

(12) PATENT
(19) AUSTRALIAN PATENT OFFICE

(11) Application No. AU 199919373 B2
(10) Patent No. 745104

(54) Title
Granule with hydrated barrier material

(51)⁷ International Patent Classification(s)
C11D 003/386 C11D 003/38

(21) Application No: **199919373**

(22) Application Date: **1998.12.21**

(87) WIPO No: **WO99/32595**

(30) Priority Data

(31) Number	(32) Date	(33) Country
60/068382	1997.12.20	US

(43) Publication Date : **1999.07.12**

(43) Publication Journal Date : **1999.09.16**

(44) Accepted Journal Date : **2002.03.14**

(71) Applicant(s)
Genencor International, Inc.

(72) Inventor(s)
Nathaniel T Becker; Robert I. Christensen Jr.; Alfred L. Gaertner; Mahmood M. Ghani; Douglas A. Dale

(74) Agent/Attorney
PHILLIPS ORMONDE and FITZPATRICK, 367 Collins Street, MELBOURNE VIC 3000

(56) Related Art
US 4689297
US 5698510
US 4417994

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶: C11D 3/386, 3/38	A1	(11) International Publication Number: WO 99/32595 (43) International Publication Date: 1 July 1999 (01.07.99)
(21) International Application Number: PCT/US98/27214 (22) International Filing Date: 21 December 1998 (21.12.98) (30) Priority Data: 60/068,382 20 December 1997 (20.12.97) US (71) Applicant (for all designated States except US): GENENCOR INTERNATIONAL, INC. [US/US]; 4 Cambridge Place, 1870 South Winton Road, Rochester, NY 14618 (US). <i>925 Page Mill Road, Palo Alto, CA 94304 USA.</i> (72) Inventors; and (75) Inventors/Applicants (for US only): BECKER, Nathaniel, T. [US/US]; 2116 Hillside Drive, Burlingame, CA 94010 (US). CHRISTENSEN, Robert, I., Jr. [US/US]; 2156 Blue Jay Circle, Pinole, CA 94564 (US). GAERTNER, Alfred, L. [DE/US]; 432 Maple Avenue, San Bruno, CA 94066 (US). GHANI, Mahmood, M. [US/US]; 870 Alcosta Drive, Milpitas, CA 95035 (US). DALE, Douglas, A. [US/US]; 1135 Encanto Way, Pacifica, CA 94044 (US). (74) Agent: ANDERSON, Kirsten, A.; Genencor International, Inc., 925 Page Mill Road, Palo Alto, CA 94304-1013 (US).		(81) Designated States: AL, AM, AT, AT (Utility model), AU (Petty patent), AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), EE, EE (Utility model), ES, FI, FI (Utility model), GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> IP AUSTRALIA 12 JUL 1999 RECEIVED
(54) Title: GRANULE WITH HYDRATED BARRIER MATERIAL (57) Abstract A granule having high stability and low dust is described. The granule includes a hydrated barrier material having moderate or high water activity. Also described are methods of producing the granules.		

GRANULE WITH HYDRATED BARRIER MATERIAL

Background of the Invention

5 Recently the use of enzymes, especially of microbial origin, has become more and more common. Enzymes are used in several industries including, for example, the starch industry, the dairy industry, and the detergent industry. It is well known in the detergent industry that the use of enzymes, particularly proteolytic enzymes, has created industrial hygiene concerns for detergent factory workers, particularly due to the health risks
10 associated with dustiness of the available enzymes.

Since the introduction of enzymes into the detergent business, many developments in the granulation and coating of enzymes have been offered by the industry. See for example the following patents relating to enzyme granulation:

U.S. Patent 4,106,991 describes an improved formation of enzyme granules by
15 including within the composition undergoing granulation, finely divided cellulose fibers in an amount of 2-40% w/w based on the dry weight of the whole composition. In addition, this patent describes that waxy substances can be used to coat the particles of the granulate.

U.S. Patent 4,689,297 describes enzyme containing particles which comprise a particulate, water dispersible core which is 150 - 2,000 microns in its longest dimension, a
20 uniform layer of enzyme around the core particle which amounts to 10%-35% by weight of the weight of the core particle, and a layer of macro-molecular, film-forming, water soluble or dispersible coating agent uniformly surrounding the enzyme layer wherein the combination of enzyme and coating agent is from 25-55% of the weight of the core particle. The core material described in this patent includes clay, a sugar crystal enclosed in layers of corn
25 starch which is coated with a layer of dextrin, agglomerated potato starch, particulate salt, agglomerated trisodium citrate, pan crystallized NaCl flakes, bentonite granules or prills, granules containing bentonite, Kaolin and diatomaceous earth or sodium citrate crystals. The film forming material may be a fatty acid ester, an alkoxylated alcohol, a polyvinyl alcohol or an ethoxylated alkylphenol.

