Review Article

Utilization of Biodiesel By-Products for Biogas Production

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This contribution reviews the possibility of using the by-products from biodiesel production as substrates for anaerobic digestion and production of biogas. The process of biodiesel production is predominantly carried out by catalyzed transesterification. Besides desired methylesters, this reaction provides also few other products, including crude glycerol, oil-pressed cakes, and washing water. Crude glycerol or g-phase is heavier separate liquid phase, composed mainly by glycerol. A couple of studies have demonstrated the possibility of biogas production, using g-phase as a single substrate, and it has also shown a great potential as a cosubstrate by anaerobic treatment of different types of organic waste or energy crops. Oil cakes or oil meals are solid residues obtained after oil extraction from the seeds. Another possible by-product is the washing water from raw biodiesel purification, which is an oily and soapy liquid. All of these materials have been suggested as feasible substrates for anaerobic degradation, although some issues and inhibitory factors have to be considered.

1. Introduction

Renewable energy sources and biofuels, including biodiesel, have been gaining increasing attention recently as a replacement for fossil fuels [1]. However, their implementation in the general market depends on making these fuels more competitive. A convenient way to lower the costs of biofuels is to use the by-products as a potential source of energy, rather then treat them as waste.

Biodiesel is a prominent candidate as alternative diesel fuel. It is offering few advantages compared to conventional diesel, including the status of renewable energy source and lower emissions. Advances against petroleum diesel fuel are represented by the terms of sulfur content, flash point, content of aromatic substances, and biodegradability [1].

With approximately 245 processing plants and annual production of about 9 million tons, European Union has had the leading position in both production and consumption of biodiesel [2]. These plants are mainly located in Germany, Italy, Austria, France, and Sweden. Production of biodiesel has been expanding rapidly also on the other continents, mainly in the USA and developing countries, such as India, Brazil, Argentina, Malaysia, and Fiji.

As a primary feedstock, vegetable oils, animal fats, or waste cooking oils can be used for the production of biodiesel. In Europe, rapeseed oil is predominantly used, while, in the world extent, highest quantities of biodiesel are produced from soya oil [3].

The process of biodiesel production is usually carried out by catalyzed transesterification with alcohol, most likely methanol (Figure 1). A catalyst is usually involved to improve the reaction rate and yield [3]. Alkalies (sodium hydroxide, potassium hydroxide, carbonates, and corresponding sodium and potassium alkoxides), acids (sulfuric acid, sulfonic acid or hydrochloric acid), or enzymes can be used to catalyze the reaction. Base-catalyzed transesterification is much faster than the acid-catalyzed one (base catalyzed transesterification is basically finished within one hour) and is most often used commercially [4–6].

The desired methylesters this reaction provides also few other products. Isolation of oil from the oil seed plants by pressing and extraction provides oil cakes or oil meal as a by-product. In the reaction of transesterification, triglycerides are converted into glycerol and methylesters.
Biodiesel production is also increasingly studied [18–20]. A mixture of free fatty acids and water in the raw material does not affect the reaction [12]. Base catalysis, including mainly the easier purification of catalysts, provides few advantages over the homogeneous catalysis, however it can be integrated than homogeneous catalysis, but it can be more easily integrated with the separation of the homogeneous catalyst from the reaction mixture have also called for development of new catalysts [12].

Although the present production of biodiesel is mainly carried out by homogeneous base-catalyzed transesterification, implementation of alternative approaches has been increasingly studied [7–24]. Homogeneous base catalysis has a few disadvantages, such as the high requirement for the purity of oil. The high consumption of energy and costly separation of the homogeneous catalyst from the reaction mixture have also called for development of new catalysts [12].

Different alternative techniques have been applied for biodiesel production [7–11]. The most promising ones include employing heterogeneous catalyst, lipase catalyst or supercritical methanol [12].

Catalysis using solid heterogeneous catalysts runs slower than homogeneous catalysis, however it can be integrated with continuous processing technologies. A great variety of catalysts in catalytic transesterification of vegetable oils have been used recently, including zeolites, hydroxides, and so forth, [13–17]. Utilization of heterogeneous catalysts provides few advantages over the homogeneous base catalysis, including mainly the easier purification of methyl esters from glycerol and impurities. Also, the content of free fatty acids and water in the raw material does not affect the reaction [12].

Enzyme catalyzed transesterification using lipases for biodiesel production is also increasingly studied [18–20]. Similarly to heterogeneous catalysis, it also provides a solution of avoiding difficult recovery of glycerol and methylesters purification. Although this technology offers an attractive alternative, the industrial application has been slow due to feasibility aspects and some technical challenges, resulting from the low solubility of methanol and glycerol in biodiesel and high cost of lipases as catalyst.

Transesterification with supercritical methanol provides several advantages, compared to traditional methods [21–24]. The reaction is fast, in addition no catalyst is needed and therefore the separating process of the catalyst and saponified products becomes unnecessary. Generation of washing water can also be avoided. However the high pressure and temperature (239–385°C) is required, which leads to high energy consumption and production costs.

Commonly the most important by-products from biodiesel production are pressed cakes from oil extraction, crude glycerol and washing water [5, 7, 18]. The nature of these products is highly dependent on the character of raw material and processing technique, although generally they present suitable substrates for anaerobic digestion with the production of biogas.

**2. Crude Glycerol**

Crude glycerol (g-phase) is heavier separate liquid phase, composed mainly by glycerol. In general for every 100 kilograms of biodiesel about 10 kilograms of g-phase is produced.

Crude glycerol generated by homogeneous base-catalyzed transesterification contains approximately 50–60% of glycerol, 12–16% of alkalis especially in the form of alkali soaps and hydroxides, 15–18% of methyl esters, 8–12% of methanol, 2–3% of water and further components [25, 26].

Table 1 and 2 summarize the characteristics of g-phases based on the source of oil used for the production of biodiesel. Analytical results from the macroelement screening tests are listed in Table 1. Crude glycerol contains a variety of elements, such as sodium, potassium, phosphorus or sulfur, originating from the primary oil. Larger quantities of sodium or potassium are also contained, coming from the catalyst.

