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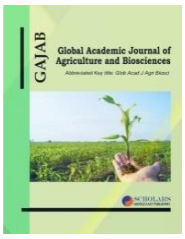

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Original Research Article

Phycoremediation Potentials of *Chlorella vulgaris* in Degradation of Crude Oil Spill

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Abstract: The contamination of aquatic ecosystems by crude oil spills is a pervasive issue in Nigeria's oil-producing regions, where waterways are frequently polluted with hydrocarbons, a primary constituent of crude oil. This study examined the phycoremediation capabilities of *Chlorella vulgaris* using established protocols. The algae isolated from freshwater samples were confirmed to be *C. vulgaris*. The results of the screening test revealed the organism's capacity to degrade heavy crude oil, as evidenced by varying levels of turbidity in the mineral salts-oil medium. Over a 47-day period, the pH of the mineral salts-oil medium decreased progressively, transitioning from neutral to acidic levels, ultimately reaching a pH of 4.1. Conversely, the absorbance of the medium increased over the same period, indicating *C. vulgaris* growth. Notably, the residual oil content in the mineral salts-crude oil medium decreased substantially, with a degradation rate of 80%. This study demonstrates the potential of *C. vulgaris* in phycoremediation of crude oil spills in Nigeria.

Keywords: Phycoremediation, Potentials, *Chlorella vulgaris*, Degradation, Crude oil, Spill.

INTRODUCTION

Biodegradation is a process by which substance are broken down into smaller compounds by living organisms and when such biodegradation is complete the process is called mineralization [1, 2]. Organic materials can be degraded aerobically, with oxygen or anaerobically, without oxygen [3]. Aquatic ecosystem has been contaminated as a result of oil spills and the public health actually threatened. The clean-up will take up to 30 years to go back to full recovery with all the environmental damages it has caused [4].

The affected individuals constitute an exposed population whose health may be potentially affected by the noxious properties of the air which includes liver necrosis and congestion of the liver [5]. Due to the detrimental drawbacks of oil spills incidents which are becoming more rampant in developing countries, environmentalist have developed new methods and forms of technology to facilitate the tasks of cleaning up oil spills [6]. Bioremediation is one promising treatment method is to exploit the ability of microorganisms to remove these organic pollutants from contaminated sites which is effective, minimally hazardous, economical, versatile and environmental friendly [7].

Biodegradation of oil contaminants can be described as the conversion of chemical compound by microorganisms into energy, cell mass and biological products [8]. The key component in bioremediation is the microorganisms, which produce enzymes involved in the degradation

reaction leading to the elimination or detoxification of the chemical pollutant [8]. Most of the biological treatment technologies involve the use of bacteria, but microalgae have already been applied for effluent treatment, either as a single species, as in the case of Chlorella, Scenedesmus or Arthrospira [9-11].

Raposo *et al*., [12] analyzed the capacity of *C. vulgaris* and the autochthonous flora of the effluents to remove some of the compounds present in the effluents. Microalgae are not unique in their bioremoval capabilities but they offer advantages over other biological materials in some conceptual bioremoval process schemes. Absorption of heavy metals by algae can be an effective process for the removal and recovery of heavy metals ions from aqueous solution [13].

Biodegradation is the general use of microorganisms to degrade contaminated sites while Phycoremediation is the specific use of algae for the same purpose. An investigation was conducted to assess the phycoremediation potential of *C. vulgaris* in mitigating the environmental impact of crude oil spills in Nigeria. The study focused on evaluating the microalgae's capacity to degrade total petroleum hydrocarbons (TPH) and polycyclic aromatic hydrocarbons (PAHs), which are primary constituents of crude oil.

MATERIALS AND METHODS

Samples Collection

The crude oil spill was obtained at an Ogoni River Rivers State, Nigeria, while microalgae samples were obtained from a fresh water in Enugu State using sterile screw-capped bottles which were opened and inserted into the fresh water at a depth of 30cm below the water surface with their mouths downward. They were thereafter turned so that the water flowed into them. The bottles were thereafter aseptically closed and transported to the laboratory in an ice packed container for the isolation, characterization and identification of *C. vulgaris.*

Isolation of *Chlorella Vulgaris*

This was carried out using the spread plate method described by Anderson [14]. An aliquot (0.1ml) of the fresh water was spread on the surface of Petri dishes containing sterile Chlorella agar using a sterile glass rod. The plates were prepared which were covered and incubated in an inverted position at 28^{0}C for 7 days.

