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(54) **Enzymatic dishwashing and rinsing process**

Verfahren zum enzymatischen Waschen und Spülen von Geschirr

Procédé enzymatique pour le lavage et le rinçage de la vaisselle

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## Description

[0001] The present invention relates to enzymatic dishwashing or rinsing processes using compositions comprising lipolytic enzymes.

5 [0002] EP-A-0 130 064 (Novo) concerns detergent additives based on Fusarium oxysporum lipase (e.g. 20,000-100,000 LU/g, used in detergent at e.g. 0.2-2% w/w).

[0003] DE-A-1 930 636 (= GB 1 273 545) (Albright & Wilson) concerns cleaning compositions based on protease, lipase and/or decarboxylase (of unspecified type) and surfactant, used inter alia for dishwashing.

10 [0004] EP-A-0 205 208 (Unilever) (not prepublished) discloses enzymatic detergent compositions comprising lipase with 1-40% anionic surfactant and builders. The lipases disclosed are those that cross-react with antibody against lipase from Pseudomonas fluorescens 1AM 1057.

[0005] EP 0 206 930 (Unilever) (not prepublished) also discloses enzymatic detergent compositions comprising similar lipases together with combinations of anionic and nonionic detergent.

15 [0006] Thus, enzymatic dishwashing compositions have been proposed in the art. As enzymes, mainly amylases and/or proteases have been proposed for inclusion in dishwashing compositions. Lipases have also been suggested, but have received far less attention than the amylases and/or proteases. Dishwashing compositions, in particular machine dishwashing compositions which are used in the main wash step of a machine dishwashing operation, have in general a satisfactory cleaning performance. However, frequently the article cleaned with such products still do not have a satisfactory visual appearance after rinsing and drying, showing film or spots. Some main wash liquor is usually carried over from the main wash step to the rinse step, causing some deposition of soil resulting in insoluble calcium salts on the articles to be rinsed, which results in visible film or spots on the articles when they are dry. In particular with glass articles, this causes an unsightly visual appearance.

[0007] We have now found that the addition of lipases to a main wash dishwashing composition or to a rinse composition significantly reduced the formation of film or spots on the articles cleaned or rinsed with such a composition.

25 [0008] Accordingly the invention provides the use of a lipolytic enzyme as a spot reducing agent a process for machine dishwashing or rinsing, comprising treating dishes with an aqueous liquid having dissolved or dispersed therein an effective amount of an enzymatic dishwashing or rinsing composition comprising from 0.5-10% by weight of a detergent surfactant and from 0-90% by weight of a builder and from 0.005-100 LU/mg of a lipolytic enzyme.

30 [0009] The lipases, used according to the present invention, may be of any suitable origin such as yeasts, fungi and bacteria. Preferably, however, they are of bacterial origin, and particularly preferably they belong to the class of bacterial lipases which show a positive immunological cross-reaction with the antibody of the lipase, produced by the microorganism Chromobacter viscosum var lipolyticum NRRL B-3673.

[0010] This lipase has been described in Dutch Patent Specification 154,269 of Toyo Jozo, and the microorganism is available to the public at the United States Department of Agriculture, Agricultural Research Service, Northern Utili-  
35 sation and Development Division at Peoria, Illinois, under the number NRRL B-3673. This lipase will hereinafter be referred to as "Toyo Jozo" lipase. The preferred lipases to be used in the present invention should show a positive immunological cross-reaction with the Toyo Jozo lipase antibody, using the standard and well-known immunodiffusion procedure according to Ouchterlony (Acta. Med. Scan., 133, pages 76-79 (1950)).

[0011] The preparation of the antiserum is carried out as follows:

40 Equal volumes of 0.1 mg/ml antigen and of Freund's adjuvant (complete or incomplete) are mixed until an emulsion is obtained. Two female rabbits are injected with 2 ml samples of the emulsion according to the following scheme:

day 0 : antigen in complete Freund's adjuvant  
day 4 : antigen in complete Freund's adjuvant  
45 day 32 : antigen in incomplete Freund's adjuvant  
day 60 : booster of antigen in incomplete Freund's adjuvant.

[0012] The serum containing the required antibody is prepared by centrifugation of clotted blood, taken on day 67.

50 [0013] The titre of the anti-Toyo Jozo-lipase antiserum is determined by the inspection of precipitation of serial dilutions of antigen and antiserum according to the Ouchterlony procedure. A 2<sup>5</sup> dilution of antiserum was the dilution that still gave a visible precipitation with an antigen concentration of 0.1 mg/ml.

