Baking yeast enterprises are a source of environmental pollution by concentrated wastewater. They are usually diluted with water and discharged into sewerage. It is rational to use methane fermentation, which will enable the removal of pollutants and make the process cost-effective through the use of biogas and digestion. Therefore, it is relevant to study the question of the influence of fermentation parameters on the cleaning efficiency.

The aim of the work is to study the methane fermentation of yeast production effluents in a continuous mode. Objectives: analysis of solving the problem of wastewater treatment of yeast plants, study of the influence of process parameters (dilution rate, addition of cobalt salts) on the efficiency of treatment; gas generation; vitamin production.

Initial COD of effluents 4500 mg O₂/dm³, pH 6. Cultivation regime – continuous, dilution rate 4,1·10⁻³, 6,2·10⁻³, 8,2·10⁻³, 12,4·10⁻³ hours⁻¹. Cleaning efficiency 78,9 %. High values of dilution rate cause overload of activated sludge, which leads to reduced cleaning efficiency. The presence of cobalt also has a depressant effect. A significant amount of biogas (up to 5,2 dm³/dm³) is produced with a high content of methane (up to 85 %), which is an alternative fuel. As the dilution rate increases, the biogas and methane content decrease. With increasing dilution rate from 4,1·10⁻³ to 12,4·10⁻³ hours⁻¹, biogas decreased from 1,11 to 0,94 dm³/g COD loading, and from 1,43 to 1,39 dm³/g COD fermentation. Similarly to the effect on the depth of purification, the inhibitory effect of cobalt on methanogenesis is observed. Digestion is a valuable fertilizer with a significant content of cobalamin vitamins (up to 95 μc/g). The addition of cobalt salts stimulates the synthesis of vitamins, providing an increase of 26,7 to 51,6 %, improving the ratio between active and inactive forms.

Keywords: biogas, vitamins, digestion, yeast production, methane fermentation, wastewater.

1. Introduction

There are now enterprises for the production of baker's yeast in almost every region of Ukraine. At different stages of the technological process, a significant amount of concentrated wastewater is formed. Water consumption at enterprises is quite high, depends on the features of technology, water management schemes, etc. and ranges from 100 to 110 m³ per 1 t of compressed yeast.

Wastewater from yeast plants is formed due to the culture fluid as a result of yeast separation, washing of technological equipment and discharge from yeast cultivators. Wastewater from baker's yeast enterprises has a significant negative impact on the environment (Poshtarenko, 2015). Most companies dilute sewage with water and discharge it into the sewer system or use aeration tanks, which is irrational. Methane fermentation technology is the most expedient, because the COD of wastewater

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from the production of baker's yeast exceeds 2000 mg O$_2$/dm$^3$. This will allow not only to remove pollution from wastewater, but also to obtain an environmentally friendly fuel – biogas (Kamyab et al., 2021). Therefore, it is not only environmentally friendly but also cost-effective, as biogas can be used to meet the thermal needs of a methane tank or the main production or production of electricity (Sajad Hashemi et al., 2021). In addition to biogas, digestion is also formed, which is a product of bioconversion of organic components in the process of methane fermentation (Song et al, 2021). Digestion is characterized by a high content of cobalamin vitamins and other biologically active substances (Angouria-Tsorochidou et al, 2021). Such fermented mass can be used as a fertilizer or growth stimulant in the treatment of seeds of different crops (Lu et al, 2021, Tampio et al, 2016). Therefore, it is important to study not only the modes of methane fermentation in order to obtain biogas, but also to stimulate the processes of vitamin production.

The aim of the work is to study the methane fermentation of yeast production effluents in a continuous mode using a stimulant for vitamin production (cobalt chloride).

The task of the work is to study the influence of the parameters of the process of continuous methane fermentation (dilution rate, addition of cobalt salts) on the efficiency of purification, gas generation, vitamin formation.

