IOSR Journal of Applied Chemistry (IOSR-JAC)
e-ISSN: 2278-5736.Volume 4, Issue 2 (Mar. – Apr. 2013), PP 50-66
www.iosrjournals.org

Experimental Studies on Bioregeneration of Activated Carbon Contaminated With Hydrocarbon

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Abstract: *The search for a more cost effective, environmentally friendly and less cumbersome process of regenerating used activated carbon in a petroleum polluted site necessitated this research work. Spent Granular Activated Carbon was regenerated having been initially characterized using cultured Pseudomonas Putida. The rate of Bioregeneration was studied by varying the volume of bacteria from 10ml, 20ml, 30ml and 40ml. The regeneration temperature was also varied from 25^{o}C to ambient temperature of 27^{o}C, 35^{o}C and further at 40 and 45^{o}C over a period of 21 days. The regeneration experiment improved as the quantity of bacteria used increased. Increasing the temperature of regeneration also increased the rate of regeneration due to chemisorptions action. There was no significant improvement when the temperature was increased to 45^{o}C suggesting that increasing temperature beyond 40^{o}C would not be economical. The regenerated GAC was characterized to determine efficiency of regeneration. Bioregeneration was impacted by variation in temperature and bacteria volume. Bioregeneration spent Granular Activated Carbon is shown by the study to be an effective and cost efficient way to remediate polluted soil and still reuse the adsorbent.*

Keywords: *Bioregeneration, Chemisorption, GAC, Hydrocarbon and Nigeria*

I. Introduction

Despite the huge economic benefits of oil exploitation, there are many associated primary and secondary problems that could impacted negatively on the habitants and the environment in Nigeria. Some of which include contamination of streams and rivers within the exploration and processing areas, oil spillage, destruction of forests and bio-diversity loss, gas flaring and environmental pollution (Nwankwo and Ifeadi, 1988; Bayode *et al*, 2011; Eregha & Irughe, 2009). Among the outlined menaces of oil exploration and exploitation, oil spillage has been reported as one with the most significant impact (Oghifo, 2011; Afinotan & Ojakorotu, 2009; Achebe *et al*, 2012; Kadafa, 2012), thus, there is the need to improve on the processes and mechanisms that can facilitate quick correction of identified spill cases.

The use of activated carbon (AC) has proven to be one of the best adsorbents for organic pollutants due to its hydrophobicity, and microporous structure (Vasilyeva *et al*, 2006). Addition of activated carbon to the polluted soil leads to a process of sorption and biodegradation of the pollutants. This adsorption process is suitable for use in a lot of other processes like the remediation of soils contaminated with hydrocarbon. Activated carbon successfully reduces the bioavailability of organic contaminants due to its strong sorption properties (Bucheli & Gustafsson, 2000). Among the advantages of the use of activated carbon for oil spill cleanup is the fact that it has a high sorptional capacity with a relative low viscosity for 1g of the carbon. It also possesses a high rate of sorption and can also achieve reasonable level of cleanup at a relatively lower cost without serious negative impact on the environment (Amer and Hussein, 2006). It is worthy of note however that activated carbon will not remove any heavy metal from the cleanup site and it will also lose its sorptional capacity when it becomes saturated. The use of activated carbon as a cleanup medium provides a less costly option compared to the other available techniques (Sivakumar *et al*, 2011; Stenzel & Merz, 1989; Vasilyeva *et al*, 2006).

Bioregeneration is widely used in solving problems of pollutant contamination of the soil and water body. According to Coelho *et al* (2006), the main disadvantage inherent in the use of activated carbon to achieve the above is the issue of contamination by the pollutant. There are various methods that can be used to remove the pollutants from the adsorbents. The advantage of using biological regeneration over thermal as espoused by Coelho *et al* (2006) include the avoidance of loss of volatile compounds as well as pyrolysis of the non-volatile adsorbents at higher temperature.

The use of activated carbon plays a vital role in the cleanup of spill sites, the attendant secondary pollution created by its dumping can be eliminated by regenerating the adsorbent. The process of thermal regeneration is very expensive and energy consuming. Hazardous by-products are also produced and there is always tendency of imposing or introducing negative effect(s) on natural properties of the product. These have necessitated the search for a more cost effective, environmentally friendly and less cumbersome process of

regenerating used activated carbon. It is against this background that experimental studies on Bioregeneration of activated carbon contaminated with hydrocarbon is imperative.

II. Research Methodology

Three samples of polluted soil of different concentration of hydrocarbon were treated with virgin activated carbon. Characterization of the granular activated carbon was carried out by determining it surface area, bulk density, pore volume, pH, moisture content, ash content and particle size before and after regeneration. The optimum degradation temperature was determined from literature. The total hydrocarbon concentration of the used activated carbon was determined and the saturated activated carbon was extracted from the soil sample using a physical sieve of 1.7 - 2.4 mm. This is necessary because the particles size of granular activated carbon is larger than the soil granules. Extracted used activated carbon was then treated with pseudomonas putida bacteria culture. The treatment take place in a Bioreactor set up the laboratory. The rate of hydrocarbon degeneration was measured at intervals of 24 hours for 21 days by collecting samples and testing for hydrocarbon content and concentration. Evidence of activated carbon regeneration occurred by the reduction in the total hydrocarbon content (THC) in the sample over the 21 days.

III. Results And Discussions

The use of Granular Activated Carbon is one of the methods in achieving site remediation. It can also be used in the removal of organic constituents in waste water due to the important advantage of not adding anything detrimental to the water (DeSilva, 2000). For economic reasons, recovering of GAC already saturated with the hydrocarbon pollutant needs to be regenerated. Amongst the various methods for achieving this, the use of bioregeneration is chosen to ensure preservation of the structure of the GAC as well as protect the environment.

The commercially obtained Granular Activated Carbon was characterized before and after use for the bioregeneration exercise as shown by the results on Table 1. The results obtained indicated that the surface area of the virgin GAC was $738m^2/g$. The surface area for the regenerated GAC was measured to be $730m^2/g$. This implies minimal distortion and impact on the surface area of the GAC during the remediation and bioregeneration experiment. The surface area also falls within the acceptable standard range of 500 - $1500m^2/g$ (DeSilva, 2000) or 600 - $1200m^2/g$ (Jabit, 2007). The bulk density measured for the virgin GAC was $386kg/m^3$. After regeneration, the bulk density was measured at $379kg/m^3$ indicating the recovery of the quality of the GAC (SAJ Holdings SDN BDH, 2002). The pore volume of the virgin GAC was measured as $0.098cm^3/g$. The regenerated GAC also had a pore volume measured as $0.097cm^3/g$. The pore volume determines the size of molecules of the substance the GAC can adsorb (Jabit, 2007) and the results obtained indicates a high efficiency of regeneration. The value is also in alignment with the standard of $1.109cm^3/g$ (Hameed *et al*, 2006). The pH of the virgin GAC was measured as 6.0. After regeneration, the pH of the GAC was measured to be 6.4. Most of the adsorbed hydrocarbon was removed from the GAC and this manifested in the attainment of 6.4 as the pH. The pH of the regenerated GAC conforms to the standard of 6.0 - 7.0 (Metcalf & Eddy, 2003) or 6.8 (Ekpete and Horsfall, 2011). The percentage moisture content was measured to be 2.72 in the virgin GAC. The regenerated GAC however has a result of 2.71 and this falls within the acceptable moisture content limit of <5% (SAJ Holdings SDN BDH, 2002). The ash content was measured as shown on the table to be 3.69% for the virgin GAC and 3.58% for the regenerated GAC. This falls within the range of 2 - 10% (Jabit, 2007) and <15% (SAJ Holdings SDN BDH, 2002). The lower the ash value, the better the GAC for use as a adsorbent (Ekpete and Horsfall, 2011). The measured particle size for the virgin GAC and the regenerated GAC was the same with the value of 1.8mm.

Plates I and II pictorially show the GAC sample in its virgin state and also after regeneration respectively.

