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TECHNOLOGY FOR TREATMENT OF LIPID-RICH WASTEWATER AND PIPELINES CLOGGED BY LIPIDS USING BACTERIAL PREPARATION

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Abstract. The complex, effective and innovative cleaning technology for lipid-rich wastewater and pipelines contaminated by lipids, was developed. For this purpose, laboratory experiments were performed to verify the efficiency of bacterial preparation (*Enterobacter aerogenes* E13, *Arthrobacter sp.* N3 and *Bacillus coagulans* S1) to degrade the grease in water and in drainpipes. The results showed that selected microorganisms intensively degrade grease to light odourless precipitate, water and CO_2, thus could be applied in industry. For optimization of technological cleaning processes, the response surface methodology was used. The optimal parameters for biological model wastewater treatment were determined: concentration of grease – 4.5–6.0 g/*l*, amount of bacterial preparation – 5.5–6.0%, pH – 8–9. Due to optimization, the grease degradation rate increased by 20–30%. The optimization of drainpipe cleaning technology was achieved in two stages. During the first stage, the experiments were performed in laboratory flasks; during the second stage, optimized cleaning process was tested in a pilot plant. The following optimal parameters were set: pH – 8, amount of bacterial preparation – 1.25 *l*/m^2 and harness of water – 0.0 mmol/*l*. In water of medium hardness, the rate of biodegradation process is 15–20% less. A satisfactory efficiency of grease biodegradation was achieved in the pilot plant: the 86.7% of grease were digested in 21 days. Besides, living microorganisms were detected inside the clean drainpipe.

Keywords: lipids, grease, wastewater, bacterial preparation, biodegradation, mathematical model, optimization.

1. Introduction

Lipids (characterized as oils, greases, fats and fatty acids) are one of the most important components of natural foods and many synthetic compounds and emulsions. Further, lipids constitute one of the major types of organic matter found in municipal wastewater (Quemeneur, Marty 1994). The amount of lipid-rich wastewater increases every year due to urbanization and development of factories. Grease can originate from a wide variety of commercial establishments, such as slaughterhouses, sausage and meet product factories, restaurants, and fish processing plants, industrial undertakings in which oils and fats are processed and barrel-washing plants (Lorenz *et al.* 2004; Pollution prevention… 2007).

Suspended lipids can be readily removed from wastewater by physical methods. Nevertheless, chemically and/or physically stabilized lipid/water emulsions should be managed in an appropriate manner. This is necessary because lipids that pass through physic-chemical treatment processes contribute to the levels of biological oxygen demand (BOD) and chemical oxygen demand (COD) in the effluents (Keenan, Sabelnikov 2000; Chang *et al.* 2001; Chipasa, Mędrzycka 2006).

Many manufacturing, food processing and industrial facilities dispose of liquid waste into sewer lines. Liquid waste often contains fats, oils and grease and other organic contaminants which, over time, lead to clogs in pipes. Pipes should be cleaned with caustic drain cleaners, routed mechanically, or replaced completely (Dale, Hill 1999a; Pollution prevention… 2007). High concentrations of grease in wastewater can lead to grease build-up on rocks in the drain-field, which eventually forms a seal over the rocks preventing water flowing into the drain-field. Thus leading, the drain-field should be removed and replaced with new materials (Dale, Hill 1999a).

Thus, biological treatment processes are commonly used to remove emulsified lipids from wastewater and drain pipes. All biological methods could be grouped in two major classes: aerobic and anaerobic processes. During anaerobic treatment, fats are hydrolyzed to glycerol and long-chain fatty acids (LCFA) followed by subsequent β-oxidation (Batstone *et al.* 2000; Ivanov *et al.* 2002; Cirne *et al.* 2007; Sousa *et al.* 2007). Fat hydrolysis is not the rate-limiting step of treatment; however, millimolar concentrations of long-chain fatty acids are capable of inhibiting the growth of numerous microorganisms. Consequently, the occurrence of LCFA presents a serious problem for anaerobic cleanup systems (Rinzema *et al.* 1994; Ivanov *et al.* 2002). During aerobic treatment, grease is converting into harmless solids, CO_2 and H_2O (Ratledge 1992).

The efficiency of fat removal by physicochemical methods, in BOD units, can reach 90%; however, only

biological cleanup allows withdrawing the emulsified and colloid lipids that remain in water (Perle *et al.* 1995). The alternative of using specific enzymes (lipases) has recently gained potentially more attention because of stringent environmental regulations and clean and friendly application of enzymes (Gandhi 1997; Mendes, Castro 2005). Lipases (triacylglycerol acylhydrolases,) are enzymes that catalyze the hydrolysis of triacylglycerols to glycerol and free fatty acids at the water–lipid interface and the reverse reaction in non-aqueous media (Borgström, Brockman 1984; Mendes, Castro 2005). These enzymes showed potential applications for degrading of oil and fats in wastewater generated by dairy industries, slaughterhouses, edible oils, fat refineries and others (Mendes, Castro 2005).

