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RESPONSE OF MICROBIAL ENZYMES SYNTHESES TO TOXICITY OF WEATHERED AND BIODEGRADED OILS.

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ABSTRACT

The toxicity of eight weathered and biodegraded Nigerian crude oils to the biosyntheses of four bacterial enzymes were examined. The constitutive intracellular enzyme, nitritase was the most sensitive to all toxicants. Two extracellular inducible enzymes tryptophanase and α-glucosidase were less, sensitive to the toxicants. These extracellular enzymes, however, were more sensitive than the intracellular inducible enzyme β-galactosidase. Inhibition of bacterial enzymes syntheses could be a useful, cheap, simple and rapid ecotoxicity assay for monitoring oil pollution in the Nigerian environment.

Key words; Sensitivity, Response, Microbial Enzymes, Toxicity, Weathered crude oil, Biodegraded crude oil

INTRODUCTION

The environmental transformations of an organic chemical in many natural aquatic systems may be induced by physical, chemical and microbial factors within the system (Plimmer, 1978: Smith and Dragun, 1984; Okpokwasili and Odokuma, 1994: Okpokwasili and Nnubia, 1994). Chemically and biological, induced changes in the composition of a crude oil are known collectively as weathering (Atlas, 1981). Microbial degradation contributes significantly to the ultimate removal of oil from the environment (Atlas, 1981; Amanchukwu *et al.*, 1989). Some of the components of crude oils may be toxic to microorganisms. Short-chain- paraffins (>C10) generally are assumed to be toxic to microorganisms because of their water solubility and dual interactions with lipid membranes (Teh, 1974; Teh and Lee, 1974). Griffin and Calder (1977) showed that weathered oils produced water-soluble fractions (WSF) that were more toxic to *Serratia marinorubra* than the parent oils.

Many toxicity tests are accomplished by using microorganisms as test organisms (Williamson and Johnson, 1981; Wang, 1984; Giesy *et al.*, 1988; Jardim *et al., 1990;* Xu and Schurri, 1990). Such bioassays must provide rapid and reliable data so they can be extrapolated to infer human risk of exposure as well as to trigger adequate waste management. These tests are based on the inhibition of some microbial vital function. Examples include inhibition of nitrite utilization by *Nitrobacter* (Williamson and Johnson 1981; Wang, 1984). Microtox tests (Giesy *et al,* 1988), Ames Salmonella assay (Vandermeulen, 1986; Vandermeulen and Lee, 1986), SOS chromotest (Quillardet *et al.*, 1982) and carbon dioxide production (Jardim *et al.*, 1990). Others include the monitoring of internal pools of DNA and ATP (Jardim *et al.*, 1990) These techniques have rapidity and simplicity in achieving results as notable advantages.

Because enzymes are key catalysts of metabolic reactions in cells the inhibition of their activity or biosyntheses has been recently explored as a basis for ecotoxicity testing (Dutton *et al.*, 1990). Beta galactosidase, an enzyme that confers *Escherichia coli* with the ability to break down lactose to galactose and glucose is constitutive while the β galactosidase in *E. coli is* intracellular and induced by the presence of lactose. Tryptophanase, an inducible enzyme produced by *E. coli* in the presence of L-tryptophan, is extracellular. Both β-galactosidase and tryptophanase production are controlled by different operons in *E. coli* (Magasanik, 1988). Alpha-glucosidase, another inducible extracellular enzyme, is produced by *Bacillus licheniformis* in response to maltose (Magasanik, 1988). Studies on the effects of some toxicants on the biosyntheses of the three enzymes β-galactosidase, tryptophanase and α-glycosidase have been initiated (Dutton *et al., 1990).*

Weathering involves the physical, chemical and biological degradation of materials (Plimmer, 1978). It involves physical factors such as the effect of wind, rain, temperature and the abrasive actions of rocks. It includes chemical factors such as hydrolysis, salinity, solution effect etc. The biological aspects include the degradative

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activities of micro and macroorganisms on materials. Thus weathering may be physical and chemical i.e. without biological imput. It may also be biological. In this study however, weathered oils are regarded as oils that have been exposed mainly to physical and chemical forces, while biodegraded oils are those that have been exposed to physical, chemical and biological (microbial) forces.

