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(54) Title: IN SITU TREATMENT FOR CONTAMINATED SURFACE WATERS AND PRODUCTS THEREFOR (57) Abstract This invention is directed to a method for the prevention of growth of aquatic algae which method comprises adding a quantity of timed-release tablets of a dry particulate composition into the surface water, wherein said tablets comprises: (a) an inner-core comprising at least one live microorganism strain in dormant condition; (b) an inner-coating over the inner-core of a water soluble substance selected from the group consisting of polyethylene glycol and hydroxypropyl methylcellulose; (c) an outer-layer over the inner-coating comprising sodium sulfate coated sodium carbonate peroxyhydrate particles and a colorant that reflects light at the same wavelength used in photosynthesis by the algae, thereby prevents the necessary light from penetrating the surface water, and (d) an outer-coating over the outer-layer of a water soluble substance selected from polyethylene glycol and hydroxypropyl methylcellulose.		

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IN SITU TREATMENT FOR CONTAMINATED SURFACE WATERS AND PRODUCTS THEREFOR

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Field of the Invention

This invention relates to a novel non-toxic in situ method for the accelerated
10 biological degradation of organic matter in the form of sewage sludge or petroleum
hydrocarbons on the surface of aquatic objects submerged in bodies of saltwater,
brackish, or freshwater. The invention includes novel non-toxic compositions and novel
products that are particularly useful for practicing the novel methods.

15

Description of the Prior Art

The delicate balance of our planets fragile aquatic ecosystems is being disturbed at
an alarming rate. Industrial, agricultural, and residential effluents enter our waterways
polluting these systems with organic, metallic and inorganic compounds. Current
methods of remediating aquatic sediments contaminated with organic pollutants such as
20 sewage, oil, pesticides, herbicides and polychlorinated biphenyls involve dredging up
the sediment and treating it elsewhere then returning it to the removal site. These
existing methods are both expensive and damaging to benthic ecosystems by killing
organisms. The need for an inexpensive, in situ and noninvasive method to remediate
such situations has lead to the development of this novel method and compositions.
25 Industrial, agricultural and residential effluents and storm water runoff has
degraded the water quality around the world. Larger and more frequent fish kills are

reported every year, such as the one occurring in the summer of 1996 in a tributary of the Chesapeake in which one billion fish of nineteen different genera were reported killed.

5 The over abundance of nutrients, as well as contaminants, in lakes and streams and estuaries has created a crisis for aquatic organisms. The high concentrations of dissolved phosphorus, has resulted in a rapid increase in growth of aquatic algae and plants.

10 The problem of lake and stream eutrophication is increasing due to the increase in nutrients available to aquatic weeds from residential and agricultural runoff. There has been speculation that agricultural runoff has a detrimental affect on estuarine water quality leading to the increase in HAB's (Harmful Algal Blooms) such as Pfiesteria.

15 Larger and more frequent fish kills are reported every year, such as the one occurring in the summer of 1996, in a tributary of the Chesapeake River, in the United States, in which one billion fish of nineteen different genera were reported killed. The over abundance of nutrients, as well as contaminants, in lakes, streams and estuaries has created a crisis for aquatic organisms. The degradation of water quality has resulted from an increase in nutrient concentrations (NO_3 , PO_4), increased oxygen demand (BOD, COD), turbidity and raised bacterial counts (T-Coli, F-Coli). This has resulted in the closure of shellfish bed harvests, a reduction in number and health of commercial fish populations that spawn in estuarine waters. Aside from accidentally
20 spills of pollutants, the run-off from agricultural operations has been the targeted in recent legislative restrictions on CLO's.

Current methods of remediating aquatic sediments contaminated with organic pollutants, such as agricultural and residential sewage, fuel oil, PCB's and other industrial chemicals, involve dredging up the sediment, treating it elsewhere, and then returning it to the removal site. Surface water treatments, such as the treatment of lakes for alga blooms, require the addition of poisonous chemical herbicides and pesticides. The need for inexpensive alternative treatments is clearly evident and has encouraged our research into this field.

Most conventional primary and secondary treatment facilities are inadequate in terms of the complete removal of many inorganic and organic chemicals leading to the eutrophication in lakes, rivers, and bays. A typical sanitary analysis of an uncontaminated flowing river requires optimally:

pH 6.5-6.8 (and not < 8.5)

Dissolved oxygen 8.0-9.0 ppm (and not >4.00-5.00 ppm)

Color 5ppm

Turbidity 5ppm

BOD (Biological Oxygen Demand at 20° C) 1.2ppm

Total solids 500 ppm

Chloride 10 ppm as Cl

By comparison analysis of the recent analysis of hog farm manure which is incorporated into farm soil showed very high values.

Supernatant

pH 7.53

COD 14,000 mg/L

Settled sludge portion

pH 7.10

COD 25,500 mg/L

TDS 7,562 mg/L

TDS 7,760 mg/L

TSS 1,880 mg/L

TSS 4,560 mg/L

These values do not reflect what will be found in the streams containing agricultural runoff. These values will be diluted by rain and irrigation water and filtered by the soil.

- 5 The potential for environmental damage exists, if this type of waste is accidentally discharged into waterways undiluted.

The activated sludge method is the most commonly used secondary wastewater treatment system for human waste. After primary treatment in which the majority of solids are settled out of the water column, these solids are diverted into an activated
10 sludge reactor while the overlaying water is sent to an aerobic treatment system before discharge. In the aerobic treatment system, oxygen is supplied to the water by aeration incorporating either surface aerators or diffusers which utilize a mechanical process requiring energy input the amount of which is dependent upon the BOD (Biological Oxygen Demand) or COD (Chemical Oxygen Demand). However the activated sludge
15 chamber, that treats the solids portion, is devoid of oxygen and anaerobic degradation is enhanced by and an increase in temperature and bacteria and nutrients.

A variation of this method is used in the treatment of concentrated livestock waste such as at hog facilities. The raw waste at these facilities is stored in what are called anaerobic sludge lagoons or pits. They consist of large earthen or cement lined
20 enclosures into which the raw slurry (manure and water) is pumped. The slurry remains in this enclosure, which is usually open to the air, for several weeks or months before it is removed and incorporated into the crop field soil. For the most part these lagoons remain anaerobic even though that are exposed to the air at the water surface. In some

cases aerators are used to diffuse oxygen into the lagoon to promote aerobic degradation which reduces that amount of noxious gases created in the anaerobic breakdown of the manure.

Our time-release tablets disclosed and claimed in U.S. Patent No. 5,275,943 for
5 biological degradation provides an efficient method for aerating wastewater sufficiently in cooperation or in place of secondary treatment systems. The incorporation of advantageous microorganisms and various nutrients as well as dissolved oxygen (by the breakdown of hydrogen peroxide) can be added into almost any wastewater environment through the use of time release tablets specifically designed for that
10 system to reduce BOD and noxious gases and establish a harmonious Eco-balance.

The economy of treatment relies on the efficiency of the wastewater treatment system to provide an environment, which supports the activity and growth of a treatment microflora along with factors such as the balance between oxygen and substrate supply. Our system designed under the U.S. Patent # 5,275,943 can incorporate appropriately
15 required nutrients, microorganisms and Oxygen via hydrogen peroxide to balance the organic and inorganic nature and control the biodegradability of the waste.

Similar studies have also been done by Higa (An Earth Saving Revolution, Japan: Sunmark Publishing Inc. 1993, p. 154) in Japan, who combines synthesizing microorganisms with zymogenic microorganisms. He defines zymogenic
20 microorganisms as those that reduce organic matter to a soluble state while creating large quantities of antioxidants. Higa developed his own process of autolysis where the digestion of organisms takes place by enzymes naturally present and has used it for breaking down agricultural synthetic chemicals as well as harmful bacteria such as

E.coli. He has identified upwards of 80 different strains of microorganisms known to have the capacity to eradicate agricultural chemicals.

In both field and laboratory studies, Cellinite Technologies (U.S. patent No. 5,275,943) has now created controlled degradation systems. Successful use of these systems involve the utilization of specific microorganisms, nutrients, and oxygen introduction through time-released aeration capsules for controlled degradation of manure and decaying detritus (plant matter). The changes in physicochemical parameters in a static system and the passage of bacterial pathogens have also been analyzed demonstrating beneficial results.

10 A recent study in Iowa (Stanley Buman, The Advantage of Manure, Proceedings from the Conference on Manure Management by the Soil and Water Conservation Society, February 10-12, 1998) stated that a typical hog production facility (CFO) of 2000 head generates 820,000 gallons of manure per year. Using industry standard calculations a producer could then land apply 3500 gallons per acre, therefore 235
15 acres were needed to utilize the manure from this facility. Depending on the crop, some need only be fertilized every other year so that the land requirement could be 470 acres. In 1996 Iowa produced 24,000,000 hogs or 9,840,000,000 gallons of manure requiring 5,622,857 acres in order to land apply it. This data just covers hog manure, not other livestock. Combining all the types of livestock manure data together, then looking at the
20 area needed to land apply it, one will find that the needed area far exceeds the available land for crops. These figures will only increase as the demand for U.S. pork products goes up worldwide.

