present invention further relates to methods for treating a biofilm using peroxyformic acid, including peroxyformic acid generated *in situ*.

In one aspect, the present disclosure is directed to a peroxyformic acid forming composition comprising: a) a first reagent that comprises an ester of a polyhydric alcohol and formic acid, and b) a second reagent that comprises hydrogen peroxide or that comprises a substance that generates hydrogen peroxide when in contact with a liquid, wherein 1) said first reagent and said second reagent are kept separately prior to use, and when it is time to generate peroxyformic acid, said first reagent and said second reagent are configured to be contacted with each other to form a liquid that comprises peroxyformic acid and has a pH below about 11, and the pH of the formed liquid becomes about 8 or lower within about 1 minute after the contact between said first reagent and said second reagent; or 2) said second reagent comprises a substance that generates hydrogen peroxide when in contact with a liquid, said first reagent and said second reagent are comprised in a solid composition, and when it is time to generate peroxyformic acid, said solid composition is configured to be contacted with a liquid to form a liquid that comprises peroxyformic acid and has a pH below about 11, and the pH of the formed liquid becomes about 8 or lower within about 1 minute after the contact between said solid composition and said liquid.

In another aspect, the present disclosure is directed to a method for forming peroxyformic acid comprising: a) contacting a first reagent that comprises an ester of a polyhydric alcohol and formic acid, and a second reagent that comprises hydrogen peroxide or that comprises a substance that generates hydrogen peroxide when in contact with a liquid to form a liquid that comprises peroxyformic acid and has a pH below about 11, wherein said first reagent and said second reagent are kept separately prior to said contacting and pH of said formed liquid becomes about 8 or lower within about 1 minute after the contact between said first reagent and said second reagent; or b) contacting a solid composition that comprises a first reagent that comprises an ester of a polyhydric alcohol and formic acid, and a second reagent that comprises a substance that generates hydrogen peroxide when in contact with a liquid with a liquid to form a liquid that comprises peroxyformic acid and has a pH below about 11, and pH of said formed liquid becomes about 8 or lower within about 1 minute after the contact between said solid composition and said liquid.

In some embodiments, the use of the above peroxyformic acid forming compositions and the above methods for forming peroxyformic acid are at least based on the surprising finding that the formic acid esters of polyhydric alcohol, such as glycerol formates, can quickly or instantly generate peroxyformic acid in the presence of H_2O_2 under very mild pH conditions in the absence of an enzyme. Furthermore, the pH of the solution quickly or instantly moved significantly lower after the mixing of the esters and H_2O_2 solution, thus eliminating the need for adjusting pH of the formed liquid.

In another aspect, the present disclosure is directed to peroxyformic acid formed using the above method.

In an embodiment, the present disclosure is directed to a method for treating a surface or a target, which method comprises a step of contacting a surface or a target with an effective amount of peroxyformic acid formed using the above methods, preferably *in situ*, to form a treated target composition, wherein said treated target composition comprises from about 0.1 ppm to about 10,000 ppm of said peroxyformic acid, and said contacting step lasts for sufficient time to stabilize or reduce microbial population in and/or on said surface or target or said treated target composition.

In another aspect, the present disclosure is directed to a method for treating a target, which method comprises contacting a target with an effective amount of peroxyformic acid as disclosed herein to form a treated target composition, wherein said treated target composition comprises from 0.1 ppm to 10,000 ppm of said peroxyformic acid, and said contacting lasts for sufficient time to bleach, remove soils and/or stabilize or reduce microbial population in and/or on said target or said treated target composition, wherein said target is selected from the group consisting of: a food item; a plant item; at least a portion of a medium, a container, an equipment, a system or a facility for growing, holding, processing, packaging, storing transporting, preparing, cooking, or serving the food item or the plant item; and water.

In another aspect, the present disclosure is directed to the use of an effective amount of the peroxyformic acid as disclosed herein in the manufacture of a medicament, for reducing microbial population in and/or on a target,

wherein the medicament comprises from 0.1 ppm to 10,000 ppm of said peroxyformic acid, wherein the target is selected from the group consisting of: a part of a urinary tract; a part of a middle ear; a part of gums; and skin or other external or mucosal surfaces of an animal.

