

Wastewater treatment bacterial additive

Abstract

The use of an inorganic azide compound as an inhibitor for a wastewater treatment bacterial additive composition. The inorganic azide compounds used in the invention serve to confer a useful life for the bacterial additive composition, yet a relatively short reactivation time which is of great advantage in wastewater treatment systems with short detention times. The inorganic azide compounds employed as an inhibitor in the bacterial composition additives of the invention include alkali metal azides, alkaline earth metal azides, lead azide and hydrazoic acid.

BACKGROUND AND FIELD OF THE INVENTION

A variety of products of a bacterial composition are commercially available for use in improving performance in biological wastewater treatment facilities. In order to be practical for application, each of these products must be provided with a shelf life through some means. Most of the products are freeze dried. These freeze dried products are dormant in the dry state, and require reactivation in aqueous media prior to addition to wastewater. Other products are liquid suspensions. Liquid products require the addition of an inhibitor at a concentration sufficient to confer self life. When the liquid suspension with chemical inhibitor is added to wastewater, dilution occurs which lowers the concentration of the inhibitor below that required for dormancy of the bacteria. Once the level of inhibitor is diluted to below the minimum level required, the bacteria reactivate, metabolize, and grow. The liquid suspension inhibited as above has an advantage over the freeze-dried product since no separate reactivation stage is required. One simply adds the liquid suspension to the media requiring treatment.

A key consideration for either freeze dried or liquid bacterial preparations is the time lag between addition to the wastewater and subsequent release of inhibition and resumed growth.

For the liquid product, release of inhibition begins with addition to wastewater. For flow-through systems, in which the detention time of any material added to the system may be a matter of hours, a short time lag before reactivation is absolutely necessary.

U.S. Pat. No. 3,963,576 of Horsfall, et al. is directed to an inhibitor for a liquid bacterial suspension and discusses the use of sulfides of sodium or potassium for this purpose.

The present invention is directed to the use of an inhibitor that confers a useful shelf life, but also provides a reactivation time which is approximately one half of the time associated with similar sulfide inhibited systems, providing a substantial advantage in systems with short detention times.

DETAILED DESCRIPTION OF THE INVENTION

This invention encompasses the choice of bacterial compositions and growth conditions to create a liquid suspension and method for rendering the suspension dormant, yet capable of rapid regeneration. Specifically, the invention includes:

1. Growth of a combination of various bacteria, for example, Bacillus, Pseudomonas, Serratia, Myxobacter, Nitrosomonas, Nitrobacter, and/or Thiobacillus bacteria.
2. Addition of a chemical inhibitor, specifically a sufficient amount of an *azide* (.sup.-N.sub.3) compound to cause the suspension to be metabolically dormant. The amount of chemical inhibitor to be added to the suspension of microorganisms is suitably 0.002% to 0.02% *azide* ion by weight of the liquid bacterial suspension. The amount of *azide* ion employed as the inhibitor provides a useful shelf life to the bacterial suspension while permitting a short reactivation time upon introduction of the suspension to the wastewater.
3. A factor influencing rate of reactivation of Bacillus bacteria is spore formation. The use of a sulfide inhibitor, as in U.S. Pat. No. 3,963,576, induces spore formation in Bacillus bacteria within several hours of sulfide addition. When Bacillus bacteria in the spore form are added to wastewater, up to 12 hours are required before the Bacillus bacteria germinate, i.e., change from spore form to the actively metabolizing form. The addition of the *azide* inhibitor in accordance with the present invention does not induce spore formation in the Bacillus bacteria, thereby permitting a very short reactivation time of, e.g., about 30 minutes.

The chemical additives used for inhibition in the present invention are inorganic *azide* compounds. Suitable *azide* compounds include alkali metal azides such as sodium *azide* and potassium *azide*, alkaline earth metal azides such as calcium *azide* and magnesium *azide*, other metal azides such as lead *azide* and hydrazoic acid (HN.sub.3).

The bacterial suspension to which the *azide* compound is added is produced by techniques well known in the art. The bacteria are grown under aerobic conditions in an aqueous nutrient medium which includes appropriate sources of carbon, nitrogen and phosphorus as exemplified below. The *azide* compound additive is added to the bacteria, whether a single or mixed culture, upon attainment of stationary growth. As stated above, the amount of *azide* additive is about 0.002% to 0.02% by weight based on the weight of the bacterial suspension.

The inhibited bacterial suspension may be stored for at least about 60 days depending upon the amount of *azide* added, the species of bacteria in the suspension, the temperature of storage, exposure to air during storage and like factors. The inhibited bacterial suspension may typically be stored in 55 gallon drums and transported to the wastewater treatment site for use. Upon addition of the inhibited bacterial suspension to the reaction vessel used for treating the wastewater, the bacteria are reactivated within

about 30 minutes within the treatment facility.

Use of the inhibited bacterial suspension of the invention enhances wastewater treatment objectives such as removal of biochemical oxygen demand (BOD), oxidation of ammonia, conversion of organic acids such as acetic acid in the presence of nitrates to molecular nitrogen and CO₂, etc. Therefore, the invention provides a convenient means for introducing specific bacteria with specific capabilities into wastewater treatment systems with specific pollutant removal requirements.

