



## CHANGES IN THE EXPRESSION OF CATABOLIC GENES DURING CRUDE PETROLEUM BIODEGRADATION BY MICROBIAL CONSORTIUM

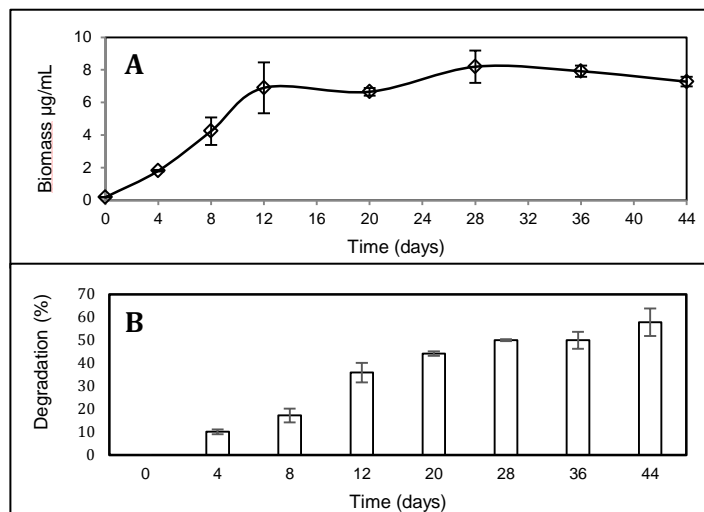
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**Introduction.** Recently, the characterization and expression of genes involved bacterial organic pollutant degradation has promoted the development, research and application of molecular techniques to improve the understanding of the microbiology ecology of contaminated environments<sup>1</sup>. Numerous studies have demonstrated that analysis of catabolic genes could play an important role in the understanding of biodegradation pathways of chemical pollutants<sup>2</sup>. Crude petroleum is a key pollutant found in the environment which is defined like a mixture of these compounds. The aim of this work was to evaluate the changes in catabolic genes expression during biodegradation process.

**Methods.** Liquid microcosm with Bushnell Hass was inoculated with 0.5 µg/mL of the microbial consortium and 4.8 g/L of crude petroleum as carbon and energy source. Degradation systems were incubated at 25°C and 150 rpm during 44 days. Microbial growth was measured through cellular protein using Peterson method. Hydrocarbons degradation was evaluated using gas chromatography with mass detection. Samples were obtained from liquid systems in the days 4, 8, 12 and 16 days. RNA was extracted using Trizol method. The RNA obtained was visualized in 1% agarose gel in 1X buffer TAE. cDNA was synthesized using SuperScript<sup>®</sup> II Reverse Transcriptase Invitrogen<sup>™</sup> following the manufacturer recommendation. Primers targeting catabolic genes were selected using bibliography information<sup>2,3</sup> and through reverse transcription polymerase chain reaction (RT-PCR) will be analyzed change in the expression of genes. RT-PCR was done using a PCR mix adjusted from the different primers employed and the melting temperature. The PCR products were visualized in 1% agarose gel in 1X buffer TAE.

**Results.** Microbial growth progress is shown in figure 1. The biodegradation process was capable to remove the 59% of the total hydrocarbons present in the crude petroleum. Maximum growth and degradation percentage were observed in the day 20. The RNA samples obtained from the lag and exponential phase were used to evaluate the catabolic genes expression. The selection of catabolic genes primers were selected by function of the main components of crude petroleum (table 1). Three primer pairs of the aromatic components were selected and one of related to alkane biodegradation. Lag phase reported the presence of the XyE1 and Be genes. The presence of the XyE1 was detected in the exponential phase too. This could be explained by the early expression of the catabolic genes as many reports have indicated early expression of the catabolic genes.



**Figure 1.** A, Microbial growth in crude petroleum. B, Degradation percentage of crude petroleum hydrocarbons

**Table 1.** Catabolic genes evaluated during the biodegradation process.

Catabolic Gene	Substrate	Exposition time (days)			
		4	8	12	20
Catechol 2,3 dioxygenase ( <i>XyE1</i> ) <sup>2</sup>	Catechol	+	+	+	+
Toluene dioxygenase ( <i>Tod</i> ) <sup>2</sup>	Benzene, toluene	-	-	-	-
Benzene dioxygenase ( <i>Be</i> ) <sup>3</sup>	Benzene, Toluene	+	+	-	-
Alkane monooxygenase ( <i>alkM</i> ) <sup>3</sup>	n-alkane	-	-	-	-

+ Detected - No detected

**Conclusions.** The presence of the XyE1 and Be genes were detected in the lag phase. This could be due to the initial presence of the aromatic compounds. The maximum percentage of degradation was observed during the lag and exponential phase of the microbial growth.

### References

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