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(54) Title: METHOD FOR INCREASING THE EFFICIENCY OF GREASE TRAPS

(57) Abstract: The present invention relates to methods of enhancing the efficiency of grease traps, or other similar devices, by subjecting FOG in the grease trap to fungal lipase.



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**TITLE: METHOD FOR INCREASING THE EFFICIENCY OF GREASE TRAPS****Field of the Invention**

5 The present invention relates to methods for enhancing the efficiency of grease traps or other similar devices.

**Background**

10 FOG (i.e., Fats, Oils and Grease) are discharged from especially restaurants and other commercial food service facilities in huge amounts. In order to reduce the FOG ending up in the public wastewater collection system, grease traps (or grease interceptors) are installed between the FOG discharging facilities and the public wastewater collection system. A grease trap works by slowing down the flow of warm/hot greasy water and allowing it to cool. As the water cools, the grease and oil separate and float to the top of the grease trap. The cooler water (less grease) 15 continues to flow down the outlet pipe to the sewer. Grease traps often contain baffles, which cover the inlet and outlet of the grease trap tank. The baffles trap the FOG and prevent FOG from flowing out of the trap. For the FOG to float to the top it is necessary to calm the water as turbulence only perplexes the separation. The FOG floating on the surface is periodically removed by hand or pump truck to avoid clogging of the grease trap. 20

US patent no. 6,649,401-B2 discloses gram-positive *Bacillus megaterium* producing extracellular lipases that degrades fats, oils and grease (FOG). The bacterium may be used in grease traps where fatty acid or grease needs to be degraded.

25 There is a need for enhancing the efficiency of grease traps, or other similar devices, in order to, e.g., reduce the FOG passing through grease traps into the public wastewater collection system.

**Brief Description of The Drawings**

30 Fig. 1 shows the dose response of FOG treated with Lipase A dosed from 4.23 to 423 KLU/L.

Fig. 2 shows the response of Lipase A dosed at 2,112 KLU/L and the dose response of FOG treated with Phospholipase A dosed from 5 to 486 KLU/L.

**Description of the Invention**

35 The present invention relates to methods for enhancing the efficiency of grease traps, or other similar devices.

The inventors have found that when adding fungal lipase to a grease trap the FOG layer that floats to the top of the grease trap is stabilized and hardened. This results in a decreased amount of FOG in the effluent leaving the grease trap and thus less FOG entering the public wastewater system. The hardening of the FOG also facilitates handling and removal of FOG in the grease trap.

In the first aspect the invention relates to a method of enhancing the efficiency of grease traps, or other similar devices, wherein FOG in the grease trap is subjected to fungal lipase.

In an embodiment the fungal lipase and optionally other enzyme activities are added as mono-component enzyme products. Further, the FOG is not subjected to bacteria, especially lipase producing bacteria.

### Fungal Lipases

Lipases: Suitable lipases according to the invention are of fungal origin. Chemically modified or protein engineered mutants (i.e., variants) are included.

In a preferred embodiment the lipase belongs to the EC 3.1.1.3. Examples of useful lipases include lipases from *Humicola* (synonym *Thermomyces*), e.g. from *H. lanuginosa* (*T. lanuginosus*) as described in EP 258 068 and EP 305 216 or from *H. insolens* as described in WO 96/13580. Other examples are fungal lipase variants are those described in WO 92/05249, WO 94/01541, EP 407 225, EP 260 105, WO 95/35381, WO 96/00292, WO 95/30744, WO 94/25578, WO 95/14783, WO 95/22615, WO 97/04079, WO 97/07202, and WO 00/60063.

In specifically contemplated embodiments the lipase used for treating FOG in the grease trap is a *Humicola lanuginosa* lipase variant disclosed in WO 00/60063 selected from the group consisting of:

|                                                         |
|---------------------------------------------------------|
| T231R+ N233R                                            |
| N94K+ D96L+ T231R+ N233R+ Q249R+ 270P+ 271G+ 272L       |
| D96L+ T231R+ N233R                                      |
| G91A+ E99K+ T231R+ N233R+ Q249R                         |
| (N33Q) +D96L +T231R +N233R +Q249R +270 PGL              |
| R209P +T231R +N233R                                     |
| (N33Q) +E99N +N101S +T231R +N233R +Q249R +270 PGL       |
| K24C +(N33Q) +D96S +T231R +N233R +Q249R +270 PCL        |
| (N33Q) +G91A +E99K +T231R +N233R +Q249R +270 PGL        |
| E1A +(N33Q) +G91A +E99K +T231R +N233R +Q249R +270 PGL   |
| (N33Q) +G91A +E99K +G255R +T231R +N233R +Q249R +270 PGL |
| (N33Q) +G91A +E99K +T231R +N233R +T244R +Q249R +270 PGL |

