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Original article

ENHANCED DEGRADATION OF CRUDE OIL WITH *Alcaligenes faecalis* ADY25 AND IRON OXIDE NANOPARTICLE

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ABSTRACT

Crude oil is composed of hydrocarbons, which forms a large group of chemicals that have caused a major concern to various forms of life. The advent of nanotechnology in conjunction with microbial cells produced nano-scale products with more efficient reactivity and larger surface area than its bulk phase facilitating the degradation of petroleum products. This study was conducted to determine the synergistic effect of *Alcaligenes faecalis* ADY25 and iron oxide nanoparticle on biodegradation of crude oil. The iron oxide nanoparticle was synthesized using corn silk extract. Pure isolate of *A. faecalis* ADY25 was collected from microbiology laboratory, Federal University of Technology Minna, Nigeria. The culture was grown on mineral salt medium containing crude oil as carbon source. This was supplemented with different amounts (0, 50, 100, 150, 200 mg) of iron oxide nanoparticles. The rate of biodegradation was determined by spectrophotometry and bacterial count was determined during biodegradation. The highest absorbance (1.418) and bacterial count (9.4 x 10^6 cfu/g) were obtained on day 9 and 12 by *A. faecalis* ADY25 supplemented with 200 mg of iron oxide nanoparticle. The results of this study showed that different amounts of iron oxide nanoparticle influenced the biodegradation of crude oil by *A. faecalis* ADY25.

Key words: *Alcaligenes faecalis* ADY25, crude oil, iron oxide, nanoparticles, biodegradation

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INTRODUCTION

Crude oil also known as petroleum is composed of hydrocarbons, which forms a large group of chemicals that have caused a major concern to humans due to their widespread distribution into the environment causing harmful effects to various forms of life [1]. The presence of petroleum and its derivatives in the environment impair the ecosystem and human as well through the transfer of toxic organic materials including polycyclic aromatic hydrocarbons (PAHs) into the food chain [2]. Presence of polycyclic aromatic hydrocarbons (PAHs) in the environment has raised great concern to the health of humans since most of them are recalcitrant in nature. Petroleum and its derivatives being an important energy resources used by industries and in our daily life, at the same time, become a major pollutant of the environment [3]. Because of its complicated nature, petroleum can produce multiple types of toxic effects; it can cause acute lethal toxicity, sub-lethal chronic toxicity, or both depending on the dosage, and metabolism of the organism. Many petroleum components have the ability to bioaccumulate within susceptible aquatic organisms and can be transferred to other food chain levels by trophic transfer [4, 5].

As such, the importance of cleaning a contaminated petroleum environment has led to the development of techniques involving physical and chemical methods such as volatilization, photooxidation, chemical oxidation, and bioaccumulation that are rarely successful in rapid removal and clean-up of PAHs. However, physical and chemical methods to remove and clean PAHs are expensive, rarely successful, and produce toxic by-products [6, 7].

Nanotechnology is an emerging field to produce nano-scale products with more efficient reactivity and larger surface area than its bulk phase. These unique attributes of nanoparticles offer immense potential for their application to clean up hydrocarbons, pesticides and metals contaminated sites. The use of these nanoparticles in the physical and chemical methods of synthesizing iron oxide nanoparticles such as sol-gel, anodization, hydrothermal and microwave assisted process, have shortcomings such as long synthesis procedure, high energy requirement and production of toxic unreactive materials. Also, most of the available commercial reducing agents are expensive and toxic [8, 9, 10].

Microbial bioremediation simply involves the use of microorganisms such as bacteria, fungi and algae in the remediation of pollutants from the environment. Among the aforementioned microorganisms, bacteria have long been considered as one of the predominant hydrocarbons degrading agents found in the environment, which are free living and ubiquitous [6, 11]. Bioremediation has been drawing an increasing attention due to its economical, eco-friendly and self-propelling attributes as compared to the conventional physico-chemical methods of remediation of contaminated sites [6].

The success of bioremediation technologies applied to hydrocarbon-polluted environments highly depends on the biodegrading capabilities of native microbial populations or exogenous microorganisms used as inoculants [3]. The presence of microorganisms with the appropriate metabolic capabilities is the most important requirement for oil spill bioremediation. Unlike higher organisms,

some microorganisms possess the catabolic capacity to use hydrocarbons as carbon and energy source. Bioremediation has been identified as a potential emerging technology for removing different spills, where physical washing and collection could not help. Highly hazardous oily materials can be mineralized to harmless products using suitable microorganisms [12]. The communities that were exposed to hydrocarbons become adapted, exhibiting selective enrichment and genetic changes. The adapted microbial communities can respond to the presence of hydrocarbon pollutants within hours and exhibit higher biodegradation rates than communities with no history of hydrocarbon contamination [13]. Crude oil biodegradation involves a mixture of various bacterial groups or consortia to degrade a wider range of hydrocarbons. The ability to isolate high numbers of certain oil-degrading microorganisms from an area is generally taken as evidence that these microorganisms are the most active oil degraders in such environment and can be used in the bioremediation of contaminated sites [14].