Storing it in open-air anaerobic sludge lagoons for a period of time, is the typical way in which a hog farm treats its manure. Periodically the surface liquid fraction (supernatant) is then pumped off and sprayed onto crop fields. The solids (sludge) on the bottom of these pits are later removed and tilled into the cropland. Large corporate farms, which have concentrated huge amounts of manure into vast outdoor sludge lagoons, discharge a great deal of noxious gasses. These gases, byproducts of anaerobic degradation of the manure, include compounds such as Hydrogen Sulfide, Methane, Ammonia, and Methyl Mercaptan, as well as Carbon Dioxide. These gases cause a nuisance to local neighbors as well as having a detrimental effect on the atmosphere, by contributing to global warming. New government restrictions will force farms to convert their treatment systems from anaerobic to aerobic treatment. Typically this would involve large capital expenditure on the part of the farmer to construct batch reactors or place high-powered electric air pumping systems into their lagoons, to insure aerobic degradation.

In lakes and ponds herbicides are commonly used to reduce the growth of aquatic weeds. The most commonly used are the inorganic copper compounds such as Copper Sulfate. These compounds tend to block portions of the photosynthetic process, preventing the production of carbohydrates, thereby killing the weeds. Organic herbicides, such as Diquat dibromide, also interfere with photosynthetic processes to kill the weeds, but are biologically persistent because they are difficult to biodegrade and may bioaccumulate in the tissues of other aquatic organisms.

Colorants have been used as herbicides with varying degrees of success. Studies by Spencer have shown that their effectiveness is due to the blocking of the

light in the 610nm to 650nm range to the plants them selves. Visible light is the portion of the electromagnetic spectrum with wavelengths between 400 and 700 billionths of a meter (400 to 700 nanometers). The colorants used are themselves non-toxic and are similar to food colorings. In principle the colorant defracts the light preventing the wave-
5 lengths necessary for photosynthesis from reaching the algae, causing its decline in growth.

All herbicides including the colorants cause the death and subsequent decay of the algae and plants. As these dead plants start to decay they cause an increase in the waters demand on available dissolved oxygen. As dissolved oxygen levels in the water
10 drop below 5.0 mg/L fish heath and fecundity are affected. In some cases of this situation has resulted in fish kills. The need for method of preventing weed production without persistent herbicides and that does not cause fish kills is evident.

Oxygen depletion nuisances are caused by the release of excessive levels of nutrients into waterways, which enhance eutrophication, and then finally, oxygen
15 depletion. Oxygen depletion can arise from the primary effect of direct organic matter inputs to the lake. In addition secondary effects of dying plankton and decaying algae can cause sudden death of fishes as well as he release of odors caused by CH₄, H₂S, and NH₃ gases. The contemporary approach is to change the direct inputs of organic matter by anaerobic or mechanical waste treatment systems or by rerouting such
20 contaminated wastes to other locations such as flowing streams.

The BOD of a system is a function of the number and types of microorganisms present as well as the rate of addition and type of substrates. Whether this demand can be satisfied or not depends on a balance existing between the fixed rate of O₂ supply

and the variable rate of demand unless methods of introducing oxygen can be introduced. Thus, in aerobic biological growth, a sufficient amount of available oxygen is essential. Mechanical aeration processes are the contemporary solution to satisfying oxygen requirements of the system whereby a gas-liquid mass transfer process takes place in which the driving force in the gas phase is the partial pressure of the gas P_g in accordance with Henry's law. In the liquid phase, the concentration gradient $C_s - C$ in which C_s is used as the saturation concentration at the gas-liquid interface where C is the concentration in the body of the liquid.

With a single timed-release layered tablet, several active steps will occur. First, the outer layer will dissolve releasing a compound that liberates both oxygen, in the form of bubbles, and a combination of blue and yellow water-soluble dyes. These bubbles act to both disburse the dye into the water column, raise DO level in the water, and loosen particles at the sediment-water interface causing the tablet to bury itself deeper into the sediment.

Next, the inner layers of the tablet disintegrate to release a combination of enzymes, buffers and aerobic and facultative anaerobic microorganisms. The dye acts to block the wavelength of light necessary for photosynthesis. This causes the death of the nuisance algae or plants, which sink to bottom as they decay. The microorganisms consume the detritus at a high rate, enhanced by the nutrients and enzymes and dissolved oxygen. The high DO level also helps to prevent the fish kills associated with the increased demand created by the decaying plant matter. The source of the oxygen is a dry form of hydrogen peroxide which, in solution, quickly degrades to molecular oxygen and water, to oxygenate the water. Since micro-organisms receive O_2 from DO

in the liquid phase instead of the usual replacement of DO from the atmosphere through a process of stirring or agitation, we introduce it directly into the system therefore relocation or extensive mechanical processing systems are not required for the gas/liquid interface. The oxygen transfer rate differs depending on the characteristics of the water by such variables as dissolved solids, organics, surface-active agents, the amount needed can be determined by using the following standard equation:

$$dC/dt = K(C_s - C_1)$$

Where K = O_2 transfer rate; C_s = concentration of dissolved O_2 at saturation; C_1 = concentration of dissolved O_2 at time t .

Through inputting the time-release tablet into the aquatic environment the rate of O_2 supply and the buffering capacity provided by a body of liquid containing dissolved O_2 is able to be in excess of the O_2 required for microbial metabolism so that the development of anaerobic conditions is restrained. Thus, we have been able to show that in nonmechanical systems, for example, barrier ditches and lagoons, O_2 supply is no longer limited to transfer at the liquid surface. Previous limitations of treatment by the low rate of O_2 transfer can be compensated for either by an increase in oxygen introduced by time-release tablets created a reduction in the substrate load.

A way of analyzing the system is to examine the carbonaceous demand, which can be estimated, using a basic approach whereby the oxygen requirement is estimated as: O_2 required per day = soluble BOD_u removed per day - BOD_u of solids leaving the system per day.

It should also be noted that alga multiplication usually has a beneficial effect on the oxygen balance in an aquatic environment since during photosynthesis most algae

produce more oxygen during daylight hours than they consume by respiration during all 24 hours. For algae contained in the effluent of a aquatic system, Toms *et al.* (Journal Institute of Water Pollution Control, United Kingdom 1975 in Arceivala, p. 801) found that the respiratory demand to be about 0.007 mg O₂ per mg as per hour, the oxygen
5 production to be about 0.1 mg O₂ per mg per hour, that is, 15 times greater than the amount consumed. Thus, the role of algae in waste stabilization in aquatic environments is beneficial to BOD for aerobic bacterial activity when the algae provides excess of oxygen required by bacteria, thus creating an aerobic environment.

The applicant is aware of prior art which makes the novel method possible. The
10 particles of the sodium carbonate peroxyhydrate mentioned as a part of the novel composition are created in a commonly used practice by spraying the pure compound with a solution containing sodium sulfate in a device know as a fluid bed dryer. The mixture then is heated to evaporate the solvent from solution. The size of the resulting particles is controlled by the length of time the resulting reaction is allowed to proceed
15 before it is arrested by the drying process, the longer the reaction the larger the resulting particle size will become.

The aerobic bacteria, yeast and facultative anaerobic bacteria used in this method are placed into a dehydrated form by a commonly used method of thermal drying in which the incubator raised strain of bacteria are placed in a device which quickly
20 evaporates the moisture from the culture without killing the bacteria or yeast. The resulting organisms are in a suspended state and will become active upon rehydration.

The enzymes used in the formulation of the novel composition are in dry crystallized form and can be chosen from the entire range of available enzymes. The

enzyme(s) chosen will be particularly suited to help breakdown the contaminant being remediated, an example being protein kinases to breakdown proteins. Lyophilized enzymes are preferred.

The applicant is further aware of the following U.S. and Foreign Patents listed
5 below and whose contents are herein incorporated by reference. U.S. Patent No. 1,057,281 teaches the use of all peroxy compounds in an oxygen bath for medicinal purposes with people. U.S. Pat. No. 1,917,489 discloses the use of a solvent such as trichloroethylene, sodium peroxide and potassium carbonate in a system used to remove deposits from the inside walls of automotive radiators. U.S. Pat. No. 3,441,388
10 describes the use of sodium perborate and salts of peroxyacids and their use as oxygen generating agents to decrease the dissolving time of solid materials in water (i.e. laundry detergents). U.S. Pat. No. 3,502,429 features the use of sodium peroxide and potassium superoxide in a system which removes excess carbon dioxide from the atmosphere of a closed room, and replenished the oxygen taken up by respiration. U.S.
15 Pat. No. 4,248,642 relates to the use of microsites of effervescence of a hypochlorite-peroxide interaction to loosen micro-deposits of debris and organic matter from reaction cells in an automated analytical instrument. U.S. Pat. No. 4,293,426 discloses the use of calcium peroxide particles coated with an insoluble organic compound, having a melting point of at least 50° C., used for water oxygenation. See in particular Col. 5,
20 lines 45-58. U.S. Pat. No. 4,395,344 describes the use of percarbonate to mix the caustic substances with the water above the clog, in a dry drain opener preparation. U.S. Pat. No. 4,156,039 teaches the use of sodium percarbonate particles coated with sodium perborate and their use as oxygen generating agents in water. U.S. Pat. No.

