Toxicity Assessment of Treated Meat Industry Wastewater in the Anaerobic Process

Ocena toksyczności ścieków z przemysłu mięsnego oczyszczonych w procesie beztlenowym

Major pollutant components of meat processing wastewater are biodegradable organic compounds, fats and proteins in both particulate and dissolved forms. Because of the possible pollution of water sources, the efficient disposal of effluent from meat plants is important. The treatment of industrial wastewater is a highly complex process that generally involves factors associated with load fluctuations and high concentrations of organic matter. Toxic effects on aquatic organisms and plants may be caused by numerous nitrogen compounds, as well as detergents and antibiotics, in the meat industry wastewater. The aim of the research was to determine the toxicity of anaerobic treated meat industry wastewaters. The level of toxicity was determined with algae growth inhibition test and Lepidium test. The values of \(E_{rC_{50}}\) (0÷96) and \(E_{bC_{50}}\) of indicators were 18.4 and 8.6% respectively. TU value for \(E_{rC_{50}}\) was 5.51 which meant acute toxicity of wastewater. The value of TU for \(E_{bC_{50}}\) was 11.62 (high toxicity of wastewater). The values of indicators RS G (relative seed germination), RRG (relative root growth) and GI (germination index) were 92, 19.5 and 17.62% respectively. Treatment efficiency meat industry wastewater during fermentation process was very high. The COD and BOD removal efficiency were on 82.3 and 80% respectively. Effluent from ASBR reactor had following parameters: COD = 206 mg O\(_2\)/dm\(^3\) and BOD = 130 mg/dm\(^3\). TOC value after anaerobic process was 75 mg C/dm\(^3\) (78.1%). The concentration of ether extract and proteins were 188 and 74 mg/dm\(^3\) respectively. Generated biogas in the methane fermentation process of wastewater from meat industry plants was characterized by a high methane content (77.5% vol.). Carbon dioxide and the ballast in the analyzed biogas were 20 and 2.5% respectively. In order to enrich biomass with methane by removal of CO\(_2\) from its content, the gas generated in the anaerobic process was subjected to the processes of chemisorption and adsorption on the granulated active carbons and molecular sieve. Purification the raw biogas by a molecular sieve has contributed to the increase of methane in enriched from 77.5 to 92.6% and the removal of CO\(_2\) from 20 to 5.7%. Due to poor quality and its high toxicity, effluent from ASBR reactor can not to be discharged into natural water. In the future it is suggested to incorporate RO or UF into the technological system in order to posttreatment the wastewater.

Keywords: meat industry wastewater, toxicity, Lepidium test, reactor ASBR, biogas purification

Introduction

The Polish meat industry started to grow rapidly in 2004. EU subsidies allowed numerous meat processing plants to make multimillion investments, which, in turn,
led to increased production and helped raise the quality of products [1]. The meat industry in the country currently comprises about 3,500 enterprises of various fields and business profiles. This section of the economy is characterized by a strong fragmentation and dispersal and includes both small family companies dedicated exclusively to the slaughter, as well as big establishments and companies. Slaughtering animals and/or the production of related products are coupled with the need for a lot of clean water and related to the emission of polluted water, which has to be purified before it can be drained off. Therefore, slaughterhouse processes in the industrialized countries are coupled with strict legislation and control to protect public health and the environment [1-9].

Slaughterhouses and meat processing plants generate a large volume of effluents. The consumption of water per slaughtered animal varies according to the animal and the process employed in each industry, and ranges from 1.0 to 8.3 m³. Meat processing plants use approximately 62 Mm³/y of water [8, 10-12]. Meat industry wastewaters composition is strong compared to domestic wastewater. The wastewater generated in meat processing plants contain high amounts of biodegradable organic matter, usually varying from 1100 to 2400 mg/dm³ in terms of BOD, with the soluble fraction varying from 40 to 60% [10, 13]. The physical nature of these wastewaters has been studies by Sayed et al. [14], who have shown that if the COD of screened (1 mm mesh) effluent, 40÷50% was present as coarse, suspended matter, which was insoluble and slowly biodegradable, and the remainder is present as colloidal and soluble matter. This varies considerably from domestic wastewater, in which the COD is present mainly in the colloidal form [14]. The main contributors of organic load to these effluents are fecal, fat, blood, suspended material, urine, loose meat and soluble proteins. The wastewater contain pathogenic and non-pathogenic viruses, bacteria and parasite eggs. Prior to discharge from the plant, poultry processors are required to remove the majority of the soluble and particulate organic material in their wastewater in order to achieve compliance with environmental regulation [14, 16-19]. The treatment of meat industrial wastewater is a highly complex process that generally involves factors associated with load fluctuations and high concentrations of organic matter. These factors are often due to inhibitors in biological processes that have not been properly introduced in the environmental or contaminants that have not been treated before being discharged into water reservoirs. Toxic effects on aquatic organisms and plants may be caused by numerous nitrogen compounds, as well as detergents and antibiotics, in the meat industry wastewater [20, 21].