30 U.S. Patent 4,740,469 describes an enzyme granular composition consisting essentially of from 1-35% by weight of an enzyme and from 0.5-30% by weight of a synthetic fibrous material having an average length of from 100-500 micron and a fineness in the range of from 0.05-0.7 denier, with the balance being an extender or filler. The granular composition may further comprise a molten waxy material, such as polyethylene
35 glycol, and optionally a colorant such as titanium dioxide.

U.S. Patent 5,254,283 describes a particulate material which has been coated with a continuous layer of a non-water soluble, warp size polymer. U.S. Patent 5,324,649

describes enzyme-containing granules having a core, an enzyme layer and an outer coating layer. The enzyme layer and, optionally, the core and outer coating layer contain a vinyl polymer.

WO 91/09941 describes an enzyme containing preparation whereby at
5 least 50% of the enzymatic activity is present in the preparation as enzyme crystals. The preparation can be either a slurry or a granulate.

WO 97/12958 discloses a microgranular enzyme composition. The granules are made by fluid-bed agglomeration which results in granules with numerous carrier or seed particles coated with enzyme and bound together by a
10 binder.

The above discussion of documents, acts, materials, devices, articles and the like is included in this specification solely for the purpose of providing a context for the present invention. It is not suggested or represented that any or all of these matters formed part of the prior art base or were common general
15 knowledge in the field relevant to the present invention as it existed in Australia before the priority date of each claim of this application.

However, even in light of these developments offered by the industry (as described above) there is a continuing need for low-dust enzyme granules which have additional beneficial characteristics. Additional beneficial
20 characteristics needed in the enzyme granulation industry are low-residue granule formulations (where low residue is defined as a reduced tendency to leave noticeable undissolved residues on clothes or other material), and improved stability formulations. Accomplishing all these desired characteristics simultaneously is a particularly challenging task since, for example, many
25 delayed release or low-dust agents such as fibrous cellulose or warp size polymers leave behind insoluble residues.

Therefore, it is desired to provide low-dust, low residue, highly soluble enzyme granules having increased stability. It is also desired to provide processes which afford the formation of such improved granules.



Summary of the Invention

One embodiment of the present invention is a granule that includes a protein core and a hydrated barrier material with moderate or high water activity. The hydrated barrier material can be in one or more layers and/or can
5 be included in the protein core.

A further embodiment of the present invention is a granule that includes an enzyme core and a hydrated barrier material with moderate or high water activity. The hydrated barrier material can be in one or more layers and/or can be included in the enzyme core.

10 Another embodiment is a method of producing the above granule.

Detailed Description of the Invention

The present invention provides a granule with improved stability having low dust. The granule includes a protein core and a hydrated barrier material
15 with moderate or high water activity.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100

101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200



-- 3 --

A "protein core" or an "enzyme core" can be homogenous such as that described in US patent application 08/995,457 or layered as described in U. S. Pat. No. 5,324,649.

Proteins that are within the scope of the present invention include pharmaceutically important proteins such as hormones or other therapeutic proteins and industrially important
5 proteins such as enzymes.

Any enzyme or combination of enzymes may be used in the present invention. Preferred enzymes include those enzymes capable of hydrolyzing substrates, e.g. stains. These enzymes are known as hydrolases which include, but are not limited to, proteases (bacterial, fungal, acid, neutral or alkaline), amylases (alpha or beta), lipases, cellulases and
10 mixtures thereof. Particularly preferred enzymes are subtilisins and cellulases. Most preferred are subtilisins such as described in U.S. Patent 4,760,025, EP Patent 130 756 B1 and EP Patent Application WO 91/06637, which are incorporated herein by reference, and cellulases such as Multifect L250™ and Puradax™, commercially available from Genencor International. Other enzymes that can be used in the present invention include oxidases,
15 transferases, dehydratases, reductases, hemicellulases and isomerases.

As noted, the barrier material can be coated over the protein core in one or more layers or made part of the protein core in order to insulate or to impede transport of water and inactivating substances to the protein. When the barrier material is part of the protein core, it can be dispersed throughout the core or as a layer in the core.