The following Examples are given merely as illustrative of the present invention and are not to be considered as limiting. Unless otherwise noted, the percentages recited therein are by weight.

Comparative tests

EXAMPLE 1

In order to test for stability of the product as well as relative reactivation time, the following experiments were performed. A mixture of *Bacillus subtilis*, *Bacillus amyloliquefaciens* and *Pseudomonas aeruginosa* was grown using the following aqueous nutrient medium:

0.2%	Sodium Citrate
0.1%	KH ₂ PO ₄
0.05%	MgSO ₄
0.1%	Yeast Extract
0.1%	NH ₄ Cl

The nutrients were dissolved in 10% tap water and 90% deionized water to make up 500 gallons of the above solution. The solution was sterilized at 15 pounds steam pressure for 15 minutes. After cooling to 98.degree. F., the nutrient medium was inoculated with the mixture of *Bacillus* and *Pseudomonas* bacteria noted above and aeration was begun.

At 30 hours of growth, three 55 gallon drums of the bacterial suspension were filled from the aerobic reactor. Characterization of the suspension at the 30 hour sampling time was as follows:

1. Total bacterial count, 1.times.10⁷ cells/ml
2. Soluble Chemical Oxygen Demand (COD) of filtrate after passing suspension through 0.5 micron filter, 650 mg/l

The three 55 gallon drums were then inhibited with chemical additives as follows:

Drum Number	Chemical Added	Grams Added
1	Sodium Sulfide	20
2	Sodium Azide	20
3	No Addition	--

The drums were sealed, and the suspension in each drum was allowed to age for 30 days.

After aging for 30 days, the drums were agitated for 15 minutes to provide thorough mixing, and were retested for bacterial count and soluble Chemical Oxygen Demand (COD). The results were as follows:

Drum Number	Inhibitor Used	Bacterial Count/ml	Soluble COD, mg/l
1	Sodium Sulfide	9.6 .times. 10.sup.6	625
2	Sodium Azide	9.1 .times. 10.sup.6	575
3	None	6.0 .times. 10.sup.6	130

As shown above, Drum 3 with no inhibitor continued to metabolize, consuming 80% of soluble COD present from initial sampling time to the end of the 30 day aging period (650 mg/l at initial sampling, 130 mg/l after 30 days of aging). Further, the bacterial count decreased from 1.times.10.sup.7 to 6.0.times.10.sup.6, indicating 40% death rate during the 30 day period. In contrast, the sulfide treated suspension and **azide** treated suspension consumed only 3.8% and 11.5% respectively of the soluble COD, while showing a death rate of 4% for the sulfide treated suspension and 9% for the **azide** treated solution. These data obviously show that treatment with both **azide** and sulfide is effective in creating a dormant suspension of bacteria relative to an uninhibited suspension.

In order to test for time lag between dilution into wastewater and reactivation, the following experiment was performed. A nutrient broth was prepared, consisting of:

0.1%	NH.sub.4 Cl
0.1%	KH.sub.2 PO.sub.4
0.05%	MgSO.sub.4
0.05%	Sodium Acetate

0.05%	Glucose
0.05%	Sodium Citrate
0.1%	Yeast Extract

The above ingredients were dissolved in 10% tap water and 90% deionized water, sterilized for 15 minutes at 15 pounds steam pressure, placed under aeration and inoculated with 1000 ppm (volumetric) of suspension from each of Drums 1, 2 and 3. At the time of inoculation, each drum had been aged for 35 days.

Absorbance was used to determine reactivation time. Distilled water was used to zero the apparatus, and the sterilized nutrient broth had an absorbance of 85. Subsequent to inoculation, the aerated samples were checked for absorbance at 20 minute intervals. An increase in absorbance indicates that growth (reactivation) is occurring. The data are presented in Table 1:

TABLE 1

Time (Hours)	Drum 1 (Sulfide)	Drum 2 (<i>Azide</i>)	Drum 3 (No Inhibitor)
0	85	85	85
1	84	85	82
2	86	92	84
3	85	148	87
4	90	225	98
5	112	272	122
6	152	335	145
7	185	362	151
8	228	402	160

From the above, it is clear that the suspension inhibited with *azide* reactivated within 2 to 3 hours, that the suspension inhibited with sulfide reactivated within 4 to 5 hours, and that the sample aged 35 days with no inhibitor showed substantially less growth than either of the inhibited samples. It is important to note that the sample inhibited with *azide* not only reactivated in half the time as did the sulfide sample, but demonstrated a more rapid growth rate upon release of inhibition. As previously explained, this shorter reactivation time is a tremendous advantage of the *azide* inhibitor system relative to the prior art sulfide system, especially in flow through systems with short retention times.

It is clear from the foregoing that the use of inorganic *azide* compounds as inhibitors confers a useful shelf life, maintains a high viable bacterial population for at least 60 days and, in the case of Bacillus, provides a significant advantage in terms of reactivation time in a wastewater treatment system.

The invention being thus described, it will be obvious that the same may be varied in

many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention, and all such modifications as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.