(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 November 2013 (14.11.2013)

WIPO | PCT

(10) International Publication Number
WO 2013/167613 A1

(51) **International Patent Classification**:
C11D 3/386 (2006.01) ***D06M 16/00*** (2006.01)

(21) **International Application Number**:
PCT/EP2013/059527

(22) **International Filing Date**:
7 May 2013 (07.05.2013)

(25) **Filing Language**: English

(26) **Publication Language**: English

(30) **Priority Data**:
12167440.2 10 May 2012 (10.05.2012) EP

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(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

WO 2013/167613 A1

(54) **Title**: CARE ENZYME SYSTEM

(57) **Abstract**: A low temperature active enzymatic fabric care composition comprising the combination of one or more primary care enzymes and one or more auxiliary care enzymes.

CARE ENZYME SYSTEM

The present invention concerns combinations of fabric care enzymes, and in particular but not exclusively fabric care enzymes in a fabric treatment composition.

WO95/02675 discloses detergent compositions comprising cellulases capable of providing improved particulate soil removal and cellulases providing a colour clarification. Disclosed are compositions comprising a first cellulase component having retaining type activity and being capable of particulate soil removal a second cellulase component having multiple domains comprising at least one non-catalytic domain attached to a catalytic domain and being capable of colour clarification.

Low temperature active enzyme formulations are needed for low temperature washing conditions. However, mesophilic and even more so thermophilic enzymes are less effective at low temperatures so that levels added to washing formulations need to be increased which makes the formulation more expensive. In the case of care enzymes which act to hydrolyse the fabric material itself, high levels can lead to increased fabric damage which is counter productive. An objective is to provide a low temperature enzymatic composition and process with improved care but with no/minimal attendant fabric damage.

Accordingly, in a first aspect, the present invention provides a low temperature active enzymatic fabric treatment composition comprising the combination of:

1. one or more primary care enzymes; and
2. one or more auxiliary care enzymes.

In a second aspect the present invention provides a low temperature enzymatic fabric treatment process comprising the step of treating a fabric with the composition of the first aspect of the invention.

In a third aspect, the present invention provides use of an auxiliary care enzyme in combination with a primary care enzyme in the treatment of fabrics for a care benefit.

With the composition of the present invention, care of the fabric is improved using a low level of fabric care enzyme whose performance is synergistically improved at low temperature by the use of an auxiliary care enzyme.

Preferably the total amount of the enzymes of the invention ie. the total amount of the primary care enzyme and the auxiliary care enzyme is 0.01 – 1.75 mg per Litre of the wash liquor

Washing machines vary in wash liquor volume from 10 L to 48 L. Accordingly the total amount of enzymes of the invention per dose may be from 0.1 – 84 mg .

For hand washing, the wash liquor may be around 4 – 5 litres in which case the dose may be 0.04 – 8.75 mg of total enzymes of the invention.

The amount of detergent composition per dose will itself vary (e.g. 35 ml, 10 ml) depending on water levels, concentration of e.g. surfactants.

The primary care enzyme may be 0.003 mg/L of wash liquor.

Preferably the auxiliary care enzyme is therefore present either in equal weight (of enzyme protein) or, more preferably greater amounts than the primary care enzyme.

Preferably the primary care enzyme and the auxiliary care enzyme are present in a weight ratio of enzyme protein in the range of from 1:1 to 1:29, more preferably 1:1 to 1:9 more preferably 1:5, more preferably 1:1.9. The preferred ranges of the invention exclude 1:30, 1:10 and 1:2.

Preferably, the one or more primary care enzymes comprises one or more enzymes of the Glycoside Hydrolase Family 45.

Preferably the one or more primary care enzymes comprises one or cellulolytic enzymes (commonly known as cellulases but not restricted to the class EC 3.1.2.4) active in restoring colour to cotton or cotton-based fabrics by removal of fuzz and pills from the surface of the fabric.

The one or more auxiliary care enzymes preferably comprise one or more glycosyl hydrolases of Family 5 and/or Family 7.

DEFINITIONS

In this patent specification:

"Primary care enzymes" means enzymes active at restoring colour to fabrics by removing fuzz and pills from the surface of the fabric.

"care benefit" means restoration of colour to or improvement of feel of fabrics by removing fuzz and pills from the surface of the fabric.

