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# Fungi in an Oilfield Wastewater in Nigeria

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**Abstract:** Occurrence of fungi in produced water of an oilfield location was investigated by cultivation of the organisms on Potato Dextrose Agar (PDA) and Oil Agar (OA) media. Counts of heterotrophic and hydrocarbon-utilizing fungi were relatively low. Mean counts ranged from $0.3\times10^1$ to $8.8\times10^1$ cfu $mL^{-1}$ for Total Heterotrophic Fungi (THF) and from $0.0\times10^1$ to $2.4\times10^1$ cfu $mL^{-1}$ for Hydrocarbon-Utilizing Fungi (HUF). Fungal species were isolated from the wastewater and in varying frequencies (percentage ratio of THF: HUF), which include Aspergillus *fumigatus* (10:0%), *A. niger* (15:10%), *Fusarium* sp. (27:0%), *Mucor* sp. (5:2%), *Pencillium* sp. (10:0%), *Rhizopus* sp. (7:7%) and *Saccharomyces* sp. (0:5%). Growth of *Saccharomyces* sp. (yeast) was suppressed in PDA while *A. fumigatus*, *Fusarium* sp. and *Penicillium* sp., which grew on PDA, were suppressed on OA medium. The study confirmed that fungi can thrive in produced water but in low number. Also, the hydrocarbon-utilizing fungi (*A. niger*, *Mucor* sp., *Rhizopus* sp. and *Saccharomyces* sp.), can be used for crude oil clean-up.

**Key words:** Occurrence, fungi, cultivation, water, oil

## INTRODUCTION

Produced waters are oilfield wastewater produced along side with crude oil. They are subsurface waters associated with the reservoir rock (Odeigah *et al*., 1997; Wills, 2000). They have variations in their chemical composition and behavior, compared with the surface waters, because they are constrained within an aquifer. Its vast amount of formation on subsurface waters has been compiled by the petroleum industry (Odeigah *et al*., 1997; Wills, 2000). Of major concern to petroleum production operations in the disposal of this oilfield wastewater, is the concentration of salts, hydrocarbons and heavy metals found in mud additives (Odeigah *et al*., 1997; Obire and Wemedo, 1996). Even during treatment, chemicals such as water clarifier and biocides were added to reduce microbial populations (Obire and Wemedo, 2002).

Produced water contains dispersed or free oil, dissolved oil and other dissolved organic compounds referred to water soluble organics; naphthenic acids, fatty acids, low molecular weight hydrocarbons and other compounds that have not been well defined (Odeigah *et al*., 1997; Wills, 2000). Indiscriminate discharge of oilfield wastewater on terrestrial and aquatic environments has been decried by Federal Environmental Protection Agency (FEPA) and Department of Petroleum Resources (DPR) by Decree 58 of December 30, 1988, with statutory responsibility of overall protection of the environment (Obire and Wemedo, 1996). The current oil and grease limit for the general permits of outer continental shelves region is 48 mg $L^{-1}$ monthly average and 72 mg $L^{-1}$ daily maximum.

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The aim of this study was to enumerate and isolate total heterotrophic fungi and hydrocarbon-utilizing fungi associated with produced water; ascertain the extent of occurrence of these fungi in the wastewater and highlight the environmental significance of presence of fungi in the produced water.

## MATERIALS AND METHODS

### Sampling

Sampling was done at two-week intervals for a period of two months (November and December, 2006). Samples were collected from Elf Nigeria Limited oilfield flow station located at Obagi in Ogba/Egbema/Ndoni Local Government Area of Rivers State, Southern Nigeria. The oilfield situates in a freshwater swamp forest vegetation of the Niger Delta and the choice of the field was because oil operations have lasted for over 40 years in the area.

Freshly-produced wastewater samples were collected at the oilfield effluent discharge point before it made contact with the environment. Samples were collected in accordance with the method described in standard methods for water and wastewater analysis (APHA, 1995). According to the above procedures, 250 mL of sterilized sample bottles were filled from gentle stream of the wastewater after flushing the interior of the nozzle of the valve with a flow of the waste water for 2 min, in order to avoid contamination from external sources. Five replicate samples were collected at each sampling period.

### Reagents and Culture Media

Engine oil and diesel in sealed containers were purchased from a filling station in Port Harcourt, Rivers State. The culture media used were Potato Dextrose Agar (PDA) and Oil Agar (OA). Potato dextrose agar had the following composition: potato extract 4.0 g, dextrose 20.0 g, agar 15.0 g, pH 5.6±0.2 and distilled water 1 L. Oil agar medium had the following composition: mineral salts medium (Amadi *et al*., 2004): ammonium chloride 0.5 g, dipotassium hydrogen phosphate 0.5 g, disodium hydrogen phosphate 2.5 g, agar 15.0 g, distilled water 1liter, pH 6.5, engine oil/diesel (1:3v/v) 5 mL. Streptomycin (0.5 g) was suplemented to the oil agar medium to suppress the bacterial growth.

### Method of Isolation of Fungi

Ten-fold serial dilution method of Ofunne (1999) was used. Decimal dilutions of the samples were made by adding 1.0 mL of wastewater to 9.0 mL of sterile normal saline (diluents) to give an initial dilution of 1:10. Further serial ten-fold dilution was made up to $10^{-2}$ dilution. Appropriate dilutions were spread in duplicate on freshly prepared dry Potato Dextrose Agar (PDA) medium for Total Heterotrophic Fungi (THF) and Oil Agar (OA) Medium for Hydrocarbon-Utilizing Fungi (HUF). The spread plate technique using a sterile bent glass rod was used for all inoculations. The inoculated plates were incubated at 28±2°C for 2 to 7 days. After incubation, colonies developed were counted and taken as total heterotrophic and hydrocarbon-utilizing fungal counts, respectively.

