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Modeling and Optimization of Aerobic Biodegradation Conditions of Gasoline and Diesel by a Bacterial Consortium

Leme Banock Lucie[1], Ntsama Mbala Claudine[2], Bilong Bilong Charles Félix[3]

[1]The University of Yaounde 1, Faculty of Sciences, Laboratory of Hydrobiology and Environment, P.O. Box 812 Yaounde, Cameroon
lemelucie[at]yahoo.com

[2]The University of Yaounde 1, Faculty of Sciences, Laboratory of Parasitology and Ecology, P.O. Box 812 Yaounde, Cameroon
ntsamaclaudine[at]yahoo.fr

[3]The University of Yaounde 1, Faculty of Sciences, Laboratory of Parasitology and Ecology, P.O. Box 812 Yaounde, Cameroon
bilong_bilong[at]yahoo.com

Abstract: ***Crude oil and its by-products are sources of environmental pollution. Their elimination by biological processes is part of the bioremediation of ecosystems. This study aims at developing models of aerobic biodegradation of gasoline and diesel and specifying the factors allowing the optimization of this biological process. The response surface methodology (RSM) was used with a centered 4-factors composite plan. The factors selected were the bacterial consortium concentration (x_1), temperature (x_2), dissolved oxygen (x_3) and the quantity of nutrients (x_4). The response measured was the rate of aerobic biodegradation. The experimental design consisted of 27 trials carried out in 10 days of incubation. The two second-order mathematical models obtained, for the rate of degradation of gasoline and diesel, were validated using different parameters. These are the values of the coefficient of determination R^2 (95.88%, 95.32%) close to 100%, the low average values of the residues (1.70 and 1.72) and the polarized factors between 0. 91 and 1.19 which indicate a strong correlation between the values of the percentage of measured biodegradation and those predicted by the models. The concentration of the bacterial consortium and the temperature in their linear (x_1 and x_2) and interactive ($x_1 x_2$) forms have a significant positive effect on the rate of biodegradation of gasoline and diesel ($P<0.05$). The aerobic biodegradation of gasoline is optimized at 80% and that of diesel at 70% when the bacterial concentration is 10^8 CFU/ml, the temperature is 30°C and the dissolved oxygen content is between 150 and 200 mg/l.***

Keywords: Modeling, Optimization, Biodegradation, Bacteria

1. Introduction

Hydrocarbons have an inherent toxic effect on several biological processes [1]. They differently affect the animal and plant kingdoms of the sea surface and depth. Fishery resources are mostly affected [2]. Regarding the consequences on human health, the massive inhalation or ingestion of oil products, the consumption of certain marine animals (fish, crustaceans, shellfish) which have been in contact with hydrocarbons may be dangerous for humans due to cumulative effects [3].

Gasoline and diesel are produced by refining crude oil. Automotive gasolines are mixtures of hydrocarbons that may have 4 to 10 carbon atoms [4] and lead-based anti-knock agents. Their composition varies with the origin of the crude oil, the refining processes and the additive substances. Gas oil or diesel is a diesel engine fuel. Obtained from the refining of crude oil, it is physically considered to be light fuel oil. Diesel is heavier than gasoline; it does not contain alkenes but rather is made up of less volatile products. It has 2000 to 4000 different hydrocarbons with carbon atoms ranging from 11 to 25 [5]. Its composition varies depending on the crude oil used and the refining process.

Table 1: Hydrocarbon constituents of gasoline and diesel

Hydrocarbon constituents	Gasoline [5]	Diesel [3]
Linear saturated hydrocarbons (n-alkanes or n- paraffins)	20-30%	24 %
Cyclic saturated hydrocarbons (cycloalkanes or naphthenes) and branched chain saturated hydrocarbons	7%	46%
Unsaturated hydrocarbons (alkenes)	30-45%	0%
Monocyclic (MAH) and polycyclic aromatic hydrocarbons (PAH)	30-45%	30%

Biodegradation is a set of mechanisms by which a compound is transformed into different by-products by the action of microorganisms [5]. These are molds, yeasts, microscopic fungi, bacteria. Biodegradation is the main natural process for decontaminating ecosystems. Its improvement is the basis of the techniques of bioremediation of pollutants in an environment [6].

