RESULTS

Isolation, Characterization and Identification of Bacteria isolates

In the isolation of bacteria present in the produced water, which are petroleum hydrocarbon degraders five colonies of the bacteria which showed higher growth rates were insolated. For further characterization and identification selected isolates were characterized by colony morphology and biochemical characteristics according to Cappuccino and Sherman (2010). The isolated bacteria are pseudomonas aeruginosa, staphylococcus aureus, Escherichia coli etc. as shown in Table1, 2 and 3 below.

Cultural and Morphological Characteristics

The colonies were culturally and morphologically identified with the aid of a high power magnifying lens in light microscope after gram staining and the shape, margin, elevation, size, colour, cell arrangement, cell type and colony surface and gram reactions resulted in the suspicion of *E. coli, Micrococcus Letus, Staphylococcus Aureus, Basillus substilis and Pseudomonas aeruginosa* was made. Results of theseexperiments are as shown in Table 1.1.

TABLE 1: CULTURAL AND MORPHOLOGICAL CHARACTERISTICS

Isolates	Shape	Margin	Elevation	Size	Colour	Colony surface	Gram reaction	Cell type	Cell arrangement	Suspected bacteria
A	Round	Rough	Flat	1mm	Green	Moist	-ve	Rod	Singly	*Pseudomonas aeruginosa*
B	Round	Smooth	Raised	1mm	Golden yellow	Moist	+ve	Cocci	Cluster	*Staphylococcus aureus*
C	Round	Smooth	Raised	0.5mm	Pink	Moist	-ve	Rod	Chain	*E. coli*
D	Round	Rough	Raised	0.5mm	Yellow	Dry	+ve	Cocci	Cluster	*Micrococcus Letus*
F	Round	Smooth	Raised	1mm	White	Dry	+ve	Cocci	Cluster	*Staphylococcus Epididymis*

Biochemical characterization of hydrocarbon degrading bacteria

The morphologically identified bacterial strains were confirmed with biochemical tests by performing Catalase test, coagulase test, oxidase test, urease test, glucose test, citrate test, and indole test and the experiments further confirmed the presence of *B. substilis, M. letus, E. coli, and P. aeruginosa* in the hydrocarbon contaminated water samples. Result of this experiment is as shown in Table 2

TABLE 2: Biochemical Characteristics

Isolates	Catalase	Coagulase	Oxidase	Urease	Glucose	Citrate	Motility	MR	Spore	Indole	Suspected Bacteria
A	-ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	-ve	-ve	*Pseudomonas aeruginosa*
B	+ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	*Staphylococcus Aureus*
C	+ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	*E. coli*
D	+ve	-ve	+ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	*Micrococcus Letus*
F	+ve	-ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve	*Staphylococcus Epididymis*

Identification of the isolates

Table 3 represent a summary of identification of five (5) bacterial isolates, which have been suspected from cultural and morphological characteristics and biochemical characteristic, the results compare with Bergey‟s Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974) identified the bacteria as shown in table 3

Table 3: Identification of Bacteria Isolate

ISOLATES	IDENTIFICATION
A	**Pseudomonas aeruginosa**
B	**Staphylococcus Aureus**
C	**Escherichia coli**
D	**Micrococcus Letus**
F	**Staphylococcus Epididymis**

Nitrate-nitrogen (NO_3-N) content

The reduction in the concentration of NO_3-N in the contaminated PAHs polluted water suggests that the process of nitrification might have reduced crude oil contamination. Hydrocarbon- utilizing microbes such as *Azobacter* spp normally become more abundant while nitrifying bacteria such as *Nitrosomonas* spp become reduced in number. This probably explains the relatively lower values of NO_3-N obtained after degradation.

Calibration of UV-Visible Spectrophotometer in concentration

The UV-Visible spectrophotometer records the concentration of hydrocarbon content of the produced water in absorbance (nm). This absorbance in terms of concentration is shown in

Table 4. The plotting of the values in a straight line helps to get the concentration of any absorbance through the equation of a straight line in fig 1

