BIOTRANSFORMATION RESULTS IN PRESENCE OF CRUDE OILS
A REPEAT OF RESULTS

EXPERIMENTAL DESIGN

This study was aimed at determining the growth rate of recombinant E.coli BL21(DE3) strains containing the phenanthrene catabolic pathway and the fluorene catabolic pathway together when using minimal medium and crude oil as a source of carbon (0.01%). Controls consisted of E.coli strain containing the corresponding vectors with no insert and culture growth on minimum medium with or without glucose (0.4%).

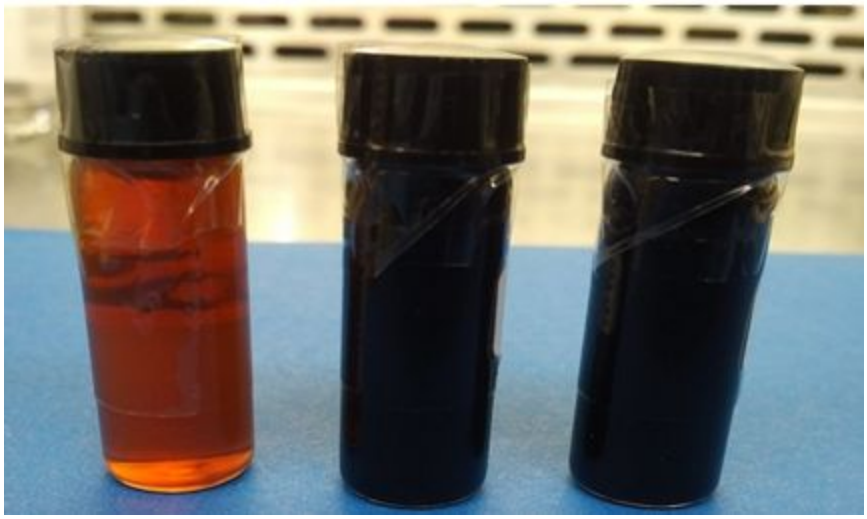


Figure 5. Crude oil samples from Pennsylvannia, Ecuador, and Saudi Arabia (left to right).
Table 15.Crude-oils description.

Properties	Pennsylvania	Ecuador (EC), Saudi Arabia (SA)
Type of crude oil	paraffin based crude oil	asphalt based crude oil
Color	green-brown , sweet	brown-black, sour
Density	light, 0.810 g/mL	heavy, over 0.910 g/mL
Sulfur	0.14 %	EU=1.31%, SA=2.48%
Composition	paraffin, hydrocarbons	Aromatics, asphalt
Products after Refining	gasoline, kerosene	gasoil, asphalt

CULTURE SETUP

E.coli BL21(DE3) recombinant cells containing both plasmids CCA-57 and CCA-48 were first grown at 37°C from glycerol stock in 4 mL of LB medium with both chloramphenicol and. CCA-57 harbored the phenanthrene pathway on vector pSB1C3 and clone CCA-48 harbored the fluorene pathway on vector pSB3T5. Recombinant cell cultures of the corresponding vectors with no catabolic inserts were initiated in a similar fashion. Overnight cultures were spun and

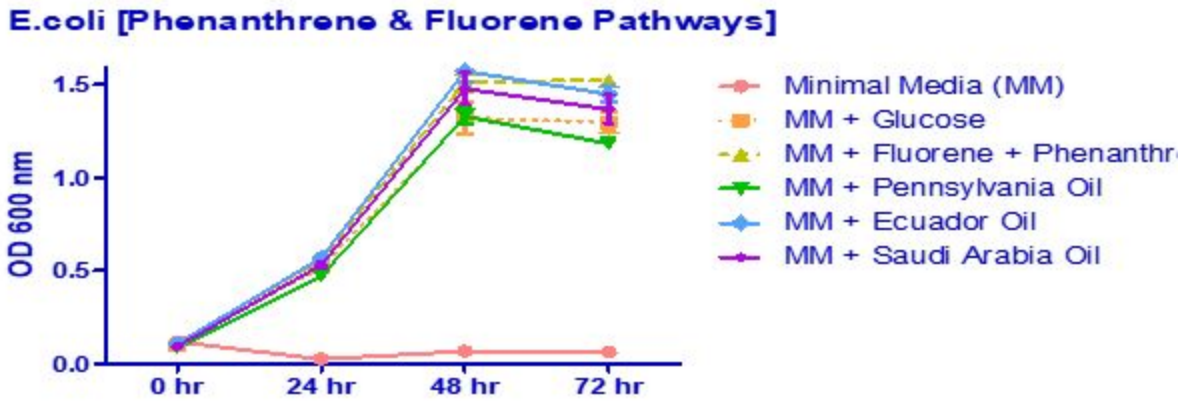
resuspended in 4 mL of minimal medium. OD of all cultures was measured at 600 nm using a spectrophotometer. All cultures were then diluted to 0.02 using the volume determined in the table below.
Growth experiments were initiated in minimal medium with no carbohydrate source, with glucose (0.4%), with phenanthrene and fluorene (0.1 mg/mL each) from a stock solution of 100 mg/mL prepared in DMSO, or with crude oil (0.01%). No antibiotics were added to the minimum medium. Three sources of crude oils were tested: Pennsylvania, Ecuador, and Saudi Arabia.