Table 1 Results on the characterization of granular activated carbon (GAC)

S/No	Property	Before Regeneration	Before Regeneration	Standard	Reference
1.	Surface area	738 m^2/g	730 m^2/g	500 - 1500m^2/g	DeSilva (2000)
2.	Bulk density	386 kg/m^3	379 kg/m^3	> 180 kg/ m^3	SAJ Holdings SDN BDH (2002)
3.	Pore volume	0.098 cm^3/g	0.097 cm^3/g	1.109	Hameed *et al.*, 2006
4.	pH	6.0	6.4	6.0 - 7.0	Metcalf and Eddy (2003)
5.	Moisture content	2.72%	2.71 %	<5% (AWWA)	SAJ Holdings SDN BDH (2002)
6.	Ash content	3.69 %	3.58%	<15%	SAJ Holdings SDN BDH (2002)
7.	Particle size	1.8 mm	1.8 mm	N/A	N/A

Plate I: Granular Activated carbon before remediation

Plate II: Granular Activated Carbon after remediation

Bioregeneration Experiment with Varying Bacteria Volume

Figure 1 is obtained from the values in Table 2. The results were obtained in the bioregeneration experiment by varying the volume of bacteria. As shown on the graph, there was a sharp decrease in THC after the first 24 hours of the experiment. This initial effect of the bacteria on the hydrocarbon was equally evident for 10, 20, 30 and 40ml bacteria volume experiments. There is a very fast production of CO_2 during the first phase of interaction between the bacteria and the hydrocarbon pollutant leading to the phenomenon above (Jonge *et al*, 1995). It also brings to the fore the fact that the volume of substrate as well as the kinetics of desorption of the hydrocarbon decreases as the contact time between both increases (Jonge *et al*, 1995). However, significant differences were noticed from day two to the twelfth day between the rate of desorption in the various samples for the bioregeneration experiment. The decrease in THC for the 10 and 20ml bacteria volume became very slow while the rate of decrease was very evident for the 30 and 40 ml bacteria volume. The reason for this is due to the presence of more bacteria considering the volume used. By implication, the samples with lower volume of bacteria had their bacteria used up earlier thereby reducing the rate of desorption over the same period of time. After day twelve, there was noticeable decrease in THC for all bacteria volume. On the last day of the experiment, the final THC for the 10, 20, 30 and 40ml bacteria volume was 7.308, 1.988, 0.526 and 0.339 respectively. It was evident that increasing the concentration of the micro organism would increase the efficiency of bioregeneration within the same time duration as confirmed by Nath *et al* (2011). At 40ml, the rate of decrease in THC was steady and evident through the duration of the experiment.

Table 2 Variation of bacterial volume in saturated GAC (100g)

	Bact. 10ml	Bact. 20ml	Bact. 30ml	Bact. 40ml
INITIAL	25.48	25.48	25.48	25.48
4/5/15	23.930	23.880	22.914	22.271
5/5/15	23.701	23.820	22.401	21.508
6/5/15	23.462	23.510	21.987	20.333
7/5/15	23.255	23.070	21.533	20.164
8/5/15	22.794	22.820	21.188	19.897
9/5/15	22.749	22.400	21.110	19.016
10/5/15	22.708	22.366	21.102	19.000
11/5/15	22.688	22.363	20.001	18.582
11/5/15	22.645	22.358	20.668	18.133
12/5/15	22.617	22.351	20.183	17.674
13/5/15	22.585	22.346	20.112	17.611
14/5/15	22.500	22.341	20.011	17.489
15/5/15	22.466	22.100	19.600	17.066
16/5/15	20.771	19.886	16.981	14.591
17/5/15	18.708	17.237	13.660	11.796
18/5/15	16.931	16.944	10.814	8.844
19/5/15	15.884	14.188	7.716	5.533
20/5/15	14.930	11.894	5.842	2.994
21/5/15	13.533	9.629	3.770	1.877
22/5/15	11.220	6.535	1.077	1.087
23/5/15	9.781	3.358	0.933	0.621
24/5/12	7.308	1.988	0.526	0.339

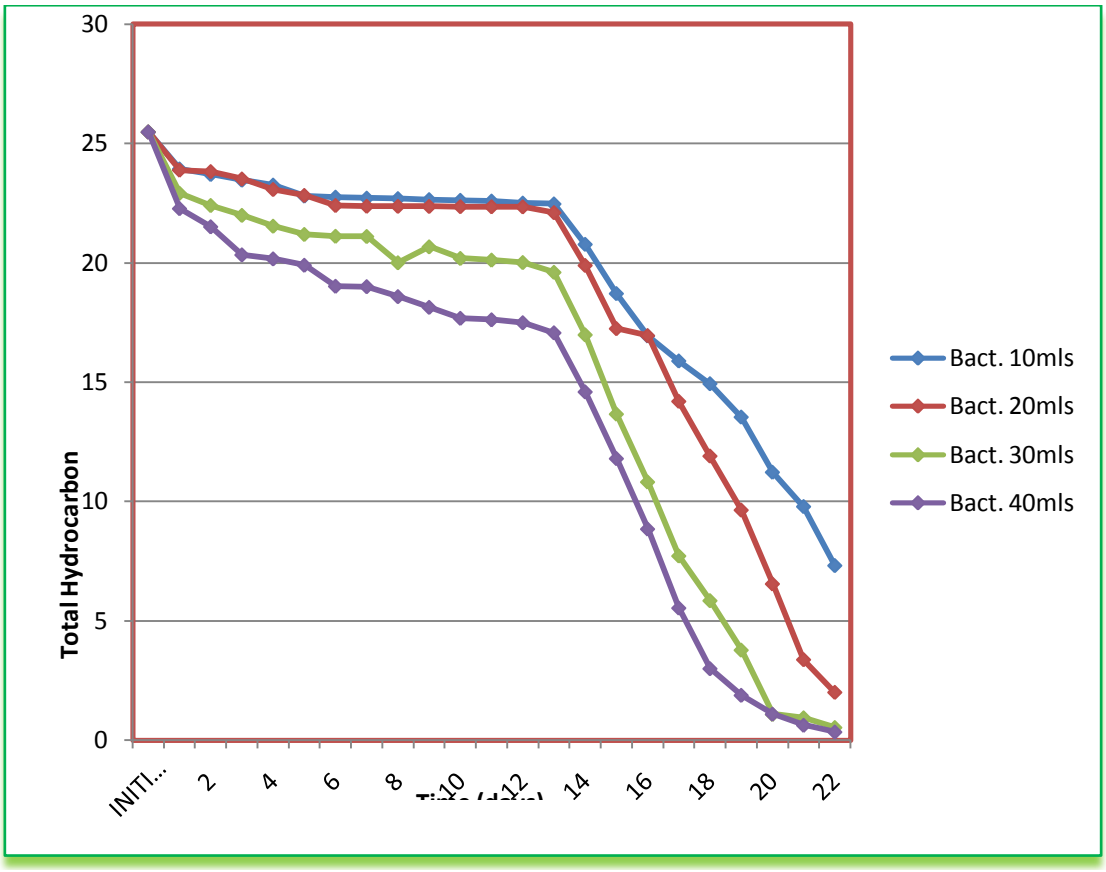

Figure 2: Bioregeneration at various volume of bacteria

Bioregeneration at Varying Temperatures

The Figure 2 below is developed from the Table 3. The graph shows the impact of change in temperature on the rate of bioregeneration. The initial THC was 24.349 for all the experimental temperatures. At 27^{o}C which was the ambient temperature, there was no significant drop in THC until the 6th day. This lag phase is possibly due to the inhibitory effect of the phenol constituent in the hydrocarbon (Ullhyan & Ghosh, 2012). Noticeable drops in THC content were observed at 25 and 35^{o}C and this was consistent for the 21 days of the experiment. Increasing the temperature above the ambient of 27^{o}C led to increase in the regeneration. This could be attributed to chemisorption (Lashaki *et al*, 2012). Final THC for the 25, 27 and 35^{o}C were 0.785, 0.599 and 1.535 respectively. There was need to probe the impact of temperature further considering that 25^{o}C was below ambient and at ambient temperature, there was an unfavourable impact on the bacteria for the first six days of lag phase (Ullhyan & Ghosh, 2012).

Table 3 Bioregeneration at Different Temperatures

	35^{0}C	**25^{0}C**	**27^{o}C**
INITIAL THC	24.349	24.349	24.349
6/4/12	20.934	20.188	24.344
7/4/12	19.880	18.835	24.338
8/4/12	19.839	18.196	24.334
9/4/12	19.274	17.886	24.214
10/4/12	19.175	17.513	24.166
11/4/12	17.800	15.159	22.616
12/4/12	15.734	12.228	18.980
13/4/12	13.990	9.741	16.841
14/4/12	13.400	9.212	15.002
15/4/12	12.656	8.884	13.629
16/4/12	11.172	7.808	12.254
17/4/12	9.534	5.791	11.060
18/4/12	7.877	4.664	8.361
19/4/12	6.690	3.990	6.574

20/4/12	5.880	2.830	5.080
21/4/12	4.109	2.526	3.208
22/4/12	3.770	1.944	2.894
23/4/12	3.502	1.606	1.979
24/4/12	2.183	1.207	1.526
25/4/12	1.774	0.962	0.880
26/4/12	1.535	0.785	0.599

