



(51) International Patent Classification:

A01N 33/04 (2006.01) A01N 31/02 (2006.01)

A01N 47/44 (2006.01) A01P 1/00 (2006.01)

A01N 59/00 (2006.01) A01P 15/00 (2006.01)

(21) International Application Number:

PCT/FI2017/050367

(22) International Filing Date:

12 May 2017 (12.05.2017)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

20165408 13 May 2016 (13.05.2016) FI

(71) Applicant: KEMIRA OYJ [FI/FI]; Porkkalankatu 3, 00180 Helsinki (FI).

(72) Inventors: AHOLA, Juhana; Orminkuja 2 G 23, 01200 Vantaa (FI). KOLARI, Marko; Palttintie 22, 01260 Vantaa (FI).

(74) Agent: BERGGREN OY; PO Box 99, Tykistökatu 2-4 B, FI-20521 Turku (FI).

(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, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, 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, SA, 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, ST, 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, KM, ML, MR, NE, SN, TD, TG).

Published:

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

(54) Title: USE OF A COMPOSITION AND METHOD FOR REDUCING BACTERIAL SPORES IN PULP SUSPENSION

(57) Abstract: The invention relates to use of a composition comprising a cationic surfactant, which comprises a primary or secondary ammonium head group and a linear unsubstituted C12-alkyl tail, for inducing germination of bacterial endospores in pulp suspension for making of paper, board or the like. The invention relates also to a method for quantitatively reducing bacterial endospores in pulp suspension for making of paper, board or the like. The method comprises obtaining of aqueous pulp suspension, and administering the said composition to the pulp suspension. The cationic surfactant is allowed to interact with bacterial endospores in the pulp suspension. Germination of endospores and destroying microorganisms is thus induced.

USE OF A COMPOSITION AND METHOD FOR REDUCING BACTERIAL SPORES IN PULP SUSPENSION

The present invention relates to a use of a composition and a method for
5 quantitatively reducing bacterial spores in pulp suspension for making of paper,
board or the like according to the preambles of enclosed independent claims.

Bacterial cells are normally present in the aqueous environments of pulp mills as
well as paper and board mills. Bacterial growth in the process is commonly
10 monitored and limited by using various measures, e.g. feeding of biocides into the
process. However, certain bacterial cells form endospores, which are highly
resistant to typical bacterial destruction methods, such as heat, disinfectants,
chemical biocides, desiccation, ultraviolet light and ionizing radiation. The
endospores may remain viable but dormant for prolonged periods, even for years,
15 until the external conditions become favourable, after which the transformation, i.e.
germination, of bacterial endospore takes place.

The amount of endospores in the final paper or board product may be a problem,
especially if the product is intended for hygiene purposes, food or beverage
20 packaging. Consequently there is an interest in the paper and board production to
reduce not only the bacteria but also the dormant endospores.

An object of this invention is to minimise or possibly even eliminate the
disadvantages existing in the prior art.
25

Another object of the present invention is to provide an effective method for
quantitatively reducing microorganisms and/or bacterial spores in the aqueous
environment of pulp mill, paper mill or board mill.

30 A further object of the present invention is to provide an effective composition for
inducing germination of bacterial endospores in the aqueous environment of pulp
mill, paper mill or board mill.

These objects are attained with the invention having the characteristics presented below in the characterising parts of the independent claims.

Some preferred embodiments of the invention are presented in the dependent
5 claims.

All the described embodiments and advantages apply all aspects of the present invention, i.e. use and method, when applicable, even if not always explicitly
10 stated so.

Typical use of a composition, which comprises a cationic surfactant comprising a primary or secondary ammonium head group and a linear unsubstituted C12-alkyl tail according to the present invention, is for inducing germination of bacterial endospores in pulp suspension for making of paper, board or the like.

15 Typical method according to the present invention for quantitatively reducing bacterial spores in pulp suspension for making of paper, board or the like, the method comprising

- obtaining aqueous pulp suspension,

20 - administering a cationic surfactant comprising a primary or secondary ammonium head group and linear unsubstituted C12-alkyl tail to the pulp suspension,

- allowing the cationic surfactant to interact with possible microorganisms, such as bacteria, and/or bacterial endospores in the pulp suspension, and

- inducing germination of endospores and/or destroying microorganisms.

25 Now it has been surprisingly found that a cationic surfactant, which comprises a primary or secondary ammonium head group and linear unsubstituted C12-alkyl tail, effectively interacts with dormant endospores and induces their germination to vegetative form in a pulp suspension. After germination the microorganisms, such
30 as bacteria, are present in vegetative form in the pulp suspension, and they can be easily destroyed, either by using a biocidal agent, heat or by the cationic surfactant itself. It is surprising that the cationic surfactant can successfully interact with the dormant endospores in the complex pulp suspension matrix, which comprise

various interfering substances and solid particles, e.g. free cations, mineral particles and fibres. Furthermore, the cationic surfactant may also interact and destroy vegetative microorganisms, such as bacteria, present in the pulp suspension. The present invention thus enables manufacture of hygienic paper or
5 board from spore-containing pulp suspension, which has been difficult or impossible with conventional methods.

The present invention comprises obtaining of an aqueous pulp suspension, which comprises lignocellulosic fibres. The aqueous pulp suspension may comprise
10 virgin fibres from mechanical pulping process and/or chemical pulping process, for example, from kraft pulping, chemi thermomechanical pulping process. The pulp suspension may additionally or alternatively comprise recycled fibres and/or broke from paper or board making process. The fibres may be bleached or unbleached.

15 The present invention is suitable for use in a pulp mill, a board mill or a paper mill.

According to one preferable embodiment of the invention the cationic surfactant is n-dodecylamine, dodecylguanidine salt or their mixture, more preferably n-dodecylamine or dodecylguanidine salt, such as dodecylguanidine hydrochloride
20 or dodecylguanidine acetate. Even more preferably the cationic surfactant may be n-dodecylamine or dodecylguanidine hydrochloride.

As described above, the cationic surfactant is allowed to interact with bacterial endospores, and optionally also with possible microorganisms, such as bacteria, in
25 the pulp suspension. The cationic surfactant induces the germination of the bacterial endospores. The germinated endospores can then be destroyed by free unconsumed cationic surfactant, additional oxidative biocide or by heat. The pulp suspension is subjected to heat for example when the fibre web formed from the pulp suspension is dried at the drying section of paper or board machine. In
30 addition, the cationic surfactant may directly interact with microorganisms, such as vegetative bacteria, and destroy them.

According to one embodiment of the invention the composition may comprise

- at least 10 weight % of cationic surfactant comprising a primary or secondary ammonium head group and a linear unsubstituted C12-alkyl tail,
- a organic solvent, which comprises at least one –OH group, and
- optionally water.

