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Functional redundancy promotes functional stability in diverse microbial bioreactor communities

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ABSTRACT

Strategies for the inoculation of bioreactors for long-term space missions include communities of diverse composition or, alternatively, communities of a few organisms selected for their ability to efficiently catalyze reactions of interest in the reactor. The concept of functional redundancy states that in a diverse community, several different organisms may be present that are capable of effecting processes necessary to the maintenance of the system function. The concept implies that if some members of the community are lost, others will be able to keep the system from failing in the critical reactions that take place therein. In a sewage reactor in the laboratory, a diverse community at steady state was perturbed by elimination of aeration for seven days. Chemical pools (NH_4^+, NO_3^-, dissolved O_2), pH, and CO_2 evolution were monitored before, during, and after the perturbation. CO_2 evolution remained relatively stable, throughout the one-month incubation, although there were strong deviations at the time when the oxygen was initially excluded, and again when O_2 was reintroduced. During the anoxic period, NO_3^- disappeared, and ammonia increased substantially, along with the pH. When the aeration was resumed, reactor conditions approached those of the pre-disturbance period. The microbial community, analyzed by TRFLP fingerprinting, changed substantially during the anaerobic period, and changed again when aeration was resumed; however, the final community was not similar in composition to the initial community, even though the functional ability of the post-disturbance community became similar to that of the initial assemblage. We conclude that redundancy of function within the community members accounted for the similarity of function under similar environmental conditions, although the community composition did not recover its original form.

INTRODUCTION

The use of bioreactor systems in human habitats in space or in planetary colonies begs the question of stability of function over long time periods during which structural changes in the resident community are likely to occur. One approach for establishing such reactors includes the use of only selected strains of microbes in the reactors. While this approach maximizes control of the organisms in the system in which the crew exists, alteration of a single population or loss of a population from the reactor could mean failure of the system to function in the way it was intended. Such an event could jeopardize the success of the mission and put the crew at substantial risk. An alternative to the gnotobiotic approach is to use natural communities that have been acclimated for bioreactor purposes. Natural communities of microbes comprise substantially more types than communities of higher organisms; the term hyperdiverse has been applied to these communities to indicate the tremendous variety of types of organisms existing together as a community. Hyperdiverse microbial communities exhibit substantial congeneric homotaxis, which imparts to them high functional redundancy, or degeneracy. For common functions such as metabolism of glucose, acetate, citrate, etc., even extreme dilution, which eliminates most of the types from the community, does not remove the function from the community (Franklin and Mills, 2003b). Furthermore, if the biomass is made similar among the dilution treatments, the rates of the catabolic reactions (determined by ^{14}C uptake) are not significantly different. These results suggest that degeneracy in hyperdiverse communities provides a level of functional stability to the system in which the community resides.