"Auxiliary care enzymes" means any enzyme which is active to hydrolyse cellooligosaccharides.

"Low temperature active" means active at temperatures of 25 degrees Celcius or lower.

"Cellooligosaccharides" means any subchain of cellulose which is a reaction product of the hydrolysis cellulose by the primary care enzyme. Preferably the sub chain is soluble and preferably it contain 2 - 8 glucose units: cellobiose (DP=2), cellotriose (DP=3), cellotetraose (DP=4), cellopentaose (DP=5), cellohexaose (DP=6), celloseptose (DP=7), cellooctanose (DP=8); more preferably 4-8 such units. (DP means degrees of polymerisation).

"Glycoside Hydrolase Family" means any Glycoside Hydrolase Family (designated by number) of the Glycoside Hydrolase Family Classification system, based on amino acid similarities, being part of the Carbohydrate-Active Enzymes database (CAZy) developed by the Glycogenomics group at **Architecture et Fonction des Macromolécules Biologiques,** Unité Mixte de Recherches UMR6098, CNRS,Université de Provence Université de la Méditerranée.

"Glycoside Hydrolase Family 5" includes the retaining enzymes of chitosanase (EC 3.2.1.132); • -mannosidase (EC 3.2.1.25); cellulase (EC 3.2.1.4); glucan • -1,3-glucosidase (EC 3.2.1.58); licheninase (EC 3.2.1.73); glucan endo-1,6-• -glucosidase (EC 3.2.1.75); mannan endo-• -1,4-mannosidase (EC 3.2.1.78); endo-• -1,4-xylanase (EC 3.2.1.8); cellulose • -1,4-cellobiosidase (EC 3.2.1.91); • -1,3-mannanase (EC 3.2.1.-); xyloglucan-specific endo-• -1,4-glucanase (EC 3.2.1.151); mannan transglycosylase (EC 2.4.1.-); endo-• -1,6-galactanase (EC 3.2.1.164); endoglycoceramidase (EC 3.2.1.123); • -primeverosidase (EC 3.2.1.149)

"Glycoside Hydrolase Family 7" includes endo-• -1,4-glucanase (EC 3.2.1.4); reducing end-acting cellobiohydrolase (EC 3.2.1.176); chitosanase (EC 3.2.1.132); endo-• -1,3-1,4-glucanase (EC 3.2.1.73)

"Glycoside Hydrolase Family 45" includes the inverting enzymes of endoglucanase (EC 3.2.1.4)

The enzymes of the invention may be from bacterial or fungal origin. Chemically modified or protein engineered mutants are included.

Preferably the primary care and/or the auxiliary care enzymes are cellulases.

Preferred cellulases include cellulases from the genera *Bacillus*, *Pseudomonas*, *Humicola*, *Fusarium*, *Thielavia*, *Acremonium*, e.g. the fungal cellulases produced from *Humicola insolens*, *Thielavia terrestris*, *Myceliophthora thermophila,* and *Fusarium oxysporum*.

Especially preferred primary care cellulases are the alkaline or neutral cellulases having color care benefits. Such preferred enzymes include those having a molecular weight of from 17kDa to 30 kDa, for example the endoglucanases sold under the tradename Biotouch(R) NCD, DCC and DCL (AB Enzymes, Darmstadt, Germany). Other preferred commercially available cellulases include Celluzyme™, Carezyme™, , Renozyme™ (Novozymes A/S), Clazinase™ and Puradax HA™, Puradax(R) EG-L and Puradax(R) HA (Genencor International Inc.), and KAC-500(B)™, KAC(R)-500(B) (Kao Corporation).

Especially preferred auxiliary care cellulases include those active to active to hydrolyse cellooligosaccharides and may include Endolase™ Celluclean™. Whitezyme(R) (Novozymes A/S, Bagsvaerd.

Other enzymes could be included in the fabric treatment composition such as proteases, lipases, phospholipases, amylases, pectate lyases, mannases, peroxidases/oxidases.

Whilst mesophilic enzymes are preferred, psychrophilic enzymes may be used and are included in the scope of the invention. Such enzymes include the cellulases and xylanase from e.g. *Clostridium sp*. PXYL1 (G. Akila, T.S.Chandra

(2003) FEMS Microbiol. Letters 219, 63-67). Psychrophilic xylanases include *E.coli* phagemid (Lee et al. 2006b).