Nanoparticles (NPs), which is gradually gaining wide prominence in bioremediation can be applied directly for removal of organic contaminants through adsorption or chemical modification [9]. It can also serve as a facilitator in microbial remediation of contaminants either by enhancing the microbial growth or by immobilizing the remediating agents or through induced production of remediating microbial enzymes. Besides, nanoparticles induced enhanced production of biosurfactants in microorganisms, also contribute to improved solubility of hydrophobic hydrocarbons and thereby, create a conducive environment for microbial degradation of these compounds in environment [9].

Bacteria used in biodegradation are ubiquitous and readily available compared to commercial reducing agents and can degrade crude oil compare to the use of physical and chemical methods of degradation, and the method of synthesizing iron oxide nanoparticle is simple, cheap and eco-friendly and requires short reaction time compare to physical and chemical methods. In addition, Plant materials used for reduction, capping and stabilization of nanoparticles are in abundance. This technique provides a great advantage over chemical and physical methods since it is cost-effective, relatively reproducible and often result in more stable materials [13]. The aim of this study was to determine the synergistic effect of *Alcaligenes faecalis* ADY25 and iron oxide nanoparticle on biodegradation of crude oil.

MATERIALS AND METHODS

Sample Collection and Pre-treatment

Corn silk was randomly collected from different locations within Bosso, Minna, Nigeria. This was followed by removal of sands from the corn silk, and other debris after which the corn silks were air dried for three days at $28^{o}C \pm 2$.

Collection of Bacterial Isolate

Pure isolate of *A. faecalis* ADY25 was collected from Microbiology laboratory, Federal University of Technology Minna, Nigeria. The bacterium was isolated from soil and was reported to have a huge potential for biosurfactant production [15]. The purity of the isolate was confirmed by streaking on nutrient agar plate and incubation at $37^{o}C$ for 24 h.

Aqueous Extraction of Corn Silk

An aqueous extract of corn silk was prepared by weighing 30 g of the corn silk into a 1000 ml beaker followed by addition of 500 ml of distilled water. The mixture was heated at 80°C for 30 min and a dark brown coloration of the aqueous solution was obtained. The extract was then allowed to cool to room temperature ($28^oC \pm 2$) and filtered using Whatman No1 filter paper and the quantity produced was measured using a measuring cylinder [16].

Synthesis of Iron Oxide Nanoparticle

The aqueous extract from a corn silk was added to 0.1 M of iron sulphate solution at the volume ratio of 2:1 in a sterile conical flask with constant stirring at room temperature ($28^oC \pm 2$) [17]. Then 1.0 M of NaOH solution was added to the above mixture to raise the pH (HANNA instrument) to 9 and the solution was heated at 60-65°C for 4 h and cooled. The resulting solution was centrifuged at 4000 rpm for 5 min and the dark greenish sediment was dried at 50°C using a hot air oven for 24 h and a brownish black residue of iron oxide was collected and kept in a universal bottle. The UV spectrophotometry of the synthesized iron oxide nanoparticles was carried out using UV-1800 series spectrophotometer at 200 to 800 nm

Preparation of Mineral Salt Medium

Mineral salt medium (MSM) was prepared using the following composition: K_2HPO_4 – 1.8g, KH_2PO_4 - 1.2g, NH_4CL – 4.0g, $NaCl_2$– 0.1g, $FeSO_4.7H_2O$– 0.01g. This was dissolved in 1000 mL distilled water in a conical flask [6].

Biodegradation of Crude Oil using *A. faecalis* ADY25 and Iron Oxide Nanoparticles

Nutrient broth was prepared and autoclaved at 121°C for 15 minutes, the broth was allowed to cool and 1 ml culture of *A. faecalis* ADY25 was inoculated inside 25 ml of the medium and incubated at 37°C for 24 hours in the incubator. Different amounts (50, 100, 150, 200 mg) of iron oxide nanoparticles were dissolved in four different conical flasks containing 50 ml of mineral salt medium. One milliliter (1 ml) of crude oil was introduced using a syringe into test tubes containing 9 mL of minimal salt medium with the different amounts of iron oxide nanoparticles after which it was autoclaved at 121°C for 15 minutes. The composition was allowed to cool and 0.1 mL of 24 h old broth culture of the organism was introduced in the test tubes, a control was set up without the organism and the absorbance was taken using a spectrophotometer (Model 752, China), every 3 days for 21 days.

