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OPTIMIZATION OF COMPLEX TECHNOLOGY FOR GREASE WASTES UTILIZATION

KOMPLEKSĀS SMĒRVIELU ATKRITUMU UTILIZĀCIJAS TEHNOLOĢIJAS OPTIMIZĀCIJA

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Abstract. *Lipids constitute one of the major types of organic matter found in municipal wastewater. 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. The treatment of this problem is to clean pipes with caustic drain cleaners, mechanically rout the pipes or to replace the pipes completely. The second problem was the utilization of solid waste. JSC "Biocentras" suggest a very effective and innovative complex technology. Firstly, lipids from wastewater and from drain pipes surfaces are removing mechanically. Remained lipids and solid wastes were treated with a composition of active microorganisms. The novel composition is nonpoisonous, no corrosive, no caustic and ecologically advantageous. The invented technology ensures maximum results at the lowest cost in the shortest period of time.*

Keywords: *bacterial composition, biodegradation, greases, mathematical model, optimization*.

Introduction

Sustainable technologies promote the development of green techniques and products in order to live in clean and healthy environment. New effective waste recycling and recovery technologies are introduced. Our goal is to develop an effective and affordable complex-technology for recovery of fatty pollutants from meat, fish and oil processing enterprises. At the same time, it will reduce the quantity of biodegradable waste disposed in landfills.

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 [1]. The amount of lipid-rich wastewater increases every year due to urbanization and the development of factories. The incidence of grease in different businesses is quite considerable. These applies, for example, in slaughterhouses, sausage and meet product factories, restaurants, and fish processing plants, industrial undertakings in which oils and fats are processed and barrel-washing plants [2; 3]. 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 [4; 5; 6].

Many manufacturing, food processing and industrial facilities dispose of liquid waste into sewer lines. Liquid waste often contains grease and other organic contaminants which, over time, lead to clogs in pipes. The treatment of this problem is to clean pipes with caustic drain cleaners, mechanically rout the pipes or to replace the pipes completely. Even when grease-traps are included in a drainage system, the grease traps can form a permanent, solid grease layer over the top of the water which requires "pump-out" of the grease-trap [3; 7]. In other situations, liquid waste is disposed into septic tanks and drain-fields. High concentrations of FOG in the waste water can lead to grease build-up on rocks in the drain-field which eventually form a seal over the rocks preventing water flowing into the drain-field. The treatment of this problem requires out the drain-field and replacing it with new materials [7].

Industrial and household fatty waste can be divided into two categories – "yellow" and "brown" fat. The first category fatty waste can be used for the secondary processing of animal feed additives, production of soaps, oils, cosmetics and skin care products. This type of waste can be composted either. The second type of fat is collected in fat catchers or generated as waste in other industrial processes, collected from pipes by mechanical. This fat is not recycled and must be handled according to the existing waste management requirements. Biodegradable organic waste of this origin can be incinerated, decayed or composted [8].

Thus biological treatment processes are commonly used to remove emulsified lipids from waste water 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 [9; 10; 11]. 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 [10; 12]. During aerobic treatment grease are converting into harmless solids, CO_2 and H_2O. Moreover, LCFA are degraded by sequential removal of two-carbon atoms via the β-oxidation pathway, resulting in release of a fatty acid shorter by two carbons and acetyl-CoA, which is then subsequently oxidized to carbon dioxide by the tricarboxylic acid cycle [13].

JSC "Biocentras" suggest a very effective and innovative lipid-rich wastewater and pipelines clogged by lipids cleaning and composting of wastes rich in grease complex technology. Firstly, lipids from wastewater and from drain pipes surfaces are removing mechanically, and remaining lipids are treated with a culture of active microorganisms *Enterobacter aerogenes* E13 (a stem degrading lipids), *Arthrobacter sp.* N3 (the stem degrading aliphatic compounds) and *Bacillus coagulans* Š1 (the stem degrading complex peptide linkage). Item, this mixture of microorganisms could be using in grease traps and septic tanks, so it's able to avoid repeated cleaning of theirs. Air pollution by compounds, emitting unpleasant odors is reduced by using microorganisms. Furthermore, microorganisms compose a lively biofilm and protect the clogging of drainpipe.

