PN-ABG-423

Best available copy -- 2 pages from one of attached publications missing

PN-ABG-423

Final Report to the Agency for International Development

Grant # DAN-5053-G-SS-7067-00

Development of Fish Feeds for the Dominican Republic

August 12, 1990

Submitted by
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Atlanta, GA 30314

FORWARD

This report was prepared by Judith Bender, Principal Investigator of AID Grant No. DAN-5053-G-SS-7067-00. It is the product of the combined efforts of a number of consultants, assistants, volunteers and Institutions. Their contributions are gratefully acknowledged.

Research staff in the Dominican Republic

Lic. A. Russell and student assistants

Government agencies and private organizations in the Dominican Republic

AID Mission in the Dominican Republic; CIMPA (Agricultural Research Station), Ms. W. Briggs, Peace Corps Volunteer of CRIAR (a private agricultural research and development station)

Department of Fisheries and Allied Aquaculture, Auburn, Alabama

Morehouse College and Clark Atlanta University research staffs.

Technical staff: Dr. P. Phillips, Ms. Y. Vatcharapijarn; Student research assistants: Dr. I. Ekpo (Nigerian student, who earned his Ph.D. with this project) and undergraduate students, Mr. R. Murray and Mr. D. Buttler.

INTRODUCTION

The long-range objective of this research project was to investigate the silage microbe mat (SMM) feed system as a low-cost, practical aquaculture feed and to assess this system in terms of potential for field application in developing countries.

The SMM system of feed production was developed with the support of an earlier AID/HBCU grant (No. DAN-5053-G-55-5046-00) and further investigated in this project. This feed is based on microbial bio-conversion of ensilaged grass clipping, which serve as the feed stock for nitrogen-fixing bacteria and cyanobacteria (blue-green algae). Rapid production of high quality protein is generated in shallow ponds after silage enrichment of the water column.

SUMMARY OF RESULTS

Specific data, which indicates the potential of the SMM feed system, are summarized below and detailed in the attached publications:

1. Protein of 26% are generated in approximately a week of culture time (Bender et al., 1989). The feed shows good digestibility and protein availability with T. nilotica and silver Carp (Ekpo and Bender, 1989; Ekpo and Bender, 1990, a and b). Carbohydrate levels of 43% indicate efficient photosynthesis of the cyanobacteria.

2. The ecological pattern of development of the mat involves a short bacterial phase, followed by establishment of a predominantly cyanobacterial climax community. Nitrogen-fixation is highest during the cyanobacterial stage.

3. System dynamics and productivity are similar in the laboratory experiments and the Dominican field ponds.

4. T. nilotica grew more rapidly on the SMM than commercial catfish feed under laboratory conditions.

5. Oxygen depression during the bacterial phase requires a change in pond design, if this feed production system is to be incorporated into the growth pond. A suggested pond design is diagramed in the attached publication (Bender et al., 1989).

6. The SMM may be appropriate for crustacean production, since the ecosystem dynamics (microbial bio-conversions and successional changes) seems to be similar to those in the Louisiana rice/crawfish ponds (Jay Hunter, Director of the Crawfish Center; see attached letter).

7. Two unexpected discoveries were made in the course of this research: (1) The SMM sequesters heavy metals and metalloids from contaminated water; see attached publication, "Lead Removal from Contaminated Water by a Mixed Microbial Ecosystem" and manuscript "Uptake, Transformation and Fixation of Se(VI) by a Mixed Selenium Tolerant Ecosystem". (2) The SMM system lowers the bacterial population in the water column within 2-3 days. This effect may be relevant to low-cost water purification in developing countries. Preliminary investigations of this potential are presently underway in the Morehouse Laboratory and a Nigerian Field station under the direction of Dr. Archibold and Dr. Ekpo.

8. Publications resulting either directly or indirectly from this research are attached. In addition, Lic. A. Russell is in the process of preparing a summary of the 1989 feed publication in spanish for publication in the science section of the Santiago, DR newspaper.

In summary, the SMM system indicates a good practical potential for low-cost, high quality feed. Since it is based on grass clippings, that can be harvested from the pond region and transformed into high quality protein by microbes in the pond, the overall environmental impact is only positive. In addition, the technology for producing this feed is relatively simple and easily transferable to the farming communities (as was demonstrated by the ease of training the Dominican CIMPA staff).

All relevant data and discussions are included in the attached publications. These documents provide the technical phase of this report.

ADDITIONAL BENEFITS RESULTING FROM THIS GRANT

The institutional and governmental linkages as well as the personal contacts provided by this grant had a number of spin-off results. Since these effects relate to the broader mission of AID they are described in this report.

Invitation to participate in the conference on ENVIRONMENT AND DEVELOPMENT IN AFRICA AND LATIN AMERICA. This conference, supported by the Ford Foundation and the Pew Charitable Trusts, will be held at the University Michigan. Three persons, involved with this grant (Russell, Phillips and Bender) will be guest speakers at the conference (see attached flyer: CICALS). The conference supports all expenses, including the trip of A. Russell from the Dominican Republic. Conference Director, K. Wylie, expressed enthusiasm for the HBCU participation and for the speaker from the Dominican Republic (A. Russell).
CREAR pond. Aquaculture ponds, using SMM and compost feeds, was developed with our assistance in Rio Limpio, DR. Peace Corps

volunteer, W. Briggs, assisted with the construction of the pond and the training of the Dominican participants. Although she has completed her service and left the DR, her last report indicated that two of the three ponds were functional and the compassino staff at CREAR was trained in pond management. We do not have fish production data at this time. However, aquaculturist, Dr. P. Phillips took a group of Atlanta University Center students to Rio Limpio this summer and reported that the ponds seemed to be in good condition.

Student Development. All Morehouse College and Clark Atlanta University students made presentations at the Region IV American Society for Microbiologists Meeting. Data resulting from this project were reported. Undergraduate student research assistant in our laboratory, D. Buttler, graduated from Morehouse College this spring and entered the Peace Corps in Africa. It is my personal opinion that this research project provided technical skills and a level of personal inspiration that will be important in his Peace Corps service.

Undergraduate student research assistant, R. Murray, from St. Vincent Island, completed his undergraduate degree at Morehouse College. He has applied to Clark Atlanta University for graduate and hopes to continue to work in our laboratory for his M.S. degree in biology.

Development of an International Environmental Training Program for the Institutions of the Atlanta University Center. The contacts provided by this research experience resulted in an invitation by Morehouse College/Clark Atlanta University to develop an international environmental training program for undergraduates and graduates. The plan is to develop a center-wide course of studies, integrating a course program, a travel experience to a developing country and a research program in problems relevant to developing country situations. As a result of this interest a proposed course of studies was developed by J. Bender and P. Phillips (see Attachment 1: DEVELOPMENT OF AN INTERNATIONAL ENVIRONMENTAL STUDIES PROGRAM AT THE ATLANTA UNIVERSITY CENTER).

Student environmental studies program in the Dominican Republic. This research project generated significant interest in the Morehouse Department of Biology. As a result, the Department Chairman, Dr. J.K. Haynes contributed part of his Packard Grant to support student travel to the Dominican Republic to study environmental issues in developing countries. The trip was organized by Dr. P. Phillips (see Attachment 2: REPORT FROM THE INTERNATIONAL ENVIRONMENTAL STUDIES PROGRAM DOMINICAN COURSE, May-June, 1990).

Contributions to Sustainable Agriculture. Clark Atlanta University has received an AID Grant to provide management training to participants from the Francophone countries. Since natural resources management is part of the program, J. Bender

and P. Phillips have been invited to participate in t training. The program of studies is described in the enclosure: PROPOSAL FOR APPROPRIATE TECHNOLOGY SEMINARS AND FIELD TRIPS.

A short article was published by J. Bender on the subject of sustainable agriculture. This article was written on the invitation of the editor of SEEDS magazine (see enclosure: SUSTAINABLE AGRICULTURE).

ACKNOWLEDGEMENTS

This project resulted in a number of benefits to the recipient Institution, other HBCU Institutions, as well as the agriculturists/aquaculturists and students in several developing countries. The AID/HBCU Program is gratefully acknowledged for this support.

The BIBLIOGRAPHY, presented below, represents the list of publications resulting from this research project.

BIBLIOGRAPHY: FISH FEEDS

Bender, J.A., Vatcharapijarn, Y. and Russell, A. (1989). Fish Feeds from Grass Clippings. Aquaculture Engineering, 8, 407-419.

Ekpo, I. and Bender, J.A. (1989). Digestibility of a Commercial Fish Feed, Wet Algae, and Dried Algae by tilapia nilotica and Silver Carp. The Progressive Fish-Culturist, 51, 83-86.

Ekpo, I. and Bender, J.A. (1990). An Emerging Technology I: Conglomerate of Algae, Bacteria and Silage as Alternative Source of Fish Feed. Proceedings of the International Conference on 3rd World Strategies for Technological Development, Yola, Gongola State, Nigeria, August 20-26, 1990.

Ekpo, I. and Bender, J.A. (1990). An Emerging Technology II: Nitrogen-Fixing Bacteria, a major ingredient in fish food production. Proceedings of the International Conference on 3rd World Strategies for Technological Development, Yola, Gongola State, Nigeria, August 20-26, 1990.

Bender, J.A. (1990). Sustainable Agriculture. Seeds: Technology and Hunger Special Issue, 12, 16.

BIBLIOGRAPHY: METAL SEQUESTERING*

Bender, J.A., Archibold, E.R., Ibeanusi, V. and Gould, J.P. (1989). Lead Removal from Contaminated Water by a Mixed Microbial Ecosystem. Water Science and Technology, 21, 1661-1664.

Bender, J.A., Gould, J.P., Vatcharapijarn, Y., Saha, G. (1990). Uptake, Transformation and Fixation of Se(VI) by a Mixed, Selenium-Tolerant Ecosystem. Manuscript in review for publication in The International Journal of Water, Air and Soil Pollution.

*List of publications related to metal uptake are included, since this work resulted indirectly from this grant.

ATTACHMENTS DESCRIBING PROGRAMS RESULTING FROM THIS PROJECT

ATTACHMENT 1

DEVELOPMENT OF AN INTERNATIONAL ENVIRONMENTAL STUDIES PROGRAM AT THE ATLANTA UNIVERSITY CENTER

Judith Bender, Ph.D., Research Scientist, Clark Atlanta University, Atlanta, GA

Peter Phillips, Ph.D., Director of International Environmental Studies, Morehouse College and Research Associate, Clark Atlanta University, Atlanta, GA

OUTLINE

Introduction

Project Goals

Integration of Overseas Students into the International Environmental Studies Program

Addendum 1: Environmental Biology at Morehouse College, Course Outline

Addendum 2: International Environmental Issues at Morehouse College, a Proposal for a Multidisciplinary Course Treating Problems of Global Environmental Deterioration

Addendum 3: Ecology at Morehouse College, a Course Outline Proposal

Addendum 4: Multidisciplinary Natural Resources course in the Dominican Republic

Addendum 5: Appropriate Energy Technology Training at Southface Energy Institute, Course Outline

Addendum 6: Curriculum revision for the Biology Department, Clark Atlanta University.

INTRODUCTION

Global ecology is the study of ecological concepts and problems on a worldwide basis. It has many components; some are related simply to the accumulation of data on local and regional events until they assume global importance, such as acid rain and soil erosion. Other global events are of such general planetary impact that they cannot be viewed locally. New approaches, involving international cooperation, will be required to evaluate them. Such environmental concerns as global heat balance and changing atmospheric chemistry are significant primarily in terms of planetary effects and involve more than the summation of local processes.

Global ecology may be examined from many directions. One approach involves the interactions of the biological and physical ecosystems and is generally evaluated by analytical scientific methods. Another is a more directly humanistic approach, involving a holistic look at populations, resources, food supplies, sociology and economics. It includes a consideration of the conditions of all living organisms with which we share the life support systems of this world. In this view, global ecology does not attempt to cover or even survey the major environmental problems of the world, but is rather a type of approach. It includes scientific analyses, but also incorporates information from other disciplines, such as sociology, economics and politics. These areas contribute to a more comprehensive understanding of the environmental problems and potential solutions. An interdisciplinary, holistic approach will be emphasized in the environmental program proposed here.

Less than 2% of the environmental professionals in the United States are Black (McCormick and Barrett, 1979). This statistic becomes very significant when considering international issues, since most developing country environmental problems involve non-white populations. Active participation of minority populations in global environmental problems is an urgent need and must begin with an active participation of undergraduates in a program that inspires and motivates, as well as provides theoretical classroom information. The proposed course of studies will include basic environmental course work from a variety of resource persons, as well as options for student research and an opportunity for participation with inhabitants from developing countries. It is hoped that this program will expand to include overseas students coming to Atlanta University Center member schools (Morehouse College, Clark

Atlanta University, Spelman College and Morris Brown College). This contact will assist all participants in appreciating the complex causes of environmental degradation and to develop a sense of environmental responsibility. With such understanding, students can begin their careers in seach of effective, long-term environmental solutions. The field-study component is fundamental to the program, and represents a new approach in undergraduate education.

This program was developed by Dr. J. Bender, microbial ecologist at Clark Atlanta Univeristy while at Morehouse College and by Dr. P. Phillips, aquaculturist and environmental biologist at Morehouse College and Clark Atlanta University. Competent field staff are available in the Dominican Republic and have participated in a cooperative aquaculture research effort with J. Bender and P. Phillips (research supported by the U.S. Agency for International Development). Field staff have also been identified in Costa Rica, Belize, Nigeria and Kenya.

McCormick, J.F.and G.W. Barrett. 1979. Ecological manpower and employment opportunities. Bioscience 29:419-423.

PROJECT GOALS

The long-range objective of this proposal is to develop an international environmental studies program which addresses the specific educational needs of Atlanta University Center students and provides training and experience in the complex global environmental issues for participants from developing countries. Specifically, the aims of this project are as follows:

1) to provide a unique environmental educational experience which is both theoretical and practical.

2) to facilitate the process of studying third world environmental problems and solutions in a developing country, from local inhabitants. The field stations are staffed by local citizens who have developed specific areas of expertise and who are familiar with the complex problems of the developing world.

3) to provide a fluid program of studies with a variety of options and resource personnel. In this way a unique program can be designed by each participant in a way which best meets his/her needs.

4) to increase the minority representation in careers relating to environmental research and policy making.

Present Status

1) At present, Environmental Biology is offered within the Biology Department at Morehouse College (see Addendum 1). The course emphasizes today's environmental problems using current research publications as resource materials.

2) Groundwork is being laid for a multidisciplinary course revolving around environmental issues at Morehouse College involving several departments (see Addendum 2).

3) Clark Atlanta University offers a basic ecology course. Additionally, Morehouse College will offer Ecology as part of an emphasis to draw Morehouse and Spelman College students to environmental conservation careers through a developing research program with Zoo Atlanta (see Addendum 3).

