IV. RENEWABLE ENERGY SOURCES

FUNCTIONAL CONTROL OF THE TECHNOLOGIES FOR BIOGAS PRODUCTION

Nora Dinova, Mihaela Belouhova, Irina Schneider, Yana Topalova

Abstract. The technologies for biogas production, based on anaerobic digestion, become more and more widely applied in the practice in Bulgaria and worldwide. Most of them have problems such as ineffective biogas production and/or production of biogas with low quality. The monitoring of the processes solely by chemical, physical and technological parameters is not sufficient and is the reason for the ineffective performance of the technologies. There is need to be implemented strategies for functional control of the biological system carrying out the process of anaerobic digestion. In this review the most modern worldwide enzymatic, fluorescent and molecular methods and parameters for functional control of the technologies for biogas production are discussed.

Key words: anaerobic digestion, biogas production, functional control, molecular methods, fluorescent methods

1. INTRODUCTION

The technologies for biogas production, based on anaerobic digestion, are widely applied in the practice. Firstly, they are applied in the transformation of the energy, containing in the biomass, into a useful fuel (biogas), which can be stored and transported. Secondly, as a result of these technologies, except of biogas, fertilizers are also generated. Thirdly, the technologies based on anaerobic digestion allow wastes treatment reducing their harmful impact on the environment (3; 30; 33). Till now in Europe there are 14 000 biogas installations, 28% of which treat wastewater sludge, municipal and industrial wastes, and the other 72% use agricultural wastes as a substrate (21).

The technologies for biogas production become widely put into practice in Bulgaria. Particular examples for industrial biogas installations in the area of Sofia city are Sofia wastewater treatment plant (SWWTP) "Kubratovo" and Biological treatment plant (BTP) „Han Bogrov". SWWTP „Kubratovo", part of „Sofia water"AD, treats the redundant sludge from the Sofia wastewater treatment and disposes of four anaerobic digesters. The quantity of the produced biogas in the summer allows 115 % covering the needs for heat and warming the digesters (40). The process control is carrying out with analysis of technological parameters such as pH, temperature, yield and specific methane yield, etc., which do not provide information about the functional activity of the biological system in these reactors and makes the early diagnostic of a problem in the system impossible. Similar is the problem in all the wastewater treatment plants (WWTP) in Bulgaria.

BTP „Han Bogrov", part of "Municipal enterprise for waste treatment", produces biogas from the treatment of food bio-waste. The parameters, which are analyzed for process control, are pH, temperature, dry matter (DM), organic dry matter (ODM), volatile fatty acids (VFA), alkalinity and other technological parameters. Implementation of a strategy for functional control of the biological system is needed for providing stability, effectiveness and efficiency of the technology for biogas production. This is not concerning only the technologies for biogas production by the treatment of food bio-waste but by the treatment of all kind of wastes.

The installations for biogas production by agricultural wastes – plant and animal, become more and more distributed in Bulgaria. The agricultural wastes are a convenient substrate for anaerobic digestion due to their widely distribution. Especially valuable as a raw material are wastes containing lignocellulose. The republic of Bulgaria and the countries from the Balkan region possess enough quantity of them. This makes them waste – resource, which is a perspective alternative energy source. Their microbial biodegradation in anaerobic conditions is still not enough examined process (15). The implementation of a profound functional control is needed for clarifying the process in details, for its rational management and for providing high effectiveness and efficiency of the available technologies for biogas production by agricultural wastes.

Every technology for biogas production consists of several components: an equipment, a biological system, process parameters, control and management system (Fig. 1). The control systems are essential part of these technologies, but they are still not enough developed in a national scale, especially if we talk about the functional process control which involves the deep mechanisms of microbiological and enzymatic processes. The market has strong need and expect the solution of this problem. The implementation of scientific and innovative approaches will contribute to the construction of algorithms and rational strategies for control of the technologies for biogas production.