5

The composition may comprise 10 – 60 weight-%, preferably 20 – 47 weight-%, more preferably 25 – 45 weight-%, of the cationic surfactant, preferably n-dodecylamine, dodecylguanidine salt or their mixture, more preferably n-dodecylamine or dodecylguanidine salt, such as dodecylguanidine hydrochloride
10 or dodecylguanidine acetate, preferably dodecylguanidine hydrochloride. Preferably the composition comprises as high amount of cationic surfactant as possible, taking into account is solubility and avoiding phase separation.

The composition may comprise an organic solvent, which comprises at least one
15 OH-group for providing solubility of the cationic surfactant. The organic solvent may be any organic solvent having at least one OH-group, but preferably the organic solvent is selected from glycols or alcohols, and even more preferably the organic solvent is selected from ethanol, isopropyl alcohol and dipropylene glycol. According to one preferable embodiment the organic solvent is dipropylene glycol,
20 which is advantageous in industrial applications due to its low inflammability. Dipropylene glycol is here understood as a mixture of isomers of oxybispropanol. Organic solvent in general improves the stability and pumpability of the composition. Further, the composition may comprise an additional stabiliser, if needed.

25

The composition may comprise 10 – 90 weight-%, preferably 10 – 60 weight-%, more preferably 10 – 50 weight-%, even more preferably 20 – 47 weight-%, sometimes even more preferably 25 – 45 weight-%, of organic solvent. The ratio of cationic surfactant to the organic solvent may be from 10:90 to 60:40 in the
30 composition. According to one preferable embodiment the ratio of cationic surfactant to the organic solvent may be about 1:1.

According to one preferable embodiment the composition comprises water in addition of the cationic surfactant and organic solvent, as well as optional biocidal agent as explained below. Water is used to add the total amount of composition in the various recipes up to 100 weight-%.

5

In some embodiments the solubility of the cationic surfactant may be improved by an addition of an acid, such as hydrochloric acid.

The composition may further comprise a biocidal agent, such as water-soluble biocide. The biocidal agent may be a non-oxidizing biocide. In an exemplary embodiment, the non-oxidizing biocides can include glutaraldehyde, 2,2-dibromo-3-nitrilopropionamide (DBNPA), 2-bromo-2-nitropropane-1,3-diol (Bronopol), carbamates, 5-chloro-2-methyl-4-isothiazolin-3-one (CMIT), 2-methyl-4-isothiazolin-3-one (MIT), 1,2-dibromo-2,4-dicyano butane, bis(trichloromethyl)sulfone, 2-bromo-2-nitrostyrene, 4,5-dichloro-1,2-dithiol-3-one, 2-n-octyl-4-isothiazolin-3-one, 1,2-benzisothiazolin-3-one, ortho-phthaldehyde, quaternary ammonium compounds ("quats"), such as n-alkyl dimethyl benzyl ammonium chloride, didecyl dimethyl ammonium chloride (DDAC) or alkenyl dimethylethyl ammonium chloride, other guanidines than dodecylguanidine salts, biguanidines, pyrithiones, 3-iodopropynyl-N-butylcarbamate, phosphonium salts, such as tetrakis hydroxymethyl phosphonium sulfate (THPS), dazomet, 2-(thiocyanomethylthio)benzothiazole, methylene bithiocyanate (MBT), and any combinations thereof. Preferably non-oxidizing biocides are selected from 5-chloro-2-methyl-4-isothiazolin-3-one (CMIT) and 2-methyl-4-isothiazolin-3-one (MIT). The composition may comprise 0.01 – 20 weight-%, preferably 1 – 20 weight-%, more preferably 5 – 15 weight-%, even more preferably 7 – 10 weight-%, of biocidal agent. The biocidal agent improves the antimicrobial effect of the composition by slowing down the metabolism of the vegetative microorganisms and germinated endospores.

30

In one embodiment of the invention an oxidizing biocide is added to the pulp suspension, preferably after the administering of the composition comprising cationic surfactant, and downstream from the point of administration of the

composition. The addition of the oxidizing biocide provides thus an efficient post-treatment after addition of the composition comprising cationic surfactant. The oxidizing biocide destroys quickly the possible vegetative microorganisms, such as bacteria, present after the induced germination and/or remaining in the pulp suspension after treatment with the composition. The oxidizing biocide also reduces the risk for excessive growth of vegetative microorganisms, which might in some circumstances result from germination of the endospores. The composition comprising the cationic surfactant is administered to the pulp suspension at least 15 min, preferably at least 30 min, more preferably at least 90 min, before administration of oxidizing biocide. In this manner it is possible to guarantee a sufficient reaction time with the cationic surfactant and the dormant endospores for germination to vegetative form.

According to one embodiment of the invention the oxidizing biocide may be an oxidant selected from chlorine, alkali and alkaline earth hypochlorite salts, hypochlorous acid, chlorinated isocyanurates, bromine, alkali and alkaline earth hypobromite salts, hypobromous acid, bromine chloride, chlorine dioxide, ozone, hydrogen peroxide, peroxy compounds, such as peracetic acid, performic acid, percarbonate or persulfate salts, halogenated hydantoins, e.g., monohalodimethylhydantoins such as monochlorodimethylhydantoin, or dihalodimethylhydantoins such as chlorobromodimethylhydantoin, monochloramines, monobromamines, dihaloamines, trihaloamines, or any combinations thereof. Alkali and alkaline earth hypochlorite salts, such as sodium hypochlorite, are being preferred. The oxidizing biocide is administered to the pulp suspension in amount of 1 – 30 ppm, preferably 5 – 20 ppm, more preferably 7 – 10 ppm, as total active chlorine or percarboxylic acid.

The composition comprising cationic surfactant may be administered as a single dose, as constant feed or periodically as separate doses administered at predetermined time intervals. According to one embodiment the composition comprising the cationic surfactant is administered continuously to the pulp suspension. Single dose or periodical administration may be performed at the time of a process change, such as machine start-up after production stop, either at pulp

mill or board mill or paper mill. Single dose administration may also be used if it is known or suspected that the raw material may be contaminated by dormant endospores. In this case the raw material may be treated with the composition before its introduction to the paper or board making process.

5

According to one preferable embodiment the composition comprising cationic surfactant is administered to pulp suspension having a consistency of 1 – 10 weight-%, preferably 3 – 10 weight-%, more preferably 3 – 7 weight-%. In other words the composition is preferably administered to so-called thick stock.

