

anaerobic tubes were grown in anaerobic jars. The average growth cycle was 6 days. A total of ten experiments were conducted of which 8 were run concurrently. 1 was invalidated.

As the growth of the organism progressed, the culture tubes were monitored on a daily basis using a photospectrometer to measure the density of the culture. At the completion of the cycle the cultures were frozen for nitrogen absorption analysis at a later date. The data gained from these readings were compared and yielded the following results:

#### Aerobic findings

On average, the organisms experienced the greatest growth in the aerobic tubes containing less than 5 mL of Perchlorate solution in nitrated media. There was less than a .2 peak density difference on average between the tubes containing pure nitrated media and the cultures grown in tubes containing .5 and 2.5 mLs of perchlorate solution. When compared with the aerobic cultures grown in non-nitrated media with less than 5 mL of Perchlorate, the average non-nitrated peak density readings were 1.65 as compared to 2.0 for the nitrated groups.

The aerobic tubes containing 5 mLs of .01, .2, and .4 M concentrations of Perchlorate solution also experienced significant organism growth, however, the peak densities would lag the tubes containing less of a Perchlorate concentration by 2 to 3 days. The peak average density readings of these tubes were approximately 1.45 as compared to the 2.0 measurements of the cultures grown in nitrated solutions containing less perchlorate. The peak readings for the cultures grown in non-nitrated solutions with higher percentages of Perchlorate were an average 1.25 compared to the 1.65 of the lesser concentration non-nitrate groups.

#### Anaerobic findings

The peak density averages of the cultures grown under anaerobic conditions were far more varied than the results of the aerobically grown cultures. While the peak densities of the anaerobic cultures were far lower than those grown with oxygen, the same growth trends were evident.

The highest peak density averages belonged to the organisms grown in the nitrated mediums containing less than 5 ml of

Perchlorate solution. The average peak density of this category was a 1.0 compared to the 2.0 peak of the aerobic category, or about half. The highest readings for the entire anaerobic group came from the cultures grown in pure nitrated media with an average peak of 1.2. The average of the non-nitrated anaerobic group was a 0.5 peak compared to the 1.65 of the aerobic category.

The performance of the cultures grown in concentrations of 5 mL and .1 M of perchlorate and greater were the most impacted categories when compared to the aerobic findings. In these categories, the cultures experienced very little growth with a .04 being the average peak of the nitrated media groups as compared to the 1.45 of the aerobic counterparts. The peak average of the non-nitrated tubes was only a .03 compared to 1.25 in the aerobic groups.

#### Conclusions

After an analysis of the data, it would appear that although the Perchlorate had an initial inhibitory effect on the growth of the *P. fluorescens* organism, by itself it only delayed and in fact did not prevent the growth of the organism. Only the combination of an anaerobic environment and high levels of Perchlorate may have prevented growth. The density levels measured in those categories may have been false readings due to the high levels of dissolved compounds in the medium. The Perchlorate inhibitory effect may also have been limited due to the breakdown of the compound in the solution. It may have reacted with the other components forming a non-inhibitory substrate.

It is possible that experiments conducted in a soil medium with a minimum of solvent would prevent the quick break down of the inhibitor and could yield different results. The case for the loss of efficacy of the perchlorate is supported by the data from the measurements of the non-nitrated medium categories. While the organism did not reach the high densities seen in the nitrated groups, the average peak readings were less than 25% below those of the highest nitrate peaks. Since the *P. fluorescens* is an obligate anaerobe only, the combination of anaerobic conditions and high inhibitor levels may have prevented its growth. These conclusions are tentative and will require further study.

**EFFECT OF PERCHLORATE INHIBITOR ON THE GROWTH OF THE PSEUDOMAS FLUORESCENS ORGANISM.** J.H. Wright Undergraduate (Microbiology) University of Arkansas, Fayetteville AR. (jhw04@uark.edu), Dr Timothy A. Kral, Associate Professor of Microbiology, University of Arkansas, Fayetteville. (tkral@uark.edu). Arkansas-Oklahoma Center for Space and Planetary Science. UARK.

After three successful Mars lander missions and in-situ analysis of collected soil samples in several planetary locations, no sign of micro-organismal life has been detected. Although these samples have returned several false positive readings, the final verdict has been that these surface locality samples were sterile and devoid of life as we know it. But why? The purpose of this project was to explore one of the possible explanations for these findings.

The objectives of the project were to introduce a selected organism (*Pseudomonas fluorescens*) into a denitrifying medium containing various levels of an inhibitor. The growth cycles of the organism in this medium were compared with control groups grown without the inhibitor. These experiments were conducted at standard temperature and pressure under both aerobic and anaerobic conditions and in nitrated and non-nitrated media.

The terrestrial soil chemical model for the experiment was based on the Atacama Desert of Chile. It has conditions similar to that found on Mars. It is very dry and arid. Some areas have not seen rain for centuries. At ten to fifteen million years old, it is the oldest desert on Earth. At its core, the desert is almost sterile. The soil composition is oxidative with a chemical make-up similar to martian soil samples. Although it contains the world's richest deposits of nitrate, little in the way of expected organisms are found. There is even a lack of cyanobacteria, the most basic of autotrophic organisms. Is this due to the oxidative nature of the soil preventing denitrification by organisms? This experiment tested this hypothesis using a chemical inhibitor similar to that found in Atacama soil.

The *Pseudomonas fluorescens* organism is an obligate anaerobe, which is widely studied as a biological control agent. It is hardy and is able to survive under a variety of environmental and chemical conditions which made it an ideal candidate for this study.

The chemical composition of the basic growth media consisted of glycerol, yeast

extract,  $(\text{NH}_4)_2\text{SO}_4$ ,  $\text{K}_2\text{HPO}_4$ ,  $\text{MgSO}_4$ ,  $\text{KH}_2\text{PO}_4$ , and  $\text{CaCl}_2$  in solution. The media was then divided into a nitrated and a non-nitrated group with the addition of  $\text{NO}_3$  to 50% of the solution. Each of these two groups were further divided into two more groups, Perchlorate ( $\text{ClO}_4^-$ ) solutions in concentrations of .1, .2, and .4 M were mixed from one of each of the groups. The Perchlorate was used as the denitrification inhibitor in this experiment. The four groups of media were then divided into test tube sets of 4 apiece and combined in various concentrations of 5 mL total volumes. The following table documents the specific concentrations of media.

	<u>Nitrated media</u>		<u>Non-Nitrated media (mL)</u>	
	$\text{ClO}_4^-$	$\text{NO}_3^-$	media	Plain media
	0.1 M			
1.	0.5	4.5		
2.	2.5	2.5		
3.	5.0	0		
4.	0	5.0		
	0.2M			
5.	5.0			
	0.4M			
6.	5.0			
	0.1M			
7.	0.5			4.5
8.	2.5			2.5
9.	5.0			0
10.	0			5.0
	0.2M			
11.	5.0			
	0.4M			
12.	5.0			
Each set x4			total 96 tubes	

Each tube was inoculated with  $100\mu\text{m}$  of cultured organism from stocks grown in nitrated and non-nitrated mediums. Each group of 4 tubes were divided into aerobic and anaerobic groups. The aerobic groups were grown in mixing tables to ensure constant circulation; the