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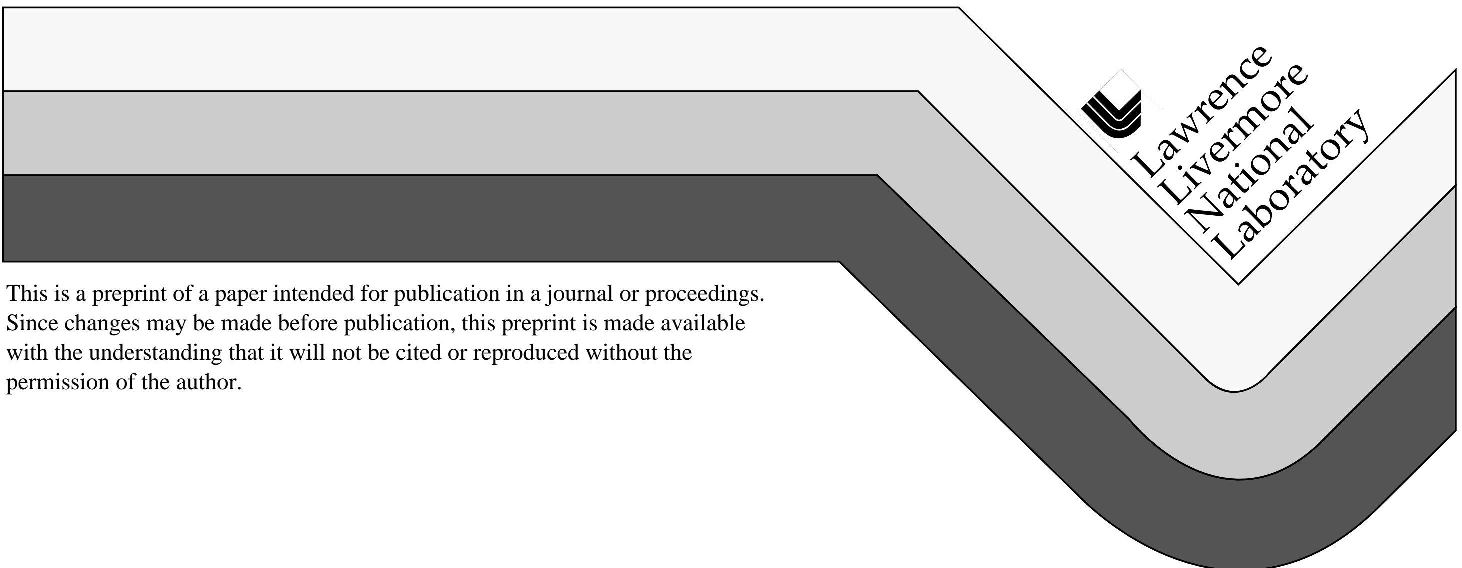

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ASSESSING MICROBIOLOGICALLY INDUCED CORROSION OF WASTE PACKAGE MATERIALS IN THE YUCCA MOUNTAIN REPOSITORY

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ABSTRACT

The contribution of bacterial activities to corrosion of nuclear waste package materials must be determined to predict the adequacy of containment for a potential nuclear waste repository at Yucca Mountain (YM), NV. The program to evaluate potential microbially induced corrosion (MIC) of candidate waste container materials includes characterization of bacteria in the post-construction YM environment, determination of their required growth conditions and growth rates, quantitative assessment of the biochemical contribution to metal corrosion, and evaluation of overall MIC rates on candidate waste package materials.

INTRODUCTION

The U.S. Department of Energy is engaged in a suitability study for a potential geological repository at YM, Nevada, for the containment and storage of commercially generated spent fuel and defense high-level nuclear waste. There is growing recognition of the role that biotic factors could play in this repository, through MIC of waste packages and other repository components.

Assessment and prediction of the impact of MIC on nuclear waste containment requires determination of the range of probabilities, rates, and required conditions for MIC of candidate container materials. Therefore, a program has been established that seeks to determine, 1) whether microorganisms contained within the post-construction YM environment have activities that have been associated with metal corrosion, 2) boundary conditions and rates for these activities under varying conditions, and 3) the rate of MIC of candidate waste package (WP) container materials under accelerated testing conditions.

The potential for MIC within the repository will depend on the presence of microorganisms that are capable of performing corrosion-related activities, the conditions under which these organisms can grow, and conditions under which MIC activities are operative. Thus, a survey of YM microorganisms for corrosion activities will partly establish MIC potential in the repository. Quantification of the rates of microbial growth and MIC activities under varying conditions will permit accurate prediction of MIC over the long term anticipated to be necessary for adequate waste containment, and ultimately target those periods over the evolution of the repository when MIC can occur. Finally, accelerated

testing will provide the overall degree to which corrosion is accelerated by microbial activities, and determine the resistance of various candidate materials to microbial corrosion. The experimental results obtained in pursuit of these goals are presented in this report.

METHODS

Isolation and Enrichment of YM Bacteria

Rock samples were aseptically collected from rock excavated during construction of the Exploratory Studies Facility (ESF), YM, and from an excavated outcropping of repository horizon geologic material at Fran Ridge, YM. Microorganisms were isolated both aerobically and anaerobically from whole and aseptically-crushed (1.7-2.4mm) rock samples at room temperature either by plating directly, or washing rock samples with Artificial Pore Water (APW, equivalent in composition to J13 well water in the YM area; Amy *et al*., 1992), and plating onto low nutrient R2 agar (Difco). Organisms that survive in nutrient-depleted environments and at elevated temperature were isolated from crushed samples (1.0g) after extended growth (aerobic incubation, 72 h.; anaerobic incubation, 17 days) in R2 broth (R2B; Reasoner, 1985) at room temperatures and 50°C.

Iron oxidizing microorganisms were cultured from YM tuff by inoculating 5g samples of crushed tuff into media previously reported to support the growth of these organisms (i.e., ATCC Media #64, Gherna, 1989). Three known and characterized iron-oxidizing microbial strains were grown in parallel with those enriched from YM tuff (*Thiobacillus ferrooxidans* ATCC#21834, #14119, #33020; Gherna, 1989). 5g samples of aseptically ground tuff were inoculated into two types of sulfate reducing bacteria (SRB) media (Baar's media and sulfate-reducing media; Gherna, 1989; Atlas, 1997) to encourage the growth of SRB from YM rock. The suitability of growth conditions was judged by the growth of a known sulfate-reducer, *Desulfovibrio vulgaris* ATCC#29579 (Gherna, 1989), grown in parallel with test samples. All cultures were incubated at room temperature and periodically, 5-10% of the culture was transferred to fresh media; a total of four transfers were made over several months of incubation.