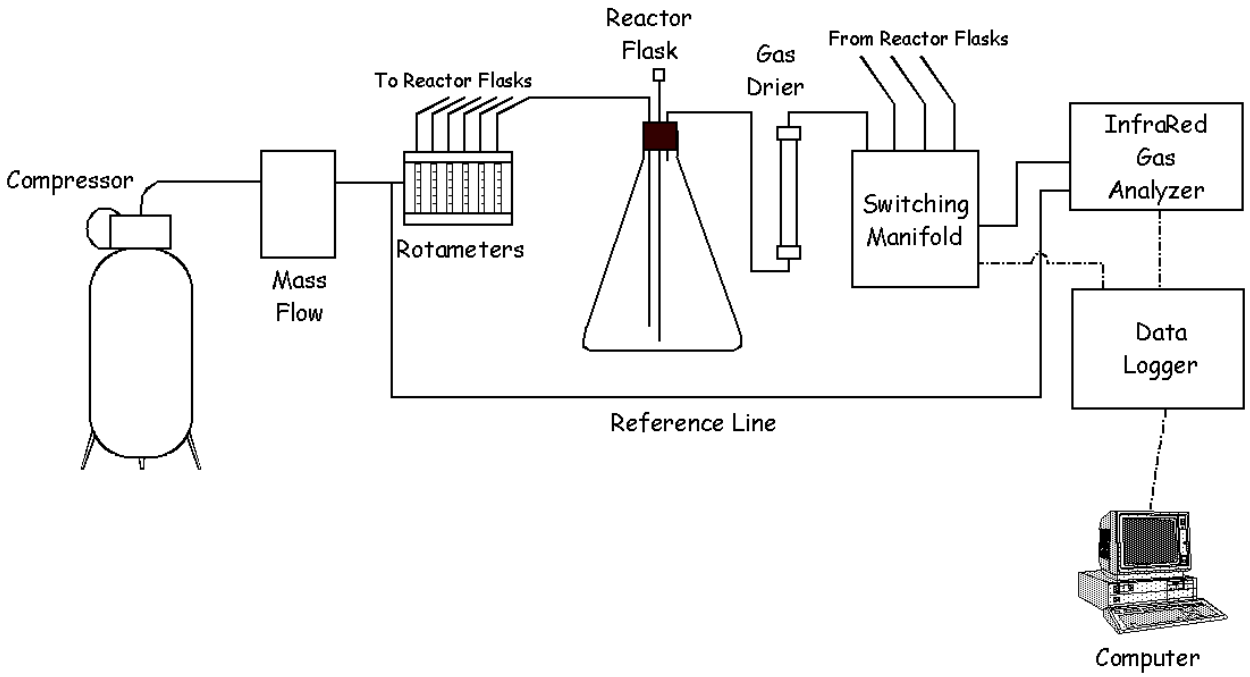

Figure 1. Design of the bioreactor system. Compressed air was fed through a series of flasks fitted with an additional tube for sampling and medium replenishment. After passing through a drying tube to remove water, the air from each flask was passed, in sequential rotation, through an IRGA that measured CO_2 content relative to a reference line that had not passed through any flask. Readings from the IRGA were passed by a Campbell 21x data logger (which also controlled the switching manifold controlling which flask was passing to the IRGA) and then to a computer for storage and readout.

The present report describes a study in which a moderately diverse community thought to possess high degeneracy was disturbed and allowed to recover from the disturbance. The experiments tested the hypothesis that disturbance of a degenerate community would result in a return to the functional state exhibited prior to the disturbance, but that the structure of the community could differ from the original in composition or distribution of individuals among the types. The variables examined centered on the total turnover of carbon, and the nitrogen cycle in the reactor flasks.

METHODS

The experimental system consisted of a community of sewage organisms maintained in a series of replicate fed-batch bioreactors (Fig 1). Six reactors, assembled from 1-L flasks) filled with 500 mL of sterile, autoclaved sewage as growth medium, were inoculated with a microbial community obtained from a raw sewage sample obtained from the equilibration basin of a wastewater treatment plant. The reactors were fed daily by removing 100 mL of the contents of the flask and replenishment with an equal volume of sterile sewage. Aerobic conditions were maintained by agitation of the flasks on a rotary shaker (100 rpm) and filtered airflow (100 mL min^{-1}) through each flask. CO_2 was measured in the effluent gas by passing the gas from each flask in sequence through an infrared gas analyzer (IRGA). Because of a limited number of connections through the switching manifold, only 4 flasks could be connected to the IRGA. After eight days of incubation, CO_2 levels and concentrations of other measured constituents appeared stable (Fig. 2). At that time, 2 reactors (connected to the IRGA) were made anaerobic by replacing the air flow with N_2 (at 100 mL min^{-1}). A third reactor was simply disconnected from the air stream. The remaining 3 reactors were maintained as before. Feeding of all the reactors continued unchanged. Eight days later, air was reintroduced to the three anaerobic flasks to return them to aerobic conditions. Samples were withdrawn from each reactor periodically and analyzed for pH, O_2, and concentrations of NH_4^+-N, and NO_3^--N. The experiment was terminated after the samples were withdrawn on day 27.

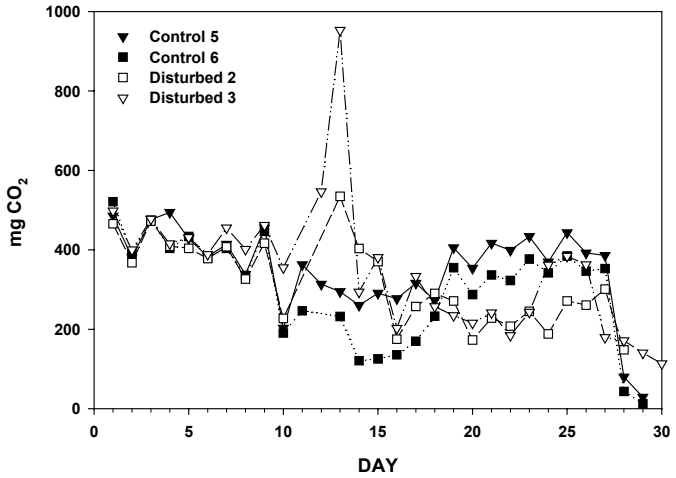

Fig. 2. Daily CO_2 production in disturbed and undisturbed bioreactors. The air supply to the disturbed flasks was replaced with N_2 between day 9 and day 16.

