CHARACTERIZATION OF PREPARATION FOR FAT SEPARATORS

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Abstract: Fats, oils and greases in waste water frequently cause serious environmental problems. A commercial preparation was tested for its ability to degrade oil and grease in wastewater. As substrates, two fats (lard and beef fat) and four oils (rape seed oil, sunflower oil, palm oil and olive oil) were chosen. The degradation ability of commercial preparation for different substrates was investigated by the determination of lipid degradation at pH 7, temperature 25 °C, aerobic condition and agitation at 160 rev/min for 14 days in Erlenmeyer flasks. Simultaneously, the lipolytic activity was spectrophotometrically determined at 420 nm. All tested substrates were degraded by the different rate to basic units (fatty acids). Rape seed oil and lard were decomposed by bacterial lipase from commercial preparation.

Key words: fats, oils, waste water, lipid degradation, preparation, lipolytic activity

1. Introduction

A wide variety of industries (e.g. the dairy industry and food processing) produce the effluents rich in fats, oils and greases (FOGs). High concentrations of fats, oils and greases in wastewater often initiate problems in wastewater treatment processes (BECKER *et al.*, 1999; Stoll and Gupta, 1997). FOGs often cause foul odours, the blockage of pipes and sewer lines. These problems are solved by the preliminary refining equipment, so-called grease traps. Grease traps may sometimes fail to retain dissolved and emulsified FOGs, allowing them to enter the water treatment system. An interesting strategy in present time is the use of lipase producing microorganisms in wastewater treatment system.

Lipases are hydrolytic enzymes that catalyze the cleavage of ester bonds in triglycerides, resulting in production of glycerol and free fatty acids. Microbial lipases are produced by yeasts, fungi and bacteria, as extracellular and intracellular enzymes. The extracellular lipases from bacteria are interesting for their abilities to degrade FOGs.

In the present study, the degradation ability of commercial microbial product to degrade FOGs under laboratory conditions was investigated. As substrates, four oils and two fats were used.

2. Materials and methods

The degradation ability of the commercial microbial products for grease separators was investigated. As substrates, four plant oils (palm oil, sunflower oil, olive oil and rape seed oil) and two animal fats (lard and beef fat) were used.

2.1 Commercial microbial product

This commercial product is the mixture of bacteria, enzymes and detergents (ILLKOVÁ *et al.*, 2008). On the basis of previous study, it was found out that the product in the grease traps contain mixture of spore gram-positive bacteria, obviously *Bacillus sp.* (GOJKOVIĆ, 2009).

2.2 Medium and growth conditions

Surface cultivation proceeded on spirit blue agar plates, to which tributyrin and Tween 20 were added as a lipase substrate in ratio 1:1. Incubation time was three days by 27 °C. This agar was used for the verification of the presence of spores of the lipolytic bacteria.

Medium for submerged cultivation contain per litre of distilled water 1.12 g K_2HPO_4; 0.48 g KH_2PO_4; 5 g NaCl; 0.1 g $MgSO_4$. $7H_2O$; 2 g $(NH_4)_2SO_4$ and 0.002 g EDTA. Medium was autoclaved at 121 °C for 20 min. Then the medium was supplemented with 1 ml plant oil and 1 g animal fats, as the natural substrate. Medium was inoculated by commercial microbial product and was incubated under aerobic conditions at 25 °C, agitated at 160 rev/min for 14 days in Erlenmeyer flasks in a shaker (EL-BESTAWY *et al.*, 2005).

2.3 Verification of the presence of spores of lipolytic bacteria

This verification was carried out on spirit blue agar plates. Commercial product was diluted to the concentration 10^{-3}, and then was filtered through bacteriological filter. Filter was placed on the agar plates (GOJKOVIĆ, 2009). A presence of spores of the lipolytic bacteria shows itself as a transparent halo around the colonies.

2.4 Growth of the biomass

Growth of the biomass on various fats and oils substrates was examined spectrophotometrically at 650 nm. Samples were taken away in definite time interval for four days and absorbencies were measured against blank (GOJKOVIĆ, 2009).

2.5 Enzyme assay

The lipolytic activity was determined by using 0.5 ml culture in 0.65 ml 0.05 M phosphate buffer (pH 7.2) a 0.1 ml 0.0025 M p-nitrophenyllaurate in ethanol. The hydrolytic reaction was carried out at 37 °C for 30 min, after which 0.25 ml 0.1 M Na_2CO_3 was added. The mixture was centrifuged and the activity determined at 420 nm. One unit of lipase activity is defined as the amount of enzyme which liberates 1 μg p-nitrophenol from p-nitrophenyllaurate, as a substrate in 30 min under assay condition (NAWANI *et al.*, 1998; SIGURGÍSLADÓTTIR *et. al.*, 1993).

Lipolytic activity was calculated on 1 mg of protein and expressed in the form of the specific activity.

