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(57) Abstract

There is disclosed methods and compositions for controlling a broad spectrum of infectious diseases in aquacultural operations, including the treatment of aquatic animals infected with pathogens associated with the infectious diseases. There is also disclosed methods and compositions for reducing pathogens in aquaculture media, and for disinfecting surfaces in contact therewith. Aquatic animals infected with a pathogen are treated by contacting the aquatic animal with a therapeutically effective amount of chlorine dioxide. Such treatment may be accomplished by immersing the aquatic animal for sufficient period of time in an aquatic medium containing a suitable amount of chlorine dioxide.

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Description

# USE OF CHLORINE DIOXINE FOR CONTROLLING INFECTION DISEASES IN AQUACULTURE

## Technical Field of the Invention

This invention is generally directed to methods and compositions for controlling a broad spectrum of infectious diseases in aquaculture operations and, more specifically, to a method of controlling infectious diseases caused by various pathogens associated with aquatic animals, aquaculture media and surfaces in contact therewith.

## Background of the Invention

World-wide fish consumption is increasing annually, and American fish consumption alone has increased 22% in the last decade. The average American now consumes 15.5 pounds of fish per year and, by the beginning of the next century, this figure is expected to increase to 20 pounds per person. In 1990, the world fish catch was approximately 85 million tons. Approximately 70% of the catch was reportedly processed for human consumption. It has been estimated that by the year 2000 well over a 100 million tons of fish per year will be required to meet demand.

As a result of expanding demand, many countries are using aquaculture to produce large quantities of fish. China and Japan, currently among the most sophisticated users of aquaculture, produced an estimated $12 billion worth of product in 1991. The United States, currently the fifth leading aquaculture producer, produced over $750 million worth of product in 1991, reflecting an increase of more than 15% per year since 1980. Catfish are responsible for the largest production volume, making up

50% of the U.S. aquaculture industry. The four most popular fish species--catfish, trout, salmon, and crawfish--account for 80% of the production. However, a total of over 100 aquatic species are cultured, including: eel, abalone, lobster, carp, tilapia, alligator, striped bass, crab, and a wide variety of mollusks.

Aquaculture of channel catfish (Ictalurus Punctatus) typifies an aquaculture operation. Eggs produced by brood stock fish are placed in controlled hatching tanks with oxygenated water of suitable temperature and quality. Under optimum conditions, the eggs hatch in 7 to 8 days. Eighteen days after hatching, the young catfish, or "fry", are transferred to outdoor ponds to mature. Pond sizes typically vary from 5 to 20 acres and are generally 4 to 5 feet deep. At this stage, the fry are less than 1 inch long and are stocked at densities ranging from 70,000 to upwards of 200,000 per acre. The catfish are harvested when they reach a market weight of 1¼-1½ pounds which, under ideal conditions, occurs approximately 18 to 24 months after hatching.

In view of the close proximity of the fish to each other, and the impossibility of segregation, such aquaculture operations are extremely vulnerable to pathogens. For example, in 1989 an outbreak of the viral disease Hemorrhagic septicmeia in salmon farm operations of some northwestern fisheries prompted the destruction of about 3,000,000 eggs, and nearly 1,000,000 young coho salmon and steelhead trout, representing a tremendous monetary loss.

Bacterial pathogens which commonly affect fish populations include: Aeromonus salmonicida, Aeromonas hydrophila, Edwardsiella ictaluri, Vibrio anguillarum, Aeromonas sobria, Pseudomonas sp., Edwardsiella tarda, Plesiomonas spp. and Flexibacter columnaris. Pathogenic fungi, which affect both fish and incubating eggs, include: Saprolegnia hypogyna, S. ferax, and

Achlyaflagellata. Pathogenic viral infections include infections by the infectious hematopoeitic necrosis virus (IHNV), the rhabdovirus, and white sturgeon iridovirus (WSIV). In addition, a common protozoan parasite, Ichthyophthirius multitilis, can cause ichthyophthiriasis, one of the most contagious diseases in aquatic species.

Since most aquatic animals produced in aquaculture are intended for human consumption, disinfectant agents for use in aquaculture must receive Food and Drug Administration (FDA) approval before application. Moreover, even if an agent is approved, the FDA may impose restrictions on its use. Such restrictions may range from prohibition from use for a specified time prior to harvesting to restrictions on water temperature and flow conditions.