Microbiological assays of chemicals are insufficient in deciding whether a particular chemical is toxic since the ecosystem contains both macro-and microorganisms. If the objective of the bioassay test is to ensure protection of all aquatic species then the inclusion of microorganisms is necessary as they clearly represent a greater biomass in natural systems. Their use also offers rapid, low cost and simple alternatives for achieving bioassay results, (Williamson and Johnson, 1981).

The objective of this study was to compare the inhibitory effects of weathered and biodegraded Nigerian crude oils on the biosyntheses of β- galactosidase, tryptophanase, α- glucosidase and nitritase. The goal was to identify the operon- enzyme system most sensitive to these toxicants which will thus serve as a rapid and economic method for assessing the toxicity of spilled oil in the Nigeria environment.

MATERIALS AND METHODS

Test Bacteria

Toxicity assays were conducted with *Escherichia coli, Bacillus licheniformis* and *Nitrobacter* sp. *E. coli* and *Nitrobacter* sp. were both isolated from the New Calabar River in Rivers State Nigeria while *B. licheniformis* was isolated from the soil near the Microbiology laboratory in the University of Port Harcourt. To ensure adaptation to laboratory conditions all strains were maintained by continuous subculture every 48h into fresh media.

Characterization and identification of Test Bacteria

Isolates were examined for colony, morphology and biochemical characteristics. For *E. coli* and *B. lincheniformis*, the tests included Gram reaction, spore staining, oxidase test, oxidative/fermentative. (O/F) utilization of glucose, motility test, indole test, Voges-Proskuaer catalase test, urea utilization, aerobic and anaerobic growth, growth in 7% NaCl and at 55^0 to 60^{0}C, which were carried out according to the methods described by Cruicshank *et al.*(1975). For *Nitrobacter* sp., Gram reaction, colony morphology and sole utilization of nitrite as energy source, were used for its identification according to the method of Colwell and Zambruski (1972). Identification of bacteria to species level followed the scheme in Laskins and Lechevalier (1977).

Chemical Reagents and Toxicants

All reagents employed in the study were of analytical grade and were purchased from Sigma Chemical Company, St. Louis, Missouri. Parent Crude oils assessed for toxicity were Antan Heavy (AH), Antan Medium (AM), Bonny Medium (BM) and Bonny light (BL). Others were Ughelli Quality Control Centre crude (UQCC), Trans Niger Pipeline (TNP), Brass River (BR) and Qua Iboe (Q1). All crude oils were provided by the Nigerian National Petroleum Corporation (NNPC), Port Harcourt. Nigeria.

Weathered (physical and Chemical) Crude Oils

Weathered crude oils in this study consisted of a mixture of crude oil and sterile deionized water. There weres no microorganisms (seed) from external sources.

Biodegraded (Physical, Chemical and Biodegraded) Crude Oils.

This consisted of a mixture of crude oil with sterile deionized water into which microorganism (seed) from the New Calabar River had been introduced.

Preparation of Toxicant Concentrations

Toxicant concentrations of 1000, 100, 10,1.0. and 0.1 mg/L were prepared . For liquid toxicants an amount equivalent to one thousand milligrams of liquid toxicant was weighed and transferred into distilled water (1000ml of distilled water minus the equivalent volume of 1000mg of liquid toxicant). A similar procedure was repeated for other concentrations (100,10,1.0,0.1). For example an amount equivalent to 100mg of the liquid toxicant was weighed and transferred into 100ml of distilled water minus the equivalent volume of 100mg of the liquid toxicant to produce 100mg/l of the toxicant.