4) Morehouse College, in cooperation with the Universidad Catolica Madre y Maestra (UCMM) in Santiago, Dominican Republic, is offering a summer program starting in 1990 based on a course developed by UCMM professors on "Natural Resources and Energy Use in a Developing Country" (see Addendum 4). This is a multidisciplinary team-taught course in English is based on a successful model of a course taught yearly for Dominican students. An English translation of the course outline is appended. UCMM has the capability of also offering Spanish at different levels simultaneously through their Spanish for Foreigners program. A course on Latin American Culture and Society is also available. Field trips will serve to illustrate the important points of the lecture sessions. Students will be able to visit and participate in the aquaculture research project of Dr. Phillips, travel to an energy farm based on agroforestry, wastewater treatment plants, and several sustainable development-type agricultural communities.

5) A second major field site for the International Environmental Sciences Program will be in Costa Rica. Dr. Phillips was a professor at the Costa Rican National University for several years and is currently working out the logistics of using a large rural development and natural resources management project as a "living laboratory" for Atlanta University Center students. The location of this project is the Caribbean coast of Costa Rica. It includes three groups of inhabitants; a black English-speaking population directly on the coast, an indigenous population located inland on an indigenous reserve and a mixed Spanish-Indian (Spanish-speaking) populatin. The area also includes a national wildlife refuge. Teams of biologists, foresters and community development experts are working with the local populations in order to aid them in the rational exploitation of their natural resources. These themes are entirely appropriate in view of the current worldwide interest in natural resources, tropical forest destruction and its

relation of global warming - the greenhouse effect. The goal for Atlanta University Center students will be a hands-on experience whereby they would have the opportunity to observe and participate in ongoing community development projects involving all three population groups and in projects involving natural resource management schemes applied toward the different ecosystems within the region. The respective team leaders could lecture Atlanta University Center students while in the field.

6) In addition to, or as an alternative to the summer program in the Dominican Republic, students may enroll in the Appropriate Energy Technology Training course at the Southface Energy Institute in Atlanta (see Addendum 5).

7) In conjunction with current construction of the Clark Atlanta University Research Center for Science and Technology, graduate programs will be developed in the environmental sciences and environmental health.

Whereas most course work is being offered or developed at Morehouse College, an active program of environmental research is underway at Clark Atlanta University. The research has a double focus: (1) the recovery of heavy metals from contaminated soils and water supplies using blue-green algae (Archibold et al., 198 ; Bender, 1985) and (2) the development of low-cost nutritional protein for fish feeds in developing countries (Bender, 1988; Ekpo, 1987). Both projects employ microbial processes and simple methods to achieve the objective of low-technology production. The fish feeds project involves laboratory development of mixed microbial systems for rapid production of cellular protein from simple waste materials in the Dominican Republic. The laboratory phase will be followed by field testing in 1990 in the Dominican Republic (Phillips, 1989). These projects will provide a basis for environmental research participation by both undergraduates and graduates participating in the program.

Archibold, E., V. Ibeanusi, J. Bender and J. Gould. 198 . The use of a mixed microbial system in removal of lead from contaminated waters. In: C.E. O'Rear and G.C. Lewellyn, Editors, pp. 161-174. Biodeterioration Research II, Plenum Press, New York, NY.

Bender, J. 1985. Metal recovery from processing wastewater. Final report to the U.S. Bureau of Mines Grant GO145031.

Bender, J. 1988. Final report to the U.S. Agency for International Development Grant DAN5053G55504600.

Ekpo, I.A. 1987. Production and digestibility of mixed

microbial plankton raised on silaged grass. Doctoral Dissertation. Atlanta University, Atlanta, GA.

Phillips, P. 1989. The role of a silage-microbe mat in conjuction with the detrital trophic level as a complete natural fish feeds system for tilapia aquaculture in the Dominican Republic. Proposal submitted for funding to USAID, Washington, D.C.

OVERSEAS STUDENT INTEGRATION

INTO THE INTERNATIONAL ENVIRONMENTAL STUDEIES PROGRAM

AT THE ATLANTA UNIVERSITY CENTER

Overseas students may take advantage of the International Environmental Studies Program by one of two routes:

1) The student may elect to enroll at one of the Atlanta University Center member schools for their entire four year baccalaurate program in biology.

2) The student may elect to spend one year as an exchange student at one of the Atlanta University Center member schools while being enrolled in their home institution. Between their fall and spring semesters, the student would take Ecology, Environmental Biology and the multidisciplinary course in Global Environmental Issues. During the summer session, the student would take either or both course offered in the Dominican Republic and the Southface Energy Institute. In the long term, it is anticipated that students would have the option of carrying out field research in Costa Rica, Kenya or several other developing countries in addition to training and laboratory research opportunities in Atlanta.

3) The student would have an opportunity to conduct experimental research in microbial ecology, recovery of heavy metals from contaminated soils and water via microbial methods or recycled water aquaculture in the Clark Atlanta University Environmental Biology Laboratory.

4) With the near-term graduate program development at Clark Atlanta University in environmental sciences and environmental health, study and research opportunities for students in these areas will emerge.

ADDENDUM 1:

MOREHOUSE COLLEGE BIO497

ENVIRONMENTAL BIOLOGY

Course Outline

Environmental Biology is designed to give students an overview of environmental science. After an introductory section of basic ecological principles, subsequent emphasis is placed on today's environmental problems, using current research publications, resource professionals and a series of guest speakers. Topics covered include: air and water quality, demographics, waste disposal, problems of food production, energy resources, and such current newsworthy topics as ozone depletion and the greenhouse effect.

1. Ecosystem

   concept
   structure
   pyramid of biomass and food chain/web
   succession
   adaptation and evolution
   energy flow
   biomass

2. Cycles in Ecosystems: Carbon

   carbon flow through the ecosystem
   photosynthesis, respiration
   role of producers, consumers and descomposers

3. Cycles in Ecosystems: Nitrogen

   nitrogen flow through the ecosystem
   nitrogen fixation
   role of decomposers, chemistry of decomposition
   denitrification
   nitrogen/protein shortages
   ecological impact of commercial fertilization

4. Cycles in Ecosystems: Phosphorus & Sulfur

   role of phosphorus in organ structure and cell metabolism
   flow of phosphorus in the ecosystem
   sources and fate of phosphorus in the ecosystem
   role of estuaries in phosphorus and nitrogen distribution in oceans

eutrophication
sulfur cycle and industrial smog formation

5. Cycles in Ecosystems: Hydrologic

water cycle and losses in the cycling process
water supplies: aquifer, water table, surface water use and shortages
water pollution: survey of pollutants in rural and urban supplies
mutagens in water, sources of pollutants and health effects

6. Populations

population dynamics and controls
human growth and economic growth
world populations
endangered species

7. Air Quality

Survey of air pollutants and their potential health effects
Control technology, economic implications
Air quality standards, Quality Act of 1967

8. Soil and Land Use

soil structures within specific biomes
erosion
land use and: agriculture, urbanization, endangered species, forest clearing

9. Mineral and Water Resources

mineral resource abundance
mining
mineral reuse and conservation
water supply
water diversion
groundwater
conservation

10. Waste Management

wastewater treatment
solid waste disposal
landfills and leachate
toxic wastes
economics of waste disposal
recycling

11. Food and Hunger

food production
world food supply problems
world agriculture systems
fisheries and aquaculture

12. Energy

resources: fossil fuel, solar, nuclear, biomass, geothermal
consumption and conservation
energy as related to agriculture and industry
energy as related to various types of pollution

13. Current Issues

Acid rain
Greenhouse Effect
Ozone
Rainforest Destruction & Desertification
Global Synthesis: Interrelationships of Technologically Developed & Developing Countries
Environmental Careers

ADDENDUM 2:

MOREHOUSE COLLEGE

INTERNATIONAL ENVIRONMENTAL ISSUES

A Proposal for a Multidisciplinary Course Treating Problems of Global Environmental Deterioration

Themes to consider for inclusion in the Multidisciplinary Course:

1. Natural Resources

   international natural renewable resources; a survey of biological, economic, political and sociological implications of natural resources management
   premise of scarity and problem of resource distribution
   limits of the environmental and social resources available to sustain both human environments and natural systems

2. Land Resources

   land use
   land degradation
   land degradation and social/economic/political/biological consequences of.....
   role of parklands in maintainence of biological (genetic) diversity

3. Population

   population and health
   fertility
   global trends in human settlement
   politics of population control

4. Agriculture

   agricultural output
   agroecology

5. Hunger

   population and development
   food production systems
   history and geography of famines

ecology and political economy of malnutrition

6. Economics

applying basic economic models to environmental dilemmas
international movement of production by multinational corporations

7. Politics

comparative effectiveness of various styles of government and differing societal value systems in confronting and solving environmental problems
environmental politics
international environmental law; regional and global agreements

8. Sociology

social ecology
cultural values and philosophies shaping environmental behaviors

9. Technology

consequences of technological change
social impacts of technology

10. Policy

educated opinions and rational decisions about the environmental controversy
skills necessary to handle resources wisely
environmental ethics
from a frontier society to a sustainable society

ADDENDUM 3:

MOREHOUSE COLLEGE

ECOLOGY

Course Outline Proposal

As an introductory course in ecology, emphasis is placed on reviewing pertinent definitions in ecology and surveying traditional ecological concepts and principles. Ecosystem structure and function is covered in depth as well as energy flow and trophic levels in natural communities.

The latter part of the course reviews the various types of aquatic and terrestrial ecosystems.

1. Historical Development and Application of Ecology

THE ECOSYSTEM

1. Ecosystem Concept

2. Nature of Energy

   climate
   currents
   circulation

3. Laws of Thermodynamics

4. Limiting Factor Principles

   minimum
   tolerance
   temperature and distribution

5. Productivity Concept

   primary productivity
   secondary productivity

6. Food Chains, Food Webs and Trophic Levels, Ecological Pyramids and Energy Flow

7. Biogeochemical Cycles

nutrient cycling
gaseous cycles
sedimentary cycles

8. Water

water properties
cycle, global water cycle
moisture, regarding plant distribution

9. The Community Concept

species dominance
species diversity
community gradients and patterns
ecotone and edge

10. Species and Individuals in Ecosystem

habitat ecological niche
natural selection
speciation, allopatry and sympatry
interspecific competition
predation
coevolution

11. Population Ecology

age structure
population growth
population regulation
population dispersal
density
distribution

12. Succession

mechanisms of succession
disturbances, role and type
time and succession
stability and climax

ECOSYSTEM SURVEY

1. Life Zones

2. Biomes

3. Terrestrial Ecosystems

grasslands, savannas
forests

tundras
deserts

4. Freshwater Ecosystems

lentic systems
lotic systems
wetlands

5. Marine Ecosystems

estuaries and intertidal areas
marshes and mangrove swamps
coral reefs
open ocean

ADDENDUM 4:

PROPOSAL FOR A MULTIDISCIPLINARY NATURAL RESOURCES COURSE TO BE OFFERED TO MOREHOUSE STUDENTS (a group of 5 - 10) IN THE DOMINICAN REPUBLIC STARTING IN THE SUMMER OF 1990

With the objective of developing an environmental sciences area with an international component at Morehouse College, the Catholic University (UCMM) of Santiago, Dominican Republic is in a position to offer Morehouse College students a course in English on "Natural Resources and Energy Use in a Developing Country" in the summer session of 1990. This would be a multidisciplinary team-taught course based on a successful model of a course taught yearly for Dominican students. Attached is an English translation of the current course. It is general and not geared only to biology students. Morehouse students have attended UCMM in past years so that the precedent is in place for receiving them. We have discussed the pros and cons of requiring a background in Spanish for each student. If necessary, UCMM has the capability of offering Spanish at different levels simultaneously through their Spanish for Foreigners program. Finally, a course on Latin American Culture and Society could be offered through the same language department. Since UCMM is recognized by the Council on International Educational Exchange (New York), credits granted by UCMM should be transferrable for Morehouse students. As an aside, I am soliciting funding from the US Agency for International Development for 1990 to build a series of fish ponds in order to test Judith Bender's silage-microbe mat system in vivo at a site near UCMM. If the proposal is approved, I could coordinate a research trip with a Morehouse student group and engage the students in my research project.

Lic. Arturo Russell, the potential UCMM coordinator for this program, and myself drew up a cost estimate for each student if the group was initially comprised of six students. We concluded that the cost for a four to five week course, including air fare, would be $1,200.00 per student. This cost would increase, perhaps in the neighborhood of $200.00 - 300.00, if we offer the Spanish and Latin American Culture and Society courses. In view of the fact that this price includes air fare, this program is highly competitive with overseas programs offered by other US colleges and universities.

PONTIFICIA UNIVERSIDAD CATOLICA MADRE Y MAESTRA
COLLEGE OF SOCIAL AND ADMINISTRATIVE SCIENCES
DEPARTMENT OF ECONOMICS

EC-470- NATURAL RESOURCES AND ENERGY

This course will present to the students an overview of the natural resources of the Dominican Republic as an economic resource, with a view toward their sustained use. A natural resource inventory will be presented, its quantity and quality. Exploitation techniques will be indicated as will the necessary criteria for long-term sustainable use.

1. Introduction

   Nature-society relationship
   Definition of a resource
   Historical development of natural resources
   Current panorama of natural resource utilization

2. Mining

   Mining potential of the Dominican Republic

3. Ecology

   Dimension of the concept
   Ecosystem structure and function
   Soil management
   National parks

4. Forests and Rivers

   Life zones in the Dominican Republic
   Potential of agroforestry systems
   Pine forest exploitation
   Problems in the production of firewood and charcoal in dry forests

5. Energy Resources

   Energy balance of the Dominican Republic
   Present situation with respect to exploitation techniques
   Potential of renewable and nonrenewable resources

6. Agricultural Exploitation

   Alternative uses for soils and their ecological effects
   Methods of agriculture exploitation, protection and fertilization

Nationwide water use
Agricultural potential of the Dominican Republic
Agricultural sector development policies

7. Tourism

Zones with tourist potential
Tourism and economic, social and cultural development
Perspectives in the Dominican Republic

8. Human Population as a Resource

Man as a production factor
Location of human resources
Society-nature relationship

ADDENDUM 5:

SOUTHFACE ENERGY INSTITUTE

APPROPRIATE ENERGY TECHNOLOGY TRAINING

Course Outline

This training course is sponsored by the International Environmental Studies Program based in the Atlanta University Center in cooperation with the Southface Energy Institute. The goals of the program are to provide both theortical information and practical training about appropriate energy technologies for economic development and resource conservation. Appropriate technologies are environmentally sound and rely on the use of renewable energy, and locally available resources and labor.

Introduction

This agenda assumes a rigorous, three-week training program with both classroom and hands-on learning experiences, and site visits to innovative appropriate energy demonstration projects. The program will be offered in the summer (flexible schedule) of each year in Atlanta.

