Desalination and Water Treatment
www.deswater.com

doi: 10.5004/dwt.2018.22978

128 (2018) 341–350
October

The influence of temperature of methane fermentation combined with pressure driven membrane techniques on treatment efficiency and toxicity of meat industry wastewater

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Received 30 March 2018; Accepted 13 August 2018

ABSTRACT

The purpose of the research was to determine the influence of temperature of the anaerobic degradation process on the efficiency of treatment and toxicity of meat industry wastewater in the UASB-UF-RO system. Batch anaerobic decomposition of meat industry wastewater was performed at psychrophilic (20°C), mesophilic (35°C) and thermophilic (45°C) temperatures to determine the influence of temperature on biogas production rate and composition of wastewater. The experiment was run for 40, 42 and 38 d at 20°C, 35°C and 45°C, respectively. Treatment efficiency of meat industry wastewater during mesophilic fermentation process was very high. Rates of removal of chemical oxygen demand (COD), total organic carbon (TOC) and BOD were 77%, 76% and 69%, respectively. The biogas produced in these temperature conditions characterized with the largest methane content (77%). It was found that wastewater from the meat processing plants treated by fermentation conducted in psychrophilic conditions pointed to the highest effect on inhibition of the algae growth rate (almost four times). The least toxic effect on algae growth was observed for wastewater anaerobically treated at temperature of 35°C ± 2°C (inhibition level –91%). During reverse osmosis, the rates of removal of COD, TOC, BOD and total nitrogen were 67%, 71% 78% and 34%, respectively. In the case of wastewater after the RO process, the value of TU was below 0.4. It is planned in the future to extend the technological system with final ammonia stripping as its final concentration in the effluent from the presented system exceeded the permissible standard over six times (RO permeate).

Keywords: Meat industry wastewater; UASB reactor; Anaerobic process; Toxicity wastewater; Ultrafiltration process; Reverse osmosis process

1. Introduction

Slaughterhouses and meat processing plants generate a large volume of effluents. The average amount of wastewater produced in the meat plant is 150 m^3/d with the BOD load that corresponds to the population equivalent (PE) of 9,500. Meat processing plants use approximately 62 Mm^3/year of water. The consumption of water per slaughtered animal depends on the animal type and the process employed in each industry, and ranges from 1.0 to 8.3 m^3 per unit [1–4]. The meat industry wastewater typically contains manifold impurities and a high organic matter level, including blood, fat, fur as well as detergents, hormones, antibiotics, preservatives and pathogenic microbes [4–6]. The physical nature of these wastewaters was studied by Sayed et al. [7] who showed that in the screened (1 mm mesh) effluent, 40%–50% of COD was present as coarse, suspended matter, which was insoluble and slowly biodegradable, and the remained COD was present as colloidal and soluble matter. This differs considerably from domestic wastewater, in which the COD is present mainly

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Presented at the XII Scientific Conference 'Membranes and Membrane Processes in Environmental Protection' – MEMPEP 2018
13–16 June 2018, Zakopane, Poland

in the colloidal form [7,8]. Table 1 presents the qualitative characteristics of wastewater produced in the meat industry. These characteristics make slaughterhouse wastewater very contaminated and specific. Hence, its discharge may cause deoxygenation of rivers and contamination of groundwater [9,10]. Thus, the slaughterhouse wastewater must be treated before its discharge into receiving bodies to eliminate its critical effects on the environment and human health.

Wastewaters from slaughterhouses and meat processing have been classified by Environmental Protection Agency (EPA) as one of the most harmful to the environment [11]. Meat industry wastewater treatment methods are similar to technologies currently used in municipal wastewater and may include preliminary, primary, secondary and even tertiary treatment. Biological treatment is used to remove organics and eventually pathogens from meat industry effluents using microorganisms. Furthermore, the biological treatment is able to remove up to 90% BOD from processing plants effluents by either aerobic or anaerobic processes [6,12]. Typically, anaerobic treatment is used because of the high organic compounds concentrations present in meat industry wastewater [14,15]. Moreover, anaerobic process seems to be the better solution for organic wastewater treatment due to several reasons. It helps to transform a large part of degradable organic compounds into biogas, a renewable source of energy, it characterizes with low value of excess sludge generation, it does not require aeration, eliminates pathogens and reduces the emission of odors (closed reactors) [16,17]. Technologically, treatment of wastewater from food processing industry is based on the use of UASB bioreactors, designed by Letting et al. in the seventies of the last century in the Netherlands. Biomass used in this bioreactor is in the form of granulated sludge, characterizes with very good sedimentation properties, resistance to fluctuation in the volume of contaminants in the wastewater supplied to the bioreactor and low unit growth of the sludge (from 0.11 to 0.22 $\text{kg}_{\text{v.s.s}}$/kg COD_{rem}) [18,19]. However, anaerobic or aerobic processes should not be used as the single treatment because of the final effluents characteristics, which needs to correspond to the current effluent discharge limits and standards [6]. Therefore, a new combined technology is necessary to sufficiently treat slaughterhouse wastewater before its discharge into receiving waters. For several years, much attention has been addressed to the development of unconventional methods for the wastewater treatment, such as pressure-driven membrane operations, for example, ultrafiltration, which removes colloids, suspended and macromolecular matter, or reverse osmosis, which rejects minerals and low-molecular-weight organic compounds [20–24]. Wastewater treated in a conventional treatment plant is usually discharged into the environment, while membrane filtration technology can produce sufficiently pure water suitable to be reused in, for example, crop irrigation and industrial processing [14,20,21]. The aim of the conducted research was to determine the influence of temperature change of the anaerobic process on the efficiency of meat industry wastewater treatment in the UASB bioreactor. Due to the poor quality of wastewater treated in the anaerobic process, wastewater was posttreated using pressure driven membrane techniques. The study also evaluated the change in the toxicity of the wastewater treated in the anaerobic process under different temperature conditions and the bioreactor effluent treated by ultrafiltration and reverse osmosis.