Table 2 shows the content of protein, fat, ash, carbohydrates in percents and caloric value for kg. G-phase is mostly composed of carbohydrates, represented by glycerol. The ash content of avoiding di

**Table 1:** Analysis results of macroelements, carbon and nitrogen in crude glycerol from different feedstocks (BDL indicates values that are below the detection limit for the corresponding analytical method) [25].

| Feed stocks       | Ida Gold Mustard | Pac Gold Mustard | Rapeseed | Canola | Soybean | Crambe | Waste vegetable oils |
|-------------------|------------------|------------------|----------|--------|---------|--------|----------------------|
| Calcium, ppm      | 11.7 ± 2.9       | 23.0 ± 1.0       | 24.0 ± 1.7 | 19.7 ± 1.5 | 11.0 ± 0 | 163.5 ± 11.6 | BDL                  |
| Potassium, ppm    | BDL              | BDL              | BDL      | BDL    | BDL     | 216.7 ± 15.3 | BDL                  |
| Magnesium, ppm    | 3.9 ± 1.0        | 6.6 ± 0.4        | 4 ± 0.3  | 5.4 ± 0.4 | 6.7 ± 0.2 | 126.7 ± 5.8  | 0.4 ± 0              |
| Phosphorus, ppm   | 25.3 ± 1.2       | 48 ± 2.0         | 65 ± 2.0 | 58.7 ± 6.8 | 53.0 ± 4.6 | 136.7 ± 57.7 | 12.0 ± 1.5           |
| Sulfur, ppm       | 21.0 ± 2.9       | 16.0 ± 1.4       | 21.0 ± 1.0 | 14.0 ± 1.5 | BDL     | BDL     | BDL                  |
| Sodium, % wt      | 1.17 ± 0.15      | 1.23 ± 0.12      | 1.06 ± 0.07 | 1.07 ± 0.12 | 1.2 ± 0.1  | 1.10 ± 0.10  | 1.40 ± 0.16          |
| Carbon, % wt      | 24.0 ± 0.00      | 24.3 ± 0.58      | 25.3 ± 0.58 | 26.3 ± 0.58 | 26.0 ± 1  | 24.0 ± 0.00  | 37.7 ± 0.58          |
| Nitrogen, % wt    | 0.04 ± 0.02      | 0.04 ± 0.01      | 0.05 ± 0.01 | 0.05 ± 0.01 | 0.04 ± 0.03 | 0.06 ± 0.02  | 0.12 ± 0.01          |

**Figure 1:** Reaction of biodiesel production by base-catalyzed transesterification with methanol.
contained in crude glycerol is mainly sodium or potassium from the catalyst.

Considering that the processing technology of biodiesel production affects the characteristics of by-products, the new technologies and modern catalysts can be expected to influence the composition and utilization of crude glycerol. For example, g-phase originating from biodiesel production using rapeseed oil with heterogeneous catalyst is limpid and colorless, containing at least 98% of glycerin and neither ash, nor inorganic compounds were detected in it [27].

As the biodiesel production is increasing exponentially, the crude glycerol generated in this process has also been generated in a large quantity. Despite the wide applications of pure glycerol in pharmaceutical, food and cosmetic industries, the refining of crude glycerol to a high purity is too expensive, especially for small and medium biodiesel producers [28]. The investments for the construction and startup operation of crude glycerol purification facility make according to Singhabhandhu [29] roughly 65 million Euros (facilities with production capacities of 1.4–2 ML/y). Weber [30] mentions 27% of the capital investment costs going to construction of the technical glycerin facility in a 12 ML biodiesel refinery (Aschach, Austria).

To improve the economic feasibility of biodiesel industry, new alternate ways of utilization of g-phase have been studied recently. Possibilities such as combustion, co-burning, composting, animal feeding, thermochemical conversions and biological conversion have been applied for crude glycerol processing [31–46].

One of the possible applications is utilization of g-phase as carbon and energy source for microbial growth in industrial microbiology. Microbial conversion of glycerol to various compounds has been investigated recently, with particular focus on the production of 1,3-propanediol [47–50], which has been considered as a main product of glycerol fermentation [28]. 1,3-propanediol presents several interesting applications, it can be used as a monomer for polycondensation to produce plastics with special properties, (polysteres, polyethers and polyurethanes) [51–54] as a monomer for cyclic compounds, as a polyglycol-type lubricant [55] and it also may serve as a solvent [56]. The biotechnological production of 1,3-propanediol from glycerol has been demonstrated for several bacteria, *Klebsiella pneumoniae*, *Clostridium butyricum* and *Citrobacter freundii* have been most commonly used in the studies [47–50, 57–59].

Besides the production of 1,3-propanediol, glycerol can also be used as a carbon source to obtain other valuable microbial products, such as recombinant proteins and enzymes, microbial lipids (single-cell oils), medicinal drugs, antibiotics and fine chemicals. Bioconversion of g-phase into chemicals, such as dihydroxyacetone, 1,2-propanediol, ethanol, hydrogen, citric acid, propionic acid, polyglycerols, succinate, have been also increasingly studied recently [60–77].

Another option offers biological production of methane from crude glycerol using anaerobic sludge [78–88]. Besides the production of methane, the advantages include low nutrient requirements, energy savings, generation of low quantities of sludge and excellent waste stabilization. Glycerol is a readily digestible substance, which can be easily stored over a long period. High energy content in g-phase makes it an interesting substrate for anaerobic digestion as well, since it offers high production of biogas in smaller reactor volumes. A great variety of microorganisms is able to use this substrate as a carbon source for the growth under anaerobic conditions, such as *Citrobacter freundii*, *Klebsiella pneumoniae*, *Clostridium pasteurianum*, *Clostridium butyricum*, *Enterobacter agglomerans*, *Enterobacter aerogenes* or *Lactobacillus reuteri* [28, 34, 58, 67]. The production of biogas through anaerobic digestion offers significant advantages over other forms of crude glycerol treatment. It requires lower investments and simpler operational conditions compared to more sophisticated preprocessing technologies, which makes it ideal for local applications. Less biomass sludge is produced in comparison to aerobic treatment technologies. The digestate is an improved fertilizer in terms of both its availability to plants and its rheology. A source of carbon neutral energy is produced in the form of biogas.

### 3. Anaerobic Digestion of Crude Glycerol

Considering anaerobic treatment of crude glycerol, potential of its main component glycerol has been well-known for a longer period [89–91]. Digestion of pure glycerol has been investigated both as a primary substrate [89, 90], and as an intermediate product of anaerobic degradation of fats [91]. Biodegradation have been carried out using either pure cultures of microorganisms [90] or sludge composed of mixed cultures from wastewater treatment plant [89].

Few studies focused on biogas production from g-phase [78–88] have also been realized recently. Anaerobic treatment of g-phase as a single substrate [78–81] was carried out as well as coprocessing of crude glycerol with different substrates [82–88].