Concentrated fatty waste obtained by mechanical removal, are composted in specially equipped places. These wastes are neutralized using active cultures of these microorganisms. Previous research has shown that this bacterial compositions created by JSC "Biocentras" breakdown effectively the fat in the water and soil and can therefore be applied to speed up composting. Developed technology is attractive for cleaner production/pollution prevention approach, as well as meets strict environmental and hygiene requirements.

The aim of experiments was to optimize complex grease wastes utilization complex technology, i.e. to set main optimal technological parameters of lipid-rich wastewater, drainpipes, contaminated by grease, cleaning and composting process.

Materials and methods

Cultivation of bacterial composition of *Enterobacter aerogenes* E13, *Arthrobacter sp.* N3 and *Bacillus coagulans* Š1 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.) at 200 rpm for 16 h. All 3 cultures of microorganisms were used in the same amount.

As model fatty substrate were set the most common by composition of fatty acids fats in wastes such as porky fat, beefy suet and sunflower oil in the same amount by weight. All experiments were divided into three major groups, i.e. the selection of optimal parameters of wastewater and pipelines biological treatments, and the determination of optimal parameters of composting process.

To set optimal biological wastewater treatment technological parameters of lipid-rich 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.) at 200 rpm for 48 and 72 h. Main technological parameters of cleaning process, were vary: pH (6-9), concentration of bacterial mixture (2-7 %) and concentration of fats (1-6 g/L).

For optimization of drainpipe, contaminated by grease, cleaning parameters four series of factorial experiments with 16 parallel tests in each were done. Near to D-optimal factorial plans of experiments were implemented in 750 mL Erlenmeyer flasks, placed in rotary shaker KS501 digital (Kika Labortechnik). Fatty substrate (2.0 g) was overspread on wall of plastic pipe, which interior area 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 got 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 composition (2-7 %) and the hardness of water (0-3 mmol/L). The term of washing process was 1 and 3 days.

In all experiments the same mineral medium was used, prepared from water wherein salts (NH_4NO_3 and KH_2PO_4), containing N, P, K elements, were dissolved in amount of 3 % of fat concentration. The pH of mineral medium was controlled by using 1 mol/l sodium hydroxide solution and 1 mol/l hydrochloric acid solution.

The fat content, humidity, pH and content of live microorganisms were assessed in compost mixtures during the composting optimization process. The composting process proceeded in 16 compost boxes with the natural ventilation, close to D-optimal (B3) factorial experimental design. The manipulated factors were: X_1 – percentage of fat mixture (5-20 %), X_2 – cell concentration of bacterial composition (10^7 – 10^9 *CFU*/g); X_3 – percentage of peat (5-15 %). For the measurement of the pH, the suspension of 1 mol/L potassium chloride solution in the ratio 1 : 2.5 (inoLab 720, Germany) was prepared [14]. Fats were biodegraded by bacterial composition. Reaction surface methodology based on factor experimental was used for optimization of technological parameters of fatty waste composting [15; 16].

In the end of experiments the amount of not-digested lipids was measured and the percentage of not-digested lipids was calculated. The amount of lipids was measured by method described Alef and Nannipieri [17] with some modification. The amount of digested lipids was given as a percentage of initial amounts of lipids in the wastewater, drainpipe or compost.

The cell concentration, defined as colonies forming units per gram (*CFU/*g), was determined by plating diluted samples of lipids on nutrient agar (Oxoid) plates and incubating at 30 °C for 24 h [18].

Process optimization technique

A new complex technology for grease wastes utilization was created (Fig.1.). During optimization procedure the optimal technological parameters of wastewater and drainpipe cleaning were determined and optimal composition of composting mixture was set. There were optimized technological processes of cleaning wastewater and drainpipes contaminated by grease parameters and composting process optimization was to determine the composition of composting mixture.

