



## SURVIVAL RESPONSE OF CONSORTIUM ISOLATES FROM DIESEL CONTAMINATED SOIL WITHIN KATSINA STATE, NIGERIA

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### Abstract

Environmental increase in the spilled diesel creates serious damages to our natural ecosystems. Biodegradation provide the best removal alternative for such diesel contamination. This study was carried out to monitor the bacteria survival response during diesel oil biodegradation. Bacteria isolation was carried out using plating technique and screened at varying diesel concentrations (0 to 10%, v/v) before being identified using the 16S rRNA gene sequencing. Bacteria consortia were formulated using the screened isolates through mathematical permutation approach. Prepared bacteria resting cells of the selected consortium was grown at 2%, v/v diesel oil which was co-contaminated with varying concentrations of Mg<sup>++</sup>, Mn<sup>++</sup>, Zn<sup>+</sup>, Co<sup>++</sup> and Fe<sup>++</sup> (0 to 4 g/L) in mineral salts medium. Total of 47 different bacteria strains were isolated and the finally screened isolates were identified as *Alcaligenes* sp., *Ochrobactrum* sp., *Alcaligenes aquatilis* and *Alcaligenes faecalis* UMYU001 (MN519483.1). These bacteria were found to be compatible with one another despite being obtained from different environments. Of the prepared consortia, combination 11 (CST11) was found to produce the highest population of  $1.6 \times 10^4$  cfu/mL after 48 hours at 2% v/v diesel oil. This CST11 survived optimally at 35<sup>0</sup>C, 7.0, and 2% v/v of the temperature, pH and diesel oil respectively. Also, the resistance thresholds of the metals for CST11 include Mg<sup>++</sup> (3.5 g/L) while Mn<sup>++</sup>, Zn<sup>++</sup>, Co<sup>++</sup> and Fe<sup>++</sup> were only resisted at 1 g/L. This recommends the consortium as good enough in surviving at the sole expense of diesel oil even in the presence of heavy metals co-contaminants.

Keywords: Biodegradation, Diesel, Metals resistance.

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## 1. Introduction

Human civilisation enhances global technological advances in the field of rising energy demand that majorly depends on fossil fuels (Somee *et al.*, 2018). Certain percentage of such fuels ended up being spilled onto the natural environment through variety of processes ranging from exploration to utilisation while affecting the natural ecosystems adversely (Spini *et al.*, 2018). To deter and dissuade these spillage-induced catastrophic aftermaths, reclamation methods are devised (Wu *et al.*, 2019). Among such remediating techniques are the physico-chemical approaches which nonetheless may have inherently debilitating side effects. Bioremediation is the most promising alternative to remediating these contaminated soils, and it involves the application of microorganisms such as fungi and bacteria to remediate polluted soils efficiently, effectively and efficaciously (Umar *et al.*, 2016; Habib *et al.*, 2018). However, heavy metals are found as co-contaminants in the spillage environments frequently in higher concentrations which further increase the remediating challenge (Sharma *et al.*, 2018; Umar *et al.*, 2018a).

The use of single microbial strain in biodegradation is usually faced with certain challenges due to unilateral nature of the organism and complexity of the chemical substrate (Lee *et al.*, 2019). However, microbial consortia could provide better opportunity due to co-metabolism, synergy and enzymatic diversification (Umar *et al.*, 2018a; 2017; Bradacova *et al.*, 2019). Moreover, well performing strains in the laboratory studies may fail to perform in the field experiments as optimal conditions are necessary for effective microbial performance (Umar *et al.*, 2018b). Furthermore, the use of heavy metal tolerant strains in the field of biodegradation is advantageous due to rising levels of inorganic co-contaminants in the affected sites (Nwagu *et al.*, 2017). This study was therefore aimed at assessing the survival nature of bacteria consortium at the sole expense of diesel oil. Such assessments include bacteria identification, consortia formulation, physico-chemical parameters optimization, and heavy metals tolerance of the isolates.

## 2. Materials and Method

### 2.1 Chemicals and Media

Diesel oil used during the experiments was purchased from the Nigerian National Petroleum Company (NNPC) petrol station in Katsina metropolis, Nigeria. All other chemicals used were obtained from the standard manufacturers and are of analytical grade. Reagents and media were prepared using de-ionised and distilled water respectively (Atlas, 2005). Bushnell Haas (BH) Agar content of  $\text{KH}_2\text{PO}_4$ ,  $\text{K}_2\text{HPO}_4$ ,  $\text{NH}_4\text{NO}_3$  with 1 g/L each and  $\text{MgSO}_4$  (0.2 g/L),  $\text{FeCl}_3$  (0.05 g/L),  $\text{CaCl}_2$  (0.02 g/L), Agar-Agar (15 g/L) were used (Atlas, 2005). Phosphate Buffer Saline (PBS) was also prepared using the protocols of Atlas, (2005) having the content of  $\text{NaCl}$  (8g/L),  $\text{KCl}$  (0.2g/L),  $\text{Na}_2\text{HPO}_4$  (1.44g/L) and  $\text{KH}_2\text{PO}_4$  (0.24 g/L). All media were sterilized at  $121^\circ\text{C}$  for 20 minutes before being used and experiments were conducted in replications.

## 2.2 Soil sampling

Diesel contaminated Soil were sampled from 12 different locations covering the entire three zones of Katsina State (Katsina, Funtua and Daura) which are located between 12°59' N latitude and 7°36' E longitude. Samples from all the sampling points designated as letters A to L were collected in sterile polythene bags within 0 to 10 cm depth after clearing the surface litter. Temperature of the sampling points were taken using handheld digital thermometer (ST9283B, MexTech) while the pHs were recorded with pH meter (pH 100, EXTECH, USA) after preparing a slurry of each sample. All samples were processed at the Microbiology research laboratory of Umaru Musa Yar'adua University Katsina within few hours of collection.

