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(54) ANTIFOULING COMPOSITION COMPRISING AN ENZYME IN THE ABSENCE OF ITS SUBSTRATE

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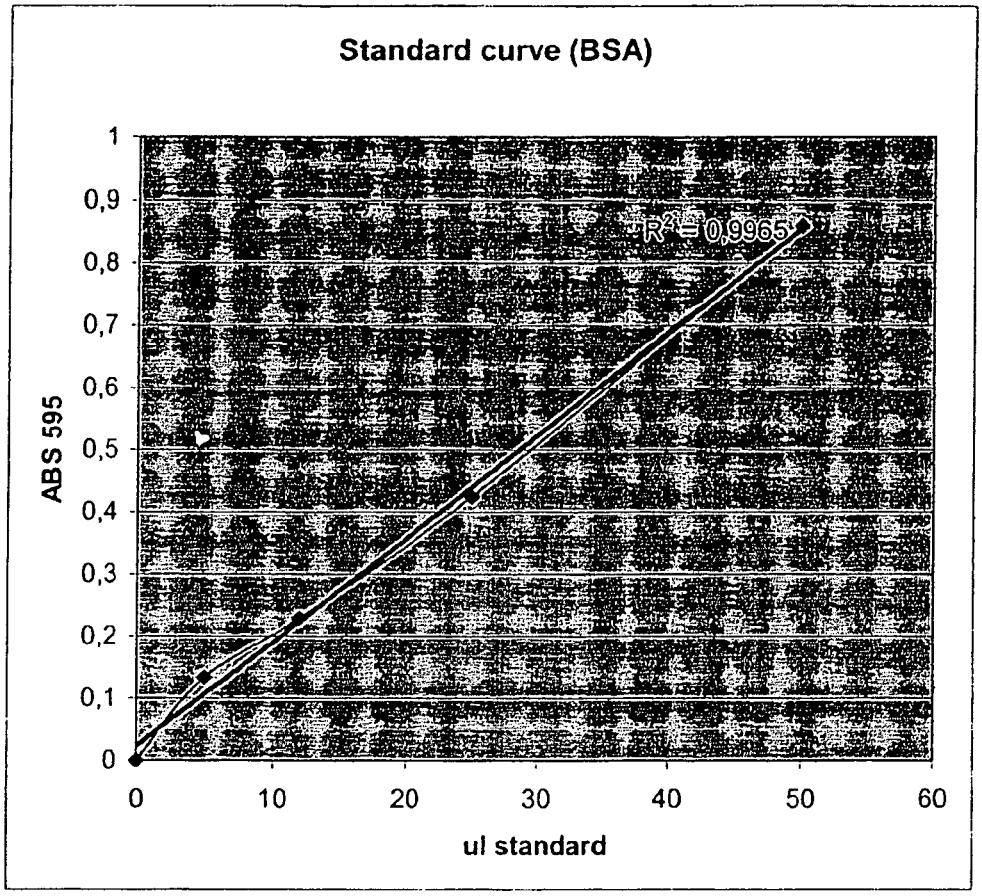
(57) **ABSTRACT**

The present invention in one aspect relates to a coating composition comprising at least one enzyme capable of acting on a compound, wherein said action results in the formation of an antifouling species comprising an antifouling activity, and wherein said compound does not form part of said coating composition. The coating composition preferably comprises at least one oxidase capable of acting on a compound, such as a substrate for said oxidase, wherein said action results in the formation of an antifouling species including an antimicrobial species comprising an antimicrobial activity. More preferred, the oxidase comprises an activity which results in the formation of a peroxide. The oxidase can be present in said coating composition in combination with one or more additional enzymes including, but not limited to, an esterase, including a lipase, an amidase, including a protease, and a polysaccharide degrading enzyme, wherein said one or more additional enzyme(s), alone or in any combination, can be included in the presence or absence of one or more substrates for one or more of said enzymes.

Fig. 1



ANTIFOULING COMPOSITION COMPRISING AN ENZYME IN THE ABSENCE OF ITS SUBSTRATE

[0001] All patent and non-patent references cited in this application are hereby incorporated by reference in their entirety.

FIELD OF THE INVENTION

[0002] The present invention relates to a coating composition comprising at least one enzyme and no substrate for said at least one enzyme. When the coating composition is applied to an object and comes into contact with an external environment wherein said substrate is present, the at least one enzyme catalyses said substrate and generates an antifouling species, including an antimicrobial species having an antimicrobial activity. The enzyme is preferably an oxidase the activity of which results in the formation of a peroxide compound.

