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Co-Metabolism Kinetics of Bioremediation of Lambda Cyhalothrin, Chlorpyrifos and Malathion Contaminated Loam Soil Using Bio-Slurry Microbes

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ABSTRACT

The indiscriminate use of insecticide in agricultural soils causes significant soil and water pollution and poses a serious threat to the global community. Degradation of these pollutants is therefore vital in pollution control. Microbial fuel cells have been employed in bio-remediation of organic pollutants due to its environmental friendliness and low cost. The occurrence of pesticides in soil has become a highly significant environmental problem, which has been increased by the vast use of pesticides worldwide and the absence of remediation technologies that have been tested at full-scale.

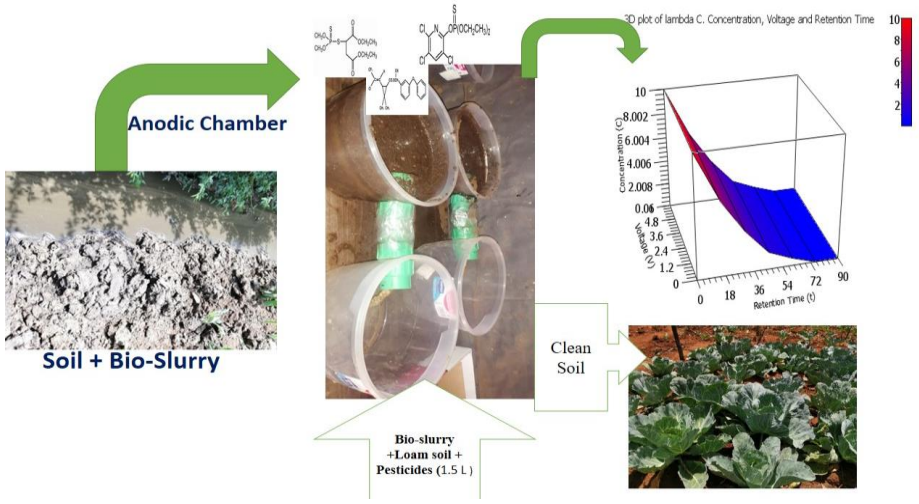
In this study, bioremediation experiments were conducted at ambient temperature of 28-32 0C and pH 5.6-8.9 to investigate the effectiveness of the process in the clean-up of pesticide contaminated loam soils. The loam soil was assessed for macro and micro properties prior to the experiments in control procedures. A H-shaped double chamber microbial fuel cell was fabricated where the anodic chamber was loaded with 750 mL loam soil inoculated with 750 mL bio-slurry doped with 10 mL of 10 ppm lambda cyhalothrin, chlorpyrifos and malathion pesticide solutions. The cathodic chamber was loaded with 1500 mL distilled water. The setup was incubated for a 90 days' retention time where voltage and current were recorded daily using a multi-meter. The degradation level was assessed using a GC-MS after sample extraction using standard QuEChERs method.

The voltage generated from the pesticide doped loam soil showed an upward trend from day 0 to day 15 in lambda cyhalothrin and malathion and from day 0 to day 20 in chlorpyrifos and pesticide mixture after which constant readings were observed for three days with downward trends thereafter. The maximum generated voltage was 0.537 V, 0.571 V, 0.572 V and 0.509 V in chlorpyrifos, lambda cyhalothrin, malathion and pesticide mix (MCL)

respectively. The bioremediation levels for chlorpyrifos and malathion were 65.80 % and 71.32 %, respectively while no detectable, lambda cyhalothrin was observed after day 60 of the study. This study concludes that bioremediation of lambda cyhalothrin, chlorpyrifos and malathion in Limuru loam soil can be achieved using microbial fuel cells.