[0014] All lipases showing a positive immunological cross reaction with the Toyo Jozo lipase antibody as hereabove described are preferred lipases for use in the present invention. Typical examples thereof are the lipases ex Pseudomonas fluorescens IAM 1057 (available under the trade name Amano-P), the lipase ex Pseudomonas fragi FERM P 1339 (available under the trade name Amano-B), lipase ex Pseudomonas nitroreducens var. lipolyticum FERM P 1338, the lipase ex Pseudomonas sp. available under the trade name Amano-CES, the lipase ex Pseudomonas cepacia, lipases ex Chromobacter viscosum, e.g. Chromobacter viscosum var. lipolyticum NRRLB 3673, commercially available from Toyo Jozo Co., Tagata, Japan; and further Chromobacter viscosum lipases from US Biochemical Corp., U.S.A. and

Diosynth Co., The Netherlands, and lipases ex *Pseudomonas gladioli*.

**[0015]** The lipases used in the present invention are included in the detergent composition in such an amount that the final detergent composition has a lipolytic enzyme activity of from 100 to 0.005 LU/mg preferably 25 to 0.05 LU/mg of the composition.

**[0016]** A Lipase Unit (LU) is that amount of lipase which produces 1  $\mu\text{mol}$  of titratable fatty acid per minute in a pH stat. under the following conditions: temperature 30°C; pH = 9.0; substrate is an emulsion of 3.3 wt.% of olive oil and 3.3% gum arabic, in the presence of 13 mmol/l  $\text{Ca}^{2+}$  and 20 mmol/l NaCl in 5 mmol/l Tris-buffer.

**[0017]** Naturally, mixtures of the above lipases can be used. The lipases can be used in their non-purified form, or in a purified form, e.g. purified with the aid of well-known adsorption methods, such as a phenylsepharose-adsorption techniques.

**[0018]** The composition used according to the invention may furthermore comprise the usual ingredients of dishwashing or rinse compositions. Thus it may contain one or more alkali salts commonly used in dishwashing compositions. Thus, it may contain organic and/or inorganic builders such as the alkali metal ortho-, pyro and tripolyphosphates and hexametaphosphates, silicates, carbonates, zeolites, borates, citrates, carboxymethyloxy-succinates, nitrilotriacetates and ethylenediamine-tetraacetates, polymeric polyelectrolytes such as polyacrylates, polymaleates, and other known organic and inorganic builder compounds.

**[0019]** Usually, the amount of builders in the composition varies from 10-90% by weight, generally from 30-70% by weight.

**[0020]** The composition may also contain a detergent-active compound. If a detergent-active compound is included, it usually is in an amount of from 0.5-10%, usually 1-5%. Any well-known type of detergent active compound may be used, such as soaps, synthetic anionic, non-ionic, amphoteric detergent surfactant and mixtures thereof. Preferably, a nonionic detergent surfactant is used, especially a low-foaming one. Suitable examples of such nonionic detergent surfactants can easily be found in M.Schick "Nonionic Surfactants" (1967).

**[0021]** The composition may furthermore contain other useful additives such as bleaching agents, bleaching agent activators, hydrotropes, fillers, perfumes, colouring agents, germicides, soil-suspending agents, aminopolyphosphonic acids and alkali metal or alkaline earth metal salts thereof, clays such as hectorites, anti-corrosion agents such as fatty acids, benzotriazole and so on. Other enzymes such as proteases, amylases, oxidases may also be included.

**[0022]** As bleaching agents the peroxygen type bleaching agents, preferably with a bleach precursor such as TAED are suitable for inclusion in the machine dishwashing compositions.

**[0023]** A typical example of a conventional machine dishwashing composition usually contains an alkali metal tripolyphosphate in an amount of from 20-60%, an alkali metal silicate in an amount of from 40-80%, or an alkali metal disilicate in an amount of 5-30% by weight a peroxy type bleaching agent in an amount of from 1-15%, a low-foaming detergent surfactant in an amount of from 0.5-5%, and minor ingredients such as perfumes, colouring agents, hydrotropes, fillers, etc.

**[0024]** When formulated as a rinse composition, it may contain from 0.5-10% by weight of a nonionic surfactant, from 5-25% by weight of an inorganic or organic acid, from 1-5% by weight of a hydrotrope, dyes, germicides, clays, silica sols, etc., the balance being water. The products of the invention can be formulated in any desirable form, such as powders, granulates, cakes, bars, pastes, liquids etc. The invention will further be illustrated by way of example.