Quantification of total bacterial biomass contained in collected rock samples was achieved by employing fatty acid analysis of membrane phospholipids extracted from crushed YM rock (Welch, 1991).

Screening for MIC-Related Activities

Identification of acid-producing strains was achieved by growing all isolated strains separately in R2B media with or without amendment of 0.5% glucose, using the pH indicators either bromocresol purple ($pK_{indicator}$=5.3), or methyl red ($pK_{indicator}$=4.1). All strains were incubated at either room temperature or 50°C., depending on their temperature of isolation. Sterile, uninoculated controls were included in all experiments.

Detection of desulfurylating microbes was performed by inoculating individual YM strains into test tubes containing R2 agar supplemented with 0.75% proteose peptone #3 (Difco), and 0.05% lead acetate which precipitates as lead sulfide when sulfide is produced. The resulting lead sulfide is detected by observation of blackening of the agar medium. Sterile, uninoculated control tubes were monitored in parallel with inoculated ones. All samples were incubated either at room temperature or 50° C., depending on the temperature of strain isolation. Growth and (lead) sulfide generation was monitored over a 34 day period.

YM bacterial isolates were screened for slime production initially on R2 agar and then R2 + 0.5% glucose agar, R2 + 0.75% peptone agar, and R2 + 0.5% glucose + 0.75% peptone agar. All isolates were incubated anaerobically or aerobically either at room temperature or 50° C., depending on their mode of isolation. Slime production was assessed by observation of gross colony morphology: the appearance and quantity of a viscous slime layer was noted and scored.

MIC Corrosion Rates

Corrosion cells consisted of a cylindrical glass flange with an O-ring seal to which the working electrode test specimen was clamped to form the bottom of the vessel. The disc-shaped metal coupons had a total exposed area of 28.3cm^2. 450ml of growth media consisting of R2 broth amended with 0.75% proteose peptone and 0.5% glucose in 100X J13 water was used to fill the cell after the coupon had been inoculated. A Saturated Calomel Electrode (SCE) reference was directly immersed in the cell, along with the platinum counter electrode. All elements which came into contact with the growth media were previously sterilized.

Working electrodes of 1020 carbon steel (which is similar in composition to the candidate outer container material, A 516) and Alloy 400 were wet-polished with abrasive paper progressively to 600-grit, cleaned with acetone and distilled water, and then autoclaved before being inoculated with a mixture of 12 strains of YM bacteria (Table 1). The YM bacteria used for inoculation included acid and slime producers, as well as SRB and iron oxidizers. Cell densities were established before aseptically combining and spreading a defined number (at least 10^8 bacterial cells of each strain) of all isolates on coupons, coupons were then air dried before they were exposed to growth media in corrosion cells.

The DC linear electrochemical polarization technique was used to conduct polarization resistance (Rp) measurements in corrosion cells. A potentiostat (EG&G Model 283) was used to perform potential scans within +/-20 mV of the corrosion potential (E_{corr}), at a scan rate of 0.04 mV/sec. The Rp value was calculated by EG&G Model 252/352 Softcorr II software. Rp measurements were used to determine i_{corr} values by the method developed by Stern (1958). Finally corrosion rates [expressed as mils (0.001 in.) per year, mpy] were calculated from i_{corr} values using Faraday's Law.

Growth Rate Determinations

Growth rates of YM-derived microbial communities were determined by adding 10g of crushed rock samples (1.7-2.4mm grain size) to 50ml of R2B. Samples were incubated aerobically by agitating them in covered flasks at ambient temperature, 30°C, and 50°C. Sterile controls were prepared by repeated cycles of autoclaving (120°C) and incubation. Growth was monitored by periodic live plating of appropriate dilutions on R2 agar.

Growth studies to determine the nutrient(s) limiting *in situ* YM microbial growth were carried out using a growth media, "High-pH7.2", which was comparable to M9 bacterial growth media (Miller, 1972), but contained 10-fold J13 levels (Delany, 1985) of chloride and carbonate. Other media were variations of High-pH 7.2: "Low media" was 0.1X High-pH 7.2. Other media variations of High-pH7.2 individually contained 1XJ13 concentrations of sulfate, nitrogen and organic carbon: "No Organic Carbon " media contained no added glucose; and sulfate, nitrogen, and phosphate levels in "J13-SO_4", "J13-NO_3", and "No-PO_4" media, respectively, were individually decreased to match those found in 1X J13. Growth rates of YM bacteria contained in YM tuff were determined by periodic live plating of batch flask cultures and continuously-fed microcosms containing YM tuff as bacterial inoculum. YM tuff was aseptically collected from YM Exploratory Studies Facility alcoves 5, 6, and 7, then aseptically crushed (1.68mm-2.38mm) and homogenized. Batch cultures were contained in 125ml flasks with 5g of crushed tuff and 20ml of media, and incubated at 30∞C., with continuous agitation (150rpm); media were not replenished in these batch systems. Microcosms initially contained 50g of crushed YM tuff and 200ml media, and were continuously supplied with fresh media (5ml/hr). All microorganisms were contained within the microcosm vessel through the use of filters (0.2mm) fitted on outlets, and were incubated at 22∞C. Sterile controls for both batch and microcosm systemscontained crushed YM tuff that was gamma-irradiated (3 Mrads) by exposure to a ^{60}Co source; these were incubated in High-pH 7.2 media.

RESULTS

Diversity of Microorganisms at YM

Previously, characterization of the native microbial communities in the YM region showed a wide array of endogenous subsurface microorganisms (Haldeman, 1993; Russell, 1994). Sampling, isolation and identification of microorganisms contained in the YM post-excavation environment, likewise showed a diversity of organisms, some of which may have been introduced as a result of construction activities.