Characterization and Identification of the Isolates

The colonial and microscopic characteristics of the isolates were determined according to the scheme of Janse *et al*., [15]. The isolates were placed in a drop of sterile distilled water on microscopic slides and a drop of iodine was applied to them. The slides were thereafter examined under a compound microscope. The isolates were identified using a catalogue of algae as done by Anderson [14].

Screening Test for Petroleum Hydrocarbons Utilization by *Chlorella vulgaris*

The test was carried out using mineral salts medium as described by Olukunle [16]. The medium was composed of the following (g/l): NaCl, 10.0; MgSO4. 7H20, 0.42; KCl, 0.29; KH2P04 0.83; NaHP04, 1.25, Agar, 20.0 and distilled water, 1 litre. A mineral salts medium was prepared and dispensed into test tubes in 9 mL aliquots. Subsequently, 1 mL of crude oil was added to each test tube, which were then sealed and autoclaved at 121°C for 15 minutes. Following cooling, the tubes were inoculated with C. vulgaris and incubated at 28°C for 14 days on a rotary shaker operating at 75 revolutions per minute. Turbidity was then assessed. Uninoculated tubes, containing mineral salts and crude oil, served as controls.

Determination of the Residual Petroleum Hydrocarbons Content

The residual crude oil content was quantified according to the method described by Amadi [17]. Upon completion of the 47-day incubation period, the contents of each flask were sterilized at 121°C for 15 minutes to inactivate *C. vulgaris*. The sterilized contents were then transferred to a separating funnel, where the residual crude oil was extracted with toluene. The funnel was sealed and tilted for 5 minutes to facilitate phase separation. The mixture was then shaken, refluxed intermittently to release trapped air, and allowed to settle for 30 minutes. The resulting toluene-oil mixture was collected in a sample bottle and analyzed spectrophotometrically at 420 nm after dilution with a known volume of toluene. A calibration curve was generated using known volumes of toluene, enabling the determination of residual hydrocarbon content.

Gas Chromatographic Numbers of the Petroleum Hydrocarbons before and after Degradation by *Chlorella vulgaris*

The gas chromatographic profile of the petroleum hydrocarbons before and after degradation by *C. vulgaris* was determined as done by Onuorah *et al*., [18] using Agilent 6890 plus gas chromatograph equipped with ionizing detector, split injector and fused silica capillary column HP-1 of 30m length, 0.35mm internal diameter and 0.5m film thickness. The detector and injector temperatures were maintained at 300^{0}C and 250^{0}C respectively. The column temperature was programmed to rise from 80^{0}C to 300^{0}C with a rate of 30C/min and final time of fifteen minutes. Nitrogen (02 free) was used as a carrier gas at a flow rate of 2ml per minute.

RESULTS

Table 1: Colonial and Microscopic Characteristics of the Alga

Colonial Characteristics	Microscopic Characteristics	Identification
Appeared greenish in colour, small and round in shape	The characterized colonies were small, spherical, non-motile, lacking flagella with a cup-shaped chloroplast	*Chlorella vulgaris* identified

Table 2: Screening Test for Petroleum Hydrocarbon by *C. vulgaris*

Petroleum	Utilization
Crude oil	+ + +

Key: + + + = heavy utilization.

