## FEATURE ARTICLE

# Biogas and its opportunities—A review

Panagiotis G. Kougias ( $\boxtimes$ ), Irini Angelidaki

Department of Environmental Engineering, Technical University of Denmark, Kgs. Lyngby DK-2800, Denmark

#### HIGHLIGHTS

- A comprehensive description of the biogas process is presented.
- Main operational parameters influencing the biogas process are reviewed.
- A historical overview of the biogas development is extensively presented.
- The current status of anaerobic digestion for biogas production is discussed.
- New horizons for exploitation and utilisation of biogas are proposed.

## ARTICLE INFO

Article history: Received 11 December 2017 Revised 22 March 2018 Accepted 23 March 2018 Available online 30 April 2018

Keywords: Anaerobic digestion Biogas Biowastes Solid waste Manure Industrial waste

# 1 Introduction

Anaerobic degradation or digestion (AD) is a microbialmediated process in which organic carbon is converted, by subsequent oxidations and reductions, to its most oxidized state  $(CO_2)$ , and to its most reduced form  $(CH_4)$ . This biological route is catalyzed by a wide range of microorganisms acting synergistically in the absence of oxygen. It is well known that AD is responsible for carbon recycling in different environments, including wetlands, rice fields, animals' intestines, aquatic sediments and manures. This process is also extensively applied in industrial scale for valorisation of organic residues. Waste

#### GRAPHIC ABSTRACT



#### ABSTRACT

Biogas production is a well-established technology primarily for the generation of renewable energy and also for the valorization of organic residues. Biogas is the end product of a biological mediated process, the so called anaerobic digestion, in which different microorganisms, follow diverse metabolic pathways to decompose the organic matter. The process has been known since ancient times and was widely applied at domestic households providing heat and power for hundreds of years. Nowadays, the biogas sector is rapidly growing and novel achievements create the foundation for constituting biogas plants as advanced bioenergy factories. In this context, the biogas plants are the basis of a circular economy concept targeting nutrients recycling, reduction of greenhouse gas emissions and biorefinery purposes. This review summarizes the current state-of-the-art and presents future perspectives related to the anaerobic digestion process for biogas production. Moreover, a historical retrospective of biogas sector from the early years of its development till its recent advancements gives an outlook of the opportunities that are opening up for process optimisation.

© Higher Education Press and Springer-Verlag GmbH Germany, part of Springer Nature 2018

and wastewater treatment has become a political priority in several countries. Biowastes, i.e. sludge, manures, agricultural or industrial organic wastes, as well as contaminated soils etc., have been traditionally applied in soils untreated as biofertilisers or deposited in landfills or even in worst cases dumped into the environment. However, environmental awareness has introduced strict legislations preventing such practices. For example, European Union set specific permitting rules for disposal of biodegradable organic matter to landfills. Treatment of biowastes by AD processes is in many cases the optimal way to convert organic waste into useful products such as energy (in the form of biogas) and soil conditioner (fertilizer). This practically means that after stabilizing the biowastes, by extraction of the energy potential, the remaining residues, can be returned to the agricultural soils providing all the necessary beneficial nutrients and maintaining humus and

<sup>✉</sup> Corresponding author

E-mail: panak@env.dtu.dk

structure in soils. The main advantages of the industrial AD process rely on the production of a versatile energy carrier and the high degree of organic matter reduction with small increase-in comparison to the aerobic processof the bacterial biomass.

This review aims to summarize the current knowledge on biogas production process and to describe innovative trends that are envisioned to have a strategic role in the near future.

# 2 Biogas and its utilization for energy production

#### 2.1 Feedstock strategies

There are different ways to classify the operation mode of biogas plants depending on the influent feedstock, the applied temperature and reactor configuration. The reactor type for anaerobic digestion is largely determined by the consistence and dry matter content of the influent to be treated. For influent substrates below 500 mg/L Total Suspended Solids (TSS) reactors with flocculent sludge can be used. For higher TSS content in the influent substrates (0.5 to  $2-3$  g TSS/L) immobilised granular sludge type reactors such as UASB, or EGSB can be used.

Table 1 Methane yield of various organic residues

Finally, Continuous Stirred Tank Reactors (CSTR) are most commonly employed for slurries, such as manures, with TSS in the range of 30 to 70–80 g/L). For higher dry matter content substrates  $(>100 \text{ g/L})$  special types of rector configurations have been developed taking into account mixing and transportation of the solid influents. An initial variation can be defined among dry and wet fermentation. The term "dry fermentation" describes the degradation process, which is characterized by high solids content ranging from 15% to 35% (or even higher for batch garage type reactors using solid waste), while on contrary, during "wet fermentation", the solids content is up to 10%, and thus the liquid content is comparatively higher [[1](#page-8-0)]. The initial design of the plant's configuration is dependent on the selection between these two fermentation processes. It has to be noted that the methane yield varies significantly among different substrates based on their chemical composition (Table 1).

The theoretical methane yields of typical substances suitable for anaerobic digestion are presented in Table 2. Very few biogas plants apply a mono-digestion operation (i.e. the digester processes only a single feedstock). The majority of the biogas plants follow co-digestion feeding strategies due to poor methane potential, high concentration of inhibitors (e.g. phenols, ammonia etc.) or seasonal availability of specific substrates. During the co-digestion



Notes: a) Results are based on biochemical methane potential tests. Differences in values may be attributed to the specific chemical composition of the tested substrates

Compounds	$\checkmark$ $\sim$ 1 COD/VS (g/g)	$\mathbf{r}$ $CH4$ yield <sup>a)</sup> $(mL-CH4/g VS)$	$CH4$ yield <sup>a)</sup> $(mL-CH4/gCOD)$	CH <sub>4</sub> content <sup>a)</sup> (%)
Protein <sup>b)</sup> $C_5H_7NO_2$	1.42	497	350	50
Lipids $C_{57}H_{104}O_6$	2.90	1015	350	70
Ethanol	2.09	732	350	75
Acetate	1.07	375	350	50
Propionate	1.51	529	350	58
Iso-butyrate/Butyrate	1.82	637	350	63
Iso-valerate/Valerate	2.04	714	350	65

Table 2 Theoretical methane yield of typical compounds

Notes: a) Methane yields are calculated at standard temperature and pressure conditions, i.e. 0°C and 1 atm. It is assumed that all the organic matter is converted to methane and carbon dioxide. b) Nitrogen is converted to ammonia  $(NH_3$  or  $NH_4^+)$ 

concept, various organic residues, which usually have dissimilar characteristics, are simultaneously treated in the same anaerobic digester. The advantages of the codigestion process can be summarized as follows:

• Increases loading of readily biodegradable matter depending on the chemical composition of the used substrates [[12](#page-8-0),[18](#page-8-0)].