Table 4: Calibration Table

TPH (g/200ml)	Absorbance @ 480nm
0.016	0.157
0.030	0.389
0.055	0.786

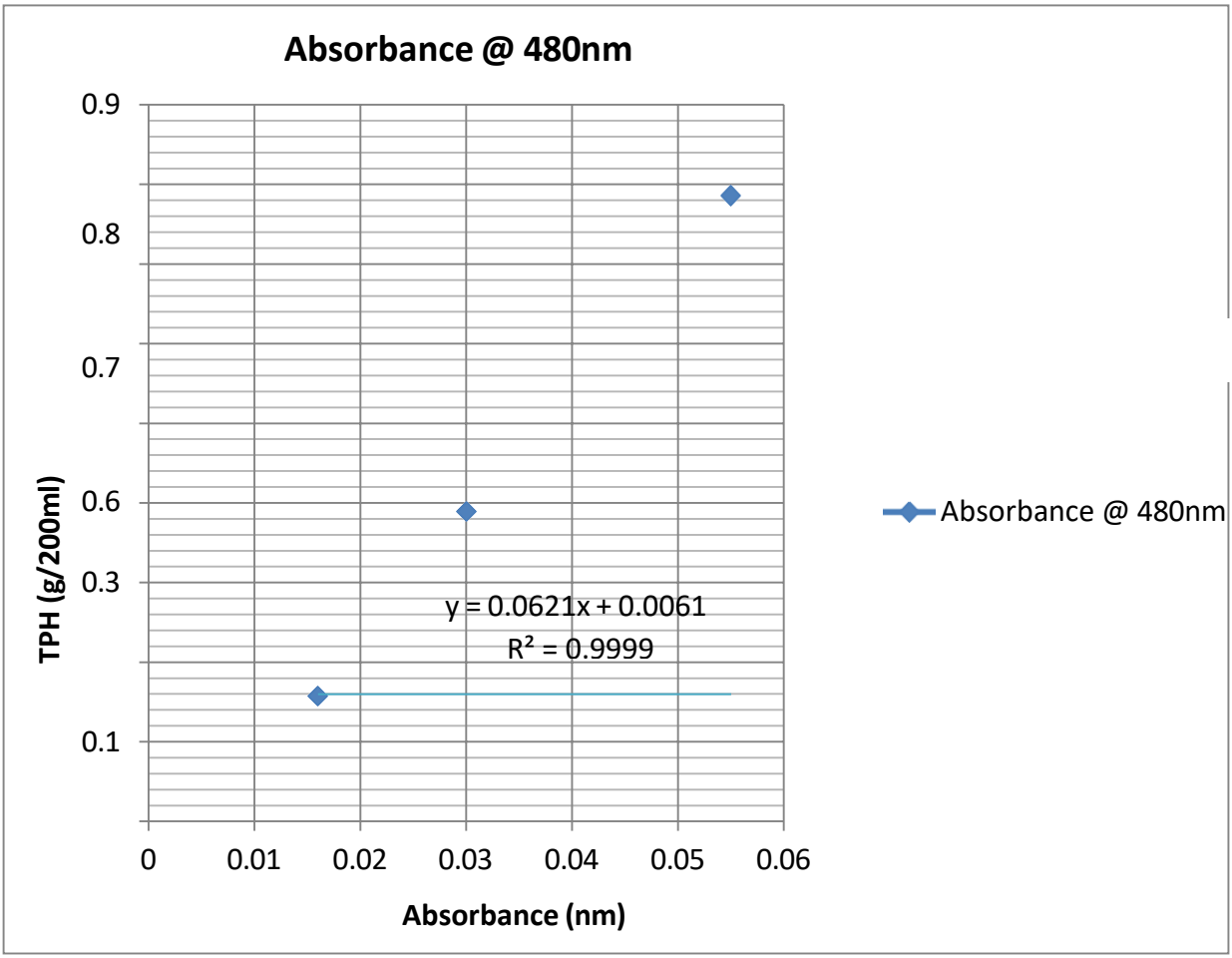

Fig 1: Calibration Curve

Hydrocarbon Biodegradation of produced water

The concentration of hydrocarbon present in the produced was measured at 3 days interval using spectrophotometer as show in the table 5 below.

