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Declarations under Rule 4.17:

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

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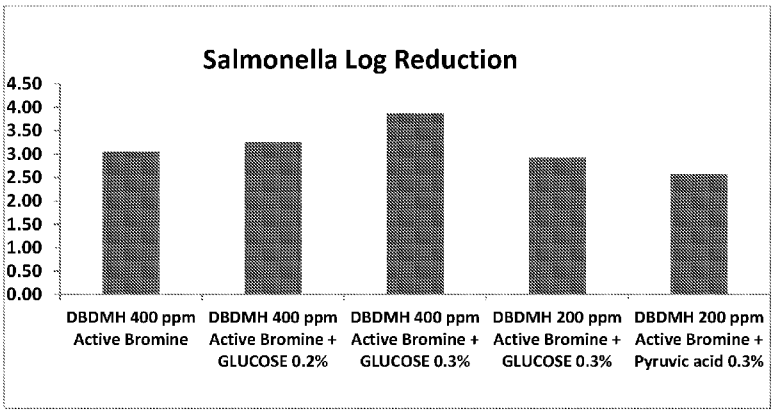
— *with international search report (Art. 21(3))*

(54) Title: ANTIMICROBIAL COMPOSITIONS CONTAINING ACTIVE HALOGEN COMPOUND FOR DISINFECTION OF FOOD PRODUCTS

Figure 1



(57) Abstract: This invention provides compositions suitable for reducing the amount of bacteria associated with a food product, said composition comprising a biocide and a non- biocidal agent, wherein the composition is capable of eliminating at least 25% more of the bacteria than the biocide alone would eliminate; and further provides methods for reducing the amount of bacteria asso - ciated with a food product using such compositions.

WO 2016/182684 A1

ANTIMICROBIAL COMPOSITIONS CONTAINING ACTIVE HALOGEN COMPOUND FOR DISINFECTION OF FOOD PRODUCTS

BACKGROUND

[0001] This invention relates to biocide compositions for use in the food industry. In particular, this invention relates to such biocide compositions having enhanced efficiency compared to the biocide by itself.

[0002] Food safety is a fundamental concern for consumers of meat and poultry. Two genera of bacteria, *Salmonella*, a gram-negative facultative rod-shaped bacterium in the family *Enterobacteriaceae* and *Campylobacter*, a gram-negative spiral or cork-screw bacterium in the family *Campylobacteriaceae* cause the majority of illnesses and deaths associated with the consumption of contaminated meat and poultry products. Antimicrobial interventions are a necessary step for food industries to ensure that the products they are selling to consumers are safe. The United States Department of Agriculture Food Safety and Inspection Service (USDA FSIS) provides a list of substances that may be used in the production of meat, poultry and egg products. The USDA FSIS list of approved substances includes organic acids such as acetic acid, citric acid and lactic acid, mixtures and blends of peroxyacetic acid, hydrogen peroxide, acetic acid and diphosphonic acids such as 1-hydroxyethylidene-1,1-diphosphonic acid (HEDP), as well as halogenated compounds such as acidified sodium chlorite, calcium hypochlorite, cetylpyridium chloride, chlorine dioxide, chlorine gas, sodium hypochlorite and hypobromous acid. The halogenated compound, 1,3-dibromo-5,5-dimethylhydantoin (DBDMH) is also a common biocide used in meat and poultry processing facilities. When dissolved in water, DBDMH combines with water to form hypobromous acid (HOBr), which is the active ingredient of this biocide. These biocides are used in varying stages of meat and poultry processes. They are used on beef, swine, goat and sheep carcasses, parts and organs, as well as whole or eviscerated poultry carcasses and poultry parts. They are used in sprays or process waters including poultry chiller waters for washing, rinsing or cooling. They are also used in

water supplied to ice machines to make ice that is added to the poultry chill tanks or added to containers to extend shelf life of product.

[0003] Bacteria have a number of survival techniques to help them thrive in adverse conditions such as in the presence of biocides. Some form biofilms, a group of cells that adhere together by forming an extracellular polymeric substance. Other specialized bacteria are able to become dormant under stress and form spores which are resistant to both chemical and physical adversities. Often bacteria that contaminate food are found in biofilm communities which are thought to contain cells in a spectrum of growth states from rapidly growing to non-growing, metabolically dormant cells. Growth retardation is particularly true in the case of the low, suboptimal temperature environment of the chill tank used in poultry processing. Phenotypically, these bacteria resemble the phenomenon of persister cells in that they become less susceptible to antibacterial challenges by temporarily shutting down their metabolism, ceasing to replicate and becoming dormant. In an article published in the journal "Nature", Kyle R. Allison et al., investigated the use of metabolites such as glucose, mannitol and fructose, as well as pyruvate in combination with select antibiotics to eradicate bacterial persisters in both Gram-negative bacteria such as *Escherichia coli* and Gram-positive bacteria such as *Staphylococcus aureus*. The results are promising for the therapeutic use of this combination to eradicate antibiotic resistant persisters, but non-therapeutic uses in food-producing animals are currently being phased out by the United State Food and Drug Administration.