Current Polish legislature does not regulate the problem of wastewater toxicity. The determination of the quality of wastewater takes into consideration only selected parameters that provide information about quantitative amount of certain substances defined as pollutants. In order to determine the possible effect of wastewater on a natural reservoir, especially on the organisms living in this reservoir, it is necessary to examine wastewater toxicity. Examinations of the toxicity are performed using dedicated toxicity tests. These tests are performed by observation of the noticeable effect of the toxic substances present in the examined sample revealed towards sensitive living organisms [8,25,26]. In the case of wastewater from the meat industry, both phytotoxic and toxic effect can be observed due to the presence of the hormones, antibiotics and high contents of nitrogen and phosphorus.

2. Material and method

2.1. Material

The material for the examination was the wastewater generated in the meat processing plant near Czestochowa (Poland). The wastewater was a mixture of streams obtained

Table 1
Pollutants concentration in raw wastewater (for a treatment plant of wastewater flow below 2,000 m^3/d) [3,6,13]

Pollution indices	Concentration of pollutants in raw wastewater, mg/dm^3		Permissible standards, mg/dm^3*	
	Range	Mean value	Sewage system	Natural receiver
pH	6–10	–	6–9.5	6–9.5
COD	860–12,000	3,200	1,000	125
BOD	640–5,000	2,400	700	25
Total nitrogen	50–600	250	50	30
Total phosphorus	15–100	50	15	5
Total suspended solids	100–3,000	1,200	350	50
Ether extract	800–2,500	1,400	100	50

*Regulation of the Ministry of Environmental Protection, Natural Resources and Forestry, dated 18 November 2014, on the classification of water and conditions the sewage discharged to waters and soil should satisfy, J. Law No. 0, item 1800.

from individual stages of the technological cycle, that is, pig slaughter and washing the slaughtering rooms and equipment. The raw wastewater had a brown colour and a tendency to rot and foaming. It was sampled from the equalization tank located on the crates, sieves and a degreaser before the aeration tank. COD of raw meat industry wastewater varied from 1,690 to 1,720 mg/dm^3 and BOD was in average equal to 1,240 mg/dm^3. High concentration of ammonia nitrogen (193 mg/dm^3) and TOC (920 mg/dm^3) was also measured. Lipid content was 1,185 mg/dm^3.

2.2. Apparatus

The UASB (*Upflow Anaerobic Sludge Blanket)* reactor with the volume of 5 dm^3 was made of the organic glass (PMMA). Stable temperatures of the anaerobic fermentation (20°C–45°C ± 2°C) were ensured using an ultrathermostat that pumped the heated water to the heating jacket of the bioreactor. The UASB effluent (anaerobically treated wastewater) was collected in the upper part of a reaction chamber, whereas the generated gas was collected in a calibrated container. The granulated sludge used in examinations was collected from the anaerobic IC bioreactor at the wastewater treatment plant in Żywiec S.A. brewery. The sludge was in the form of granules with the diameter from 2 to 5 mm and dry mass of 77.45 g/dm^3 (including the content of organic matter of 64.25 g/dm^3 and mineral substances of 13.20 g/dm^3).

The process of the final treatment of biologically pretreated wastewater was performed using membrane separation techniques. The apparatus equipped in a plate-frame membrane module SEPA CF–NP (Osmonics, USA) was used. The module was composed of two steel plates between which a flat membrane in the form of a rectangular sheet of dimensions of 190 × 140 mm was placed (total surface of the membrane was 155 cm^2, and the filtration area 144 cm^2). A membrane installation equipped in a feed material container with the volume of 8,000 cm^3 was operated using the cross-flow system. The module was designed so that the filtration process could be performed over a wide range of pressure (from 0.05 to 8 MPa) and linear velocity over the membrane surface from 0.1 to 4.5 m/s. Commercial polyamide membranes (ADF) were used during the reverse osmosis process, whereas ultrafiltration was performed using membranes made of polysulfone (PSF-16). Table 2 presents the characteristics of the osmotic membrane.

Polysulfone membrane was prepared by the phase inversion method. Casting solution consisted of 15 wt.% of PSF and 85 wt.% of DMF (*N*,*N*-dimethylformamide). The solution was shaken for 20 h to obtain homogeneous solution. Next, membranes were casted using doctor blade with 0.2 mm thickness on a glass plate and immediately immersed in deionized water at ± 20°C. Solidified membranes were stored in deionized water at temperature 8°C for 24 h for their stabilization. Suitable porosity of the PSF-16 membrane was 73% [27].