4,025,453 relates to the activation of peroxide-based dry laundry bleaches in an aqueous medium with pH above 7.5 through the use of cyanamide. U.S. Pat. No. 4,026,798 describes the use of peroxygen compounds in a process for treatment of dirty dry cleaning bath solutions. U.S. Pat. No. 4,073,888 relates to the use of peroxy compounds as stabilizers for use with chlorine dioxide and quaternary ammonium salts as sterilizing agents. U.S. Pat. No. 4,086,175 discloses the activation of peroxide-based dry laundry bleaches in a buffered aqueous medium through the use of cyanamide and magnesium. U.S. Pat. No. 4,120,650 features the use of dry peroxygen compounds in conjunction with chlorine releasing compounds in a laundry detergent composition. U.S. Pat. NO. 4,197,198 teaches the use of peroxygen compounds as stabilizing agents for use with chlorine dioxide to degrade phenol compounds in a waste water stream. U.S. Pat. No. 4,251,486 features the use of sodium carbonate in a waste water treatment process for decomposing injurious substances. U.S. Pat. No. 4,253,971 teaches the use of peroxygen compounds as secondary algicide in the process of water treatment through the use of a linear polymeric biguanide. JP Patent 49-27799 relates to the use of calcium peroxide in fish culture to oxygenate breeding ponds. JP Patent 88-156001 discloses the use of oxygen generating compounds packed in gas-permeable nonwoven cloth bags for use in live fish transportation. JP Patent 89-51302 teaches the use of oxygen generating compounds packed in gas-permeable nonwoven polyethylene-coated cloth bags for use in live fish transportation.

SUMMARY OF THE INVENTION

This invention combats several aspects of the pollution problem in the environment. All involve treating the contamination at the site without disturbing the sediment and the animals and plants living there.

5 The first aspect of the invention is the accelerated degradation of organic matter on the submerged sediment surfaces. In the environment there is an enormous problem with the accidental or intentional introduction of organic matter in the form of raw or partially treated sewage into waterways. Pipe damage, rain water overflow, and out dated or overburdened sewage treatment facilities are the causes. The sewage sludge places a
10 tremendous demand on the available dissolved oxygen levels resulting in a hazardous situation of low oxygen known as hypoxia. A typical hypoxic situation has dissolved oxygen levels of 2.0 milligrams per liter or less. This creates an environment which is toxic to many fish and aquatic invertebrates. As these fish and invertebrates die and fall to the bottom their decomposition adds to the oxygen depletion of the surrounding
15 water. In the past the only way to remediate such mishaps would be to dredge up the contaminated sediment, treat it, and return it to the environment. This process is both expensive and potentially damaging to the organisms which are crucial to the balance of the sediments ecosystem. The most common alternative chosen is to do nothing which would be viable if the spills were not chronic and frequent. If left alone naturally
20 occurring anaerobic bacteria would breakdown these compounds over a long period of time. However the huge quantity and frequency of these spills overburdens the sediment's ecosystem leading to oxygen deprivation and the death of many organisms necessary to the proper balance of the ecosystem. This type of damage can destroy

ecosystems necessary to the propagation of commercially important species of fish and invertebrates. This invention proposes an in situ approach to help remediate this problem. Its approach is one in which the ability of naturally occurring and/or seeded microorganisms to breakdown these contaminants is enhanced by the timed-release of oxygen gas, via chemical reaction, and chemical additives such as buffering agents and enzymes. This method proposes that situations, where aerobic bacteria no longer exist due to low oxygen levels, can be reseeded with timed-release tablets having an inner-core of dehydrated living bacteria. The choice of which additional additives to enhance the process, buffer the pH, and control the dissolving rate of the tablet, will depend upon each particular situation. The catabolic processes which could normally take months now occur in hours or days due to the accelerated growth of the aerobic organisms. Once the organic matter is completely broken down, the bacterial food source is depleted. Then the bacteria start to die off, eventually returning their number to precontamination balanced levels.

The second aspect of the invention deals with the problem of pH balance in aquatic systems. At present acid-contaminated lakes and streams have been remediated by the introduction of large quantities of alkali such as lime $\text{Ca}(\text{OH})_2$ in a process known as liming. Liming produces a sharp rise in pH, causing a shock to the ecosystem resulting in the death of algae and invertebrates. These dead organisms fall to the bottom adding to the layer of decomposing organic matter created by the death of organisms already killed by the acid contamination itself. This layer decaying organic matter puts an increased demand on the available oxygen levels in the water above the sediment. This bottom water quickly becomes hypoxic, thereby causing the death of

additional animals and plants, starting a cycle of death. Eventually, over a period of time the lake will recover after liming to its proper pH level. This is the goal of liming, but it is short lived because of the chronic input of acid rain. The lake will have to be retreated periodically.

5 The death cycle can be broken, however, by the use of the novel composition, which will act to buffer and stabilize the pH in a slow, time-release manner rather than a sharp rise as in traditional liming methods. The novel composition also simultaneously raises available oxygen levels preventing hypoxia, and seeds aerobic bacterial growth in anaerobic sediments which are overburdened with decaying organic matter.

10 In some freshwater lakes, the problem of pH balance is different then that found in saltwater lakes, because of a lack of buffering compounds in the water matrix and surrounding soils and sediment. This reduced buffering capacity of the lake water results in wide fluctuations of pH after acid rain events. These fluctuations can prove to be deadly to aquatic organisms. The optimum pH level is in the 6.8 to 7.8 range
15 depending upon the type of fish and invertebrates found in the lake. This situation requires the addition of a weak acid to the novel composition to achieve the proper pH in the final solution and a buffering agent to stabilize it. Other buffering agents which serve to control pH can be used. They include acetates such as calcium magnesium acetate, borates, and phosphate buffering agents.

20 A third aspect of the invention deals with the oxygenation and seeding of hypoxic bottom waters, referred to as hypolimnion, with aerobic and/or anaerobic bacterial cysts and/or yeast cysts. During prolonged, warm, calm weather, a thermocline usually develops separating cold, dense bottom water from the warm surface layer and from

atmospheric oxygen. Bacterial degradation of organic matter on the seabed is likely to reduce oxygen levels in bottom water or hypolimnion under these circumstances. There is also a difference in dissolved oxygen saturation points between fresh and saltwater. In freshwater the saturation level of dissolved oxygen at 77° F. is approximately 5.9 milligrams per liter. This is much higher than that of saltwater at the same temperature. Because of this there can be a greater potential for hypoxia, low oxygen levels, in marine bottom water overburdened with decaying organic matter. Dangerously low oxygen concentrations can result thereby damaging the ecosystem and the environment. Low levels of oxygen in bottom waters can be raised by dispersing on to or below the surface of the water, above the zone in question, a quantity of timed-release tablets made of a dry particulate composition consisting essentially of an outer-coating of a water soluble substance such as hydroxypropyl methylcellulose or polyethylene glycol, over an outer-layer of an oxidative alkali such as sodium sulfate coated sodium carbonate peroxyhydrate particles, additives including enzymes such as protein kinases, buffering agents such as magnesium carbonate, acetates, borates, and phosphates and acids such as citric or sulfamic acid, sugars such as dextrose, oxidation catalysts such as manganese dioxide and, an inner-coating of a water soluble substance such as hydroxypropyl methylcellulose or polyethylene glycol and an inner-core of dehydrated aerobic or facultative anaerobic and yeast bacterial cysts in a paraffin or gelatin binder and dextrose.

A fourth aspect of this invention deals with treating aquarium gravel or aquaculture pond sediment, which is contaminated with an overload of decaying organic matter. This situation can be remediated using an approach similar to the one

mentioned above. This approach is one in which the ability of naturally occurring and/or seeded aerobic microorganisms ability to breakdown these contaminants is enhanced by the timed-release of oxygen gas, via chemical reaction, and chemical additives such as buffering agents and enzymes. The choice of which additional additives to enhance the process, buffer the pH, and control the dissolving rate of the tablet, will depend upon each particular situation. The rising bubbles of oxygen also act to mechanically loosen and resuspend the particles of organic matter around the tablet, allowing for greater exposed surface area of the particle accessible to bacteria and other microorganisms which can break it down at an accelerated pace.

An object of the present invention is to provide an novel non-toxic in situ method for the accelerated biological degradation of organic matter in the form of sewage sludge on the surface of aquatic sediments in water by dispersing on to or below the surface of the water a quantity of timed-release tablets. Said tablets sink, then dissolve in layers, releasing oxygen bubbles which mechanically loosen and resuspend the organic matter increasing the surface area available to bacteria. When the inner-core of the tablets dissolve aerobic bacteria are released. The bacteria then feed on the sewage sludge at an accelerated pace.

An additional object of this invention is to provide novel nontoxic in situ method for the accelerated biological degradation of organic matter in the form of petroleum hydrocarbons on the, surface of aquatic sediments in water by dispersing on to or below the surface of the water a quantity of timed-release tablets. Said tablets sink, then dissolve in layers, releasing oxygen bubbles which mechanically loosen and resuspend the organic matter increasing the surface area available to bacteria. When the inner-

core of the tablets dissolve aerobic bacteria are released. The bacteria then feed on the petroleum hydrocarbons at an accelerated pace.

Another object of this invention is to provide novel nontoxic compositions and novel products that are particularly useful in practicing the novel methods.