A number of biological processes and compositions have been developed, which are directed at specific contaminants, for example: *Xanthomonas maltophilia* and *Bacillus thuringiensis* have been used to degrade polar organic solvents (Middleditch, Lee 1994); a combination of amylase, lipase and/or protease have been used to digest colloidal materials such as starch, grease, fat and protein (Tobey, Stapleton 2001); and a yeast fermentation combination has been described as effective in deodorizing sewage and ponds and in the degradation of organic waste (Dale, Hill 1999b). However, some combinations have been found to be unstable and yielded variable results from one batch to another. Other combinations described above are directed at only a specific contaminant and do not address the problems presented by waste containing high grease.

It is desirable to provide a non-toxic and non-polluting combination for emulsification and digestion of fats, oils and grease and other organic contaminants that clog up pipes.

JSC "Biocentras" suggests a very effective and innovative technology for cleaning of lipid-rich wastewater and pipelines, contaminated by lipids. Firstly, lipids from wastewater and drainpipe surfaces are removed mechanically, and remaining lipids are treated with a preparation of active microorganisms *Enterobacter aerogenes E13* (a stem degrading lipids), *Arthrobacter sp. (N3)* (the stem degrading aliphatic compounds) and *Bacillus coagulans (S1)* (the stem degrading complex peptide linkage). This combination of microorganisms could be used in grease traps and septic tanks to avoid repeated cleaning. Air pollution by compounds, emitting unpleasant odours is reduced by using microorganisms. Furthermore, microorganisms compose a live biofilm and protect drainpipes against clogging.

The last scientific experiments showed that our developed bacterial combination effectively digests grease in water, so could be widely used in treatment of wastewater and pipelines clogged by grease.

The aim of experiments was to create a technology for biological cleaning of wastewater and drainpipes, contaminated by grease; and to evaluate optimal parameters of technological cleaning processes.

2. Materials and methods

Cultivation of bacterial preparation of *Enterobacter aerogenes* E13, *Arthrobacter sp.* N3 and *Bacillus coagulans* S1 was carried out in flasks. The 750 ml Erlenmeyer flasks containing 70 ml of complex nutrient medium were incubated at 30 °C in a rotary shaker Innova 43 (New Brunswick Scientific Co., UK) at 200 rpm for 16 h. All 3 cultures of microorganisms were used in the same amount.

The mixture of pork fat, beef suet and sunflower oil (1:1:1) was chosen as a model substrate because of the analogous fatty acid composition to the fatty contaminants in wastewater.

The same mineral medium, prepared from water wherein salts (NH_4NO_3, KH_2PO_4) containing N, P, K elements were dissolved, was used in all experiments. The concentration of salts in mineral medium comprised 3% of fat concentration. The pH of mineral medium was controlled using 1M NaOH and 1M HCl.

All experiments were divided into two major groups, i.e. the creation of wastewater and pipelines, contaminated by grease, cleaning technology and the selection of optimal parameters of biodegradation. The performed experiments are presented in Fig. 1.

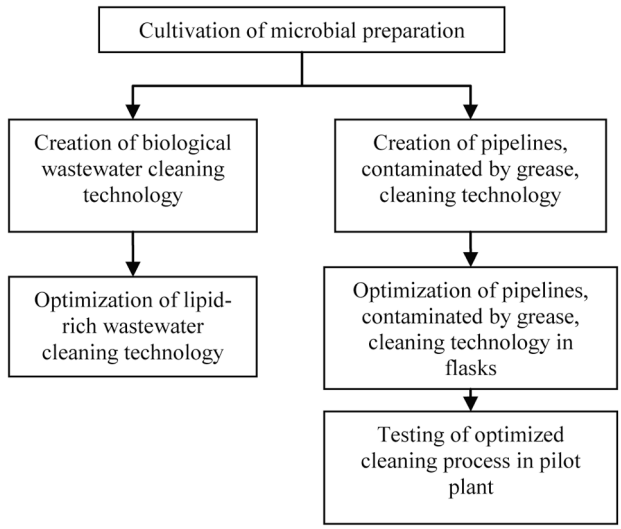

Fig. 1. Design of experiments

Firstly, the technology of biological wastewater treatment was created. For this purpose, some experiments were performed to verify the efficiency of bacterial preparation to degrade the grease in lipid-rich model wastewater. The 750 ml Erlenmeyer flasks containing 150 ml of mineral medium were sterilized at 121 °C for 20 min. The final cell concentration was $4 \cdot 10^8$ CFU/ml after the injection of bacterial preparation. The amount of added fatty substrate was ranged from 20 to 80 g/*l*. Erlenmeyer flasks containing experimental solution were incubated at 25 °C in a rotary shaker Innova 43 (New Brunswick Scientific Co., UK) at 100 rpm for 28 h.

To set optimal technological parameters of lipid-rich model wastewater cleaning, 4 series of factorial experiments with 16 parallel tests in each were done. Factorial

plans of experiments near to D-optimal experimental designs were used, invoking a methodology of reaction surface. The 750 ml Erlenmeyer flasks containing 200 ml of experimental solution were incubated at 30 °C in a rotary shaker Innova 43 (New Brunswick Scientific Co., UK) at 200 rpm for 48 and 72 h. Main technological parameters of cleaning process were: pH (6–9), concentration of bacterial combination (2–7%) and concentration of fats (1–6 g/*l*).