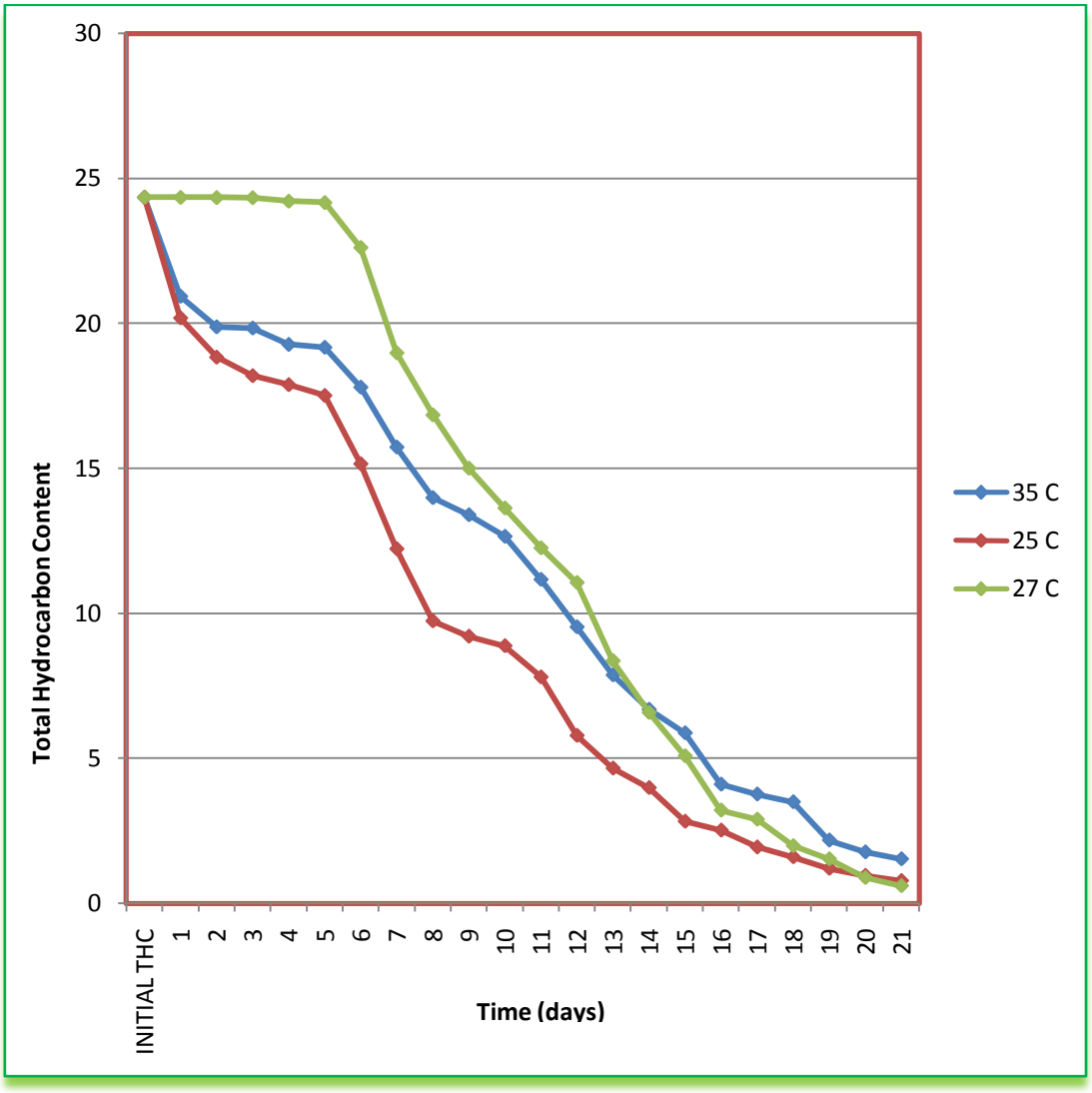

Figure 2: Bioregeneration at various temperatures

As mentioned above, there was need to further study the impact of higher temperature on the rate of bioregeneration. The final THC at 35^{o}C was used as the initial THC for the extended experiment at 40 and 45^{o}C. The results shown on Figure 3 shows that at 45^{o}C, there was initially no significant impact for about 10days unlike the situation at 40^{o}C where noticeable and steady decrease in THC was observed all through the experiment. At the end of the experiment on the 21st day, the final THC was almost equal for both temperatures. The optimum experimental temperature for bioregeneration is suggested to be 35^{o}C to 45^{o}C (Lashaki *et al*, 2012). It is important to consider the energy used at 45^{o}C and associated cost if same regeneration efficiency can be achieved at 40^{o}C.

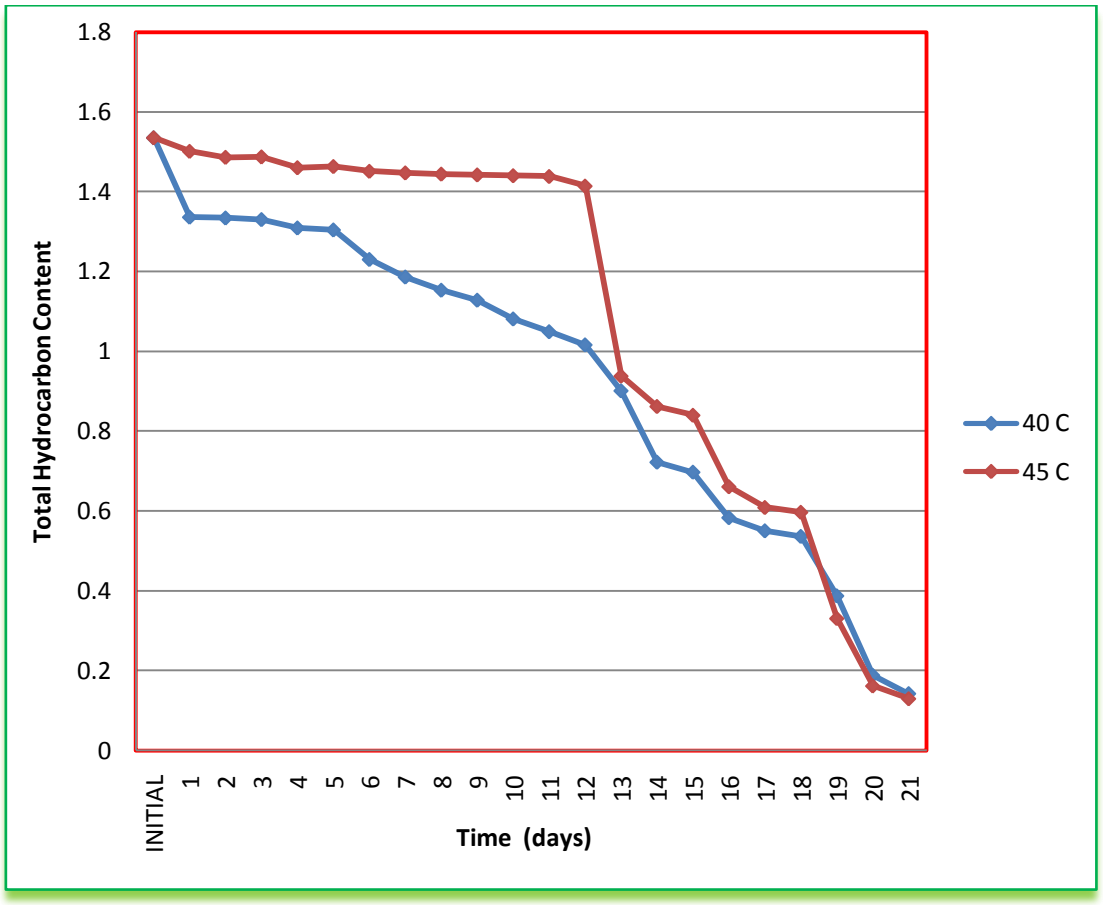

Figure 3: Bioregeneration expanded to 40 and 45^{o}C

VI. Conclusions

Bioregeneration is very effective in recovering spent granulated activated carbon (GAC) for reuse considering the quality of the regenerated GAC in comparison to the virgin sample. Increasing the volume of bacteria increased the rate of Bioregeneration. Also, temperature plays an important role in Bioregeneration efficiency and increasing the temperature improved the efficiency in as much as it is beyond the temperature that will incapacitate the bacteria colony. Effective Bioregeneration was achieved at 40^{o}C as such it is concluded that increasing the temperature of Bioregeneration to 45^{o}C was not cost effective.

Acknowledgments

My immense gratitude goes to my Project Supervisor, Dr. Abdulfatai Jimoh, my co-Supervisor, Dr. A. S Abdulkareem who was always available to support this cause from the very first day. I feel delighted that your support has brought this dream to fruition.