The biodegradation of hydrocarbons by microorganisms called hydrocarbonoclasts was demonstrated in 1946 by ZoBell. Ever since, the number of bacterial species identified as possessing this property has continued to increase. In general, a bacterial species turns out to be unable to significantly metabolize a pollutant but a bacterial consortium would be more efficient because in bioremediation, the importance of the use of mixed cultures

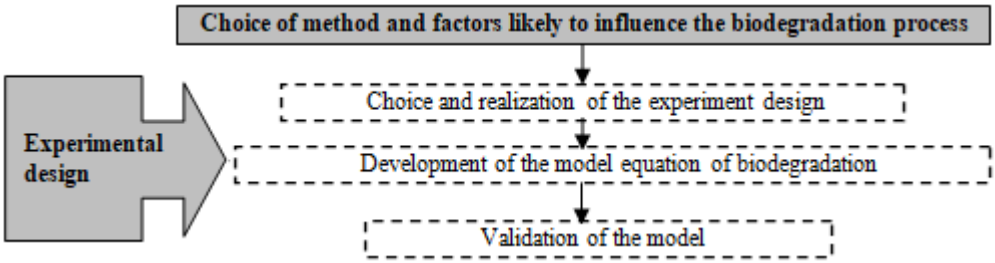
rather than pure cultures has been proved to be pollutants [7]. Their advantage is mainly related to the synergistic effects of interactions between microorganisms [8].

Several biological and physico-chemical factors influence the biodegradation process of petroleum hydrocarbons. Response surface methodology (RSM) is heavily used in process optimization, as it allows the effects of each factor to be determined separately and in interaction [9]. It uses the experiment designs that are effective experimental strategies to determine the optimal conditions for carrying out a process. This is a set of statistical and mathematical techniques used for the development, improvement and optimization of certain processes in which, an interest response is affected by several process variables and the objective being to optimize this response [10].

The aim of this study is to use the RSM method to determine the optimal experimental conditions for the degradation of gasoline and diesel by bacteria, and to examine both the individual and combined effects of the factors that influence this biological process.

2. Material and methods

The algorithm below presents the main steps in modeling and optimizing the biodegradation process inspired by [11] and [12]



Algorithm 1: Main steps in modeling and optimization

2.1. Origin of the bacterial consortium and petroleum products

The bacterial mixed culture used for hydrocarbons biodegradation contains six bacterial species collected from the beach waters of Kribi and Limbe cities in Cameroon. The criteria for selection were: the capacity of each bacteria to live into the pollutants (gasoline and diesel) for 35 days, as well as its weak pathogenic capacity according to the literature [13], [14]. Thus, selected bacteria were: *Achromobacter xylosoxidans*, *Alcaligenes denitrificans*, *Bacillus* sp., *Pseudomonas cepacia*, *Pseudomonas putida* and *Sphingomonas paucimobilis*. The bacterial consortium used for this work consisted of these six species.

In this study, we used the commercial sample of gasoline and diesel.

2.2. Experimental design of the aerobic biodegradation model and optimization

2.2.1. Choice of method and factors

To get the optimal conditions for biodegradation of gasoline and diesel, the response surface methodology (RSM) was used with a 4-factor centered composite plan adopted as the experimental design. Four variables were chosen. These are factors which according to the literature [15], [16] and our preliminary works had an influence on the process of biodegradation of pollutants (gasoline and diesel). These are: bacterial consortium concentration (x_1), temperature (x_2), dissolved oxygen (x_3) and the quantity of nutrient (x_4).

2.2.2. Choice and realization of the experiment design

The experiment design therefore consisted of 27 trials carried out within 10 days of incubation. The expected outcome (y) is the percentage of aerobic biodegradation of gasoline or diesel. During the tests, control samples and test samples were made.

Sample control: two types of control samples were made. The first contained 100 ml of Bushnell Haas Broth (BHB) Medium with 7.5 ml of gasoline or diesel. The second contained different concentrations of bacterial consortium (0, 10^4, 10^8CFU/ml) and 100 ml of BHB medium.

Sample tests: different concentrations of the bacterial consortium (0, 10^4, 10^8 CFU/ml) were introduced into an Erlenmeyer flask containing 100 ml of BHB medium with 7.5 ml of gasoline and diesel. Depending on the test conditions, these Erlenmeyer flasks were incubated at different temperatures (10, 15, 30°C), different oxygenation (50, 150, 300 mg/l) and different nutrient contents (10, 50, 100 ml).