10

In general, it is possible to administer the composition comprising cationic surfactant to at least one of the process locations selected from a pulp and/or broke storage tower, a pulp thickener, a pulp and/or broke pulper. The exact location depends, inter alia, if the administration is performed in a pulp mill or in a board/paper mill. Preferably the composition is administered early in the process, in order to guarantee an effective time for interaction between the cationic surfactant of the composition and the dormant endospores. For example, the composition may be administered to circulation line, which feeds thick pulp suspension comprising broke and having consistency of ≥ 3 % to a broke tower. In this manner the time of interaction is prolonged, even up to several hours.

According to one embodiment of the invention the composition comprising cationic surfactant is administered in amount that provides a negative delta spore value between inlet and outlet of the selected process location. Negative delta spore value means that the endospore value measured from the pulp suspension at the inlet of a process location is higher than the endospore value measured from the pulp suspension at the outlet of the same process location, i.e. the difference between the two values is negative. The composition may be administered in amount that provides a dosage of the cationic surfactant to the pulp suspension in the range of 1 – 1000 ppm, preferably 10 – 500 ppm, more preferably 50 – 200 ppm, calculated as active cationic surfactant.

According to one embodiment of the invention a chelating agent, such as DTPA or EDTA, is added to the pulp suspension at the latest with the administration of the cationic surfactant. Chelating agent interacts with the possible cations present in the pulp suspension and thus inhibits their interference to the interaction between the cationic surfactant and the endospore cortex. In another embodiment it is possible to add a chelating agent, such as DTPA or EDTA, to the pulp suspension before the administration of the cationic surfactant in order to guarantee effective interaction between the chelating agent and the cationic surfactant. The chelating agent may be added to the pulp suspension in amount of 1 – 100 ppm, preferably 10 – 50 ppm, more preferably 15 – 25 ppm, calculated as active agent. Preferably the chelating agent is selected from EDTA or DTPA. Use of chelating agent is especially advantageous when the concentration of cations in the pulp suspension is at least 5 ppm. The use of chelating agents is especially advantageous if the pulp suspension comprises Fe^{3+} or Cu^{2+} cations. Usually use of chelating agents is preferable if the pulp suspension comprises recycled fibres.

The temperature of the fibrous pulp suspension during the administration of the composition comprising the cationic surfactant may be at least +30 °C, preferably +40 – +70 °C. It has been observed that the elevated temperature of the pulp suspension improves the germination effect obtainable by the cationic surfactant.

The pH of the pulp suspension is preferably in the range of 4 – 12 in order to guarantee the optimal interaction with the cationic surfactant and microorganisms and/or the bacterial endospores.

The pulp suspension treated with the composition comprising cationic surfactant may be formed into a fibrous web of paper, board or tissue and dried. The present method enables, for example, production of board which contains a microorganism level, i.e. bacterial level, which is less than about 2500 cfu/g, preferably less than about 2000 cfu/g, more preferably less than about 1500 cfu/g, even more preferably less than about 1000 cfu/g, or more preferably less than about 500 cfu/g, or most preferably less than about 250 cfu/g.

EXPERIMENTAL

Some embodiments of the invention are described more closely in the following non-limiting examples.

5 **Example 1**

This on-site side-flow test was performed at an alkaline board machine, which produces 3-ply food packaging board, in order to test efficacy of Composition 1-1 according to the invention and comprising cationic surfactant against total aerobes and aerobic spores of broke tower (consistency 3.5%, pH 7.2, Oxidation Reduction
10 Potential, ORP, +158 mV). Cationic surfactant was dosed (contact time 90 min, 180 rpm) as 150 ppm dodecylamine into broke sample, which after total aerobes and aerobic spore counts were quantitated (plate count agar, +45 °C/+37°C, 2 days incubation) at an external laboratory. Prior to the bacterial spore determination, samples were pasteurized at +80 °C for 20 min. Results are shown
15 in Figure 1. The left hand column shows the broke reference sample with no added biocide, the right hand column the broke sample dosed with Composition 1-1.

Figure 1 shows that the untreated broke (3.5 % consistency, pH 7.2, ORP +158
20 mV) contained much aerobic bacteria, 14 000 000 cfu/ml, as well as aerobic spores, 300 cfu/ml. The use of Composition 1-1 according to the invention markedly decreased total aerobes from 14 000 000 cfu/ml to 200 cfu/ml and aerobic spores from 300 cfu/ml to 40 cfu/ml. Obtained results thus indicate that the Composition 1-1 can effectively hygienize broke with an economically feasible
25 dosage. Eventually such broke hygienization treatment enables food packaging board mills to produce the highest hygiene board grades with maximum bacteria content of 250 cfu per gram of dry final board.

Example 2

30 This on-site side-flow test was performed at an alkaline board machine, which produces 3-ply food packaging board, in order to test efficacy of Composition 1-2 according to the invention and comprising cationic surfactant against total aerobes

and aerobic spores of incoming mechanical pulp (consistency 5.4 %, pH 6.5, ORP -190 mV). The cationic surfactant was dosed (+48 °C, 75 min, 180 rpm) as 150 ppm dodecylamine into pulp sample. Total aerobes and aerobic spore counts were quantitated (plate count agar, +45 °C/+37°C, 2 days incubation) at an external
5 laboratory. Prior to the bacterial spore determination, samples were pasteurized at +80 °C for 20 min. Results are shown in Figure 2. The left hand column shows the pulp reference sample with no added biocide, the right hand column the pulp sample dosed with Composition 1-2.

10 Figure 2 shows that the untreated incoming pulp (5.4 % consistency, pH 6.5, ORP -190 mV) contained much aerobic bacteria, 1 000 000 cfu/ml, but no spores, <10 cfu/ml. Due to the dosing of Composition 1-2, total aerobes were effectively killed as the level decreased from 1 000 000 cfu/ml to 200 cfu/ml. Obtained results thus indicate that Composition 1-2 can effectively hygienize pulp despite the high fibre
15 content (5.4% consistency) and low ORP (-190 mV) at economically feasible dosage rate. Eventually the hygienisation of pulp enables food packaging board mills to produce highest hygiene board grades where bacteria content is max 250 cfu per gram of dry final board.

20 **Example 3**

This on-site side-flow test was performed at an alkaline board machine, which produces 3-ply food packaging board, in order to test efficacy of the method according to the invention against total aerobes and aerobic spores of broke tower (consistency 3.5%, pH 7.2, ORP +65 mV) . The cationic surfactant, i.e.
25 Composition 1-3, was dosed (75 min, 180 rpm) as 150 ppm dodecylamine into broke sample, and there after post-treated (30 min, 180 rpm) with sodium hypochlorite (as 10 ppm active chlorine) in order to improve killing effect against vegetative and germinated cells. Total aerobes and aerobic spore counts were quantitated (plate count agar, +45 °C/+37°C, 2 days incubation) at an external
30 laboratory. Prior to the bacterial spore determination, samples were pasteurized at +80 °C for 20 min. Results are shown in Figure 3. The left hand column shows the broke reference sample with no added biocide, the middle column shows the

broke sample dosed with Composition 1-3, and the right hand column the broke sample dosed with Composition 1-3 and with sodium hypochlorite.