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APPENDICES

APPENDIX I
GRAPHICAL AND TABULAR PRESENTATION OF DATA

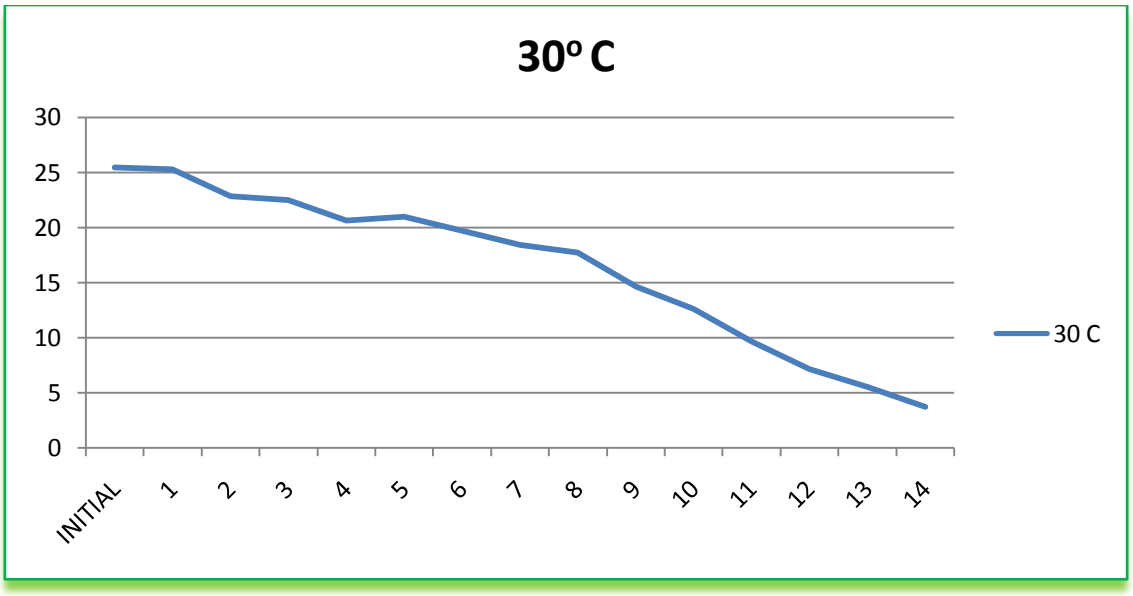

Fig I-A: Remediation at 30°C

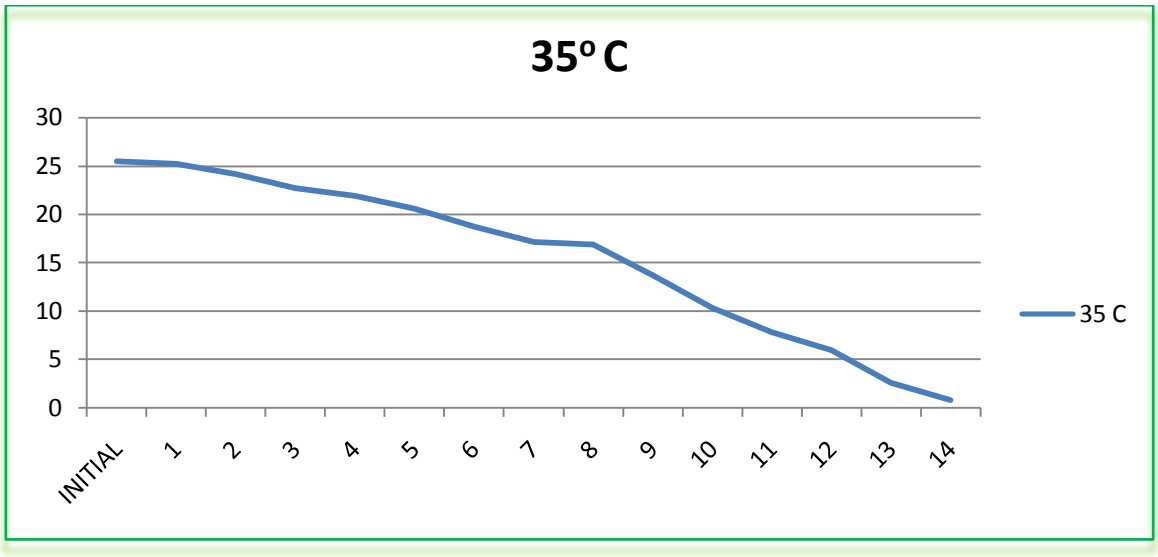

Fig I-B: Remediation @ 35°C

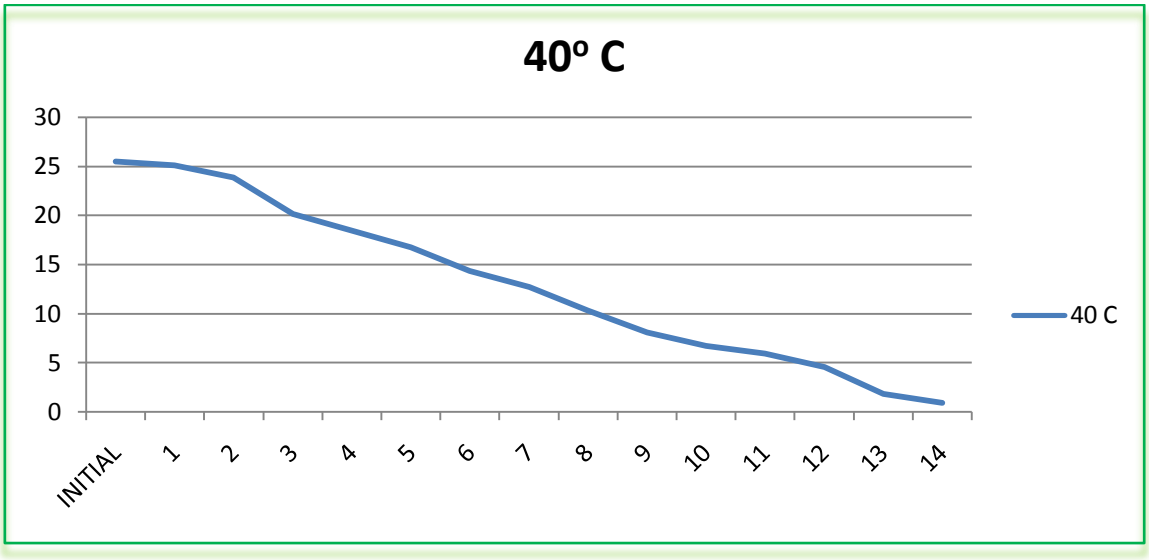

Fig I-C: Remediation @ 40°C

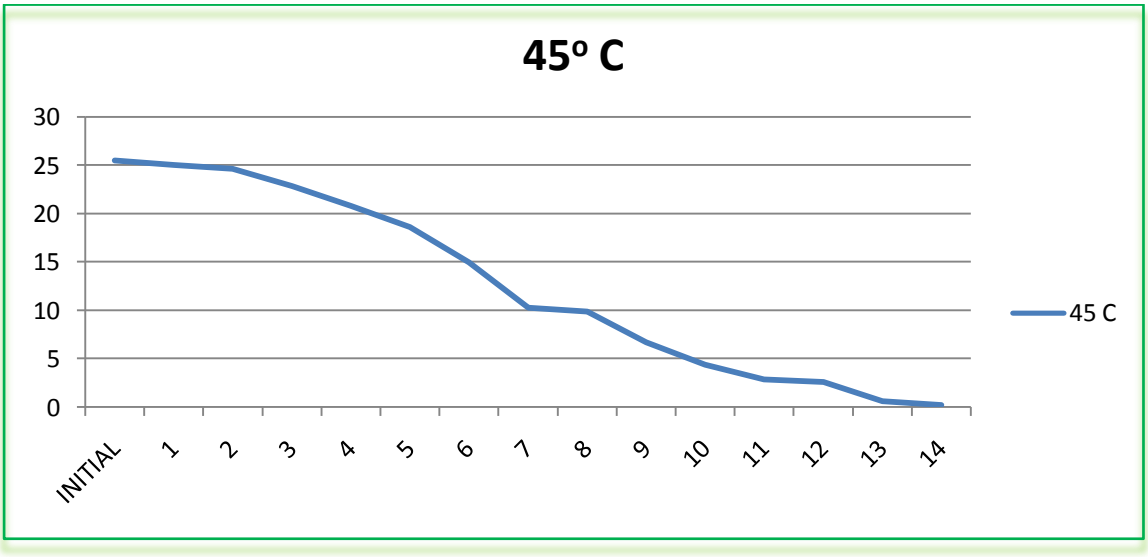

Fig I-D: Remediation @ 45°C

Table I-A: Variation of bacterial volume in saturated GAC (100g)

	Bact. 10ml	Bact. 20ml	Bact. 30ml	Bact. 40ml
INITIAL	25.48	25.48	25.48	25.48
4/5/15	23.930	23.880	22.914	22.271
5/5/15	23.701	23.820	22.401	21.508
6/5/15	23.462	23.510	21.987	20.333
7/5/15	23.255	23.070	21.533	20.164
8/5/15	22.794	22.820	21.188	19.897
9/5/15	22.749	22.400	21.110	19.016
10/5/15	22.708	22.366	21.102	19.000
11/5/15	22.688	22.363	20.001	18.582
11/5/15	22.645	22.358	20.668	18.133
12/5/15	22.617	22.351	20.183	17.674
13/5/15	22.585	22.346	20.112	17.611
14/5/15	22.500	22.341	20.011	17.489
15/5/15	22.466	22.100	19.600	17.066
16/5/15	20.771	19.886	16.981	14.591
17/5/15	18.708	17.237	13.660	11.796
18/5/15	16.931	16.944	10.814	8.844
19/5/15	15.884	14.188	7.716	5.533
20/5/15	14.930	11.894	5.842	2.994
21/5/15	13.533	9.629	3.770	1.877
22/5/15	11.220	6.535	1.077	1.087
23/5/15	9.781	3.358	0.933	0.621
24/5/12	7.308	1.988	0.526	0.339

