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Full Length Research

Application of microbial fuel cells in the degradation of 2,4,5,6-tetrachloroisophthalonitrile (chlorothalonil)

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ABSTRACT: Pesticide's persistence in the environment due to the relatively slow degradation mechanism leads to their bio-accumulation which in turn has adverse impacts on human health. Bio-remediation involves utilization of microbes from nature to the breakdown of organic molecules. The purpose of this study is to investigate the potential of microbes in degrading chlorothalonil. Aerobic-anaerobic combined conditions in an H-shaped double chamber microbial fuel cell (MFC) were employed for the breakdown of chlorothalonil. Decomposing tomatoes were used as the major substrate with their proximate properties being analyzed using standard method. Glucose loaded with different concentrations of chlorothalonil was introduced to the cells on day 10 when voltage production had stabilized. The voltage and current generated were monitored using a digital multi-meter while pesticide concentrations were obtained using a UV-Vis spectrophotometer. The highest voltage readings were obtained on day 9 of degradation, with values ranging from 0.463 to 0.537 V. The current ranged from 0.002 to 0.076 mA. Higher voltage and current values were recorded in solutions with lower pesticide concentration. The obtained degradation level was highest in 10 g glucose at 95.95 and 98.75% for day 10 and 20 respectively. The lowest breakdown was observed in the cells without glucose at 10.54 and 31.04% on day 10 and 20 respectively. The results demonstrate that MFC technology can be employed in mineralization of chlorinated pesticides as an alternative for incineration and photo-degradation.

Keywords: Bio-remediation, chlorothalonil, MFC, microbes, power, voltage.

INTRODUCTION

Tomato production in greenhouses involves the employment of pesticides to curb pest and diseases. Some of the sprayed pesticides do not reach the target site thereby contaminating the soils. Pesticide molecules in the soil or plant surface can undergo degradation via the various route e.g, by light, water or microbe. Microbial fuel cells (MFC) employs microbial activities in the oxidation of degradable matter which results in generation of current (Logan et al., 2006). Electrons produced by bacteria from these substrates are transferred to the anode and flow to the cathode linked by a conductive material containing a resistor, or operated under a load (Logan 2009, 2010). MFC technology has been employed widely in mineralization of pesticides by doping substrates like glucose and/or acetate with the pesticide molecule of interest in the anaerobic-anodic chamber (Amel et al., 2014, Maria et al., 2013). The microbes feed on the organic matter together with the pesticide leading to its breakdown (Huang et al., 2011).

According to US EPA (1999), the main mode of chlorothalonil disintegration in the environment results from the microbes. Chen et al. (2001) examined the impacts of micro-organisms on fungicides in three different soil types (silt-loam, fresh and soils with the leafy matter). The results indicated that the activities of the micro-organism were highly inhibited by the pesticide molecule in the soil.

Mori et al. (1996) assessed the microbial debasement of chlorothalonil in unfertilized and prepared farmstead compost or soil. In the study, degradation of the applied