### Microscopic Examination and Identification of Fungal Isolates

Microscopic examination of mould growth was done by observing the colonial morphology: color of colony, texture, shape and surface appearance; cultural characteristics: asexual and sexual reproductive structures like sporangia, conidial head, arthrospores, septate or non-septate vegetative mycelia, (Winn *et al*., 2006; Burnett, 1976). The needle mount method as described by Wemedo *et al*. (2002) was used for microscopic examinations of the moulds.

All identifications of pure isolates were made on the basis of their cultural and morphological characteristics (Alexopoulos and Sun, 1962; Burnett, 1976; Winn *et al*., 2006).

## RESULTS AND DISCUSSION

Mean fungal counts of the produced water ranged from $0.3\times10^1$ to $8.8\times10^1$ cfu $mL^{-1}$ for total heterotrophic fungi and from $0.0\times10^1$ to $2.4\times10^1$ cfu $mL^{-1}$ for hydrocarbon-utilizing fungi (Table 1). Fungal populations of the wastewater are low. The low fungal counts may be due to the stress to which the organisms were exposed to in the wastewater (Obire and Wemedo, 1996, 2002). Once separated from the oil, the wastewater is subjected to various forms of treatments including removal of oil; and addition of chemicals such as biocides to reduce the populations of microorganisms in the wastewater before final discharge (Obire and Wemedo, 2002). The presence of fungi in the produced water revealed that the chemical treatment introduced into the water did not completely eliminate the fungi but reduced their numbers to a minimal level.

Fungal species occurred in oilfield wastewater and can survive in it. The fungi isolated in this study included the *Aspergillus fumigatus*, *A. niger*, *Fusarium* species, *Rhizpous* species and *Saccharomyces* species. Occurrence of these fungi in the produced water (Oily water) suggests that the fungal species have the ability to utilize the traces of oil present in this water and able to grow in the oil agar medium. In this connection, Okpokwasili and Olise (1991) and Wemedo and Obire (1998) also reported the ability of similar organisms to utilize crude oil, which confirmed the ability of the fungi to thrive in the oily water. It has been noted that after treatment, the wastewater still contains dispersed or free oil, dissolved oil and other hydrocarbons (Obire and Wemedo, 1996, 2002; Wills, 2000).

The frequency of occurrence of the fungal genera is relatively high in the case of *Fusarium* species and *A. niger* and low in the case of the other genera (Table 2). This revealed that different fungal types have different rate of thriving in the oily water.

Growth of the organisms on the media was noted. The growth of fungi in oil agar took longer time than in potato dextrose agar. Of the seven genera isolated, only *Saccharomyces* species did not grow on potato dextrose agar. *Aspergillus nigar*, *Mucor* species, *Rhizopus* species and *Saccharomyces* species (yeast) grew on oil agar medium and were taken as hydrocarbon-utilizing fungi in this study. Wemedo and Obire (1998) affirmed similar organisms as hydrocarbon-utilizing fungi. They stated that fungal organisms were capable of utilizing the major hydrocarbon components of the oily water. Growth of *A. fumigatus*, *Fusarium* species and *Penicillium* species on oil agar were suppressed. *Aspergillus niger* had the highest frequency of growth on oil agar medium (Table 2).

Table 1: Mean densities of fungi in wastewater samples

| Sampling periods | Counts of heterotrophic fungi (cfu$\times10^1$ $mL^{-1}$) | Counts of hydrocarbon-utilizing fungi (cfu$\times10^1$ $mL^{-1}$) |
|---|---|---|
| Day 1 | 8.8 | 0.0 |
| Week 2 | 1.3 | 2.0 |
| Week 4 | 1.2 | 2.4 |
| Week 6 | 0.3 | 1.6 |

Table 2: Fungi types isolated from wastewater samples and their frequencies

| Fungal types | Frequency of THF | Frequency of HUF |
|---|---|---|
| *Aspergillus fumigatus* | 4(10) | 0(0) |
| *Aspergillus niger* | 6(15) | 5(12) |
| *Fusarium* species | 11(27) | 0(0) |
| *Mucor* species | 2(5) | 1(2) |
| *Penicillium* species | 4(10) | 0(0) |
| *Rhizopus* species | 3(7) | 3(7) |
| *Saccharomyces* species | 0(0) | 2(5) |

Values in parenthesis are frequency percentage

Previous reports have highlighted the environmental consequences of release of oilfield wastewater (Oily water) into the environments (Odeigah *et al*., 1997; Obire and Wemedo, 2002). In this context, Obire and Amusan (2003) reported a significant effect of formation water on the microbial population of a freshwater stream in Nigeria. On the other hand, Obire and Wemedo (1996, 2002) concluded that oilfield wastewater had no adverse effects on the microorganisms of a soil in Nigeria. However, the contradivity in results may be due to the fact that the environmental consequence of oilfield produced water may be more devastating than what was reported.

The significance of presence of fungi in the produced water may be related to their capability to utilize hydrocarbons in the water, which was confirmed by their growth on oil agar. The hydrocarbon-utilizing isolates in this study can be used in bioremediation exercise if introduced onto a crude oil polluted environment. Moreover, since the wastewater contained these hydrocarbon-utilizing fungi, it can be either minimally applied to crude oil-contaminated environment to decontaminate the polluted ecosystem instead of isolating the fungi. Fungi can be used for cleaning up crude oil-contaminated environment.

In conclusion, the present investigation revealed the occurrence of viable fungi in oilfield wastewater though in low rate and can be useful in crude oil clean-up exercise.

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