While any given growth media (e.g., R2) permits only the growth of a small fraction of a microbial community (Atlas, 1982; Roznak and Colwell, 1987), a multiplicity of microbial types were still detected from whole and crushed post-excavation YM rock on low nutrient R2 media. A representative group of distinct individual isolates were purified and identified by fatty acid analysis (Welch, 1991; Analytical Services, Inc., VT). Identified and preserved YM bacterial isolates included representatives of the following genera: *Bacillus, Arthrobacter, Cellulomonas, Corynebacterium, Pseudomonas, Staphylococcus, Xanthomonas,* and *Flavobacterium*. These bacterial classes collectively contain members that are capable of forming spores, producing acids, degrading a wide variety of organic compounds, and remaining active under both oxic and anoxic conditions.

The oxidation of Fe(II) to Fe(III) and the reduction of sulfate to sulfide are elements of different microbial metabolic pathways that have been linked to metal corrosion processes (Lee *et al*., 1994; Borenstein, 1994). Therefore, efforts were made to culture organisms that carry out these transformations from YM geologic samples. All control strains grew well under the conditions provided, showing that the media and conditions used did allow growth of these types of organisms. The iron-oxidizing enrichments from YM geologic samples, likewise produced iron-oxidizing cultures. However,sulfate-reducing bacteria (SRBs, which use transform sulfate to sulfide as a terminal product) were not recovered from all YM geologic samples, despite repeated attempts using two types of sulfate reducing media, and the growth of the control strain under the same conditions. SRB were recovered using the same enrichment techniques from some select sites in the YM region however, demonstrating that the presence of SRB, key players in the corrosion process, are not ubiquitous.

Quantification of extracted fatty acids revealed approximately twice as much biomass, was contained on exposed tunnel walls as was found in deeper, unexposed rock. Biomass contents of these YM rock samples correlated with approximately 4 -7 X 10^4 cells/gram of dry rock.

Screening YM Bacteria for MIC Activities

All isolated YM bacterial strains were screened for their abilities to produce acid, exopolysaccharide slime, and sulfide, all microbial activities associated with metal corrosion.

Results revealed that both growth and acid production were generally more rapid when the media was amended with glucose. When 46 bacterial isolates were tested with glucose amendment, 10 (21.7%) of these displayed a decrease in pH (from about pH 7.0) to pH 5.3 or less after a period of growth lasting from 3 to 16 days. When glucose was not added to the media, only 5 strains tested showed similar extents of acid production. Sterile control cultures maintained the initial pH of the media. The lowest pH attained was 4.46 after a 76 hour incubation by a *Pseudomonas stutzeri* isolate in media with added glucose and the methyl red indicator.

Microbially-produced sulfide may be corrosive to metals, as are the ferrous sulfides that form when produced sulfide reacts with soluble ferrous ions. Most often these activities are associated with SRB, which directly promote corrosion by depolarizing the cathode through consumption of available

hydrogen from the metal surface (Borenstein, 1994). Attempts to isolate classical SRB from the immediate YM environs demonstrated that these organisms are not uniformly distributed. However, microbes can also produce sulfide by the decomposition of proteins, a process known as "putrefaction" or "desulfurylation", in which the sulfur-containing amino acids methionine and cysteine are broken down, releasing sulfide in the process.

When individual YM isolates were examined for the capacity to desulfurylate, 21 of the 45 aerobic strains tested (46.7%) demonstrated sulfide production in the oxic area of the culture, whereas only three of the aerobically-isolated strains (all 50° C. isolates) showed sulfide production in the anoxic area of the culture. Three of the 17 (17.6%) anaerobically-isolated bacteria tested showed sulfide production in the anoxic butt of the tube, one of these had significant sulfide production under aerobic conditions as well. A total of 7 of the 17 (41.2%) anaerobic isolates displayed some detectable sulfide production under aerobic conditions. No sulfide production was detected in any of the sterile controls.

The production and export of polysaccharides (long chain sugar polymers) to the exterior of cells is characteristic of many bacterial species. Exopolysaccharide production results in the build-up of a slime layer which protects, embeds, and allows anchorage of cells to solid surfaces. Generally, it can be expected that differential nutrient/endproduct concentrations, relative humidity, and oxygen tensions exist throughout the film (Costerton and Geesey, 1985). Slime producing organisms probably indirectly contribute to corrosion by permitting adherence and facilitating interactions among the different types of organisms that cause corrosion. Direct effects of slime on corrosion include the creation of differential aeration cells and localized elevations of ions, which in turn form crevices under which corrosion is accelerated (Borenstein, 1994; Costerton and Boivin, 1991).

When YM isolates were screened for slime production, it was found that prodigious amounts of polysaccharide were produced by two *Bacillus* isolates and two as yet unidentified isolates with very similar colony morphologies. All of these strains were identified as sulfide producers in former screening analyses, however none of these were identified acid-producers (above, Table I). Moderate quantities of slime were generated by five other YM strains that included representatives of the genera *Arthrobacter* and *Pseudomonas*. Retesting of six of these slime generators on R2 agar supplemented with peptone and glucose revealed that slime production varied with media type. The greatest degree of slime production occurred on media containing glucose for two of the strains tested. The other isolates showed little difference in the quantity of polysaccharide produced between media types. A single anaerobic isolate demonstrated copious slime generation on R2 agar.

Table I summarizes the overlap between corrosion activities among a representative group of YM bacterial isolates.

PLACE TABLE I HERE

Real-Time Measurement of MIC by Polarization Resistance

A subset of the characterized YM bacteria were spread on candidate WP material coupons in systems designed to collect polarization resistance (Rp) data for corrosion rate calculations, and to determine cathodic and anodic potentiodynamic polarization behavior to assess corrosion mechanisms.

The observed corrosion rate of Alloy 400, as expected, was lower than those observed for 1020 carbon steel (CS) (Fig. 1). However, the corrosion rate of Alloy 400 increased nearly 3-fold during the first two weeks of incubation, while that of 1020 CS decreased. Thus, even though there was an initial differential of 80-fold in corrosion rates (0.012 mpy for Alloy 400 and 0.98 mpy for 1020 CS), this was decreased to 7-8-fold after a steady state was achieved (approximately 0.04 mpy for Alloy 400 and 0.3 mpy for 1020 CS). Even though the corrosion rates were low for Alloy 400 coupons, Rp measurements did not show noise or passive film effects.

