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Bioaugmentation Approach using *Pseudomonas* and *Bacillus* for Malodour Reduction in Poultry Feacal Waste Management

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Abstract

Introduction. A workable strategy is bioaugmentation, which involves introducing certain bacteria in sufficient quantities to promote biodegradation. This study focuses on isolating and utilizing malodor-reducing bacteria from fecal wastes obtained from a poultry farm in Ashi, Ibadan. **Methods**. Standard methods were employed to isolate and identify species of Pseudomonas and Bacillus. Quantitative detection of hydrogen sulfide gas and other relevant parameters was performed using MSA Orion and Multi Gas Detector. Hydrogen sulfide (H2S) release was quantitatively monitored during fermentation, considering varying loads of inocula. **Results**. The bacterial isolates comprised *Pseudomonas aeruginosa, P. fluorescens, P. putida, Bacillus fastidiosus, B. licheniformis, B. megaterium, B. subtilis, B. sphaericus*, and *B. thuringiensis*. Odor levels varied based on inocula load and fermentation duration. In batches with Pseudomonas, hydrogen sulfide was undetectable after two days, while Bacillus-inoculated batches required ten days. The formation of microbial mats and subsequent decrease in H_2S content contributed to malodor reduction. Notably, fluorescent pseudomonas exhibited successful mineralization during the treatment of fecal waste. **Conclusion**. *Pseudomonas* isolates demonstrated superior effectiveness in odor reduction compared to *Bacillus* isolates.

Key word: Bioaugmentation, Seeding, Poultry, odor, *Pseudomonas, Bacillus.*

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Introduction

The utilization of pre-grown microbial cultures to increase microbial populations at a specific location is referred to as bioaugmentation. This approach has been shown to enhance pollution cleanup, reduce cleanup times, and minimize costs (1, 2). A relatively recent strategy for mitigating environmental waste is using specific strains of microorganisms sourced from the environment to improve standard waste treatment procedures (3, 4). In order to maintain or enhance the biodegradation potential within a reactor, adding certain bacteria to a bioreactor may be necessary. This process involves introducing native or non-native microbial cultures, or "inocula," to the system to replace or enhance the natural microbial population (5-9).
Bioaugmentation offers the possibility of providing specific bacteria in sufficient quantities to complete the biodegradation process. This ensures the adequate supply of appropriate microorganisms without posing any risks (10-12). Microbial inocula are prepared in the laboratory using soil or groundwater samples obtained either from the intended site of application or from another location where the biodegradation of the target compounds is known to occur. Only microorganisms capable of metabolizing the target chemicals can grow on the media. The isolated soil or groundwater microbes are then introduced to a medium containing the compounds to be broken down (2, 12).

Poultry waste stands out as one of the most significant sources of odor emissions and customer complaints among various types of operations, leading to odor-related issues (13, 14). It has been established that exposure to odors can have a negative impact on the psychophysical well-being and behavior of individuals, even if the odorous chemicals themselves are not necessarily harmful or hazardous to human health (14-16). Due to the volatile nature of the intermediate molecules formed during the biological degradation process of this material, odors are generated at poultry waste sites. Odorous chemicals are produced in liquid and solid phases as organic material undergoes biological degradation. When chicken manure is not properly managed, gases such as ammonia, hydrogen sulfide, and methane are released (3, 17).

This study highlights the use of *Pseudomonas* and *Bacillus* isolates, indigenous organisms obtained from poultry waste samples, for bioaugmentation studies. The primary objective of this work is to outline practices, strategies, and

techniques that can be implemented to mitigate malodor in poultry fecal waste management, ensuring environmental sustainability as a safer alternative to other chemical odor-neutralizing agents that may pose risks to human health.

Materials and Methods

Sample Collections

Samples of poultry fecal waste were collected in Ashi, Ibadan. The pH of the poultry fecal waste samples was determined using a pH meter - HANNA photometer (HI 9813-6). Before measuring the samples' pH, the pH meter was calibrated with buffers of pH 4.0 and pH 7.0.