Mesophilic anaerobic digestion of crude glycerol was studied in work Lopez et al. (2009). The substrate was

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#### Table 2: Food nutrient analysis for crude glycerol samples [25].

| Feed stocks            | Ida Gold Mustard | Pac Gold Mustard | Rapeseed | Canola | Soybean | Crambe | Waste vegetable oils |
|------------------------|------------------|------------------|----------|--------|---------|--------|---------------------|
| Fats, %                | 2.03             | 1.11             | 9.74     | 13.1   | 7.98    | 8.08   | 60.1                |
| Carbohydrates, %       | 82.8             | 83.8             | 75.5     | 75.2   | 76.2    | 78.6   | 26.9                |
| Protein, %             | 0.14             | 0.18             | 0.07     | 0.06   | 0.05    | 0.44   | 0.23                |
| Calories, kJ/kg         | 14.6             | 14.5             | 16.3     | 17.5   | 15.8    | 16.3   | 27.2                |
| Ash, %                 | 2.8              | 1.9              | 0.7      | 0.65   | 2.73    | 0.25   | 5.5                 |

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previously treated in two different ways: (1) acidification with phosphoric acid and centrifugation (so-called acidified glycerol) or (2) acidification followed by distillation (so-called distilled glycerol) [78]. Either granular sludge from anaerobic reactor treating brewery wastewater or nongranular sludge from anaerobic reactor treating urban wastewater was used for inoculation of batch laboratory-scale reactors, having the working volume of one liter. The variations in the methane production were studied, considering the different ways of substrate pretreatment and different types of sludge. The use of the combination of granular sludge with acidified glycerol was found to be the best option for anaerobic treatment of glycerol [78]. The organic loading rates for each substrate and sludge type were in the range of 0.92–2.0 kg/m²·d (COD). Organic loading rate (ORL) is presented as the weight of organic matter per day applied over a specific volume of reactor. The parameter COD (chemical oxygen demand) represents indirectly the amount of organic compounds in the sample. It is a measure of the oxygen needed to degrade organic matter. A decrease in specific methane production was observed when the ORL was increased further. Considering the biomass production and cell maintenance null, 0.382 m³ of methane are theoretically produced per kilogram of removed COD. Experimentally, the effectiveness of the process in each case was: 76% using granular sludge-acidified glycerol, 75% using nongranular sludge-acidified glycerol and 93% with granular sludge-distilled glycerol (0.292; 0.288 and 0.356 m³/kg COD removed, resp.). Besides the methane production coefficient, the removed COD percentage is also important in order to determine biodegradability. This was found to be around 100% using granular sludge-acidified glycerol, 75% with nongranular sludge-acidified glycerol and 85% using granular sludge-distilled glycerol.

Crude glycerol was processed in anaerobic laboratory-mixed reactor under mesophilic conditions for several months by Bodik et al. [79]. The anaerobic reactor achieved stable operation at the volume loading of 4 kg/m³·d with biogas production ca0.980 m³/L of dosed g-phase. The maximal reached volumetric loading was 8–10 kg/m³·d, but the loading was considered to be very sensitive and unstable, because it caused decrease of the specific methane production and increase of concentrations of volatile fatty acids (VFA) and dissolved COD. Very effective transformation of g-phase into biogas was measured (more than 95%) which gives very good assumptions for posttreatment of sludge water. The concentration of dissolved inorganic substances increased during the monitored period very slowly but continuously from 1.3 g/L up to 15 g/L. Higher concentrations of dissolved salts could cause inhibition of anaerobic degradation, however no significant influence was observed during this experiment.

In the work Huthan et al. (2009) results of crude glycerol treatment in the laboratory-mixed reactor (with effective volume of 4 liters) and in the laboratory UASB (upflow anaerobic sludge blanket) reactors (volume of 3.7 liters) are described [80]. From this work resulted that the operation of mesophilic anaerobic degradation of crude glycerol as the only organic substrate is feasible, however the process operation is very sensible to organic overloading of reactor. The laboratory-mixed reactor achieved stable operation at ORL of 4 kg/m³·d (COD). The specific production of biogas achieved ca0.980 m³/L of glycerol added. The laboratory UASB reactor with granulated biomass achieved stable operation at ORL of 6.5 kg/m³·d and the specific biogas production was ca0.840 m³/L of glycerol added. Inoculation of the UASB reactor with suspended biomass showed that this type of sludge is not suitable for this purpose because of sludge flotation during the reactor operation.

Yang et al. (2008) examined biodegradation of glycerol-containing synthetic wastes using a fixed-bed laboratory bioreactor packed with polyurethane under mesophilic and thermophilic anaerobic conditions [81]. Better performance was obtained from the reactor under the thermophilic conditions. When increasing the ORL from 0.25 to 1 kg/m³·d, the COD removal efficiency was decreasing under mesophilic conditions, however under thermophilic conditions higher COD removal was achieved corresponding to higher loading rate. After 516 days of reactor operation, the bed materials under the thermophilic reactors were removed to measure the quantity of attached biomass and for microscopic observation. The polyurethane immobilization carrier retained more biomass than did the liquid phase of reactor. About 95% of the microbes were maintained on the fixed-bed. The immobilized microorganisms present in the thermophilic reactor were primarily Methanobacterium sp., Methanosarcina sp., Bacillus sp., Clostridium sp., Desulfotomaculum sp. and Ruminococcus.