5 A further object of this invention is to provide novel nontoxic in situ methods to oxygenate and seed with aerobic bacteria the hypoxic bottom waters of lakes, streams, bays, and estuaries in a timed-release manner by dispersing on to or below the surface of the water a quantity of timed-release tablets. Said tablets sink then dissolve in layers, releasing oxygen bubbles which raise the dissolved oxygen in a controlled manner and
10 reseed aerobic bacterial populations.

Still a further object of this invention is to treat acid-contaminated lakes and streams by buffering the pH and raising dissolved oxygen levels and seeding aerobic bacteria via the timed-release tablets of the novel composition.

Another object of this invention is to provide novel compositions and novel
15 products containing chemical ingredients for aiding in achieving the proper pH and dissolved oxygen and aerobic bacterial levels of surface and bottom water and sediment in acid-contaminated lakes and streams.

A still further object of the present invention is the prevention of growth of aquatic algae by dissolving a water-soluble colorant that reflects light at the same wavelength
20 used in photosynthesis by the algae and also to aid in the breakdown of dead algae which has settled at the sediment-water interface, by adding oxygen, nutrients and additives first and then adding bacteria which breakdown the dead algae.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Sodium carbonate peroxyhydrate particles coated with sodium sulfate, are dry solids at room temperatures, are nontoxic to humans and to aquatic life, and react with water to release oxygen in such form that matter is loosened and removed from surfaces in the water, but does not remove the protective mucous coatings on fish that are in the water. The size of the particles chosen for use in the tablets is decided according to the rate of oxygen release that is desired. In addition, binding agents such as polyethylene glycol and coating agents such as methylcellulose can be added to achieve a time-released, tabletized version of the novel composition.

Various dry-powder additives can be included in the novel composition, which do not interfere with the cleaning action of the oxidant, but provide beneficial effects in the aquatic environment. For example, magnesium carbonate may be included because it helps to maintain the proper balance of magnesium-to-calcium in the saltwater, so that magnesium is not leached from sensitive invertebrates such as anemones. Citric acid can be included in the novel composition to adjust the pH of the water in a safe range. Other buffering agents which serve to control pH can be used. They include acetates such as calcium magnesium acetate, borates, and phosphate buffering agents.

The tablets themselves can be created by several methods including the following example. The following is only one example and is not intended to limit the scope of the invention to the preferred embodiments mentioned. This example involves the use of several steps. In the first step is the creation of the inner core of the tablet. This is accomplished by dry mixing the heat-dried bacterial cysts with binding agents such as a paraffin or a gelatine, and/or a sugar such as dextrose. Any strain of aerobic

or facultative anaerobic bacteria or yeast that is capable of forming cysts, endospores or ascospores in adverse conditions can be used. The strain or strains chosen will depend upon their availability to breakdown the contaminant being remediated as well as their ability to survive in the particular aqueous environment. These strains include the
5 bacterial genera *Bacillus*, *Sporolactobacillus*, *Sporosarcina*, *Sphaerotilus*, *Beggiatoa*, and *Micrococcus*. Also any yeast may be included, such as those within the genera *Saccharomyces*.

The formulation of the protective coat, cyst, endospore, and ascospore enable the bacteria or yeast to survive long periods of time without food or moisture. When
10 placed in a hydrated environment, the organism breaks out of its protective coating and grows and reproduces. These types of organisms can be artificially induced to form cysts, endospores or ascospores by a commonly used method of dehydration known as heat drying. A common example of a product created by this method is the dry powdered yeast used in baking and brewing.

15 This mixture is then pressed into small tablets of approximately 50 milligrams by the use of any known compactor such as a roller-type or rotary pelletizer. The small tablets can then be coated with a water soluble substance, such as hydroxypropyl methylcellulose, in a device such as a drum dryer. In this process the tablets are sprayed while moving with a liquid solution containing the coating material. The solvent
20 is then heat evaporated in the dryer. The second step involves compressing the core tablet within the center of the larger final tablet. This is accomplished by a device known as rotary double tabletizer such as the Korsch Pharmakontroll 2.03. This device allows for the core tablet to be positioned in the center while the outer-layer of approximately

600 milligrams of the oxidative alkali/additive mixture is compressed around it. The third and final step involve coating the final tablet with a water soluble coating such as hydroxypropyl methylcellulose in a drum dryer. In this process the tablets are sprayed while moving with a liquid solution containing the coating material. The solvent is then
5 heat evaporated in the dryer.

The preferred embodiment of the instant invention is timed-release tablets of a dry particulate composition consisting essentially of an inner-core of dehydrated bacterial cysts, an inner-coating of a water soluble substance such as hydroxypropyl methylcellulose, an outer-layer of an oxidative alkali such as sodium sulfate coated
10 sodium carbonate peroxyhydrate particles, and an outer-coating of a water soluble substance such as hydroxypropyl methylcellulose.

The timed-release tablets according to the instant invention may contain 10% to 95% by weight of the inner-core additives such as a paraffin or a gelatin bind, and/or sugars such as dextrose.

15 The timed-release tablets according to the instant invention may also contain from 0.1% to 20% by weight of the outer layer additives including enzymes such as protein kinases, buffering agents such as magnesium carbonate, acetates, borates and phosphates and acids such as citric or sulfamic acid, sugars such as dextrose, and oxidation catalysts such as manganese dioxide.

20 The outer-coating timed-release tablets according to the instant invention is a water soluble compound comprising from 0.1% to 5% by weight of the entire tablet. The inner-core coating of the timed-release tablets according to the instant invention is from 0.1% to 5% by weight of the inner-core.

The present invention is specifically designed to provide a better solution to combat the problems associated with lake eutrofication. With a single timed-release layered tablet, several active steps will occur. First, the outer layer will dissolve releasing a compound that liberates both oxygen, in the form of bubbles, and a combination of blue and yellow water-soluble dyes. The bubbles act to both disburse the dye into the water column, raise DO level in the water, and loosen particles at the sediment-water interface causing the tablet to bury itself deeper into the sediment. Next, the inner layers of the tablet disintegrate to release a combination of enzymes, buffers and aerobic and facultative anaerobic microorganisms. The dye acts to block the wavelength of light necessary for photosynthesis. This causes the death of the nuisance algae or plants, which sink to bottom as they decay. The microorganisms consume the detritus at a high rate, enhanced by the nutrients and enzymes and dissolved oxygen. The high DO level also helps to prevent the fish kills associated with the increased demand created by the decaying plant matter. The source of the oxygen is a dry form of hydrogen peroxide which as a solution quickly degrades to molecular oxygen and water, to oxygenate the water. Since micro-organisms receive O₂ from DO in the liquid phase instead of the usual replacement of DO from the atmosphere through a process of stirring or agitation, we introduce it directly into the system therefore relocation or extensive mechanical processing systems are not required for the gas/liquid interface. The oxygen transfer rate differs depending on the characteristics of the water by such variables as dissolved solids, organics, surface-active agents, the amount needed can be determined by using the following standard equation:

$$dC/dt = K(C_s - C_t)$$

Where $K=O_2$ transfer rate; C_{sub-s} = concentration of dissolved O_2 at saturation; C_{sub-1} = concentration of dissolved O_2 at time t .

Through inputting the time-release tablet into the aquatic environment the rate of
5 O_2 supply and the buffering capacity provided by a body of liquid containing dissolved
 O_2 is able to be in excess of the O_2 required for microbial metabolism so that the
development of anaerobic conditions is restrained. Thus, we have been able to show
that in nonmechanical systems, for example, barrier ditches and lagoons, O_2 supply is
no longer limited to transfer at the liquid surface. Previous limitations of treatment by
10 the low rate of O_2 transfer can be compensated for either by an increase in oxygen
introduced by time-release tablets created a reduction in the substrate load.

A way of analyzing the system is to examine the carbonaceous demand, which
can be estimated, using a basic approach whereby the oxygen requirement is estimated
as: O_2 required per day = soluble BOD_u removed per day – BOD_u of solids leaving the
15 system per day.

It should also be noted that alga multiplication usually has a beneficial effect on
the oxygen balance in an aquatic environment since during photosynthesis most algae
produce more oxygen during daylight hours than they consume by respiration during all
24 hours. For algae contained in the effluent of a aquatic system, Toms *et al*
20 ("Observations on the performance of polishing lagoons" Journal Institute of Water
Pollution Control, United Kingdom 1975 in Arceivala, p. 801) found that the respiratory
demand to be about 0.007 mg O_2 per mg as per hour, the oxygen production to be
about 0.1 mg O_2 per mg per hour, that is, 15 times greater than the amount consumed.

Thus, the role of algae in waste stabilization in aquatic environments is beneficial to BOD for aerobic bacterial activity when the algae provides excess of oxygen required by bacteria, thus creating an aerobic environment.

The tablets of the present invention release components over time that accomplish three tasks. The first task is the prevention of growth of aquatic algae by dissolving a water-soluble colorant that reflects light at the same wavelength used in photosynthesis by the algae. The second and third are to aid in the breakdown of dead algae which has settled at the sediment-water interface, by adding oxygen, nutrients and additives first and then adding bacteria which breakdown the dead algae. This is accomplished by the addition of tablets to the surface water, which sink to the bottom releasing their components. The tablets quickly release a water-soluble colorant, which diffuses into the surface water, and acts to block the wavelength of ambient light necessary for photosynthesis to occur in growing algae. The colorant also prevents the necessary light from penetrating the surface water and to the existing algae thereby killing it. Sinks dead alga sinks to the bottom where it decays. The bacteria, nutrients and additives help to breakdown the dead algae.