### 2. Materials and Methods

Methane fermentation of wastewater from the baker's yeast production plant took place in a laboratory apparatus – a methane tank with a volume of 3 dm$^3$ and a water gasholder – storage of biogas. A thermostat was used to ensure the mesophilic fermentation regime.

Process indicators were determined by standard methods (Muravyov, 2010).

The pH was determined using a pH-340 device. The volume of biogas was recorded by the volume of water displaced by biogas from the gasholder into the receiving tank. The concentration of methane and carbon dioxide in biogas was determined by an accelerated method: passing biogas through a 10 % solution of sodium hydroxide (Semenova, 2019).

### 3. Results and Discussion

The Department of Environmental Safety and Labor Protection of the National University of Food Technologies conducts research on methane fermentation of concentrated wastewater treatment processes in the food industry, as well as the utilization of agricultural waste.

Concentrated wastewater from baker's yeast production was subjected to methane fermentation in a 3 dm$^3$ methane tank placed in a thermostat to maintain a temperature of 37 ± 1 °C. This temperature corresponds to the mesophilic regime of methane fermentation. Anaerobic activated sludge from the operating bioreactor of the Yuzefo-Mykolaiv Biogas Station (YMBS) was used.

Wastewater had a light brown color, a characteristic odor of yeast, the rate of contamination by COD (chemical oxygen demand) was 4500 mg O$_2$/dm$^3$, dry matter content 12 g/dm$^3$, pH 6.

The cultivation regime was continuous, the rate of dilution was 4,1·10$^{-3}$, 6,2·10$^{-3}$, 8,2·10$^{-3}$, 12,4·10$^{-3}$ hours$^{-1}$.

The process was monitored by the following indicators: temperature, COD,
volume of biogas, methane content in biogas, content of cobalamin vitamins (B12, factor III, factor B), purification efficiency. Volume of biogas was also calculated by the amount of COD contaminants loaded into the methane tank (dm³/g COD_{loading}) and the difference in the amount of contaminants between the initial and final COD values (dm³/g COD_{fermentation}).

Since one of the objectives of the study was to identify the effect of stimulants (cobalt chloride) on the process of vitamin production, experiments were conducted in parallel with or without salt addition. This made it possible to identify the effect of cobalt not only on the intensity of vitamin formation, but also in general on the process of purification and gas generation.

Methane fermentation of effluents caused a high degree of purification. The results obtained during the study are shown in table 1.

The maximum degree of purification (78,9 %) was achieved at the lowest flow rate (without the addition of cobalt), and the minimum (66,7 %) – at the highest flow rate (with the addition of cobalt). That is, at high concentrations of substrate and high values of dilution rate, there is an overload of anaerobic sludge microorganisms with wastewater components, which reduces the assimilation of contaminants, and, accordingly, the efficiency of treatment. The presence of cobalt also has a depressant effect on the purification process.

| Dilution rate, hours⁻¹ | Without cobalt chloride | With cobalt chloride |
|------------------------|-------------------------|---------------------|
| COD ending, mg O₂/dm³ | cleaning efficiency, %  | COD ending, mg O₂/dm³ | cleaning efficiency, % |
| 4,1·10⁻³               | 950                     | 78,9                | 1 000               | 77,8 |
| 6,2·10⁻³               | 1 050                   | 76,7                | 1 120               | 75,1 |
| 8,2·10⁻³               | 1 100                   | 75,6                | 1 200               | 73,3 |
| 12,4·10⁻³              | 1 400                   | 68,9                | 1 500               | 66,7 |

The intensity of gas generation, depending on the dilution rates and the effect of cobalt salts was also studied (table 2).

There is a relationship between volume of biogas and dilution rate. As the dilution rate increases, the volume of biogas decreases. Obviously, increasing the rate of dilution leads to the fact that the components of the substrate during this time do not undergo complete cleavage to the components that form the basis of biogas. That is, the high content of components in the bioreactor does not allow the culture to carry out methanogenesis in full. Similarly to the effect on the depth of purification, the inhibitory effect of cobalt on methane generation is observed.