20 Suitable hydrated barrier materials with moderate or high water activity can include salts of an inorganic or organic acid, sugars, polysaccharides, lipids, proteins or synthetic polymers; preferably salts.

The term "water activity", symbolized a_w , refers to the fractional relative humidity of an atmosphere in equilibrium with a solid or liquid phase material, i.e., the ratio of the partial
25 pressure of water vapor to that present above pure water at the same temperature. In all phases between which water distribution has reached equilibrium, it is by definition equal. The term "relative humidity" is generally used to describe the water in the atmosphere or gas phase in equilibrium with the solid, and is expressed as a percentage, with 100% as the relative humidity of pure water in a closed system. Thus, for any water activity value, there
30 is a corresponding relative humidity given by $\%RH = 100 * a_w$.

Water activity can be readily measured by methods known in the art, typically by placing a sample of the material inside the temperature-controlled chamber of a water activity meter, such as the Water Activity System Model D2100 available from Rotronic Instrument Corp. (Huntington, NY), and allowing the measurement to reach equilibrium as
35 indicated on the display.

A "hydrated" barrier material contains water in a free or bound form, or a combination of the two. The water of hydration can be added either during or after the coating process. The degree of hydration will be a function of the material itself and the temperature, humidity and drying conditions under which it is applied.

5 "Moderate or high" water activity includes a water activity of at least 0.25, preferably greater than 0.30, most preferably greater than 0.35. The water activity referred to herein is that of the granule itself once it has the barrier material--but no further coatings--coated onto it. Further coatings may mask accurate measurement of the water activity of the barrier material as a distinct layer.

10 Without wishing to be bound by theory, it is expected that materials with a water activity greater than 0.25 will have a reduced driving force for picking up water under storage conditions in which the relative humidity is greater than 25%. Most climates have relative humidities above 25%. Many detergents have water activities in the range of about 0.3 to 0.4. If the water activity of the granule is actually higher than that of the surrounding
15 detergent or storage climate, the driving force for pick up of water by the granule should be eliminated, and in fact water may be given up by the granule to its surroundings. Even if the water activity of the granule is lower than that of the detergent or the corresponding relative humidity, the water present in the barrier layer would act as a shield limiting the amount of water and hence in activating substances being picked up by the granule and affecting the
20 protein core.

In the case of salt hydrates, the hydrated material is a crystalline salt hydrate with bound water(s) of crystallization. The hydrate should be chosen and applied in a manner such that the resulting coated granule will have a water activity in excess of 0.25, or as high as possible while still providing a granule which is dry to the touch. By applying a salt
25 hydrate, or any other suitable hydrated barrier material, in such a manner, as noted above, one expects that this would eliminate any driving force for further uptake of water by the granule. As an important consequence, the driving force for transport of substances which may be detrimental to enzyme activity, such as perborate or peroxide anion, is removed. Without water as a vehicle, these substances are less likely to penetrate the enzyme core.
30 Empirical data demonstrates that enzyme activity in the granule is substantially enhanced by coating the enzyme core with stable salt hydrates.

Preferred salts include magnesium sulfate heptahydrate, zinc sulfate heptahydrate, copper sulfate pentahydrate, sodium phosphate dibasic heptahydrate, magnesium nitrate hexahydrate, sodium borate decahydrate, sodium citrate dihydrate and magnesium acetate
35 tetrahydrate.

The granules of the present invention can also comprise one or more coating layers. For example, such coating layers may be one or more intermediate coating layers, or such coating layers may be one or more outside coating layers or a combination thereof. Coating layers may serve any of a
5 number of functions in a granule composition, depending on the end use of the granule. For example, coatings may render the protein resistant to oxidation by bleach, bring about the desirable rates of dissolution upon introduction of the granule into an aqueous medium, or provide a barrier against ambient moisture in order to enhance the storage stability of the enzyme and reduce the
10 possibility of microbial growth within the granule.

Throughout the description and claims of the specification the word "comprise" and variations of the word, such as "comprising" and "comprises", is not intended to exclude other additives, components, integers or steps.

13

14



Suitable coatings include polyvinyl alcohol (PVA), polyvinyl pyrrolidone (PVP), cellulose derivatives such as methylcellulose, hydroxypropylmethyl cellulose, hydroxycellulose, ethylcellulose, carboxymethyl cellulose, hydroxypropyl cellulose, polyethylene glycol, polyethylene oxide, chitosan, gum arabic, xanthan, carrageenan, latex polymers, and enteric coatings. Furthermore, coating agents may be used in conjunction with other active agents of the same or different categories.