Some experiments were done to check a potentiality of bacterial preparation to degrade the lipids in drainpipe in the process of creation of cleaning technology for pipelines, contaminated by lipids. Fatty substrate (2.0 g) was overspread on the inner surface of a plastic pipe, the interior area of which was 45.22 cm^2. Fragments of pipes, contaminated by lipids, were placed into 750 ml Erlenmeyer flasks, containing the mineral medium. Final volume of experimental solution was 200 ml. The pH of medium was held at 6.5, 7.0 and 8.0. The amount of bacterial preparation was 0 (control), 2, 5 and 7 percent. The flasks were incubated at 24±1 °C in rotary shaker KS501 digital (Kika Labortechnik, Germany) for 1, 3, 7 and 14 days. Three cycles of shaking for 1 hour each at 150 rpm were done every day. The first cycle of shaking was performed using bacterial combination and tap water was used for second cycles.

Experiments were divided in two stages to set optimal technological parameters for cleaning of drainpipes, contaminated by grease. The first stage was performed in Erlenmeyer flasks and after that, optimized cleaning process was tested in a pilot plant.

Four series of factorial experiments with 16 parallel tests in each were done in first stage. Near to D-optimal factorial plans of experiments were implemented in 750 ml Erlenmeyer flasks, placed in the rotary shaker KS501 digital (Kika Labortechnik, Germany). Fatty substrate (2.0 g) was overspread on internal surface of plastic pipe, the interior area of which was 45.22 cm^2. Fragments of pipes clogged by lipids, were dipped in flasks, containing washing solution. Washing solutions were prepared using deionized water, and different hardness of water was achieved by using corresponding amounts of $MgSO_4$ and $CaCl_2$. The temperature was maintained at 24 °C and main technological parameters, of cleaning process were changed: pH (6.5–10.5), concentration of bacterial preparation (2–7%) and the hardness of water (0–3 mmol/*l*). The term of washing process was 1 and 3 days.

The testing of cleaning process at optimal conditions was set in a pilot plant presented in Fig. 2. The pilot plant was made from drainpipes of 50 mm in diameter. The length of experimental sewerage was 2 meters. Fatty substrate was overspread on internal surface of elbow bends (Figs. 2. 3a, 3b) and the drainpipe (Figs. 2. 3c). Contaminated and not contaminated by grease sewerage fragments were weighed and the amount of lipids was then obtained by difference. Three cycles of pumping for 1 hour each at 450 ml/min (30 rpm) were done every day. The first cycle of washing was performed using bacterial preparation and tap water was used for second cycles. One cycle of pumping using a model wastewater, wherein

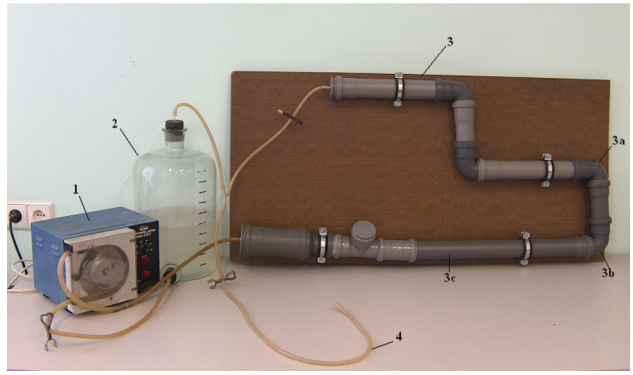

Fig. 2. A pilot plant for analysis of technological cleaning process of drainpipes clogged by grease: 1 – a peristaltic pump; 2 – a container of working solution; 3 – a pipeline (3a, 3b, 3c – elbow bends and a pipe clogged by grease); 4 – a tap for sample collection

the concentration of grease was 50 g/*l* was carried out after 7 days. The dosage of bacterial preparation was repeated after 14 days.

The amount of not-digested lipids was measured and the percentage of not-digested lipids was calculated at the end of experiments. The amount of lipids was measured by method described by Alef and Nannipieri (1995) with some modifications. Not-digested lipid fraction was extracted with a mixture of chloroform and methanol. For purification of the extracted lipids, 10 ml $CHCl_3$ was added to the monophasic solvent system and shaking was continued for 15 min. Then 30 ml of 0.1 M aqueous KCl was added and shaking was continued for 15 min. The mixture was then filtered under suction through glass microfibre filters on a Büchner funnel. The filtrate was transferred to a separation funnel and allowed to separate (15 min) into a methanolic aqueous phase and chloroform phase. The chloroform phase, which contains lipids, was evaporated to the dryness and weighed. Also the weight of the evaporating flask was determined. The amount of not-digested lipids was then obtained by difference. The amount of digested lipids was given as a percentage of initial amounts of lipids in the model wastewater.

The amount of not digested lipids in pilot plant was determined gravimetrically. The air-dried elbow bends and drainpipe were weighed and the amount of not-digested lipids was then obtained by difference.

The cell concentration, defined as colony forming units per milliliter (*CFU/ml*) in liquid samples and per gram (*CFU/g*) in samples of grease, was determined by plating diluted samples of lipids on nutrient agar (Oxoid) plates and incubating at 30 °C for 24 h (Levišauskas *et al.* 2004).