Feasibility of utilization of crude glycerol as a cosubstrate has been proven for example in the work of Fountoulakis (2009). The effects of g-phase on the performance of anaerobic reactor treating different types of organic waste (organic fraction of municipal solid waste, mixture of olive mill wastewater and slaughterhouse wastewater) were examined, in order to enhance methane production and increase the yield of hydrogen [82]. Digestion was carried out in a single-stage reactor with a working volume of 3 liters, inoculated with anaerobic sludge from municipal sewage treatment plant and the share of crude glycerol made 1% (v/v) of the dose. The supplementation of the feed with crude glycerol had a significant positive effect and the methane production rate in both cases increased close to the theoretical values given total biodegradation of glycerol. Addition of g-phase to a reactor treating the organic fraction of municipal solid waste resulted in the increase of methane production to 2.094 L/d, compared to 1.400 L/d. An enhanced methane production was also observed when a mixture of olive mill wastewater and slaughterhouse wastewater was supplemented with crude glycerol. Specifically, by adding 1% of g-phase to the feed, the methane production rate increased from 0.479 L/d to 1.210 L/d. Stable concentration levels of COD indicated that COD attributed to glycerol in the feed was totally digested in the reactor. The estimated yield of methane generated from the digestion of glycerol was in both cases almost reaching the value of theoretical methane production. The theoretical methane production from digestion of glycerol was estimated at 0.751 L/d (using the Buswell formula and the ideal gas law).
Fountoulakis et al. studied also the feasibility of adding crude glycerol to the anaerobic digesters treating sewage sludge in wastewater treatment plants [83]. Both batch and continuous experiments were carried out at 35 ºC. It was observed that glycerol addition up to 1% (v/v) in the feed increased methane production in the reactor above the expected theoretical value, as it was totally digested and furthermore enhanced the growth of active biomass in the system. On the other hand, any further increase of glycerol caused a high imbalance in the anaerobic digestion process. The reactor treating the sewage sludge produced 1.106 ± 0.036 L/d of methane before the addition of glycerol and 2.353 ± 0.094 L/d after the addition of glycerol (1% in the feed). The extra glycerol-COD added to the feed did not have a negative effect on reactor performance, but seemed to increase the active biomass (volatile solids) concentration in the system. Also, the kinetic experiments have shown that glycerol biodegradation took place significantly faster than propionate (which is an intermediate product) biodegradation, and it was therefore suggested that the glycerol overload in the reactors increased propionate concentration.

Ma et al. studied the improvement of anaerobic treatment of potato processing wastewater in a laboratory UASB reactor by codigestion with crude glycerol [84]. Influence of three types of glycerol was tested: pure glycerol, crude glycerol and high conductivity glycerol. All 3 types of glycerol are generated as a by-product by the production of biodiesel. They are obtained by different processing technologies and their characteristics differ. Supplement of pure glycerol of 2 mL/L of potato processing wastewater resulted in increase of the specific biogas production by 0.740 m³/L of glycerol added. High COD removal efficiencies (around 85%) were obtained. Moreover, a better in-reactor biomass yield (surplus of active biomass in the reactor) was observed for the UASB reactor supplemented with so-called pure glycerol (0.012 g VS (volatile solids) per gram of COD removed) compared to the reactor without added glycerol (0.002 g VS per gram of COD removed), which suggests a positive effect of glycerol on the sludge blanket growth.

Álvarez et al. carried out a laboratory study, aimed at maximizing methane production by anaerobic codigestion of three agroindustrial wastes: crude glycerol, pig manure and tuna fish waste [85]. Experiments were performed by batch (discontinuous) assays and 500 mL reactors were operated under the temperature of 35 ºC. Different blends composed by various percentages of these substrates were fed into the reactors. Compositions of these blends were specified using linear programming optimization method to find most suitable ratios of cosubstrates which would achieve highest biodegradation potential or highest methane production rate. The highest biodegradation potential (methane production of 0.321 m³/kg COD) was reached with a mixture composed of 84% pig manure, 5% fish waste and 11% biodiesel waste, while the highest methane production rate (16.4 L/kg-d (COD)) was obtained by a mixture containing 88% pig manure, 4% fish waste and 8% biodiesel waste. Mixture composed of 84% pig manure, 5% fish waste and 11% biodiesel waste and mixture of 79% pig manure, 5% fish waste and 16% biodiesel waste have also achieved very high methane production rates (14.4 L/kg-d (COD) and 12.8 L/kg-d (COD) resp.) compared to the control sample using pig manure substrate (8.3 L/kg-d (COD)).

Anaerobic codigestion of crude glycerol in the reactors processing maize, maize silage and pig manure as main substrates, is described in work of Amon et al. (2004). Laboratory digesters under mesophilic conditions were processing a basic mixture, which included 31% of maize silage, 15% of corn maize and 54% of pig manure, together with addition of various levels of g-phase (3, 6, 8 and 15%). The methane yield from the basic mixture without glycerine addition reached 0.335 m³/kg VS [86]. Addition of 3% of glycerine increased the methane yield by 20% and achieved 0.411 m³/kg VS. The addition of 6% of glycerine resulted in the highest methane yield of 0.440 m³/kg VS. Addition of more than 6% glycerine to the basic mixture had only a low positive influence on the methane yield. Addition of 15% glycerine even decreased the methane yield to 0.400 m³/kg VS and the duration of fermentation increased. Methane formation at the start of the experiments was delayed. Analysis of the VFA concentrations in the mixture during the experiments resulted in the hypothesis that the inhibition of methane formation was caused by increased concentration of propionic and butyric acids. The large amounts of these acids were built during decomposition of methanol. VFA accumulation reflects a kinetic uncoupling between acid producers and consumers and is typical for stress situations [92]. The main cause of the toxic effects of high VFA concentrations on the anaerobic digestion process is generally considered to be the resulting drop in pH.

Long-term operation of anaerobic digester for cofermentation of maize silage and crude glycerol was studied in work Špaková et al. (2009). Two laboratory models of a volume of 6 liters were fed by maize silage and a mixture of maize silage with crude glycerol and operated under mesophilic conditions [87]. During the operation period, no negative influence of supplementation of the feed with crude glycerol was observed. Biogas production as well as the sludge water quality (pH, concentrations of COD, VFA, ammonia and phosphate) was similar in both reactors. Maximum portion of g-phase added formed 41.5% of total daily COD dose (together with maize silage). Specific biogas production achieved was approximately 0.40 m³/kg (COD) in the case of both sole maize silage and a mixture of maize silage with g-phase, meaning that both the maize silage and g-phase had similar specific biogas productions per unit quantity of COD.

A positive effect of glycerol as a cofermentation medium is supported by Amon et al. (2006). Biogas productions from pig manure, crude glycerol and a mixture of 94% of manure with 6% of glycerol were compared in the study [88]. A 6% supplementation of glycerol to pig manure and maize silage resulted in a significant increase in methane production from 0.569 to 0.679 m³/kg (VS). The methane yield of the mixture supplemented with glycerine was higher than the combined methane yields of both substrates if digested separately. Increase in the specific methane production could not be just corresponding to supplemented glycerol, but was also result of the improved anaerobic degradation caused by the effect of codigestion. Co-digestion of various substrates provides
in many cases suitable option for anaerobic processing for various technical reasons. One of the main reasons is the stability of pH and sufficient buffer capacity. Lack of nutrients or high concentration of inhibitory agents can also be improved by sensible choice of cosubstrates. A particularly strong reason for codigestion of feedstock is the adjustment of the carbon-to-nitrogen (C/N) ratio. Digestion of hardly degradable substances was found to be faster by the addition of easily degradable substrates. Moreover some previously problematic wastes were found digestible if digested in a mixture of other waste.