Figure 3 shows that the untreated broke (3.5 % consistency, pH 7.2, ORP +65mV) contained much aerobic bacteria, 37 000 000 cfu/ml, and some aerobic spores, 70 cfu/ml. The composition 1-3 markedly decreased level of total aerobes from 37 000 000 cfu/ml to 1 600 cfu/ml, and showed also sporicidal effect as the aerobic spore counts decreased from 70 cfu/ml to 30 cfu/ml. Bacteria and spore killing effect was further improved by introducing a sodium hypochlorite post-treatment, as 10 ppm active chlorine. The microbiological quality of the broke was drastically improved; total aerobes content decreased from 37 000 000 cfu/ml to 400 cfu/ml, and aerobic spore counts from 70 cfu/ml to 10 cfu/ml. Obtained results thus indicate that the method according to the invention provides an economically feasible solution for broke hygienisation, which eventually enables food packaging board mills to produce the highest hygiene board grades with maximum bacteria content of 250 cfu per gram of dry final board.

Example 4 (reference)

This laboratory test was performed in order to test killing effect of sodium hypochlorite against total aerobes and aerobic spores in broke sample (consistency 1.0%, pH 7.8, ORP +146 mV) taken from an alkaline board machine, which produces 3-ply food packaging board. Total aerobes and aerobic spore counts were quantitated by using plate count agar (+45 °C/+37°C, 2 days incubation). Prior to the bacterial spore determination, samples were pasteurized at +80 °C for 20 min. Results are shown in Figure 4. The left hand column shows the broke reference sample with no added biocide with 1 day contact time, the right hand column the broke sample dosed with sodium hypochlorite with 1 day contact time.

Figure 4 shows that the untreated broke (1.0 % consistency, pH 7.8, ORP +146 mV) contained much aerobic bacteria, 650 000 cfu/ml, and some aerobic spores,

70 cfu/ml. Sodium hypochlorite treatment, as 10 ppm active chlorine, showed only weak killing effect, as total aerobes level remained at high level, 390 000 cfu/ml, despite the chlorine treatment. Similarly, the sodium hypochlorite treatment, as 10 ppm active chlorine, was ineffective against mature spores, as aerobic spore content remained at close to initial level of 60 cfu/ml. Obtained results thus indicate that sodium hypochlorite, as 10 ppm active chlorine, alone may not be used to effectively hygienize broke.

Example 5

This test was performed in a pilot paper machine producing 1-ply paper. Aim of the test was to verify efficacy of the invention to provide a low quantity of aerobic bacteria and aerobic bacterial spores in dry paper end-product. Composition 1-5 comprising 40 weight-% of dodecylamine, 40 weight-% of dipropylene glycol and 20 weight-% of water was used according to the invention. It was dosed as 100, 150 or 300 mg/l into pulp suspension with 2.25 % consistency. The pulp suspension was then mixed 120 rpm for 30 min at +40 °C. The pulp suspension was made from a batch of authentic, spore-contaminated folding-box board with average spore content of 7000 cfu/gram of dry board. All pulp suspensions in the pilot paper machine were also post-treated (5 min, 120 rpm) with glutaraldehyde (dose: 50 ppm as active agent) in order to maintain control of vegetative bacterial cells in the pilot machine. Total aerobic bacteria (without pasteurization) and aerobic spores (with pasteurization step at +82 °C for 10 min) were quantified from the dry paper end product according to the ISO 8784-1 standard for microbiological examination of paper and board.

25

Figure 5 as well as Table 1 show results from microbiological examination of the dry paper samples that were produced in the pilot paper machine with different dosages of Composition 1-5 in the pulp slurry.

Table 1 Results of example 5.

	Total aerobic bacteria (cfu/g)	Aerobic spores (cfu/g)
Reference paper, without added composition to the pulp	1 973	1 423
Paper 1 Dosage: 100 mg/l composition 1-5	523	414
Paper 2 Dosage: 150 mg/l composition 1-5	108	20
Paper 3 Dosage: 300 mg/l composition 1-5	70	18

Figure 5 and table 1 show that the reference paper contained high quantity of aerobic bacteria (1973 cfu/g) and aerobic bacterial spores (1423 cfu/g). This indicates that the contaminated raw material with high initial endospore content caused subsequent contamination of the produced paper. The treatment with the glutaraldehyde biocide was not alone capable to lower spore contamination in the produced paper.

Use of Composition 1-5 according to invention markedly decreased level of endospores in final paper at every tested dosage level. The method according to the invention showed sporicidal effect lowering the spore counts 1423 cfu/g (reference) to 414 cfu/g (with 100 mg/l treatment) or to 20 cfu/g (with 150 mg/l treatment) or to 18 cfu/g (with 300 mg/l treatment). Similarly, the total aerobic bacteria counts from the dry paper were significantly lower. Obtained results thus indicate that the invention provides a feasible solution for paper end-product hygienisation at a reasonable dosage level, which eventually enables food-packaging paper and board mills to produce the highest hygiene grades with maximum bacteria content of 250 cfu per gram of dry final paper or board.

20

Example 6

This laboratory test was performed to compare biocidal efficacy of three test products, namely a conventional biocide Composition R comprising glutaraldehyde (reference), and Compositions 1-6 and 2-6, which comprised a cationic surfactant comprising a primary ammonium head group and linear unsubstituted C12-alkyl tail, and a cationic surfactant comprising a secondary ammonium head group and

25

linear unsubstituted C12-alkyl tail. Composition R comprised 50 weight-% of glutaraldehyde. Composition 1-6 comprised 40 weight-% of dodecylamine, 40 weight-% of dipropyleneglycol and 20 weight-% of water. Composition 2-6 comprised 35 weight-% of dodecylguanidine hydrochloride and 65 weight-% of
5 dipropyleneglycol.

Products were tested against Total aerobic bacteria and Aerobic spores in authentic process water taken from a paper machine producing food-packaging board. At the moment of sampling from process, the authentic process water
10 contained high amounts of Total aerobic bacteria, >100 000 cfu/ml, but very few Aerobic spores, <100 cfu/ml. In order to be better capable to evaluate product efficacies against Aerobic spores, the process water was spiked with additional spores, targeting for Aerobic spore level of ca. 100.000 cfu/ml. This spore solution for spiking was prepared as follows: Authentic spore-forming bacteria that were
15 isolated from a paper making process were pre-grown for 2 days at +37 °C in modified nutrient broth, after which the mature spores were harvested, and washed by using centrifugation and cold water.

