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Reduction of organic load from palm oil mill effluent (POME) using selected fungal strains isolated from POME dump sites

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Environmental concerns are becoming important global tasks. Palm oil mill effluent (POME) contains oil and grease and also rich in organic matter in the form of total suspended solids which can increase biochemical oxygen demand (BOD) and chemical oxygen demand (COD) of POME. It is generated in huge quantities during the production of crude palm oil and pollutes land, water and atmosphere if left untreated. The current study mainly focuses on evaluating the efficacy of fungal isolates screened and isolated from POME dump sites in the bioremediation of POME. Five fungal species used in the present study were previously isolated by the author from POME dump sites of Pedavegi palm oil mill industry. Out of these, *Emericella nidulans* NFCCI 3643 was proven to be an excellent biological agent in reducing the organic load of POME. The organism showed 80.28% reduction in COD, 88.23% in BOD and 87.34% in oil/grease content at their optimal environmental and nutritional conditions. The mixed cultures showed better reduction efficiency as compared to individual pure cultures. The natural inhabitants of POME dump sites showed their lipolytic ability and *E. nidulans* was found to be an excellent agent in the bioremediation of POME. Fungal isolates in consortium can function better in bioremediating POME than individual pure cultures.

Key words: Palm oil mill effluent (POME), oil and grease, biochemical oxygen demand (BOD), chemical oxygen demand (COD), organic load, *Emericella nidulans*, bioremediation.

INTRODUCTION

Palm oil mill effluent (POME) is the waste that is being released by the palm oil mills during oil extraction process from palm fruit bunches. In recent years, a lot of attention has been drawn towards environmental hazards that are caused by direct release of industrial effluents without proper treatment. It was estimated that about 1.5 tons of POME were generated for a tone of fresh palm fruit bunches during oil extraction process (Ahmad et al., 2003). POME is a thick brown colored liquid rich in total solids, oil and grease (Poh and Chong, 2008; Mahzad et al., 2009). The chemical oxygen demand (COD) and biological oxygen demand (BOD) of POME is also high

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(Pogaku and Sarbatly, 2013). A high concentration of organic matter, COD (45,000 to 65,000 mg/L) and BOD (18,000 to 48,000 mg/L) of POME was reported by Chin et al. (1996). Soleimaninanadegani and Manshad (2014) reported a COD and BOD in the range of 80,100 to 95,000 and 23,400 to 52,100 mg/L, respectively. A COD and BOD of 40,000 to 50, 000 and 20,000 to 25,000 mg/L were reported by Najafpour et al. (2006). In addition to oil and grease, POME also contains various complex polymers like carbohydrates, lipids, proteins, certain minerals and nitrogenous compounds (Ohimain et al., 2013a). Discharge of untreated POME into aquatic bodies can cause dark coloration of water, eutrophication which further makes the water unsuitable for consumption (Tubonimi et al., 2007; Cheng et al., 2010; Foo and Hameed, 2010). The nature of POME also causes odour pollution (Er et al., 2011). A high COD value of POME also distracts the aquatic life (Maygaonkar et al., 2012), resulting in loss of biodiversity (Singh and Pandey, 2009). As direct discharge of such effluents into the environment without proper treatment might cause considerable environmental problems (Cheng et al., 2010; Awotoye et al., 2011; Jameel and Olanrewaju, 2011; Lam and Lee, 2011), there is need to treat POME from palm oil mills before they are discharged into the environment.