Preliminary range finding test was undertaken. Organisms were exposed to logarithmic concentrations of toxicants. The least toxicant concentration that produced total inhibition of the enzyme system (or death of organism) was taken as 100% inhibition (the highest toxicant concentration). Also the highest toxicant

Table 1: Characteristics of the hydrocarbon fuels

Fuel name	Boiling point Ranges (°C)	API gravity Ranges
Gasoline(Premium Motor Spirit)	40-180	70.6-45.4
Kerosene(Dual Purpose Kero)	180-230	45.4-40.0
Aviation Turbine Kerosene(High Pour Fuel Oil).	180-230	45.4-40.0
Diesel(Automotive Gas Oil).	230-405	61.2-19.8

Port Harcourt Refinery Company

concentration that could produce no inhibition of enzyme synthesis was taken as the lowest toxicant concentration. Toxicant concentrations were then produced between these two limits.

Concentrations varied according to the toxicity of the toxicant To 50ml of distilled water, 0.1g of solid toxicant (or its equivalent in volume for liquid toxicants) was added. The volume was made up to 100 ml to produce a concentration of 1000 mg/L. Serial tenfold dilutions of this stock were produced till required minimum concentration (concentration that will have no effect on the enzyme biosynthesis) was achieved.

Toxicity Assay Protocol

The method of Dutton *et. al.* (1990) was employed for assays for the biosyntheses of β-galactosidase, tryptophanase and α-glucosidase . They had the following basic steps in common: (1) cell growth (2) cell washing (3) cell exposure to toxicant, (4) induction of enzyme biosynthesis and (5) measurement of enzyme activity. Nitritase biosynthesis assay also followed the same steps except that the measurement of enzymatic activity was done indirectly by measuring the loss of substrate (nitrite) as in APHA (1985).

Cell Growth and preparation

Escherichia coli was grown in nutrient broth overnight at 37°C.
Cells were diluted with medium to A_{550} = 0.2. (Absorbance 0.2 at 550 nm). Medium without organisms was used as blank. Cells were allowed to grow to A_{550} =0.6 after which were washed thrice with 0.8% NaCl.

Exposure to Toxicant

Cell (0.1 ml) of washed cell suspension at A_{550} = 0.6) were exposed to 0.9ml of toxicant and incubated for 30min.

Control

Control contained cell plus medium with toxicant.

Enzyme Induction.

To 1ml each of the reaction mixture, 0.1 ml Isopropyl-β-D-thiogalactoside (IPTG) 0.1%(w/v) (Sigma Chemical Company,) 0.5 - buffer and 0.5 ml growth medium were added. The mixture was incubated for 30 min.

Beta – galactosidase Measurement.

Since β-galactosidase is intracellular, it was necessary to lyse the cells. This was performed by the addition of 0.1 ml 10% Sodium Dodedcyl Sulphate (SDS) 10% (w/v) to the reaction mixture. This was followed by the addition of 0.2 ml O-nitrophenyl-*D*-galactoside (ONPG) 0.4% (w/v) Sigma Chemical Company. The mixture was incubated until colour developed (15 min). The reaction was stopped with 1 ml or cold Na_2CO_3 (1M) and the absorbance at 542nm using a blank consisting of all reagents minus IPTG was determined (Dutton *et al*, 1990).

Tryptophanase Biosynthesis Assay

(i) L-tryptophan (250 mg/L) in KH2PO3 (13.6 g/L) adjusted to pH –7.8

(ii) Erhlich's reagent consisting of 6 parts of p- dimethylaminobenzaldehyde (5%, w/v/)Sigma Chemical Company, in 95 % ethanol and 12 parts acid

alcohol (16 ml conc. H_2SO_4 in 200 ml 95% ethanol).

Cell growth and preparation

Escherichia coli was grown in casein hydrolysate without tryptophan (10g/1), yeast extract (5 g/L) and NaCL (10g/l) overnight at 37°C.