At the start of the test, the spore spiked process water was divided into eight
20 individual samples. Two samples were treated with Composition 1-6, two with Composition 2-6 and two with a conventional Composition R. Compositions were dosed in such amount that the active amount of active agent was the same (90 mg/l) in each sample. In addition two samples were stored as such and used as 0-reference samples, without any added biocide. Samples were stored at 45 °C, 180
25 rpm mixing, for 50 minutes, followed by quantification of Total aerobic bacteria (non-pasteurized samples) and Aerobic spores (plate count agar, +37°C, 2 days incubation). Prior to the aerobic spore determination, samples were pasteurized at +82 °C for 10 min. Results are shown as average values of two parallel samples in Figure 6 and Table 2.

Table 2 Results of example 6.

	Total aerobic bacteria (cfu/ml)	Aerobic spores (cfu/ml)
0-reference (no added biocides)	379 000	92 000
Composition 1-6 (90 mg/l Dodecylamine)	5 050	2 500
Composition 2-6 (90 mg/l Dodecylguanidine hydrochloride)	3 300	760
Composition R, reference (90 mg/l Glutaraldehyde)	655 000	115 000

Results in Figure 6 and Table 2 for 0-reference sample clearly show that the process water, after spiked with spores, contained a large amount of Total aerobic bacteria, 379 000 cfu/ml, and Aerobic spores, 92 000 cfu/ml. Composition 1-6 effectively decreased Total aerobic bacteria level from 379 000 cfu/ml to 5050 cfu/ml and Aerobic spore level from 92 000 cfu/ml to 2500 cfu/ml. Composition 2-6 effectively decreased Total aerobic bacteria level from 379 000 cfu/ml to 3300 cfu/ml and Aerobic spore level from 92 000 cfu/ml to 760 cfu/ml. It is seen that both Compositions 1-6 and 2-6 were surprisingly effective in decreasing Total aerobic bacteria as well as Aerobic spores. On the contrary, reference Composition R was non-effective against Total bacteria and Aerobic spores. Obtained results thus indicate that the use of two tested Compositions 1-6 and 2-6 according to the invention, containing dodecylamine or dodecylguanidine hydrochloride as active agent, can be effective for hygienization of paper making process streams with high total bacteria and aerobic spore content.

Example 7

This laboratory test was performed to evaluate efficacy of a Composition 1-7 according to the invention and comprising a cationic surfactant comprising a secondary ammonium head group and linear unsubstituted C12-alkyl tail, against Total aerobic bacteria and Aerobic spores in authentic process water taken from a paper machine producing food packaging board. Composition comprised 35 weight-% of dodecylguanidine hydrochloride and 65 weight-% dipropyleneglycol.

At the start, the process water was divided into four individual samples; two were treated with Composition 1-7 and two samples were stored as such and used as 0-reference (process water without added biocides). Samples were stored at 45 °C, 180 rpm mixing, for 30 minutes, followed by quantification of Total aerobic bacteria (non-pasteurized samples) and Aerobic spores (plate count agar, +37°C, 2 days incubation). Prior to the aerobic spore determination, samples were pasteurized at +82 °C for 10 min. Results of two parallel samples are shown as average values in Figure 7 and Table 3.

Table 3 Results of example 7.

	Total aerobic bacteria (cfu/ml)	Aerobic spores (cfu/ml)
0-reference (no added biocides)	1 162 500	70
Composition 1-7 (90 mg/l Dodecylguanidine hydrochloride)	<100	25

Results in Figure 7 and Table 3 show that the process water as such contained a large amount of Total aerobic bacteria, 1 162 500 cfu/ml, and some Aerobic spores, 70 cfu/ml. Composition 1-7, at active agent dosage of 90 mg/l Dodecylguanidine hydrochloride, showed excellent hygienisation effect of the process water. Total aerobic bacteria level dropped from 1 162 500 cfu/ml to < 100 cfu/ml, which is below practical detection limit, and the level of Aerobic spores decreased from 70 cfu/ml to 20 cfu/ml. Obtained results thus indicate that the Composition 1-7, with dodecylguanidine hydrochloride as the active agent, may effectively be used to hygienize paper making process streams containing aerobic bacteria and bacterial spores.

Even if the invention was described with reference to what at present seems to be the most practical and preferred embodiments, it is appreciated that the invention shall not be limited to the embodiments described above, but the invention is intended to cover also different modifications and equivalent technical solutions within the scope of the enclosed claims.

CLAIMS

1. Use of a composition comprising a cationic surfactant, which comprises a primary or secondary ammonium head group and a linear unsubstituted C12-alkyl tail, for inducing germination of bacterial endospores in pulp suspension for making of paper, board or the like.
2. Use according to claim 1, **characterised** in that the composition comprises
- at least 10 weight % of the cationic surfactant,
 - an organic solvent, which comprises at least one OH group, and
 - optionally water.
3. Use according to claim 1 or 2, **characterised** in that the cationic surfactant is n-dodecylamine or dodecylguanidine salt, such as dodecylguanidine hydrochloride.
4. Use according to claim 1, 2 or 3, **characterised** in that the composition comprises 10 – 60 weight-%, preferably 20 – 47 weight-%, more preferably 25 – 45 weight-%, of the cationic surfactant.
5. Use according to any of preceding claims 1 – 4, **characterised** in that the composition comprises 10 – 90 weight-%, preferably 10 – 60 weight-%, more preferably 10 – 50 weight-%, even more preferably 20 – 47 weight-%, sometimes even more preferably 25 – 45 weight-%, of organic solvent.
6. Use according to any of preceding claims 1 – 5, **characterised** in that the organic solvent is selected from glycols and alcohols, such as ethanol, isopropyl alcohol and dipropylene glycol.
7. Use according to any of preceding claims 1 – 6, **characterised** in that the composition comprises biocidal agent, such as a water-soluble biocide.
8. Use according to claim 7, **characterised** in that the biocidal agent is a non-oxidizing biocide selected from group comprising glutaraldehyde, 2,2-dibromo-3-

