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Anaerobic digestion assisted by composting to deodorize and eliminate pathogenic microorganisms in sludge from a municipal WWTP

Anabel Altamirano Guerrerosa, Edison A. Romero-Cabellob , Daniel Neciosup Gonzalesa , Maribel Rodríguez Rodrígueza, Eduardo Espinoza Farfána, Rita Cabello Torresa\*

aUniversidad César Vallejo, Research Group ICAMB, San Juan de Lurigancho, Lima, Perú

cUniversidad Nacional Agraria la Molina, Av. La Molina,La Molina Lima Peru

rcabello@ucv.edu.pe

The use of wastewater sludge has potential for application as soil quality improvers, but not without first removing bad odours (H2S) and sanitizing to avoid the presence of pathogens. This research aimed to eliminate H2S and sanitize wastewater sludge through two defined stages of anaerobic digestion and pre-composting. For this, four treatments applied in two stages were proposed. In the first stage, doses of beneficial microorganisms were applied as inoculum (T0= BMOs; T1=5 ml BMOs; T2= 10 ml BMOs; T3= 15 ml BMOs) to the sludge load in the anaerobic digestion. The second stage consisted of the formation of pre-compost piles to eliminate pathogenic microorganisms. The results showed a significant difference between the treatments, especially T2 which achieved almost 100% removal of H2S and pathogens. The anaerobic digestion conditions showed greater efficiency for a more stable pH close to 7.5 and for a mesophilic temperature of 30oC, while the pre-compost temperature was a critical factor that reached 66oC in T2, sufficient to eliminate a significant load of *Salmonella spp*., *E. coli* and helminth eggs. It was demonstrated that the application of integrated anaerobic digestion and pre-compost methods can be efficiently applied to remove H2S and eliminate pathogens from waste sludge.

* 1. Introduction

The inadequate stabilization of the residual sludge in the Municipal Wastewater Treatment Plant (WWTP) can generate an environmental and health contamination problem; a viable alternative is anaerobic digestion assisted by pre-composting, favouring the economic use of waste (Jacobo et al., 2023). A simple technology with a lower investment cost is the use of beneficial microorganisms to accelerate the process of transformation of organic material and elimination of bad odours from waste sludge to restrict sulphate-reducing bacteria and eliminate the formation of H2S (Rathnayake et al., 2021). The application of these microorganisms in the development of anaerobic digestion eliminates hydrogen sulphide. This technology can be assisted by the composting process until the thermophile stage, allowing in turn the elimination of pathogens from the sludge (Chen, et al. 2022). The objective of the research was to stabilize the residual sludge of a municipal WWTP, through the application of beneficial microorganisms (BM) for the elimination of H2S in an anaerobic digestion process assisted by a subsequent pre-compost to eliminate pathogenic microorganisms.

* 1. Methods

**2.1 Raw material collection:**

500 L of residual sludge were collected from the municipal WWTP of Cieneguilla (Lima) and stored for 2 days, separating the solid part for the deodorization process. Likewise, 1 ton of green floral waste was collected at collection points in the “Padre Eterno cemetery” (Lima) for its composting process.

**2.2 Physicochemical and microbiological characterization**

Physicochemical and microbiological parameters were measured at the beginning and at the end of the treatments. Moisture (M%), organic matter (OM%), pH in the mud mixtures were measured and H2S was also measured using a multigas detector (4500-S 2-s method). Similarly, in the pre-composting process, H%, pH and T(oC) were measured, in addition to pathogenic microorganisms: *Eschearia coli* MPN, *Salmonella spp*. MPN and viable eggs of HH helminths. The Viable Mesophilic Aerobic Count (VMAC) (CFU/ml) was also performed; mould and yeast count (MYC) (CFU/ml); Actinomycetes (UFC/ml) present in the BMOs inoculum to determine its influence on the elimination of H2S. Wang et al. (2022) defines beneficial microorganisms as useful inocula for soil and crop management, bio-controls, and used in bioremediation. However, the authors prefer to define MOBs as microorganisms that can be used to accelerate or improve biochemical processes in eco-friendly technologies, this includes their application in anaerobic digestion, compost, remediation processes, etc. for the benefit of human beings and the environment.

