ECOLOGICALLY AND ECONOMICALLY EFFICIENT STRATEGY OF WASTEWATER TREATMENT

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Abstract: A strategy of winery and distillery waste water treatment is proposed, having the ecologic and economic effect, based on application and intensification of anaerobic fermentation of organic substrate in waste water, with biogas production as energetic agent with high contents in methane. The technology applied combines the following processes: additive of exogenous hydrogen to ensure the most complete transformation in methane of carbon dioxide, forming on the acetogenic stage of anaerobic fermentation, introduction of supplementary CO_2 in bioreactor in mixture with hydrogen, acceleration of methanogenesis with biochemical stimulants, introducing of additives to enhance the contents in vitamin B_{12} in fermentation sludge.

Key words: waste water, biogas, electrolytic cell, fermentation, biostimulant.

Introduction

Surface water pollution by organic compounds from leachates of wastes generated by industries of agricultural products processing can become a serious environmental problem when treatment is impaired by the absence of treatment facilities, high maintenance cost of these stations or lack of energy resources for the treatment through aerobic fermentation. Such situations are quite common for developing countries or those with transition economies. For example, some small rivers from the territory of the Republic of Moldova, tributaries of Dniester river that serve as sources of water for several million people from Moldova and Ukraine, due to discharges from wine enterprises, fruit or vegetable processors and cattle farms are "dead" already before the inflow in Dniester, therefore they can bring with them substantial amounts of organic pollutants. This situation, as well as the deficit of energy [1] in the country has motivated the development and proposal of a new approach to management of liquid wastes generated by producers of wine, beer, sugar, alcohol, yeasts, meat, dairy, potatoes, fruits, vegetable and other raw materials containing carbohydrates (mono- and oligosacharides, starch, pectin, etc.) and other organic substances [2, 3] that in certain conditions undergo fermentation.

At the same time, anaerobic fermentation of organic substrates in waste waters from the aforementioned industries, can be not only the way to reduce the pollutants contents, but the source of biogas as well. The technologies and equipment available so far do not ensure the biogas obtaining from the above materials with the maximal possible yield. Only 30-60% of organic admixtures are transformed in biogas, and the methane contents in biogas in most cases does not exceed 60%. To reach these indicators, the thermophilic conditions are most often needed for anaerobic fermentation (45-55^{o}C), which requires to return and consume of the most part of the energy obtained.

The resolving of the biogas production increase problem from agricultural wastes must be based on improvement of technological parameters (reducing of expenditures, efficiency increase, etc.), equipment and flow sheets, and also the development and application of novel technologies, considering the ecological safety.

The scope of the present work is intensification of biochemical process of the distillery grains anaerobic fermentation, in order to remove the organic pollutants removal, with the increase of the biogas yield and biomethane contents in it, reducing of power consumption and labour contents of the process.

Materials and methods

Anaerobic fermentation of grains with the additional introduction of carbon dioxide and hydrogen in the reaction mixture

The study of the biologically active substances influence on methanogenesis was carried out in the bench-scale installation with four reactors.

Researches of the influence of biologically active substances on the process of methanogenesis were carried out on a specially designed set of four bioreactors (Fig. 1).

The equipment consists of a bench-scale bioreactor 1, placed in the thermostatically controlled volume, devices to remove and control the volume of obtained biogas, formed sludge and purified liquid. This equipment makes it possible to study of biogas purification process from admixtures of CO_2 and H_2S simultaneously. The total volume of each bioreactor is 5000 cm^3, the useful volume - 3500 cm^3.

The gas obtained in bioreactor was accumulated in calibrated reservoir 9, displacing water into the reservoir 10. Parameters of accumulated biogas were controlled – volume (V_1) and pressure (P_1), which was determined by the heights difference of water level.

After the amount of biogas necessary for the purification experiment was accumulated, the biogas valve (4) was closed. With the increasing the volumes heights difference 9 and 10, in volume 9 the pressure was created, necessary for the gas passing through the purification device 12. The liquid introduced from the water reservoir 10, displaced the gas from the calibrated reservoir and with the necessary pressure and discharge has directed it to the purification device. The purified gas was collected in the similar reservoir, consisting of the calibrated reservoir 9 and water reservoir 10. At the outlet of gas purification device, the volume (V_2), pressure (P_2) and gas temperature were controlled again.