BACKGROUND OF THE INVENTION

[0003] Antifouling species such as e.g. antimicrobial species, antibacterial species, antifungal species, biocides, and biorepellents, are in broad use today. The importance of protecting various objects with such compounds against the attack of fouling organisms, bacteria, and fungi continues to increase.

[0004] For example, structures in contact with seawater, for example ships, oceanic constructions, fish farming nets, buoys and industrial water systems, are constantly exposed to water inhabited by various organisms. Therefore, as time passes by, microorganisms such as bacteria and diatoms and, further, fouling organisms of larger size, for example barnacles, mussels and sea lettuce, adhere to and grow on said structures.

[0005] Marine organisms covering a surface of a structure exposed to seawater result in e.g. corrosion of the covered part; decreased marine fuel efficiency due to increased frictional resistance of the ship bottom against seawater; deaths of fish and shellfish, or decreased working efficiency, due to clogging of fish farming nets; and sinking buoys due to reduced buoyancy. It is thus important to apply an antifouling treatment to such structures exposed to seawater.

[0006] Meanwhile, as can be easily understood from the serious problem posed by an increasing incidence of e.g. nosocomial infection due to meticillin-resistant staphylococci, it is also very important to treat interior walls, fixtures, furnishings, upholstery, etc. against the growth of bacteria and fungi in order to protect the internal environment of places such as hospitals, schools, and hotels against such microorganisms.

[0007] The antimicrobial technology for the structures exposed to seawater or the interior walls of a hospital, for instance, includes a method which comprises incorporating a compound having antimicrobial activity in the very object to be protected and a method which comprises coating the surface of an object with a coating composition containing a compound having antimicrobial activity.

paint is designed to release such a compound gradually from the film into water by utilizing its solubility to thereby provide a sustained antifouling effect.

[0009] As the technology for keeping the interior environment of hospitals, etc., against bacteria and fungi, it is common practice to apply a coating containing a compound having antibacterial/antifungul activity to the surface of the interior walls, fixtures, furnishings, upholstery, etc.

[0010] When an object is treated with a compound having antifouling or antibacterial/antifungal activity, it is of course expected that the effect of the treatment will be expressed steadily over as long a time as possible. However, according to the above technology comprising coating an object surface with a coating composition containing an antimicrobial compound itself, satisfactory effect is obtained only for a limited time period following the treatment. Even when the content of those active compound is high, the effect declines rapidly with time, thus failing to ensure a sustained long-term effect.

[0011] As compounds having antifouling activity for incorporation in antifouling paints, organotin compounds have been mostly employed. A variety of other compounds such as aliphatic carboxylic acids, aromatic carboxylicacids, aliphatic alcohols, phenolic compounds and e.g. hydrogen peroxide are also known to have antimicrobial activity.

[0012] However, as pointed out frequently, organotin compounds have high toxicity and, when formulated in antifouling paints, find their way into the seawater to contaminate the marine environment. In addition, the protection of workers against hazards adds to the difficulty of use of those compounds.

[0013] Aliphatic carboxylic acids, aromatic carboxylic acids, aliphatic alcohols, and phenolic compounds are free from safety and pollution problems just as is hydrogen peroxide. However, when those compounds are directly formulated into an antifouling paint and applied to the structures in water, they are eluted from the films into the surrounding water in a very brief period of time because of their highly solubility. It is thus impossible to maintain an elution level necessary for displaying antifouling property for a long period of time. When these compounds are formulated into an antibacterial/antifungal paint and applied to the interior walls of hospitals, they are readily evaporated off or driven off by the water contained in the atmosphere as it is the case with said antifouling paint. Thus it also fails to provide a long-term antibacterial/antifungal effect. Besides, carboxylic acids in general emanate intense foreign odors so that they are difficult to use just as are toxic compounds.

[0014] Although hydrogen peroxide is highly safe and free from the above problems, this species is a relatively unstable compound and it has so far been regarded as practically impossible to use it directly as an ingredient in antifouling or antibacterial/antifungal paints.

[0015] The demand exists for a new technology for sustained long-term effect without using excessive an unnecessary amounts of hazardous chemicals posing a risk to human health. It is one objective of the present invention to

[0016] U.S. Pat. No. 6,004,510 (Lever Brothers) discloses a process for the treatment of a surface with a hygiene agent which can include the steps of: a) providing at the surface a non-photochemical catalyst (such as a transition metal compound) which catalyses the formation of the hygiene agent from one or more precursors, whereby the catalyst becomes deposited at the surface, and, b) subsequently treating the surface with a treatment agent (such as a solution of hydrogen peroxide) having the or each hygiene agent precursor, such that the hygiene agent is generated at the surface. The disclosure also provides a process which includes the step of treating the surface which has a non-photochemical catalyst bound thereto with a treatment agent having at least one hygiene agent precursor which forms said hygiene agent in the presence of the catalyst, and a process for the manufacture of an article which includes the step of incorporating therein, at the time of manufacture, a non-photochemical catalyst capable of transforming at least one hygiene agent precursor into a hygiene agent. All of the examples relate to inorganic metal compounds.