PLACE FIG. 1 HERE

Initially, at the start of the incubation period, redox potentials of bulk solution in Alloy 400 cells (as measured by a bare platinum electrode) were more noble, at about -180 mV versus the reference electrode, compared to about -400 mV in 1020 CS-containing cells. The E_{corr} for 1020 CS was on the order of -660 mV versus the reference electrode, while that of the Alloy 400 increased from -380 mV to -300 mV (became more noble) during continuous incubation.

Growth Rates and Parameters of Whole YM Bacterial Communities

Growth rates of whole communities of YM-derived microorganisms in low nutrient R2B varied somewhat, depending on the temperature of incubation. While communities grown at room temperature or 30°C. showed altered doubling times with respect to those grown at 50°C., all cultures demonstrated significant increases in cell numbers, to over 10^{7} cells/ml of media at the conclusion of the 30 h. growth period (Fig. 2). Additionally, it was found that some bacterial isolates, most likely spore-forming species, survived repeated exposure to 120°C.

PLACE FIG. 2 HERE

Extended growth at room temperature and 50°C generally showed a low diversity of microbial forms (one to three cell types). However, high cell numbers were reached after extended growth under aerobic conditions (2 x 10^{8}-10^{9} cells/ml), while anaerobic conditions produced low cell densities (e.g., 140 cells/ml), and no growth was evident after extended anaerobic incubation at 50°C.

Growth studies were also carried out to determine the nutrient(s) limiting *in situ* YM microbial growth. For this purpose, a growth media, High-pH7.2 (see Methods) was employed. Growth rates were determined in both shake flasks (which were not replenished), and in continuously fed systems. High-pH7.2 media supported significant growth of YM microorganisms at both the initial and 0.1X (Low) concentrations. When concentrations of the individual macronutrients organic carbon, phosphate, sulfate and nitrogen were decreased to 1X J13 levels, significant bacterial growth was still evident. Final cell concentrations after 6 days of incubation were in excess of 10^{6} cells/ml in all cases (Fig. 3).

PLACE FIG. 3 HERE

DISCUSSION

Extant Microorganisms in the Post-Construction Repository Environment

Samples of Topopah Springs tuff were aseptically collected from a mined Fran Ridge outcrop [the Large Block (LB) Test Site], rock rubble excavated during construction of the ESF, and from the tunnel walls of the ESF. Thus, collected rock samples are representative of the post-construction repository environment. Bacteria introduced by construction machinery, on repository construction materials, and by human intervention were thus included in the isolations and testing described here.

Identification of bacteria contained in excavated samples on a low nutrient source shows good agreement with organisms previously isolated from undisturbed subsurface areas in the YM region (Haldeman, 1993). However, two bacterial genera found in the post-excavation environment, Flavobacteria and Cellumonads, were not reported to be native to this environment; these may have been introduced by construction activities. Even with the limitation imposed by growth on a single nutrient source (which

only allows detection of a fraction of the extant types of microorganisms), the range of bacteria found include some of those associated with MIC.

When samples collected from the ESF tunnel walls were quantitatively analyzed for their content of fatty acids, an indicator of the total bacterial biomass, twice as much was found on the exposed tunnel walls as that detected on unexposed rock. This could indicate that the walls of the tunnel are inoculated with bacteria introduced as part of the excavation process, and thus agree with the finding that organisms were found in the mined rock which were not present in the native community. Alternatively, since quantitative analysis of total fatty acids does not distinguish between the types of bacteria present, this finding could indicate that the alteration in environment caused by excavation produced growth of native organisms.

Use of alternative media specific for growth of iron oxidizers and SRB showed the prevalence of acid-tolerant iron oxidizers, but sulfate reducers were not found in the rock samples collected from the ESF. SRB have been found in other areas adjacent to YM (Amy, personal communication). Therefore, it must be assumed that SRB distribution is not ubiquitous. Since sulfate reducers have been described as important bacterial components of MIC (Lee, 1995), the non-uniform distribution of SRBs indicates that sulfide production may not be a major factor in potential MIC in the potential repository environment.

Both higher temperatures and anoxic conditions could result in lower diversity of microbes obtained under these culture conditions. Topopah Springs tuff and its associated pore water has been characterized as generally containing a significant amount of oxygen (Buscheck and Nitao, 1994), such that strictly anaerobic, or even facultatively anaerobic organisms, might be expected in lower abundance relative to strict aerobes. Since elevated temperatures are not the norm in the subsurface, those organisms adapted to growth at 50°C. may not likewise be present in high abundance. However, the demonstration that at least some organisms at the YM site are capable of growth at higher temperatures and under oxygen pressures lower than that found in the atmosphere, shows that a subset of the extant YM microbial community harbor the potential to grow and survive at elevated temperatures and under reducing conditions.

Capabilities of YM Microorganisms to Promote MIC

Screening isolated YM organisms for their abilities to produce acid, slime, and generate sulfide by putrefaction (desulfurylation) showed that, at least under some conditions among given bacterial isolates, these capabilities are present.

The greatest degrees of slime and acid production generally occurred when organic carbon was available. Since organic carbon-containing compounds (*e.g.*, rubber, fuels, plastics) are expected to be introduced into the potential repository, these may act to increase MIC rates, if they are bioavailable. Subsequent experiments showed that rates of acid production over a three to seven day period are measurable and result in orders of magnitude differences in proton concentration. The rates of acid production, in view of the long time spans that must be considered to evaluate the integrity of the YM repository, are significant and could contribute to MIC of WP materials.

Screening for sulfide production by individual YM isolates demonstrated that a significant proportion of YM bacterial isolates are capable of producing sulfide *via* putrefaction under both oxic and anoxic conditions, at least when provided with excess protein substrate. Isolates of *Pseudomonas*, *Flavobacterium*, *Bacillus*, *Arachnia*, and *Cellulomonas* were all found to produce sulfide; four of these isolates were found to produce both acid and sulfide using these screening techniques (Table I). The use of these putrefying bacteria in accelerated corrosion tests should allow a more precise determination of whether hydrogen sulfide and associated metal sulfides are directly capable of causing corrosion to WP materials. Since these microbes generate sulfides by cleaving reduced sulfide groups from proteins and do not engage in sulfate reduction and hydrogen utilization, the effects of *only* sulfides on corrosion can

be assessed absent cathodic depolarization effects caused by hydrogen stripping from the metal surface (Borenstein, 1994).

