Biological Treatment of aTritiated HPLC Mixed Waste to Meet RCRA Requirements

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Background and Regulatory Constraints. As both the U.S. Environmental Protection Agency (EPA) and the Nuclear Regulatory Commission (NRC) have acknowledged in regulatory guidance documents and in final and proposed rulemakings, dual regulation of mixed waste under the Atomic Energy Act and the Resource Conservation and Recovery Act (RCRA) presents a number of difficulties of mixed waste management. In the case of tritiated mixed waste, the United States biomedical R&D community faces very limited treatment and disposal options for many of the mixed wastes generated by their research activities.

Under RCRA, D-coded organic solvents must be treated to meet concentration-based and/or technology-specific land disposal restrictions (LDRs) before they may be disposed. However, available offsite commercial or DOE options for performing the RCRA-required treatment (e.g., combustion; see Table 1) are not desirable in Lawrence Berkeley National Laboratory's (LBNL's) view, based both on environmental consequences (i.e., tritium release to the atmosphere), and on expense (estimated to be $30-100 per mCi). When faced with unsatisfactory treatment and disposal options for the tritiated mixed wastes, the LBNL decided to develop an alternative treatment method that can efficiently destroy the organic compounds in the wastes and confine the tritium.

Waste Composition and EPA Treatment Standards. The spent solvents generated from the high performance liquid chromatography (HPLC) purification process may consist of the following compounds: water, acetonitrile, methanol, hexane, tetrahydrofuran, ethanol, isopropanol, trifluoroacetic acid, or chloroform. Table 1 lists the EPA codes of these solvents. Table 2 lists examples of the targeted solvent wastes for this study.

Table 1. EPA Codes of HPLC Solvents and Universal Treatment Standards

EPA Code	HPLC Solvent Ingredients	Universal Treatment Standard (UTS) for Nonwastewater (high TOC)
D001	Acetone, ACN, EtOH, EtOAc, ether, MeOH, THF, IPA, DMF	CMBST, RORGS, or POLYM
D022	chloroform (> 6 ppm TCLP)	6 mg/kg

Note: CMBST = combustion, RORGS = recovery of organics, POLYM = polymerization, TOC = total organic compounds

Proposed Biodegradation Process. In order to prevent pollution to the environment, identify fiscally preferable methods, and potentially recover tritium from the HPLC mixed waste, LBNL would like to explore some other treatment alternatives. The proposed biodegradation process has the capability to destroy the organic chemicals and confine the tritium in the

treatment residue. Biodegradation processes have been successfully used in treating hazardous chemical contaminants in many site remediation projects. However, there were only a few cases that involved degradation tests for low-level mixed wastes.

The proposed biodegradation process is either a sequencing batch reactor (SBR) or a chemostat reactor. This process will consist of a continuously stirred tank reactor and a vapor phase emission reduction system. The sample will be metered into this reactor along with nutrient solution and oxygen. The biomass may be recycled and reused for the next batch. The vapor stream will then be passed through several absorbent beds to remove any tritium. Tritium monitoring systems will be installed at the outlets of the biodegradation process.

Preliminary Study. In this phase of study, we are conducting experiments to determine the biodegradation kinetics of the mixed waste stream generated from the HPLC purification process of a biomedical research facility. Our HPLC liquid waste samples normally have high water content (up to 80%) and relatively low organic content (less than or equal to 20%).

In this study, we focus on the degradation of hazardous chemicals (mainly acetonitrile and methanol) in the waste stream (see Table 2). We will try to answer the following questions:

(a) what are the treatable concentration ranges (is there any substrate inhibition on culture at high concentrations)?
(b) what are the process limitations (oxygen demand, biomass production, production of metabolites, etc.)? and
(c) does one component have an effect on degradation of another component?

In order to find out answers for the above questions, we will develop mixed cultures capable of degrading acetonitrile and methanol in the HPLC mixed waste and determine the kinetic parameters governing the mixed culture cell growth using either acetonitrile or methanol as a substrate. To date, two different approaches have been performed: conducting cell growth rate analysis in batch reactions and examining the bacterial oxygen uptake rates.

Table 2. Examples of HPLC Waste Composition

Water Content	Solvent Content
60 - 80%	ACN (20 - 40%) with trace of THF, TFA, MeOH, IPA, hexane, EtOAc,
60 – 80%	MeOH (20–40%), with trace of THF, TFA, CAN.
80%	ACN (10%) and MeOH (10%).
35 – 80%	ACN (20–65%), with trace of THF, MeOH, EtOH, IPA, TFA, DMF

Note: ACN = acetonitrile, DMF = dimethyl formamide, EtOAc = ethyl acetate, EtOH = ethanol, IPA = isopropanol, MeOH = methanol, THF = tetrahydofuran, TFA = trifluoroacetic acid

Cell growth rate as a function of methanol or acetonitrile concentration was measured by optical density using a spectrophotometer. We found that with methanol as a substrate,

(a) it showed no inhibition effect on the culture at or below 16,000 mg/liter, and
(b) the Monod model fit the data.

Similar results were also obtained when acetonitrile was used as substrate (when concentrations were less than 10,000 mg/liter). However, when a concentration of 100,000 mg/liter was included, the Haldane model fit the acetonitrile data, which suggests that acetonitrile has inhibitory effects on the culture at higher concentrations.

To confirm the results obtained in the growth curve experiments, we also gathered information on oxygen uptake rate by the mixed culture. Bacteria oxygen uptake rates are determined by using a respirometer that measures the amount of dissolved oxygen in the mixed culture when substrates were introduced. A substrate (methanol or acetonitrile) of predetermined concentration was injected into the reaction cell of the respirometer and oxygen uptake by the mixed culture was recorded. The oxygen uptake rate is then fit into a reaction model (either the Monod or Haldane equation) to determine the kinetic parameters. From our data, we found that acetonitrile has growth inhibition effects on the bacteria when high concentration of acetonitrile (>10,000 mg/liter) was injected, where methanol does not. These results support the conclusion that we obtained from the growth curve experiments.

Our results indicate that biological treatment has potential to serve as an alternative technology for the HPLC mixed waste treatment. The results from our preliminary experiments will be used in the design of our biological treatment process (using either a SBR or a chemostat). We will focus on (a) the degradation of major components (methanol and acetonitrile) as a mixture, (b) the degradation of minor components (trifluoroacetic acid, ethanol, tetrahydrofuran, ieopropanol, etc.), and (c) confirming the obtained kinetics with a SBR or chemostat.