[0004] Therefore there is a need for a biocide composition and a method of using such that is capable of achieving good efficacy at a relatively low level of biocide for use in the food industry.

THE INVENTION

[0005] This invention provides compositions suitable for reducing the amount of bacteria associated with a food product, said composition comprising a biocide and a non-biocidal agent, wherein: (I) the biocide comprises one or more 1,3-dibromo-5,5-dialkylhydantoins, one or more N,N'-bromochloro-5,5-dialkylhydantoins, chlorine dioxide, chlorine, hypochlorous acid that was formed by electrolysis, one or more alkali

metal hypochlorites, one or more alkaline earth metal hypochlorites, monochloramine, chlorinated cyanuric acids; or a bromine-based biocide formed in water from: (i) bromine chloride or bromine chloride and bromine, with or without conjoint use of chlorine, and (ii) overbased alkali metal salt of sulfamic acid and/or sulfamic acid, alkali metal base, and water, where (i) and (ii) are in relative proportions such that there is an atom ratio of nitrogen to active bromine greater than 0.93, and where in the bromide based biocide has a pH of greater than 7; or (iii) one or more bromide sources selected from ammonium bromide, hydrogen bromide, one or more alkali metal bromides, one or more alkaline earth metal bromides, and mixtures of any two or more of the foregoing, (iv) a chlorine source, (v) optionally at least one inorganic base, and (vi) optionally sulfamic acid and/or a metal salt of sulfamic acid; (II) the non-biocidal agent comprises D-glucose, D-erythrose, D-fructose, D-galactose, D-mannose, D-xylose, L-fucose, N-acetyl-D-glucosamine, N-acetyl-D-mannosamine, or pyruvate; (III) the composition is capable of eliminating at least about 25% more of the bacteria than the biocide alone would eliminate; and (IV) the food product comprises meat or poultry carcasses, meat or poultry parts, fruits, vegetables, or eggs.

[0006] Further, this invention provides methods of reducing the amount of bacteria associated with a food product, said methods comprising contacting the food product with a composition comprising a biocide and a non-biocidal agent, wherein: (I) the biocide comprises one or more 1,3-dibromo-5,5-dialkylhydantoins, one or more N,N'-bromochloro-5,5-dialkylhydantoins, chlorine dioxide, chlorine, hypochlorous acid that was formed by electrolysis, one or more alkali metal hypochlorites, one or more alkaline earth metal hypochlorites, monochloramine, chlorinated cyanuric acids; or a bromine-based biocide formed in water from: (i) bromine chloride or bromine chloride and bromine, with or without conjoint use of chlorine, and (ii) overbased alkali metal salt of sulfamic acid and/or sulfamic acid, alkali metal base, and water, where (i) and (ii) are in relative proportions such that there is an atom ratio of nitrogen to active bromine greater than 0.93, and where in the bromide based biocide has a pH of greater than 7; or (iii) one or more bromide sources selected from ammonium bromide, hydrogen bromide, one or more alkali metal bromides, one or more alkaline earth metal bromides, and mixtures of any two or more of the foregoing, (iv) a chlorine source, (v) optionally at

least one inorganic base, and (vi) optionally sulfamic acid and/or a metal salt of sulfamic acid; (II) the non-biocidal agent comprises D-glucose, D-erythrose, D-fructose, D-galactose, D-mannose, D-xylose, L-fucose, N-acetyl-D-glucosamine, N-acetyl-D-mannosamine, or pyruvate; (III) the composition eliminates at least about 25% more of the bacteria than the biocide alone would have eliminated, and (IV) the food product comprises meat or poultry carcasses, meat or poultry parts, fruits, vegetables, or eggs.

[0007] As used herein, a "biocide" means a chemical compound capable of reducing or eliminating bacterial associated with food products, and to "treat" or "treating" of a food product with a biocide means to contact at least a portion of the food product with the biocide.