**2.3 Deodorization Process**

Cultivation and activation of BMOs. Cabbage pieces, cooked chicken liver, molasses and salt were added to 1L of distilled water. This mixture was grown at room temperature in airtight bags for 7 days, until a whitish appearance (like cotton) and a fermentation smell were observed. The supernatant was decanted and filtered to be used as inoculum (Meza, 2019). The inoculum was activated at a ratio of 100 ml/30 L of diluted molasses before use.

**2.4 Anaerobic Reactor**

The anaerobic digestion reactor had the following dimensions: 200 cm long x 50 cm wide x 50 cm high and 1 cm thick. The reactor had four divisions, where each mixture or treatment was arranged (T0, T1, T2 and T3), each division had an orifice connected by pipes to containers containing 1N sulfuric acid to capture ammonia gas.

**2.5 Residual sludge deodorization:**

The operating capacity of each compartment was 100L, for which the residual sludge and BMOs inoculum were added in proportions (v/v) of 5%(T1), 10%(T2), 15% (T3) and a control without BMOs (T0), for 15 days at 30 oC. The reason is that in 15 days the bad smell of rotten eggs disappeared.

**2.6 Pre-composting:**

Each biosolid obtained in the anaerobic digestion process was mixed with 250 kg of green waste in composting piles of 1.5 m long x 0.5 m wide x 1 m high for the elimination of pathogens. This process lasted 15 days, involving the mesophilic and thermophilic or sanitization phase.



Figure 1. H2S elimination process and sludge sanitation applied in the research

**2.7 Data analysis**

Microsoft Excel was used to prepare graphs and tables, the Shapiro-Wilk test was applied to verify the normality of the data; Analysis of variance (ANOVA) was used to distinguish significant differences between treatments. The Tukey Test was applied to compare the standard errors using the number of treatments as the numerator and the degrees of freedom of the error as the denominator.

* 1. Results and discussion
		1. Removal of hydrogen sulphide from waste sludge

Table 1 shows the analytical results of the physicochemical parameters of the original residual sludge sample, of the inoculum of beneficial microorganisms (BMOs), the values of pH, organic matter (O.M.), moisture and H2S content of day 1 and day 15 for each treatment applied in the removal of H2S from the sludge through anaerobic digestion (T0, T1, T2, T3). The absence of BMO at T0 did not favor alkaline conditions; on the other hand, the original microorganisms required more time to assimilate the organic matter and generated a higher content of fatty acids, decreasing the pH of the medium.

Table 1. Physicochemical parameters of waste sludge, BMOs, and treatments applied by anaerobic digestion to eliminate H2S

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Parameter | Sludgeo | BMOso | To Day1 | To Day15 | T1 Day1 | T1 Day15 | T2 Day1 | T2 Day15 | T3 Day1 | T3 Day15 |
| pH | 6.81 | 3.45 | 7.16 ± 0.01 | 6.37 ± 0.02 | 7.13 ± 0.01 | 7.71 ± 0.01 | 7.42 ± 0.02 | 7.48 ± 0.01 | 7.37 ± 0.02 | 7.46 ± 0.01 |
| E.C. (dS/cm) | 3.06 | 8 | - | - | - | - | - | - | - | - |
| O.M. (%) | 54.17 | 81.45 | 53 ± 0.01 | 36 ± 0.01 | 53 ± 0.01 | 24 ± 0.02 | 54 ± 0.004 | 9 ± 0.02 | 54 ± 0.01 | 15 ± 0.01 |
| Moisture (%) | 55.75 | - | 65 ± 0.01 | 26 ± 0.01 | 62 ± 0.02 | 56 ± 0.02 | 66 ± 0.01 | 51 ± 0.02 | 68 ± 0.02 | 57 ± 0.01 |
| N(%) | 2.87 | 1208 | - | - | - | - | - | - | - | - |
| P(%) | 0.94 | 32.63 | - | - | - | - | - | - | - | - |
| K(%) | 0.199 | 700 | - | - | - | - | - | - | - | - |
| Ca(%) | 10.06 | 81 | - | - | - | - | - | - | - | - |
| Mg(%) | 0.82 | - | - | - | - | - | - | - | - | - |
| Na(%) | 0.11 | 6.1 | - | - | - | - | - | - | - | - |
| BOD (mg/L) | 116 | - | - | - | - | - | - | - | - | - |
| TSS (mg/L) | 220 | - | - | - | - | - | - | - | - | - |
| VSS (mg/L) | 3000 | - | - | - | - | - | - | - | - | - |
| H2S (ppm) |   |   | 140.3 ± 0.6 | 68 ± 1.5 | 137.7 ± 2.5 | 54 ± 1 | 139 ± 1 | 1 ± 1 | 140.3 ± 1.5  | 47 ± 1 |

