluble and non-soluble organic compounds of a high-molecular weight (Mata-Alvarez, 2003). By definition, hydrolysis causes a molecule split and the hydrogen and hydroxide ions from the water molecule can be attached to the separate products. Three main mechanisms exist for the release of enzymes and the subsequent hydrolysis of the complex substrate during anaerobic digestion:

1. the microorganism produces enzymes to the liquid, where they adsorb to a particle and react with a soluble substrate,
2. the organism attaches to a particle, secretes enzymes into its immediate environment and then gains the benefits from the dissolved substrates released,
3. the organism has attached enzymes which may also act as transport receptors to the cell interior; such a mechanism indicates an adsorption onto the surface of the particle.

It is difficult to describe the whole process, thus hydrolysis of complex organic matter depends on several factors such as particle size, pH, enzyme production, as well as their diffusion and adsorption to the particles. There are three main types of compounds that can be hydrolyzed: carbohydrates, proteins and lipids. Analyzing their mass balance throughout the system, inert organic compounds should also be included, whereas mineral compounds are excluded. Many groups of fermentative bacteria are able to produce extracellular enzymes necessary to hydrolyse complex organic matter. Hydrolysis of proteins into amino acids depends on proteolytic bacteria which produce protease enzymes. The cellulolytic and xylanolytic microorganisms generate cellulase

and xylanase enzymes that convert carbohydrates (cellulose and xylan) to glucose and xylose. Lipase which is obtained from lipolytic bacteria degrades lipids into glycerol and long-chain fatty acids (Eriksson et al., 2002; Wong et al., 1999; Cammarota et al., 2006). Considering the susceptibility of substrates to enzymatic conversion it should be noted that hydrolysis of the lignocellulose-based compounds needs an enhancement pretreatment, using such technology as steam explosion, providing the biomass fractionation (Shimizu et al., 1998, Fernandez-Bolanos et al, 2001, Sun et al., 2004).

Acidogenesis

Acidogenesis represents a second stage of the anaerobic digestion process. During this phase, also called acid fermentation or simply fermentation, sugars and amino acids are converted into alcohols, volatile fatty acids (propionic, butyric and valeric acid), acetate, hydrogen and carbon dioxide. Ammonia is also produced by degradation of amino acids. The microorganisms involved in acidic decomposition are called fermentative bacteria. Taking into account that efficiency of anaerobic degradation depends mainly on the coordinated activity of acidogens and methanogens, any imbalance in the metabolism of the consortia mentioned leads to disturbances of the process. Accumulation of the intermediary products (such as VFAs) in a high concentration can induce inhibition in a methanogenic microorganism's activity. Acidogenesis is surely sensitive to many factors such as substrate character, hydraulic retention time (HRT), pH, temperature and organic loading rate. Temperature is an extremely important inhibitory factor; it has been observed that lower operational temperature causes a decrease of the activity of the microorganism. Additionally it has been reported that biogas yield drops with decreasing temperature (Shin et al., 2005). Another essential factor affecting acidogenesis is pH. According to Yu (2003) the degree of acidification increased with pH from the value of 32% at pH 4.0 to 71.6% at pH 6.5, whereas it dropped when pH increased to 7.0. The optimum pH for acidogens was found to be around 6.0.

Acetogenesis

Acetate is one of the most important intermediates in anaerobic digestion process. During acetogenesis, obligatory hydrogen-producing acetogens (OHPAs) oxidize reduced compounds such as VFAs (propionate, butyrate) and alcohol (ethanol) to hydrogen, carbon dioxide and acetate. Acetate can also be formed during homoacetogenesis when homoacetogenic bacteria (HA) use the CO₂, H₂ and multibicarbonate compounds to acetate production (Mata-Alvarez et al., 2003). It was observed that homoacetogenesis occurred only in psychrophilic environment (Siriwongrungson et al., 2007). Homoacetogenic microorganisms have an ability for easier adaptation in lower temperatures than hydrogenotrophic methanogens which are their competitors for hydrogen. Under mesophilic and thermophilic conditions HA cannot compete with methanogenic bacteria due to more energy generated by them.

