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Assessment of indigenous yeast from biodiesel effluents contaminated site

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Abstract

Biodiesel is a clean renewable fuel that is sold as a small component in diesel blends as an alternative energy source to diesel. The spills are source of pollution for the ecosystem, necessitating the development of appropriate cleanup techniques. This study described the use of yeasts as a bioremediator of biodiesel effluent. The yeast was isolated from soil samples collected from University of Benin (Ugbowo Campus), Benin City, contaminated with biodiesel effluent, and were identified and characterized considering morphological and biochemical properties by standard methods. The yeasts were inoculated at a final concentration of 0.02 optical density unit at 600 nm in 60 mL flasks containing 10 mL of MMF plus 1% (v/v) of biodiesel as the sole source of carbon and cultured for 5 days at 28°C, with agitation at 180 rpm. All bottles were sealed with rubber stoppers and sealed with plastic film to prevent the evaporative loss of hydrocarbons. After this period, the residual was extracted from the medium for analysis. All tests were performed in triplicate. Therefore, *S. unisporum* and *S. exigus* were able to thrive in medium containing only 1 % (v/v) biodiesel with the greatest capacity to degrade biodiesel wastewater. This suggests that these yeasts can employ in the bioremediation of petroleum hydrocarbon contaminated environment.

1. Introduction

Petroleum products which are extensively prevalent all over the planet and their demanding use is powerfully connected to the anthropogeneous expulsion these hydrocarbons into the environment [1].

Strategies for controlling environmental contamination with petroleum and its derivatives have been the subject of various studies over the past three decades. There is a great diversity of microorganisms able to utilize hydrocarbons as a sole carbon source, including bacteria, yeasts and molds. Bacteria are among the best-described hydrocarbon utilizing microorganisms [2][3]. In addition, some yeast strains display an excellent capacity to degrade oil-related compounds [4][5][6][7].

Bioremediation using selected microorganisms provides a good opportunity because it is environmentally friendly and cost effective. Some microbial strains can degrade hydrocarbons and utilize the resulting carbon compounds as food and energy sources for growth and reproduction. Simultaneously, the hydrocarbons are hydrolyzed from toxic into non-toxic compounds and simple

inorganic compounds, such as CO_2 and H_2O, along with microbial biomass accumulation, through oxidation under aerobic and anaerobic conditions. To degrade organic pollutants, microorganisms must have metabolic processes to optimize the contact between microbial cells and organic pollutants, such as the production of biosurfactants, intracellular pathways to initiate the attack on organic pollutants, usually mediated by the activation and incorporation of oxygen by oxygenases and peroxidases, as well as peripheral degradation pathways to convert organic pollutants step by step into the intermediates of central intermediary metabolism[3].

The objective of this study was to isolate, identify and investigate the potential of yeasts to degrade biodiesel effluents for use in the bioremediation of contaminated areas.

2. Methodology

2.1 Sampling site and isolation of yeasts potentially able to degrade petroleum hydrocarbons

The yeast used in this study were isolated from soil samples collected from University of Benin (Ugbowo Campus) contaminated with biodiesel effluent. The samples were thereafter preserved on ice and transported to the laboratory for the isolation of microorganisms. Aliquots of 100 µL from serial dilutions (10^{-1} to 10^{-5}) were plated on Sabouraud Dextrose Agar (SDA), and the media after they were autoclaved and homogenized in a sterile blender before being distributed onto the plates. The plates were incubated at 28°C for up to 5 days. Individual colonies on the plate's representative of each morphotype were purified by streaking on SDA medium.

2.2 Identification of yeasts

The yeasts were characterized considering morphological and biochemical properties by standard methods [8] and identification followed the keys of Kurtzman and Fell [9].

2.3 Assays of yeast growth in Mineral Salt Medium (MSM)

The yeasts were inoculated at a final concentration of 0.02 optical density unit at 600 nm in 60 mL flasks containing 10 mL of MMF plus 1% (v/v) of biodiesel as the sole source of carbon.

2.4 Biodiesel degradation assays

The yeasts were inoculated at a final concentration of 0.02 optical density unit at 600 nm in 60 mL flasks containing 10 mL of MMF plus 1% (v/v) of biodiesel as the sole source of carbon.