According to the results, a decrease in the initial pH is observed for T0 (-0.79 units), unlike the other treatments, where the pH increased in the following order: T1 (+0.58) >T3 (+0.09) >T2 (+0.06), this means that T2 has maintained almost stable pH, which allowed favourable conditions for microbial development. The reason behind this is that the buffer system of volatile fatty acids and CO2 dissolved in N2 and endogenous free NH3 regulates the pH and alkalinity in the mixture (Zhao et al., 2020). In this case, a greater buffer capacity was produced, which favoured the absorption of H2S by the sulfoxidizing bacteria and greater development of these microorganisms. That is, the pH of the organic load has had a determining effect on the process, T2 was closer to a system in almost steady state, similar to other desulfurization studies, which reached pH = 8, eliminating 99% of H2S (Ou et al., 2020). Greater stationary stability favoured greater consumption of organic matter and water that followed the following order: T2>T3>T1>T0. The decrease in organic matter is part of the mineralization in the process (López-González et al. 2021). In addition, the reactor design included 4 divisions, each division with a small hole of 0.5 cm in diameter to discharge ammonia gas and limited entry of oxygen. It is important to highlight those traces of oxygen in the system, in turn, allowed O2 to act as an electron acceptor from the available donor H2S (Andreides et al. 2020), which favoured the biological oxidation of T2 sulphides. A minimal amount of oxygen favours the oxidation of HS- to elemental sulphur; while for T0, unfavourable secondary reactions would have occurred that transformed the HS- ion into less alkaline forms (Chen et al. 2022). Likewise, Table 1 consequently demonstrates the decrease in H2S load in the following order: T2>T3>T1>T0, produced by the following general equation:

|  |  |
| --- | --- |
| 2H 2HS- + O2   →  2So + 2OH- | (1) |

On the other hand, table 2 shows the results of the microbiological analysis on day 1 and day 15 of anaerobic digestion.

Table 2. Microbiological parameters resulting from Anaerobic Digestion

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Parameter | Sludge Day1 | BMOs Day1 | T0 Day15 | T1 Day15 | T2 Day15 | T3 Day15 |
| Count of viable mesophilic aerobes (CFU/ml) | 5.4x103 | 2.0x105 | 9.2x103 | 2.5x104 | 65x107 | 1.3x106 |
| Mould and yeast count (CFU/ml) | 2.0x 103 | 5.30x105 | 7.4x103 | 8.8x107 | 7.0x108 | 8.1x106 |
| Actinomycetes (CFU/ml) | 0 | <10 | 0 | 7.9x103 | 2.2x103 | 3.0x103 |
| Number of total coliforms (MPN/10 g) | >11x106 |  |  |  |  |  |
| Number of faecal coliforms (MPN/10 g) | >11x106 |  |  |  |  |  |
| Number of *Escherichia coli* (MPN/10 g) | >11x106 |  |  |  |  |  |
| Detection of *Salmonella spp*. in 10g | Presence |  |  |  |  |  |
| Counting helminth larvae and eggs (No/4g) | 180 |   |   |   |   |   |

The H2S removal calculation in each system followed the equation:

|  |  |
| --- | --- |
| 2H Removal of H2S = ([H2S]o - [H2S]f/ [H2S]o )\*100%=  | (2) |