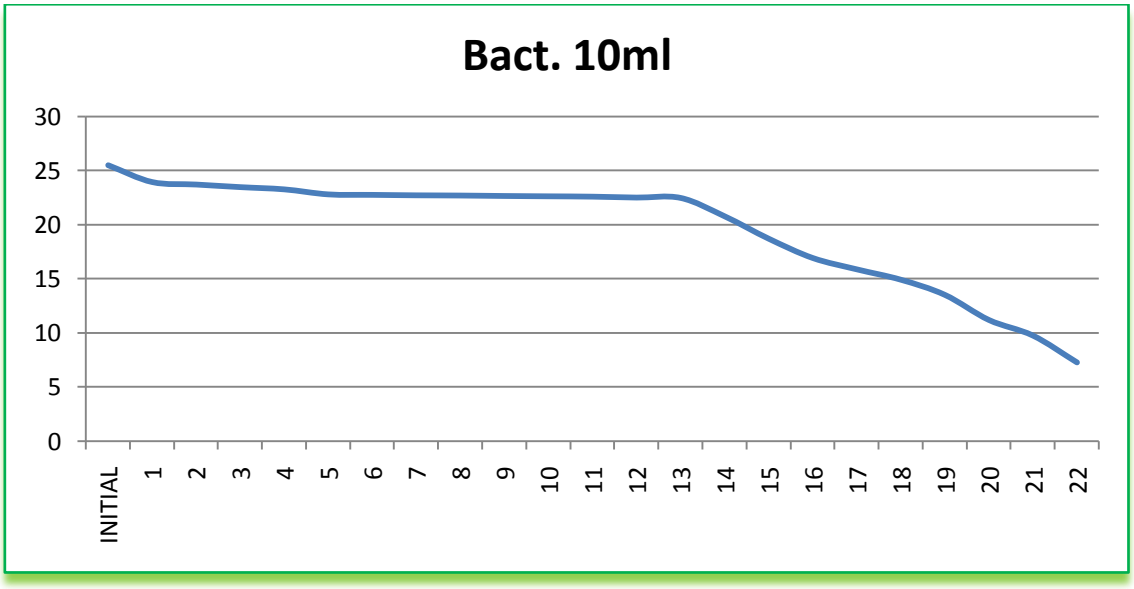

Fig I-E: Bioregeneration @ 10ml of bacteria

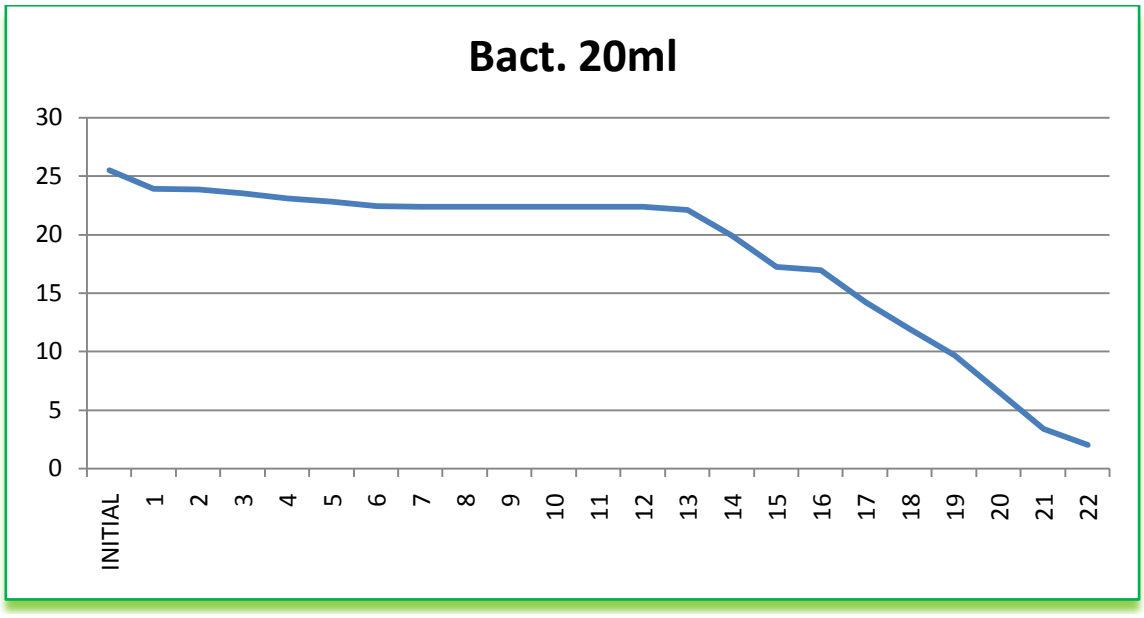

Fig I-F: Bioregeneration @ 20ml of bacteria

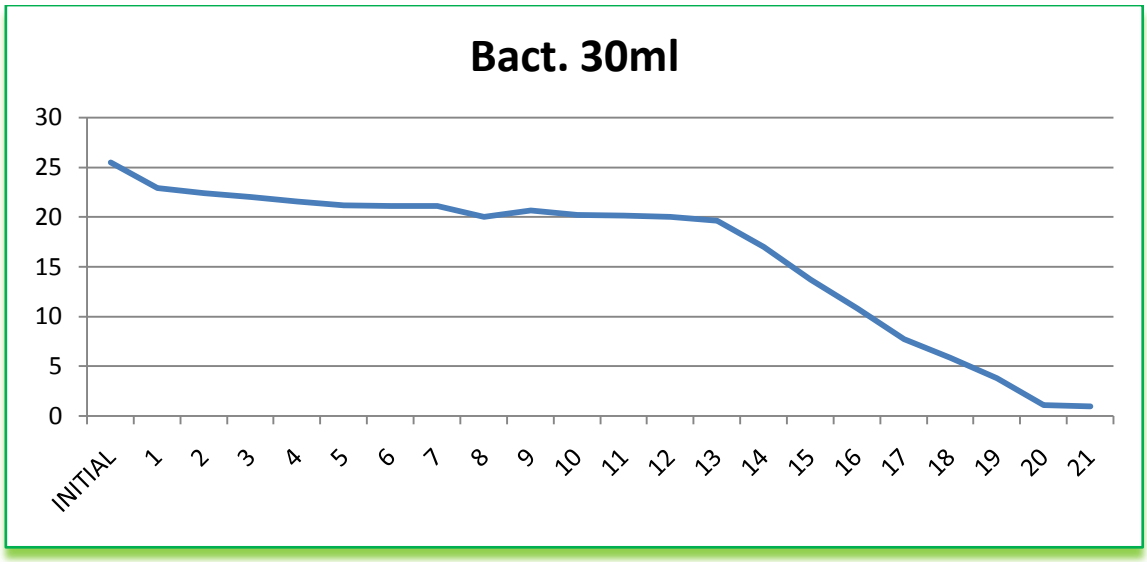

Fig I-G: Bioregeneration @ 30ml of bacteria

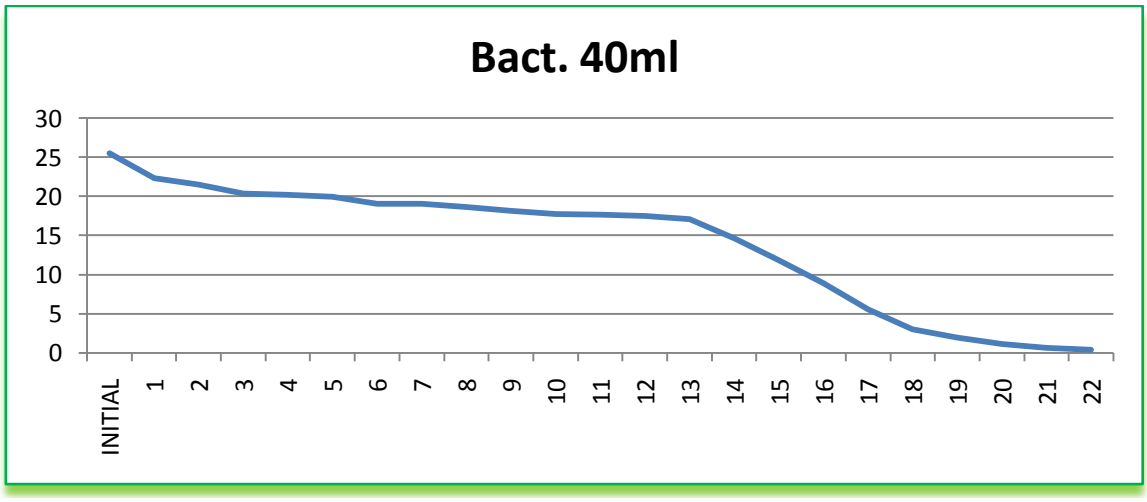

Fig I-H: Bioregeneration @ 40ml of bacteria

Table I-B: Bioregeneration at Different Temperatures

	35^0C	25^0C	27^oC
INITIAL THC	24.349	24.349	24.349
6/4/12	20.934	20.188	24.344
7/4/12	19.880	18.835	24.338
8/4/12	19.839	18.196	24.334
9/4/12	19.274	17.886	24.214
10/4/12	19.175	17.513	24.166
11/4/12	17.800	15.159	22.616
12/4/12	15.734	12.228	18.980
13/4/12	13.990	9.741	16.841
14/4/12	13.400	9.212	15.002
15/4/12	12.656	8.884	13.629
16/4/12	11.172	7.808	12.254
17/4/12	9.534	5.791	11.060
18/4/12	7.877	4.664	8.361
19/4/12	6.690	3.990	6.574
20/4/12	5.880	2.830	5.080
21/4/12	4.109	2.526	3.208
22/4/12	3.770	1.944	2.894
23/4/12	3.502	1.606	1.979
24/4/12	2.183	1.207	1.526
25/4/12	1.774	0.962	0.880
26/4/12	1.535	0.785	0.599