Table 2
Characteristics of the osmotic membrane (ADF)

Type of membrane	RO ADF
Polymer	Polyamide
Retention coefficient, *R*%	99.5
pH	4–11
J_v, psi	15–800
Pressure, bar	54
Cl$^-$, ppm	1,000
Temp., °C	50

2.3. Research methodology

The examinations were divided into three stages. The first stage involved examinations of the initially pretreated wastewater in the UASB bioreactor (Stage 1: anaerobic process). The process was continued for 120 d. Anaerobic granulated sludge of concentration of 20 g/dm^3 was supplied to the bioreactor. The effect of the temperature of the anaerobic process (from 20°C ± 2°C to 45°C ± 2°C) on the effectiveness of treatment of such the industrial wastewater was examined. Hydraulic retention time (HRT) was constant (3 d). The raw wastewater and the wastewater treated in the particular temperature conditions were usually analyzed every 2 d with regard to changes in COD, TOC, pH, alkalinity and VFA (volatile fatty acids). Once a week the measurements of BOD, NH_4^+ and ether extract were made. The composition and amount of the biogas generated in the process were continuously monitored. At the moment of determination of the most beneficial conditions of anaerobic biodegradation, the effluent from the UASB reactor was directly supplied to the plate-frame membrane module in order to ensure final treatment (Stage 2: membrane treatment). In the first step, the polymeric ultrafiltration membranes (PSF-16) and commercial osmotic membranes (ADF) were subjected to conditioning and their transport characteristics were determined. Ultrafiltration treatment of the effluent from the UASB reactor was conducted at the pressure of 0.4 MPa, whereas the permeate after ultrafiltration process was treated in a high-pressure driven process at the pressure of 2.0 MPa. Linear flow velocity of the filtered medium over the membrane surface was 2.0 m/s for both processes. The efficiency of the wastewater treatment using both separation methods was assessed using the relationships between experimental temporary flux, the time filtration and permeability of membranes. In ultrafiltration and reverse osmosis permeates, the content of pollutants as BOD, COD, TOC, NH_4^+ and ether extract was determined. Raw wastewater and wastewater treated at particular stages of the experiment were also subjected to microbiological analysis to determine the general number of psychrophilic and mesophilic bacteria and those from the *coli* group.

The last step attempted to demonstrate whether the wastewater treated in the process of methane fermentation and wastewater treated using pressure driven membrane techniques showed a toxic effect on, for example, water flora and fauna (Stage 3: wastewater toxicity). Garden cress (*Lepidium sativum*) and algae (*Chlorella vulgaris*) were used for this purpose. Growth inhibition test for the *C. vulgaris* algae was performed according to the OECD guidelines [28]. The test consisted of the incubation of algae for 72 h in the examined samples of wastewater. The control sample was grown on a medium recommended by the OECD. Cellular biomass density was measured at 24 h intervals by counting cells under the microscope using the Thoma chamber. After the standard screening test run on undiluted samples, a series of five dilutions was performed with a decreasing geometrical progress. The proper test was conducted by measuring density of incubated cells for individual concentrations. Algae growth rate (μ) was compared for each dilution of wastewater and a percentage of algae growth inhibition was computed. Next, ErC50 (0–72 h) value was computed as an effective wastewater concentration, which corresponded to a 50% inhibition of algae growth rate after 72 h, using the regression line, equations of which were computed for each sample. The ErC50 (0–72 h) values were computed per toxicity units (TU) and assigned to specific toxicity class according to the classification by Persoone et al. [8,26,29].

The *Lepidium* test was performed in accordance with the methodology proposed by Walter [30]. A paper disc was placed on a Petri plate, then 5 cm^3 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 this time, the length of the germinated seeds was measured [8].

2.4. Physical and chemical analyses

The HACH DR/4000 spectrophotometer was used to perform the measurements of COD. BOD was determined using the respirometric method by means of the measurement set OXI Top WTW. Kiper TOC 10C analyzer PX-120 with AS40-Dione3.11 autosampler was used for the determination of TOC. CP-401/CP-40 ph-meter was used to measure pH during the process of anaerobic biodegradation of wastewater from a meat processing plant. Total alkalinity was determined according to PN90/C-04540/03 standard. Ammonia nitrogen and VFA were determined by the distillation method on Büchi 323-Distillation. Lipid content (ether extract) was determined by two methods: direct extraction and Soxhlet extraction. Koch method was used to determine the total number of bacteria. The standard test for the coliform group was carried out by the multiple-tube fermentation technique [31]. The composition of the generated biogas was analyzed by means of the SR2-DO (Germany) biogas analyzer.

3. Results and discussion

3.1. Examinations of the effect of temperature of the methane fermentation process on the efficiency of wastewater treatment and the amount and the quality of biogas

Anaerobic treatment of wastewater from the meat processing plant was performed at a constant organic loading rate (OLR – 0.57 kg COD/m^3d) and sludge loading rate (SLR – 0.028 kg COD/$kg_{v.s.s.}$ d). During treatment of wastewater under psychrophilic conditions (1st to 40th day of the experiment) mean rate of removal of COD, TOC and BOD was 69%, 72% and 61%, respectively. The amounts of COD, TOC and BOD for wastewater treated at temperature of 20°C ± 2°C were 529, 258 and 470 mg/dm^3, respectively. Concentration of ether extract in these process conditions was reduced from 1,185 to 438 mg/dm^3 (63%). The increase of temperature to 35°C ± 2°C (41st–82nd day of the experiment) resulted in the increase in COD removal efficiency by 8% (to the level in effluent- 393 mg/dm^3), TOC by 5% (to the level in effluent- 194 mg/dm^3) and BOD by 9% (to the level in effluent- 337 mg/dm^3). In the case of ether extract, the removal efficiency was higher by 16% (the level in effluent- 248 mg/dm^3). On the 83rd day of the experiment, the temperature in the UASB reactor was rapidly increased to 45°C ± 2°C. For the first day, the effluent from the UASB reactor was characterized by substantially higher values of COD (490 mg/dm^3) and TOC (336 mg/dm^3) compared with those obtained during wastewater treatment in mesophilic conditions. From the 90th day of the experiment (7th day of the process in thermophilic conditions), quality of the treated wastewater was gradually improving. After 36 d of methane fermentation under thermophilic conditions, COD, TOC and BOD and ether extract were 420, 215, 398 and 198 mg/dm^3, respectively.