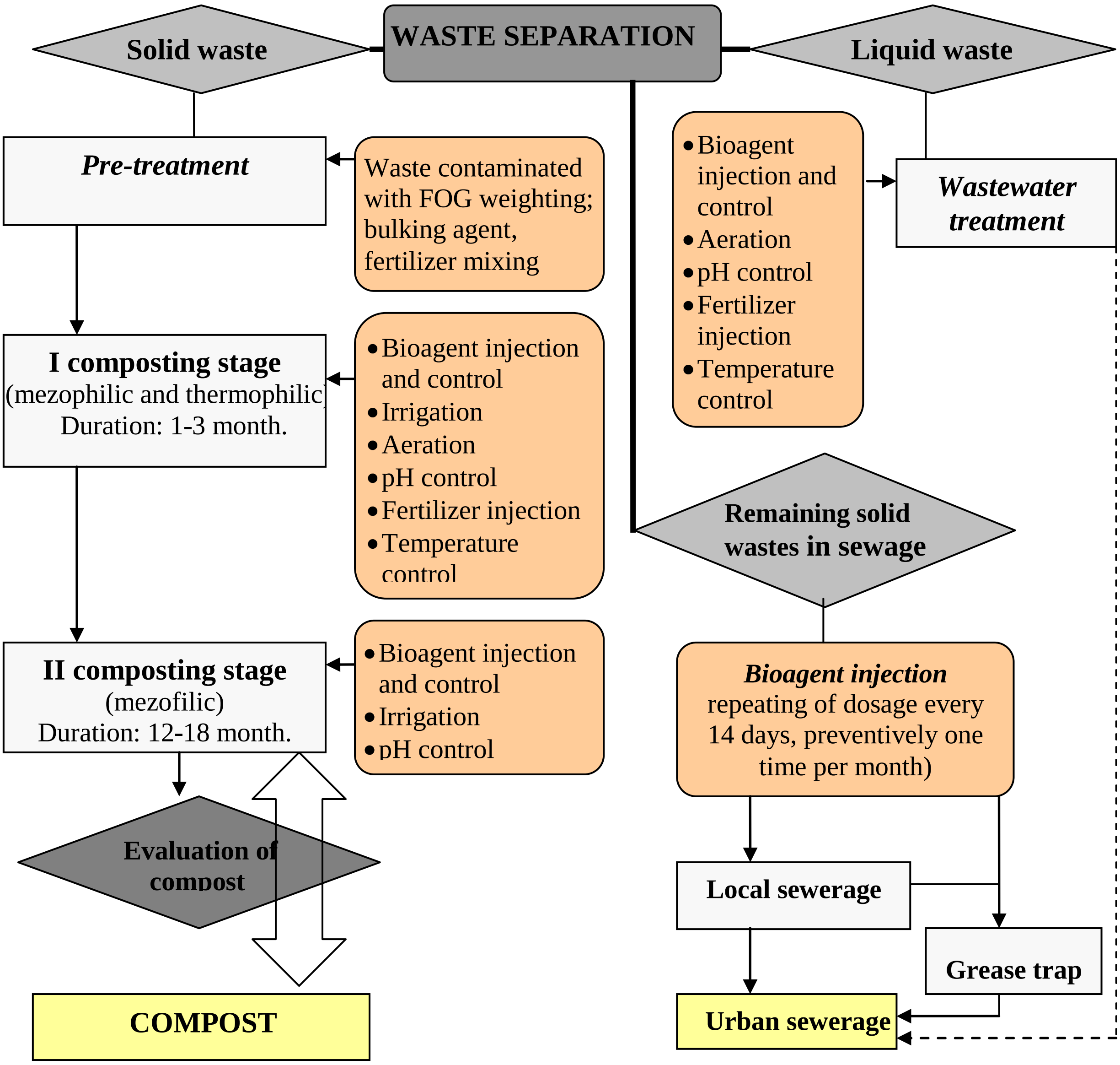

Fig.1. **Complex technological for grease wastes utilization diagram**

For all procedures optimization the response surface methodology was used [16, 18, 19], which includes design of factorial experiments, development of models for the response surface estimation and model-based search of optimum point.

