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(54) Title: COMPOSITIONS EXHIBITING SYNERGY IN BIOFILM CONTROL

(57) Abstract: A method of controlling and removing biofilm on a surface in contact with an aqueous industrial system comprising the step of adding an effective amount of biofilm-disrupting agent and adding a biocide to the aqueous system being treated to reduce and remove biofilm forming microbes from a surface in contact with the aqueous system is disclosed. A synergistic biocidal composition is also disclosed.



WO 2019/079106 A1

## COMPOSITIONS EXHIBITING SYNERGY IN BIOFILM CONTROL

## CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to Provisional Patent Application No. 62/573,871, filed October 18, 2017, which is incorporated herein by reference in its entirety.

## TECHNICAL FIELD

[0002] The present disclosure pertains to the control of microorganisms in an aqueous environment.

## BACKGROUND

[0003] Microbial biofilms in industrial, commercial, and civic systems and structures have substantial negative impacts on the functioning and operation of those systems and structures, including reducing heat transfer, plugging pipes and lines, serving as a reservoir of pathogens, causing mechanical and structural failure, promoting corrosion, contaminating and degrading products, drinking and recreational water, and reducing aesthetic values.

[0004] Biofilms are defined in the context of this document as microbes which settle, attach, and then grow or exist on surfaces. They may be composed of a single species or they may be poly specific, and may consist of bacteria, viruses, fungi, algae, and micro- or macro-eukaryotic organisms such as amoeba, diatoms, nematodes, and worms. Biofilms may exist submerged in liquid, in splash zones, moist environments, and even dry environments such as those found on the surfaces of statuary and buildings. Biofilms are structurally composed of microbial cells encased in a molecularly diverse polymeric matrix composed of polysaccharides, protein, DNA, and numerous small molecules. In natural environments they also can entrain dirt, soil, vegetable matter, and other environmental components. This material is often referred to as slime. The anatomy of a biofilm is extensively influenced by the composition of the environment and the shearing force supplied by the movement of the matrix over the film.

[0005] The consequences of microbes living in a fixed environment as opposed to free-floating in the bulk fluid are extensive with the microbes differentiating expression of their genome ranging from a few genes to almost 50% of their genome. These changes have an immense effect on the susceptibility of the biofilm cells to chemical biocides, antibiotics, and other

environmental stressors. In addition to the widespread physiological alterations the biofilm cells exist in the polymeric matrix which can interfere with the access of biocides or antibiotics to the cells, further reducing their susceptibility. Changes in biocide and antibiotic susceptibility of over one thousand-fold have been documented.

[0006] The most common approach to the control of biofilms has been the application of chemical biocides including oxidizing, reactive, and membrane-active biocides. Regardless of the mechanistic class of biocides biofilms have proven far more recalcitrant to their inhibitory and cidal action for the reasons discussed in the previous paragraph resulting in the need to apply high concentrations of biocide to achieve a desired effect.

[0007] Oxidizing biocides are commonly used as biofilm control agents in a wide variety of industrial, commercial, and civic areas because they are inexpensive and effective against planktonic microbes. They can be effective microbial control but high application rates, costs to treat, and the corrosive effect of the oxidants on materials of construction, as well as regulatory limitations in some cases, often make it difficult to apply them at rates effective for long-term biofilm control.

[0008] Oxidizing biocides, although they can kill substantial portions of the biofilm population, are not effective in removing biofilms from the surface. This is not satisfactory since some of the negative effects of biofilms derive from their physical presence on the surface. For instance, biofilms are excellent insulators and vastly impede heat transfer in cooling towers and chillers and although a treated biofilm may be substantially dead it will still insulate the surface. In addition, the large numbers of dead cells provide the surviving fragment of the treated population with a ready source of nutrients and biofilms tend to quickly re-grow to their original density.

[0009] Adjunct treatments in the form of biofilm-disrupting materials have been administered in conjunction with biocides to increase efficacy in both killing the microbes and removing them from the surface. These biofilm disrupting agents are most often anionic, cationic, or non-ionic surfactants whose presumed mechanism is to interact with the biofilm structure which both allows a more efficient penetration of the biofilm by the biocide and to remove biofilm by their surface-active properties. Despite the long presence of these biofilm disrupting agents in the market they are most often underutilized likely due to the efficacy of treatment programs using both oxidizing and non-oxidizing biocides. However, market, cost, and environmental concerns have brought about a desire to reduce the use of biocides without a reduction in the efficacy of

microbial control programs and interest in dispersants has been increasing in many markets, particularly industrial cooling waters. As one would expect relative abilities of these biofilm disrupting agents ranges from poor to good and their efficacy can be influenced by the composition of the bulk matrix. One would also expect that some combinations of oxidizing biocides and biofilm disrupting agents would be more efficacious than others based on the interaction of their chemistry and effect on the biofilm structure.