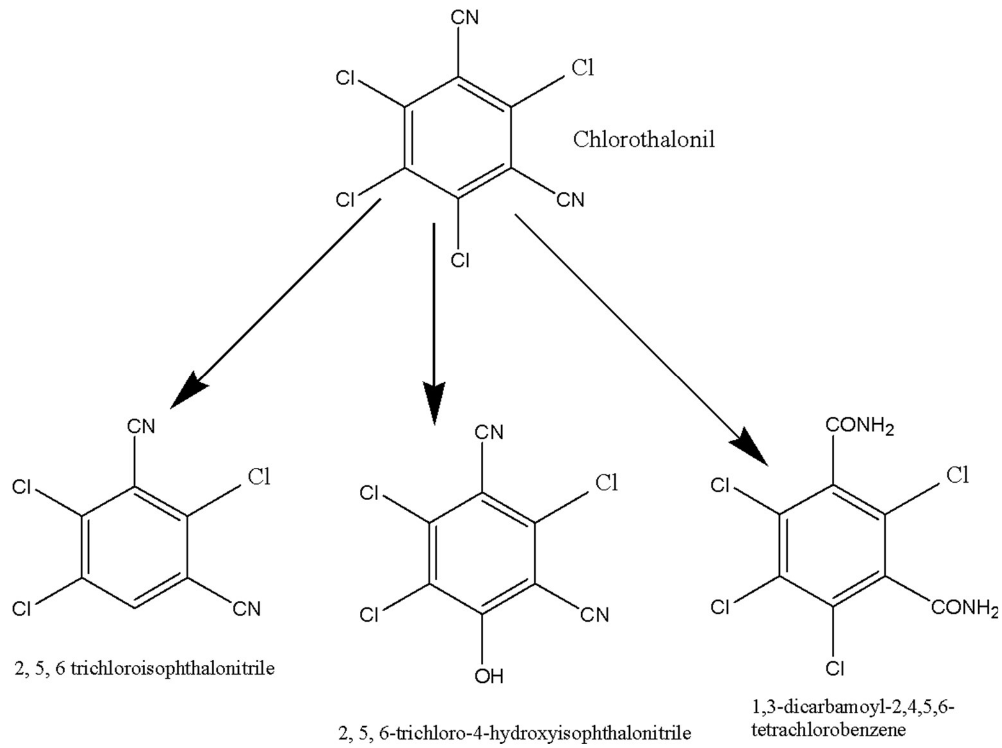


Figure 1. The proposed microbial degradation pathway for chlorothalonil (Ukai et al., 2003).

pesticide was influenced by the organic matter content of the soil, pH and organic carbon content. Manure application in the soil influenced the microbial activities with the addition of substrate rich in carbon content (Mori et al. 1996). Wang et al. (2011) investigated the anaerobic breakdown of chlorothalonil in four paddy soils. The results obtained show a significance dependence of bio-degradation rate on pH and carbon content. In addition, degradation of chlorothalonil was optimal at neutral pH (6.3 to 6.6) and in soil containing 3 to 4% total carbon (Wang et al. 2011).

Motonaga et al. (1996) identified the gram-negative rod bacterium, from the chlorothalonil-treated soil. This bacterium hydrolysed more than 75% of chlorothalonil into 4-hydroxy-2, 5, 6 trichloroisophthalonitrile and a chloride anion. This strain was reported to deliver hydroxylated metabolite (Motonaga et al. 1996). *Bacillus cereus* degraded chlorothalonil based on co-metabolism while carbon sources enhanced its degradation as observed by Zhang et al. (2007). In another study by Liang et al. (2010), *Ochrobactrum sp* was identified from chlorothalonil-contaminated soil which is reported to breakdown chlorothalonil to undetectable levels within 48 hours at 20 to 40ºC and a pH range of 6 to 9. In oxygen free set up, hydrolytic de-chlorination take place resulting in a stable hydroxyl by-product (Liang et al., 2010). In a study by Ukai et al., (2003), influence of the chlorine atoms on mineralization of chlrothalonil was examined. The results showed that the resulting metabolites contain 3 to 4 chlorine atoms only. The two major degrade products are 2, 5, 6-trichloro-4-hydroxyisophthalonitrile and 2, 5, 6 trichloroisophthalonitrile as shown in Figure 1. Sato and Tanaka (1987) identified other metabolites which were as a result of de-chlorination and partial substitution. Carlo-Rojas et al. (2004) reported 4-hydroxychlorothalonil, Metylthiotrichloroisophtalonitrile and Trichloroisophtalonitrile in aerobic soils as well as 3-carbamyl-2,4,5-trichlorobenzoic acid and 3-cyano-2,3,4,5,6-tetrachlorobenzoamide as degradation metabolites in aerobic acidic soils.

In the current study, MFC technology was employed in the breakdown of chlorothalonil pesticide. Thus, the purpose of this study is to investigate the potential of microbes in degrading chlorothalonil.

MATERIALS AND METHODS

Chlorothalonil standard (IOBA Chemmie, 99% pure), Acetonitrile (HPLC analytical grade 85% from Fisher Scientific Co. (Fairlawn, NJ)). Analytical grade Un-hydrous magnesium sulphate and sodium acetate were used as received. Graphite rods from dry batteries, plastic bowls, agarose and sodium chloride were used in the assembly of H-shaped double chamber fuel cells. The inoculums used was rumen fluid from Dagoretti slaughter house (Nairobi, Kenya). Tomato waste from Kangemi market, a grocery store in Nairobi County, Kenya was used as the primary substrate while glucose doped with pesticide was the co-substrate.