[0017] U.S. Pat. No. 5,998,200 (Duke University) discloses a method for preventing fouling of an aquatic apparatus by an aquatic organism which comprises affixing a biologically active chemical to a surface intended for use in contact with an aquatic environment containing the organism, wherein the chemical is an enzyme, repellant, chelating agent, enzyme inhibitor, or non-metallic toxicant capable of hindering the attachment of the organism to the surface while affixed to the surface, is disclosed along with improved apparatuses which are produced using the method. The present invention in one preferred aspect relates to an enzyme in the form of an oxidase the activity of which results in the formation of a peroxide.

[0018] FR 2562554 A1 (Noel) discloses a anti-fouling coating composition comprising a protease and/or an endopeptidase. The present invention in one preferred aspect relates to an enzyme in the form of an oxidase the activity of which results in the formation of a peroxide.

[0019] U.S. Pat. No. 6,150,146 (Nippon Paint) discloses a method for controlled release of compounds having antimicrobial activity and a coating composition capable of controlled release of compounds having antimicrobial activity is provided. The disclosure relates to a method for releasing a compound having antimicrobial activity from a matrix at a controlled rate, which comprises incorporating an enzyme and a substrate in said matrix beforehand to allow said enzyme and said substrate to react with each other in said matrix to thereby produce said compound having antimicrobial activity; and further relates to a coating composition comprising a film-forming resin, an enzyme, and a substrate, said enzyme being capable of reacting with said substrate to produce a compound having antimicrobial activity. The present invention in one aspect relates to a composition, wherein an enzyme is present in the absence of its substrate.

[0020] WO 00/68324 (Novo Nordisk) relates to a preserved and/or conserved water based paint composition comprising an oxidoreductase, an oxidizing agent, a binder and at least 10% w/w water. The present invention in one aspect relates to a composition, wherein an enzyme is enzyme obtained or obtainable from a marine organism; and (iii) (a) a substrate for the enzyme; and/or (b) a precursor enzyme and a precursor substrate, wherein the precursor enzyme and the precursor substrate are selected such that a substrate for the enzyme is generatable by action of the precursor enzyme on the precursor substrate; wherein the enzyme and the substrate are selected such that an antifoulant compound is generatable by action of the enzyme on the substrate. The present invention in one aspect relates to a composition, wherein an enzyme is present in the absence of its substrate.

[0022] WO 0027204 (Novo Nordisk) discloses a phenol oxidising enzyme system, including a peroxidase an a peroxide source. The present invention in one aspect relates to a composition, wherein an enzyme is present in the absence of its substrate.

[0023] U.S. Pat. No. 6,221,821 (Novo Nordisk/Novozymes) in one embodiment relates to a paint comprising for conservation purposes a variant of a haloperoxidase. Haloperoxidases consume peroxides when oxidising halides. The present invention in one aspect relates to a composition, wherein an enzyme is present in the absence of its substrate.

[0024] U.S. Pat. No. 6,251,386 (Novo Nordisk) relates to an antimicrobial composition comprising a haloperoxidase and hydrogen peroxide. The present invention in one aspect relates to a composition, wherein an enzyme is present in the absence of its substrate.

[0025] U.S. Pat. No. 5,919,689 (Selvig) discloses marine antifouling compositions and/or paints containing, microorganism(s), or mixtures of hydrolytic enzyme(s) and microorganism(s), wherein the microorganism or hydrolytic enzyme reduce fouling of a surface coated by the marine antifouling composition and/or paint. Such compositions and/or paints may contain a catalytically effective amount of an inorganic salt. Also disclosed are articles coated with the composition and/or paint. Finally, methods are disclosed for reducing fouling of a marine surface, for reducing marine corrosion, for limiting absorption of water by a marine surface, for reducing the coefficient of drag of a marine surface, removing marine growth from a marine surface, and for reducing mildew fungus on a marine surface. The present invention does not employ a coating composition comprising a microorganism.

SUMMARY OF INVENTION

[0026] The present invention in one aspect relates to a composition comprising at least one enzyme capable of acting on a compound, wherein said action results in the formation of an antifouling species comprising an antifouling activity, and wherein said compound does not form part of said composition.

[0027] The composition is preferably a coating composition further comprising a pigment, or a hygienic composition further comprising a fragrance, or a composition as stated herein above further comprising both a pigment and a fragrence.