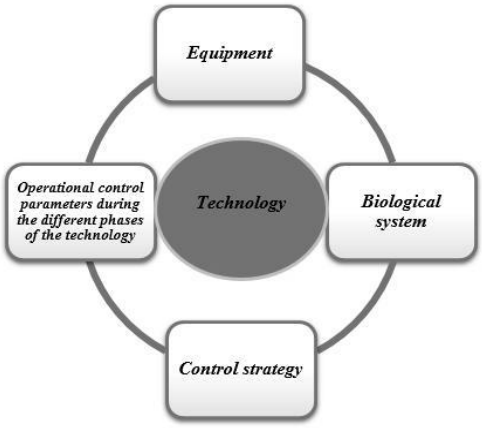

Fig. 1. Components of the technologies for biogas production

2. PROBLEMS IN CONTROL STRATEGIES

Anaerobic digestion is a multistep process which involves different groups of microorganisms that transform the organic substances into methane and carbon dioxide and reduce the organic matter with 35 to 60% depending on the operational conditions. A consortium of microorganisms, especially bacteria and methanogens, participates in the transformation of high organic compounds to methane (8).

Process problems in anaerobic digestion systems often go unnoticed until they severely affect the treatment and deplete biogas production because there is not enough monitoring and information for the plant operator to work on in order to properly regulate the feed flow rate, composition, and operational condition (29). Common problems related to the process of anaerobic digestion are ineffective production of biogas or production of biogas with low quality. Common operational problems with microbiological origin in the anaerobic digesters are: acidogenic conditions (acid media of the digester), which can be result of different reasons (for example hydraulic and organic loading; toxicity; insufficiently loading) and biological foaming.

The whole performance of the anaerobic digestion depends on many factors such as temperature, pH, chemical content of the substrate, retention time, competition for protons and electrons donors with the sulfate-reducing bacteria, toxicants, etc. (22). According to Gasch et al. the monitoring of biogas plants only by chemical and physical variables is apparently not sufficient and because of that the efficiency of numerous plants is not satisfactory or even indeterminable. Therefore and because the biogas production is a biological process, the analysis of microbiological parameters is very important (12). The anaerobic digestion stability depends on the active groups of microorganisms which are involved in it. The detailed understanding of the way on which the anaerobic digester functions requires quantitative information about the number of microorganisms, the biomass and the activity of the different groups of microorganisms. The number, the biomass and the activity are single ecological parameters but although they are interconnected by specific algorithms, they cannot be used interchangeably (22).

According to Yu et al. in anaerobiosis, the methanogens play a key role in stabilizing pollution load by participating in the terminal step, methanogenesis. Because methanogenesis is commonly the rate limiting step in most anaerobiosis, the majority of the attention has been given to investigating the most favorable conditions to ensure efficient methanogenesis (39).

For effective and efficient performance of the processes in the technologies for biogas production is required objective management of the biological system. According to Topalova (37) the management is a harmonic combination between analysis, decisions, actions and organizational measures whose goal is to provide sustainable and competitive advantages of the system they are applied to. The components of the management are

three: 1/ analysis strategy; 2/ strategy for decisions formulation; 3/ application strategy. The main management law is the Deming cycle, which includes four main steps – plan, do, check and action. The control is assumed as one of the essential parts of the management. The control strategy includes specific indicators and indicative relations, critical control points (CCP) by time, place and expansion of the control (Fig. 2). Here is also the reverse control which gives an opportunity the technology to be corrected depending on the final result and effectiveness (37).

Usually the CCP are the key places in the processes and this is the reason why it is possible on one side to obtain information from them about the speed, the scale and the mechanism of the process and on the other side this information is valuable for the adequate management of the process. The information we receive from the CCP analysis gives us the opportunity to estimate the functioning of the system, to prevent eventual risk events and to correct the technology depending on the final result and effectiveness.