Statistical models for approximation the desired response surfaces are developed using data of factorial experiments that were carried out according to close to D-optimal experimental designs B_n [15; 20]. Statistical test for model adequacy proved that the second order polynomial models were suitable for prediction the desired responses:

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

where Y is predicted response (grease degradation/removal efficiency), x_i are independent variables (technological parameters subjected to optimization), $a_{\bullet}$ are the model parameters, n is a number of independent variables [15].

The model (1) parameters are identified using the least squares method [15].

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:

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

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

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 [16; 18]. 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.

Results and Discussion

Optimization of main technological parameters of lipid-rich wastewater cleaning

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 rates at which oxygen is transferred to biofilms, thereby depriving the microorganisms of oxygen. This effect results in reduced microbial activity [6]. Also the presence of lipids in wastewater is related to occurrences of troublesome foam. JSC "Biocentras" suggest the modern biological treatment technology, according that the composition of microorganisms is placed in bioreactor filled of lipid-rich wastewater. To reduce the foaming problems the discontinuous aeration is used.

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

The response surface (amount of degraded grease depending on the technological parameters: grease concentration, amount of bacterial composition and pH) predicted by statistical model at the final stage of optimization procedure is presented by the isoresponse contour plots in Fig. 2.

The following optimal values of technological parameters are determined: concentration of grease – 4.5-6.0 g/L, amount of bacterial composition – 5.5-6.0 %, pH – 8-9. By realization the process at the optimal conditions, about 30 % of grease has been degraded in 2 days and about 60 % in 3 days. At the initial technological conditions: concentration of grease – 3.5 g/L, amount of bacterial composition – 4.5 %, pH – 7 (centre point of experimental design plan), from which the optimization procedure started amounts of degraded grease totaled 25 % and 45 %, respectively.

Due to optimization, the grease degradation (splitting) rate increased by 20-30 %.

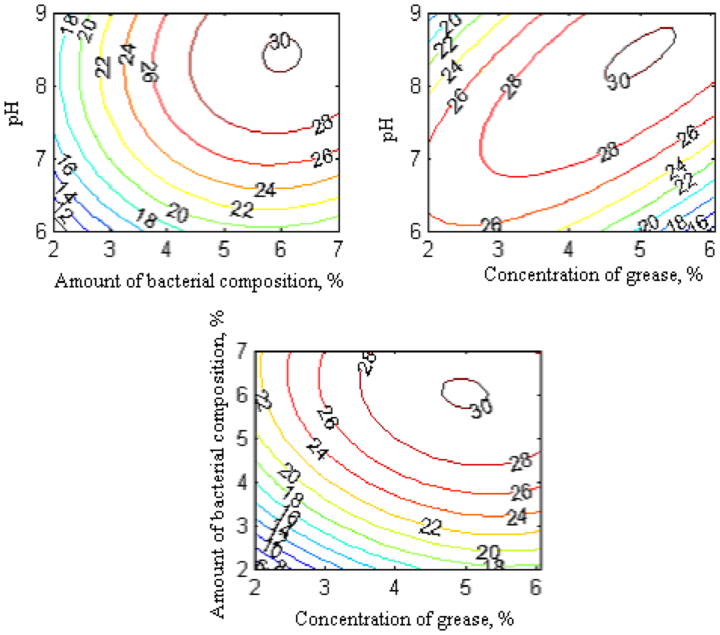

Fig.2. **Response surface of the amount of degraded grease depending on technological parameters**

Optimization of main technological parameters of sewage pipelines, contaminated by grease

To effectively use the developed technology in industry, it is necessary to optimize main technological parameters, such as pH and the amount of bacterial composition estimating both economically and in opinion of efficiency of grease biodegradation.

