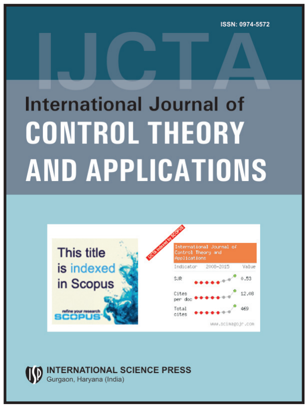

International Journal of Control Theory and Applications

ISSN : 0974-5572

Volume 9 • Number 44 • 2016

Biodegradation of Polychlorinated Biphenyls (PCBs) by Native Microbial Consortium in Contaminated Dielectric Oils: A Regression Analysis

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Abstract: Reducing the levels of PCB-contaminated dielectric oil is a crucial aspect in the electrical sector. In this regard, both clients and workers of this industry may suffer organic and genetic affectations due to the significant toxicity of this substance. In addition, this problem will continue to be greater due to the growing electricity demand. In this respect, the presence of some microbial consortiums may be used to address this issue. However, it is necessary to count on suitable and effective mathematical approaches to finally validate and predict the degradation performance of these microorganisms. Therefore, this paper focuses on the application of regression analysis to measure the biodegradation process of different microbial consortiums. First, the effects of two factors: Observation period (4 weeks, 8 weeks and 12 weeks) and Microbial consortium (Aerobic bacteria, Fungus and Anaerobic Bacteria) were assessed via applying hypothesis tests. Then, a correlation analysis was performed to define how the observation period and PCB concentration are associated. Finally, the regression equations are obtained and the degradation ratios of each consortium are identified In this case, the results demonstrated that both factors were found to be statistically significant (P-value < 0.05). Additionally, it is shown that the *Aerobic bacteria* were found to be the consortium with the highest PCB-degradation ratio (-23.088 ppm/week) which highlights the relevance of the microbial aerobic metabolism in the degradation process of PCB.

Keywords: Biodegradation, PCBs, Microbial Consortium, Dielectric Oil, Regression Analysis.

1. INTRODUCTION

The Polychlorinated Biphenyls (PCBs) are synthetic molecules composing of carbon, hydrogen and chlorine atoms. These are generated by replacing between one and ten hydrogen atoms by chlorine atoms in the biphenyl molecule. As a result, 209 PCB combinations can be potentially achieved [1]-[2]. Due to their physical and chemical properties, they are fire-resistant, very stable, slightly volatile and does not conduct electricity and under normal temperature. Therefore, they have been widely used in different industrial sectors, e.g. electrical industry regarding the dielectric flow of power transformers [3]. Additionally, several studies have demonstrated the adverse effects of exposing to these compounds in humans and other living organisms [4] [5] [6] [7].

In Colombia, based on the preliminary inventory of PCBs, there are between 11585 ton and 19256 ton of PCBs in dielectric power transformers and other contaminated equipment. From these, approximately a third part (2662 ton) is liquid which can be found in transformers and storage bins of electrical, manufacturing and transportation sectors [2]. Thus, the storage of inactive equipment and PCB oils is a very critical feature for any public or private firm with PCB inventory. In this regard, the Decree No. 4741/2005 established that the storage of hazardous wastes in the surroundings of power transformers cannot exceed one year [8]. Furthermore, the Resolution 222/2011 sets that the owners of PCB-contaminated inventory and wastes should adequately eliminate them not later than 2028 according to the goals established in this document [9]

Based on the aforementioned events, it is necessary to develop chemical, physical and biological treatments deleting the presence of PCBs. In this respect, the microbial biodegradation is an effective and low-cost process. Herein, living organisms are used (e.g. bacterial and fungal species) due to their ability of anaerobically or aerobically metabolizing the PCBs by transforming them into CO_2, H_2O or both less complex and easy-to-degradation organic compounds. This can be explained with the great metabolic versatility of the microorganisms to obtain energy and biomass from toxic and highly recalcitrant compounds (e.g. PCBs). In addition, they have a high metabolic and reproductive rate through which they obtain high quantities of biomass for degradation process [10]-[11]. In this regard, several microorganisms have been reported as PCB degradators. These strains include groups of gram-negative bacteria (e.g. *Pseudomonas Achromobacter, Burkholderia, Comamonas, Sphingomonas, Ralstonia,* and *Acinetobacter*) and gram-positive genus (e.g. *Rhodococcus, Corynebacterium* and *Bacillus*) which use biphenyls as a unique source of energy and carbon; and co-metabolize the PCBs by the biphenyl pathway enzymes [12] – [13] – [14]. Other studies can be found at [15] – [16] – [17] – [18]. Nevertheless, just a few reports on degradation by individual strains and microorganism consortiums with complex contaminated PCB matrixes (e.g. dielectric oils of heavily PCB-contaminated transformers) were found.