The course covers a broad range of energy technologies, including passive solar cooling, more efficient cooking methods, community electrical systems and alternative fuel production. In developing the agenda, we have identified the following energy needs and targeted energy technologies:

1. Residential Energy Needs

   Cooling - shading and ventilation strategies, radiant heat barriers, and low energy cooling
   Electrical appliances - photovoltaic (PV) systems
   Refrigeration - either alternative fuel-powered refrigerators or PV-powered units
   Hot water - solar hot water systems
   Drying - solar food and wood dryers
   Cooking - solar cook stoves
   Lighting - PV systems with efficient lights

2. Commercial/Small Industrial Energy Needs (in addition to above)

Process heat - solar hot water systems, solar food dryers, solar drying kilns
Sterilization - solar hot water systems
Water pumping - PV powered pumping systems
Exterior lighting - PV systems with efficient lights

3. Agricultural Energy Needs

Process heat - solar hot water systems, solar dryers and kilns
Sterilization - solar hot water systems
Irrigation - PV powered pumping stations
Exterior lighting - PV systems with efficient lights
Crop protection - PV powered electric fences

4. Fuel Needs

Methanol fuels from wood and wood products
Ethanol fuels from crops

Addendum 6: Curriculum revision for the Biology Department, Clark Atlanta University.

Working with the present curriculum, this is a suggested alternate biology program for a concentration in environmental science.

The CORE CURRICULUM should be modified in the following manner:

(1) Core courses that remain in place:

General Biology 111 and 112
Genetics 312
Physiology (one semester only) 475
Cell Biology Laboratory 478

(2) Movement of present elective courses to the core:

Botany (= Plant Morphology) replaces one semester of Physiology 321
Comparative Vertebrate Anatomy (= combined Vertebrate Anatomy and Vertebrate Embryology) 251
Invertebrate Zoology 255

(3) Movement of present core courses to the elective category:

Research in Biology 480 and 482

The following are ENVIRONMENTAL SCIENCE electives leading to a concentration in environmental science:

(1) Courses presently offered:

Environmental Biology 261

(2) New courses: (see attachment for course descriptions)

General Ecology
Environmental Biology Laboratory
Topics in Environmental Science
Population and Community Ecology
Aquatic/Marine Biology
Summer Internship I and II

(3) New courses for the Chemistry Department:

Environmental Pharmacology/Toxicology
Analytical Environmental Chemistry
Perspective in Waste Management

(4) Note: Statistics should be offered as part of the environmental option.

ENVIRONMENTAL SCIENCE OPTION AT CAU
FEBRUARY 1990

CORE CURRICULUM

Recommendations/considerations

Diversity the core curriculum to include invertebrate/ vertebrate zoology, plant biology and others? This might be done by substituting these courses in the environmental option for present courses which relate directly to the medical sciences. Research in Biology might be moved from the core curriculum to an elective option.

ELECTIVES

General Ecology

As an introductory course in general ecology, emphasis is placed surveying the traditional ecological concepts and principles of ecosystem structure and function, energy flow and trophic levels in natural communities.

Environmental Biology

2 Possibilities:

(1) An environmental science course reviewing current problems of society as they relate to the environment: water and air quality, conservation, waste management, environmental mutagenesis, food production and pest control. Review of the problems and potential solutions will be addressed from national and international perspectives.

(2) An overview of forest/fishery/wildlife biology.

Possibly both content options could be offered as 2 courses.

Environmental Biology Lab

This course surveys current field and laboratory techniques in environmental science.

Topics in Environmental Science

2 Possibilities:

(1) Multidisciplinary course in Global Environmental Issues (in conjunction with Political Science, Economics, Sociology and coordinated by Biology).

(2) Research problems in environmental science.

Population and Community Ecology

Study of the interrelationships of organisms with in a population, community and ecosystem will be studied. Factors such as social interactions, population growth and distribution will be addressed.

Aquatic/Marine Biology

Analysis of major freshwater (streams, rivers, lakes, impoundments, inland wetlands), estuarine (estuary, salt marsh, mangrove) and marine (littoral, shelf, reef, open ocean) ecosystem types, with a field trip to each major ecosystem type.

Summer Internship I and II

3 Possibilities:

(1) Summer research in EPA laboratories.

(2) Marine research in Jamaica.

(3) Dominican Republic course: Natural Resources and Energy in Developing Countries.

The following 3 courses could be offered in the Chemistry Department as part of the environmental science option:

Environmental Pharmacology/Toxicology

Basic principles of interactions between environmental chemicals, hazardous wastes and toxins with human and animal systems will be studied. Natural defenses in response to xenobiotics will be reviewed.

Analytical Environmental Chemistry

Methods of detection and analyses of hazardous environmental chemicals will be addressed. Background of analytical methods and current instrumentation will be included.

Perspectives in Waste Management

Current status of waste disposal, problems from a chemical perspective and potential solutions will be included in this course.

Note: Statistics should be offered as part of the environmental options.

ATTACHMENT 2

REPORT FROM THE INTERNATIONAL ENVIRONMENTAL STUDIES PROGRAM
DOMINICAN REPUBLIC COURSE, May-June 1990

Judith Bender and Peter Phillips, Coordinators

The program activities were coordinated from the campus of the Catholic University of Santiago (UCMM), Dominican Republic (DR). The program duration was four weeks, from May 26 to June 23, 1990. The cost per student, including air fare, was approximately $1,500.00. Much of the program for this year was subsidized by the Packard Grant to the Biology Department's International Environmental Institute.

Our in-country coordinator was Lic. Arturo Russell, Dean of the College of Sciences and Humanities at UCMM. The structured activities included:

1. Natural Resources and Energy Use. This class met for 2.5 hours during most weekday afternoons. This three-credit hour course is required for all UCMM students and is offered during their fall semester. The full-semester course content was given to our Morehouse and Spelman students in English. The multidisciplinary team included a biologist, a chemist, a social geographer, an economist, a civil engineer, a mining engineer and a visiting social science professor from the University of Sherbrooke, Canada. Themes covered were ecological principles, ecological life zones of the DR, environmental degradation in the DR, land tenure, small-scale farming and its environmental impact, deforestation, mining and mineral resources, energy resources and use, human resources and its link to environmental degradation. Students were required to
write two papers during the course. Final grades for this course will be sent by the UCMM Registrar during July.

2. Natural Resources Field Trips. Three major field trips were conducted to illustrate the classroom lectures:

a. Ferronickel mines and the company's land reclamation and water pollution containment programs. This was a demonstration of how this company reclaimed land after the open-pit mining process, through topsoil replacement and several reforestation steps. Water seepage from the mined areas are contained until suspended solids settle and effluent water quality is monitored before it reaches local waterways.

b. Central mountain chain land management project supported by the US-based Kellogg Foundation. Peasant farmers are taught, through example at several local pilot projects, how to farm on hillsides to minimize soil erosion, principles of reforestation and sustainable forest management, fish culture and agriculture without the use of agrochemicals.

c. Biodynamic agricultural project supported by the US-based Inter-American Foundation. This was a peasant training center for teaching sound soil management, water conservation and non-synthetic chemical agriculture practices. This was also a training center for US Peace Corps volunteers.

3. Intensive Spanish for Foreigners Course. On the first weekday of our program, each student received a Spanish language assessment. Spanish was not a prerequisite for this program and our students, indeed, had little prior preparation in Spanish. UCMM is well-equipped, through its Spanish for Foreigners program, to handle the Spanish novice. Each student received 3 hours of instruction each weekday morning, except when field trips were planned during a weekday. In addition to daily homework assignments, a final exam was given. Final grades for this course will be sent by the UCMM Registrar during July.

4. Latin American Culture and Society Seminars. A Dominican anthropologist presented two afternoon lectures on Latin American society; past (i.e., links to west African cultures) and present (structure of present day hispanic societies), and human resources as a component of the ecological landscape in the Dominican Republic.

5. Cultural Enrichment and Sightseeing Field Trips. Two field trips were completed on weekends outside of classroom activities:

a. Santo Domingo Colonial Zone; the capital of the DR, Santo Domingo has restored colonial areas dating from the earliest Spanish colonization of the Americas.

b. Atlantic coast weekend trip.

Each student was placed with a different middle-class Dominican family so that they would be immersed in the Spanish language and could more effectively integrate into the whole foreign experience and cross the cultural barrier. Though all students were initially very apprehensive principally due to the language barrier, this proved to be unanimously rewarding and helped to round-out the entire month's experience. We have videotaped the lectures, field trips, student neighborhood, student families, the university campus, the city of Santiago and final student interviews. The tapes will be edited to be used as a promotional tool for funding-raising activities and as a recruitment tool for future student groups.

In summary, this was a highly structured and intensive four-week program which effectively illustrated the environmental dilemmas facing a typical developing country and which presented the student with a holistic experience including environmental science, foreign language and hispanic society.

PROPOSAL FOR APPROPRIATE TECHNOLOGY SEMINARS AND FIELD TRIPS

Date	Seminar	Field Trip	Leader
Aug 27 a.m.	Sustainble Use of Resources: Unifying Theme		Bender
Aug 27 a.m.	Case Study of Natural Resource Mismanagement		Phillips
Aug 27 p.m.	Appropriate Technology: Microbial Mat System		Bender
Aug 27 p.m.	Appropriate Technology: Dom.Rep. Fish Culture		Phillips
Aug 27 p.m.	Appropriate Technology: Costa Rica Fertilizer		Phillips
Aug 28 a.m. & p.m.		Aquaculture: Auburn Univ., AL	Phillips & Bender
Aug 29 a.m.	Appropriate Technology: Wastewater Treatment Malaria Control with Fish		Bender
Aug 29 a.m.	Appropriate Technology: Metal Contamination		Bender
Aug 29 a.m.		Laboratory Visit	Bender & Phillips
Aug 29 p.m.		Clayton County Wastewater Treat. Plant	Bender & Phillips
Aug 30 a.m.	Solid Waste & Waste-water Treatment - GA Tech		Gould
Aug 30 p.m.		Solid Waste & Waste-water Treatment - GA Tech	Gould

PUBLICATIONS RESULTING FROM THIS PROJECT

Aquacultural Engineering 8 (1989) 407–419

Reprints not yet available

Fish Feeds from Grass Clippings

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&

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(Received 18 April 1988; accepted 15 December 1989)

ABSTRACT

The objective of this research was to develop a quality fish feed that could be produced from simple plant material by way of microbial processing. Silaged grass clippings, applied to shallow ponds, produced floating mats of microbial biomass containing 26% protein and 43% carbohydrate in 7–10 days. The final silage–microbe mat (SMM) showed increases of over 300% in biomass and 100% in protein content over the silage feedstock. Productivity and microbial succession patterns were similar in laboratory ponds and tropical field ponds located in the Dominican Republic. In laboratory experiments SMM-fed tilapia, Oreochromis niloticus, *showed significant growth increases over controls (fed commercial catfish fish feed), if they were allowed to graze the SMM together with the gelatinous detrital deposited during SMM culture. SMM produced little fish growth if it was harvested and fed separately in clean-bottom ponds without detrital buildup. These data show that the microbial mat produced from processed grass clippings can produce a nutritious natural fish food in the pond. The detrital trophic level in the sediment (stimulated by SMM culture), is a critical component in this diet.*

INTRODUCTION

The development of a low-cost fish feed, which can be produced in simple and practical ways, is important to both large and small aqua-

Aquacultural Engineering 0144-8609/90/$03.50 — © 1990 Elsevier Science Publishers Ltd, England. Printed in Great Britain

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culture systems. Although pond enrichment with animal manures is a well-established method of natural fish food production in ponds, manures are not always available and often have competitive uses in developing countries. A feed-production system based on simple, vegetative starter materials has the potential of expanding natural feed production in remote rural areas, where resources are low and human protein deficiencies are often high.

Schroeder (1987) reports that various trophic levels in the water column and sediments become involved in effective nutrient production after manure enrichment. Unique microbial ecosystems work in concert to (1) produce protein and carbohydrate macromolecules and (2) process these nutrients into molecular species easily assimilated by tilapia. Schroeder concludes that it is specifically the low molecular weight component which stimulates the rapid growth in tilapia *Oreochromis niloticus*. However, these components (products of anaerobic processing of detritus in the sediments) are generally soluble and would be lost in the water column without appropriate entrapment mechanisms. Gelatinous layers, deposited by copious slime producers in the sediments, may trap these effective nutrients, making them available to the grazing fish.

These conclusions are supported by Bowen's observations of nutrient production in Lake Valencia, Venezuela (Bowen, 1980). High levels of amino acids produced and retained within the detrital systems of the lake correlated with rapid growth of *Oreochromis mossambicus*.

The observations of Schroeder and Bowen signify an important direction in the production of natural fish feeds: the importance of employing all trophic levels in the process. The primary intent of this research was to design a comprehensive natural fish feed system, which systematically targets the various trophic levels (identified by Schroeder and Bowen) and develops a simple method of maximizing those environmental factors critical to fish growth.

The specific approach of this project was to (1) generate a high-quality microbial biomass in the water column from simple, vegetative starter material, (2) stimulate microbial systems for nutrient processing and storage in the detritus at the pond bottom, and (3) define the appropriate fish feeding scheme which utilizes the nutrients in all trophic levels.

MATERIALS AND METHODS

Overview

The following strategies were followed in the development of the natural fish feeds: (1) selection of a low-protein vegetative material having no

competitive uses in the Dominican Republic (DR), (2) pre-treatment of the material by silaging (anaerobic processing), (3) application of the silage to laboratory ponds, followed by observation of the microbial ecology in the water column and sediment region after silage enrichment, (5) determination of the SMM productivity in terms of rate of microbial biomass production and percent protein in the final product, (6) reconstruction of the processes in a Dominican field pond, (7) laboratory investigation of effective nutrient value for *O. niloticus* and definition of specific fish feeding regimes, which utilize all trophic levels on the pond.

Silaging pre-treatment

Native grasses were cut and silaged by packing the green cuttings into air-tight enclosures (McDonald, 1981). Spontaneous microbial processing was allowed to continue for 15–20 days.

Laboratory pond enrichment and ecosystem assessment

Laboratory ponds (Fig. 1) measuring 30 cm × 14 cm × 12 cm were layered with 2 liters of sandy-loam soil, filled to a depth of 4·5 cm with conditioned tap water, and placed in a controlled environmental chamber at 28–30°C. A shallow water column was maintained throughout the experiments to allow for increased microbial communication between the soil and pond surface, and to maximize the incident radiation to the pond sediments. Cyanobacteria was inoculated as *Oscillatoria* sp. in the first culture and emerged spontaneously in all subsequent trials.

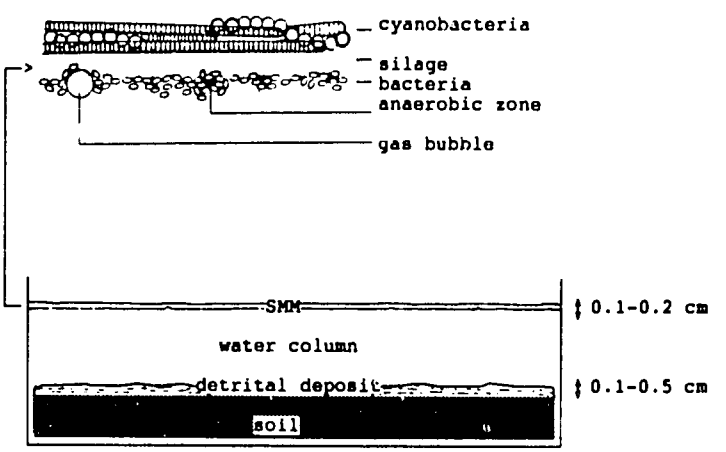

Fig. 1. Model of SMM floating on the laboratory pond and detrital gel deposit in the sediment.

