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Research Article

Bio-Remediation of Lambda Cyhalothrin, Malathion and Chlorpyrifos Using Anaerobic Digestion Bio-Slurry Microbes

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Abstract

Anaerobic digestion of degradable material yields biogas used as fuel and bio-slurry widely employed as organic fertilizer as well as chicken and fish supplements. The bio-slurry is rich in microbes which makes it applicable in the bio-remediation of persistent pollutants in soil. In the current research work, the bacterial forming unit was determined using the standard plate method at microbiology laboratory, University of Nairobi after-which the microbes in biogas bio-slurry was applied in microbial fuel cells bio-remediation of lambda cyhalothrin, malathion and chlorpyrifos. The bio-slurry was doped with 10 mL 10 ppm pesticide solution and subjected to voltage generation via a H-shaped dual chamber microbial fuel cell for a 90 days' retention period. Daily voltage and current were recorded using a multi-meter while pesticide levels were determined using GC-MS after QuEChERS method extraction. The microbial counts result showed a $3.15\pm0.01 * 10^{10}$ CFU/ml from the biogas bio-slurry sample. The observed maximum voltage in bio-slurry was 0.568 V in day 28 while the maximum generated voltage on doping the biogas bio-slurry with the chlorpyrifos, lambda cyhalothrin, malathion and the pesticides mix (CLM) were 0.551, 0.565, 0.538 and 0.533 V respectively. The bio-degradation levels achieved were 73.40% malathion, 87.70% chlorpyrifos while no lambda cyhalothrin was detected on the 90^{th} day of incubation.

Keywords: Bioremediation; Bio-slurry; MFC; Voltage; Pesticides

Introduction

Bio-slurry is an anaerobic processed natural material discharged from biogas reactor after generation of methane gas for cooking, lighting, and running hardware (Islam, 2006). The bacterial pathogens, including *Salmonella*, *Staphylococcus*, *Listeria monocytogenes*, *Mycobacterium avium subsp. Paratuberculosis, Clostridium spp*., *Streptococci*, *Bacillus spp*., *Campylobacter, L. monocytogenes*, and *Yersinia enterocolitic* are present in digestate and are harmful to human health(Baggeet al., 2005; Masse et al., 2011). Livestock manure treated with anaerobic digestion system reduced the bacteria population drastically(Aitken et al., 2007). Sidhu and Toze (2009) reported that some pathogens exist after oxygen depletion while others take habitat in agricultural soil (Johansson et al., 2005) after bio-slurry administration. Another study by Goberna et al. (2011) observed that *E. coli* was absent from bio-slurry which had been subjected to a 60 days retention at 37°C anaerobic degradation while *Listeria spp* was present.

Employment of digestate in agricultural activities is a cost-effective way to reduce environmental risk while also utilizing biogas slurry nutrients (Islam et al., 2019). The quality of ecological environment is widely shown microbial responses to additives like fertilizers (Zhang et al., 2021). Conversion of wastes to biogas is a successful technology in waste treatment (Abubaker et al., 2012; Wang et al., 2019). Halfway incubated biogas slurry released into the environment can pollute the water and air (Nkoa, 2014; Insam et al., 2015). Also, biogas slurry is rich in nutrients and trace elements and can be utilized as a high-quality organic fertilizer, either alone or

in combination with conventional fertilizers (Möller and Müller, 2012; Baral et al., 2017).