Anaerobic meat industry wastewater treatment combined with proper posttreatment represents the ideal solution for environmental protection. The main advantages of anaerobic treatment such as little sludge produced, production of methane gas as a source of energy, it is a low energy process making it more environmentally friendly and has lower running costs as a result of a low energy inputs [20]. The Anaerobic Sequencing Batch Reactor (ASBR) is a technology for wastewater treatment that combines different cycles and stages of operation depending on the quality required of the effluent water [20, 21]. In recent years, the
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Need to implement effective systems for the treatment of industrial effluents has been established in order to reduce toxic wastewater. Studies have shown that many pharmaceutical compounds and detergents are not completely removed by conventional wastewater treatment technology. While many persistent pollutants break down relatively quickly in the environment many others are highly resistant to degradation. Toxicity is usually determined by the capacity of a substance to have an adverse effect on an organism [20-22]. There are many methods and indicators used for determining the toxicity of wastewater. A toxicity test can determine the relationship between the dose of toxic substance and the reaction of organisms. One of the toxicity indicators is EC$_{50}$, which determines the effective dose of toxic sample causing the effect in 50% of the tested population [22]. The organisms most frequently used for toxicity testing are bacteria, fish, algae and Daphnia. For trials of this nature these types of organisms have the advantage of presenting biochemical pathways similar to those of higher organisms. Furthermore, they have short life cycles and respond quickly to changes in the environment [20, 21].

The aim of the conducted research was to determine the effectiveness of wastewater treatment from the meat industry in the ASBR type bioreactor. Together with raw wastewater anaerobic biodegradation of pollutants, the possible toxicity for natural water reservoir was determined as well as an attempt to purify the biogas produced during anaerobic processes.

1. Material and methods

1.1. Meat industry wastewater

The wastewater came sampled from the meat-processing plant near Czestochowa whose activity covers the slaughtering and processing of pigs. The raw wastewater had a brown color and smelled bad and was also characterized by a tendency to rot and foaming. COD of raw meat industry wastewater varied from 1163 to 1175 mg/dm$^3$ and BOD was average at 650 mg/dm$^3$. High concentration of total nitrogen (250 mg N/dm$^3$) and chloride (1000 mg Cl$^-$/dm$^3$) was also observed. Lipid content and proteins were respectively 875 and 269 mg/dm$^3$ [7].

1.2. Reactor ASBR and characteristics of anaerobic granular sludge

In the experiment, an anaerobic sequencing batch reactors (ASBR) was used. The ASBR reactor has a cylindrical shape with a total volume of 12 dm$^3$. The reactor tank was made of plexiglass. The produced biogas was collected in a calibrated glass cylinder which was filled with acidified aqua deionized water [7]. Produced biogas were sent to the scrubber filled with adsorbents or absorbents. The stages of the research are presented in Figure 1.
The anaerobic granular sludge used in the research, was picked up from an anaerobic IC reactor at the wastewater treatment plant at Zywiec SA brewery. Granules typically have a spherical form with a diameter from 3 to 5 mm, where the value of organic matter concentration was 68.28 g/dm$^3$ and mineral compounds achieved value of 12.56 g/dm$^3$ (total suspensions - 80.84 g/dm$^3$).

### 1.3. Characteristics of used adsorbents and absorbents

In the process of CO$_2$ adsorption from biogas, granulated carbon and a molecular sieve was used. The granulated active carbons with the following symbols were used (Table 1): AG-5, BA-10, NG-1 (Gryfiskand Sp. z o.o. from Hajnówka). A molecular sieve (13xHPx8x12) was provided by the Shanghai BOJ Molecular Sieve company.