Currently only four disinfectants - Terramycin (oxytetracycline), Romet-30, MS-222 (tricaine), and formalin - are registered and available for use on aquatic species intended for human consumption. Their application, however, is limited to the treatment of a very few diseases and to a small number of aquatic species, and have significant restrictions on withdrawal time (i.e., withdrawal prior to fish harvesting). In addition to the above disinfectants, other compounds have been used to improve aquaculture yields. These compounds include such materials as acetic acid (used as a parasitic dip), sodium and calcium chloride (used as osmoregulators), sodium carbonate and carbon dioxide (used for anesthetization of fish); sodium sulfite (used to improve egg hatchability); and povidone iodone (used as an egg surface disinfectant).

Despite FDA scrutiny, numerous disinfecting chemicals that were previously deemed acceptable have recently been delisted after widespread use. For example, the compounds furazolidone, nitrofurazone, chloramphenicol, carofur and silvex, were primary

ingredients of various disinfecting substances used in the aquaculture industry and have now been withdrawn from use due to suspected carcinogenic activity.

Accordingly, there is a growing need in the aquaculture field for an effective agent to control pathogens that limit productivity of commercial fisheries. Such agents should control a broad spectrum of infectious diseases in aquaculture operations, and meet existing safety guidelines. The present invention satisfies this need, and provides further related advantages.

## Summary of the Invention

In brief, the present invention is generally directed to methods and compositions for controlling a broad spectrum of infectious diseases in aquaculture operations, including the treatment of aquatic animals and reducing pathogens in an aquaculture medium and surfaces in contact therewith.

In one embodiment, a method of treating an aquatic animal is disclosed which includes contacting an aquatic animal infected by one or more pathogens with a therapeutically effective amount of chlorine dioxide.

Another embodiment of this invention discloses a method of reducing pathogens in an aquaculture medium by increasing the concentration of chlorine dioxide in the aquaculture medium.

In yet a further embodiment, a method is disclosed for disinfecting a surface exposed to an aquaculture medium by contacting the surface with a solution having an elevated chlorine dioxide concentration.

Other aspects of this invention will become apparent upon reference to the following detailed description.

and the animal, and should generally be in the range of 4°C to 35°C, and preferably 10°C to 30°C.

In another embodiment of the present invention, there is disclosed a method of reducing pathogens in an aquaculture medium by adding chlorine dioxide to the aquaculture medium to a concentration ranging from 1 ppm to 500 ppm, preferably from 2 ppm to 250 ppm, and more preferably from 2.5 ppm to 100 ppm. In this embodiment, chlorine dioxide may be added to the aquaculture medium by any of the methods identified above. With regard to timing of the addition, the chlorine dioxide may be added to an aquaculture medium which already contains aquatic animals, may be added to an aquaculture medium prior to the introduction of aquatic animals thereto, or may be added to an aquaculture medium after contact with aquatic animals.

In another embodiment of this invention, a method is disclosed for disinfecting a surface that has been or will be exposed to an aquaculture medium. In this embodiment, the surface is contacted with an aqueous solution containing chlorine dioxide at a concentration ranging from 1 ppm to 500 ppm, preferably from 2 ppm to 250 ppm, and more preferably from 2.5 ppm to 100 ppm. Moreover, the surface is in contact with the chlorine dioxide solution for a period of time sufficient to disinfect the surface, which generally ranges from about 1 minute to 1 hour. Suitable aqueous chlorine dioxide solutions for use in this embodiment may be produced using any of the methods described above.

The following examples are offered by way of illustration, not limitation.

EXAMPLES

Example 1

This Example illustrates the inhibition of the growth of bacterial pathogens using an aqueous chlorine dioxide solution.

An aqueous chlorine dioxide solution was prepared by mixing an equal volume of a 3.0% sodium chlorite solution with a 16.7% lactic acid solution. This mixture contains approximately 1200 parts per million (ppm) of chlorine dioxide thirty minutes after combination. From the chlorine dioxide solution, an aqueous stock solution of 100 ppm chlorine dioxide was prepared.

Various cultures of bacteria pathogenic to fish were incubated in tryptic soy broth for 24-48 hours at 24°C, and then serially diluted in phosphate buffered saline. The resulting bacterial test cultures contained approximately $1.2 \times 10^{10}$ bacterial cells per milliliter (ml).

