TECHNOLOGICAL SOLUTION OF BIOGAS OUTPUT INCREASING AT GRAIN DISTILLERY SPENT WASH FERMENTATION

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Background. Distillery spent wash is the main waste of the alcohol industry. This is highly concentrated wastewater, which is characterized by high chemical oxygen demand (COD) values – up to 60–120 g O₂/dm³ – and low pH values – up to 3.7. It makes its processing a complicated task. One of the methods of its utilization is anaerobic fermentation with the production of biogas. Inert carriers for microorganism immobilization or granulation are used to intensify the process of anaerobic treatment and increase sludge concentration. Due to the high concentration of anaerobic microorganisms in granules, compared to the number of microorganisms in free-floating active sludge, the process of methanogenesis is more intensive with a high yield of biogas and a decrease in COD.

Objective. To determine the possible centres of anaerobic sludge granulation in highly concentrated waste of alcohol production to increase biogas output in the process of waste treatment.

Methods. Activated carbon, modified carbon on which surface calcium ions were precipitated, FeCl₃, silica gel based sorbent, talc based sorbent were used to form pellets as the centres of microorganism accumulation.

Results. Pellets have not been formed with the use of activated carbon; modified carbon with calcium ions; silica gel based sorbent and ferrum compounds. Pellets of activated sludge were received with the use of talc based sorbent – formation of initial biofilm on a carrier was observed on the third day of fermentation and then it was growing in pellets.

Conclusions. It was demonstrated that for highly concentrated waste effluents of a distillery (grain distillery spent wash), which after co-fermentation with poultry manure contain volatile fatty acids in concentration 800–2000 mg/dm³, it is needed to use sorbents to receive microorganism pellets as a centre of granulation. Sorbents must contain donors and proton acceptors. It was established that the use of granular activated sludge for the distillery waste purification improves the efficiency of COD and Biochemical Oxygen Demand removal by 15–17%, increases biogas output by 26 ± 2% and biogas methane content by 8 ± 1%.

Keywords: granulation; biogas; distillery spent wash; fermentation; methane.

Introduction

Grain distillery spent wash (GDSW) as a highly concentrated wastewater is characterized by the high values of COD – up to 60–120 g of O₂/dm³ and low pH values – up to 3.7. It influences a choice of methods and technological solutions of waste treatment. One of the methods of waste utilization used is an anaerobic fermentation with receiving of biogas [1]. Based on the fact that the hydrogen value influences on methane production by its producers and the optimal pH value for the methanogenesis process makes up 6.8–8.0 [2], the technology assumes a minor content of spent wash (2–3%) in the fermenter. It has an effect on the process cost effectiveness and a target product output [3].

The proposed two-stage technology [4] allows for adjusting the pH value in the first reactor due to introduction of poultry manure with the concurrent obtaining of biogas and its output increase. The process is characterized by the balance of nutrition components of microbial association and rational correlation C:N:P. Biogas is also produced from dissolved high- and low-molecular compounds during the subsequent anaerobic stage of the advanced waste treatment. But the process rate and the waste purification level depend, first of all, on the concentration of free-floating microorganisms, which varies in reactors of different type from 2 to 4 g/dm³ [2].

Inert carriers for microorganism immobilization or granulation are used to increase sludge concentration. This results in the increase of process rate for substance destruction, the decrease of hydraulic period for waste retention in the reactor and its volume that lowers operating costs with no loss in quality of waste purification [5], [6].

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The high load on the granular sludge is achieved through the improvement of sedimentation properties. It guarantees its presence in the reactor zone and makes impossible to wash out sludge with the upward water flow. The high specific activity of methanogenic bacteria (2 kg of COD/kg of dry organic substance per day, upon the load of 50 kg COD/m³) is also conditioned by interspecific transfer of hydrogen between the pelletled colonies of acetogenic bacteria and hydrogenotrophic methanogenic archaea and their protection with pellet matrix from negative impacts resulting in the raised destruction rate of organic substances and their transformation into a desired product – biogas [6], [7].