Microbial Isolation

Microbial isolation was performed after agitating the waste with the electrode of a pH meter. The temperature of the poultry waste was measured by inserting a mercury-in-glass thermometer, and the recorded temperature readings were noted. The media utilized for microbial isolation included Nutrient Agar, Nutrient Yeast Broth, Pseudomonas Agar (Kings Medium A and B) developed by AOAC (18) and Omojasola et al. (19). Serial dilutions of poultry fecal samples were prepared using Nutrient Agar and Pseudomonas Agar, and pour plate procedures were employed. The diluted samples were plated out in duplicate. Incubation of all plates was carried out at 35 °C for 24 hours. Bacterial colonies from infected plates were randomly selected and streaked with wire loops to obtain pure cultures. The pure cultures were then placed on a slant using Nutrient Agar for further testing. Subsequently, the pure cultures were refrigerated and subcultured (19). To determine the likely identities of the bacterial isolates, the cellular and morphological characteristics, biochemical and physiological traits, and results of sugar fermentation tests were analyzed. Cheesbrough (20) was used to assess the isolates' likelihood based on each test's results.

Inoculum preparation

To promote the rapid growth of microbial cells, the bacterial isolates were cultured in a nutritional yeast broth fortified with yeast extract to enhance its nutrient content. The broth was then inoculated with the target organisms and incubated using an incubator shaker to promote optimal growth. Following the method described by Onajobi et al. (4), the cultured broth was centrifuged, and the cell count was determined using the plate count technique, resulting in a dilution factor 10^6 per milliliter. This concentrated inoculum was added to a 250 ml conical flask containing chicken excrement. Different methods, including the addition of 10 ml of nutrient cultured broth and the supernatant obtained from centrifuging the cultured broth, were employed to inoculate the poultry fecal waste. Hydrogen sulfide gas and other parameters were quantified using MSA Orion Multi Gas Detector at intervals on the fifth and tenth day. Additionally, lead acetate testing strips were used to qualitatively assess the decrease in H_2S levels at 48-hour intervals (20).

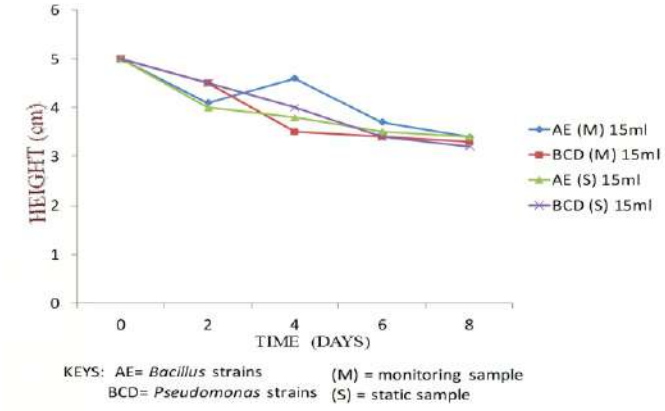
Results

The isolated cultures were determined and identified as *Pseudomonas aeruginosa, P. fluorescens, P. putida, P. alcaligens, P. Syringae*, which were designated as KBA2, 11P, KAA1, KAA2, and A4 respectively and *Bacillus astidiosus, B. polymyxa, B. thuringiensis, B. anthracis, B. subtilis, B. lichenliformis* and *B. Sphaericus* were denoted by 2.4KB, 1.1KB, 1.2KA, 2.1NA, BKB, 1.6KB and 12NA respectively.

Figure 1 below illustrates changes in the height of poultry waste in degradation bottles containing bacteria cultured broth during degradation. The sludge was reduced from 5.0 cm to 3.2 cm in static samples and to 3.3 cm in monitoring samples inoculated with 15 ml of *Pseudomonas* strains KBA2, 11P, KAA1, KAA2, and A4 and reduced to 3.4 cm from 5.0 cm in both static samples and monitoring samples inoculated with 15 ml of *Bacillus*.