Suitable PVAs for incorporation in the coating layer(s) of the granule include partially hydrolyzed, fully hydrolyzed and intermediately hydrolyzed PVAs having low to high degrees of viscosity. Preferably, the outer coating layer comprises partially hydrolyzed PVA having low viscosity. Other vinyl polymers which may be useful include polyvinyl acetate and polyvinyl pyrrolidone. Useful copolymers include, for example, PVA-methylmethacrylate copolymer and PVP-PVA copolymer.

The coating layers of the present invention may further comprise one or more of the following: plasticizers, extenders, lubricants, pigments, and optionally additional enzymes. Suitable plasticizers useful in the coating layers of the present invention are plasticizers including, for example, polyols such as sugars, sugar alcohols, or polyethylene glycols (PEGs), urea, glycol, propylene glycol or other known plasticizers such as triethyl citrate, dibutyl or dimethyl phthalate or water. Suitable pigments useful in the coating layers of the present invention include, but are not limited to, finely divided whiteners such as titanium dioxide or calcium carbonate or colored pigments or dyes or a combination thereof. Preferably such pigments are low residue pigments upon dissolution. Suitable extenders include sugars such as sucrose or starch hydrolysates such as maltodextrin and corn syrup solids, clays such as kaolin and bentonite, and talc. Suitable lubricants include nonionic surfactants such as Neodol, tallow alcohols, fatty acids, fatty acid salts such as magnesium stearate and fatty acid esters.

The outer coating layer of the present invention preferably comprises between about 1-25% by weight of the coated granule.



-- 6 --

Adjunct ingredients may be added to the granules of the present invention. Adjunct ingredients may include: metallic salts; solubilizers; activators; antioxidants; dyes; inhibitors; binders; fragrances; enzyme protecting agents/scavengers such as ammonium sulfate, ammonium citrate, urea, guanidine hydrochloride, guanidine carbonate, guanidine sulfamate, thiourea dioxide, monoethanolamine, diethanolamine, triethanolamine, amino acids such as glycine, sodium glutamate and the like, proteins such as bovine serum albumin, casein and the like etc.; surfactants including anionic surfactants, ampholytic surfactants, nonionic surfactants, cationic surfactants and long-chain fatty acid salts; builders; alkalis or inorganic electrolytes; bleaching agents; bluing agents and fluorescent dyes and whiteners; and caking inhibitors.

The granules described herein may be made by methods known to those skilled in the art of enzyme granulation, including pan-coating, fluid-bed coating, fluid-bed agglomeration, prilling, disc granulation, spray drying, extrusion, centrifugal extrusion, spheronization, drum granulation, high shear agglomeration, or combinations of these techniques.

The following examples are representative and not intended to be limiting. One skilled in the art could choose other proteins, protein cores, enzymes, enzyme cores, seed particles, methods and coating agents based on the teachings herein.

Examples

Example 1. Stability of Magnesium Sulfate Coated Protease Granules

A. In a Deseret 60 fluidized bed coater, 54.1 kg of sucrose/starch non pareil seeds were charged and fluidized. Onto these cores, 75.8 kg of protease UF concentrate containing 62.9 g/kg subtilisin protease were sprayed under the following conditions. (Ranges indicate initial and final values over the course of the specified ramp time):

Ramp time:	80 minutes
Fluid feed rate	0.6-1.0 liter/min
Atomization pressure	75 psi
Inlet air temperature	85-92 degrees C
Outlet air temperature	50 degrees C
Fluidization air rate	18 m3/min

A solution of magnesium sulfate was prepared by adding 22.2 kg of magnesium sulfate heptahydrate into 22.2 kg of water, and this was sprayed onto the enzyme-coated cores under the following conditions in order to provide that 20% of the final granule would

-- 7 --

be magnesium sulfate heptahydrate, with care being taken to keep the bed temperature close to, but slightly below, 50 degrees C:

Ramp time: 40 minutes
Fluid feed rate 0.6-1.7 liter/min
Atomization pressure 45 psi
Inlet air temperature 70-84 degrees C
Outlet air temperature 48-50 degrees C
Fluidization air rate 18 m3/min

Finally, a polymer coating solution was prepared by dissolving 6.35 kg of Elvanol 51-05 polyvinyl alcohol, 7.94 kg titanium dioxide and 1.59 kg Neodol 23-6.5T nonionic surfactant in 50.12 kg water and spraying over the salt-coated enzyme cores under the following conditions:

Ramp time: 10 min, then constant for 100 min
Fluid feed rate 0.6 liter/min
Atomization pressure 75 psi
Inlet air temperature 50 degrees C
Outlet air temperature 75-80 degrees C
Fluidization air rate 18 m3/min

The harvested granules had an enzyme concentration of approximately 40 g/kg.