Several of the identified sulfide producers were further quantitatively analyzed for their rates and extents of sulfide production. Preliminary results showed sulfide concentrations increased from zero to about 500ppb for all strains grown under anaerobic conditions, and to approximately 330ppb for the same isolates incubated aerobically. Thus, microbially-induced sulfide production increased significantly above background levels, and was detected among a wide variety of YM isolates under both aerobic and anaerobic conditions when strains were provided with an excess protein source (in the form of added peptone). These strains could, therefore, conceivably contribute to MIC given permissible environmental conditions.

Currently, a more representative approach is being undertaken by analyzing these chemical factors in a continuously fed system, with YM rock (including the endogenous microorganisms), and material coupons. Comparison with identical systems containing sterilized rock will allow determination of the chemical contribution of YM bacteria to metal corrosion. These results, along with growth rate data, will be compiled into a predictive model to better assess the performance of WP materials over the long term.

Assessment of YM Bacterial Growth Rates and Conditions
The potential for MIC in the YM repository will partly depend on the ability of the microorganisms that are present to grow. Even under permissive growth conditions, MIC rates will be determined by microbial growth rates, as well as by other factors.

Determination of whole YM communities growth rates in R2B, using unsterilized crushed YM rock as inoculum, revealed that even a modest nutrient source supports significant logarithmic growth of native and introduced YM bacteria, and total microbial cell densities reach similar levels regardless of the temperature of incubation, up to 50°C. (Fig. 2). It was found that some YM bacteria (most likely sporulating species) were able to survive repeated autoclaving at 120°C . This finding demonstrates that even at the highly elevated temperatures expected to occur after waste emplacement in the repository, some YM microorganisms may be able to survive through the predicted periods of intense heat.

Extended growth in R2B resulted in the maintenance of high cell numbers despite depletion of nutrients, under oxic conditions. This might be expected of organisms adapted to a low nutrient environment such as YM rock; they demonstrate the ability to survive through depleted conditions. The low cell densities achieved under anoxic extended growth in R2B are indicative of energetically unfavorable anaerobic metabolic processes, and may also indicate that nutrients specific for anaerobic organisms are either in low abundance or are unavailable for use.

Cell densities in the ESF were estimated to be on the order of $4\text{-}7 \times 10^4$ cells/g of dry rock, this is low when compared to a more nutrient rich environment where microorganisms can achieve densities of 10^9 cells/g (Alexander, 1997). While the cell density of native microorganisms is low in the pristine or even mined YM environment, this environment will be greatly perturbed upon emplacement of nuclear waste and other repository components. Therefore, central to assessing the contribution of MIC to containment of nuclear waste, is whether MIC-causing organisms will grow in the altered repository environment. Another way of posing this question is to determine the factors that are now limiting growth in the mountain; presumably definition of limiting factors will define permissive growth conditions. Initial experiments to determine these limiting factors used a simple, defined growth media (M9; Miller, 1972) which was altered slightly to more closely conform to the composition of YM pore water (J13). Significant bacterial growth was observed in this media (Fig. 3). Nutrients that are used for bacterial metabolism (in the greatest quantities) were individually reduced to levels found in YM pore water, and significant growth was still observed. These findings indicate that bacterial growth in YM is not

limited by a lack of organic carbon, phosphate, nitrogen, or sulfate, at least under saturated, aerobic conditions. Sterilized YM tuff produced no growth, indicating that the observed bacterial growth in non-sterilized cultures originated from YM tuff.

The growth of microorganisms in the absence of added organic carbon (YM tuff contains no organic sources of carbon), suggests that bacterial carbon dioxide fixation accounted for growth under these conditions. Nitrogen and sulfate concentrations in YM pore water is also apparently sufficient to support microbial growth, as growth was also observed when the concentrations of these species was reduced to J13 levels.

J13 pore water contains trace levels of phosphate, which is used in significant quantities for microbial metabolism. However, bacterial growth was still observed in both batch and microcosm cultures when phosphate was eliminated from growth media. YM microorganisms thus obtained the necessary phosphate for growth under the described culture conditions. YM geologic material reportedly contains trace amounts of phosphate (Broxton, 1987), and phosphate dissolution from the tuff could explain the source of phosphate.

These last results are preliminary, but they strongly suggest that nutrient levels are sufficient to support growth of native bacterial communities in YM, under some environmental conditions. Thus, avoiding the use of materials that contain microbial nutrients may not preclude microbial growth in the potential YM repository. Currently, experiments using simulated J13 pore water as a basis for media formulations is ongoing.

Assessing Overall MIC Rates by Polarization Resistance

The goals of these studies were to design and implement a system whereby the contribution of YM bacteria to overall corrosion rates of candidate WP materials could be quantitatively determined. Further, attempts were made to generate a system whereby environmental factors could be altered, and candidate materials could be tested for their relative susceptibilities to MIC. The signal to noise ratio was greatly improved from earlier utilized systems by enlarging the size of the coupon and integrating the reference electrode directly into the same cell containing the working electrode. Development of this new cell type has permitted the evaluation of MIC of more corrosion resistant WP candidate materials.

Since the coupon is entirely submerged, and the media was not aerated throughout the incubation period, electrochemical conditions at the coupon surface were uniform and more anoxic, as oxygen was presumably progressively consumed by aerobic microbial activities in the improved system. Thus, the enhancing of differential oxygen concentrations by microbial activities was not observed in this system, and corrosion rates were correspondingly reduced. Future modifications to this system will therefore include continual aeration of the media to better simulate the prospective YM repository conditions, and obtain better estimates of MIC rates.

Conditions in the 1020 CS-containing corrosion cells were more reduced than those in the Alloy 400 cells. Since all other conditions between these cell types were equivalent (except the type of metal coupon) , the increased Fe^{+2} in cells containing 1020 CS may have caused a greater consumption of oxygen by iron oxidizing bacteria. 1020 CS may also be more favorable to microbial growth generally, causing a greater degree of oxygen uptake by aerobic organisms. Alternatively, chemical oxidation of solubilized Fe^{+2} would deaerate the solution in 1020 CS cells, and thereby lower redox potential.