Keywords: Bio-Remediation, Bio-Slurry, Pesticides, Loam Soil, Co-Metabolism

Graphical Abstract



I. INTRODUCTION

Soil pollution is a worldwide problem that draws its origins from anthropologic and natural sources (Kumar *et al.,* 2022). Urbanization, industrialization, and food-demand increases have required the use of compounds, substances, and chemical agents, which, over the years, have brought on the dispersion and accumulation of pollutants in the environment. The common pollutants present in the soil are heavy metals, polycyclic aromatic hydrocarbons (PAHs), or pesticides (Chen *et al.,* 2015). Soil can be used to generate electrical power in microbial fuel cells (MFCs), which convert chemical energy from soil organic compounds into electricity via catalysis by soil source exoelectrogenic microorganisms (Jiang Y-B et *al.,* 2016). The process of soil power generation has several potential applications. Firstly, the pollutant toxicity and soil microbial activity could be monitored by the generated electrical signals of the MFCs, such as peak voltage, quantity of electrons and start-up time (Deng *et al.,* 2015; Jiang *et al.,* 2015). Secondly, the use of MFCs would lead to the elimination of soil pollutants including phenol, petrol and oil (Huang *et al.,* 2011; Wang *et al.,* 2012). Thirdly, the operation of MFCs mitigates methane emissions from paddy soil and sediment (Arends *et al.,* 2014). MFCs do not need energy input, instead, a small amount of electrical power is generated. Therefore, MFCs are considered a sustainable technology. The performance of these MFCs is largely related to the magnitude of electrical current generated by the exoelectrogenic bacteria in soil. However, little is known about the character of power generation and the diversity of exoelectrogenic bacteria in different soils. To date, around 50 bacteria belonging to three phyla *Proteobacteria, Firmicutes*,

and *Acidobacteria* have been identified as exoelectrogenic (Zhi *et al.,* 2014).

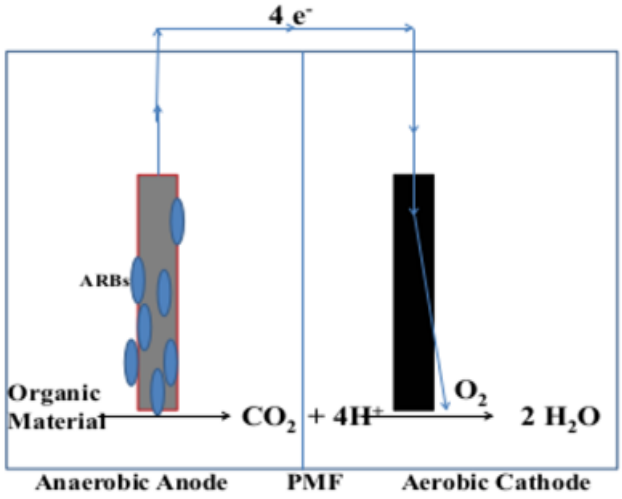


Figure 1: A dual chamber MFC (Pisciotta and Dolceamore, 2016).

The application of chlorpyrifos in the field leads to various negative impacts on soil, water, and plant systems. Due to high hydrophobicity, chlorpyrifos adheres to the soil particles and forms clumps that entrap nutrients and restrict its movement toward the plant root (Kumar *et al.,* 2022). The rate of biodegradation in soil depends on four variables are as follows: (i) Availability of pesticide or metabolite to the microorganisms (ii) Physiological status of the microorganisms (iii) Survival and/or proliferation of pesticide degrading microorganisms at contaminated site (iv) Sustainable population of these microorganisms (Dileep, 2008).

Co-Metabolism Kinetics

Co-metabolism recently has emerged as a powerful method for the biodegradation of refractory pollutants (Goudar, 2012). The microbial degradation efficiency of refractory compounds could be improved by altering the substrate structure of carbon and energy sources (Wang *et al.,* 2015).Co-metabolic kinetics was modeled for better understanding the effect of cyanide (non-growth substrate) and sodium acetate (growth substrate) on the microbial growth, which could also explore the interaction between cyanide and sodium acetate in the process of co-metabolism(Lv et al., 2016).Microbial growth kinetics and substrate degradation kinetics in the co-metabolism process were fitted using nonlinear least squares method by Origin 8.5. The adopted models along with their mathematical form have been described below. In the early 1940s, Monod, (1949) model was proposed, which relates specific growth rate (μ) and substrate concentration (CS) of a single growth controlling substrate represented by the equation 1.

$$\mu = \mu_{max} \frac{C_s}{K_s + C_s} \dots\dots\dots\dots\dots\dots\dots\dots\dots (1)$$

Where μ is the specific growth rate, CS is the limiting substrate concentration (mg L^{-1}), μmax is the maximum specific growth rate (h^{-1}), *and KS* is the half saturation coefficient (mg L^{-1}).