Using biogas slurry effectively improve soil quality, reduce gases emmisions, minimize plant disease, and bring other benefits, according to previous studies (Terhoeven-Urselmans et al., 2009; Louro et al., 2013; Wang L. et al., 2018; Xu M. et al., 2019). The microbial population indicates the pollution status of a given system (Bell et al., 2012; Gonthier et al., 2014) which is adversely influenced by bio-slurry dosage. In rice-rape rotation systems, Xu M. et al. (2019); Xu Z. et al. (2019) discovered that a moderate dosage of digestate might positively enhance soil bacterial diversity, however too much or too little application had the reverse effect. Biogas residues boosted the microbial activity in wheat soil, according to Abubaker et al. (2012). Wentzel et al. (2015), on the other hand, found that using biogas slurry as a fertilizer lowered soil microbial activity and that the ratio of fungal to bacterial C dropped as soil clay concentration increased. Digested materials hardly little impacted the soil microbial community composition in an incubation experiment conducted by Johansen et al. (2013). The impact of biogas slurry addition on the soil microbial population was variable and varied on a number of factors, including application method, usage dose, soil type, and crop type. In the soil nutrient cycle, bacteria and fungi have a lot of interaction and cooperation, and they play a big role in ecological function (Liu et al., 2016; Wang H. et al., 2018). Despite extensive research, only a few studies have looked at how biogas slurry affects both bacterial and fungal communities. By examining the impacts of various strategies on soil microbial populations, it is possible to improve and optimize the application of biogas slurry.

Bio-remediation uses biological agents, mainly microorganisms i.e. yeast, fungi or bacteria to clean up contaminated soil and water (Strong and Burgess, 2008). This technology relies on promoting the growth of specific microflora or microbial consortia that are indigenous to the contaminated sites that can perform desired activities (Agarwal, 1998). The establishment of such microbial consortia can be done in several ways e.g. by promoting growth through the addition of nutrients, by adding terminal electron acceptor or by controlling moisture and temperature conditions (Hess et al., 1997; Agarwal, 1998; Smith et al., 1998). In bioremediation processes, microorganisms use the contaminants as nutrient or energy sources (Hess et al., 1997; Agarwal, 1998; Tang et al., 2007). Biogas plants produce huge quantities of organic residues and biogas. Cow dung is widely used in the plant as a part of waste management and biogas production. The residues are used as organic fertilizer and biogas is used as fuel in agricultural areas. therefore, in this research work, we investigate the efficiency of biogas bio-slurry microbes in bio-remediation of lambda cyhalothrin, malathion and chlorpyrifos using microbial fuel cell technology.

Methodology

Sampling

The biogas bio-slurry was obtained from a running biogas digester using cow dung as the substrate initiated with cow's rumen matter from Dagoretti slaughterhouse. The was done after 10-20 days' inoculation at psychrophilic conditions of 23-27°C. For microbial analysis, 1 ml of the sample was homogenized with 9 ml of phosphate-buffered saline solution. After mixing, serial dilution was made from 10^{-1} to 10^{-8} for culturing in different types of bacteriological media.

Bacteria Total Count

The spread plate technique was used to enumerate the total viable bacteria, *E. coli*, *Salmonella spp*., and *Staphylococcus spp*. (International Standards Organization (ISO-6579), 2002). All the media were prepared according to manufactures instructions. For enumeration of total viable count (TVC), nutrient agar media (NA) were used. From each dilution, 0.1 ml was inoculated on the center of the respective agar media by sterile pipette and spread by a sterile glass rod. After that, the plates were incubated at 37°C for 24 h. Following incubation, colonies that appeared on NA were counted and calculated by multiplying the average number of colonies in particular dilution with dilution factors and recorded as colony-forming unit per gram of samples.

Microbial Fuel Cells Construction

Two 1.5 liter containers were prepared as anode and cathode chambers. Two small holes were made on the caps of the containers

to insert the wire through. One end of the copper wire was attached to 5.7cm long and 0.7cm diameter graphite rod electrodes. A salt bridge was prepared using 2.5 liters of 1M NaCl, 3% agarose solution and lamp wicks. The wicks were boiled in NaCl and 3% agarose solution for 10 minutes after which it was kept in the freezer at -4°C for solidification. The solidified salt bridge was passed through PVC pipes and attached to the chambers using Araldite adhesive, which makes them leak-proof. The electrodes used in this study were spent battery carbon rods stuck together using a zero-resistance copper wire as shown in figure 1. The carbon rods were obtained from batteries after which they we thoroughly cleaned using water and later scrub using a sandpaper. They were then socked in concentrated Sulphuric acid for 24 hours before stacking them together. The was 0.00399m^2 operating electrodes surface area. The assembly of the H-shaped MFC was done, as shown in figure 1 as earlier described by Kamau et al., (2018). A digital voltmeter was attached to the copper wires from the cathodic and anodic chambers, and the voltage and current were monitored daily.