To determine the effect of chlorine dioxide on bacterial cell growth, reaction mixtures were prepared containing 0.1 ml of the bacterial test culture, 1 ml of the 100 ppm chlorine dioxide solution, and 8.9 ml of a growth medium (tryptic soy). These reaction mixtures were then incubated for 24-48 hours at 35°C. In all reaction mixtures, bacterial growth was inhibited at 10 ppm chlorine dioxide (i.e., 1 ml of 100 ppm chlorine dioxide diluted to 10 ml).

To determine the lowest level of chlorine dioxide necessary to inhibit bacterial growth, the 100 ppm stock chlorine dioxide solution was further diluted, and mixed with the bacterial test cultures in the manner identified above. The lowest concentration of chlorine dioxide that inhibited bacterial growth was found to be species dependent, and ranged from 2.5 ppm to 9 ppm. The results of this experiment are presented in Table 1.

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In another embodiment of this invention, a method is disclosed for disinfecting a surface that has been or will be exposed to an aquaculture medium. In this embodiment, the surface is contacted with an aqueous solution containing chlorine dioxide at a concentration ranging from 1 ppm to 500 ppm, preferably from 2 ppm to 250 ppm, and more preferably from 2.5 ppm to 100 ppm. Moreover, the surface is in contact with the chlorine dioxide solution for a period of time sufficient to disinfect the surface, which generally ranges from about 1 minute to 1 hour. Suitable aqueous chlorine dioxide solutions for use in this embodiment may be produced using any of the methods described above.

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Table 1

Minimum Levels of Chlorine Dioxide Required to Inhibit Bacterial Growth

| Bacterium | Chlorine Dioxide (ppm) |
|---|---|
| Aeromonas salmonicida | 3.8 |
| Aeromonas hydrophila | 3.9 |
| Aeromonas sobria | 2.5 |
| Pseudomonas sp. | 3.0 |
| Edwardsiella tarda | 7.0 |
| Edwardsiella ictaluri | 3.0 |
| Plesiomonas spp. | 3.0 |
| Plesiomonas shigelloides | 9.0 |

Example 2

This Example illustrates the inhibition of the growth of viral pathogens using an aqueous chlorine dioxide solution.

A 100 ppm stock chlorine dioxide solution, and dilutions thereof, were prepared in the manner described in Example 1, and evaluated against a viral pathogen which affects salmonids, infectious hematopoeitic necrosis virus (IHNV) (U.S. Fish and Wildlife Service).

Reaction mixtures were prepared which contained 0.1 ml of virus (i.e., about $10^6$ pfu/ml) and 1.0, 2.5, 10, 25, and 50 ppm of chlorine dioxide stock solution, respectively. The reaction mixtures were then incubated for 48 hours at 14°C and a pH 7.0.

Viable virus remaining after incubation with chlorine dioxide was detected by serially diluting each virus-chlorine dioxide mixture, adding Chinook salmon embryo (CHSE 214) cells to each mixture and plating in 96-well microtiter plates. After incubation at 14°C for 10 days, the titer of virus treated with a 10 ppm chlorine dioxide solution at pH 7.0 was reduced by about 99%.

Example 3

This Example illustrates inhibition of the growth of protozoan pathogens using a chlorine dioxide solution.

A 100 ppm stock chlorine dioxide solution was prepared in the manner described in Example 1 and evaluated against the common contagious ectoparasite Ichthyophthirius multifilis (Ich) (U.S. Fish and Wildlife Service - obtained from skin scrapings of channel catfish).

A reaction mixture was prepared by combining 9 ml of an aqueous solution containing the ectoparasite and 1 ml of 100 ppm chlorine dioxide solution (10 ppm chlorine dioxide solution at pH 5.0). In all cases cilia and intercellular movement was inhibited within 30 minutes of mixing (as observed by microscope), and in some cases the parasites were observed bursting.

Example 4

This Example illustrates alternative methods of preparing aqueous chlorine dioxide solutions of this invention.

An aqueous solution of chlorine dioxide was prepared by dissolving 13.5 g of powdered, technical grade sodium chlorite (ca. 80% pure) and 29 g citric acid in 1.5 liters of water, and stirring. After 15 minutes, the aqueous solution contained approximately 3000 ppm chlorine dioxide as measured spectrophotometrically at 360 nm. This aqueous chlorine dioxide solution, as well as dilutions thereof, may be used in place of the aqueous chlorine dioxide solutions of Examples 1-3.

Alternatively, gaseous chlorine dioxide may be bubbled into water to yield aqueous chlorine dioxide solutions containing up to 3000 ppm chlorine dioxide from which appropriate dilutions may be made.