- nitrilopropionamide, 2-bromo-2-nitropropane-1,3-diol, carbamates, 5-chloro-2-methyl-4-isothiazolin-3-one, 2-methyl-4-isothiazolin-3-one, 1,2-dibromo-2,4-dicyano butane, bis(trichloromethyl)sulfone, 2-bromo-2-nitrostyrene, 4,5-dichloro-1,2-dithiol-3-one, 2-n-octyl-4-isothiazolin-3-one, 1,2-benzisothiazolin-3-one, ortho-
- 5 phthaldehyde, quaternary ammonium compounds, other guanidines than dodecylguanidine salts, biguanidines, pyrithiones, 3-iodopropynyl-N-butylcarbamate, phosphonium salts, dazomet, 2-(thiocyanomethylthio)benzothiazole, methylene bithiocyanate and any combinations thereof.
- 10 9. Use according to claim 7 or 8, **characterised** in that the composition comprises 0.01 – 20 weight-%, preferably 1 – 20 weight-%, more preferably 5 – 15 weight-%, even more preferably 7 – 10 weight-%, of the biocidal agent.
- 15 10. Method for quantitatively reducing bacterial endospores in pulp suspension for making of paper, board or the like, the method comprising
- obtaining aqueous pulp suspension,
 - administering a composition comprising a cationic surfactant, which comprises a primary or secondary ammonium head group and linear unsubstituted C12-alkyl tail to the pulp suspension,
- 20 - allowing the cationic surfactant to interact with bacterial endospores in the pulp suspension, and
- inducing germination of endospores and destroying microorganisms by using a biocidal agent, heat or by the cationic surfactant itself.
- 25 11. Method according to claim 10, **characterised** in that the cationic surfactant is n-dodecylamine or dodecylguanidine salt, such as dodecylguanidine hydrochloride.
- 30 12. Method according to claim 10 or 11, **characterised** in administering the composition comprising cationic surfactant to at least one of the process locations selected from a pulp and/or broke storage tower, a pulp thickener, a pulp and/or broke pulper.

13. Method according to claim 12, **characterised** in administering the composition comprising cationic surfactant in amount that provides a negative delta spore value between inlet and outlet of the selected process location.

- 5 14. Method according to any of preceding claims 10 – 13, **characterised** in administering the composition comprising cationic surfactant in amount that provides a dosage of the cationic surfactant to the pulp suspension in the range of 1 – 1000 ppm, preferably 10 – 500 ppm, more preferably 50 – 200 ppm, calculated as active cationic surfactant.

10

15. Method according to any of preceding claims 10 – 14, **characterised** in administering an oxidizing biocide to the pulp suspension.

- 15 16. Method according to claim 15, **characterized** in administering oxidizing biocide to the pulp suspension in amount of 1 – 30 ppm, preferably 5 – 20 ppm, more preferably 7 – 10 ppm, as total active chlorine or percarboxylic acid.

- 20 17. Method according to any of preceding claims 10 – 16, **characterised** in adding a chelating agent, such as DTPA or EDTA, to the pulp suspension at the latest with the administration of the cationic surfactant.

18. Method according to claim 17, **characterised** in adding a chelating agent, such as DTPA or EDTA, to the pulp suspension in amount of 1 – 100 ppm, preferably 10 – 50 ppm, more preferably 15 – 25 ppm, given as active agent.