Process optimization technique

For optimization of cleaning processes used for technological wastewater and drainpipes contaminated by grease, the response surface methodology was used (Theodore, Panda 1995; Mayers, Montgomery 2002; Levišauskas *et al.* 2004), which includes design of factorial experiments, development of models for the response

surface estimation and model-based search of optimum point.

Statistical models are developed using data of factorial experiments that were carried out according to close to D-optimal experimental designs B_n (Hartmann *et al.* 1974; Montgomery 2001). Statistical test for model adequacy proved that the second order polynomial models were suitable for prediction the desired responses:

$$Y = a_0 + \sum_{i=1}^{n} a_i x_i + \sum_{i=1}^{n} a_{ii} x_i^2 + \sum_{i=1}^{n} \sum_{j=i+1}^{n} a_{ij} x_i x_j , \quad (1)$$

where Y is predicted response (grease degradation/removal efficiency), x_i – independent variables (technological parameters subjected to optimization), $a_\bullet$ – the model parameters, n – a number of independent variables (Montgomery 2001).

The model (1) parameters are identified using the least squares method (Montgomery 2001).

Using the identified model (1) the point $\mathbf{x}^* = \left[x_1^* \quad \dots \quad x_n^* \right]^{\mathrm{T}}$ is calculated, at which the predicted response takes maximum value.

If the maximum point lies on the boundary of experimental design area, a normalized gradient vector at this point is calculated:

$$grad_n Y\left(\mathbf{x}^*\right) = \frac{\nabla Y\left(\mathbf{x}\right)}{\left\| \nabla Y\left(\mathbf{x}\right) \right\|_{\mathbf{x}=\mathbf{x}^*}}, \quad (2)$$

$$\nabla Y\left(\mathbf{x}\right) = \left[\frac{\partial Y\left(\mathbf{x}\right)}{\partial x_1} \quad \dots \quad \frac{\partial Y\left(\mathbf{x}\right)}{\partial x_n} \right]^T .$$

The gradient vector (2) determines the search direction of the optimum point outside the experimental design area. Along the calculated direction, the expected location of optimum point is predicted and the new cycle of factorial experiment and response surface estimation around the predicted point is performed (Mayers, Montgomery 2002; Levišauskas *et al.* 2004).

If the calculated maximum point lies inside the experimental design area, this point is an optimum point and the test experiment is carried out at that point.

Calculations related to the statistical model identification and the response surface analyses are performed using Matlab/Simulink tools.

3. Results and discussion

3.1. The creation of lipid-rich wastewater cleaning technology and the optimization of main technological parameters

Oxidative biological processes in aqueous environments are limited by the low solubility of oxygen in water. However, lipids have detrimental effects on oxygen transfer. They reduce the oxygen transfer rates to biofilms, thereby depriving the microorganisms of oxygen. This effect results in reduced microbial activity (Chipasa, Mędrzycka 2006). The presence of lipids in wastewater is related to occurrences of troublesome foam also. According to the JSC "Biocentras" – producer of biological treatment technology – the combination of microorganisms is placed in bioreactor filled with lipid-rich wastewater. The discontinuous aeration is used to reduce foaming problems.

The preparation of microorganisms degraded model substrate more effectively when the initial concentration was 2–4% of amount of water (to 0.77% and 2.4%, respectively). The process of biodegradation was marginal when initial concentration of substrate was 6–8%. The biodegradation of model substrate was faster and deeper using the selected composition of microorganisms (*Enterobacter aerogenes E13, Arthrobacter sp. N3 and Bacillus coagulans S1*). Initial concentrations of substrate were markedly reduced (from 2–6% to 0.11–0.3%, respectively) in 2–3 weeks. The initial (8%) concentration of substrate was decreased to 1.3% after 4 weeks. Obtained results showed great efficiency of the selected bacterial combination in the degradation of grease in model wastewater. The bacterial preparation can be applied in a wide variety of fields: septic tanks, grease traps, drain fields, garbage disposals and farms.

To apply this technology in industry, it is necessary to set optimal technological parameters, such as pH, initial concentration of grease and initial amount of bacterial preparation.

Experimental data of degraded grease at final series of factorial experiments (mean values of 2 repeated experiments) and the statistical model predictions are given in Table 1. The adequacy of the model (1) was tested with the F (Fisher) statistic (Myers, Montgomery 2002). Since the computed value of the F-statistic $F = 2.62$ does not exceed the critical value $F_{\nu 1,\, \nu 2,\, \alpha} = F_{5,\, 1,\, 0.05} = 2.90$, we admit that the identified model (1) for prediction of the percentage of degraded grease is adequate.