Methanogenesis

Methane is produced via two pathways of methanogenesis: hydrogenotrophic and acetoclastic. Hydrogenotrophic methanogenic bacteria (HMB) convert hydrogen and carbon dioxide to methane (autotrophic conversion) whereas acetoclastic methanogenic bacteria (AMB) represented mainly by *Methanosarcina* and *Methanosaeta* produce methane and carbon dioxide from acetate, formate or other organic compounds (heterotrophic metabolism).

Hydrogenotrophic methanogenesis generates about 30-40% of the overall production of CH₄ originated from the biomethanization process in according to the reaction:



Hydrogenotrophic methanogens compared to AMB are characterized by higher growth rates, with the doubling time of 4-6 hours. On the contrary, AMB grow slowly with the doubling time around

24 hours. Moreover, they can suffer from the presence of an intermediary product such as hydrogen or sulfates entering the digester (Bohn et al., 2002).

Aceticlastic methanogenesis typically accounts for 60-70% of the overall methane production and requires an activity of two *Archaea* groups: *Methanosaeta* and *Methanobacteriales*. In such a pathway methane is produced from organic substrates according to the reaction:



However the four-stage anaerobic digestion is commonly accepted, it should be noted that Pohland (1992) identified three additional phases of the anaerobic degradation processes. The distinguished stages include: oxidation of reduced compounds by sulfate-reducing bacteria (SRB), acetate oxidation by sulfate-reducing or nitrate-reducing bacteria (NRB) and oxidation of hydrogen (or formate) by SRB or NRB. With the presence of sulfate or nitrate the activity of SRB or NRB can be observed, resulting in oxidation of butyric and propionic acids, as well as alcohols, to carbon dioxide and acetate, in subsequent acetate oxidation to carbon dioxide and in hydrogen oxidation to methane.

Organic matter includes both substrates of various character (carbohydrates, proteins and lipids) and of diverse susceptibility to enzymatic conversion. As a result, the compounds in question are degraded differently via specific pathways; therefore for their proper understanding a detailed characteristic of the decomposition has to be known.

Anaerobic biodegradation of carbohydrates

Carbohydrates are homo- and heteropolymers of pentoses, hexoses or sugar derivatives which occur in soluble form or as particles. Hydrolysis and subsequent acidogenic fermentation of its products are catalyzed by the same trophic group of microorganisms. The hydrolysis rate of polymers can vary significantly. Pectin and hemicellulose are converted ten times faster than lignin-encrusted cellulose. The rate of cellulose biodegradation depends mainly on the forms of the cellulose in the substrate. If cellulose is lignin-encrusted, lignin prevents access of cellulases to the fibers of cellulose and thus restricts hydrolysis. When cellulose has mainly a crystalline form, cellulases can easily attach to it and then hydrolysis can be a relatively fast process. High concentration of crystalline cellulose in the reactor feedstock leads to an inhibition of the biomethanization on account of propionate and butyrate formation during acetogenesis. Due to the narrow access to lignin-encrusted material by cellulases, hydrolysis of such a substrate is the limiting stage in its anaerobic degradation to methane and carbon dioxide (Schwarz, 2001). Figure 2 presents pathways of degradation of lignocellulose and cellulose.

Anaerobic biodegradation of protein

Proteins are macromolecules which can be either solid or soluble. The pathways of protein biodegradation are shown in Figure 3. Hydrolysis is catalyzed by many types of proteases which cleave dipeptides, oligopeptides or membrane-permeable amino acids. Hydrolysis of proteins, in contrast to hydrolysis of carbohydrates, requires a neutral or weakly alkaline pH. Fermentation of carbohydrates decreases the pH value due to the formation of volatile fatty acids. However, fermentation of amino acids does not lead to a pH change due to ammonia formation. The presence of ammonium ions together with the CO₂-bicarbonate-carbonate buffers the system well during acidogenesis by a pH stabilization of protein-containing feedstock. Acetogenesis of proteinaceous medium requires a low partial H₂ pressure which can be obtained by a syntrophic interaction between fermentative protein-degrading bacteria and acetogenic and methanogenic or sulfate-reducing consortia (Gallert and Winter, 2005).

