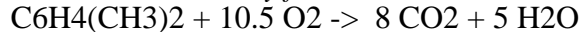


BIOREMEDIATION EXAM - 1999 SOLUTION

Point values are shown in [brackets]. List assumptions used and show units.

1. [2] Write a balanced stoichiometry for the aerobic bio-mineralization of ortho-xylene.



2. [2] The Michaelis-Menten biokinetics for aerobic bacteria degrading o-xylene are: $K = 1 \text{ g/g-d}$, $K_s = 1 \text{ mg/L}$, and net yield = $0.4 \text{ g VSS/g o-xylene}$. If the initial biomass concentration is 10 mg VSS/L , and 50 mg/L o-xylene is completely biodegraded, what is the final biomass concentration (in mg VSS/L)?

$$Y * (\text{So} - \text{Sf}) = \text{biomass growth}; 0.4 \text{ g VSS/g oX} (50 \text{ mg/L oX}) = 20 \text{ mg/L VSS}$$

$$10 \text{ mg/L VSS initial} + 20 \text{ mg/L VSS growth} = 30 \text{ mg/L VSS final}$$

3. [4] If no other nitrogen source is available, how many grams of nitrate-N would be consumed per gram of ortho-xylene biomineralized under aerobic conditions (for the bacteria in question 2)?

of the dry cell weight, 50% is carbon, 14% is nitrogen.

Use the nitrate-N for cell material. $1 \text{ g oX biomineralized} = 0.4 \text{ g VSS}$

assume that only carbon was combusted away: $0.4 \text{ g C} * 14 \text{ g N} / 50 \text{ g C} = 0.112 \text{ g NO}_3\text{-N}$

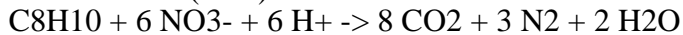
OR assume $\text{VSS} = 0.8 * \text{total dry mass}$; then $0.4 \text{ g VSS} / 0.8 = 0.5 \text{ g dry mass} * 0.5 \text{ g C/g dry}$

$$0.25 \text{ g C} * 14 / 50 = 0.07 \text{ g NO}_3\text{-N}$$

4. [4] How many mg/L of nitrate-N would be consumed as an electron acceptor to biodegrade 50 mg/L o-xylene if no oxygen is available?

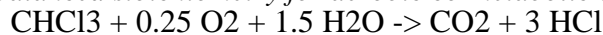


$$\text{O: } 3x = 16 + (10-x)/2 \Rightarrow x = 6$$



$$50 \text{ mg/L oX} * 1 \text{ mol} / 106 \text{ g} * 6 \text{ mol} / 1 \text{ mol} * 14 \text{ g/mol} = 39.6 \text{ mg/L NO}_3\text{-N}$$

5. [2] Write a balanced stoichiometry for aerobic co-metabolic biodegradation of chloroform (CHCl_3).



6. [5] What are 6 co-substrates for aerobic cometabolic TCE degradation?

methane, ammonia, propane, isoprene, butane, toluene, phenol

For 2 of these, give 1 advantage and 1 disadvantage (compared to the alternate co-substrates).

methane: gas, non-toxic, fastest rate TCE deg., low solubility, potentially explosive; intermediate toxicity

phenol: high solubility, potential to avoid intermediate toxicity (high T_c), toxic itself,

toluene: toxic to humans to a low effluent conc. allowed; supports a range of microbes

7. [6] What are the three primary purposes of the additives used in composting?

rich organics for co-substrates

bulking agent (better oxygen supply)

fast degrading organics produce heat to increase bioavailability and biokinetic rates

nutrients (N, P)

list the purpose(s) of each of the following:

cow manure - co-substrate; heat generation

straw - bulking

crushed oyster shells - buffer pH changes

8. [4] List 4 different types of ex-situ biological reactors used to treat contaminated gases.

biofilter biotrickling filter bioscrubber suspended-growth reactor

9. [4] Given the following data from a 4 cm diameter x 10 cm tall soil core: weight = 218.9 g , dry weight = 217.14 g , ignited (post 550°C) weight = 216.88 g . When the final (post ignited) soil was added into a beaker containing 100 mL of water, the final water level in the beaker was 190 mL . Calculate the soil porosity.