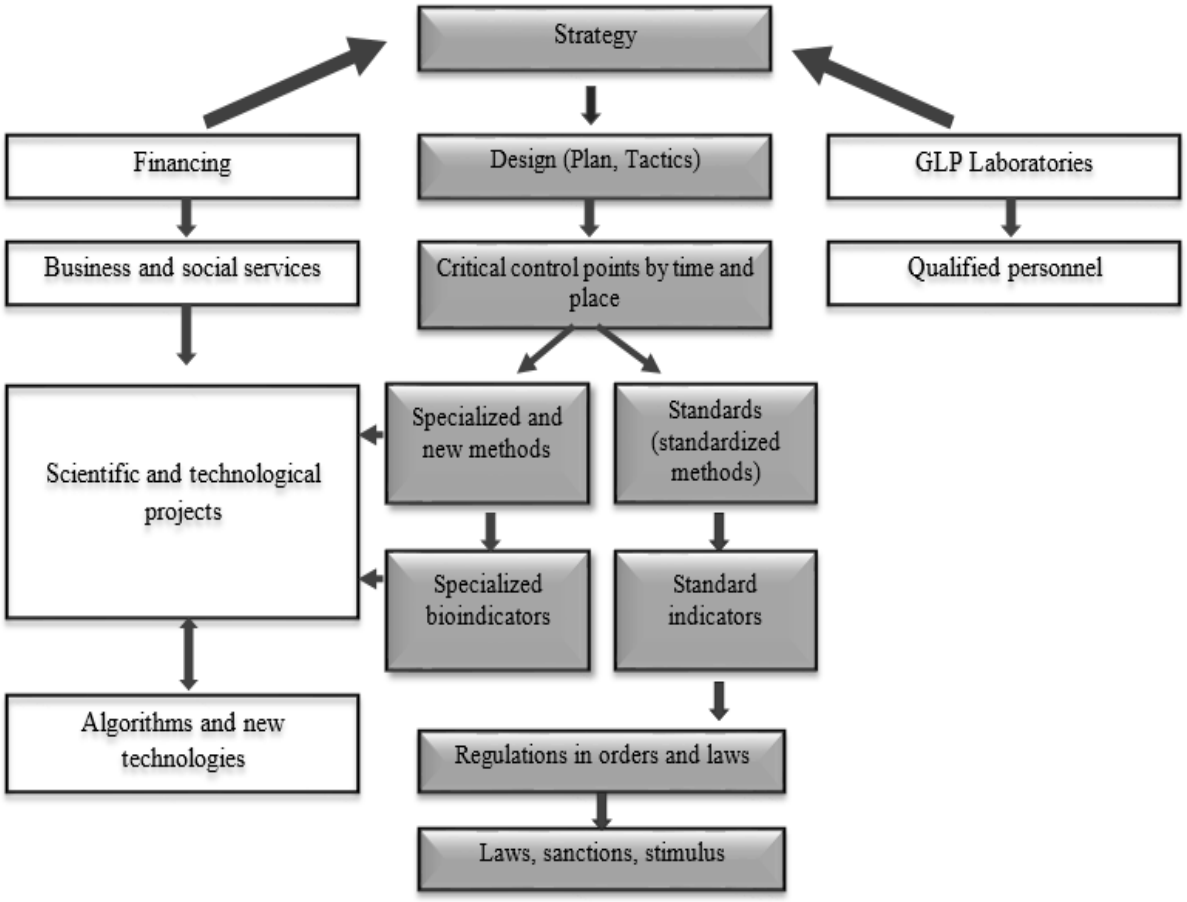

Fig. 2 Control means (37)

The most commonly used parameters in the control strategies in the technologies for biogas production are presented in Table 1. The change in any of the listed indicators is a signal for a system problem but if it comes to that the system usually is already strongly affected or inhibited.

Others commonly used parameters giving information about the process effectiveness are the ratio volatile organic acid content/buffer capacity (FOS/TAC) and the biochemical methane potential (BMP).

Table 1. Currently used key indicators for problems in the process of biogas production (32)

Indicator	**Decreasing**	**Increasing**
Biogas production	**X**	
Methane content in the biogas	**X**	
Alkalinity	**X**	
pH	**X**	
Fatty acids concentration		**X**
CO_2 content in the biogas		**X**

FOS/TAC

The result of this analysis presents the connections between two parameters – volatile fatty acids (FOS) and buffer capacity (TAC). TAC is an abbreviation for total inorganic carbon (basic buffer capacity measured as mg $CaCO_3$/l). The stability of the process can be evaluated by these two parameters separately (volatile fatty acids and buffer capacity). If the level of the organic acids is too high (for example >10 g/l), it indicates that the metabolism is not finished and that can result in process inhibition. However this effect is not representative if at the same time there is enough buffer capacity in the system (20).