Powell (1967) revised the Monod equation by introducing the maintenance rate (m). But Powell and Monod models do not consider the effect of self-inhibition, which all were non-inhibition dynamics models.

$$\mu = \frac{(\mu_{max} + m)C_s}{K_s + C_s} - m \dots\dots\dots\dots\dots\dots\dots (2)$$

To account for deficiency of the above models, an improved model (Haldane) was proposed which contains the substrate inhibition effect. Owing to its importance and mathematical simplicity, the Haldane model (Andrews, 1968) was commonly accepted by researchers. The Model equation is shown in equation 3, where $K1$ is the substrate inhibition constant (mg L^{-1}).

$$\mu = \frac{\mu_{max} C_s}{K_s + S + \left(\frac{S^2}{K_1}\right)} \dots\dots\dots\dots\dots\dots\dots\dots\dots (3)$$

Based on the ideal growth state of microorganism, Levenspiel (1988) proposed a kinetic model of substrate inhibition as equation 4. The model calculates the values of the critical inhibitor concentration (S_m). Above the value of S_m, the culture growth is completely inhibited.

$$\mu = \frac{\mu_{max} C_s}{K_s + S}\left(1 - \frac{C_s}{S_m}\right)^n \dots\dots\dots\dots\dots\dots\dots\dots (4)$$

Considering the diffusion-controlled substrate, Teissier (De pra *et al.,* 2016) proposed an exponential kinetic model as per equation 5.

$$\mu = \mu_{max}\{exp\left(\frac{-C_s}{K_1}\right) - exp\left(\frac{-C_S}{K_s}\right)\} \dots\dots\dots\dots\dots (5)$$

Aiba model (Dey and Mukherjee, 2010) shown in equation 6 which correlates the growth inhibition data with substrate degradation was also proposed.

$$\mu = \mu_{max}\frac{C_s}{K_s + C_s} exp\left(\frac{-C_s}{K_1}\right) \dots\dots\dots\dots\dots\dots\dots\dots\dots (6)$$

The above models are all inhibition kinetic models of the single substrate. To further analyze the synchronous effect of the non-growth substrate (cyanide) and growth substrate (sodium acetate) on microbial growth, the SKIP (Sum Kinetics with Interaction Parameters) model (Singh and Balomajumder, 2016) was adopted to simulate co-metabolism.

$$\mu = \frac{\mu_{max,S1} C_{s1}}{K_{S1} + C_{s1} + \left(\frac{C_{S1}^2}{K_1}\right) + I_{2,1} C_{2s}} + \frac{\mu_{max,S2} C_{s2}}{K_{S2} + C_{S2} + \left(\frac{C_{S2}^2}{K_2}\right) + I_{1,2} C_{s1}} \quad \dots (7)$$

Where the interaction parameter $I_{2,1}$ indicates the effect of substrate 2 on the utilization of substrate 1 and *vice versa*. If the values of $I_{2,1}$ and $I_{1,2}$ are zero, there is no interaction between the two substrates.

In the current study, the bio-remediation kinetics of co-metabolic breakdown of three commonly used pesticide in central Kenya inoculated with biogas bio-slurry was studied via eco-friendly microbial fuel cell technology.

II. Methodology

Sampling

The loam soil sample used in this study was collected from Mbugua Farm in Ndeiya Ward, Limuru Sub-County, Kiambu County, Kenya. Top soil was scoped after removing the vegetation about 1.5-3.0 cm deep. No known record of pesticide usage on this soil for over thirty years. The microbial community count was done using the standard plate method as described by Kinyua *et al.,* 2022 while soil analysis was carried out as described by Mbugua *et al.,* 2022.