In addition, the high level of organic matter of POME as indicated by its high COD, BOD, grease and oil content, serves as good substrate for growth of a wide variety of microbes (Roux-Van Der Merwe et al., 2005; Md Din et al., 2006). POME being rich in oil content also serves as a habitat for several groups of lipase producing microorganisms as well as hydrocarbon degraders (Rahman et al., 2007; Ohimain et al., 2013b). Use of fungi in the bioremediation of POME has drawn the attention of researchers since last two decades, as most of the previous works involved the use of bacteria in the bioremediation of POME. Several fungi like *Rhizopus, Mucor, Candida rugosa, Geotrichum candidum, Aspergillus*, etc. have been well studied for their ability to produce lipolytic enzymes (Burkert et al., 2004; D'Annibale et al., 2006; Grbavcic et al., 2007; Nwauche and Ogbonna, 2011). Though, POME is a major environmental concern, only limited studies were reported its bioremediation (Oswal et al., 2002; Wu et al., 2010; Soleimaninanadegani and Manshad, 2014). Therefore, screening and isolation of fungi from POME dump sites provides an alternative way to clean up environmental pollutants as these microbes use the organic compounds present in the POME as supplements and thereby degrade these substances into simpler compounds like methane, carbon dioxide and water. The bioconversion of POME by microorganisms also has additional advantages in that, it makes the POME to be useful in the production of a variety of compounds such as antibiotics, biofertilizer, solvent, bio-insecticides, biohydrogen, polyhydroxyalkanoates, organic acids and enzymes (Wu et al., 2009). Hence, the present study was carried out to investigate the ability of the selected indigenous fungi in the bioremediation of POME.

The results obtained in the present study clearly demonstrate that POME dump sites are potential sources of lipase producing microorganisms and can be used to treat POME.

MATERIALS AND METHODS

Collection and preservation of POME

POME was collected from Pedavegi palm oil mill in sterile plastic bottles, sealed and transported in ice box to the laboratory. The sample was stored at -20°C until further use. The physico-chemical characteristics of the POME were studied using the standard methods published by APHA (American Public Health Association, 2005).

Characterization of POME and analytical methods

POME collected from Pedavegi palm oil industry was characterized by determining the physicochemical parameters such as chemical oxygen demand (COD), biochemical oxygen demand (BOD5), total suspended solids (TSS), oil and grease (O&G) and pH. The treatment efficiency was determined by characterizing the POME before and after the treatment. Reduction in organic load COD was determined spectrophotometrically, BOD5 was used to measure the biodegradability, TSS were determined as dry weight (mg/L), partition-gravimetric method (Kirschman and Pomeroy, 1949) was employed to determine oil and grease content and pH meter was used to measure the pH. The above methods were carried out as per the standard procedures described in the Standard Methods for the Examination of Water and Wastewater (Clesceri et al., 1999; APHA, 1995, 2005).

Reduction efficiency (RE %) of COD was defined as the amount of COD that decreased as compared to the initial COD amount. Reduction efficiency (RE %) of BOD in terms of BOD5 was defined as the amount of BOD that decreased as compared to the initial BOD amount. The experiments were carried out in triplicates.

Fungal isolates

Five fungal species out of 12 isolates that were screened and isolated from the POME dump sites of Peda vegi palm oil mill, West Godavari District, A.P., India (Suseela et al., 2014) were selected for POME inoculation. They include *Emericella nidulans* NFCCI 3643, *Trichoderma reesei*, *Trichoderma harzianum*, *Aspergillus niger* and *Aspergillus fumigatus*. The fungi used for POME inoculation were identified based on morphological characteristics and microscopic observation of fungal spores using lactophenol cotton blue staining. For morphological characterization, the fungal isolates were cultivated on czepakdox agar medium. The shape, size, arrangement and development of conidiophores, phialides and conidiospores were studied using the taxonomic tools of Hoog et al. (2000).

Inoculation of sterile POME

The five fungal isolates that showed highest lipase producing activity were selected in the present study to test their applicability in the bioremediation of POME. The raw POME sample transferred into 250 mL of Erlenmeyer flask was autoclaved at 121°C for 20 min. The cooled autoclaved sample was inoculated with five percent of spore suspension containing 10^6 cells/mL and incubated at 30°C with shaking at 150 rpm. At an interval of 24 h, samples were collected under aseptic conditions up to 5 days and analyzed for BOD5, COD, oil and grease. Control flasks were not inoculated. The efficiency for organic load reduction and the percentage reduction was measured by using the following equation (Piro et al., 2011):

$$\text{Reduction \%} = 100 - \frac{C_{raw}\ \text{POME} - C_f}{C_{raw}\ \text{POME}} \times 100$$

Where C_{raw} POME is the concentration of COD, BOD5, oil and grease of raw POME and C_f is the concentration of the above said parameters after treatment. All the experiments were performed in triplicates.

