Original article

Biodegradation of Crude Oil in the Soil by Bacterial Consortium in Heglig Oil Field, Sudan

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ARTICLE INFO

Article history:
Received 2016 December 7th
Reviewed 2016 June 6th
Accepted 2017 April 1st

Keywords:
constorium
biodegradation
Heglig isolates
pollutants

Abstract

This work focuses on isolating the indigenous microorganisms in petroleum contaminated sites and investigating their efficiency to degrade the crude oil of Heglig oil field in Sudan. The selected isolates HP, HWH and HAC (which were identified as *Bacillus safensis*, *Bacillus pumilus* and *Actinomycetes*) were studied for 90 days to assess their ability to degrade crude oil (1%, 3%, 5% and 10%) individually and in consortia. The potential isolates HP, HWH and HAC were identified as *Bacillus saefnsis*, *B. pumplis* and *Actinomyses*, respectively. The ability of consortium isolates to degrade crude oil was increased. The biodegradation of consortium isolates HP+HWH comparing with HP as individual of 3 % crude oil from 56.1% to 90.02%, and of 5% crude oil from 38.7% to 64.5% respectively. The consortium of HP+HWH was the best among the other consortia in Heglig isolates. This consortium was followed by the consortium of three isolates HAC+HP+HWH. All consortia which included isolate HAC (HAC+HP, HAC+HWH and HAC+HP+HWH) showed low efficiency compared to individual isolates.

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Introduction

The contamination of soils with mineral oil hydrocarbons is a widespread environmental problem. Strictly speaking, mineral oil hydrocarbons are not xenobiotic, but their large-scale use and various applications lead, in many cases, to environmental contamination (Gallego *et al.,* 2001).

Bioremediation methods are currently receiving increasing publicity as promising environmental friendly treatment technologies for the remediation of hydrocarbons. Biodegradation of complex hydrocarbons usually requires the cooperation of more than a single species. This is particularly true in pollutants that are made up of many deferent compounds such as crude oil or petroleum where complete mineralization to CO_2 and H_2O is desired (Olajire and Essien, 2014). Individual microorganisms can metabolize only a limited range of hydrocarbon substrates, so assemblages of mixed populations with overall broad enzymatic capacities are required to bring the rate and extent of petroleum biodegradation further (Kelechi *et al.,* 2014). This strongly suggests that each strain or genera have their roles in the hydrocarbon transformation processes. The rates of uptake and mineralization of many organic compounds by a microbial population depend on the concentration of the compound (Olivera *et al*., 1997). Inhibition of biodegradation by nutrients or oxygen limitation or through toxic effects exerted by volatile hydrocarbons may occur due to high concentrations of undispersed hydrocarbons (Rahman *et al*., 2002). In this paper, the role of microbes as a consortium in the bioremediation of oil-polluted soils and the extent of biodegradation they can exert was evaluated.

Materials and Methods

Collection of soil samples

Twenty crude oil contaminated subsurface soil samples, were collected from lagoon I of the oil production site of Heglig field in Southern Kordofan, Sudan and were used for isolation of hydrocarbon- utilizing microorganisms. The dimensions of the lagoon were 880 m width; 2240 m length and 2.5 m depth). The samples were collected in whirl-pack bags and labeled.

Isolation of microorganisms from contaminated soils

Ten grams of crude oil-contaminated soil were dissolved in 90 ml of sterilized distilled water in a 250 ml conical flask. 0.2 ml inoculated from dilutions 10-3 and 10-4 were transferred to the agar surface of Modified Nutrient Agar (MNA). The inoculums were spread over the agar surface using sterilized glass spreaders and the plates were incubated for two days at 28 ºC.

Growth of the recovered isolates on crude oil as the only carbon source.

