ISSN 1996-3351

Asian Journal of Biological Sciences

http://knowledgiascientific.com

Knowledgia SCIENTIFIC
A Place to Publish Outstanding Research

Asian Journal of Biological Sciences 4 (4): 369-374, 2011
ISSN 1996-3351 / DOI: 10.3923/ajbs.2011.369.374

Glucose Level and Amylase Activity in Crude Oil Contaminated Soil Bioremediated with Poultry Manure and Sawdust

[1]E. Jeroh, [2]N.J. Tonukari and [2]A. Anigboro
[1]Department of Biology, Federal Government College, P.M.B. 1014, Warri, Delta State, Nigeria
[2]Department of Biochemistry, Delta State University, Abraka, Nigeria

Corresponding Author: E. Jeroh, Department of Biology, Federal Government College, P.M.B. 1014, Warri, Delta State, Nigeria Tel: +2348059634317, +2347040060587, +2348137936850

ABSTRACT
The analysis of soil glucose level and amylase activity in crude oil treated soil following remediation with Poultry Manure (PM) and Sawdust (SD) were studied for four weeks using standard bioremediation techniques. The aim of this research was to analyse the effect of sawdust and poultry manure on the bioremediation of crude oil contaminated soil. Amylase activity was determined by the Richard and Norbert method while glucose was determined by the Barham and Trinder method of analysis respectively. The aim of this study was to determine the role sawdust and poultry manure play in the process of bioremediation of crude oil contaminated soil. The result showed a statistically significant decrease in soil glucose level after bioremediation ($p<0.05$). The introduction of sawdust, led to increase in soil glucose level on day 14 (0.09±0.222, 0.07±0.242 and 0.08±0.242 (mmol g^{-1}), respectively, while the drop experienced on day 28 may be due to the utilization of available glucose by microorganisms for metabolic activities that led to biodegradation of crude oil. The amylase activity in the soil upon crude oil contamination reduced from 151.40±1.22 to 150.80±1.22 IU g^{-1}. Statistical analysis shows that treatment of crude oil contaminated soil with sawdust and poultry manure increased soil amylase activity ($p<0.05$). The results of this study suggest that a combination of poultry manure and sawdust in bioremediation of crude oil contaminated soil is a solution to the problem of oil spillage in the Niger Delta region of Nigeria.

Key words: Crude oil, bioremediation, glucose, amylase activity, pollution

INTRODUCTION
Advance in science and technology since the industrial revolution has increasingly enabled humans to exploit natural resources. Soil is one of the most vital natural resources which produce food for millions of people and supplies raw materials for a large number of industries on which the world economy is sustained. The soil is a key component of natural ecosystem because environmental sustainability depends largely on a sustainable soil ecosystem (Adriano *et al*., 1998). The soil is a veritable tool in agricultural practices. Agricultural practices that reduce soil degradation and improve agricultural sustainability are needed particularly for tropical and sub tropical soils.

Balota *et al*. (2004) observed that there is correlation of soil enzyme activity with total organic carbon, carbon and nitrogen biomass. Soil enzymes (amylases, acid and alkaline phosphatases, cellulases and arylsulfatases, among others) regulate ecosystem functioning and in particular, play a key role in nutrient cycling. They catalyses certain important reactions necessary for the life processes of microorganisms in soil and the stabilization of soil structure (Makoi and Ndakidemi, 2008). Soil enzymatic activity which can be determined quite promptly and precisely is a reliable indicator reflecting the current biological state of the soil (Wyszkowska *et al.*, 2002). According to Achuba (2006), crude oil induced changes in the activities of starch degrading enzymes. Atuanya (1987) stated that, Invertase activity reduced on addition of crude oil to the soil, due to the unfavourable conditions created by the crude oil and also, cellular destruction by toxic substances, contained in the crude oil and that, crude oil contamination decreased the activity of soil Invertase and glucose level. Anigboro and Tonukari (2008) also revealed that crude oil in soil inhibited the germination of seeds as well as reduce invertase and amylase activities in *Vigna unguiculata* and in cassava leaf extract which may have been accompanied by reduction in nutrient mobilization. This change in amylase and Invertase activity has been attributed to pH and temperature (Kathiresan and Manivannan, 2006). They also observed that, carbon and organic nitrogen sources are preferred for the production of amylase. Mishra and Behera (2008) also revealed that amylase activity increased with increase in temperature.

Crude oil contamination has caused critical environmental and health defects and increasing attention has been paid to developing and implementing innovative technology for cleaning up such contamination. Crude oils are composed of mixture of paraffin, alicyclic and polyaromatic hydrocarbon (PAH) (Okerentugba and Ezeronye, 2003). Crude oil has been a major contaminant of soil and water in oil producing communities the world over during exploration and transportation and its spillage has caused critical environmental and health hazards. Crude oil spills from pipelines and refineries leads to oil pollution which causes damage to the environment (Ogbo and Okhuoya, 2008). Oil pollution is a major environmental concern in many countries and this has led to a concerted effort in studying the feasibility of using oil-degrading bacteria for bioremediation (Akoachere *et al.*, 2008).