The percentage of aerobic biodegradation of the hydrocarbon constituents of gasoline and diesel was evaluated using Fourier's Infrared Spectroscopy for transformation, with Attenuated Total Reflection (IRTF-ATR). For this purpose, spectral data were collected. The spectra were pretreated and a chemometric analysis was performed [17]. During the 27 experiments in the experimental design, an operation to transform the real variables into coded ones (1) was performed (table 2). This operation made it possible to make the effects of the real variables, which are not necessarily expressed in the same units, comparable. The transformations were made using the following methods [18], [22]:

$$x_j = \frac{U_j - U_j^\circ}{\Delta U_j} \text{ et } \Delta U_j = \frac{U_{j\max} - U_j^\circ}{x_{j\max}} = \frac{U_j^\circ - U_{j\min}}{x_{j\min}} \quad (1)$$

With: xj = value of the coded variable, Uj = value of the natural variable,
ΔUj = amplitude variation, $Uj\ min$ = minimum value of the natural variable,

Uj ° = value of the natural variable j at the center of the domain,
Uj max = maximum value of the natural variable j,
xjmax = maximum value of the coded variable, *xjmin* = minimum value of the coded variable.

Table 2: Transformation of the values of real variables into values of coded variables

Parameters	Variables	Real Values	Coded Values
Bacterial concentration (CFU /ml)	x_1	Maximum : 10^8	+1
		Center : 10^4	0
		Minimum : 0	-1
Temperature (°C)	x_2	Maximum : 30	+1
		Center : 15	0
		Minimum : 10	-1
Dissolved oxygen (mg/l)	x_3	Maximum : 300	+1
		Center : 150	0
		Minimum : 50	-1
Nutrient content (ml)	x_4	Maximum : 100	+1
		Center : 50	0
		Minimum : 10	-1

The matrices of real variables (Mr) were thus obtained using each xj matching the matrix of coded variables (Mc) (tables 3 and 4).

Development and validation of the model equation of biodegradation

After carrying out the experimental design, the model equation and its coefficients were defined (2). The general equation of the second-degree polynomial model for response Y (rate of biodegradation) is as follows:

$$y = \beta_0 + \sum_{j=1}^{k} \beta_j x_j + \sum_{j=1}^{k} \beta_{jj} x_j^2 + \sum\sum_{i<j}^{k} \beta_{ij} x_i x_j + \varepsilon \quad (2)$$

β*o** = constant, **ε** = error, ***βj, βjj et ***βij*** = model coefficients, ***y*** = response (biodegradation rate), **xj** = coded value of parameter j.

The following equation is the matrix expression of this model : $y = M\beta + \varepsilon$ which, in terms of matrix i (3) :

$$\begin{bmatrix} y_1 \\ y_2 \\ . \\ . \\ . \\ y_n \end{bmatrix} = \begin{bmatrix} 1 & x_{11} & x_{12} & . & . & x_{1k} \\ 1 & x_{21} & . & . & . & x_{2k} \\ . & . & . & . & . & . \\ . & . & . & . & . & . \\ . & . & . & . & . & . \\ 1 & x_{n1} & x_{n2} & . & . & x_{nk} \end{bmatrix} \times \begin{bmatrix} \beta_0 \\ \beta_1 \\ . \\ . \\ . \\ \beta_k \end{bmatrix} + \begin{bmatrix} \varepsilon_0 \\ \varepsilon_1 \\ . \\ . \\ . \\ \varepsilon_k \end{bmatrix} \quad (3)$$

The resolution of this system of equations was performed by the method of least squares (MLS), assuming that random errors were identically distributed with mean zeros and an unknown common variance, independent of one another. For each observation *i*, the difference between the obtained value *yi* and the adapted one *ŷi* was obtained by this method (4):

$$\varepsilon_i = y_i - y_i \quad (4)$$

The evaluations of the ßj were made so that, as much as possible, they reduce the sum of squared errors (SSE) which are expressed as follows (5):

$$SSE = \sum_{i=1}^{n} \varepsilon_i^2 = \sum \left(y_i - \hat{y}_i \right)^2 \quad (5)$$

The evaluation of the residues (errors) was carried out with the following equation (6):

$$\varepsilon = y - M\beta \quad (6)$$

Considering the above equation, SSE is written as follows (7):

$$SSE = \varepsilon^T \varepsilon = \left(y - M\beta \right)^T \left(y - M\beta \right) \quad (7)$$

Finding out the partial derivative of SSE with respect to that of β, the following family of equations is obtained: $y = M\beta$. The resolution of this last equation leads to this solution (8):

$$\beta = \left(M^T M \right)^{-1} M^T y = C M^T y \quad (8)$$

where C is the square matrix expressed as $C = (M^T M)^{-1}$

The validation of the mathematical models of biodegradation of gasoline and diesel was carried out by the analysis of the determination coefficient R^2, the mean value of the residues and the comparative method of polarized factors.