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Fish growth in laboratory ponds: SMM feed versus commercial feed

Growth of *O. niloticus* fed with SMM was compared with that of the controls, fed with Purina catfish ration. Fish ranged in weight from 0·5 to 3·5 g/fish (with smaller fish used in the initial trials). Five fish were stocked in each of four pond types: (1) soil-bottom pond in which the SMM had been grown for several weeks previous to the experiment. Fish were allowed to graze the SMM at the surface and the deposits in the sediments; (2) soil-bottom pond only. In this case SMM was grown in separate ponds, harvested and broadcast daily in the feeding pond; (3) clean-bottom pond (without soil), in which SMM (cultured separately) was again broadcast in the feeding pond; (4) soil-bottom pond, excluding SMM culture, in which the gelatinous sediment material was added as fish food. This material resulted from sediment build-up during SMM culture in a separate pond; it was harvested and added daily to pond (4). In summary, pond type (1) contained the complete ecosystem: SMM (floating biomass) and the detrital component deposited during SMM culture; pond type (2) excluded only the microbial/detrital deposits resulting from SMM culture; pond type (3) excluded all soil, sediments and bottom deposits; and pond type (4) excluded the floating SMM and utilized only the detrital material in the sediments as a food supply.

In pond type (1), SMM was cultured until a thick biomass covered the pond surface and a gelatinous layer developed in the sediments. The water level was then increased to 11 cm and fish were allowed to graze until all of the SMM and microbial build-up on the soil bottom was completely consumed.

A two-week growth period was generally used in all experiments, except those with pond type (1). In these experiments the growth period was determined by the time required for fish to consume all the standing SMM, previously cultured in this pond (2–3 weeks).

In order to provide comparable quantities of available feeds in the four ponds (SMM sufficient for satiation grazing), excess SMM or sediment material was added daily in ponds (2), (3) and (4). Since the added material did not decompose, this feeding regime had no impact on the pond water quality. Identical water quality conditions were maintained in all ponds during the experiments (temperature: 26°C, pH: $7 \pm 0{\cdot}5$, ammonia: $<0{\cdot}1$ mg/liter, oxygen: 3–4 mg /liter, night and 6–8 mg/liter, daytime).

Control and test fish were weighed and measured at the end of the growth period. The resulting data were analyzed for significance with the Student's *t*-test (Snedecor & Cockran, 1979).

RESULTS AND DISCUSSION

SMM productivity

The results of this research show that native wild grass cuttings, if appropriately treated, stimulate microbial processes which generate a nutritious biomass. A mixed matrix of silaged grass, bacteria and cyanobacteria, containing 26% protein and 43% carbohydrate was produced in shallow, enriched laboratory ponds. Although several samples of native vegetation (tree prunings and green agricultural wastes) from the DR produced similar results, grasses were selected as the biomass because they are available around the ponds, grow rapidly, and can be harvested repeatedly without impacting the natural environment.

Table 1 presents a summary of the SMM feed production and nutritional value. Rapid increases in SMM weight, together with the potential for weekly harvest, result in a projected production level of 15 $g/m^2/d$. This rate of biomass production significantly exceeds that of the legume crop, alfalfa, which produces a growing season average of 5 $g/m^2/d$.

In Fig. 2 the grass silage feed stock (used to generate the SMM in the pond) is compared to the final SMM biomass after 8 days of culture. The rapid production of nutrients, illustrated in Fig. 2 and Table 1, suggest that the SMM method shows good potential for low-cost feed production.

Field production of SMM in the DR was similar to the laboratory in microbial pattern of development and final nutrient value. The field system was highly predictable and reproducible with little pond management.

TABLE 1
Characterization of the SMM[a]

Component	*Value*
Biomass production ($g/m^2/d$)	14·96 ± 5·26
Protein in biomass at 7–10 d (%)	26·01 ± 1·77
Increase SMM over grass-silage input, 9-d culture (%)	
(a) biomass weight	321 ± 148
(b) protein content	113 ± 24
Digestibility by *O. niloticus* (% of protein[b])	80·73 ± 1·23

[a]Values represent an average, with SD, of a minimum of five samples.
[b]Digestion data is from Bender & Ekpo (1989). SMM was similar in digestibility to Purina catfish ration (digestibility differences not significant, $p < 0·05$).

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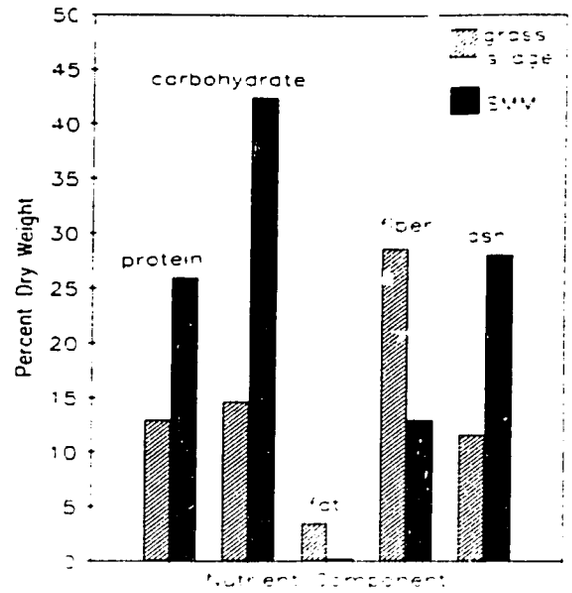

Fig. 2. Comparison of silage feed stock to final SMM. All nutrients represent a single, paired sample except protein, which is an average of 10 samples. SD for protein values: silage = 0·92; SMM = 1·77.

Microbial ecology of the silage–microbe mat production

An important directional movement of nitrogen-fixing bacteria to the pond surface, induced by the floating silaged material, was critical to the final SMM production. Positive chemotactic response ($p < 0{\cdot}02$) was confirmed by microbial growth patterns toward the grass silage on soft agar plates. The lactic and acetic acids in the silage were identified as the specific chemotaxins for motile, nitrogen-fixing microbes emerging spontaneously in the system. This physiological response probably initiated the primary microbial community at the pond surface and established this location for the subsequent successional stages.

Electron micrographs (Fig. 3) illustrate the various phases of mat culture, from early bacterial colonization of the silage to the mature mat, dominated by cyanobacteria. The specific population changes, identified during the microbial succession ((1), (2) and (3) in Fig. 4), correspond with the A, B, C series of the micrographs. Bacterial populations (approximately 40% nitrogen-fixers) emerge from the soil, bloom in the water column (Fig. 4(1)), colonize the grass at the pond surface (Fig. 3(A)) and produce slimy exudates, which anneal the microbes to the silage (Fig. 3(B)). This stage is followed by invasion of cyanobacteria

Fig. 3. Electron micrographs of the SMM system in three stages of development. A: Bacteria populating the grass on day 1; arrow = bacteria clustering around grass stoma; B: Slime production, annealing the bacteria to the grass; arrow = entrapped gas bubble within the matrix. C: Cyanobacteria filaments invading after day 4. Magnifications: A, × 1148; B, × 1813; C, × 780.

laboratory). However, the large amounts of microbial biomass involved in the nitrogen-fixation process may account for the high overall protein productivity. This factor represents the significant difference between the SMM system and that of a manure-based feed, which is based on nitrogen-concentration and conversion, rather than fixation.

O. niloticus feeding experiments

SMM, when applied under the feeding conditions of pond type (1), supports significantly higher growth in *O. niloticus* than the controls fed on costly commercial fish feed (Fig. 5). However, fish showed weight gains only if they were allowed to graze in the environment in which the SMM had been cultured. Experimental trials in which SMM or sediment material were cultured separately, harvested, and fed to the fish in either the soil-bottom or lined ponds produced no significant weight gains in the fish. Control fish showed similar growth increases in either pond type (no differences between soil-bottom or lined ponds ($p = 0{\cdot}02$)), but rates

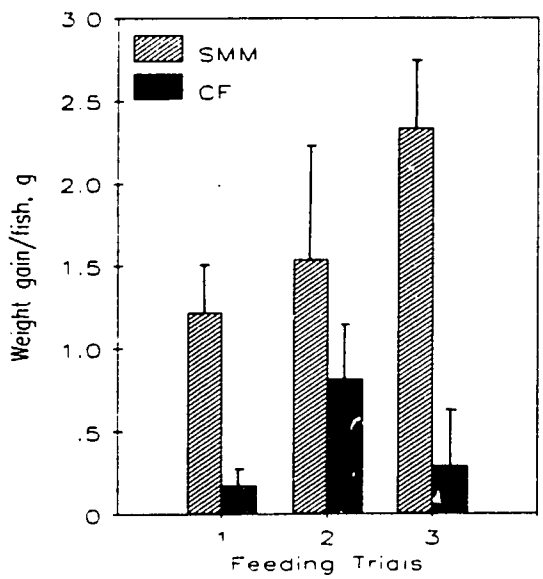

Fig. 5. Weight gain of *O. niloticus* fed SMM compared to those fed Purina catfish ration (CF). Pond conditions for feeding were for SMM: soil-bottom pond in which mat had been cultured; for CF: clean, lined pond without natural food source. Growth days/trial were: 1 = 21, 2 = 13, 3 = 15. Percent increases in weight/trial were for SMM and CF respectively: 1 = 183, 13; 2 = 81, 119, 3 = 68, 20 (all significant at $p < 0{\cdot}02$). In pond types (2) and (3), representing incomplete pond ecosystems, fish gains were significantly lower than the CF controls (data not shown).

were lower than the experimental fish grazing in pond type (1) (significant at $p < 0{\cdot}02$).

The history of the pond ecosystem, in terms of primary production and detrital processing, is clearly an important factor in the production of a balanced diet of natural food within the pond. The integration of the detrital materials with the primary biomass seems to be critical to total fish nutrition and signals the importance of the total environment in natural food production.

SUMMARY OF CONCLUSIONS AND FUTURE RESEARCH

The results of the fish feeding experiments identify the nutritional value of the SMM system and signify the importance of the detrital trophic level in *O. niloticus* growth. Low-level additions of silage to shallow ponds rapidly generated nutrient-rich biomass at the pond surface and stimulated a bloom of slime-producing bacteria within the sediment region. Cell turn-over in the heavy microbial biomass at the pond surface presumably contributed a continuous supply of cell debris for anaerobic processing in the sediments. Gelatinous deposits at the bottom of the pond may have provided an appropriate matrix for the sequester and storage of these nutritious products, thereby making them available for fish grazing. When consumed together, both components of the ecosystem (surface biomass and bottom detritus) provided a complete natural food for the fish. Since this system is based on simple and available biomass (grass) and is driven by natural microbial processes, it may hold important potential for cost-effective feed production in developing countries.

Fish growth data in Fig. 5 is considered to be significant in relative terms only (comparisons of test versus control populations). Small laboratory ponds and low water volumes represent significant experimental constraints; absolute fish production in g/ha/d will require further research in field ponds.

Environmental surveys of green materials appropriate for silaging and subsequent pond enrichment should be made. Evaluation of the materials should include the possible impact of repeated harvest of the vegetation from the environment, as well the potential of the silaged product in stimulating microbial biomass. The culture of leguminous trees, which regenerate at the pruning cut (such as the Pinion in the DR), may provide a renewable supply of silaging material within the pond area.

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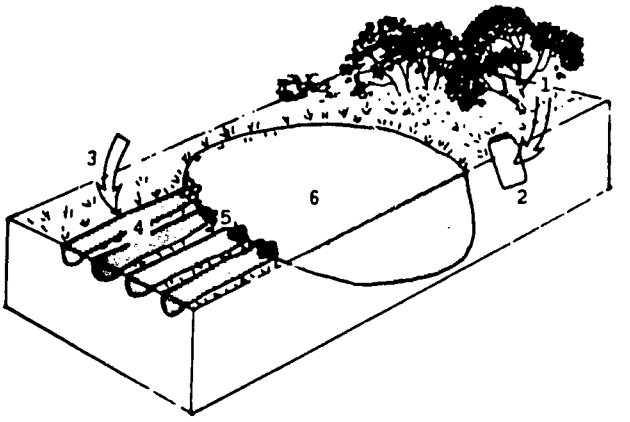

Fig. 6. Proposed experimental design for SMM production integrated with fish grow-out pond. (1) Renewable sources of green silaging material (grass, tree prunings); (2) air-tight silaging enclosure (above-ground bin or underground trench); (3) mature silage is added to SMM culture pond (arrow); (4) SMM culture lanes and grazing areas; (5) exclusion barriers during SMM culture. Barriers are removed, one lane at a time, for fish grazing and replaced when SMM is consumed. Lane is then reseeded with silage. (6) = Deep pond for fish grow-out.

Field production of SMM feed will require a change in pond design. SMM culture must be done in shallow ponds, which can be opened to deeper grow-out areas. Although the specific surface area ratio of feed production to grow-out pond is not known, laboratory data indicates that it is approximately 1:1. A method of excluding the fish from the SMM culture area must be designed into the pond system, since the fish will eat the silage directly before it can mature into the SMM. Figure 6 illustrates one pond design, which might provide a basis for further field research.

ACKNOWLEDGEMENTS

This project was supported by US Agency for International Development, Grant No. DAN-5053-G-SS-7067-00 and the National Institutes of Health, MBRS Program, Grant No. RR8006. We thank the following institutions and individuals in the Dominican Republic for their special contributions to this project: Director Hernandez and the staff of the Centro de Investigacion y Mejoramiento de la Produccion Animal (CIMPA), Director of International Programs at the Universidad Catolica Madre y Maestra, Dr Peralta: Peace Corps Volunteer, W. Briggs, of Centro Regional de Estudios de Alternativas Rurales (CREAR).

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The Progressive Fish-Culturist 51:83–86, 1989

Digestibility of a Commercial Fish Feed, Wet Algae, and Dried Algae by *Tilapia nilotica* and Silver Carp

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Abstract.—We stimulated growth of microbial food in ponds by silage grass enrichment and investigated the digestibility by fish of a harvestable mat of biomass produced in this way. Synthetic ponds, enriched with silaged grass, produced a high-protein microbial bloom (19–24% dry weight), which attached to the silage forming a slimy matrix at the pond surface. Silver carp (*Hypophthalmichthys molitrix*) and Nile tilapia (*Tilapia nilotica*) were fed rations of the microbial mat, and digestibility of fresh and dried samples was compared with that of commercial fish feeds. The fresh microbial mat was 81% digestible by Nile tilapia and 75% digestible by silver carp. The dried form was significantly less digestible by both species. Fresh microbial mat compared favorably with commercial catfish feed in digestibility by Nile tilapia.

