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ISOLATION AND CHARACTERIZATION OF BACTERIAL CONSORTIA WITH CAPACITY TO DEGRADE HYDROCARBONS

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Graphical Abstract

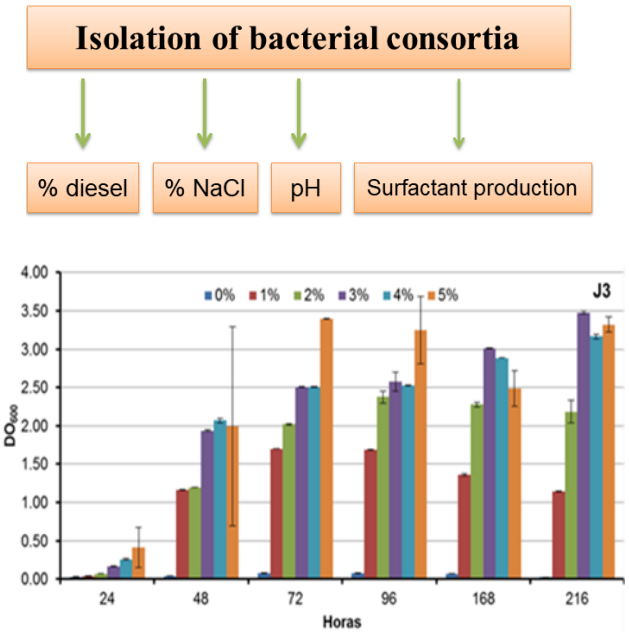


Figure 1. Growth of bacterial consortium J3 in different concentrations of diesel.

Abstract.

The increasing use of hydrocarbons has generated several environmental problems due to accidents during the extraction, transportation, refining, storage and use of said compounds. Diesel is a complex mixture of alkanes and aromatics, which due to its wide use has become a very frequent pollutant of water and soil. Therefore, it is necessary to explore alternatives for the degradation of said pollutants. The purpose of this work is to analyze the optimal conditions for the degradation of diesel by bacterial consortiums from polluted soils, as well as to evaluate the production of surfactants. The bacterial consortiums (J3 and S3) were obtained from soil samples and selected in diesel as the sole carbon source, in addition, the data of the growth kinetics obtained to date in different concentrations of diesel indicate that the two bacterial consortiums use it more at 3 and 4%. On the other hand, at a pH of 7 and 8 in the

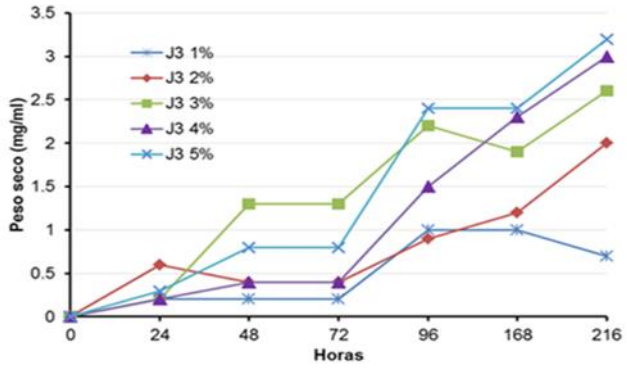


Figure 2) Determination of the biomass produced during bacterial growth by the J3 consortium.