Figure 1: Set-up of H-shaped microbial fuel cells with a multi-meter.

The control experiment was run by loading the bio-slurry into the anodic chamber and reading the daily voltage and current for 90 days.

Bioremediation studies

The study involved the investigation of the efficiency of microbial fuel cells in the degradation of lambda cyhalothrin, malathion and Chlorpyrifos pesticide residues. The anodic chamber was fed with 1500 L of microbe rich bio-slurry from biogas reactor spiked with 10ml, of 100ppm lambda cyhalothrin, malathion and Chlorpyrifos and a mixture solution of lambda cyhalothrin, malathion and Chlorpyrifos. The degradation levels were determined by measuring the concentration of the pesticide after every 5 days for 90 days. The QuEChERS method (Anastassiades et al., 2003). The sample extracts were placed onto a tray for automated GC/MS analysis as described by (Amirahmadi et al., 2013). The Voltage and current generated were recorded on daily basis.

Results and Discussions

The microbial counts' in the digestate was 3.15±0.01 * 1010 CFU/ml. Similar results had been observed by Mbugua (2021) using rumen waste from slaughterhouse samples. Microbial counts in bio-slurry sample in this study agree with previously conducted research studies (Bonetta et al., 2011; Watcharasukarn et al., 2009). In bio-slurry samples of natural bio-slurry pits, the total viable counts ranged from 7.26 to 8.65 logcfu/gm observed in bio-slurry by Islam et al., 2019. Proteobacteria have been shown to be capable of degrading refractory organic compounds, a process that is heavily impacted by the presence of suitable organic carbon (Goldfarb et al., 2011; Hamm et al., 2016). Further, it was evident that the microbial count decreased ($1.8x10^{10}$CFU/ml – $6.3x10^{8}$CFU/ml) over the 28-day retention period (Akubuenyi and Achor, 2018). The microbial analysis of different bacterial species responsible for anaerobic breakdown of matter indicates presence of aerobic, facultative anaerobic and strict anaerobe in days 1, 14 and 28 respectively. *Pseudomonasspp*, *Bacillusspp*, *Lactobacillusspp*, *Klebsiellaspp*, *Proteusspp*, *Escherichiacoli* and *Staphylococcusspp* were among the organisms isolated on day 1, which indicates that the initial microbial hydrolytic activities on the waste materials are mediated by aerobic and

facultative anaerobic bacteria. The presence of *Staphylococcusspp*, *Enterococcusspp*, *Peptostreptococcusspp*, *Micrococcusspp* and *Fusobacteriumspp* were present in the sample analysed on day 14 showing that the digester was becoming anaerobic. Isolation of *Propionibacteriumspp*, *Listeriaspp*, *Erysipelothrixspp* and *Clostridiumspp* on day 28 indicating that the digester has turned anaerobic, the stage at which biogas is produced (Akubuenyi and Achor, 2018; Abenaet al., 2019). The slurry contains water at a range of 93.27 – 96.53%, 4.50 -7.63 % of dry solids and 2.5 – 3.78% of inorganic matter. Other content in the digestate includes scum, liquid effluent, sludge and many other organic and inorganic substances (Devarenjanet al., 2019).

The voltage and current obtain on running the control experiment are shown in figure 2. An upward increase in both voltage and current were observed for the first days of the experiments. In voltage for stance, the voltage increased linearly from day zero to day 28 after which it plateaued and then started dropping. The observed maximum voltage in the control setup was 0.568 V in day 28.