<table>
<thead>
<tr>
<th>Carbon</th>
<th>Specific surface area m$^2$/g</th>
<th>Bulk density g/dm$^3$</th>
<th>Typical grain size, mm</th>
<th>Ash content %</th>
<th>Mechanical strength, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>AG-5</td>
<td>950–1050</td>
<td>390–410</td>
<td>1</td>
<td>8±10</td>
<td>min. 90</td>
</tr>
<tr>
<td>BA-10</td>
<td>min. 1000</td>
<td>490±30</td>
<td>3</td>
<td>10</td>
<td>97</td>
</tr>
<tr>
<td>NG-1</td>
<td>min. 850</td>
<td>max 550</td>
<td>3.8</td>
<td>max 20</td>
<td>min. 98</td>
</tr>
</tbody>
</table>

In the process of chemisorption, 3 absorbents were applied, that is, a 3% NaOH solution, a 3% KOH solution and a 10% monoethanolamine solution (NH$_2$CH$_2$CH$_2$OH).
1.4. Analytical procedures

Raw meat industry wastewater and effluent from the ASBR were sampled periodically for pH value, alkalinity, chloride, COD, BOD, total organic carbon (TOC), total nitrogen (TN), volatile fatty acids (VFA), ether extract and protein. Summary of analytical test results:

- the pH value was determined with a pH-meter Cole Parmer 59002-00,
- the COD value was determined using the colorimetric method by PN-85/C-04578/02,
- OXITop® (WTW GmbH) was used to determine the value of BOD,
- TOC and TN values were measured by Kiper TOC 10C analyser PX-120 with autosampler,
- the alkalinity and chloride were measured according to standard method [23, 24],
- VFA was determined with the distillation method on Büchi 323-Distillation Unit by PN-75/C-04616/04,
- lipid content (ether extract) was determined by two methods: direct extraction and Soxhlet extraction,
- protein content of meat industry wastewater was estimated by Lowry's method,
- total suspensions, organic matter concentration and mineral compounds were determined by direct weight method according to PN-75/C-04616/01.

The composition of the biogas was analyzed using Geotechnical Instruments GA 2000. Determination of the total number of bacterial used by Koch method. The standard test for the coliform group was carried out by the multiple-tube fermentation technique [23-25].

1.4.1. Toxicity test - algae growth inhibition

The tests were performed in accordance with the OECD 201 [26] guidelines and according to the annex to the resolution of the Minister of Health dated July 28th, on the methods of conducting studies of physico-chemical properties, toxicity and eco-toxicity of substances and chemical preparations [26, 27].

The principle of the performance of the test is based on the incubation of algae in tested samples of wastewater for a specific period of time and on the measurement of the number of algae cells per 1 cm$^3$ of a sample from each sample, which corresponds to the density of the cell biomass. In accordance with the recommendations, the initial density of the algae cells amounted to $10^{-4}$ per 1 cm$^3$. The unicellular algae - *Chlorella vulgaris* was used in the experiment. The following concentrations of wastewater samples were prepared: 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.57% and a control sample - in the pure algae culture medium. From the moment of commencement of the test, the number of algae cells was measured in each sample in hours 48, 72 and 96 using the Thoma counting chamber for this purpose. In order to present a correlation between the concentration and the effect, the speeds of algae growth ($\mu$) were compared for the respective concentrations of wastewater at specific times (48, 72 and 96 h) using the formula (1):
\[ \mu_{0-n} = \frac{\ln N_n - \ln N_0}{t_n - t_0} \]  \(1\)

where:
\( \mu_{0-n} \) - the average specific growth rate from moment time 0 to n,
\( N_n \) - the biomass concentration (cm\(^3\)) at time \( t_n \),
\( N_0 \) - the biomass concentration (cm\(^3\)) at time \( t_0 \),
\( t_0 \) - the moment time for the start of the period,
\( t_n \) - is the moment time for the end of the period.

Next, calculate the percent inhibition of growth rate for each treatment replicate from the equation:
\[ \%I = \frac{\mu_c - \mu_t}{\mu_c} \times 100 \]  \(2\)

where:
\( \%I \) - percent inhibition on average specific growth rate,
\( \mu_c \) - mean value for \( \mu \) in the control,
\( \mu_t \) - value for growth rate in the treatment.