Physicochemical analysis

Ash, moisture and fiber contents were determined using AOAC (1990) method and as describe by ASTM D (1989). Fat, crude nitrogen and protein contents were determined using Soxhlet extraction and Kjedhal methods described in Pearson (1976). Energy content was carried out using the AOAC method described in Onwuka (2005) while total and volatile solids were determined using Renewable technologies (2005) method. The analysis was done at the Department of Food Science, Nutrition and Technology, College of Agriculture and Veterinary Sciences, University of Nairobi, Kenya.

Bio-degradation studies

Fuel Cell Assembly

Double chamber microbial fuel cells were fabricated using the locally available material as described by Kamau et al., 2017. Studies of the amount of chlorothalonil degraded were done by adding 1, 5 and 10 g glucose to 10 ml of 100 ppm stock solution to the anodic chamber containing blended rotten tomatoes 10 days after voltage stabilization. A set without glucose was used as a control.

To study the effect of different concentration of chlorothalonil, 10 and 20 ppm of chlorothalonil mixed with 2.5 g glucose was added to the anodic chamber. A control was set using blended tomatoes.

Analysis

Chlorothalonil degradation levels were obtained using Shimadzu UV-Vis spectrophotometer. Voltage and current were recorded daily using DT9205A digital multimeter for 30 days. The mean of obtained data was used to make the plots using Minitab 17.

RESULTS AND DISCUSSIONS

Proximate analysis parameters are very important when considering a feedstock to be used for the Energy Recovery Systems (Rominiyi, 2015). Table 1 shows the proximate properties of tomatoes in wet and dry weight basis. The moisture content was 95.16 and 4.84% on wet and dry basis respectively. All the other properties were higher on dry basis compare to wet basis. Moisture content reported in this study is slightly higher but in range with previous studies by Oko-Ibom and Asiegbu, (2007), Adubofuor et al. (2010) and Hossain et al. (2010) who have reported the moisture content in the range of 88.19 to 90.67%. The energy values of tomato waste are 19 times higher on dry basis compare to wet basis.

Table 1. Proximate properties of tomatoes.

Property	Wet weight	Dry weight
Moisture	95.16±1.23	4.84±0.06
Volatile Matter	4.38±0.03	85.63±1.09
Carbohydrates	2.93±0.02	55.42±0.56
Protein	0.57±0.01	11.89±0.69
Fat	0.12±0.01	2.57±0.02
Ash	0.46±0.02	9.53±0.32
Mineral Matter	0.51±0.03	10.48±0.25
Energy (Kcal/100g)	15.08±0.09	292.37±1.56

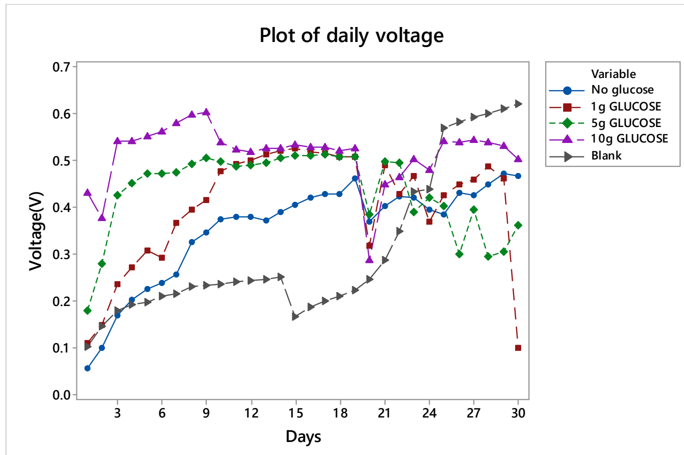


Figure 2. Daily voltage production.

The daily voltage in all the samples increased from day 1 to 9. There was a drop in the recorded voltage on day 10 when the pesticide solutions were introduced apart from the set where no pesticide was added after which the voltage starts to increase. On day 20, the voltage reduced a factor attributed to destabilization of anaerobic conditions during sampling. An upward trend was thereafter observed eventually forming a plateau on day 27 (Figure 2). This could be due to the diminishing substrate levels translating to decreasing microbial activities and subsequent death of microbes. 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.

The recorded voltage on on days 9, 19 and 30 were 0.603, 0.527 and 0.502 V respectively for the set containing 10 g glucose in 100 ppm chlorothalonil solution. Glucose serves as a good substrate in the breakdown of chlorinated pesticides as earlier observed by Huang et al.