Bioremediation is any process that uses microorganism, fungi, green plant or their enzymes to return the environment altered by contaminants to its original or close to its original condition before contamination. The addition of organic waste material such as poultry manure, sawdust and dry leaves to the soil, facilitates aeration and increase the water holding capacity of the soil thus enhancing bioremediation. Bioremediation is an economical and safe method for cleaning up of oil spills. Bioremediation of crude oil polluted soil is becoming increasingly important as most exploration and distribution of crude oil and its products are usually environmentally non friendly (Odokuma and Dickson, 2003). Measuring the success of bioremediation of oil spills is based on several parameters. This includes the degradation of polycyclic aromatic hydrocarbon (PAHs) in the crude oil (Igwo-Ezikpe *et al.*, 2006). The measurement of soil enzyme activities before, during contamination and after bioremediation is used to determine the success of bioremediation (Wyszkowska and Wyszkowski, 2006).

This study, investigates the glucose level and amylase activities of crude oil contaminated soil remediated with poultry manure and sawdust.

MATERIALS AND METHODS

Materials: Soil samples were collected from a farmland in Abraka, the sawdust (SD) was collected from a sawmill in Abraka, the Poultry Manure (PM), from a poultry farm in Federal Government College, Warri, while the crude oil was collected from Shell Petroleum Development Company (SPDC), Warri. Other materials and apparatus used in this study included, test tubes, test tube rack measuring cylinder conical flasks, beakers, universal bottles, spectrophotometer, laboratory coats, spatula, weighing balance, electronic digital weighting balance, micropipette, filter paper cotton wool, masking tape and plastic bowls. This study was carried out in the Biochemistry Department of the Delta State University, Abraka- Nigeria, between 26th April, 2008 and 23rd June, 2008.

Methods: 7.3% (v/w) crude oil was added to the treated soil while the control soil had 7.3% (v/w) of distil water added to it. The experimental soil for bioremediation separated into two samples had 7.3% (v/w) crude oil. 22% (v/w) of sawdust was added to one sample while 22% (v/w) of poultry manure was added to the other. The last experimental set up had the combination of sawdust and poultry manure of 7.3 and 11.1% (v/w), respectively.

For the control sample, 10 g of soil was measured into a measuring cylinder and made up to 100 mL with sterile deionized water. The measurement of enzyme activity and glucose level was done using the filtrate solution. Amylase activity was determined by the method of Richard and Norbert (1980) while glucose level was determined by method of Barham and Trinder (1972).

Statistical analysis: The results were expressed as mean±SD. The one way analysis of variance (ANOVA) was used for the evaluation of statistical significance.

RESULTS AND DISCUSSION

Upon contamination of soil with crude oil, the glucose level of the crude oil contaminated soil (COTS) decreased when compared with control soil (NC) (Table 1). Bioremediation with sawdust and poultry manure increased the glucose level on day 14, when compared with crude oil treated soil and normal (control) soil. Thereafter, the glucose level dropped with time (day 28). It was observed that remediation with sawdust on day 14 had the highest glucose level 0.09±0.222 mmol g^{-1}. This was statistically significant ($p<0.05$).

The result from Table 2 shows that upon crude oil contamination, amylase activity in the soil decreased. However, bioremediation with sawdust increased the amylase activity significantly ($p<0.05$) on day 14, when compared with crude oil contaminated soil.

After several years of oil exploration and exploitation activities in this country, the Nigerian public has come to realize that oil business does not only generate financial benefits but also pollution problems (Osuno, 1983).

Crude oil spills from pipelines and refineries cause damage to the environment, with the contamination, changing the physicochemical and biological properties of the soil because the oil may be toxic to some microorganisms and plants (Ogbo and Okhuoya, 2008). Pollution of the natural environment has been observed to have adverse effects on the soil. In other to ameliorate these effects on the soil, the concept of bioremediation was initiated.