The work by Hutnan et al. (2009) showed that crude glycerol is also a suitable cosubstrate in the full scale biogas plant for anaerobic treatment of maize silage [80]. Reactor was operated under mesophilic conditions and the effective volume of a full scale anaerobic reactor was 2450 m³. Evaluated specific production of biogas from crude glycerol was about 0.890 m³/kg of crude glycerol added. Dose of crude glycerol, which represented only 5.2% of overall dose to biogas plant, produced almost 15% of overall biogas production. A significant influence on positive economical balance of biogas plant using this cosubstrate has been demonstrated in this study. At the electrical power output of cogeneration unit 300 kW is the daily share of electricity produced from crude glycerol 1067 kWh. At the current price of 0.15 € per 1 kWh, this represents a daily profit of 156.55 € and a saving of almost 15% silage (1865 kg at a price around 60 € for 1 ton).

The possible inhibition effects, resulting from the substrate composition, have to be considered by anaerobic treatment of crude glycerol. Metabolism of the anaerobic microorganisms may be negatively affected mainly by the high salinity of g-phase [79, 80]. The relatively high content of sodium or potassium salts (ca 20–100 g/L) originates from the catalyst, used for the biodiesel production. Higher concentrations of sodium in the anaerobic reactor can seriously inhibit the microbial activity [93].

Biological processing of organic materials in the presence of salts have been studied mainly as an alternative possibility of treating wastewater from industrial processes [94–97] (meat canning, pickled vegetables, dairy products, olive and fish processing industries, petroleum, textile and leather industries). The anaerobic digestion of industrial saline effluents, predominantly from seafood processing, at salt concentrations ranging from 10 to 71 g/L has been studied recently, using different processes, such as an anaerobic filter, UASB reactor and an anaerobic contact system [94, 95, 98]. The COD removal efficiencies obtained, generally remained between 70% and 90%, with OLR ranging from 1 to 15 kg/m²·d (COD).

The concentration of sodium exceeding 10 g/L was for a long time generally considered to strongly inhibit methanogenesis [94]. However, the anaerobic digestion in the high salinity level was proven to be possible for treatment of fish-processing effluent [99, 100], if a suitable strategy for adapting the methanogenic biomass was applied. Furthermore, it was shown, that the toxicity of sodium in sludge depends on several factors, such as the type of methanogenic substrate used, the antagonistic or synergistic effects of other ions, the nature and the progressive adaptation of sludge to high salinity and reactor configuration [93, 101].

These factors may be the cause of different results achieved in different studies. Some of the researchers reported the concentrations from 0.9 g/L to 8 g/L to be slightly inhibitory (reduction in methane production by 10%), using different types of substrates [102]. In other experiments, the concentrations in the range of 5.6–53 g/L, depending on the conditions, have been documented to cause the decrease of methane production to a half [93, 101–103].

Adequate adaptation of the sludge appears to be of extreme importance, hence the continuous exposure of methanogenic sludge seems to lead to the tolerance of a higher salinity compared to the sludge exposed to salt shocks. The adaptation includes gradual increase of salt concentrations in the sludge, by low organic loading, providing adequate conditions for internal structural changes in the predominant species of methanogens, to adapt to higher osmolarity [104]. Hence the startup period may take several months [94]. According to Gebauer [97] is the adaptation to high sodium concentrations more likely to happen as a result of selection of tolerant species than by adaptation of every single microorganism.

Methanogenic microorganisms seem to be more affected by sodium toxicity than other populations, such as prokionate utilizes [101]. The most sensitive appeared to be the nitrogen removing microorganisms [98]. Provided the biomass is acclimated, high salinity is reportedly not an obstacle to its growth and it has no negative influence on sedimentation properties of the sludge or the granulated sludge viscosity. The lack of macronutrients (nitrogen, phosphorus, sulfur) in the medium was found to have a more pronounced negative effect on biomass under the saline conditions. On the other hand, the absence of micronutrients did not further reduce biomass activity under salinity [101, 105].

Another important concern about the anaerobic digestion of crude glycerol should be the concentration of nitrogen-rich substances. Ammonium concentration up to 200 mg/L in the anaerobic reactor is considered to be beneficial [93], since nitrogen is an essential nutrient for microorganisms. Considering the low concentration of nitrogen in the crude glycerol, it may be necessary to supply the nitrogen-rich substances into the reactor. Urea or NH4Cl are most frequently used as external source of ammonium nitrogen.

4. Oil Cakes

Oil cakes or oil meals are solid residues obtained after oil extraction from the seeds. Their composition widely varies depending on the quality of seeds or nuts, growing conditions and extraction methods. Oil cakes can be either edible or nonedible. Edible cakes have a high protein content ranging from 15 to 50%. The chemical compositions of oil cakes originating from different types of plants are listed in Table 3 [106].

In our geographic area (EU), rapeseed and sunflower are the most frequently used substrates, hence we are focusing on the by-products from their processing.
Table 3: Composition of oil cakes.

| Oil cake       | Dry matter % | Crude protein % | Crude fibre % | Ash % | Calcium % | Phosphorus % |
|----------------|--------------|-----------------|---------------|-------|-----------|--------------|
| Canola oil cake| 90           | 33.9            | 9.7           | 6.2   | 0.79      | 1.06         |
| Coconut oil cake| 88.8        | 25.2            | 10.8          | 6.0   | 0.08      | 0.67         |
| Cottonseed cake  | 94.3        | 40.3            | 15.7          | 6.8   | 0.31      | 0.11         |
| Groundnut oil cake| 92.6      | 49.5            | 5.3           | 4.5   | 0.11      | 0.74         |
| Mustard oil cake  | 89.8        | 38.5            | 3.5           | 9.9   | 0.05      | 1.11         |
| Olive oil cake     | 85.2        | 6.3             | 40.0          | 4.2   | —         | —            |
| Palm kernel cake   | 90.8        | 18.6            | 37            | 4.5   | 0.31      | 0.85         |
| Sesame oil cake    | 83.2        | 35.6            | 7.6           | 11.8  | 2.45      | 1.11         |
| Soy bean cake      | 84.8        | 47.5            | 5.1           | 6.4   | 0.13      | 0.69         |
| Sunflower oil cake | 91          | 34.1            | 13.2          | 6.6   | 0.30      | 1.30         |