$\text{pb} = (1-n) \text{ ps}$; $\text{pb} = \text{dry weight} / \text{total cylinder volume}$; $\text{ps} = \text{ignited weight} / \text{water volume displaced}$

$$217.14 \text{ g} / 126.66 \text{ cm}^3 = (1-n) (216.88 \text{ g} / 90 \text{ cm}^3)$$

$$1.714 = (1-n) 2.41$$

$$n = 0.289$$

10. [8] Given the following kinetic coefficients for a pure culture of aerobic bacteria: maximum specific benzene degradation rate 5 g benz/g VSS-d; benzene half-saturation coefficient 0.1 mg/L; net yield 0.5 g VSS/g benz; substrate inhibition coefficient 100 mg benz/L; endogenous decay rate 0.01 d⁻¹; maximum specific growth rate on xylene 4 d⁻¹; xyl half-saturation concentration 1 mg/L; substrate inhibition coefficient 500 mg xylene/L; net yield 0.4 g VSS/g xyl.

a. Find the initial benzene degradation rate with 100 mg/L VSS and 20 mg/L benzene

$$dS/dt = K S X / (K_s + S + S^2/K_i)$$

$$dS/dt = 5 \text{ g/g-d} * 20 \text{ mg/L} * 100 \text{ mg/L} / (0.1 \text{ mg/L} + 20 \text{ mg/L} + 20 \text{ mg/L}^2 / 100 \text{ mg/L})$$

$$dS/dt = 10000 \text{ mg/L-d} / 24.1 = 415 \text{ mg benz / L soln -d}$$

b. the initial xylene degradation rate with 200 mg/L VSS, 20 mg/L benzene, and 10 mg/L xylene

$$dS/dt = K S X / (K_s (1 + B/K_{s-b}) + S + S^2/K_i)$$

$$\mu_m / Y = K \Rightarrow K = 4 \text{ d}^{-1} / 0.4 \text{ g VSS/g oX} = 10 \text{ g oX/g VSS-d}$$

$$dS/dt = 10 \text{ g/g-d} * 10 \text{ mg/L} * 200 \text{ mg/L} / (1 \text{ mg/L} (1 + (20 \text{ mg/L}/0.1 \text{ mg/L})) + 10 \text{ mg/L} + 100 \text{ mg/L}/500)$$

$$dS/dt = 20000 \text{ mg/L-d} / 211.2$$

$$dS/dt = 94 \text{ mg oXylene / L soln -d}$$

11. [3] List three compounds that can be degraded by anaerobic bacteria but can not be degraded by any known aerobic bacteria under **any** conditions (including potential co-substrate availability).

tetrachloroethene, hexachloroethane, carbon tetrachloride, >6 Cl-PCB, hexachlorobenzene

12. [6] List three methods that you could use to prove that the bacteria present in a soil sample have the capability to degrade BTEX compounds (be as specific as possible).

plate on agar with BTEX present as the sole carbon source, look for growth

use a DNA probe to look for TOL, TMO, TOM, TBU, TOD coding sequence in DNA

use an enzyme assay to look for above listed enzymes

use a lab test in closed flask, add radiolabeled BTEX, look for ¹⁴CO₂ production; compare to “killed” controls

13. [6] The following compounds are all in a gas stream bubbled into a 2 m deep liquid reactor.

Cmpd	Inlet Gas Conc, mg/L	MW	Solubility, mg/L	H	Kow, L/L
A	10	100	1000	0.2	1000
B	10	120	500	0.2	100
C	10	110	2000	0.1	10

a. If bioactivity maintains the liquid concentration of all compounds below 0.05 mg/L, which compound (A, B, or C) will have the lowest concentration in the effluent gas?

predominant problem is mass transfer of compound into liquid out of the gas

CMPD C has the lowest Henry's and therefore greatest desire to partition into liquid

b. If bioactivity maintains the liquid concentration of cmpd A at 0.2 mg/L, cmpd B at 2 mg/L, and cmpd C at 20 mg/L, which will have the lowest concentration in the effluent gas?

Here, the high liquid conc prevents a high “driving force” to get the compound to partition out of the gas and into the liquid. “H * Cl” term is highest for C (0.1*20 = 2) so it will transfer the worst. H * Cl is the lowest for compound A (0.2*0.2 = 0.04) so it will transfer the best and have the lowest concentration in the effluent gas.