1/4

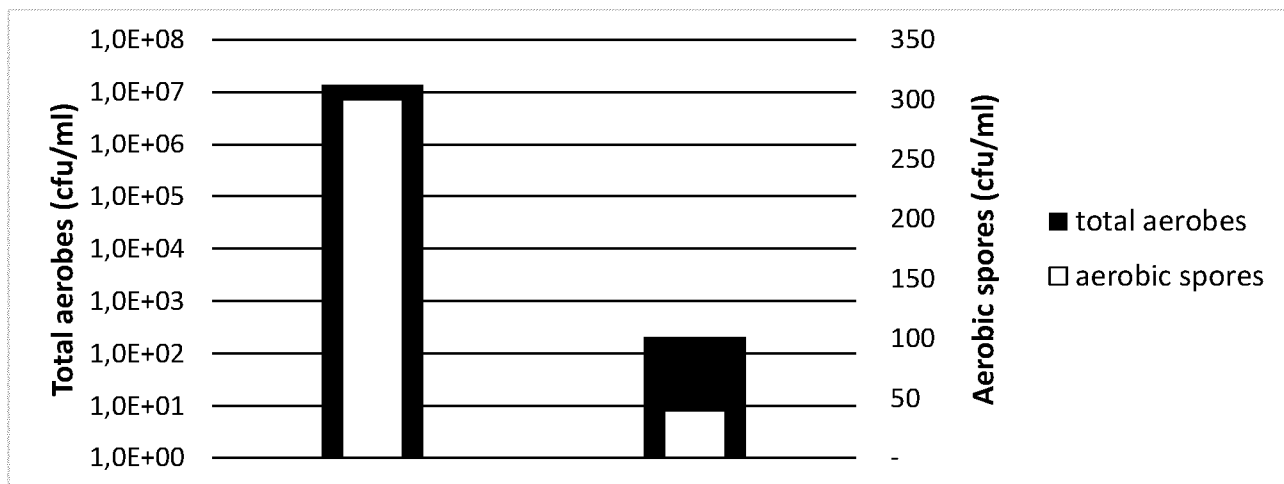


Fig. 1

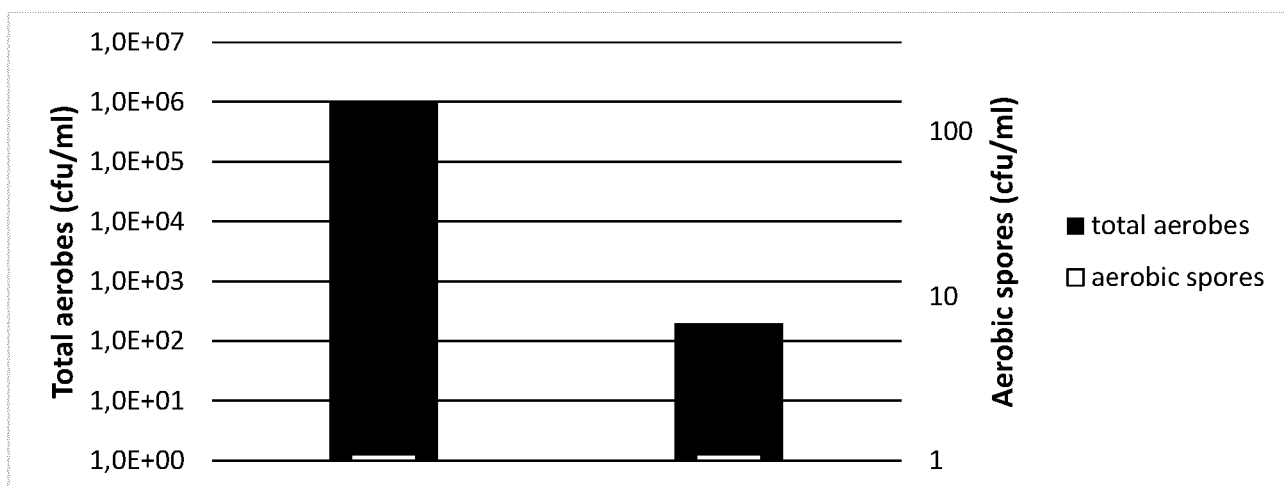


Fig. 2

2/4

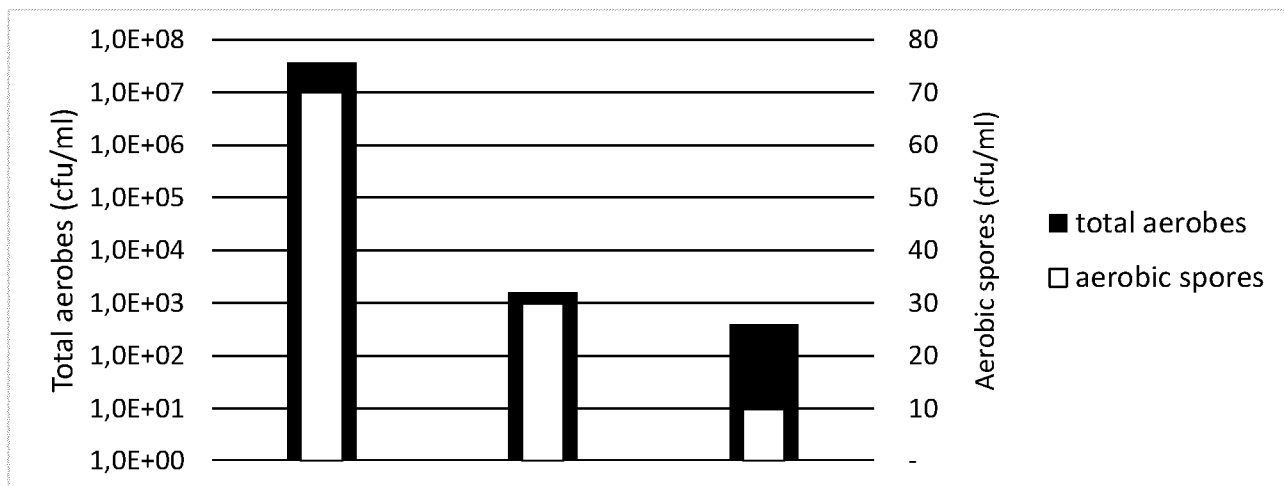


Fig. 3

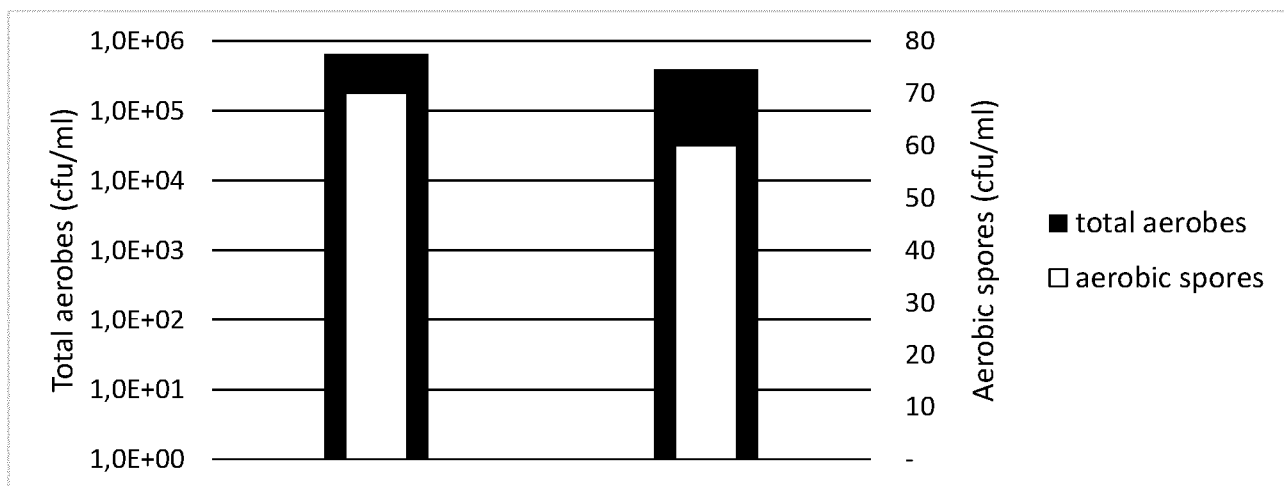


Fig. 4

3/4

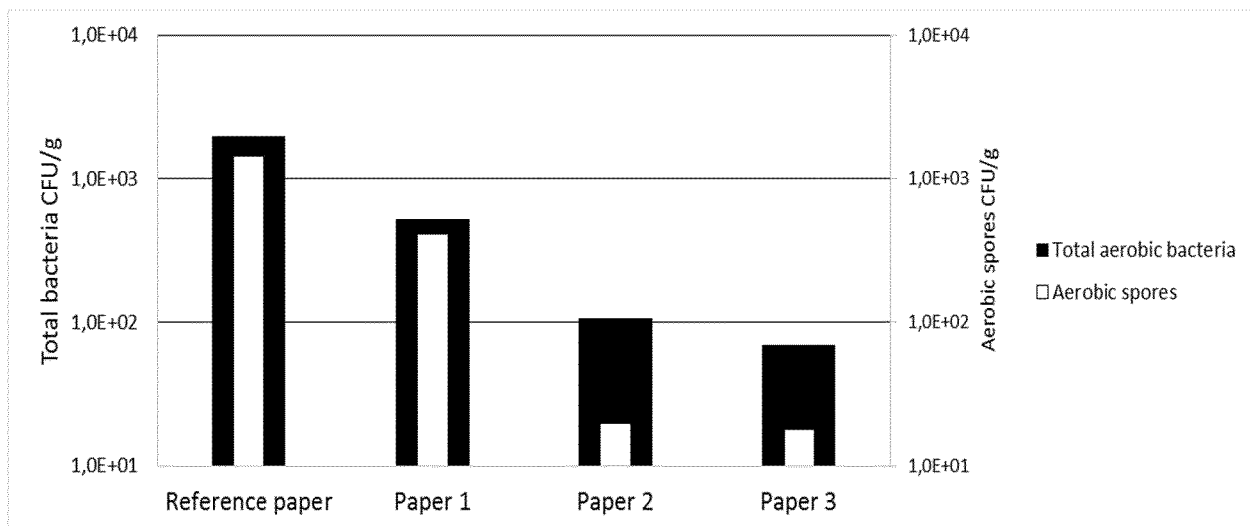


Fig. 5

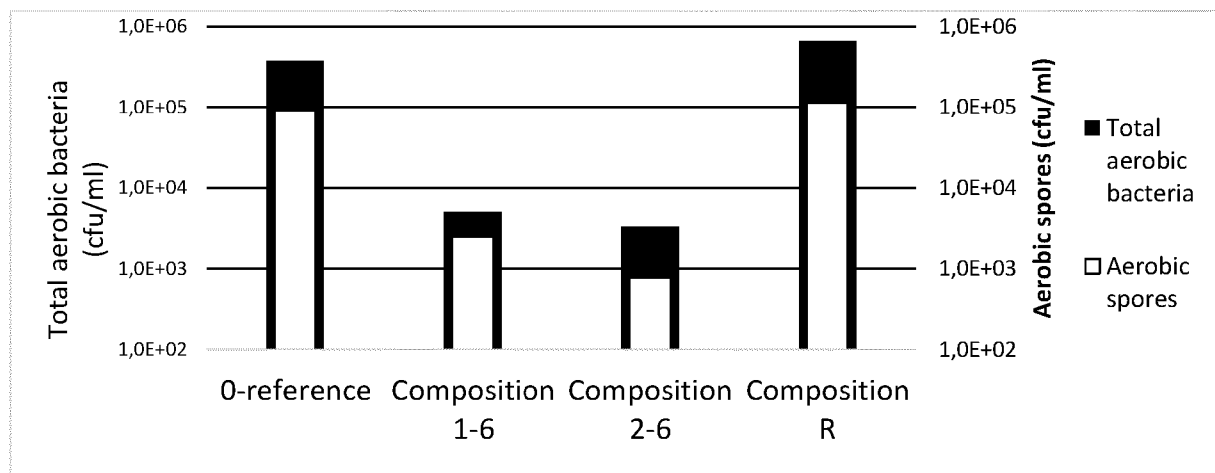


Figure 6

4/4

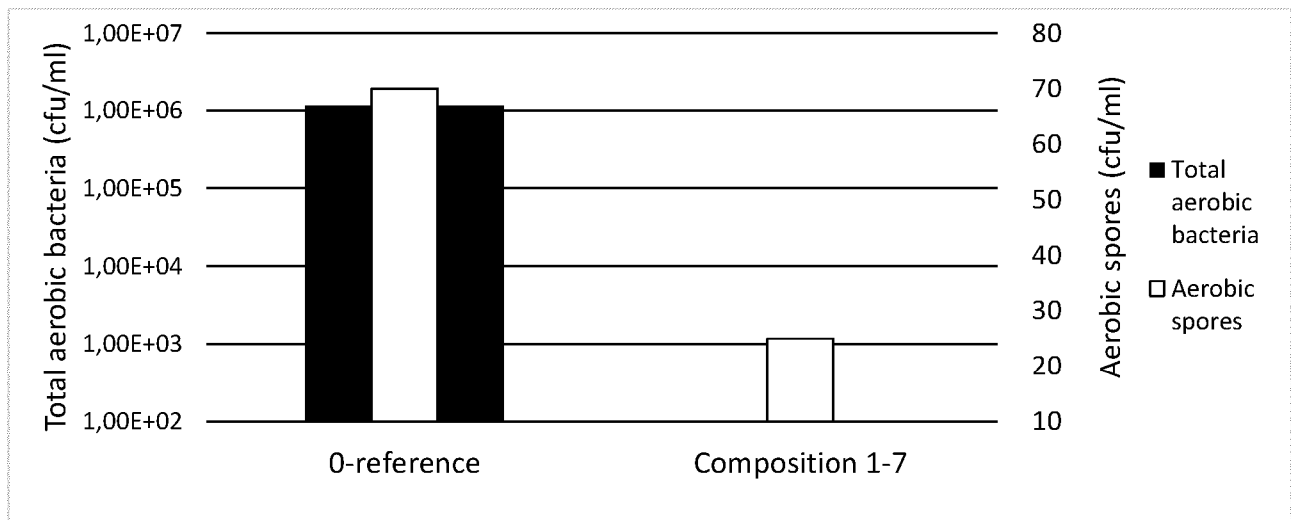


Figure 7

INTERNATIONAL SEARCH REPORT

International application No
PCT/FI2017/050367

A. CLASSIFICATION OF SUBJECT MATTER

INV. A01N33/04 A01N47/44 A01N59/00 A01N31/02 A01P1/00
A01P15/00

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)

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, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 01/66471 A2 (HERCULES INC [US]) 13 September 2001 (2001-09-13) claim 1 examples	1-18
Y	----- LUO MI ET AL: "Chemical insights into dodecylamine spore lethal germination", CHEMICAL SCIENCE, vol. 5, no. 8, 1 January 2014 (2014-01-01) , pages 3320-3324, XP055389779, United Kingdom ISSN: 2041-6520, DOI: 10.1039/C4SC01613K Results and Discussion; page 3321 - page 3324 ----- -/--	1-18



Further documents are listed in the continuation of Box C.



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

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

11 July 2017

Date of mailing of the international search report