The development of a low-cost, high-quality fish feed for aquaculture systems is particularly important in developing countries. In these regions, the effective use of simple materials in the production of feeds can determine the success of marginal aquaculture operations. In general, use of natural ecosystem processes often allows less energy-intensive technologies to be used. Therefore, it is important to investigate the environmental systems that might produce natural feeds and to determine those feeds that best suit the particular physiology of the cultured fish. It is known that tilapia (*Tilapia* spp.) and silver carp (*Hypophthalmichthys molitrix*) graze on the complex web of plankton available in natural waters. When ponds are fertilized, microbial levels increase, and the enriched pond becomes an effective source of nutrients for the fish (Schroeder 1980). Although organic fertilizer, such as manure, is a poor direct feed, it can be used to enrich a pond, thereby providing a sufficient and balanced diet. Schroeder (1980) correlated the isotopic carbon ratios of the various microbial species present in a fertilized pond with the fish harvested from the pond. His results suggest that the bacterial component is the primary nutrient source for tilapia cultured in a fertilized pond, and that cyanobacteria (blue-green algae) provide the second most important nutrient source. Protozoa and other heterotrophs showed little carbon correlation (although they may be necessary for trace amino acids missing in microbes). According to Lovell (1980), feeding habits of tilapia vary by species. Most species cultured for food are continuous feeders that graze efficiently in the water column and on the pond bottom. Tilapia benefit from highly fertile ponds because they consume plankton as well as benthic micro- and macroorganisms (Lovell 1980).

Enhancement of fish-food production by pond fertilization has generally depended on the availability of commercial fertilizers or animal manure. These resources are not always available in developing countries, or they have competitive uses in land agriculture systems, so the direction of our research has been to use simple waste plant materials to stimulate microbial production in ponds. Plant materials (and their silaged forms) contain insufficient protein levels (9–13%) to be used as direct feed.

According to McDonald (1981), silaging releases water-soluble carbohydrates, which are used to produce a bloom of lactic acid bacteria. The subsequent lowering of the pH in the environment to approximately four discourages other competitive microbes. The final silaged product is rich in lactic acid and in other nutrients in their available, mineralized form. This enriched medium, when added to a pond, supports the development of a mixed microbial system (including cyanobacteria and nitrogen-fixing bacteria) at the pond surface. We

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speculated that a community of nitrogen-fixing microbes, spontaneously emerging in the silaged-enriched pond, could substantially elevate the protein levels of the pond biomass and provide a digestible product for the fish. The specific objectives of our research were (1) to analyze the protein levels of the microbial biomass produced by ponds enriched with silaged grass and (2) to determine the digestibility of the microbial conglomerate annealed to the grass after 7–14 d of culture.

Methods

Experimental Design

The overall design of this research was to enrich simulated laboratory ponds with silaged grass, to harvest the resulting algal mat in 7–14 d, and to investigate the digestibility of this product by Nile tilapia (*T. nilotica*) and silver carp. Laboratory ponds (clear plastic tanks 58 cm × 15 cm × 8 cm) were layered with 8,000 g of sandy–loam soil. The soil was taken from compost and estuarine environments (Savannah, Georgia) in order to insure inoculation with a wide variety of soil microbes, including anaerobes that might be expected to function well in waterlogged soil. Additions of phosphate (1.25 g/kg) and lime (2.5 g/kg) were made to the soil. Three liters of brackish water were added (the medium contained, in g/L: $CaCl_2$, 0.07; $MgSO_4 \cdot 7H_2O$, 0.055; NaCl, 0.35; KCl, 0.029; K_2HPO_4, 0.0005). Initial pH was adjusted to 7.5 and generally rose to 9.5 in 7–10 d. Silaged grass clippings (3.4 g/tank, dry weight) were added to the surface of the water column together with 10 mL of log-phase *Anabaena* sp. culture. All ecosystems were maintained at 30°C. Illumination was provided intermittently (day–night cycle) by incandescent lights (one 60-W bulb per tank, placed 25 cm from the pond surface).

Silage was prepared from unfertilized, natural southern grasses, such as Kentucky fescue (*Festuca elatior*). Fresh-cut clippings were packed tightly into 1-L jars and allowed to ferment anaerobically for 20 d at room temperature (McDonald 1981). Finished silage was used to enrich the water column to stimulate the microbial bloom. The bacterial component of the bloom emerged spontaneously in the system. Cyanobacteria were supplied by inoculating *Anabaena* sp. in the water column (10 mL of a log-phase culture). The cyanobacterium strain was isolated in Tampa, Florida, and stock cultures were maintained at 30°C in 100 mL of Allen–Arnon (1955) medium (Allen and Arnon 1955) at pH 7.5.

The mixed microbial system was allowed to develop for 7–14 d. Harvest was facilitated because the microbes annealed tightly to the buoyant silage in slimy layers that could be easily harvested by raking the mat from the surface of the tank. Protein levels of all samples were determined by the micro-Kjeldahl method, as adapted by Lovell (1981). For digestibility determinations, the quantities of control and test feeds were adjusted in terms of protein equivalency.

The experimental animals used in this research were obtained from the Auburn University (Alabama) Department of Fisheries and Allied Aquacultures. Each experimental group consisted of five to six juvenile Nile tilapia weighing 3–8 g or five to six juvenile silver carp weighing 5–10 g. Fish were maintained in a 220-L aquarium containing under-gravel and side filter systems. Each group was allowed a minimum of 6 d acclimation with the test or control feeds before the digestion experiments were begun. All fish were given the test diet (microbial mat) or control feed (commercial ration) at a rate of 4% body weight (adjusted by protein equivalency), offered in four equal portions per day.

The diets were prepared with the following main feedstuffs: Purina Catfish Chow 5136 (used as the commercial fish feed, CFF), dry algal mat (DAM), and wet algal mat (WAM). Although the microbial mat at the time of harvest (7–14 d) contained silage and bacteria, it was predominantly cyanobacteria and is, therefore, referred to as an algal mat feed. The CFF diet contained 93.50% Purina Catfish Chow, 2.00% carboxymethylcellulose (CMC) as a binder, 4.00% vitamin premix (Popma 1982), and 0.50% chromic oxide as an indicator. The DAM diet contained 98.00% dry algal mat and 2.00% CMC, and the WAM diet contained 100.00% plankton mat. Chlorophyll *a* was used as a natural indicator in both DAM and WAM feeds.

A feces collection chamber was constructed by fitting a 40-L plastic bucket (bottom removed) with a large funnel and suspending it in a cylindrical fiberglass tank (Figure 1). A 50-mL syringe was used to draw out the fish feces, which collected at the bottom of the funnel. Fish were fed in a separate aquarium and transferred to the feces collection chamber 20 min after they were fed; they were returned to the aquarium after feces collection. Popma (1982) confirmed that there is negligible nutrient leaching from the feces within 30 min after defecation. Feces were collected and dried in an oven (60°C) for 24 h and stored in desiccators until analyzed.

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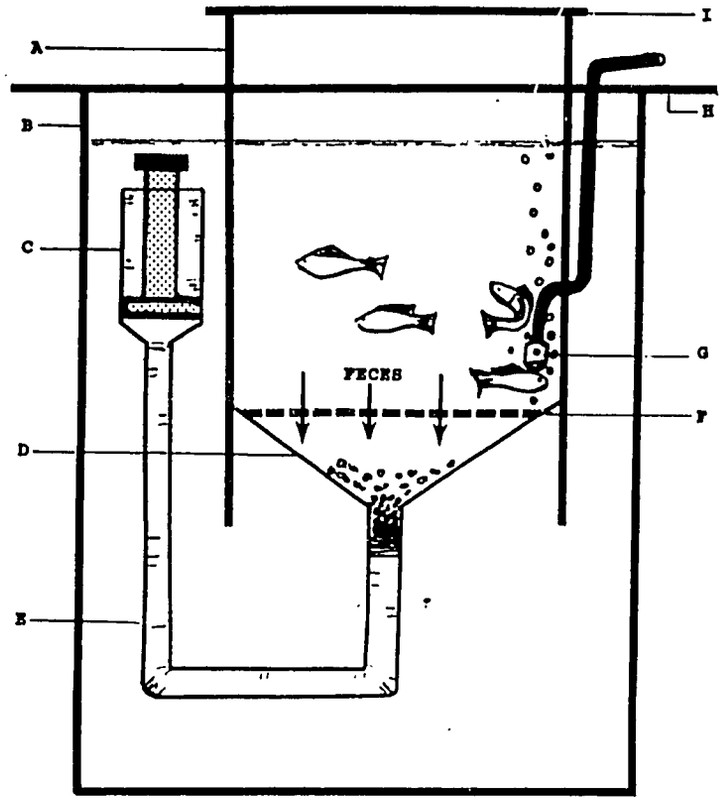

FIGURE 1.—Fish feces collection chamber. (A) 40-L plastic bucket with bottom removed, (B) 120-L fiberglass tank, (C) 50-mL syringe, (D) funnel, (E) flexible hose, (F) mesh screen (2.3 cm), (G) air stone, (H) rod for suspension of bucket, (I) Styrofoam cover.

Digestibility Determinations

Digestion determination methods for the algal mat samples followed those of Reid et al. (1950), as adapted by Popma (1982). In this method chlorophyll *a*, an indigestible pigment, is converted to pheophytin *a* during acid hydrolysis. Optical density of the hydrolysate was measured at 410 nm (Anderson and Calvin 1962); chromic oxide was used as a spectrophotometric standard. Six samples of each feed or feces were hydrolyzed to completion with an acid–acetone preparation (2.5 mL of 6 N HCl per liter of 90% acetone, used at a rate of 100 mL hydrolysis solution/50 mg of sample).

Digestion determinations of commercial fish feed samples employed the standard chromic oxide procedures of Furukawa and Tsukahara (1966). Like chromogen pigment, chromic oxide is not digestible or absorbed in the alimentary canal, and the quantity recovered in the feces serves as a marker of the amount of feed digested. In this method, chromic oxide was added to the feed and the corresponding feces samples were collected. Optical density of the hydrolysate was measured at 350 nm. Protein determinations of feces samples were identical to those used for the feeds. Percent digestibility was determined with the equations of Maynard and Loosli (1969). Data were analyzed for statistical significance by either Student's *t*-test or analysis of variance.

Results and Discussion

Production of Microbial Protein

The addition of the silaged grass to the pond surface produced a large bacterial bloom (10^5–10^8 cells/mL) in the water column during the first 3 d. The bacterial bloom was followed by a predictable succession to the cyanobacteria stage, which remained stable during 14 d of culture. The microbes annealed to the silage, forming a self-buoyant mat that maintained its floatation by trapped gas bubbles within the biomass. Protein levels generally increased twofold, from 9–13% in the initial silage to 20–25% in the final microbial biomass at the pond surface. In 8 d of culture, the dry weight increase of the surface biomass was 150% (gain over the initial silage used for enrich-

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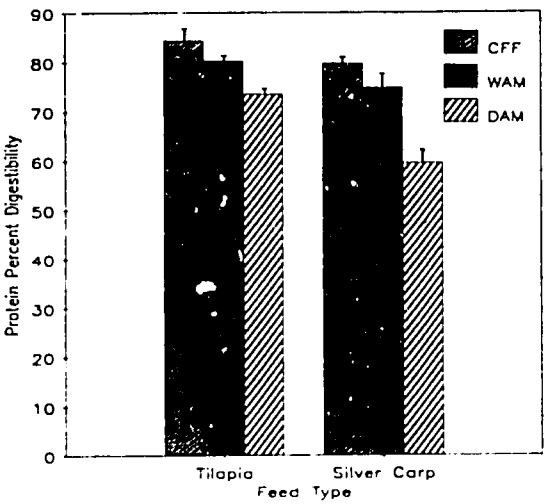

FIGURE 2.—Percent protein digestibility of natural and commercial fish feeds by Nile tilapia and silver carp. CFF = commercial fish feed; WAM = wet algal mat; DAM = dry algal mat.

ment). After the tray soil was spent by three sequential harvests, the 8-d biomass weight increase was 75%, but the protein values remained approximately the same. In certain trials, the protein and biomass productivity appeared to decrease significantly. This effect seemed to correspond to blooms of algal grazers in the water column.

Digestibility of the Microbial Mat

Nile tilapia and silver carp have similar feeding niches, so a similarity in the digestibility of natural microbial food was expected and was realized (Figure 2; Table 1). Generally, Nile tilapia, with a stomach pH of 1.2 (Bowen 1976), was more efficient in digesting all feeds than was silver carp ($P < 0.005$). Drying significantly lowered feed digestibility in terms of both protein and dry matter

TABLE 1.—Crude protein and dry matter digestibility by Nile tilapia and silver carp fed commercial fish feed, wet algal mat, and dry algal mat.

Feed stuff[a]	% protein	% digestibility of crude protein (mean ± SE; N = 3)	% digestibility of dry matter (mean ± SE; N = 3)
		Nile tilapia	
CFF	30.54	84.38±2.51	50.46±1.89
WAM	25.39	80.73±1.28	62.05±1.35
DAM	19.25	73.62±1.38	49.98±2.48
		Silver carp	
CFF	30.54	79.78±1.60	63.51±2.00
WAM	25.39	74.96±2.10	59.66±1.88
DAM	19.25	59.62±2.40	44.48±2.80

[a] CFF = commercial fish feed; WAM = wet algal mat; DAM = dry algal mat.

($P < 0.005$) and would probably compromise the effective nutritional value for the fish. Nile tilapia digested the wet algal mat as well as the commercial feed (Figure 2). This was an important finding because it demonstrates that nutrients in the algal mat will be available to the fish. However, the data do not provide information about how well the algal mat feed is assimilated. The similarity in digestibility of commercial feed and wet algal mat suggests that the substitution of cultured microbes for all or part of a commercial feed may have potential value. The question of nutritional balance, not addressed in this research, remains an important issue for further investigation.

Acknowledgments

Richard T. Lovell, Department of Fisheries and Allied Aquacultures, Auburn University, and S. A. Meyer, Microbiology Department, Georgia State University, generously gave advice and recommendations in this research.

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INTERNATIONAL CONFERENCE ON 3RD WORLD STRATEGIES FOR TECHNOLOGICAL DEVELOPMENT

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Date: 18th January, 19X

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PAPER FOR PUBLICATION

On behalf of the Conference Planning Committee we wish to inform you that your paper AN EMERGING TECHNOLOGY I: CONGLOMERATE OF ALGAE, BACTERIA AND SILAGE AS ALTERNATIVE SOURCE OF FISH FEED.

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M.A. Madusolumuo
Secretary
Conference Planning Committee

An Emerging Technology I: Conglomerate of Algae, Bacteria and Silage as Alternative Source of Fish Feed

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ABSTRACT

Nitrogen-fixing bacteria and blue-green algae were raised on silage grass and fed to Tilapia nilotica and Hypophthamicthys molitrix (Silver Carp). The objective of this research is to investigate the digestibility of plankton mat by silver carp and a tropical species of cultured fish, T. nilotica. The crude protein content of the conglomerate was analyzed using micro-Kjeldahl determination of nitrogen method and found to be 25.39%. The results show that fresh microbial mat is 80.73% digestible by tilapia and 74.96% by silver carp. This compared favourably with commercial catfish feed in digestibility by tilapia.