Changes in COD and TOC in the wastewater treated during the experiment are presented in Figs. 1(a) and (b). Fig. 1(c) presents changes in the quality of wastewater treated versus temperature of methane fermentation process.

During the anaerobic process, nitrogen-containing compounds removal rate was insignificant. However, during the examination, the removal efficiency of ammonium nitrogen under mesophilic and thermophilic conditions was 36% and 30%, respectively. Explanation could be provided by the appearance of purple sulphur-free bacteria (probably *Rhodobacter, Rhodobium, Rhodopseudomonas, Rhodospirillum*) on the walls of the UASB reactor. Some purple nonsulphur bacteria, could denitrify, that is, reduce nitrate (which was reduced to nitrite then to nitrogen, with each denitrifying bacterium capable of one or both stages) by respiring it anaerobically instead of oxygen [32].

Level of VFA/alkalinity was monitored during methane fermentation for all the temperature conditions (Fig. 1(d)). With undisturbed process, this index should not exceed 0.3 [33]. According to Zhao and Viraraghavan, inhibition of methanogens occurs when VFA/alkalinity value exceeds 0.8 [34]. On the first day of the experiment in psychrophilic conditions, the VFA/alkalinity ratio was very high: 0.67 (2nd day) and 0.38 (6th day). From 9th to 40th day, its value ranged from 0.28 to 0.32. It was observed that the increase in temperature of methane fermentation to 35°C ± 2°C contributed to the reduction in the index value and its level ranged between 0.26 and 0.28. Further increase in temperature of the process (45°C ± 2°C) resulted in the increase in its value (0.29–0.31).

Volume and composition of the released gas were also evaluated during this stage of the examinations. During thermophilic fermentation, daily biogas yield was the highest (2,640 cm^3). The performance of the process at lower temperatures was also correlated with lower daily biogas yield. Under psychrophilic and mesophilic conditions, mean daily biogas yield was 1,840 and 2,100 cm^3, respectively (Fig. 2). A very important parameter monitored during anaerobic treatment of wastewater from the meat processing plant was

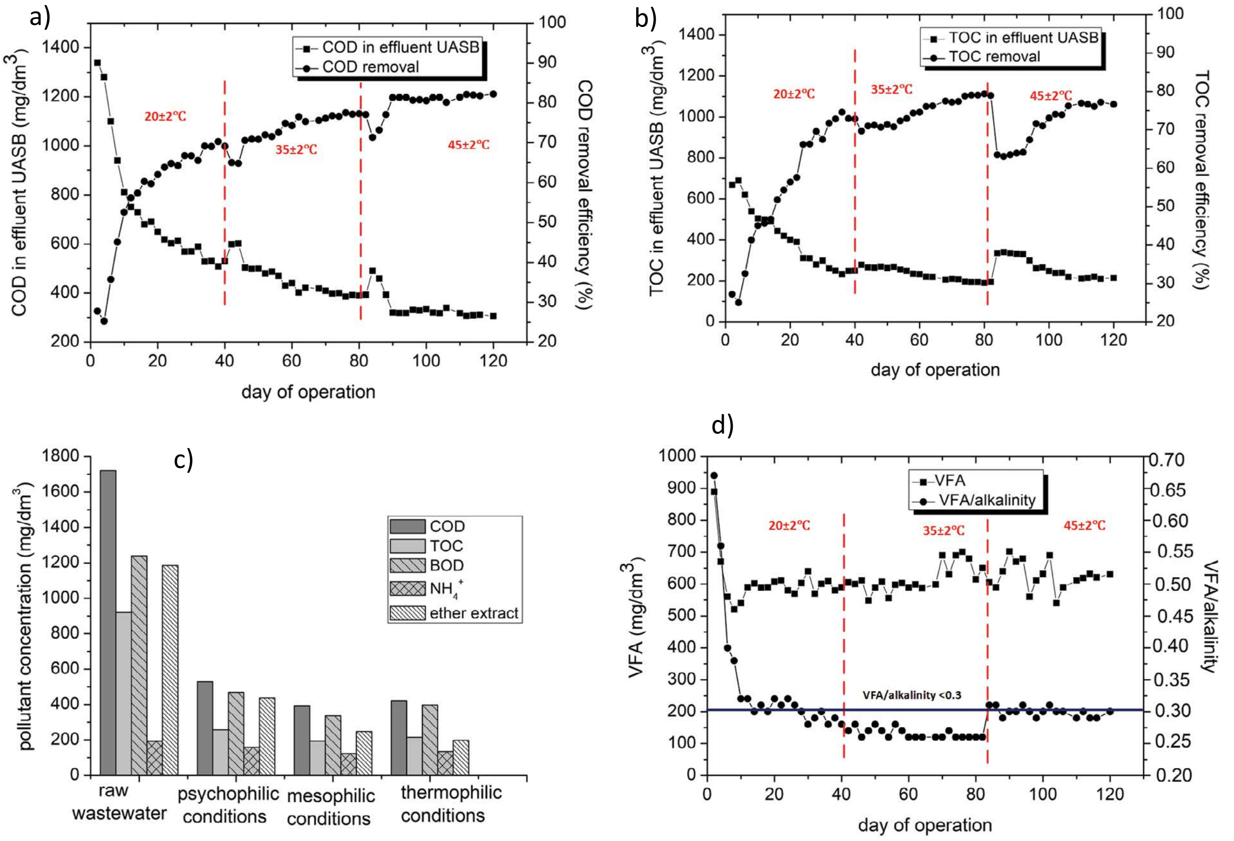

Fig. 1. The effect of temperature conditions the anaerobic process on the value of COD (a), TOC (b), effluent quality (c) and VFA/alkalinity (d) in meat industry wastewater treatment.