Example 5

This Example illustrates the non-toxicity of chlorine dioxide treatment to steelhead trout eggs as measured by the survival rates of trout fingerlings.

Freshly spawned and fertilized steelhead trout eggs (Onchorhynchus mykiss) were water-hardened at pH 4.5 in the 100 ppm stock chlorine dioxide solution of Example 1, or in one of four dilutions thereof (50 ppm, 33 ppm, 25 ppm, and 10 ppm). The eggs were then immersed in one of the five aqueous chlorine dioxide solutions, or a water control, and held for 30 minutes at 10°C. At the end of the immersion period, the chlorine dioxide solutions were diluted to 5 times their original volume with additional water.

Eggs exposed to the 10 ppm dilution resulted in an 85% survival rate of swim-up fry compared to the 95% survival rate of those exposed to the 0 ppm dilution.

Example 6

This Example illustrates the non-toxicity of chlorine dioxide treatment to catfish eggs as measured by alteration of hatching percentages.

Catfish eggs which were spawned and fertilized 6 hours were water-hardened at pH 4.5 in the 100 ppm stock chlorine dioxide solution of Example 1, or in one of four dilutions thereof (50 ppm, 33 ppm, 25 ppm, and 10 ppm). The eggs were then immersed in one of the five chlorine dioxide solutions, or a water control, and held for 30 minutes at 10°C. At the end of the immersion period, the chlorine dioxide solutions were diluted to 5 times their original volume with additional water.

Catfish eggs at the eyed stage were not adversely affected by the immersion. The percentage of hatch-out after 30-minute immersion was similar to control values for all concentrations of chlorine dioxide.

Example 7

This Example illustrates the non-toxicity of chlorine dioxide treatment to steelhead trout and catfish fingerlings as measured by their survival rates after immersion.

Approximately 100 steelhead trout fingerlings and 100 catfish fingerlings were each separately immersed in about 40 liters of 10 ppm chlorine dioxide solution at pH 5.5 and 10°C for 24 hours. At the end of the immersion period, the chlorine dioxide solutions were diluted to 5 times their original volume with additional water. Such chlorine dioxide immersion resulted in 100% survival rate.

Example 8

This Example illustrates the non-toxicity of chlorine dioxide treatment to one-pound channel catfish as measured by their survival rates after immersion.

Five one-pound channel catfish were immersed in 100 liters in each of a 10 ppm or 25 ppm chlorine dioxide solution at pH 5.5 and 15°C for 30 minutes. At the end of the immersion period, the chlorine dioxide solutions were diluted to 5 times their original volume with additional water.

There was a 100% survival rate for fish exposed to both the 25 ppm chlorine dioxide solution and the 10 ppm chlorine dioxide solution.

Example 9

This Example illustrates the non-toxicity of chlorine dioxide treatment to healthy, ornamental koi as measured by their survival rates after immersion.

Groups of 65 adult ornamental koi of varying age and weight were immersed in 10 liters of one of five chlorine dioxide solutions with set concentrations of 50, 33, 25, 10 and 0 ppm at a pH of about 4.5 - 5.0 and 15°C for 30 minutes. At the end of the immersion period, the chlorine dioxide solutions were diluted to 5 times their original volume with additional water. The results of this experiment are presented in Table 2.

Table 2

Percent Survival of Ornamental Koi After Immersion in Chlorine Dioxide Solutions

| Post-Treatment Time | Survival Rates | | | | |
|---|---|---|---|---|---|
| | 0 ppm | 10 ppm | 25 ppm | 33 ppm | 50 ppm |
| 30 minutes | 100% | 100% | 100% | 100% | 100% |
| 2 hours | 100% | 100% | 66% | 50% | 0% |
| 6 hours | 100% | 100% | 66% | 33% | 0% |
| 24 hours | 100% | 100% | 66% | 17% | 0% |

Based on the results presented in Table 2, immersion of the ornamental koi for 30 minutes in an aqueous solution containing 10 ppm chlorine dioxide did not adversely effect survival of the fish.

Example 10

This Example illustrates the treatment of diseases caused by various pathogens (i.e., microorganisms and protozoa) associated with aquaculture.

Ornamental fantail goldfish (Carassius auratus) were infected by direct skin contact with Ichthyophthirius multifilis (Ich) and two kinds of trematodes (parasite

flatworms). Untreated fish (i.e., fish not treated with chlorine dioxide) showed no survivability after 72 hours.