Table 3: Changes in pH of the Mineral Salt oil Medium in forty-seven (47) days

Petroleum	Initial pH	Final pH
Crude oil	7.0	4.1

Table 4: Changes in Absorbance and Residual Salt oil Medium during forty-seven (47) days of Degradation by *C. vulgaris*

Petroleum	Initial Absorbance	Final Absorbance	Initial Oil Content	Final Oil Content	Degradation (%)
Crude oil	0.463	0.931	1.0	0.2	80

Table 5: Total Petroleum Hydrocarbons Content of the Unused Crude Oil

Peak No	Peak Name	Ret Time (min)	Result (ppm)	Peak Area (counts)	Sep. Code
1	C8	2.288	51.7754	93665	TF
2	C9	3.563	47.5136	104262	VV
3	C10	4.204	102.6131	222853	VV
4	C11	5.417	283.4754	578118	VV
5	C12	6.166	260.2037	563082	VV
6	C13	6.484	321.9747	698102	VV
7	C14	6.746	651.4872	1389109	VV
8	C15	7.913	399.4311	843900	VV
9	C16	8.261	1064.8687	2387873	VV
10	C17	8.776	181.0118	379634	VV
11	Pr	8.828	332.1787	693845	VV
12	Ph	9.269	245.4649	468080	VV
13	C18	9.312	221.6834	459842	VV
14	C19	9.824	238.9113	497046	VV
15	C20	10.316	199.8598	411096	VV
16	C21	10.785	202.4454	388116	VV
17	C22	11.236	175.2851	330656	VV
18	C23	11.670	226.7178	410079	VV
19	C24	12.087	204.7097	352622	VV
20	C25	12.491	219.5586	369869	VV
21	C26	12.878	168.3433	276130	VV
22	C27	13.254	164.9786	256877	VV
23	C28	13.616	165.6586	261789	VV
24	C29	13.969	195.5036	308811	VV
25	C30	14.234	227.7272	395371	VV
26	C31	14.436	15.9866	28631	VV
27	C32	14.485	122.6598	206102	VV
28	C33	14.874	33.9041	57060	VV
29	C34	15.665	0.7032	1138	TF
30	C35	16.105	0.0949	140	TF
31	C36	16.697	0.0933	80	TS
32	C37	17.957	0.0344	44	TS
33	C38	18.605	0.2453	259	VV
34	C39	19.013	0.4124	361	VV
35	C40	20.652	6.4319	80	TF
	Total		**6733.9463**	**13434722**	

Table 6: Polyaromatic Hydrocarbons Content of the Unused Crude Oil

Peak No	Peak Name	Ret Time (min)	Result (ppm)	Peak Area (counts)	Sep. Code
1	Naphthalene	6.423	401.6422	475995	VV
2	Acenapthene	8.193	1722.6167	1290081	BV
3	Acenaphthylene	8.763	184.8440	235271	VP
4	Fluorene	9.249	106.8113	112083	VV
5	Phenanthrene	10.252	126.9304	119040	BB
6	Anthracene	11.606	148.8202	97529	BB
7	Fluoranthene	12.023	102.0345	88263	BB
8	Pyrene	12.427	86.9386	71777	BB
9	Benzo[a]anthracene	12.816	101.5148	66882	BB
10	Chrysene	13.192	80.4657	57012	BB
11	Benzo[b]fluoranthene	13.556	139.1202	72159	BV
12	Benzo[k]fluoranthene	13.909	103.7429	57332	PB
13	Benzo[a]pyrene	14.165	122.0223	54509	BP
14	Dibenzo[a,h]anthracene	14.462	152.4289	58093	VB
15	Indeno[1,2,3-cd]pyrene	14.885	16.5319	7303	PB
16	Benzo[ghi]perylene	16.629	126.0225	55705	BB
	Totals		**3722.4875**	**2919034**	