|                                                                                       |
|---------------------------------------------------------------------------------------|
| G91A +E99K +T231R +N233R +Q249R                                                       |
| E87K +G91D +D96L +G225P +T231R +N233R +Q249R +N251D                                   |
| G91A +E99K +T231R +N233R +Q249R +270AGVF                                              |
| G91A +E99K +T189G +T231R +N233R +Q249R                                                |
| D102G +T231R +N233R +Q249R                                                            |
| T231R +N233R +Q249R +270AGVF                                                          |
| R209P +T231R +N233R                                                                   |
| N33Q +N94K +D96L +T231R +N233R +Q249R +270PGLPFKRV                                    |
| N33Q +N94K +D96L +T231R +N233R +Q249R                                                 |
| N33Q +D96S +T231R +N233R +Q249R                                                       |
| N33Q +D96S +V228I + +T231R +N233R +Q249R                                              |
| E1A +N33Q +G91A +E99K +T231R +N233R +Q249R +270PGLPFKRV                               |
| N33Q +S83T +E87K +G91A + E99K +T231R +N233R +Q249R<br>+270PGLPFKRV                    |
| N33Q +G91A + E99K +T231R +N233R +Q249R +270PGLPFKRV                                   |
| T231R +N233R +270CP                                                                   |
| T231R +N233R +270RE                                                                   |
| N33Q +E99N +N101S +T231R +N233R +Q249R +270PGLPFKRV                                   |
| D62A +S83T + G91A +E99K +T231R +N233R +Q249R                                          |
| E99N +N101S +T231R +N233R +Q249R                                                      |
| R84W +G91A +E99K +T231R +N233R +Q249R                                                 |
| G91A +E99K +T231R +N233R +Q249R +270SPG                                               |
| G91A +E99K +T231R +N233R +Q249R +270VVVP                                              |
| G91A +E99K +T231R +N233R +Q249R +270LLASSGRGGHR                                       |
| G91A +E99K +T231R +N233R +Q249R +270VTT                                               |
| G91A +E99K +T231R +N233R +Q249R +270VLQ                                               |
| G91A +E99K +T231R +N233R +Q249R +270TST                                               |
| G91A +E99K +T231R +N233R +Q249R +270LRI                                               |
| V60G +D62E +G91A +E99K +T231R +N233R +Q249R                                           |
| G91A +D96W +E99K +T231R +N233R +G263Q +L264A +I265T +G266S<br>+T267A +L269N +270AGGFS |

Preferred commercially available lipase enzymes include Lipolase™, Lipoprime, Lipolase Ultra™, Lipomax™, Liposam™, and Lipex™ (Novozymes A/S).

In an embodiment the fungal lipase is a phospholipase. The term phospholipase is an enzyme which has activity towards phospholipids.

- 5 In a preferred embodiment the phospholipase is a phospholipase A<sub>1</sub> (EC. 3.1.1.32). In another preferred embodiment of the invention the phospholipase is a phospholipase A<sub>2</sub> (EC.3.1.1.4.).

The fungal phospholipase may be derived from a strain of the class *Pyrenomycetes*, such as the genus *Fusarium*, such as a strain of *F. culmorum*, *F. heterosporum*, *F. solani*, or a strain of *F. oxysporum*.

In an embodiment the phospholipase is derived from a strain of *Fusarium*, especially *Fusarium oxysporum*. The phospholipase may be the one concerned in WO 98/026057 displayed in SEQ ID NO: 2 derived from *Fusarium oxysporum* DSM 2672, or variants thereof. The phospholipase may also be from a filamentous fungus strain within the genus *Aspergillus*, such as a strain of *Aspergillus awamori*, *Aspergillus foetidus*, *Aspergillus japonicus*, *Aspergillus niger* or *Aspergillus oryzae*. Preferred phospholipases are derived from a strain of *Humicola*, especially *Humicola lanuginosa*. The phospholipase may be a variant, such as one of the variants disclosed in WO 00/32758, which are hereby incorporated by reference. Preferred phospholipase variants include variants listed in Example 5 of WO 00/32758, which is hereby specifically incorporated by reference. In another preferred embodiment the phospholipase is one described in WO 04/111216, especially the variants listed in the table in Example 1.