DRAWINGS

[0008] Figure 1 is a graphical depiction of the logarithmic ("LOG") reduction in the number of colony forming units of *Salmonella* following the antimicrobial treatment of poultry parts using DBDMH as the biocide mixed with either D-glucose or pyruvate as the enhancer.

DETAILED DESCRIPTION

[0009] Biocides for use in compositions of this invention comprise (1) one or more 1,3-dibromo-5,5-dialkylhydantoins; (2) one or more N,N'-bromochloro-5,5-dialkylhydantoins; (3) chlorine dioxide; (4) chlorine; (5) hypochlorous acid formed by electrolysis; (6) one or more alkali metal hypochlorites and/or one or more alkaline earth metal hypochlorites; (7) monochloramine; (8) peracetic acid; (9) chlorinated cyanuric acids; or (10) a bromine-based biocide formed in water from

(A) (i) bromine chloride or bromine chloride and bromine, with or without conjoint use of chlorine, and (ii) overbased alkali metal salt of sulfamic acid and/or sulfamic acid, alkali metal base, and water, where (i) and (ii) are in relative proportions such that there is an atom ratio of nitrogen to active bromine greater than 0.93, and where in the bromide based biocide has a pH of greater than 7; or

(B) (i) one or more bromide sources selected from ammonium bromide, hydrogen bromide, one or more alkali metal bromides, one or more alkaline earth metal bromides, and mixtures of any two or more of the foregoing, (ii) a chlorine source, (iii) optionally at least one inorganic base, and (iv) optionally sulfamic acid and/or a metal salt of sulfamic acid.

Most preferred is the use of 1,3-dibromo-5,5-dimethylhydantoin. Methods for producing such biocides are known and reported in the literature. Preferably, such biocides are used in methods of this invention in microbiocidal amounts.

[00010] When contacting poultry carcasses or edible parts thereof with aqueous solutions formed from a 1,3-dihalo-5,5-dialkylhyantoin used pursuant to this invention, it is especially preferred to use in the chill tank, a microbiocidal effective amount of active bromine. Food Contact Substance Notification No. 1190 states that DBDMH is to be used at a level not to exceed that needed to provide 900 ppm available or active bromine in water or ice applied to meat products and 450 ppm available or active bromine in water or ice applied to poultry products. This active or available bromine is determinable by the DPD test procedure. This test procedure is designed for measuring active halogen concentrations.

[00011] Biocidal compositions of the present invention include a non-biocidal agent to enhance or improve the antimicrobial activity especially against Gram negative bacteria such as *Salmonella* or *Campylobacter*. This non-biocidal agent or enhancer is a component that enhances the effectiveness of the microbiocide such that when the composition without the enhancer component is used separately, the effectiveness of the microbiocide is not as good. The enhancing effect can be with respect to the level of kill, the speed of kill and/or the spectrum of microorganisms killed. An enhancer may be a synergist that when combined with the remainder of the composition, it causes the composition as a whole to display an activity greater than the sum of the activity of the composition without the enhancer component.

[00012] Non-biocidal agents are selected from monosaccharides that include D-glucose, D-erythrose, D-fructose, D-galactose, D-mannose, D-xylose, L-fucose, N-acetyl-D-glucosamine, N-acetyl-D-mannosamine and pyruvate. Glucose is a monosaccharide that is important in both prokaryotic and eukaryotic organisms. Cells

of both classes of organisms use carbohydrates such as glucose as a source of energy and it is also a metabolic intermediate. Glucose is one of the main products of photosynthesis and it is needed for cellular respiration in both prokaryotes and eukaryotes. Salmonella converts glucose into pyruvate in a process known as glycolysis which releases energy needed to fuel growth and reproduction. Pyruvate is an organic acid that plays a key role in bacterial metabolism.

[00013] Biocide compositions and methods of this invention are useful for treating food products such as, without limitation, meat or poultry carcasses, meat or poultry parts, fruits, vegetables, and eggs.

[00014] A mode of application of these food-grade biocides pursuant to this invention involves contacting the meat or poultry itself with a mixture of an aqueous solution containing the biocide and an effective amount of the enhancer in the chill tank.

EXAMPLES

CONTROL AND EXAMPLE 1

[00015] A study was conducted to determine the effectiveness of 1,3-dibromo-5,5-dimethylhydantoin (DBDMH) mixed with an enhancer, D-glucose, as a food-grade biocide, when used in the poultry chill tank for the control of microorganisms associated with raw poultry.