In order to indicate the value of EC\(_{50}\) (\( E_bC_{50} \)) as the effective wastewater concentration, which causes a 50% inhibition in the growth of algae biomass, two methods were used:
- probit method (95% confidence interval),
- graphic interpolation method in the linear scale.

The first stage of the probit method was to calculate the value of the density growth inhibition of the algae cells in accordance with equation:
\[ \%I = \frac{B_c-B_n}{B_c-B_0} \times 100 \]  \(3\)

where:
\( \%I \) - the percentage inhibition,
\( B_c \) - the number of algal cells in 1 cm\(^3\) of control sample at time \( t \),
\( B_n \) - the number of algal cells in 1 cm\(^3\) of test sample at time \( t \),
\( B_0 \) - the number of algal cells in 1 cm\(^3\) of control sample after the time \( t_0 \).

Then, the regression coefficient \( b \) - in accordance with the formula presented below.

The concentrations for which probit \( y \) ranged between: 3.5 ≤ \( y \) ≤ 6.5 were used in the calculations:
\[ b = \frac{\sum_{i=1}^{k} x_i y_i - x \sum_{i=1}^{k} y_i}{\sum_{i=1}^{k} x_i^2 - x \sum_{i=1}^{k} x_i} \]  \(4\)

where:
\( k \) - the number of concentrations included in the calculations,
\( x_i \) - the logarithm of the concentration of the \( i \)-th concentration,
\( y_i \) - probit corresponding to the percentage of mortality for the \( i \)-th concentration,
\( x \) - the average concentrations of individual logarithms.
The next stage was to calculate the effective concentration $EC_{50}$, using the formula below:

$$EC_{50} = \text{Nlg} \frac{5 - \bar{y} + \bar{x}^2}{a}$$  \hspace{1cm} (5)

where:
- $\text{Nlg}$ - antilog,
- $\bar{x}$ - values corresponding to the standard probit percent inhibition for various concentrations,
- $\bar{y}$ - the average concentrations of individual logarithms.

The last stage in allowing the sample to be classified in the appropriate toxicity class according to Persoone was to calculate the value of $EC_{50}$ in reference to toxicity units (TU) in accordance with equation (6) [28]:

$$TU = \frac{1}{EC_{50}} \times 100$$  \hspace{1cm} (6)

### 1.4.2. Phytotoxicity test

The *Lepidium* Test was performed in accordance with the methodology proposed by Walter et al. [28]. A paper disc was placed on a Petri plate, then 5 ml of tested wastewater was added and 10 grains of garden cress were sown. The control sample was prepared similarly, using distilled water. Each variant of the experiment was repeated 10 times, for raw and treated wastewater from the meat processing plant respectively. After the performance of seeding, the plates were placed in an incubator (25°C) and incubated for 48 h without access to light. After the expiration of the allotted time, the length of the germinated seeds was measured. On the basis of the obtained data, the percentage indicators of RSG, RRG and GI were calculated according to the given formulas:

- the percentages of relative seed germination (RSG)
  \[ %\text{RSG} = \left( \frac{S_E}{S_K} \right) \times 100 \]  \hspace{1cm} (7)

  where:
  - $S_E$ - numer of seeds germinated in wastewater extract,
  - $S_K$ - numer of seeds germinated in control,

- relative root growth (RRG)
  \[ %\text{RRG} = \left( \frac{R_E}{R_K} \right) \times 100 \]  \hspace{1cm} (8)

  where:
  - $R_E$ - mean root length in wastewater extract,
  - $R_K$ - mean root length in wastewater control,
\[
GL = \frac{RSG \times RRG}{100}
\] (9)

2. Results and discussion

2.1. Treatment of meat industry wastewater in ASBR reactor

The anaerobic process in ASBR reactor was carried out with organic loading rate (OLR) 0.969 kg COD/m³d and sludge loading rate 0.097 kg COD/kgvssd. The concentration of anaerobic granular sludge was at the level 10 g/dm³. To obtain the preset objective, the anaerobic granular sludge and the raw wastewater were proportioned once per 24 h to the cyclic bioreactor in which their detention time was 24 h. The study was conducted until the cycle repeated. The times of particular cycles of bioreactor operation were:
- tank filling (0.5 h),
- reaction phase (22 h),
- sedimentation phase (0.75 h),
- wastewater drainage (0.75 h).

