Effects of Crude Oil on Biomass and Protein Production by Aquatic Yeasts

[1]Nwokoro, O. and [2]Chukwu, B. C.
[1]Industrial Microbiology and Biotechnology Laboratory, Department of Microbiology, University of Nigeria, Nsukka, Nigeria
[2]Department of Biochemistry, University of Nigeria, Nsukka, Nigeria
***Corresponding Author:** E-mail: ogbonnaya.nwokoro@unn.edu.ng; Tel No: +2348034402414

Abstract

Toxic effects of Bonny light crude oil on the growth of three aquatic yeasts namely Yarrowia lipolytica, Candida tropicalis and Debryomyces hansenii were studied based on their biomass and protein production. The species showed different responses to the toxic influences of various crude oil concentrations. The growth response was measured spectrophotometrically using optical density (OD) at 600nm. Yarrowia lipolytica responded positively to different crude oil levels. A general assessment indicated that 2% (v/v) crude oil concentration stimulated maximum growth and protein production of this organism. Lower yields were observed at reduced crude oil levels. Growth decreased gradually among Candida tropicalis and Debryomyces hansenii cultures in comparison to the control. Biomass of Candida tropicalis increased from 0.1 (OD_{600nm}) at 0 h to 0.49 after 20 h at 0.5% crude oil concentration. This level gradually declined to 0.04 after 20 h cultivation at 1.5 % crude oil concentration. Maximum decline in optical density of this organism was observed at crude oil concentration of 2.0%. Protein levels for Candida tropicalis decreased from 0.13 mg/mL after 20 h at crude oil concentration of 0.5% to 0.04 mg/mL after 20 h at maximum crude oil concentration of 2%. The biomass of Debryomyces hansenii increased slightly from 0.1(OD_{600nm}) at 0 h to 0.44 after 20 h at 0.5% crude oil level. Further decreases in OD values of this organism occurred progressively as the crude oil concentration was increased. Lowest protein yield was observed at a crude oil concentration of 2% at which the least protein production of 0.05 mg/mL was produced after 20 h.

Key words: Crude oil, yeasts; protein; biomass.

Introduction

Petroleum – based products are the major sources of energy for industry and daily life and as the world's dependence on crude oil and its derivatives increases so does the level of exploration and this has created the conditions for the potential distribution of large amounts of toxins associated crude oil into the environment (Obahiagbon, *et al.*, 2009). Crude oil is a complex substance consisting mainly of petroleum hydrocarbon and it is the major source of pollution in the marine environments as a result of its release from activities such as offshore drilling, natural oil seepage, washing of oil tankers as well as production, transportation and ruptured pipeline accidents (Hasanuzzaman *et al*., 2007; Hidayat and Tachibana, 2012). The water soluble fraction of crude oil has been reported to reduce the growth of biomass in the contaminated environment as a result toxicity of its components (Kishore and Mukherjee, 2006). There are few reports on the toxic effects of crude oil on yeasts. Most reports in literature are concerned with the degradation of crude oil by yeasts (Martins *et al*., 2012). The toxicity of crude oil or petroleum products varies widely, depending on their composition and concentration, on environmental factors and on the biological state of the organisms at the time of the contamination.