In another embodiment, the present disclosure is directed to a method for treating a biofilm, which method comprises contacting a biofilm on a surface with an effective amount of peroxyformic acid for a sufficient time to stabilize, reduce and/or remove microbial population in and/or on said treated biofilm, or to stabilize, reduce and/or remove said biofilm on said surface.

While multiple embodiments are disclosed, still other embodiments of the present disclosure will become apparent to those skilled in the art from the following detailed description, which shows and describes illustrative embodiments of the invention. Accordingly, the drawings and detailed description are to be regarded as illustrative in nature and not restrictive.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates perhydrolysis of various esters according to embodiments of the invention.

The claimed invention is:

1. A peroxyformic acid forming composition comprising:

a) a first reagent that comprises an ester of a polyhydric alcohol and formic acid, and

b) a second reagent that comprises hydrogen peroxide or that comprises a substance that generates hydrogen peroxide when in contact with a liquid,

wherein

1) said first reagent and said second reagent are kept separately prior to use, and when it is time to generate peroxyformic acid, said first reagent and said second reagent are configured to be contacted with each other to form a liquid that comprises peroxyformic acid and has a pH below 11, and the pH of the formed liquid becomes 8 or lower within 1 minute after the contact between said first reagent and said second reagent; or

2) said second reagent comprises a substance that generates hydrogen peroxide when in contact with a liquid, said first reagent and said second reagent are comprised in a solid composition, and when it is time to generate peroxyformic acid, said solid composition is configured to be contacted with a liquid to form a liquid that comprises peroxyformic acid and has a pH below 11, and the pH of the formed liquid becomes 8 or lower within 1 minute after the contact between said solid composition and said liquid.

2. The peroxyformic acid forming composition of claim 1, wherein the ester comprises glycerol formates, ethylene glycol formates, pentaerythritol formates, mannitol formates, propylene glycol formates, sorbitol formates or sugar formates, wherein the sugar formates comprise sucrose formates, dextrin formates, maltodextrin formates, or starch formates.

3. The peroxyformic acid forming composition of any of claims 1-2, wherein the second reagent has a pH below 11.

4. The peroxyformic acid forming composition of any one of claims 1-3, wherein the first reagent and the second reagent are configured to be contacted with each other to form a solution that has a pH ranging from 4 to 9, and comprises peroxyformic acid, or the solid composition is configured to be contacted with a liquid to form a solution that has a pH ranging from 4 to 9, and comprises peroxyformic acid, wherein the first reagent and the second reagent are configured to be contacted with each other to form a solution that comprises peroxyformic acid under ambient conditions, or the solid composition is configured to be contacted with a liquid to form a solution that comprises peroxyformic acid under ambient conditions.

5. The peroxyformic acid forming composition of any of claims 1-4, which further comprises a catalyst or an enzyme that catalyzes formation of peroxyformic acid from the ester of a polyhydric alcohol and formic acid, and hydrogen peroxide, and wherein the catalyst or enzyme comprises a perhydrolytic enzyme, lipase, coronase, termanyl or esperease.

6. The peroxyformic acid forming composition of any of claims 1-5, which further comprises a stabilizing agent for peroxyformic acid, a stabilizing agent for hydrogen peroxide, and/or a pH buffering/adjusting agent, and wherein the stabilizing agent is a phosphonate salt(s) and/or a heterocyclic dicarboxylic acid.

7. The peroxyformic acid forming composition of any of claims 1-6, which comprises multiple dosages of the first reagent that are kept separately prior to use, and are used to contact hydrogen peroxide sequentially, and wherein the multiple dosages of the first reagent comprise the same ester of a polyhydric alcohol and formic acid or different esters of polyhydric alcohols and formic acid.

8. The peroxyformic acid forming composition of any of claims 1-7, which comprises in the formed liquid from 1 ppm to 500,000 ppm of an ester of a polyhydric alcohol and formic acid, or

wherein the solid composition comprises from 1 ppm to 500,000 ppm of an ester of a polyhydric alcohol and formic acid in the formed liquid, and which comprises in the formed liquid 0.1 ppm to 300,000 ppm of hydrogen peroxide or a substance at an amount that generates from 0.1 ppm to 300,000 ppm of hydrogen peroxide upon contact with a liquid, or wherein the solid composition comprises a substance at an amount that generates from 0.1 ppm to 100,000 ppm of hydrogen peroxide in the formed liquid.