The following examples serve to provide a better understanding of the invention, without however limiting the scope of the invention to the embodiments described.

EXAMPLE 1

The following are the key steps required to carry out an environmental clean-up operation:

First the design of the initial sampling and testing program is customized to fit the situation and will include four general areas:

1. Sampling Grid Pattern--is the actual location of the sample sites and is dependent upon the size shape and depth of the location as well as proximity to possible sources or inputs of pollutants.

2. Diurnal Study--is the sampling of the chosen number of sampling points over a period of 24 hours. The test parameters include temperature, pH, dissolved oxygen, specific conductivity, tidal flux, and weather conditions.

3. Depth Profile--this type of sampling involves the taking of samples at different depths and includes parameters such as biochemical oxygen demand (BOD), chemical oxygen demand (COD), total organic carbon (TOC), ammonia, nitrate, nitrite, total Kjeldahl nitrogen (TKN), hydrogen sulfide (H₂S), total phosphate (T-PO₄), ortho-phosphate (O-PO₄), specific conductivity, temperature, total dissolved solids (TDS), total suspended solids (TSS), turbidity and chlorophyll A.

4. Sediment Analysis--the biological portion of this type involves the identification of benthic organisms macroinvertebrates and bacteria, as well as chemical analysis including parameters as Total Organic Carbon (TOC), redox potential, total metals (As, Cd, Cr, Pb, Hg, Zn, Cu, Fe).

The initial testing will be carried out EPA guidelines and safe laboratory practices. Sample will be taken using EPA guidelines and in properly preserved containers. A chain-of-custody will be maintained throughout the analysis. Water samples will be collected using non-contaminating hand dippers at the surface or Nanson or Niskin sampling devices at the surface depth. Sediment samples will

obtained using a hand corer if the water is shallow and a Ekman or ponar grab sampler if the water is deep.

Results of the analysis are then plotted graphically in order to determine a baseline and to see any indication of either over acidification due to acid precipitation or oxygen demand overload due organic pollution such as sewage. If it is found that the treatment will reduce the BOD and or COD and breakdown the organic matter with out producing toxic residuals such as the oxidized form of mercury, than the project will move to the next phase if not alternatives will be discussed with the client.

The decision to proceed will depend on what effects adding oxygen gas would in compensating for the demand or in the acid situation what the buffering capacity of the product may due to the pH. If any of the heavy metals are found in high concentration in solution and the solution is in the acid range (below 7.0) than the treatment would be beneficial in precipitating the metals out of solution. It might be advisable to add additional compounds (specific to the metal) to actively chelate the metal into a complex which is biologically inactive. If the Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the contaminated zone are 50% higher than that of a non-contaminated site in the area and the Dissolved Oxygen (DO) level is below 2.0 milligrams per liter, then the site is good candidate for treatment.

The custom design of the treatment will depend on the type and extent of pollution and the chemistry of the water. If for example, the problem is acid rain contamination, then the product must be buffered to achieve the proper pH. If the pH of the water is lower than 6.3 than this particular method would not be recommended

especially is high metal concentrations such as mercury, are found because the oxidation of metallic complexes can cause them to become even more toxic.

If the problem is untreated sewage sludge which has accumulated on the sediment surface then the product should be pressed into tablets. Their size and shape will depend upon the conditions of the site and include; depth of contaminant sludge
5 depth to the sediment surface, current strength and direction, water surface conditions, and weather. In order to predict where a tablet will land on the bottom once dropped from the surface. To determine this the currents at the site must be studied. The faster the current, the quicker the tablet will need to sink. This is accomplished by pressing
10 smaller rounder tablets. An example would be 500 milligram spherical tablets. In slow, calm water flat disk-like or elongated tablets can be used.

The actual treatment can take three forms. The first will involve spreading the granular product over the surface of the contaminated area. This will be accomplished using a boat with a hopper/spreader device as shown in my prior U.S. Patent No.
15 5,275,943. The device has a hopper which is loaded with granular product or tablets and as the boat moves along its course the spreader shoots the product into the water. The rate at which the product is added to the water can be controlled by the amount of product being released from the hopper to the spreader and on the speed at which the boat travels. The course or pattern which the boat takes will depend upon the site
20 conditions and the type and location of the contamination. If the water column is being treated, as is the case when granular product is required, a high boat speed and loose pattern can be used.

The second situation involves spreading tabletized product into the water using the same device as above if the contamination is concentrated in the sediment. In that case tablets should be applied at a slow speed and tight overlapping pattern is required.

5 The third situation involves spreading tabletized product into the water using the device also shown in my prior U.S. Patent No. 5,275,943 if the contamination is concentrated in the sediment. This device is used when the contamination is concentrated in small specific area. It enables the crew to place tablet on to a specific spot on the sediment surface with great accuracy.

10 Testing should be continue during the treatment process. This is done to see if the treatment is having a beneficial effect and to see if any adjustments need to be made. The parameters at least include dissolved oxygen, BOD, COD, pH, temperature, conductivity, TSS, TDS, Turbidity, and total metals. If dissolved metal concentrations are rising then the process should be stopped and reevaluated. A temporary rise in
15 BOD, COD, TSS, and Turbidity should be followed by a drop and leveling off at a value which is within the acceptable range for the specific situation. If the pH is raising to quickly the process should be stopped and the concentrations being added should be reduced accordingly.

20 Follow-up testing should be continued until a steady baseline is achieved. It should be noted that there are daily and seasonal variations which must be accounted in the evaluation of the steady baseline and the decision to terminate the testing. The parameters should include as many of the ones listed above.

EXAMPLE 2

Aquatic Field Test

A small private pond in New York State was used for field trials from 4/21/98 to 5/2/98. The pond is approximately one foot-acre in size and has a viable population of
5 North American bass and freshwater sunfish. It also has had in past years a chronic problem with blooms of the filamentous alga Lyngbia sp. This algae is considered a nuisance because of its enormous growth rate and propagation capabilities and its tendency to completely overrun a ponds surface with thick mats of filamentous growth. Another nuisance plant in the study area is Myriophyllum sp., a non-indigenous water
10 milfoil.

A small area of the pond (6'x3'x2') was segregated using black plastic material waited on the bottom and with floats above. Within the area enclosed there is a viable mass of the filamentous alga Lyngbia sp.

Our Tests were conducted to determine the dye concentrations necessary to
15 achieve a reduction in growth in this algae and the effect of dissolved oxygen levels on the growth of aerobic micro organisms in breaking down the settled detritus.

3200 mg layered tablets were produced containing a mixture of dyes (acid blue #9 and acid yellow #23) and oxidative alkali enzymes (including cellulase) and bacteria cultivated for their ability to breakdown decaying plant matter.

20 YSI model 6920 remote monitoring probe sondes is being used to measure the test parameters as well as separate tests for turbidity. Each probe is designed to take readings for Depth, temperature, Dissolved oxygen, conductivity, Total Dissolved solids,

Oxidation-Reduction Potential, and at set intervals. The weather conditions are also monitored over the duration of the test.

The results of the first field trial are displayed on Figure 1. That data shows that the tablets were able to raise DO levels above saturation. The dissolve time ranged from 30 to 45 minutes. The water stayed saturated for 30 minutes and it took 9 hours and 45 minutes for the oxygen to be consumed. The growth of the algae was reduced as compared with the algae in the control area. As this particular algae grows the mats move closer to the surface. This distance was used as a measure growth rate. The control group grew at an average of 20mm per day as opposed to the treated area which a average rate of 6mm per day over 97 hour period. There were fluctuations in weather and temperature as well as rain during the test period. The rainfall affected the concentration of the dye. The long as the concentration of the dye remained above visually perceptible levels there seems to be a negative effect on the growth rate of the Lyngiba sp. in the field. The effect of the tablets on the sediment surface due to the bacterial breakdown of the detritus was noticeable. There areas surrounding the where that tablets (10cm radius) settled showed the most signs of change. The larger bits of detritus was reduced to fine particles giving the the impression of a clearer area.

EXAMPLE 3

20 Manure Treatment Experimental Design

Microbial treatment or "purification" may be regarded as a process by which the pollutants, in the raw waste, are converted to microbial cell biomass or insoluble substances. This biomass can then be separated from the final end produce which is

water containing a suitable BOD, resulting in the satisfactory achievement of the reduction of pollution associated with agricultural wastes.

The treatment we have developed however, does not rely on mechanical means to aerate the manure. It uses the byproduct of a chemical reaction, hydrogen peroxide, which in solution quickly degrades to molecular oxygen and water, to oxygenate the water. Based on Applicant's U.S. patented methodology (U.S. patent No. 5,275,943) it uses a timed-release tablet to both oxygenate the water column slowly, and introduce aerobic bacteria, enzymes, buffers and additives. These components help to speed up the degradation of the manure and reduce gasses which would have otherwise been created during anaerobic degradation. Because our process is aerobic, the noxious gases are not produced, thereby reducing the foul odors associated with these pits. The tablets are custom manufactured into layers to incorporate the chemical requirements of a particular situation such as the inclusion of a phosphate precipitant like ferrous chloride. The key to its success is providing a source of molecular oxygen on a continuous basis, gently disturbing the sludge particles at the bottom of the lagoon with oxygen bubbles. The raised particles of sludge create a greater surface area on which the aerobic bacteria can attach speeding up the degradation process.