• Improves buffer capacity of the influent mixture maintaining the pH levels within the range for methanogenesis [[21](#page-8-0),[22](#page-8-0)].

 Provides better nutrient balance, especially for improving the C/N ratio [[7,23,24\]](#page-8-0).

• Dilutes inhibitory compounds avoiding deterioration of the anaerobic digestion process [\[2,25](#page-8-0)].

Leads to higher volumetric methane production [[3,26](#page-8-0)].

• Promotes synergistic effects leading to advanced biodegradation [\[14,16](#page-8-0)[,27\]](#page-9-0).

 Contributes in solving problems related to the digesters' stirring or pumping, especially while processing solid wastes [\[28](#page-9-0)].

• Improves the economics of biogas plants [[29](#page-9-0),[30](#page-9-0)].

• Provides better hygienic stabilization [[31](#page-9-0)].

2.2 Main operational parameters influencing the biogas process

#### 2.2.1 Temperature

The overall digestion process occurs in anaerobic reactors that operate under mesophilic (30°C–40°C, mainly 35°C– 37°C) or thermophilic (50°C–60°C, mainly 52°C–55°C) temperature conditions. The selection of the operating temperature and its control at stable levels is of outmost importance as these parameters are strongly affecting the development of the digesters' microbial structure [[32](#page-9-0)–[34](#page-9-0)].

Temperature fluctuations cause process imbalances associated with accumulation of Volatile Fatty Acids (VFA) and concomitant decrease in biogas production [\[35\]](#page-9-0). It is well known that thermophilic conditions present a number of advantages compared to mesophilic ones, namely:

 Can withstand higher organic loads due to faster reaction rates [\[36,37](#page-9-0)].

 Shorter hydraulic retention time (HRT) of the reactor which typically lasts 15 days at thermophilic and 20–25 days at mesophilic conditions [\[28\]](#page-9-0).

• Can achieve better degradation of Long Chain Fatty Acids (LCFA) [[38](#page-9-0)].

• Produces lower amount and more qualitative effluent digestate depending on the chemical composition of the used substrates [[37](#page-9-0)].

• Improves the energy balance of the process and lowers the initial capital cost for investment due to the smaller reactor size [\[37,39\]](#page-9-0).

 Achieve better sanitation of the effluents [\[40,41\]](#page-9-0). This is the main reason for choosing thermophilic temperatures. For ensuring good effluent quality certain regulations have to be fulfilled such as a minimum guaranteed holding time at a specific thermophilic temperature. Therefore, many thermophilic biogas plants are leading the effluents through a holding tank where the effluents are retained for number of hours to ensure good sanitation.

On the contrary, the drawbacks of thermophilic operation are associated with the requirement of more energy for covering the increased thermal needs. The energy needs are significantly reduced with good insulation of the tanks and efficient heat exchanging. Other drawbacks are higher risk of process instability especially in case of high ammonia loads and reduced dewaterability [[42,43](#page-9-0)]. Finally, another issue that was considered to prevent the thermophilic operation of biogas plants was related to the

inoculation and start-up process [[28](#page-9-0),[44](#page-9-0)]. Nevertheless, nowadays, improved start-up strategies are developed to alleviate this problem [[34](#page-9-0),[43](#page-9-0),[45](#page-9-0)].

#### 2.2.2 pH and volatile fatty acids

The biogas production process occurs in a defined narrow pH interval ranging from approximately 6 to 8.5. In case the pH of the reactor exceeds these limits then the process is deteriorated resulting in a dramatic decrease in methane production. Changes in the pH values can be correlated with other operational parameters; thus, an accumulation of organic acids (acidification) will typically lower the pH, while increased ammonia concentrations or  $CO<sub>2</sub>$  removal will lead to an increment of pH values. It has to be mentioned that the pH drop due to VFA accumulation is additionally dependent on the used substrate. Some organic residues, as for example cattle manure, have high buffer capacity, and thus, are able to maintain the pH of the system balanced. A pH drop will occur only in cases that the concentration of VFA is remarkably high exceeding a certain point and frequently the process is already severely influenced. Therefore, the VFA accumulation can be seen as a result of an already inhibited process, and is not considered as the actual reason.

Currently, it is widely accepted that the VFA are recognized as one of the state indicators for the biogas process [\[46\]](#page-9-0). More specifically, the concentration profile of individual VFA and especially the ratio between them can provide essential information for process monitoring and can serve as early indicators for potential imbalances. For example, in a deliberate inhibitory shock of a manurebased biogas reactor with Long Chain Fatty Acids (LCFA), it was shown that the acetate to propionate ratio was reversed shortly after the LCFA injection, reflecting the process disturbance [\[47\]](#page-9-0). Neither the concentration of the total VFA nor the pH was found to be changed highlighting the importance of individual fatty acids as key intermediates for detection of upsets during the biogas production process [\[46,47\]](#page-9-0).

#### 2.2.3 Inhibitors of the process

During AD, there are some compounds that, if their concentration exceeds certain limits, can reduce the biogas production or in worst conditions can cause fatal deterioration of the process. These compounds are either toxic substances or intermediate metabolic products. In general, methanogens are considered to be more sensitive to a potential exposure to toxicants compared to bacteria.

One of the most common inhibitors of AD process is the increased ammonia concentration. Ammonia is present in a wide variety of organic residues, as for example swine or poultry manure and high proteinaceous sludge [[9,10](#page-8-0),[48](#page-9-0)]. Moreover, ammonia can also be formed during protein

degradation or can originate from other compounds, such as urea [\[49,50\]](#page-9-0). It is well documented that the inhibitory effects are attributed to the free ammonia  $(NH<sub>3</sub>)$  and not to the ammonium ion  $(NH_4^+)$ . In general, it is reported that a concentration of total ammonia nitrogen between 1.7 to 14 g/L can cause 50% reduction in the methane production [[51](#page-9-0)]. However, the absolute concentration value above which ammonia leads to process inhibition is difficult to be quoted as this is additionally dependent on other factors, such as temperature, pH or inoculum source. More specifically, free ammonia is in equilibrium with ammonium ion and its concentration depends on the pH value. Similarly, the equilibrium is affected by the operational temperature; higher temperature leads to higher concentration of free ammonia, resulting in more intense toxicity phenomena. Ammonia inhibition causes also VFA accumulation, which will in turn decrease the pH of the reactor. The lowering of the pH will partially alleviate the toxicity effect of ammonia as the concentration of free ammonia will be decreased. However, this homeostatic mechanism will maintain the operation of the reactor in a relapsed phase, which is called "inhibited steady state" condition. It has been previously reported that many full-scale biogas plants that operate under inhibited steady-state conditions due to high ammonia loads are losing up to 30% of their maximum methane production yield, which obviously lead in serious operational problems and significant economic losses [\[52\]](#page-9-0).