BMP

The maximum methane quantity, which can be produced from 1 g COD in a wastewater, indicates how much the wastewater is amenable for anaerobic treatment As well as that BMP is an indicator for the kinetics and the efficiency of the anaerobic digestion process. Its determination takes up the time of 40 to 60 days (9). The results from the BMP test are sensitive to many factors, some of which are operational conditions such as temperature, pH and mixing (19).

The use of methods and parameters for early indication of the activity and its functional specifics is required to sustain a stable methanogenic bio-system. The functional parameters are assumed as much faster and more accurately representing the dynamic of the system state (37).

FUNCTIONAL CONTROL

One of the most commonly used parameters are the enzyme activities that catalyse the main, the alternative and the additional metabolic pathways (37).

Phosphatase activity

The phosphatase activity is proposed as a biochemical means for early indication of failures or serious operational problems in the anaerobic digesters and in the whole technology for biogas production. Increasing of the concentration of alkaline or acid phosphatases can predict instability of the process in the digesters earlier than the conventional indicators (pH, VFA biogas production) (8). The phosphatase activity is an indicator for the heterotrophic activity level of the microorganisms induced by the presence of organic substrates. Thus**,** it is a potential indicator for the speed of the metabolism of the biodegradable organic matter, the presence of toxicants and the organic loading (35).

Adenosine triphosphate (ATP) and anaerobic dehydrogenase activity (anDHA)

In the process of anaerobic digestion of organic compounds the biogas production, the biodegradation of the organic matter and the activity of the microorganisms are the three factors that are used for evaluation of the anaerobic biodegradability. The microbial activity of the anaerobic biological system can be defined by measuring the ATP concentration and the anDHA. These parameters correspond well

with other classical parameter such as biogas production rate. The analysis for defining the ATP concentration is more complex than this for defining the dehydrogenase (14). The determination of ATP gives information about the total physiological condition of the biomass (28). ATP is assumed as an indicator for the changes in the metabolic activity of the methanogen consortium and the functioning of the anaerobic digesters as well as for eventual toxic inhibition (35). The dehydrogenase activity is an approved and many times verified indicator for the total metabolic condition of the biological systems for the speed of transformation of the conventional and xenobiotic pollutants (37). In contrast to the aerobic dehydrogenases, the measuring of the anaerobic dehydrogenases requires another tetrazolium salt – iodonitrotetrazolium chloride (INT), which has lower redox-potential and implements forward in the electron transport chain right after the anaerobic dehydrogenases. (14).

Specific methanogenic activity (SMA)

SMA defines the methane producing capability of the sludge for a gram specific substrate when the availability of the substrate is not a limiting factor. The dynamic studying of the activity of the methanogen population is excessively important indicator for the control and for achieving effectiveness of the anaerobic digestion. The SMA is a key test in the studying of the operational conditions in the anaerobic technology, an important factor for its management and sustainability.

In the beginning of the start-up of a technology the determination of SMA is exclusively important for defining the appropriate start-up organic loading. During the different phases a regular measurement of SMA can give information about the different stages of development of the biological system. As well as that a change in SMA indicates an inhibition or accumulation of low degradable or even not degradable organic matter from the influent (16).

The substrates used in the SMA test usually are intermediate products of the anaerobic digestion process. The supply of every intermediate product separately to the biomass can enable the evaluation of every trophic group (6).

Esterase activity

The determination of the esterase activity has been proven in practice to be a good indicator for the general heterotrophic degradation activity in biological systems. It has been used particularly for wastewater and soil analytics. This analytical method till now has not been applied for monitoring of biogas plants, although several relationships between the esterase activity and other process parameters can be identified in other bioengineered plants. This makes the esterase activity also interesting for the analysis and monitoring of biogas plants (12). In some of the first researches related to this subject Lebuhn et al. observed a positive correlation between esterase activity and substrate conversion rate into methane, revealing that the process disruption is reflected by decreased enzyme activities. Furthermore, Lebuhn et al. found out a negative correlation of esterase as well as aminopeptidase activities and substrate quality, providing fermentability indications regarding silage as substrates (18).