Table 4: Composition of rapeseed, rapeseed cake after extraction of 60, 70, and 75% of oil and rapeseed meal, in percents of total solids [111].

| Feed stock            | Rapeseed | Rapeseed cake | Rapeseed meal |
|-----------------------|----------|---------------|---------------|
| Portion of extracted oil | 60%      | 70%           | 75%           |
| Crude oil             | 45       | 24.7          | 19.7          | 17   | 4.5 |
| Crude protein         | 23       | 31.5          | 33.6          | 34.7 | 40  |
| Crude fibre           | 7        | 9.6           | 10.2          | 10.6 | 12.3|
| Ash                   | 5        | 6.8           | 7.3           | 7.5  | 7.7 |
polypeptides by extracellular enzymes. The hydrolysis of carbohydrates takes place within a few hours, while the hydrolysis of proteins within few days. Rate and readiness of degradation of different types of carbohydrates can quite vary. Fats are often decomposed completely. Hemicellulose and lignin, forming the shells of rapeseed, could be quite difficult to decompose in the process of biogas production.

Accumulation of free fatty acids can cause a problem by digestion of materials with higher content of oil, considering that the fats decomposition step is faster than the methanogenesis. Generally, hydrolytic and acidogenic microorganisms are growing about ten times faster than methanogens. Co-digestion of rapeseed cake or meal with other feedstocks, such as manure, provides an alternative solution. Improvement of the biogas production is expected, based on the high oil content.

Rapeseed cake and rapeseed meal are nitrogen-rich media, they contain about 35–40% of nitrogen substances [106]. These substances are predominantly proteins, containing amino acids. Expressed in the terms of carbon-to-nitrogen ratio, this makes about 5–8 in case of rapeseed cake. Compared to other materials, the C/N ratio of lignocellulosic materials is in the range of 60–400, grass and silages have C/N about 20–40, swine manure about 12–15 and sunflower meal about 6–13 [118]. The C/N ratio of substrate is an important parameter to be considered by anaerobic degradation, since high content of nitrogen may cause too high content of ammonium nitrate in the biogas reactor. Ammonium nitrogen levels of about 4 g/L of wet sludge bring the risk of process inhibition. If the ammonium content is too high, it is necessary to dilute the substrate with water or nitrogen-poor material.

Phytotoxic effects of rapeseed cake, caused by the content of glucosinolates, must also be considered. They play an important role in the process of digestion, since in higher concentrations they may have harmful effect on methanogenic. The risk of inhibition is getting less serious with decreasing of the glucosinolates level in the rapeseed meal and cake.

There is not much information about experimental anaerobic processing of rapeseed cake or meal in the available literature.

Bernesson et al. estimated the potential biogas production from rapeseed, rapeseed meal and rapeseed cake after 60–75% extraction of oil [111]. Table 5 indicates, that with the increased amount of oil extracted in the process, the possible biogas production from rapeseed cake is decreasing.

Antonopoulou et al. carried out batch mesophilic biochemical methane potential tests using rapeseed and sunflower residues as a substrate [119]. The experiments indicated that the biological methane potential of rapeseed and sunflower meal were 0.450 m^3/kg and 0.481 m^3/kg, respectively. Compared to commonly used substrate maize silage, the potential of these oil meals are about 40% higher, so it suggests interesting substrates for the production of biogas. Various pretreatment methods, such as thermal, chemical (through alkali or acid addition) or combination of the above methods were also tested in the effort to enhance the methane productivity and yield. Thermal pretreatment method was conducted at 121°C for 60 minutes in a pressure cooker. Acid or alkali pretreatment of the feedstocks was conducted by the addition of 2% w/v H_2SO_4 or NaOH, respectively, for 60 minutes at a temperature of 25°C or at 121°C for 60 min in a pressure cooker (thermal acid or thermal alkali pretreatment). The experiments showed that the pretreatment methods tested did not enhance the methane potential of the rapeseed and sunflower residues. This could be attributed to the inhibitory compounds which were possibly released during the pretreatment.

### Table 5: Methane production potential from rapeseed, rapeseed cake and meal in m^3/kg VS [111].

| Feed stock     | Rapeseed | Rapeseed cake | Rapeseed meal |
|----------------|----------|---------------|---------------|
| Portion of extracted oil | 60% | 70% | 75% |
| Saccharides    | 0.11     | 0.17          | 0.17          | 0.20 |
| Lipids         | 0.43     | 0.19          | 0.16          | 0.04 |
| Proteins       | 0.12     | 0.17          | 0.18          | 0.20 |
| Together       | 0.66     | 0.53          | 0.51          | 0.45 |
| Together (kg)  | 0.47     | 0.37          | 0.36          | 0.32 |
| Calorific value (MJ) | 23.4   | 19.5          | 18.6          | 18.1 |

### 6. Anaerobic Digestion of Sunflower Oil Cake

Sunflower oil cakes and meals are also feasible feedstocks for anaerobic digestion. Raposo et al. examined their anaerobic degradability, biochemical methanogenic potential and the influence of substrate to inoculum ratio in batch laboratory-scale digesters [120, 121]. High stability of the anaerobic digestion process of sunflower oil cake under mesophilic conditions was demonstrated.

The experimental study, with the duration of 7 days, was carried out in a multibatch reactor system [120, 121], which consisted of continuously stirred flasks with an effective volume of 250 mL. The six different inoculum to substrate ratios were tested: 3.0, 2.0, 1.5, 1.0, 0.8 and 0.5. The ultimate methane yield decreased considerably with the inoculum to substrate ratio. The yield of methane was in the range from 0.227 m^3/kg for the ratio of 3.0 to 0.107 m^3/kg (VS) for the ratio of 0.5. Biodegradability copied this trend, from 86% to 41% was achieved. Higher contribution of substrate may cause lower methane yield due to higher energy consumption.
in the hydrolytic-acidogenic stage. However, the net VS removed only varied from 42% to 36%, when the ratio decreased from 3.0 to 0.5, which demonstrated the adequate operation of the hydrolytic-acidogenic stage.