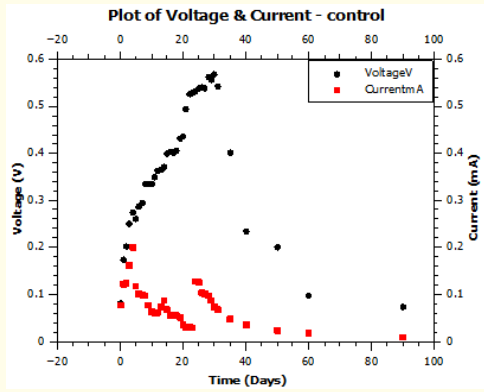

Figure 2: Plot of voltage and current generated from the control experiment.

The maximum generated voltage on doping the biogas bio-slurry with the chloripyrifos, lambda cyhalothrin, malathion and the pesticides mix (CLM) were 0.551, 0.565, 0.538 and 0.533 V respectively. The voltage generated increased steadily from the initial setup from day 0 to day 17 with low increasing rate up to day 31 where a downward voltage generation was observed (figure 3). The results obtained correlate with those observed by Kamau et al., (2019) in microbial fuel cell degradation of chlorothalonil using fresh biogas bio-slurry from the abattoir. In that study, voltage increased with time with0.603, 0.527 and 0.502 V voltage recorded on days 9, 19 and 30, respectively for the set containing 10 g glucose in 100 ppm chlorothalonil solution.

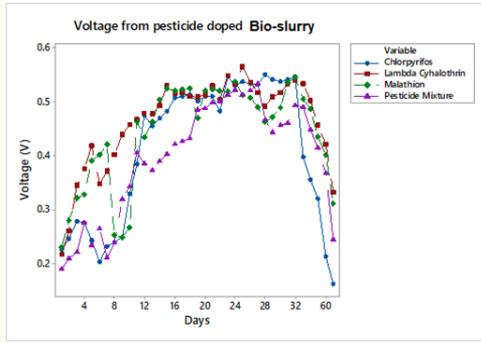

Figure 3: Daily voltage generated cabbage doped with Chlorpyrifos, Lambda Cyhalothrin, Malathion and pesticide mix.

The 3D plot of pesticides concentration, voltage and retention time in biogas bio-slurry is shown by figures 4, 5, and 6.

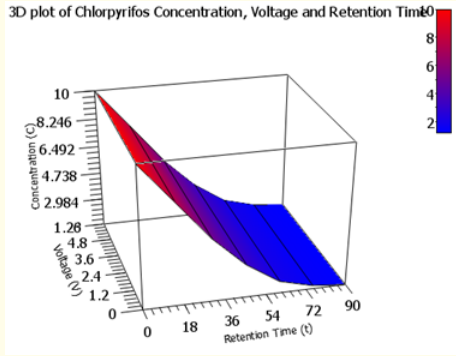

Figure 4: 3D plot of chlorpyrifos Concentration, Voltage and Retention Time in biogas bio-slurry.

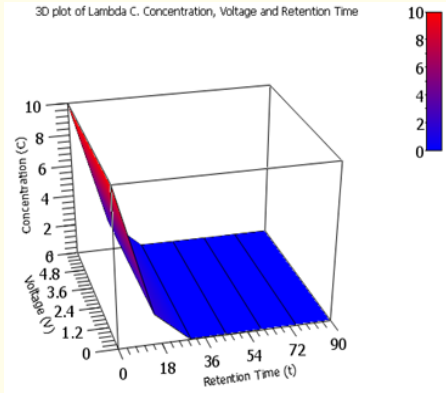

Figure 5: 3D plot of lambda cyhalothrin Concentration, Voltage and Retention Time in biogas bio-slurry.