INTRODUCTION

In commercial fish farming, protein-based commercial fish feeds have generally been relied upon as the main source of fish food. Unfortunately, commercial fish feed has proved to be the single most costly item in fish culture, determining the viability of aquacultural projects. In developing countries such high quality plant protein as soybean, corn, peanut, and cottonseed meal are either not available or are given priority for human or farm animal use and are not generally used for fish feeds (Stickney, 1986).

The artificial system of microbial production, used in this study, is not the one commonly used to stimulate microbial fish feeds (that is, integrating the pond with minimial production and using the manures for microbe production). In this research, free-living nitrogen-fixing microbes are used to increase the protein content employing silage grasses or leaves as a source of available carbohydrates. The littoral biomass or silage/plankton mat is used as a substrate in thus investigation rather than allowing the microbes to float in the water column or culture in the benthic region. The specific objectives of this work are: (1) To produce a microbial biomass from waste organic materials (2) To ascertain the digestibility of the microbial conglomerate mixture.

MATERIALS AND METHODS

Blue-green Algae (BGA) Culture: The BGA used in this study were non-axenic species of Anabaena, NB19 strain isolated in Tampa, Florida. Microscopic examination of the strain revealed that it is filmentous and heterocyst-forming cyanobacteria, similar in morphology to our standard strain of Anabaena (Carolina Biological Supply Co., Burlington, North Carolina). Stock cultures were grown and maintained at 30°C in either 50 or 100 ml of modified Allen-Arnon (1955) medium at pH 7.5-8.0.

Grass silage: Many tropical and temperate plant materials were ensilaged according to the method of McDonald (1981).

Estuary soil: The estuary soil used in this research was taken from the seashores of Savannah, Georgia. It was carried in heavy-duty plastic bags to the laboratory, air-dried and stored in a greenhouse.

Plankton tank: Several plankton tanks were constructed using plexiglass about 0.5 cm thick each measuring 65.5 x 14.5 x 17.5 cm. The estuary soil was crushed into small sizes and 8 kg of the soil was spread to cover the bottom of the tank evenly (about 5.5 cm from the bottom on all sides). On top of the soil was laid either one or two thickness(es) of fiber glass mesh. A layer of oyster shells was last to cover the bottom. A strip of black paper was taped to the outside of the tank to cover it from soil level to the bottom; this prevented light from reaching the soil except from the top.

Biomass production: To produce the conglomerate of BG algae, grass silage, and nitrogen-fixing bacteria a pond ecosystem was simulated and plankton mat generated. The plankton tank was filled with enough brackish water, made up according to Schroeder (1980), untill the level of water was 3.5-4.0 from the top of the tank. The mixed microbial system was allowed to develop for 7-14 days. Protein levels of all samples were determined by the micro-Kjeldahl method as adapted by Lovell, 1981. The quantities of control and test feed were adjusted in terms of protein equivalency.

Experimental animals: The animals used in this work were obtained from Auburn University, Fisheries Department. Each experimental group consisted of 5-6 juvenile tilapia weighing 3-8 g, or 5-6 silver carp fingerlings weighing 5-10 g. Fish were maintained in 330 liter aquarium, containing underground and side filter purification systems. Each group was allowed a minimum of 6 days acclimation with the test or control feeds before beginning the digestion experiments. All fish were given the test (microbial mat) or control (commercial fish feed) at a rate of 4% body weight, offered in 4 equal portions per day.

Diet preparation: The diets were prepared using the following main feedstuffs: Purina Catfish Chow #5136 used as the commercial fishfed (CFF), dry algae mat (DAM) and wet algae (WAM) as shown in Table 1.

Table 1. Diet composition (expressed as percentages of dry diet)

INGREDIENTS	TEST FEEDSTUFFS		
	CFF	DAM	WAM
Catfish Chow	93.50	0.00	0.00
Plankton mat	0.00	98.00	100.00
Carboxymethyl Cellulose (CMC)	2.00	2.00	0.00
Vitamin premix[1]	4.00	0.00	0.00
Chromic oxide indicator	0.50	0.00	0.00

[1]The quantity of vitamin premix components included in each kg of dry diet were: Thiamine 4 mg. riboflavin 4 mg, pyrodixine 4 mg, folic acid 2 mg, pantothenate 16 mg, chlorine chloride 1 mg, niacin 59 mg, B_{12} 0.01 mg, vitamin A in acetate form 2 mg, vitamin D_3 1 mg, menadodoine-Na-bisulfite 20 mg, inositol 156 mg, biotin 1 mg, and ethoxyquin 80 mg (Popma, 1982).

The pellets of the CFF were ground in a Waring blender for 20 min with the other ingredients listed in Table 1 added and thoroughly blended after distilled water (30-35%) was added to form a paste. DAM was prepared by drying the harvested plankton mat over anhydrous $CaSO_4$ (Drierite) in a 60°C oven for 48 h. Previously most of the water had been soaked out using paper towels and the mat cut with scissors into very small pieces. Enough water was added (30-35%) to make a paste and strings were made by forcing the paste through a 50 ml stringe. The final product was stored at -15°C in freezer bags until needed for feeding.

WAM was prepared by removing most of the water with paper towel as described earlier and then cutting with scissors into a 2-4 mm pieces. This product was stored at -15°C in airtight plastic bowls. The dry weight/wet weight ratio was determined.

A feces collection chamber constructed by using a ten-gallon plastic bucket with the bottom removed. This was fitted with large funnel and suspended in a cylindrical aquarium. A 50-ml syringe was used to draw out the feces which collected at the bottom of the funnel. The feeds were dried in an oven (60°C) for 24 hr and stored in dessication until analysed. After the fourth (last) feeding the fish were transferred into the feces collection chamber and held there for 20 mins. After this time, the fish were returned to the feeding chamber and the feces collected. It had been established by Popma (1982) that there is negligible nutrient leaching from the feces within 30 min following defecation.

Digestibility Determinations: Digestion determinations of the algal mat samples were those of Reid *et al*. (1950) as adapted

by Popma (1982). This method relies on the fact that chlorophyll a, an indigestible pigment with optical density peaking at 430 nm, is converted to pheophytin a (peaks at 410 nm), during acid hydrolysis. Absorbance of the hydrolysate was read at 410 nm, using chromic oxide as a spectrophotometric standard. Hydrolysis of feed or feces samples were accomplished by using a mixture of 2.5 ml of 6 N HCl per liter of 90% acetone. Digestion determinations of commercial fish feed samples were done according to the method of Furukawa and Tsukahara (1966). In this method, like chromogen pigment, chromic oxide is not digestible or absorbed in the alimentary canal, and the quantity recovered in the feces serves as a marker of the amount of feed digested. Crude protein content of samples was estimated by multiplying Kjeldahl nitrogen (Horwitz, ed. 1970) by 6.25.

Statistical Analysis: Statistically significant differences in comparisons of some of the data were analysed by either a Student t-test or analysis of variance (Snedecor and Cochran, 1979).

RESULTS AND DISCUSSION

Biomass Production: The addition of the silaged grass to the pond surface produced a large bacterial bloom (10^5 - 10^6 cells/ml) in the water column during the first three days. This was followed by the cynobacterial stage, which remained stable during 14 days of culture. The microbes annealed to the silage forcing air bubbles which enabled the mat to become self bouyant.

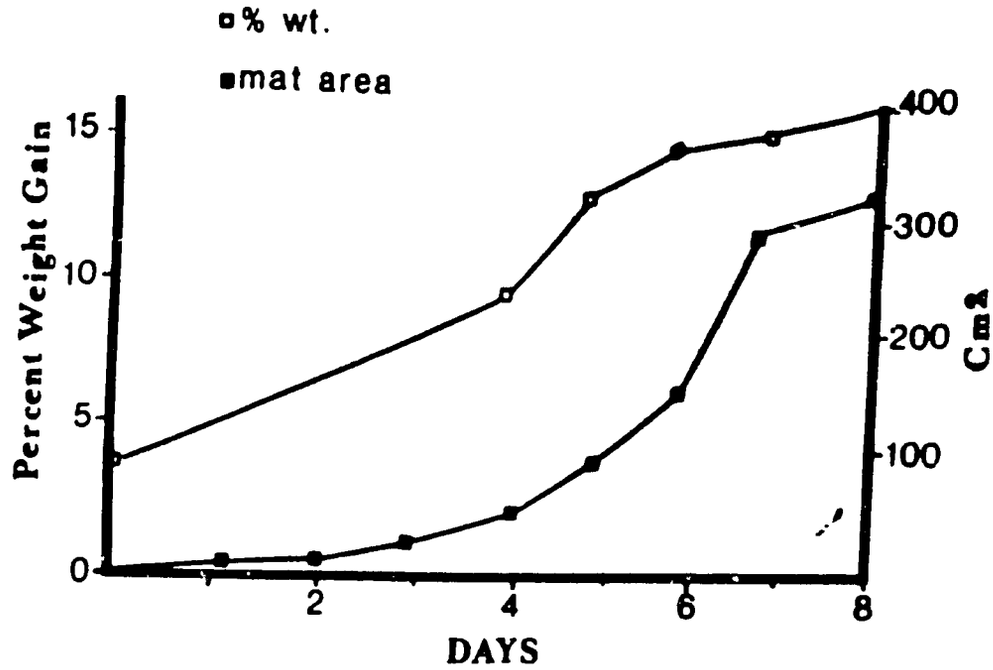

Figure 1. Percent weight gain (□) of plankton mat and mat area (■) in cm^2 in simulated pond ecosystem.

In 8 days of the culture the weight increase of the surface biomass was 150% (gain over the initial silage used for enrichment). The protein levels generally increased two-fold, from 9-13% to 20-25% in the final biomass (Figure 1).

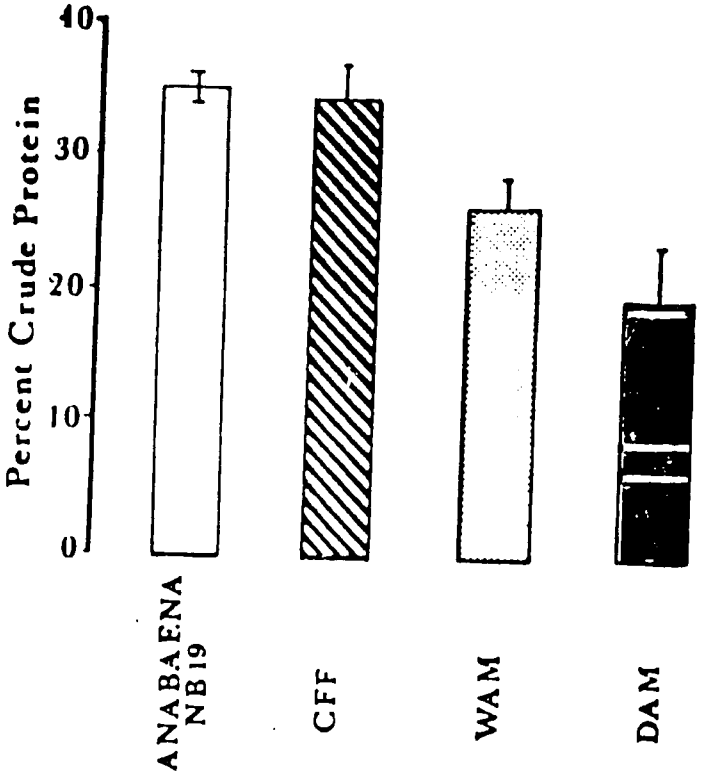

Figure 2: Histogram of the percent crude protein content for the different fish feeds.

The protein content in pure algae (Anabaena NB19) is comparable to that in commercial fish feeds 35% and 34.80%, respectively. (Figure 2). However, the protein content of wet algae mat is 25%. It has been found that 25% protein feed was as effective as 35% protein feed in mainatining weight of channel catfish in ponds over winter. The protein content in algae mat might be increased by reducing the amount of silage, though this might result in longer turnover time for optimal weight gain. This increased percent protein in algae mat could be used in feeding younger fish since high-prtein diets are generally used as fry starters (Subcommittee on Warmwater Fish Nutrition, 1983). The CFF without indicator contained more protein than CFF diluted by indicator ($P<0.01$). The protein difference between WAM and DAM is not statistically significant. Table 2 shows the protein digestibility of different fish feeds in tilapia and silver carp. Tilapia and silver carp are known to have identical feeding habits, therefore the closeness of digestibility is expected. Tilapia digests CFF protein better than silver carp, but silver carp on the other hand digests CFF dry matter better (63.51%) than tilapia's 50.46%. The differences may be attributed to

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Our Ref: CPC.2/Vol.I

Date: 18th January, 1990

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An Emerging Technology II: Nitrogen-fixing bacteria as an ingredient in fish food production

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ABSTRACT

Isolation of free-living nitrogen-fixing bacteria from Georgia soil had been achieved for the first time. Employing lactic acid from silaged grass 40% of all the bacterial population was found to be nitrogen fixers. The separation scheme by Krieg and Holt (1984) was used in the isolation and identification of the bacteria. Microbial mat and formation was not possible without the bloom of the nitrogen fixers firstly, by day three following inoculation. The specific objective of this research had been to isolate and use free-living nitrogen-fixing microbes to increase the protein content employing silage grasses as a source of available carbohydrates and to identify the major microbial genera in the biomass.