Soil analysis

The properties of the loam soil used in this study was analyzed as described by Mbugua *et al.,* 2022 for the determination of pH, micro and macro nutrients.

Microbial Fuel Cells Construction

Two 1.2liter 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 litres 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. Graphite rods were obtained from batteries after which they we thoroughly cleaned using water and later scrub using a sand paper. They were then socked in concentrated sulphuric acid for 24 hours before stacking them together with 0.00399m^2 operating electrodes surface area. The assembly of the H-shaped MFC was done, as shown in figure 3.33 as earlier described by Mbugua *et al.,* (2020). A digital voltmeter was attached to the copper wires from the cathodic and anodic chambers, and the voltage and current were monitored daily.

Figure 2 : Set-up of H-shaped microbial fuel cells with a multi-meter

Bioremediation studies

The study involved investigation of efficiency of microbial fuel cells in degradation of lambda cyhalothrin, malathion and Chlorpyrifos pesticide residues. The anodic chamber was fed with 750g tomato, cabbage and loam soil inoculated with 750ml rumen wastes 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 Voltage and current generated were recorded on daily basis.

The models of degradation kinetics

In addition to the growth kinetics of substrate inhibition, the degradation of the pesticides in co-metabolism MFC was modeled using zero order, pseudo-first, pseudo-second and three-half order equations as described by Costa *et al.,* 2012; Saravanan *et al.,* 2009). The equations of these models are given in table 1.

Table 1: The models of degradation kinetics

Models	Equations	Reference
Zero order model	$C_S = C_{S0} - k_0 t$	Park *et al.,* 2008
First order model	$C_S = C_{S0} exp\,(-k_1 t)$	Boucabeille *et a*l., 1994
Second order model	$K_s ln\left(\frac{C_S}{C_{S0}}\right) + C_s - C_{S0} = -K_2 t, k_2 = \mu_{max} X_0$	Raybuck, 1992
Three-half order kinetic model	$Y = -k_{3,1} - \frac{k_{3,1}}{2} = \frac{1}{t} ln\left(\frac{C_{s0} - P + K_0}{C_{S0}}\right)$ $P = C_{S0} - C_S + k_0 t$	Karavaiko *et al.,* 2000

Where $k0$, k_1, k_2, $k_{3,1}$, and $k_{3,2}$ are rate constants of zero-order, first-order, second-order, three-half order respective, X_0 is the initial biomass concentration in MFC reactor, S_0 is the initial substrate concentration (mg L^{-1}) in MFC reactor.

III. Results

Loam soil properties

The loam soil was collected not deeper than 2cm from the surface after removing the plant matter. The properties of the loam soil employed in this experiment are listed in table 2. The soil's pH was determined to be 6.60. When compared to data from Mbugua *et al.* (2014)

on Limuru loam soil, it was found to be comparable to the loam soil used in this experiment, the soil moisture content is alike and measured at 43.36 percent.

Table 2: Properties of the loam soil

Profile	Properties	Profile	Properties
Soil depth cm	Top	Calcium milli-equivalent%	44.4±2.11
Soil pH-H_2O (1:2.5)	6.5±0.51	Magnesium me%	3.1±0.09
Elect. Cond. ms/cm	0.3±0.01	Potassium me%	1.5±0.66
Carbon %	2.7±0.32	Sodium me%	3.6±1.11
Sand %	40±3.56	Sum me%	52.6±3.44
Silt %	40±4.55	Base %	100+
Clay %	20±2.88	ESP	14.4±6.74
Texture Class	Loam	Total nitrogen %	0.25±0.08
Cat. Exch. Capacity. me%	24.8±2.67	Phosphorus ppm	44±5.00
Zinc ppm	62.9±10.22	Iron ppm	96.2±12.90
Copper ppm	1.22±0.11	me is milli-equivalent	

The soil carbon percentage was recorded at 2.69±0.31 %. Soil fertility is highly influenced by the macro and micro nutrients like calcium, magnesium, potassium and iron, phosphorous and nitrogen. The CEC in the loam soil was noted to be 24.80% which compares well to what was reported by Mbugua *et al.,* 2014 at 24.05% for loam soils.