The Count of *A. faecalis* ADY25 during Biodegradation

One milliliter of the 24 h old MSM broth culture of *A. faecalis* ADY25 was serially diluted tenfold, using a sterile distilled water. The 0.1 mL of 10^{-4} dilution factor was placed on a sterile Petri dish and 15 ml of sterile Nutrient agar medium was poured on it. It was gently swirled and allowed to gel after which it was incubated at 37°C for 24 h and the count was taken and expressed as cfu/mL of the sample. This procedure continues every 3 days for 21 days.

RESULTS

The wet iron oxide nanoparticle and oven dried iron oxide nanoparticles is shown in Plates 1 and 2 while the UV spectrum of

synthesized iron oxide nanoparticle is shown in Figure 1.

Plate 1. Wet iron oxide nanoparticle, Plate 2: Oven dried iron oxide nanoparticles

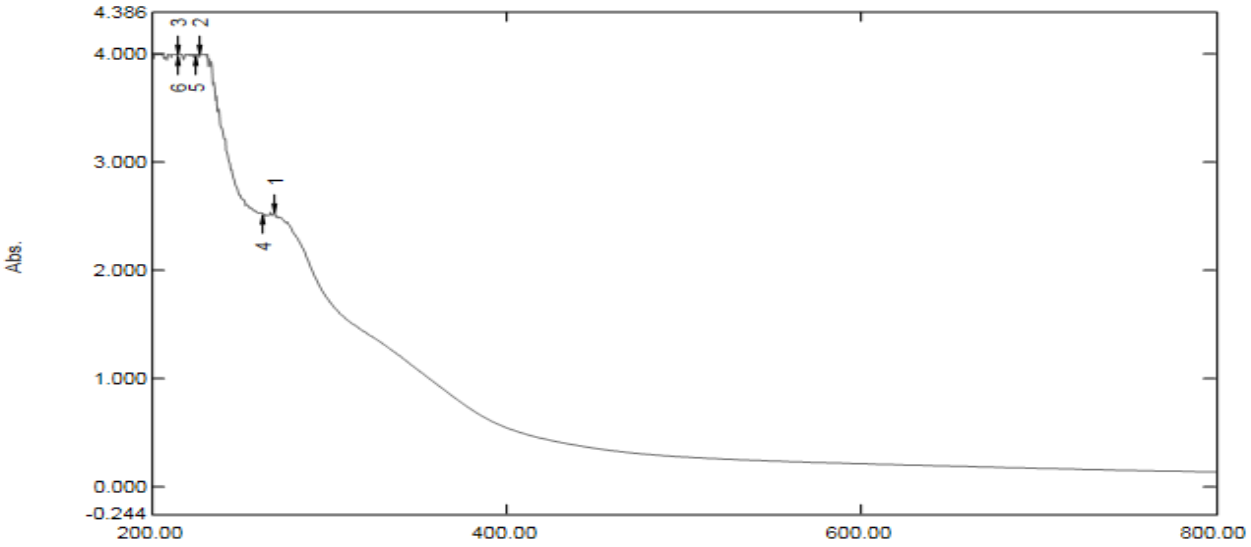

Figure 1: UV Spectrum of synthesized iron oxide

The effects of varying amount of iron-oxide nanoparticles (50 mg, 100 mg, 150 mg, 200 mg) on the growth of *A. feacalis* ADY25 during biodegradation of crude oil is shown in Figure 2. The highest absorbance (1.418) was obtained at the concentration of 200 mg on day 9. There was a gradual increase in the absorbance as the concentration of the iron oxide nanoparticles increases. The control had the least absorbance compared to the samples treated with different amount of iron oxide nanoparticles throughout the treatment period.

The effects of varying amounts of iron-oxide nanoparticle on the count of *A. faecalis* ADY25 during biodegradation of crude oil is shown in Figure 3. The growth of *A. faecalis* ADY25 was best supported by 200 mg iron oxide nanoparticles with the highest cell densities of 9.4×10^{6} cfu/g on day 18 of incubation.

