

INHIBITORS FOR THE PROTECTION OF GRAIN AND FEED AGAINST MOLD

Background of the Invention

[0001] The invention relates generally to mold inhibitors and, more specifically, to improved propionic acid-based inhibitors for protecting grain and feed against molds. This unique mold inhibitor formulation combines a salt of propionic acid and a solubilizing agent.

[0002] The widespread occurrence and multiplication of microorganisms in the environment leads to degradation of organic material including grain and feed ingredients. Cereals constitute an important source of energy and protein in animal diets. Managing raw materials under adverse climatic conditions is crucial for retaining acceptable feed quality. Grains can develop molds that produce mycotoxin compounds that develop while in storage. High moisture content in the grain promotes the development of molds and mycotoxins.

[0003] Propionic acid-based mold inhibitors are widely accepted as cost effective and efficacious products for the protection of grains and animal feed against mold. Propionic acid is Generally Recognized As Safe (GRAS) by the United States Federal Department of Agriculture (USFDA). A number of companies offer a range of propionic acid-based mold inhibitor products for the varying needs of the feed industry. Most of these products contain very high levels of propionic acid-which may be corrosive to the equipment used in applying the mold inhibitor and to the equipment used in handling and storing treated feed and grain.

[0004] Accordingly, there is needed a low cost mold inhibitor with good mold-inhibiting properties and with improved spreadability on the substrates. Formulations of the present invention are cheaper, have a pH above about 4.0, and a reduced amount of propionic acid than the existing products in the market.

Summary of the Invention

[0005] The invention consists of formulations for inhibiting mold in grains and feed. The formulations are based on propionic acid, and include a base for increasing the pH, a solubilizing agent, and an essential oil. The amount of propionic acid is reduced from known propionic acid-based mold inhibitors. A solubilizing agent with good spreading properties (i.e., a high HLB number) is included in the formulations, and is believed to improve the distribution of the formulation across the substrate being protected. An essential oil, preferably cinnamon oil, is included in the formulations. Cinnamon oil has antimicrobial properties and reduces the corrosiveness of the formulations. The formulations have a reduced amount of the primary active ingredient (propionic acid) yet, surprisingly, are more effective at inhibiting mold. The addition of the solubilizing agent enhances the mold inhibiting property of the formulation.

Brief Description of the Figures

[0006] Fig. 1 is a chart of the effect of sodium lauryl sulfate against mold growth in samples of corn using a carbon dioxide production test.

[0007] Fig. 2 is a chart of the effect of mold inhibitor formulations with different concentrations of sodium lauryl sulfate against the mold growth in samples of corn using a carbon dioxide production test.

[0008] Fig. 3 is a chart of the effect of mold inhibitor formulations with and without a solubilizing agent against *Mucor*, *Aspergillus* and *Penicillium* species.

Detailed Description of Preferred Embodiments

[0009] Propionic acid-based mold inhibitors include mold inhibitors that contain propionic acid either in the un-neutralized form or in combination with one or more bases.

[00010] Bases used for neutralizing propionic acid in mold inhibitors of the present invention include hydroxides of alkali elements, specifically ammonium hydroxide, potassium hydroxide, calcium hydroxide and sodium hydroxide, and preferably ammonium hydroxide.

[00011] Solubilizing agents for inclusion in formulations of the present invention include surfactants with a high HLB number, preferably above 7, and specifically include anionic surfactants, and more specifically include N-cetyl-N-ethyl morpholinium ethosulfate, N-soya-N-ethyl morpholinium ethosulfate, polyoxyethylene sorbitan monolaurate, polyoxyethylene lauryl ether, sodium oleate, potassium oleate, and sodium lauryl sulfate.

[00012] Essential oils for inclusion in formulations of the present invention include lemongrass oil, palmarosa oil, clove oil, cinnamon oil, eucalyptus oil, sage oil, thyme oil, and cassia oil.

EXAMPLE 1

[00013] Materials and Methods

[00014] All the chemicals or reagents used were of technical grade. All tests were performed in duplicate.

[00015] A carbon dioxide production method was selected for use as a method to evaluate the efficacy of the formulations. Field corn samples were used for the tests. One hundred and fifty gram samples of corn were taken and the moisture content of the samples adjusted to 16%. The mold inhibitor was added to the sample at a dosage equivalent to 1 kg/Ton. The sample was then mixed thoroughly. An untreated sample was used as the control. Samples were transferred into sterile 500 ml glass bottles. Each container was screwed tight with fabricated caps attached having both inlet and outlet valves. The containers were incubated at 25°C. The carbon dioxide concentration in the headspace was measured every 2 days using a Riken Keiki (Tokyo, Japan) Infrared Gas

Analyzer (Model RI-555). The carbon dioxide concentration was plotted against time and the area under the curve was calculated.

[00016] Three types of mold were selected for use in the examples, *Mucor*, *Aspergillus*, and *Penicillium*. Samples of *Mucor* spp. were isolated from decaying bread. The other two microorganisms, specifically *Aspergillus flavus* ATCC 24125 and *Penicillium chrysogenum* ATCC 48908), were obtained from the American Type Culture Collection (Manassas, Virginia).

[00017] A Disk Diffusion Method was used to evaluate and compare the different formulations. Potato Dextrose Agar (PDA) was procured from Becton Dickinson and Company USA. Agar plates (14 cm in diameter) were prepared and allowed to set at room temperature. The spore concentrations of *Penicillium*, *Aspergillus* and *Mucor* in the suspension were 1.725×10^7 , 1.45×10^6 and 2.85×10^6 , respectively. Each 300 μ L volume of spore suspension of the tested microorganism was subsequently spread on the PDA solid media plates. Sterile paper disks (6 mm in diameter) were placed onto the dried inoculated plates, and 15 μ L of each test substance was gently added onto the disks. The plates were subsequently incubated at 25°C for 48 h. The diameters of inhibition zones were measured and reported.