Once a suitable enzyme has been selected, it is relatively easy for the skilled man to isolate a suitable micro-organism capable of producing the enzyme under washing conditions. To that end, micro-organisms are screened for their capability of producing the desired enzyme under washing conditions, in an assay that resembles the washing conditions as closely as possible.

For all enzymes of the invention, enzyme variants (produced, for example, by recombinant techniques) are included within the meaning of the term "enzyme". Examples of such enzyme variants are disclosed, e.g., in EP 251,446 (Genencor), WO 91/00345 (Novo Nordisk), EP 525,610 (Solvay) and WO 94/02618 (Gist-Brocades NV).

The fabric treatment composition may comprise a laundry/fabric cleaning/care composition and may comprise one or more surfactants and/or optionally other ingredients.

Such compositions of the invention may be in dry solid form e.g. powdered, granules or tableted powders or liquid or gel form. It may also be in the form of a solid detergent bar. The composition may be a concentrate to be diluted, rehydrated and/or dissolved in a solvent, including water, before use. The composition may also be a ready-to-use (in-use) composition.

The present invention is suitable for use in industrial or domestic fabric wash compositions, fabric conditioning compositions and compositions for both washing and conditioning fabrics (so-called through the wash conditioner compositions). The present invention can also be applied to industrial or domestic non-detergent based fabric care compositions, for example spray-on compositions.

Other detergent ingredients may be included including surfactants, builders, sequestring agents, hydrotropes, preservatives, complexing agents, polymers, stabilizers, perfumes, optical brighteners, or other ingredients such as e.g. fabric conditioners including clays, foam boosters, suds suppressors (anti-foams), anti-corrosion agents, soil-suspending agents, anti-soil redeposition agents, anti-microbials, tarnish inhibitors, or combinations of one or more thereof, provided that these ingredients are compatible with the enzymes.

The fabric wash compositions may comprise a fabric wash detergent material selected from non-soap anionic surfactant, nonionic surfactants, soap, amphoteric surfactants, zwitterionic surfactants and mixtures thereof. The surfactants may be present in the composition at a level of from 0.1% to 60% by weight.

Any enzyme present in a composition may be stabilized using conventional stabilizing agents, e.g., a polyol such as propylene glycol or glycerol, a sugar or sugar alcohol, lactic acid, boric acid, or a boric acid derivative, e.g., an aromatic borate ester, or a phenyl boronic acid derivative such as 4-formylphenyl boronic acid.

Examples Of Non Limiting Embodiments Of The Invention

1. Assay to determine the level of reducing sugars released from cotton linters by cellulases. This shows the

Part I - Enzyme hydrolysis:

Enzymes concentrations/ratios A,B and C were prepared as follows.
A total concentration of 4mg/L: 1:1 ratio of primary : auxiliary
B. total concentration of 1.74 mg/L: 1:1.9 ratio of primary : auxiliary
C. total concentration of 1.5 mg/L: 1:5 ratio of primary : auxilary

The enzymes A,B and C were incubated in 200• l volume in microplate wells with 2% (w/v) cotton linters in 15mM MOPS buffer (pH 7) for 1 hour at room temperature/25°C. Rapid agitation was provided by a Heidolph microtitre plate shaker platform at 1,050rpm. No enzyme and no substrate controls, as well as a reducing sugar standard (glucose) range from 0 - 200• M, were included in the assays. At the end of the incubation, cotton linters were allowed to settle for 10 minutes before 100• l aliquots of supernatant solution were transferred to clean microplates.

Part II - BCA assay:

For concentration as in A Bicinchoninic Acid (BCA) method was used to determine the release of reducing sugars into solution. BCA reagent solution was prepared by mixing BCA reagents A and B in a 50:1 (v/v) ratio. BCA reagent solution was then mixed in a 1:1 (v/v) ratio with 100µl aliquots of sample supernatant solutions in sealed microplate wells and incubated at 65-70°C for 40 minutes. Microplates were then allowed to cool to room temperature in a refrigerator, before well seals were carefully removed. Absorbance of reaction products was measured at 540nm in a spectrophotometer. Interference by protein was accounted for by subtracting protein only (no cotton linters) control readings. Total reducing sugar released was calculated using a standard curve of glucose from 0 - 200µM.

