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ANALYSIS OF SYNAGRO BIOSOLID PELLETS AND PELLETS APPLIED TO
HAWAIIAN SOIL FOR DETECTION AND GROWTH OF *SALMONELLA*

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EXECUTIVE SUMMARY

The Department of Environmental Services (ENV), City and County of Honolulu need to upgrade its sludge treatment system at the Sand Island Wastewater Reclamation Treatment Facility (SIWRF). ENV selected the Synagro System as the new treatment to treat sludge at SIWRF because the Synagro System is approved under EPA Part 503 Biosolids Rule to produce a Class A biosolid product. Moreover, since the biosolid product is dried and pelletized, problems related to vector attraction, re-growth of sewage pathogens in biosolid, as well as safety issues related to storage, transporting and application of biosolid to land are minimized. However, due to concerns related to the reliability and applicability of the Synagro System to Hawaii, the City Council passed Resolution No.03-193, FD1, which conditionally approved ENV's application to use the Synagro System. The approval was conditioned on completing some testing of the Synagro pellet and this pellet applied to a Hawaiian soil for a sewage pathogen (*Salmonella*). This Resolution directed Roger Fujioka of the University of Hawaii to develop the appropriate test protocol, to complete the testing and to submit a final report. Based on the guidelines of the Resolution, two goals of the study were formulated and the study protocol devised to address these two goals.

Statement of Goal One

To develop a scientifically valid test protocol to obtain monitoring data that the Pinellas, Synagro pellet had been sufficiently treated to disinfect pathogenic virus, helminth ova, and *Salmonella* bacteria based on concentrations of less than 3 MPN of *Salmonella*/4 g of total solids biosolids (dry weight basis).

To address this goal, a test protocol was developed based on testing Synagro pellets obtained from the Synagro Pinellas Plant for *Salmonella*, total coliform, *E. coli* and heterotrophic bacteria. *Salmonella* was assayed as the representative pathogen in sludge with a potential to multiply in soil. Total coliform and *E. coli* were selected because they represent bacteria that are normally found in high concentrations in untreated sludge and the residual levels of these bacteria in treated sludge (biosolid) are indicative of the efficiency by which that particular sludge treatment system is disinfecting sludge. The test for heterotrophic bacteria enumerates the culturable population of bacteria in any sample. The concentration of heterotrophic bacteria in any sample characterizes the microbial load of that sample. For treated sludge (biosolids), this test will measure residual bacteria surviving the sludge treatment process. The test protocol was designed to answer three relevant questions.

1. Is the concentration of *Salmonella* in the Synagro pellet below the EPA approved limit of 3 MPN/4 g of biosolids?

This question must be answered because it addresses a current EPA regulation. To answer this question, Synagro pellets were initially assayed for *Salmonella* using EPA approved method and the 5-Tube MPN method. For this test, 10 g, 1 g, and 0.1 g of Synagro pellet were assayed and all tests were negative for *Salmonella*. Under these conditions, the calculated concentration of *Salmonella* was less than 0.02 MPN/g of Synagro pellet (dry weight). The non-detectable level of *Salmonella* in the pellet was confirmed by determining that larger sample sizes (4 g, 40 g, 80

g) of Synagro pellet samples were also negative for *Salmonella* when the Presence/Absence test was used. These results show that the concentration of *Salmonella* in the Synagro pellet was in the non-detectable level and was well below the regulatory level of 3 MPN/4 g of biosolids. A limitation of this assay is that concentrations of *Salmonella* in the untreated sludge are variable and may be low. Thus, tests that only determine non-detectable level of *Salmonella* do not provide information as to the efficiency and consistency of the disinfection process.

2. What additional assurance can be obtained to show that the Synagro treatment system is effective in disinfecting sewage microorganisms and pathogens?

Another test was needed to obtain additional data to directly measure the disinfection efficiency of the Synagro System. To answer this question, Synagro pellets were assayed for total coliform and *E. coli* because these bacteria are consistently present in high concentrations in the untreated sludge and consistent reduction of *E. coli* by the treatment system is one gauge used to describe the effectiveness of a disinfection system. For this test, the 5-Tube MPN method was used to assay 10 g, 1 g, and 0.1 g of powdered Synagro pellets. All samples were negative for total coliform and *E. coli*. The minimum detectable level of total coliform and *E. coli* was calculated to contain less than 0.02 MPN/g dry weight of Synagro pellet. Since untreated sludge consistently contains *E. coli* at concentrations of at least 10^6 MPN/g of sludge, the absence of *E. coli* in the treated sludge or biosolid shows that the Synagro System disinfected at least 6 logs of *E. coli*. A disinfecting system that inactivates 6 logs of *E. coli* is considered a reliable disinfecting system. Since *Salmonella* was not detected in the same biosolid sample, it can be assumed that other pathogens (viruses, helminth ova) would have been inactivated as well. In summary, the results of this test provide an independent measurement that the Synagro System is effective in disinfecting most pathogens.