Cell preparation was as for β - galactosidase biosynthesis assay.

Exposure to Toxicant

Cells (0.1 ml of washed cell suspension at A550 = 0.6) were exposed to 0.9 ml toxicant and incubated for 30. min.

Enzyme induction

To the reaction mixture 0.4 ml buffer, 0.1 L-tryptophan and 0.5 ml nutrient broth were added. The mixture was incubated for 120 min.

Trytophanase Measurement

One milliliter of Erhlich's reagent was added to the reaction mixture and incubated for 15 min. Absorbance at 568nm using a blank consisting of all reagents minus L-tryptophan, was determined.

Alpha-glucosidase Biosynthesis Assay

Maltose (4% w/v) and autoclaved p-nitrophenyl β-D – glucoside (PNG) 0.5% (w/v) were used. This solution was filter – sterilized (0.22um pore size) and stored at 4°C in the dark.

Cell Growth, Preparation and Enzyme Induction

Bacillus licheniformis – was grown in tryptone soya broth without dextrose overnight at 37°C. Cell preparation and toxicant exposure were as in β - galactosidase biosynthesis assay.

To the reaction mixture 0.4 ml Z –buffer,(Na_2HPO_4 $7H_2O$ 16.1g/L NaH_2 PO_4 H_2O,5.5. g/L KCL,O.75 g/L; and $MgSO_4$. $7H_2O$ O.25 g/L) 0.1 ml maltose and 0.5 fresh medium were added. The mixture was incubated for 60 min.

Alpha-glucosidase Measurement

O nitrophenyl-*D*-galactoside (ONPG) (0.2 ml) was added and the mixture incubated for 60 min. Reaction was stopped by the addition of 1ml Na_2C0_3. The absorbance of the mixture at 420nm was measured. A solution consisting of all other reagents minus maltose was used as blank for the reading.

Nitritase Biosynthesis Assay

The reagents used were as in method for nitrite determination APHA (1985). The other steps growth, cell preparation, exposure to toxicant, enzyme induction and nitritase measurement were as reported earlier (Okpokwasili and Odokuma,1994).

Toxicity And Analysis

The method employed was adopted from Dutton *et al* (1990). One hundred milliters of serial dilutions of each fresh crude oils were placed in 250 ml cotton wool-plugged shake flasks. The flasks were incubated for 4

Table 2: Composition and Functions of the drilling Chemicals tested.

Trade name	Composition	Function
Huile Clean	Clean oil	Lubricant
Chaux (Lime)	Calcium Hydroxide	Acidity reducer
Carbotrol A9	Organic Polymer blend Containing methanol And asphalt	Filtration Control agent
Carbotec. HW	Polymerized organic acids	Alkalinity reducer
Carbotec Sea	Polymerized organic acids	Alkalinity reducer
Carbovis	Quaternary amine – treated bentonite	Viscosifier
Carbomul Sea	Amide polymer containing 2.-methoxyethanol	Emulsifier/wetting agent.

weeks. Enzyme biosynthesis assays for nitritase, tryptophanase β- galactosidase and α-glucosidase were carried out on each of the crude oil concentrations. Periodicity of assays was immediately after preparation of toxicant concentration (day O) and thereafter once a week for four weeks. This served as base-line control data.

Water samples containing a mixed population of hydrocarbonoclastic microorganisms was obtained from the New Calabar River at a site located about 1 km southwest of the University of Port Harcourt, Nigeria

Seeding of crude oil samples

To 75 ml of each crude oil concentration, 25 ml of the New Calabar River water was added.