Results

Tables 1,2 and 3 show the results from A, B and C (respectively) as described above : synergistic release of reducing sugars from cotton linters by a 1:1 mix of three different Family 45 EG-V type endoglucanases (Carezyme, Renozyme and Biotouch) and a Family 7 EG-I type endoglucanase (Endolase). The assay was carried out at 25°C. No enzyme control values have been subtracted from all data.

Table 1

Enzyme	A (4mg/L total; 1:1 primary : care ratio) Reducing sugars released (• M)
50% Endolase	11.4
100% Endolase	16.4
50% Carezyme	53.5
100% Carezyme	72.6
Carezyme + Endolase	108.5
50% Renozyme	67.0
100% Renozyme	83.9
Renozyme + Endolase	130.1
50% Biotouch	21.7
100% Biotouch	51.1
Biotouch + Endolase	113.6

Table 2

	Reducing sugars released (• M)
1.14mg/L Endolase	19.5
1.74mg/L Endolase	20.9
0.6mg/L Renozyme	30.8
1.74mg/L Renozyme	57.6
0.6:1.14mg/L Renozyme:Endolase	90.0
0.6mg/L Biotouch	8.3
1.74mg/L Biotouch	26.1
0.6:1.14mg/L Biotouch:Endolase	56.9

Table 3

	Reducing sugars released (• M)
1.25mg/L Endolase	15.3
1.5mg/L Endolase	16.1
0.25mg/L Renozyme	9.3
1.5mg/L Renozyme	40.8
0.25:1.25mg/L Renozyme:Endolase	51.8
0.25mg/L Biotouch	0.0
1.5mg/L Biotouch	8.4
0.25:1.25mg/L Biotouch:Endolase	30.3

Table 4– (corresponds to Figure 4) showing synergistic release of reducing sugars from cotton linters by a 1:1 mix of two different Family 45 EG-V type endoglucanases (Carezyme and Biotouch) and a Family 5 EG-I type endoglucanase (Celluclean). The assay was carried out at 25°C. No enzyme control values have been subtracted from all data.

	Reducing sugars released (• M)
50% Celluclean	7.7
100% Celluclean	15.5
50% Carezyme	35.1
100% Carezyme	47.7
Carezyme + Celluclean	60.8
50% Biotouch	7.7
100% Biotouch	34.4
Biotouch + Celluclean	71.3

Conclusions

- A 1:1 mix of the two types of cellulase according to the invention was found to act synergistically in the release of reducing sugars from cotton linters at 25°C. The first cellulase component in this mix is a primary care enzyme, being a Family 45 EG-V type endoglucanase.

- The secondary cellulase component is an auxiliary care enzyme with low activity towards cotton linters. Family 45 endoglucanase. The action of the secondary cellulase is thought to reduce the pool of soluble oligo- / poly-saccharides which effectively inhibit the activity of the Family 45 endoglucanase towards the insoluble cotton linters.

Exemplary Fabric Treatment Compositons (% wt)

Alkybenzenesulfonic (LAS) acid	12.8
NaOH	2.58
C12-14 alcohol 7-ethoxolate	8.6
C12-13 alcohol 3-ethoxolate Sulphate Na salt	8.6
Citric acid	2.9
C12-18 fatty acid	5
Total Enzymes according to the invention	1
Other enzymes (e.g. amylase and/or protease)	1
Triethanolamine	3.1
Sequestrant (Dequest 2066)	0.5
Fluorescor	0.3
Propylene glycol	8.6
Glycerol	4.78
Perfume	1.6
Water	22.1
Perfume, dyes, miscellaneous minors	balance.

In further embodiments, the enzymes of the invention are included at 0.02 wt % .

The viscosity of the above exemplary composition is 2700 cps at 20 s^{-1}. However, lower viscosity formulations are possible.

Unless stated otherwise, all proportions are given in weight percent by weight of the total composition.

It is of course to be understood that the invention is not intended to be restricted to the details of the above embodiment which are described by way of example only.

CLAIMS

1. A low temperature active enzymatic fabric care composition comprising the combination of one or more primary care enzymes and one or more auxiliary care enzymes.

2. A low temperature active enzymatic fabric care composition according to any preceding claim, wherein the total amount f enzymes of the invention ie. the total amount of the primary care enzyme and the auxiliary care enzyme is 0.01 – 1.75 mg per Litre of the wash liquor.