Akonye and Onwudiwe (2004) reported that, the addition of vegetative component such as sawdust as well as dry grass helped in the remediation process of the soil. Adedokun and Ataga (2007) also revealed that the addition of sawdust and waste cotton as soil amendment and bioaugmentation with *Pleurotus pulmonarius* on soil polluted with crude oil increased seed germination. Okolo *et al.* (2005) observed that, treatment of the soil with poultry manure alone, enhanced oil degradation but the extent of this was influenced by the incorporation of alternate carbon substrates or surfactants and that the addition of glucose encouraged crude oil degradation, while addition of starch reduced the extent of degradation obtained. Sumera *et al.* (2009) observed

Table 1: The glucose levels (mmol g^{-1}) of crude oil contaminated soil treated with poultry manure and sawdust

Samples	Day 0	Day 14	Day 28
Normal control soil	0.05±0.212b	0.05±0.232b	0.05±0.224b
Crude oil treated soil (COTS)	0.04±0.222b	0.04±0.242b	0.03±0.222b
COTS+sawdust (SD)	0.06±0.222b	0.09±0.222a	0.06±0.225b
COTS+poultry manure (PM)	0.04±0.242b	0.07±0.242a	0.05±0.226b
COTS+PM+SD	0.06±0.232b	0.08±0.242a	0.06±0.222b

Mean±SD: Values with different letter are significantly different

Table 2: Amylase activities (IU g^{-1}) of crude oil contaminated soil treated with poultry manure and sawdust

Samples	Day 0	Day 14	Day 28
Normal control soil	151.40±1.22a	156.36±1.23a	157.78±1.35a
Crude oil treated soil (COTS)	150.80±1.22a	114.40±1.42c	115.54±1.33c
COTS+sawdust (SD)	162.40±1.24a	142.48±1.43b	109.07±1.33c
COTS+poultry manure (PM)	162.40±1.42a	123.98±1.44b	100.51±1.44c
COTS+PM+SD	162.40±1.22a	136.70±1.44b	122.22±1.22b

Mean±SD: Values with different letter are significantly different

that the presence of alternative carbon source enhanced glucose production which in turn enhanced biodegradative abilities of the microorganisms. Ibekwe *et al.* (2006) stated that the addition of organic nutrient enhanced microbial utilization of hydrocarbons.

The results from this investigation show that upon contamination of the soil, there was a decrease in the glucose level on day o when compared with control soil (NC) (Table 1). However, upon bioremediation with sawdust and poultry manure there was an increase of glucose level on day 14 which was statistically significant ($p<0.05$). The increase in day 14 is due to the presence of sawdust which introduces carbon source into the soil. This result is supported by the findings of Achuba (2006) and Sumera *et al.* (2009). Achuba (2006) stated that upon crude oil contamination there is induced stress in seedling which leads to free sugar while Sumera revealed that the presence of alternative carbon source enhanced glucose production. Okolo *et al.* (2005) also revealed that the addition of glucose encouraged crude oil degradation. The drop at day 28 may be due to the utilization of the produced glucose by the microorganism for the generation of energy required for the various metabolic activities taking place as a result of biodegradation because microbial population prefer carbon source that requires less exergonic reaction, to obtain carbon and energy required for cellular metabolism.

The use of sawdust in the treatment of crude oil contaminated soil was observed to significantly increase amylase activity on day 14 when compared with normal control soil ($p<0.05$) (Table 2). This research finding is supported by Achuba (2006) who stated that crude oil contamination induced change in starch degrading enzyme thus inducing stress which leads to decrease in amylase level of activity in the soil. The research finding is also been reported by Anigboro and Tonukari (2008), who observed that crude oil contamination reduced amylase activity in *Vigna unguiculata*. The reduction in amylase activity upon the initial contamination is due to stress created in the soil resulting in the unavailability of nutrients as a result of the contamination. The increase in amylase activity is due to the introduction of sawdust as a bioremediation option. Sawdust is known to introduce cellulose into the soil thereby enhancing the activity of amylase. Day 14 witnessed the highest level of activity 142.48±1.43 IU g^{-1} when compared with the crude oil treated soil 114±1.42 IU g^{-1}. This finding is supported by Kathiresan and Manivannan (2006), who observed that amylase, is an inducible enzyme which is induced in the presence of carbon source.

CONCLUSION

This present research has shown that glucose level of soil decreased upon crude oil contamination. However, bioremediation with sawdust made soil glucose available for crude oil degraders present in the soil and enhanced amylase activity that was reduced upon crude oil contamination. The results of this study clearly showed that sawdust can be used as a good bioremediation material on a crude oil contaminated soil.

ACKNOWLEDGMENT

We are grateful to Mr. Atori, John of the Biochemistry Department, Delta State University, Abraka-Nigeria, for his technical assistance in carrying out this study.

REFERENCES

Achuba, F.I., 2006. The effect of sub lethal concentrations of crude oil on the growth and metabolism of cowpea (*Vigna unguiculata*) seedlings. Environmentalist, 26: 17-20.

Adedokun, O.M. and A.E. Ataga, 2007. Effects of amendments and bioaugmentation of soil polluted with crude oil, automotive gasoline oil and spent engine oil on the growth of cowpea (*Vigna unguiculata* L. Walp). Sci. Res. Essays, 2: 147-149.

