

BIODEGRADATION OF CRUDE OIL IN
SEAWATER USING A MIXTURE OF OIL AND
HETEROTROPHIC BACTERIA

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May 2007

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The project aims to enhance oil biodegradation in seawater. Retardation of crude oil biodegradation in seawater is hypothetically due to the inhibiting metabolites produced by the oil bacterium which inhibit its enzymes. For this purpose, the consortium bacteria with an active oil-utilizing bacterium (AR3) and two oil-resistant and active heterotrophic bacteria (OG1) and (OG2) was formed.

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Heterotrophic bacteria, OG1 and OG2 were able to remove metabolites produced during oil degradation. It found that AR3 was retarded by metabolites, while OG1 and OG2 were able to grow in the metabolites. OG1 and OG2 were together to enhance growth and removal of the metabolites. Once the metabolites are removed by OG1 and OG2, AR3 can degrade oil in seawater. OG1 and OG2 will enhance AR3 to grow and degrade crude oil in seawater. The maximum specific growth rate of AR3 and consortium bacteria were 0.008 h^{-1} and 0.011 h^{-1} respectively. Presence of OG1 and OG2 in consortium was able to increase the maximum specific growth rate of

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This project aims to enhance oil biodegradation in seawater. Retardation of crude oil biodegradation in seawater is hypothetically due to the inhibiting metabolites produced by the oil bacterium which inhibit its enzymes. For this purpose, the consortium bacteria with an active oil-oxidizing bacterium (AR3) and two oil-resistant and active heterotrophic bacteria (OG1 and OG2) was formed. Heterotrophic bacteria, OG1 and OG2 were able to remove metabolites produced during oil degradation. It found that AR3 was retarded by metabolites, while OG1 and OG2 were able to grow in the metabolites. OG1 and OG2 were together to enhance growth and removal of the metabolites. Once the metabolites are removed by OG1 and OG2, AR3 can degrade more oil in seawater. OG1 and OG2 will enhance AR3 to grow and degrade crude oil in seawater. The maximum specific growth rate of AR3 and consortium bacteria were 0.068 h^{-1} and 0.091 h^{-1} respectively. Presence of OG1 and OG2 in consortium was able to increase the maximum specific growth rate of AR3 for 33.8%. A 59.9% of crude oil degradation was degraded by AR3 pure culture, while 68.6% by consortium bacteria. There was a 8.7% increasement in oil degradation by consortium bacteria as compared to that found in the AR3. A 31.4% of

Tapis blended crude oil remained in the seawater was due to the asphaltenes and resin hydrocarbons. Experiments have shown that asphaltenes and resin hydrocarbons in Tapis blended crude oil was 291.1 ppm (30.6%). Consortium bacteria was able to degrade 84.1% of total hydrocarbons while 67.0% was degraded by AR3. A 99.8% of total aliphatic and 38.4% of the total aromatic hydrocarbons were degraded by consortium bacteria, while merely 79.4% of total aliphatic and 31.0% of total aromatic were degraded by AR3 under the same experimental conditions. For targeted 16 aliphatic and 16 aromatic hydrocarbons, 86.704 ppm (95.9%) of aliphatic and 9.260 ppm (67.7%) of aromatic hydrocarbons were degraded by consortium bacteria, while only 76.104 ppm (84.2%) aliphatic and 7.001 ppm (51.2%) aromatic were degraded by AR3 under the same experimental conditions. This consortium bacteria was able to degrade the carcinogenic aromatic hydrocarbons in crude oil listed by USEPA. Results obtained from this study support the hypothesis that retardation of AR3 in degrading oil is due to metabolites. Specific inhibiting metabolites are not known, most probably there are organic acids.

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**BIODEGRADASI PADA MINYAK MENTAH DALAM AIR LAUT
MENGUNAKAN CAMPURAN BAKTERIA MINYAK DAN
HETEROTROFIK**

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Mei 2007

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Projek ini bertujuan untuk mempertingkatkan biodegradasi minyak di dalam air laut. Perencatan biodegradasi minyak mentah di dalam air laut adalah disebabkan oleh metabolit merencat yang dihasilkan oleh bakteria minyak yang boleh merencatkan enzimnya. Bagi tujuan ini, bakteria konsortium yang terdiri daripada bakteria pengoksidaan minyak yang aktif (AR3) dan dua bakteria heterotrofik yang aktif dan tahan dalam minyak (OG1 dan OG2). Bakteria heterotropik (OG1 dan OG2) boleh menyingkirkan metabolit semasa penguraian minyak. Ia didapati bahawa AR3 telah direncatkan oleh metabolit manakala OG1 and OG2 mampu tumbuh di dalam metabolit. OG1 dan OG2 bersama-sama untuk meningkatkan pertumbuhan dan menguraikan metabolit. Sebaik sahaja metabolit disingkirkan oleh OG1 dan OG2, AR3 boleh mengurai lebih banyak minyak di dalam air laut. OG1 dan OG2 akan mempertingkatkan pertumbuhan AR3 dan mengurai minyak mentah di dalam air laut. Kadar pertumbuhan spesifik maksimum bagi AR3 dan bakteria konsortium adalah 0.068 h^{-1} dan 0.091 h^{-1} masing-masing. Kehadiran OG1 and OG2 di dalam konsortium boleh meningkatkan kadar pertumbuhan spesifik maksimum bagi AR3 sebanyak

33.8%. Sebanyak 59.9% daripada minyak mentah telah diurai oleh kultur tulen AR3, manakala 68.6% oleh bakteria konsortium. Terdapat peningkatan sebanyak 8.7% di dalam penguraian minyak oleh bakteria konsortium jika dibandingkan dengan AR3. Sebanyak 31.4% daripada minyak mentah Tapis blended (Tapis blended crude oil) yang masih tinggal di dalam air laut adalah hidrokarbon asphaltenes dan resin. Eksperimen telah menunjukkan bahawa terdapat sebanyak 291.1 ppm (30.6%) hidrokarbon asphaltenes dan resin di dalam minyak mentah Tapis blended (Tapis blended crude oil). Bacteria konsortium berupaya mengurai 84.1% daripada jumlah hidrokarbon manakala 67.0% diurai oleh AR3. 99.8% daripada jumlah hidrokarbon alifatik dan 38.4% daripada jumlah hidrokarbon aromatik telah diurai oleh bakteria konsortium manakala hanya 79.4% daripada jumlah hidrokarbon alifatik dan 31.0% daripada jumlah hidrokarbon aromatik diurai oleh AR3 di bawah keadaan eksperimen yang sama. Bagi 16 alifatik dan 16 hidrokarbon aromatik, 86.704 ppm (95.9%) daripada hidrokarbon alifatik dan 9.260 ppm (67.7%) daripada hidrokarbon aromatic telah diurai oleh bakteria konsortium manakala hanya 76.104 ppm (84.2%) hidrokarbon alifatik dan 7.001 ppm (51.2%) hidrokarbon aromatik diurai oleh AR3 di bawah keadaan eksperimen yang sama. Bacteria konsortium ini berupaya untuk mengurai hidrokarbon aromatik yang berkarsinogenik di dalam minyak mentah yang telah disenaraikan oleh USEPA. Keputusan yang diperolehi daripada kajian ini telah menyokong hipotesis bahawa perencatan AR3 dalam penguraian minyak adalah disebabkan oleh metabolit. Kespesifikan metabolit merencat ini tidak diketahui dan kebanyakan daripadanya adalah asid organik.