Another compound that is associated with toxicity effects of biogas production process is the Long Chain Fatty Acids (LCFA). Various agro-industrial residues, as for example slaughterhouse wastes, food wastes and olivemill wastewater, contain high concentrations of LCFA. The inhibition caused by LCFA is attributed to the accumulation of compounds produced during β-oxidation, which can not be further oxidised as the required reactions are thermodynamically unfavorable [\[38\]](#page-9-0). Therefore, it has been previously reported that LCFA affect negatively the activity of hydrolytic, acidogenic, acetogenic bacteria and methanogenic archaea [[53](#page-9-0),[54](#page-9-0)]. It was found that methanogens are more tolerant to the inhibitory effect of LCFA compared to the bacterial community [\[55\]](#page-9-0). Moreover, hydrogenotrophic methanogens are more resilient to LCFA toxicity than acetoclastic methanogens [\[53\]](#page-9-0). It has been proven that LCFA inhibition does not necessarily lead to a fatal deterioration of the process, but is rather a reversed phenomenon [[47](#page-9-0),[54\]](#page-9-0). A recent metagenomic study demonstrated that a biogas microbial community, which is previously exposed to LCFA, recovers faster from the inhibitory shock compared to a non- adapted microbial consortium and also that the process is less deteriorated [[47](#page-9-0)].

Finally, another problem of biogas plants is related to foaming incidents, which are caused by operational problems (e.g. poor mixing, organic overload etc.) or by specific biosurfactants produced during AD process

[\[14,](#page-8-0)[56\]](#page-9-0). A survey reported that foaming incidents in fullscale biogas plants often lasted from one day to three weeks, resulting in 20%–50% biogas production loss [[57](#page-10-0)]. Foaming is not leading to VFA accumulation or acidification of the rector like the other inhibitors. However, the process imbalance is attributed to the thick layer that is formed on the reactors surface, entrapping the produced biogas, reducing the reactor active volume and thus creating dead zones. Several strategies, mainly based on the addition of chemical agents, have been employed to counteract foaming incidents either by preventing the formation of foam or by destructing it once it is created [\[58\]](#page-10-0).

#### 2.3 End-use of biogas in the energy sector

Biogas is mainly composed by carbon dioxide (25%–50%) and methane (50%–75%); however, minor quantities of nitrogen, hydrogen, ammonia and hydrogen sulphide (usually less than 1% of the total gas volume) are also present [\[59](#page-10-0)].

Traditionally, biogas is exploited for generation of heat or combined heat and power (CHP). Especially in developing countries, in which electrical power is limited and people rely on biomass utilization for covering their energy needs, biogas is extensively employed for fuelling cooking stoves [[60,61](#page-10-0)] and for providing lightning [[62](#page-10-0)]. The biogas reactors in these areas are household scaled with a typical size of only  $2-10$  m<sup>3</sup>, which does not allow the accommodation of CHP or purification processes [[63](#page-10-0)]. On the contrary, in farm-scale or centralised biogas plants, the generated gas is burned in a CHP unit, and depending on the efficiency of the engine, is transformed to approximately 35%–40% electrical energy, 45%–50% heat, while 15% are energy losses. It has to be noted that the impurities contained in biogas, and especially hydrogen sulphide, must be removed to avoid any damage or corrosion of the combustion engines. Moreover, as a result of carbon transformation, organic bound minerals and salts are released and contained in the reactor's effluent stream, which can be further utilized as soil conditioners *(i.e.*) biofertilisers). As it will be further discussed, more attention is given nowadays to the expansion of biogas utilization as transportation fuel or as substitute to natural gas. To do so, the contained impurities in the biogas and mainly the  $CO<sub>2</sub>$  have to be removed. This led to an increment of cleaning and purification processes contributing to a greater market potential for biogas sector [\[64\]](#page-10-0).

# 3 Anaerobic digestion — Past

The biogas process has been recognized since ancient times. The first reference related to biogas was mentioned by Plinius, and was referring to mysterious flames

appearing from swamps or other subsurface locations. At those times, this observation was considered to be caused by dragons or other mythical phenomena [[65](#page-10-0)]. Moreover, anecdotal evidence suggests that biogas was used in Assyria during the 10th century before Christ (BC) in order to heat bath water [\[61\]](#page-10-0). The first attempt to describe biogas was made by the Italian physicist and chemist Alessandro Volta in 1777, who found methane in the marshes of Maggiore Lake [\[66\]](#page-10-0). Afterwards Cruikshank in 1801 proved the absence of oxygen molecules in methane, while Dalton provided the correct methane formula in 1804 [\[65\]](#page-10-0). Systematic investigations initiated in the second half of the nineteenth century, during which the microbiological basis for the AD process was founded. The first one that demonstrated that methane was derived from a microbiological process was Béchamp in 1868 [[67](#page-10-0)]. Shortly after, it was established that the polymers were hydrolysed by enzymatic activity, and that organic acids were produced as intermediates. In the beginning of the 20th century, and more specifically in 1906, Omelianski [[68](#page-10-0)] and particularly the Dutch microbiologist Söhngen [[69](#page-10-0)] showed that methane-reducing bacteria can directly utilize the products from cellulose fermentation (e.g. formate, acetate, ethanol, hydrogen and carbon dioxide). Some attempt was also given to establish knowledge about the microorganisms responsible for the different steps of the AD food chain. It is impressive that the mechanisms of<br>recent trends in anaerobic digestion related with the biogas<br>upgrade were first formulated more than a 100 years ago;<br>Söhngen's experiments in 1910 using enriched cul recent trends in anaerobic digestion related with the biogas upgrade were first formulated more than a 100 years ago; Söhngen's experiments in 1910 using enriched cultures [[67](#page-10-0)] concluded to the formulation of stoichiometric equation of hydrogenotrophic methanogenesis:

$$
CO2 + 4H2 \rightarrow CH2 + 2H2O
$$

Later, in 1933, with the work of Buswell and Boruff [[70](#page-10-0)] theoretical calculations of the methane potential were established. The first methane producing microorganisms were isolated in 1936 and were Mathanobacillus omelianskii, Methanobacterium formicicum, Methanosarcina barkerii, and Methanococcus vannielli [[67](#page-10-0)]. Since then, a much deeper knowledge about the AD process has emerged.