The following optimal values of technological parameters using the identified statistical model (1) are

Table 1. Experimental design, results and model predictions of degraded grease (concentration of grease – x_1; Amount of bacterial preparation – x_2; pH – x_3)

Exp No	Experimental conditions			Experimental results, %	Model predictions, %
	x_1, g/*l*	x_2, %	x_3		
1	6.0	7.0	9.0	31.0	28.7
2	2.0	7.0	9.0	16.2	17.3
3	6.0	2.0	9.0	19.1	17.5
4	2.0	2.0	9.0	0.01	0.0
5	6.0	7.0	6.0	11.4	12.0
6	2.0	7.0	6.0	23.3	25.1
7	6.0	2.0	6.0	4.77	3.93
8	2.0	2.0	6.0	8.15	10.7
9	6.0	4.5	7.5	20.5	24.6
10	2.0	4.5	7.5	27.5	22.3
11	4.0	7.0	7.5	30.1	28.8
12	4.0	2.0	7.5	15.8	16.0
13	4.0	4.5	9.0	21.8	24.8
14	4.0	4.5	6.0	26.0	21.9
15	4.0	4.5	7.5	25.2	27.4

calculated: concentration of grease – 5.0 g/*l*, amount of bacterial preparation – 6.0%, pH – 8.5.

The results of cleaning experiments performed at the optimal conditions showed, that about 30% of grease has been degraded in 2 days and about 60% – in 3 days.

Amounts of degraded grease totalled 25% and 45%, respectively at the initial technological conditions (centre point of experimental design plan, where the optimization procedure started; concentration of grease – 3.5 g/*l*, amount of bacterial preparation – 4.5%, pH – 7.

The degradation (splitting) rate of grease increased by 20–30% due to optimization.

The response surface (amount of degraded grease depending on the technological parameters: grease concentration, amount of bacterial preparation and pH) predicted by the statistical model (1) in the vicinity of the calculated optimum point is presented by the isoresponse contour plots in Fig. 3.

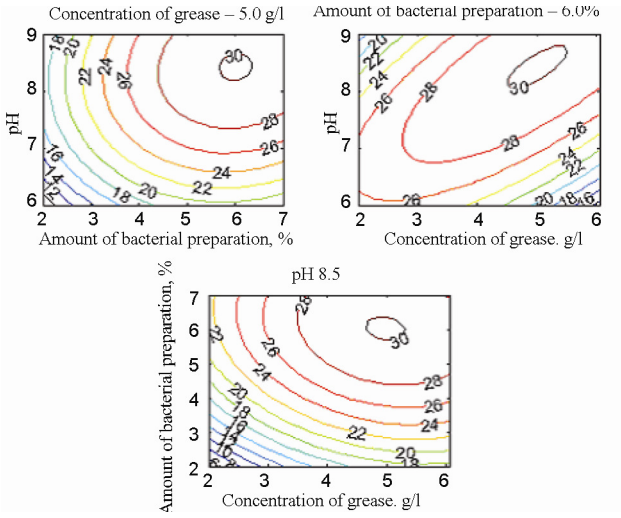

Fig. 3. Response surface of percentage of grease degraded in two days, depending on initial values of technological parameters in the vicinity of maximum degradation rate point

3.2. The creation of cleaning technology for sewage pipelines contaminated by grease, and the optimization of main technological parameters

The results of experiments are showed in Table 2. The degradation and washing of grease from fragments of drainpipes was set in all studied examples. It was determined, that the degradation of grease accelerates by increasing of the amount of bacterial preparation and pH. The maximum biodegradation of grease (68%) by using 2% (0.5 *l*/m^2) of bacterial preparation was obtained after 14 days at pH 8.0. Whereas by using 5% (1.1 *l*/m^2) and 7% (1.5 *l*/m^2) of bacterial preparation, respectively 78.5 and 92.0% of grease has been degraded after 14 days at the same conditions.

The effect of pH on efficiency of grease biodegradation was estimated using 2 and 5% of bacterial preparation.

On average, the efficiency of degradation of grease decreases by 8%, by decreasing pH from 8.0 to 7.0, and 7%, by decreasing pH from 7.0 to 6.5. The effect of medium pH on biodegradation process is lower using higher amounts of bacterial preparation (7%).

After injection of the bacterial preparation in drainpipe with a grease clog, microorganisms start actively pullulate in 12–24 hours, effectively converting grease into eco-friendly products of microbial metabolism.

The concentration of microorganisms in grease after 1 day was not high (10^7 CFU/g) due to an adaptation period of microorganisms (Table 2). The cell concentration was higher (10^9 CFU/g) after 3 days, but the efficiency of grease biodegradation was low. Microorganisms actively pullulated and used small amounts of grease as a source of energy needed for growth at this period. The quantity of bacterium after 7 days increased fractionally but fatty substrate was degraded more effectively due to the fact, that biomass reached the peak of growth. The cell concentration in fatty substrate was decreased to 10^8 CFU/g after 14 days, because microorganisms might degenerate under the influence of environmental factors or might be washed