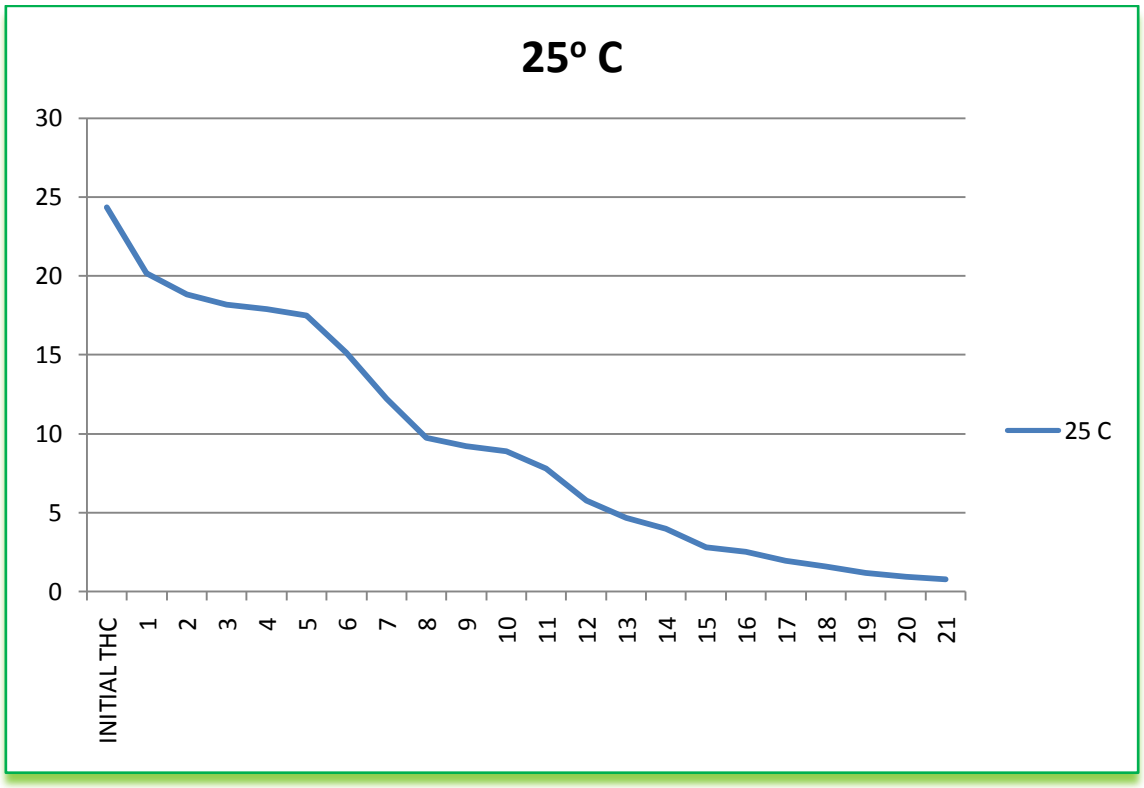

Fig I-J: Bioregeneration experiment at 25^oC

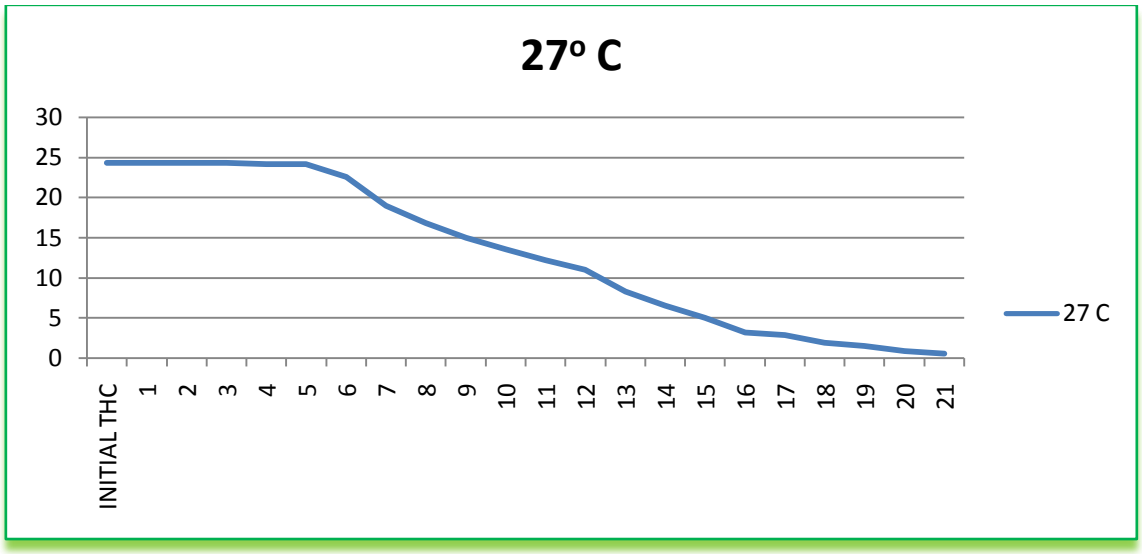

Fig I-K: Bioregeneration experiment at 27°C

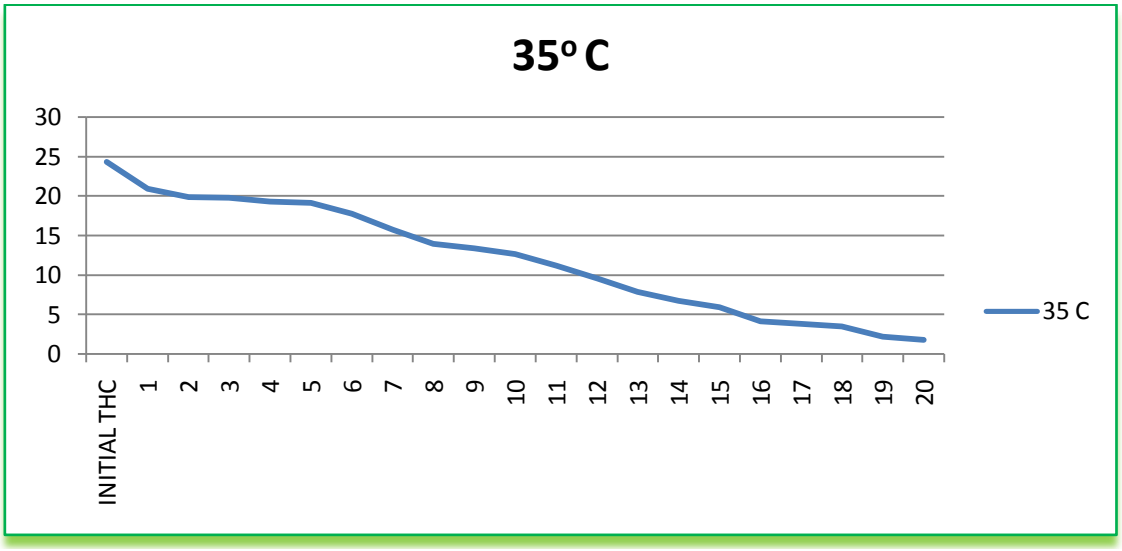

Fig I-L: Bioregeneration experiment at 35°C

Table I-C: Further bioregeneration at higher temperatures

	40^{0}c	**45^{0}c**
INITIAL	1.535	1.535
4/5/15	1.336	1.501
5/5/15	1.334	1.486
6/5/15	1.330	1.487
7/5/15	1.309	1.460
8/5/15	1.304	1.463
9/5/15	1.230	1.451
10/5/15	1.186	1.447
11/5/15	1.153	1.444
12/5/15	1.128	1.442
13/5/15	1.081	1.440
14/5/15	1.049	1.438
15/5/15	1.016	1.414
16/5/15	0.901	0.938

17/5/15	0.722	0.862
18/5/15	0.697	0.840
19/5/15	0.583	0.661
20/5/15	0.550	0.609
21/5/15	0.536	0.597
22/5/15	0.387	0.331
23/5/15	0.188	0.162
24/5/12	0.142	0.130