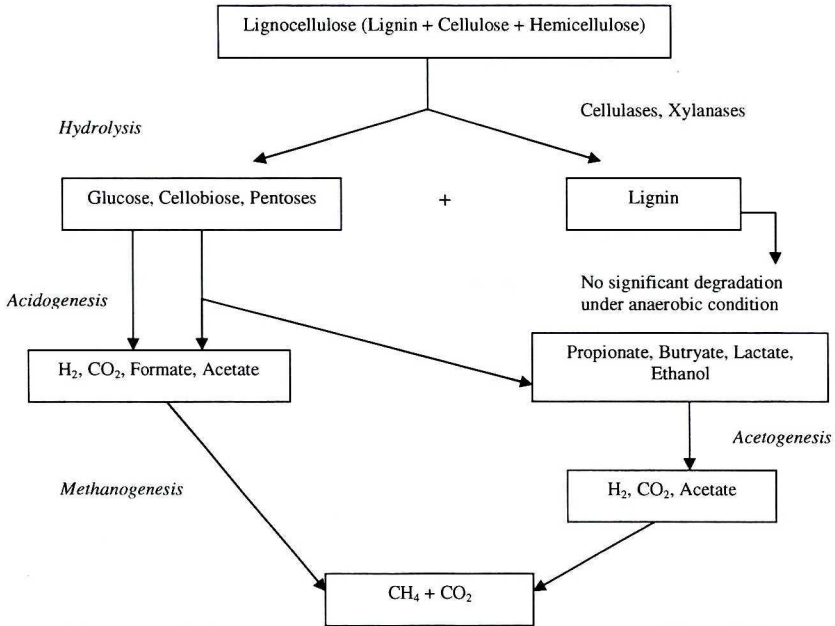


Fig. 2. Anaerobic degradation of lignocellulose and cellulose (Gallert and Winter, 2005).

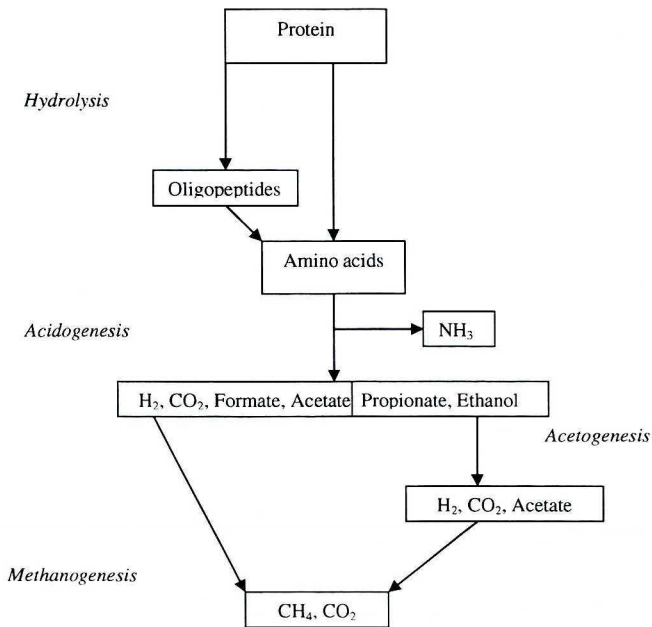


Fig. 3. Anaerobic degradation of protein (Gallert and Winter, 2005).