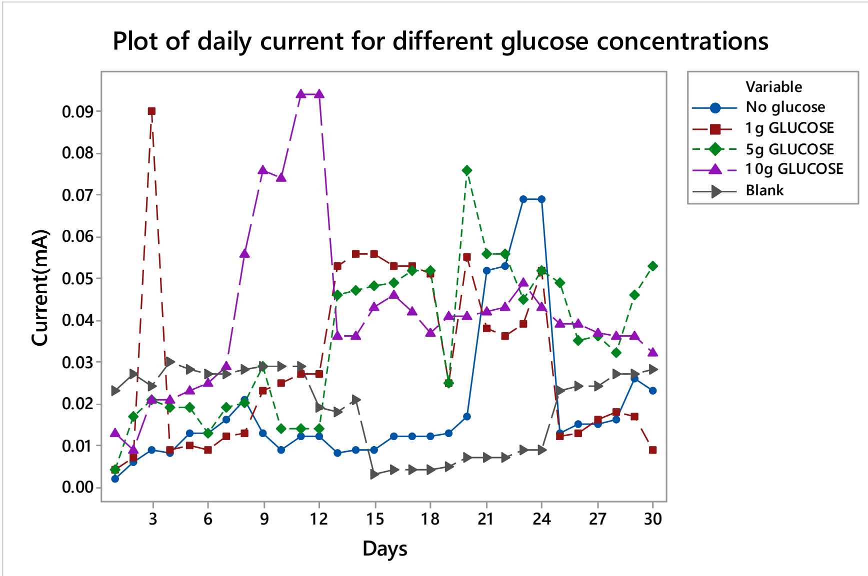
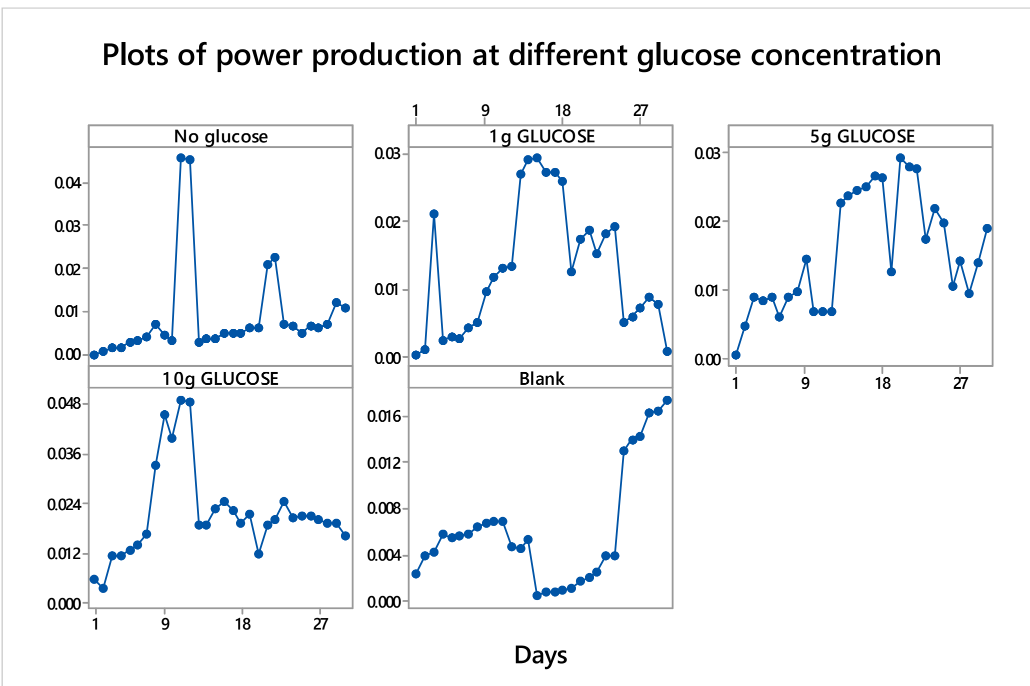


Figure 3. Time plots of daily current for different glucose levels.



