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Biodegradation of Crude Oil by *Saccharomyces cerevisiae* Isolated from Fermented Zobo (Locally Fermented Beverage in Nigeria)

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Abstract: The increase in demand for crude oil as a source of energy and as a primary raw material for industries has resulted in an increase in its production, transportation and refining, which in turn has resulted in gross pollution of the environment. In this study, *Saccharomyces cerevisiae* isolated from a commercially prepared local fermented beverage 'zobo' (prepared from *Hibiscus* flower) was tested to determine its potential to degrade crude oil for a period of 28 days under aerobic condition. The percentage of oil biodegradation was determined using weight loss method and gas chromatography mass spectroscopy (GC/MS) of the residual crude oil after 28 days. At the end of 28 days 49.29% crude oil degradation was recorded. The result suggests the potential of *Saccharomyces cerevisiae* for bioremediation of oil polluted sites.

Key words: Crude oil, biodegradation, bioremediation, fermented zobo

INTRODUCTION

Crude oil is a natural product, comprising a complex mixture of various hydrocarbons, created by the decomposition of plant remains from the carboniferous period under high temperature and pressure (Van Hamme *et al*., 2003). The broadest environmental pollutants classification is into two major categories: organic and inorganic pollutants (Jim *et al*., 2005). Quantitatively organic pollutants that are of most concern are the hydrocarbons in their various forms. The most common are petroleum hydrocarbons (mixtures of n-alkanes, mono-, di-and polyaromatic compounds, heterocyclic aromatics and other minor constituents) and host of other compounds (Jim *et al*., 2005). The petroleum industry is a major contributor of organic contaminants to the natural environment, releasing hydrocarbon contaminants into the environment in a number of ways. Accidental and deliberate crude oil spills have been and still continue to be, a significant source of environmental pollution and poses a serious environmental problem, due to the possibility of air, water and soil contamination (Trindade *et al*., 2005). There is also food chain disruption following the loss of phytoplankton, shellfish, fish and birds. Microbial degradation of crude oil as a means of clearing oil spills in the natural environment is a slow process and therefore, stimulated biodegradation through microbial seeding, application of fertilizer, tilling and liming (if the soil is acidic) or a combination of all these methods may be the answer (Isinguzo and Odu, 1987; Ekundayo and Obire, 1987). The aim of this study is to isolate *Saccharomyces cerevisiae* from fermented Zobo and to use the isolate to degrade crude oil and identify the component of the crude oil that can be degraded by this organism.

MATERIALS AND METHODS

Collection of samples: The crude oil was collected with 750 mL container from Port Harcourt refining company, Alesa-Eleme, Rivers state, Nigeria. The zobo drink was collected from shop 3 beside boys hostel in Bosso campus Federal University of Technology Minna, Niger state.

Isolation of *Saccharomyces cerevisiae* from fermented zobo: The freshly prepared zobo drink was bought from commercial retailer in Bosso market Minna, Nigeria and kept to ferment for 2 days, 1 mL of the fermented zobo was serially diluted, 0.1 mL of the serially diluted sample was plated on sabauroud dextrose agar by spread plate method and incubated in at 25°C for 48 h. The colonies were counted and express as colonies forming units per gram (CFU g^{-1}). The colonies were sub-cultured repeatedly on sabauroud dextrose agar to get a pure culture and was later stored in an agar slants in their pure forms for further characterization.

Characterization and identification: The yeast isolate was gram stained and characterized based on its, colonial

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morphology, colony shape, mycelium formation and following biochemical test; nitrate reduction test and carbohydrate fermentation test.

Biodegradation studies with the yeast isolates: The biodegradation studies was carried out by inoculating 1 mL of 24 h broth culture of the yeast isolate into 50 mL of sterile Mineral Salt Medium (MSM) [1.8 g K_2HPO_4, 4.0 g NH_4Cl, 0.2 g $MgSO_4.7H_2O$, 1.2 g KH_2PO_4, 0.01 g $FeSO_4.7H_2O$] that contained 1 g of crude oil in a Erlenmeyer flask. The experiment was set up in triplicate with control flasks which contained 50 mL of sterile mineral salt medium plus 1 g of crude oil but without added microorganisms. The flasks were incubated at 30°C for 28 days. At seven days intervals, duplicate flasks per organisms plus control flasks were removed from incubator and the residual crude oil extracted with 20 mL of diethyl ether and dried with anhydrous sodium sulphate. The solvent was evaporated using rotary evaporator and the weight of the residual oil was measured and recorded. The percentage biodegradation of the crude oil was calculated using the formula of Ijah and Ukpe (1992) as stated below.

$$\text{Biodegradation (\%)} = \frac{\text{Weight of oil (control)-weight of oil (degraded)}}{\text{Weight of oil (control)}} \times 100$$

The residual oil was diluted with diethyl ether and cleaned up with silica gel. One micro litre of the extracted oil sample was analyzed using gas chromatography mass spectroscopy (GC/MS) QP-2010 in scan mode. The GC was equipped with cross linked 5% phenyl methyl siloxane capillary column, QP-5MS, helium was used as carrier gas. The temperature program was started at 60°C and raised by 10°C min^{-1} until 200°C, which was maintained for 8 min.