INTRODUCTION

The organic fertilizer, such as manure, is a poor direct feed. Schroeder (1979), in using carbon ration analyses, suggests that it is the bacterial component primarily and cynobacteria (blue-green algae) secondarily that provide the nutrient content on which they grow. Protozoa and other heterotrophs, according to his report, showed little carbon correlation (although they may be necessary for trace amino acids missing in bacteria). There had not been any efforts at producing and using free-living nitrogen-fixing bacteria as the main ingredient in fish food formulation. According to Moriarity (1973) the acidity in the stomach of tilapia actually reaches concentrations capable of lysing the cell walls of certain vegetable cells and bacteria. Since bacteria and blue-green algae have similar cell walls, the low pH of 1.25 in S. mossambicus may be effective in lysing of bacteria (Bowen 1976). Several studies have been done on sources of protein and other nutrients for tilapia. Though absolute protein requirements have not been completely outlined for any species as yet (Stickney, 1986); the nutritive value of detritus has been demonstrated in terms of amino acid balance (Bowen, 1976, 1980 and 1981). Since it is now established that tilapia can lyse the cell-wall of bacteria; this project aims to capitlize on this by using readily available organic base-lactate, the end product of lactic acid bacteria metabolism. The

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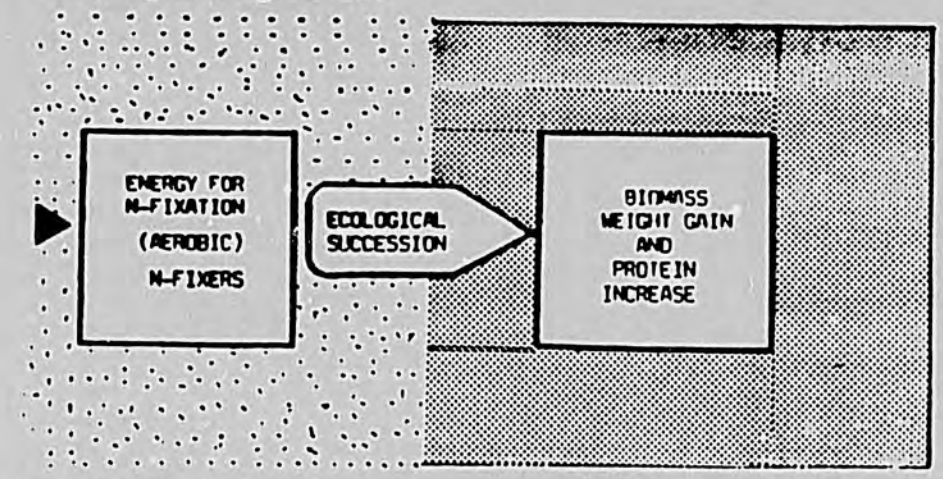

lerial Phase 1-3 days Algal Phase 4-10 days

cological succession in simulated pond.

ilm covered most of the water surface for the first 3
e sharply followed by the algae bloom on day 4, with
daily increase in size of biomass to day 7 when the
o brown on top.

opulation and some parameters

count and concentration of CO_2, O_2, reducing sugar,
nitrate were as shown in Figures 2 and 3.
bacterial population was 10^7, peaking on day 3 at
r day 3 the bacterial count reduced steadily to 10^1
ys. The initial CO_2 concentration was 10 mg ml^{-1} up to
ly the 4th day when there was a sharp drop to almost
or the rest of the days stayed at this level. Oxygen
slight decrease from 4.3 mg l^{-1} on day zero to about
one, after which there was a gradual increase to level
9th day at approximately 7 mg l^{-1}. There was steep
the level of reducing sugar from 10 mg l^{-1} initially
the 10th day. The NH_3 concentration showed initial
1.2 mg l^{-1} to 12 mg l^{-1} on day one, from there fell
almost zero on the 13th day. Nitrate rose from zero to
most 4 mg l^{-1} on day 3 and then gradually dropped to
on the 11th day.
Lactic acid bacteria used the water-soluble
es (glucose, fructose and fructans) as energy source
quickly and change the pH of the environment to about
uraging other competitors like Clostridia. The final
actate, supported the bacterial-algal system in the
harp fall in CO_2 content to almost zero and rise in O_2

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Figure 4. Concentrations of reducing sugar (●), ammonia, and nitrate (★) in estuary soil-brackish water ecosystem.

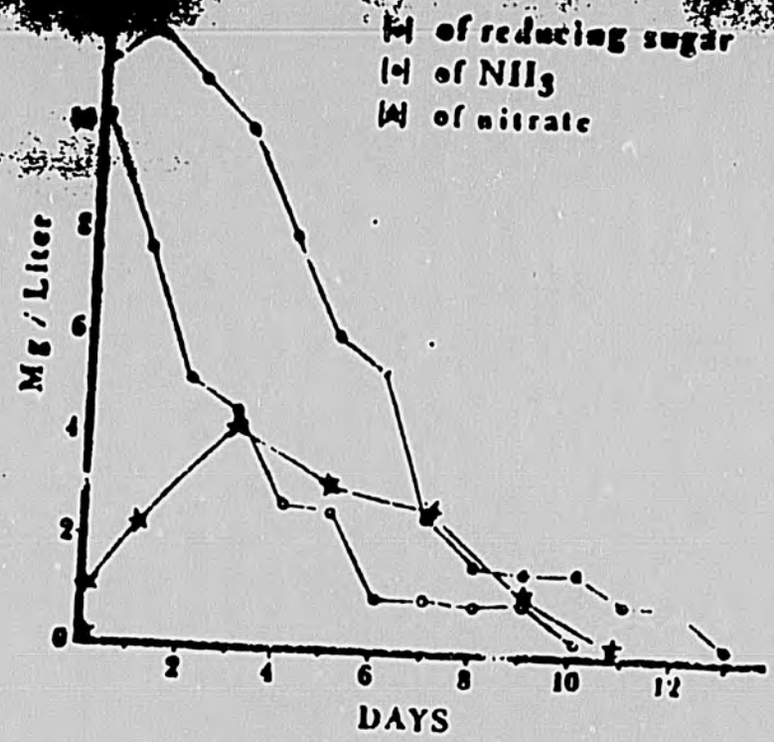
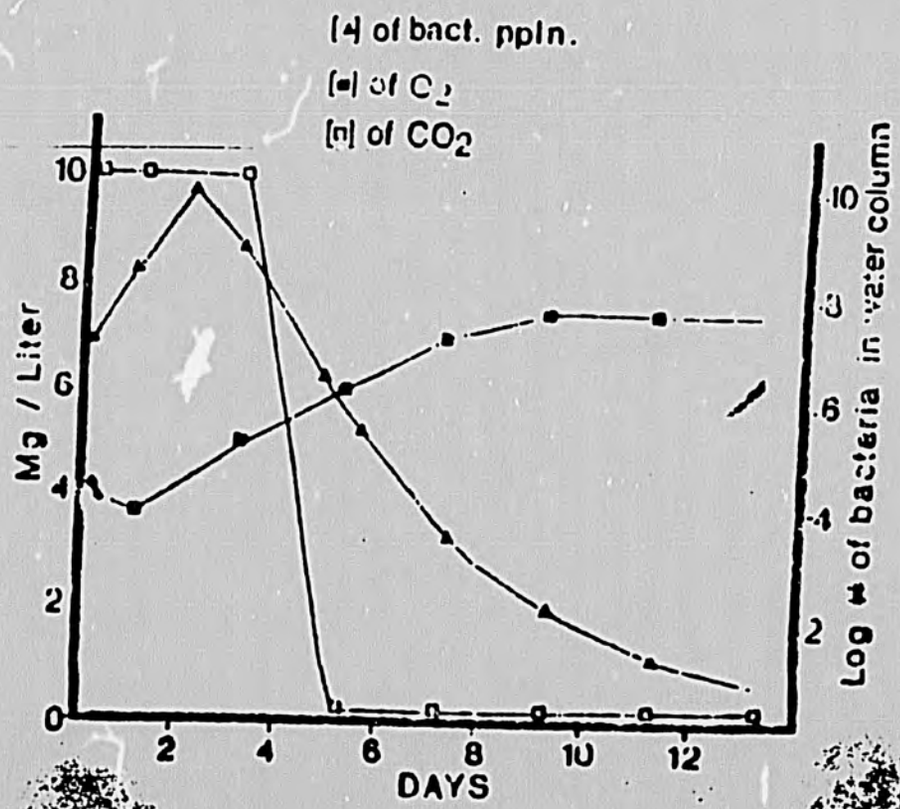


Figure 5. Bacterial population (▲), concentrations of CO_2 (□), and O_2 (■) in estuary soil-brackish water simulated pond ecosystem.



58

content might indicate that the system was being changed from anaerobic to micro-aerobic and this was an ideal environment for free-living nitrogen-fixing microbes and BGA. Nitrogenase functions better under low O_2 tension (Stewart and Tex, 1970; Anderson et al., 1980). While BGA uses the specialized organelles, heterocysts, to protect the nitrogenase, Azotobacter produces slime around the cell and respires at a very high rate, decreasing O_2, to achieve the same objective (Fogg et al., 1973; Anderson et al., 1980). The fall in the level of reducing sugar further illustrates the activity of these microbes (in this case the nitrogen-fixing bacteria) which can use this as an energy source for the production of NH_3. The initial nitrate content was close to zero in the pond water column, but rose to 4 mg l^{-1} by day 3. This corresponded with the rapid increase of the nitrogen-fixing bacteria.

Identification of free-living nitrogen-fixing bacteria

It was noted that morphologically, Azotobacter is larger than Azomonas and can also form cysts, while Azomonas cannot. The most prevalent kinds of bacteria separted from the water column consisted of 40% aerobes, 20% anaerobes, 27% aerobic nitrogen-fixers and 13% anaerobic nitrogen-fixers. Of the aerobic nitrogen-fixers the gram negatives made up 60% and the gram positives, 40%. The 60% for the gram negative was composed of 35% genus Beijerinckia, genus Derxia 40% and family Acetobacteraceae 25%.

The apparent high percentage of free-living nitrogen-fixing bacteria may be due to the aquatic environment, which was specifically designed to favour the development and growth of these organisms in the system. The percentages, therefore, do not necessarily reflect those typical of natural environment which has not been enriched. Isolation of free-living nitrogen-fixing bacteria from Georgia soil has not been reported,however, the estuary soil used in this research came from Savannah, Georgia, a subtropical region. This may represent the source of these organisms in the simulated ponds. In addition, Azotobacter had been known to be dormant for more than 2300 years (Abd-al-Malek and Ishac, 1966) and will replicate when conditions become conducive to growth. This may also explain why these organisms were also isolated from very old compost soil in Atlanta, Georgia. It is important to note the potential of generating Azotobacter blooms by simply adjusting the environmental condition. These had been used to generate rich fish food in the conglomerate of algae mt.

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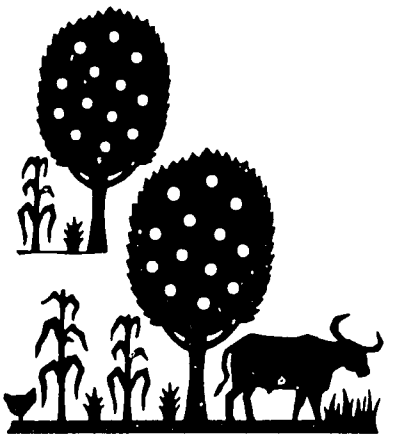
SUSTAINABLE AGRICULTURE



Developing a variety of agricultural strategies that are productive and sustainable is critical not only to third world people, but to the survival of natural ecosystems worldwide.

Sustainable agriculture can be considered from a purely technical point of view, or from the vantage point of the attitudes and philosophies that underlie specific agricultural practices.

From a technical point of view, sustainable agriculture – in which productivity can be maintained indefinitely – generally includes the basic techniques of nutrient conservation and recycling. It maximizes species well suited to the natural environment, does not overload the carrying capacity of the ecosystem and avoids the use of synthetics.

The foundations of sustainable agriculture can often be identified within third world cultures since the methods frequently used are associated with labor-intensive agriculture.

In contrast, the familiar chemical-dependent western systems of agriculture, so widely accepted as the only viable methods for the 21st century, often exclude considerations of long-term sustainable potential. The underlying attitudes reflect a position of domination over nature – a scientific philosophy that evolved with the revolutionary advances in agricultural technologies.

Systems associated with both sustainable and chemical-dependent methods of agriculture have certain inherent problems as well as potentials. For example, green revolution strategies, which depend on inputs of commercial fertilizers, insecticides and irrigation water, can count on rapid increases in productivity, while certain traditional and ancient low technology methods can be environmentally devastating when practiced on a larger scale by rising populations.

In the process of developing new ways of producing food that both support human needs and sustain the natural environment, it will be critical to review our basic attitude toward nature.

One model of sustainable agriculture in Rio Limpio, Dominican Republic, is particularly impressive because it remains highly productive while reclaiming and restoring a seriously damaged environment. Deforested hillsides that have been denuded of top soil by water erosion, are terraced and replanted with indigenous trees, shrubs, and vegetables.

Rain water is channeled through trenches, designed within the terraces, and stored in reservoirs for the dry season. A variety of trees, shrubs and vegetables are placed for maximum use of sun and soil nutrients. Fertility is replenished by frequent intercropping of legumes and by recycling all vegetative and animal residues.

Since the tools used at Rio Limpio are simple hoes and shovels, these methods of agriculture bring human hands back in touch with nature. Resident farmers, who now serve as teachers in the project, explained that careful tending to an eroded hillside can reclaim the agricultural productivity in a single season. The brown and barren mountains of Haiti, visible across the Dominican border from Rio Limpio, stand in sharp contrast to these lush, green productive slopes – setting a new standard for hope in the region.

Baobab Farm near Mambasa, Kenya, provides a unique example of environmental recovery linked to sustainable agriculture. There, near the Indian Ocean, all surface vegetation and soil were systematically removed in limestone mining operations by a concrete factory. Because of the atypical environment, the team of scientists and naturalists working in the region developed their own unique methods of agriculture.

They approached each problem as a student of the ecosystem, rather than as a dominant human technician. The tree farm is one of their many successes. As I examined the tall and healthy pine trees growing from crusty limestone rock on Baobab Farm, I noticed the conspicuous absence of all soil. The methods used to accomplish such a feat revealed more than a technology. "Microbes" my guide explained. "I go into the forest and just watch for the most successful trees, then prepare simple microbe mixtures from their root soil."

Assuming that the old trees in the forest had linked successfully with the microbial community, he applied their soil samples to each of the cultured seedlings. The resulting rapid growth produced the first harvest of poles for housing construction in three years, thereby relieving some of the pressure on nearby forests.

As I stood in the shady, cultured pine woods, it was hard to imagine that several years earlier this was a barren, rocky field baking in the sun.

There were many unexpected lessons in sustainable agriculture to be learned from this ingenious group. The one that applies to all is that of forming alliances with nature, assuming a posture of respectful partnership.

I realized that in some indescribable way the attitude of the Kenyan naturalist is an important component of their success story. For years he had cared for a blind, highly poisonous Black Mamba snake, "because the fields belonged to him long before they came to us."

By Judith Bender

Wat. Sci. Tech. Vol. 21, Brighton, pp. 1661–1664, 1989.
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0273–1223/89 $0·00 + ·50
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Ponds/Vegetation

LEAD REMOVAL FROM CONTAMINATED WATER BY A MIXED MICROBIAL ECOSYSTEM

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ABSTRACT

The research reported here explored the advantages of using an integrated ecosystem for the uptake of lead and examined some of the possible mechanisms for the transfer of the metal through the system with eventual binding in the microbial biomass. Strategies were applied to (1) stimulate enhanced production of biomass and consequent lead recovery by simple enrichments of the microbial environment, and (2) increase the microbial tolerance and lead uptake capacity by adaptation of the component species.

The microbial ecosystem employed here was one which arose spontaneously after pond enrichment with silaged grass clippings and moved through a predictable microbial succession. Ecosystem processes and microbial relationships resulted in the mobilization of metal in the soil bed and water column with ultimate deposition in the surface biomass. The stable silage-microbe biomass, floating at the top of the pond, bound the metal for extended periods of time.

MATERIALS AND METHODS

Clear plastic tanks were layered with 300 g of sandy-loam soil and filled with 3 liters water. Initial pH was adjusted to 7.5 and generally rose to 9.5 in 7-10 days. Grass clippings, prepared by anaerobic digestion (silaging process), were added to the water column at a rate of 48 g/m^2. Blue-green algae (Anabaena sp, NB-19-Pb) and a mixed population of bacteria (initially selected from the pond water column) were adapted for lead tolerance, then inoculated into the pond. Adaptation procedures were uv exposure (5 min at 56 cm) and step-wise exposure to increasing lead concentrations. Lead nitrate was added at a level of 300 mg/tank. Precipitation was allowed to occur, since it did not interfere with subsequent transport of metal. In lead spiking experiments 300 mg/tank were added nine times over the 30-day test period. Ecosystem samples of the water column, soil bed, surface biomass were acid hydrolyzed and analyzed for lead with a Varian 700 atomic absorption unit.