Similar experiments were also carried out using mixed cultures of organisms (MC1 (*E. nidulans* + *A. niger* + *A. fumigatus*) and MC2 (*T. harzianum* + *T. reesei*)) to test whether the organisms in group can perform better as compared to individual pure cultures. For preparing mixed cultures, spores of all fungi were mixed in equal ratio and inoculated into sterile POME.

RESULTS AND DISCUSSION

Characteristics of POME

POME collected from the Pedavegi palm oil mill was a thick dark brown colored viscous oily liquid with abhorrent odour. The raw POME contains a BOD of 39,476 mg/L, COD at a concentration of 79,980 mg/L, TSS 15,238 mg/L, oil and grease 209 mg/L and pH 4.28. Similar findings were reported by Najafpour et al. (2006), Vijayaraghavan et al. (2007), AbdulKarim et al. (2011), Lam and Lee (2011) and Bala et al. (2015).

Fungal isolates from POME dump sites

A total of 12 different fungal members were screened and isolated from POME dump sites (Suseela et al., 2014). Out of them, 5 fungal isolates that showed good lipase producing activity were selected for further studies.

Oil and grease removal (%) from POME using selected fungal isolates

The use of fungi and yeast such as *Trichoderma viride*, *Saccharomyces cerevisiae* and *Yarrowia lipolytica* for the treatment of POME has not been extended to the removal of oil and grease (Jameel and Olanrewaju, 2011) despite their high potential in removing COD from POME. This may be due to the fact that these microorganisms are not indigenous to POME. In the present study, the ability of 5 fungal members isolated from POME dump sites were investigated for the removal of oil and grease from POME.

The removal (%) of oil and grease from POME is shown in Figure 1. From the results, it is evident that reduction efficiency of *Emericella nidulans* was highest with 87.34% followed by *A. niger*, *T. harzianum*, *A. fumigatus* and *T. reesei* with 71.23, 68.21, 61.17 and 59.09%, respectively. There was only 16.85% reduction efficiency with the control indicating the potentiality of our POME isolates in the removal of oil and grease. The results obtained are in agreement with the reports of Oswal et al. (2002) in his work on treatment of POME with *Y. lipolytica* NCIM 3589. A 93.3% reduction in oil and grease was reported by Lan et al. (2009) using *Y. lipolytica* W29.

Reduction efficiency (RE %) of COD using selected fungal isolates (Individual pure cultures)

Figure 2 shows the reduction efficiency of COD for selected fungal isolates. From the results, it is evident that reduction efficiency of *E. nidulans* was highest with 80.28% followed by *A. niger*, *T. harzianum*, *A. fumigatus* and *T. reesei* with 71.08, 64.83, 61.86 and 59.26%, respectively.

There was only 13.88% reduction efficiency with control indicating that the POME isolates are effective in COD reduction. Similar findings were reported regarding COD reduction by El-Bestawy et al. (2005), Takeno et al. (2005), Lan et al. (2009) AbdulKarim et al. (2011), Abass et al. (2012), Mohammed et al. (2014), Soleimaninanadegani and Manshad (2014) and Bala et al. (2015) in their studies using different microorganisms. The present study is significant in understanding the role of fungi in the bioremediation of oil contaminated effluents such as POME.

Reduction efficiency (RE %) of COD using mixed cultures (combination of fungal isolates)

The reduction efficiency of COD by mixed cultures is shown in Figure 3. From the results, it is clearly evident that there is enhanced organic load reduction with mixed cultures as compared to pure cultures and is as follows: MC1 (*E. nidulans* + *A. niger* + *A. fumigatus*) (91.43%) > MC2 (*T. harzianum* + *T. reesei*) (73.14%) > control (17.23%).

This study provides an understanding on the role of mixed cultures in the treatment of waste waters such as those from oil processing industries. The results reported are in good agreement with the results of previous workers who also used mixed cultures for the effluent treatment (Chigusa et al., 1996; Wakelin and Forster, 1997; AbdulKarim et al., 2011).