3. A low temperature active enzymatic fabric care composition according to any preceding claim, wherein the total amount of enzymes of the invention ie. the total amount of the primary care enzyme and the auxiliary care enzyme is 0.1 – 84 mg per dose of total composition.

4. A low temperature active enzymatic fabric care composition according to any preceding claim wherein the auxiliary care enzyme is present either in equal or, more preferably greater amounts than the primary care enzyme.

5. A low temperature active enzymatic fabric care composition according to any preceding claim wherein the primary care enzyme and the auxiliary care enzyme are present in a weight ratio of enzyme protein in the range of from 1:1 to 1:29, more preferably 1:1 to 1:9 more preferably 1:5, more preferably 1:1.9. The preferred ranges of the invention exclude 1:30, 1:10 and 1:2.

6. A low temperature active enzymatic fabric care composition according to any preceding claim wherein the one or more primary care enzymes comprises one or more enzymes of the Glycoside Hydrolase Family 45.

7. A low temperature active enzymatic fabric care composition according to any preceding claim wherein one or more primary care enzymes comprises one or cellulolytic enzymes active in restoring colour to cotton or cotton-based fabrics by removal of fuzz and pills from the surface of the fabric.

8. A low temperature active enzymatic fabric care composition according to any preceding claim wherein one or more auxiliary care enzymes comprise one or more glycosyl hydrolases of Family 5 and/or Family 7.

9. A low temperature enzymatic fabric care process comprising the step of treating a fabric with the composition of any preceding claim.

10. Use of an auxiliary care enzyme in combination with a primary care enzyme in the treatment of fabrics for a care benefit.

11. Use of a low temperature active enzymatic fabric care composition comprising the combination of one or more primary care enzymes and one or more auxiliary care enzymes, in the treatment of fabrics for a care benefit.