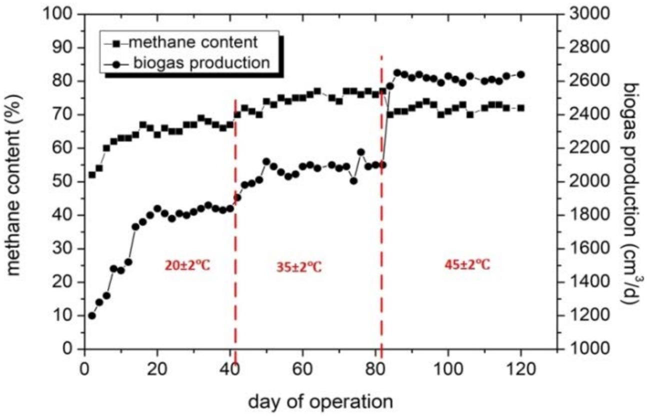

Fig. 2. The impact of temperature conditions of the anaerobic process on the biogas production/methane content in meat industry wastewater treatment.

biogas yield efficiency (Y), which was determined based on the following formula:

$$Y = \frac{V_b}{a} \quad \left(\mathrm{dm}^3 / \mathrm{g\ COD_{rem}}\right) \tag{1}$$

where V_b – volume of biogas generated during the day, dm^3/d, a – the amount of removed COD during the day, g/d.

The level of biogas yield efficiency coefficient during wastewater treatment under psychrophilic conditions was in average 0.32 dm^3 biogas/g COD$_{\mathrm{rem}}$. The Y coefficient had greater values at higher temperatures and it was 0.39 dm^3 biogas/g COD$_{\mathrm{rem}}$. (mesophilic conditions) and 0.37 dm^3 biogas/gCOD$_{\mathrm{rem.}}$ (thermophilic conditions). On the first day of the process in psychrophilic conditions, percentage of methane content in the biogas generated ranged from 52% to 64% (2nd to 8th day). It was also found that the biogas produced in these temperature conditions was characterized by the lowest methane content (67%). The best biogas composition in terms of quality was obtained during treatment of wastewater under mesophilic conditions. Methane percentage share in biogas was 77% with respect to CO$_2$ and other gases, but biogas production was much lower than in thermophilic conditions. In thermophilic temperature, the content of methane in the generated biogas was 72%. Mean content of H$_2$S and CO during the entire experiment was respectively 84 and 57 ppm.

The attempts were made to evaluate the efficiency of removal of microbiological pollutants from wastewater generated in the meat processing plant. Raw wastewater was characterized by a high total count of mesophilic bacteria (21.4 × 10^5) and psychrophilic bacteria (37.2 × 10^5). The bacteria of the coliform group were also present in this waste (10^{-4}). The highest reduction in total count of mesophilic bacteria, psychrophilic bacteria and coliform group bacteria were observed during wastewater treatment under thermophilic conditions. This was connected with wastewater hygienisation which occurred in high temperatures. The coliform index for the *coli* group was reduced from 10^{-4} to 10^{-7}, whereas general count of mesophilic and psychrophilic bacteria reduced to 16.2×10^3 and 14.5×10^3, respectively.

The results are similar to those obtained by previous researchers during anaerobic treatment of this type of industrial wastewater. In a study by Gannouna et al. [17] COD removal rate in mesophilic conditions (37°C ± 1°C) ranged from 80% to 90%. The increase in temperature of the anaerobic reactor to 55°C ± 1°C (thermophilic conditions) resulted in the decrease in COD removal rate to the level of 70%–72%. Biogas yield efficiency under thermophilic and mesophilic conditions was 0.32–0.45 dm^3 biogas/g $COD_{rem.}$ and 0.2–0.15 dm^3 biogas/g COD_{rem}, respectively. The content of methane in the gas generated in thermophilic conditions ranged from 63% to 73%. Insignificantly higher content of methane was observed during fermentation process at lower temperatures (65%–75%) [16,17].

3.2. Treatment of biologically pretreated wastewater from meat processing plant using membrane processes

As shown by the results obtained at the previous stage of the examinations, wastewater treated in the anaerobic process in all temperature conditions was characterized by the concentration of pollutants that disabled its direct discharge to the natural reservoir. Therefore, the attempt was made to treat the wastewater pretreated under mesophilic conditions in the process of ultrafiltration and reverse osmosis.

Transport properties of the used polymeric membranes were determined before the final treatment of the liquor from the UASB reactor. This consisted of passing the deionized water through the membranes at transmembrane pressures in the range of 1–3 MPa (for RO) and from 0.2 to 0.8 MPa (for UF). The volumetric permeate flux was determined for each pressure according to the formula:

$$J = \frac{v}{S \times t} \quad \left(\mathrm{m}^3 / \mathrm{m}^2\mathrm{s}\right) \tag{2}$$

where J – volumetric permeate flux, m^3/m^2 s, v – permeate volume, m^3, S – membrane surface, m^2 and T – time, s.

It was found in both cases that with the increase in pressure, the flux of received permeate also increased, and this relation character was represented by a linear function. At the next stage, both types of membranes were subjected to conditioning, which consisted of the flow of pure water at constant pressure through their surfaces. In the case of ultrafiltration membranes, conditioning was performed at the pressure of 0.4 MPa, whereas for the osmotic membrane the pressure was 2.0 MPa. The stage of membrane conditioning was conducted for 300 min and it was found in both cases that volumetric fluxes of water reached a steady level of 1.54×10^{-5} m^3/m^2 s (UF) and 3.80×10^{-6} m^3/m^2 s (RO). After the transport properties determination and conditioning, wastewater treatment was carried out.