Enhanced organic load reduction with mixed cultures

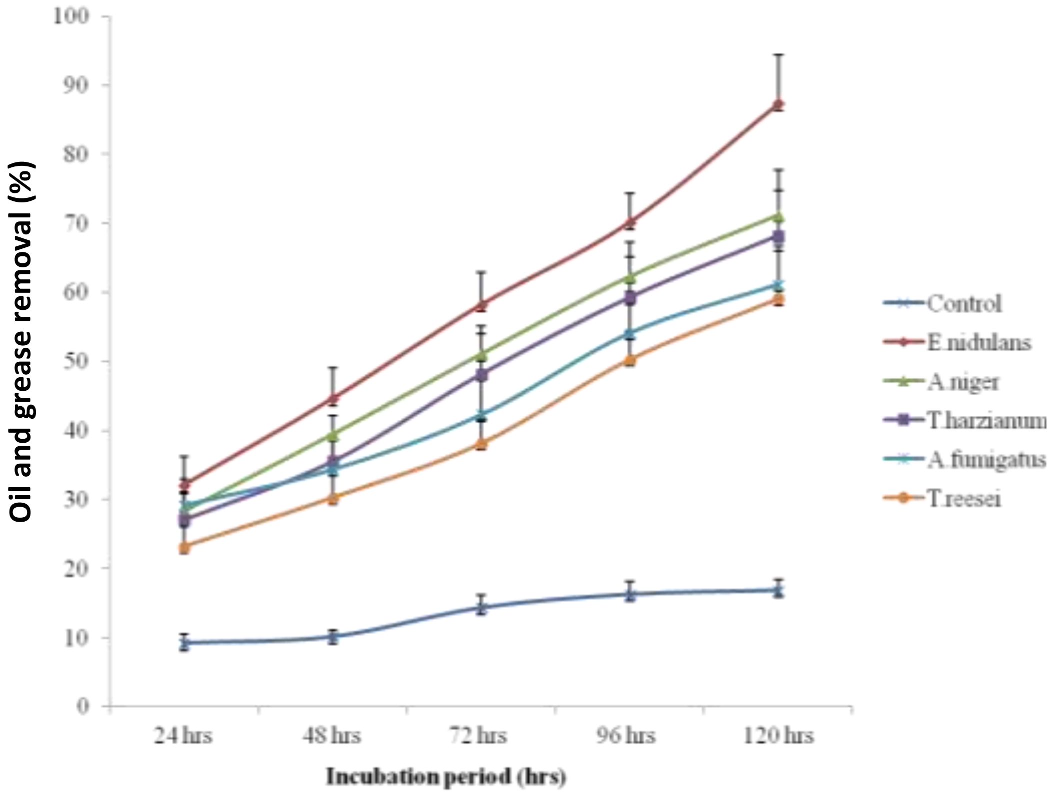

Figure 1. Oil and grease removal (%) in POME**.**

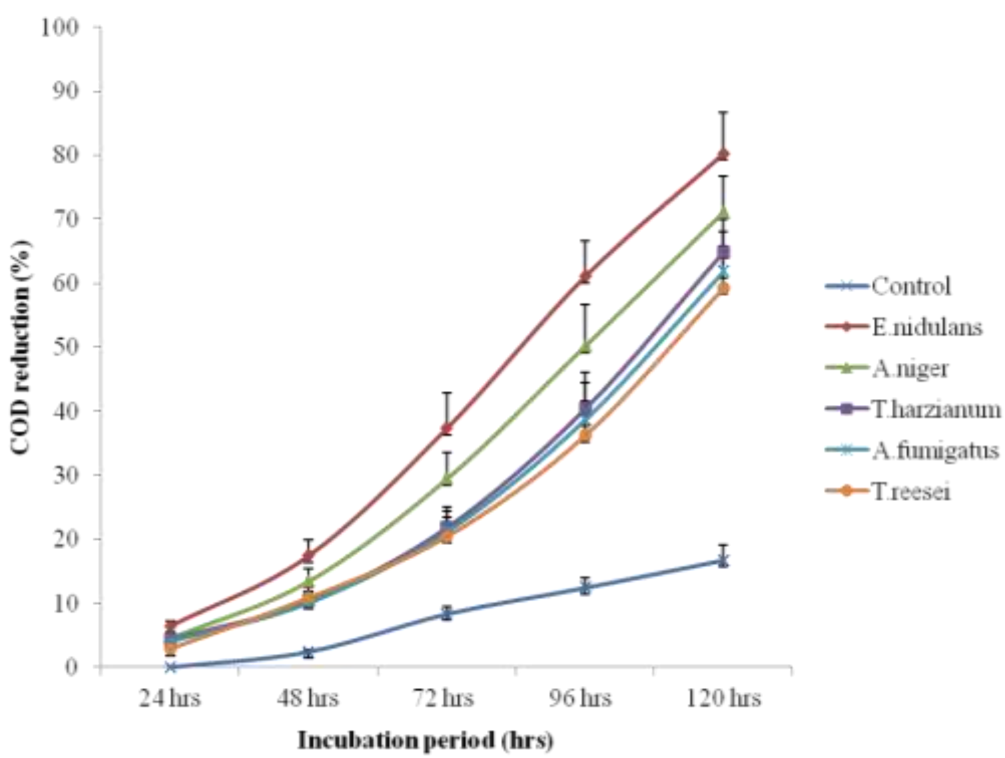