Back to the technological progresses, it is known that since 1860, septic tanks were introduced for sewage stabilization. It is reported that in 1890 a septic tank was designed by Donald Cameron from which the produced biogas was collected and used for street lightning in the city of Exeter, England [\[71\]](#page-10-0). The first AD plant was built in a leper colony in 1859 at Bombay, India [[72\]](#page-10-0). In China, the commercial use of biogas was attributed to Guorui Luo who constructed in 1921 a  $8 \text{ m}^3$  biogas tank fed with garbage to supply the energy for cooking and lighting his family house [\[73\]](#page-10-0). The same period started the commercialisation of biogas utilization in the western world. In 1920, the first sewage treatment plant in Germany was supplying the gas grid with biogas [[61](#page-10-0)]. However, the biogas momentum occurred in the 1970s, as a result of high oil prices that motivated research work in finding new alternative energy sources [\[61\]](#page-10-0). Increasing trend was also seen in research activities, which started growing after the first energy crisis mid-seventies, but mainly, when the awareness to climate changes and renewable energy at the end-nineties was settled. This can be verified by the number of research articles that are annually published in scientific journals as illustrated in Fig. 1.

## 4 Anaerobic digestion — Current status

#### 4.1 Fundamentals about the AD process

The general model for anaerobic degradation of organic matter includes four consecutive steps, namely hydrolysis, acidogenesis, acetogenesis and methanogenesis. Initially, fermentative bacteria hydrolyze the organic polymers to soluble oligomers and monomers by the action of extracellular enzymes. Subsequently, the dissolved products are further utilized by bacterial species in order to form short-chain fatty acids (i.e. lower than 6 carbons), acetate, alcohols, hydrogen and carbon dioxide. Shortchain fatty acids with more carbons than acetate and alcohols are oxidised by acetogenic bacteria resulting in the production of acetate, formate, hydrogen and carbon dioxide. Finally, the latter compounds are utilized by archaea (or by syntrophic interaction between syntrophic acetate oxidising bacteria and hydrogenotrophic archaea) to produce methane. This flow depicts a simplified representation of the process, which still requires intensive research so as to be fully elucidated.

Nevertheless, the knowledge about the biogas process has significantly increased during the recent years. New substrates (e.g. algae [\[74\]](#page-10-0)), novel applications (e.g. biogas upgrading [[75](#page-10-0)–[77](#page-10-0)]), solutions of AD problems (e.g.

ammonia toxicity [\[78,79\]](#page-10-0), accumulation of Long Chain Faty Acids [[80](#page-10-0),[81](#page-10-0)]), new tools for process monitoring (e.g. VFA sensors [[82\]](#page-10-0), modeling [[83\]](#page-10-0)), different reactor configurations (e.g. serial [\[35\]](#page-9-0), membrane reactors [\[84\]](#page-10-0)) are among the technological and methodological advancements that have been recently achieved. Especially, the significant reduction in the cost and required time of high throughput sequencing techniques enabled a rapid progression in understanding the complex AD microbial process. Not only information about the complex microbial composition, but also about the expression of the different genes at various environmental conditions has been enlightened, giving enormous possibilities which can be explored in the future. Nowadays, advanced –omic tools are employed to decipher the AD black box. Thus, genome-centic metagenomics coupled with metatranscriptomics, metaproteomics, metabolomics or stable isotope probing are used in order to associate specific metabolic processes with microbial species [\[85](#page-10-0)–[91](#page-11-0)]. Apart from the syntrophic interactions between members of the AD microbiome, it was recently demonstrated that the AD food chain resembles a funnel concept (Fig. 2), involving novel microbes with broad functional roles at the initial steps of the process; subsequently, the community becomes steadily more specialized while reaching the last step of methanogenesis [[88](#page-10-0)].

#### 4.2 Statistics of biogas plants

The biogas process has been known and utilized for many years, but especially after the rise of energy prices during the 1970s, the process has received renewed attention due to the wish of finding alternative energy sources to reduce the dependency on fossil fuels. Although the price of fossil fuels decreased in 1985, and since 2015, the interest in the biogas process still remains due to the environmental benefits of anaerobic waste degradation. The main applications of biogas are in the area of treatment of



Fig. 1 Annual number of scientific articles indexed in "Scopus" and "Web of Science" databases based on the keyword "biogas". The decreased number of "Web of Science" for year 2017 is attributed to the time needed for the database to be updated



Fig. 2 Representation of the functional roles of the microbial species involved in the different steps of AD process resembles a funnel as reported by Campanaro and collaborators [\[88\]](#page-10-0). The species involved at each step of AD can be found at the original Figure of the cited article [[88](#page-10-0)]

primary and secondary sludge from domestic wastewaters, household solid wastes, manures, industrial wastes and agricultural residues. In respect to the energy output, the main contributors are however, mainly manures, industrial wastes, and agricultural residues, while municipal biomasses (sludges and household wastes) are playing only a minor role and the biogas process can be more seen as waste treatment methods for these waste streams, rather than bioenergy production factories.

Biogas plants are constantly proliferating in most parts of the world. Nowadays, the main contributor in biogas is China. China has an impressive number of biogas plants. It is estimated that there are around 50 million family scale, 4000 farm scale around, 2500 industrial (mainly for highstrength wastewater), and a few biogas plants treating wastewater sludge. Moreover, India has a considerable number of biogas plants mostly small scale and family owned. In Europe there is an increasing tendency in construction and expansion of biogas plants (Fig. 3). This is strongly motivated by the EU legislation which set as target 20% renewable energy contribution by year 2020.

## 5 Anaerobic digestion — Future perspectives

Despite AD has been known and applied for hundreds of years, both the technology and the applications are still relatively simple. So far, the process has been largely seen as a "black box" and the microbiology behind the process was taken as given, not able to manipulate. However, with the recent progress in microbial ecology, taking advantage of the huge development in sequencing technologies and by using bioinformatics, the biogas microbiome has started to be deciphered. Currently, several studies identify novel uncultivated microbes along with clarification of several of their metabolic interactions. It is certain that new information is progressively gained in tremendous high speed. This new knowledge will be used in the future for steering the AD in a more specialized way, tailored to the specific needs of the process. It is envisioned that the biogas sector will soon be in the era, in which advanced microbial resource management, interventions in microbial composition, and in some cases, entirely customised



Fig. 3 Biogas plants in Europe by end 2015 [\[92\]](#page-11-0)

microbial consortia will be applied. This will result in more efficient AD process with enhanced utilization of biomass. Moreover, it can not be disputed that microbiology will play a great role in diagnostics and monitoring of AD process by the exploitation of specific biomarkers.

