INVESTIGATION INTO THE MECHANISM(S) WHICH PERMIT THE HIGH-RATE, DEGRADATION OF PAHS AND RELATED PETROLEUM HYDROCARBONS IN SEQUENCING BATCH REACTORS BY ATTACHED CELLS IN A CONTROLLED MIXED BACTERIAL COMMUNITY.

by

Emad Hussein

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INVESTIGATION INTO THE MECHANISM(S) WHICH PERMIT THE HIGH-
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Emad Hussein

Under the direction of George E. Pierce

Abstract:
A stable mixed culture, deposited as ATCC 55644, previously shown to degrade
petroleum hydrocarbons at relatively high concentrations was used as the source of
inoculum. This culture was grown in Stanier’s minimal media, either in the presence of
different concentrations of naphthalene, nitrobenzene and toluene (NNT) or naphthalene
and toluene (NT) as the sole source of C and/or N. Results showed that the majority of the
strains isolated from the mixed culture were able to grow in the presence of NNT or NT.
A total of 20 different isolates were isolated from the mixed culture. Individual isolates
were grown in Stanier’s minimal medium containing a single hydrocarbon as the source of
carbon or carbon and nitrogen. Only one strain was found to grow solely in the presence
of nitrobenzene as the source of C and N. Most of the other isolates were able to grow in
the presence of naphthalene, toluene, acenaphthene, anthracene, fluoranthene and
phenanthrene, n-dodecane, hexadecane, n-pentadecane, n-tetradecane, and n-octadecane.
Planktonic and immobilized cells of the controlled mixed culture (ATCC 55644) were
grown in separate Sequential Batch Reactors (SBR) using Stanier’s media, to which
naphthalene, nitrobenzene and toluene were added as the sole source of C and/or N.
Biodegradation was determined by measuring the residual hydrocarbon in the SBR and
the amount of trapped volatile organic carbon (VOC) and the evolved CO₂. Gas
chromatography data showed that immobilized cells were able to degrade NNT faster than
the planktonic cells. This observation was confirmed by CO₂ evolution. Over time the
loading of hydrocarbon was significantly increased from a starting level of 400 ppm
(Naphthalene), 100 ppm (Nitrobenzene), and 500 ppm (toluene), to a final level of 3000
ppm (Naphthalene), 400 ppm (Nitrobenzene), and 1600 ppm (toluene). While increasing
nutrient loading, the frequency of re-feeding with hydrocarbons was changed from an
initial re-feeding every 60 hrs to a final re-feeding frequency of 18 hrs.
The experiments clearly showed that the attached, mixed microbial community was able
to effectively and rapidly degrade high concentrations of hydrocarbons. This demonstrated
the practical advantages of employing attached, mixed microbial cultures in a SBR.

INDEX WORDS: Jordan Oil Refinery, Middle East, Jordan, CO₂ trap, synthetic waste,
titration, Dehydrogenase enzyme, Naphthalene oxygenase, Evolved CO₂, Volatile organic
carbons, Triphenyl-Tetrazolium chloride, Biomass, Granular activated carbon (GAC),
Bioreactors, Fermentor, DAP 2, VOC trap