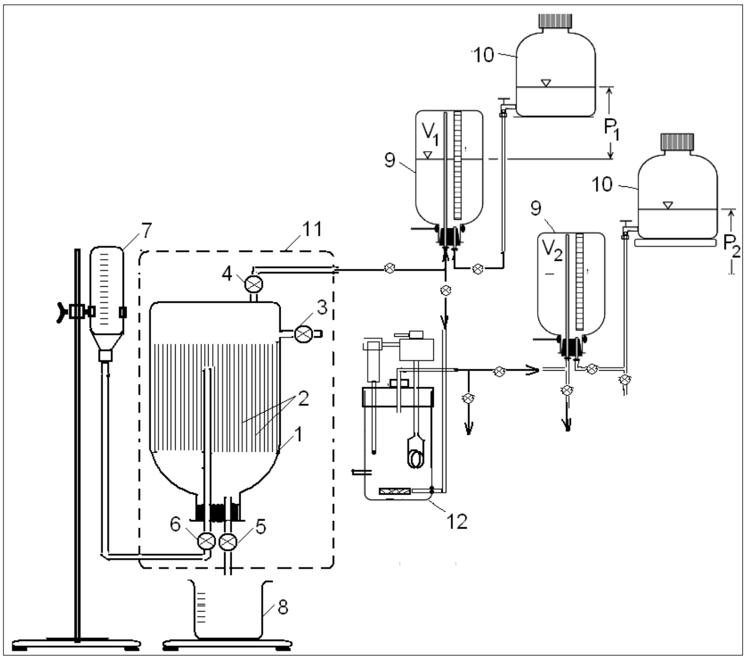

Fig. 1. The scheme of bench-scale reactor for biochemical generation of biogas and its purification from the admixtures: 1 – bioreactor; 2 – package for the immobilization of microorganisms; 3 - 6 – valves; 7 – reservoir for nutrient solution; 8 – glass; 9 – reservoir with calibrated capacity; 10 – water reservoirs; 11 – thermostat; 12 – device for biogas purification

The equipment makes it possible to control the operation of different devices on biogas purification, one-by-one logging them into the scheme.

To ensure the additional surface for the development of immobilized methanogenic bacteria, the grapevine rods have been introduced in bioreactor, with the total package surface of 8000 cm^2 [4-7]. The experiment was conducted under anaerobic conditions, the temperature was maintained as of $33,0 \pm 0,1^0$C.

The cultivation and adaptation of anaerobic microorganisms was carried by a known procedure with the sludge inoculation from the municipal waste water treatment plant of Chisinau. During one month the accumulation of fixed microorganisms proceeded in bioreactors, and process parameters were under the control, which promoted the development of methanogenic microorganisms. We used nutrient mixture containing sugar and milk (3:1). On the first stage the intensive CO_2 emission occurred, the liquid in the bulk of bioreactor was acidified due to the acidophilic fermentation [8]. The indicators of the transition from the acidophilic process to methanogenic one in bioreactors were the achievement of values pH = 7.4-7.6 and the stable amount of evolved gas. To accelerate this process during 15 days the nutrient mixture was basified with NaON to pH = 13.0. Later on, the regime of nutrition mixture introduction remained the same during 20 days, and pH control and its correction ensured pH=7.4-7.6 maintenance in bioreactor. In parallel, the volumes of emitted gas were measured. Once this indicator is stable for all the bioreactors during 5-7 days, this means that the microflora growing process is finalized.

The next stage of bioreactors preparation for the research was the nutrition of microorganisms with the distillery grains. For this scope the nutrition mixture (milk + sugar) was gradually substituted with the distillery grains (BOD_5 = 17800-18400 mg/l). After each substitution 25% of nutrition mixture was kept till the stable indicators of gas yield were reached.

The bioreactors prepared by this way can be used to determine the effect of stimulating substances on methanogenic process. Three concentrations of substances were studied at the same time, and one of bioreactors was the control. In each bioreactor the biogas yield and composition was measured, the constant dose of stimulating additive, added to the nutrition mixture, was thus maintained. Following the first series of tests, the bioreactors were fed with the same nutrition mixture, and the stable indicators (gas yield and composition) were reached in all of them. Thus, the bioreactors were ready for the next set of experiments.

The effect of biologically active substanses (BAS) was estimated following the rate of gas emission and the methan content in it. These data were measures within the interval of 10-54 hours, following the fresh grains was introduction, in the regime of stationary growth phase of methanogenic culture. The indicators were measured after the two nutrition cycles and following three nutrition cycles. The total length of one experiment was 300 hours.