According to this, the order of H2S removal achieved was: T2 (99%)>T3 (67%)>T1 (61%)>To (51%). For T2, the dose of beneficial microorganisms represented 10% of the reactor load; the mixture was subjected to a mesophilic temperature of 30oC, which is recommended for the reduction of H2S in the anaerobic digestion process (Zhao and Liu, 2019). T2 showed an efficient performance in sulfoxidation, evidenced by a higher content of mesophilic aerobic microorganisms, molds and yeasts, which were related to a higher consumption of OM (83.2%) and a more stable pH increased only by 0.06 units throughout the period. process (final pH: 7.48). While T3, performance decreases with an increase in pH to 7.46 (Δ=0.09), lower MO consumption (72.2%) and a decrease in humidity. The sulfoxidizing bacteria present in anaerobic digestion need to degrade organic matter and use CO2 as a source of carbon and energy to oxidize H2S. Obviously, the alkaline pH and the temperature maintained in the appropriate range (20–43 ◦C) were convenient for mesophilic microbial development (Nie et al. 2021). This result was comparable to that reported by Oh et al. (2024), who applied a direct chemical component such as Fe powder to reduce the content of H2S formed in the biogas during anaerobic digestion for 10 to 20 days, thus managing to eliminate just over 98% of the H2S (Oh, et al. 2024). Furthermore, as seen in Table 2, the mesophilic microbial community of T2 increased notably from 5.4 x103 to 65 x 107 CFU/ml, this consumed 83% of the organic matter present in the reactor. Consequently, the content of moulds and yeasts increased from 2 x 103 to 7x108 CFU/ml and that of actinomycetes from 1.2 x 102 to 2.2 x 103 CFU/ml. Likewise, the significant presence of pathogenic microorganisms of the order of 106 was observed.

* + 1. Sanitation of sludge through pre-composting

Table 3 shows the results of the pre-composting stage.

Table 3. Physicochemical and microbiological results in the pathogen elimination stage in pre-composting

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Parameter | To Day16 | To Day30 | T1 Day16 | T1 Day30 | T2 Day16 | T2 Day30 | T3 Day16 | T3 Day30 |
| pH | 6.74 ± 0.01 | 7.88 ± 0.02 | 6.95 ± 0.02 | 7.86 ± 0.02 | 7.18 ± 0.01 | 7.78 ± 0.02 | 7.38 ± 0.02 | 7.84 ± 0.01 |
| Temperature (oC) | 27.03 ± 0.06 | 18 ±0.1 | 30.07 ± 0.12 | 59 ± 0.1 | 29.13 ± 0.06 | 66 ±0.25 | 26.43 ± 0.12 | 62 ± 0.15 |
| Moisture (%) | 73 ± 0.02 | 41 ± 0.01 | 63 ± 0.02 | 47.7 ± 0.02 | 56 ± 0.01 | 64.7 ± 0.02 | 58 ± 0.02 | 64 ± 0.01 |
| C/N | 20 | 16 | 23 | 20 | 36 | 27 | 46 | 39 |
| *E. coli* (MPN/10g TS) | 1.1E+07 | 53 ± 15 | 1.1E+07 | 20 ± 10 | 1.1E+07 | 1 ± 1 | 1.1E+07 | 33 ± 21 |
| *Salmonella spp*. (MPN/10 g TS) | 4E+07 | 42 ± 4 | 4E+07 | 8 ± 2 | 4E+07 | 1 ± 1 | 4E+07 | 13 ± 3 |
| Viable helminth eggs (he/4g TS) | 160 | 86 ± 4 | 150 | 20 ± 2 | 120 | 1 ± 1 | 98 | 26 ± 2.6 |

According to table 3, the following emerges:

Pezzolla, et al. (2021) points out that sewage sludge has a C/N ratio lower than 25-35, this is due to the excess of N over degradable C, which is why it has been necessary to mix it with green waste or pruning to balance the ratio within the range 25-35 which is considered optimal for the initial mix. In this case we find two defined groups, the first group with initial values lower than the expected C/N range: T0 (20) < T1 (23), then T2 (36) which remained in a better situation, and T3 (46) which presented a value that exceeded what was expected. This C/N relationship is relevant because it delimited the availability of unconsumed nutrients in the piles formed (Chen et al., 2022). Green waste served to especially provide the carbon source and improve the C/N ratio (Khadra, et al. 2021). Likewise, one way to guarantee high performance in pre-composting is to have a humidity between 40 and 60%, with an initial temperature from 35 until 40 °C, particle size of 3.1 to 12.7 mm and pHs in the range of 5.5 to 8 (Amuah et al. 2022). Furthermore, the pre-composted material must meet the NPK ratio from 1%-4%(N), 0.1-0.4%(P) and 1-4%(K). Regarding humidity, it can be seen that with the exception of T0 (73%), the other treatments maintained the necessary level: T2 (56%) <T3 (58%) <T1 (63%) and remained in the appropriate range. Likewise, although the pH remained in the expected range for all treatments, however the first two treatments showed a greater gradient in the increase T0 (+1.14) and T1 (+0.91) due to a greater presence of organic acids; as intermediate products of bacterial decomposition of easily degradable substrates (Chen et al. 2022). The highest but most stable pH values of T2 (+0.6) and T3 (+0.46) were associated with the decomposition of organic matter rich in nitrogen (Chen et al., 2020). T2 and T3 achieved slight increases as indicated by López-González et al. (2021).