Figure 1

Changes in the height of the poultry waste with different cultured broths of the bacteria during degradation



The change in the height of the poultry waste with different bacterial cell concentrations during degradation is shown in Figure 2. There were reductions to 2.6cm and 2.8cm from 5.0cm in the samples inoculated with 4ml and 8ml of *Pseudomonas* spp.; KBA2, 11P, KAA1, KAA2, and A4, respectively, while 4ml and 8ml of *Bacillus* strains 2.4cm KB, 1.1KB, 1.2KA, 2.1NA, BKB, 1.6KB, and 12NA reduced the samples to 4.2cm and 4.9cm respectively from 5.0cm. The increase in sludge size after six days was a result of water formed during the process of hydrolysis of degradation.

Figure 2

Changes in height of the poultry waste with different bacterial cell concentrations during degradation

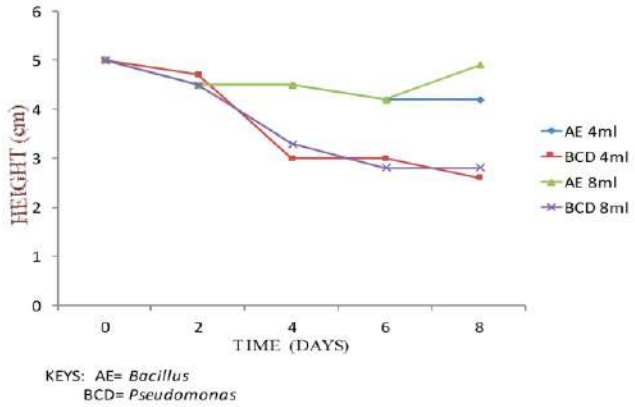
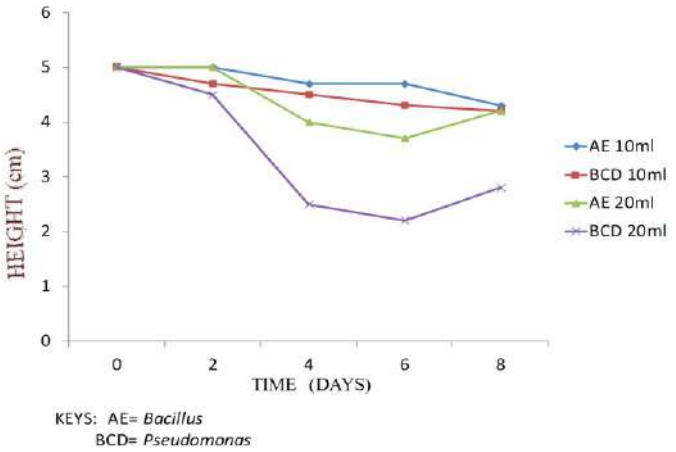


Figure 3 shows changes in the height of the poultry waste with different supernatant concentrations during degradation. There were reductions to 2.8cm and 4.2cm from 5.0cm in the samples inoculated with 20ml and 10ml of supernatant from *Pseudomonas* spp. KBA2, 11P, KAA1, KAA2, and A4 respectively while 20ml and 10ml of supernatant from *Bacillus* spp. 2.4KB, 1.1KB, 1.2KA, 2.1NA, BKB, 1.6KB, and 12NA reduced the samples to 4.2cm, 4.3cm, and 5.0cm respectively. The increase in size resulted from the process of hydrolysis of the sample.

Figure 3

Changes in the height of the poultry waste with different supernatant concentrations during degradation



On exposure to the vapor of a slightly acidified sample, the lead acetate testing strips become blackened by the formation of lead sulfide (PbS). Table 1 shows a qualitative H_2S test in poultry fecal waste samples after treatment with *Bacillus* and *Pseudomonas* strains and compared with peptone treatment samples. The H_2S gas disappeared in samples inoculated with *Pseudomonas* spp. KBA2, 11P, KAA1, KAA2, and A4 after 48 hours while it was reduced to trace levels in samples inoculated with *Bacillus* strains 2.4KB, 1.1KB, 1.2KA, 2.1NA, BKB, 1.6KB, and 12NA after 48 hours. The peptone-treated samples showed a reduction in H2S gas to a trace level after 8 days, which is a representative sample of biostimulation, and remained at a high level in an uninoculated sample (control). The formation of H_2S gas by the supernatant's components was the cause of the rise in H2S gas in the samples that had been exposed to it for 24 hours.