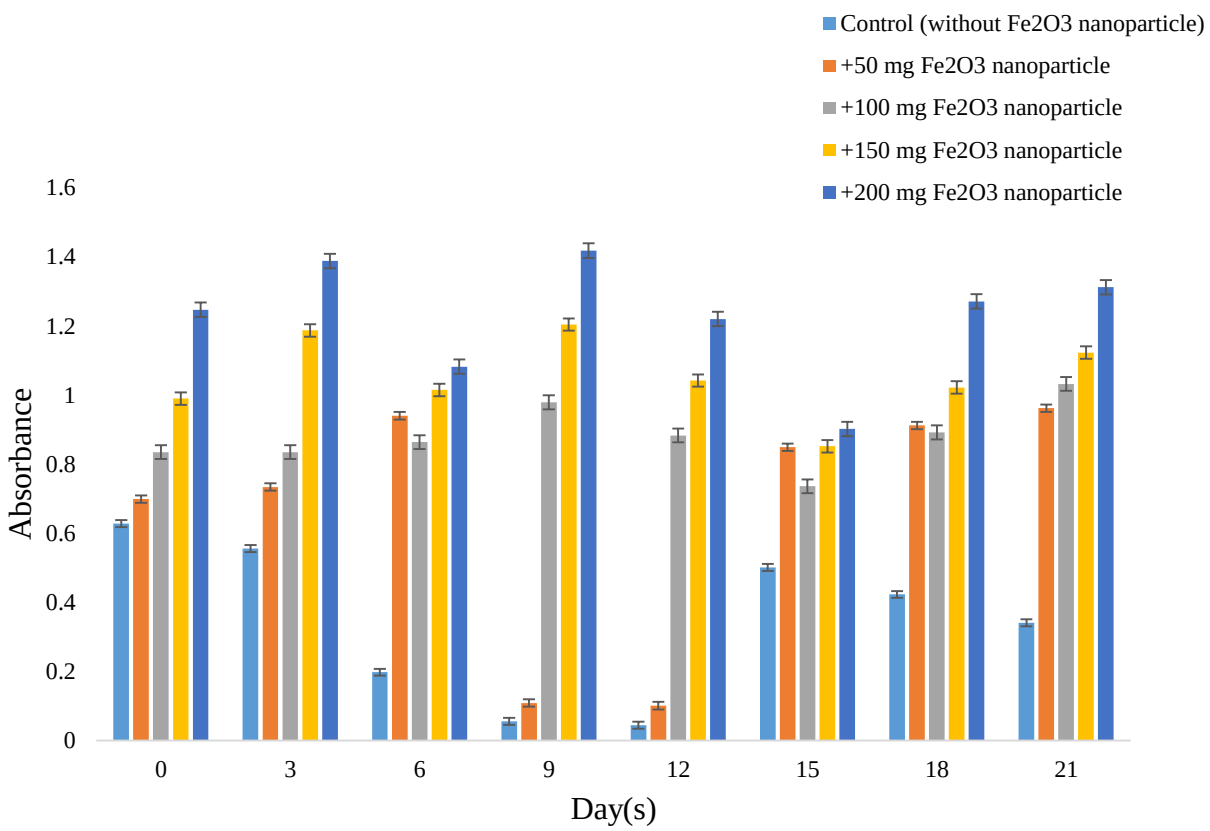

Figure 2: The effects of varying amounts of iron-oxide nanoparticles on the growth of *A. faecalis* ADY25 during crude oil degradation.