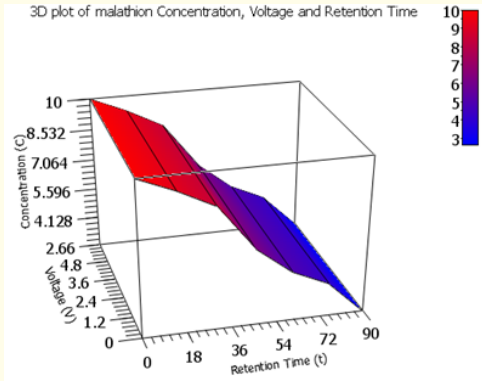

Figure 6: 3D plot of malathion Concentration, Voltage and Retention Time in biogas bio-slurry.

The bio-degradation levels achieved were 73.40% malathion, 87.70% chlorpyrifos while no lambda cyhalothrin was detected on the 90th day of this study as per figures 4, 5 and 6. In lambda cyhalothrin for example, it means that the bacterial load in the bio-slurry is able to achieve close to a hundred percent bioremediation. Using rumen fluid from slaughterhouse, Kamau et al., 2019 were able to achieve over 79.09% degradation of chlorothalonil depending on the main substrate. A study by Katayama et al. (1991) and Regitano et al. (2001) showed that available of carbon during mineralization of chlorothalonil affects microbial activities. In addition, they recorded that the highest mineralization reached was possibly due to greater metabolic activity in those soils with higher organic matter content.

Bio-remediation Decay Kinetics

The bio-remediation decay plots were simulated using the first order, second order and third order decay curves fitted onto the experimental curves. The first, second and third order curves are shown by equation 1, 2 and 3 respectively.

$$Y = Y_0 + Ae^{\frac{-x}{t}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$Y = Y_0 + A_1e^{\frac{-x}{t_1}} + A_1e^{\frac{-x}{t_2}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

$$Y = Y_0 + A_1e^{\frac{-x}{t_1}} + A_2e^{\frac{-x}{t_2}} + A_3e^{\frac{-x}{t_3}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Where Y_0 corresponds to the initial pesticide concentration, t_1 is the first decay time, t_2 is the second decay time and t_3 is the third decay time.

The fitted plots are displayed in figures 7, 8 and 9 with the first, second and third order decay curves. The resultant statistical analysis for fitness showed regression values range between 0.9387 to 0.9997 based on pesticide properties.

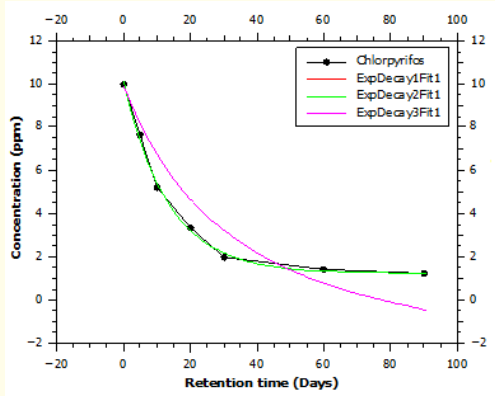

Figure 7: Fitted plots of chlorpyrifos decay curves in biogas bio-slurry.

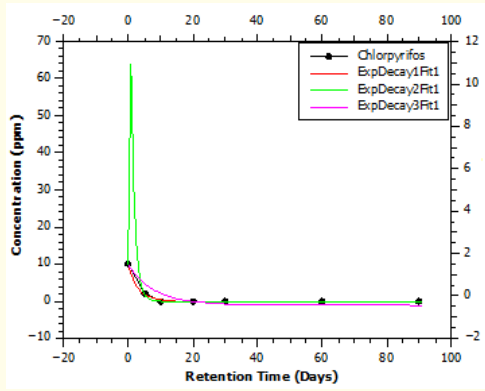

Figure 8: Fitted plots of lambda cyaholthrin decay curves in biogas bio-slurry.