Figure 2. COD reduction (%) in POME sample (pure cultures).

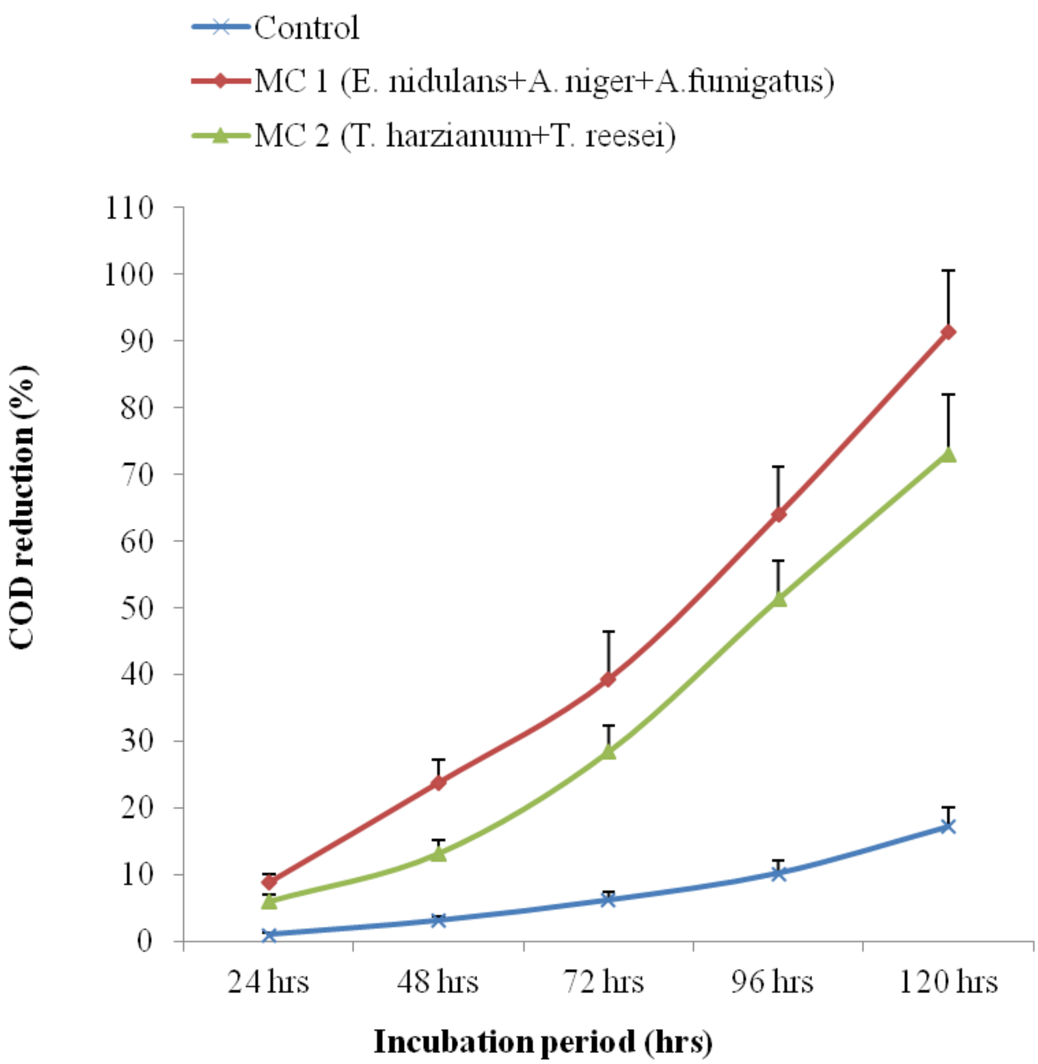

Figure 3. COD reduction (%) in POME sample (mixed cultures).