It was shown [8] that the granulation rate and the characteristics of granular sludge depend on microorganism species composition and their ratio in pellets, substrate specificity, hydrophobicity, surface charge, and capability for extracellular polymers synthesis.

Under real-life conditions, the granulation process might be disturbed due to different factors which result in a sludge washout from the reactor, pellet destruction, reduced biogas output and efficiency of wastewater treatment. Therefore, the study of granulation process in a course of anaerobic treatment of highly concentrated wastewater with methane production is a relevant task.

The work objective is to determine the possible centres of anaerobic sludge granulation in highly concentrated waste of alcohol production to increase biogas output in the process of waste treatment.

The following tasks need to be solved to meet the objective:
- to study formation of pellets of anaerobic sludge in distillery waste using the granulation centres of different nature;
- to establish an impact of granular activated sludge on efficacy of methane production and wastewater treatment.

Materials and methods

Nowadays, there is no any accepted model or theory of pellets formation. Table 1 demonstrates the models which explain the current theories of pellets formation from microorganisms [6]–[10].

As is seen from Table 1, all the models based on physical granulation theory explain the formation of granular sludge by the effect of physical factors which are intrinsic to the biomass fermentation process with the biogas production and run in the reactor: liquid and gas flow rate, ageing of sludge and its excessive volume removal from the reactor, quantity and qualitative composition of suspended solids in initial sludge, etc.

The models, which describe granulation from the view point of physiological peculiarities of microorganism species and strains (e.g., *Methanobacterium AZ* strain, *Methanothrix* bacteria) as well as their interaction, are attributed to microbiological [6].

Within the frames of thermodynamic theory, there are four granulation stages defined: transport of microorganism cells of activated sludge to the surface of inert material or other cells; initial reversible adsorption of substrate by means of physical and chemical interaction; irreversible attachment of cells to substrate with microbial appendages or exopolymers; cell propagation and pellet development. The microorganism granulation mechanisms are also explained proceeding from thermodynamic conditions of the grain growing processes influenced by many factors such as: hydrophobicity and electrophoretic mobility, proton translocation on the surface of bacterial membranes which stimulate its activation.

The use of granular sludge is expected at Stage 2 of GDSW treatment [4]. That’s why liquid fraction formed after sedimentation and release of solids, fermented (within 20 days) mixture of GDSW, poultry manure and industrial wastes of a distillery at the ratio of 1:1.7:0.06, relatively, on a dry organic substance (DOS), served as a standardized test solution [4]. The phase separation was carried out using centrifugation (centrifuge CLK-1) for 15 min at the velocity of 3000 rpm. Characteristic water values at entry to reactor 2 are given in Table 2.

The following has been used for granulation in capacity of the centres of microorganism accumulation:
- activated carbon, grade 207EA “Ecofilter” (Kyiv, Ukraine), with surface area 950–1100 m²/g and bulk density 0.48–0.52 g/cm³, grain size – 4.75–2.00 mm;
- modified carbon to which surface calcium ions were precipitated. Precipitation was carried out by means of keeping activated carbon in calcium chloride solution with concentration 0.05%;
- FeCl₃ which is often used for granulation in methane tanks during the treatment of waste effluents of breweries, yeast plants, sugar beets, and potatoes processing plants. Since hydrolysed FeCl₃ is produced in the form of oxo- and hydroxycarboxylic compounds, their aggregation serves as a primer for granulation;
### Table 1: Theories and models of the anaerobic sludge granulation process [6]–[10]