No. of days = 21

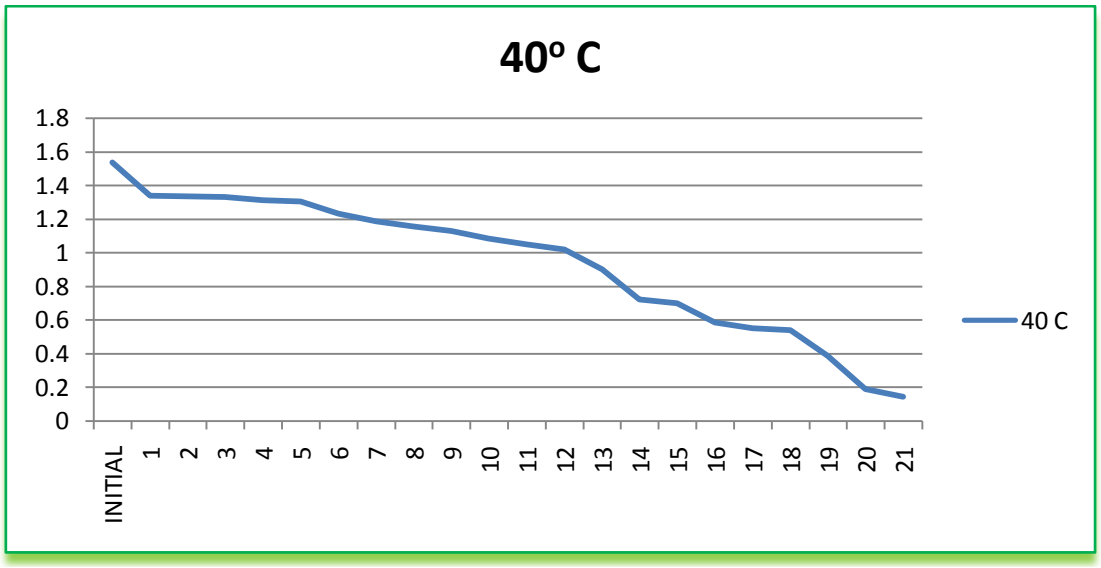

Fig I-M: Further bioregeneration at 40^{o}C

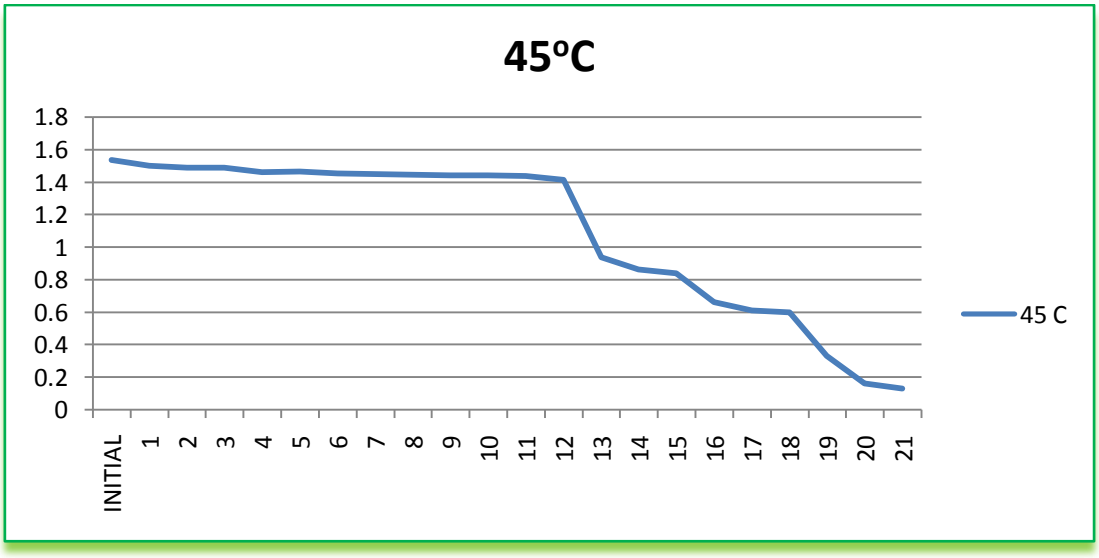

Fig I-N: Further Bioregeneration at 45^{o}C

Table I-D: Model simulation at 10ml of bacteria

S/No	Days	t	CAO	CA	rA	R	CA^2	R.CA	rm
1	4	24	25.48	23.93	0.064583	370.529	572.6449	8866.76	0.077582
2	5	48	25.48	23.701	0.037063	639.4874	561.7374	15156.49	0.043619
3	6	72	25.48	23.462	0.028028	837.0981	550.4654	19640	0.029753
4	7	96	25.48	23.255	0.023177	1003.362	540.795	23333.18	0.023234
5	8	120	25.48	22.794	0.022383	1018.347	519.5664	23212.2	0.015466
6	9	144	25.48	22.749	0.018965	1199.508	517.517	27287.6	0.014967

7	10	168	25.48	22.708	0.0165	1376.242	515.6533	31251.71	0.014537
8	11	192	25.48	22.645	0.014766	1533.63	512.796	34729.04	0.013921
9	12	216	25.48	22.617	0.013255	1706.347	511.5287	38592.45	0.013663
10	13	240	25.48	22.585	0.012063	1872.332	510.0822	42286.61	0.013378
11	14	264	25.48	22.5	0.011288	1993.289	506.25	44848.99	0.012673
12	15	288	25.48	22.466	0.010465	2146.718	504.7212	48228.17	0.01241
13	16	312	25.48	20.771	0.015093	1376.206	431.4344	28585.17	0.018499
14	17	336	25.48	18.708	0.020155	928.2174	349.9893	17365.09	0.019457
15	18	360	25.48	16.931	0.023747	712.9676	286.6588	12071.25	0.020583
16	19	384	25.48	15.884	0.02499	635.6248	252.3015	10096.27	0.021444
17	20	408	25.48	14.93	0.025858	577.3877	222.9049	8620.398	0.022414
18	21	432	25.48	13.533	0.027655	489.3493	183.1421	6622.364	0.024301
19	22	456	25.48	11.22	0.031272	358.7882	125.8884	4025.604	0.029838
20	23	480	25.48	9.781	0.032706	299.056	95.66796	2925.067	0.037117
21	24	504	25.48	7.308	0.036056	202.6872	53.40686	1481.238	0.10416

Table I-E: Model simulation at 20ml of bacteria

S/No		t	CAO	CA	rA	R	CA^2	R.CA	rm
1	4	24	25.48	23.88	0.066667	358.2	570.2544	8553.816	0.049851519
2	5	48	25.48	23.82	0.034583	688.7711	567.3924	16406.53	0.045902372
3	6	72	25.48	23.51	0.027361	859.2487	552.7201	20200.94	0.032422989
4	7	96	25.48	23.07	0.025104	918.971	532.2249	21200.66	0.022667899
5	8	120	25.48	22.82	0.022167	1029.474	520.7524	23492.59	0.019273498
6	9	144	25.48	22.4	0.021389	1047.273	501.76	23458.91	0.01530741
7	10	168	25.48	22.366	0.018536	1206.644	500.238	26987.79	0.015051586
8	11	192	25.48	22.358	0.01626	1374.996	499.8802	30742.15	0.014992519
9	12	216	25.48	22.357	0.014458	1546.305	499.8354	34570.75	0.014985166
10	13	240	25.48	22.346	0.013058	1711.244	499.3437	38239.47	0.014904706
11	14	264	25.48	22.341	0.01189	1878.95	499.1203	41977.62	0.014868392
12	15	288	25.48	22.1	0.011736	1883.077	488.41	41616	0.01329029
13	16	312	25.48	19.886	0.017929	1109.123	395.453	22056.01	0.022392794
14	17	336	25.48	17.237	0.024533	702.6122	297.1142	12110.93	0.023047841
15	18	360	25.48	16.944	0.023711	714.6017	287.0991	12108.21	0.023135696
16	19	384	25.48	14.188	0.029406	482.4825	201.2993	6845.461	0.02418938
17	20	408	25.48	11.894	0.033299	357.1877	141.4672	4248.39	0.025570292
18	21	432	25.48	9.629	0.036692	262.4269	92.71764	2526.908	0.027886339
19	22	456	25.48	6.535	0.041546	157.2953	42.70623	1027.925	0.035991126
20	23	480	25.48	3.358	0.046088	72.8614	11.27616	244.6686	0.249507322
21	24	504	25.48	1.988	0.046611	42.65077	3.952144	84.78974	-0.03367417

Table I-F: Model simulation at 30ml of bacteria

S/No		t	CAO	CA	rA	R	CA^2	R.CA	rm
1	4	24	25.48	22.914	0.106917	214.3164	525.0514	4910.847	0.095968
2	5	48	25.48	22.401	0.064146	349.2199	501.8048	7822.874	0.062206
3	6	72	25.48	21.987	0.048514	453.2104	483.4282	9964.738	0.048003
4	7	96	25.48	21.533	0.041115	523.7314	463.6701	11277.51	0.038083
5	8	120	25.48	21.188	0.035767	592.3952	448.9313	12551.67	0.032747
6	9	144	25.48	21.11	0.030347	695.6156	445.6321	14684.44	0.031722
7	10	168	25.48	21.102	0.02606	809.7615	445.2944	17087.59	0.03162
8	11	192	25.48	20.668	0.025063	824.6584	427.1662	17044.04	0.026841
9	12	216	25.48	20.183	0.024523	823.0183	407.3535	16610.98	0.022812
10	13	240	25.48	20.112	0.022367	899.1952	404.4925	18084.61	0.022309
11	14	264	25.48	20.011	0.020716	965.9726	400.4401	19330.08	0.021624
12	15	288	25.48	19.6	0.020417	960	384.16	18816	0.019166
13	16	312	25.48	16.981	0.02724	623.3759	288.3544	10585.55	0.032033
14	17	336	25.48	13.66	0.035179	388.3046	186.5956	5304.24	0.032658