RESULTS AND DISCUSSION: Time course study of culture growth of recombinant E.coli BL21(DE3) in minimal medium in presence of crude oils

In order to evaluate whether the recombinant cells had the ability to transform PAHs in crude oil samples, growth experiments were set up with a recombinant clone expressing both the fluorene and the phenanthrene catabolic pathways. The clone with the catabolic pathway under the control of the strongest constitutive promoter [CCA-48 for fluorene and CCA-57 for phenanthrene] was set in cultures using M9 minimal medium supplemented with fluorene (0.1 mg/mL) and phenanthrene (0.1 mg/mL) as sole source of carbon in presence of Tween (0.1%), or with crude oils from three sources.

As illustrated in the table above reporting the average of 8 data points (Optical Density) of cultures, the recombinant E.coli containing the biodegradation pathway could utilize crude oils whereas the control bacteria containing the vector only could not. Minimal media was used as medium for culture. Strains could all grow in presence of glucose when used as a carbon source.

This acts as a proof of concept because the fact that our bacteria are degrading PAHs are an indication of our methods' effectiveness.



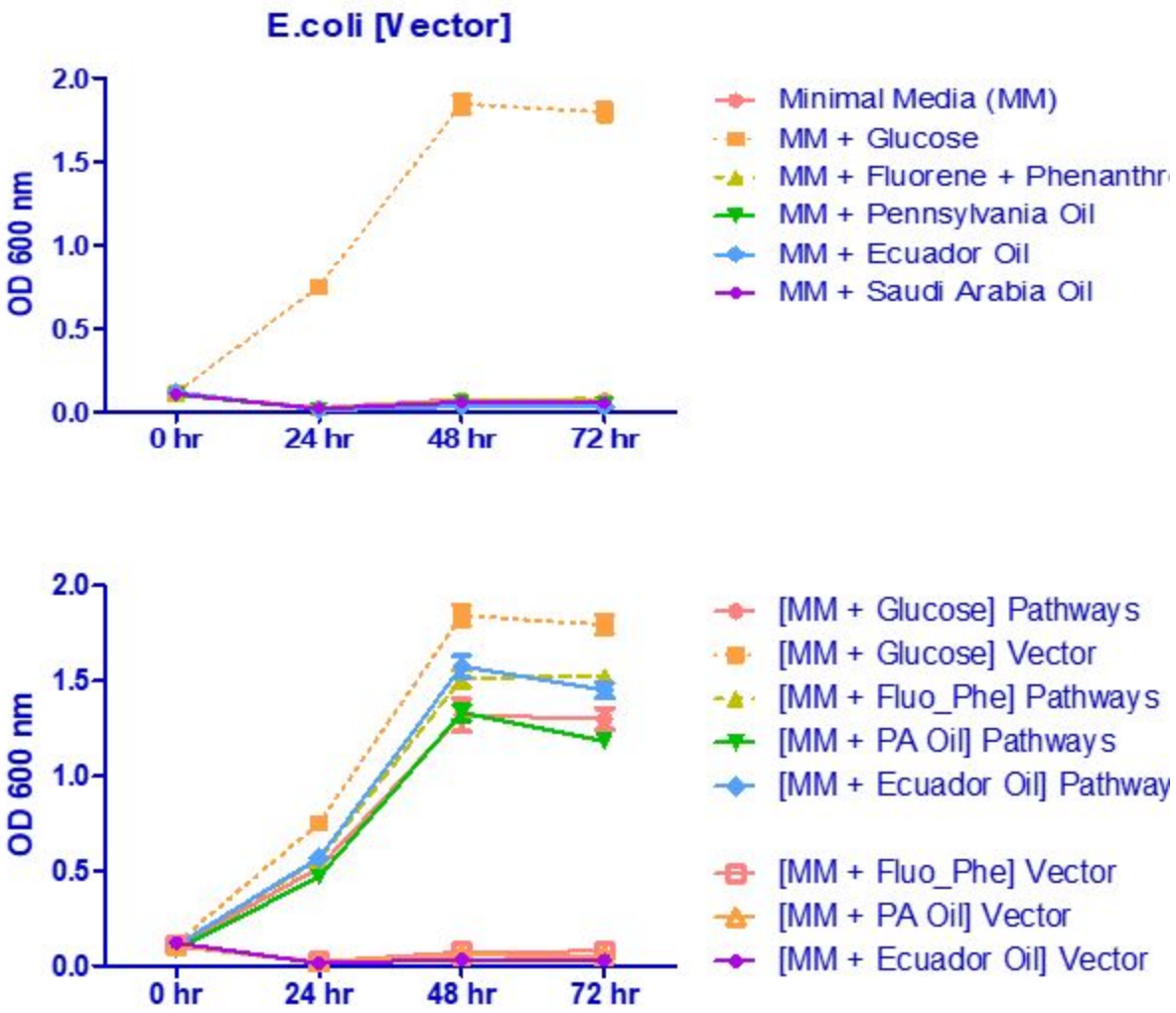


Figure 6. Time course biotransformation experiments using crude oil samples from Pennsylvania (PA), Saudi Arabia, and Ecuador, measuring absorbance at 600nm of E.coli BL21 recombinant cultures containing the fluorene and phenanthrene catabolic pathways or control vectors. MM=M9 minimal medium.

Because the Bacteria are degrading in Oil, our project's goal is complete. It is a proof of Concept.