Table 7: Total Petroleum Hydrocarbons Content of the Residual Crude Oil

Peak No	Peak Name	Ret Time (min)	Result (ppm)	Peak Area (counts)	Sep. Code
1	C8	2.693	1.8066	3268	BP
2	C9	3.592	2.0212	4435	VV
3	C10	4.218	1.6451	3573	VV
4	C11	5.407	0.8924	1820	TF
5	C12	6.138	0.1423	308	TS
6	C13	6.428	2.9715	6443	VV
7	C14	6.707	8.1785	17438	VP
8	C15	7.888	1.9417	4102	VV
9	C16	8.188	15.0309	33705	PV
10	C17	8.703	4.2754	8967	VV
11	Pr	8.771	21.6991	45324	VP
12	C18	9.254	1.7615	3654	TF
13	Ph	9.302	0.5377	1025	TF
14	C19	9.758	2.7002	5618	TF
15	C20	10.296	0.4173	858	VV
16	C21	10.731	1.3687	2624	PB
17	C22	11.209	0.2966	559	BV
18	C23	11.634	1.7515	3168	VV
19	C24	12.050	0.6408	1104	VV
20	C25	12.444	1.1460	1931	VV
21	C26	12.865	0.2970	487	VV
22	C27	13.211	0.5935	924	VV
23	C28	13.593	0.7872	1244	VV
24	C29	13.961	0.6663	1053	VV
25	C30	14.256	3.4345	5963	VV
26	C31	14.506	0.3421	613	VV
27	C32	14.898	2.9754	4999	VV
28	C33	15.327	1.4419	2427	VV
29	C34	15.619	5.4247	8778	VV
30	C35	16.067	6.4100	9438	VV
31	C36	16.640	69.3449	59587	VB
32	C37	17.237	0.1479	190	PV
33	C38	17.827	0.3975	419	VV
34	C39	18.836	0.2531	222	VV
35	C40	20.653	1.3426	17	VV
	Totals		**165.0836**	**246285**	

Table 8: Polyaromatic Hyrdrocarbons Content of the Residual Crude Oil

Peak No	Peak Name	Ret Time (min)	Result (ppm)	Peak Area (counts)	Sep. Code
1	Naphthalene	6.413	3.3442	7927	BB
2	Acenapthene	8.168	30.0689	45038	BB
3	Acenaphtylene	8.750	3.9366	10021	BB
4	Benzo[ghi]perylene	16.603	52.4870	46401	BB
	Totals		**89.8367**	**109387**	

DISCUSSION

Table 1 showed that the algae isolated from freshwater samples were identified as *C. vulgaris.* This finding is consistent with previous studies, including Onuorah *et al*., [19], who identified *C. vulgaris* from a pond, and Janse *et al*., [15], who also isolated *C. vulgaris* from freshwater environments.

Table 2 presents the results of the screening test for petroleum hydrocarbon utilization by *C. vulgaris*, which demonstrated the organism's ability to utilize heavy crude oil. The degree of turbidity produced in the mineral salts-oil medium varied, indicating the level of hydrocarbon utilization by *C. vulgaris*.

Table 3 illustrates the changes in pH of the mineral salts-oil medium during 47 days of petroleum hydrocarbon degradation by *C. vulgaris*. A progressive decrease in pH was observed, shifting from neutral to acidic levels, with the mineral salts medium inoculated with *C. vulgaris* ultimately reaching a pH of 4.1.

Table 4 demonstrates that the absorbance of the mineral salts-oil medium increased over 47 days, indicating growth of *C. vulgaris* as it utilized crude oil as a carbon and energy source and also reveals that the residual oil content in the mineral salts-crude oil medium decreased significantly, with a degradation percentage of 80%.

Tables 5-8 reveal a significant decrease in the peak areas and peak numbers of total petroleum hydrocarbon and polyatomic hydrocarbon in crude oil spill after forty-seven days of phycoremediation using the alga *C. vulgaris*. This suggests that extending the degradation period beyond forty-seven days would likely result in even greater reduction and removal of pollutants. This finding is consistent with the results of Onuorah [19], who demonstrated that *C. vulgaris* effectively reduced the levels of total petroleum and polyatomic hydrocarbons in crude oil, as well as in refined oil products such as kerosene and petrol.

The findings of this study are consistent with the results reported by Onuorah *et al*., [18, 19], which demonstrated a reduction in peak numbers and peak areas of residual oil after 42 days of bacterial utilization and degradation of crude oil spill by *C. vulgaris* isolated from a pond.

CONCLUSION

In conclusion, this study demonstrates that *C. vulgaris* isolated from freshwater habitats exhibits significant phycoremediation potential for biodegrading crude oil spills, and can be cultured and applied to polluted sites by local communities or government agencies.

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