## 2.3 Bacteria isolation, screening and identification

Bacteria within the soil samples were isolated using serial dilution and plating techniques (Barrow and Feltham, 1993). Each dilution was cultured in replications on the prepared Nutrient Agar (NA) plates for 24 hours at 37°C. Colonies were purified on freshly prepared NA medium using the same culture conditions and pure isolates were preserved. Bacterial resting cells (BRC) for the individual strains were prepared, counted and separately used as inoculums during the screening process (Sogani *et al.*, 2012). The BRC counting was performed using British standard Naubeur haemocytometer with 0.4% w/v trypan blue reagent where  $1.5 \times 10^5$  cell/mL was obtained for each isolate. Based on the microscopic BRC counting, living cells were calculated using the following formula adopted from Doyle and Bryan (1998):

$$\text{Total viable cells (cells/mL)} = X \times Y \times Z \times 10^4 \quad (1)$$

Where,

X = volume of the inoculums used in microscopy (0.1 mL)

Y = dilution factor of cells in trypan blue

Z = average number of living cells counted per square

$10^4$  = conversion factor of  $0.1 \text{ mm}^3$  (area of square) to mL

One hundred micro liter of each BRC in PBS suspension was inoculated separately on BH Agar containing different diesel quantities (0%, 2%, 4%, 6%, 8% and 10% v/v) as the sole carbon source. These were grown at 37°C aerobically and plates were monitored on 24 hours' basis where colonies formed were used to indicate the survival ability of the organisms at the sole expense of diesel oil (Umar *et al.*, 2016). Identification of the diesel degrading bacteria was based on a three-tiered procedure adapted from Cowan and Steel's Manual and Bergy's Manual of Deterministic Bacteriology (de Vos *et al.*, 2003; Barrow and Feltham, 1993). Biochemical tests involving catalase, oxidase, urease, casein, starch, citrate, H<sub>2</sub>S, mannitol and MR-VP were also carried out (Barrow and Feltham, 1993; Kamzolova *et al.*, 2010; Hemraj *et al.*, 2013; Nikhil *et al.*, 2013; Karthika *et al.*, 2014). Further identification was carried out using 16S rRNA gene sequencing using the universal primers (Forward; 5'-GGACTACAGGGTATCTAAT-3' and Reverse: 5'-AGAGTTTGATCCTGG-3').

## 2.4 Formulation of diesel degrading consortia

Purity test was initially carried out from the screened bacteria using plating culture for 24 hours at 37°C (Wang *et al.*, 2011). Bacteria resting cells involving the screened strains at  $1.5 \times 10^5$  cells/mL for each strain was prepared separately and used as inoculums. These inoculums were used to prepare diesel degrading consortia based on the protocols adopted from Sarkar *et al.*, (2011). Initially, compatibility testing was performed by cross spreading all the screened strains simultaneously on a single NA plate and incubated for 24 hours at 37°C (Raja *et al.*, 2006). Mixed appearance of colonies indicates all the screened bacteria as compatible to one another. Experimental repetitions for the consortia formulation was calculated using the combined mathematical permutation. This calculation was represented by the following formula as adopted from Rosen, (2007).

$$E = \frac{n!}{(n-r)!r!} \quad (2)$$

Where,

E = total number of experimental repetitions,

n = total number of bacteria involved (4),

r = number of bacteria per consortium,

! = factorial sign.

The component for each consortium was evaluated by the combination formula adopted from Laisin *et al.* (2012). Hence, 11 variegated consortia named CST1 to CST11 were formulated for each of 2% and 4%, whereby the isolates were arbitrarily represented by the alphabets A34, A35, A36 and A38. These were made by combining equal proportions of the individual bacteria resting cells ( $1.5 \times 10^5$  cells/mL each). From each formulated consortium, 100µL aliquots of the inoculums was inoculated onto BHA medium containing 2% v/v and 4% v/v diesel concentration separately (Umar *et al.*, 2017). The plates were incubated at 37°C for up to 192 hours with constant monitoring on 24 hours' basis for colony appearance (Karthika *et al.* 2014). This criterion was used in selecting the final consortium.

## 2.5 Optimization of the diesel degradation culture

During initial screening, the culture conditions used were temperature (37°C), salinity (0 g/L), pH (7.0), diesel concentration (2%, v/v). These factors were optimized using one factor at a time analyses (Chan *et al.*, 2009). The parameters range used include pH (5, 6, 7, 8, 9, 10, 11, 12), Temperature (20°C, 25°C, 30°C, 35°C, 40°C, 45°C, 50°C), salinity (0g/L, 5g/L, 10g/L, 15g/L, 20g/L, 25g/L), and diesel concentration (0%, 2%, 4%, 6%, 8%, 10%, v/v), as modified from Chen *et al.* (2012). For each optimization analysis, best performing parameter value replaces the previously used value for the subsequent experiments. Initially, the diesel degradation pH was optimized by adjusting the BH medium pH using filter sterilized hydrochloric acid (0.1M) and sodium hydroxide solution (0.1 M). Wide pH range of 5 to 12 was selected due to rare documentation of pH effect on diesel biodegradation and results were recorded on 24 hours basis up to 7 days (Pawar, 2015).

The temperature optimization was performed using the best selected pH while incubation temperature was varied as 25<sup>0</sup>C to 50<sup>0</sup>C with the remaining culture conditions being maintained as those in the previous experiments. The plates were subsequently incubated and the growth of the bacteria was measured at 24 hours intervals for 5 days. Salinity optimization was conducted using 0 to 25 g/L sodium chloride (w/v) at 24-hours intervals for 7 days. Substrate optimization was done by incorporating different concentrations of diesel oil (0% v/v to 10% v/v) into the prepared BH agar. The pH, salinity and temperature were adjusted to reflect the optimum values obtained. Bacteria population was measured at 24 hour intervals for 7 days.

### **2.6 Heavy metals tolerance on diesel degrading Consortium**

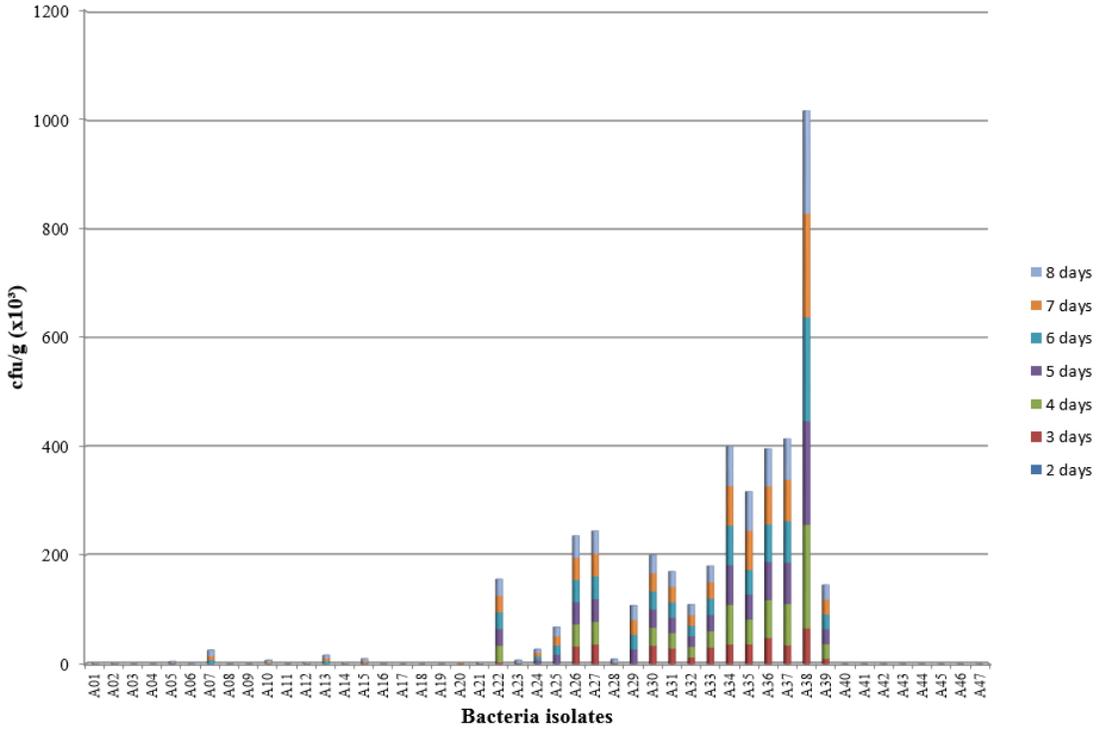
The effects of Mg<sup>++</sup>, Mn<sup>++</sup>, Co<sup>++</sup>, Fe<sup>++</sup> and Zn<sup>++</sup> were tested on the diesel degrading consortium from the previous experiments based on the method adapted from Nwagwu *et al.* (2017). This was performed using the BH agar supplemented with 2% filter sterilized diesel oil. Experimental treatments were performed using different concentrations (1 to 4 g/L) of the selected heavy metals at the optimum conditions.

