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# Varying Conditions for Hexanoic Acid Degradation with BioTiger™

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Koji Foreman, Charles Milliken, Robin Brigmon



## Abstract

BioTiger™ (BT) is a consortium of 12 bacteria designed for petroleum waste biodegradation. BT is currently being studied and could be considered for bioremediation of the Athabasca oil sands refineries in Canada and elsewhere. The run off ponds from the petroleum extraction processes, called tailings ponds, are a mixture of polycyclic aromatic hydrocarbons, naphthenic acids, hydrocarbons, toxic chemicals like heavy metals, water, and sand. Due to environmental regulations the oil industry would like to separate and degrade the hazardous chemical species from the tailings ponds while recycling the water. It has been shown that BT at 30 °C is able to completely degrade 10 mM hexanoic acid (HA) co-metabolically with 0.2% yeast extract (w/v) in 48 hours when starting at 0.4 OD 600nm. After establishing this stable degradation capability, variations were tested to explore the wider parameters of BT activity in temperature, pH, intermediate degradation, co-metabolic dependence, and transfer stability. Due to the vast differences in temperature at various points in the refineries, a wide range of temperatures were assessed. The results indicate that BT retains the ability to degrade HA, a model surrogate for tailings pond contaminants, at temperatures ranging from 15°C to 35°C. Hexanamide (HAM) was shown to be an intermediate generated during the degradation of HA in an earlier work and HAM is completely degraded after 48 hours, indicating that HAM is not the final product of HA degradation. Various replacements for yeast extract were attempted. Glucose, a carbon source; casein amino acids, a protein source; additional ammonia, mimicking known media; and additional phosphate with Wolfe's vitamins and minerals all showed no significant degradation of HA compared to control. Decreasing the yeast extract concentration (0.05%) demonstrated limited but significant degradation. Finally, serial inoculations of BT were performed to determine the stability of degradation over several generations. Overall, BT has shown to be moderately flexible for HA co-metabolic biodegradation.

## Background



Figure 1. Dead ducks floating in tailings pond water

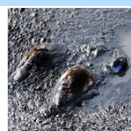
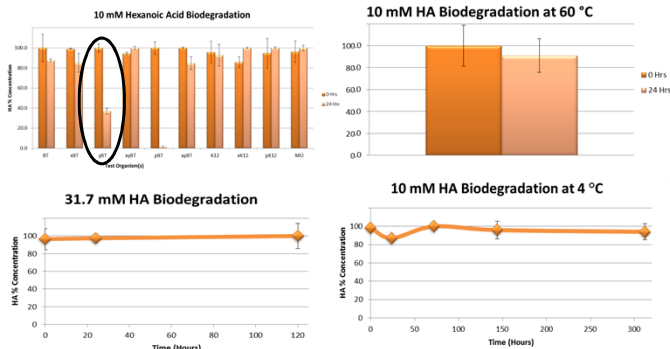


Figure 2. Tumors found in fish exposed to tailings pond runoff

- Oil sands are petroleum and hydrocarbon containing sands mostly found in various sites in Canada
- The extraction process for oil sands produces huge amounts of contaminated water and sand called tailings.
- The tailings are dumped into man-made ponds and may leak into the environment
- Extraction byproducts in tailings include recalcitrant polycyclic, or polynuclear, aromatic hydrocarbons (PAHs) and naphthenic acids (NAs) some of which are carcinogenic and toxic
- HA is a variety of NA which is relatively harmless, it is being used as a model for NA degradation

Component	Identification	ATCC Accession Number
BPB	<i>Flexibacter cf. sancti SRS</i>	PTA-5570
BPC	<i>Pseudomonas fredriksbergensis SRS</i>	PTA-5571
BPE	<i>Staphylococcus warneri Imp 19417 SRS</i>	PTA-5572
BPF	<i>Sphingomonas SRS</i>	PTA-5573
BPH	<i>Sphingomonas sp s37 SRS</i>	PTA-5574
BPI	<i>Phylobacterium SRS (α Proteobacterium TA-A1)</i>	PTA-5575
BPJ	<i>Serratia ficaria SRS (α Proteobacterium TA 12-21)</i>	PTA-5576
BPK	<i>Agrobacterium tumefaciens SRS</i>	PTA-5577
BPL	<i>Rhizobium sp. sdw045 SRS</i>	PTA-5578
BPS	<i>Pseudomonas-putida Biotype B SRS</i>	PTA-5581
BPZ	<i>Alcaligenes-Piechaudii SRS</i>	PTA-5580
BP-20	<i>Ralstonia pickettii SRS</i>	PTA-5579