Indicators like SMA assays, methane production rates, biogas composition, chemical oxygen demand (COD) removal, pH, granule morphology, acetate utilization rates, methanethiol concentration, quantification of VFA, BMP, etc. have all been suggested or used to evaluate digester function. These parameters are closely related to the metabolic functions of the microbial community but they do not directly quantify microorganisms. According to Morris et al. a successful removal of organic waste from the influent wastewater and methane production depend upon the collaborative efforts of the members of an interdependent microbial community, so knowledge of the structure and function of the community in anaerobic wastewater digesters can be very useful when attempting to stabilize or increase the efficiency of waste removal and biogas production (23).

There are different techniques for a quantitative determination of methanogens. Some of these methods, their advantages and disadvantages are presented in Table 2.

Table 2 Comparison between different methods for quantitative characterization of methanogens (4; 5; 34)

Method	**Advantages**	**Disadvantages**
Direct quantification of autofluorescent methanogens	Fast quantification of methanogens	The cells of *Methanosaeta* cannot be quantified; It is not applicable for cell aggregates
Determination of specific coenzymes	Detection of specific groups of methanogens	Content of coenzyme varies depending on the growth conditions of the species
Most probable number method	Verification of the results received by other methods	Continuous cultivation in strong anaerobic conditions and incomplete information because of the impossibility of the methanogens to be cultivated as a separate group (out of the community)
Cultivation of methanogen on/in a solid media	Verification of the results received by other methods	Continuous cultivation in strong anaerobic conditions and incomplete information because of the impossibility of the methanogens to be cultivated as a separate group (out of the community due to their syntrophic nature)
Real-time (quantitative) PCR	Analysis of microbial suspension with low quantity of methanogens	Probably it is not convenient for suspensions with high concentration of methanogens
Fluorescent in situ hybridization (FISH) with rRNA targeted fluorescent oligonucleotide probes detected by confocal scanning laser microscope	Analysis of biofilm and disperse structures of cell aggregates	The FISH protocol for direct quantitative measurement is too complicated but it provides easier qualitative determination of methanogens, their localization and their ability to form consortiums with synergetic and syntrophic interactions. In combination with digital measurements the received information gives sufficiently accurate quantitative and functional information
FISH – differentiation between specific (hybridized) and unspecific binding of rRNA – targeted oligonucleotide probe, using labeled and nonlabeled probes and fluorescence spectrometer	Quantitative determination of methanogens in attached and dispersed microbial aggregates such as biofilms and aggregates	If the quantity of the methanogens is small, the sample should be first concentrated on filter

Indicative potential of F_{420}

Different methods can be used for studying the microorganisms in the process of methanogenesis. The methane-producers can be distinguished by the other microorganisms due to their unique cell structure. The methanogens belong to a special domain microorganisms, *Archaea*, and thus they have unique components in their cell membranes. One of these components is cofactor F_{420}, which emits bluish fluorescent light when it is elucidated with ultraviolet (UV) light. Thus the methane-producers can be distinguished by the other microorganisms using microscope with a UV lamp as they fluoresce in a blue-green color (32) in 420 nm (31). Cofactor F_{420} is the primary electron acceptor of the hydrogen and serves as an electron carrier for different dehydrogenases and NADP oxireductases. When it is oxidized cofactor F_{420} exhibits an absorption peak in 420 nm, but in its reduced form the absorption in 420 nm and the

fluorescence disappear. Cofactor F_{420} encounters in different concentrations in all the methanogens. In anaerobic environment coenzyme F_{420} is associated exclusively with methanogenic bacteria. The quantitative methods for F_{420} determination are based mainly on its fluorescent characteristics and thence they have been developed for determination of the methanogens number or methanogenic activity in anaerobic digesters. It has been found out that the content of cofactor F_{420} does not correlate with the total methanogenic activity but just with this of the hydrogenotrophic methanogens. This discoveries are due to the essential differences in the content of the cofactors in acetotrophs and hydrogenotrophs. It has been found out also that these trophically different methanogens contain structurally different types of F_{420} (13).

According to Yu et al. the study of nonculturable organisms has benefited enormously from recent advances in the environmental molecular genetics. They propose different hybridization methods, such as fluorescence in situ hybridization (FISH) and dotblot and whole-cell hybridization, which have been valuable for detecting the presence of methanogens in different environments, including laboratory- or full-scale anaerobic bioreactors (39).