Conversion of volume of biogas per unit of loaded (dm³/g COD_{loading}) and fermented contaminants by COD (dm³/g COD_{fermentation}) confirmed the opposite trend in terms of dilution rate. Thus, with increasing dilution rate from 4,1 · 10⁻³ to 12,4 · 10⁻³ hours⁻¹ volume of biogas decreased from 1,11 to 0,94 dm³/g COD_{loading}, and from 1,43 to 1,39 dm³/g COD_{fermentation}. That is, at lower dilution rates, the transformation of contaminants was more efficient.
Table 2. Intensity of gas generation and methane content in biogas during methane fermentation of wastewater

| Dilution rate, hours\(^{-1}\) | Without cobalt chloride | With cobalt chloride |
|-------------------------------|-------------------------|----------------------|
|                               | Volume of biogas, dm\(^3\)/dm\(^3\) effluents | Methane content in biogas, % | Volume of biogas, dm\(^3\)/dm\(^3\) effluents | Methane content in biogas, % |
| 4,1·10\(^{-3}\)               | 5,2                      | 85,0                 | 4,9                      | 83,5                 |
| 6,2·10\(^{-3}\)               | 5,0                      | 83,8                 | 4,7                      | 82,8                 |
| 8,2·10\(^{-3}\)               | 4,7                      | 83,0                 | 4,5                      | 82,0                 |
| 12,4·10\(^{-3}\)              | 4,1                      | 80,5                 | 3,8                      | 79,0                 |

Biogas was characterized by a high methane content (up to 85 %). Such biogas is a high-quality alternative fuel that can be used to meet the thermal needs of the methane tank, the main technological process of the enterprise or for the production of electricity.

The highest methane content (85 %) was observed at the minimum flow rate. An increase in this indicator, as well as the addition of cobalt salts, led to a decrease in the amount of methane in biogas to 79 %.

In addition to identifying the energy value of concentrated wastewater from yeast production, we studied the peculiarities of the accumulation of vitamins in the fermented culture fluid (digestion). Namely, the influence of process parameters – the rate of dilution and the presence or absence of cobalt salts.

Analysis of the digestion showed that in the process of methane fermentation of wastewater accumulates a fairly high content of vitamins of the cobalamin group. Compared with the initial content, the amount of these vitamins increased by 6,46 – 13,2 times (table 3).

Table 3. The content of cobalamin vitamins in the initial substrate and digestion

| The content of vitamins in the initial substrate, μcg/g | Dilution rate, hours\(^{-1}\) | The content of vitamins in the digestion, μcg/g |
|-------------------------------------------------------|-------------------------------|-----------------------------------------------|
|                                                       |                               | without cobalt chloride | with cobalt chloride |
| 7,2                                                   | 4,1·10\(^{-3}\)              | 46,5                      | 70,5                      |
|                                                       | 6,2·10\(^{-3}\)              | 69,5                      | 87,0                      |
|                                                       | 8,2·10\(^{-3}\)              | 71,7                      | 92,4                      |
|                                                       | 12,4·10\(^{-3}\)             | 75,0                      | 95,0                      |
The highest content of vitamins in the non-baltic medium reached 75 μcg/g at the maximum value of the dilution rate. There is a clear trend of increasing the content of vitamins with increasing dilution rate. But with increasing this figure, the increase in vitamin content becomes less intense. When changing the dilution rate from $4.1 \cdot 10^{-3}$ to $6.2 \cdot 10^{-3}$ hours$^{-1}$, the increase was 49,5 %, and from $8.2 \cdot 10^{-3}$ to $12.4 \cdot 10^{-3}$ hours$^{-1}$ – only 4,6 %. Obviously, too significant an increase in the rate of dilution causes a gradual complication of the transformation of waste components into vitamins.