| Group | Model | Model idea |
|-------|-------|------------|
| **Physical and chemical theories** | **Model of inert cores** | Anaerobic bacteria, which result in formation of initial biofilms, in particular, embryonic granules, are attached to inert micro particles by means of surfaces structures |
| | **Selection pressure theory** | A washout of dispersed sludge and a selection of heavy sludge take place depending on the rates of hydraulic load and biogas production |
| | **The growth of colonized suspended particles** | Pellets are formed by means of growing of small fraction biofilm (microorganism colonization) that was produced due to friction, or flown into a reactor with waste water |
| **Microbiologic/ecologic theories** | **Consolidation of microfloccules of microorganisms of Methanothrix genus** | Methanothrix filament serves as a matrix for cocci and coli. First, micro-floccules are formed and then they are consolidated in pellets due to surface properties and morphology of Methanothrix bacteria |
| | **Capetown's model** | Granulation takes place by means of Methanobacterium AZ, which under the conditions of high partial pressure of Н₂, unlimited source of nitrogen in the form of ammonium, limited quantity of cysteine and in neutral media, releases extracellular polypeptide that binds Methanobacterium AZ and other bacteria in pellets |
| | **Spaghetti model** | Pellets are formed by microorganisms of Methanothrix genus. In the beginning, they resemble the spaghetti swirls formed by the Methanothrix long filaments |
| | **Model of many layers** | Methanothrix grouping is the centres of granulation. The layer-by-layer growth of pellets is performed with a spatial separation of various trophic groups of microorganisms: H₂-producing acetogenic bacteria and H₂-consuming bacteria |
| | **Syntrophic micro colony model** | Micro colonies, by means of syntrophic relations, may lead to the creation of consortiums and subsequent formation of pellets, because consortiums are characterized by stable conditions, co-evolution, and protection against the effects of microorganism media participating in the given consortium |
| | **Model of three types of pellets which split volatile fatty acids (VFA)** | Methanothrix, Methanosarcina bacteria participate in the granulation process and they form 3 types of pellets which split VFA: 1 – compact spherical pellets comprising mainly of rod-like bacteria which resemble Methanothrix soehngenii in short chains or separate cells; 2 – spherical pellets comprising mainly of poorly twisted filamentous bacteria which are attached to inert particles. Predominating bacteria resemble Methanothrix soehngenii; 3 – compact spherical pellets comprising mainly of bacteria of Metaphosarcina type |
| | **Model of Methanothrix bundles** | Pellets are formed from the aggregates of Methanothrix and other bacteria. By means of filaments, Methanothrix forms specific bundles separated by an external matrix. When bundle sizes get larger, the external matrix is deactivated, and the centre of pellets is formed. This centre is made of compact filament of Methanothrix |
| | **Model of anaerobic granulation of defined species** | Pellet formation is based on the capability of bacterial species to produce dense aggregates in an anaerobic reactor and/or ensure surface binding for other bacteria, which are unable to form aggregates and pellets. It is believed that methanogenic microbes are key species for granulation. In particular, the species such as Methanothrix, Methanobrevibacter, and Methanosarcina |
| **Thermodynamic theories** | **Surface tension model** | Bacteria receive the maximum possible free adhesion energy under the low or high liquid surface tension. Pellets formed under the low liquid surface tension values and which contain acytogenes around methanogenic association contribute to more stable characteristics of the process, because they are less sensitive to gas bubble adhesion and subsequent washout |
| Model                                    | Description                                                                                                                                                                                                                                                                                                                                 |
|-----------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Four-stage model                        | 4 stages of granulation: 1. Cells transfer to the surface of uncolonized inert material or other cells. 2. Initial reversible adsorption by means of physical and chemical force on substrate. 3. Irreversible adhesion of cells to substrate with the use of microbial appendages and/or polymers. 4. Cell propagation and pellet development                                                                                     |
| Proton translocation – dehydration model | The (molecular) mechanism of sediment granulation is based on proton translocation activity on the surfaces of bacterial membranes. According to theory, the sludge granulation process takes four stages: 1. Dehydration of bacterial surfaces; 2. Formation of embryonic pellets; 3. Pellet maturation; 4. Post-maturation                                                                                         |
| Crystallized core formation model       | Granulation stages: 1. Growth and generation of different bacterial species; 2. Bacterial attachment or adhesion with the cells of suspended substances or other bacteria with the formation of matrix or the pellet core as a crystallization centre. 3. Seizure or integration of bacteria, microcolony growth and, finally, formation of a spherical pellet 1–5 mm in diameter |
| Cellular automation model               | Cellular automation model is defined as a spatial discrete-time system in which the state of automation is defined by a set of rules acting locally, but are applied across the system. This model is aimed at the reproduction of microbial structures under the conditions of limited substrate transfer. The substrate gradients created by local substrate consumption allow bacteria placed on the “top” to have more accessible substrates compared to those placed at the bottom. Therefore, the structure of microcolony or biofilm is related to the resource availability |
| Cell–cell interconnection model         | Some bacteria by virtue of intercellular interactions form a biofilm, which under certain conditions, initiates granulation                                                                                                                                                                                                                  |
| Other                                   |                                                                                                                                                                                                                                                                                                                                            |
| Cluster model                           | The precise mechanism has not been defined yet, but it is known that the interconnection between substrate composition and/or concentration and kinetic properties of microorganisms plays a key role. There are several hypotheses available: 1. Physical aspects associated with the space limitation for microorganisms upon growing a biofilm. When divided, daughter cells occupy certain space and push away neighbouring cells. The clusters were received at this process modelling; 2. Under the conditions of substrate exhaustion in the deeper layers of biofilm or aggregate, microbes are divided actively into the upper areas only and form a new biomass. Therefore, the growth of biofilm or aggregate becomes unidirectional, and the colonies are grown as the "fingers" toward volume liquid; 3. Anaerobes produce signalling molecules from cell-to-cell, which may stimulate formation of pores and channels. These signalling molecules or their structural analogues may also be presented in waste effluents |
| General four-stage model                | 1. Initiation of bacteria-bacteria contact or bacteria attachment to the cores by means of physical relocation; 2. Support to stable multicellular contacts by means of initial attracting forces; 3. Maturation of cell aggregations by virtue of microbial forces; 4. Formation of steady three-dimensional structure of microbial aggregate by hydrodynamic displacement forces |
Table 2: Water parameters after GDSW and poultry co-fermentation