#### DETAILED DESCRIPTION

**[0010]** The following detailed description is merely exemplary in nature and is not intended to limit the invention or the application and uses of the invention. Furthermore, there is no intention to be bound by any theory presented in the preceding background of the invention or the following detailed description.

**[0011]** It has been surprisingly found that some combinations of biocides, preferably oxidizing biocides, and biofilm disrupting agents exhibit synergistic control of biofilms in terms of both killing them and removing them from the surface. The total effect of the combination of biocides and biofilm disrupting agents is far greater than the mere additive effect of the two chemicals such that the amounts of one, or both, chemicals can be greatly reduced and still achieve the desired endpoint of biofilm control. This synergistic interaction has not been found for all combinations of chemicals, nor at all ratios of the two chemicals.

**[0012]** Disclosed is a method of controlling and removing biofilm on surfaces in contact with an aqueous industrial system comprising the step of adding an effective amount of biofilm-disrupting agent and adding a biocide to the aqueous system being treated to reduce and remove biofilm forming microbes from a surface in contact with the aqueous system.

**[0013]** The invention also provides for a synergistic composition comprising a biofilm-disrupting agent and a biocide.

**[0014]** Oxidizing biocides useful in the invention include sodium hypochlorite, calcium hypochlorite, and other hypochlorite salts, hypochlorous acid, hypobromous acid, monohaloamine biocides derived from ammonium hydroxide, ammonium chloride, ammonium sulfate, ammonium acetate, ammonium bicarbonate, ammonium bromide, ammonium carbonate, ammonium carbamate, ammonium sulfamate, ammonium nitrate, ammonium oxalate,

ammonium persulfate, ammonium phosphate, ammonium sulfide, urea and urea derivatives, and other nitrogen containing compounds capable of donating an ammonium ion, being reacted with a chlorine or bromine moiety such as a chlorinated or brominated oxidant preferably hypochlorous acid or hypochlorite, preferable hypochlorite; and blends of ammonium-derived chloramine compounds such as monochloramine and dichloramine. Such haloamine biocides are known in the art see for example US 7285224, US 7052614US 7837883, US 7820060. Other oxidizing biocides include dibromonitrilo propionamide, bromochloro-dimethyl hydantoin and other halogenated hydantoins, and trichloroisocyanuric acid. Non-oxidizing biocides used against biofilms and expected to work with the dispersant include isothiazolone biocides, glutaraldehyde, formaldehyde and formaldehyde-releasing compounds, tetrakis-hydroxy phosphonium chloride, as well as other non-cationic biocides.

**[0015]** The biofilm-disrupting agent used in the invention is an anionic surfactant, preferable an anionic sulfonate surfactant. Anionic sulfonate surfactants for use in the present invention include alkyl sulfonates, linear and branched primary and secondary alkyl sulfonate and the linear or branch alkyl aromatic sulfonate. Particularly preferred are alkyl benzene sulfonate surfactants, such as sodium dodecyl benzene sulfonate. Other salts of dodecyl benzene sulfonate may also be used as the counter ion (sodium in this case) has no bearing on the mechanism of the disrupting agent.

**[0016]** Linear alkylbenzenesulfonates (sometimes also referred as LABS) are a family of organic compounds with the formula  $C_6H_5C_nH_{2n+1}$ . Typically, the average  $n$  lies between 10 and 16. Linear alkylbenzenes are generally available as an average alkyl range, such as the average alkyl group can be  $C_{12}$ - $C_{15}$  or  $C_{12}$ - $C_{13}$  or  $C_{10}$ - $C_{13}$ .

**[0017]** Sodium dodecylbenzenesulfonates (“SDBS”) are alkylbenzenesulfonates. Most sodium dodecylbenzenesulfonates are a member of the linear alkylbenzenesulfonates, meaning that the dodecyl group ( $C_{12}H_{25}$ ) is unbranched. This dodecyl chain can be attached at the 4-position of the benzenesulfonate group.