Table 2. Biodegradation of fatty substrate in drainpipe fragments

No	Conditions of experiment		Results of experiments after 1 day		Results of experiments after 3 days		Results of experiments after 7 days		Results of experiments after 14 days	
	Amount of biomass %	pH	Cell concentration, CFU/g	Digested lipids, %	Cell concentration, CFU/g	Digested lipids, %	Cell concentration, CFU/g	Digested lipids, %	Cell concentration, CFU/g	Digested lipids, %
1	0	6.5	$<10^2$	14.0	$<10^2$	14.0	$<10^2$	14.0	$<10^2$	15.0
2		7.0	$<10^2$	15.0	$<10^2$	15.0	$<10^2$	15.0	$<10^2$	15.5
3		8.0	$<10^2$	16.5	$<10^2$	16.5	$<10^2$	16.5	$<10^2$	16.0
4	2	6.5	$3.7\cdot10^7$	20.5	$1.5\cdot10^9$	21.5	$2.1\cdot10^9$	36.5	$4.2\cdot10^8$	56.5
5		7.0	$2.5\cdot10^7$	22.5	$2.0\cdot10^9$	25.0	$2.5\cdot10^9$	42.5	$3.8\cdot10^8$	62.5
6		8.0	$5.6\cdot10^7$	22.0	$2.9\cdot10^9$	26.0	$3.3\cdot10^9$	47.0	$5.1\cdot10^8$	68.0
7	5	6.5	$5.2\cdot10^7$	20.5	$3.1\cdot10^9$	25.5	$3.4\cdot10^9$	41.0	$4.5\cdot10^8$	66.5
8		7.0	$3.7\cdot10^8$	23.5	$2.7\cdot10^9$	24.0	$4.7\cdot10^9$	48.5	$4.9\cdot10^8$	73.0
9		8.0	$9.8\cdot10^7$	30.5	$9.0\cdot10^9$	35.0	$1.0\cdot10^{10}$	51.0	$1.5\cdot10^9$	78.5
10	7č	6.5	$4.4\cdot10^8$	25.0	$3.1\cdot10^9$	29.0	$3.7\cdot10^9$	54.0	$6.8\cdot10^8$	84.0
11		7.0	$2.8\cdot10^8$	27.0	$1.6\cdot10^9$	29.5	$2.1\cdot10^9$	59.5	$6.2\cdot10^8$	89.5
12		8.0	$7.4\cdot10^8$	29.5	$1.2\cdot10^9$	37.0	$2.2\cdot10^9$	62.0	$6.0\cdot10^8$	92.0

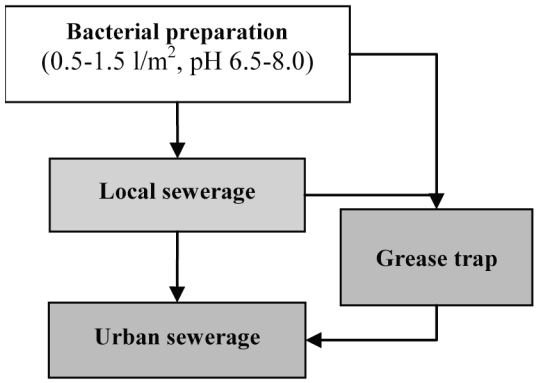

Fig. 4. The technological scheme of sewerage cleaning

out performing cycles of shaking with tap water. Due to decreasing of quantity of remaining live microorganisms and the efficiency of biodegradation of grease, the dosage of bacterial preparation was repeated after 14 days.

A technological scheme of cleaning of sewage pipelines is represented in Fig. 4. The bacterial preparation is poured into local sewerage or grease trap (of a manufactory or private house). The introduction of selected bacterial combination then takes place preferably as close as possible to the grease enter point in the drainage system, and at latest at the point where a significant deposition of grease from the wastewater is to be expected, this being the grease trap if fitted or the point at which a grease trap would most sensibly be positioned. Microorganisms compose a live biofilm on walls of drainpipes, which protects from formation of new grease clogs.

The bacterial preparation should be poured into sewerage when the load of sewage system is minimal (overnight or for a weekend). It is not recommended to use disinfectants or chemical detergents in 24 hours after the introduction of selected combination, because they eliminate the activity of microorganisms. The results of accomplished experiments showed, that the quantity of microorganisms was decreased from 10^8 CFU/g to 10^5 CFU/g, when the drainpipes were washed with tap water immediately after introduction of bacterial preparation, and only to 10^7 CFU/g, when the drainpipes were washed after one day. Also, an additional experiment using 1% solution of dish abstergent "Fairy" after 5 hours and 24 hours post introduction of bacterial combination was performed Using abstergent after 5 hours, the quantity of microorganisms decreased from 10^8 CFU/g to 10^5 CFU/g, while after 24 hours the substantial effect of cell concentration (10^7 CFU/g) was not detected.

Therefore, it is necessary to optimize main technological parameters, such as initial pH and the initial amount of bacterial combination estimating both economical effect and efficiency of grease biodegradation for the successful application of developed technology.

Experimental data of percentage of degraded grease after 3 days (mean values of 2 repeated factorial experiments) and the statistical model predictions are given in Table 3. The computed value of the F-statistic $F = 2.53$ is less than critical value $F_{v1,\, v2,\, \alpha} = F_{5,\, 15,\, 0.05} = 2.90$;

Table 3. Experimental design, results and model predictions of degraded grease (pH – x_1; amount of bacterial preparation – x_2; hardness of water – x_3)

Exp No	Experimental conditions			Experimental results, %	Model predictions, %
	x_1	x_2, %	x_3, mmol/*l*		
1	9.0	7.0	2.0	22.1	22.9
2	7.5	7.0	2.0	19.3	19.6
3	9.0	4.0	2.0	20.9	19.8
4	7.5	4.0	2.0	14.8	16.2
5	9.0	7.0	0.0	25.0	23.4
6	7.5	7.0	0.0	14.3	15.2
7	9.0	4.0	0.0	22.4	21.9
8	7.5	4.0	0.0	14.4	13.3
9	9.0	5.5	1.0	20.6	23.0
10	7.5	5.5	1.0	18.4	17.1
11	8.25	7.0	1.0	14.6	14.3
12	8.25	4.0	1.0	10.4	11.8
13	8.25	5.5	2.0	24.0	22.7
14	8.25	5.5	0.0	19.4	21.6
15	8.25	5.5	1.0	20.0	18.1

therefore, the established model (1) for prediction of the percentage of degraded grease is satisfactory.