2.3. Other statistical analyzes

The matrix operations to evaluate the β vector were performed using Statgraphics Centurion software which also helped to do the Durbin-Watson (DW) test of serial autocorrelation of the residue. The graphs were plotted using Sigmaplot software.

3. Results and discussion

3.1. Results of the experimental design

The results of the realization of the experimental design (Tables 3 and 4) made it possible to develop mathematical models represented by the following second degree polynomials:

-for gasoline: **Y** = 64,5789 + 28,25x_1 + 12,*9167*x_2 + 6,25x_3 + 3,58333x_4 - 27,4803x_1^2 + 11,3553x_1x_2 + 8,60526x_1x_3 + 3,8552x_1x_4- 5,48026x_2^2 + 4,60526x_2x_3 + 8,85526x_2x_4–8,48026x_3^2- 9,89474x_3x_4 - 8,23026x_4^2.

-for diesel: **Y**= 50,0 + 25,0x_1 + 11,8333x_2 + 4,58333x_3 + 1,08333x_4 -19,875x_1^2 + 11,25x_1x_2 +7,5x_1x_3 + 3,75x_1x_4 - 8,375x_2^2 + 3,754,60526x_2x_3 + 7,0x_2x_4 - 3,0x_3^2+ 0,0x_3x_4 - 4,0x_4^2.

With x_1 = bacterial concentration; x_2 = temperature; x_3 = dissolved oxygen;
x_4 = the quantity of nutrients; **Y** = percentage of biodegradation

Table 3: Experimental results of the composite central plan of gasoline degradation rate

N° Exp.	Coded values making a matrix (Mc)				Natural values making a matrix (Mr)				Biodegadation rate Y (%)	
	x_1	x_2	x_3	x_4	x_1	x_2	x_3	x_4	Actually measured (**Yrel**)	Predicted by model (**Ypre**)
1	0	-1	0	-1	10^4	10	150	10	45.5	50.6
2	0	-1	1	0	10^4	10	30	50	43.7	42
3	-1	0	0	1	0	10	150	100	0	0.1
4	0	1	1	0	10^4	30	300	50	50.1	53.9
5	1	1	0	0	10^8	30	150	50	81.8	78
6	-1	0	0	0	0	10	150	50	0	0.1
7	0	0	0	0	10^4	10	150	50	44.7	42.9
8	1	1	1	1	10^8	30	300	100	58.3	60.13
9	-1	0	0	1	0	10	150	10	0	0.2
10	-1	0	-1	0	0	15	50	50	0	0.1
11	1	-1	0	0	10^8	10	150	50	62.4	59.7
12	1	0	0	-1	10^8	15	150	10	68.7	67.8
13	-1	-1	0	0	0	10	150	50	0	0.2
14	0	0	1	-1	10^4	15	300	10	48.7	48.8
15	1	0	-1	0	10^8	10	50	50	61.5	60.9
16	0	1	-1	0	10^4	30	50	50	49.4	48.7
17	-1	1	0	0	0	30	150	50	0	0.2
18	0	-1	-1	0	10^4	10	50	50	40.1	40.8
19	1	0	0	1	10^8	15	150	100	72.8	77.82
20	0	-1	0	1	10^4	10	150	100	41.4	40.7
21	0	-1	0	-1	10^4	30	150	10	50.1	48.7
22	0	0	0	1	10^4	15	150	100	47.2	42.6
23	-1	-1	-1	-1	0	10	50	10	0	0
24	1	0	0	1	10^8	15	150	100	70.7	69.7
25	0	0	1	1	10^4	15	300	100	48	45.3
26	0	0	-1	-1	10^4	15	50	10	47.4	50.7
27	0	1	0	10	10^4	30	150	100	46.6	50.1

Table 4: Experimental results of the composite central plan of diesel degradation rate