**[0018]** The invention also provides for a synergistic composition comprising a biofilm-disrupting agent and a biocide, wherein the biofilm-disrupting agent is sodium dodecylbenzenesulfonates and the biocide is a haloamine preferable selected from monohaloamine, dihaloamine and combinations thereof. The haloamine can be chloramine. Preferably the ratio of biofilm disrupting agent to the oxidizing biocide is from 1-part biocide to

greater than 1-part biofilm disrupting agent. The weight ratio of biocide to biofilm-disrupting agent can be from 1:1 to 1: 20, more preferable for 1:1 to 1:8.

[0019] The interactions of two chemicals in a composition can occur in three possible manners. In the first manner the two chemicals interact in a negative manner to diminish the combined effect of the composition such that the result achieved is less than what one would expect from their combined activities. Thus, if one agent by itself achieves a value of 50 in a measured variable and the second agent by itself achieves a value of 50, in a negative interaction the combined reduction value for the two would be less than 100. Another manner in which they can interact is additive, in which the final result is the simple addition of the two values. Thus, two agents, each capable of achieving a value of 50, are combined their total combined value would be 100. In the third manner, which is the most desirable in the case of microbial control, the result of combining two agents, each capable of achieving a value of 50, would be some value greater than 100.

[0020] Researchers have developed formula for measuring the nature and extent of interactions between components in a composition. In the area of microbial control, the most commonly used equation is that described in Kull et al (Kull et al., 1961, J. Appl. Microbiology 9:538) which by reference is incorporated into this document. Recent examples of the use of this equation in patents are US #9555018, Synergistic combinations of organic acids useful for controlling microorganisms in industrial process, and US #8778646, method of treatment of microorganisms during propagation, conditioning, and fermentation using hops acid extracts and organic acid. The original Kull equation used the minimal inhibitory concentration of antimicrobial agents (MIC) as the endpoints of determination. The MIC values is the lowest measured concentration of antimicrobial agent that results in the inhibition of a microbial culture. Inhibition may be determined visually by examining turbidity of a microbial culture, it may be determined by counting viable cells by culture-based or microscopic methods, or by some measure of metabolic activity, among other possible means. The equation is presented below:

[0021] Synergy Index = (Endpoint a/Endpoint A) + (Endpoint b/Endpoint B) in which Endpoint A is that of agent A by itself, Endpoint a is that of agent A in combination with agent B, Endpoint B is that of agent B by itself, and Endpoint b is that of agent B in combination with agent A.

[0022] In this work the efficacy of the agents alone and in combination were determined by measuring the number of viable cells in model biofilms remaining after treatment. The Minimum Biofilm Eradication value (MBEC) is defined as a 95% reduction in the number of viable cells compared to the untreated control. The relatively non-toxic dispersants are unable to reach that level of killing with physically possible concentrations, thus for those agents the MBEC is considered the highest value tested. Since this value is used as the divisor in the synergy index equation this highest tested value is actually an underestimate of the MBEC and thus synergy index values are also underestimated.

[0023] This invention is primarily intended for use in industrial process waters, particularly cooling towers, evaporators, chillers, and condensers, but will be of utility in any industrial process where biofilms form in aqueous matrices to the detriment of the process. It is anticipated that the invention can be also be used in geothermal fluid processing, oil and gas extraction, and processes using clean-in-place systems.

[0024] The concentration of the biofilm-disrupting agent, such as SDBS, to be used is in the range of 1 to 100 mg per Liter (ppm) of water in the aqueous system being treated, or 1-50 mg/L, preferentially from 1 to 15 mg/L, preferentially from 2 to 10 mg/L, and most preferred from 2-6 mg/L.

[0025] Biocide on an active level basis as  $\text{Cl}_2$  is generally dosed in amount of from at least 1.0 ppm as  $\text{Cl}_2$  or at least 1.5 ppm as  $\text{Cl}_2$  or preferable at least 2 ppm as  $\text{Cl}_2$  or greater, or at least 2.5 ppm as  $\text{Cl}_2$  or greater and up to 15 ppm as  $\text{Cl}_2$  or more preferable up to 10 ppm as  $\text{Cl}_2$  based on mg of biocide per Liter of water being treated. Preferably the dosage of biocide is from 1.5 mg to 10 mg biocide per liter of water being treated.