Anaerobic degradation of lipids and neutral fats

Fats and lipids are the third group of biopolymers that contribute substantially to the COD value. A scheme of anaerobic degradation of lipids is shown in Figure 4. Solid fats, lipids or oils must previously be emulsified to provide a maximum surface for hydrolytic cleavage by lipases or phospholipases. Glycerol, saturated and unsaturated fatty acids are generated from neutral fats. Lipolysis of phospholipids produces fatty acids and amino alcohols, whereas lipolysis of glycolipids forms fatty acids, amino alcohols and hexoses. Lipids and neutral fats can be degraded to biogas by cooperation between fermentative and methanogenic microorganisms in low-loaded systems, or by interaction between fermentative acetogenic and methanogenic bacteria in high-loaded systems. The long-chain fatty acids are decomposed by acetogenic microorganisms via β -oxidation to acetate and hydrogen (Gallert and Winter, 2005). Odd-numbered fatty acids are degraded to acetate, propionate and hydrogen. Even-numbered fatty acids are converted to acetate and hydrogen. Methanol and ethanol, which are formed from choline, are decomposed to acetate, hydrogen and carbon dioxide. During the methanogenic phase acetate, hydrogen and CO_2 are converted to biogas.

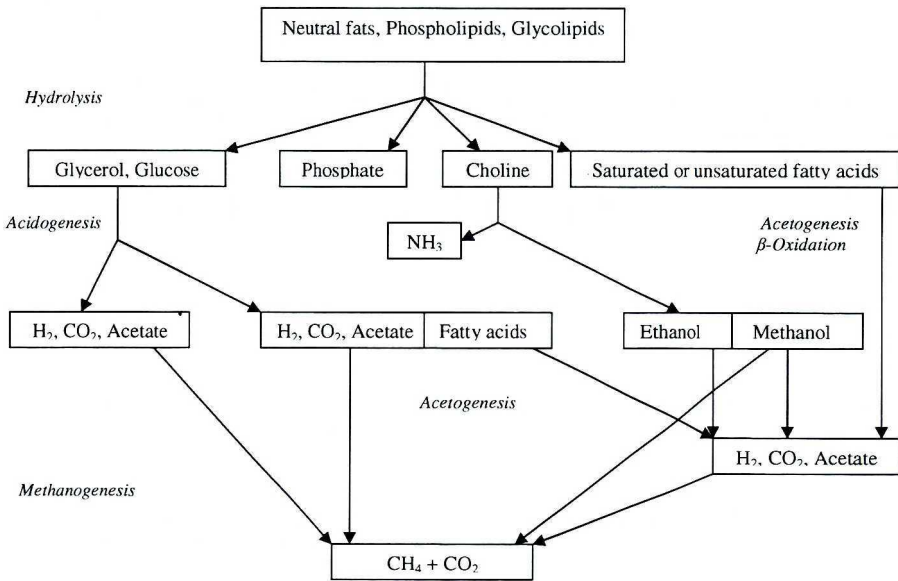


Fig. 4. Anaerobic degradation of fats (Gallert and Winter, 2005).

It should be noted that fats, proteins and carbohydrates can be degraded both under mesophilic and thermophilic conditions. However, the microorganism consortia responsible for their transformations (involved in a substrate and intermediate conversion) are quite different.

Co-digestion

Co-digestion is an anaerobic degradation of at least two organic wastes originating from different sources (Fig. 5). The process in question, also called the co-fermentation or biomethanization of selected waste mixture, enables the decomposition of organic substrates with high biogas potential and various characteristics. This makes conversion more profitable in comparison with one-source

waste degradation both from an economical and environmental point of view. There are some wastes recognized as suitable substrates for co-digestion and suggested for improving biogas production. They include sewage sludge, source-sorted organic fraction of municipal solid waste, manure, farm waste and some industrial organic wastes (e.g. food waste). Such co-substrates could effectively be degraded under specific environmental conditions (pH, temperature, hydrogen concentration) in suitably chosen mixtures. Some co-substrates require a comprehensive and costly pretreatment (e.g. source-separated organic fraction from municipal waste or lignocellulose-based waste); some, due to their potential hygienic risk, have to be treated by thermal hygienization. The bio-components and bio-waste which can be used in the co-fermentation process, as well as those with poor properties, are presented in Table 1.