The response surface (amount of degraded grease depending on the technological parameters: hardness of water, amount of bacterial composition and pH) predicted by statistical model at the final stage of optimization procedure is presented by the isoresponse contour plots in Fig.3.

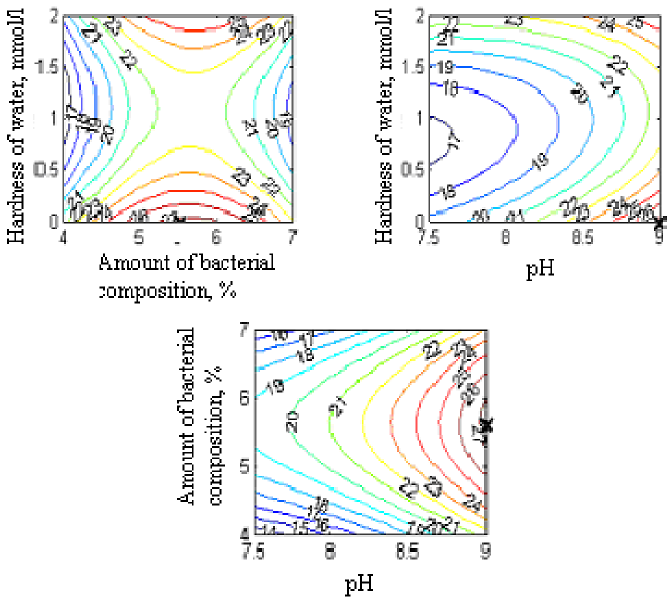

Fig.3. **Response surface of the amount of degraded grease depending on technological parameters**

The following optimal values of technological parameters were determined: hardness of water – 0.0 mmol/L, amount of bacterial composition – 5.5-6.0 %, pH – 9. At the optimal technological conditions, about 24 % of grease has been degraded in 1 day and about 40 % in 3 days.

Due to the use of tap water, whose total hardness 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. Using the tap water in compare with deionized water, the same efficiency of drainpipe cleaning is enriching over longer period of time.

Optimization of main technological parameters of composting process

A visible reduction in the quantity of microorganisms in composting boxes, where the initial concentration of the mixture of fat was 12.5 % and 20 % was observed after 2 weeks. Microorganisms require a long adaptation period because fat is heavily degradable substrate. After the adaptation period lipolytic activity arises and fat degradation as well. The maximum fall in pH was detected in the same compost boxes.

The mathematical model for approximation the response surface (dependence of the fat degradation rate on the composition of composting mixture) was developed using data of the factorial experiment. The second-order multiple polynomial model demonstrates an adequate approximation of the response surface over the investigated region of the factor variations:

$$Y = a_0 + a_1 x_1 + a_2 x_2 + a_3 x_3 + a_4 x_1^2 + a_5 x_2^2 + a_6 x_3^2 + a_7 x_1 x_2 + a_8 x_1 x_3 + a_9 x_2 x_3 \qquad (3)$$

where Y is predicted response (percentage of degraded fat), x_1 is percentage of fat mixture, %, x_2 is cell concentration of bacterial composition, *CFU*/g, x_3 is percentage of peat, %, a_i are the model parameters. The model parameters are identified using the least squares method and the statistical test for model adequacy [16] proved that the model (3) was suitable for prediction the fat degradation rate.

The calculated sectional views of the percentage of degraded fat after two weeks of composting in the vicinity of the predicted maximum percentage point are presented by isolines in Fig.4.