[0026] Preferably the weight ratio of biofilm disrupting agent to the biocide, preferably oxidizing biocide, is from 1-part biocide to greater than 1-part biofilm disrupting agent. The weight ratio of biocide to biofilm-disrupting agent can be from 1:1 to 1:40, preferably from 1:1 to 1:20, more preferable from 1:1 to 1:8. Each component as measured by weight.

[0027] A person skilled in the art would be able to determine the best dosing point but in general directly upstream of the fouled location is preferred. For instance, the invention could be applied to a cooling tower sump or directly to the cooling tower distribution box or head box thereby treating the cooling water system.

[0028] The biofilm disrupting agent and the oxidizing biocide can be added sequentially or simultaneously or the components can be blended together and added as a single composition.

## EXAMPLES

### Example 1. Synergistic effects of Monochloramine and SDBS

[0029] Dose response studies were performed to determine the Minimum Biofilm Eradication Concentration (MBEC) for monochloramine and SDBS alone. The MBEC is defined as the concentration of agent that reduces the viable biofilm population by 95% of the untreated control value as measured by viable plate counts. Experiments were then performed to determine the result of combining the two agents, oxidizing biocide monochloramine and dispersant SDBS, on biofilm populations. The experiments examined three concentrations of monochloramine with four concentrations of SDBS. The SDBA used in the examples was Bio-Soft™ D-4 (Stapan Company, Northfield, IL).

[0030] M9YG media is a simple minimal salt medium supplemented with 500 mg/L glucose and 0.01% yeast extract. The salts composition is intended to mimic a typical cooling tower water composition. The composition of the media is made using the following procedure: 5XM9 salt composition is mixed using 64 gm Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O, 15 grams KH<sub>2</sub>PO<sub>4</sub>, 2.5 gm NaCl and 5 grams NH<sub>4</sub>Cl in one liter of water. This is divided into 200 ml aliquots and sterilized (by autoclave). To 750 ml of sterile deionized water is added the sterile supplement solutions while stirring. A white precipitate will appear on addition of the CaCl<sub>2</sub> but will dissolve with stirring. Supplement solution is 200 ml of 5XM9 composition, 2ml of 1M MgSO<sub>4</sub>, 0.1 ml of 1M CaCl<sub>2</sub>, 20 ml of 20% glucose, 1 ml of 10% yeast extract, and enough water to make 1000 ml of solution. See reference: Molecular Cloning - A Laboratory Manual (Second Edition). 1989. J. Sambrook & T. Maniatis. Cold Spring Harbor Press

[0031] The inoculum used in the examples were overnight cultures of *Pseudomonas putida*. *Pseudomonads* are common cooling water contaminants, and while cooling water populations are polymicrobial the *pseudomonads* are often used in such studies as representative of the population as a whole.

[0032] Biofilms were grown on stainless steel 316 coupons in a CDC Biofilm reactor using M9YG minimal salts growth media for a period of twenty-four hours. SDBS alone,



monochloramine alone, and combinations of the oxidizer and dispersant were added to the wells of a 12-well cell culture plate. A control was done with M9YG media. After the biofilms were grown, each coupon from the rods in the CDC reactor was unscrewed and dropped into a well of the plate. The plate was then incubated for two hours at 28°C with shaking. Following the incubation, the coupons were removed from the wells and placed into 5 mL of phosphate-buffered saline (PBS) and sonicated for six minutes. Viable cells released into the fluid were then determined by a plating method.

**[0033]** Synergy indices were calculated as described in Kull et al. as in example 1.

**[0034]** Table 1 shows, monochloramine alone required a concentration of 20 mg/L to achieve a reduction in the viable biofilm population of greater than 90%, and 800 mg/L of SDBS achieved a reduction of 48.62%. However, many ratios of the two agents examined exhibited greater activity than could be expected from merely adding that of the two agents alone. For instance, a combination of 2.5 mg/L MCA (1/8 of the value of MCA alone) and 25 mg/L, 1 SDBS (1/32 of the value of SDBS alone) are able to achieve the MBEC goal of 95% reduction in viable biofilm cells. This synergistic effect is obtained with ratios of MCA to SDBS from 1:1.25 to 1: 31.2.