RESULTS

The results in Table 1 show the percentage of crude oil degradation by *Saccharomyces cerevisiae*. At the end of 28 days, 49.29% oil biodegradation was recorded by the test organisms compared to 2.5% biodegradation recorded in the uninoculated control.

Table 2 show different hydrocarbon fractions present in crude oil at zero day before subjection to degradation by *Saccharomyces cerevisiae*. About twenty one different hydrocarbon compounds were identified in the crude oil sample ranging from C_7 to C_{24}.

Biodegradation of hydrocarbon fractions present in the crude oil was analyzed after 28 days of incubation to determine the extent of biodegradation of hydrocarbons using GC/MS. At the end of 28 days, the hydrocarbon peaks drastically reduced from 24 to 16 with complete degradation of hydrocarbon fractions within the range of C_{19} to C_{24}. However, a new complex compound with 35 carbon (Hexadecanoic acid, 2-hydroxy esther) chain was introduced during the process of biodegradation by the yeast isolate (Table 3).

The GC/MS chromatogram of the crude oil before it was subjected to biodegradation by *Saccharomyces cerevisiae* is shown in Fig. 1.

Figure 2 shows the GC/MS chromatogram of the residual crude oil after 28 days biodegradation by *Saccharomyces cerevisiae*. The result shows a reduction

Table 1: Percentage of crude oil biodegradation by *Saccharomyces cerevisiae*

	Oil biodegradation (%)			
Microbial isolate (days)	7	14	21	28
S. cerevisiae	15.45±1.7	24.69±1.8	35.1±14.7	49.29±5.0
Control	1.60±0.8	1.90±0.9	2.3±1.20	2.50±1.4

Table 2: Hydrocarbon present in crude oil before degradation

Peak formula	Compound name
C_7H_8	Toluene
$C_{10}H_{22}$	Decane
C_9H_{12}	Benzene
$C_{11}H_{24}$	Undecane
$C_{12}H_{26}$	Dodecane
$C_{11}H_{24}$	Undecane
$C_{13}H_{28}$	Tridecane
$C_{16}H_{30}Br_2O_2$	Indene
$C_{14}H_{30}$	Tetradecane
$C_{15}H_{26}$	1H-Indene
$C_{15}H_{28}$	Naphthalene
$C_{15}H_{32}$	Pentadecane
$C_{16}H_{34}$	Hexadecane
$C_{15}H_{32}$	Pentadecane
$C_{17}H_{36}$	Heptadecane
$C_{18}H_{38}$	Octadecane
$C_{19}H_{40}$	Nonadecane
$C_{20}H_{42}$	Eicosane
$C_{22}H_{40}$	Docosane
$C_{21}H_{40}$	Heneicosane
$C_{24}H_{50}$	Tetracosane

Table 3: Hydrocarbon present in crude oil after 28 days biodegradation by *Saccharomyces cerevisiae*

Peak formula	Compound name
C_7H_8	Toluene
$C_{11}H_{24}$	2,3,7-Trimethyloctane
$C_{15}H_{32}$	2,6,11-Trimethyldodecane
$C_{15}H_{28}$	IH-indene
$C_{13}H_{28}$	Tridecane
$C_{21}H_{44}$	2,6,10,15-Tetramethylheptadecane
$C_{15}H_{32}$	pentadecane
$C_{15}H_{24}O$	Butylated Hydrotoluene
$C_{16}H_{34}$	Hexadecane
$C_{18}H_{38}$	2,6,10-Trimethylpentadecane
$C_{19}H_{40}$	Pentadecane 2,6,10,14-tetramethyl
$C_{18}H_{38}$	Octadecane
$C_{20}H_{42}$	Hexadecane,2,6,10,12-tetramethyl
$C_{19}H_{36}O_2$	11-Octadecenoic acid,methyl esther
$C_{18}H_{34}O_2$	6-octadecenoic acid
$C_{35}H_{68}O_5$	Hexadecanoic acid,2-hydroxy esther