14. [9] Given that an FBR at steady-state is treating 100 m³/min contaminated water with inlet concentration of 100 mg/L toluene and an effluent concentration 0.1 mg/L toluene. If the biodegradation kinetics of toluene are 1st order below 5 mg/L toluene with k = 10 L/mg biomass-d:

a. What is the total biomass concentration in the reactor (in g biomass)?

Mass Balance TOLUENE:

$$100 \text{ mg/L} * 100 \text{ m}^3/\text{min} = 0.1 \text{ mg/L} * 100 \text{ m}^3/\text{min} + 10 \text{ L/mg-d (BIO)} 0.1 \text{ mg/L tol}$$

$$1 \text{ E}7 \text{ mg/min} - 1 \text{ E}4 \text{ mg/min} = 1(\text{BIO}) / \text{d}$$

$$9.99 \text{ E}6 \text{ mg/min} * 60 * 24 \text{ min/d} = \text{BIO} / \text{d}$$

$$\text{BIO} = 1.43856 \text{ E}10 \text{ mg biomass} = 1439 \text{ kg bio}$$

b. This reactor contains 1500 kg of sand packing, the packed bed volume is 1 m³ with porosity of 0.4, and the reactor is operated with a recycle rate that causes 200% bed expansion. What is the final depth of reactor needed, assuming that the reactor has a cross-sectional area of 1 m²?
 packed bed 1 m³ + expansion of 1 m³(2) = 3 m³ total expanded bed
 add 10% safety factor => 3 * 1.1 = 3.3 m³ / 1 m² = 3.3 m deep

c. What is the HRT in your reactor?

$$\text{HRT} = V / Q = (3.3 \text{ m}^3 \text{ total} - 0.6 \text{ m}^3 \text{ solid}) / 100 \text{ m}^3/\text{min} = 0.027 \text{ min}$$

15. [6] A soil slurry reactor operated with an HRT of 50 days has an inlet concentration of 1000 mg TOC/L slurry, the solids content in the slurry is 10% by volume, and the effluent concentration is 50 mg TOC/L solids and 10 mg TOC/L liquid. (assume soil solids have a density of 2 g/mL)

a. If the biodegradation rate is first order with respect to the liquid concentration, write an equation(s) to calculate the biodegradation rate constant.

MASS BALANCE TOC

$$100 \text{ mg TOC/L} * Q_{\text{sl}} = 50 \text{ mg TOC/L solids (0.1 L solids/L slurry)} Q_{\text{sl}} + 10 \text{ mg TOC/L liq (0.9 L liq/L slurry)} Q_{\text{sl}} + k (10 \text{ mg/L}) \text{ VOL}$$

dividing both sides by slurry flowrate (Q_{sl}): 100 = 5 + 9 + 10 K HRT

b. If the system is at equilibrium, what is the soil:water partitioning coefficient of the TOC?

$$K_d = C_{\text{soil}} / C_{\text{water}} = 50 \text{ mg TOC/L solids} / 10 \text{ mg TOC/L liq}$$

$$K_d = 5 \text{ L liq} / \text{L solids}$$

16. [5] Given the results below from gas samples taken from 8 ft deep in the vadose zone at 3 sites.

sample	SITE 1			SITE 2			SITE 3		
	% O ₂	% CO ₂	HC, mg/L	% O ₂	% CO ₂	HC, mg/L	% O ₂	% CO ₂	HC, mg/L
1	18	1	0	3	1	15	8	3	1
2	5	8	10	1	1	20	16	1	0
3	6	7	8	2	1	10	4	8	3
4	7	6	6	2	1	5	1	10	10
5	6	7	8	15	1	0	2	9	8

HC = hydrocarbons; assume that the average H of the HCs is 0.1.

At which sites would you further investigate the potential for remediation by bioventing? BRIEFLY justify.