The experiments were performed under anaerobic conditions, under the constant temperature 33.0 ± 0.1^{o}C. The grains with the initial COD = 28250 mgO_2/l, and BOD = 19370 mgO_2/l was used. Betulinol extracted from the birch-tree crust was introduced in the reaction medium. The gas with CO_2 contents of 96.5 % was collected in rubber gasholder. At the same time, hydrogen was electrochemically generated in electrolytic cell. The cell was operating with flow-through porous cathode, recirculation of 18% NaOH solution with the linear flow rate of 0.5-1.0 cm/s, under the voluminous cathodic current density of 0.5 A/dm^2 [9, 10]. Hydrogen thus obtained was mixed with carbon dioxide in the ratio 1:4. After the biochemical methane formation process reached the operating regime, the gas mixture CO_2/H_2 was dosed in the reactor. Power consumption for the process were estimated on the base of the summary heat consumption for maintenance of optimal process temperature, and the expenses for hydrogen production dosed in the biochemical process, considering anaerobic fermentation time.

Anaerobic fermentation of grains with the additional introduction of biologically active substances

Micro-additives of bioactive substances were introduced into the distillery grains, with pH=5.2, COD = 25350 mgO_2/l and BOD_5 - 17650 mgO_2/l, and the mixture was subjected to anaerobic fermentation under the mesophilous regime in the thermostat with the temperature 32±2^{o}C. The process was carried out under the flow-through conditions in bioreactor with separated acetogenic and methanogenic compartments. CO_2 evolved on the acetogenic stage, was mixed with electrolytic hydrogen 1:4 and dosed in the methanogenic compartment.

Increase in the B_{12} vitamin contents in fermentation sludge

The grains was used, having pH=5.2, COD = 28250 mgO_2/l and BOD_5 = 19125 mgO_2/l. The tests were carried out with amaranth extract – 50 mg/l, cobalt tartrate-ammonia complex – 50 mg/l and potassium ferrocyanide – 40 mg/l. The control test was carried out for comparison.

Results and discussion

The research was aimed at the resolving of two problems: maximal removal of organic admixtures from the waste waters, resulted from the agricultural products processing, and improving the ecological and economic efficiency of the aforementioned wastes utilization for the production of biogas with the maximal methane contents. To reach this scope, the scheme (Fig. 2) was elaborated, envisaging the combination of various approaches with elaboration of special modified equipment to improve the efficiency of novel technological procedures.