In the 30-min treatment wastewater, UF permeate flux was 1.29×10^{-5} m^3/m^2 s, whereas after its stabilization after 210 min, it reached 0.94×10^{-5} m^3/m^2 s. Changes in volumetric flux of pure water and permeate during ultrafiltration process are illustrated in Fig. 3(a). Based on the determined value of the initial volumetric pure water flux and a mean value of volumetric flux of permeate, the relative permeability of ultrafiltration membrane was also determined. The reduced permeability was connected with the phenomenon of deposition of pollutants present in treated wastewater on the surface and/or in membrane pores (fouling). After 210 min of filtration process, relative permeability of the membrane was 61%.

During final treatment of wastewater after UF in a high-pressure membrane process, it was found, that the initial value of permeate flux (RO) was 2.54×10^{-6} m^3/m^2 s. The value of the flux with the process progress was reduced, and, after 90 min, it became steady at the level of 1.81×10^{-6} m^3/m^2 s. Because of the deposition of the pollutants on the surface of osmotic membrane, during 180 min of filtration, the level of flux was reduced by 23% (Fig. 3(b)). Relative permeability of the osmotic membrane was also reduced with time for the same reason (fouling and biofouling). After 30 min of the filtration process, relative permeability of the membrane was 53% and after 90 min of filtration process, relative permeability of the membrane was 47%.

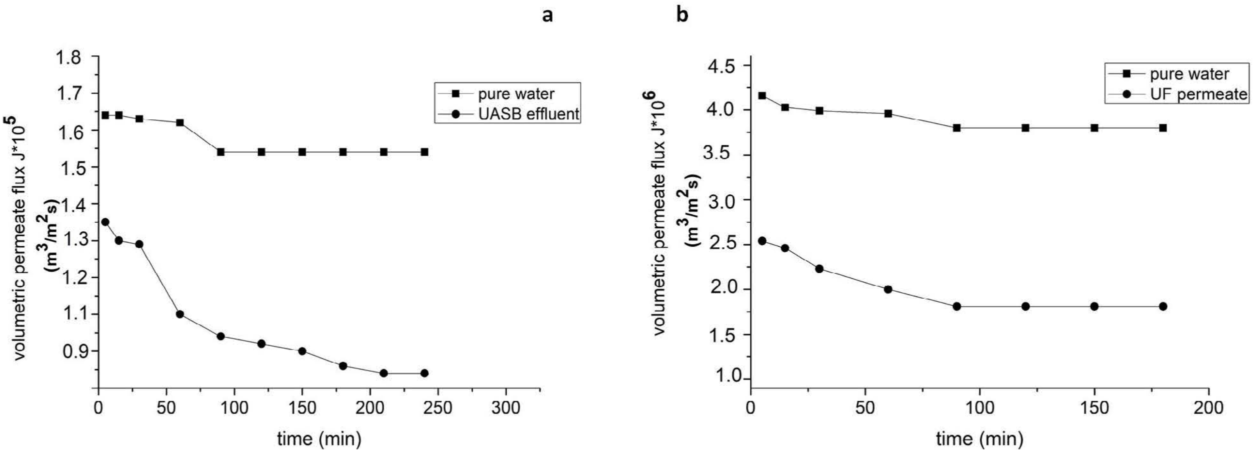

Fig. 3. The changes of the permeate flux in UF process (a) and RO process (b).

As expected, the ultrafiltration process ensured a high removal of the contaminants from biologically treated wastewater. Rejection coefficients for COD, TOC, BOD and ammonium nitrogen were 65% (concentration UF permeate- 131 mg/dm^3), 73% (concentration in UF permeate- 52 mg/dm^3), 71.2% (concentration in UF permeate- 97 mg/dm^3) and 25% (concentration in UF permeate- 93 mg/dm^3), respectively. The value of ether extract in the permeate after ultrafiltration treatment was equal to 68 mg/dm^3. The results obtained in the study demonstrated that wastewater treated in the examined system was still unsuitable for direct discharge to a natural reservoir. The values of indices for treated wastewater, according to Polish standards, were exceeded nearly four times (BOD), twice (TOC) and nine times (NH_4^+). The value of ether extract and COD was exceeded insignificantly [13]. Probably the pore size of the UF membrane was too big. The membrane with more suitable characteristics (lower pore size) could be applied.

During reverse osmosis, the rejection of COD, TOC, BOD and total nitrogen was 67% (concentration in RO permeate- 43 mg/dm^3), 71% (concentration in RO permeate- 15 mg/dm^3), 78% (concentration in RO permeate- 21 mg/dm^3) and 34% (concentration in RO permeate- 61 mg/dm^3), respectively. The value of ether extract after high-pressure driven separation was reduced to the level of 13 mg/dm^3. It is planned in the future to extend the technological system with final ammonia stripping as its final concentration exceeded the permissible concentration over six times. The efficiency of treatment of the wastewater in the integrated UASB-UF-RO system is presented in Fig. 4.

The use of pressure driven membrane processes for biologically treated wastewater treatment contributed to a substantial reduction in the content of pollutants and offered opportunities for using the generated water for industrial and washing purposes at the plant. It was found that the use of membrane separation technologies allowed for a substantial reduction in microorganisms present in the effluent from the UASB reactor. The reduction in mesophilic bacteria count for ultrafiltration and reverse osmosis was 98.1% and 99.4%, respectively. An analogous situation was found for psychrophilic bacteria (98.6% after UF and 99.3% after RO). The results obtained from the experiment and the examinations conducted by Yordanov [20] demonstrated that pressure driven membrane technologies could be successfully used as an alternative or a support of the conventional methods used to treat wastewater generated during slaughtering. Their results showed that the ultrafiltration could be an efficient purification method. The efficiency of COD and BOD removal exceeded 94% [20].