Indicative potential of PCR

PCR-methods, using specific for methanogens primers, are widely distributed in the characterization of methanogenic communities. The quantitative PCR (qPCR) and the HOPE - method (Hierarchical Oligonucleotide Primer Extension) provide sensitive quantitative information about the targeted gene with a high enough dynamic range for quantitative detection.

The HOPE technique combines the advantages of the PCR-methods and the fluorescent methods. These methods can be used for monitoring of different taxonomic groups of methanogens in microbial communities. The results produced by the PCR-based methods can be influenced by different factors or process steps such as cell lysis, DNA extraction, the choice of primer and the amplification step. This is a precondition the results received by the HOPE – technique to be carefully interpreted and to be interpreted in a parallel with the results produced by other methods (25).

Indicative potential of FISH

The fluorescence *in situ* hybridization analysis (FISH) is commonly used and popular method for studying microbial populations in natural and in biological systems in different technologies (10**).** FISH is useful for many applications in all fields of the microbiology because it is a technique that allows a moment visualization, an identification, a quantification and a localization of different microbial cells. FISH allows the identification not only of cultiurable microorganisms but also of non-culturable that helps for the understanding of complex microbial communities. The cultivation methods are time-consuming and often too selective especially for fastidious and non-culturable bacteria and thus they do not represent the exact content of the mixed microbial communities (24; 27). The non-cultivating techniques based on PCR and *in situ* hybridizations are more and more widely applied. Although the PCR techniques give information about the type of the microorganisms (culturable or non-culturable), they do not give the opportunity to study the spatial distribution and the localization of the targeted microorganisms in the microbial consortiums.

FISH detects nucleic sequences with a fluorescently labeled probes which specifically hybridize to the complementary targeted sequence in the intact cell (24). FISH markers can be fluorochromes or other molecules which are detected with fluorescently labeled similar reagents (2). The most commonly used target molecule for FISH in the microbiology is 16S rRNA due to its genetic stability, the presence of conservative and variable sequences and the high number of copies in the cell (24). 16S rRNA with its genes are also the most commonly used biomarkers for determination and quantitative detection of methanogenic communities in the environment. A big number of oligonucleotide probes for specific and hierarchic identification of methanogens has been used for clarifying the diversity and the presence of the different methanogenic communities in wastewater treatment sludge, in the solid bio-waste, in the rumen of herbivores, in sediments, in the human gut, in wetlands, in lakes, in rice fields, in soils, etc. (25).

FISH can be used for visualization of the spatial distribution of certain communities in biofilms such as the methanogenic communities in granular sludge (26). Tabatabei et al. have observed that the localization of microorganisms by FISH can prove the symbiotic relationship between specific microorganisms (36). The hydrogenotrophic methanogens often are localized near syntrophic substrate-degrading bacteria such as the propionate-oxidizing bacteria from the genera *Syntrophobacter* and *Pelotomaculum*; such a proximity between the syntrophic bacteria and the methanogens has been observed with FISH by a confocal laser scanning microscope (26).

DGGE/ rRNA clone library

According to Tabatabei et al. the denaturing gradient gel electrophoresis (DGGE) of 16S rRNA has been used for the characterization of bacterial communities in activated sludge. The rRNA clone library and DGGE method can provide direct sequence information useful for the assessment of phylogenetic groups of the present methanogens. However, the reliability and reproducibility of these methods are affected by technical factors like efficiency of DNA extraction, PCR biases, and selection of clones. Also, the relative abundance of different 16S rRNA clones amplified from a mixed population depends upon genome size and the rRNA gene copy number of bacteria present, and thus are not necessarily reflective of the relative population of different taxa. The combined use of FISH, DGGE and 16S rDNA-cloning methods allowed analyzing of bacterial communities more precisely (36). The catalyst for the methane-forming step in methanogenic archaea metabolism is Methyl-coenzyme M reductase (MCR), and the gene – *mcr*A, present in all methanogens is a functional marker (38). According to Alvarado et al. one of the advantages of *mcr*A gene is that only one or two copies of *mcr*A have been found in sequenced methanogens genomes, making it a more precise tool for estimating the number of these archaeas in the digesters than the 16S RNA gene, which can have up to four copies per genome. Also, they found out a strong correlation between *mcr*A copy number and methane production has been reported in H_2/CO_2^- enriched cultures. Moreover, transcription of *mcr*A has been used to demonstrate that methanogens are metabolically active, as it is well known that these microorganisms are capable of dormancy when conditions are not optimal. Thus, the identification of active members of the methanogenic population can provide a real insight into the digester performance (1). Some researchers examined the methanogen community composition by utilising DGGE and direct clone library analysis on PCR products obtained with *mcr*A-GC and *mcr*A primers (38).