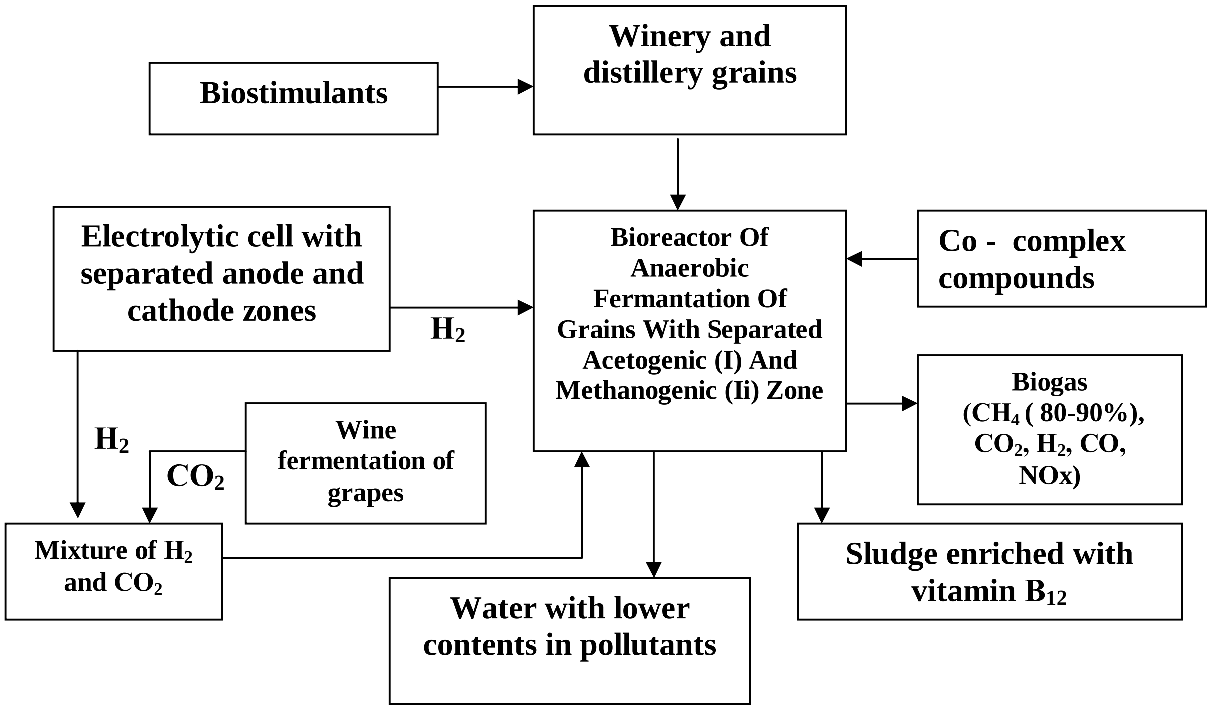

Fig. 2. Scheme of intensified anaerobic treatment of winery grains with the enhanced biogas yield

The selection of the concrete solutions regarding the improving of treatment degree and efficiency of biogas production from agricultural processing industries wastes is based on the knowledge of biochemical mechanism of biogas formation during the anaerobic fermentation. The specifics of methanogenesis should be considered in biotechnology of biogas obtaining from the organic wastes. Transformation of the organic wastes in biogas occurs as the result of an entire conversions, due to the microorganisms. It is well known that anaerobic fermentation with methane formation involves two stages: acetogenic and methanogenic. At the first stage, as a result of the hydrolytic decomposition of organic substances, along with the alcohols, acids, CO_2 and CO, hydrogen (H_2) is formed. Concentration of hydrogen formed plays further a decisive role in the regulation of quantitative composition of the products of methane formation process, according to the following scheme [11]: $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$.

However, according to balance, the amount of hydrogen formed during the anaerobic process, is insufficient, therefore the noncombustible carbon dioxide is practically always present in the biogas composition. This entails the task to increase the amount of hydrogen in the reaction mixture. The solution proposed in the present work, is the introduction of additional amount of hydrogen in methanogenesis process.

Equipment modification and additional introduction of H_2/CO_2 mixture

To ensure the higher efficiency of CO_2 mixing with exogenous H_2, the bioreactor was developed with separated acetogenic and methanogenic compartments, so as to separate the evolving CO_2 from other products of acetogenous stage. The system was equipped with the additional devices for the obtaining and introduction of hydrogen into the methanogenic compartment. To generate hydrogen, electrolytic cell was used with anodic and cathodic compartments, separated with diaphragm.

To obtain hydrogen with low specific energy consumption, a special flow-through porous cathode with modified surface was elaborated. Alloys applied on the cathode surface, ensure the low overvoltage of hydrogen evolving. The diluted electrolytic solution with high electro-conductivity was subjected to electrolysis. Hydrogen was evolved on the cathode, whereas oxygen

– on anode, with almost 100% current yield, under the experimentally determined linear flow rate and current density. Hydrogen, electrochemically generated, was mixed with carbon dioxide, and once the methane formation process reached the operation regime, the gas mixture CO_2/H_2 was introduced into the methanogenic compartment of bioreactor.

Application of this method for intensification of winery grains fermentation, due to the introduction of additional CO_2 and H_2 in fermentation mixture of winery and distillery grains, made it possible to increase by 12% the amount of the evolving biogas, compared to the conventional conditions. The methane contents in biogas was increased by apprx. 50%, which testifies on the higher efficiency of anaerobic fermentation. Energy consumption was more than two-times reduced.

Stimulation of fermentation with bioactive substances

To intensify the microbiological activity and accelerate the anaerobic methanogenic process, as well as to increase the methane yield, we have essentially broadened the circle of fermentation stimulants with natural bioactive substances. A selection and testing of novel phytochemical substances and biochemical vegetable components was carried out from the available vegetable material, including the agricultural processing wastes (Table 1). The substances were selected due to their biological activity, simple and cheap obtaining (synthesis) or extraction and availability of raw material.

Table 1

Components, tested as anaerobic fermentation stimulants

No.	Supposed stimulant	Active substance	Raw material source
1	Triterpenoids	Betulinol and its derivatives	Birch-tree crust
2	Furostanol glucoside	Tomatoside	Tomato seeds
		Capsicoside	Sweet pepper seeds
3	Extract of amaranth seeds	Oil enriched with squalene	Amaranth seeds
4	Extract of chestnut seeds	Oil-containig terponoids	Chestnut seeds
5	Saponins	Escyne	Chestnut seeds

Bioactive substances promote the intensification of anaerobic fermentation of organic substrates in grains, and as a consequence, general increase of the biogas yield, and in some cases the contents in methane in it (Table 2). COD and BOD values were 1.5-times decreased. The time of anaerobic fermentation of winery grains in the presence of bioactive substances was 2.4-times reduced, compared to control, COD and BOD valued were accordingly 1.2- and 1.3-times reduced. These results testify on higher efficiency of anaerobic fermentation, with the reduced energy and labor consumption. The degree of waste water treatment is thus improved, due to the more complete assimilability of organic substances by microorganisms.