| Indicator                              | Value |
|----------------------------------------|-------|
| Chemical oxygen demand, COD, mg/dm³   | 2800  |
| Biological oxygen demand, BOD, mg/dm³  | 1500  |
| Suspended substances, SS, mg/dm³       | 2300  |
| Volatile fatty acids, VFA, mg/dm³      | 1230  |
| pH                                     | 7.58  |

- silica gel based sorbent, grain size – 2-3 mm (Institute of New Materials of Shandong Academy of Sciences, China)
- talc based sorbent, grain size – 2-3 mm (Institute of New Materials of Shandong Academy of Sciences, China).

Anaerobic sludge from a fermenter for receiving biogas from GDSW was used as inoculum in the Ecobiotechnology and Bioenergetics Department of the National Technical University "Igor Sikorsky Kyiv Polytechnic Institute". The GDSW used was provided by Chervonoslobodskyi Distillery, Kyiv Oblast.

The granulation process with the use of different centres of adsorption has been carried out in the laboratory shaking flask with heating at the temperature of 37 ± 2 °C, stirring rate is 60 rpm. Cone flasks with the gas outlet system for removal of biogas produced were used as reactors as well as they made impossible to penetrate the air to the reactor medium.

The degree of anaerobic sludge granulation was evaluated using the microscope XSP-139TP (Ulab TM, China) with 20× zoom and the energy-dispersive spectrometer (EDS) Oxford X-Max 50.

Wastewater treatment using free-floating and granular activated sludge has been carried out under the anaerobic conditions in the mesophilic mode at the temperature of 40 ± 2 °C in reactors with capacity of 1.5 dm³, fill factor – 0.8. The substrate was stirred using paddled impeller sat the rate of 60 rpm. Each reactor was connected to the wet type gas receiver for biogas collection.