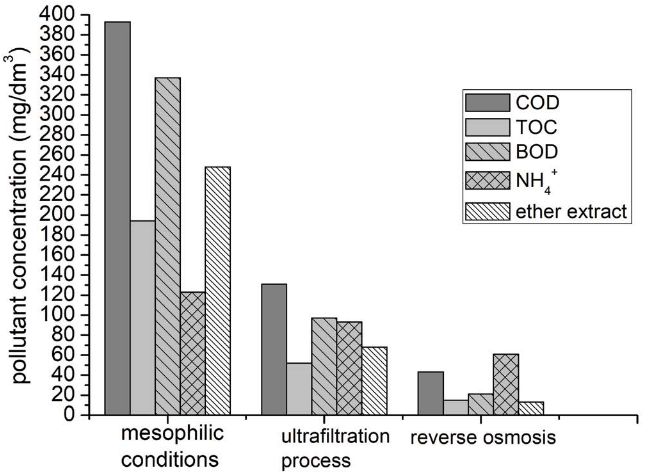

Fig. 4. The changes of the quality of meat industry wastewater purified in the UASB-UF-RO system.

3.3. The assessment of the toxicity of the wastewater treated in the anaerobic process associated with pressure-driven membrane processes

The algal growth inhibition test was used for determination of the samples, concentration of which inhibited algal growth-rate by *50*%.

The following wastewater concentrations were used for the samples: 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.57% and the control sample (in pure algae growth medium). Algal cells were counted four times. First, before addition of wastewater samples, then after 48, 72 and 96 h of test. The curves that represented the increase in algae biomass processes are given in Table 3. Due to the short time of incubation, the shape of growth curves was not consistent with the shape of a typical biomass growth curve. A logarithmic growth phase could be observed in the most frequent microorganisms growth curve, during which the number of algae cells increased logarithmically. The next stage comprised of the rest and the dying phases, which pointed to the depletion of growth of nutrients in the growth medium, accompanied by the accumulation of metabolites and a slow reduction in biomass growth rate. Graphical presentation of the relationships between the samples concentration and inhibition effect is shown in Fig. 5(a).

It was found that wastewater from the meat processing plants treated through fermentation conducted in psychrophilic conditions pointed to the highest effect on the inhibition of the algae growth rate (almost four times) in comparison with the blank sample. In the case of biologically treated wastewater, the least toxic effect on algae growth was observed for wastewater treated at temperature of 35°C ± 2°C. In this case, undiluted wastewater caused inhibition at the level of 91%. The ErC50 (0–72 h) value was computed based on the above charts. According to the adopted methodology, the values of efficient concentration were computed per units of toxicity and adequately classified. Values of effective concentrations leading to 50% inhibition of algae growth and the corresponding units of toxicity with the classification description are shown in Table 4. In the case of wastewater after the UF and RO process, the value of TU was below 0.4. The results of the toxicity of such treated wastewater were similar to the control tests carried out on distilled water.

Several studies have been focused on the examination of toxicity of wastewater from meat processing plants. Rodríguez-Loaiza et al. analyzed how aerobic processing in the SBR reactor impacted on changes in toxicity of this type of industrial waste [25]. These researchers demonstrated that before treatment, wastewater was highly toxic (EC < 60%), whereas the results after the treatment showed low or none toxicity (EC50 > 82%). The researchers also documented a

Table 3
The effect of temperature changes in methane fermentation on the algae cells number at temperature of 20°C (a), 35°C (b) and 45°C (c)

(a) Temperature 20°C

Concentration (%)	Incubation time (h)			
	0	48	72	96
100	304,684	3,125	Total lack of algae growth	Total lack of algae growth
50		3,906		
25		14,844	35,156	8,594
12.5		23,438	65,625	14,844
6.25		38,281	73,438	55,469
3.125		40,625	82,813	59,375
1.5625		43,750	85,938	62,500
Control		437,500	476,563	718,750

Number of algae cells in 1 cm^3

(b) Temperature 35°C [8]

Concentration (%)	Incubation time (h)			
	0	48	72	96
100	304,684	210,938	324,219	62,500
50		82,031	140,625	144,531
25		156,250	324,219	335,938
12.5		347,656	828,125	484,375
6.25		484,375	980,469	503,906
3.125		484,375	1,015,625	634,375
1.5625		562,500	1,101,563	796,875
Control		437,500	476,563	718,750

Number of algae cells in 1 cm^3

(c) Temperature 45°C

Concentration (%)	Incubation time (h)			
	0	48	72	96
100	304,684	140,625	35,156	11,719
50		54,688	85,938	156,250
25		234,375	105,469	257,813
12.5		335,938	390,625	531,250
6.25		363,281	453,125	621,094
3.125		406,250	507,813	601,563
1.5625		445,313	531,250	617,188
Control		437,500	476,563	718,750