### **2.7 Data analysis**

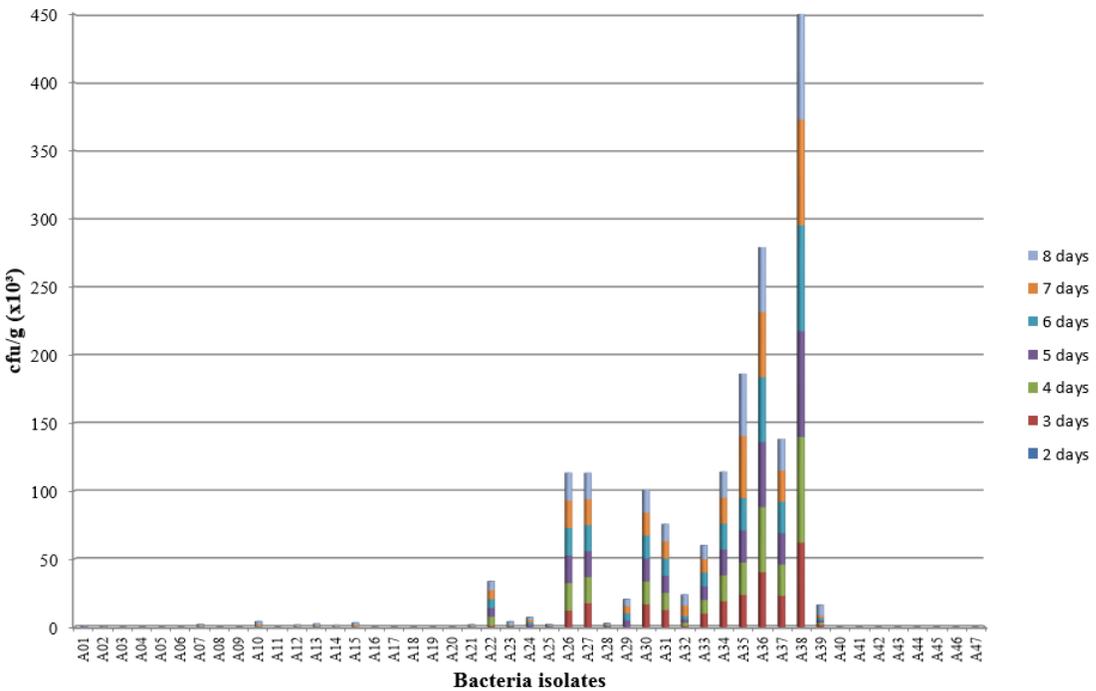
The data obtained from the diesel biodegradation were statistically analyzed using one way analyses of variance (ANOVA) at 95% confidence limit. Replicates data were presented as mean ± standard deviation based on the Tukey's HSD and Duncan's Multiple Range Test (DMRT).

## **3. Results**

During bacteria isolation, 47 different strains were obtained from the 12 samples of diesel contaminated soil based on plating techniques where profuse populations were observed with different morphology. These bacteria were screened on varying concentrations of filter sterilized diesel oil as the sole carbon source in BH agar. A total of 30 different strains produced colonies range of 2.0 x 10<sup>2</sup> cfu/mL to 1.9 x 10<sup>6</sup> cfu/mL in 2%, v/v diesel oil after 192 hours (Fig 1). When subjected to higher diesel concentration of 4% v/v, only 27 strains produced a population range of 3.0 x 10<sup>2</sup> cfu/mL to 7.8 x 10<sup>4</sup> cfu/mL which shows tremendous reduction from what was obtained at 2% diesel oil (Fig 2). The entire 47 isolates were also subjected to increased diesel oil of 6% which indicates no colony was produced even after 192 hours. Based on the screening results, four isolates designated as A34, A35, A36 and A38 produced 7.3 x 10<sup>4</sup>, 7.2 x 10<sup>4</sup>, 7.0 x 10<sup>4</sup>, 1.9 x 10<sup>5</sup> cfu/mL and also 1.9 x 10<sup>4</sup>, 4.6 x 10<sup>4</sup>, 4.8 x 10<sup>4</sup>, 7.8 x 10<sup>4</sup> cfu/mL at 2% and 4% v/v diesel oil respectively (Fig 1 and 2). These were found to be the highest recorded bacteria response and the strains were selected for further analyses.



**Figure 1:** Screening of bacteria isolates using 2% v/v diesel oil as sole carbon source in BHA at 37°C incubation temperature.



**Figure 2:** Screening of bacteria isolates using 4% v/v diesel oil as sole carbon source in BHA at 37°C incubation temperature.