B. Accelerated Stability Test

The stability of many enzyme granules formulated into bleach-containing detergents is generally excellent, showing generally no more than about 10 to 20% loss in activity over 6 weeks storage at 30 to 37° C and 70% to 80% R.H. However, to aid in the development and screening of granular formulations, it is desirable to have an accelerated means of determining relative granule stability. The conditions of the accelerated stability test (AST) are far more severe than enzyme granules or detergents would ever encounter in realistic storage or transport. The AST is a "stress test" designed to discriminate differences between formulations which would otherwise not be evident for weeks or months.

In this test, a test detergent base was made from the following ingredients:

72% WFK-1 detergent base

(WFK, Forschungsinstitut fuer
Reinigungstechnologie e.V.,

-- 8 --

Krefeld, Germany)

25%	sodium perborate monohydrate	(Degussa Corp., Allendale Park, New Jersey.)
3%	TAED bleach activator (= tetraacetythylenediamine)	(Warwick International, Mostyn, UK)

For each enzyme sample to be tested, three identical tubes were prepared by adding 1 gram of the test base and 30 mg of enzyme granules to a 15 ml conical tube and mixed by inverting the capped tube 5-8 times by hand. A hole was drilled in the tube cap with a 1/16 inch drill bit. One of the three tubes was assayed immediately and the other two were stored in a humidity chamber set at 50° C and 70%R.H. One of the two stored tubes was assayed after 1 day of storage; the second, after 3 days of storage. Storage stability was reported for Day 1 and Day 3 by dividing the remaining activity by the original activity at Day 0, expressed as a percentage.

The enzyme activity was determined by adding to each tube 30 ml of 0.25M MES pH 5.5 buffer containing 20 µl Catalase HP L5000 (Genencor International, Rochester, NY) and incubating for 40 minutes to inactivate the perborate. After this, the enzyme was assayed by adding 10 µl of the test tube mixture and 10 µl of sAAPF protease substrate to 980 µl of 0.1M Tris pH 8.6, then incubating at 25°C over 3 minutes, and measuring the optical absorbance at 410 nm. The slope of the absorbance vs. time was then multiplied by the dilution factor and the known extinction coefficient for the specific protease to obtain an enzyme activity as concentration in mg/ml.

The process described in A above was repeated three more times, the only difference being that the outlet air temperature was controlled at a setpoint of 40, 60 and 70 degrees C in each of the three separate runs. Samples were removed from all four batches after the magnesium sulfate barrier coating had been applied, and water activities of the granules were measure in a Rotronic Water Activity System, as reported in Table 1. Two of the granules, after application of the final polymer coating, were placed in WFK-1 detergent formula and stored in tubes with drilled caps for three days at 50 degrees C and 70% relative humidity, according to the accelerated stability test method described above. Tubes were withdrawn from the humidity chamber and assayed after 1 day and 3 days. The percent retained activities are reported in Table 1. The results indicate the granules in which magnesium sulfate was coated at 50 degrees C outlet temperature are significantly more stable than those coated at 70 degrees C, and that the more stable granules had a water activity above 0.35, while the less stable granules had a significantly lower water activity.

Table 1. Stability of Magnesium Sulfate Coated Enzyme Granules

Outlet Temp (C)	A _w of MgSO ₄ Coated Protease Cores	Percent Retained Activity of Granules Stored in Bleach Detergent			
		0 days	1 day	3 days	
40	0.374				
50	0.409	100%	108%	97%	
60	0.140				
70	0.165	100%	94%	63%	

Example 2. Stability of Sodium Citrate Coated Protease Granules

5 A. In a Vector 60 coater, 25 kg of sucrose/starch nonpareil seeds were fluidized and 30.9 kg of subtilisin protease concentrate with a concentration of 65.9 g/L and 18.3% total solids were sprayed onto the fluidized cores under the following conditions:

Ramp time: 55 minutes
 10 Fluid feed rate 0.5-0.9 liter/min
 Atomization pressure 75 psi
 Inlet air temperature 60-95 degrees C
 Outlet air temperature 50 degrees C
 Fluidization air rate 24 m³/min