Examples of commercial phospholipases include LECITASE™ and LECITASE™ ULTRA, or LIPOPAN™ F (available from Novozymes A/S, Denmark).

According to the invention the lipase may be dosed in the range from 1-1,000 mg per kg FOG, preferably 5-800 mg/kg FOG, especially 10-600 mg/kg FOG; or 10-100,00 KLU per kg FOG, preferably 50-80,000 KLU per kg FOG, especially 100-60,000 KLU per kg FOG.

According to the invention the lipase may be dosed in the range from 0.1-10,000 KLU/L grease traps influent, preferably 1-8,000 KLU/L grease traps influent, especially 2-5,000 grease traps influent.

## Proteases

Proteases: According to the invention a protease may advantageously be added to disperse the FOG.

Suitable proteases include those of animal, vegetable or microbial origin. Microbial origin is preferred. Chemically modified or protein engineered mutants are included. The protease may be a serine protease or a metallo protease, preferably an alkaline microbial protease or a trypsin-like protease. Examples of alkaline proteases are subtilisins, especially those derived from *Bacillus*, e.g., subtilisin Novo, subtilisin Carlsberg, subtilisin 309, subtilisin 147 and subtilisin 168 (described in WO 89/06279). Examples of trypsin-like proteases are trypsin (e.g. of porcine or bovine origin) and the *Fusarium* protease described in WO 89/06270 and WO 94/25583.

Examples of useful proteases are the variants described in WO 92/19729, WO 98/20115, WO 98/20116, and WO 98/34946, especially the variants with substitutions in one or more of the following positions: 27, 36, 57, 76, 87, 97, 101, 104, 120, 123, 167, 170, 194, 206, 218, 222, 224, 235 and 274. Preferred commercially available protease enzymes include Alcalase™, Savinase™, Primase™, Duralase™, Dyrazym™, Esperase™, Everlase™, Polarzyme™, and Kannase™, (Novozymes A/S), Maxatase™, Maxacal™, Maxapem™, Properase™, Purafect™, Purafect OXP™, FN2™, and FN3™ (Genencor International Inc.).

## 10 Other Enzymes

Other enzyme activities may be present when practicing a method of the invention. Examples of such other enzymes are cellulases (endo-glucanases), beta-glucanases, hemicellulases, alpha-amylases, cutinases, pectinases, pectate lyases, or mixtures thereof.

15

## Materials & Methods

Chemicals used as buffers and reagents were commercial products of at least reagent grade.

## 20 Enzymes:

Lipase A is a variant of *Humicola lanuginosa* disclosed as SEQ ID NO: 2 in US patent no. 5,869,438 with the following amino acid substitutions: T231R + N233R. The variant is also disclosed in the Example in WO 00/60063. Lipase A is available from Novozymes A/S, Denmark.

25

Phospholipase A is a variant of *Humicola lanuginosa* sequence disclosed as SEQ ID NO: 2 in US patent no. 5,869,438 with the following amino acid substitutions and C-terminal extension: G91A +D96W +E99K +G263Q +L264A +I265T +G266D +T267A +L269N+270AGGFS It is expressed in *A. oryzae*. The variant is described in Example 5 in WO 0032758 which reference is hereby incorporated by reference.

30

FOG: Actual FOG collected from a sanitary liftstation. The FOG used in Example 2 was collected from a Zebulon, NC, USA, liftstation directly downstream from a commercial "Hardee's" restaurant. The liftstation is #151 under the care of the City of Raleigh Waste Water Authority; the physical address is 615 West Gannon Ave. Zebulon, NC 27597.

35

## Lipase activity (LU)

The lipolytic activity was determined using tributyrine as substrate. This method

was based on the hydrolysis of tributyrin by the enzyme, and the alkali consumption is registered as a function of time.

One Lipase Unit (LU) is defined as the amount of enzyme which, under standard conditions (i.e. at 30°C; pH 7.0; with Gum Arabic as emulsifier and tributyrine as substrate) liberates 1 micro mol titrable butyric acid per minute.

