US006150146A

United States Patent [19]

Hamade et al.

[11] **Patent Number: 6,150,146**

[45] **Date of Patent: Nov. 21, 2000**

[54] **METHOD FOR CONTROLLED RELEASE OF COMPOUNDS HAVING ANTIMICROBIAL ACTIVITY AND COATING COMPOSITION**

[75] Inventors: **Ryoji Hamade**, Kadoma; **Naoki Yamamori**, Kyotanabe, both of Japan

[73] Assignee: **Nippon Paint Co., Ltd.**, Osaka, Japan

[21] Appl. No.: **09/042,570**

[22] Filed: **Jan. 17, 1998**

[30] **Foreign Application Priority Data**

Mar. 17, 1997 [JP] Japan 9-084505

[51] **Int. Cl.**[7] **C12P 7/02**; C12P 13/00; C12P 7/40; C12N 9/18; A61K 31/74

[52] **U.S. Cl.** **435/155**; 435/128; 435/136; 435/197; 435/212; 424/78.09

[58] **Field of Search** 435/128, 132, 435/136, 155, 189, 192, 197, 212; 106/14.34; 424/78.09

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,297,137	10/1981	Sachetto et al.	514/493
5,262,151	11/1993	Montgomery	424/50
5,770,188	6/1998	Hamade et al.	424/78.09
5,801,226	9/1998	Cummins et al.	530/388.2
5,919,689	7/1999	Selvig et al.	435/202
5,998,200	12/1999	Bonaventura et al.	435/264

FOREIGN PATENT DOCUMENTS

0 518 445 A1	12/1992	European Pat. Off. .
04337369	5/1991	Japan .
04279677	10/1992	Japan .
95/27009	10/1995	WIPO .
96/38548	12/1996	WIPO .

OTHER PUBLICATIONS

Kennedy et al. J. Chem. Soc. Perkin Trans. 1 (1978) 9: 1058–66 (abstract only).

Primary Examiner—Jon P. Weber
Attorney, Agent, or Firm—Pollock, Vande Sande & Amernick

[57] **ABSTRACT**

A novel method for controlled release of compounds having antimicrobial activity and a novel coating composition capable of controlled release of compounds having antimicrobial activity is provided.

This invention 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.