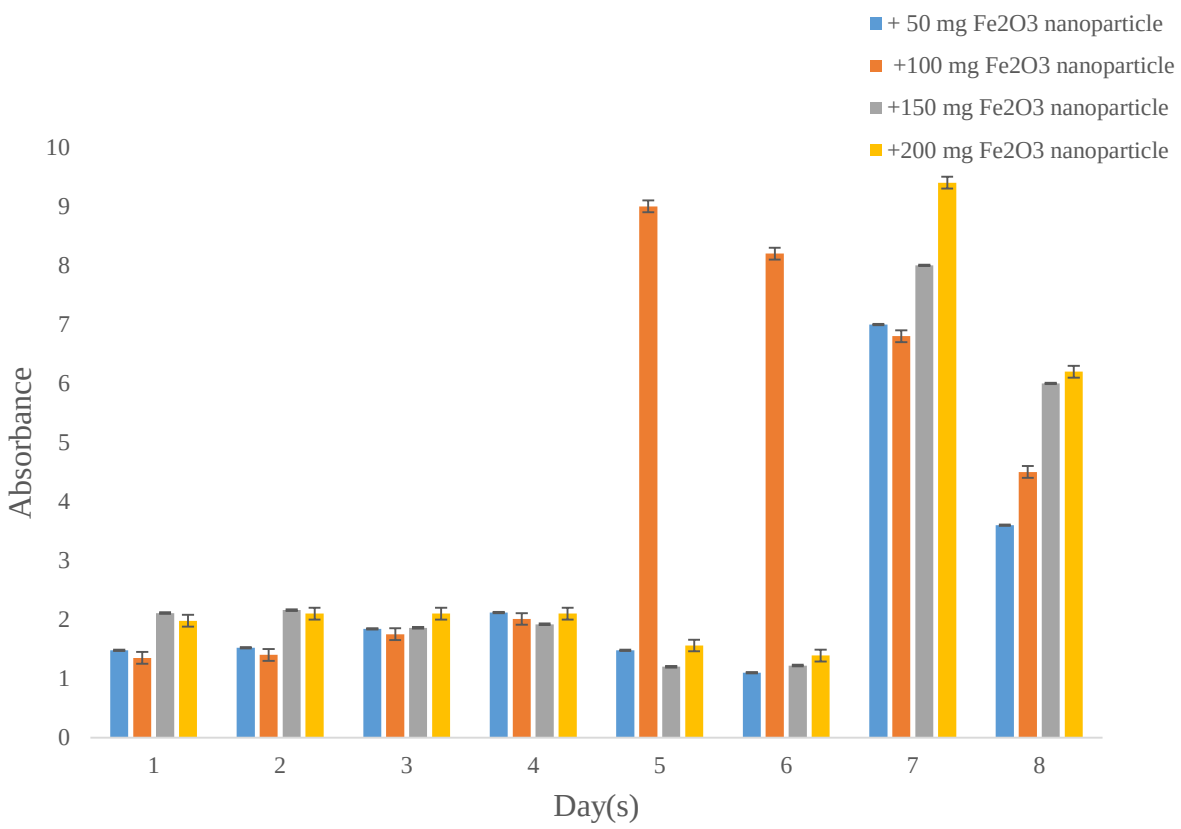

Figure 3: The effects of varying amount of iron-oxide nanoparticle on the count of *A. faecalis* ADY25 during crude oil degradation

DISCUSSION

Alcaligenes faecalis ADY25 used in this study is a Gram-negative rod previously identified as crude oil degrading bacterium with potential to degrade petrochemical products [18]. The isolate was able to degrade crude oil utilizing it as carbon source. The reason for this may be that the organism has enzyme system to degrade and utilize hydrocarbons and it is also capable of attaching to the substrate and produce biosurfactants, which help to break the oil into tiny micelle [15]. When the medium was supplemented with different amounts of iron oxide nanoparticles, an increase in the rate of degradation was observed. This indicates the ability of the iron oxide nanoparticles to enhance the biodegradable ability of the isolate. Report from [19] indicates that nanoparticles are a supportive nutritional component, which influences lag phase and stationary phase and acts by reducing the duration of lag phase and increasing the duration of exponential and stationary phase. From the result, it was observed that the highest absorbance observed when 200 mg of iron oxide nanoparticles was used. This suggests that increase in amounts of iron oxide nanoparticles may increase the rate of biodegradation. This work is similar to that of [20] who obtained the best degradation using a microcosm containing 0.2 g of iron oxide nanoparticles and biosurfactant to degrade paraffins.

The organism was able to adapt and grow in the presence of crude oil multiplying in cell densities, however bacterial growth was best supported by 100 mg and 200 mg of iron oxide nanoparticle. The highest count (9.4×10^6 cfu/mL) was observed on the 18th day by cultures supplemented with 200 mg of iron oxide nanoparticle, this was followed by 100 mg (9.0×10^6cfu/mL) after 12 days' incubation period. The test organism utilized crude oil as carbon and energy source and as the quantity of the crude oil decreases, microbial cell generation increases. This suggest that the test organisms utilize the crude oil for their growth and metabolism thereby removing the crude oil from the environment [21]. The observed growth and degradation potential of the isolate on the concentration of crude oil may be as a result of its previous exposure to pollutant. Furthermore, the growth of *A. faecalis* ADY25 in crude oil induced the secretion of extracellular protein and carbohydrate [22]. Previous studies have also shown hydrocarbon degrading bacteria to produce extracellular emulsifying agents generally consisting polysaccharide associated with protein. Some examples include alasan and emulsan, derived from *Acinetobacter*.

CONCLUSION

This study showed the potential of *A. faecalis* ADY25 and iron oxide nanoparticles to degrade crude oil. Different amount of iron oxide nanoparticles (50, 100, 150 and 200 mg) enhance the process of degradation by the isolate. The use of the isolate and 200 mg of iron oxide nanoparticle to clean up crude oil contaminated environments is encouraged.

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