9. The peroxyformic acid forming composition of any of claims 1-8, which is configured to form a solution that comprises from 0.1 ppm to 10,000 ppm of peroxyformic acid in the formed liquid.

10. The peroxyformic acid forming composition of any of claims 1-9, which is configured to form a solution that comprises at least 1 ppm peroxyformic acid within 1 minute of the contact time, and/or which is configured to form a solution that comprises at least 80% of the peak concentration of peroxyformic acid within from 5 minutes to 15 minutes of the contact time, and/or wherein the formed peroxyformic acid maintains at least 50% of the peak concentration from 5 minutes to 25 minutes after the contact between the first reagent and the second reagent.

11. The peroxyformic acid forming composition of any of claims 1-10, which further comprises a C_2-C_{22} percarboxylic acid, and wherein the first reagent, the second reagent or the solid composition comprising the first reagent and the second reagent are kept separately from the C_2-C_{22} percarboxylic acid prior to use.

12. A method for forming peroxyformic acid comprising:

a) contacting a first reagent that comprises an ester of a polyhydric alcohol and formic acid, and a second reagent that comprises hydrogen peroxide or that comprises a substance that generates hydrogen peroxide when in contact with a liquid to form a liquid that comprises peroxyformic acid and has a pH below 11, wherein said first reagent and said second reagent are kept separately prior to said contacting and pH of said formed liquid becomes 8 or lower within 1 minute after the contact between said first reagent and said second reagent; or

b) contacting a solid composition that comprises a first reagent that comprises an ester of a polyhydric alcohol and formic acid, and a second reagent that comprises a substance that generates hydrogen peroxide when in contact with a liquid with a liquid to form a liquid that comprises peroxyformic acid and has a pH below 11, and pH of said formed liquid becomes 8 or lower within 1 minute after the contact between said solid composition and said liquid.

13. The method of claim 12, wherein the ester comprises glycerol formates, ethylene glycol formates, pentaerythritol formates, mannitol formates, propylene glycol formates, sorbitol formates or sugar formates, and wherein the sugar formates are sucrose formates, dextrin formates, maltodextrin formates, or starch formates.

14. The method of any of claims 12-13, wherein the second reagent has a pH ranging from 0 to 11, or from 5 to 9, and wherein the first reagent and the second reagent are contacted with each other to form a solution that has a pH ranging from 4 to 9, and comprises peroxyformic acid, or the solid composition is contacted with a liquid to form a solution that has a pH ranging from 4 to 9, and comprises peroxyformic acid.

15. The method of any of claims 12-14, wherein the first reagent and the second reagent are contacted to form a solution that comprises peroxyformic acid under ambient conditions, or the solid

composition is contacted with a liquid to form a solution that comprises peroxyformic acid under ambient conditions.

16. The method of any of claims 12-15, wherein the first reagent and the second reagent are contacted with each other or the solid composition is contacted with a liquid in the presence of a catalyst or an enzyme that catalyzes formation of peroxyformic acid from the ester of a polyhydric alcohol and formic acid, and hydrogen peroxide, and wherein the catalyst or enzyme comprises a perhydrolytic enzyme, lipase, coronase, termanyl or esperease.

17. The method of any of claims 12-16, which further comprises using a stabilizing agent for peroxyformic acid, a stabilizing agent for hydrogen peroxide, and/or a pH buffering/adjusting agent, and wherein the stabilizing agent is phosphonate salt(s) and/or a heterocyclic dicarboxylic acid.

18. The method of any of claims 12-17, which comprises contacting hydrogen peroxide with multiple dosages of the first reagent sequentially, wherein the multiple dosages of the first reagent comprise the same ester of a polyhydric alcohol and formic acid, or wherein the multiple dosages of the first reagent comprise different esters of polyhydric alcohols and formic acid.

19. The method of any of claims 12-18, wherein the first reagent in the formed liquid comprises from 1 ppm to 500,000 ppm of an ester of a polyhydric alcohol and formic acid, wherein the second reagent in the formed liquid comprises 0.1 ppm to 100,000 ppm of hydrogen peroxide, and wherein the reagents form a solution that comprises from 0.1 ppm to 100,000 ppm of peroxyformic acid.