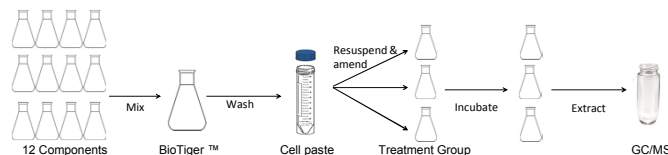
## Prior Works



## Method

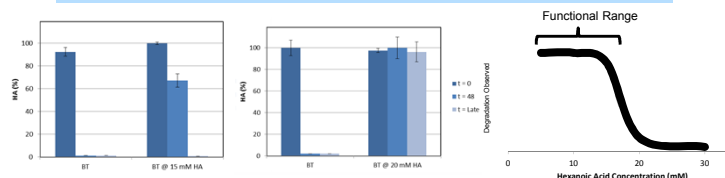
To test the various conditions for degradation, the same process as previous students was used.

- Grow 12 components of BioTiger and mix to make the mother
- Spin down enough cells to create 25 mL media at 0.4 OD @ 600 nm
- Resuspend in BH + Cometabolites and substrates
- Add 1 mL Ethyl Acetate, shake, and run through the GC

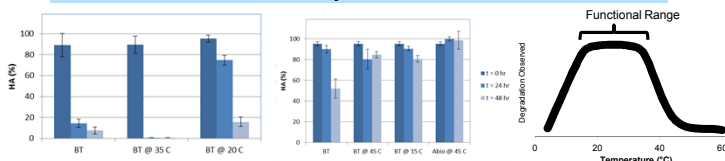


The various conditions were all prepared identically to the control of 10 mM HA in BH and 0.2% (m/v) YE at 30°C, pH 7, and 0.4 at OD600 except for the variable of interest. Cometabolite testing replaced YE and substrate testing replaced HA.

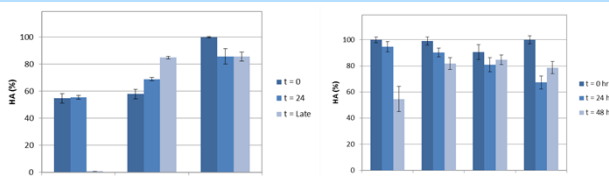
## Concentration



## Temperature

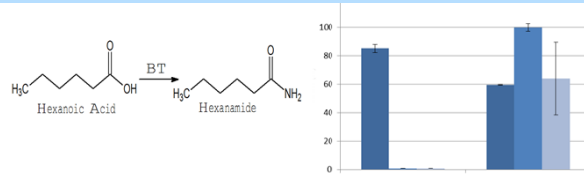


## Cometabolites



Test	Cometabolite
BT	0.2% (m/v) Yeast Extract
BT + NH4	0.01 M Ammonium Chloride
BT + Gluc	0.2% (m/v) Glucose
BT + CA	0.2% (m/v) Casein Amino Acids
BT + VMP	Contains 0.05M KH <sub>2</sub> PO <sub>4</sub> and Wolfe's Vitamins and Minerals

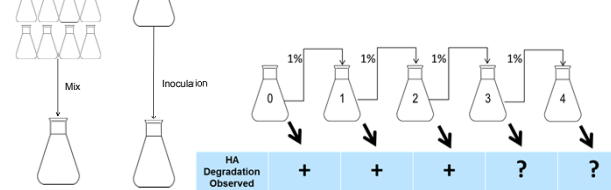
## HAM Cometabolism



## Serial Transfer

Each mother is inoculated from the previous mother using a 1% inoculation

After 2-4 days growth (random to simulate a working environment), the mother is used to set up treatment sets as before



## Conclusion

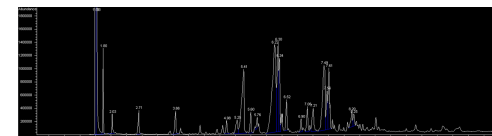


Figure 3. GC chromatograph of NA

Temperature (°C)	Degradation Observed	Cometabolite	Degradation Observed
60	-	0.2% (m/v) Glucose	-
55	-	0.2% (m/v) Casein	-
45	-	0.1 M Ammonium	-
30	+	0.5 M KH <sub>2</sub> PO <sub>4</sub> + Vitamins & Minerals	-
35	+	Substrate	
20	+	HAM	+
15	+	HDA	?
HA Concentration (mM)		NA	?
5	+	pH	
10	+	3	?
15	+	5	?
20	-	7	+
30	-	9	?
		11	?

## Planned Work

- Patent or Manuscript submission
- pH Extraction Technique
- Naphthenic Acid Degradation detection method
- Toluene detection method development
- BT community analysis
- Growth studies

## Acknowledgements

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