Fig. 1

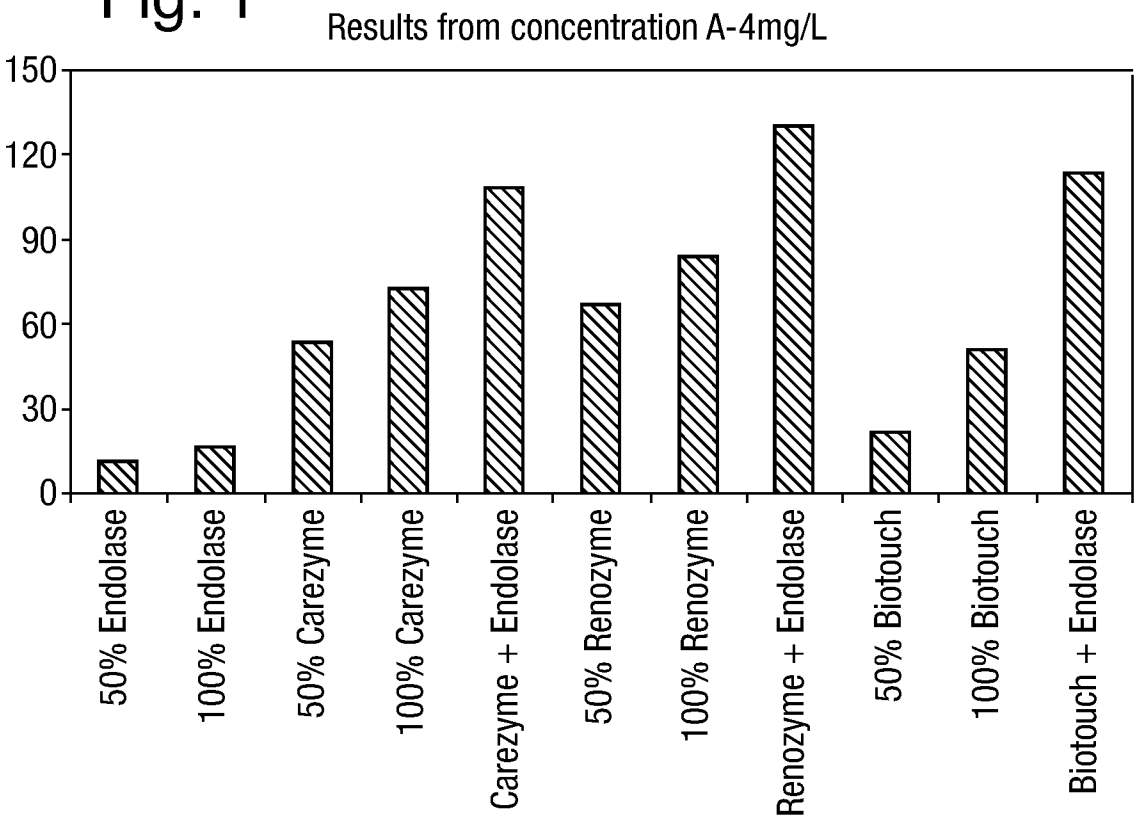

Results from concentration A-4mg/L
150
120
90
60
30
0
50% Endolase
100% Endolase
50% Carezyme
100% Carezyme
Carezyme + Endolase
50% Renozyme
100% Renozyme
Renozyme + Endolase
50% Biotouch
100% Biotouch
Biotouch + Endolase

Fig. 2

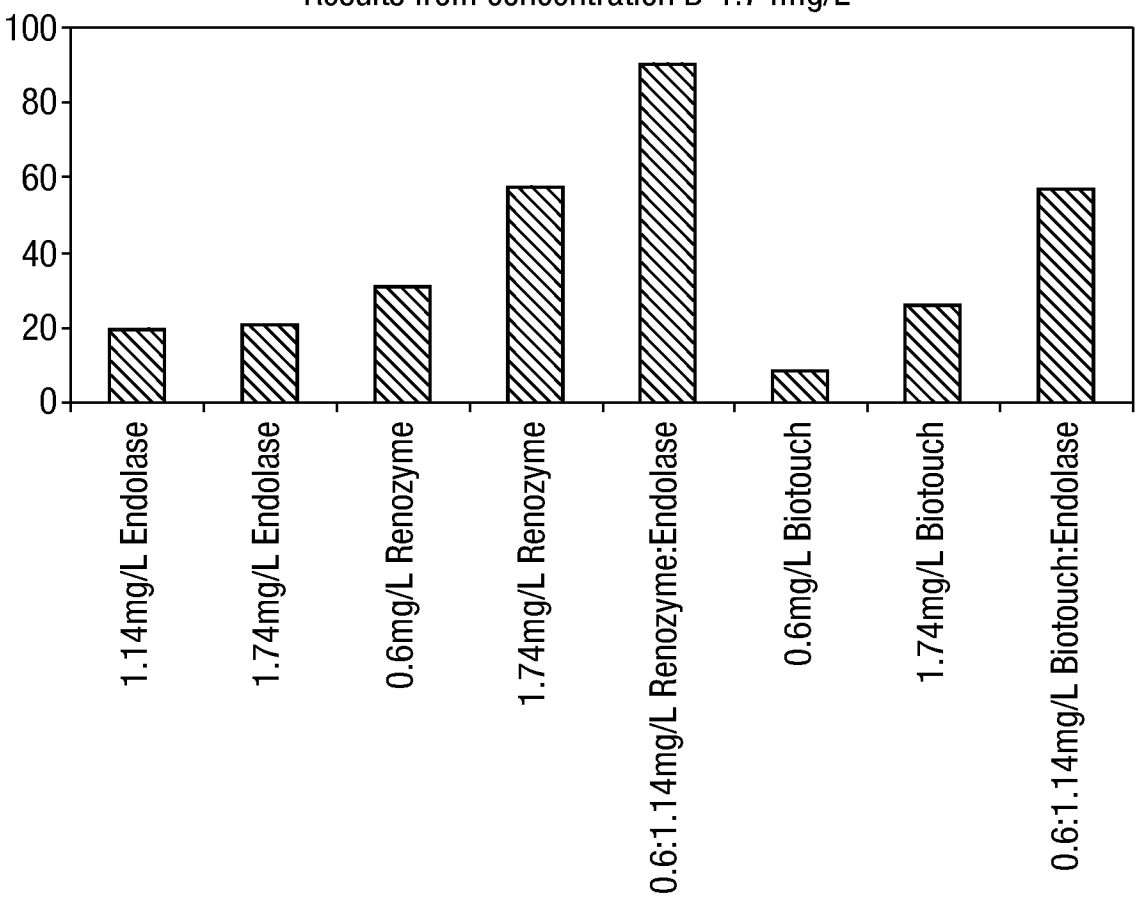

Results from concentration B-1.74mg/L
100
80
60
40
20
0
1.14mg/L Endolase
1.74mg/L Endolase
0.6mg/L Renozyme
1.74mg/L Renozyme
0.6:1.14mg/L Renozyme:Endolase
0.6mg/L Biotouch
1.74mg/L Biotouch
0.6:1.14mg/L Biotouch:Endolase