Crude oil consists of a complex mixture of organic compounds that include volatile and non volatile compounds such as saturated hydrocarbons, cycloalkanes, aromatic compounds, polycyclic aromatic compounds, polar compounds and resins/asphaltene compounds (Kim *et al*., 2012). The Niger Delta eco region of Nigeria has been associated with frequent oil spills resulting from oil pipeline vandalization, tanker accidents and accidental rupture of oil pipelines and these mishaps result in the release of crude oil and refined petroleum products into the terrestrial and aquatic environments (Okpokwasili and Amanchukwu, 1988). Despite more stringent environmental regulations, the risk of an oil spill affecting the ecosystems is still high and which we must accept as inevitable (Tanee and Kinako, 2008). Toxicity of petroleum hydrocarbons is highly variable depending on the type and concentration, exposure time, state and environmental conditions. Yeasts are widely distributed in the terrestrial and aquatic environments, as well as in wine and various foods (Chen *et al*., 2009). However, yeasts with different metabolic attributes have been reported to occur in aquatic environments, such as oceans and seas, estuaries, lakes, and rivers (Kutty and Philip 2008). Previous studies reported that marine yeasts do not belong to a specific genus or group, but are represented by a wide variety of well-known genera, such as *Candida*, *Cryptococcus*, *Debaryomyces*, *Pichia*, *Hansenula*, *Rhodotorula*, *Saccharomyces*, *Trichosporon*, and *Torulopsis* (Kutty and Philip 2008). Many potentially toxic chemicals enter the environment and each act on ecological targets through their own specific mode of action. Ecological risk assessment is usually based on toxicity data obtained from laboratory tests. There are a number of studies on responses of some fungi to crude oil pollution (Masaphy *et al*., 1996). With regards to literature, yeast morphology (Ferretti de Lima, 2004; Farag and Soliman, 2011); its molecular characteristics (Sood, and Lal, 2009);

metabolism (Teh, 1975; Junior *et al*., 2009); growth and biomass synthesis (Obire and Anyanwu, 2009) and protein production (Iida *et al*., 1998) have been affected. The objective of the study was to investigate the potential effects of crude oil on aquatic yeasts biomass and protein production as part of assessing the potential risk of exposure of yeast populations to hydrocarbon residues in aquatic microbiota.

Materials and Methods

Yeast Isolation: Water sample was collected from Nembe River in Bayelsa State, Nigeria. This river is periodically polluted with crude oil. The sample was collected into a screw – capped conical flask at a depth of about 10cm from the surface and was taken into the laboratory for microbiological analysis. About 0.1ml of the water sample was plated onto Sabouraud Dextrose agar (Oxoid, Ltd., UK) plates containing 0.1% chloramphenicol to suppress bacterial contaminants. Plates were incubated at 30±2°C for 48 h. Pure cultures were obtained by streaking on fresh agar plates and the isolated yeasts were identified based on the taxonomic scheme given by Lodder (1970) and de Hoog *et al.* (2000).

Inoculation and Biomass Production: Into Sabouraud dextrose broth (100 ml) contained in conical flasks was each added 0.5, 1.0, 1.5 and 2 % (v/v) Bonny light crude oil. A control experiment devoid of crude oil was separately prepared. The medium was dispensed in 10 mL aliquots in test tubes and autoclaved at 121°C for 15 min. The isolates were diluted to optical density (OD) 0.1 at 600nm measured in a Spectrum lab 23A spectrophotometer and 0.5 mL aliquot was each added into the tubes. The inoculated tubes were incubated for 20 h at 30±2°C on a shaker (Fisher Roto Rack Model 343). Optical density readings were measured at 600nm.

Protein Determination: Yeast protein was precipitated with 10% trichloroacetic acid and determined according to the method of Lowry *et al.* (1951) using bovine serum albumin (Sigma-Aldrich) as a standard.

Statistical Analysis: Analysis of variance (ANOVA) and least significant difference (LSD) were used to determine the significant differences among mean values where by $p \leq 0.05$ was considered significant.

Results and Discussion

Three yeasts were isolated from Nembe River which periodically receives crude oil pollution. The isolates were identified as *Yarrowia lipolytica*, *Candida tropicalis* and *Debryomysces hansenii*. The effects of various concentrations of crude oil on biomass and protein production by the isolates were tested in broth culture. Toxicological crude oil analysis is usually performed on the basis of the water-soluble fraction. However, this yields only a partial estimate of the damage caused by these contaminants because a substantial hydrophobic amount can be adsorbed by suspended solids (biotic and abiotic) which directly affects species (Martinez-Jeronimo *et al.,* 2005). Water soluble fraction also tend to underestimate the toxic damage that can be produced in natural environments. We therefore tested the effects of the entire crude oil sample on yeast biomass and protein production. Yeast samples were collected from polluted river and these samples were ideal candidates because the microbial communities have been acclimatized to conditions of frequent contamination of crude oil.

Biomass of *Yarrowia lipolytica* attained in a medium containing crude oil after 20 h incubation is shown in Fig 1. Maximum cell biomass increased with increasing concentrations of crude oil.