Groups of 3 infected ornamental fantail goldfish were immersed in 100 liters of one of four chlorine dioxide solutions or a control with set concentrations of 50, 33, 25, 10 and 0 ppm at a pH 7.0 and 18°C for 30 minutes. At the end of the immersion period, the chlorine dioxide solutions were diluted to 5 times their original volume with additional water.

Table 3 details the survival rates of the exposed fish.

Table 3

Percent Survival of Infected Fantail Goldfish After Immersion in Chlorine Dioxide Solutions

| Post-Treatment Time | Survival Rates | | | | |
|---|---|---|---|---|---|
| | 0 ppm | 10 ppm | 25 ppm | 33 ppm | 50 ppm |
| 30 minutes | 100% | 100% | 100% | 100% | 100% |
| 6 hours | 100% | 67% | 67% | 100% | 67% |
| 24 hours | 67% | 67% | 67% | 100% | 67% |
| 48 hours | 0% | 0% | 33% | 100% | 67% |
| 72 hours | 0% | 0% | 0% | 67% | 0% |

From this data, a concentration of about 33 ppm chlorine dioxide is beneficial in treating fish infected with the pathogens used in this experiment. Further optimization of the chlorine dioxide concentration and contact time may be achieved by employing, for example, a greater number of fish per treatment group.

Example 11

This Example illustrates the treatment of diseases caused by fungal pathogens associated with aquaculture.

A 3/4-pound channel catfish (Ictalurus punctatus), which was infected with an unidentified water-borne fungus, was immersed in 50 liters of a 10 ppm chlorine dioxide solution at a pH of 5.1 and 18°C for 30 minutes. At the end of the immersion period, the chlorine dioxide solution was diluted to 5 times its original volume with additional water. The catfish was cleared of all visible signs of infection four days after the one time immersion.

Example 12

This Example illustrates the ability of chlorine dioxide to substantially reduce the number of pathogens in an aquaculture medium.

One part of an aqueous 2.64% sodium chlorite solution was combined with 100 parts of an aqueous mixture containing 0.016% of tartaric acid, 0.1% of ribose, and 0.90% of sodium chloride. The resulting solution forms about 100 ppm of chlorine dioxide as a reaction product within a few minutes of mixing. One part of the resulting solution was added to 20 parts of fisheries water (either fresh or saltwater) containing the bacterial, fungal or protozoal pathogens.

After 6 hours, the fisheries water was plated out on trypticase soy agar and incubated for 24 hours at 35°C. The fisheries water was found to be sterile, where as untreated control samples yielded agar plates with confluent organism growth which was too numerous to count.

Example 13

This Example illustrates the use of the present invention to disinfect surfaces that contact the aquaculture medium.

The 1200 ppm chlorine dioxide solution of Example 1 is diluted 1:10 with fresh or salt water. The resulting combination is directly applied, without

neutralization, to dry or damp surfaces of water tanks, vats, and other equipment in aquaculture to eliminate the hazard of pathogens, prior to subsequent contact with water.

Example 14

This Example illustrates the treatment of large volumes of water associated with aquaculture, either to eliminate harmful pathogens and/or to treat fish within the water.

One volume of an aqueous solution containing 41.5% sodium chlorate and 6.66 sodium chloride is combined with 0.58 volumes of 90% sulfuric acid, under constant mixing. When the exothermic reaction is complete, a quantity of chlorine dioxide is produced equivalent to 57 parts for every 100 parts of initial sodium chlorate. The level of chorine dioxide that is produced may be measured by titration or spectrophotometrically to determine the proper quantity to be introduced into the aquaculture waters.

Introduction of the chlorine dioxide can be achieved by proportionate addition of the liquid to the aquaculture waters (with or without neutralization of its acidity) or by passage of air over the reaction mixture and bubbling that air through the aquaculture waters.

From the foregoing it will be evident that although specific embodiments of the invention have been described herein for the purposes of illustration, various modifications may be made without deviating from the spirit or scope of the invention.

Claims

1. A method of treating an aquatic animal infected by a pathogen, comprising contacting the aquatic animal with an aquaculture medium containing a therapeutically effective amount of chlorine dioxide.

2. The method of claim 1 wherein the aquaculture medium containing the therapeutically effective amount of chlorine dioxide is formed by addition of chlorine dioxide to the aquaculture medium in the presence of the aquatic animal.

