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REVIEW PAPER ON HARNESSING NATURE'S POWER: TREATMENT OF OILY WASTEWATER BY USING MARINE FUNGI

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ABSTRACT
Oily wastewater poses a significant environmental challenge for maritime industries, necessitating innovative and sustainable treatment methods. This article explores the promising approach of utilizing marine fungi for the remediation of oily bilge wastewater. By examining the potential of these fungal organisms, we aim to shed light on a sustainable and eco-friendly solution to address the pollution concerns associated with maritime activities. Oily bilge waste waters released from ships cause an emerging environmental problem in the seas because they are toxic to many sea organisms. Bilge waters need new treatments that are efficient, economic, and local. The crude oil degradation efficacy of five marine fungal isolates (Acremonium sp., Ceratocystis sp., Cladosporium sp., Emericellopsis sp., Fusarium magnifereae) was studied in controlled conditions. The plate culture technique was used to measure the degradation of crude oil in five concentrations (100, 200, 300, 400, 500 mg/L). In addition, the degradation of oily bilge water (320 mg/L) collected from ships was measured using broth culture technique. The fungi F. magnifereae had the highest crude oil degradation efficacy; the crude oil concentration of bilge water was reduced to one-tenth: from the original 320 mg/L to 32 mg/L. Turbidity (350 NTU) and biological oxygen demand (380 mg/L) were reduced to one-tenth of the original. Acremonium sp. and Ceratocystis sp. degraded crude oil slightly less (to 1/7) and two of the isolates poorly (to 1/5) (Emericellopsis sp. and Cladosporium sp.). The phytotoxicity of bilge water was studied with a seed germination test. The seeds of mung bean did not germinate in bilge water (0% germinated) while 90% of the seeds germinated in F. magnifereae-treated bilge water. The germination in tap water was 81% and in distilled water 74%. Seed germination and crude oil concentration correlated strongly (r = 0.90) giving a significant regression equation. F. magnifereae could degrade crude oil in bilge water and remove phytotoxic compounds
Keywords: Harnessing Nature's, sustainable, eco-friendly, Marine Fungi, Waste water treatment.

Introduction:
Oily bilge wastewater, a byproduct of ship engine operations, contains a complex mixture of hydrocarbons and contaminants, presenting a serious threat to marine ecosystems. Traditional methods of treating bilge water often fall short in effectively removing these pollutants. The introduction of marine fungi as a bio-remediation agent offers a promising alternative that aligns with the principles of green and sustainable technology. The organization of the International Convention for the Prevention of Pollution from Ships (MARPOL) has set regulations for the discharge of oil residues into the marine environment. The oil concentration of the effluent without dilution must not exceed 15 mg/L. The limit set by MARPOL has not succeeded to limit pollution enough. In many cases, the treatment of oily waste is not efficient enough compared to the MARPOL requirements and more efficient onboard treatment techniques must be developed. Due to the enormously increasing maritime traffic also smaller discharges such as oily bilge waters from vessels must be cleaned throughout the world. Numerous techniques have been developed to treat industrial oily wastewater as reviewed recently (Adetunji and Olaniran, 2021). Chemical and physical techniques such as chemical- and

electrocoagulation, carbon adsorption, membrane filtration, and gravity separation are commonly used. Biological treatments use microorganisms that can degrade crude oil using it for their growth.

The Role of Marine Fungi:

Marine fungi, with their unique metabolic capabilities, have demonstrated a remarkable ability to break down hydrocarbons and other pollutants found in oily bilge wastewater. These fungi, adapted to thrive in marine environments, exhibit a natural affinity for hydrocarbon-rich substrates, making them ideal candidates for remediation efforts.

2. Materials and methods

2.1. Fungi : The hydrocarbon degradation study was conducted using previously isolated and identified marine fungi (Ameen et al., 2022). The marine fungi Fusarium magnifereae (OM487085 NCBI accession number), Acremonium sp. (OM510382), Emericellopsis sp. (OM510377), Cladosporium sp. (OM510301), Ceratocystis sp. (OM510413) had been collected from Red sea coast, Saudi Arabia and maintained in the laboratory of the Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia. The fungi were first aseptically transferred on potato dextrose agar (PDA) (HiMedia) and incubated at 37 C for 3–4 days. Single colonies were then subcultured on PDA plates and preserved.

2.2. Oil degradation experiment: Bilge water (10 L) was collected from ships located in Aljubail port, east of Saudi Arabia, and transferred aseptically to the laboratory. Crude oil was collected from Saudi Aramco, Ltd., Riyadh refinery. All experiments were carried out as three replicates. In the experiments, the crude oil concentration was measured using a crude oil analyzer (Bruker ASTM D7575), turbidity using an electronic turbidity meter (Hach turbidimeter) in NTU (nephelometric turbidity units), BOD (biological oxygen demand) using a BOD meter (BD600) and COD (chemical oxygen method) using a COD meter (Lovibond). Minimal salt (MS) (HiMedia) medium was used for both agar plate and broth techniques. The degradation of five different concentrations of crude oil (100, 200, 300, 400, and 500 mg/L) was tested using agar plate technique. MS agar media were prepared with five different concentrations of crude oil. The five fungi were inoculated on their plates and incubated at 37 C for 4 days. After the incubation, the zone of clearance was measured with a ruler. For the bilge water treatment experiment, the fungi were first inoculated into 500 mL of MS broth and incubated in a rotary shaker at 37 C for 4 days. The fungal mycelia were separated using a sterile cheesecloth and the spores (300 mL) were collected. Then the spore suspension was inoculated into 5 L of experimental bilge water, mixed thoroughly, incubated at 37 C in an orbital shaker for 4 days, and measured for the crude oil concentration and other water properties mentioned above.