Inoculum properties

The outcomes obtained for the bacteria amounts obtained from samples of the loam soil and the bio-slurry is presented in table 3, whereby bacteria concentrations were 3.01±0.02 * 10^9 cfu/g in loam soil, and 3.15± 0.01* 10^{10} cfu/ml in the bio-slurry.

Table 3: Total microbes count from loam soil and bio-slurry samples.

Sample	Count	Unit
Bio-slurry	3.15±0.01 * 10^{10}	cfu/ml
Loam soil	3.01 ±0.02* 10^9	cfu/g

The speed at which the substrate is broken down is well explained by the number of bacteria community in a sample. A good example is that of Wieder et al., (2013), which concluded that higher bacterial activity leads to higher rates of bio-degradation of organic matter. It is also notable that for rumen usage in tasting bio-degradation, the time duration should not exceed 4 days. Due to varying bacterial load and diversity levels, inoculum sources affect the substrate's ability to degrade (Mwaniki et al, 2016).

Control

The voltage generated from the pesticide doped loam soil showed an upward trend from day 0 to day 15 in lambda cyhaolthrin and malathion and from day 0 to day 20 in chlorpyrifos and MCL mixture after which constant readings were observed for three days with downward trends thereafter. The maximum generated voltage was 0.537 V, 0.571 V, 0.572 V and 0.509 V in chlorpyrifos, lambda cyhalothrin, malathion and MCL respectively, as shown in figure 3.

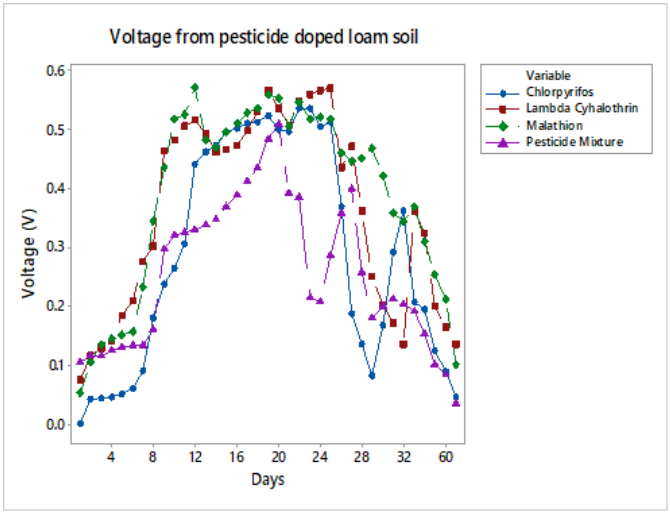


Figure 3 : Daily voltage generated cabbage doped with Chlorpyrifos, Lambda Cyhalothrin, Malathion and pesticide mix

The voltage and current generated from the co-metabolism of different substrates is as a result of microbial breakdown of carbon material in biomass in anaerobic condition. The voltage is highly influenced

by microbial community and operation conditions as reported by Mbugua *et al.,* 2021.

Bioremediation studies

Bio-remediation is a promising technology which utilizes the ability of microorganisms to remove pollution from the environment and are eco-friendly, economical and versatile (Finley *et al.,* 2010). During biodegradation processes, pesticides are transformed into degradation products or completely mineralized by microorganisms, which use the pollutant compounds as nutrients for their metabolic reactions. A key role in the biotransformation mechanisms is carried out by enzymes, such as hydrolases, peroxidases, and oxygenases, that influence and catalyze the biochemical reactions (Raffa & Chiampo, 2021). Major reactions in pesticide destruction include mineralization and co-metabolism. Pesticide degradation is influenced by many factors such as type of pesticide, type of microorganism, temperature, humidity, and acidity in the environment (Sehrawat *et al.,* 2021).