The microorganisms which appeared in the medium used were purified and streaked on Mineral Salt Agar (MSA) (Abdualdaim *et al.* 2008). After sterilizing, 1% crude oil was added as sole source of carbon and energy for screening. The plates were incubated at 35°C for one week. The isolates that showed vigorous growth in a few days were inoculated in Mineral Salts Medium (MSM) (Kennedy et al., 1975) with 1% crude oil as the carbon source for testing their potentiality. The inoculated medium was kept on the shaker at 200 rpm at 30°C for a period of three weeks. The growth of the isolates was recorded and categorized spectrophotometrically.

Preparation of consortium culture

The three bacterial cultures which have an excellent growth in MSM were mixed, four groups of mixed cultures. The cultures were inoculated in MSM medium and after incubation 1 % of crude oil (v\v) was added as a source of carbon. The flasks were incubated on a reciprocal shaker at room temperature for 15 days.

Determination of the ability of isolates to degrade different concentrations of crude oil in soil.

Different concentrations of crude oil were applied each to 100g of uncontaminated soil samples obtained from the field site. The humidity was adjusted to approximately 60% of field capacity. Ten ml of consortium isolates broth and the different concentrations of crude oil (1 %, 3 %, 5 %, and 10%) were applied on top of watered soil and well mixed. Each bottle (500 ml) was sealed with a plastic lid and fixed with a rubber. Some pores were made on the top of the cover to facilitate aeration. The bottles were incubated at room temperature in the dark for 12 weeks. The residue of crude oil in the soil samples was determined at two weeks interval by the weight loss method (Bossert and Bartha, 1984).

Results and Discussion

Eighty percent of the isolates obtained from Heglig site showed ability to grow in Mineral Salt Agar (MSA) containing 1% crude oil. This flora reflects the normal native heterotrophic bacteria present in soil and seems to be crude oil utilizers.

The potential crude oil degrading isolates were selected using a spectrophotometer to measures the turbidity. From the results, it was noted that there were three isolates in Heglig soil which had excellent oil-degrading potential. These were HP, HWH and HAC which recorded 2, 1.2 and1.4 OD (Optical density), respectively.

Fig.1. showed that the biodegradation of consortium isolates HP+HWH comparing with HP of 1 % crude oil increased from 70.9% to 98.5%, and of 3 % crude oil from 56.1% to 90.02%. The efficiency of the isolates was decreased when the concentration of crude of was increased and this was shown when the concentration was increased to 5 and 10 %. The biodegradation % of 5% of crude oil was increased from 38.7% to 64.5% and of 10% crude oil from 28.9% to 30.6% after 12 weeks of incubation. Also the result revealed that when we comparing the isolate HWH

with HP+HWH we found that the efficiency of biodegradation was increased from 67.3% to 98.5 % for 1 %, and from 55.6 % to 79.5 % for 3 %.

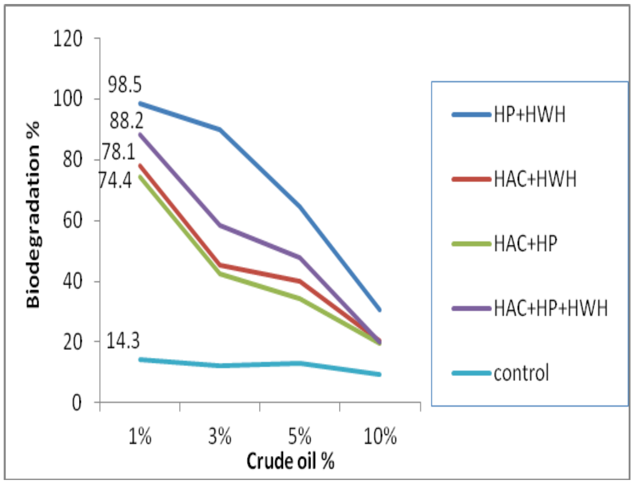

Fig.1. Comparison of biodegradation by consortium isolates and control for Heglig crude oil at different concentrations