Membrane hybridization

The analysis of different communities through methods based on RNA give information about the *in situ* activity of the different groups in the ecosystems because the synthesis of the RNA (with some exceptions) influence the growth rate of the organisms and the RNA metabolism is much faster than this of DNA. The membrane hybridization allows exact counting of the different types of rRNA molecules but this method requires multiple lab steps, very often radioactive labeled DNA probes, reference rRNA as an external standard for every experiment. This method can be replaced by others much faster and easy to be accomplished (25; 26).

Microarrays

According to Franke-Whittle et al. the core innovation of the microarray technique is the ability to attach nucleic acids to a solid matrix in a precise location to create a densely packed array. The DNA-microarray technology offers the possibility to be analyzed a whole order of microorganisms defining their presence or absence and their metabolic activity in some sample (13). Recently ANAEROCHIP has been created for analysis of the methanogenic communities in anaerobig digesters. It contains oligonucleotide sequences that detect 16S rRNA of the most of the mesophilic and thermophilic methanogens in an anaerobic biomass (7; 11).

Immunological techniques

Immunological analyses with polyclonal or monoclonal antibodies have been used as a tool for determining the numbers and identity of methanogens in anaerobic digesters. It has been shown that the methanogenic microflora of anaerobic digesters was more diverse than previously thought using methods

such as indirect immunofluorescence (IIF) and slide immunoenzymatic assays (SIA) (7). According to Lange et al. although the antibody probes are not able to reach the same detailed level of specificity as nucleic acid probes, and the production of antibodies is laborious and requires that the immunizing strain is isolated, some qualities are superior. They observed that binding of antibodies to the cell happens on the cell surface, leaving no need for permeabilization of the cells, as opposed to hybridization with nucleic acid probes, which takes place inside the cell. It was found out that the signal obtained with nucleic acid probes is dependent on the level of ribosomal RNA in the cell, which is related to the physiological state of the cell (17).

SEM

According to Lebuhn et al. apart from molecular biology depending light microscopy techniques, biofilms and single cells can be also investigated by scanning electron microscopy with unequalled magnifications of up to 500,000-fold. Scanning and transmission electron microscopy (SEM/TEM) have been used to study overall biofilm organization patterns and also to investigate cell-to-cell interactions of anaerobic digestion process innate syntrophic microbial partners on a nano-scale level such as the interspecies electron transfer (18).

CONCLUSION

Control strategies that include CCP defined on purpose, use of a complex of standard and specialized methods, analysis of specific for the biological system bioindicators with an accent on the functional parameters for control would provide stability, effectiveness and efficiency of the technologies for biogas production (Fig. 3).