2.5 Assays of yeast growth in Mineral Salt Medium (MSM)

The yeasts were cultured for 5 days in 60 mL flasks containing 10 mL of MMF plus 1% (v/v) biodiesel at 28°C, with agitation at 180 rpm. All bottles were sealed with rubber stoppers and sealed with plastic film to prevent the evaporative loss of hydrocarbons. After this period, the residual was extracted from the medium for analysis. All tests were performed in triplicate

3. Results and Discussion

The availability of fatty acid methyl esters (biodiesel), which are a superior supply of carbon to sustain microbial growth than petroleum hydrocarbons, might explain the microbial preference for biodiesel-containing fuels [10]. Biodiesel effluent contains a high concentration of oils, and its microbiota may metabolize these compounds.

In this study, the growth of yeasts observed from Day one to Day fourteen (Fig. 1) is an indication that the yeasts were able to utilize some components of the biodiesel effluent, which served as the sole

source of carbon accessible in the culture medium. Osarumwense and Igiebor [11] and Winquist *et al*. [12] reported on the capacity of fungi to biodegrade petroleum hydrocarbons. Biodiesel biodegradation investigations, on the other hand, have primarily used bacteria [1][13][14][15], despite the fact that fungi have great bioremediation capability. The significance of bioaugmentation in pollution removal and site remediation has been discussed in scientific circles. Morais and Tauk-Tornisielo [16] utilized a mixed-culture inoculum (bacteria and fungus) to breakdown oil sludge in soil, but found no enhancement in the rate of biodegradation. Similarly, Kauppi *et al*. [17] did not have success with bioaugmentation in bioremediation of diesel-contaminated soil. In contrary to Soares *et al*. [18], who established the capacity of *Candida viswanathii* to considerably enhance (about 50%) the biodegradation of biodiesel effluent in soil. Other authors have used bioaugmentation with promising results [11][19][20].