Table 1. Synergistic effects of Monochloramine and SDBS	% Biofilm reduction	Synergy Index	ratio MCA:SDBS
untreated control	0		
20 mg/L MCA	93.58		
312 mg/L SDBS	48.62		
10 mg/L MCA : 78 mg/L SDBS	95.93	0.75	1:7.8
10 mg/L MCA : 39 mg/L SDBS	99.8	0.625	1:3.9
10 mg/LMCA : 19.5 mg/L SDBS	99.59	0.563	1:1.95
10 mg/L MCA : 9.8 mg/L SDBS	99.8	0.531	1:0.98
5 mg/L MCA : 78 mg/L SDBS	98.91	0.5	1:15.6
5 mg/L MCA : 39 mg/L SDBS	97.98	0.375	1:7.8
5 mg/L MCA : 19.5 mg/L SDBS	98.91	0.313	1:3.9
5 mg/L MCA : 9.8 mg/L SDBS	97.98	0.281	1:1.95
2.5 mg/L MCA : 78 mg/L SDBS	97.98	0.375	1:31.2
2.5 mg/L MCA : 39 mg/L SDBS	95.93	0.25	1:15.6
2.5mg/L MCA : 9.8 mg/L SDBS	97.14	0.156	1:3.9

Example 2. Synergistic effects of Monochloramine/Dichloramine Blend and SDBS

[0035] Dose response studies were performed to determine the Minimum Biofilm Eradication Concentration (MBEC) for monochloramine/dichloramine blend (MCA/DCA) and SDBS alone. The MBEC is defined as the concentration of agent that reduces the viable biofilm population by 95% of the untreated control value as measured by viable plate counts. Experiments were then performed to determine the result of combining the two agents, oxidizing biocide MCA/DCA and dispersant sodium benzenesulfonate, on biofilm populations. The experiments examined two concentrations of MCA/DCA with four concentrations of sodium benzenesulfonate.

[0036] Briefly, biofilms were grown on stainless steel 316 coupons in a CDC Biofilm reactor using M9YG minimal salts growth media for a period of twenty-four hours. SDBS alone,

monochloramine alone, and combinations of the oxidizer and dispersant were added to the wells of a 12 well cell culture plate. A control was done with M9YG media. After the biofilms were grown, each coupon from the rods in the CDC reactor was unscrewed and dropped into a well of the plate. The plate was then incubated for two hours at 28°C with shaking. Following the incubation, the coupons were removed from the wells and placed into 5 mL of phosphate-buffered saline (PBS) and sonicated for six minutes. Viable cells released into the fluid were then determined by a plating method.

[0037] Synergy indices were calculated by the method of Kull et al. as in example 1.

[0038] As table 2 below shows, MCA/DCA alone required a concentration of 10 mg/L to achieve a reduction in the viable biofilm population of greater than 90%, and 312 mg/L of SDBS achieved a reduction of 84.58%. However, many ratios of the two agents examined exhibited greater activity than could be expected from merely adding that of the two agents alone. For instance, a combination of 2.5 mg/L MCA/DCA (1/8 of the value of MCA alone) and 9.8 mg/L SDBS (1/32 of the value of SDBS alone) are able to achieve the MBEC endpoint of 99% reduction in viable biofilm cells. This synergistic effect is obtained with ratios of MCA/DCA to SDBS of from 1:1.6 to 1:31.6.

Table 2: Synergistic effects of Monochloramine/Dichloramine Blend and SDBS	% Biofilm reduction	Synergy Index	ratio MCA-DCA:SDBS
Control			
10 mg/L MCA-DCA	97.03		
312 mg/L SDBS	84.58		
2.5 mg/L MCA-DCA : 79 mg/L SDBS	99.94	0.5	1:31.6
2.5 mg/L MCA-DCA :39 mg/L SDBS	99.9	0.38	1:15.6
2.5 mg/L MCA-DCA : 19.5 mg/L SDBS	99	0.31	1:7.8
2.5 mg/L MCA-DCA : 9.8 mg/L SDBS	99.96	0.28	1:3.9
2.5 mg/L MCA-DCA : 3.9 mg/L SDBS	99.9	0.26	1:1.6
5 mg/L MCA-DCA : 39 mg/L SDBS	97.31	0.63	1:7.8
5 mg/L MCA-DCA :19.5 mg/L SDBS	99.59	0.56	1:3.9
5 mg/L MCA-DCA : 9.8 mg/L SDBS	99.3	0.53	1:1.96

[0039] While at least one exemplary embodiment has been presented in the foregoing detailed description, it should be appreciated that a vast number of variations exist. It should also be appreciated that the exemplary embodiment or exemplary embodiments are only examples, and are not intended to limit the scope, applicability, or configuration of the invention in any way. Rather, the foregoing detailed description will provide those skilled in the art with a convenient road map for implementing an exemplary embodiment, it being understood that various changes may be made in the function and arrangement of elements described in an exemplary embodiment without departing from the scope of the invention as set forth in the appended claims and their legal equivalents.