Table 5: Biodegradation of hydrocarbon in produced water concentration

Day	Date	Absorbance (nm)	TPH (g/200ml)	TPH (mg/L)
1	18/6/2021	0.306	0.025103	125.513
2	21/6/2021	0.247	0.021439	107.194
3	24/6/2021	0.203	0.018706	93.532
4	27/6/2021	0.182	0.017402	87.011
5	30/6/2021	0.178	0.017154	85.769
6	03/7/2021	0.174	0.016905	84.527

Biodegradation efficiency

The efficiency of the biodegradation shown that the rate of degradation of the hydrocarbon increases with time (table 6) and the biodegradation curve decreases (fig 2)

Table 6: biodegradation efficiency

Day	TPH (mg/L)	Biodegradation efficiency (%)
1	125.513	-
2	107.194	14.595
3	93.532	25.48
4	87.011	30.676
5	85.769	31.665
6	84.527	32.655

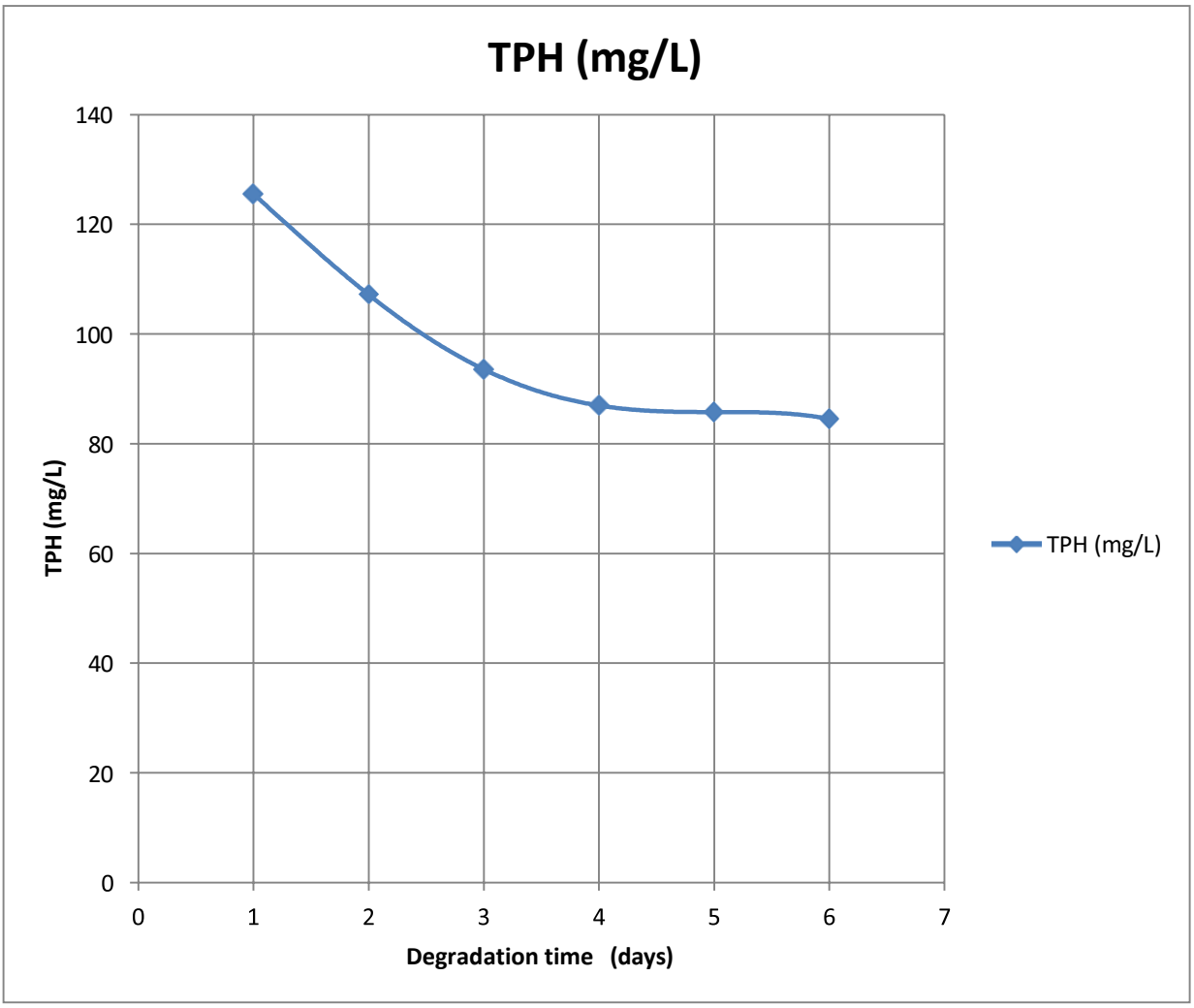

Fig 2: biodegradation curve