Fig. 3

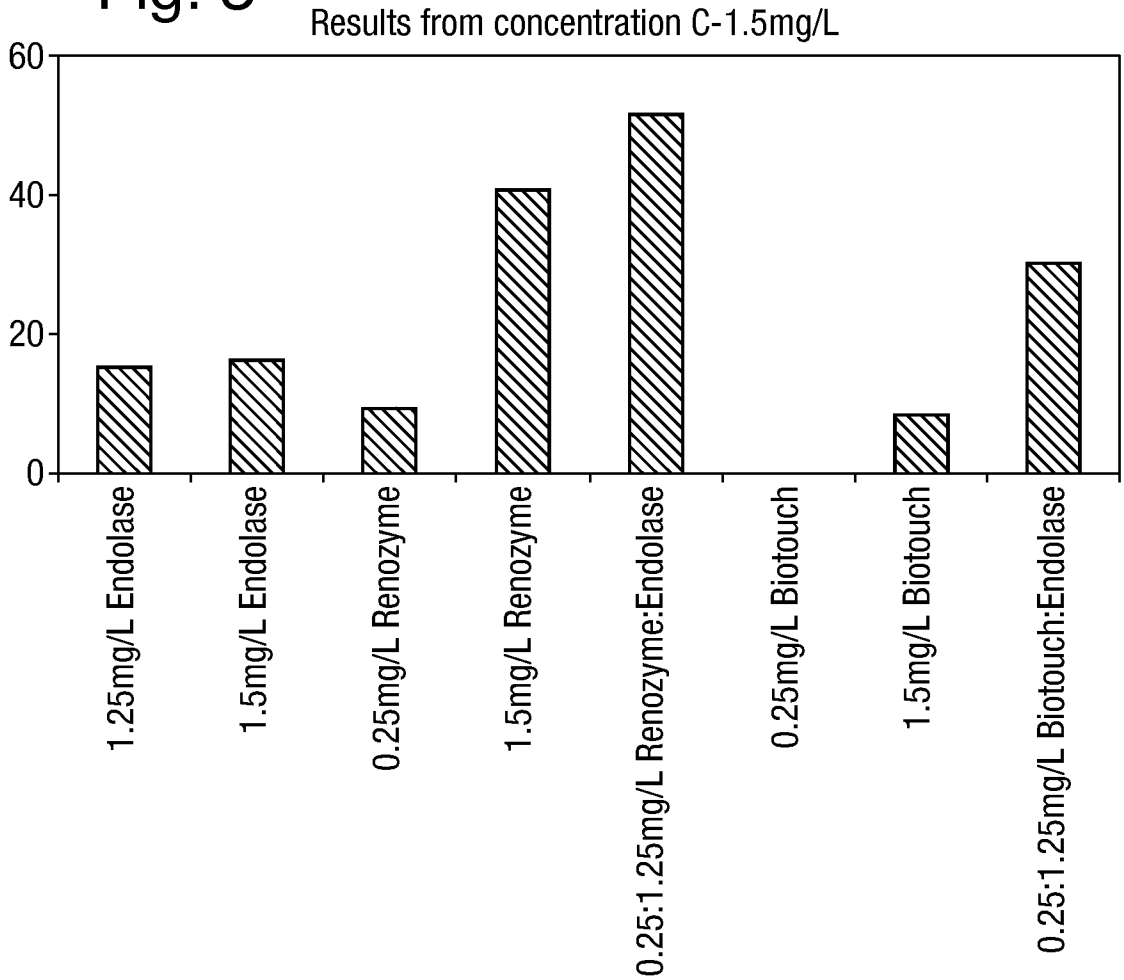
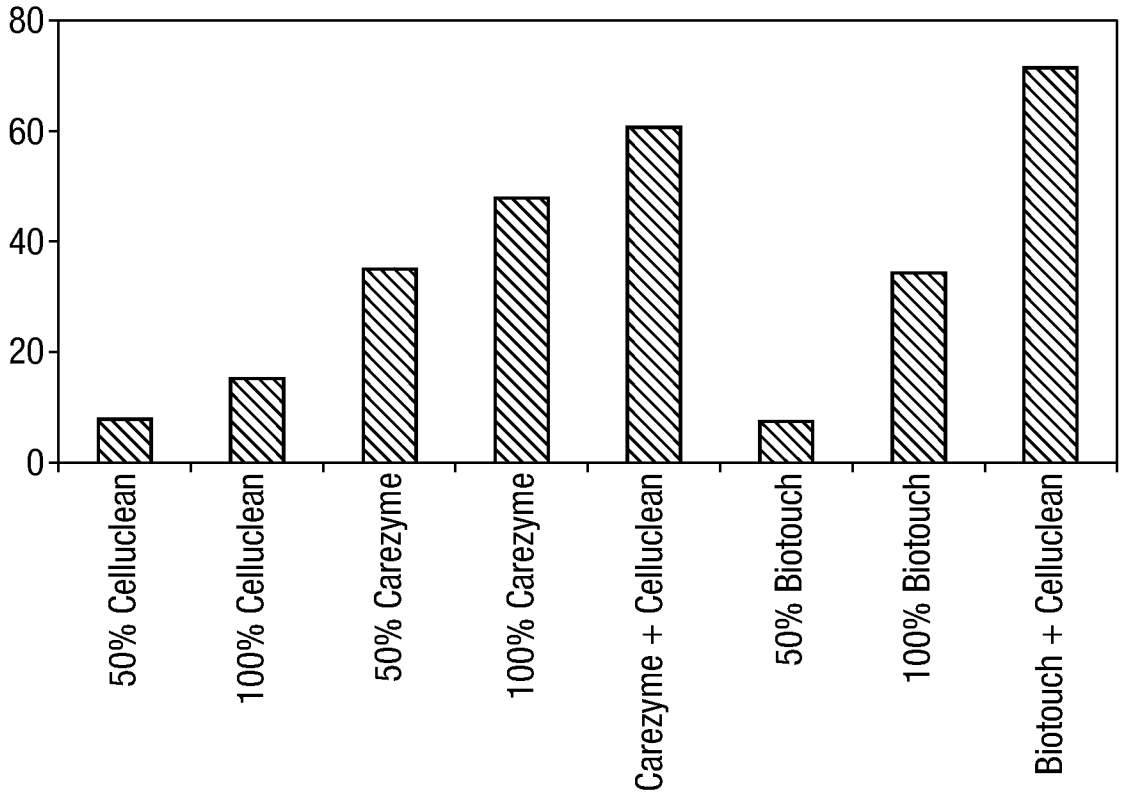