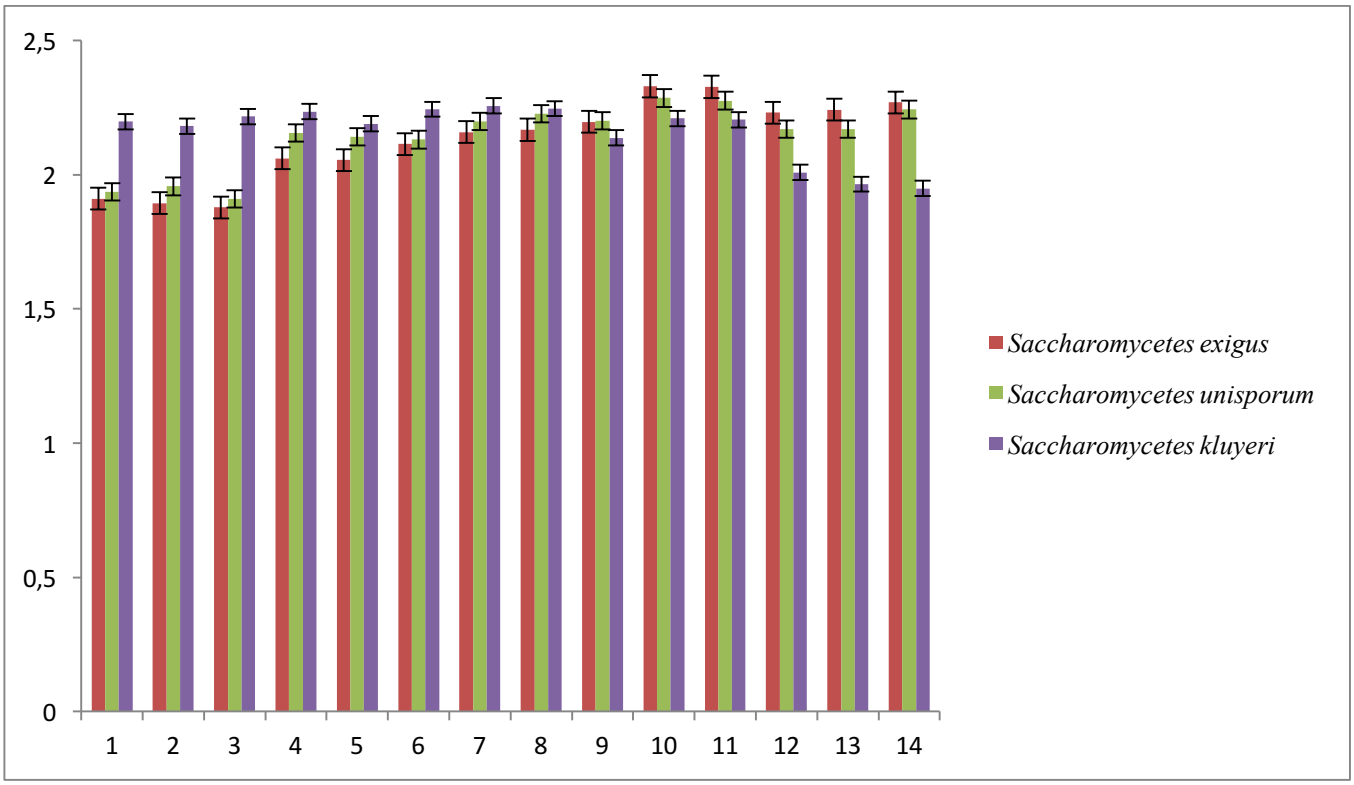

Figure 1: In vitro biodegradation studies of isolates

Using Ward's method of cluster analysis, it was observed that *S. exigus* and *S. unisporum* were most similar (Fig. 2) in enhancing remediative capacity of the biodiesel effluent than *S. kluyeri*, which was a stand-alone. However, this suggests that *S. exigus* and *S. unisporum* have the capability to degrade the effluent. Lahav [21] isolated *Meyerozyma* (Pichia) *guilliermondii* and *R. mucilaginosa* in evaporation ponds contaminated with industrial wastes, which were able to utilize diverse hydrocarbons as carbon sources, including anthracene and phenanthrene.

Margesin [22] isolated 28 strains of yeast from diverse uncontaminated cold environments. These strains used representative fractions of oil hydrocarbons for their growth, such as n-alkanes and monoaromatic and polyaromatic hydrocarbons, at low temperatures.Yeasts have shown tolerance values depending on the organism studied and the site of isolation. Zahir [23] tested the toluene tolerance of bacteria isolated from soil contaminated with hydrocarbons and from rhizospheric soil. In

this study, the yeasts showed good potential for the degradation of biodiesel. The degradation of gasoline by microbiota isolated from urban wastewater activated sludge was investigated by Solano-Serena [24], who observed a degradation rate of 74% (400 mg L-1) with two days of incubation, and 94% after 23 days of incubation.