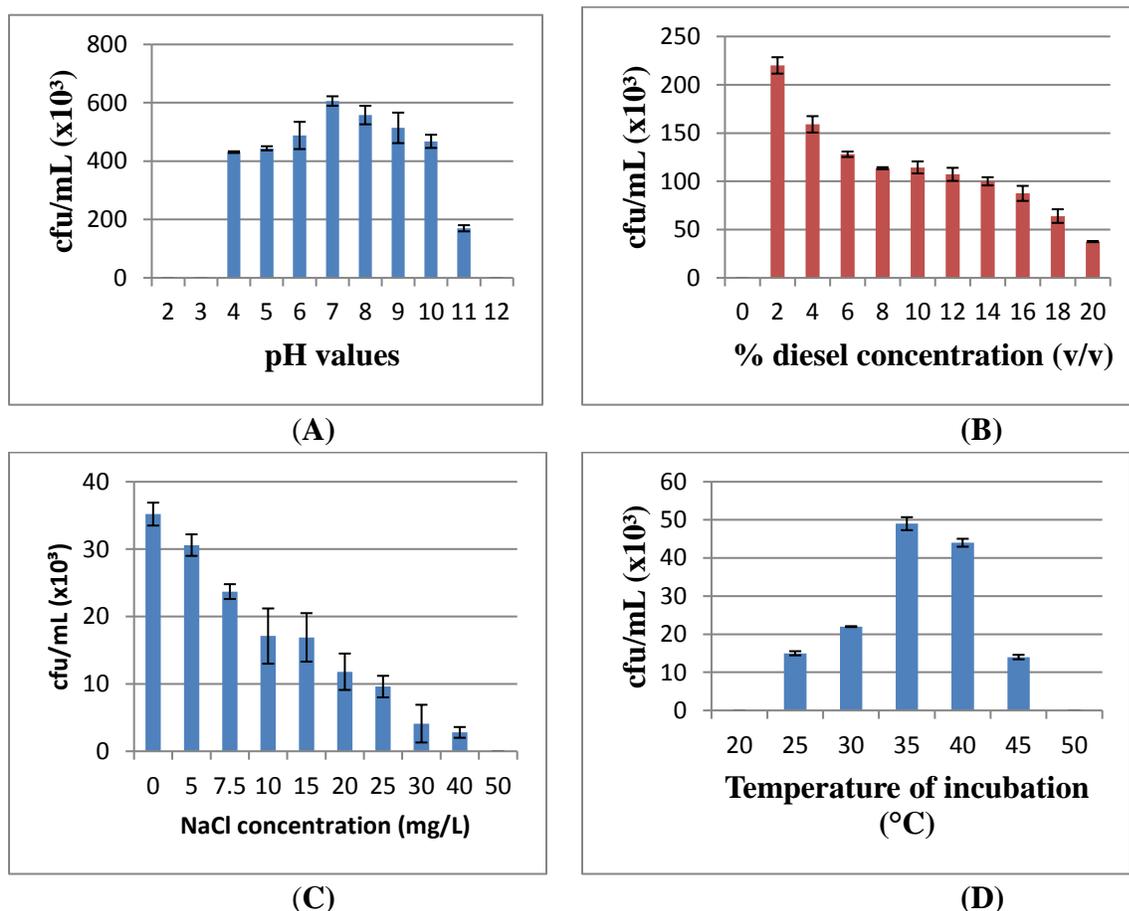
During the initial 48 hours of incubation, the screened isolates started diesel degradation while most of the remaining isolates started after 96 hours of incubation. These differences might be due to the initial biodegradation resistance of 2% v/v concentration. The screened isolates A34, A35, A6, and A38 were identified as *Alcaligenes* sp., *Ochrobactrum* sp., *Alcaligenes aquatilis* and *Alcaligenes faecalis* UMYU001 (MN519483.1) respectively. The screened and identified isolates were used in the formulation of effective diesel degrading consortia (Table 1). All the four bacteria were found to be compatible with one another despite being isolated from different environments. The mathematical permutation applied suggested 11 different consortia which were formulated in a mixed combination of two, three, and four strains separately having equal volumes of  $1.5 \times 10^5$  cells/mL resting cells for each strain (Table 1). The combined degradation strength of the individual consortium was found to produce tremendous colonies even at 4%, v/v diesel oil. Hence, consortium 11 (CST11) was selected to be the best for diesel biodegradation.

**Table 1:** Consortia formulation containing equal ratio of different bacteria strains ( $4.0 \times 10^4$  cells/mL) during diesel degradation after 96 hours at 2%, v/v diesel oil.

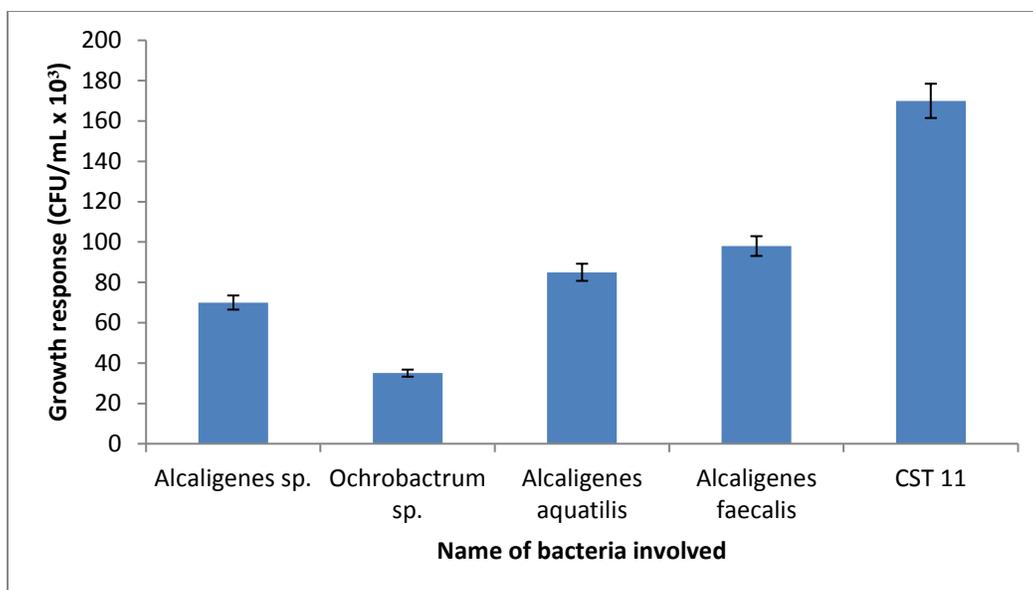
Name	Combination	48 hrs growth (CFU/mL)	72 hrs growth (CFU/mL)	96 hrs growth (CFU/mL)
CST1	A and B	$2.0 \times 10^2 \pm 7.07^e *$	$3.0 \times 10^2 \pm 7.07^f *$	$16.48 \pm 4.30^b$
CST2	A and C	$3.0 \times 10^3 \pm 2.83^b$	$3.0 \times 10^3 \pm 2.83^b$	$1.99 \pm 0.04^c$
CST3	A and D	$4.4 \times 10^2 \pm 14.14^e *$	$6.4 \times 10^2 \pm 14.14^d *$	$5.67 \pm 1.83^d$
CST4	B and C	$1.6 \times 10^3 \pm 22.63^c$	$1.9 \times 10^3 \pm 22.63^c$	$4.56 \pm 0.79^d$
CST5	B and D	$3.0 \times 10^2 \pm 33.94^e *$	$7.8 \times 10^2 \pm 33.94^d *$	$23.12 \pm 1.36^a$
CST6	C and D	$4.8 \times 10^2 \pm 13.94^e *$	$6.7 \times 10^2 \pm 13.94^d *$	$5.56 \pm 2.88^d$
CST7	A, B and C	$1.6 \times 10^3 \pm 11.31^c$	$1.7 \times 10^3 \pm 11.31^c$	$18.76 \pm 4.81^b$
CST8	A, B and D	$1.1 \times 10^3 \pm 22.63^c$	$1.4 \times 10^3 \pm 22.63^c$	$9.12 \pm 3.51^c$
CST9	A, C and D	$0.6 \times 10^2 \pm 22.7^f *$	$3.3 \times 10^3 \pm 22.7^b *$	$4.89 \pm 3.27^d$
CST10	B, C and D	$4.8 \times 10^2 \pm 22.63^d *$	$8.0 \times 10^2 \pm 22.63^e *$	$13.96 \pm 1.41^{bc}$
CST11	A, B, C and D	$1.6 \times 10^4 \pm 90.51^a$	$1.7 \times 10^4 \pm 90.51^a$	$8.32 \pm 0.68^c$