## CLAIMS

What is claimed is:

1. A method of controlling and removing biofilm on a surface in contact with an aqueous system comprising the step of adding a biofilm-disrupting agent and a biocide to the aqueous system.
2. The method of claim 1, wherein the biocide is an oxidizing biocide.
3. The method of claim 1 or 2, wherein the oxidizing biocide is selected from the group consisting of dibromonitrilo propionamide, halogenated hydantoin, for example bromochloro-dimethyl hydantoin, hypobromous acid, trichloroisocyanuric acid, haloamine based biocides, dihaloamine based biocides and combinations thereof.
4. The method of claim 3, wherein the biocide is selected from the group consisting of haloamine based biocides and dihaloamine based biocides.
5. The method of any one of claims 1-4, wherein the biocide comprises at least one of a monochloramine or a dichloramine.
6. The method of claim 1, wherein the biocide is derived from the reaction of a chlorine moiety with a nitrogen containing compound capable of donating an ammonium ion.
7. The method of claim 6, wherein the biocide is derived from the reaction of a chlorine moiety with ammonium hydroxide, ammonium chloride, ammonium sulfate, ammonium acetate, ammonium bicarbonate, ammonium bromide, ammonium carbonate, ammonium carbamate, ammonium sulfamate, ammonium nitrate, ammonium oxalate, ammonium persulfate, ammonium phosphate, ammonium sulfide, urea and urea derivatives and combinations thereof.
8. The method of claim 7, wherein the chlorine moiety is hypochlorite.

9. The method of claim 1, wherein the biocide comprises blends of ammonium-derived chloramine compounds.
10. The method of claim 1, wherein the biocide is a non-oxidizing biocide.
11. The method of claim 10, wherein the non-oxidizing biocide is selected from isothiazolone biocides, glutaraldehyde, formaldehyde and formaldehyde-releasing compounds, tetrakis-hydroxy phosphonium chloride and combinations thereof.
12. The method of any one of the previous claims wherein the biofilm-disrupting agent is an anionic sulfonate surfactant or an anionic alkyl sulfonate surfactant.
13. The method of any one of the previous claims, wherein the biofilm-disrupting agent comprises an anionic sulfonate surfactant selected from the group consisting of alkyl sulfonates, linear or branched primary alkyl sulfonates, linear or branched secondary alkyl sulfonates, linear or branch alkyl aromatic sulfonate and combinations thereof.
14. The method of any one of the previous claims, wherein the biofilm-disrupting agent comprises a linear alkyl benzene sulfonate surfactant, preferably a salt of dodecyl benzene sulfonate.
15. The method of any one of the previous claims, wherein the concentration of biofilm-disrupting agent to be dosed is in the range of from about 1 mg per Liter (mg/L) to about 100 mg per Liter (mg/L) of water in the aqueous system being treated, or from about 1 mg/L to about 50 mg/L, preferentially from about 2 to about 15 mg/L, more preferentially from about 2 mg/L to about 10 mg/L of water in the aqueous system being treated.
16. The method of any of the previous claims, wherein the concentration of biofilm-disrupting agent to be dosed is in the range of from about 2-6 mg/L of water being treated.

17. The method of any of the previous claims, wherein the biocide is dosed in an amount of from at least about 1.0 mg/L as Cl<sub>2</sub> based on mg of biocide per liter of water being treated, or 1.5 mg/L as Cl<sub>2</sub> based on mg of biocide per liter of water in the aqueous system being treated, preferably at least 2 mg/L as Cl<sub>2</sub> or greater, preferably the biocide is added in an amount of from about 2.5 mg/L as Cl<sub>2</sub> to about 15 mg/L as Cl<sub>2</sub>, and more preferably from about 2.5 mg/L as Cl<sub>2</sub> to about 10 mg/L as Cl<sub>2</sub> based on mg of biocide per liter of water in the aqueous system being treated.