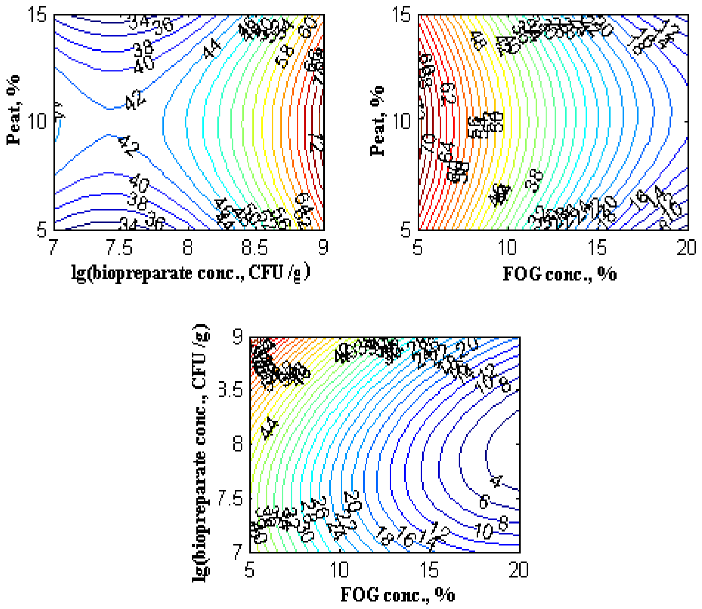

Fig.4. **Dependence of the degraded fat percentage (after 2 weeks) on the technological parameters: percentage of fat, inoculate cell concentration of bacterial composition and the percentage of peat in the vicinity of maximum degraded fat percentage point.**

The response surface based analysis has shown that the maximum rate of fat degradation (over 73 % within 2 weeks) was observed at the following technological conditions: the fat content – 5.0 %, cell concentration in bacterial compositions – 10^9 *CFU*/g, peat content – 9.5 %. The character of the response surface shows that the percentage degradation rate of fat increases with

decreasing the initial fat concentration: by decreasing the initial concentration from 20 % to 5 % the percentage degradation rate rose over 3 times.
However, in economic terms it is reasonable to treat the compost with higher initial percentage of fat, at which the higher degradation rate over a longer period of time can be achieved in terms of absolute units. At the initial fat concentration 20 % in 6 weeks about 70 % of total fat was degraded.

Conclusions

Developed complex greases wastes treatment technology was optimized. Firstly, lipids from wastewater and from drain pipes surfaces remove mechanically. Remaining lipids were treated with the composition of active microorganisms. Solid phase was composted using the composition grease oxidizing microorganisms.
The application of selected bacterium *Enterobacter aerogenes* E13, *Arthrobacter sp.* N3 and *Bacillus coagulans* Š1 in lipid-rich wastewater and in solid wastes composting treatment is very perspective process. The novel composition is nonpoisonous, no corrosive, no caustic and ecologically advantageous. By substantially reducing big amounts of grease which cause clogging of drainpipes, sewage pipelines was effective in deodorizing sewage/composting mixtures and eliminates clogging.
The optimal parameters for biological wastewater treatment were set: concentration of grease – 4.5-6.0 g/L, amount of bacterial composition – 5.5-6.0 %, pH – 8-9. In optimal conditions, the grease degradation process runs 20-30 % faster.
The optimal parameters for biological cleaning of pipelines clogged with grease were set: Ph – 8, amount of bacterial composition – 1.25 L/m^2 and harness of water – 0.0 mmol/L. At the medium hard water the rate of biodegradation process is 15-20 % less, it is recommended to equip the equipment for softening of water.
The optimal experimental composting mixture composition was determined: the initial fat content – 5 %, the concentration of bacterial composition's cells – 10^9 *CFU*/g, the quantity of structural materials – 9.5 %. Fat degradation rate slows down 3 times if the initial fat concentration increases from 5 % to 20 %.
The invented technology ensures maximum results at the lowest cost in the shortest period of time.