3. Results

3.1. Oil degradation: The test with different concentrations of crude oil showed that the higher the concentration the lower the degradation of oil measured as the zone of clearance (ZOI) (Table 1). The reduction in the degradation efficiency was observed for all fungal species. The highest crude oil degradation efficiency was obtained by Fusarium magnifereae: the agar plate assay gave ZOI of 55 mm in 100 mg/L crude oil and 25 mm in 500 mg/L crude oil (Table 1). The poorest degrader, Emericellopsis sp, reached half of the efficiency, the respective clearance zone values being 23 mm and 6 mm. Bilge water contained 320 mg/L crude oil. Turbidity was 350 NTU, BOD 210 mg/L, COD 1088 mg/L. Fusarium magnifereae reduced the values to 34 mg/L, 33 NTU, 40 mg/L, and 188 mg/L, respectively (Table 2). Acremonium sp. and Ceratocystis sp. reduced the crude oil concentration to 43 mg/L and 47 mg/L, respectively. The rest of the fungi reduced the values less. **3.2. Seed germination**: The seeds did not germinate (0%) in raw bilge wastewater of which crude oil concentration was 320 mg/L (Fig. 1). Germination was 81% and 74% in tap water and distilled water, respectively. The highest germination was observed after the F. magnifereae treatment, when 90% of the seeds germinated (Fig. 2). The germination after the Acremonium sp. treatment was 82%, and after Ceratocystis sp. 78%. Seed germination and crude oil concentration correlated strongly (r = 0.90, n =

15). The significant regression model explained 81% of the variation, and thus, showed high dependence between the variables (Table 3). The equation was as follows. Seed germination = 2.5 Crude oil concentration + 183. The equation indicated a significant, strong, and relatively sharp decrease in germination when the crude oil concentration increased.

4. Discussion:

The treatment of oily bilge wastewater must be developed to be more efficient due to the increasing oil pollution caused by the increasing motorized traffic in all seas. Around the Arabian Peninsula, many busy ports with high traffic pollute coastal waters. The seas around the Arabian Peninsula suffer from oil accidents and also from oil pollution originating from ordinary operating ships (Suneel et al., 2019). Local solutions to treat bilge waters onboard the vessels and near the ports with indigenous microbes are needed. Economic and local solutions to treat bilge wastewater are offered by microorganisms such as fungi. Previously, sixty different fungal isolates from 13 genera were isolated from crude oil. contaminated fields in Iran (Dawoodi et al., 2016). This shows that the potential to find local fungi to be utilized in bilge wastewater treatment is high. Efficient oil degraders were found also in India; the marine fungi Fusarium sp. and Acremonium sp. (Barnes et al., 2018). In our experiment, the same genera were the most efficient degraders. F. magnifereae reduced the crude oil concentration of bilge water to ca. one-tenth of the original concentration (320 mg/L) in bilge water. Other bilge wastewater properties (turbidity, biological and chemical oxygen demand) were also reduced to ca. one-tenth of the original values. These values confirm that the oil has been degraded to one-tenth of the original concentration. In our experiment, the crude oil concentration was originally 320 mg/L. It appeared that the concentration was suitable to be degraded efficiently. Surprisingly, the germination of seeds was slightly lower in distilled and tap water than in cleaned bilge water. This may be due to the lack of nutrients and other elements in distilled or tap water. Bilge water was shown to contain nitrogen and other elements (Olorunfemi et al., 2012) that possibly improved the germination. The germination below 90% was observed also previously in distilled water (Olorunfemi et al., 2012, Olorunfemi and Idada, 2013). Regarding cleaned bilge wastewater, we found no studies on phytotoxicity. Phytotoxicity seemed to be reduced only slightly when experimental petroleum refinery wastewater was treated using an electrochemical process; the germination of wheat seeds increased from 17% to 23% (Gousmi et al., 2016). Our microbial treatment increased the germination from 0% to 90%, and thus, was shown to reduce phytotoxicity remarkably. The relation between the crude oil concentration and seed germination was negative and strong. The statistically significant regression analysis indicated the remarkable difference between ca 50 – 65 mg/L concentration and 30–40 mg/L concentration. While the former concentration inhibited the germination remarkably, the latter concentration allowed almost all seeds to germinate. It shows that the oil concentration needs to be reduced to 30 – 40 mg/L to be satisfactory. Our results show that the treatment with F. magnifereae was successful and efficient in reducing phytotoxic compounds. Our F. magnifereae-treatment seemed to remove the phytotoxic compounds present in bilge wastewater. This result is outstanding in the arid Arabian Peninsula where the possibility to utilize treated wastewater will be of great value

5. Conclusion:

Many methods are used to treat and detoxify bilge wastewater. We applied five marine fungi and found that F. magnifereae had a high crude oil reduction ability. F. magnifereae was the most efficient fungus not only to decrease the crude oil concentration but to detoxify bilge water. Bilge water was detoxified. Relation of seed germination and crude oil concentration after the treatments with five different fungi. The line refers to the linear regression equation. ANOVA-table of the regression analysis between seed germination (%) and crude oil concentration (mg/L) after the treatments with different fungi. ANOVA df SS F P Regression 1 8720 57.3 4.04E-06 Residual 13 1975 Total 14 10,696 F. Ameen and A.A. the seed germination test. The phytotoxicity of untreated bilge wastewater was high and shown to be reduced remarkably by the treatment with the marine fungi F. magnifereae. The result gives promising implications that the cleaned water could be used for irrigation in farming.

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