Table 2

Intensification of anaerobic fermentation of winery grain due to the application of bioactive substances

No.	Indicator	Control	Adding of bio-active substance
1	Time of anaerobic fermentation, H	36	15
2	COD after anaerobic treatment, mg O_2/l	680	560
3	BOD_5 after anaerobic treatment, mg O_2/l	255	195
4	Ratio BOD_5:COD after anaerobic treatment	0.375	0.348
5	Specific amount of emitted biogas, dm^3/1 kg COD	0.51	0.59
6	CO_2 contents in biogas, vol.%	69.2	94.8

This effect is a result of various stimulating and physiological properties of bioactive substances, which, in dependence on fermentation conditions and chemical structure, manifest themselves as anti-oxidants, anto-hypoxants and anti-mutagens, membrane-protectors etc. They facilitate the stabilization of cell membranes of microorganisms, increase the cells stability, reduce the peroxide oxidation of lipids and prevent the deterioration of cell membranes. This provokes the acceleration of biochemical methanogenesis running and increase in the biogas yield.

Increase in the vitamin B_{12} contents in the fermentation sludge

To improve the economic and ecological efficiency of the proposed winery grains treatment, a new improved method was be tested on the obtaining of feeding concentrate of vitamin B_{12}, along with methane, during the grains fermentation [6]. Besides the bioactive substances as anaerobic fermentation stimulants, cobalt tartrate-ammonia complex and potassium ferrocyanide was introduced in the grains, as a catalyst of vitamin (Table 3).

Table 3

Stimulation of methanogenesis and formation of vitamin B_{12} due to the complex use of bioactive substances, cobalt tartrate-ammonia complex and potassium ferrocyanide

No.	Indicator	Control	Amaranth + Co(III) tartrate-ammonia complex + potassium ferrocyanide
1	Time of biochemical treatment, H	12	6
2	COD, mgO_2/l	975	725
3	BOD_5, mgO_2/l	539	424
4	Specific amount of emitted biogas, $dm^3/1$ kg COD	0.49	0.68
5	CO_2 contents in biogas, ol.%	34.5	3.8
6	Contents in vitamin B_{12} in protein-vitamin concentrate, mgB_{12}/kg	27	784

It was found that the joint application of bioactive substances, cobalt tartrate-ammonia complex and potassium ferrocyanide made it possible to reduce twice the time of anaerobic fermentation of grains, compared to control. At the same time, the contents in vitamin B_{12} in protein-vitamin feeding concentrate was increased by more than two orders (Table 3). This is a result of both the effect of stimulating additive on biochemical process, and enriching of treated medium with the potassium and cobalt complex compounds to feed the bacteria, producing vitamin B_{12}. This is confirmed also by the reducing of COD and BOD_5, and the higher methane yield, compared to the control test, which is the result of the balanced contents in hydrogen and carbon dioxide.

Conclusions

The innovative approach to the winery wastewater treatment with the acceleration of organic pollutants decomposition, increase in biogas yield and methane contents in it, reduction in energy intensity and process complexity has been proposed. The approach is based on intensification of anaerobic fermentation of winery grains, using additives of biologically active substances, cobalt compounds and potassium ferrocyanide, increasing the amount of hydrogen in the reaction mixture and the improvement of equipment, to ensure the efficient methanogenesis.

The improvement in the fermentation significantly reduces the amount of organic pollutants in the winery wastewater and, consequently, the load on wastewater treatment facilities and negative impact on the environment.

The integrated use of biologically active substances, complex compounds of cobalt and potassium ferrocyanide to enhance the process of methanogenesis, as well as additional supply of hydrogen and carbon dioxide mixture provided the 2 times reduction of the anaerobic digestion of winery grains and increased methane contents in the biogas by 50%. The process of methane-producing bacteria stimulation led to the increase of the vitamin B_{12} contents in the sediment more than two orders of magnitude compared with the control, that allows to use it as a protein-vitamin feeding concentrate.

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