At three times during the incubation, part of each collected sample was set aside for analysis of the microbial community composition. The samples were collected and the microbial cells removed by centrifugal sedimentation (50,000 × g) for 20 min. The pellet was collected an frozen at –80°C until analysis. Community composition was examined by terminal restriction fragment length polymorphism (TRFLP) analysis (Liu *et al.*, 1997). Bacterial community PCR was performed in 100-µl reaction volumes using a fluorescently labeled forward primer (Bac-SSU27F_6-FAM™) and an unlabeled reverse primer (Bac-SSU1492R) targeting the small subunit 16S rDNA region of the ribosomal DNA operon. Final PCR reagent concentrations were: 2.0 mM $MgCl_2$, 1x Applied Biosystems Buffer II, 200 µM of each dNTP, 1.0 µM of each primer, 0.4 µg/µl BSA (Bovine Serum Albumin), and 1.25 U AmpliTaq DNA polymerase. All amplification reactions were performed in an MJ Research PTC-200 Thermocycler (Waltham, MA, USA) with reagents obtained from Applied Biosystems (Foster City, CA, USA), with the exception of BSA that was obtained from Roche Diagnostics (Indianapolis, IN, USA). Thermocycler reaction conditions for fungal ITS amplification were: 5 min initial denaturation at 94°C followed by 35 cycles of 0.5 min at 94°C, 2 min of annealing at 50°C, and 3 min extension at 72°C followed by a final extension step of 5 min at 72°C. Bacterial 16S-rDNA PCR amplifications were identical except that the annealing temperature was 55°C. Reaction yield was determined by 1.5% agarose gel electrophoresis. PCR products were purified and concentrated using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany), digested with the appropriate restriction endonuclease at 5U per reaction according to manufacturer's instructions (New England Biolabs, Beverly, MA, USA). The restriction endonucleases HaeIII and RsaI were used to digest 16S-rDNA targets while the enzyme Hinf I was used for digestion of fungal ITS targets. Following restriction, samples were purified and desalted with QIAquick Nucleotide Removal Kit (Qiagen, Hilden, Germany), denatured at 95°C for 10 min, and separated by capillary electrophoresis in an ABI 310 Genetic Analyzer in GeneScan mode (Applied Biosystems, Foster City, CA, USA).

Electropherograms from the TRFLP analyses were converted to binary format representing the presence or absence of a peak (representing a single ribotype) at a given molecular weight. A cutoff of 100 relative fluorescence units was used to distinguish peak signals from background noise. The matrices (ribotype × sample) were examined with Principal Components Analysis to visualize differences among the community fingerprints determined for each site and dominant plant type (Franklin et al., 2001; Franklin and Mills, 2003a; Franklin et al., 1999; Wikstrom et al., 1999). Patterns of ribotype presence create a fingerprint of the communities.

RESULTS

CO_2 production in the flasks behaved similarly and was stable until the aeration regime was altered after sample collection on 11/6. At that point, CO_2 in the anaerobic reactors initially spiked 3 days into the anaerobic period then returned to a level consistent with that from the aerobic treatments. During that same period, the CO_2 production in the aerobic reactors decreased from the level before and after the disturbance. When re-aeration was started, CO_2 in the anaerobic flasks was less than that in the aerobic treatments until very near the end of the experiment when the levels were again similar. The pH of all reactors was tightly grouped between 6.0 and 6.5 at the outset of the incubation (Fig. 3). In the reactors treated anaerobically, the pH increased during the anaerobic period to about 8.75. When the air stream was reconnected to the anaerobic treatments, the pH decreased slowly until the end of the experiment when it had reached about 6.2. It is interesting to note that the pH of all the flasks followed a gentle downward trend during the initial stabilization period and leveled off around pH 5 at about the time that the anaerobic conditions were released from those treatments. The anaerobic flask that was not connected to the N_2 stream behaved differently from its replicas. After an initial increase in pH, the acidity increased such that by the end of the anaerobic period it was indistinguishable from that of the 3 aerobic flasks, suggesting that the reactor was not anaerobic.