The value of COD of the raw wastewater amounted to 1163 mg/dm³. After the first cycle of treatment, a degree of removal of COD was obtained at a level of 36.5% (739 mg O₂/dm³). After the next cycle, a significant decrease in the value of COD up to 346 mg/dm³ was noted. During the seven cycle, COD decreased to the level of 210 mg O₂/dm³. The average degree of removal of COD in the repeatable cycles amounted to 82.3%. During the anaerobic treatment process, the value of BOD of the raw wastewater was decreased from 650 mg/dm³ to the level of 130 mg/dm³ (degree of BOD removal - 80%). Total organic carbon (TOC) in raw wastewater was 343 mg C/dm³. TOC value after anaerobic process (after VII cycle) was 75 mg C/dm³ (78.1%).

The characteristic of pollution in meat industry wastewater are the ether extract and proteins. Ether extract removal efficiency was 78.5% (188 mg/dm³). It was concluded that the content of proteins in the methane fermentation process was decreased by 72.4% (up to the level of 74 mg/cm³ in the treated wastewater).

During experiment the VFA/alkalinity the ratio, which properly represents fermentation, was estimated. The maximum value above which the process inhibition takes place is assumed on the level of 0.3. The highest value of VFA/alkalinity ratio 0.28 and 0.26 was respectively in I and VII cycle. In cycles from II to VI were constant level in the range of 0.23÷0.25.

In effluent ASBR reactor COD, BOD, TOC, proteins and ether extract value were nearly 2-fold (COD), 5-fold (BOD), 2.5-fold (TOC), and almost 4-fold (ether extract) exceeded in relation to permissible standards [29]. The value of total nitrogen in effluent ASBR (312 mg/dm³) was 11-fold exceeded in comparison to permissible standards (30 mg/dm³). Concentration of pollution in raw and treated wastewaters present Table 2.
Table 2. Concentration of pollution in raw and treated wastewaters

<table>
<thead>
<tr>
<th>Indicator of pollution</th>
<th>Raw wastewater</th>
<th>Treated wastewater in subsequent cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cycle I</td>
<td>Cycle II</td>
</tr>
<tr>
<td>pH</td>
<td>7.28</td>
<td>7.24</td>
</tr>
<tr>
<td>COD*</td>
<td>1163</td>
<td>739</td>
</tr>
<tr>
<td>BOD*</td>
<td>650</td>
<td>-</td>
</tr>
<tr>
<td>Alkalinity*</td>
<td>320</td>
<td>660</td>
</tr>
<tr>
<td>VFA/alkalinity</td>
<td>0.75</td>
<td>0.28</td>
</tr>
<tr>
<td>TOC*</td>
<td>343</td>
<td>95</td>
</tr>
<tr>
<td>TN*</td>
<td>205</td>
<td>301</td>
</tr>
<tr>
<td>Chloride*</td>
<td>1000</td>
<td>100</td>
</tr>
<tr>
<td>Proteins*</td>
<td>269</td>
<td>-</td>
</tr>
<tr>
<td>Ether extract*</td>
<td>875</td>
<td>-</td>
</tr>
</tbody>
</table>

An attempt at assessment of the effectiveness of microbiological pollution removal from wastewater generated at the meat processing plant was made during the experiment. The analysis involved raw wastewater as well as wastewater treated after the seventh cycle of operation in the anaerobic sequencing batch reactor (ASBR). The raw wastewater was characterized by a high overall number of mesophile bacteria’s ($2.14 \times 10^5$), which became lower after the methane fermentation process by up to $7.4 \times 10^5$. The index of the coliform bacteria was decreased from $10^{-7}$ to $10^{-4}$.

### 2.2. Biogas enrichment

Generated biogas in the methane fermentation process of wastewater from meat industry plants was characterized by a high methane content (77.5% vol.). Carbon dioxide and the ballast in the analyzed biogas were 20 and 2.5% respectively. The biogas were also tracers such as unwanted hydrogen sulfide (125 ppm) and carbon monoxide (62 ppm).