The values of BOD, COD, and SS were calculated using the standard method [11], medium pH was measured using the ion meter 1–160 MI (RF) [12].

Biogas composition was determined with the gas chromatograph LHM-8-MD (RF) [13] using the standard method, VFA liquid concentration was established with the liquid chromatograph HPLC Shimadzu (Kyoto, Japan) using the standard method [14].

Results

Pellets have not been formed with the use of activated carbon, silica gel based sorbent and fer-rum compounds that can be explained by specifics of GDSW composition and the microorganism association properties.

By using talc based sorbent as a granulation centre, the formation of initial biofilm on a carrier has been observed on the third day of fermentation and then it was grown in an activated sludge pellet. The obtained pellets are given in Fig. 1.

![Figure 1: Anaerobic sludge before granulation (a), pellets formed on talc based sorbent (b)](image-url)
processing. The ratio of inoculate and substrate on a DOS basis is 1:5, relatively.

The water treatment parameters at Stage 2 of distillery waste disposal per day with the initial COD content – 2800 mg/dm³ are given in Table 3.

Table 3: Water purification degree at Stage 2 of GDSW processing

| Indicator | Indicator value with the use of free-floating anaerobic sludge | Indicator value with the use of granular sludge |
|-----------|---------------------------------------------------------------|-------------------------------------------------|
| COD, mg/dm³/removal efficiency, % | 780 ± 46.87/72 ± 1.5 | 310 ± 43.39/89 ± 1.77 |
| BOD, mg/dm³/removal efficiency, % | 400 ± 36.06/73 ± 2.12 | 180 ± 17.39/88 ± 1.5 |
| SS, mg/dm³/removal efficiency, % | 600 ± 44.91/74 ± 2.12 | 200 ± 29.75/91 ± 1.4 |

Discussion

The choice of granulation centres is based on heterogeneity of their surfaces on which aggregation of suspended solids and microorganisms may have place.

Not all microorganism species interact with the inert surface of activated carbon [15], [16]. The availability of high concentration of volatile fatty acids in water also hinders the aggregation of substances and microorganisms, because they prevent neutralization of particle charges and, correspondingly, their aggregation as on carbon surface, so against each other. The presence of acids in solution explains the absence of granulation on other above mentioned sorbents. Silica gel pellets were destroyed in 7 days of being in wastewater, which confirms the studied medium aggressivity.

The aggregation of hydroxyl compounds between themselves and the formation of adsorption centres do not take place with the use of ferrum compounds and the availability of acids. Hydroxyl compounds are transformed into soluble state by reaction:

\[
\text{Fe(OH)}_3^+ + \text{CH}_3\text{COOH} \rightarrow 2 \text{CH}_3\text{COO}^- + \text{Fe}^{3+} + 2\text{H}^+. \]

The carbon activation with calcium ions for the creation of activated carbon interaction centres with microorganisms did not demonstrate positive results. Perhaps, this is associated with the availability of competition between negatively charged surface of microorganisms and the anions of volatile fatty acids for the attachment centres, which reduces or makes impossible the microorganism interaction with activated carbon.

So far as the surface of talc based sorbent particle, except the extended surface (of pores), contains the oxygen and magnesium atoms, this allows for sedimentation of charged and uncharged particles. The pellets are grown due to the formation of layers which from the beginning contain nutrients in suspended particles, which precipitate on the pellet surface, and various microorganism species that are capable as to destruct particles, so to produce methane. Moreover, in the process of granulation, other species, which are not adsorbed on sorbent, are attached to the pellets due to the formation of polymeric polysaccharidic or polypeptide matrix that can be produced by Methanobacterium and other species [16]. As described in literature sources [6], [9], [10], [17], methanogenic bacteria that form the bedded structures, Methanohrix soehngenii (Methanoseta concilii), Methanosarcina spp., Methanobrevibacter, play a special role in the formation and functioning of anaerobic sludge pellets.