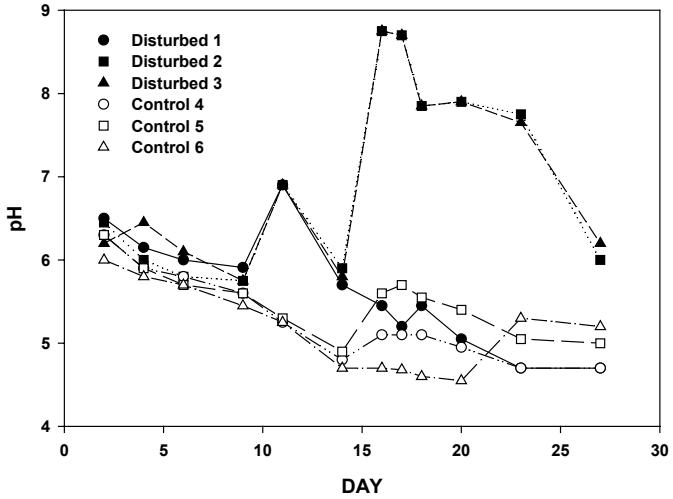

Fig. 3 Change in pH in disturbed and undisturbed bioreactors. The air supply to the anaerobic flasks was replaced with N_2 between day 9 and day 16.

Concentrations of NH_4^+-N were initially low for all reactors (Fig. 4a). When the anaerobic treatment began, NH_4^+-N concentration increased in anaerobic reactors to a level around 20 mg L^{-1}, where it remained for the duration of the experiment. NO_3^--N levels were much higher than $NH4^+$-N throughout the initial aerobic incubation period and stayed high (the levels were variable with time, but were similar among flasks) in the aerobic reactors throughout the entire incubation. In anaerobic reactors, the NO_3^--N concentration dropped

rapidly with the onset of anaerobiosis, and they remained at a very low level throughout the incubation until the very end, when they began to increase to the levels observed in the aerobic treatments. The anaerobic reactor that behaved like the aerobic ones with respect to pH also mimicked the behavior of the aerobic treatments with respect to both NH_4^+ and NO_3^-. In the reactors that were incubated anaerobically, there was an overall loss of combined nitrogen ($NH_4^+ + NO_3^-$) during and after the anaerobic period as compared with the pre-disturbance period. This loss of inorganic nitrogen (above that accounted for by dilution during replenishment) in the anaerobic reactors can be explained by denitrification, and perhaps some NH_3 loss by volatilization due to the high pH. The pH of those reactors was around 8.7, and the pK_a for the ammonia-ammonium equilibrium is about 9.3.