NOT site 1, since not oxygen limited (O₂ conc > 5% everywhere)

SITE 2 since oxygen is depleted significantly where there is HC contamination compared to bkg

SITE 3 since oxygen is depleted <5% at some high HC contaminated areas, and more than bkg

What are 2 further tests that you would conduct in the field prior to implementing full-scale bioventing?

on site OUR test

in situ air permeability test

17. [4] Given a bioventing well screened from 5 ft below ground to 15 ft below ground. The soil porosity (coarse gravel) is 0.5; the soil is 10% water saturated, 10% NAPL saturated. You operate an air injection well at 10 cfm which gives a radius of influence of 50 ft. What is soil gas exchange rate in pore volumes per day?

$$\text{pore volume of gas within radius of influence} = b * \pi * r^2 * n_{\text{air}}$$

$$10 \text{ ft} * \pi * (50 \text{ ft})^2 * (0.5 * 0.8)$$

$$31416 \text{ ft}^3 = 1 \text{ pore volume}$$

$$\text{pore volumes} / \text{day} = 10 \text{ cfm} / 31416 \text{ cf/pore vol} = 0.0003 \text{ pore vol} / \text{min} = 0.46 \text{ pore vol/day}$$

18. [9] A site is contaminated with jet fuel that has penetrated the vadose zone and is now floating on the water table and dissolving into the unconfined aquifer. Background groundwater concentrations of sulfate, Fe⁺³, nitrate and oxygen are 50 mg/L, 50 mg/L, 50 mg/L, and 8 mg/L, respectively.

a. Under conditions of natural attenuation, most of the jet fuel (by mass) likely be degraded by bacteria using which of the available electron acceptors?

utilization factors: 3 mg O₂/mg HC; nitrate 4.9, iron 21.5, sulfate 4.7

Therefore, sulfate!

Also, remember on the “pie graph” from lecture that methanogenesis was 39%, sulfate reduction 29%, nitrate reduction 14%, aerobic 10%, iron 8% (as an average over sites)

=> also credit if you said nitrate (more preferred for energy and almost as much available)

b. If there is a drinking water well downgradient that is at risk, in which scenario below (a) or (b) would natural attenuation be more likely to attenuate the plume before it reaches the well, or no difference predicts (assume that all non-listed site parameters are the same for A and B):

	OPTION A	OPTION B	your answer + briefly why
b1	groundwater velocity 1 m/yr	groundwater velocity 10 m/y	Option A better chance to attenuate faster GW velocity gives less time for attenuation and bioreactions;
b2	the foc of the soil is 0.1%	the foc of the soil is 1%	B better chance to attenuate higher foc = more sorption, slower plume movement (bigger Retardation)
b3	longitudinal hydrodynamic dispersion is 1 m ² /d	longitudinal hydrodynamic dispersion is 10 m ² /d	A; less dispersion will slow the movement ahead of the “Average” velocity of the plume
b4	transverse hydrodynamic dispersion is 0.1 m ² /d	transverse hydrodynamic dispersion is 1 m ² /d	B; more dilution and “sideways” movement of plume
b5	10 kg of fuel spilled	100 kg fuel spilled	A; less source, greater chance that it will be attenuated sooner, maybe before plume hits receptor

19. [8] To determine whether natural attenuation would be sufficient to protect a down-gradient groundwater well from a plume of chloroform contamination, would you be more likely to use an instantaneous biodegradation model based on electron acceptors or a first-order model? justify your answer.

CF only degrades aerobically cometabolically; it is also a “slow degrading” compound anaerobically. Therefore, first-order model probably better than instantaneous (also more typical to model chlorinated cmpds with 1st order and fuel hydrocarbons with inst. model)

What tests could you run to provide data to support this claim regarding the appropriate kinetic model?

look for production of by-products

look for slow tailing decrease in concs with distance (inst model more blocky)

look for depletion in electron acceptors/donors slowly down the plume rather than “sudden”

blocky change

try to fit Bioscreen model to the current plume

What other data would you gather (in the field and/or the lab) to support a case for natural attenuation to EPA?

bacteria naturally present in the site soil can degrade CF (in the lab)

CF-degrading bacteria active on site (DCM, CM, Cl- by-products)

gene probes?

run a risk model to prove enough attenuation occurring for minimal risk to receptors

20. [3] The addition of a non-ionic surfactant into the groundwater injected into a soil column (foc 2%, porosity 0.3, 5% saturated with PCB NAPL) failed to increase the biodegradation removal of PCBs from the column (compared to a control without surfactant addition). List 3 possible causes for the lack of enhanced PCB biodegradation.

surfactant was toxic to the bacteria

PCB in the micelle not available for biodegradation

biodegradation of PCB not bio-availability limited (limited by electron acceptor or donor, nutrients, etc)

higher PCB solubility was toxic to the bacteria