The sorbent due to the availability of oxygen atoms on the surface and the bacterial cells with a negative charge of the surface leads to the formation of hydration shell. The change of a pellet surface charge and its partial dehydration take place by means of proton transfer to the membrane surface upon substrate oxidation with acetogenic bacteria. The absence of hydration shell results in hydrophobic interaction between the cells and pellet compaction. The metabolite transport between the cells facilitates this process also [18]–[20].
A spherical pellet shape is produced under the effect of hydraulic force of rising biogas bubbles. The separation of microorganism species in a pellet is determined by the velocity of nutrients transport between them.

Therefore, for wastewater with concentration of volatile fatty acids within 800–2000 mg/dm³, it is possible to use the granulation centres of sorbents, which contain proton donors and acceptors, to form the anaerobic sludge pellets.

As is seen from Fig. 2, biogas output is higher by 26 ± 2%, if granular anaerobic sludge is used. At the same time, biogas methane content is increased by 8 ± 1%. It means the granulation of anaerobic sludge provides the possibility to increase both biogas output and biogas methane content.

As is seen from Table 3, the wastewater treatment process, with the use of granular activated sludge, is characterized by a higher removal efficiency of polluting substances. Recovery rate of organic substances is increased, and removal degree reaches 89%, which is by 17% higher compared to the use of free-floating sludge.

Conclusions

It was demonstrated that for highly concentrated waste effluents of the distillery (GDSW), which after co-fermentation with poultry manure contain volatile fatty acids in concentration 800-2000 mg/dm³, it is necessary to use sorbents containing both proton donors and acceptors to obtain microorganism pellets in capacity of granulation centres. Talc can serve as a basic substance for such sorbents.

It was established that the use of granular activated sludge for the distillery wastewater treatment increases the removal efficiency of COD and BOD by 15–17%, increases biogas output by 26 ± 2% and biogas methane content — by 8 ± 1%.

Further study of the abiotic factors influence (temperature, pH of the medium, availability of nutrients) on the formed active sludge granules in the conditions of our technological process.

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ТЕХНОЛОГИЧЕСКОЕ РЕШЕНИЕ ПОВЫШЕНИЯ ВЫХОДА БИОГАЗА ПРИ ФЕРМЕНТАЦИИ ЗЕРНОВОЙ ПОСЛЕСПИРТОВОЙ БАРДЫ

Проблематика. Основным отходом спиртовой промышленности является послеспиртовая барда. Она является высококонцентрированным сточным водам и характеризуется высокими значениями химического потребления кислорода (ХПК) – до 60–120 г О2/дм3 – и низкими значениями рН – до 3,7, что делает ее переработку непростой задачей. Одним из методов ее утилизации является анаэробное брожение с применением активного ила как доноров формирования гранул. За счет высокой концентрации анаэробных микроорганизмов в гранулах, процесс метаногенеза происходит с высоким выходом биогаза и снижением ХПК.

Методика реализации. Для формирования гранул как центры акумуляции микроорганизмов использовали: активированный уголь, сорбент на основе силикагеля, FeCl3, сорбент на основе силикагеля и щелочное высококонцентрированное сточное воду, на которой проводилась культивация микроорганизмов.

Результаты. Показано, что для высококонцентрированных сточных вод спиртзавода (послеспиртовая барда), которые после коферментации с птичьим пометом содержат летучие жирные кислоты в концентрациях 800–2000 мг/дм3, для получения гранул микроорганизмов как центры грануляции необходимо использовать сорбенты, содержащие в своем составе как доноры, так и акцепторы протонов. Установлено, что использование гранулированного активного ила для очистки стоков спиртзавода увеличивает эффективность удаления химического и биохимического потребления кислорода на 15–17 %, повышает выход биогаза на 26 ± 2 %, а также увеличивает выход биогаза на 8 ± 1 %. Ключевые слова: грануляция; биогаз; послеспиртовая барда; ферментация; метан.