In the aerobic environment the bacterial genera Nitrosomonas and Nitrobacter convert Ammonium Nitrogen to Nitrite then Nitrite to Nitrate. Once the micro-environment at the sediment-water interface is adjusted to promote aerobic bacterial growth (Nitrification) the bacterial portion of the tablet dissolves. As these bacteria mature and reproduce they consume the sludge without producing the noxious gases. There are several odor-reducing steps occurring simultaneously, aside from the aerobic

bacterial degradation of the manure. Physically the rising bubbles of oxygen tend to purge any dissolved gases out of solution there by removing them from manure. Chemically the dissolution of the dry oxidative alkali raises the pH of the manure thereby preventing the volatilization ammonia nitrogen. Such biological treatment
5 degrades the polluting organic matter as a result of the activity of a mixture of microorganisms being cultivated and introduced by our patented methodology. The introduced bacteria, after moving from their suspended state to the growth phase, absorb and consume the existing organic matter as food provided they have the proper surrounding environment. Our method ensures that the environment is sufficiently
10 oxygenated to promote the growth of the aerobic and facultative anaerobes.

The fundamental principle is that wild microorganisms will multiply if they are provided with the organic matter in sewage and DO (dissolved oxygen). In the process, most of the biodegradable carbon compounds are converted to CO₂. In our research we introduce beneficial microorganisms, oxygen and nutrients sufficiently able to repair
15 any imbalance created in a high BOD system.

EXAMPLE 4

20 Bench-scale test

An experimental design for the bench-scale testing based on one developed by Dr. Bundy of Iowa State University (Lorimor, J., Bundy D., Manure Odor Reduction from Pit Additives Iowa State University Department of Agricultural & Biosystems Engineering May 1996) was utilized. These were completed to narrow down the choices
25 between 12 variations of the prototype tablets, containing different concentrations of components, to be used in the field test onsite at the hog farm sludge lagoon. Six

reactors constructed of 4" diameter PVC 40" long with and end cap and treaded top cap and pressure relief hose to remove gas produced from the laboratory. The reactors were filled with 5 liters of Distilled water and sealed. Fresh hog manure was acquired from a local 1400 head facility that maintains a 125,000-gallon cement lined anaerobic sludge lagoon. The percent moisture of the manure was determined to be 20% using AWWA standard method 2540b ("Method 2540 Solids" in Standard Methods for the Examination of water and Wastewater. 17th edition. APHA-AWWA-WPCF ed. By Clesceri, L., Greenburg, A.E. and Trussell, R.R. 1989. Pgs. 2.71-79). Although the "Bundy method" suggests using manure that has been diluted to 4% solids with water, we chose to adjust the test solution to 10% solids. This is because on actual farms the manure supernatant's test at between 10% and 20 % solids and our result would be closer to the field conditions. At the start of each test, a one-liter volume of water and manure was added to each container to bring the percent Solids up to 10%. Total liquid volume in each container was 6 liters. The mixture was then stirred with a modified paint stirrer to ensure the homogenization of the sample. A YSI model 6920 probe sonde was used to measure the test parameter. The probe is designed to take readings for Depth, temperature, pH, Dissolved oxygen, conductivity, Total Dissolved solids, Oxidation-Reduction Potential, and Ammonium-Nitrogen at set intervals. The initial interval was set at one minute, but later modified to every 15 minutes over a two-day period. The initial test determined that there was a lag time of 22 hours between the saturation of the water with dissolved oxygen and the consumption of the oxygen by the bacteria (Figure 2).

This was followed by a sharp decline in the Dissolved Oxygen level down to a hypoxic level below 2.0mg/l. The pH rose from 3.0 to 8.4 preventing the volatilization of ammonia, which does not volatilize at a pH over 5.0. After the initial burst of off gasses there was a noticeable reduction in odor between the control and the treated samples. A
5 modification of the standard methods protocol will be used to quantify the field test results. Odor is very subjective and can vary from person to person so a panel will be used to standardize the results.

Robinson (Robinson, K. "Aerobic Treatment of Agricultural Wastes" in *Microbial Aspects of Pollution*. (ed.) G. Sykes and F.A. Skinner, London: Academic Press, 1971,
10 p. 94) also found the benefits of raising pH in a study. He found, in hog waste, that an alkaline pH value (8.5-9.0) could be maintained when the substrate has a high N content. He also found that the maintenance of such pH levels corresponds with a high rate of reduction of O2 demand of the substrate; lower rates of substrate supply lead to the production of acid conditions (pH 5.5-6.0).

EXAMPLE 5

Field Test

The field test of the tablets in the manure lagoon at the hog facility was implemented from 4/9/98-4/16/98. From the bench scale testing a quantity of tablets
20 was added at intervals over a 7-day period. A preliminary sample was taken at the start of the test and one at the end. Test parameters include pH, Volatile Fatty Acids, COD, BOD, Total solids, Total Volatile Solids, Ammonia Nitrogen, Total Kjeldahl Nitrogen, and Total Phosphorus and Odor. The results are shown in Figure 3.

EXAMPLE 6

Preparation of Tablets Containing Dyes

In order to produce a final tablet that has layer (delayed-release) properties, a method of production involves a process called slug and grind which includes several steps.

The first requires the blending of ingredients below:

- 1 Dried bacteria or microorganisms (such as Nitrosomas sp., Nitrobacter sp. and Bacillus sp.) on a bran flake powder
 2. Enzymes (such as amylase, cellulase)
 3. Binders - Microcrystalline Cellulose (such as Avicil NT200/Lattice NT200)
 4. Lactose (slows disintegration of Tablets)
 - 5) Gums (such as Xanthan Fines)
 6. Sodium Lauryl Sulfate (Surfactant)
- to create a premix slug.

The second step after blending for 5 minutes, tablets, such as 1.5" round, are pressed using a press such as a Rimic set at between 20-30 psi. These tablets are added to grinding machine with an #8 mesh screen. This produces preweight slug granules.

The third step involves mixing the Dye powders below

1. Acid Blue #9
2. Acid Yellow #23

with a binder such as Microcrystalline Cellulose to make it more flowable and easier to handle. This step helps to prevent the formation of dust, which makes the handling of these dye more difficult.

The fourth step in the manufacture process involves the mixing of the final ingredients
5 below:

1. Sodium Carbonate Peroxyhydrate (Sodium Percarbonate)
2. Corn Starch
3. Binder (Microcrystalline Cellulose such as Avicel NT200/Lattice NT200)

with the resulting mixtures from steps two and three for 5 minutes.

- 10 The fifth step involves taking the the final mixture from step four and pressing a tablet . Tablets range from 0.5 to 10,000 grams can be produced using the standard method for a single station or rotary press. Prototypes were produced with the weight cam on a single station stokes press set to 59-62 grams and hardness adjusted to 90-110# Rimac (1-1/2" rack). After pressing the tablets needed to relax 30 minutes in which 25-30 of
15 their original hardness is lost. After that period the tablets hardness remains stable.

The above tablets can be prepared in a similar way using other cellulose derivatives as blending ingredients and also as coatings to taylor made time release profiles.

EXAMPLE 7

- 20 Tablets containing the components below can be manufactured as shown in Example 6 above using conventional tableting methodology utilized in the pharmaceutical industry which includes coatings so as to control the release aspects of the tablets.

Coatings – corn based sugar, or lactose, or water soluble synthetic polymers

Oxygen provider – sodium percarbonate

Nutrients or enzymes – cellulase, amylase, lactase,

Microbials- family Nitrosomas, and Nitrobacter and Bacillus, Sporolactobacillus,
5 micrococcus Arthrobacter, Flavobacterium, pseudomonas, Rhizomas, Rhizobium,
Rhizobacter, Azoarcus, Ralstonia, Sporosarcina, Sphacrotillus, Beggiatoa,
Saccharomyces

Precipitants- Ferric Chloride, Alum, Lime

Buffers –magnesium carbonate

10 Colorants – acid blue #9, acid yellow #23 other water soluble non-toxic dyes

Binders and additives – microcrystalline cellulose, magnesium stearate

The foregoing description thereof is provided as illustrative of some of the
15 preferred embodiments of the concepts of this invention. While these embodiments
represent what is regarded as the best modes for practicing this invention, they are not
intended as delineating the scope of the invention, which is set forth in the following
claims. It will be understood that the above descriptions of the present invention are
susceptible to various changes, modifications and adaptations, and the same are
20 intended to be comprehended within the meaning and range of equivalents of the
appended claims.