In relation to temperature, sanitization is achieved in thermophilic conditions above 65°C (stabilization) and can reach 67°C as a result of the intense microbial activity generated by the degradation of easily biodegradable molecules. This condition is capable of eliminating *Ascaris* eggs (Khadra, et al 2021). In our case, during the 14 days of pre-composting, clearly T0, presented some instability in the process, since the temperature decreased to 18°C, while the others managed to reach higher values in the following order: T1 (59°C) < T3 (62 °C) < T2 (66°C). Temperatures greater than 44 °C were sufficient to inhibit the activity of most pathogenic microorganisms (Chen et al. 2022).

On the contrary, Cardoso et al. (2021) recommends a minimum temperature of 71.1 °C for 10 s, to eliminate approximately 8 log of Salmonella cells, while Avidov et al. (2021) points out that temperature is the main factor that significantly influences the decomposition rates of Salmonella spp. However, the water content and the initial pH maintain a second place of influence with significant effects mainly at 30 and 40°C. The temperatures reached in the experiments significantly eliminated these pathogens, recording a survival range of *Salmonella* spp. between 1 to 42 MPN/10 g TS. Likewise, Okada et al. (2024) verified that temperatures in the thermophilic phase of 45 ◦C to 55 oC during the composting process are capable of eliminating *E. coli.* In the present investigation, the inactivation of these three microorganisms was achieved in 15 days with temperatures from 27°C to 65°C. It can be concluded that temperature and pre-composting time are factors that influence the inactivation of microorganisms. The implementation of this process on a full scale requires a gradual scaling up at a pilot level, which must be adjusted as the matter and MOB content increases before taking it to an industrial scale, especially due to the marked sulfoxidant microbial sensitivity.

The Analysis of variance (Table 4) has shown significant differences (p < 0.05) between the treatments for the elimination of H2S in anaerobic digestion applying beneficial microorganisms as inoculum providing sulfoxidizing bacteria. Of all the treatments, it was shown that the T2 conditions were effective for the removal of H2S and the elimination of the pathogens *Salmonella, E. coli*. and eggs and helminths, almost 100%.

Tabla 4. ANOVA: Anaerobic digestion and Pre-composting

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|   |   | Sum of squares | DF | Mean Squares | F | Sig. |
| Anaerobic digestion |  |  |  |  |  |  |
| H2S measurements | Between groups | 7626.25 | 3 | 2542 | 1906.56 | 0 |
| Deodorization | Within groups | 10.667 | 8 | 1.333 |  |  |
|  | Total | 7636.9 | 11 |  |  |  |
| Pre-composting |  |  |  |  |  |  |
| *Eschearichia coli* | Between groups | 4375.6 | 3 | 1458.5 | 7.6 | 0 |
|  | Within groups | 1535.3 | 8 | 191.9 |  |  |
|  | Total | 5910.9 | 11 |  |  |  |
| *Salmonella spp* | Between groups | 2900.3 | 3 | 966.7 | 111.5 | 0 |
|  | Within groups | 69.333 | 8 | 8.667 |  |  |
|  | Total | 2969.6 | 11 |  |  |  |
| *Helminth eggs* | Between groups | 12073.5 | 3 | 4024.5 | 862.4 | 0 |
|  | Within groups | 37.3 | 8 | 4.667 |  |  |
|   | Total | 12110.9 | 11 |   |   |   |

**Conclusion**

Waste sludge from the local wastewater treatment plant has been stabilized through the application of beneficial microorganisms that mitigated the activity of **sulphate**-reducing bacteria through the anaerobic digestion process. The optimal dose of BMOs used as inoculum was established at 10% volume with respect to the volume of the organic load in the anaerobic reactor. This reactor operated over a period of 15 days, under almost stationary conditions where the alkaline pH remained close to 7.5 and the temperature remained stable at 30 oC, eliminating 99% of H2S, thus demonstrating the efficiency of the technique. Subsequently, the assisted application of precomposting eliminated almost 100% of the pathogenic microorganisms such as *Salmonella, E. coli* and helminth eggs. In this case, temperature was the determining parameter, which reached 66ºC, ideally suited to sanitize the sludge.

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