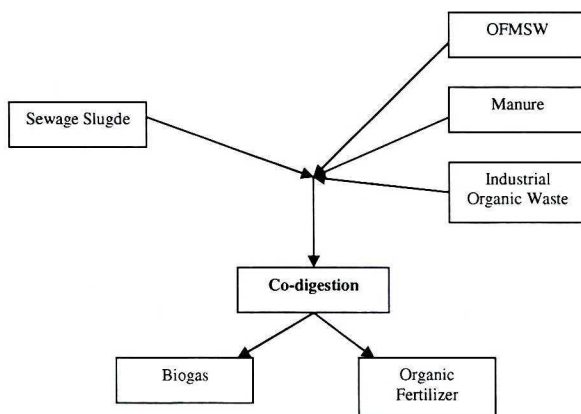


Fig. 5. Substrates and products of the biomethanization process of selected waste mixtures

Considering the general problems related to one-source waste fermentation, co-digestion seems to be a promising solution. Benefits of co-digestion consist in (Cecchi et al., 1996):

- dilution of toxic substances coming from any of the substrates involved,
- improved nutrient balance,
- higher biogas yield and an increased load of biodegradable organic matter,
- synergetic effects on microorganisms,
- high digestion rate,
- possible removal of some xenobiotics (detoxification based on co-metabolism process).

Taking into account the large amount of sewage sludge (SS) produced in wastewater treatment plants and the number of existing digesters to stabilize it, the co-fermentation of SS with another type of organic waste is very attractive. Anaerobic digestion of SS as a single substrate faces many problems due to its characteristics such as low C:N ratio, low content of total solids, high concentration of macro- and micronutrients and low content of dry matter. Co-digestion of sewage sludge and OFMSW is an alternative to the conventional separate one-substrate degradation.

Table 1. Advantageous and poor components for co-fermentation process (Braun et al., 2003)

Material	Excellent	Good	Poor
Biogenic materials from agriculture - straw and other fibrous plant residues - green plant material, crops, grain, silages		+	+
Harvest residues		+	
Animal manure		+	
Yeast and yeastlike products	+		
Fruit, corn, potato (slops, skins, seeds)	+		
Animal and slaughterhouse waste: - animal fat - blood - fish waste - chicken waste - animal parts	+	+	+
Waste from plant and animal fat products: - spoiled plant oils - oil seed residues - fats - edible oil sludge - edible fat sludge	+	+	
Pulp and paper industry wastes		+	
Sludge from gelatin production	+		
Sludge from starch production	+		
Residues from potato starch production	+		
Residues from maize starch production	+		
Residues from rice starch production	+		
Slaughterhouse wastes - animal fat - flotation sludge - blood - fish waste - chicken waste	+	+	
Food industry waste		+	

OFMSW, due to its composition, requires more complex metabolic pathways to be converted to methane. Furthermore, such a substrate induces a lot of technological (i.e. ammonia deficit) and technical (i.e. transport of dense medium) problems due to the following features: high content of dry matter (30-50%), high C/N ratio, deficit of micro- and macronutrients, or presence of toxic compounds (heavy metals and phthalates) (Hartmann et al., 2003). On the other hand OFMSW contains a high amount of easy biodegradable matter resulting in a methane yield of up to 330 dm³/kg VS (Rintala and Jarvinen, 1996). In accordance with this, organic fraction of municipal solid waste should be degraded together with another component, such as sewage sludge, livestock waste or other organic wastes to attain possible benefits. Nowadays only 7% of the overall OFMSW in Europe is utilized in co-fermentation and the percentage share of co-fermented OFMSW will certainly be rising.