[00016] All tests were conducted in a laboratory-based poultry chill tank system, which was simulated in one gallon containers. In the "Control" treatment, a first container was filled with 2,100 ml of a solution of 1,3-dibromo-5,5-dimethylhydantoin (DBDMH) that contained 400 ppm of active bromine. The bacterial reduction from this Control treatment was compared to the other treatments to determine the effect of the additives. In the first example treatment, a second test group container was filled with 2,100 ml of a solution of DBDMH that contained 400 ppm of active bromine mixed with 0.2% D-glucose. Five bacterially challenged chicken drumsticks were immersed into each container. The containers were placed on an orbital shaker set at 200 rpm at 4°C. The total immersion time was 60 minutes. After 25 minutes of immersion, the solution in each container was replaced by emptying the solutions out of each container and

refilling each container with the same volume of the same solution that it had previously contained. After a total of 60 minutes of immersion, the drumsticks were each transferred to separate Ziploc bags. Each Ziploc bag was filled with 36 ml of a peptone rinse solution. The drumsticks were rinsed according to the Whole Bird Rinse Method as outlined in USDA Food Safety and Inspection Service Section 4.5.5. One ml of the rinsate was removed and serially diluted in peptone buffer, followed by plating on APC (Aerobic Plate Count) media for enumeration of colony forming units (CFU). A recovery control group consisted of 3 drumsticks that were sampled for enumeration of CFU right after the bacterial challenge. The example 1 treatment was conducted twice.

[00017] The logarithmic reduction of Salmonella for the Control treatment was determined by subtracting the average logarithm of CFU obtained from the drumsticks that had been immersed in the Control treatment solution from the average logarithm of CFU obtained from the drumsticks of the recovery control group. The logarithmic reduction of Salmonella for the first example treatment was determined by subtracting the average logarithm of CFU obtained from the drumsticks that had been immersed in the first example treatment solution from the average logarithm of CFU obtained from the drumsticks of the recovery control group. The results are tabulated in Table 1. The results show that the addition of 0.2 % D-glucose enhanced the efficacy of the biocide by 0.21 logarithmic units compared to the biocide by itself.

EXAMPLE 2

[00018] The procedure of Example 1 was repeated except that for the example 2 treatment, 0.3% D-glucose was mixed with 2100 ml of a solution of DBDMH containing 400 ppm of active bromine instead of 0.2%. The results are tabulated in Table 1. The results show that the addition of 0.3% D-glucose enhanced the efficacy of the biocide by 0.84 logarithmic units compared to the biocide by itself.

EXAMPLE 3

[00019] The procedure of Example 1 was repeated except that for the example 3 treatment, 0.3% D-glucose was mixed with 2100 ml of a solution of DBDMH containing 200 ppm of active bromine (sub-lethal dose) instead of 400 ppm. The results, tabulated

in Table 1, showed that even with half the biocide the log reduction was similar to the test using 400 ppm solution.

EXAMPLE 4

[00020] The procedure of Example 1 was repeated except that for the example 4 treatment, 0.3% pyruvic acid was mixed with 2100 ml of a solution of DBDMH containing 200 ppm of active bromine instead of D-glucose. The results are tabulated in Table 1. The logarithmic reduction of Salmonella for the example 4 treatment was significantly lower than using 400 ppm active bromine as the biocide.

TABLE 1

LOGARITHMIC "LOG" REDUCTION APC NORMALIZED AGAINST POSITIVE

		LOG REDUCTION
Control	**DBDMH 400 ppm Active Bromine**	3.04
Ex. 1	**DBDMH 400 ppm Active Bromine + D-Glucose 0.2%**	3.25
Ex. 2	**DBDMH 400 ppm Active Bromine + D-Glucose 0.3%**	3.88
Ex. 3	**DBDMH 200 ppm Active Bromine + D-Glucose 0.3%**	2.93
Ex. 4	**DBDMH 200 ppm Active Bromine + Pyruvate 0.3%**	2.57