35 Claims, 1 Drawing Sheet

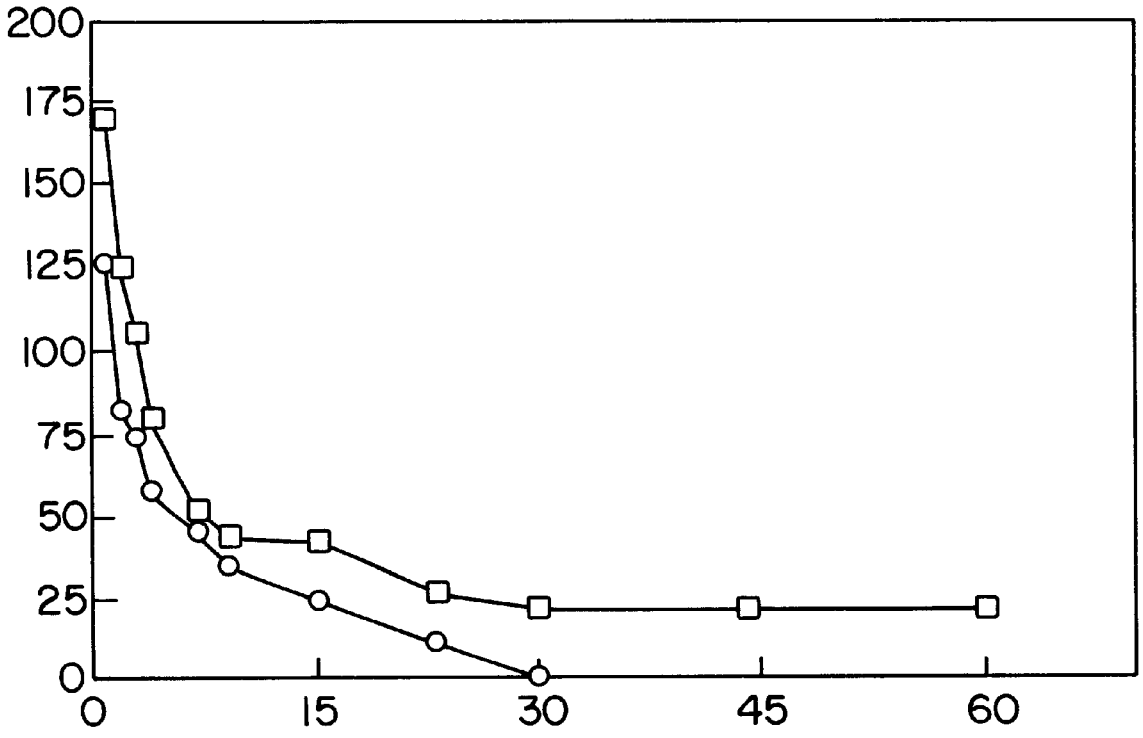

FIG. 1

METHOD FOR CONTROLLED RELEASE OF COMPOUNDS HAVING ANTIMICROBIAL ACTIVITY AND COATING COMPOSITION

TECHNICAL FIELD

The present invention relates to a method for controlled release of compounds having antimicrobial activity and to a coating composition.

PRIOR ART

Compounds having antimicrobial activity, inclusive of antifouling agents, antibacterial agents, antifungal agents, 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 has increased greatly in recent years.

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 such animals and plants as barnacles mussels and sea lettuce, adhere to and grow on said structures. When the surfaces of the structures exposed to seawater are covered with such marine organisms, there take place corrosion of the covered part; decreased marine fuel efficiency due to increased frictional resistance of the ship bottom against seawater; massive deaths of fishes and shellfishes or decreased working efficiency due to clogging of fish farming nets; and sinking buoys due to reduced buoyancy. It is therefore very important to apply an antifouling treatment to such structures exposed to seawater.

Meanwhile, as can be easily understood from the serious problem posed by an increasing incidence of 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 hospitals, schools, and hotels against such microorganisms.

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.

For example, the conventional antifouling technology for structures exposed to seawater comprises coating the surface of structures with an antifouling paint containing a compound having antimicrobial activity. This antifouling 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.

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.

When an object is treated with a compound having antifouling or antibacterial/antifungal activity, it is of course

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 insure a sustained long-term effect. The demand therefore exists for a new methodology for insuring a sustained long-term effect.

There are many compounds having antimicrobial activity. As compounds having antifouling activity for incorporation in antifouling paints, organotin compounds have been mostly employed. Moreover, as described in Bohkin Bohbai Handbook [Handbook of Antibacterial and Antifungal Technologies (Japanese Society for Protection Against Bacteria and Fungi ed., Gihodo Publishing Co., 1986)], it is known that hydrogen peroxide shows high antimicrobial/biocidal activity against a broad spectrum of bacteria and other microorganisms. Furthermore, a variety of compounds such as aliphatic carboxylic acids, aromatic carboxylicacids, aliphatic alcohols, and phenolic compounds are also known to have antimicrobial activity.

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.

Hydrogen peroxide is highly safe and free from the above problems. Because hydrogen peroxide is an unstable compound, however, it has been practically impossible to use it directly as an ingredient in antifouling or antibacterial/antifungal paints.

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 fir water. 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.

In the above state of the art, the present invention has for its object to provide a novel method for sustained release of compounds having antimicrobial activity and a coating composition capable of releasing a safe and effective compound having antimicrobial activity at a controlled rate.

SUMMARY OF THE INVENTION

The present invention 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.