In order to enrich biomass with methane by removal of CO$_2$ from its content, the gas generated in the anaerobic process was subjected to the processes of chemisorption and adsorption on the granulated active carbons and molecular sieve.

After making the biogas pass through the washer filled with granulated active carbon AG-5, a 6.3% increase in methane and 3.3% decrease in the content of carbon dioxide were noted. In the case of active carbon BA-10, changes in the composition of the treated biogas were observed to the least extent, the methane content in the enriched biogas increased by only 1.2%. Among the tested active carbons, the best effects were obtained using carbon NG-1. Upon conducting the adsorption process, the methane content in the treated biogas increased by 10.5%, and the carbon dioxide content fell by 9.1%. The replacement of the granulated...
active carbons with the 13xHPx 8x12 molecular sieve contributed to an increase in the methane content of 15.1% and removal of as much as 14.3% of carbon dioxide.

The conducted chemisorption processes demonstrated similar effects on the removal of CO₂ from biogas to those obtained in the molecular sieve. Among the used absorbents, the greatest effectiveness of CO₂ removal from biogas was obtained with the use of the 3% NaOH solution. A 11.6% increase in the quantity of methane in the treated biogas was observed, with a simultaneous decrease in the carbon dioxide content by half (from 20 to 10%). An almost identical effect was obtained when the raw biogas was passed through washers filled with a 10% solution of monoethanolamine (an increase in methane by 13%). On top of this, monoethanolamine was effective in the removal of CO₂, whose content fell from 70 ppm to 40 ppm. The poorest effects were obtained with the use of a 3% KOH solution, where CO₂ was hardly removed, only 6% (Table 3).

### Table 3. Comparison of methods used purification biogas

<table>
<thead>
<tr>
<th>Component</th>
<th>Raw biogas</th>
<th>Purification biogas</th>
<th>The used absorbents</th>
<th>Granular activated carbon</th>
<th>Molecular sieve</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>3% KOH</td>
<td>3% NaOH</td>
<td>10% NH₂CH₂CH₂OH</td>
</tr>
<tr>
<td>CH₄</td>
<td>77.5</td>
<td>84.7</td>
<td>89.1</td>
<td>90.5</td>
<td></td>
</tr>
<tr>
<td>CO₂</td>
<td>20</td>
<td>14</td>
<td>10</td>
<td>8.5</td>
<td></td>
</tr>
<tr>
<td>Ballast</td>
<td>2.5</td>
<td>1.3</td>
<td>0.9</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>AG-5</td>
<td>BA-10</td>
<td>NG-1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>82.7</td>
<td>78.7</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>16.7</td>
<td>18.9</td>
<td>10.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.6</td>
<td>2.4</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 2.3. Algae growth inhibition

The algae growth inhibition test was based on the measurements of algae cell density per 1 cm³ of wastewater. After testing all the concentrations of the respective wastewater samples, the results were specified in Table 4, taking into consideration the test time.

### Table 4. Change in the number of algal cells during the test

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Incubation time, h</th>
</tr>
</thead>
<tbody>
<tr>
<td>% s</td>
<td>0</td>
</tr>
<tr>
<td>100</td>
<td>210 938</td>
</tr>
<tr>
<td>50</td>
<td>82 031</td>
</tr>
<tr>
<td>25</td>
<td>156 250</td>
</tr>
<tr>
<td>12.5</td>
<td>347 656</td>
</tr>
<tr>
<td>6.25</td>
<td>484 375</td>
</tr>
<tr>
<td>3.125</td>
<td>484 375</td>
</tr>
<tr>
<td>1.5625</td>
<td>562 500</td>
</tr>
<tr>
<td>0</td>
<td>437 500</td>
</tr>
</tbody>
</table>

304 684
The obtained results allowed the dependency charts related to the density of Chlorella vulgaris cells per 1 cm$^2$ to be created, depending on the time of cultivation for each concentration of the sample of anaerobically treated wastewater. In order to depict the correlation between the concentration and the effect, the speeds of algae growth were compared and the percentage of algae growth speed inhibition in the tested wastewater samples was calculated (Fig. 2).

The undiluted wastewater (100% treated wastewater) led to the algal growth inhibition at the level of 91%.