All concentrations for the enzyme assay were prepared in triplicate. Preliminary range finding test was carried out where possible (because some toxicants did not inhibit the enzyme systems). To determine toxicant concentration causing between 10 and 100% inhibition, the degree of enzyme inhibition was determined by measuring absorbance with respect to control (assigned 0% inhibition). Control contained no toxicant. For nitritase biosynthesis assay, the ratio of the absorbance in control to that of toxicant multiplied by 100 was taken as percent toxicant inhibition. For the three other enzyme systems, the ratio of the absorbance of the toxicant to that of the control multiplied by 100 was taken as the percent toxicant inhibition. The mean of triplicate samples was obtained and used to plot a graph of percent inhibition versus concentration of toxicant. The chemical concentration giving 50% inhibition (IC_{50}) for weathered and biodegraded crudes was derived from the line of regression of the plot.

RESULTS

The results of the effect of eight Nigerian parent crude oils on the inhibition of biosynthesis of four enzymes are presented in Table 1. The data revealed that nitritase was the most sensitive to the crude oils tested. This was followed by tryptophanase and α- glucosidase. Antan Heavy (AH) was the most toxic (IC_{50} values were the least except in α - glucosidase).

The effects of weathering and biodegradation of eight crude oils on the biosyntheses of four enzymes (nitritase, tryptophanase, α- glucosidase and β-galactosidase) are presented in Tables 2-5. The data indicated that nitritase was the

TABLE 3: MEDIAN INHIBITION CONCENTRATION OF HYDROCARBON FUELS AND ORGANIC SOLVENTS TO THE INDUCTION OF FOUR BACTERIAL ENZYMES.

	IC_{50} OF ENZYMES			
Chemicals	β-galactosidase	Tryptophanase	α-glucosidase	Nitritase
Jet fuel	NT	NT	NT	0.8
Kerosene	NT	70,000	30,00	0.5
Diesel	NT	NT	2,000	0.8
Gasoline	NT	1.0	NT	7.0
Hexane	850	0.9	400	9.0
Heptane	900.000	3000	100,000	100
Toluene	700	5.0	100	0.05
Xylene	NT	2500	9000	0.009

NT (Not toxic) = above 1,000 000 mg/L

most sensitive followed by tryptophanase, (the IC_{50} values of nitritase and tryptophanase were the least compared to the IC_{50} of the other enzymes). . Beta –galactosidase showed the least sensitivity (see Table 4). There was an increase in the toxicity of all crude from day 0 to the seventh day. By the 4th week, biodegraded crude become slightly more toxic to nitritase than weathered crude.

An increase in the sensitivity of nitritase to both the biodegraded and weathered oils throughout the incubation period was observed. Results in Tables 3, 4 and 5 showed that weathered, crude oils were slightly more toxic to β-galactosidase and tryptophanase than their biodegraded counterparts. This was obvious in the 4th week. However, for both enzymes there was a decrease in toxicity from day 0 to the 4th week of both weathered and biodegraded oils.

DISCUSSION

Nitritase biosynthesis was the most sensitive to all toxicants. This was followed by tryptophanase and α-glucosidase biosyntheses. Beta –galactosidase biosyntheses showed the least sensitivity to these toxicants. There was no significant difference between the sensitivity of the enzymes to weathered crude oils and to biodegraded crude oils. Weathering is the physical, chemical and biological degradation of substances in –situ (Plimmer, 1978). Therefore weathering includes the effects of radiation, wind, rocks, solution, hydrolysis, microorganisms and macroorganisms on materials. In this study-weathered crude oils meant oils that were exposed o both physical and chemical factors. While biodegraded crude oils referred to crude oils that were exposed to physical chemical and biological (microbial degradation) factors. A number of factors such as solubility, substitution, chain length and concentration are responsible for the toxicity of an organic pollutant (Babich and Stotzky, 1977: Vandermeulen and Lee, 1986; Petibone and Cooney. 1988). Griffin and Calder 1977) have revealed that the addition of crude oil to water produces water soluble fractions (WSF). The production of WSF concentrations of between 17.5-26.0ug/L by three Nigerian crudes, Bonny