The present invention further relates to a coating compo-

BRIEF DESCRIPTION OF THE DRAWING

FIG. **1** is a diagrammatic representation of the amount of release versus the duration of release of butyric acid in Example 1 and Comparative Example 1, where the ordinate represents the amount of release of butyric acid ($\mu g/cm^2$/day) and the abscissa represents the duration of release (day).

DETAILED DESCRIPTION OF THE INVENTION

The present invention is now described in detail.

According to the method for controlled release of a compound having antimicrobial activity according to the present invention, an enzyme and a substrate are incorporated in a matrix beforehand and allowed to react with each other in the matrix to thereby produce a compound having antimicrobial activity.

The matrix is not particularly restricted in kind but includes a paint film, a self-supporting film, a textile fabric, a synthetic resin film or sheet, and an inorganic wall paint, among others.

The compound having antimicrobial activity is not restricted, provided it is produced as the result of enzyme-substrate reaction. As such, there can be mentioned many compounds having antifouling activity, compounds having antibacterial/antifungal activity, compounds having biocidal activity, and compounds having biorepellent activity, etc.

As mentioned above, the compound having antimicrobial activity is produced by enzymatic reaction between an enzyme and a substrate. It should be understood that said compound having antimicrobial activity may be a compound obtained as the direct result of enzymatic reaction between the enzyme and the substrate or a compound formed from the product of such enzymatic reaction through further enzymatic or chemical reaction. The former case in which the compound having antimicrobial activity is the direct product of enzymatic reaction typically includes the case in which said substrate is a precursor of the compound having antimicrobial activity. Typical of the latter case in which the compound having antimicrobial activity is formed from such an enzymatic reaction product through further enzymatic or chemical reaction is the case in which such an enzymatic reaction product is a precursor of the objective compound having antimicrobial activity.

Furthermore, said compound having antimicrobial activity may be other than products whose major structural portions are originated from the substrate among the products obtained by said enzyme-substrate reaction. Falling into this category is the case in which the substrate is deaminated or oxidized/reduced by deamination or oxidation/reduction reaction to produce an amino-containing compound or a peroxide.

The above-mentioned compound having antimicrobial activity includes but is not limited to carboxyl group-containing compounds, hydroxyl group-containing compounds, amino group-containing compounds, aldehyde group-containing compounds, hydrogen peroxide, and decomposition products of chitosan.

The carboxyl group-containing compound is not particularly restricted in kind but includes a variety of organic acid compounds, e.g. aliphatic acids such as formic acid, acetic dibasic acids such as oxalic acid etc.; aromatic carboxylic acids such as benzoic acid, p-chlorobenzoic acid, p-hydroxybenzoic acid, salicylic acid, cinnamic acid, etc.; and their derivatives and halides.

There is no particular limitation on an enzyme-substrate combination capable of producing such a carboxyl group-containing compound. Typical are the case in which the enzyme is an esterase and the substrate is an ester bond-containing compound and the case in which the enzyme is an amidase and the substrate is an amide bond-containing compound.

The esterase is not particularly restricted in kind but includes esterases such as carboxylesterase, arylesterase, acetylesterase, etc.; lipases such as triacylglycerol lipase, lipoprotein lipase, etc.; and proteases such as subtilisin, chymotrypsin, tripsin, elastase, cathepsin, papain, chymopapain, pepsin, etc., and so forth.

The ester bond-containing compound mentioned above is not particularly restricted in kind but includes, among others, esters of any of said carboxyl group-containing compounds with aliphatic alcohols such as methyl alcohol, ethyl alcohol, propyl alcohol, butyl alcohol, pentyl alcohol, caproyl alcohol, caprylyl alcohol, capryl alcohol, lauryl alcohol, myristyl alcohol, palmityl alcohol, oleyl alcohol, etc.; esters of any of said carboxyl group-containing compounds with aromatic alcohols such as phenol, benzyl alcohol, etc.; esters of any of said carboxyl group-containing compounds with polyhydric alcohols such as ethylene glycol, glycerol, etc.; and esters of any of said carboxyl group-containing compounds with derivatives or halides of said aliphatic alcohols, aromatic alcohols, or polyhydric alcohols.