2.6 Lipid degradation

From each Erlenmeyer flasks 20 ml culture medium was aseptically drawn and transferred to a separating funnel, where it was mixed with 20 ml of hexane. The mixture was agitated for 2 min and the upper layer was put into the clean and weighted beaker. The lower layer was re-extracted by a fresh 20 ml of hexane and the upper layer after the extraction was collected to the beaker again. The extract in the beaker was evaporated by heating at 100 °C. Then the dry extracted lipids were weighted and dissolved in 50 ml of alcohol in the presence of phenolphthalein indicator. The solution was titrated with 0, 1 M KOH until the developing of pink colour. The same procedure was repeated within 2, 6, 8, 10 and 14 days. The free fatty acids (%) in the sample, which indicates the lipid degradation and fatty acids utilization, was calculated according to this equation:

$$\%\ free\ fatty\ acids\ = \frac{V_{KOH}\ .c_{KOH}\ .\ M\ .\ 100}{1000\ .m}$$

Here, V_{KOH} is the volume of 0.1 M KOH at the end point, c_{KOH} actual concentration of the 0.1 M KOH, M is the molecular weight of the oleic acid and m is the weight of the dry extract (ILLKOVÁ *et al.*, 2008; EL-BESTAWY *et al.*, 1998).

3. Results and discussion

The ability of commercial product to degrade various lipid substrates, occurring in wastewater, was tested. Lipolytic activities, growth of the biomass, presence of spore and lipid degradation were investigated.

3.1 Presence of spore

For this test spirit blue agar was used. After three days of incubation it was found out that all bacteriological filters caught spore from commercial product. This effect was detected as a transparent halo around the colonies, Fig. 1.

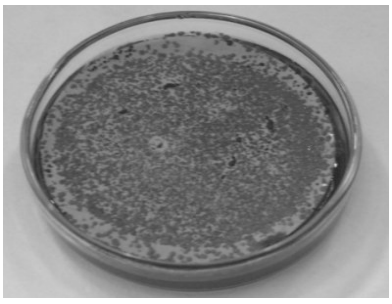

Fig. 1. Appearance of colonies at the bacteriological filter.

3.2 Growth of the biomass

Growth of the biomass was investigated by four plant oils (olive, palm and sunflower oil and rape seed oil) and two fats (lard and beef) during four days (Fig. 2,3).

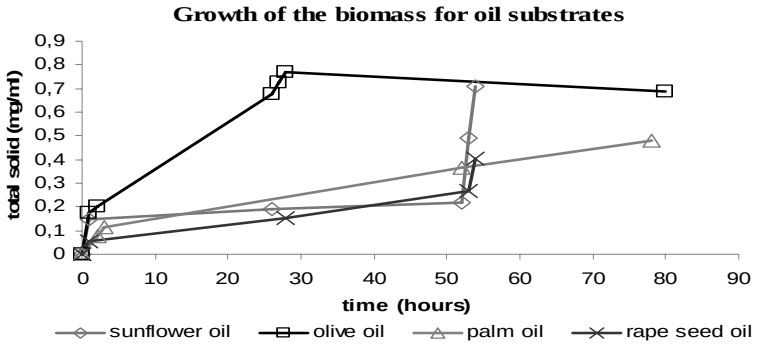

Fig. 2. Growth of the biomass for various plant oil substrates.

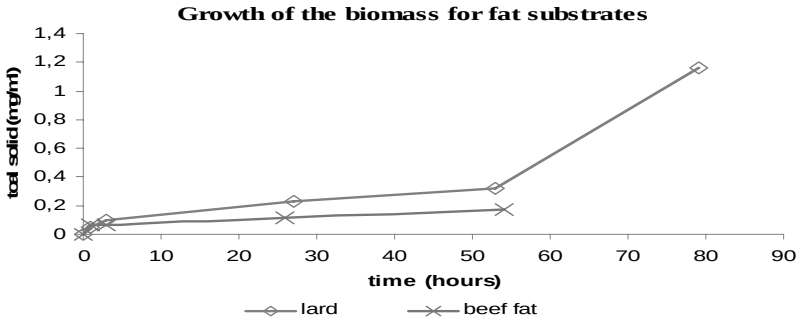

Fig. 3. Growth of the biomass for various animal fat substrates.

3.3 Lipolytic activity

As substrate for lipase 0, 0025 M p-nitrophenyllaurate in ethanol was used, activity was measured spectrophotometrically at 420 nm. Activity of lipases was determined as a dependence of absorbance on the time in the definite time interval at 37 °C. Results for plant oils were expressed at mg of protein at Table 1 and Fig. 4 and for animal fats at Table 2 and Fig. 5.

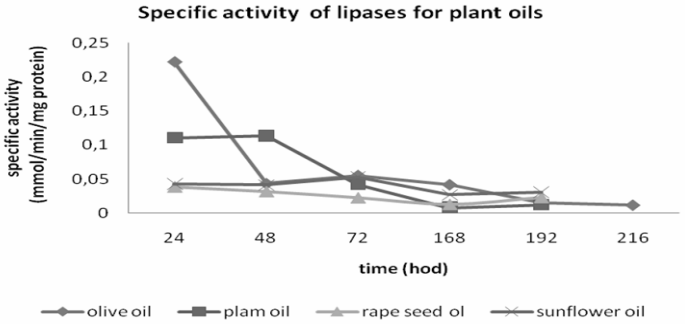

Fig. 4. Specific activity of lipases (mmol/min/mg protein).