Currently, there is a lack of automatic control in biogas plants and the process is mainly laying on empirical practices and man-made decisions. Therefore, the biogas digesters are occasionally overloaded or sometimes the biogas plant operators decide to follow a more conservative feeding strategy; in both cases, the profitability of the biogas plant is reduced due to the loss of potential methane production or due to imbalances. Advanced monitoring and control is going to play an essential role in the future biogas plants contributing significantly in process optimisation. Several monitoring sensors are already emerging and it is foreseen that their coupling with control models and algorithms will dominate the decision making process of the biogas plants.

Based on existing statistical data, it is projected that the number and the energy capacity of the full scale biogas plants will increase. Also, standardised family scale biogas reactors are going to be deployed in rural areas, avoiding the frequently occurring cases of abandoned digesters. It is estimated that around 50% of these household scale biogas reactors were abandoned in the past due to either poor construction or improper operation. Therefore, there is an increased market potential for the construction of turn-key biogas facilities with standardised plug-in modules.

In respect to novel applications, biogas will play a significant role in the creation of a sustainable circular economy where not only the organic matter but also nutrients (N, P) are recycled, returning the organic residues back to the societal community as energy, fuels and bioproducts. Therefore, besides heat and electricity biogas is going to be widely applied (after upgrading to biomethane) as vehicle fuel and will be added into the natural gas grid. To fulfil this goal, synergies with other renewable energy systems (e.g. wind or solar) through Power-to-Gas (P2G) concepts are projected, supported by

the sharp decrease in the production cost of renewable electricity, which has been recorded during the last decade. The new P2G technologies will aim to balance the ondemand electricity supply and also to provide cost effective solutions for the development of autonomous smart grids, especially in areas (e.g. islands) that are disconnected from the centralised electricity grid. The time frame in which such concepts will be demonstrated at full-scale is depending on various parameters, as for example the fluctuating electricity price, the CAPEX of the technologies and incentives provided by the local governments (e.g. energy strategy, feed-in tariff policies etc).

Moreover, apart from the conversion of  $CO<sub>2</sub>$  to biomethane, it expected that biogas will be exploited for the production of more advanced molecules. Under these concepts methane will be used as source for specific microorganisms (e.g. methanotrophic bacteria) to generate valuable compounds. High value added products like proteins (single cell proteins; both microalgae, methylotrophic bacteria and hydrogen oxidising bacteria), extracellular polysaccharides, bioplastics (e.g. polyhydroxyalkanoates), platform chemicals (e.g. biosuccinic acid, hexanol, lactic acid) are going to be targeted. Again, the implementation of the aforementioned processes at industrial scale relies on the successful addressing of biotechnological challenges, which can be overcome by interdisciplinary research. Thereby, the wastes and organic residues will get much higher economic value compared to their conventional conversion to biogas and in turn, biogas production will open new horizons for expanding its end use.

# 6 Conclusions

Biogas production process is an established technology for energy generation. However, recent trends open new horizons for exploitation of biogas, expanding its potential applications. Since the biogas market is facing rapid development, it is envisioned that more advanced <span id="page-8-0"></span>monitoring and control of the process is going to provide better utilization of the treated biomasses. A deeper understanding of microbial insights is going to play a more important role for tailoring the biogas process and for deciphering the anaerobic digestion "black box". Finally, it is foreseen that in the future the biogas plants are going to constitute advanced bioenergy factories with more secure and stable operation.

Acknowledgements This work was supported by the Innovation Fund Denmark under the project "SYMBIO–Integration of biomass and wind power for biogas enhancement and upgrading via hydrogen assisted anaerobic digestion" (Contract 12-132654).

## References

- 1. Stolze Y, Zakrzewski M, Maus I, Eikmeyer F, Jaenicke S, Rottmann N, Siebner C, Pühler A, Schlüter A. Comparative metagenomics of biogas-producing microbial communities from production-scale biogas plants operating under wet or dry fermentation conditions. Biotechnology for Biofuels, 2015, 8(1): 14
- 2. Tsapekos P, Kougias P G, Angelidaki I. Anaerobic mono- and codigestion of mechanically pretreated meadow grass for biogas production. Energy & Fuels, 2015, 29(7): 4005–4010
- 3. Søndergaard M M, Fotidis I A, Kovalovszki A, Angelidaki I. Anaerobic co-digestion of agricultural byproducts with manure for enhanced biogas production. Energy & Fuels, 2015, 29(12): 8088– 8094
- 4. Kougias P G, Boe K, Tsapekos P, Angelidaki I. Foam suppression in overloaded manure-based biogas reactors using antifoaming agents. Bioresource Technology, 2014, 153(2): 198–205
- 5. Labatut R A, Angenent L T, Scott N R. Biochemical methane potential and biodegradability of complex organic substrates. Bioresource Technology, 2011, 102(3): 2255–2264
- 6. Zarkadas I, Dontis G, Pilidis G, Sarigiannis D A. Exploring the potential of fur farming wastes and byproducts as substrates to anaerobic digestion process. Renewable Energy, 2016, 96(2): 1063– 1070
- 7. Tsapekos P, Kougias P G, Treu L, Campanaro S, Angelidaki I. Process performance and comparative metagenomic analysis during co-digestion of manure and lignocellulosic biomass for biogas production. Applied Energy, 2017, 185(1): 126–135
- 8. Li Y, Zhang R, Liu G, Chen C, He Y, Liu X. Comparison of methane production potential, biodegradability, and kinetics of different organic substrates. Bioresource Technology, 2013, 149(2): 565–569
- 9. Kougias P G, Fotidis I A, Zaganas I D, Kotsopoulos T A, Martzopoulos G G. Zeolite and swine inoculum effect on poultry manure biomethanation. International Agrophysics, 2017, 27(2): 169–173
- 10. Fotidis I A, Kougias P G, Zaganas I D, Kotsopoulos T A, Martzopoulos G G. Inoculum and zeolite synergistic effect on anaerobic digestion of poultry manure. Environmental Technology, 2014, 35(9–12): 1219–1225
- 11. Frigon J C, Guiot S R. Biomethane production from starch and lignocellulosic crops—A comparative review. Biofuels, Biopro-