18. The method of any one of the previous claims, wherein the ratio of biofilm disrupting agent to the biocide is from about 1-part biocide to greater than 1-part biofilm disrupting agent.

19. The method of any one of the previous claims, wherein the weight ratio of biocide to biofilm disrupting agent is from about 1:1 to about 1:40, preferably from about 1:1 to about 1:20, more preferable from about 1:1 to about 1:8 by weight.

20. The method of any one of the previous claims, wherein the amount of biofilm-disrupting agent added is from about 1 mg/L to about 100 mg/L, preferable from about 1 to about 50 mg/L, or more preferably from about 1 mg/L to about 15 mg/L based on the volume of water being treated, wherein the biofilm disrupting agent comprises sodium dodecyl benzene sulfonate.

21. The method of claim 20, wherein the amount of biofilm disrupting agent is from about 1 to about 10 mg/L of water in the aqueous system being treated and the biocide dosage is from about 1 mg/L to about 10 mg/L based upon active chlorine, and the weight ratio of biocide to biofilm disrupting agent is from about 1:1 to about 1:8, and wherein the biofilm disrupting agent comprises sodium dodecyl benzene sulfonate.

22. The method of any one of the previous claims, wherein the aqueous system is selected from the group consisting of cooling towers, evaporators, chillers, condensers, pulp and paper mills, boilers, wastewater, reclaimed wastewater, mineral slurries, starch slurries, clay slurries, biorefining waters, sludge, colloidal suspensions, irrigation waters, oil and gas waters and combinations thereof.

23. A composition comprising a biofilm-disrupting agent and a biocide, wherein the biofilm-disrupting agent is sodium dodecylbenzenesulfonates and the biocide is a haloamine, preferable selected from the group consisting of monohaloamine, dihaloamine and combinations thereof.
24. The composition of claim 23, wherein the haloamine is selected from the group consisting of chloramine, dichloramine and combinations thereof.
25. The composition of claim 23 or 24, wherein the weight ratio of biofilm disrupting agent to the oxidizing biocide is from about 1-part biocide to greater than 1-part biofilm disrupting agent.
26. The composition of any one of claims 23 to 25, wherein the weight ratio of biocide to biofilm-disrupting agent is from about 1:1 to about 1:20.



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US18/55526

**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3.  Claims Nos.: 5, 12-22, 26  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

- Remark on Protest**
- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
  - The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
  - No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US18/55526

A. CLASSIFICATION OF SUBJECT MATTER  
 IPC - A01N 25/30, 33/02, 33/14, 41/04; C02F 1/50, 1/68, 1/76; C01B 21/09; A61L 2/16 (2018.01)  
 CPC - A01N 25/30, 33/02, 33/14, 41/04; C02F 1/50, 1/68, 1/76; C01B 21/09; A61L 2/16, 31/16

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)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2012/0232153 A1 (GRIESE, GG et al.) 13 September 2012; paragraphs [0009], [0064]	1
Y	US 2006/0231505 A1 (MAYER, MJ et al.) 19 October 2006; paragraphs [0017], [0019], [0022], [0027], [0058], [0065], [0095]	1-2, 3/1-2, 4/3/1-2, 6-11, 23-24, 25/23-24
Y	US 5,670,055 A (YU, FP et al.) 23 September 1997; column 2, lines 23-24, 55-56; column 3, lines 65-66; Table VII	1-2, 3/1-2, 4/3/1-2, 6-11, 23-24, 25/23-24
A	WO 2010/072257 A1 (OBOE IPR AB) 01 July 2010; entire document	1-2, 3/1-2, 4/3/1-2, 6-11, 23-24, 25/23-24
A	US 2008/0317702 A1 (EDGINGTON, G et al.) 25 December 2008; entire document	1-2, 3/1-2, 4/3/1-2, 6-11, 23-24, 25/23-24
A	WO 2009/143511 A1 (KEMIRA OYJ) 26 November 2009; entire document	1-2, 3/1-2, 4/3/1-2, 6-11, 23-24, 25/23-24

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

27 November 2018 (27.11.2018)

Date of mailing of the international search report

20 DEC 2018

Name and mailing address of the ISA/

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