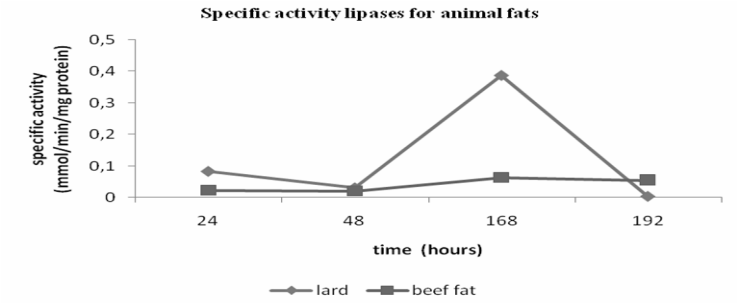

Fig. 5. Specific activity of lipases (mmol/min/mg protein).

Table 1. Specific activity for plant oils (mmol/min/mg protein).

oil /time (h)	24	48	72	168	192	216
olive	0.222	0.043	0.055	0.042	0.015	0.012
palm	0.111	0.114	-	0.042	0.008	0.012
rape seed	0.039	-	0.032	0.023	0.012	0.023
sunflower	0.043	-	0.042	0.053	0.027	0.030

Table 2. Specific activity for fats (mmol/min/mg protein).

fats/hours	24	48	168	192
lard	0.084	0.032	0.387	0.005
beef fat	0.023	0.021	0.064	0.056

3.4 Lipid degradation

From the determination of lipid degradation and fatty acid utilization follows that commercial product has the ability to degrade the lipids and then utilize the fatty acids The highest increase of the free fatty acids (FFA) percentage for olive (47.6 %), palm (58.5 %) and rape seed oil (49.8 %) was observed after 72 hours. For sunflower oil (52.2 %) after 120 hours and for lard (88.6 %) and beef fat (86.5 %) after 96 hours, Table 3.

Table 3. Percent of free fatty acids during lipid degradation.

Quantity of the free fatty acids (%) during lipid degradation						
oils or fats/hours	**48**	**72**	**96**	**120**	**192**	**216**
olive	14.8	47.6	16.8	18.8	13.9	9.9
palm	14.2	58.5	49.8	26.1	12.4	10.9
rape seed	21.3	49.8	20.3	29.3	12.8	6.9
sunflower	18.2	36.6	12.5	52.5	10.3	7.9
lard	40.5	63.7	88.6	12.7	66.5	33.9
beef fat	74.6	83.4	86.5	70.9	33.5	27.3

4. Conclusions

From the experimental results follows that the studied commercial microbial product for grease traps has the ability to degrade various plant oils and animal fats with different efficiency. Best lipolytic activity was observed at palm oil, olive oil and lard, but best degrading substrates are rape seed oil and lard. Growth of biomass was found out by all used substrates. Total concentration of the biomass during cultivation differs, according to the sort of the substrate. It depends on the emulsifier properties of oils or fats. Verification of the presence will be investigated in the other study.

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References

BECKER, P., KOSTER, D., POPOV, M.N., MARKOSSIAN, S., ANTRANIKIAN, G., MARKL, H.: The biodegradation of olive oil and treatment of lipid – rich wool wastewater under aerobic thermophilic condition. Water Res., 33, 1999, 653-660.

EL-BESTAWY, E., EL-MASRY, M.H., EL-ADL, N.E.: The potentially of free Gram – negative bacteria for removing oil and grease from contamined industrial effluents. World J. Microbiol. Biotechnol., 21, 2005, 815-822.

GOJKOVIĆ, Ž.: Studium přípravků do odlučovačů tuků. Diplomová práce, Vysoké učení technické v Brně, Fakulta chemická, 2009, 59 p.

ILLKOVÁ, K., OMELKOVÁ, J., VLČKOVÁ, B.: Lipid degradation and its application in lipid – containing wastewater. Chem. Listy, 99, 2008, 1234-2345.

NAWANI, N., DOSANJH, N. S., KAUR, J.: A novel thermostable lipase from a thermophilic *Bacillus sp.*: Characterization and esterification studies. Biotechnol. Lett., 20, 1998, 997-1000.

SIGURGÍSLADÓTTIR, S., KONRÁÖSDÓTTIR, M., JÓNSON, Á., KRISTJÁNSSON, J.K., MATTHIASSON, E.: Lipase activity of thermophilic bacteria from Icelandic hot springs. Biotechnol. Lett., 15, 1993, 361-366.

STOLL, U., GUPTA, H.: Management strategies for oil and grease residues. Waste Manage. Res., 15, 1997, 23-32.