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OAK RIDGE RESEARCH INSTITUTE
CENTRAL OFFICE
December 8, 1993

MEMORANDUM

FROM: Irene McMullen

TO: Eleanor Blue

SUBJECT: Contract #21607; Final Report Task #3

As per your request here is another copy of the final report on Task Order #3. If you should need additional information, please do not hesitate to call.

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OSTI

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Task #3

Final Report

Proposal for Optimizing a Biological Treatment System for
Dentrification of Y-12 Waste Stream.

Dates covered - March 16, 1987 - Sept. 15, 1987.

From: Grenetta McKinstry, Tanya Osborne, Adolf King,
and Cynthia Tolman.

To: I. Jeter

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MASTER

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yes

Introduction

At the start of this project (inception), very little was known about microbial activity in Y-12 denitrification of plating rinse waste (PRW) process tanks. Due to research efforts at Oak Ridge Research Institute (ORRI) and with the helpful assistance of engineers Jeter and Zablesky, we were able to accumulate a wealth of knowledge about the tank process itself.

Biological denitrification as a metabolic process in nature is not new. There are numerous reports in the literature on the isolation of denitrifying bacteria from soil (3, 5, 6), sewage-sludge (4), contaminated waters (2,4), lakes, rivers, and streams (1, 6.), and etc. Biological denitrifications in large batch style, 10,000 gallon, process tanks, however, such as those at Y-12, used for nitrate reduction of PRW, is relatively new. That's why this project was started. Much of the information we've generated under the contract is revolutionary for the type of system in question.

This report will provide a summary of what we've accomplished at ORRI under the contract proposal "Optimizing a Biological Treatment System for Denitrification of Y-12 Waste Stream", from inception to contract end.

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General Objectives

To learn as much as possible about microbial activities in Y-12 waste stream process tanks in 6 months or less. There were some directives, however, and these were:

1. Isolation, purification, and characterization, of isolates present in F4 process material. F4 was a denitrification process tank that went to completion in terms of NO_3 N_2 . Sample material from the tank was given to ORRI for research efforts under this contract. F5 was a denitrification process tank that had not gone to completion at the time ORRI received process sample material. F5 contained high amounts of NO_3 and NO_2 .
2. To determine which of the purified microorganisms isolated from F4 were denitrifiers and of those denitrifiers which were most important to the denitrification of PRW in Y-12 tanks.
3. Determine the upper and lower limits of several physiological parameters important to the success of denitrification in Y-12 waste stream tanks. These parameters included pH, DO, Temp., phosphate, nitrogen content, carbon source, and metal content.
4. Detect any toxic inhibitors in plating rinse waste such as various metals, specifically Cu, Ni, Zn, Pb, and Cr, that may slow down or inhibit denitrification of Y-12 plating rinse waste (PRW). In addition, we included Al, U and Tl for investigation.

5. Provide seeding methodology for seeding Y-12 denitrification waste stream process tanks.

Materials and Methods

Left out of this report. See milestone 1, 2, and 3 progress reports.

Project Accomplishments and Discussion

There were many. They included:

1. Development of laboratory ecosystems (microcosms) closely resembling the Y-12 denitrification process tanks in so far as ingredients and microbial consistency. Any methodologies developed under this contract are the ownership of Martin Marietta and can be used for any future work on this subject matter.
2. Laboratory protocols specifically designed for experimentation with PRW (see milestones 1, 2, and 3 progress reports).
3. The isolation, purification, and characterization of 17 denitrifiers and other bacteria from F4 and F5 process material. Duplicate agar slants of each denitrifier and the other bacteria were provided to Y-12 engineer, Jeter, on May 16, 1987.
4. Provided data on several physiological parameters of interest to Y-12, (a e below).
 - a) DO - Determined the importance of keeping oxygen out of the tanks during "denitrification" itself. At process

start-up, when the microorganisms enter the tank ecosystem with sewage-sludge inoculum, oxygen is needed for growth. However, denitrification is an anaerobic process. Oxygen inhibits. This could explain the abrupt stopping periods during the denitrification cycle that has occurred in the past in Y-12 process tanks. If oxygen is introduced at any time during denitrification, brief interruptions of the process may occur.

b) C - source - We found succinate to be a more efficient carbon source than Ca Acetate. Also, when using acetate, peptone assists in growth of microorganisms and denitrification in minimal medium containing PRW and in the mimic tank ecosystems. Without peptone, poor to no growth occurred in minimal medium.

c) pH - Alkaline pH proved to be preferential for the process, optimums between 8.5 - 9.5. Undesired pH conditions were acidic, below 6.5.

d) Temperature - Optimums were between 35°-39°C, although 25°C would allow the process to go to completion. The rate was much faster at the higher temperature than 25°C and therefore, a temperature control unit on the Y-12 process tanks to maintain the tanks at the higher temperature was recommended (milestone 2 progress report).

e) Phosphorus and nitrogen content - we could not find

a direct relationship between phosphate content in the process tanks and denitrification itself. However, phosphate is normally added to medium to assist in microbial cell growth. Thus, we suggested it remain an additive to the process tanks.

Nitrogen content in plating rinse waste (PRW), nitric acid, and NaNO_3 were studied. Microorganisms from F4 process material could denitrify all NO_3 sources. However, longer time was spent denitrifying PRW than the other two sources. This lead us to believe that PRW contained possible inhibitors that may slow down the denitrification process in Y-12 tanks. Low to high nitrogen amounts were, also, tested. F4 denitrifiers could reduce starting levels of NO_3 in PRW as high as 100,000 ppm to less than 10 ppm (EPA approval), and even to 0.