ducts & Biorefining, 2010, 4(4): 447–458

- 12. O-Thong S, Boe K, AngelidakiI. Thermophilic anaerobic codigestion of oil palm empty fruit bunches with palm oil mill effluent for efficient biogas production. Applied Energy, 2012, 93(5): 648– 654
- 13. Menardo S, Cacciatore V, Balsari P. Batch and continuous biogas production arising from feed varying in rice straw volumes following pre-treatment with extrusion. Bioresource Technology, 2015, 180(36): 154–161
- 14. Kougias P G, Boe K, Einarsdottir E S, Angelidaki I. Counteracting foaming caused by lipids or proteins in biogas reactors using rapeseed oil or oleic acid as antifoaming agents. Water Research, 2015, 79(1): 119–127
- 15. Li Y, Zhang R, Liu X, Chen C, Xiao X, Feng L, He Y, Liu G. Evaluating methane production from anaerobic mono- and codigestion of kitchen waste, corn stover, and chicken manure. Energy & Fuels, 2013, 27(4): 2085–2091
- 16. Pagés-Díaz J, Pereda-Reyes I, Taherzadeh M J, Sárvári-Horváth I, Lundin M. Anaerobic co-digestion of solid slaughterhouse wastes with agro-residues: Synergistic and antagonistic interactions determined in batch digestion assays. Chemical Engineering Journal, 2014, 245(5): 89–98
- 17. Davidsson A, Gruvberger C, Christensen T H, Hansen T L, Jansen J. Methane yield in source-sorted organic fraction of municipal solid waste. Waste Management (New York, N.Y.), 2007, 27(3): 406– 414
- 18. Borowski S, Domański J, Weatherley L. Anaerobic co-digestion of swine and poultry manure with municipal sewage sludge. Waste Management (New York, N.Y.), 2014, 34(2): 513–521
- 19. Cabbai V, Ballico M, Aneggi E, Goi D. BMP tests of source selected OFMSW to evaluate anaerobic codigestion with sewage sludge. Waste Management (New York, N.Y.), 2013, 33(7): 1626–1632
- 20. D'Este M, Alvarado-Morales M, Ciofalo A, Angelidaki I. Macroalgae Laminaria digitata and Saccharina latissima as potential biomasses for biogas and total phenolics production: Focusing on seasonal and spatial variations of the algae. Energy & Fuels, 2017, 31(7): 7166–7175
- 21. Zhang C, Xiao G, Peng L, Su H, Tan T. The anaerobic co-digestion of food waste and cattle manure. Bioresource Technology, 2013, 129(2): 170–176
- 22. Wei Y, Li X, Yu L, Zou D, Yuan H. Mesophilic anaerobic codigestion of cattle manure and corn stover with biological and chemical pretreatment. Bioresource Technology, 2015, 198(1): 431– 436
- 23. Kougias P G, Kotsopoulos T A, Martzopoulos G G. Effect of feedstock composition and organic loading rate during the mesophilic co-digestion of olive mill wastewater and swine manure. Renewable Energy, 2014, 69(3): 202–207
- 24. Liu C, Li H, Zhang Y, Liu C. Improve biogas production from loworganic-content sludge through high-solids anaerobic co-digestion with food waste. Bioresource Technology, 2016, 219(1): 252–260
- 25. Mata-Alvarez J, Dosta J, Macé S, Astals S. Codigestion of solid wastes: a review of its uses and perspectives including modeling. Critical Reviews in Biotechnology, 2011, 31(2): 99–111
- 26. Dennehy C, Lawlor P G, Gardiner G E, Jiang Y, Cormican P, McCabe M S, Zhan X. Process stability and microbial community

<span id="page-9-0"></span>composition in pig manure and food waste anaerobic co-digesters operated at low HRTs. Frontiers of Environmental Science & Engineering, 2017, 11(3): 4

- 27. Macias-Corral M, Samani Z, Hanson A, Smith G, Funk P, Yu H, Longworth J. Anaerobic digestion of municipal solid waste and agricultural waste and the effect of co-digestion with dairy cow manure. Bioresource Technology, 2008, 99(17): 8288–8293
- 28. Angelidaki I, Ellegaard L. Codigestion of manure and organic wastes in centralized biogas plants: Status and future trends. Applied Biochemistry and Biotechnology, 2003, 109(1–3): 95–105
- 29. Hosseini Koupaie E, Barrantes Leiva M, Eskicioglu C, Dutil C. Mesophilic batch anaerobic co-digestion of fruit-juice industrial waste and municipal waste sludge: Process and cost-benefit analysis. Bioresource Technology, 2014, 152(152C): 66–73
- 30. Banks C J, Salter A M, Heaven S, Riley K. Energetic and environmental benefits of co-digestion of food waste and cattle slurry: A preliminary assessment. Resources, Conservation and Recycling, 2011, 56(1): 71–79
- 31. Sosnowski P, Wieczorek A, Ledakowicz S. Anaerobic co-digestion of sewage sludge and organic fraction of municipal solid wastes. Advances in Environmental Research, 2003, 7(3): 609–616
- 32. Levén L, Eriksson A R B, Schnürer A. Effect of process temperature on bacterial and archaeal communities in two methanogenic bioreactors treating organic household waste. FEMS Microbiology Ecology, 2007, 59(3): 683–693
- 33. Luo G, De Francisci D, Kougias P G, Laura T, Zhu X, Angelidaki I. New steady-state microbial community compositions and process performances in biogas reactors induced by temperature disturbances. Biotechnology for Biofuels, 2015, 8(1): 3
- 34. Zhu X, Treu L, Kougias P G, Campanaro S, Angelidaki I. Converting mesophilic upflow sludge blanket (UASB) reactors to thermophilic by applying axenic methanogenic culture bioaugmentation. Chemical Engineering Journal, 2018, 332(1): 508–516
- 35. Angelidaki I, Boe K, Ellegaard L. Effect of operating conditions and reactor configuration on efficiency of full-scale biogas plants. Water Science and Technology, 2005, 52(1–2): 189–194
- 36. Suhartini S, Heaven S, Banks C J. Comparison of mesophilic and thermophilic anaerobic digestion of sugar beet pulp: performance, dewaterability and foam control. Bioresource Technology, 2014, 152(1): 202–211
- 37. Bouallagui H, Haouari O, Touhami Y, Ben Cheikh R, Marouani L, Hamdi M. Effect of temperature on the performance of an anaerobic tubular reactor treating fruit and vegetable waste. Process Biochemistry, 2004, 39(12): 2143–2148
- 38. Labatut R A, Angenent L T, Scott N R. Conventional mesophilic vs. thermophilic anaerobic digestion: A trade-off between performance and stability? Water Research, 2014, 53(8): 249–258
- 39. Ghasimi D S M, Tao Y, de Kreuk M, Zandvoort M H, van Lier J B. Microbial population dynamics during long-term sludge adaptation of thermophilic and mesophilic sequencing batch digesters treating sewage fine sieved fraction at varying organic loading rates. Biotechnology for Biofuels, 2015, 8(1): 171
- 40. Watanabe H, Kitamura T, Ochi S, Ozaki M. Inactivation of pathogenic bacteria under mesophilic and thermophilic conditions. Water Science and Technology, 1997, 36(36): 25–32
- 41. Pandey P K, Soupir M L. Escherichia coli inactivation kinetics in