At the same time the regression curve equation was generated for the obtained data and the effective concentration value was calculated. Taking advantage of the generated regression curve equations ($y = 55.3x-19.6$), the value of ErC$_{50}$ (0÷96 h) was calculated. This value was 18.4%. The next stage of the toxicity test was to indicate the value of EC$_{50}$ (ErC$_{50}$), that is, the effective wastewater concentration, which causes a 50% inhibition of the algae biomass growth. For this purpose, the values of the density growth inhibition for the algae cells were calculated. During the conversion of the percentage value of inhibition into probits, the values of probits ranging between $3.5 \leq y \leq 6.5$ were taken into account. In order to calculate the value of the regression coefficient "b", a series of calculations for each sample was performed, and the results are presented in Table 5.

In accordance with equations (3) and (4) given in the methodology, the regression coefficient and the effective concentration ErC$_{50}$ were calculated. The value of the regression coefficient amounted to 2.28, and the effective concentration - ErC$_{50}$ was 8.6.

In order to classify the wastewater into the appropriate toxicity classes according to Persoone et al. [30], the values of EC$_{50}$ converted into toxicity units (TU) were calculated. TU value for the ErC$_{50}$ (0÷96 h) and ErC$_{50}$ was respectively 5.51 (acute toxicity) and 11.62 (high acute toxicity).
Table 5. The probits method of determining the effective concentration \( EC_{50} \)

<table>
<thead>
<tr>
<th>Concentration %</th>
<th>( X_i )</th>
<th>( I )</th>
<th>( y_i )</th>
<th>( \log) concentration</th>
<th>percent inhibition</th>
<th>for inhibition probit</th>
<th>( (x_i)^2 )</th>
<th>( X_i/Y_i )</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>2.00</td>
<td>158.49</td>
<td>-----</td>
<td>4.00</td>
<td>----</td>
<td>4.00</td>
<td>----</td>
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Rodríguez-Loaiza et al. [31] evaluated, the toxicity of wastewater from a meat by-products processing industry before and after treatment using the Sequencing Batch Reactor (SBR). They reported that the effluents prior to treatment were highly toxic (EC < 60%) whereas post-treatment results showed low or no toxicity (EC\(_{50} > 82\%\)). They also showed a high correlation between the ammonia nitrogen and the toxicity of wastewater. In the anaerobic process (in contrast to the aerobic process), there is no significant oxidation of nitric pollutants (total nitrogen or ammonium nitrogen). In the effluents from the ASBR reactor, the concentration of ammonium nitrogen was 124 mg/dm\(^3\) which could cause the achieved toxicity values of treated wastewater.

2.4. Lepidium test

It was found that in the case of raw wastewater, the average number of germinated seeds was greater than (9.7) in comparison with the wastewater treated in the fermentation process (9.6). The average length of the root in the case of the raw wastewater was also greater (3.2 mm) in comparison to the seeds cultivated in the treated wastewater (0.95 mm).

The values of indicators RSG (relative seed germination), RRG (relative root growth) and GI (germination index) were 92, 19.5 and 17.62% respectively.

This can be explained by the fact that the substances that enable the proper germination of plants (substances with the nature of plant hormones) may be found in wastewater. This can also be related to the presence of a significant number of biogenic compounds which contribute to the growth activity of cress. Studies, reporting that increased N content in industrial effluents is beneficial for plant growth [32, 33]. Our results agree with other studies being conducted on toxicity of industrial effluents using lettuce and other seeds as bioindicators. Gerber et al. [34], evaluated the phytotoxic effects of raw and treated effluents from a swine slaughterhouse on cucumber and lettuce seeds and determined correlations among physicochemical characteristics of such effluents and the germination of seeds used as
bioindicators. The effluents treatment system was efficient to reduce the concentration of some physicochemical parameters to levels within those recommended by the Brazilian legislation, except for P, ammoniacal N and TKN concentration. Although phytotoxicity of the treated effluent was less in comparison to the raw effluent, the GI for cucumber and lettuce seeds submitted to each of the tested effluent was lower than 80% [34].