3. The method of claim 1 wherein the aquatic animal is added to the aquaculture medium containing the therapeutically effective amount of chlorine dioxide.

4. The method of claim 1 wherein the therapeutically effective amount of chlorine dioxide in the aquaculture medium ranges from 1 to 500 ppm.

5. The method of claim 1 wherein the therapeutically effective amount of chlorine dioxide in the aquaculture medium ranges from 2 to 250 ppm.

6. The method of claim 1 wherein the therapeutically effective amount of chlorine dioxide in the aquaculture medium ranges from 2.5 to 100 ppm.

7. The method of claim 1 wherein the aquatic animal is contacted with the aquaculture medium containing a therapeutically effective amount of chlorine dioxide for a period of time ranging from 5 minutes to 48 hours.

8. The method of claim 1 wherein the aquatic animal is contacted with the aquaculture medium containing a

therapeutically effective amount of chlorine dioxide for a period of time ranging from 10 minutes to 12 hours.

9. The method of claim 1 wherein the aquatic animal is contacted with the aquaculture medium containing a therapeutically effective amount of chlorine dioxide for a period of time ranging from 30 minutes to 5 hours.

10. The method of claim 1 wherein the aquatic animal is contacted with the aquaculture medium containing a therapeutically effective amount of chlorine dioxide at a temperature ranging from 4°C to 35°C.

11. The method of claim 1 wherein the aquatic animal is contacted with the aquaculture medium containing a therapeutically effective amount of chlorine dioxide at a concentration ranging from 1 to 500 ppm.

12. The method of claim 1 wherein the aquatic animal has been infected by at least one pathogen selected from bacteria, viruses, fungi and protozoan parasites.

13. The method of claim 12 wherein the pathogen is a bacteria and is selected from the group Aeromonas salmonicida, Aeromonas hydrophila, Aeromonas sobria, Pseudomonas sp., Edwardsiella ictaluri, Edwardsiella tarda Vibrio anguillarum, Flexibacter columnaris and Plesiomonas sp.

14. The method of claim 12 wherein the pathogen is a virus and is selected from the group rhabdovirus, white sturgeon iridovirus, and infectious hematopoeitic necrosis virus.

15. The method of claim 12 wherein the pathogen is a fungi and is selected from the group Saprolegnia hypogyna, S. ferax and Achlya flagellata.

16. The method of claim 12 wherein the pathogen is the protozoan parasite Ichthyophthirius multifilis.

17. A method of reducing pathogens in an aquaculture medium, comprising adding chlorine dioxide to the aquaculture medium to a concentration ranging from 1 to 500 ppm.

18. The method of claim 17 wherein the chlorine dioxide is added to the aquaculture medium by bubbling chlorine dioxide gas into the aquaculture medium.

19. The method of claim 17 wherein the chlorine dioxide is added to the aquaculture medium by mixing an aqueous solution containing chlorine dioxide with the aquaculture medium.

20. The method of claim 19 wherein the aqueous solution containing chlorine dioxide is formed by the generation of chlorine dioxide produced by the reaction of a metal chlorite and a strong acid.

21. A method for disinfecting an aquatic surface, comprising contacting the surface with a solution containing chlorine dioxide at a concentration ranging from 1 to 500 ppm.

22. The method of claim 21 wherein the solution contains chlorine dioxide at a concentration ranging from 2 to 250 ppm.

23. The method of claim 21 wherein the solution contains chlorine dioxide at a concentration ranging from 2.5 to 100 ppm.

# INTERNATIONAL SEARCH REPORT

Interr ıal Application No
PCT/US 95/00219

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A01N59/00 A01N59/08 A01K61/00

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)
IPC 6 A01N A01K

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | EP,A,0 544 950 ( JAPAN PET DRUGS CO LTD) 9 June 1993<br>see the whole document<br>--- | 1-23 |
| X | US,A,4 946 690 (JAPAN PET DRUGS CO) 7 August 1990<br>see the whole document<br>--- | 1-23 |
| X | PROG. FISH-CULT.,<br>vol. 50,no. 1, 1988<br>pages 51-55,<br>R.P. DEMPSTER, P. MORALES AND F. X. GLENNON 'Use of sodium chlorite to combat anchorworm infestations of fish.'<br>see the whole document<br>---<br>-/-- | 1-23 |

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| 5 May 1995 | 15. 06. 95 |
| Name and mailing address of the ISA<br>European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax (+ 31-70) 340-3016 | Authorized officer<br>Klaver, J |

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