**Note:** Different superscripts (a, b, c, d, e, f) represent significant differences within columns while (\*) denotes significant differences between columns ( $p \leq 0.05$ ). The letter A represent *Alcaligenes* sp., B: *Ochrobactrum* sp., C: *Alcaligenes aquatilis*, and D: *Alcaligenes faecalis* UMYU001 (MN519483.1).

Regarding the culture conditions optimization based on single factor at a time analyses, wide pH range of 2 to 12 was selected so as to observe the response of the selected consortium. The result indicates the consortium has an excellent performance at neutral pH of 7.0 which indicate all the bacteria performance are pH dependent (Figure 3A). As for the diesel oil concentration, it was evident that 2% diesel quantity provides the best concentration for the biodegradation experiments (Figure 3B). The salinity optimization showed that sodium chloride only possesses inhibitory effect to the formulated consortium (Figure 3C). This was observed from the decreased bacteria population as the salinity concentration increases. The optimum temperature recorded was 35°C which is very close to the initial 37°C used for the isolation. At this 35°C temperature, consortium population reaches up to  $4.9 \times 10^4$  cfu/mL  $\pm 1.7$  (Figure 3D). When all the optimum parameters were used at a time to degrade 2% v/v diesel concentration, the result obtained indicate a very high increase of  $1.7 \times 10^4$  cfu/mL while the individual bacteria attained 7.0, 3.0, 15.0 and 9.0  $\times 10^2$  cfu/mL for *Alcaligenes* sp., *Ochrobactrum* sp., *Alcaligenes aquatilis* and *Alcaligenes faecalis* UMYU001 (MN519483.1) respectively (Figure 4).

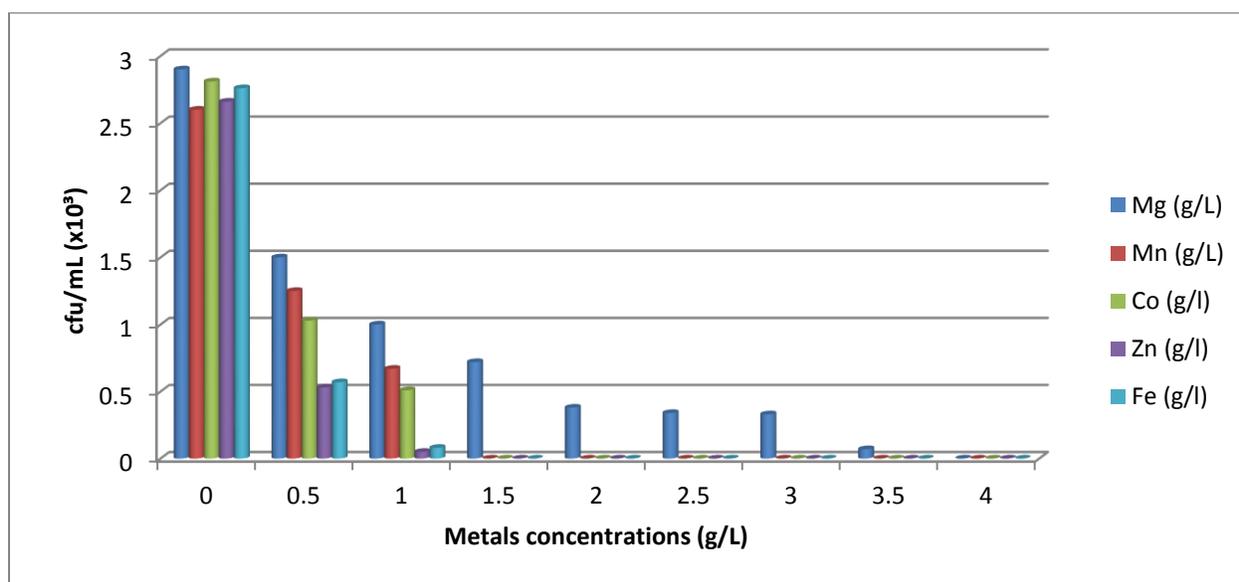


**Figure 3:** Optimization of biodegradation conditions using bacteria consortium CST11 indicating optimum (A) pH, (B) substrate concentration, (C) salinity and (D) temperature.



**Figure 4:** Comparison of the bacteria growth population that survived at the sole expense of 2%, v/v diesel oil between the formulated consortium and the individual isolates.

The heavy metal resistance thresholds exhibited by the isolates in the chosen consortium (CST11) for  $Mg^{++}$ ,  $Mn^{++}$ ,  $Zn^{++}$ ,  $Co^{++}$  and  $Fe^{++}$  was presented in Figure 5. Magnesium was the highest tolerated heavy metal by the consortium where  $70 \pm 0.01$  cfu/mL was produced at 3.5 g/L. As for the  $Mn^{++}$ ,  $Zn^{++}$ ,  $Co^{++}$ , and  $Fe^{++}$  only 1 g/L was tolerated by the consortium with a total population of  $670 \pm 0.12$ ,  $50 \pm 0.06$ ,  $510 \pm 0.08$ ,  $80 \pm 0.02$  cfu/ mL, respectively.



**Figure 5:** Bacteria population obtained when CST11 was grown at 2% v/v diesel oil which was co-contaminated with varying concentrations (0 to 4 g/L) of Mg, Mn, Co, Zn and Fe.

#### 4. Discussion

Persistent contamination is associated with buffering ability of petrochemical compounds to neutralize basic pH (Essien and John, 2010). Study by Harrot-Paw, (2012) demonstrated the ability of diesel, at high concentrations, to reduce microbial growth in soils up to 55%, depending on the soil type. This could be as a result of the differences in the persistence of diesel contaminants over time, and microbial survival capability which corresponds with those of the optimized parameters in Figure 3. The study findings on the microbial counts are also within the range of the reported studies by Karthika *et al.* (2014), and Nikhil *et al.* (2013) who obtained bacteria growth ranges of  $2.64 \times 10^7$  -  $7.88 \times 10^8$  cfu/g from diesel contaminated soil. This can be explained by the selection pressure that the heavy diesel contamination subjects the organisms. Musa, (2019) on the other hand reported an average value of about  $1.0 \times 10^7$  cfu/mL of diesel degrading bacteria from engine-oil contaminated soil.