N° Exp.	Coded values making a matr (Mc)				Natural values making a matrix (Mr)				Biodegradation rate Y (%)	
	x_1	x_2	x_3	x_4	x_1	x_2	x_3	x_4	Actually measured (**Yrel**)	Predicted by the model (**Ypre**)
1	-1	0	-1	0	0	15	50	50	0	0
2	1	-1	0	0	10^8	10	150	50	58.9	54.5
3	0	0	0	0	10^4	15	150	50	35.7	40.72
4	0	0	1	1	10^4	15	300	100	35.6	35.68
5	-1	0	0	-1	0	15	150	10	0	0
6	1	1	0	0	10^8	30	150	50	72.5	70
7	1	1	0	-1	10^8	30	150	10	68.8	68.17
8	0	0	-1	-1	10^4	15	50	10	36	36.7
9	0	1	1	0	10^4	30	300	50	45	45.11
10	0	-1	0	1	10^4	10	150	100	31.1	26.64
11	-1	0	0	1	0	15	150	100	0	0.1
12	1	0	0	-1	10^8	15	150	10	59.6	58.7
13	-1	1	0	0	0	30	150	50	0	0.1
14	-1	-1	-1	-1	0	10	50	10	0	0
15	0	-1	1	0	10^4	10	300	50	30.8	28.18
16	-1	0	0	0	0	15	150	50	0	0.3
17	0	1	0	1	10^4	30	150	100	46.7	51.9
18	0	1	0	-1	10^4	30	150	10	46.8	51.41
19	0	0	1	1	10^4	15	300	100	35.7	37.3
20	0	-1	-1	0	10^4	10	50	50	30.6	31.5
21	1	0	1	0	10^8	15	300	50	59.4	55.5
22	0	0	-1	1	10^4	15	50	100	35.4	30.7
23	-1	-1	0	0	0	10	150	50	0	0.1
24	0	-1	0	-1	10^4	10	150	10	30.7	33.9

25	1	0	-1	0	10^8	15	50	50	59	60.9
26	1	0	0	1	10^8	15	150	100	59.8	58.7
27	0	1	-1	0	10^4	30	50	50	40.7	41.1

3.2. Validation of models

As Table 5 goes, the validation parameters (R^2, residues, and polarized factors) make it possible to validate the produced biodegradation models of gasoline and diesel. The reason is that the values of the coefficient of determination R^2 close to 100% indicate a strong correlation between the values of the measured biodegradation rate and those predicted by the models. Therefore, the adjusted models of our experiments respectively accounted for 95.88% and 95.32% of the variance of gasoline and diesel biodegradation. These results match those of [23] who obtained a coefficient of determination R^2 = 0.9565 during the biodegradation of petroleum hydrocarbons by a bacterium.

The relatively low average values of the residues obtained (1.70 and 1.72) reveal that at particular points chosen in the experimental field, adjusting the model to the observed data is satisfactory; therefore the deviation between Yrel and Ypre is small. In addition, the Durbin-Watson (DW) statistic gives a probability P>0.05 showing that there is no serial autocorrelation of the residues.

The polarized factors Bf and Af between 0.91 and 1.19 certify that there is not a large difference between the predicted and measured values of the percentage of biodegradation of gasoline and diesel.

Table 5: Validation parameters of the biodegradation models

	Coefficient of determination R^2(%)	Coefficient of determination adjusted R^2(%)	DMA Mean residue value	Polarized factor (B_f)	Polarized accuracy factor (A_f)
Gasoline	98.09	95.88	1.70	0.94	1.12
Diesel	97.84	95.32	1.72	0.91	1.19

3.3. Model optimization

3.3.1. Estimation of the quantitative effects of factors on biodegradation

Table 6 show that the bacterial concentration and the temperature in their linear (x_1 and x_2) and interactive (x_1 x_2) forms have a significant positive effect on the rate of gasoline biodegradation (P<0.05). For the case of diesel, a significant positive effect (P<0.05) was observed on the bacterial concentration, on the temperature in their linear (x_1 and x_2), quadratic (x_1^2, x_2^2) and interactive (x_1 x_2) forms, then dissolved oxygen in its linear form (x_3), as well as bacterial concentration-dissolved oxygen (x_1 x_3) and temperature-nutrient (x_2 x_4) interactions. Some factors have a negligible effect on the biodegradation process (P> 0.05). The bacterial concentration improved the biodegradation of gasoline and diesel thanks to the biosurfactants released by the bacteria and the efficiency of their enzyme systems [24]. The positive impact of temperature in this process would be due to the fact that on the one hand, it modifies the physical state and the chemical composition of gasoline and diesel [25] and, on the other hand, it influences the physiological activity of bacteria. This statistically significant effect of temperature on the biodegradation of hydrocarbons was also observed by Shuo *et al* [22]. Naturally, bacteria need a strong supply of oxygen and nutrients to act, but in our case they may have found another source of nutrients including hydrocarbons. This may account for the insignificant impact of these two parameters on biodegradation phenomenon.