Results from concentration C-1.5mg/L
60
40
20
0
1.25mg/L Endolase
1.5mg/L Endolase
0.25mg/L Renozyme
1.5mg/L Renozyme
0.25:1.25mg/L Renozyme:Endolase
0.25mg/L Biotouch
1.5mg/L Biotouch
0.25:1.25mg/L Biotouch:Endolase

Fig. 4


80
60
40
20
0
50% Celluclean
100% Celluclean
50% Carezyme
100% Carezyme
Carezyme + Celluclean
50% Biotouch
100% Biotouch
Biotouch + Celluclean

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/059527

A. CLASSIFICATION OF SUBJECT MATTER
INV. C11D3/386 D06M16/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C11D D06M

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	L.P. WALKER ET AL: "Fragmentation of Cellulose by the Major Thermomonospora fusca Cellulases, Trichoderma reesei CBHI, and Their Mixtures", BIOTECHNOLOGY AND BIOENGINEERING, vol. 40, 1 January 1992 (1992-01-01), pages 1019-1026, XP055051887,	1
Y	the whole document	2-9

X	WO 00/42146 A1 (PROCTER & GAMBLE [US]; SHOWELL MICHAEL STANFORD [US]; ZHU YONG [US]; W) 20 July 2000 (2000-07-20)	1,10,11
Y	Abstract; page 4	2-9

X	US 5 688 290 A (BJORK NANCY [US] ET AL) 18 November 1997 (1997-11-18)	1,10,11
Y	Abstract; column 2 - column 3	2-9

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 May 2013	05/06/2013

Name and mailing address of the ISA/	Authorized officer
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1

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/059527

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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