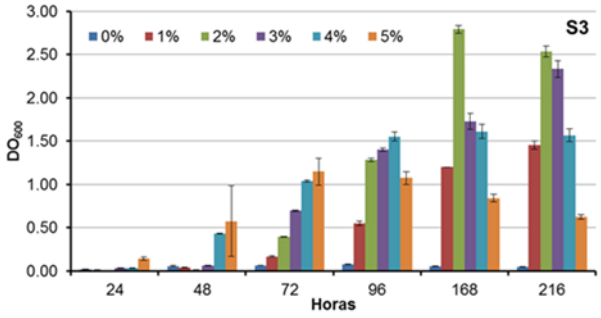
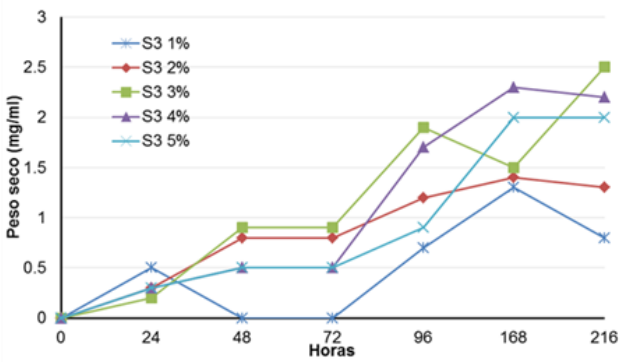


Figure 3. Growth of bacterial consortium J3 in different concentrations of diesel.



4) Determination of the biomass produced during bacterial growth by S3.

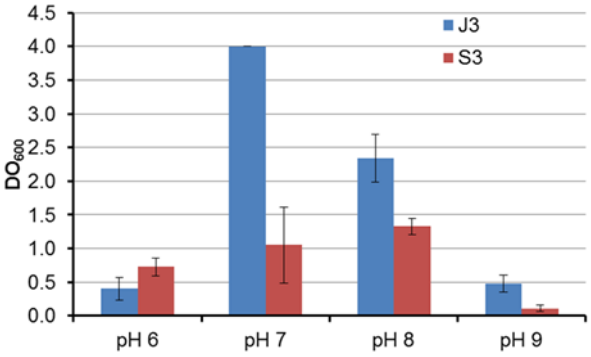
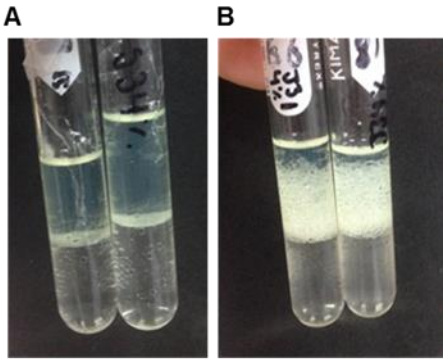


Figure 5. Growth of bacterial consortia J3 and S3 in different

presence of 3% of diesel, better bacterial growth was observed. Also, the S3 consortium showed good growth at NaCl levels of 4%. For the case of the production of surfactants only the J3 consortium, production was produced with an E24 emulsion index of 38%, which was also recorded by the hemolytic activity of the bacterial supernatant and by the collapse of the oil drop. On the other hand, the consortia are able to grow in pentadecane and citronellol, while they do not grow in naphthalene and pyrene as the sole source of carbon. Although they are partial results, they reflect the potential of these consortiums for the degradation of linear hydrocarbons, so studies are being conducted to quantify diesel degradation, identify the members of each consortium and characterize the surfactants produced.

pH.



	Índice E_{24}
S3 3%	0
S3 4%	0
J3 3%	6.3
J3 4%	37.8

Figure 6. Production of surfactants by the bacterial consortia J3 and S3.

Introduction *(optional)*

The exploration, production and transportation of oil has increased in recent years and is expected to increase further in the next. As a consequence, spills and oil pollution are expected to increase as well (Liu et al., 2017). Diesel is one of the products obtained from the refining of oil, which is a complex mixture of linear, branched, cyclic and aromatic hydrocarbons, which has been considered one of the main organic pollutants with harmful effects on living beings (Gallego et al. al., 2001). Hydrocarbons are very frequent pollutants of soil and water, so that their removal is one of the main topics in environmental sciences (Arslan et al., 2014). Although hydrocarbons have toxic effects for many organisms, many of them also have the capacity to use them as a carbon source and make bioremediation of hydrocarbons a viable option. The degradation of hydrocarbons by microbial communities represents a viable and cheap option for the removal of said compounds in diverse environments. The efficiency of bioremedication is influenced by several factors, among which are environmental factors such as pH, temperature and availability of nutrients, among others (Palanisamy et al., 2014). To date, bacteria with the capacity to degrade hydrocarbons (diesel) have been described, among which are Pseudomonas aeruginosa, Bacillus subtilis, Bacillus megaterium, Corynebacterium kutscher (Muthukamalam et al., 2017). In this work, the capacity of two bacterial consortiums for diesel degradation was analyzed, as well as the production of surfactants by said partners.