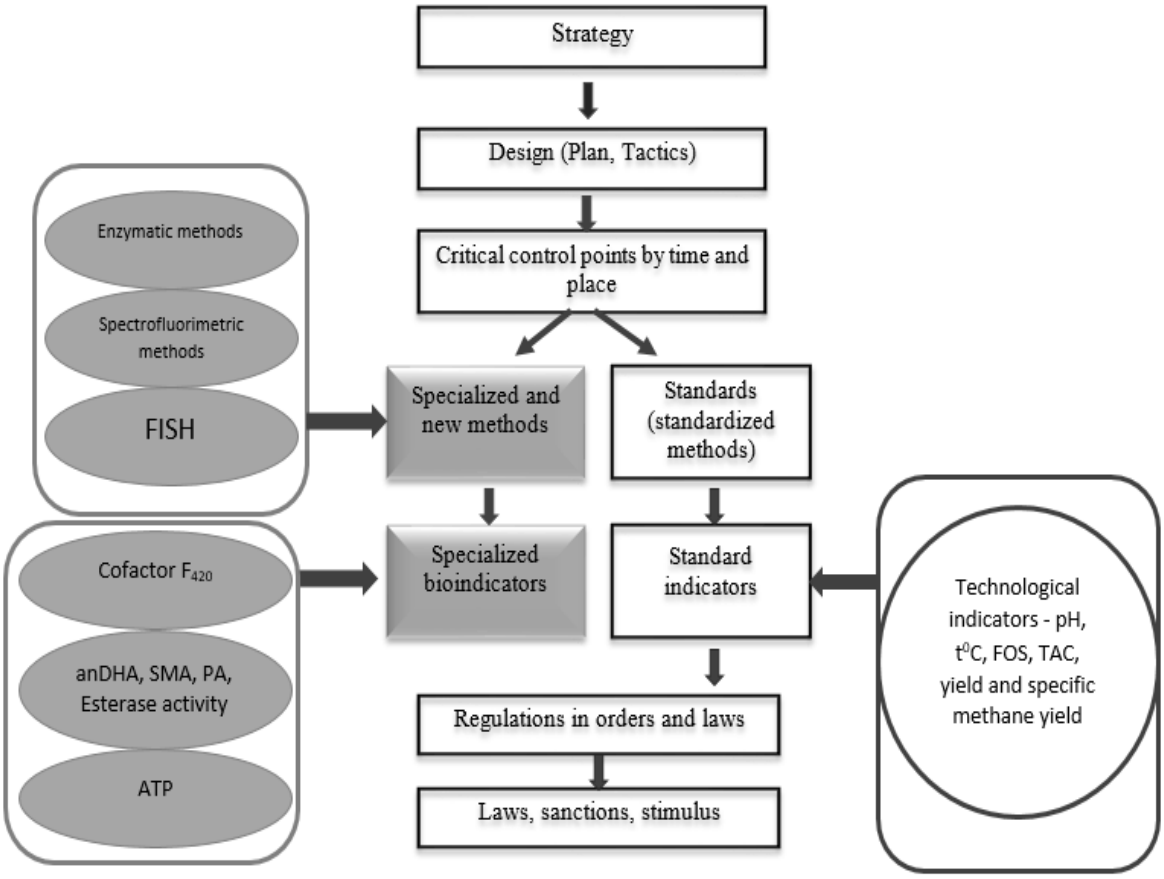

Fig. 3 Strategies for functional control of the technologies for biogas production (our modification of Topalova (37))

In addition to the enzymatic parameters that give information about the activity of the biological system the molecular-genetic and the fluorescent methods give the possibility to analyze the non-culturable microorganisms in the methanogenic syntrophic communities.

The spectrofluorometric analysis of the F_{420} concentration that in anaerobic conditions is connected solely with methanogens give information about their growth and their metabolic activity. FISH gives information about all the mentioned parameters of the microbial communities (quantitative, qualitative constitution and spatial distribution) and combines the precision of the molecular techniques with the visual information of the microscopic analysis (4).

The applications of molecular-diagnostic, enzymatic methods and methods related with the coenzyme F_{420} concentration in a parallel with the trivial chemical and technological parameters is a possibility to be created a system for functional, express, adequate to the accurate state of the technology control. Such a system allows certain elements of it to be commercialized and distributed for effective and efficient management of the technologies for biogas production as a renewable, clean energetic source. All that can be considered as a kind of a necessary innovation in the field of the circle economy.

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ФУНКЦИОНАЛЕН КОНТРОЛ НА ТЕХНОЛОГИИТЕ ЗА ПРОИЗВОДСТВО НА БИОГАЗ

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Резюме. Технологиите за производство на биогаз на принципа на анаеробната биодеградация се прилагат все по-широко и по-широко в практиката в България и по света. При повечето от тях се срещат проблеми като неефективна продукция на биогаз и/или продукци на биогаз с ниско качество. Мониторингът на тези технологии само по химични, физични и технологични показатели е недостатъчен и е причина за неефективността им. Необходимо е въвеждане на стратегии за функционален контрол на биологичната система, осъществяваща процеса на анаеробна биодеградация. В този обзор са разгледани най-модерните в световен мащаб ензимологични, флуоресцентни и молекулярни методи и показатели, използвани за осъществяване на функционален контрол на технологиите за производство на биогаз.

Ключови думи: анаеробна биодеградация, производство на биогаз, функционален контрол, молекулярни методи, флуоресцентни методи

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