3. What is the microbial load of the dried, Synagro biosolids pellets?

This test was included because there were unresolved questions related to the expected efficiency by which the Synagro System will disinfect all microorganisms in the sludge during the treatment process. To address this question, the Synagro pellet was assayed for concentration of heterotrophic bacteria using the spread plate method. Relatively high concentrations (10^6 CFU/g of pellet) of heterotrophic bacteria were detected in the Synagro pellet. These results indicate that residual concentrations of bacteria in the Synagro biosolids are relatively high and that the Synagro heat treatment process did not destroy all the bacteria in the sludge. Since the predominant bacteria was gram positive bacteria, the most likely explanation is that the residual heterotrophic bacteria in the Synagro pellet represent *Bacillus* spores because they are resistant to heat inactivation and they are found in sewage sludge.

Statement of Goal Two

To develop a scientifically valid test protocol to obtain monitoring data for presence and multiplication of *Salmonella* bacteria in two Hawaiian soils to which Synagro pellets had been added.

To address this goal, a test protocol was developed based on adding Synagro pellets to soil samples (Molokai soil, Waimanalo soil) obtained from two farms used to grow crops in the windward and central regions of Oahu and analyzing these soil samples for *Salmonella*, total coliform and heterotrophic bacteria. *Salmonella* was selected to represent the pathogen in sludge with a potential to multiply in soil. Total coliform was selected because this group of bacteria is normally found in sewage and in soil. In soil, many total coliform bacteria will multiply but their growth in soil is somewhat limited. The test for heterotrophic bacteria was selected because it enumerates populations of many soil bacteria, which are especially suited to growing in soil environments. For this protocol, the soil samples included unamended Molokai and Waimanalo soil as well as these two soils amended with 1X (highest recommended application rate of Synagro pellet) and 5X this application rate as well as Hoagland Solution, as a control on readily available source of nutrients. The eight soil samples analyzed in this protocol are listed below:

1. Waimanalo soil: unamended
2. Molokai soil: unamended
3. Waimanalo soil amended with Synagro pellet (1X recommended rate)
4. Waimanalo soil amended with Synagro pellet (5 X recommended rate)
5. Waimanalo soil amended with Hoagland Solution (control for nutrients)
6. Molokai soil amended with Synagro pellet (1X recommended rate)
7. Molokai soil amended with Synagro pellet (5X recommended rate)
8. Molokai soil amended with Hoagland Solution (control for nutrients)

The test protocol was designed to answer three basic questions.

1. Will nutrients be released from Synagro pellets during the period of the soil experiment?

This question was considered to be important because nutrient release from Synagro pellets into the soil is the mechanism to stimulate the growth of *Salmonella*. However, several documents stated that release of nutrients from Synagro pellets applied to land is a slow process that can take months. The possibility existed that nutrients would not be released during the period of the soil experiment. To address this concern, the decision was made to reduce the size of the Synagro pellet to a powdery consistency as a way to enhance the release of nutrients by increasing the interaction of the Synagro pellets with soil constituents. To obtain data that nutrients were actually being released from Synagro pellets during the period of the experiment, growth of total coliform and heterotrophic bacteria were measured in the eight soil samples shown above. The results of these experiments showed that populations of total coliform and heterotrophic bacteria did not multiply in unamended Molokai and Waimanalo soil but multiplied in these two soils when they were amended with 1X and 5X Synagro pellets. In these experiments, Hoagland Solution was added to one set of soil samples as a control on the rapid release of nutrients. This control was necessary to interpret the data if the data showed that

Synagro pellet had not released nutrients during the period of the soil experiment. Hoagland Solution primarily contained inorganic nutrients and therefore organic nutrients released from Synagro pellets appeared to be superior in stimulating the growth of bacteria.

2. Are conditions of the soil environments suitable for growth of bacteria, including *Salmonella*?

When soil experiments are conducted, there are many variables and many unknowns. Since the design of the soil experiment was to detect growth of bacteria, including *Salmonella*, controls were needed to show that soil conditions allowed for continued growth of bacteria during the period of the experiment. In this regard, the soil experiment was conducted under laboratory-controlled conditions rather than simulated field conditions to maintain conditions suitable for growth of *Salmonella*. The first condition was to maintain soil moisture above 30% so moisture would not be the limiting factor for bacterial growth. The second condition was to maintain the temperature of the soil experiment at $24 \pm 3^\circ\text{C}$ so changes in temperature would not be a factor in reducing the growth rate of bacteria during some periods of the experiment. The third condition was to secure the soil reactions to prevent contamination from external and unknown sources, which would complicate the interpretation of the data. Another important concern was the possible release of toxic chemicals by Synagro pellets or from Waimanalo or Molokai soils. Presence of toxic chemical can prevent growth of bacteria and without knowledge of the effects of toxic chemicals, the data will be difficult to interpret. Evidence for toxic chemicals can be obtained if populations of total coliform or heterotrophic bacteria are drastically reduced in some of the soil samples.