20. The method of any of claims 12-19, which is used to form a solution that comprises at least 1 ppm peroxyformic acid within 1 minute of the contact time, and/or a solution that comprises

at least 80% of the peak concentration of peroxyformic acid within from 5 minutes to 15 minutes of the contact time, and/or wherein the formed peroxyformic acid maintains at least 50% of the peak concentration from 5 minutes to 25 minutes after the contact between the first reagent and the second reagent.

21. The method of any of claims 12-20, which further comprises forming a C_2-C_{22} percarboxylic acid in the liquid.

22. The method of claim 21, wherein the C_2-C_{22} percarboxylic acid comprises peroxyacetic acid, peroxyoctanoic acid and/or peroxysulfonated oleic acid.

23. Peroxyformic acid formed using a method of any of claims 12-22.

24. A method for treating a target, which method comprises contacting a target with an effective amount of peroxyformic acid of claim 23 to form a treated target composition, wherein said treated target composition comprises from 0.1 ppm to 10,000 ppm of said peroxyformic acid, and said contacting lasts for sufficient time to bleach, remove soils and/or stabilize or reduce microbial population in and/or on said target or said treated target composition, wherein said target is selected from the group consisting of: a food item; a plant item; at least a portion of a medium, a container, an equipment, a system or a facility for growing, holding, processing, packaging, storing transporting, preparing, cooking, or serving the food item or the plant item; and water.

25. The method of claim 24, wherein the target is a surface in need of high level disinfecting, and the method comprises providing an effective amount of peroxyformic acid of claim 23 to the surface in need of treatment to form a treated surface, wherein said treated surface source comprises from 1 ppm to 2,000 ppm of said peroxyformic acid.

26. The method of claim 25, wherein the surface is a medical instrument in need of repurposing or reprocessing.

27. The method of claim 26, wherein the medical instrument is an endoscope and the treated surface comprises from 10 ppm to 1,000 ppm of the peroxyformic acid.

28. The method of claim 27, wherein the contacting step lasts for 10 minutes.

29. The method of claims 24-28, wherein the pH of the peroxyformic acid is from 7-8.

30. The method of claim 24, wherein the peroxyformic acid is used at a concentration from 1 ppm to 1,000 ppm.

31. The method of claim 24, wherein the peroxyformic acid is used at a concentration from 10 ppm to 300 ppm.

32. The method of any of claims 24-31, wherein the peroxyformic acid is applied to the target by means of a spray, a fog, or a foam, or by dipping all or part of the target is dipped in a composition comprising the peroxyformic acid, and wherein the contacting step lasts for at least 10 seconds.

33. The method of any of claims 24-32, wherein the microbial population in and/or on the target or the treated target composition is reduced by at least one $\log_{10}$, at least two $\log_{10}$ or at least three $\log_{10}$

34. The method of any of claims 24-33, wherein the microbial population comprises a prokaryotic microbial population, a bacterial population, an archaeal population, a eukaryotic microbial population, or combinations thereof.

35. The method of any of claims 24-34, which is conducted at a temperature ranging from 0°C to 70°C.

36. The peroxyformic acid forming composition of any of claims 1-11, which does not comprise a perhyrolysis enzyme member of family 7 of the carbohydrate esterases (CE-7).

37. The peroxyformic acid forming composition of any of claims 1-11, wherein the contacting step forms a solution that comprises at least 1 ppm peroxyformic acid within 45 seconds or less of the contact time, within 30 seconds or less of the contact time, within 15 seconds or less of the contact time, or within 5 seconds or less of the contact time.

38. The peroxyformic acid forming composition of any of claims 1-11, wherein the contacting step forms a solution that comprises at least 100 ppm peroxyformic acid within 5 minutes or less of the contact time.

39. The peroxyformic acid forming composition of any of claims 1-11, wherein the contacting step forms a solution that comprises at least 500 ppm peroxyformic acid within 5 minutes or less of the contact time.

40. The use of an effective amount of the peroxyformic acid of claim 23 in the manufacture of a medicament, for reducing microbial population in and/or on a target, wherein the medicament comprises from 0.1 ppm to 10,000 ppm of said peroxyformic acid, wherein the target is selected from the group consisting of: a part of a urinary tract; a part of a middle ear; a part of gums; and skin or other external or mucosal surfaces of an animal.