Adriano, D.C., A. Chlopecka and K.I. Kaplan, 1998. Role of soil chemistry in soil remediation and ecosystem conservation. Soil. Sci. Soc. Am. Special Publ., 52: 361-386.

Akoachere, J.F.T.K., T.N. Akenji, F.N. Yongabi, G. Nkwelang and R.N. Ndip, 2008. Lubricating oil-degrading bacteria in soils from filling stations and auto-machanic workshops in Buea, Cameroon: Occurrence and characteristics of isolates. Afr. J. Biotechnol., 7: 1700-1706.

Akonye, L.A.D. and I.O. Onwudiwe, 2004. Potential for sawdust and *Chromolaena* leaves as soil amendments for plants growth in oil polluted soil. Niger Delta Biol., 4: 50-60.

Anigboro, A.A. and N.J. Tonukari, 2008. Effect of crude oil on invertase and amylase activities in cassava leaf extract and germinating cowpea seedlings. Asian J. Biol. Sci., 1: 56-60.

Atuanya, E.L., 1987. Effects of waste engine oil pollution on physical and chemical properties of soil: A case study of waste oil contaminated Delta soil in Bendel State, Nigeria. J. Applied Sci., 5: 55-176.

Balota, E.L., M. Kanashiro, A.C. Filho, D.S. Andrade and R.P. Dick, 2004. Soil enzyme activity under Long-term tillage and crop rotation system in subtropical agroecosystems. Braz. J. Microbial., 35: 300-306.

Barham, D. and P. Trinder, 1972. An improved colour reagent for the determination of blood glucose by the oxidase system. Analyst., 97: 142-145.

Ibekwe, V.I., K.C. Ubochi and E.U. Ezeji, 2006. Effect of organic nutrient on microbial utilization of hydrocarbon on crude oil contamination soil. Afr. J. Biotechnol., 5: 983-986.

Igwo-Ezikpe, M.N., O.G. Gbenle and M.O. Ilori, 2006. Growth study on chrysene degraders isolated from polycyclic aromatic hydrocarbon polluted soils in Nigeria. Afr. J. Biotechnol., 5: 823-828.

Kathiresan, K. and S. Manivannan, 2006. a-amylase production by *Penicillium fellutanum* isolated from mangrove rhizosphere soil. Afr. J. Biotechnol., 5: 829-832.

Makoi, J.H.J.R. and P.A. Ndakidemi, 2008. Selected soil enzymes: Examples of their potential roles in the ecosystem. Afr. J. Biotechnol., 7: 181-191.

Mishra, S. and N. Behera, 2008. Amylase activity of a starch degrading bacteria isolated from soil receiving kitchen wastes. Afr. J. Biotechnol., 7: 3326-3331.

Odokuma, L.O. and A.A. Dickson, 2003. Bioremediation of crude oil polluted tropical mangrove environment. J. Applied Sci. Environ. Manage., 7: 23-29.

Ogbo, E.M. and J.A. Okhuoya, 2008. Biodegradation of aliphatic, aromatic, resinic and asphaltic fraction of crude oil contaminated soils by *Pleurotus tuber-regium* Fr. Singer-a white rot fungus. Afr. J. Biotechnol., 7: 4291-4297.

Okerentugba, P.O. and O.U. Ezeronye, 2003. Petroleum degrading potentials of single and mixed microbial cultures isolated from rivers and refinery effluent in Nigeria. Arf. J. Biotechnol., 2: 288-292.

Okolo, J.C., E.N. Amadi and C.T. Odu, 2005. Effects of soil treatments containing poultry manure on crude oil degradation in a sandy loam soil. Applied Ecol. Environ. Res., 3: 47-53.

Osuno, B.A., 1983. Forward the petroleum industry and the Nigerian environment. Proceedings of an International Seminar, (IS'83), Petroleum Training Institute, Warri, Nigeria, Effurun, pp: 57-61.

Richard, A.K. and W.T. Norbert, 1980. Ion effects in measurement of ionized calcium with a calcium-selective electrode. Clin. Chem., 26: 640-644.

Sumera, A.K., H. Muhammad, L.K. Abdul, A. Bashir, A. Safia and L. In-Jung, 2009. Influence of pH, temperature and glucose on biodegradation of 4-aminophenol by a novel bacteria strain, *Pseudomonas*. ST-4. Afr. J. Biotechnol., 8: 3827-3831.

Wyszkowska, J., J. Kucharski and E. Waldowska, 2002. The effluence of diesel oil contamination on soil enzymes activity. *Rostlinna Vyroba,* 48: 58-62.

Wyszkowska, J. and M. Wyszkowski, 2006. Role of compost, bentonite and lime in recovering the biochemical equilibrium of diesel oil contaminated soil. Plant Soil Environ., 52: 505-514.