Table 6: Probabilities of the factors on the biodegradation of gasoline and diesel

Factors	P (for gasoline)	P (for diesel)
x_1	**0.0023**	**0.0010**
x_2	**0.0476**	**0.0010**
x_3	0.5322	**0.0066**
x_4	0.0744	0.4532
x_1^2	0.1592	**0.0013**
x_2^2	0.4079	**0.0018**
x_3^2	0.0532	0.1779
x_4^2	0.8338	0.0806
x_1x_2	**0.0270**	**0.0006**
x_1x_3	1.0000	**0.0092**
x_1x_4	0.2870	0.1473
x_2x_3	0.0557	0.1473
x_2x_4	1.0000	**0.0135**
x_3x_4	0.2870	1.0000

x_1 = bacterial concentration; x_2 = temperature; x_3 = dissolved oxygen; x_4 = the quantity of nutrients

In the course of this study, the bacterial concentration of the medium (x_1) directly and gradually increases the percentage of biodegradation up to a maximum of 70% for gasoline (figure 1) and 58% for diesel (figure 2). This effect was potentiated when its action was associated with that of the temperature (x_1 x_2) because the biodegradation rate of gasoline then exceeded 78% (figure 1) and that of diesel over 70% (figure 2). The interaction between the bacterial concentration of the medium and its dissolved oxygen content (x_1 x_3) and the one between this same bacterial concentration and the amount of nutrients (x_1 x_4) respectively generated 74% and 70% of the biodegradation of petroleum hydrocarbons. In the case of diesel, these percentages are 68% and 64%.

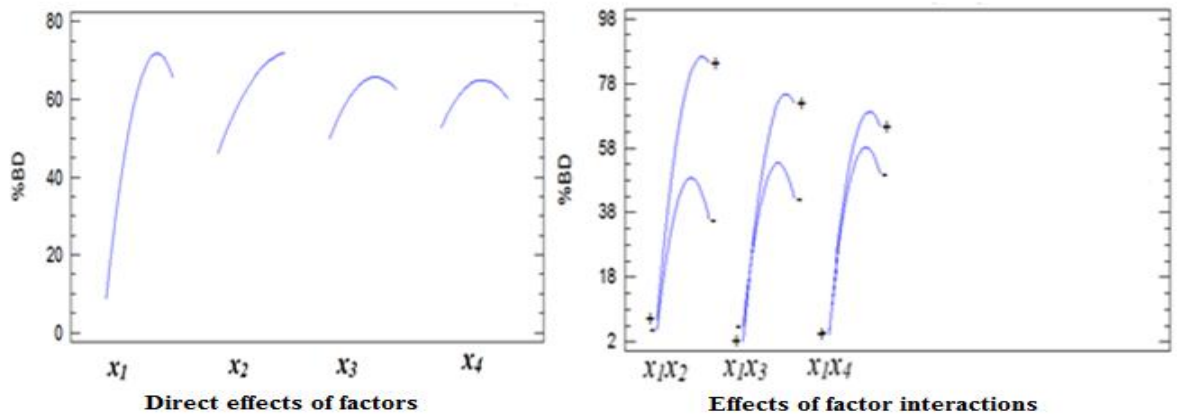


Figure 1: Direct and interactive effects of factors on the rate of gasoline biodegradation.