was also reported by various workers (El-masry et al., 2004; El-Bestawy et al., 2005). The microorganisms in the mixed cultures utilizes the organic substances of POME as nutrients and hence results in organic load reduction (Jameel and Olanrewaju, 2011; Jameel et al., 2011).

Reduction efficiency (RE %) of BOD using selected fungal isolates (individual pure cultures)

Figure 4 represents the reduction efficiency of BOD for selected fungal isolates. From the results, it is evident that reduction efficiency of *E. nidulans* was highest with 88.23% followed by *A. niger*, *T. reesei, A. fumigatus* and *T. harzianum* with 77.64, 68.17, 63.26 and 52.63%, respectively. Similar findings were reported by El-Masry et al. (2004) and El-Bestawy et al. (2005).

Reduction efficiency (RE %) of BOD using mixed cultures (combination of fungal isolates)

The reduction efficiency of BOD by mixed cultures is shown in Figure 5. From the results, it is clearly evident that there is enhanced organic load reduction with mixed cultures as compared to pure cultures and it is as follows: MC1 (*E. nidulans* + *A. niger* + *A. fumigatus*) (94.34%) > MC2 (*T. harzianum* + *T. reesei*) (78.21%) > control (16.54%).

The findings are in agreement with Qingwei et al. (1998) and Bala et al. (2015). The results are mainly attributed to the synergistic effect of different fungal members in the mixed culture (Chigusa et al., 1996; Benka-coker and Ekundayo, 1997; Odegaar et al., 1998). The present treatment process also has advantage in that no additional physical or chemical treatment was required. Similar findings were reported by El-Bestawy et

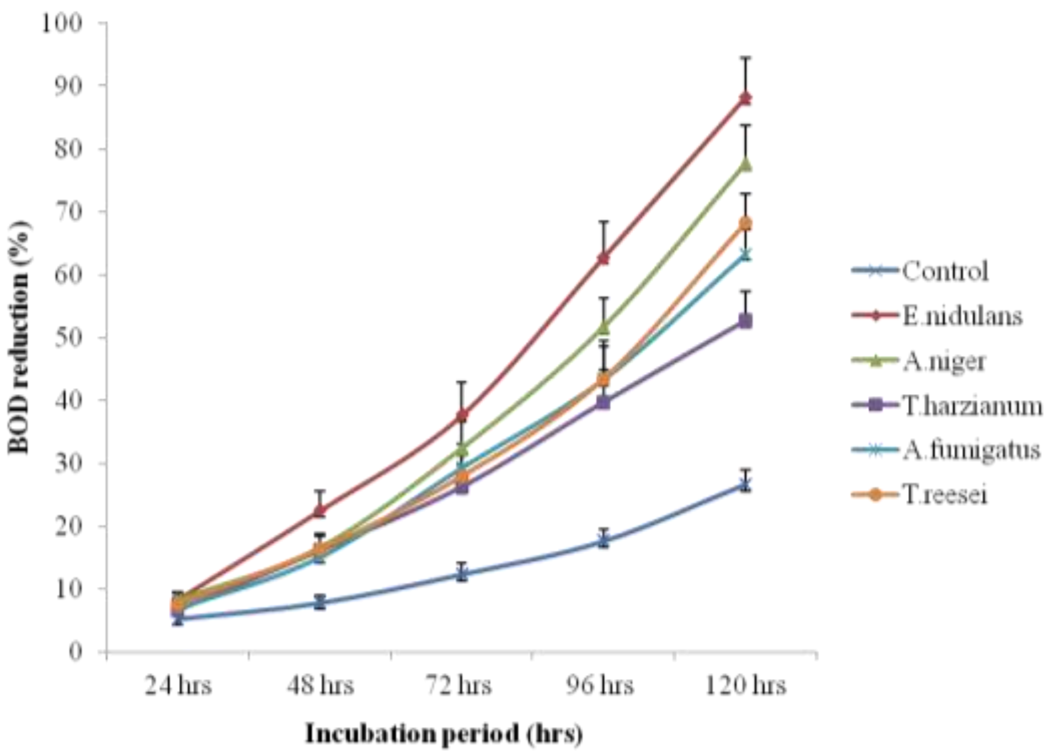

Figure 4. BOD reduction (%) in POME sample.