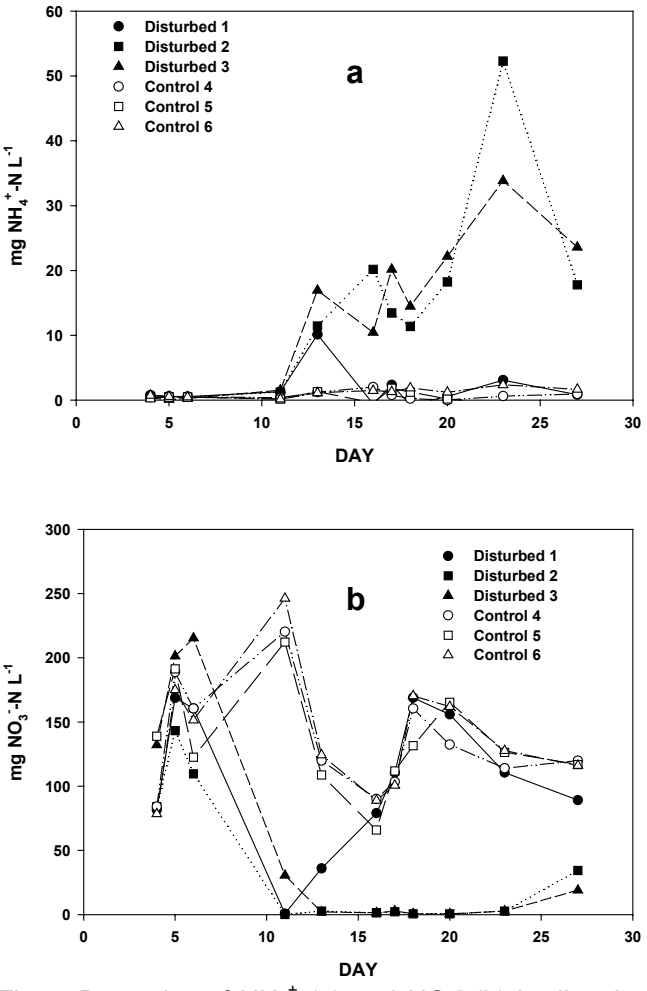

Fig. 4 Dynamics of NH_4^+ (a) and NO_3^- (b) in disturbed and undisturbed bioreactors. The air supply to the anaerobic flasks was replaced with N_2 between day 9 and day 16. Note the differences in scales of the abscissa for the two coordinate sets.

We interpret the nitrogen results to indicate that the guild of nitrifying organisms was inhibited during the anaerobic and post-anaerobic incubation periods. It is probable that the aerobic NH_4^+ oxidizers were greatly diminished in number due to the lack of oxygen, and that some time after the return of the aeration was needed to build the guild membership back to an effective level. The increase in nitrate observed at the end of the incubation combined with the loss of NH_4^+ and the reduction in pH suggests that the nitrifiers were recovering at that time. Although the incubations were, unfortunately, stopped before the systems had come to complete recovery, the information on the chemical species indicates that the treatments were, functionally very similar.

During the anaerobic portion of the incubation, the shift from nitrification to denitrification causing a loss of nitrate and a sharp increase in NH_4^+ in the anaerobic flasks. Note that the pH in those flasks increased substantially, consistent with the shift in nitrogen metabolism. When air was reapplied to the flasks, CO_2 production in all the reactors began to move back together, NH_4^+ and pH in the (formerly) anaerobic flasks began to decrease and the nitrate began to increase. In other words, all the measured parameters were returning to their pre-disturbance levels.

While function in the reactors was returning to a similar state between the disturbed and undisturbed reactors, comparison of the communities showed a different result. The pre-disturbance community composition for both treatments clustered very closely together (Fig. 5). When the N_2 was introduced to half the flasks, their community moved a substantial distance in PC space from the central cluster, whereas the aerobic community composition moved a minimal difference, consistent with the magnitude of changes in the functions contained in the reactors. When the air was reintroduced, the aerobic community maintained its position in the tight cluster near the starting point. The anaerobic community moved again moved a substantial distance in PC space, but it did not return to a position proximal to the central cluster comprising the aerobic flasks, and it was significantly different from the starting state of all the flasks and the ending state of the aerobic reactors.

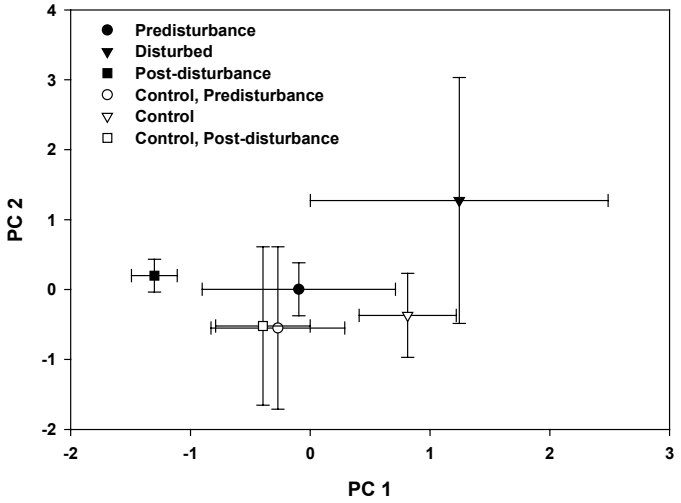

Fig 5. Principal components analysis of bacterial community composition determined by TRFLP of the pre-disturbance, disturbed, and post-disturbance periods for the disturbed and control bioreactor communities.

CONCLUSION

Despite the incomplete time course, this experiment strongly suggests that resiliency in function was uncoupled from resiliency in structure. Two different communities appeared to confer the same functional properties to the system. This observation must be explored further to understand its implications for communities in closed systems such as spacecraft and enclosed planetary colonies, however it suggests that hyperdiverse microbial communities possess a high degree of redundancy in function that confers functional resiliency even in response to major disturbance. This property of diverse communities is termed congeneric homotaxis (Hill and Weigert, 1980), and it represents a potential feature of microbial communities that will enable the maintenance of stable functional capacity in closed systems for space exploration applications.

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