The screening results showed some of the isolates can readily withstand both 2% and 4% diesel concentrations (v/v) where significant differences in the growth population was observed (Table 1). There is direct relationship between performance of diesel degrading bacteria at 2% and 4% as evaluated using the Pearson Correlation Coefficient ( $r = 0.92$ ), with R value of 0.84. The results from ANOVA indicate that the bacterial survival response in diesel oil after 8 days of incubation was significantly different ( $p < 0.05$ ). The screened and identified isolates include *Alcaligenes* sp. (A34), *Ochrobactrum* sp. (A35), *Alcaligenes aquatilis* (A36) and *Alcaligenes faecalis* UMYU001 (MN519483.1), A38. These isolates were reported in the previous literatures to be able to degrade diesel oil (Lee *et al.*, 2006; Nikhil *et al.*, 2013; Karthika *et al.*, 2014; Rehman *et al.*, 2015; Umar *et al.*, 2020).

All these bacteria are gram positive which reduces the chances of antagonism among them through the secretion of lytic enzymes and other toxic, inhibitory substances (Pelczar *et al.*, 2003). Occupation of the same ecological niche and co-metabolizing on the same substrate may also contribute to the fact that in almost all the scenarios, the consortia performed better than single strains (Figure 4). The longer time period taken before growth of the organisms can be attributable to the time lag before cellular syntheses of necessary enzymes for diesel utilization as a sole carbon source (Rehman *et al.*, 2015).

The results of the optimization studies showed that the selected consortium can survive within the mesophilic temperature range of 25<sup>0</sup>C to 45<sup>0</sup>C. The best temperature supporting the growth of the consortium was 35<sup>0</sup>C which produced a higher population of  $4.9 \times 10^4$  cfu/mL  $\pm$  1.7, followed by 40<sup>0</sup>C with  $4.4 \times 10^4$  cfu/mL  $\pm$  1.04 while 20<sup>0</sup>C and 50<sup>0</sup>C do not produced any colony. These observations are in concomitance with observed temperatures of the soils from where the samples were collected. Therefore, the organisms were comfortable with the mesophilic temperature range prior to isolation. The adversity of higher and lower temperatures to cellular physiologic functionalities and enzymatic activities are well established. Mesophilic organisms are previously

reported to carryout diesel biodegradation by Agarry and Latinwo (2015) among many others. Furthermore, higher temperatures can coagulate cellular proteins, and non-optimal temperatures may predispose cellular activities to a sluggish nature, or stop them altogether. Nonetheless, the presence of heat-shock proteins in some bacteria and endospore formation are advocated as survival mechanisms in cases of extreme temperatures (Willey *et al.*, 2013). Indeed, very few reports of degradation at low temperatures exist (Darma *et al.*, 2019).

The survival pH range of the organisms was 4 to 11 which showed the versatility of the organism and their capability to potentially biodegrade diesel irrespective of wide pH fluctuations. Additionally, changes in pH can damage the balance of microbial fluids/buffers. Extreme pH values affect microbes by breaking the DNA and catalyze protein denaturation which is directly related to survival, growth and proliferation of soil microorganisms (Rousk *et al.*, 2009). In a previous studies by Umar *et al.* (2017; 2020), the best pH for proliferation of hydrocarbonoclastic bacteria was determined to be neutral.

Regarding the salinity optimization, the consortium can survive a salinity of up to 40mg/L, with proportionate decreases in the cfu/mL of the diesel degrading bacteria as the salinity increases. When applied in excess, sodium chloride can inhibit microbial growth due to high osmotic pressure (Willey *et al.*, 2013). However, survival may be entrenched by possession of genetic factors, such as the presence of the Pro U operon, an important osmoregulatory locus. Comparatively, Riis *et al.* (2003) demonstrated the ability of diesel degrading bacteria to survive at salinities of 0-25% w/v. The substrate optimization results indicate the preference of the consortium to lower diesel quantity as higher concentration might slower the degradation process.

The results of the heavy metal tolerance experiments indicate that the highest resistance was exhibited against Mg<sup>++</sup>, Mn<sup>++</sup>, Zn<sup>++</sup>, Co<sup>++</sup> and Fe<sup>++</sup>. Generally, higher concentrations of heavy metals are known to diminish microbial activity, through inhibition of microbial enzymes, causing DNA mutation (Nordberg *et al.*, 2007; Umar *et al.*, 2018). This toxicity of the metals at the high concentration may be responsible for the rapid decrease in the consortium population upon increase in concentration (Hassen *et al.*, 1998).

The resistance threshold of the consortium was higher in Mg<sup>++</sup> and Mn<sup>++</sup>and Co<sup>++</sup> than in the Zn<sup>++</sup> and Fe<sup>++</sup>. This suggested that lower molecular weight metals are more tolerable for the formulated consortium than those with higher masses. The concentration of metals resisted by the consortium was higher to those found in the contaminated soils of Katsina (Hassen *et al.*, 1998). An explanation for the relatively high resistance thresholds of these metals centers on the fact that their natural habitats are potentially saturated with these heavy metals, and hence, the organisms have in-built mechanisms for survival in heavy metal contaminated environments (Oaikhena *et al.* 2016).

Furthermore, some of the heavy metals tested are required in low concentrations for effective survival as  $Zn^{++}$  is quintessential constituent of many metalloenzymes and transcription factors (Nordberg *et al.*, 2007). Such play roles of significance in cell functions ranging from transcription and replication to gene expression and signal transduction. However, higher concentrations of  $Zn^{++}$  may be deleterious to absorption of other equally essential nutrients and minerals. Some of these metals are used as co-factors in enzymatic reaction, and iron in particular, plays a role in the electron transport chain. However, the low tolerance to  $Fe^{++}$  in this study can be attributable to  $Fe^{++}$  insolubility and its reactivity, which may extirpate its ready availability and escalate its toxicity (Nwagu *et al.*, 2017).

The regulation of  $Fe^{++}$  is controlled by two protein subclasses during uptake and export in the event of deficiency or excess with impending cellular toxicity. Hence,  $Fe^{++}$  can rigorously monitor transportation. One of the major proteins involved in the cellular uptake of  $Fe^{++}$  is transferrin, however,  $Mn^{++}$  and  $Zn^{++}$  are known to also bind to transferrin thereby causing cellular predicaments (Nordberg *et al.*, 2007).