15	18	360	25.48	10.814	0.040739	265.4466	116.9426	2870.54	0.033539
16	19	384	25.48	7.716	0.04626	166.7949	59.53666	1286.989	0.035378
17	20	408	25.48	5.842	0.048132	121.3737	34.12896	709.0649	0.037692
18	21	432	25.48	3.77	0.050255	75.01796	14.2129	282.8177	0.044239
19	22	456	25.48	1.077	0.053515	20.12507	1.159929	21.6747	-0.19693
20	23	480	25.48	0.933	0.05114	18.24418	0.870489	17.02182	-0.09042
21	24	504	25.48	0.526	0.049512	10.62371	0.276676	5.58807	-0.02189

Table I-G: Model simulation at 40ml of bacteria

S/No		t	CAO	CA	rA	R	CA^2	R.CA	rm
1	4	24	25.48	22.271	0.133708	166.564	495.9974	3709.548	0.116643
2	5	48	25.48	21.508	0.08275	259.9154	462.5941	5590.261	0.086599
3	6	72	25.48	20.333	0.071486	284.4329	413.4309	5783.374	0.060371
4	7	96	25.48	20.164	0.055375	364.1354	406.5869	7342.427	0.05769
5	8	120	25.48	19.897	0.046525	427.6625	395.8906	8509.202	0.053831
6	9	144	25.48	19.016	0.044889	423.6238	361.6083	8055.629	0.043622
7	10	168	25.48	19	0.038571	492.5926	361	9359.259	0.043465
8	11	192	25.48	18.133	0.038266	473.8718	328.8057	8592.717	0.036094
9	12	216	25.48	17.674	0.036139	489.0576	312.3703	8643.605	0.032916
10	13	240	25.48	17.611	0.032788	537.1254	310.1473	9459.316	0.032512
11	14	264	25.48	17.489	0.030269	577.787	305.8651	10104.92	0.031748
12	15	288	25.48	17.066	0.029215	584.1464	291.2484	9969.043	0.029288
13	16	312	25.48	14.591	0.034901	418.0726	212.8973	6100.097	0.038516
14	17	336	25.48	11.796	0.040726	289.6416	139.1456	3416.613	0.038961
15	18	360	25.48	8.844	0.046211	191.3825	78.21634	1692.587	0.03976
16	19	384	25.48	5.533	0.051945	106.5159	30.61409	589.3523	0.041813
17	20	408	25.48	2.994	0.055113	54.325	8.964036	162.6491	0.047348
18	21	432	25.48	1.877	0.054637	34.35428	3.523129	64.48298	0.057162
19	22	456	25.48	1.087	0.053493	20.32026	1.181569	22.08812	0.095872
20	23	480	25.48	0.621	0.05179	11.99083	0.385641	7.446304	-0.46229
21	24	504	25.48	0.339	0.049883	6.795911	0.114921	2.303814	-0.03759

Table I-H: Model simulation at 25^{o}C temperature

S/No	Days	t	CAO	CA	rA	R	CA^2	R.CA	rm
1	4	24	24.349	20.188	0.173375	116.4412	407.5553	2350.716	0.083672
2	5	48	24.349	18.835	0.114875	163.9608	354.7572	3088.202	0.083117
3	6	72	24.349	18.196	0.085458	212.9225	331.0944	3874.337	0.08283
4	7	96	24.349	17.886	0.067323	265.6748	319.909	4751.859	0.082684
5	8	120	24.349	17.513	0.056967	307.4254	306.7052	5383.941	0.082502
6	9	144	24.349	15.159	0.063819	237.5295	229.7953	3600.71	0.081172
7	10	168	24.349	12.228	0.072149	169.483	149.524	2072.439	0.078904
8	11	192	24.349	9.741	0.076083	128.0307	94.88708	1247.147	0.076096
9	12	216	24.349	9.212	0.070079	131.4522	84.86094	1210.938	0.075339
10	13	240	24.349	8.884	0.064438	137.87	78.92546	1224.837	0.074833
11	14	264	24.349	7.808	0.062655	124.6183	60.96486	973.02	0.072937
12	15	288	24.349	5.791	0.064438	89.87003	33.53568	520.4373	0.067983
13	16	312	24.349	4.664	0.063093	73.92268	21.7529	344.7754	0.063922
14	17	336	24.349	3.99	0.060592	65.84999	15.9201	262.7415	0.060771
15	18	360	24.349	2.83	0.059775	47.34421	8.0089	133.9841	0.053316
16	19	384	24.349	2.526	0.056831	44.44778	6.380676	112.2751	0.05074
17	20	408	24.349	1.944	0.054914	35.40067	3.779136	68.8189	0.044717
18	21	432	24.349	1.606	0.052646	30.50574	2.579236	48.99222	0.040343
19	22	456	24.349	1.207	0.05075	23.78325	1.456849	28.70638	0.034016
20	23	480	24.349	0.962	0.048723	19.7443	0.925444	18.99402	0.029306
21	24	504	24.349	0.785	0.046754	16.79002	0.616225	13.18016	0.025398

Table I-J: Model simulation at 27^{o}C temperature

S/No		t	CAO	CA	rA	R	CA^2	R.CA	rm
1	4	24	24.349	24.344	0.000208	116851.2	592.6303	2844626	0.000491861
2	5	48	24.349	24.338	0.000229	106202.2	592.3382	2584749	0.000491898
3	6	72	24.349	24.334	0.000208	116803.2	592.1436	2842289	0.000491923
4	7	96	24.349	24.214	0.001406	17218.84	586.3178	416937.1	0.000492682
5	8	120	24.349	24.166	0.001525	15846.56	583.9956	382947.9	0.000492989
6	9	144	24.349	22.616	0.012035	1879.229	511.4835	42500.64	0.000503822
7	10	168	24.349	18.98	0.031958	593.8983	360.2404	11272.19	0.000539211
8	11	192	24.349	16.841	0.039104	430.6702	283.6193	7252.917	0.00057089
9	12	216	24.349	15.002	0.043273	346.6815	225.06	5200.916	0.000609863
10	13	240	24.349	13.629	0.044667	305.1269	185.7496	4158.574	0.000650855
11	14	264	24.349	12.254	0.045814	267.4705	150.1605	3277.584	0.000709307
12	15	288	24.349	11.06	0.046142	239.693	122.3236	2651.004	0.000784719
13	16	312	24.349	8.361	0.051244	163.1619	69.90632	1364.196	0.001211411
14	17	336	24.349	6.574	0.052902	124.268	43.21748	816.9379	0.003072201
15	18	360	24.349	5.08	0.053525	94.90892	25.8064	482.1373	-0.002758757
16	19	384	24.349	3.208	0.055055	58.26933	10.29126	186.928	-0.000469696
17	20	408	24.349	2.894	0.052586	55.03388	8.375236	159.2681	-0.000377476
18	21	432	24.349	1.979	0.051782	38.21761	3.916441	75.63266	-0.000195843
19	22	456	24.349	1.526	0.05005	30.48924	2.328676	46.52659	-0.000134899
20	23	480	24.349	0.88	0.048894	17.99821	0.7744	15.83843	-6.75175E-05
21	24	504	24.349	0.599	0.047123	12.71141	0.358801	7.614135	-4.3461E-05

Table I-K: Model simulation at 35^{o}C temperature

S/No		t	CAO	CA	rA	R	CA^2	R.CA	rm
1	4	24	24.349	20.934	0.142292	147.1204	438.2324	3079.817	0.06038
2	5	48	24.349	19.88	0.093104	213.5243	395.2144	4244.863	0.060113
3	6	72	24.349	19.839	0.062639	316.7202	393.5859	6283.412	0.060102
4	7	96	24.349	19.274	0.052865	364.5919	371.4871	7027.145	0.059948
5	8	120	24.349	19.175	0.043117	444.7236	367.6806	8527.575	0.05992
6	9	144	24.349	17.8	0.045479	391.388	316.84	6966.706	0.059503
7	10	168	24.349	15.734	0.05128	306.8267	247.5588	4827.611	0.058755
8	11	192	24.349	13.99	0.053953	259.2992	195.7201	3627.595	0.057972
9	12	216	24.349	13.4	0.05069	264.3529	179.56	3542.329	0.057667
10	13	240	24.349	12.656	0.048721	259.7657	160.1743	3287.594	0.057247
11	14	264	24.349	11.172	0.049913	223.83	124.8136	2500.629	0.056267
12	15	288	24.349	9.534	0.051441	185.3386	90.89716	1767.019	0.054889
13	16	312	24.349	7.877	0.052795	149.2001	62.04713	1175.249	0.053027
14	17	336	24.349	6.69	0.052557	127.2915	44.7561	851.5799	0.051251
15	18	360	24.349	5.88	0.051303	114.6137	34.5744	673.9284	0.049729
16	19	384	24.349	4.109	0.052708	77.95731	16.88388	320.3266	0.044974
17	20	408	24.349	3.77	0.050439	74.74416	14.2129	281.7855	0.043726
18	21	432	24.349	3.502	0.048257	72.56987	12.264	254.1397	0.042629
19	22	456	24.349	2.183	0.04861	44.90878	4.765489	98.03587	0.035134
20	23	480	24.349	1.774	0.047031	37.7196	3.147076	66.91457	0.03172
21	24	504	24.349	1.535	0.045266	33.91076	2.356225	52.05301	0.02935