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References

1. Quemeneur M., Marty Y. Fatty acids and sterols in domestic wastewater. Water Research, Vol. 28 No. 5, 1994. p. 1217-1226.
2. Lorenz H.E., Lang A., Lüling M., Ophardt H. Clearing waste water pipes or grease traps clogged with grease with a grease solvent. United States Patent No. 6706518 B2. 2004.03.16., 36 p.
3. Pollution Prevention Assistance Division. Georgia Department of Natural Resources [online]. 2007. Vilnius: [cited 18 December 2008]. Available from internet: <www.p2ad.org/documents/ci_pubs_fog.html>.
4. Keenan D., Sabelnikov A. Biological augmentation eliminates grease and oil in baker wastewater. Water Environment Research, Vol. 72, No. 2, 2000. p. 141-146.
5. Chang I.S., Chung C.M., Han S.H. Treatment of oily wastewater by ultra filtration and ozone. Desalination, Vol. 133, No. 3, 2001. p. 139-144.
6. Chipasa K.B., Mędrzycka K. Behavior of lipids in biological wastewater treatment processes. Journal of Industrial Microbiology and Biotechnology, Vol. 33, No. 8, 2006. p. 635-645.
7. Dale P., Hill J.E. Composition for the treatment for municipal and industrial waste-water. United States Patent No. 5879928. 1999.03.09. 15 p.
8. Gea T., Artola A., Sánchez A. Co-composting sewage sludge and fats. Optimal ratios and process evolution. Sustainable Organic Waste Management for Enviromental Protection and Food Safety. Organic Waste Treatments: Safety Implications. 2004. p. 223-228.

9. Batstone D.J., Keller J., Newell R.B., Newland M. Modelling anaerobic degradation of complex wastewater. I: model development. Bioresource Technology, Vol. 75, No. 1, 2000. p. 67-74.
10. Ivanov V.N., Stabnikova E.V., Stabnikov V.P., Kim I.S., Zubair A. Effects of Iron Compounds on the treatment of fat-containing wastewaters. Applied Biochemistry and Microbiology, Vol. 38, No. 3, 2002. p. 255-258.
11. Sousa, D. Z., Pereira, M. A., Alves, J. I., Smidt, H., Stams, A. J. M., Alves M. M. Anaerobic microbial LCFA degradation in bioreactors, in Session PP3A – Bio-electrochemical Processes 11th IWA World Congress on Anaerobic Digestion 23-27 September 2007 Brisbane, Australia, 2007, 7 p.
12. Rinzema, A., Boone, M., van Knippenberg, K., Lettinga, G. Bacterial effect of long-chain fatty acids in anaerobic digestion. Water Environment Research, No. 66, 1994, p. 40-49.
13. Ratledge, C. Microbial oxidation of fatty alcohols and fatty acids. Journal of Chemical Technology & Biotechnology, No. 55, 1992, p. 399-400.
14. LST ISO 10390:2005. Soil quality – Determination of pH. 7 p.
15. Montgomery, D.C. Design and Analysis of Experiments. John Wiley&Sons, 2001.
16. Myers, R.H., Montgomery, D.C. Responce Surface Methodology. Process and Product Optimization Using Designed Experiments. John Wiley & Sons. 2002, 798 p.
17. Ed. Alef, K., Nannipieri, P. Methdods in Aplied Soil Microbiology and Biochemistry. London: Academic Press, Harcourt Brace& Company. 1995, 578 p.
18. Levišauskas, D., Tekorius, T., Čipinytė, V., Grigiškis, S. Experimental optimization of nutrient media for cultivation of Arthrobacter sp. bacteria. Latvian Journal of Chemistry, No. 1, 2004, p. 75-80.
19. Theodore, R., Panda, T. Application of response surface methodology to evaluate the influence of temperature and initial pH on the production of beta -1,3 glucanase and carboxymetyl cellulose from Trichoderma harzianum. Enzyme and Microbial Technology, Vol. 17, No. 12, 1995, p. 1043-1049.
20. Hartmann, K., Lezki, E., Schafer. W. Statistische Versuchsplanung und Auswertung in der Stoffwirtschaft [Design of Experiments and Evaluation in the Material Economy]. Leipzig: VEB Deutscher Verlag fur Grundstoffindustrie, 1974, 552 p.