Table 1

H_2S Qualitative Test

Days	Bacterial cell						Supernatant				Control	
	4ml		8ml		10ml		20ml		30ml		P	C
	AE	BCD	AE	BCD	AE	BCD	AE	BCD	AE	BCD		
0	++	++	++	++	++	++	++	++	++	++	++	++
2	+	+	++	+	+	+++	+++	+++	+++	+++	++	++
4	+	-	+	-	-	+	+	+	+	++	++	++
6	+	-	+	-	+	+	+	+	+	+	++	++
8	+	-	+	+	+	+	+	+	+	+	+	++

Keys: Dark Black strip= +++ Trace= + AE = Bacillus C= Control Light Black= ++ No Change= ++ BCD= Psuedomonas P = Control+peptone

Figure 4 shows a graphical representation of the quantitative determination of gasses in samples inoculated with 8ml of *Bacillus* spp. and *Pseudomonas* spp. compared with the control after ten days of treatment. In the samples seeded with *Pseudomonas* strains KBA2, 11P, KAA1, KAA2, and A4; H2S, CO, CH4 gas was reduced to 2ppm, 16.2 ppm and8% respectively and O2 gas increased to 19.1%. While they reduced to 0ppm, 2ppm, and 2%, respectively, and O2 gas increased to 16.3% in samples seeded with *Bacillus* strains 2.4KB, 1.1KB, 1.2KA, 2.1NA, BKB, 1.6KB, and 12NA. The uninoculated sample (control) showed the level of the gasses to be 65ppm, 77ppm, 8%, and 17.7%, respectively.

Figure 4

Quantitative analysis of samples inoculated with bacterial cells after 10 days of treatment

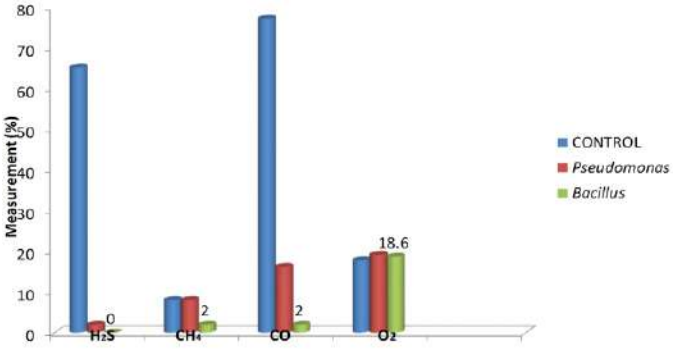


Figure 5 shows a graphical representation of the quantitative determination of gasses in samples inoculated with 20ml of *Bacillus* spp. and *Pseudomonas* spp. supernatant obtained after centrifugation and compared with control treated with peptone treated and uninoculated control after 10 days of treatment. In the samples seeded with the supernatant of *Pseudomonas* spp. KBA2, 11P, KAA1, KAA2, and A4; H_2S, CO, CH4 gas were reduced to 2ppm, 14ppm, 4% respectively and O2 gas increased to 20.4%. While they reduced to 0ppm, 2ppm, 2%, respectively, and O2 gas increased to 16.3% in samples seeded with *Bacillus* spp. 2.4KB, 1.1KB, 1.2KA, 2.1NA, BKB, 1.6KB and 12NA. The sample treated with peptone showed a reduction in gasses to 4ppm, 26.1ppm, and 5%, and O_2 gas increased to 19.1%, while the inoculated control sample showed the level of the gasses to be 65ppm, 77ppm, 8% and 17.7% respectively. The biostimulation theory, which states that the appropriate native organisms were stimulated to a population that may induce degradation in poultry waste samples after days, could explain the drop in the level of the gasses after 10 days in a control treated with peptone.

Figure 6: shows a graphical representation of the quantitative determination of gasses in samples inoculated with 20ml of *Bacillus* spp. and *Pseudomonas* spp. supernatant and compared with the control after ten days of treatment. In the samples seeded with *Pseudomonas* strains KBA2, 11P, KAA1, KAA2, and A4; H_2S, CO, CH_4 gas were reduced to 0ppm, 4ppm and 2% respectively and O_2 gas increased to 20.4%. While they reduced to 40ppm, 11.3ppm and 14%, respectively, and O2 gas increased to 18.2% in samples seeded with *Bacillus* strains 2.4KB, 1.1KB, 1.2KA, 2.1NA, BKB, 1.6KB, and 12NA. The

un-inoculated sample (control) showed the level of the gasses to be 65ppm, 77ppm, 8%, and 17.7%, respectively.