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Materials and Methods *(optional)*

Isolation of bacteria. Samples of 1 kg of soil were collected in two workshops of diesel engines, at a depth of between 5 and 15 cm. For the enrichment of bacteria with potential for the degradation of hydrocarbons, the soil samples were homogenized and 10 g of each were deposited in a flask with 90 ml of minimal M9 medium and as the only carbon source 1% of commercial diesel (Pemex). The flasks were incubated at 30 ° C and the growth monitored for 15 days. From these primary cultures 50 ml of M9 medium added with 1% diesel were inoculated and incubated for 7 days at 30 ° C, the process was repeated once more.

Effect of diesel concentration on bacterial growth. With bacterial consortiums named J3 and S3 growth kinetics were performed in basal salt medium (MBS) supplemented with 0, 1, 2, 3, 4 and 5% of diesel as the sole carbon source, bacterial growth was determined by measuring the optical density at 600 nm and dry weight at 24, 48, 72, 96, 168 and 216 h.

Effect of pH and salt. The effect of pH on diesel degradation was also analyzed, for which the J3 and S3 consortiums were grown for 7 days in MBS with 3% diesel and pH of 6, 7, 8 and 9. On the other hand, the effect of salt concentration (NaCl) on bacterial growth in the presence of 3% diesel, the concentrations tested were 0, 0.1, 0.5, 1, 2 and 4%.

Production of surfactants. The production of surfactants in the bacterial consortiums was analyzed in MBS supplemented with 3 and 4% of diesel. The quantification of the production of surfactants was carried out by means of the emulsification index at 24 h, hemolytic activity, collapse of the oil drop and oil displacement test.

Studies in process. Experiments are underway to quantify diesel degradation, analyze the types of hydrocarbons that they prefer to use as a carbon source (aromatic or aliphatic), characterize the surfactants produced by the bacterial consortia and identify the members of each consortium.

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Results and Discussion *(optional)*

Two soil samples were collected, from which the bacterial consortiums J3 and S3 were obtained. These bacteria are able to use diesel as the sole carbon source. In general, the concentrations of diesel in which the best bacterial development was observed were between 3 and 4%, which was monitored every 24 h by measuring the OD at 600 nm (Figure 1 and 3) and the determination of biomass in dry weight form (Figure 2 and 4). It was also determined that the pH at which the bacterial consortia showed better growth in the presence of 3% diesel was 7 for the J3 consortium and 8 for S3 (Figure 5). Likewise, the two consortiums were able to use diesel as the sole carbon source in the presence of up to 4% NaCl in the culture medium. While, the analysis of the production of surfactants revealed that only the J3 consortium was able to produce them in greater quantity at the 4% concentration of diesel, with an emulsification index at 24 h of 38% (Figure 6B). This was also analyzed by the hemolytic capacity and the collapse of oil droplets.

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Conclusions *(optional)*

According to the results obtained to date, the two bacterial consortiums are able to use diesel as the sole carbon source, which indicates that they have potential for the degradation of hydrocarbons. The microbial growth was better at concentrations of 3 and 4% of diesel and at a pH of 7. The data obtained so far indicate that the J3 consortium grows better than S3 in the presence of diesel, which is possibly due to the fact that it produces surfactants that favor the assimilation of hydrocarbons.

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References *(mandatory)*

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