These result was agreement with Muthuswamy *et al.,* (2007) which reported that the mixed bacterial consortium showed more growth and degradation than did individual strains. At 1% crude oil concentration, the mixed bacterial consortium degraded 77% of the crude oil. This was followed by *Pseudomonas sp*. BPS1-8 which degraded 69%; then by *Bacillus sp*. IOS1-7 which degraded 64%; then *Pseudomonas sp*. HPS2-5 which degraded 45%, and *Corynebacterium sp*. BPS2-6 which degraded 41% while the percentages of degradation by the mixed bacterial consortium decreased from 77% to 45% as the concentration of crude oil was increased from 1 to 12%. Sugiura *et al.,* (1997) noted that the biodegradation of oil products by microbial consortium was more effective compared to isolated single cultures due to the complex chemical structures of oil products. These results are in conformity with Adebusoye *et al.,* (2007) who demonstrated that mixed culture of microbial community is required to complete biodegradation of oil pollutants because the hydrocarbon mixtures differ markedly in volatility, solubility, and susceptibility to degradation and the necessary enzymes needed cannot be found in a single organism. This coincides with Al-Saleh *et al.,* (2009) who reported that individual microorganisms metabolize only a limited range of hydrocarbon substrates and crude oil is made of a mixture of compounds, so the biodegradation of it requires mixture of different bacterial groups or consortia functioning to degrade a wider range of hydrocarbons. Read and Shima (2014) reported that mixed bacterial culture could carry out a maximum degradation (88.5%) for the studied crude oil at 22°C after 28 days of incubation followed by *P. aeruginosa, B. subtilis, and A. lwoffi.*

According to these results it can be concluded that bacterial consortia were more efficient degraders of crude oil than individual isolates (Fig.2.), except for isolate HAC which was highly efficient as an individual. All consortia which included isolate HAC (HAC+HP, HAC+HWH and HAC+HP+HWH) showed low efficiency compared to individual isolates. Also the biodegradation percentage by the consortium of the three isolates HP+HAC+HWH respectively were low in comparison to individual isolates. These results are in agreement with Maqueda, *et al.,* (2008) which reported that antimicrobial substances produced by Bacillus spp. have broad antimicrobial activities against other strains. Stein, (2005) and Raaijmakers *et al*., (2010) reported that Bacillus spp. produce many types of antibiotics such as lipopeptides and bacteriocin-like substances, In particular, antibiotics produced by *Bacillus subtilis*, which have been granted "Generally Recognized As Safe" (GRAS) status, have low toxicity, high biodegradability, and are environmentally friendly (Raaijmakers *et al.,* 2010).

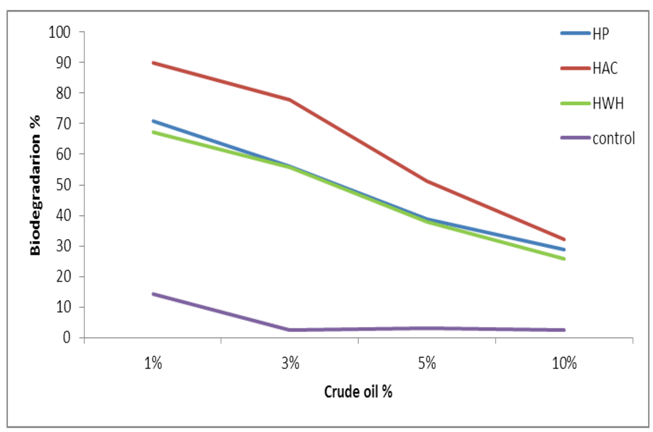

Fig. 2. Biodegradation of Heglig crude oil by individual isolates at different concentration

Conclusions

1. The bacterial consortia were more efficient degraders of crude oil than individual isolates, except for isolate (HAC)

which was highly efficient as an individual because the hydrocarbon mixtures differ markedly in volatility, solubility, and susceptibility to degradation and the necessary enzymes needed for biodegradation cannot be found in a single organism.

2. Isolate HAC produced antimicrobial substances, which have inhibitor effectiveness against other strains.

Recommendation

The selected bacterial *Bacillus safensis*, *Bacillus pumilus* and *Actinomycetes* isolates could be effective in clearing oil spills and the mixed bacterial culture can efficiently degrade the crude oil components.

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