TABLE 4: MEDIAN INHIBITION CONCENTRATION OF OIL SPILL DISPERSANTS, REFERENCE DETERGENTS AND HOUSEHOLD DETERGENTS TO THE INDUCTION OF FOUR BACTERIAL ENZYMES

	IC_{50} OF ENZYMES			
Chemicals	β-galactosidase	Tryptophanase	α-glucosidase	Nitritase
Corexit 9527	NT	0.035	500	30.0
Triton X –100	7.0	NT	700	50.0
Surflo OW-1	NT	3.5	500	0.85
Prodesolv	NT	NT	0.09	2.0
Dispolene 36 S	0.25	15,000	2.0	NT
SDS	6000	70,000	110	10.0
Tween 80	NT	150	0.9	NT
Flex	NT	50.0	NT	6.0
Spencer	NT	NT	NT	10.0
Teepol	NT	NT	NT	7.5
Rainbow	NT	NT	NT	15.0
Apollo	NT	10.0	80.0	1.5

NT (Not toxic) = above 1,000000 mg/L

TABLE 5: MEDIAN INHIBITION CONCENTRATION OF DRILLING CHEMICALS AND CRUDE OILS.

	IC_{50} OF ENZYMES			
Chemicals	β-galactosidase	Tryptophanase	α-glucosidase	Nitritase
Carbotec Sea	NT	45.0	NT	15.0
Carbomul Sea	NT	NT	NT	500
Huile Clean	NT	NT	NT	5.0
Chaux(lime)	0.1	NT	NT	600
Carbotec HW	1.0	NT	0.075	75.0
Carbotrol A9	NT	1000	NT	100
Carbovis	40.0	NT	NT	4000
Qua Iboe Crude	NT	150	85,000	15.0
Brass River	NT	140	NT	40.0
Bonny Light	NT	400	NT	10.0
Bonny Medium	80,000	18.0	NT	60.0
TNP	NT	2.0	NT	100
UQCC	NT	100	NT	7500
Antar Medium	4.5	2.0	8000	70
Antar Heavy	1.0	0.8	8000	3.5

NT (Not Toxic) = above 1,000000 mg /L.

medium Bonny light and Forcados blend has been reported (Imevbore *et al.*, 1987). The production of WSF by the crude in this study may be responsible for their toxicities. The relative insensitivity of β- galactosidase biosynthesis to most crude oils may be due to the inability of the WSF of these crudes to penetrate the outer membrane of *E. coli* either due to low concentration of WSF or lack of permeases to transport the toxicant across the cell membrane. Therefore this enzyme would make a poor toxicity index. Similar results have been obtained by Dutton *et al.* (1990). Their studies revealed the relative insensitivity of β- galactosidase by *E coli* to hydrophiles such as hydrothol. The higher sensitivity of nitritase biosynthesis may be due to the high permeability of the outer membrane of ***Nitrobacter*** sp to the WSF of the crude oils. The influx of the WSF probably inactivated the nitritase enzyme present within the cell . Nitritase would thus provide first choice as a toxicity index. The higher sensitivity of tryptophanase synthesis to crude oils than β -galactosidase biosynthesis in the same *E. coli* strain may be as a result of the extra-cellular nature of tryptophanase enzyme. This may result in the enzyme making easier contact with the WSF of the crude oil. A similar result was obtained by Dutton *et al.* (1990) who observed a higher sensitivity of tryptophanase biosynthesis to Sodium dodecyl sulphate (SDS) and polychlorinated phenyls (PCP) than β - galactosidase biosynthesis. Gram positive bacteria, such as *Bacillus licheniformis*, do not possess an outer membrane (Koch and Schaechter, 1985) and thus may be expected to be more sensitive to the WSF crude oils than Gram negative bacterial such as *E. coli* and *Nitrobacter* sp. The data, however, showed that *B. licheniformis* was relatively insensitive to most crude oils. The production of spores by *B. licheniformis* may have been responsible for its insensitivity.