#### Example 1

**[0025]** In a commercial dishwashing machine tests were carried out under the following conditions:

detergent composition dosage	30 g	
lipase dosage	2.5 g (= 15 LU/ml)	
water hardness	main wash	27° GH
	flood	9° GH
	rinse 1	9° GH
	rinse 2	9° GH
	final rinse	9° GH
wash temperature and time	55°C - 60 minutes	

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articles to be cleaned	clean dishes
	clean glasses
test soil	35 g egg-yolk, added at the beginning of the main wash.

**[0026]** The detergent composition had the following formulation:

	in gr.
sodium tripolyphosphate	11.61
sodium carbonate	2.7
TAED (59.7%)	1.81
sodium perborate	2.43
calcium-Dequest (47.6%)	0.397
sodium disilicate	3.3
low foaming nonionic (Plurafac RA40)	0.405
hectorite clay (Laponite XLS)	0.675
sodium sulphate	5.610
amylase (4.8 Maltose units/mg)	0.540

**[0027]** As lipase, the Toyo Jozo lipase was used.

**[0028]** The test was carried out in two runs, one without and one with lipase.

**[0029]** The glasses were appraised by an expert panel as to their visual appearance, and were given a score according to the following scale:

Film	Spots
1. no film	1. no spots
2. slight film	2. 1-5 spots
3. clear film	3. 5-10 spots
4. heavy film	4. 11-20 spots
	5. more than 20 spots

**[0030]** The following results were obtained:

	run 1 (no lipase)	run 2 (with lipases)
average film score	2.3	2.3
average number of spots	250	100

### Example 2

**[0031]** The test of Example 1 was repeated, but under the following different conditions:

wash temperature	65°C
soil	standard soil; at the end of the main wash, clean plates were put in the machine, so that no cleaning took place in the final rinse, but only a carry-over took place.
run 1	no lipase added in the final rinse
run 2	2.5 g Toyo Jozo lipase was added to the final rinse (15 LU/ml).

**[0032]** The main wash composition had the following formulation:

	% by weight
sodium tripolyphosphate	36
sodium carbonate	9
sodium disilicate	15
TAED	3.8
sodium perborate tetrahydrate	8.8
potassium metaborate	0.35
sodium metaborate	0.85
calcium-Dequest	0.63
Amylase (4.8 MU/mg)	1.8
Esperase (a protease; 675 GU/mg)	1.8
low-foaming nonionic (Plurafac RA 40)	1.35
hectorite (Laponite XLS)	2.25
benzotriazole	0.1
sodium sulphate	14.0
water	9.5

**[0033]** The following spot and film scores of the glasses were obtained:

	run 1 (no lipase)	run 2 (with lipases)
average film score	1	1
average spot score	5	2.7

### Example 3

**[0034]** Replacing the Toyo Jozo lipase by the Amano-P lipase or Lipase MY (ex *Candida cylindracea*) or Amano AP-6 lipase (ex *Aspergillus niger*) in Example 2 gave similar results.

### Example 4

**[0035]** The following formulation is an example of a rinse aid composition to which lipases can be added:

	% by weight
low-foaming nonionic (Plurafac RA 30)	10
citric acid	18
sodium xylene sulphonate	3.0
dye	0.003
butylated hydroxytoluene	0.1
water	q.s.

### Claims

1. Use of a lipolytic enzyme as a spot reducing agent in a process for machine dishwashing or rinsing, comprising treating dishes with an aqueous liquid having dissolved or dispersed therein an effective amount of an enzymatic dishwashing or rinsing composition comprising from 0.5-10% by weight of a detergent surfactant and from 0-90% by weight of a builder and from 0.005-100 LU/mg of a lipolytic enzyme.
2. Use according to claim 1, characterised in that the lipase is a bacterial lipase which shows a positive immunological cross-reaction with the antibody of the lipase, produced by Chromobacter viscosum var. lipolyticum NRRL B 3673.
3. Use according to claim 2, characterised in that the lipase is producible by the microorganism selected from the group consisting of Pseudomonas fluorescens, Pseudomonas fragi, Pseudomonas nitroreducens var. lipolyticum, Pseudomonas cepacia, Pseudomonas gladioli and Chromobacter viscosum.
4. Use according to any of claims 1-3 in a machine dishwashing process, characterised in that there is used a composition containing from 20-60% by weight of an alkali metal silicate, from 1-15% by weight of a peroxy type bleaching agent and from 0.5-5% by weight of a low-foaming nonionic detergent.
5. Use according to any of claims 1-3 in a machine rinsing process, characterised in that there is used a composition containing, in an aqueous medium, from 0.5-10% by weight of a nonionic surfactant, from 5-25% by weight of an organic or inorganic acid and from 1-5% by weight of a hydrotrope.