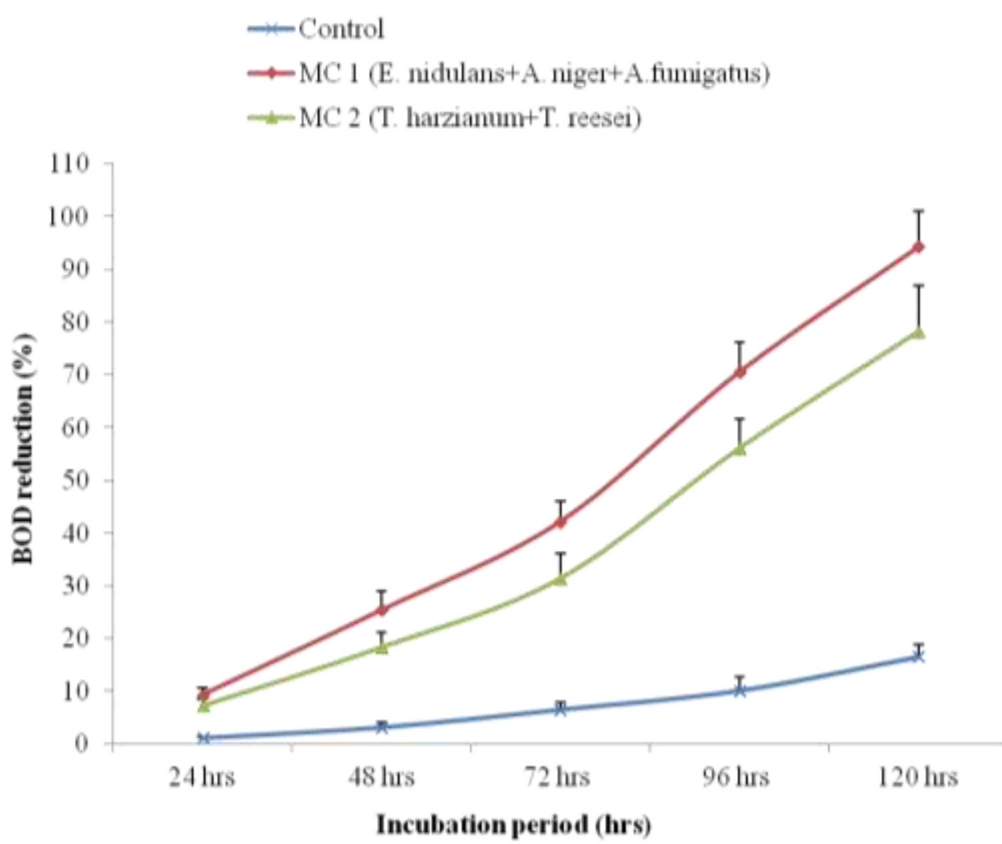

Figure 5. BOD reduction (%) in POME sample (mixed cultures).

al. (2005) in his work on treatment of contaminated industrial effluents by mixed cultures of bacteria.

Conclusion

The application of isolated fungi in the biodegradation of POME was investigated in the present study. The 5 fungal members isolated from POME dump sites were found to be effective in reducing COD, BOD, oil and grease of POME. This study is certainly useful in understanding the role of fungal members in either pure cultures or in the form of mixed cultures and in biological treatment of effluents from oil processing industries. The mixed culture (MC1) (*E. nidulans* + *A. niger* + *A. fumigatus*) is found to be most effective in the treatment of POME with a reduction efficiency of COD (91.43%) and BOD (94.34%). As high BOD and COD concentrations of POME make it unsuitable for discharge into the environment, recycling of POME by biological treatment will certainly gain importance based on its safe discharge and reuse.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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