The ester bond-containing compound mentioned above is hydrolyzed by said esterase in the above-mentioned matrix to produce said carboxylic group-containing compound. This enzymatic reaction proceeds when water is present in the reaction system, as follows.

$$R^1COOR^2+H_2O \rightarrow R^1COOH+R^2OH$$

In the above reaction schema, R^1 represents carboxylic residue and R^2 represents an alcohol residue. The water necessary for the above enzymatic reaction is supplied as follows. Thus, when the above matrix is present in the atmosphere, the water contained in the atmosphere migrates into the matrix to supply the necessary water to the reaction system. When the matrix exists in water such as seawater, the very water penetrates into the matrix to supply the necessary water to the reaction system. Therefore, even when said ester bond-containing compound and said esterase coexist in the matrix, the above-mentioned enzymatic reaction does not proceed unless water penetrates into the matrix, with the result that said carboxyl group-containing compound cannot be produced. On the other hand, when the matrix contacts air or seawater and consequently water finds its way into the matrix, the above enzymatic reaction proceeds, with the result that said carboxyl group-containing compound is produced in the matrix. Thus, in accordance with the present invention, said carboxyl group-containing compound is persistently produced within the matrix at a constant rate to obtain the objective controlled release.

The amidase mentioned above is not particularly

The amide bond-containing compound mentioned above is not particularly restricted in kind but includes, among others, amides of any of said carboxyl group-containing compounds with aliphatic amines such as butylamine, hexylamine, octylamine, decylamine, laurylamine, stearylamine, oleylamine, etc.; and amides of any said carboxyl group-containing compounds with aromatic amines such as aniline, toluidine, xylidine, and alkylanilines such as hexylaniline, octylaniline, nonylaniline, dodecylaniline, and so forth.

The hydroxyl group-containing compound mentioned above is not particularly restricted in kind but includes, among others, aliphatic alcohols such as methyl alcohol, ethyl alcohol, propyl alcohol, isopropyl alcohol, butyl alcohol, isobutyl alcohol, pentyl alcohol, isopentyl alcohol, hexyl alcohol, etc.; aromatic alcohols such as phenol, chlorophenol, and alkylphenols such as cresol, xylenol, etc., resorcinol, benzyl alcohol, etc.; and the derivatives and halides of said aliphatic or aromatic alcohols.

There is no particular limitation on an enzyme-substrate combination capable of producing the hydroxyl group-containing compound. Preferred is the case in which the enzyme is an esterase and the substrate is an ester bond-containing compound.

The esterase and the ester bond-containing compound are not particularly restricted in kind but each includes the species mentioned hereinbefore.

The amino group-containing compound mentioned above is not particularly restricted in kind, either, but includes aliphatic amines such as butylamine, hexylamine, octylamine, decylamine, laurylamine, stearylamine, oleylamine, cyclohexylamine, etc.; and aromatic amines such as aniline, toluidine, xylidine, p-n-hexylaniline, p-n-octylaniline, p-nonylaniline, p-dodecylaniline, and so forth.

There is no particular limitation on an enzyme-substrate combination capable of producing said amino group-containing compound. Preferred, however, is the case in which the enzyme is an amidase and the substrate is an amide bond-containing compound.

The amidase and the amide bond-containing compound are not particularly restricted in kind but each includes the species mentioned hereinbefore.

The aldehyde group-containing compound is not particularly restricted in kind but includes aliphatic aldehydes such as formaldehyde, glyoxal, succinaldehyde, glutaraldehyde, capronaldehyde, caprylaldehyde, caprinaldehyde, laurinaldehyde, stearinaldehyde, oleinaldehyde, etc.; benzaldehyde and its derivatives such as p-n-hexylbenzaldehyde, p-octylbenzaldehyde, p-oleylbenzaldehyde, vaniline, piperonal, etc.; salicylaldehyde, cinnamaldehyde, and so forth.