## 5. Conclusion

This study identified *Alcaligenes sp.*, *Ochrobactrum sp.*, *Alcaligenes aquatilis* and *Alcaligenes faecalis* UMYU001 (MN519483.1) as the best performing isolates that survived at the sole expense of diesel even in the presence certain heavy metals co-contaminants. The formulated consortium has surviving conditions ranges of 25 to 45<sup>0</sup>C (35<sup>0</sup>C optimum), pH of 5 to 11 (pH 7 optimum), salinity range of 0 to 20g/L (0g/L optimum) and a substrate concentration (diesel concentration v/v) of 2% to 12% (2% optimum). The heavy metal resistance thresholds of the consortium were: Magnesium (3.5 g/L), Manganese (1.0 g/L), Zinc (1.0 g/L), Cobalt (1.0 g/L) and Iron (1.0 g/L).

## Conflict of interest

The authors declared none.

## Authorship contribution statement

**Umar Zubairu Darma:** designed, conducted and supervised all experimental protocols, data collection and editing the drafted manuscript; **Aminu Musa:** assisted in collecting soil samples used for the experiments; **Yahaya Riko Yunusa:** assisted in soil samples collection, conducting the experiments and drafting the manuscript.

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## References

- Agarry, S. and Latinwo, G.K., 2015. Biodegradation of diesel oil in soil and its enhancement by application of bioventing and amendment with brewery waste effluents as bio stimulation-bio augmentation agents. *Journal of Ecological Engineering*, 16: 82–91. doi: <https://doi.org/10.12911/22998993/1861>
- Atlas, R., 2005. *Handbook of Media for Environmental Microbiology*, 2<sup>nd</sup> Edition, London, UK: Taylor & Francis Group. Pp: 42, 76. doi: <https://doi.org/10.1201/9781420037487>
- Barrow, G.I. and Feltham, R.K.A., 1993. *Cowan and Steel's Manual for the Identification of Medical Bacteria*. Third Edition. Cambridge, UK: Cambridge University Press. doi: <https://doi.org/10.1017/CBO9780511527104>
- Bradacova, K., Floria, A.S., Bar-Tal, A., Minz, D., Yerminyahu, U., Shawahna, R., Kraut-Cohen, J., Zolti, A., Erel, R., Dietel, K., Weinmann, M., Zinnerman, B., Berger, N., Ludewig, U., Neumann, G. and Poster, G., 2019. Microbial Consortia versus Single-Strain Inoculants: An Advantage in PGPM-Assisted Tomato Production? *Agronomy*, 9(105): 1-25. doi: <https://doi.org/10.3390/agronomy9020105>
- Chan, S.W., Lee, C.Y., Yap, C.F., Mustapha, W.A.W. and Ho, C.W., 2009. Optimisation of extraction conditions for phenolic compounds from limaupurut (*Citrus hystrix*) peels. *International Food Research Journal*, 16(2): 203-213.
- Chen, J., Huang, P.T., Zhang, K.Y. and Ding, F.R., 2012. Isolation of bio surfactant producers, optimization and properties of bio surfactant produced by *Acinetobacter* sp. from petroleum-contaminated soil. *Journal of Applied Microbiology*, 112: 660–671. <https://doi.org/10.1111/j.1365-2672.2012.05242.x>
- Darma, U.Z., Mansir, A.Z., and Riko, Y.Y., 2019. Compatibility and formulation of diesel degrading consortia using bacteria isolated from contaminated soil. *Bayero Journal of Pure and Applied Sciences*, 12(1), 199-208.
- de Vos, P., Garrity, G.M., Jones, D., Krieg, N.R., Ludwig, W., Rainey, F.A., Schleifer, K.H. and Whitman, W.B., 2003. *Bergey's Manual of Systematic Bacteriology. Volume III, The Firmicutes*. 2<sup>nd</sup> Edition. Georgia, USA Springer. Page 2-8.
- Doyle, A., and Bryan, G.J., 1998. *Cell and Tissue Culture: Laboratory Procedure in Biotechnology*, John Willey & Sons, Chicester, Page 58.
- Essien, O.E. and John, O.A., 2010. Impact of crude-oil spillage pollution and chemical remediation on agricultural soil properties and crop growth. *Journal of Applied Sciences and Environmental Management*, 14(4): 147–154. doi: 10.4314/jasem.v14i4.63304
- Habib, S., Ahmad, S.A., Johari, W.L.W., AbdShukor, M.Y., Alias, S.A., Khalil, K.A. and Yasid, N.A., 2018. Evaluation of conventional and response surface level optimisation of *n*-dodecane (*n*-C12) mineralisation by psychrotolerant strains isolated from pristine soil at Southern Victoria Island, Antarctica. *Microbial Cell Factories*, 17(44): 1-21. doi: 10.1186/s12934-018-0889-8.
- Harrot-Paw, N., 2012. Influence of diesel fuel on number of selected soil microorganisms group. *RoczPanstwZaklHigl*, 63(8): 367-372. <https://pubmed.ncbi.nlm.nih.gov/23173343/>

- Hassen, A., Saidi, N.J., Cherif, M. and Boudabous, A., 1998. Effect of heavy metals on *Pseudomonasaeruginosa* and *Bacillusthuringiensis*. *Bioresource Technology*, 65: 73-82. [https://doi.org/10.1016/S0960-8524\(98\)00011-X](https://doi.org/10.1016/S0960-8524(98)00011-X)
- Hemraj, V., Diksha, S. and Avneet, G., 2013. A review on commonly used biochemical tests for bacteria. *Innovare Journal of Life Sciences*, 1:1-7. <https://innovareacademics.in/journals/index.php/ijls/article/view/30>
- Kamzolova, S.V., Anastassiadis, S.G., Fatykhova, A.R., Golovchenko, N.P. and Morgunov, I.G., 2010. Strain and process development for citric acid production from glycerol containing waste of biodiesel manufacture. *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology*, 2010: 1020-1028.
- Karthika, R., Gopinath, L.R., Archaya, S. and Bhuvaneshwan, R., 2014. Isolation of diesel degrading bacteria, identification of catechol gene and its biogas production. *IOSR Journal of Environmental Science, Toxicology and Food Technology*, 8: 76-82.
- Laisin, M., Okoli, O.C., Ujumadu, R. and Nsiegbe, N., 2012. r-Permutation and r-Combination techniques for k-inclusion condition. *Journal of Physical Sciences and Innovation*, 4: 5-11.
- Lee, M., Kim, M., Singleton, I., Goodfellow, M., and Lee, S., 2006. Enhanced biodegradation of diesel oil by a newly identified *Rhodococcusbaikonurensis* EN3 in the presence of mycolic acid. *Journal of Applied Microbiology*: 325-333. doi: 10.1111/j.1365-2672.2005.02756.x
- Lee, Y., Lee, Y. and Jeon, C.O., 2019. Biodegradation of naphthalene, BTEX, and aliphatic hydrocarbons by *Paraburkholderiaaromaticivorans*BN5 isolated from petroleum-contaminated soil. *Nature Scientific Reports*, 9(860):1-12. doi: 10.1038/s41598-018-36165-x
- Musa, S.I., 2019. Isolation and identification of diesel oil-degrading bacteria in used engine oil contaminated soil. *Journal of Applied Science and Environmental Management*, 23: 431-435. doi: 10.4314/jasem.v23i3.10
- Nikhil, T., Deepa, V., Rohan, G. and Satish, B., 2013. Isolation, characterisation and identification of bacteria from garage soil and comparison of their bioremediation potential. *International Research Journal of Environment Sciences*, 2: 48-50.
- Nwagwu, E.C., Yilwa, V.M., Egbe, N.E. and Onwumere, G.B., 2017. Isolation and characterization of heavy metal tolerant bacteria from Panteka stream, Kaduna, Nigeria and their potential for bioremediation. *African Journal of Biotechnology*, 16(1): 32-40. doi: 10.5897/AJB2016.15676
- Oaikhena, E.E., Makaije, D.B., Denwe, S.D., Namadi, M.M. and Haroun, A.A., 2016. Bioremediation potentials of heavy metal tolerant bacteria isolated from petroleum refinery effluent. *American Journal of Environmental Protection*, 5(2): 29-34. doi: 10.11648/j.ajep.20160502.12
- Pawar, R.M., 2015. The Effect of Soil pH on Bioremediation of PAHs. *Journal of Bioremediation and Biodegradation*, 6, 1-14.