Special emphasis should be put on effective degradation of manure because its share in the total organic waste collected in Europe reaches 91% and significantly exceeds other shares, which amount to 4%, 2% and 3%, respectively, for OFMSW, sewage sludge and industrial organic waste (Braun et al., 2003). Manure is an excellent co-substrate, which buffers the system due to its high-ammonia content, supplies the nutrients required for bacterial growth and dilutes feedstock as a result of its low dry matter (3-9% in waste from various sources). However, in a single substrate digestion of livestock the methane yield is relatively low and depends both on the waste source and reactor loading rate, ranging from 35.6 to 290 dm³/kg VS at mesophilic conditions (Hansen et al.,

allows to improve the nutrient balance, dilutes potential toxic compounds and leads to an increase in the biogas yields and the quality of the fertilizer.

Anaerobic co-digestion of OFMSW and sewage sludge (SS) is especially attractive due to the large amount of sewage produced in wastewater treatment plants and the number of substrate characteristics of OFMSW and SS that are complementary in their combination (Fig. 6). Sewage sludge is characterized by a higher concentration of micro- and macronutrients than that of OFMSW, compensating the latter deficiency. An optimal mixture of OFMSW and SS for the co-digestion process depends on the waste characteristics and pretreatment technology. Biomethanization of a single substrate is used for both dry systems (TS content higher than 20%) and wet ones (TS less than 20%), whereas co-digestion is used mainly for the latter type. Wet digestion (or low load) systems are reported to operate with a high biogas production and VS reduction when the feedstock OFMSW:SS ratio amounts to 80:20 on a total solids basis and 25:75% as a volume ratio (Hamzawi et al., 1998). The feasibility of such a co-digestion was examined by Sosnowski et al. (2003), who later also proposed a simple kinetic model of anaerobic digestion (2008). Dinsdale et al. (1999) examined the co-digestion of waste activated sludge and fruit/vegetable waste, whereas Gomez et al. (2006) focused on the primary sludge and the fruit/vegetable fraction of the municipal solid waste as co-substrates for anaerobic digestion.

Using manure has also been beneficial in the process of decomposition of different kinds of waste. Co-digestion has been applied here with fruit and vegetable, fish offal, pig manure, dissolved air flotation sludge and brewery sludge (Callaghan et al., 1999). The co-digestion of manure and sewage sludge turned out to be the best solution in stabilizing the operational conditions and enhancing biogas production rates (Kayhanian and Rich, 1995). Capela et al. (2008) studied the technical feasibility of anaerobic co-digestion of three substrates such as OFMSW, organic industrial sludge (from pulp and paper industry) and cattle manure at mesophilic conditions. The highest value of the specific methane yield ($250 \text{ dm}^3/\text{kg}$) and the efficiency of TVS removal (57%) was obtained with the ratio of co-substrates amounting to 75%/12.5%/12.5% for OFMSW/cattle manure/organic industrial sludge.

Other organic co-substrates include different proteinaceous industrial waste. Recently, there have been many publications focused on co-fermentation using various promising organic components, such as olive mill effluent, algal sludge, animal blood and stomach content (Boubaker and Ridha, 2007, Yen and Brune, 2007, Alvarez and Liden, 2008). A comprehensive study on the co-digestion process of proteinaceous industrial waste was presented by Braun et al. (2003). Boubaker and Ridha stated that olive mill wastewater digested with olive mill solid waste increased the methane yield. Co-digestion of algal sludge and waste paper was found to offer two benefits: balancing the C/N ratio and increasing the cellulase activity leading to the biodegradation of algal sludge and subsequent supplementation of nutrients to digester. The optimized C/N ratio was estimated to be at the level of 20-25:1 (Yen and Brune, 2007). The study presented by Alvarez and Liden (2008) indicates a novel approach to treating some kinds of waste (animal blood and stomach content) which cannot be successfully degraded as separate substrates. Co-digestion of manure (cattle and swine), slaughterhouse waste (blood) and fruits/vegetable waste under mesophilic conditions reduced the volatile solids content by about 50-65% and produced about $300 \text{ dm}^3 \text{ CH}_4/\text{kg}$ volatile solids (VS) added at organic load rate up to $1.3 \text{ kg VS}/\text{m}^3\text{d}$.

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