anaerobic digestion of dairy manure under moderate, mesophilic and thermophilic temperatures. AMB Express, 2011, 1(1): 18

- 42. Angelidaki I, Ahring B K. Anaerobic thermophilic digestion of manure at different ammonia loads: Effect of temperature. Water Research, 1994, 28(3): 727–731
- 43. Tezel U, Tandukar M, Hajaya M G, Pavlostathis S G. Transition of municipal sludge anaerobic digestion from mesophilic to thermophilic and long-term performance evaluation. Bioresource Technology, 2014, 170(5): 385–394
- 44. Zhu X, Treu L, Kougias P G, Campanaro S, Angelidaki I. Characterization of the planktonic microbiome in upflow anaerobic sludge blanket reactors during adaptation of mesophilic methanogenic granules to thermophilic operational conditions. Anaerobe, 2017, 46(1): 69–77
- 45. Tian Z, Zhang Y, Li Y, Chi Y, Yang M. Rapid establishment of thermophilic anaerobic microbial community during the one-step startup of thermophilic anaerobic digestion from a mesophilic digester. Water Research, 2015, 69(1): 9–19
- 46. Boe K, Batstone D J, Steyer J P, Angelidaki I. State indicators for monitoring the anaerobic digestion process. Water Research, 2010, 44(20): 5973–5980
- 47. Kougias P G, Treu L, Campanaro S, Zhu X, Angelidaki I. Dynamic functional characterization and phylogenetic changes due to Long Chain Fatty Acids pulses in biogas reactors. Scientific Reports, 2016, 6(1): 28810
- 48. An D, Wang T, Zhou Q, Wang C, Yang Q, Xu B, Zhang Q. Effects of total solids content on performance of sludge mesophilic anaerobic digestion and dewaterability of digested sludge. Waste Management (New York, N.Y.), 2017, 62(1): 188–193
- 49. Zhang W, Heaven S, Banks C J. Continuous operation of thermophilic food waste digestion with side-stream ammonia stripping. Bioresource Technology, 2017, 244(Pt 1): 611–620
- 50. Moestedt J, Müller B, Westerholm M, Schnürer A. Ammonia threshold for inhibition of anaerobic digestion of thin stillage and the importance of organic loading rate. Microbial Biotechnology, 2016, 9(2): 180–194
- 51. Chen Y, Cheng J J, Creamer K S. Inhibition of anaerobic digestion process: A review. Bioresource Technology, 2008, 99(10): 4044– 4064
- 52. Nielsen H B, Angelidaki I. Codigestion of manure and industrial organic waste at centralized biogas plants: Process imbalances and limitations. Water Science and Technology, 2008, 58(7): 1521–1528
- 53. Lalman J, Bagley D M. Effects of C18 long chain fatty acids on glucose, butyrate and hydrogen degradation. Water Research, 2002, 36(13): 3307–3313
- 54. Pereira M A, Pires O C, Mota M, Alves M M. Anaerobic biodegradation of oleic and palmitic acids: Evidence of mass transfer limitations caused by long chain fatty acid accumulation onto the anaerobic sludge. Biotechnology and Bioengineering, 2005, 92(1): 15–23
- 55. Ma J, Zhao Q B, Laurens L L M, Jarvis E E, Nagle N J, Chen S, Frear C S. Mechanism, kinetics and microbiology of inhibition caused by long-chain fatty acids in anaerobic digestion of algal biomass. Biotechnology for Biofuels, 2015, 8(1): 141
- 56. Moeller L, Lehnig M, Schenk J, Zehnsdorf A. Foam formation in biogas plants caused by anaerobic digestion of sugar beet.