The increase in CODs concentrations presented 780–6100 mg/L for the inoculum to substrate ratios of 3.0–0.5, respectively. In case of inoculum-substrate ratio of 3.0 or 2.0, the final values of total VFA were proportional to the amount of substrate added, and no accumulation occurred. However, when the ratio was lower than 2.0, an imbalance of the process was observed, when the VFAs increased to 2050 or 5500 mg/L (for ratios 0.8 and 0.5, resp.). The dissolved CODs also increased, reaching the levels of 3380–12100 mg/L after seven days for the inoculum-substrate ratios of 3.0–0.5, respectively. The trend in the increase of COD with digestion time observed was due mainly to the accumulation of VFA, which reflects a kinetic uncoupling between acid formers and consumers and is typical for a stress situation. This means that the hydrolytic-acidogenic stage was carried out satisfactorily and the imbalance of the process was due to the stress of methanogenic microorganisms.

The net production of total ammonia nitrogen increased with the load added, as a consequence of degradation of proteins from the sunflower oil cake, achieving a maximum value of 1085 mg/L at the ratio of 0.5 (198 mg/L at the ratio 3.0). However, the specific total ammonia nitrogen reached in all ratios similar production of about 40 mg/g VS added.

Identification of the individual VFA may also provide valuable information on the metabolic pathways involved in the process. The high influence of inoculum-substrate ratio on the composition and concentration of the different VFA was shown. By the inoculum to substrate ratio of 3 and 2, the predominant VFA were valeric and butyric acids, but the residual compound was the latter. The absence of acetic and propionic acids indicated, that the methanogenic stage was not disturbed and the formation of methane from these intermediates was quick.

When the ratios of 1.5, 1.0 and 0.8 were applied, the predominant VFA during the first few days were acetic and propionic acids, followed by valeric and butyric acids. Although in the end valeric acid dominated. This performance demonstrated that the lower inoculum-substrate ratio causes the greater accumulation of the longer chain VFA.

By the ratio of 0.5 the predominant VFA were acetic and propionic acids during the first few days, followed by a decrease in acetic acid with time, with a significant residual concentration of propionic, valeric and butyric acids. The VFA profile obtained is a consequence of the imbalance in the methanogenic stage.

De La Rubia et al. investigated also influence of the hydraulic retention time (HRT, it is a measure of the length of time that sludge remains in reactor.) and OLR on the performance of the hydrolytic-acidogenic step of a two-stage anaerobic digestion process of sunflower oil cake [122]. The experiments were performed in laboratory-scale completely stirred tank reactors, with a working volume of 2 L, at mesophilic (35°C) temperature. Digesters were operated over a total period of approximately 350 days. Six OLRs (ranging from 4 to 9 kg/m³·d(VS)) for four HRTs (8, 10, 12 and 15 days) were tested to check the effect of each operational variable. Hydrolysis yields obtained for all HRTs and OLRs assayed were in the range of 20.5–30.1%.

Variations in HRT did not affect the COD solubilization of this substrate within the HRT range (15–8 days) researched. Variations in OLR affect the organic matter liquefaction slightly, the highest value (30.1%) being achieved for HRT of 10 days and OLR of 6 kg/m³·d(VS). The acidification yield increased with OLR up to 6 kg/m³·d(VS), the highest value (83.8%) being achieved for HRT of 10 days and an OLR of 6 kg/m³·d(VS). However, higher loading provokes a decrease in the acidification yield, probably due to the fact that the acidogenic bacteria could have been affected and inhibited at the highest OLR studied.

7. Washing Water

Another possible by-product from biodiesel production offers the water, generated by washing of raw biodiesel. Under the conventional process (alkali-catalyzed transesterification) for every 100 L biodiesel produced about 20 L of washing water is discharged (or more in case of prior acid pretreatment) [123].

Washing water (usually referred to as biodiesel wastewater) is a viscous liquid with an opaque white color similar to aqueous soap. It contains significant amounts of methanol, glycerol and soaps. Methyl esters bound with soap, NaOH or KOH from the catalyst, sodium or potassium salts and trace mono, di- and triglycerides bound up with the soap are also contained in the water.

A great variety of systems for biodiesel purification is available commercially and new alternative technologies are also being investigated. The possible options include dry washing. In this case, the impurities from biodiesel (free glycerol, soap, free fatty acids, catalyst, glycerides, etc.) are absorbed to form a solid waste product instead of a liquid. Dry washing replaces water with an ion exchange resin or a magnesium silicate powder. Both these methods are being used in industrial plants [124]. No regeneration is normally applied and the spent material has to be disposed of to landfill or other applications (compost, potential animal feed additive and potential fuel).

Relatively expensive ultrafiltration or reverse osmosis could also be applied for purification of biodiesel. Yet, the washing with water remains the most convenient alternative [125–127].

Besides crude glycerol, oil cakes and biodiesel wastewater, other potential by-products can be generated in the biodiesel industry. These products are specific, depending on the processing technologies in biodiesel plants. For example, some facilities utilize citric acid solutions in order to wash reactors and other equipment, which produces possible additional waste.

Like the raw glycerol, washing water has also high levels of COD, values in the range of 18–800 g/L have been reported [123, 128–130]. High content of degradable organic substances makes it a suitable source of carbon for microbiological processes, however some issues have to be considered.
The wastewater is basic (alkaline), due to the significant levels of residual KOH, and contains a high level of oil and grease and has a high solid content. Nutrients for microbial growth (such as nitrogen and phosphorus) are not abundant in washing water, except for the carbon source. Together these components inhibit the growth of most microorganisms making this wastewater difficult to degrade naturally [123]. Focusing on anaerobic degradation, long-chain fatty acids, which are present on a high level in the washing water, have been reported to be inhibitors of the digestion process [93]. To reduce this effect, electrocoagulation has been proposed as a successful pretreatment for oily wastewater with a subsequent anaerobic treatment [129, 130].

With the likely expansion of biodiesel production by plants using the conventional method, comes the inherent need to treat the wastewater. The main component of the wastewater is the residual remaining oil, thus, such wastewater should not be discharged into public drainage because the oil causes plugging of the drainage and decreases biological activity in sewage treatment. Some of the typical commercially available treatments of oily wastewater employ a dissolved air floatation technique or oil and grease trap unit [130]. Currently, several processes have been developed to treat the biodiesel wastewater, such as the use of chemical recovery approach and electrochemical treatment [128, 130], but also the employment of microbiological processes [123, 131–134] and anaerobic digestion [129].

In the work of Jaruwat et al. (2010), the management of raw biodiesel wastewater was carried out at a laboratory scale at ambient temperature by a combined protonation based chemical recovery of biodiesel followed by electrochemical treatment of the residual wastewater [128]. The combined treatment completely removed COD and oil and grease, and reduced BOD (biologic oxygen demand) levels by more than 95%.