On the other hand, several authors have proposed mathematical approaches to model the performance of PCB degradation under different conditions. In this regard, [19] used a simple pharmacokinetic model to describe the plasma concentrations of PCB congeners in 583 people working in a contaminated building. Also, in this work, the half-life of the congeners was calculated by logarithmic regression analysis. In [20], a mathematical model, capable of describing sorption, sequestration, and biodegradation in soil/water systems was applied to interpret the efficacy process of organic chemicals on remediation sites. The model was proved to be valid when comparing measurements of biodegradation of PAHs and PCBs in land treatment units. Also in [21], the authors exposed a mathematical model considering the mass balance equations aiming at describing the fate of medical degradation in soil/water microcosm systems. In this case, this framework was applied to a benzene degradation test. Other mathematical frameworks can be found in [22] – [23] – [24]. In light of these, there are no models describing the effects of several microbial consortiums and their degradation ability on PCB-contaminated dielectric oil with the use of different substratum (as a source of energy and carbon); and aerobic/anaerobic atmospheres. Therefore, this paper proposes a regression model to address this problem. The remainder of this paper is organized as follows: Section II introduces the regression analysis and describes the proposed methodology. Then, Section III presents the results and discussion. Finally, the conclusions and future work are shown in Section IV.

2. THE REGRESSION ANALYSIS

The Regression Analysis is a widely used and potent statistical method that is used to model the relationship between a response variable (dependent variable) and one or more predictor variables (independent variable) [25] – [26] – [27]. Additionally, it can be also applied to evaluate the presence of effect modification [28] – [29]. One of the main strengths of Regression Analysis is to provide a model to understand and predict the response variable by fitting a regression equation. In light of these, since origins, The Regression Analysis has been widely applied to model different biodegradation processes: In the evaluation of biological pre-treatment with white rot fungi [30], biodegradability of ionic liquids [31] – [33], biodegradation of products in aerobic soils [32], anaerobic biodegradability [34], biodegradation with intermittent aeration [35], biodegradability of acids [36], biodegradation in contaminated soils [37] and other applications.

To effectively perform the Regression Analysis, it is necessary to follow these steps:

Step 1: Define the independent factors, levels, and response variables.

Set k independent factors (x) whose significance will be assessed through hypothesis tests on a set of response variables (y). Each factor (A, B, …) may be defined by different levels according to the study focus. Each combination is denoted with k digits where the first digit indicates the A level, the second digit refers to the B level,…, and the *k-esim* digit is the K level.

Step 2: Generate the null and alternative hypothesis for each independent factor.

The hypotheses are based on literature review and the researcher's belief regarding the influence of independent factors on the response variable [38-39]. In this, the means of factor treatments are compared (refer to Eq. 1-2) to validate if these are statistically equal (P-value < 0.01).

$$\begin{aligned} &H_0: \tau_1 = \tau_2 = \cdots = \tau_a = 0 \\ &H_1: \text{At least one } \tau_i \neq 0 \end{aligned} \quad \text{(Eq. 1)}$$

$$\begin{aligned} &H_0: \beta_1 = \beta_2 = \cdots = \beta_b = 0 \\ &H_1: \text{At least one } \beta_j \neq 0 \end{aligned} \quad \text{(Eq. 2)}$$

Step 3: Data collection and random running order

After verifying if the metering system is adequate [38], the samples can be collected. Then, the measurements are performed randomly to delete the influence of strange factors in the experiment.

Step 4: Perform the Regression Analysis to assess the significance of factors on the response variables.

Step 5: Calculate the correlation coefficient (Pearson Coefficient) r to determine how strong the independent factor is associated with the response variable (refer to Eq. 3). Here, n is the number of pairs of data.

$$r = \frac{n\sum xy - \left(\sum x\right)\left(\sum y\right)}{\sqrt{n\left(\sum x^2\right) - \left(\sum x\right)^2}\sqrt{n\left(\sum r^2\right) - \left(\sum y\right)^2}} \quad \text{(Eq. 3)}$$

This coefficient ranges between –1 and 1 and quantifies the strength and direction of the linear association between the variables. If r is close to 1 (greater than 0.8), then the response and predictor variables have a strong positive correlation. On the other hand, if r is close to –1 (less than –0.8), the variables have a strong negative correlation. In this regard, a positive correlation indicates that as values of x increases, values of y also increase and vice-versa. Whilst, a negative correlation denotes that as values of x increases, values of y decrease and vice-versa.

Step 8: Set a regression equation to predict the response variable

In this step, an equation is generated to describe the relationship between the predictors and the response variables. This expression is modelled as a linear equation ($y = mx + b$).