The best biomass yield of 0.81 (OD_{600nm}) after 20 h incubation occurred at the highest crude oil concentration of 2.0%. Lower yields were observed as the crude oil concentrations were reduced (Fig. 1).

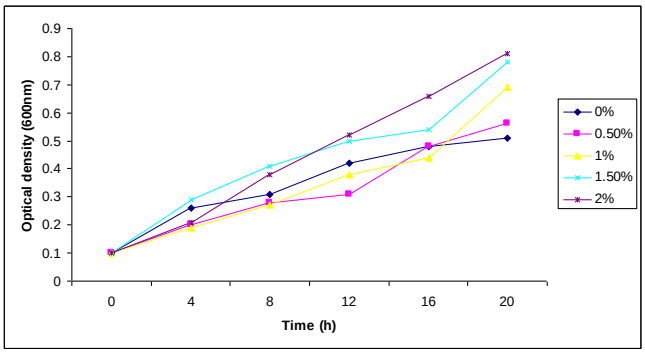

Figure 1: Effect of crude oil on biomass production by *Yarrowia lipolytica*

Biomass in broth containing crude oil did not vary significantly ($p \leq 0.05$) from control samples. *Yarrowia lipolytica* is a yeast that degrades hydrophobic substrates very efficiently (Bankar *et al*., 2009). Due to its ability, these strains have been focus of bioremediation studies, being used as promising agent for treatment of contaminated areas. The ability of *Yarrowia lipolytica* to tolerate crude oil and grow in it suggests that it can be employed as bioremediation agent and can be used in restoring the eco system when polluted by oil. In the present study, increase in the OD in crude oil contain medium indicated that fungal growth was due to the utilization of crude oil as a source of carbon for growth.

Fig. 2 illustrates an obvious effect of crude oil on the growth of *Candida tropicalis* over the 20 h culture period. The inhibitory effect of the complete medium became quite noticeable at 1 % crude oil concentration at which the biomass level was 0.09(OD_{600nm}) after 20 h. Biomass yields were lowest as crude oil concentrations were increased. The inhibitory effects of crude oil proceeded with a much higher rate compared to the control resulting in almost a complete cessation of growth at 2% crude oil concentration after 20 h (Fig. 2).

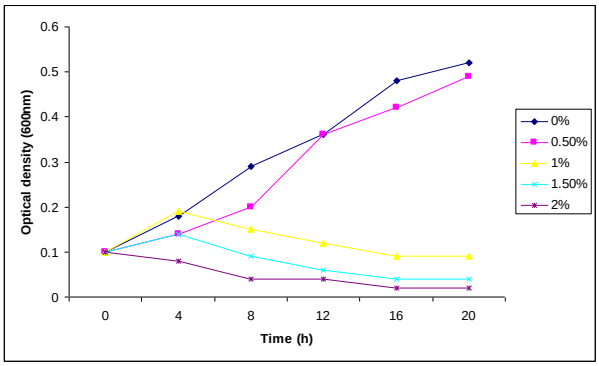

Figure 2: Effect of crude oil on biomass production by *Candida tropicalis*

The cell population of the control at this time was about 0.52 (OD_{600nm}). The effects of adding varying concentrations of crude oil to the broth containing *Debryomyces hansenii* as the test organism is shown in Fig 3. Crude oil concentrations were effective in retarding the growth rate of the yeast. The cell concentration of *Debryomyces hansenii* increased slightly from 0.1 (OD_{600nm}) at 0 h to 0.44 (OD_{600nm}) at a crude oil concentration of 0.5%. Further decreases in OD values of this organism occurred progressively as the crude oil concentrations were increased (Fig 3). Maximum biomass decline was observed after 20 h at crude oil concentration of 2.0% at which a cell concentration of 0.03 (OD_{600nm}) was produced. This result is statistically different ($p \leq 0.05$) from the control which gave a maximum OD 0.59 after 20 h.