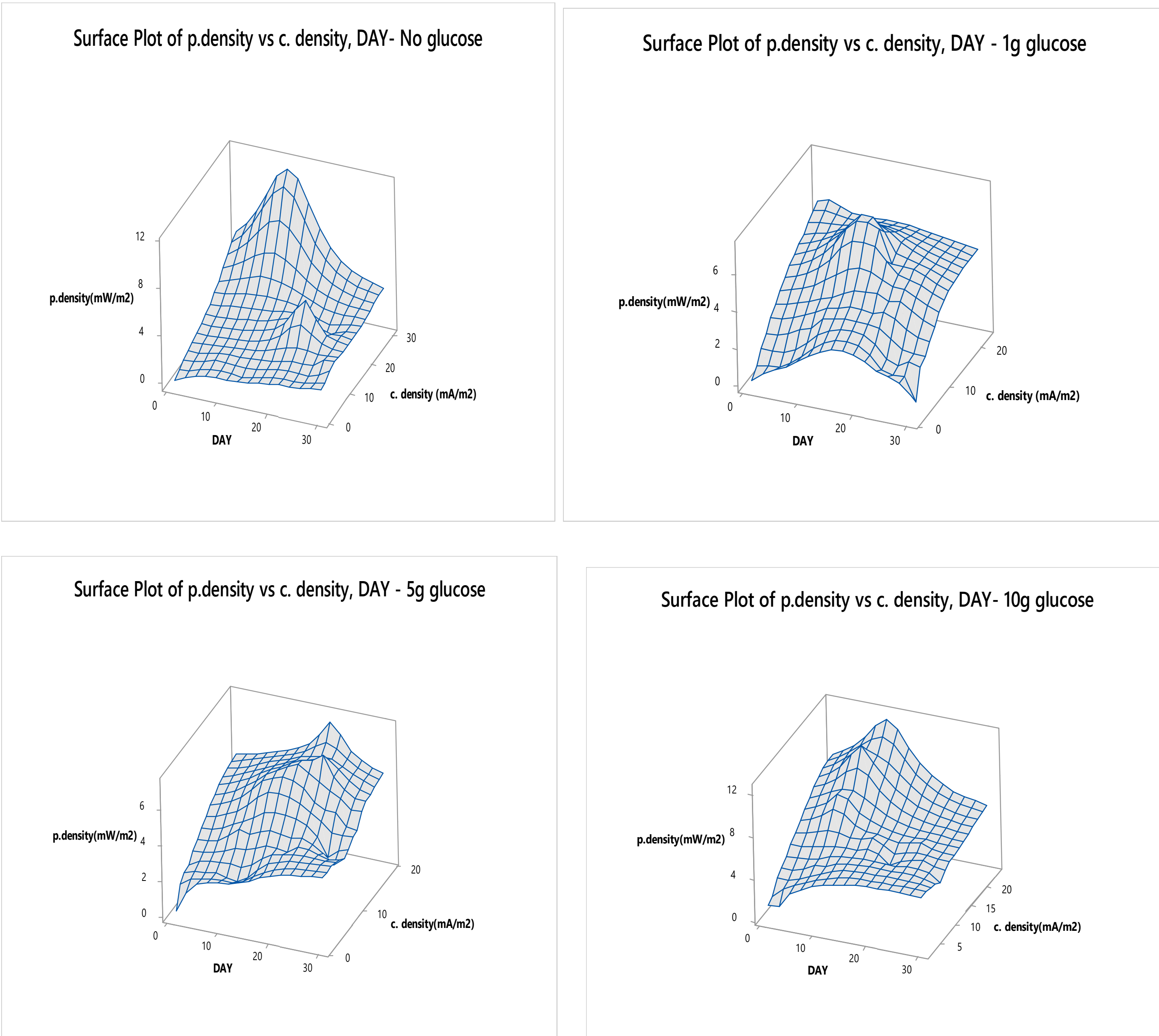
Figures 4. Daily power production.

(2012) in mineralization of pentachlorophenol.

The current generated from the set-ups is shown in Figure 3. The current was lowest on the set up with blank tomatoes since it had no inoculum. In glucose solutions, the recorded current was highest in 10 g glucose solution followed by 5 g and 1 g respectively. This is explained by availability of higher carbon source which microbes use as food.

From Figure 3, the highest current was obtained from the set-up with no glucose solution. Current is the flow of electrons therefore, the microbes fed on tomatoes and pesticide molecule at a faster rate compared to the solutions containing glucose.