[00018] Results and Discussions

[00019] New Product Composition: The new product formulations include propionic acid in an amount between about 25% and 55% and preferably between 35% and 40%; a base in an amount to bring the pH of the formulation to between about pH 4 and about pH 6, usually between about 5% and 25%, preferably including hydroxides of alkali elements, specifically ammonium hydroxide, calcium hydroxide and sodium hydroxide, and most preferably ammonium hydroxide; a solubilizing agent, preferably an anionic surfactant having an HLB number greater than about 7 and most preferably greater than about 17, in an amount between about 0.1% and about 20%, and preferably between 1% and 10%, and the balance being water. The formulations may also include

between about 0.01% and about 2%, and most preferably between about 0.1% and about 0.5% of an essential oil. The details of a preferred embodiment formulation are given in Table 1.

[00020] Table 1: Composition of a new formulation

Ingredient	% (w/w)
Propionic acid	40.35
Ammonium Hydroxide (27% strength)	10.00
Propylene glycol	1.00
Sodium Lauryl Sulfate (SLS)	1.00
Cinnamon bark oil	0.15
Water	47.49
Caramel color	0.01

[00021] Carbon Dioxide Test: The carbon dioxide test is an accelerated microbial stability test designed to measure the efficacy of mold inhibitors. More carbon dioxide production indicates more mold growth. An efficient mold inhibitor will produce less carbon dioxide in this test and protect the samples from mold. Figure 1 shows the results from the carbon dioxide test using the sodium lauryl sulfate at 1%, 5% and 10% in water and a negative control (only the corn sample).

[00022] It can be seen from Fig 1 that the corn samples treated with sodium lauryl sulfate produced the same or more carbon dioxide than the untreated corn sample (negative control). These data indicate that the addition of sodium lauryl sulfate alone to corn was not effective in inhibiting the mold growth but it could actually enhance the mold growth.

[00023] Figure 2 shows the results obtained from the carbon dioxide test using the proposed mold inhibitor formulation with different concentrations of sodium lauryl sulfate (SLS). Table 2 gives the details of the formulations used for the experiments.

[00024] Table 2: Composition of formulations used in the carbon dioxide test

Ingredient	Positive control	Positive control + 1% SLS	Positive control + 5% SLS	Positive control + 10 % SLS	Negative control
Propionic acid	40	40	40	40	Corn samples without treatment
Ammonium hydroxide	10	10	10	10	
Water	50	49	45	40	
SLS	0	1	5	10	

[00025] The results showed (Fig 2) that the untreated corn sample produced significantly higher amount of carbon dioxide on day 10 and 13 than the treated samples. The samples treated with the formulations containing sodium lauryl sulfate showed improved mold inhibiting property. It is important to note that sodium lauryl sulfate alone (Fig 1) was not effective in inhibiting mold growth but in combination with the positive control it enhanced the mold inhibiting property of the formulation.

[00026] Disk Diffusion Method: Figure 3 shows the results from the Disk Diffusion tests on *Aspergillus flavus*, *Mucor* spp., and *Penicillium chrysogenum*. The final formulation mentioned in Table 1 and the positive control and the positive control + 1% sodium lauryl sulfate mentioned in Table 2 were subjected to this Disk Diffusion method. The results showed that the formulations containing sodium lauryl sulfate performed better than the formulation without sodium lauryl sulfate.

[00027] Conclusion

[00028] The new low cost mold inhibitor combines propionic acid, ammonium hydroxide, water and sodium lauryl sulfate. The addition of sodium lauryl sulfate enhances the mold inhibiting property of the formulations. . Without being bound by any particular theory of operation, it is believed that the surprisingly high efficacy of the formulations of the present invention is due to the improved distribution of the product over the substrate to which it is applied for the inhibition of molds.

[00029] The foregoing description comprises illustrative embodiments of the present inventions. The foregoing embodiments and the methods described herein may vary based on the ability, experience, and preference of those skilled in the art. Merely listing the steps of the method in a certain order does not necessarily constitute any limitation on the order of the steps of the method. The foregoing description and drawings merely explain and illustrate the invention, and the invention is not limited thereto, except insofar as the claims are so limited. Those skilled in the art who have the disclosure before them will be able to make modifications and variations therein without departing from the intended scope of the following claims.

We claim:

1. A mold inhibitor composition of improved efficacy, comprising in aqueous solution not more than 55% by volume propionic acid; a base in an amount to adjust the pH to between about 4 and about 6, and a solubilizing agent.
2. A mold inhibitor formulation as defined in claim 1, wherein the base is selected from the group consisting of ammonium hydroxide, potassium hydroxide, calcium hydroxide, and sodium hydroxide.
3. A mold inhibitor as defined in claim 1, wherein the solubilizing agent has an HLB number greater than 7.
4. A mold inhibitor as defined in claim 3, wherein the solubilizing agent has an HLB number greater than 17.
5. A mold inhibitor as defined in claim 1, wherein the solubilizing agent is selected from the group consisting of N-cetyl-N-ethyl morpholinium ethosulfate, N-soya-N-ethyl morpholinium ethosulfate, polyoxyethylene sorbitan monolaurate, polyoxylene lauryl ether, sodium oleate, potassium oleate, and sodium lauryl sulfate.
6. A mold inhibitor as defined in claim 1, further comprising an essential oil.
7. A mold inhibitor as defined in claim 6, wherein the essential oil is selected from the group consisting of lemongrass oil, palmrosa oil, clove oil, cinnamon oil, eucalyptus oil, sage oil, thyme oil, and cassia oil.