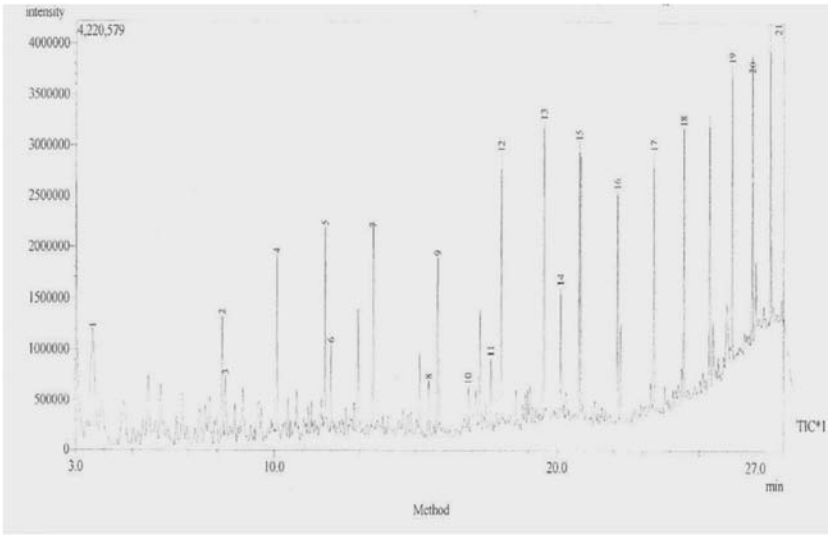

Fig. 1: Chromatogram of crude oil at 0 day

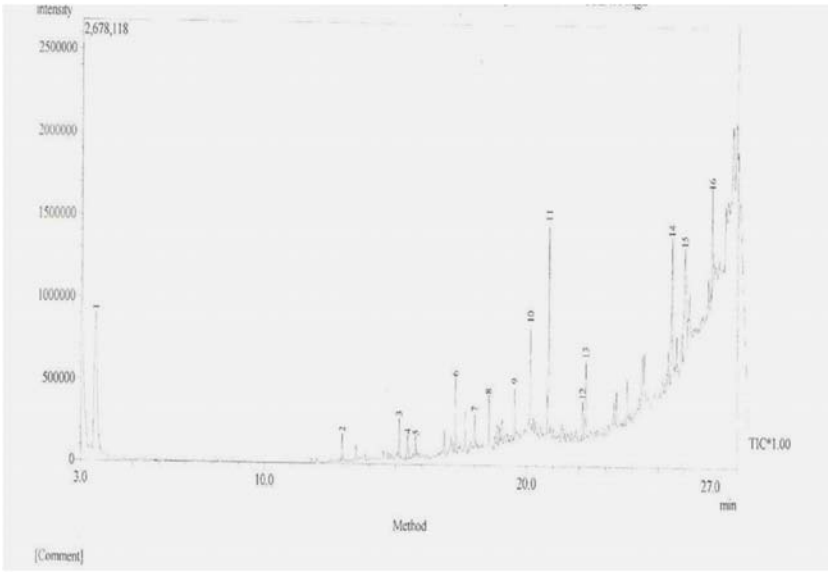

Fig. 2: Chromatogram of crude oil at day 28 after degradation

in the intensity of the hydrocarbon peaks when compared to Fig. 1. Most of the peaks decreased tremendously and few decreased only to some extent which is an indication of biodegradation of the crude oil by the yeast isolate.

DISCUSSION

The yeast isolate used in this study was identified as *Saccharomyces cerevisiae*. The microorganism has been previously reported to be found and isolated from oil polluted sites by Obire (1988) who also reported that fungi such as *Saccharomyces*, *Rhodotorula* and *Sporobolomyces* have evolved with the ability to degrade petroleum unlike other groups of microorganisms. The result of the percentage biodegradation of crude oil by *Saccharomyces cerevisiae* increases with increase in incubation period from 7 to 28 days this shows the potential of *Saccharomyces cerevisiae* to use crude oil as

a sole carbon source. This might be as a result of efficient hydrocarbon uptake via special receptor sites for binding hydrocarbons and might have a unique feature that assist in the emulsification and transport of hydrocarbons into the cell and also presence of enzymes that introduce molecular oxygen into the carbon and generate intermediates that subsequently enter common energy yielding catabolic pathway (Obire *et al*., 2008). The composition of the crude oil ranges from n-alkanes, polycyclic aromatic hydrocarbon and aromatic hydrocarbon from the result of the GC/MS of the crude oil at day 0. After an incubation of 28 days toluene and hexadecane were not degraded, this might be as a result of their toxicity or the inability of the fungus to produce enzymes that can degrade this component, while some of this component were converted into an intermediate, some were converted into a more complex compounds this might be as a result of reaction of two or more intermediates. Naphthalene was converted to pentadecane, The result agreed with the report of Muncnerova and Augustin (1994) that *Saccharomyces cerevisiae* has the ability to oxidise or transform polycyclic aromatic hydrocarbons and render them non-toxic. The n-alkane such as Nonadecane, Eicosane, Docosane Heneicosane and Tetracosane were completely degraded. This is in agreement with the review of Obuekwe *et al*. (2005) and Ashraf and Ali (2006) on the utilization of n-alkanes by yeast as a sole carbon and energy source.

CONCLUSION

Saccharomyces cerevisiae isolated from fermented Zobo has the potential to utilize crude oil as sole carbon source. The components degraded from the crude oil were majorly the n-alkane. Some of the aromatic hydrocarbons were converted into an intermediate product. *Saccharomyces cerevisae* could be a good candidate microorganism in the bioremediation of crude oil contaminated sites because of its environmental friendliness.

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