Number of algae cells in 1 cm^3

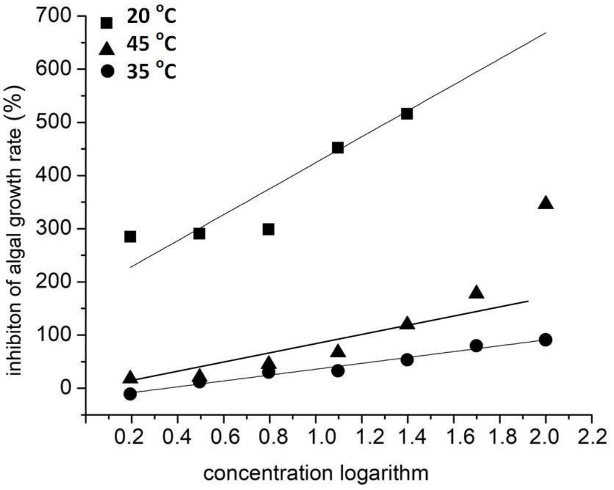

Fig. 5. The effect of temperature changes in methane fermentation on the algal growth rate inhibition.

high correlation between ammonium nitrogen and toxicity of wastewater. In the anaerobic process (contrary to aerobic), nitrogen pollutants were not oxidized (total nitrogen or ammonium nitrogen). In wastewater from the UASB reactor, the content of ammonium nitrogen was 124 mg/dm^3, which could have caused the obtained high values of toxicity of the treated wastewater.

The assessment of phytotoxicity of raw wastewater and treated wastewater in individual test phases was done with the use of *Lepidium*. It was concluded that in the case of raw wastewater, mean number of shooting seeds was greater than 9.7. For comparison, this number for the wastewater treated through fermentation was from 9.2 to 9.6. Mean length of roots in the case of raw wastewater was also greater (3.2 mm) compared with seeds grown on anaerobically treated wastewater (0.8–0.95 mm). This can be explained by the fact that substances which allow for proper seeds shooting (e.g., substances with plant hormone character) can be found in raw wastewater. This can be also connected with the presence of biogenic compounds, which contribute to the increase in activity of garden cress.

Studies have found that the increased content of N in industrial wastewater was beneficial to plant growth [35–38]. The results obtained in the paper are consistent with previous studies of toxicity of food industry wastewater using lettuce or other bioindicators. Gerber et al. [37] evaluated phytotoxicity of raw and cleaned wastewater from pig slaughter

Table 4
Toxicity of treated wastewater in the anaerobic process (20 °C–45°C ± 2°C)

Unit process	The value of the ErC50 index (0–96 h) (%)	The value of TU	Effective concentration EbC50 (%)	The value of TU
Methane fermentation 20°C ± 2°C	0.18	555.5 very high acute toxicity	–	–
Methane fermentation 35°C ± 2°C	18.14	5.51 acute toxicity	16.67	6.00 acute toxicity
Methane fermentation 45°C ± 2°C	5.63	17.76 very high acute toxicity	8.6	11.62 very high acute toxicity

Table 5
Analysis of the *Lepidium* test for raw and treated wastewater from the meat processing plant

Index	Blank sample (water)	Raw wastewater	UASB effluent (wastewater treated in the anaerobic process)			Wastewater posttreated in membrane processes	
			20°C ± 2°C	35°C ± 2°C	45°C ± 2°C	UF	RO
RSG (%)	–	97	96	92	92	99	100
RRG (%)	–	68.09	20.21	19.15	17.02	95.7	97.8
GI (–)	–	66.04	19.4	17.62	15.65	94.7	97.8
The average amount of seeds germinated	10	9.7	9.6	9.2	9.2	9.9	10
The average length of the root (mm)	4.7	3.2	0.95	0.9	0.8	4.5	4.6

RSG, relative seed germination; RRG, relative root growth; GI, germination index.

on cucumber and lettuce seeds. They established correlations between physicochemical properties of wastewater and shooting seeds used as bioindicators. The wastewater treatment in biological system efficiently reduced concentration of certain physicochemical parameters to the level recommended by Brazilian legal regulations. One exception was phosphorus and nitrogen compounds. Phytotoxicity of the treated wastewater was lower compared with raw wastewater and GI for seeds of cucumber and lettuce was lower than 80%.

In the case of wastewater treated by means of UF and RO, mean number of shooting seeds was 9.9 and 10, respectively, whereas mean root length was 4.5 mm (UF) and 4.6 mm (RO). Table 5 presents the results of the *Lepidium* test for wastewater treated using the UASB-UF-RO system.

4. Conclusion

- It is most advantageous to carry out the methane fermentation process under mesophilic conditions. Rates of removal of COD, TOC and BOD were 77% (concentration in UASB effluent- 393 mg/dm^3), 76% (concentration in UASB effluent - 194 mg/dm^3) and 69% (concentration in UASB effluent - 337 mg/dm^3), respectively. It was also found that the biogas produced in these temperature conditions was characterized by the largest methane content (77%).
- During reverse osmosis, the rates of removal of COD, TOC, BOD and total nitrogen were 67% (concentration in RO permeate- 43 mg/dm^3), 71% (concentration in RO permeate- 15 mg/dm^3), 78% (concentration in RO permeate- 21 mg/dm^3) and 34% (concentration in RO permeate- 61 mg/dm^3), respectively. It is planned in the future to extend the technological system with final ammonia stripping due to over six extension of the permissible level.
- It was found that wastewater from the meat processing plants treated through fermentation conducted in psychrophilic conditions pointed to the highest effect on inhibition of the algae growth rate (almost four times) in comparison with blank sample. In the case of biologically treated wastewater, the least toxic effect on algae growth was observed for wastewater treated at temperature of 35°C ± 2°C (inhibition level –91%). In the case of wastewater after the UF and RO process, the value of TU was below 0.4.

Acknowledgements

The study has been funded by the Polish Ministry of Sciences and Higher Education with statutory Project No. BS/PB–401–301/11 and Grant No. N523 N 56038.

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