The use of cobalt salt has a significant stimulating effect on the synthesis of vitamins. Depending on the dilution rate, the increase in the amount of vitamins ranged from 26.7 to 51.6 %. But significant values of dilution rates had an effect similar to the experiment without cobalt salts.

The study of the qualitative composition of cobalamin group vitamins in the fermented culture fluid revealed the effect of dilution rate and cobalt salt content on the ratio between active (vitamin B$_{12}$ and form III) and inactive (form B) components (table 4).

### Table 4. Influence of cultivation parameters on qualitative and quantitative composition of vitamins

| Dilution rate, hours$^{-1}$ | Without cobalt chloride | With cobalt chloride | vitamin content, μcg/g: | fB | B$_{12}$ | fIII | fB | B$_{12}$ | fIII |
|-----------------------------|-------------------------|----------------------|-------------------------|----|---------|------|----|---------|------|
| 4,1·10$^{-3}$               | 1,56                    | 30,00                | 14,94                   | 2,50 | 50,00   | 18,00 |
| 6,2·10$^{-3}$               | 2,40                    | 45,70                | 21,40                   | 2,70 | 59,00   | 25,30 |
| 8,2·10$^{-3}$               | 2,50                    | 48,00                | 21,20                   | 8,70 | 52,50   | 31,20 |
| 12,4·10$^{-3}$              | 13,28                   | 43,92                | 17,80                   | 20,60 | 40,50   | 33,90 |

The vast majority of vitamins are the active form (B$_{12}$ and fIII), and the true form of B$_{12}$ dominates. The addition of cobalt salts promotes its more intensive accumulation, except for cultivation at the highest dilution rate.

Without the addition of cobalt salts, the content of vitamin B$_{12}$ increases, reaching a maximum of 48 μcg/g at a dilution rate of $8.2 \cdot 10^{-3}$ hours$^{-1}$. Further increase in this cultivation parameter leads to a decrease in the amount of vitamin. When cobalt was added, the maximum content of vitamin B$_{12}$ (59 μcg/g) was at a dilution rate of $6.2 \cdot 10^{-3}$ hours$^{-1}$. In the future, the effect was similar to the experiment without cobalt.

Increasing the rate of dilution affects the ratio of active and inactive forms of vitamins, namely the reduction of active content. One of the reasons for this may be that methanogens do not have time to biotransform precursors into the active form of vitamins.

The highest total content of active forms of vitamin (69,2 μcg/g) was recorded at a dilution rate of $8.2 \cdot 10^{-3}$ hours$^{-1}$ in cobalt-free medium, and 84,3 μcg/g – at a dilution rate of $6.2 \cdot 10^{-3}$ hours$^{-1}$ in medium with cobalt. The content of inactive forms of vitamin (fB) is insignificant and only at the maximum selected value of the dilution rate reaches quite high concentrations. Therefore, such cultivation parameters are not rational in
the case of using methane fermentation of these effluents to obtain vitaminized fermented culture fluid. Accordingly, the use of the dilution rate in the range from $6,2 \cdot 10^{-3}$ to $8,2 \cdot 10^{-3}$ hours$^{-1}$ is optimal for the total content of active forms of vitamins, as well as for the content of true vitamin B$_{12}$.

The addition of cobalt leads to an increase in the total amount of vitamins and achieve a better ratio of active and inactive forms of vitamins relative to the non-cobalt environment.

Because, in addition to these vitamins, the digestion contains nitrogen, phosphorus, potassium and other valuable components, it is an effective fertilizer for agricultural plants and can also be used to stimulate their growth and development.

4. Conclusions

Therefore, methane fermentation of yeast production wastewater is a technology for a comprehensive solution to their disposal.

Removal of the bulk of pollutants is ensured, reaching the maximum degree of purification up to 78.9%. High values of dilution rate cause overload of anaerobic activated sludge with pollutants, which leads to a decrease in cleaning efficiency. The presence of cobalt also has a depressant effect on the purification process.