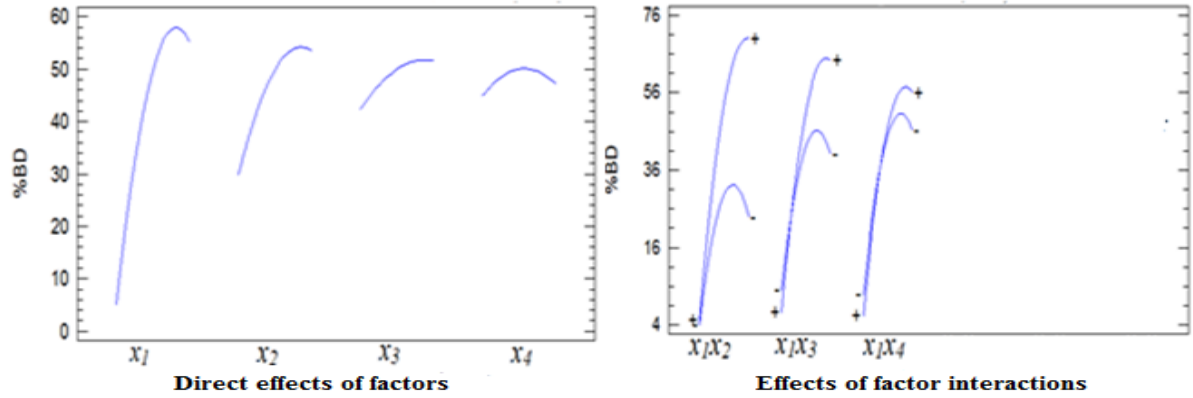


Figure 2: Direct and interactive effects of factors on the biodegradation rate of diesel.

3.3.2. Determination of the optimum biodegradation value of gasoline

The hatched area of the isoresponse curve shows that to exceed a biodegradation percentage of 80%, the temperature must be between 25°C and 30°C and the bacterial concentration of the medium must be between $8x10^7$FCU/ml et 10^8 CFU/ml (figures 3 and 5). However, by setting the temperature at 30°C and the bacterial concentration in the experimental chamber at 10^8 CFU/ml, the biodegradation rate of petroleum hydrocarbons was optimized at 80% when the dissolved oxygen content oscillates between 150 and 200 mg/l (figures 3 and 4). The results obtained by Mustafa *et al* [26] revealed that at a temperature of 30°C, 95.87% of PAHs are completely degraded by the fungus *Aspergillus flavus*.

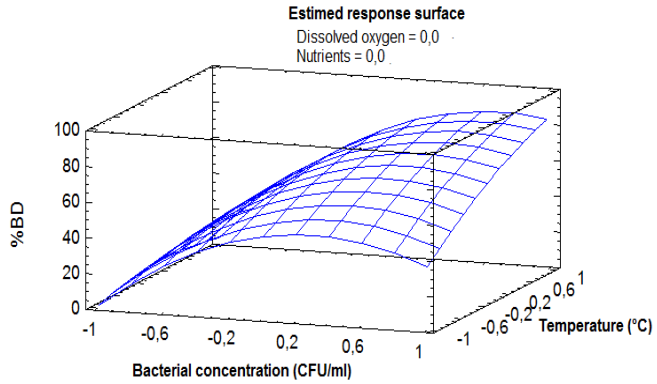


Figure 3: Response surface in coded values

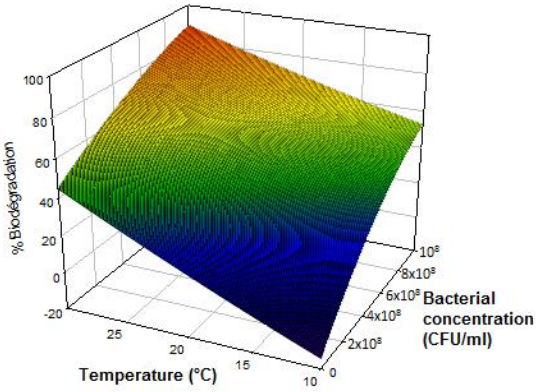


Figure 4 : Response surface in real values

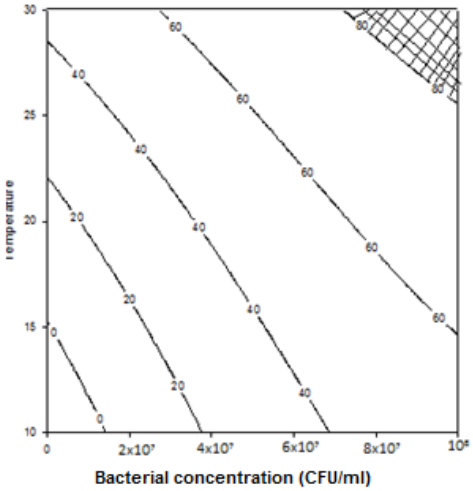


Figure 5: Isoresponse curves

3.3.3. Determination of the optimal biodegradation value of diesel

The hatched area of the isoresponse curves shows that to exceed a percentage of diesel biodegradation of 65%, the temperature must be between 22°C and 30°C and the bacterial concentration of the experimental medium must be between 9 x 10^7CFU/ml et 10^8CFU/ml (figures 6 and 8). By setting the temperature at 30°C and the bacterial concentration of the experimental medium at 10^8CFU/ml, the biodegradation rate was optimized at 70% when the dissolved oxygen content oscillates between 150 and 200 mg/l (figures 6 and 7).