Power was calculated by multiplying voltage and current. Daily plots for power obtained are shown in Figure 4. The power obtained was in the range of 0.0056 to 0.0492



Figures 5. Surface plots of daily power density and current density.

mW for 5 g glucose in 100 ppm chlorothalonil solution. The surface plot of daily power and the current density is shown in Figure 5. Current and power density were calculated as reported by Kamau et al. (2017).

The percentage levels of the amount of chlorothalonil degraded is shown in Figure 6. As expected, degradation increased with time of exposure.

Concentration variation of chlorothalonil

The results on the variation of concentration of chlorothalonil on microbial activities, are shown in Figure 7. The voltage was highest for 20 ppm pesticide solution. In this case, as opposed to earlier observations, addition of glucose doped solution had no significant effect on the voltage. Voltage recorded was highest in 20 ppm solution followed by 10 ppm and lowest in the blank set-up. Lowest voltage was observed on day 20 due to destabilization of the biofilm during sampling.

The daily current was lowest in blank set-up, 10 and 20 ppm solution respectively as shown in Figure 7(b). Figure 8 shows the power obtained from different concentrations of chlorothalonil. High concentrations of chlorothalonil

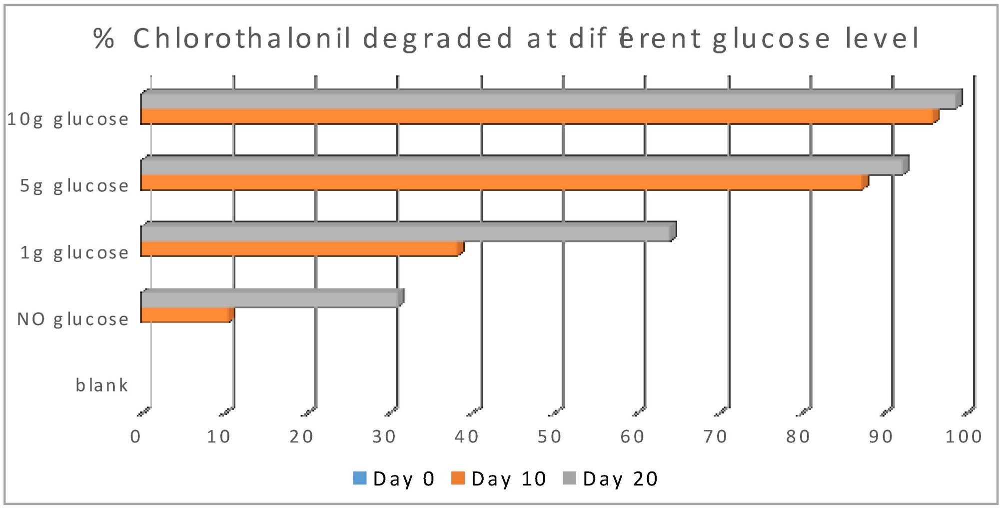


Figure 6. Percentage chlorothalonil degraded at different glucose levels.

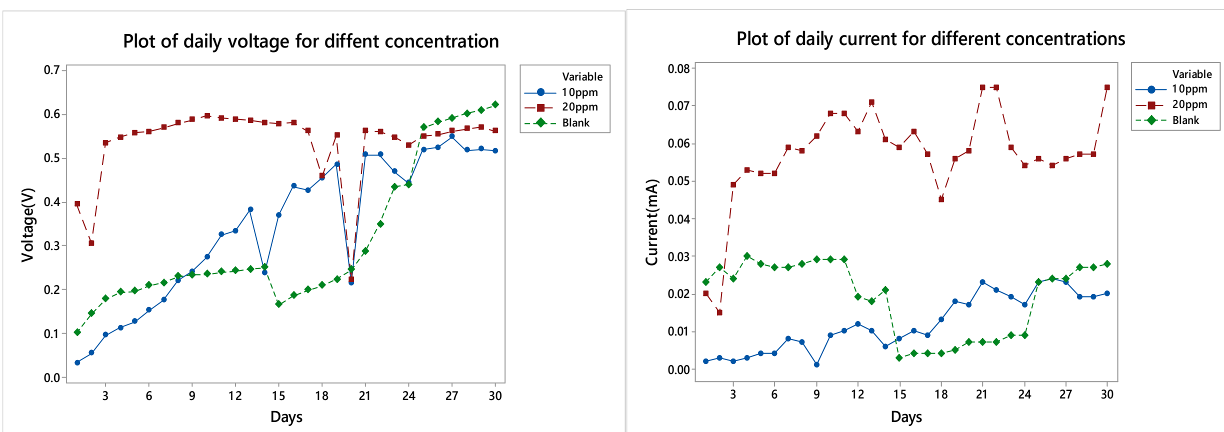


Figure 7. Daily (a) voltage and (b) current generated for varying amount of chlorothalonil.