At the same time, the process is accompanied by the formation of a significant amount of biogas (up to 5.2 dm$^3$/dm$^3$ wastewater) with a high methane content (up to 85%). Such biogas is a high-quality alternative fuel to meet the thermal needs of the methane tank, the technological process of the enterprise or for the production of electricity. As the dilution rate increases, the volume of biogas and methane content in it decrease. Thus, with increasing dilution rate from $4,1 \cdot 10^{-3}$ to $12,4 \cdot 10^{-3}$ hours$^{-1}$, volume of biogas decreased from 1.11 to 0.94 dm$^3$/g COD$_{loading}$, and from 1.43 to 1.39 dm$^3$/g COD$_{fermentation}$. Namely, at lower dilution rates, the transformation of contaminants was more efficient. Similarly to the effect on the depth of purification, the inhibitory effect of cobalt on methane generation is observed.

Methane fermentation can also be used to produce digestion, which is a valuable fertilizer and stimulant of crop growth. It contains a significant amount of cobalamin vitamins (up to 95 μg/g). By regulating the parameters of the process, the optimal indicators are achieved in terms of the total content of active forms of vitamins, as well as in terms of the content of true vitamin B$_{12}$.

The addition of cobalt salts stimulates the synthesis of vitamins, providing an increase from 26.7 to 51.6%, as well as improving the ratio between active and inactive forms of vitamins.

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БІОТРАНСФОРМАЦІЯ СТІЧНИХ ВОД ВИРОБНИЦТВА ХЛІБОПЕКАРСЬКИХ ДРІЖДЖІВ ІЗ ОТРИМАННЯМ БІОГАЗУ

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Підприємства із виготовлення хлібопекарських дріжджів є джерелом забруднення довкілля концентрованими стічними водами. Зазвичай їх розводять водою і складають у канілізаційні мережі. Раціональним є використання метанової ферментації, що забезпечить вилучення біогазу та дигестату. Тому актуальним є вивчення питання впливу параметрів ферментації на ефективність очищення. Мета роботи – дослідження метанової ферментації стічних вод хлібопекарських дріжджів у безперервному режимі. Задачі: аналіз вирішення проблеми очищення стічних вод дріжджових заводів, дослідження впливу параметрів процесу (швидкість розбиваючого процесу, завантаження солей кобалу) на ефективність очищення; газогенерацію; вітамінотворення. Початкове збільшення pH стоків 4,0 моль/л, pH 6. Режим культивування – безперервний, швидкість розбиваючого процесу 4,1·10⁻³, 6,2·10⁻³, 8,2·10⁻³, 12,4·10⁻³ год⁻¹. Ефективність очищення 78,9 %. Високе значення швидкості розбиваючого процесу зумовлює перевантаження активного мулу, що веде до значення ефективності очищення. Наявність кобалу також чинить пригнічувальну дію. Утворюється значна кількість біогазу (до 5,2 мм³/мл) із високим вмістом метану (до 85 %), якій є альтернативним паливом. При збільшенні протоку вихід біогазу і вміст метану зменшуються. При збільшенні швидкості розбиваючого процесу від 4,1·10⁻³ до 12,4·10⁻³ год⁻¹ вихід біогазу знижувався з 1,11 до 0,94 м³/г ХСК (аналіт.), та з 1,43 до 1,39 мм³/г ХСК (дигестат). Аналогічно зі збільшенням глибини очищення, прослідковується пригнічувальна дія кобалу на метаногенерацію. Дигестат є цінним добривом, має значний вміст вітамінів кобаламінової групи (до 95 мкг/г). Додавання солей кобалу стимулює біотрансформацію синтез вітамінів, забезпечуючи їх приріст від 26,7 до 51,6 %, покращуючи співвідношення між активними і неактивними формами.

Ключові слова: біогаз, вітаміни, дигестат, дріжджове виробництво, метанова ферmentація, стічні води.