The calculated values of technological parameters, at which the maximum percentage of degraded grease is gained, are as follows: hardness of water – 0.0 mmol/*l*, amount of bacterial preparation – 5.5%, pH – 9. At the optimal technological conditions, about 24% of grease has been degraded in 1 day and about 40% in 3 days.

The response surface (amount of degraded grease depending on technological parameters: hardness of water, amount of bacterial preparation and pH) predicted by the statistical model in the vicinity of the calculated maximum degradation rate point is presented by the isoresponse contour plots in Fig. 5.

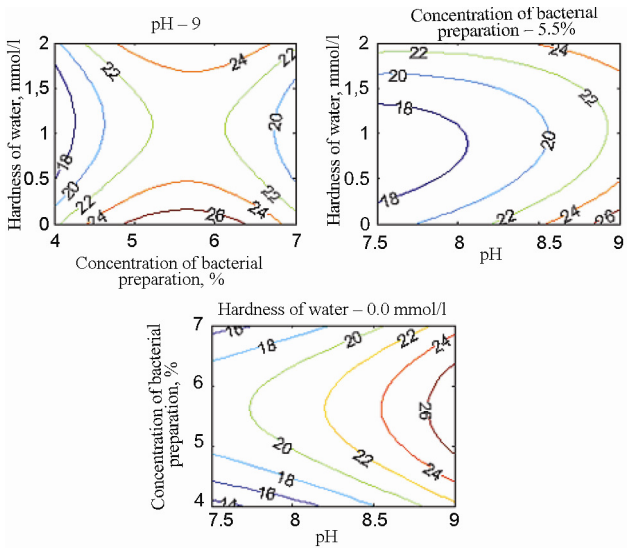

Fig. 5. Response surface of the percentage grease degraded in 3 days depending on technological parameters in the vicinity of maximum degradation rate point

A shape of the response surface shows the maximum point laying on the boundary of experimental design area and calculated normalized gradient vector at this point determines the search direction of the optimum point outside the experimental design area, i.e. to increase pH. However, the increasing of pH isn't advisable, because microorganisms don't grow and don't degrade grease in high rates of pH and, if pH of medium will change, the grease can adhere to the wall of drainpipes and clog them. Given a high concentration of alkali, fatty acids react and form salts (soaps), which dissolve in water, but in default of alkali the hydrolysis of soap intensifies and the possibility of forming acidic soaps, increases (Kuleasan, Tekin 2008). Acidic soaps compose complex structures that attract grease and over outwash, and form heavy layers inside drainpipes thus narrowing their radius. Furthermore, high concentrations of alkali cause environmental problems and cause the corrosion inside drainpipes. The cell concentration of microorganisms signally decreases, given high rates of pH. For example, the quantity of microorganisms at selected optimal conditions was 10^{10} CFU/g after 3 days, but at pH 10.5 – $<10^6$ CFU/g. Consequently, the optimal pH was set 9 on the grounds of cell concentration and the efficiency of grease biodegradation.

Due to the use of tap water, the total hardness of which reaches 3.5–4.0 mmol/*l*, for realization of biodegradation process, the grease degradation rate decreased by 15–20% compared to rate at optimal conditions. It is advisable to equip water softening systems in factories.

The results of experiments, performed in a pilot plant at optimal conditions, are presented in Table 4. The good biodegradation of grease was set. The 86.7% of grease was digested after 21 days.

Table 4. Efficiency of grease biodegradation in pilot plant under optimal conditions

Time of biodegradation, days	Cell concentration, CFU·g^{-1}	Amount of grease, g	Digested lipids, %
0	$1.2 \cdot 10^{10}$	50.16	-
1	$2.4 \cdot 10^{8}$	43.54	13.2
3	$1.5 \cdot 10^{9}$	36.29	27.7
7	$5.8 \cdot 10^{8}$	29.63	40.9
14	$3.6 \cdot 10^{6}$	16.33	67.4
21	$6.0 \cdot 10^{8}$	6.67	86.7

Inside the clean drainpipe, the live microorganisms were detected. Microorganisms compose a live biofilm and protect from the formation of new clogs of grease inside the sewerage. The model wastewater with 50 g/l of fatty substrate was passed through the pilot plant after 7 days. It was noticed that the grease didn't adhere to the walls of drainpipes.

The cell concentration significantly decreased after 14 days; therefore the dosage of bacterial preparation was repeated.

4. Conclusions

1. The application of selected bacterium *Enterobacter aerogenes E13, Arthrobacter sp. N3 and Bacillus coagulans S1* in treatment of lipid-rich wastewater is promising process.