The measured degradation rates for malathion, lambda-cyhalothrin, and chlorpyrifos were 79.32%, 99.90%, and 78.20%, respectively, as shown in figures 4. The shows the degradation kinetics rates of first, second and third order while the rate of degradation parameters are shown in table 3.

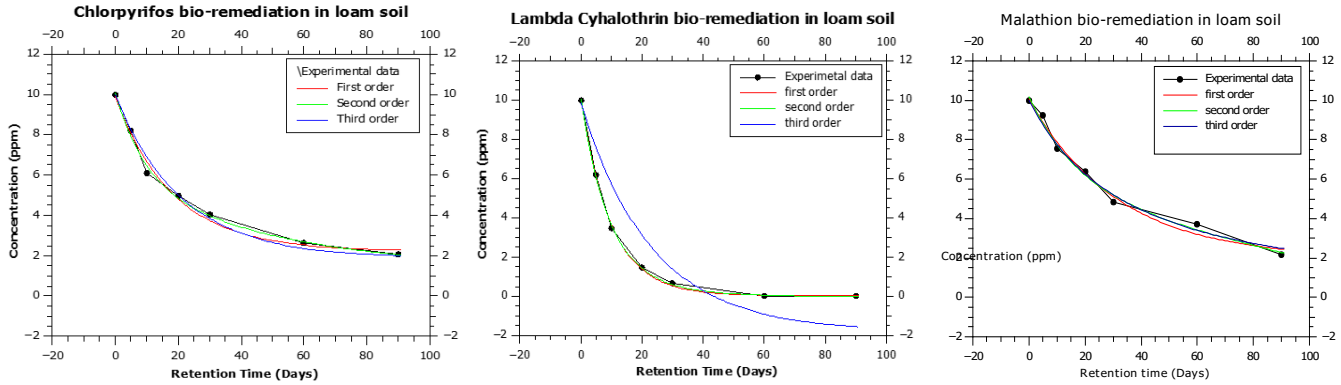


Figure 4 : The rate of degradation of different pesticides in loam soil

Table 3: Model parameters for pesticides degradation of zero order, first order, second order, three-half order models in mixed substrate MFC

Pesticide	Zero order model		First order model		Second order model			Third-half order model		
	$K0$	R^2	$K1$	R^2	$K2$	KS	R^2	$K3,1$	$K3,2$	R^2
Chlorpyrifos	0.0500	-3.429	0.076	0.989	0.0459	0.0426	0.994	0.0212	-8.131	0.984
Lambda Cyhaolthrin	0.0892	-0.788	0.099	0.999	0.0964	0.0632	0.995	0.0232	3.872	0.834
Malathion	0.0695	-4.520	0.0808	0.988	0.0492	0.0584	0.944	0.0176	0.1762	0.092

The models include zero order, first order, second order, three-half order were applied to research degradation kinetics to understand process dynamics, and model parameters were listed in table 3. By comparing with other models, the second order model was found to be most fitting for cyanide degradation with a high correlation coefficient (>0.99). But the first order and second order model have the shortcoming that they do not considerate the factors of bacterial growth. To overcome this limitation, Brunner and

Focht, (1984) proposed a three-half order model integrating the microbial growth and substrate degradation. Nevertheless, the correlation coefficient (0.977–0.998) of the three-half order model was slightly lower than the second order model. However, the three-half order model can also be used as a suitable model for prediction.

IV. Discussion

Microbial fuel cells are bio-electrochemical devices capable of using electroactive bacteria present in the environment to oxidize organic material and transfer electrons to an electrode as part of their metabolism to generate bio-electricity (Simeon *et al.,* 2022). It has been shown that practical usable energy can be derived from MFCs with the help of environmental consortia (Xu *et al.,* 2019). Therefore, MFCs technology is widely employable in bio-remediation of toxic pollutants like heavy metals (Fang & Achal, 2019) and pesticides while generating bio-energy (Cao *et al.,* 2015). In the past decade, the use of bioelectrochemical systems (BES) has received increased attention as an alternate remediation approach that can overcome many limitations of conventional (bio)treatments for contaminants, including PAHs (Mandal and Das, 2018). Kinyua *et al.,* 2022 employed slaughterhouse microbial communities in bio-degradation of different class of pesticides.