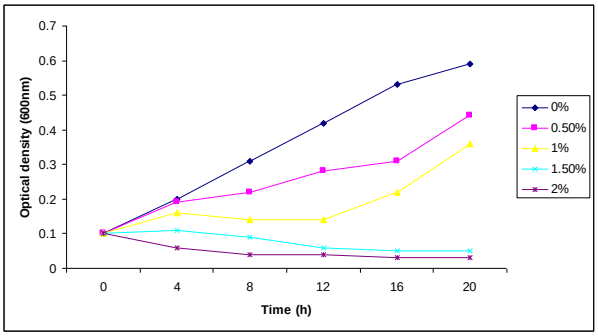

Figure 3: Effect of crude oil on biomass production by *Debryomyces hansenii.*

Yarrowia lipolytica cultures produced the best protein yields of 1.62 mg/mL at maximum crude oil level of 2% after 20 h (Fig. 4). Lower protein production by this organism occurred as the crude oil levels were reduced. These values were comparable to the control and showed no significant difference ($p \leq 0.05$). Protein yields by *Candida lipolytica* at varying crude oil concentrations is shown in Fig. 5. Yeast protein levels decreased from 0.13 mg/mL after 20 h at a crude oil concentration of 0.5% to 0.04 mg/mL after 20 h at maximum crude oil concentration of 2%. Values for the control experiment showed increases in protein production from 0.3 mg/mL at 0 h to 1.03 mg/mL after 20 h. The reduced protein content is possibly due to decreased enzyme activity which is necessary for the recovery of the organisms. Data in Fig. 6 shows protein production by *Debryomyces hansenii* at different crude oil concentrations. Lowest protein level was observed at a crude oil concentration of 2% at which the least protein yield of 0.05 mg/mL was produced. Protein production in control samples was much higher than these values and there was a statistical difference ($p \leq 0.05$) than the experimental results.

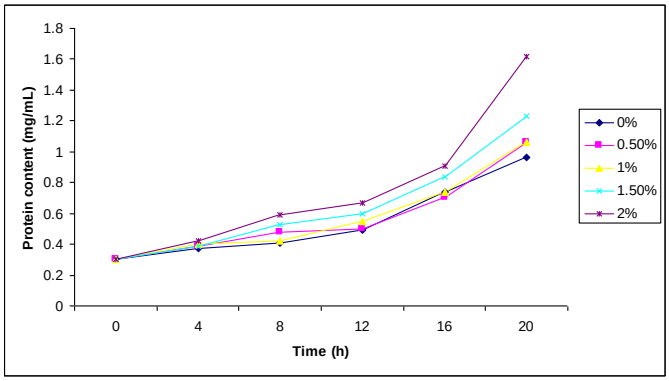

Figure 4: Effect of crude oil on protein production by *Yarrowia lipolytica.*

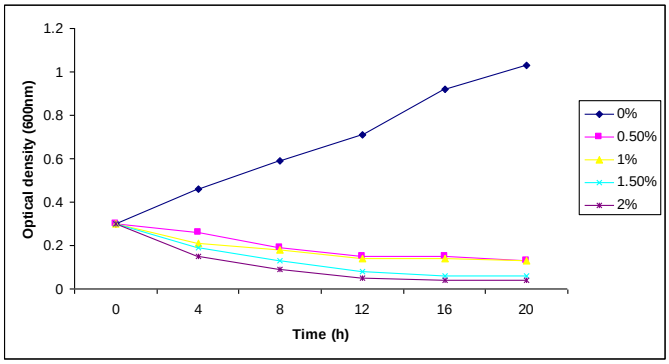

Figure 5: Effect of crude oil on protein production by *Candida tropicalis.*

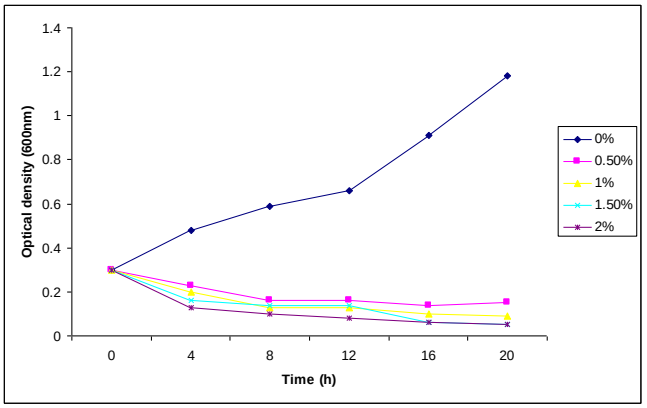

Figure 6: Effect of crude oil on protein production by *Debryomyces hansenii.*

Pollution caused by petroleum and its derivatives is the most prevalent problem in the environment. The release of crude oil into the environment by oil spills continue to receive worldwide attention. Oil pollution