Antan medium and Antan heavy were the most toxic of the test crude oils (especially to the enzyme systems β-galactosidase tryptophanase and α- glucosidase, see Table

1) The IC_{50} for Antan heavy for β - galactosidase, tryptophanase, α- glucosidase and Nitritase were 1.0, 0.8, 8000 and 3.5mg/L respectively. For Antan light the IC_{50} for these enzyme systems were 1.0, 0.8, 8000 and 0.2mg/L respectively. This may have been due to the production of a higher concentration of WSF rather thar. the presence of more toxic components in the WSFs. Bonny medium was more toxic to β - galactosidase and tryptophanase enzyme system than Bonny light. The IC_{50} of Bonny medium to β - galactosidase and tryptophanase were 80,000 and 8.0mg/l respectively while the IC_{50} of Bonny light to these enzymes systems were > 1000000 and 400 mg/l respectively. Bonny medium was more toxic than Bonny light. The observations differ from the reports of Imevbore *et al.* (1987). They observed that light crudes were more toxic to two shrimps (*Desmocaris trispinosa* and *Palaemonetes africanus)* than medium crudes. The results from the study may be due to the production of more toxic components in the WSF of heavier crude oils than a WSF concentration difference.

The effect of weathering and biodegradation on the biosyntheses of nitritase, tryptophanase and β- galactosidase is shown in Tables 2-5. The data showed that nitritase was the most sensitive, followed by tryptophanase. Beta-galactosidase showed the least sensitivity to both biodegraded and weathered oils. Explanation adduced for the toxicity of the parent oils may also be responsible for these results. The decrease in toxicity of both biodegraded and weathered oils to these enzymes from day 0 to the 7th day may be attributed to the increase in the concentration of WSF. Griffin and Calder (1977) observed that weathered oils produced WSFs that were more toxic to *Serratia marinorubra* than the parent oils. They attributed this increased toxicity to the higher concentration of hydrocarbons in the WSF. By the 4th week, biodegraded oils became slightly more toxic to nitritase biosynthesis than weathered crudes. This may be attributed to the production of degradation products or metabolites by the hydrocarbonoclastic organisms that are more toxic than the parent oils to *Nitrobacter.* The toxicity of these products to *Nitrobacter* increased with incubation time which may have been responsible for the increase in sensitivity of nitritase with time for both biodegraded and weathered crude oils. Table 3,4, and 5 revealed that weathered crudes were slightly more toxic to β-galactosidase and tryptophanase biosyntheses than biodegraded crude, especially by the end of the first and fourth weeks. This may be attributable to the high tolerance of *E. coli* to the intermediate produced by the microorgnism in the biodegraded samples. The high tolerance of *E. coli* to these intermediates may account for the decrease in sensitivity of biosyntheses of two enzymes from the second week to the fourth week for both biodegraded and weathered oils.

CONCLUSION

Nitritase biosynthesis was the most sensitive to the toxicants, followed by tryptophanase and α - glucosidase biosyntheses with similar sensitivities. Beta – galactosidase biosynthesis was the least sensitive to the toxicants. The medium inhibition concentration (IC_{50}) for nitritase for weathered Bonny medium Bonny light, Brass River, Qua Iboe, UQCC, TNP, Antan medium and Antan heavy after 4 weeks were 4.0, 0.06, 30.0, 0.08, 0.08, 0.09 , 10 and 0.008mg/L respectively . The IC_{50} for the same crude oils for tryptophanase and β-galactosidase for the same period were all greater than 1,000000mg/l except Antan heavy for tryptophanase. The IC_{50} for α-glucosidase for all crude oils for the same period were 900,000, 1000, >1000,000, 600.000, 30, >1000,000, >1000,00 and >7.0 mg/L, respectively.

The study shows that Nitritase enzyme inhibition will serve as a more sensitive indicator for the determination of toxicity levels of crude oils.

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