- Pelczar, M.J., Chan, E.C.S. and Krieg, N.R., 2003. Microbiology of Soil. In, *Microbiology*, 5th Edition. New Delhi, India: Tata McGraw-Hill Publishing Company Limited. Page 58-65.
- Raja, P., Uma, S., Gopal, H., and Govindarajan, K., 2006. Impact of bio inoculants consortium on rice root exudates, biological nitrogen fixation and plant growth. *Journal Biological Science*, 6, 815-823. doi: 10.3923/jbs.2006.815.823
- Rehman, Z.U., Khan, K., Faisal, S., Kamal, R., Ahmad, S., Irfan, M.K.H., Safa, I.U. and Kumar, T., 2015. Isolation and identification of diesel degrading bacteria from oil contaminated soil in Mansehra, Pakistan. *International Journal of Scientific and Engineering Research*, 6: 1438-1661.
- Riis, V., Kleinstuber, S. and Babel, W., 2003. Influence of high salinities on the degradation of diesel fuel by bacterial consortia. *Canadian Journal of Microbiology*, 49(11): 713-721. doi: 10.1139/w03-083
- Rosen, K.H., 2007. *Combinatorics of Permutations*. 1<sup>st</sup> Edition, Boca Raton, Florida: Chapman and Hall/CRC. Page 33.
- Rousk, J., Brookes, P.C. and Baath, E., 2009. Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. *Applied and Environmental Microbiology*, 75(6): 1589–1596. doi: 10.1128/AEM.02775-08
- Sarkar, P., Meghvanshi, M., and Singh, R., 2011. Microbial consortium: A new approach in effective degradation of organic kitchen wastes. *International Journal of Environmental Science and Development*, 2(3), 170. <http://www.ijesd.org/papers/118-D550.pdf>
- Sharma, S., Tiwari, S., Hasan, A., Saxena, V. and Pandey, L.M., 2018. Recent advances in conventional and contemporary methods for remediation of heavy metal-contaminated soils. *3 Biotech*, 8(216): 1-18. doi: 10.1007/s13205-018-1237-8
- Sogani, M., Mathur, N., Sharma, P., and Bhatnagar, P., 2012. Comparison of immobilized whole resting cells in different matrices vis-a-vis free cells of *Bacillus megaterium* for acyltransferase activity. *Journal of Environmental Research and Development*, 6(3A), 695-701.
- Somee, M.R., Shavandi, M., Dastgheib, S.M.M. and Amoozegar, M.A., 2018. Bioremediation of oil-based drill cuttings by a halophilic consortium isolated from oil-contaminated saline soil. *3 Biotech*, 8(229): 1-13. doi: 10.1007/s13205-018-1261-8
- Spini, G., Spina, F., Poli, A., Bliex, A-L., Regnier, T., Gramellini, C., Varese, G.C. and Puglisi, E., 2018. Molecular and microbiological insights on the enrichment procedures for the isolation of petroleum degrading bacteria and fungi. *Frontiers in Microbiology*. 9, 2543: 1-19. <https://doi.org/10.3389/fmicb.2018.02543>
- Umar, Z.D., Aminu, M., and Yahaya, Y.R., 2020. Optimization of Diesel biodegrading conditions using Response Surface Methodology based on Central Composite Design. *Polycyclic Aromatic Compounds*. doi: 10.1080/10406638.2020.1823859
- Umar, Z.D., Aziz, N.A.A., Zulkifli, S.Z. and Mustafa, M., 2018a. Efficiency of polycyclic aromatic hydrocarbons (PAHs) degrading consortium in resisting heavy metals during

- PAHs degradation. *International Journal of Environment*, 7(1): 14-27. doi: <https://doi.org/10.3126/ije.v7i1.21291>
- Umar, Z.D., Azwady, A.A.N, Zulkifli, S.Z., and Muskhazli, M., 2018b. Effective Phenanthrene and Pyrene biodegradation using *Enterobacter* sp. MM087 (KT933254) isolated from used engine oil contaminated soil. *Egyptian Journal of Petroleum* 27(3), 349-359. <https://doi.org/10.1016/j.ejpe.2017.06.001>
- Umar, Z.D., Aziz, N.A.A., Zulkifli, S.Z. and Muskhazli, M., 2017. Rapid biodegradation of PAHs using effective *C. sakazakii* MM045 (KT933253). *MethodsX*, 4, 104-117. doi: 10.1016/j.mex.2017.02.003. eCollection 2017
- Umar, Z.D, Aziz, N.A.A., Zulkefli, S.Z. and Muskhazli, M., 2016. Identification of phenanthrene and pyrene degrading bacteria from used engine oil contaminated soil. *International Journal of Scientific and Engineering Research*, 7, 680-686.
- Wang, Z., Liu, X., Chen, L., Hu, X. and Liu, F., 2011. Degradation of diesel with microorganisms in rhizosphere of *Carex phacota* Spr. *Procedia Environmental Sciences*, 8: 61–67.
- Willey, J., Sherwood, L.M. and Woolverton, C.J., 2013. *Prescott's Microbiology*, Eighth/International African Value Edition, New York, USA: McGraw-Hill. Page 23-70.
- Wu, Y., Xu, M., Xue, J., Shi, K. and Gu, M., 2019. Characterization and enhanced degradation potentials of bio surfactant-producing bacteria isolated from a marine environment. *ACS Omega* 2019, 4: 1645–1651.