Measured corrosion rates on both inoculated and sterile coupons changed during the incubation period until they reached a “steady state” value. Initial high rates on inoculated coupons may reflect the ready availability of nutrients. Lower rates observed later in the incubation period could indicate exhaustion of the media immediately surrounding the material coupon or buildup of toxic end products.

Since diffusion of nutrients and end products would be minimal, it is expected that the observed "steady state" may not reflect one that would occur in a continuously fed system. Water (and accompanying solutes) are expected to eventually invade the repository in a more-or-less continuous fashion, a continuously fed system may therefore be a better reflection of actual repository conditions, and so provide a better measure of MIC over a long period. Despite these caveats, it was possible to discern 7-8-fold difference in MIC between 1020 CS and Alloy 400. Microbial contributions to corrosion cannot be discerned until sterile controls are included using this system.

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REFERENCES

M. ALEXANDER, "Microbial communities and interactions:a prelude," *in* Manual of Environmental Microbiology eds. C.J. Hurst, G.R. Knudsen, M.J. McInerney, L.D. Stetzenbach, and M.V. Walter. Washington, D.C., American Society for Microbiology Press. pp. 5-13, (1997)

P.S. AMY, D.L HALDEMAN, D. RINGELBERG, D.H. HALL, AND C. RUSSELL, "Comparison of identification systems for classification of bacteria isolated from water and endolithic habitats within the deep subsurface," Appl. Environ. Microbiol.,58:3367-3373 (1992)

R.M. ATLAS, "Enumeration and estimation of biomass of microbial components in the biosphere, *in* Experimental Microbial Ecology eds. R.G. Burns and J.H. Slater. Oxford,England,Blackwell Scientific Publishers. pp.84-102 (1982)

R.M. ATLAS, Handbook of Microbiological Media, second edition, Boca Raton, FL, CRC Press,Inc., (1997)

S.W.BORENSTEIN, Microbially Influenced Corrosion Handbook. New York, NY, Industrial Press Inc. (1994).

D.E. BROXTON, D.L. BISH, R.G. WARREN, "Distribution and chemistry of diagenetic minerals at Yucca Mountain, Nye County, Nevada," Clays and Clay Minerals, 35:89-110, (1987)

T.A. BUSCHECK AND J.J. NITAO, 1994, "The impact of buoyant gas-phase flow and heterogeneity on thermo-hydrological behavior at Yucca Mountain". Proceedings of the International High Level Radioactive Waste Management Conference, Las Vegas, NV and, UCRL-JC-115351, Lawrence Livermore Natl. Laboratory Report.

J.W. COSTERTON AND J. BOIVIN, "The role of biofilms in microbial corrosion," *in* Microbially Influenced Corrosion and Biodeterioration, eds. N.J.E. Dowling, M.W. Mittleman, and J.S. Danko. Houston, TX, National Association of Corrosion Engineers, (1991).

J.W. COSTERTON AND G.G. GEESEY, 1985,"The microbial ecology of surface colonization and of consequent corrosion". Proceedings of the International Conference on BiologicallyInduced Corrosion, Gaithersburg, MD.

J.M. DELANY, "Reaction of Topopah Spring Tuff with J-13 Water: A Geochemical Modeling Approach Using the EQ3/6 Reaction Path Code," UCID-53631, Lawrence Livermore National Laboratory, (1985).

R. GHERNA, P. PIENTA, AND R. COTE, eds. Catalogue of Bacteria and Bacteriophages. 17th edition. Rockville, MD, American Type Culture Collection, (1989)

D.L. HALDEMAN , P.S. AMY, D. RINGELBERG, AND D.C. WHITE, "Characterization of the microbiology within a $21m^3$ sectioon of rock from the deep subsurface," Microb. Ecol, 6:145-159, (1993)

W. LEE, A. LEWANDOWSKI, P. H. NIELSEN AND W. A. HAMILTON, "Role of sulfate-reducing bacteria in corrosion of mild steel: a review," Biofouling,8:165-194, (1995)

J.H. MILLER. Experiments in Molecular Genetics, Cold Spring Harbor, N.Y., Cold Spring Harbor Laboratory Press, (1972)

D.J. REASONER and E.E. GELDREICH, "A new medium for the enumeration and subculture of bacteria from potable water." Appl. Environ. Microbiol. 49:1-7, (1985)

D.B. ROZNAK, AND R.R. COLWELL, "Survival strategies of bacteria in the natural environment," Microbiol. Rev., 51:365-379, (1987)

C. E. RUSSELL, R. JACOBSEN, D. L. HALDEMAN AND P. S. AMY, "Heterogeneity of deep subsurface microorganisms and correlations to hydrogeological and geochemical parameters," Geomicrobiol. J., 12: 37-51, (1994)

M. STERN, "A method for determining corrosion rates from linear polarization data,"Corrosion, 14:440t-444t, (1958)

D.F. WELCH, "Applications of cellular fatty acid analysis," Clin. Microbiol. Rev., 422-438, (1991)

Table I. Yucca Mountain Bacterial Isolates Displaying Corrosion-Related Activities

Strain#[a]	Species Identification[b]	Acid Production’	Sulfide Production[d]	Slime Production[e]
• ESF-CI	Cellulomonas flavigena	X	x (oxic)	
LB-71 h-UT-1 3	Pseudomonas stutzeri	X		
• ESF-71 h-FIT-4	Flavobacterium esteroaromaticum	X	X (oxic)	
*LBan-U7	uncharacterized	X		
LBan-UW2	Cellulomonas turbata	X		
‘LB-71 h-50-3	probably Bacillus sp.	X		
ESFan-U4	Bacillus circulans	X		
LBan-C1	Arachnia propionica (poor match)	X	X (oxic/anoxic)	
LBan-UW2	Cellulomonas turbata	X		
LBan-U1	Cellulomonas galida	X		
LBan-U2	Bacillus pabuli	X	X (anoxic)	
• LBan-U3	Bacillus pantothenticus		X (anoxic)	X
LB-71 h-50-2	Bacillus pumilus subgroup B		X (anoxic)	
*LB-71 h-50-4	Bacillus subtilus		X (anoxic)	X
‘LB-71 h-50-6	probably Bacillus sp.		X (anoxic)	X
ESF-71 h-RT-1	Flavobacterium esteroaromaticum		X (oxic)	
‘LB-CI	uncharacterized		X (oxic)	X
LB-C2	uncharacterized		X (oxic)	
LB-C7	Pseudomonas stutzeri		X (oxic)	
‘LB-71 h-RT-15	Pseudomonas pseudoflava			X
LB-71 h-RT-4	Pseudomonas pseudoflava			X
LB-CW-6	Arthrobacter oxydans			X

‘Used as inoculum for electrochemical determination of corosion rates with iron oxidizing and sulfate reducing enrichment cultures.