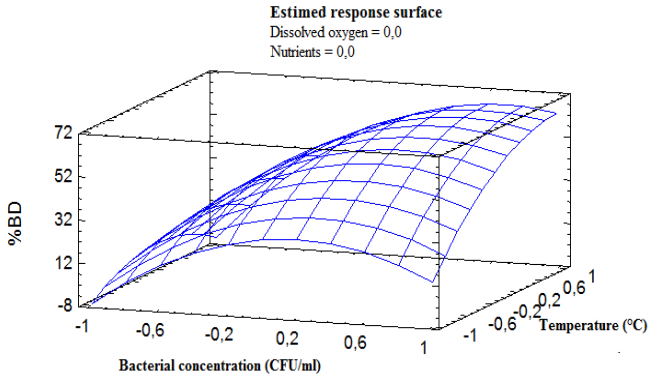


Figure 6: Response surface in coded values

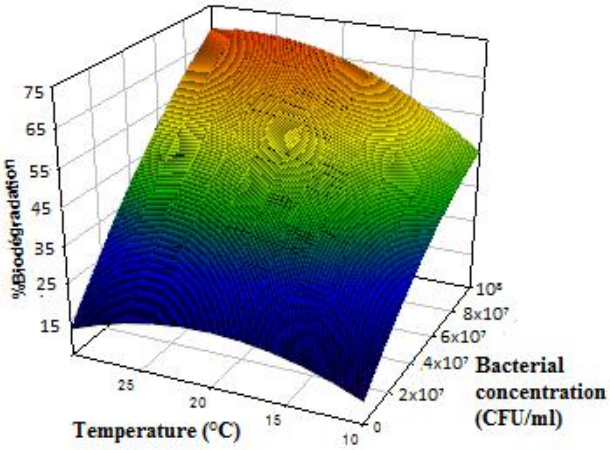


Figure 7: Response surface in real values

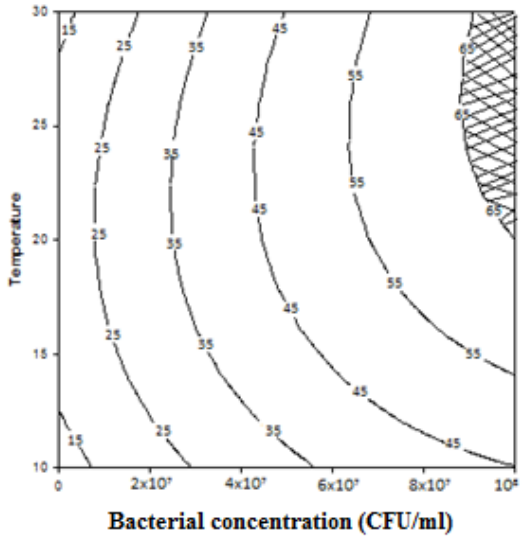


Figure 8: Isoresponse curves

Since it is difficult to degrade diesel in view of its density, viscosity and high molecular weight [5], after optimization, its rate of biodegradation was lower than that of gasoline.

4. Conclusion

The aerobic biodegradation process of gasoline and diesel is greatly influenced by temperature and bacterial concentration. Bacteria use hydrocarbons like nutrient and can sometimes take anaerobic mechanisms to degrade hydrocarbon compounds in gasoline and diesel. The two experimental designs made weakly represent this phenomenon of biodegradation.

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Author Profile

Leme Banock Lucie received the p.h.D Doctorate degree in Hydrobiology and Environment from the University of Yaounde 1, Cameroon in 2016. During 2016-2019, she was chemical engineer in water treatment at ASPAC International Company (Cameroon/Belgium). Since 2020 she is assistant lecturer at the Faculty of Sciences, University of Yaounde 1.