Figure 5

Quantitative analysis of samples inoculated with supernatant after 10 days of treatment

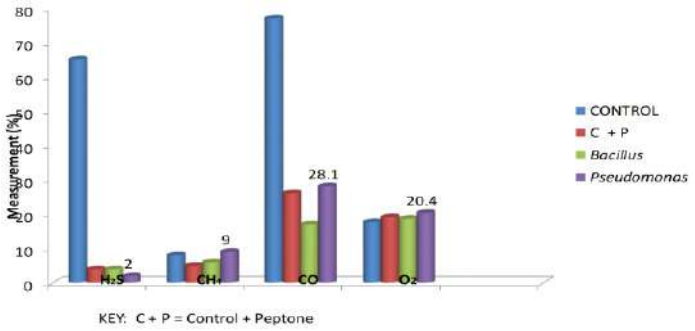
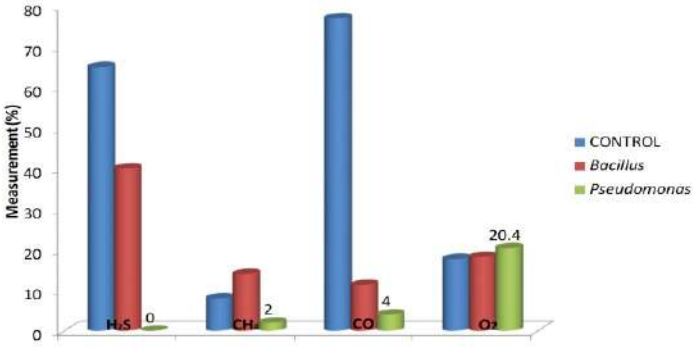


Figure 6

Quantitative analysis of samples inoculated with a cultured broth of the bacteria after 10 days of treatment



Discussion

The results of this study revealed the presence of several probable organisms, including *Pseudomonas fluorescens, P. aeruginosa, P. putida, P. alcaligens*, and *P. syringae*, designated as KBA2, 11P, KAA1, KAA2, and A4, respectively. Additionally, other identified organisms were *Bacillus fastidiosus, B. polymyxa, B. thuringiensis, B. anthracis, B. subtilis, B. licheniformis*, and *B. sphaericus*, denoted as 2.4KB, 1.1KB, 1.2KA, 2.1NA, BKB, 1.6KB, and 12NA. The gram-negative rod-shaped bacteria displayed various pigments, such as pyocyanin (blue-green) in P. aeruginosa, pyoverdin (yellow-green) in *P. fluorescens*, and pyorubin (red-brown) in *P. syringae* when grown on Kings Media A and B. These findings are consistent with the findings of Bustamante et al. (21), Venty et al. (22), and Onajobi et al. (23), who conducted similar studies on 68 bacterial isolates from soil and sawdust and reported a low percentage (6%) of isolates exhibiting significant emulsification activity. This aligns with the observed high emulsification activity in *Pseudomonas putida* SG1 and *Burkholderia cepacia* strain 717.

The current study also observed the formation of a microbial mat with a dry surface on the inoculated poultry fecal waste samples, resulting from microbial metabolic activities. The presence of air spaces increased due to gas production. However, on the seventh day, the samples collapsed due to a decrease in air spaces, resulting in a size reduction (sludge volume). Subsequently, the samples underwent hydrolysis, with an increase in watery content and a change to black color. Notably, the samples treated with *Pseudomonas strains* (KBA2, 11P, KAA1, KAA2, and A4) exhibited faster reactions compared to those treated with *Bacillus* strains (2.4KB, 1.1KB, 1.2KA, 2.1NA, BKB, 1.6KB, and 12NA). This finding is similar to the work of Meenakshisundaram et al. (24). Despite the presence of more *Bacillus* species originating from cassava pieces, similar to the findings of Meenakshisundaram et al. (24) in the pharmaceutical industry where they destroyed bacterial biosurfactants, these *Bacillus* species were isolated from all waste samples.