[0029] In one aspect of the invention there is provided a method for reducing marine corrosion comprising the step of coating a marine surface with a marine antifouling composition, whereby the composition forms at least one film that reduces adsorption of corrosive molecules to the surface. Also disclosed is a method wherein the composition impedes surface corrosion and intergranular corrosion.

[0030] Another aspect of the invention is a method for reducing marine corrosion comprising the step of coating a marine surface with a marine antifouling paint, whereby the paint forms at least one film that reduces adsorption of corrosive molecules to the surface. In yet another aspect of the claimed invention, a method is disclosed, wherein the paint impedes surface corrosion and intergranular corrosion.

[0031] Yet another aspect of the invention is a method for limiting absorption of water by a marine surface comprising the step of coating the surface with a marine antifouling composition or marine antifouling paint, whereby the composition or paint produces a film which in turn reduces the porosity of the surface.

[0032] In another aspect of the invention, a method is disclosed for reducing the coefficient of drag of a marine surface comprising the step of coating the surface with a marine antifouling composition or marine antifouling paint. The invention is also directed to methods of using the marine antifouling composition or marine antifouling paint wherein surfactants capable of acting as wetting agents are produced by microorganisms in contact with the composition or paint.

[0033] An aspect of the invention is a method for removing marine growth from a marine surface, comprising the step of coating the surface with a marine antifouling composition or marine antifouling paint. Another aspect of the invention is a method of using the marine antifouling composition or marine antifouling paint wherein the marine growth is hard or soft growth. Yet another aspect of the invention is a method of using the marine antifouling composition or marine antifouling paint, wherein e.g. hydrolytic enzymes attack exudates of existing growths and causes release of hard and soft growth.

[0034] In another aspect of the invention, marine antifouling compositions or marine antifouling paints are disclosed that comprise an inorganic salt present in a catalytically effective amount. Yet another aspect of the invention is a method of reducing the tendency of a propeller to cavitate under a load, comprising the step of coating a surface of the propeller with a marine antifouling composition or marine antifouling paint. Still another aspect of the invention is a method of using a marine antifouling composition for reducing mildew fungus on a marine surface, comprising the step of coating a marine surface with a marine antifouling composition, whereby the composition forms at least one film that reduces the adsorption or attachment of mildew fungus to the surface, or impedes the growth of mildew fungus on the marine surface.

[0035] Definitions

[0036] Anti-fouling: The effect of controlling, reducing and/or eliminating over time the number of undesirable

[0038] Bio-film: Habitation of microbial organisms on a solid or semi-solid surface.

[0039] Coating composition: Composition for coating an object, such as a paint.

[0040] Co-factor: Additional factor required by an enzyme.

[0041] Compound: Substrate for an enzyme capable of catalysing said compound, wherein said catalysis results in the formation of an antimicrobial species comprising an antimicrobial activity.

[0042] Enzyme: Biomolecule comprising a plurality of amino acids and capable of catalysing conversion of substrates into products. The terms enzyme and precursor enzyme are used interchangably unless otherwise indicated. An enzyme is acting on a compound as defined herein when said action generates an antifouling species having antifouling activity. A precursor enzyme is any enzyme capable of providing to the enzyme, by means of degradation or otherwise, a substrate for said enzyme in the form of said compound.

[0043] Marine organism: Any organism capable of habitating in an aqueous environment, including organisms capable of forming undesirable bio-films.

[0044] Microbial organism: Any organism belonging to the classes of prokaryotes and lower eukaryotes, including bacteria, yeasts, fungal cells and slime molds.

[0045] Oxidase: Enzyme the activity of which results in an oxidation, including an oxidation resulting in the formation of a peroxide, including hydrogenperoxide.

[0046] Peroxide: Product resulting from a reaction involving an oxidase.

[0047] Precursor compound: Precursor compounds are capable of being catalysed by a precursor enzyme, wherein said catalysis results in the formation of a compound capable of being catalysed by an enzyme under the generation of an antifouling species, including an antimicrobial species having an antimicrobial activity.

[0048] Secretion: Process of translocating a compound or precursor compound across the outer membrane of a microbial species. Secretion applies to compounds which remain membrane associated and to compounds which are subsequently released into an external environment.

[0049] Surface: Outer part of e.g. a microbial organism in contact with the external environment.

DETAILED DESCRIPTION OF THE INVENTION

[0050] The present invention in one preferred embodiment relates to a coating composition comprising at least one enzyme, preferably an oxidase, capable of acting on a compound, such as a substrate for said oxidase, wherein said action results in the formation of an antifouling species including an antimicrobial species comprising an antimicrobial activity, and wherein said compound does not form part

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