3. CASE STUDY

To validate the proposed approach, a case study is presented. In this case, three different microbial consortiums were considered: *Aerobic bacteria, Fungus and Anaerobic Bacteria* (All of them preserved in 4% glycerol at 70°C).

The anaerobic tests had an integrated N_2-CO_2 atmosphere on a ratio of 80/20. On the other hand, three observation periods were taken into account (*4 weeks, 8 weeks and 12 weeks*) and the response variable was defined to be the PCB concentration (obtained by the ASTM D-4059-00 method). Each consortium was previously purified and each combination was replicated twice in 1% acetate. This study was part of a project created to diminish the PCB levels derived from the dielectric oil contained in the power transformers located in a Colombian region. In this respect, the dielectric oil samples were provided by a company from electrical industry. It is also good to highlight that the potential contamination level of these devices has been identified as a high risk for both workers from electrical industry and customers. To do this, the stepwise procedure explained in Section II was effectively implemented. Initially, the independent factors, treatments and response variable were defined as cited in the above paragraph. Then, the null and alternative hypotheses were established via applying Eq. 1-2 as shown below:

$$H_0: \tau_{4\text{ weeks}} = \tau_{8\text{ weeks}} = \tau_{12\text{ weeks}} = 0$$

$$H_a: \text{At least one } \tau_i \neq 0$$

$$H_0: \beta_{\text{anaerobicbac}} = \beta_{\text{fungus}} = \beta_{\text{aerobicbac}} = 0$$

$$H_a: \text{At least one } \beta_j \neq 0$$

Afterward, the data regarding the response variable was collected as described in Table 1. Considering the information registered in this table; the regression analysis was performed with the alpha level equal to 0.05. In this respect, both *Observation period* and *Microbial Consortium* were proved to be statistically significant (P-value < 0.05).

Table 1
Average PCB concentration in different microbial consortiums and observation periods

Observation period (weeks)	*Average PCB concentration (ppm)*		
	Aerobic bacteria	*Fungus*	*Anaerobic bacteria*
4	234	199.33	163.33
8	41	76	106
12	49.3	81.3	101

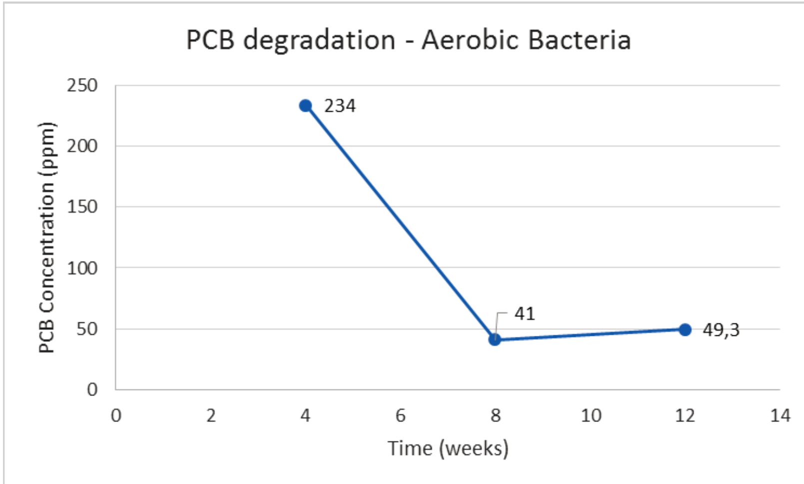
After assessing the significance of the independent factors, the correlation coefficient was calculated for each microbial consortium. Particularly, for *Aerobic Bacteria*, r was found to be -0.7164 which denotes a medium high correlation between time period and PCB concentration. On the other hand, for *Fungus*, r was calculated to be -0.8166 which represents a high negative correlation level. Finally, considering *Anaerobic Bacteria*, the Pearson coefficient was estimated at -0.8097 which denotes the same interpretation as the aforementioned consortiums. To complement this, the degradation ratios were identified by obtaining the mathematical expression for each consortium as shown below:

Aerobic Bacteria: $y = -23.088x + 292.8$ (refer to Figure 1a)