### Patentansprüche

1. Verwendung eines lipolytischen Enzyms als fleckenreduzierendes Agens in einem Verfahren zum Maschinen-Geschirrwaschen oder -Spülen, umfassend das Behandeln von Geschirr mit einer wäßrigen Flüssigkeit, in der gelöst oder dispergiert ist eine wirksame Menge einer enzymatischen Geschirrwash- oder Spül-Zusammensetzung, umfassend 0,5-10 Gew. -% eines Detergensoberflächenaktiven Materials und 0-90 Gew. -% eines Builders und 0,005-100 LU(Lipase-Einheiten)/mg eines lipolytischen Enzyms.
2. Verwendung nach Anspruch 1, dadurch gekennzeichnet, daß die Lipase eine bakterielle Lipase ist, die eine positive immunologische Kreuzreaktion mit dem Antikörper der Lipase, die durch Chromobacter viscosum var. lipolyticum NRRL B 3673 gebildet wird, zeigt.
3. Verwendung nach Anspruch 2, dadurch gekennzeichnet, daß die Lipase durch den Mikroorganismus, ausgewählt aus der Gruppe bestehend aus Pseudomonas fluorescens, Pseudomonas fragi, Pseudomonas nitroreducens var. lipolyticum, Pseudomonas cepacia, Pseudomonas gladioli und Chromobacter viscosum, gebildet werden kann.
4. Verwendung nach einem der Ansprüche 1-3 in einem Maschinen-Geschirrwash-Verfahren, dadurch gekennzeichnet, daß eine Zusammensetzung verwendet wird, die 20-60 Gew.-% eines Alkalimetallsilikats, 1-15 Gew.-% eines Bleichmittels vom Peroxytyp und 0,5-5 Gew.-% eines schwachschäumenden nicht-ionischen Detergens enthält.

5. Verwendung nach einem der Ansprüche 1-3 in einem Maschinen-Spülverfahren, dadurch gekennzeichnet, daß eine Zusammensetzung verwendet wird, die in einem wäßrigen Medium 0,5-10 Gew.-% eines nicht-ionischen oberflächenaktiven Materials, 5-25 Gew.-% einer organischen oder anorganischen Säure und 1-5 Gew.-% eines Hydrotops enthält.

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## Revendications

1. Utilisation d'une enzyme lipolytique en tant qu'agent réduisant les taches dans un procédé pour le lavage ou le rinçage de la vaisselle en machine, comprenant le traitement de la vaisselle avec un liquide aqueux dans lequel est dissoute ou dispersée une quantité efficace d'une composition enzymatique pour le lavage ou le rinçage de la vaisselle contenant de 0,5 à 10% en poids d'un tensio-actif détergent et de 0 à 90% en poids d'un adjuvant, caractérisée en ce qu'elle contient en outre de 0,005 à 100 UL/mg d'enzyme lipolytique.
2. Utilisation selon la revendication 1, caractérisée en ce que la lipase est une lipase bactérienne qui présente une réaction croisée immunologique positive avec l'anticorps dirigé contre la lipase produite par Chromobacter viscosum var. lipolyticum NRRL B 3673.
3. Utilisation selon la revendication 2, caractérisée en ce que la lipase peut être produite par le microorganisme choisi dans le groupe constitué de Pseudomonas fluorescens, Pseudomonas nitroreducens var. lipolyticum, Pseudomonas cepacia, Pseudomonas gladioli et Chromobacter viscosum.
4. Utilisation selon l'une quelconque des revendications 1 à 3 dans un procédé pour lavage de la vaisselle en machine, caractérisée en ce qu'on y utilise une composition contenant de 20 à 60% en poids de silicate de métal alcalin, de 1 à 15% en poids d'agent de blanchiment de type peroxy et de 0,5 à 5% en poids de détergent non ionique faiblement moussant.
5. Utilisation selon l'une quelconque des revendications 1 à 3 dans un procédé de rinçage en machine, caractérisée en ce qu'on y utilise une composition contenant, en milieu aqueux, de 0,5 à 10% en poids de tensio-actif non ionique, de 5 à 25% en poids d'acide organique ou inorganique et de 1 à 5% en poids d'hydrotrope.

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