problems are increasingly becoming a common theme in the world today and this has resulted in the contamination of the environment particularly in the oil producing areas of the world (Obahiagbon et al; 2009). The utilization of hydrocarbons by microorganisms as a sole carbon and energy source has been reviewed by Obuekwe et al. (2005) and Ashraf and Ali (2006). In many reports, bacteria have been identified as more efficient crude oil degraders than yeasts. Crude oil toxicity of yeast populations have also been studied (Obire and Anyanwu, 2009). Our present investigation was compared to previous research findings on responses of fungi and other plankton populations to crude oil exposure. Walker et al (1975) observed that crude and fuel oil had little noticeable effects on yeast and fungal populations. Ghita and Ardelean, 2010 reported limited growth inhibition of petroleum hydrocarbon to some marine plankton populations. Lytle (1970) reported inhibition of natural fresh water plankton populations by crude oils while Hellebust et al., 1975 reported no noticeable effects on a similar natural system. Head et al 2006; Leahy and Colwell, 1990; Nikolopoulou and Kalogerakis (2009) reported the role of microorganisms in petroleum hydrocarbon consumption. Farag and Soliman (2011) reported *Candida tropicalis* which showed a high potency in the degradation of petroleum oil and hydrocarbons. Sood and Lal (2009) isolated a novel *Candida digboiensis* strain from the Digboi refinery that was capable of utilizing hydrocarbons. The authors also observed the ability of the yeast to utilize alkanes and transform aromatic hydrocarbons to their oxidative forms. Kim et al., (2012) studied the effects of crude oil, dispersant and oil dispersant mixtures on human feacal microbiota in an *in vitro* culture system and observed that microbiota populations were affected differently by oil and dispersant oil and the influence of dispersed oil was significantly greater than that of either oil or dispersant alone compared to control cultures. Obire and Anyanwu, (2009) investigated the effects of various concentrations of crude oil on fungal populations in the soil and showed that higher concentrations of crude oil had adverse effects on fungal diversity and population, enhancing the population of only a few fungi. The authors reported that higher concentrations of crude oil had toxic effects on the cells and led to decreased fungal biomass production.

Conclusion

Results suggested the inhibitory effects on growth and protein production in crude oil treated cells of *Candida tropicalis* and *Debryomyces hansenii*. Growth stimulation and increased protein production were observed in *Yarrowia lipolytica* cultures. Maximum biomass of *Yarrowia lipolytica* occurred at 2.0% crude oil concentration. Lower yields were observed at reduced crude oil levels. *Yarrowia lipolytica* cultures produced the best protein yields of 1.62 mg/mL at maximum crude oil level of 2% after 20 h. Lower protein production by this organism occurred as the crude oil levels were reduced. Biomass of *Candida tropicalis* gradually declined to 0.04 after 20 h cultivation at 1.5 % crude oil concentration. Maximum decline biomass of this organism was observed at crude oil concentration of 2.0%. Protein yields by *Candida tropicalis* at varying crude oil levels reveal a decrease from 0.13 mg/mL after 20 h at crude oil concentration of 0.5% to 0.04 mg/mL after 20 h at maximum crude oil concentration of 2%. The cell concentration of *Debryomyces hansenii* increased slightly from 0.1 (OD_{600nm}) at 0 h to 0.44 (OD_{600nm}) at 0.5% crude oil level after 20 h. Further decreases in OD values of this organism occurred progressively as the crude oil concentration was increased. Also, lowest protein level of this organism was observed at a maximum crude oil concentration of 2%.

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