The initial H2S levels in all samples, as indicated by blackened lead acetate strips on day zero, showed a subsequent reduction after 48 hours in samples treated with bacterial cells. In the samples treated with *Pseudomonas spp.*, including KBA2, 11P, KAA1, KAA2, and A4, H2S completely disappeared after four days. However, in samples treated with *Bacillus spp.*, such as 2.4KB, 1.1KB, 1.2KA, 2.1NA, BKB, 1.6KB, and 12NA, H2S levels reduced to an average level. On the other hand, when samples were treated with centrifugation-derived supernatant, there was an initial significant increase in H2S levels, indicated by a darkening of the strips after 48 hours. Subsequently, the H2S levels decreased to trace levels after four days and throughout the entire study period. This observation can be attributed to the contribution of all supernatant components to the formation of H2S. These findings are consistent with the studies conducted by Salim et al. (3) and Nordiyana et al. (25), which also investigated the removal of *Bacillus subtilis*.

The poultry fecal waste sample control, with the addition of peptone, exhibited a decrease in H2S levels to trace levels after the treatment period. In contrast, the poultry fecal waste sample control in its natural form maintained high levels of H2S throughout the treatment period. These findings align with the research conducted by Ajao et al. (26) and Yang et al. (27). This demonstrates the concept of biostimulation, which involves adding nutrients or amendments to stimulate the growth of the existing native microbial population (assuming the correct microbes are present) (28). On the other hand, bioaugmentation involves the addition of naturally occurring microbes, ensuring that the correct microbes are added in sufficient quantities (27,29). It has been observed that fluorescent *Pseudomonas* have the capability to degrade a wide range of chemical compounds (3). They play a crucial role in the mineralization process in sewage treatment, where more than 80 different materials are used as carbon and energy sources (30).

Adeyemi et al. (2) and Onajobi et al. (4) highlighted the significance of these versatile bacterial strains, which are naturally occurring microbes specifically isolated from the environment due to their exceptional ability to eliminate a wide range of persistent compounds found in waste. The *Pseudomonas* strains are considered "versatile" because they are stable and not in the form of spores, allowing them to commence the degradation of various challenging chemical

compounds immediately. These strains efficiently and rapidly degrade pollutants, producing simple by-products such as carbon dioxide and water (30). Additionally, the biological treatment effectively eliminates most organic wastes, ensuring the generation of harmless end products and mitigating potential environmental risks or liabilities.

Conclusions

In this study, the utilization of Pseudomonas species as inoculants demonstrated higher activity compared to the samples treated with Bacillus species and supernatants. This was evident in the significant reduction of sludge volume and H_2S gas. Therefore, the bioaugmentation of poultry fecal waste with Pseudomonas species proves to be a safe and effective alternative to chemical treatments, which may pose potential environmental risks. These microorganisms possess enzymes that enable them to metabolize pollutants, making them well-suited for the breakdown of contaminants. Like their ability to clean up surfaces contaminated with oil, gasoline, and grease, microbes are also effective in remediating dirt, asphalt, concrete, wood, and metal.

Author contributions

Study conception and design: Ismail B. Onajobi; Oyindamola J. Samson
Data collection: Ismail B. Onajobi; Obasola E. Fagade; Adeniyi A. Ogunjobi; Oyindamola J. Samson
Analysis and interpretation of results: Ismail B. Onajobi; Obasola E. Fagade; Adeniyi A. Ogunjobi
Draft manuscript preparation: Ismail B. Onajobi; Oyindamola J. Samson
All authors reviewed the results and approved the final version of the manuscript. All authors agreed to be responsible for all aspects of the work to ensure the accuracy and integrity of the published manuscript.

Ethics statement

The authors declare that the published work reflects an investigation and analysis carried out truthfully and completely.

Conflict of interest

The authors declare no conflict of interest.

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Availability of data

Available from the corresponding author upon request.

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