What is claimed is:

1. A non-toxic in situ method for the accelerated biological degradation of organic
5 matter on the surface of aquatic sediments in water comprising dispersing on to or
below the surface of the water a quantity of timed-release tablets of a dry particulate
composition, dissolving said tablets in layers, releasing oxygen bubbles which
mechanically loosen and resuspend the organic matter and the bacteria contained in
the inner-core of said tablets, wherein the timed-release tablets of a dry particulate
10 composition comprises:
 - (1) an inner-core comprising at least one live microorganism strain in dormant
condition selected from the group consisting of aerobic bacteria, facultative
anaerobic bacteria and yeast which are capable of forming cysts, endospores or
ascospores in adverse conditions,
 - 15 (2) an inner-coating over the inner-core of a water soluble substance selected from
the group consisting of polyethylene glycol and hydroxypropyl methylcellulose,
 - (3) an outer-layer over the inner-coating of an oxidative alkali comprising sodium
sulfate coated sodium carbonate peroxyhydrate particles, and
 - (4) an outer-coating over the outer-layer of a water soluble substance selected from
20 polyethylene glycol and hydroxypropyl methylcellulose.
2. The method of claim 1, wherein said organic matter is sewage sludge.
3. The method of claim 1, wherein said organic matter is petroleum hydrocarbon.

4. The method of claim 1, wherein the inner-core of said tablets further contains binder selected from the group consisting of paraffin, gelatin and dextrose.

5. The method of claim 1, wherein the outer layer of said tablets further contains at least one additive selected from the group consisting of enzymes, buffering agents, sugars and oxidation catalysts.

6. The method of claim 5, wherein the enzymes are protein kinases, the buffering agents are selected from the group consisting of magnesium carbonate, acetates, borates, phosphates, citric acid and sulfamic acid, the sugar is dextrose and the oxidation catalyst is manganese dioxide.

7. The method of claim 1, wherein the live microorganism strain in said inner-core of the tablets is selected from the group consisting of bacterial genera *Bacillus*, *Sporolactobacillus*, *Sporosarcina*, *Sphaerotilus*, *Beggiatoa* and *Micrococcus* and yeast genera *Saccharomyces*, *Arthrobacter*, *flavobacterium*, *Pseudomonas*, *Nitrosomas*, *nitrobacter*, *Rhizomas*, *Rhizobium*, *Rhizobacter*, *Azoarcus*, *Ralstonia*.

8. Timed-release tablets of a dry particulate composition employed in the method of claim 1.

9. A non-toxic in situ method for oxygenating the hypoxic bottom waters of lakes, streams, bays and estuaries and seeding aerobic bacteria in a controlled manner

comprising dispersing on to or below the surface of the water a quantity of timed-release tablets of a dry particulate composition, dissolving said tablets in layers, releasing oxygen bubbles which raise the dissolved oxygen and reseed aerobic bacterial populations, wherein the timed-release tablets of a dry particulate composition

5 comprises:

- (1) an inner-core comprising at least one live microorganism strain in dormant condition selected from the group consisting of aerobic bacteria, facultative anaerobic bacteria and yeast which are capable of forming cysts, endospores or ascospores in adverse conditions,
- 10 (2) an inner-coating over the inner-core of a water soluble substance selected from the group consisting of polyethylene glycol and hydroxypropyl methylcellulose,
- (3) an outer-layer over the inner-coating of an oxidative alkali comprising sodium sulfate coated sodium carbonate peroxyhydrate particles, and
- (4) an outer-coating over the outer-layer of a water soluble substance selected from
15 polyethylene glycol and hydroxypropyl methylcellulose.

10. The method of claim 9, wherein the inner-core of said tablets further contains binder selected from the group consisting of paraffin, gelatin and dextrose.

20 11. The method of claim 9, wherein the outer layer of said tablets further contains at least one additive selected from the group consisting of enzymes, buffering agents, sugars and oxidation catalysts.

12. The method of claim 11, wherein the enzymes are protein kinases, the buffering agents are selected from the group consisting of magnesium carbonate, acetates, borates, phosphates, citric acid and sulfamic acid, the sugar is dextrose and the oxidation catalyst is manganese dioxide.

5

13. The method of claim 9, wherein the live microorganism strain in said inner-core of the tablets is selected from the group consisting of bacterial genera *Bacillus*, *Sporolactobacillus*, *Sporosarcina*, *Sphaerotilus*, *Beggiatoa* and *Micrococcus* and yeast genera *Saccharomyces*, *Sporosarcina*, *Sphaerotilus*, *Beggiatoa* and *Micrococcus* and
10 yeast genera *Saccharomyces*, *Arthrobacter*, *flavobacterium*, *Pseudomonas*, *Nitrosomas*, *nitrobacter*, *Rhizomas*, *Rhizobium*, *Rhizobacter*, *Azoarcus*, *Ralstonia*.

14. A method for treating acid-contaminated lakes and streams comprising dispersing a quantity of timed-release tablets of a dry particulate composition into the
15 lakes and streams, buffering the pH of said water in a timed-release manner, raising the dissolved oxygen levels and seeding aerobic bacterial growth, wherein the timed-release tablets of a dry particulate composition comprises:

- 20
- (1) an inner-core comprising at least one live microorganism strain in dormant condition selected from the group consisting of aerobic bacteria, facultative anaerobic bacteria and yeast which are capable of forming cysts, endospores or ascospores in adverse conditions,
 - (2) an inner-coating over the inner-core of a water soluble substance selected from the group consisting of polyethylene glycol and hydroxypropyl methylcellulose,

- (3) an outer-layer over the inner-coating of an oxidative alkali comprising sodium sulfate coated sodium carbonate peroxyhydrate particles, and
- (4) an outer-coating over the outer-layer of a water soluble substance selected from polyethylene glycol and hydroxypropyl methylcellulose.

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15. The method of claim 14, wherein the lakes are freshwater lakes.

16. The method of claim 15, wherein the tablets further contain a weak acid to achieve the proper pH level and a buffering agent.

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17. The method of claim 16, wherein the weak acid is acetic acid, the buffering agent is selected from calcium magnesium acetate, borate and phosphate.

18. The method of claim 16, wherein the proper pH level is in the range of 6.8 to 7.8.

15

19. Timed-release tablets of a dry particulate composition employed in the method of claim 14.

20. A method for the prevention of growth of aquatic algae comprising adding a quantity of timed-release tablets of a dry particulate composition into the surface water, wherein said tablets comprises:

- (1) an inner-core comprising at least one live microorganism strain in dormant condition selected from the group consisting of aerobic bacteria, facultative

anaerobic bacteria and yeast which are capable of forming cysts, endospores or ascospores in adverse conditions,

(2) an inner-coating over the inner-core of a water soluble substance selected from the group consisting of polyethylene glycol and hydroxypropyl methylcellulose,

5 (3) an outer-layer over the inner-coating comprising sodium sulfate coated sodium carbonate peroxyhydrate particles and a colorant that reflects light at the same wavelength used in photosynthesis by the algae, thereby prevents the necessary light from penetrating the surface water, and

10 (4) an outer-coating over the outer-layer of a water soluble substance selected from polyethylene glycol and hydroxypropyl methylcellulose.

21. The method of claim 20, wherein the colorant in the outer layer of said tablets is a mixture of acid blue and acid yellow.

15 22. The method of claim 20, wherein the inner-core of said tablets further contains binder selected from the group consisting of paraffin, gelatin and dextrose.

23. The method of claim 20, wherein the outer layer of said tablets further contains at least one additive selected from the group consisting of enzymes, buffering agents,
20 sugars and oxidation catalysts.

24. The method of claim 23, wherein the enzymes are protein kinases, the buffering agents are selected from the group consisting of magnesium carbonate, acetates,

borates, phosphates, citric acid and sulfamic acid, the sugar is dextrose and the oxidation catalyst is manganese dioxide.

25. The method of claim 20, wherein the live microorganism strain in said inner-core of the tablets is selected from the group consisting of bacterial genera *Bacillus*, *Sporolactobacillus*, *Sporosarcina*, *Sphaerotilus*, *Beggiatoa* and *Micrococcus* and yeast genera *Saccharomyces*.

26. A timed-release tablet of a dry particulate composition suitable for treating contaminated surface waters, wherein said tablet comprises:

- 10 (1) an inner-core comprising at least one live microorganism strain in dormant condition selected from the group consisting of aerobic bacteria, facultative anaerobic bacteria and yeast which are capable of forming cysts, endospores or ascospores in adverse conditions,
- (2) an inner-coating over the inner-core of a water soluble substance selected
15 from the group consisting of polyethylene glycol and hydroxypropyl methylcellulose,
- (3) an outer-layer over the inner-coating comprising sodium sulfate coated sodium carbonate peroxyhydrate particles and a colorant that reflects light at the same wavelength used in photosynthesis by the algae, thereby prevents the necessary light from penetrating the surface water, and
- 20 (4) an outer-coating over the outer-layer of a water soluble substance selected from polyethylene glycol and hydroxypropyl methylcellulose.