An enzyme-substrate combination capable of producing said aldehyde group-containing compound is not particularly restricted but includes the case in which the enzyme is alcohol dehydrogenase and the substrate is an aliphatic alcohol, e.g. methanol, ethanol, etc.; the case in which the enzyme is alcohol oxidase and the substrate is an aliphatic alcohol such as methanol, ethanol, etc.; the case in which the enzyme is arylalcohol dehydrogenase and the substrate is an aromatic alcohol such as phenol, cresol, etc.; and the case in which the enzyme is amine oxidase and the substrate is an aliphatic amine such as butylamine, hexylamine, and so forth.

An enzyme-substrate combination capable of producing

A combination of said oxidase with said compound to be oxidized thereby is not particularly restricted but specifically includes such combinations as malate oxidase-malic acid; glucose oxidase-glucose; hexose oxidase-glucose; cholesterol oxidase-cholesterol; arylalcohol oxidase-arylalcohol: galactose oxidase-galactose; alcohol oxidase-alcohol; lathosterol oxidase-lathosterol; aspartate oxidase-aspartic acid; L-amino-acid oxidase-L-amino acid; D-amino-acid oxidase-D-amino acid; amine oxidase-amine; D-glutamate oxidase-glutamine; ethanolamine oxidase-ethanolamine; NADH oxidase-NADH; urate oxidase (uricase)-uric acid; superoxide dismutase-superoxide radical; and so forth.

The enzymatic reaction between said oxidase and its substrate compound yields hydrogen peroxide. As shown below, this enzymatic reaction proceeds when either oxygen or oxygen and water are present in the reaction system.

1) Compound A (substrate)+oxygen→Compound B (oxide of Compound A)+hydrogen peroxide

2) Compound C (substrate)+oxygen+water→Compound D+hydrogen peroxide+ammonium ion

3) Compound E (substrate)+oxygen+water→Compound F (oxide of Compound E)+hydrogen peroxide

4) Superoxide radical+proton→hydrogen peroxide+oxygen

The above-mentioned oxygen is supplied not only from atmospheric air but also from seawater containing dissolved oxygen as the oxygen permeates into the matrix from its surface in contact with seawater or the like. Therefore, even in water such as seawater, the enzymatic reaction proceeds in the matrix as water penetrates into the matrix, with the result that hydrogen peroxide is produced in the matrix.

An enzyme-substrate combination capable of producing said decomposition product of chitosan is not particularly restricted. Preferred is the case in which the enzyme is a chitosan-decomposing enzyme and the substrate is chitosan.

The chitosan-decomposing enzyme is not particularly restricted in kind but includes chitosanase, cellulose, lysothyme, and so forth.

In the present invention, the enzyme for use as incorporated in the matrix may be any of a purified enzyme, a crude enzyme, or enzyme-containing cells. The source of the enzyme is not restricted, either, but includes microorganisms, plants, and animals. Moreover, in incorporating an enzyme in the matrix, the enzyme may be directly incorporated or it can be used after modification with another compound, or in the form of an immobilized enzyme. The specific forms of said modified enzyme and immobilized enzyme are not particularly restricted but include enzymes entrapped in reverse micelles; enzymes modified with lipids or surfactants; enzymes modified with polyethylene glycol; and enzymes immobilized on polymer matrices, among other forms.

Generally, enzymatic reaction between an enzyme and a substrate proceeds in the presence of water as the medium. Thus, even if both an enzyme and its substrate coexist, enzymatic reaction does not proceed unless they encounter each other through the water. In the method for controlled release of a compound having antibiotic activity according to the present invention, the controlled release of the compound having antimicrobial activity is achieved by exploiting the above characteristic of enzymatic reaction. Thus, an enzyme and its substrate occurring as dispersed in a matrix are brought into contact by the water penetrating into the matrix from the atmospheric air or other environment with

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.