5. Identified toxic inhibitors present in PRW that could slow down the denitrification process in Y-12 tanks. This was done by combining information obtained from agar plate metal sensitivity tests, ICP analysis, and microcosm data.
6. Obtained knowledge about the denitrification system as a whole, the synergistic relationship between the microorganisms involved in the tank process, understanding their ecosystem, and their need for genetic adaptation to their environment (see a c below).

- a) When we first received the F4 and F5 process

material, 7/8/86, we attempted to isolate denitrifiers from both process samples. Several good enrichment techniques were used. From our efforts we were able to obtain 3-4 denitrifiers, no more. Only 2 of these denitrifiers were clearly identified as being so. These were Pseudomonas stutzeri and Alcaligenes denitrificans. At that time neither of the 2 isolates gave very impressive rates of reducing NO_3 in PRW. In March, 1987, after several months of project delay and room temperature storage of both process materials, attempts were again made to isolate denitrifiers from F4 and F5 process material using the same enrichment techniques as used before. With the second enrichment we were able to isolate 17 denitrifiers plus other bacteria, and a mixed population of isolates that could easily reduce 100,000 ppm starting NO_3 amounts to 0, all obtained from the F4 process sample. Only 2 bacteria were obtained from F5, the same number as before, neither of which were denitrifiers and therefore of no significance to the process.

It appeared with the second enrichment, we had a "green thumb" for generating denitrifiers. In hind-site, however, the increased number of denitrifiers obtained from the second enrichment was probably due to long-term room storage of F4 process material between 7/8/86 and 3/16/87. In some ways the F4 process material served as an enrichment environment for denitrifiers. The

bacteria became acclimated to their environment either by taking on additional metabolic traits needed for PRW denitrification or, rather than acclimation just increased in number from the first enrichment time to the second. In either case we were able to obtain the 17 denitrifiers from the second enrichment right at the time they were needed for our research efforts.

b) We learned the importance of batch style denitrification processes over flow-thru methods. Batch style processes such as the one run by Y-12 for denitrification of PRW, are frequently found in large industry used for microbial fermentation processes. Batch style systems, whether for denitrification processes, antibiotic fermentations, or beer fermentations, provide a "stable" ecosystem for microorganisms to develop metabolic mechanisms important for making the processes go from beginning to end. Microbial stability is the key factor to keep in mind when running the denitrification process in Y-12 tanks. "Microorganisms" are performing the functions inside the tanks that make the process a success not the engineers operating the tanks. Therefore, microorganisms must be kept happy and thriving in their ecosystem so that the denitrification process will go to completion. Process tank microorganisms are happiest at optimum process conditions.

A flow-thru method would not have been as efficient

for denitrification of plating rinse waste (PRW) as the batch style process system. Microorganisms, in a flow-thru situation, would not have had the opportunity to establish a "stable" ecosystem and develop the metabolic processes or synergistic relationship needed among the tank microorganisms to denitrify plating rinse waste. Therefore, no successful denitrification of PRW would have occurred.

c) The tank served as a selective environment for the type of microorganisms ORRI isolated from the F4 process material. Microbial content of sewage-sludge is vast, containing a wide variety of microorganisms, mostly enterics. Because of Y-12 tank specifications (physiological parameters), enterics were selected against in the F4 and F5 process tanks, samples of which ORRI received from Y-12 engineers. Based on our enrichment techniques and isolation procedures, enterics would have appeared on isolation agar if they had originally been present in the F4 and F5 samples. No enterics were obtained. Therefore, it can only be concluded that the tank environment was selective against the enterics and selective for the type of microorganisms isolated.

There were several parameters that made the tank a selective environment. The carbon source was an important selective agent. Use of Ca Acetate over glucose or some other popular carbon source for microbial growth was a key factor. Plating rinse waste was another key

element. PRW has no nutrients but contains toxic substances, inhibitory to growth of most bacteria. Alkaline pH, a third factor was highly selective for Alcaligenes and Moraxella. Nitrate strength was also important because of the osmotic pressures placed on bacteria, keeping some from developing and allowing others to surface (i.e. denitrifiers). Therefore, "survival of the fittest" occurs in all ecosystems and establishes the type of microorganisms present.

7. A major finding was the significance of Pseudomonads for denitrifying plating rinse waste. The Pseudomonads are needed for Y-12 denitrification of waste stream material because they detoxify metals in PRW. This ability comes from Pseudomonads containing degradative plasmids. These plasmids carry genetic information for heavy metal reduction.
8. Developed seeding methodology for Y-12 denitrification process tanks based on data obtained from seeding laboratory mimic tank ecosystems. A three-stage seed was suggested using three isolates, Pseudomonas 1037, Alcaligenes 1021, and Moraxella 1032 for the reasons provided in milestone 2 progress report.

Conclusions

Over the past 6 months quite a bit of information was gathered on the denitrification of plating rinse waste by

microorganisms present in Y-12 process tanks. Work efforts under the contract assigned to us by Martin Marietta Y-12 engineers, was perceived by ORRI as being a successful undertaking, completed on time as targeted by three milestone progress reports and a final summary report. It is hoped that information supplied to Y-12, in all progress reports, were satisfactory and that they will seriously consider the suggestions made for improving their rates of denitrification in their waste stream process tanks. These suggestions were:

1. Substitute succinate for acetate as C-source.
2. Temperature controls on the process tanks to maintain them at 35 - 39°C.
3. Three-stage seed of their denitrification process.

We have very much enjoyed working on the project with engineers, Jeter and Zablesky. Their able assistance helped along the way to contract completion.

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