[a]ESF=Exploratory Study Facility: LB=Large Block; an=isolated under anaerobic conditions; C=crushed rock isolate; U=uncrushed rock isolate; W=isolated from after washing rock; 71 h=isolated after 71 hours of growth; RT=room temperature isolate; 50=50°C. isolate.

[b]Determined by fatty acid analysis using the MIDI/Hewlett Packard microbial identification system (MIS; Analytical Services, Inc., VT); identification of given isolates may be tentative due to a lesser degree of similarity with type organisms contained in the MIS database.

‘Determined after growth in R2 media with or without 0.5% glucose and pH indicators bromocresol purple and methyl red.

[d]Determined after growth in R2 agar media (Difco) containing 0.75% proteose peptone #3 (Difco) and lead acetate.

[e]Determined after growth on R2 agar (Difco).

Fig. 1. Corrosion of inoculated Alloy 400 (open symbols) and 1020 carbon steel (closed symbols) as a function of incubation time. Each curve depicts data collected from a single trial/corrosion cell.

Fig. 2. Effect of temperature on the growth rate of whole YM microbial communities in R2 media. Crushed YM tuff was inoculated in media and incubated aerobically at 20°C (●), 30°C (□), or 50°C (A), sterile uninoculated media incubated at 20°C., (X). Periodically samples were withdrawn and cell count was determined by live planting.

Fig. 3. Growth rates of YM communities as a function of macronutrient concentrations in minimal microbial media composed as described in the Methods. (■), High-pH7.2 media; (□),Low media; (A), No Organic Carbon media; (A), J13-PO_4 media; (●), J13-NO_3; (O), J13-SO_4; (◆), sterile control containing irradiated rock.