[00021] It is to be understood that the reactants and components referred to by chemical name or formula anywhere in the specification or claims hereof, whether referred to in the singular or plural, are identified as they exist prior to being combined with or coming into contact with another substance referred to by chemical name or chemical type (e.g., another reactant, a solvent, or etc.). It matters not what chemical changes, transformations and/or reactions, if any, take place in the resulting combination or solution or reaction medium as such changes, transformations and/or

reactions are the natural result of bringing the specified reactants and/or components together under the conditions called for pursuant to this disclosure. Thus the reactants and components are identified as ingredients to be brought together in connection with performing a desired chemical reaction or in forming a combination to be used in conducting a desired reaction. Accordingly, even though the claims hereinafter may refer to substances, components and/or ingredients in the present tense ("comprises", "is", etc.), the reference is to the substance, component or ingredient as it existed at the time just before it was first contacted, combined, blended or mixed with one or more other substances, components and/or ingredients in accordance with the present disclosure. Whatever transformations, if any, which occur in situ as a reaction is conducted is what the claim is intended to cover. Thus the fact that a substance, component or ingredient may have lost its original identity through a chemical reaction or transformation during the course of contacting, combining, blending or mixing operations, if conducted in accordance with this disclosure and with the application of common sense and the ordinary skill of a chemist, is thus wholly immaterial for an accurate understanding and appreciation of the true meaning and substance of this disclosure and the claims thereof. As will be familiar to those skilled in the art, the terms "combined", "combining", and the like as used herein mean that the components that are "combined" or that one is "combining" are put into a container with each other. Likewise a "combination" of components means the components having been put together in a container.

[00022] While the present invention has been described in terms of one or more preferred embodiments, it is to be understood that other modifications may be made without departing from the scope of the invention, which is set forth in the claims below.

CLAIMS

What is claimed is:

1. A composition suitable for reducing the amount of bacteria associated with a food product said composition comprising a biocide and a non-biocidal agent, wherein:

- the biocide comprises one or more 1,3-dibromo-5,5-dialkylhydantoins, one or more N,N'-bromochloro-5,5-dialkylhydantoins, chlorine dioxide, chlorine, hypochlorous acid that was formed by electrolysis, one or more alkali metal hypochlorites, one or more alkaline earth metal hypochlorites, monochloramine, chlorinated cyanuric acids; or a bromine-based biocide formed in water from: (i) bromine chloride or bromine chloride and bromine, with or without conjoint use of chlorine, and (ii) overbased alkali metal salt of sulfamic acid and/or sulfamic acid, alkali metal base, and water, where (i) and (ii) are in relative proportions such that there is an atom ratio of nitrogen to active bromine greater than 0.93, and where in the bromide based biocide has a pH of greater than 7; or (iii) one or more bromide sources selected from ammonium bromide, hydrogen bromide, one or more alkali metal bromides, one or more alkaline earth metal bromides, and mixtures of any two or more of the foregoing, (iv) a chlorine source, (v) optionally at least one inorganic base, and (vi) optionally sulfamic acid and/or a metal salt of sulfamic acid ,

- the non-biocidal agent comprises D-glucose, D-erythrose, D-fructose, D-galactose, D-mannose, D-xylose, L-fucose, N-acetyl-D-glucosamine, N-acetyl-D-mannosamine, or pyruvate,

- the composition is capable of eliminating at least about 25% more of the bacteria than the biocide alone would eliminate, and

– the food product comprises meat or poultry carcasses, meat or poultry parts, fruits, vegetables, or eggs.

2. A method of reducing the amount of bacteria associated with a food product, said method comprising contacting the food product with a composition comprising a biocide and a non-biocidal agent, wherein:

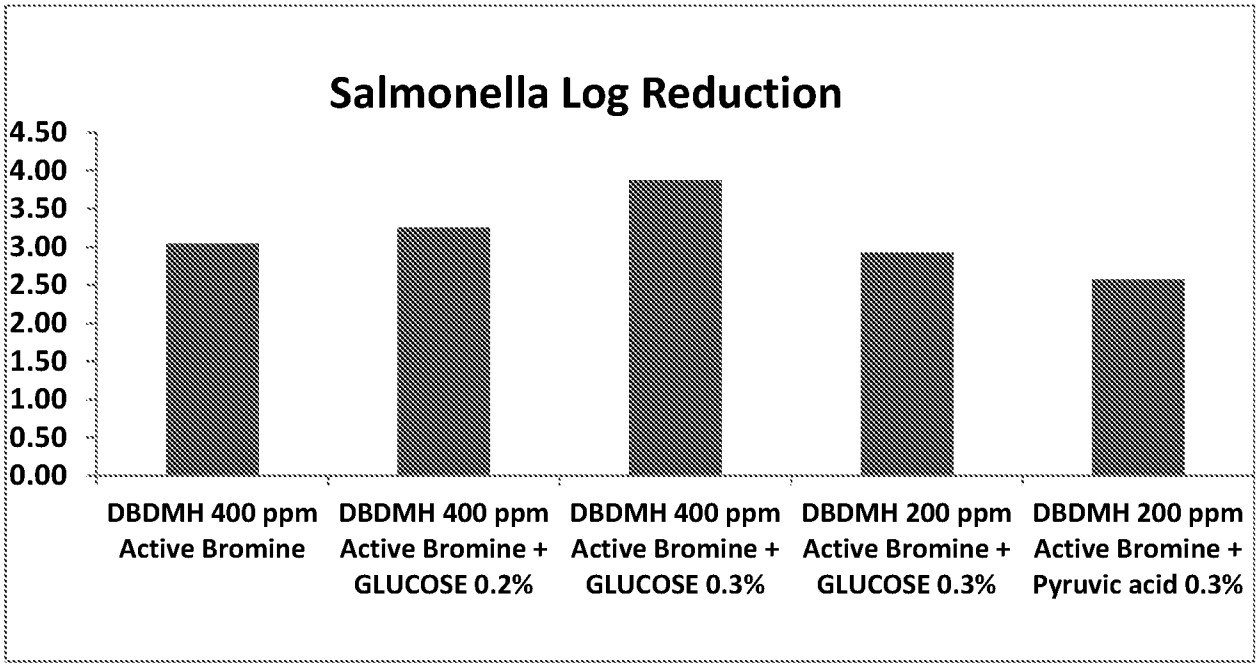
- the biocide comprises one or more 1,3-dibromo-5,5-dialkylhydantoins, one or more N,N'-bromochloro-5,5-dialkylhydantoins, chlorine dioxide, chlorine, hypochlorous acid that was formed by electrolysis, one or more alkali metal hypochlorites, one or more alkaline earth metal hypochlorites, monochloramine, chlorinated cyanuric acids; or a bromine-based biocide formed in water from: (i) bromine chloride or bromine chloride and bromine, with or without conjoint use of chlorine, and (ii) overbased alkali metal salt of sulfamic acid and/or sulfamic acid, alkali metal base, and water, where (i) and (ii) are in relative proportions such that there is an atom ratio of nitrogen to active bromine greater than 0.93, and where in the bromide based biocide has a pH of greater than 7; or (iii) one or more bromide sources selected from ammonium bromide, hydrogen bromide, one or more alkali metal bromides, one or more alkaline earth metal bromides, and mixtures of any two or more of the foregoing, (iv) a chlorine source, (v) optionally at least one inorganic base, and (vi) optionally sulfamic acid and/or a metal salt of sulfamic acid,

- the non-biocidal agent comprises D-glucose, D-erythrose, D-fructose, D-galactose, D-mannose, D-xylose, L-fucose, N-acetyl-D-glucosamine, N-acetyl-D-mannosamine, or pyruvate,

- the composition eliminates at least about 25% more of the bacteria than the biocide alone would have eliminated, and

– the food product comprises meat or poultry carcasses, meat or poultry parts, fruits, vegetables, or eggs.

Figure 1


Salmonella Log Reduction
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
DBDMH 400 ppm Active Bromine
DBDMH 400 ppm Active Bromine + GLUCOSE 0.2%
DBDMH 400 ppm Active Bromine + GLUCOSE 0.3%
DBDMH 200 ppm Active Bromine + GLUCOSE 0.3%
DBDMH 200 ppm Active Bromine + Pyruvic acid 0.3%

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/027670

A. CLASSIFICATION OF SUBJECT MATTER
INV. A23L3/358 A01N59/00 A01N35/02 A01P1/00
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)
A23L A01N

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, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2006/014527 A1 (CALLERAME JOSEPH [US]) 9 February 2006 (2006-02-09) paragraphs [0056], [0058], [0108]	1,2
X	US 4 861 514 A (HUTCHINGS RICHARD S [US]) 29 August 1989 (1989-08-29) column 10; example 5; table IX	1,2
X	WO 2006/047713 A2 (NOVOZYMES NORTH AMERICA INC [US]; NOVOZYMES AS [DK]; XU HUI [US]; PEDE) 4 May 2006 (2006-05-04) pages 4-5; example 1; table 1	1,2
X	CN 1 582 661 A (JINLING PETROCHEMICAL CO LTD L [CN]) 23 February 2005 (2005-02-23) page 4, paragraph 1	1,2

-/--

[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

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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
30 May 2016	07/06/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Sawicki, Marcin

INTERNATIONAL SEARCH REPORT

International application No
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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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X	US 2013/216293 A1 (GARNER DEWAIN [US] ET AL) 22 August 2013 (2013-08-22) paragraphs [0127] - [0131]; example 2	1,2
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2016/027670

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