2. The optimal parameters for biological wastewater treatment were set: concentration of grease – 4.5–6.0 g/*l*, amount of bacterial preparation – 5.5–6.0%, pH – 8–9. In optimal conditions, the grease degradation process runs 20–30% faster.

3. The selected microorganisms can be used to remove grease from pipelines. These microorganisms intensively degrade lipids in the pipe to light, odourless precipitate, which doesn't clog pipelines, water and CO_2. Also microorganisms form the live biofilm on the wall of a pipe, which blocks the formation of layers of contaminants inside the pipeline.

4. The optimal parameters for biological cleaning of clogged with grease pipelines were set: pH – 8, amount of bacterial preparation – 1.25 *l*/m^2 and harness of water – 0.0 mmol/l. If water hardness is medium, the rate of biodegradation process is 15–20% less, thus it is recommended to install the water softening equipment.

5. The good biodegradation of grease was set in the pilot plant at optimal conditions. The 86.7% of grease was digested in 21 days.

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NUOTEKŲ IR VAMZDYNŲ, UŽTERŠTŲ RIEBALAIS, VALYMO, NAUDOJANT BAKTERINĮ PREPARATĄ, TECHNOLOGIJA

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Santrauka

Sukurta kompleksinė, efektyvi ir inovacinė nuotekų bei vamzdynų, užterštų riebalais, valymo technologija. Atlikti laboratoriniai eksperimentai, siekiant nustatyti biopreparato, sudaryto iš *Enterobacter aerogenes* E13, *Arthrobacter sp.* N3 ir *Bacillus coagulans* S1, riebalų skaidymo efektyvumą kiek vandenyje, tiek vamzdyje. Gauti rezultatai parodė, kad atrinkti mikroorganizmai intensyviai skaido riebalus iki lengvų, neturinčių nemalonaus kvapo nuosėdų, vandens ir CO_2, todėl gali būti taikomi pramonėje. Technologiniam valymo procesui optimizuoti taikyta reakcijos paviršiaus metodologija. Nustatyti optimalūs nuotekų, užterštų riebalais, valymo technologiniai parametrai: riebalų koncentracija – 4,5–6,0 g/*l*, biopreparato kiekis – 5,5–6,0 %, pH – 8–9. Optimizavus procesą, riebalų skaidymo greitis padidėja 20–30 %. Vamzdžių, užterštų riebalais, valymo technologijos optimizavimas atliktas dviem etapais. Pirmajame etape eksperimentiniai tyrimai atlikti kolbose, antrajame etape optimizuotas procesas testuotas valant riebalinius teršalus bandomajame įrenginyje. Nustatyti optimalūs valymo parametrai: pH – 9, biopreparato kiekis – 1,25 *l*/m^2 ir vandens kietis – 0,0 mmol/*l*. Esant vidutiniam vandens kiečiui biodagradacijos proceso greitis sumažėja 15–20 %. Geras riebalų skaidymo efektyvumas pasiektas bandomajame įrenginyje, po 21 paros suskaidyta 86,7 % riebalų. Taip pat ant švaraus vamzdžio sienelių rasta gyvų mikroorganizmų.

Reikšminiai žodžiai: lipidai, riebalai, nuotekos, biopreparatas, biodegradacija, matematinis modelis, optimizavimas.

ТЕХНОЛОГИЯ ОЧИСТКИ СТОЧНЫХ ВОД И ТРУБОПРОВОДОВ, ЗАГРЯЗНЁННЫХ ЛИПИДАМИ, С ИСПОЛЬЗОВАНИЕМ БАКТЕРИАЛЬНОГО ПРЕПАРАТА

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Р е з ю м е

Создана комплексная, эффективная и инновационная технология очистки сточных вод и трубопроводов, загрязнённых липидами. С целью проверки эффективности бактериального состава (*Enterobacter aerogenes* E13, *Arthrobacter sp.* N3 и *Bacillus coagulans* S1) для расщепления жиров в воде и водосточной трубе были выполнены лабораторные эксперименты. Полученные результаты показали, что отобранные микроорганизмы интенсивно расщепляют жир до лёгкого осадка без побочного запаха воды и CO_2 и могут быть применены в промышленности. Для оптимизации технологических процессов очистки использовался метод математического моделирования. Были определены оптимальные параметры для биологической обработки сточных вод: концентрация жира – 4,5–6,0 г/л, количество бактериального состава – 5,5–6,0%, pH фактор – 8–9. После оптимизации скорость деградации жира увеличилась на 20–30%. Оптимизация технологии по очистке водосточных труб была выполнена на двух стадиях. На первой стадии эксперименты были выполнены в лабораторных колбах. На второй стадии оптимизационный процесс очистки был проведен в пилотной установке. Были установлены оптимальные параметры: pH фактор – 8, количество бактериального состава – 1,25 л/м^2 и жёсткость воды – 0,0 ммол/л. В среднежёсткой воде скорость процесса биологического распада была на 15–20% меньше. Хорошая биодеградация жира была установлена в пилотной установке. 86,7% жира было расщеплено за 21 день. Также в чистой водосточной трубе были обнаружены живые микроорганизмы.

Ключевые слова: липиды, жиры, сточные воды, бактериальный состав, биодеградация, математическое моделирование, оптимизация.

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