Fig 1

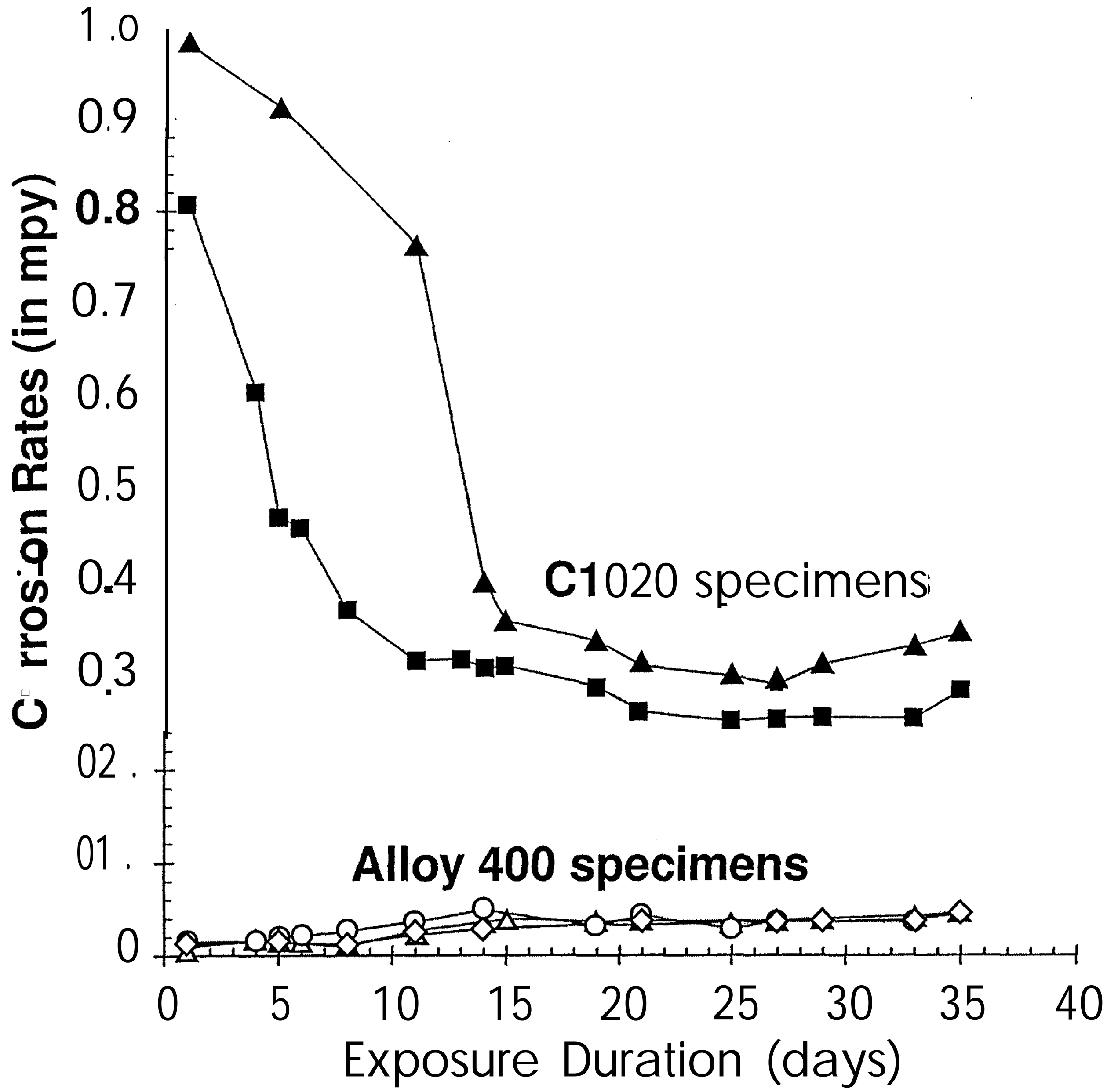
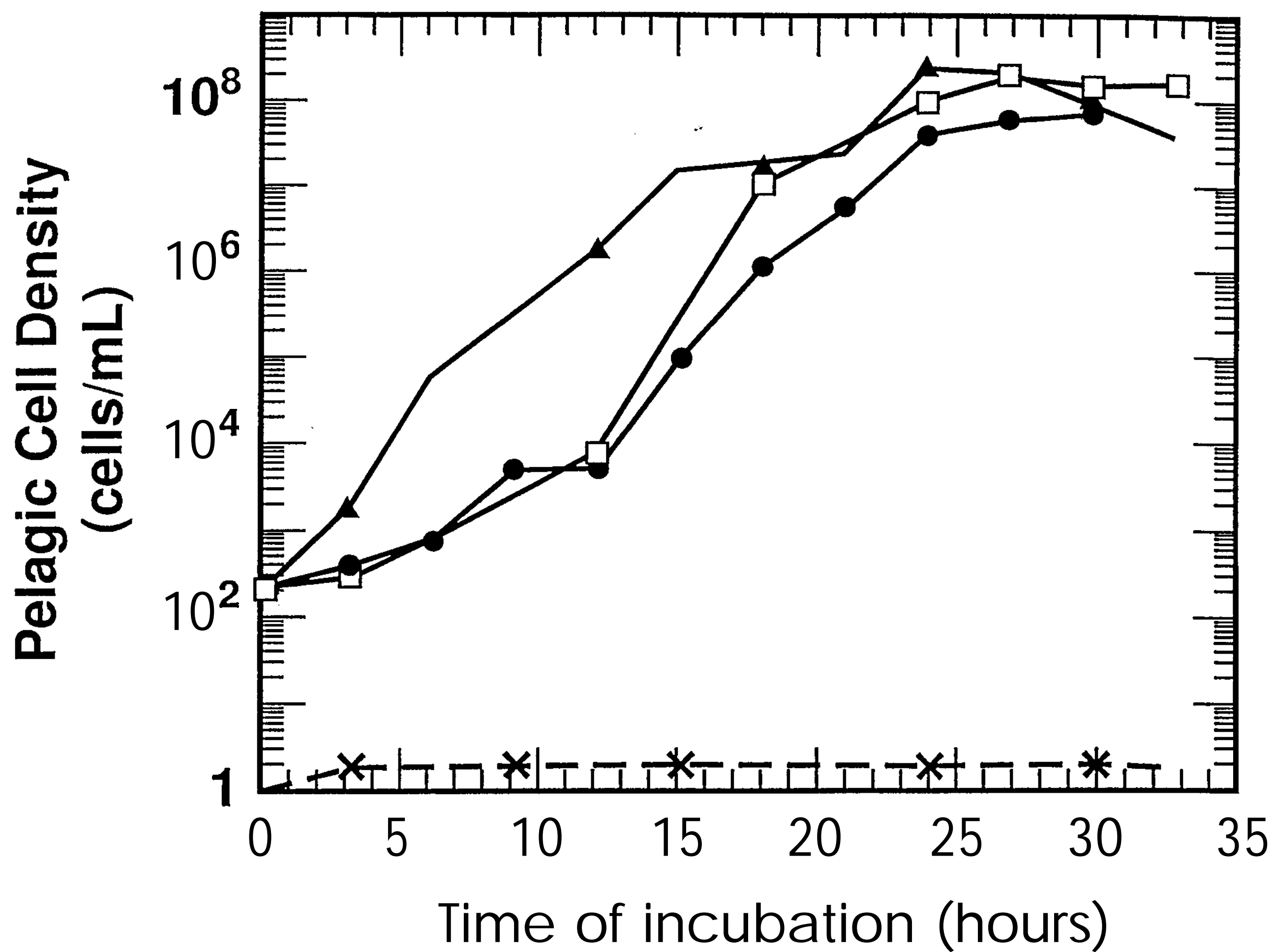


Fig 2



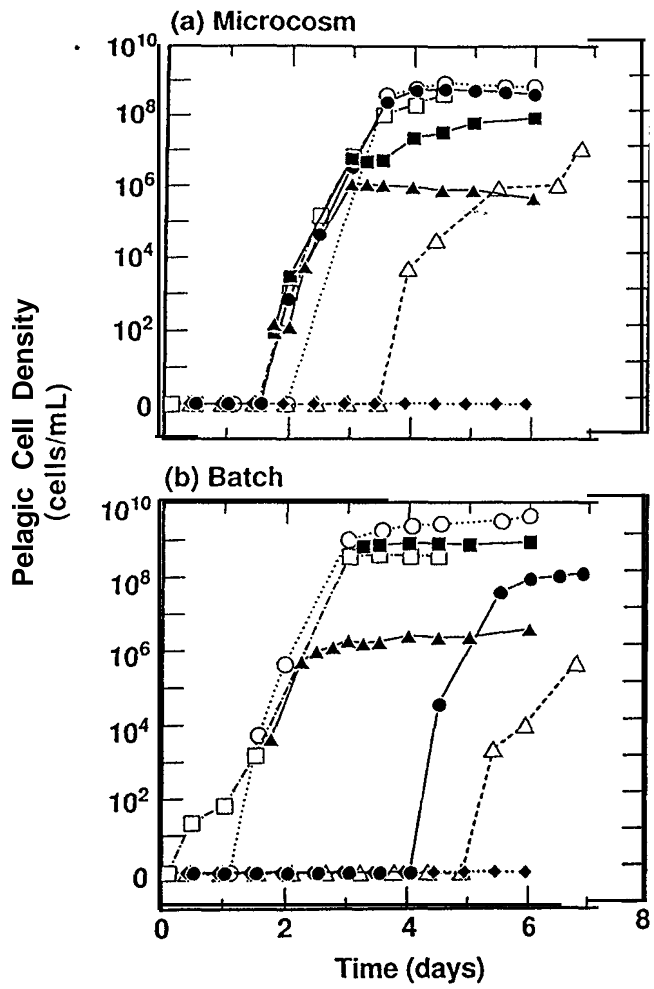


Fig 3

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