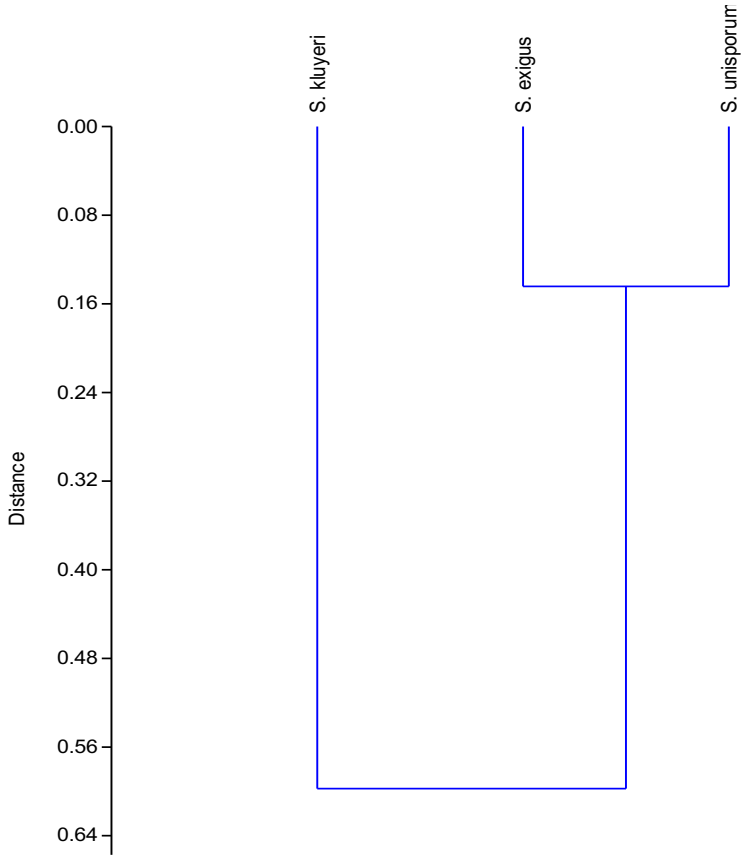

Figure 2: Dendrogram from cluster analyses (ward's method) of yeast isolates accumulated during the study

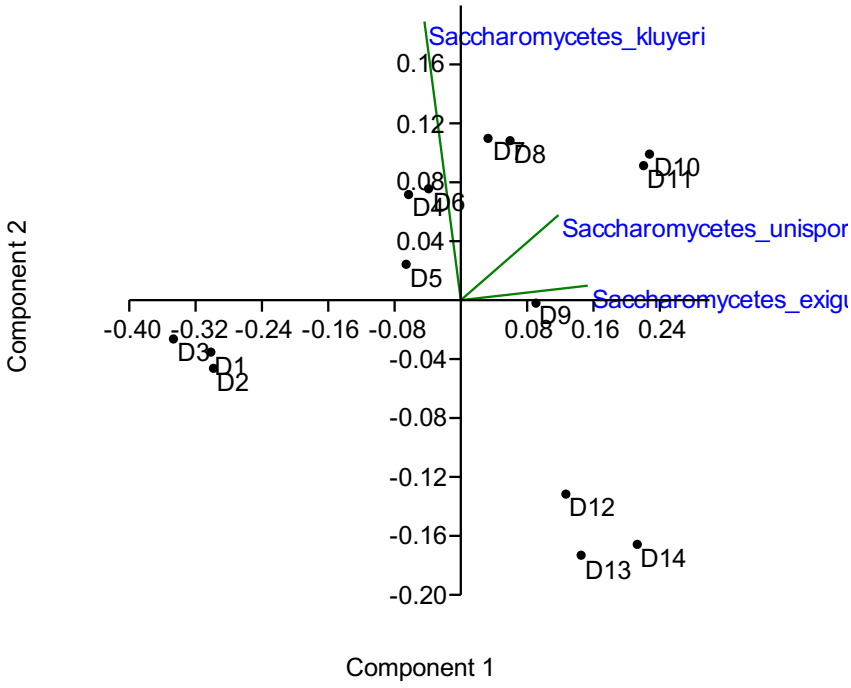

Figure 3: Principal Component Analyses biplot relationship of yeast isolates accumulated during the study.

Yerushalmi and Guiot [25] reported the complete degradation of gasoline by the microbiota of soil contaminated by this fuel, which degraded 16.1 to 600 mg L^{-1} of gasoline after 2.5 and 16 days, respectively. Microorganisms are major players in site remediation, according to Gentry *et al*. [26] and Fantroussi and Agathos [27], but their efficiency is dependent on many abiotic and biotic factors, including the chemical nature and concentration of pollutants, their availability to microorganisms, and the physical and chemical characteristics of the environment (temperature, pH, water content, nutrient availability).

Conclusion

In this study, yeasts isolated were capable to grow in medium containing 1% (v/v) biodiesel as the only carbon source; and the highest growth rates were observed in medium supplemented with gasoline. S. unisporum and S. exigus showed the highest degradation capability for biodiesel effluent. These results highlight the potential of yeast isolates to be used in the bioremediation of sites highly contaminated with petroleum hydrocarbons.

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Disclosure statement: *Conflict of Interest:* The authors declare that there are no conflicts of interest.
Compliance with Ethical Standards: This article does not contain any studies involving human or animal subjects.

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