The test results showed that populations of total coliform and heterotrophic bacteria were not inhibited in any of the eight soil samples. These results indicate that toxic chemicals were not released in the soil experiments to inhibit bacterial growth. Moreover, populations of these two bacteria increased in response to addition of 1X and 5X Synagro pellets. These results show that environmental conditions for the soil experiments were suitable for growth of bacteria, including *Salmonella*.

3. Is there evidence for presence and multiplication of *Salmonella* in any of the soil samples but especially when Synagro pellets are applied to Molokai and Waimanalo soil samples?

The tests to determine presence and multiplication of *Salmonella* in the soil experiment were especially important because they directly address the statement, which describes goal two of this study. The tests to detect the presence and multiplication for *Salmonella* in the soil samples were considered valid because results of other experiments had established that nutrients were being released by Synagro pellets during the period of the experiment and soil conditions were suitable for growth of bacteria, including *Salmonella*. In the test to detect for presence and multiplication of *Salmonella* in the eight soil samples, the EPA approved method was used and the 3-Tube MPN method was used to analyze 1 g, 0.1 g, and 0.01 g samples from each of the eight soil samples over the 12 days of the experiment. *Salmonella* was not detected in any of these soil samples, including soil samples amended with 1X and 5X Synagro pellets. Since all the test reactions were negative for *Salmonella*, the concentration of *Salmonella* in these samples could not be determined. However, based on the results obtained, the minimum detectable levels

of *Salmonella* in the soil samples were calculated to be less than 4.3 to 5.3 MPN/g of soil (dry weight). Since *Salmonella* was not detected in any of the soil samples by the MPN assay, the Presence/Absence test was then used to detect for presence of *Salmonella* in larger sized samples (4 g, 40 g) of all unamended and amended Molokai and Waimanalo soil samples. The absence of *Salmonella* in the eight soil samples when up to 40 g of soil samples were tested indicated that these two soil samples were not contaminated with *Salmonella*. Since *Salmonella* was not detected in Synagro pellets and in Molokai and Waimanalo soil, the most reasonable conclusion for the non-detection of *Salmonella* and absence of multiplication of *Salmonella* in all the soil samples was the absence of *Salmonella* in these samples. These results provide evidence that two Hawaiian soil types (Waimanalo, Molokai) used extensively for agriculture in the windward and central area of Oahu did not contain detectable levels of *Salmonella* bacteria.

The relevant question related to this study is to assess the value of the results obtained from this study. The value of this study is that it represents the first study conducted in Hawaii to analyze for natural populations of *Salmonella* in soil used for farming. The absence of *Salmonella* from soils obtained from two farms on Oahu indicates that *Salmonella* has not become established in all soil samples on Oahu in a similar way as total coliform and *E. coli* have. The results of this study present the first assessment of farm soil quality with regard to *Salmonella* and suggest that for these two farms, there is no pressing need to test more samples from these sites for *Salmonella*. However, the results are relevant because farms are one of the targeted sites for application of biosolids. The results obtained from this study are applicable to Molokai and Waimanalo soils obtained from their respective farm sites and to similar farms. However, since the results of this study came from a single soil sample obtained from the two farm sites, the direct applicability of these results are limited to those two sample sites and the results are not automatically applicable to the entire farm site and are not applicable to those farms throughout the year. The results of this study are less applicable to other sites, which differ from the farm sites where the Molokai and Waimanalo soils were obtained. A major limitation of this study is that the results cannot be applied directly to soils with different characteristics, to soils under different environmental conditions, to soils receiving different contaminating sources and to soil sites used for different purposes.

In summary, the results of this study did not resolve many questions such as: 1) Can *Salmonella* multiply in some soil environments? 2) Will the application of biosolids or other forms of nutrients to soil significantly enhance multiplication of *Salmonella*? and 3) Can *Salmonella* become established in some soil environments in Hawaii to the extent that it may cause a public health problem? To address these concerns, more soil experiments must be completed at sites where biosolids are now being applied, at sites where biosolids are being planned to be applied and at sites where there are no plans to apply biosolids. A comparison of the results at these different sites can be used to better predict the impact of land application of biosolids and the expected occurrence and fate of *Salmonella* in soil environments in Hawaii.