15 A solution of trisodium citrate was prepared by adding 13.2 kg of trisodium citrate dihydrate into 19.7 kg of water, and this was sprayed onto the enzyme-coated cores under the following conditions in order to provide that 25% of the final granule would be trisodium citrate dihydrate, with care being taken to keep the bed temperature close to 50 degrees C:

20 Ramp time: 23 minutes
 Fluid feed rate 0.6-1.9 liter/min
 Atomization pressure 75 psi
 Inlet air temperature 60-95 degrees C
 25 Outlet air temperature 50 degrees C
 Fluidization air rate 24 m³/min

Finally, a polymer coating solution was prepared by dissolving 2.94 kg Methocel HPMC, 0.98 kg polyethylene glycol, molecular weight 600, 2.06 kg titanium dioxide and 0.59 kg

-- 10 --

Neodol 23-6.5T nonionic surfactant in 55.88 kg water and spraying over the salt-coated enzyme cores under the following conditions:

Ramp time: 10 min, then 80 minutes constant

Fluid feed rate 0.5 - 0.7 liter/min

Atomization pressure 75 psi

Inlet air temperature 75-80 degrees C

Outlet air temperature 60 degrees C

Fluidization air rate 18 m³/min

The harvested granules had a weight of 49.5 kg and an enzyme concentration of approximately 40 g/kg.

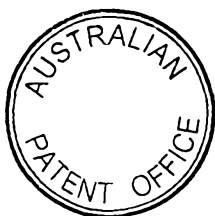
B. The above process was repeated under the same conditions, but the outlet air temperature was controlled at a setpoint of 70 degrees C. Samples were removed from both batches after the sodium citrate barrier coating had been applied, and water activities of the granules were measure in a Rotronic Water Activity System, as reported in Table 2. The two granules, after application of the final polymer coating, were placed in an automatic dish detergent base and stored in sealed tubes for 84 days at 37 degrees C. Tubes were withdrawn from the humidity chamber and assayed after 14, 42 and 84 days. The percent retained activities are reported in Table 2. The results indicate the granules in which sodium citrate was coated at 50 degrees C outlet temperature are significantly more stable than those coated at 70 degrees C, and that the more stable granules had a water activity above 0.25, while the less stable granules had a significantly lower water activity.

Table 2. Stability of Sodium Citrate Coated Enzyme Granules

Outlet Temp (C)	A _w of Na ₃ Citrate Coated Protease Cores	Percent Retained Activity of Granules Stored in Bleach Detergent			
		0 days	14 days	42 days	84 days
55	0.272	100%	90%	89%	87%
70	0.059	100%	86%	81%	75%

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A granule comprising an protein core and a barrier material, wherein the barrier material comprises a hydrated barrier material with moderate or high water activity.
5
2. The granule of claim 1, wherein the barrier material is a salt.
3. The granule of claim 2, wherein the salt is selected from the group
10 consisting of magnesium sulfate heptahydrate, zinc sulfate heptahydrate, copper sulfate pentahydrate, sodium phosphate dibasic heptahydrate, magnesium nitrate hexahydrate, sodium borate decahydrate, sodium citrate dihydrate and magnesium acetate tetrahydrate.
- 15 4. The granule of any one of claims 1 to 3, wherein the barrier material is part of the protein core.
5. The granule of any one of claims 1 to 4, wherein the barrier material is coated over the protein core.
20
6. The granule of any one of claims 1 to 5, further comprising a layer of material between the protein core and the barrier material.
7. The granule of any one of claims 1 to 6, further comprising a layer of
25 material over the barrier layer and protein core.
8. The granule of any one of claims 1 to 7, wherein the protein is an enzyme.
9. The granule of any one of claims 1 to 8, wherein the water activity is
30 greater than 0.25.



10. A method of producing a granule comprising:

- a) providing a protein core;
- b) coating a hydrated barrier material with moderate or high water activity onto the protein core.

5

11. The method of claim 10, further comprising a coating over the barrier material.

12. A granule according to claim 1, substantially as hereinbefore described
10 with reference to any one of the examples.

13. A method according to claim 10, substantially as hereinbefore described with reference to any one of the examples.

15 DATED : 5 February, 2001

PHILLIPS ORMONDE & FITZPATRICK

Attorneys For:

GENENCOR INTERNATIONAL, INC.

20