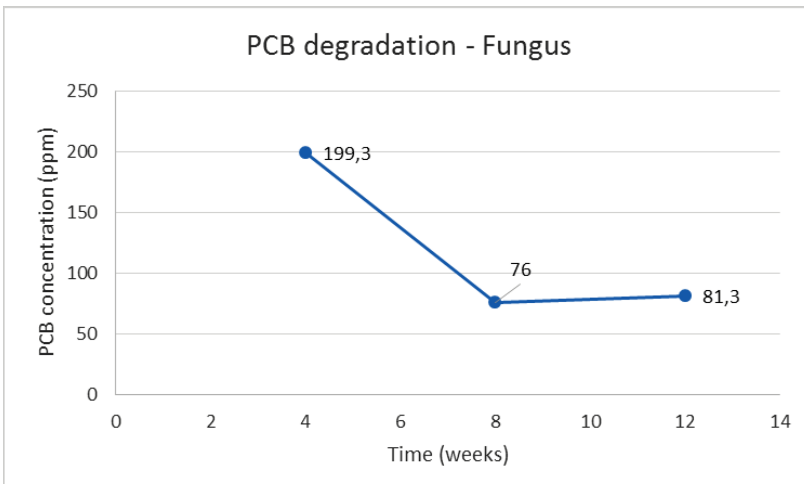
Fungus: $y = -14.75x + 236.87$ (refer to Figure 1b)

Anaerobic Bacteria: $y = -7.79x + 185.77$ (refer to Figure 1c)

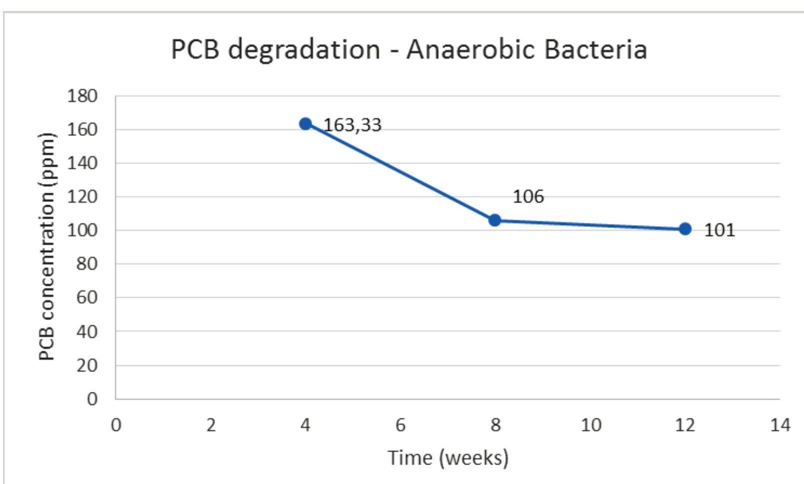
Considering the above expressions, it can be noted that the higher degradation ratio can be found in *Aerobic Bacteria* with -23.088 ppm/week which can be supported in the fact that the aerobic metabolism is more efficient than presented in both anaerobic bacteria and fungus. In this respect, the oxygen is the final receptor of electrons during the breathing process. This is very efficient due to the oxygen has a very low potential for reduction. In contrast, the anaerobic organisms use electron receptors having a higher reduction level compared to oxygen. This means that the breathing process in anaerobic organisms is less efficient compared to the aerobic which leads to slower growing ratios.



(a)



(b)



(c)

Figure 1: PCB biodegradation: (a) Aerobic bacteria, (b) Fungus and (c) Anaerobic Bacteria

4. CONCLUSION

Modelling the biodegradation processes of PCB-contaminated dielectric oils became an arduous task that must be addressed with accurate and effective statistical techniques. However, in reported literature, the studies concentrating on mathematical frameworks describing the effects of several microbial consortiums and their degradation ability on PCB-contaminated dielectric oil, with the use of different substratum (as a source of energy and carbon) and aerobic/anaerobic atmospheres, are largely limited. To cover this gap, the present paper proposed a regression analysis which was concluded to be valid when representing the biodegradation process of PCB in different consortiums. This framework can be extended and replicated with a high level of efficacy in other bacterial consortiums being PCB-tolerant. This issue is even more important for both clients and workers from electrical industry who are exposed to suffer from organic and genetic affectations due to the significant toxicity level of PCB-contaminated dielectric oils.

Considering the case study, the results evidenced that both factors (*Observation period and Microbial consortium*) were found to be statistically significant on the *average PCB concentration.* Additionally, the *Aerobic bacteria* were found to be the consortium with the highest PCB-degradation ratio (–23.088 ppm/week) which highlights the relevance of oxygen in the breathing processes of these organisms. On the other hand, high and medium-high negative correlations were identified between *Observation period* and PCB concentration which evidences that as values of *time* increases, the values of *PCB concentration* tend to decrease.

In future research, it is recommended to work on developing mathematical models representing biodegradation treatments of contaminated environments that use degraded consortiums. Additionally, it is proposed to perform statistical methods to validate the effectiveness of new treatments eliminating PCB from objects and contaminated soils.

Acknowledgment

The authors would like to thank the support and funding of Universidad Libre (Barranquilla), Electricaribe, Colciencias and the program "Virginia Gutiérrez de Pineda" for young and innovative researchers.

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