A folder AF 95/5 describing this analytical method in more detail is available upon request to Novozymes A/S, Denmark, which folder is hereby included by reference.

## 10    **EXAMPLES**

### **Example 1**

#### Treatment of FOG with Fungal Lipase

The experiment was done in 50 ml Falcon tubes in a water bath at 50°C. Lipase A was dosed and then the temperature was steadily dropped over 48 hours to measure the ability of the enzyme to keep the FOG in solution/emulsion. A dose of 55 KLU enzyme was applied to 5 g FOG/50ml DI water. The test resulted in a heat stable cap that did not release suspended solids.

### 20    **Example 2**

#### Stabilization of FOG by the use of Fungal Lipase and Phospholipase.

FOG was treated with Lipase A and Phospholipase A as follows:

1. Four grams of FOG was placed into separate 50 ml Falcon Tubes.
2. The total volume of each tube was brought to a volume of 40 ml with distilled water (DI water).
- 25    3. The FOG containing tubes were placed in a water bath at a temperature of 50°C for 24 hours to revert the FOG into its liquid state; an oily, viscous emulsion.
4. After 24 hours enzymes were dosed and left to incubate for about 12 hours.
5. After 12 hours differences were noted in the tubes according to the physical properties of FOG in the top of the tube and the capacity for suspended solids to remain in the aqueous phase.
- 30    6. The temperature was steadily dropped until stable caps of FOG began to form in enzymatically treated tubes. The ideal temperature in this case was proven to be about 35°C.

35

The result of the treatment with Lipase A and Phospholipase A are shown in Figs 1 and 2.

Fig. 1: tubes from left to right:

Control (untreated); 4.23 KLU/L of Lipase A; 21.15 KLU/L of Lipase A; 42.3 KLU/L of Lipase A; 106 KLU/L of Lipase A; 212 KLU/L of Lipase A; and 423 KLU/L of Lipase A

5 Fig. 2: Tubes from left to right:

2,115 KLU/L of Lipase A;

5 KLU/L of Phospholipase A; 25 KLU/L of Phospholipase A; 49 KLU/L of Phospholipase A; 122 KLU/L of Phospholipase A; 243 KLU/L of Phospholipase A; 486 KLU/L of Phospholipase A.

10 As can be seen from the figures FOG treated with Lipase A resulted in a heat stable cap that did not release suspended solids. The cap became harder and more stable in higher dosages. 2,115 KLU/L of Lipase A gave the most stable cap

Treatment with Phospholipase A also gave stable caps, but Phospholipase A did not perform as well at the higher dosages.

15 The invention described and claimed herein is not to be limited in scope by the specific embodiments herein disclosed, since these embodiments are intended as illustrations of several aspects of the invention. Any equivalent embodiments are intended to be within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those  
20 skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims. In the case of conflict, the present disclosure including definitions will control.

Various references are cited herein, the disclosures of which are incorporated by reference in their entireties.

## Claims

1. A method of enhancing the efficiency of grease traps, or other similar devices, wherein FOG in the grease trap is subjected to fungal lipase.
- 5 2. The method of claim 1, wherein the lipase is derived from a strain of the genus *Humicola*, preferably *Humicola lanuginosa*, especially *Humicola lanuginosa* DSM 4109.
3. The method of claims 1 or 2, wherein the lipase is a variant of the mature part of the  
10 *Humicola lanuginosa* lipase disclosed as SEQ ID NO: 2 in US patent no. 5,869,438.
4. The method of any of claim 1, wherein the lipase is a phospholipase.
5. The method of any of claims 1-4, wherein the lipase is dosed the range from 1-1,000  
15 mg per kg FOG, preferably 5-800 mg/kg FOG, especially 10-600 mg/kg FOG.
6. The method of any of claims 1-5, wherein the lipase is a mono-component product.
7. The method of any of claims 1-6, wherein the FOG is not subjected to bacteria,  
20 especially lipase producing bacteria.
8. The method of any of claims 1-7, further comprising subjected in the FOG to protease.
- 25 9. The method of claim 8, wherein the protease is a bacterial protease, preferably derived from a strain of the genus *Bacillus*, especially a strain of the species *Bacillus halodurans*.
- 30 10. Use of a fungal lipase for enhancing the efficiency of grease traps or other similar devices.



Fig. 1



Fig. 2