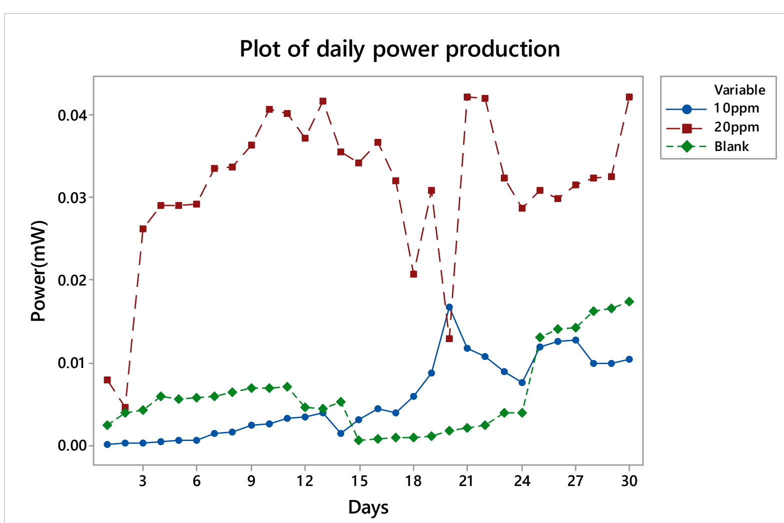


Figure 8. daily power generated for varying amount of chlorothalonil.

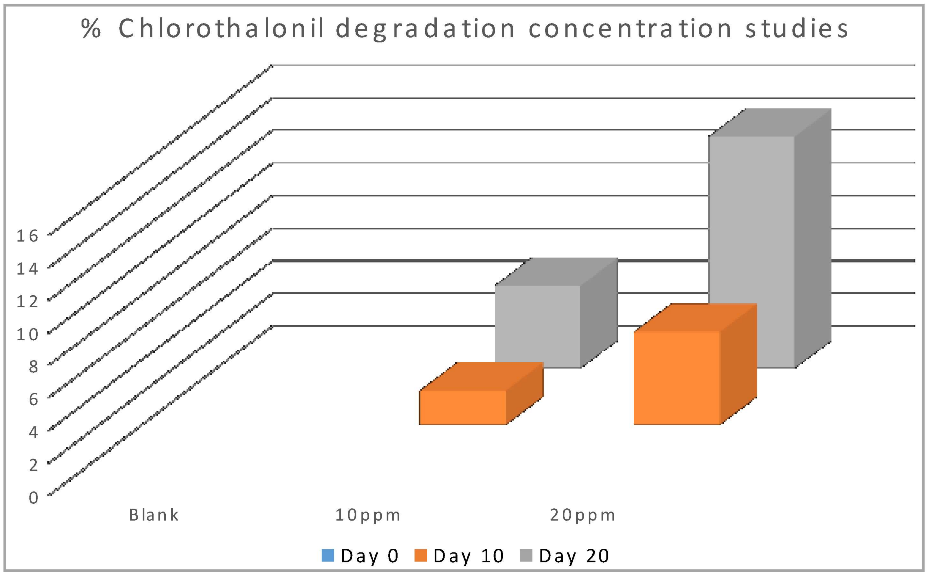


Figure 9. Percentage Chlorothalonil degradation concentration studies.

resulted in high degradation as shown in Figure 9. This has been attributed to high carbon/substrate available as microbes food. This had been observed by Carlo-Rojas et al. (2004) who observed that initial carbon and chlorothalonil dose had significant effect on removal efficiency.

Conclusion

The study concludes that chlorothalonil can be broken down to its respective metabolites by employing microbial fuel technology. Voltage and current increase with substrate concentration as the microbes degrade chlorothalonil loaded substrate.

Recommendation

From this work, mineralization of chlorothalonil fungicide used in tomato production using MFC is recommended due to its environmental friendliness and green energy production.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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