Current and voltage generation is an indication of substrate breakdown by microbes in anaerobic anodic chamber of microbial fuel cells. The rate of current generation shows the rate at which microbes are degrading the substrate/carbon releasing electrons. (Kamau *et al.,* 2019). In bio-remediation of pollutants, the pesticide molecule serves as a carbon sources and therefore it's broken down by micro-organisms in soil (Cycoń *et al.,* 2009).

In the study by Mbugua et al., 2022, the observed maximum 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 0.533V respectively with bio-degradation levels achieved were 73.40% malathion, 87.70% chlorpyrifos while no lambda cyhalothrin was detected on the 90th day of incubation.

Malathion is degraded by carboxyesterase enzyme and it is detected in several fungi like *Aspergillus sp., Penicillum sp*. and *Rhizoctonia sp.* Hasan (1999) also demonstrated the same type of fungal utilization and degradation of Malathion.

Results of bio-degradation of chlorpyrifos by environment restoring microbes showed the decrease in chlorpyrifos concentration (500 mg L^{-1}) was started on the 7th day of incubation, followed by full disappearance on the 30th day of incubation in liquid medium with some consortium treatment (Kumar *et al.,* 2022)

To reduce the environmental and public health risks associated with pyrethroid use, it is necessary to develop rapid and effective methods to remove or minimize the concentrations of insecticides in the environment. Among the variety of methods that are used for the remediation of contaminated environments, the biological approach, which is based on the catabolic activity of pesticide-degrading bacteria, seems to be the most promising and effective strategy (Chen et al., 2012; Zhao et al., 2013; Cycoń *et al.,* 2014).

The relation between the specific growth rate (μ) of a population of microorganisms and the substrate concentration (S) is a valuable tool in biotechnology. This relationship is represented by a set of empirically derived rate laws referred to as theoretical models. These models are nothing but mathematical expressions generated to describe the behavior of a given system (Okpokwasili & Nweke, 2005).

Growth kinetics, i.e., the relationship between specific growth rate and the concentration of a substrate, is one

of the basic tools in microbiology. Microbial growth kinetics, i.e., the relationship between the specific growth rate (μ) of a microbial population and the substrate concentration (*s*), is an indispensable tool in all fields of microbiology, be it physiology, genetics, ecology, or biotechnology, and therefore it is an important part of the basic teaching of microbiology. (Kovárová-Kovar & Egli, 1998).

Co-metabolism is defined as the ability of a microorganism to convert a non-growth substrate in the presence of either a growth substrate or another biodegradable substrate (Tran et al., 2013).It represents a potential alternative for the elimination of pollutants, as it separates the process of pollutant biodegradation from the growth of microorganisms, resulting in a shortened period of adaption and propagation. Co-metabolism has been confirmed to be effective in the elimination of alkanes, as well as aromatic and chlorinated compounds in natural environments, including methyl *tert*-butyl ether (MTBE)(Dalton and Stirling 1982; Horvath, 1972).

V. Conclusions

The voltage generated from the pesticide doped loam soil showed an upward trend from day 0 to day 15 in lambda cyhalothrin and malathion and from day 0 to day 20 in chlorpyrifos and pesticide mixture after which constant readings were observed for three days with downward trends thereafter. The maximum generated voltage was 0.537 V, 0.571 V, 0.572 V and 0.509 V in chlorpyrifos, lambda cyhalothrin, malathion and pesticide mix (MCL) respectively. The bioremediation levels for chlorpyrifos and malathion were 65.80 % and 71.32 %, respectively while no detectable, lambda cyhalothrin was observed after day 60 of the study. This study concludes that bioremediation of lambda cyhalothrin, chlorpyrifos and malathion in Limuru loam soil can be achieved using microbial fuel cells.

Conflicts of interest

There are no conflicts to declare.

Acknowledgments

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