Pond Dye Treatment Study (sampling at 15 minute intervals 4/21/98 - 5/2/98)

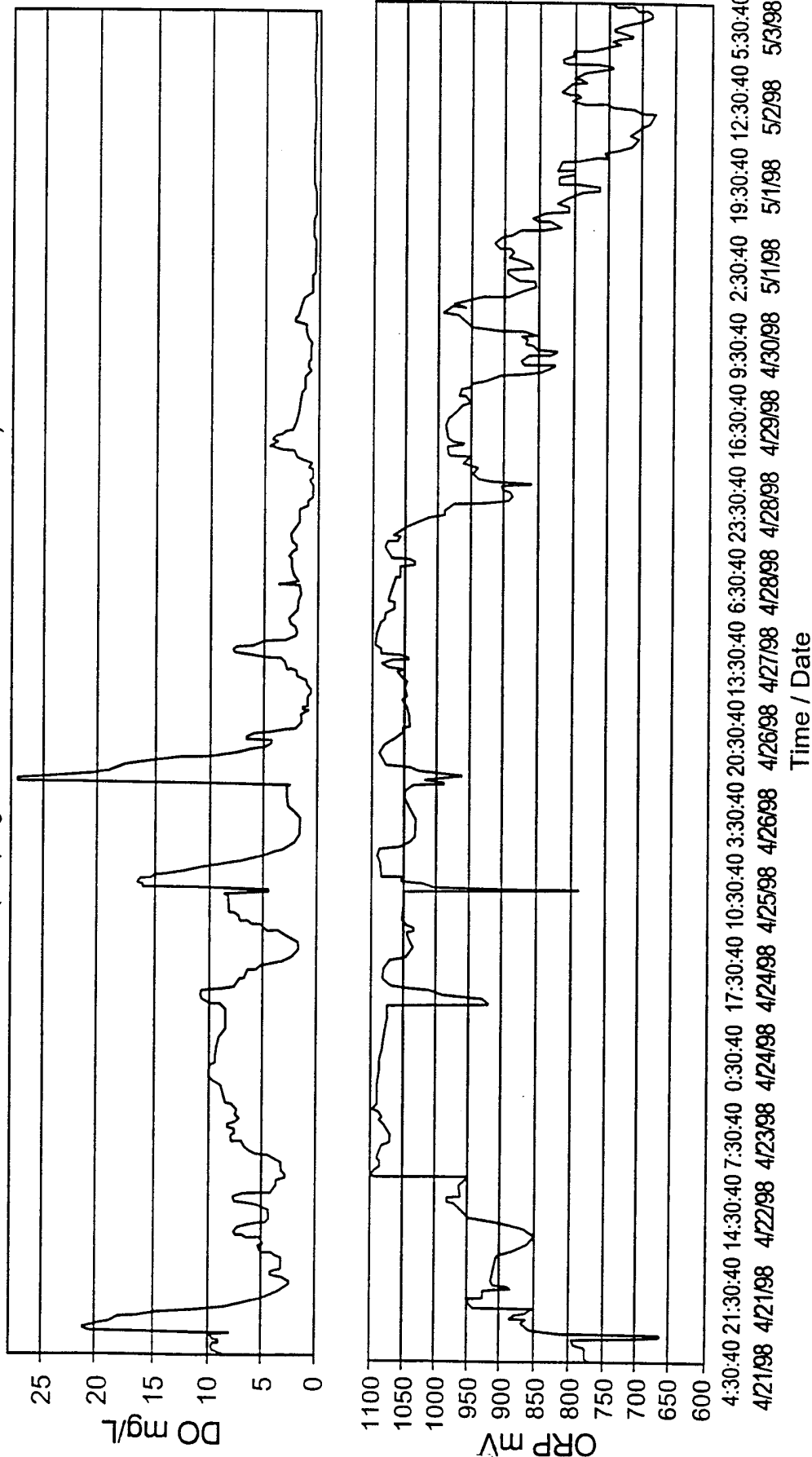
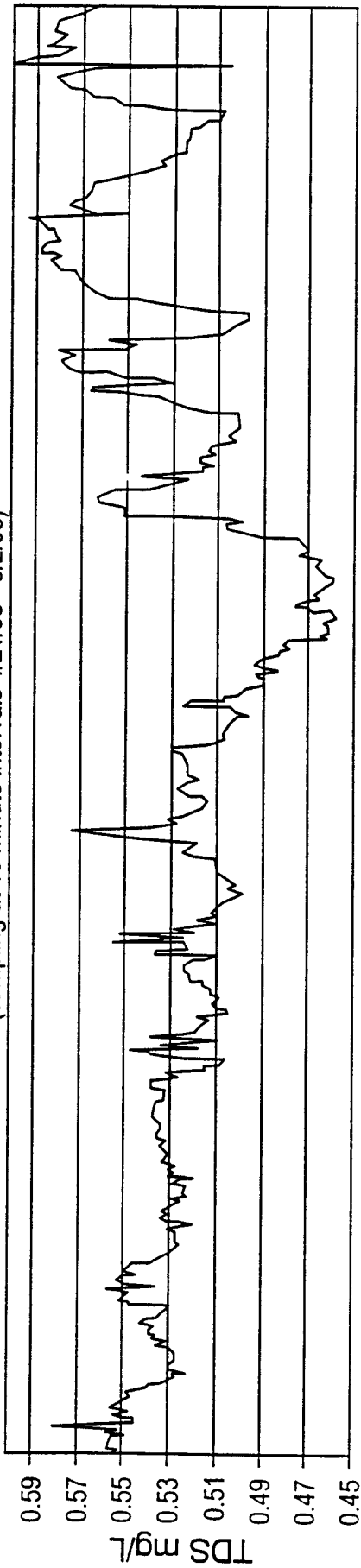


Figure 1A Addition of tablets to enclosure were made on 4/21/98 (21:00) and then on 4/25/98 (9:45)
Note: 9.7° - 9.8°C is the average temp. at which microbial growth rate slows due to enzyme kinetics.

Pond Dye Treatment Study (sampling at 15 minute intervals 4/21/98 - 5/2/98)



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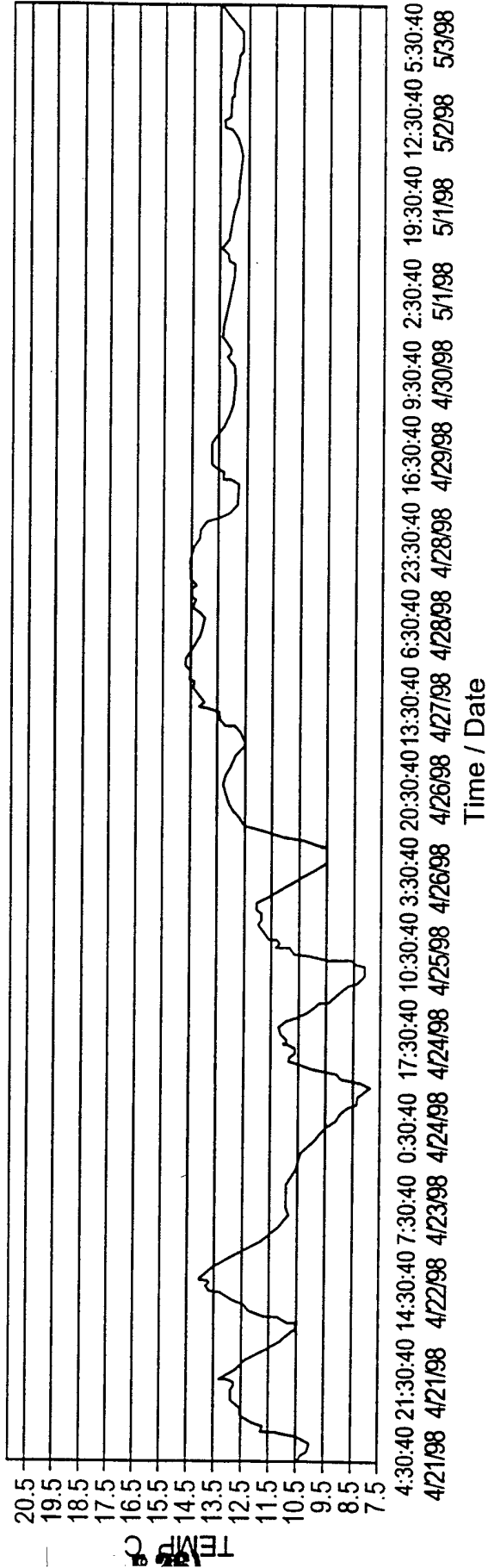


Figure 1B Addition of tablets to enclosure were made on 4/21/98 (21:00) and then on 4/25/98 (11:00) 4/26/98 (9:45)
Note: 9.7° - 9.8° C. is the average temp. at which microbial growth rate slows due to enzyme kinetics.

Figure 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/16692

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C02F 3/34; C12N 11/12

US CL : 210/606; 435/179

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)

U.S. : 210/606, 610, 611, 620,631, 632; 435/176-182, 262.5, 822, 832-839, 853-857, 911, 940-943

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 practicable, 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.
A	US 3,860,490 A (GUTTAG) 14 January 1975, see entire document.	1-26
A	US 4,582,805 A (BOZZELI et al) 15 April 1986, see entire document.	8, 19, 26
A	US 5,275,943 A (DiTURO) 04 January 1994, see entire document.	1-26
A	US 5,648,099 A (BATICH et al) 15 July 1997, see entire document.	8, 19, 26



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	*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
A document defining the general state of the art which is not considered to be of particular relevance	*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
E earlier document published on or after the international filing date	*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
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)	*&* document member of the same patent family
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	

Date of the actual completion of the international search

29 OCTOBER 1999

Date of mailing of the international search report

19 NOV 1999

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