In the study, carried out by Chavalparit and Ongwandee (2009), electrocoagulation was adopted to treat the biodiesel wastewater [130]. This study demonstrates that the electrocoagulation process using an aluminum anode and graphite cathode is effective in reducing oil and grease and suspended solids by more than 95% in the washing water. However, the COD removal is achieved by 55% due to less significant removal of glycerol and methanol. Therefore, the electrocoagulation process is possibly suitable for a primary treatment for biodiesel wastewater and it still requires a further biological treatment process. Authors believe that pretreatment with electrocoagulation followed by a biological treatment process is feasible and competitive compared with evaporation or pure physicochemical treatments. It requires less energy consumption, short process time, no chemical addition and less sludge production.

The biological treatment of washing water was investigated by Suehara et al. (2005). For the microbiological degradation using a 10-L fermentor, oil degradable yeast, *Rhodotorula mucilaginosa*, was used and the optimum conditions were determined [123]. The pH was adjusted to 6.8 and several nutrients such as a nitrogen source (ammonium sulfate, ammonium chloride or urea), KH₂PO₄ and MgSO₄·7H₂O were added to the wastewater. To avoid the inhibition of the microbial growth, the raw biodiesel wastewater was diluted with the same volume of water. The optimal initial concentration of yeast extract was 1 g/L and the optimal C/N ratio was between 17 and 68 when using urea as a nitrogen source. Authors suggest this biological treatment system to be useful for small-scale biodiesel production plants, because it is simple and no controllers, except for a temperature, are necessary.

Kato et al. (2005) proposed a continuous-type consortium bioreactor for treatment of washing water [131]. The main component of this reactor was bacteria-fixed ceramic material with high-oil degrading capability. A series of oil decomposition tests was carried out using the consortium system, in which the most important bacteria types were *Acinetobacter*, *Bacillus* and *Pseudomonas*. The optimal conditions for operation were confirmed by batch tests: air agitation, pH of around 6 and water temperature of 30°C. This reactor operated almost maintenance-free for one year. The field test results for washing water showed that oil and grease concentrations decreased from an initial 120 g/L to a treated range of 10–30 mg/L.

Papanikolaou et al. investigated valorization of soaps from washing water for the production of microbial lipids of specific structure [132–134]. Several oleaginous yeasts and molds are able to accumulate in abundance storage lipid, and at the same time modify the composition of the fat utilized as the carbon source. In the case of various crude fats or fatty wastewaters of low value, this may be an industrially and financially interesting approach. Potential production of cocoa-butter substitute by *Yarrowia lipolytica* was studied and the cell growth and lipid accumulation of *Y. lipolytica* was investigated [132].

The anaerobic codigestion of glycerol and wastewater derived from biodiesel manufacturing was studied in batch laboratory-scale reactors of 1 L volume, inoculated by granular biomass, at mesophilic (35°C) temperature [129]. The main purpose of this study was to evaluate the performance, stability, biodegradability, methane yield coefficient, kinetics of methane production, inoculum-substrate ratio and OLR of the anaerobic codigestion of these by-products derived from biodiesel manufacturing.

Prior to the biological treatment, glycerol was acidified with H₃PO₄ in order to recover the alkaline catalyst employed in the transesterification reaction (KOH) as agricultural fertilizer. Wastewater was subjected to an electrocoagulation process in order to reduce its oil content. The pretreated washing water was mixed with glycerol at a proportion of 85–15 (COD), until obtaining a final soluble COD of 300 g/L. After mixing, the anaerobic revalorization of the wastewater was studied employing inoculum-substrate ratios ranging from 5.02 to 1.48 kilogram of VSS (volatile suspended solids) per kilogram of COD and OLR of 0.27–0.36 kg/kg·d (COD/VSS). Biodegradability was found to be around 100%, while the methane yield coefficient was 0.310 m³/kg COD removed. The results showed that anaerobic codigestion reduces the clean water and nutrient requirement, with the consequent economical and environmental benefit.
8. Conclusions

The process of biodiesel production is predominantly carried out by catalyzed transesterification. Besides desired methylesters, this reaction provides also a few other products, including crude glycerol, oil-pressed cakes and washing water. Although their composition widely varies depending on the parameters and substrates used for biodiesel production, all these by-products provide valuable feedstocks for biogas generation. The possibility and performance of anaerobic digestion of these materials have been studied to various extents. The results can be summarized in few points:

(i) Crude glycerol from biodiesel production was proven to be a suitable substrate for anaerobic degradation. A couple of studies have demonstrated the possibility of biogas production, using g-phase as a single substrate.

(ii) G-phase has also shown a great potential as a cosubstrate by anaerobic treatment of different types of organic waste: organic fraction of municipal solid waste, mixture of olive mill wastewater and slaughterhouse wastewater and potato processing wastewater. Positive effect of crude glycerol on the enhancement of anaerobic processes was observed by treatment of corn maize, maize silage, and swine manure.

(iii) Oil cakes and oil meals can also be used as feasible and economically interesting substrates for biogas production. The possibility of methane production from rapeseed and sunflower oil cakes, which deserve the most interest in our area, has been suggested lately.

(iv) Tests of anaerobic degradability, biochemical methanogenic potential and influence of substrate to inoculum ratio demonstrated high stability of the anaerobic digestion of sunflower oil cake under mesophilic conditions.

(v) The potential biogas production from rapeseed meal and rapeseed cake was estimated. It was shown, that with the increased amount of oil gained in the extraction process, the possible biogas production from rapeseed cake decreases.

(vi) No significant effect of the pretreatment (thermal and chemical), of neither sunflower nor rapeseed residues, on the enhancement of methane yield has been observed so far.

(vii) Washing water from biodiesel purification is also a promising material for anaerobic degradation, considering the high content of readily degradable organic substances. However, the possibility of biogas generation has not been sufficiently studied.

(viii) The specific inhibition effects, resulting from the substrates composition, have to be considered by anaerobic treatment of biodiesel by-products. In case of anaerobic digestion of crude glycerol, high salinity of the substrates may negatively affect the methanogenic microorganisms. The concentration of ammonium should also be monitored. Since nitrogen is an essential nutrient for microorganisms, the low concentration in the crude glycerol and washing water has to be compensated by ammonium supplement. On the other hand, rapeseed cake contains a high portion of nitrogen-rich substances, which may cause inhibition of digestion due to ammonium accumulation in the reactor.

Utilization of the by-products as a potential source of energy, rather then treat them as a waste, seems to be a convenient way of lowering the costs of biodiesel and making it more competitive.

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