<span id="page-10-0"></span>Bioresource Technology, 2015, 178(1): 270–277

- 57. Kougias P G, Boe K, O-Thong S, Kristensen L A, Angelidaki I. Anaerobic digestion foaming in full-scale biogas plants: A survey on causes and solutions. Water Science and Technology, 2014, 69 (4): 889–895
- 58. Kougias P G, Tsapekos P, Boe K, Angelidaki I. Antifoaming effect of chemical compounds in manure biogas reactors. Water Research, 2013, 47(16): 6280–6288
- 59. Angelidaki I, Karakashev D, Batstone D J, Plugge C M, Stams A J M. Biomethanation and its potential. Methods in Enzymology, 2011, 494(Chapter 16): 327–351
- 60. Lansche J, Müller J. Life cycle assessment (LCA) of biogas versus dung combustion household cooking systems in developing countries—A case study in Ethiopia. Journal of Cleaner Production, 2017, 165(1): 828–835
- 61. Bond T, Templeton M R. History and future of domestic biogas plants in the developing world. Energy for Sustainable Development, 2011, 15(4): 347–354
- 62. Rajendran K, Aslanzadeh S, Taherzadeh M J. Household biogas digesters—A review. Energies, 2012, 5(8): 2911–2942
- 63. Surendra K C, Takara D, Hashimoto A G, Khanal S K. Biogas as a sustainable energy source for developing countries: Opportunities and challenges. Renewable & Sustainable Energy Reviews, 2014, 31(2): 846–859
- 64. Sun Q, Li H, Yan J, Liu L, Yu Z, Yu X. Selection of appropriate biogas upgrading technology—A review of biogas cleaning, upgrading and utilisation. Renewable  $\&$  Sustainable Energy Reviews, 2015, 51(1): 521–532
- 65. van Brakel J. The Ignis Fatuus of Biogas Small-Scale Anaerobic Digesters ("Biogas Plants"): A Critical Review of the Pre-1970 Literature. Delft: Delft University Press, 1980
- 66. Volta A. Lettere del Signor Don Allesandro Volta... sull'aria infiammabile nativa delle paludi. Marelli, 1977
- 67. Barker H. Bacterial Fermentations. New York: Wiley, 1956
- 68. Omelianski W. Über Methanbildung in der Natur bei biologischen Prozessen. Zentralblatt fuèr Bakteriol. Parasitenkd. II, 1906
- 69. Söhngen N. Über bakterien, welche methan als kohlenstoffnahrung und energiequelle gebrauchen. Zentrabl Bakteriol Parasitenk Infekt, 1906
- 70. Buswell A, Boruff C. Mechanical equipment for continuous fermentation of fibrous materials. Industrial & Engineering Chemistry Research, 2002, 25(6): 147–149
- 71. Hobson P, Bousfield S, Summers R. Anaerobic digestion of organic matter: Critical Reviews in Environmental Science and Technology, 1974, 4(1–4): 131–191
- 72. Meynell P J. Methane: Planning a Digester. Berlin: Schocken Books, 1978
- 73. He P J. Anaerobic digestion: An intriguing long history in China. Waste Management, 2010, 30(4): 549–550
- 74. Vergara-Fernández A, Vargas G, Alarcón N, Velasco A. Evaluation of marine algae as a source of biogas in a two-stage anaerobic reactor system. Biomass and Bioenergy, 2008, 32(4): 338–344
- 75. Angelidaki I, Treu L, Tsapekos P, Luo G, Campanaro S, Wenzel H, Kougias P G. Biogas upgrading and utilization: Current status and perspectives. Biotechnology Advances, 2018, 36(2): 452–466
- 76. Bauer F, Persson T, Hulteberg C, Tamm D. Biogas upgra-

ding—Technology overview, comparison and perspectives for the future. Biofuels, Bioproducts & Biorefining, 2013, 7(5): 499–511

- 77. Kougias P G, Treu L, Benavente D P, Boe K, Campanaro S, Angelidaki I. Ex-situ biogas upgrading and enhancement in different reactor systems. Bioresource Technology, 2017, 225(1): 429–437
- 78. Westerholm M, Müller B, Arthurson V, Schnürer A. Changes in the acetogenic population in a mesophilic anaerobic digester in response to increasing ammonia concentration. Microbes and Environments, 2011, 26(4): 347–353
- 79. Fotidis I A, Karakashev D, Kotsopoulos T A, Martzopoulos G G, Angelidaki I. Effect of ammonium and acetate on methanogenic pathway and methanogenic community composition. FEMS Microbiology Ecology, 2013, 83(1): 38–48
- 80. Palatsi J, Illa J, Prenafeta-Boldú F X, Laureni M, Fernandez B, Angelidaki I, Flotats X. Long-chain fatty acids inhibition and adaptation process in anaerobic thermophilic digestion: Batch tests, microbial community structure and mathematical modelling. Bioresource Technology, 2010, 101(7): 2243–2251
- 81. Sousa D Z, Pereira M A, Smidt H, Stams A J M, Alves M M. Molecular assessment of complex microbial communities degrading long chain fatty acids in methanogenic bioreactors. FEMS Microbiology Ecology, 2007, 60(2): 252–265
- 82. Boe K, Batstone D J, Angelidaki I. An innovative online VFA monitoring system for the anerobic process, based on headspace gas chromatography. Biotechnology and Bioengineering, 2007, 96(4): 712–721
- 83. Batstone D J, Keller J, Angelidaki I, Kalyuzhnyi S V, Pavlostathis S G, Rozzi A, Sanders W T, Siegrist H, Vavilin V A. The IWA anaerobic digestion model No 1 (ADM1). Water Science and Technology, 2002, 45(10): 65–73
- 84. Vyrides I, Stuckey D C. Saline sewage treatment using a submerged anaerobic membrane reactor (SAMBR): Effects of activated carbon addition and biogas-sparging time. Water Research, 2009, 43(4): 933–942
- 85. Bremges A, Maus I, Belmann P, Eikmeyer F, Winkler A, Albersmeier A, Púhler A, Schlúter A, Sczyrba A. Deeply sequenced metagenome and metatranscriptome of a biogas-producing microbial community from an agricultural production-scale biogas plant. GigaScience, 2015, 4(1): 33
- 86. Schlüter A, Bekel T, Diaz N N, Dondrup M, Eichenlaub R, Gartemann K H, Krahn I, Krause L, Krömeke H, Kruse O, Mussgnug J H, Neuweger H, Niehaus K, Púhler A, Runte K J, Szczepanowski R, Tauch A, Tilker A, Viehöver P, Goesmann A. The metagenome of a biogas-producing microbial community of a production-scale biogas plant fermenter analysed by the 454 pyrosequencing technology. Journal of Biotechnology, 2008, 136  $(1-2)$ : 77-90
- 87. Treu L, Kougias P G, Campanaro S, Bassani I, Angelidaki I. Deeper insight into the structure of the anaerobic digestion microbial community; the biogas microbiome database is expanded with 157 new genomes. Bioresource Technology, 2016, 216(1): 260–266
- 88. Campanaro S, Treu L, Kougias P G, De Francisci D, Valle G, Angelidaki I. Metagenomic analysis and functional characterization of the biogas microbiome using high throughput shotgun sequencing and a novel binning strategy. Biotechnology for Biofuels, 2016,

<span id="page-11-0"></span>9(1): 26

- 89. Mosbæk F, Kjeldal H, Mulat D G, Albertsen M, Ward A J, Feilberg A, Nielsen J L. Identification of syntrophic acetate-oxidizing bacteria in anaerobic digesters by combined protein-based stable isotope probing and metagenomics. ISME Journal, 2016, 10(10): 2405–2418
- 90. Treu L, Campanaro S, Kougias P G, Zhu X, Angelidaki I. Untangling the effect of fatty acid addition at species level revealed different transcriptional responses of the biogas microbial commu-

nity members. Environmental Science & Technology, 2016, 50(11): 6079–6090

- 91. Ziels R M, Sousa D Z, Stensel H D, Beck D A C. DNA-SIP based genome-centric metagenomics identifies key long-chain fatty aciddegrading populations in anaerobic digesters with different feeding frequencies. ISME Journal, 2018, 12(1): 112–123
- 92. European Biogas Association. 6th edition of the Statistical Report of the European Biogas Association. Brussels: European Biogas Association, 2016