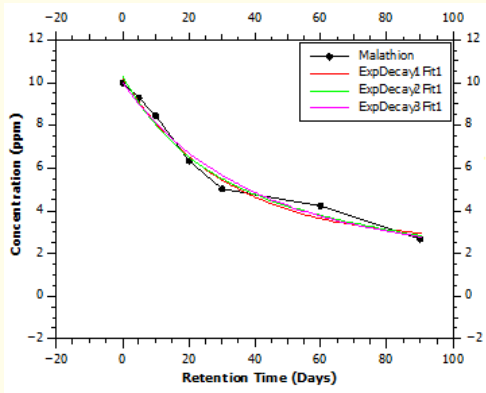

Figure 9: Fitted plots of malathion decay curves in biogas bio-slurry.

Discussions

From the results above, it was noted that the microbial communities in bio-slurry degraded lambda cyahalothrin, malathion and chlorpyprifos at different rates depending on their population amongst other factors. This had earlier been observed by Chakraborty et al., (2006). For example, the voltage obtained in the control experiments where no pesticide residue was added increases up to day 19 but on adding the pesticide, the voltage production continues up to day 32. This is an indication that the pesticide residue serves as food for the micro-organisms which enables bio-remediation. The relationship between the voltage generation and degradation levels was proportional. For example, during the first 30 days, high voltage is generated with high rate of degradation. In lambda cyhalothrin for example, the voltage ranges from day 0 to day 32 was 0.223 -0.543 V which translated to over 92 – 99.90 % remediation of the pesticide molecule. The kinetic decay curve fitting showed that the three pesticide decay could be explained by first-order exponential decay kinetics. Similar results had been observed for chlorpyrifos by Sarkouhi et al, (2016). However, in the current study, the three decay kinetics gave high regression fitting values of 0.921 – 0.998 indicating fitness to the model.

The levels of a pesticide in an environment (P) is a crucial parameter for investigating the bio-degradation rate (i.e., -d[P]/dt) in nature. Many pesticides degrade via pseudo-first-order kinetics, in which the bio-degradation rate is determined by the residual pesticide concentration (Pal et al., 2006). The bio-degradation rate declines proportionally with the pesticide concentration (i.e., d[P]/dt =

-k[P]), where d[P]/dt is the pesticide concentration gradient with time, and k is the bio-degradation rate constant. Theoretically, 0.2% of its initial concentration should be degraded after 180 days' incubation for a 20-day half-life though at high initial levels, the rate of decay (k) is low. Several pesticides (e.g., DDT, HCH, endosulfan, BHC, and atrazine) are ubiquitous pesticides which pollutes the soil and sediments as they are less bio-availability (Chowdhury et al., 2008). Odukkathil and Vasudevan (2013) reported that the half-life of less bio-available pesticides (e.g., DDT, HCH, endosulfan, BHC, and atrazine pesticides) ranges from 100 to 200 d (Pal et al., 2006). The majority of these residues are adsorbed on soil particles, making them unavailable for further breakdown by soil bacteria. Based on a few case studies, an attempt has been made in some reviews to offer a brief notion on 'major limits in pesticide bio-degradation in soil'.

Conclusion

It was concluded that the microbial community feed on the substrate thereby increasing exponentially observed by upward trend in current and voltage generation. The maximum generated voltage on doping the biogas bio-slurry with the chloripyrifos, lambda cyhalothrin, malathion and the pesticides mix (CLM) were 0.551, 0.565, 0.538 and 0.533 v respectively. The bio-degradation levels achieved were 73.40% malathion, 87.70% chlorpyrifos while no lambda cyhalothrin was detected on the 90th day. The second order decay kinetics best explained the rate of bio-remediation with over 0.9997 regression fits. This study therefore recommends employment of microbial fuel cell technology in bio-remediation of pesticides as pollutants are removed from the environment as well as renewable energy is created.

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