Conclusions

− In effluent ASBR reactor COD, BOD, TOC, proteins and ether extract value were nearly 2-fold (COD), 5-fold (BOD), 2.5-fold (TOC), and almost 4-fold (ether extract) exceeded in relation to permissible standards.
− Purification the raw biogas by a molecular sieve has contributed to the increase of methane in enriched from 77.5 to 92.6% and the removal of CO₂ from 20 to 5.7%.
− Resulted in inhibition rate of algal growth of undiluted wastewater (100% treated wastewater) at 91%.
− TU value for the \( E_{C_{50}} (0-96 \text{ h}) \) and \( E_{C_{30}} \) was respectively 5.51 (acute toxicity) and 11.62 (high acute toxicity).
− The values of indicators RSG (relative seed germination), RRG (relative root growth) and GI (germination index) were 92, 19.5 and 17.62% respectively.
− The average length of the root in the case of the raw wastewater was also greater (3.2 mm) in comparison to the seeds cultivated in the treated wastewater (0.95 mm).
− Due to poor quality and its high toxicity, effluent from ASBR reactor can not to be discharged into natural water. In the future it is suggested to incorporate RO or UF into the technological system in order to posttreatment the wastewater.

Acknowledgements

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References


[27] Regulation of the Minister of Health dated July 28th, 1003 on the methods of conducting studies of physico-chemical properties, toxicity and eco-toxicity of substances and chemical preparations.
Streszczenie

Głównymi zanieczyszczeniami obecnymi w ściekach powstających na terenie zakładu mięsnego są biodegradowalne związki organiczne, tłuszcze i białka, występujące w nich zarówno w formie cząstek stałych, jak i rozpuszczonych. Ze względu na możliwość zanieczyszczenia nimi naturalnych odbiorników ważne jest skuteczne oczyszczanie tego rodzaju ścieków poprodukcyjnych. Oczyszczanie ścieków przemysłowych jest bardzo złożonym procesem, na który wpływa wiele czynników, m.in. wysokie stężenie materii organicznej w ściekach, jak również duże ich wahania. Działanie toksyczne na organizmy wodne i rośliny może być spowodowane przez występujące w ściekach z przemysłu mięsnego związki azotu, a także detergenty i antybiotyki. Celem badań było określenie toksyczności beztlenowo oczyszczonych ścieków z przemysłu mięsnego. Poziom ich toksyczności określono za pomocą testu zahamowania wzrostu glonów oraz testu *Lepidium*. Wartości wskaźników ErC₅₀ (0,96) i E₅₀ wynosiły odpowiednio 18,4 i 8,6%. Wartość TU dla ErC₅₀ wyniosła 5,51, co oznaczało ostro toksyczność ścieków. Wartość TU dla E₅₀ wyniosła 11,62 (wysoka toksyczność ścieków). Odpływ z reaktora ASBR charakterystyka była bardzo wysoka. Stopień usunięcia ChZT i BZT wynosił na poziomie odpowiednio 82,3 i 80%. Odpływ z reaktora ASBR charakteryzował się następującymi wartościami: ChZT - 206 mg/dm³ i BZT - 130 mg/dm³. Wartość OWO po procesie beztlenowym obniżyła się do poziomu 75 mg C/dm³ (78,1%). Stężenie ekstraktu eterowego i białek w ściekach oczyszczonych wynosiło odpowiednio 188 i 74 mg/dm³. Wytworzony w procesie fermentacji metanowej ścieków z zakładu mięsnego biogaz charakteryzował się wysoką zawartością metanu (77,5% obj.). Zawartość diętnika ięga i balastu w analizowanym biogazie wynosiła odpowiednio 20 i 2,5%. W celu wzbogacenia biogazu w metan poprzez usunięcie z jego zawartości CO₂ wytworzony w procesie beztlenowym gaz poddano procesom chemisorpcji oraz adsorpcji na granulowanych węglach aktywnych oraz na siatce molecularnym. Oczyszczanie surowego biogazu za pomocą sita molecularnego przyczyniło się do wzrostu zawartości metanu z 77,5 do 92,6% przy jednoczesnym usunięciu CO₂ z 20 do 5,7%. Z powodu jednak złej jakości odpływu z reaktora ASBR oraz jego wysokiej toksyczności ścieki tak oczyszczone nie mogą być odprowadzane do odbiornika naturalnego. W przyszłości w celu ich doczyszczania sugeruje się włączenie procesu RO lub UF do układu technologicznego.

Słowa kluczowe: ścieki z zakładu mięsnego, toksyczność, *Lepidium* test, reaktor ASBR, oczyszczanie biogazu