19/07/2017

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Davies, Maxwell

INTERNATIONAL SEARCH REPORT

International application No

PCT/FI2017/050367

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2010/000744 A2 (MIRET LAB [ES]; ROCABAYERA BONVILA XAVIER [ES]; FIGUERAS ROCA SERGI [E] 7 January 2010 (2010-01-07) claims 1-11 -----	1-18
Y	WO 2007/125154 A1 (KEMIRA OYJ [FI]; KOLARI MARKO [FI]) 8 November 2007 (2007-11-08) page 5, line 1 - page 6, line 6 -----	17,18
A	US 5 041 463 A (WHITEKETTLE WILSON K [US] ET AL) 20 August 1991 (1991-08-20) claims 1-7 -----	1-18

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/FI2017/050367

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0166471	A2	13-09-2001	AU 4177401 A 17-09-2001 WO 0166471 A2 13-09-2001
WO 2010000744	A2	07-01-2010	AR 072462 A1 01-09-2010 BR PI0913835 A2 09-08-2016 CA 2728327 A1 07-01-2010 CN 102088858 A 08-06-2011 EP 2348842 A2 03-08-2011 ES 2392384 T3 10-12-2012 JP 2011526593 A 13-10-2011 RU 2011102291 A 10-08-2012 US 2011144203 A1 16-06-2011 WO 2010000744 A2 07-01-2010
WO 2007125154	A1	08-11-2007	BR PI0709563 A2 19-07-2011 CA 2645826 A1 08-11-2007 CN 101443516 A 27-05-2009 EP 1994221 A1 26-11-2008 ES 2405360 T3 30-05-2013 FI 20060247 A 29-10-2007 RU 2008140286 A 27-04-2010 US 2009294082 A1 03-12-2009 WO 2007125154 A1 08-11-2007
US 5041463	A	20-08-1991	NONE