The bacteria and cyanobacteria were allowed to progress through their natural successional stages to the stable climax community of a silage-microbe biomass floating at the water surface. Metal concentrations were monitored daily in the water column and the soil bed. The biomass, developing at the surface, was generally not disturbed until the final harvest at the end of the experiment. In specific experiments, designed to monitor the metal flow within the system, a series of 10 identical tanks were set up and the surface microbes were harvested from 1 tank daily during the various successional stages. Metal analyses of the biomass were correlated with those of the water column. These experiments provided data on the migration of the metals during the formation of the silage-microbe mat at the water surface.

RESULTS AND DISCUSSION

Figure 1 shows the results produced from a series of 10 identical tanks harvested and analyzed daily. The quantities of lead in the biomass, correlated with the lead remaining in the water column, suggested a pattern of transport from the water to the surface mat. The first 4 days of culture could be characterized as the bacterial phase, during which a bacterial bloom occurred in the water column and around the silage at the pond surface. Generally during this period, the lead lost from the water column was located in the soil bed, with little uptake occurring at the pond surface. As the algal phase initiated, with green filaments attaching to the floating silage, lead began to accumulate in the surface biomass, indicating the predominant role of algae in sequestering lead. However, the involvement of bacteria in the process appeared to be substantial. Any interference with the bacterial phase (i.e. omitting the inoculation of metal-tolerant bacteria) prevented the establishment of the surface mat and resulted in almost total deposition of the lead in the soil. It is significant that the rapid accumulation of lead in the mat corresponded with a decrease in bacteria from the water column, further reinforcing a linkage between bacterial behavior and lead transport in this system.

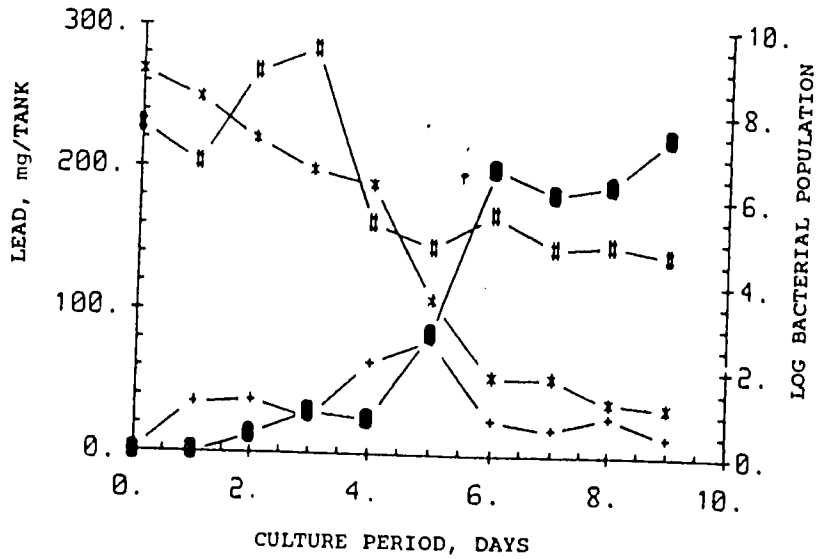

FIGURE 1. LEAD FLOW THROUGH THE ECOSYSTEM.

Lead concentrations: * = in water column, ● = in surface mat of microbes, + = in soil; # = bacterial population in water column.

Predominance area diagram (not shown), based on the composition of the water column and the solubility products compiled from Sillen and Martell (1964, 1971) predicted the deposition of basic lead chloride, $Pb_2Cl(OH)_3$, at the observed pH range in the water column. The computations based on known chloride hydroxide and sulfate equilibria indicated that equilibrium concentrations of lead in the systems should have been consistently less than approximately 2 mg/l. There were strong indications that both transport of lead from the soil and mobilization in the water column were dependent on the bacterial component of the system. Substantial water column lead levels were associated with materials retained by a 0.45 μm filter, which were probably bacterial cells. It can be postulated that the bacteria functioned in 2 ways to moderate lead behavior in these systems: (1) they took the lead up from solution by an undefined sorption mechanism and (2) they excreted an extracellular chelating agent enabling the lead to remain in solution well in excess of predicted solubility limits. Such a chelating agent might be produced by a metal tolerant mutant as a chemical function to detoxify the lead.

In Figure 2 a theoretical diagram of the floating biomass is presented. It is believed that the heterogeneous array of micro-environments in the silage-microbe mat provided conditions appropriate for the precipitation of PbS. Microprobe analyses of the mat identified anaerobic zones within the mat and sulfate-reducing anaerobes were identified in the biomass samples. In addition, acidified samples of the mat produced a positive sulfide test with lead acetate paper. It is speculated that the production of sulfide within the microbial biomass would decrease the toxicity of the lead by precipitating the metal as PbS in the intercellular spaces. This may account for the fact that the observed metal-tolerance levels of the microbes functioning in the ecosystem surpass those of the component microbial species cultured alone.

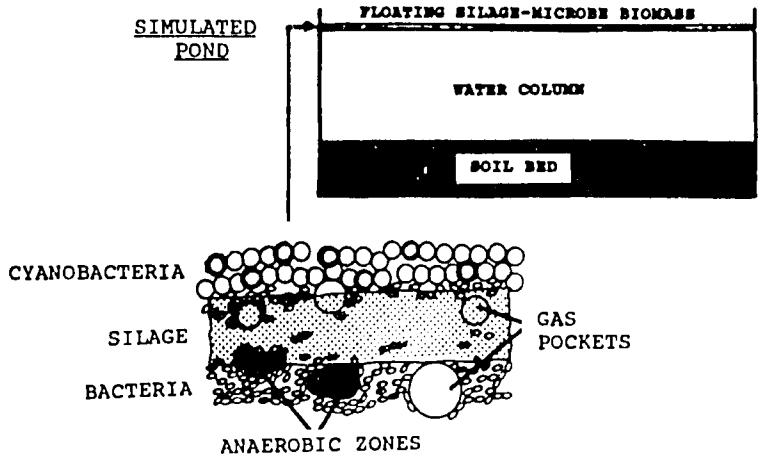

FIGURE 2. MODEL OF THE FLOATING BIOMASS AT THE POND SURFACE.

Figure 3 shows the results of a spiking experiment maintained for 30 days. After the stable climax ecosystem was established (day 6), lead uptake was

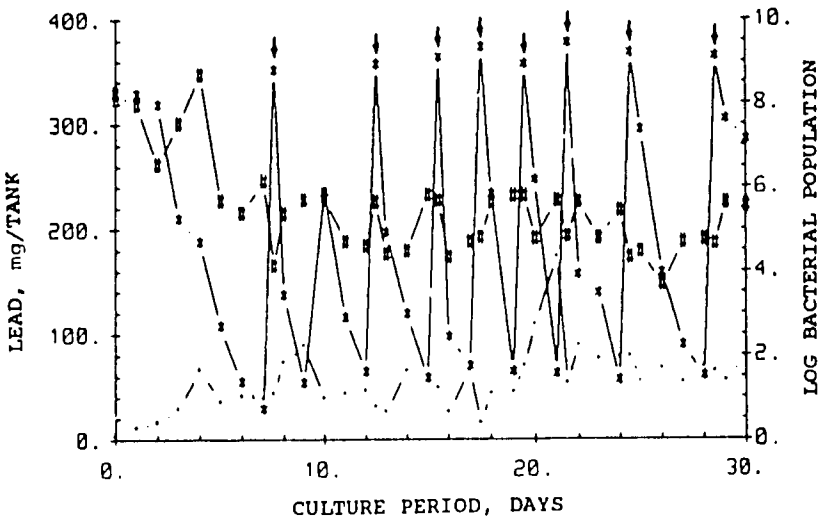

FIGURE 3. LEAD HOLDING CAPACITY OF THE ECOSYSTEM.

Lead concentrations: * = in water column, · = in soil; ‡ = log bacterial population in water column, ↓ = lead spikes.

twice as rapid with additional spikes. At the end of the experiment lead was not removed as effectively from the water column, although the biomass appeared to be healthy and functional. Lead remaining in the water column on day 30 may be a result of the sloughing off of lead-laden microbes from the undersurface of the mat. The peak at day 10 does not represent a spike, but rather dropping of lead into the water column from the mat, followed by rapid resorption.

Under optimum conditions this ecosystem sequesters lead at a rate of 23±6 mg/l/d and concentrates the metal in the biomass at levels of 0.15 mg lead/g biomass. It exceeds the uptake capacity of the algal component, NB-19-Pb, cultured alone under ideal conditions.

CONCLUSIONS

This microbial ecosystem, enriched with silaged grass and inoculated with metal-tolerant strains, shows good potential for three applications: water purification, pond soil bed decontamination and low-cost metal recovery. In this system a number of problems, generally associated with metal removal, were solved in rather simple ways. It is important to consider that the system described here is driven by microbial processes, which did not evolve in isolated culture, but rather as a consortium of microbial species functioning as an elaborate and elegantly cooperative unit. Biotechnical applications of mixed microbial, natural ecosystems, which have been adapted as a functional unit for specific operations, may have a variety of advantages. From a practical standpoint, this process offers substantial advantages over both conventional methods such as lime precipitation and less common techniques such as ion exchange. In contrast to these methods, the microbial system can be designed to display high selectivity for one or more specific metals and has been demonstrated to function efficiently over a wide range of metal concentrations. In addition, the selectivity of this system offers the potential for recovery of metals in relatively pure form from complex mixtures by use of such options as sequential treatment of a complex mixture by a series of metal-specific ecosystems. Harvesting of metal-laden mats from the pond surface would be significantly easier than settling and subsequent dewatering of sludges.

Operationally, the microbial systems would be very much less demanding in terms of complex engineered systems and consumption of chemicals for precipitation or regeneration of resins and thus less costly with respect to both capital and operating costs. While there are inadequate data available at present to perform a detailed economic analysis of these microbial systems, there is strong evidence that they will be fully competitive with physical and chemical methods of metal removal and recovery.

ACKNOWLEDGEMENT

Primary support for this research was provided by the U.S. Bureau of Mines, Grant No. G0145031, and the U.S. Department of Energy, Contract No. C86-11-0866. Partial support for students and instruments was contributed by the National Institutes of Health, Grant No. RR-8006.

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Uptake, Transformation and Fixation of Se(VI) by a Mixed, Selenium Tolerant Ecosystem

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ABSTRACT

The uptake, transformation and fate of selenium added as selenate to a mixed microbial ecosystem containing selenium tolerant bacteria and cyanobacteria has been studied in simulated ponds. In these systems evidence has been obtained of concerted activities by the microorganisms in which the bacteria are responsible for transport into a surface mat in which the combined actions of the microbial consortium provide chemical conditions under which the selenium can be reduced to the elemental form and physically entrained in the mat. Strong qualitative indications of the formation of volatile alkylselenium compounds are also observed. The removal of selenium from the water column is rapid and essentially quantitative. The advantages of such biological ecosystems for remediation of selenium contaminated aquatic ecosystems are discussed.

INTRODUCTION

Selenium is an essential dietary element in minute quantities, but is toxic to most organisms, including humans, at high concentrations. The U. S. Environmental Protection Agency has set the maximum contaminant level at 10 μg/L in drinking water and has included selenium on the list of priority pollutants in water. The anthropogenic mobilization of selenium by irrigation practices

in the western United States (Tanji et al., 1986) and by the metal mining and smelting industries (Nriagu, 1989) is a well-documented environmental problem. The ultimate fate of environmental deposits of selenium is a serious consideration, because the complex chemical nature of selenium complicates predictability in terms of environmental transport and speciation within the natural ecosystem.

Since selenium contamination is widely distributed, the economics of any proposed remediation process become an important issue. Bioremediation methods, utilizing microbial systems for decontamination of selenium deposits, are appealing in that they offer low-cost potential and are generally environmentally acceptable. Many common bacteria have been shown to reduce selenate to selenite, to generate alkylselenium compounds, hydrogen selenide, selenoamino acids and elemental selenium (Doran, 1982; Sarathchandra and Watinson, 1981; Oremland and Sehr, 1986).

Controlled and predictable microbial systems might offer methods of selenium decontamination through the generation of volatile products, biological transformation to non-toxic forms (or chemical species that are not bioavailable) and the production of economically valuable products, such as elemental selenium. The objective of this research was to examine the potential of mixed microbial mats for decontamination of selenium-infused pond water. The rate of selenium exit from the water column, the location of final deposit in the pond and the speciation of selenium, resulting from the biological transformations within the system, were examined.

Selenium Chemistry

The chemistry of selenium is quite complex. This element is a chemical analog of sulfur and has much in common with that element. In its inorganic forms therefore, selenium can exist readily in the +6 oxidation state (SeO_4^{2-}), the +4 oxidation state (SeO_3^{2-}), the -2 oxidation state (H_2Se) and, of course, as the neutral element. It differs significantly from sulfur however, in displaying a

fairly large range of conditions under which it can exist as the neutral element (Figure 1). Thus, while environmental deposition of elemental sulfur is an uncommon phenomenon, deposition of elemental selenium can occur readily in the chemical conditions of sediment, soil and water. Should reduction of selenium proceed to the selenide, a substantial sink for selenium in the form of metal selenides exists. These selenides share with sulfides the abundance of very sparingly soluble salts which can be formed with such common metals as ferrous iron.

The organic chemistry of selenium is also of very great importance in any consideration of the environmental behavior and fate of this element. Challenger (1955) has reviewed the role of fungi in the biomethylation of selenium. Of special interest with respect to the research reported herein have been studies (Reamer and Zoller, 1980, Doran and Alexander, 1977, Flemming and Alexander, 1972, Francis, and coworkers, 1974) which have demonstrated that microorganisms of several classes found in soils are able to convert selenate and selenite into such volatile selenium compounds as dimethyl selenide (CH_3SeCH_3), dimethyl diselenide ($CH_3SeSeCH_3$) and dimethyl selenone ($[CH_3]_2SeO_2$). This process has several consequences. It establishes loss through volatization as a potentially major route of selenium from one environmental phase to another. Since the alkyl selenium species tend to be significantly less toxic than many of the inorganic forms of selenium, this may represent a means of environmental detoxification of selenium. Finally, there exists the potential for the oxidation of these species with formation of elemental selenium as an alternative to the purely reductive pathway to that species.

METHODS

Overview

Mixed microbial mats were established by enriching simulated laboratory ponds with silaged grass clippings. After enrichment, mats developed spontaneously in the sediment and surface regions of the ponds by bacterial colonization of the silage, followed by a predictable succession to

cyanobacterial dominance. Microbial tolerance to selenium was enhanced by inoculating the water column with mixed populations of bacteria and cyanobacteria that had been developed for elevated resistance to selenate. The water column was infused with 40 mg/L of sodium selenate and sampled every three days to assess selenium exit from the aqueous medium. At the end of the experiment the mats, sediment and soil samples were harvested, hydrolyzed and analyzed for selenium content. Red deposits occurring in the system, were harvested and analyzed.

Simulated laboratory ponds

Clear plastic tanks (32 cm x 14 cm x 13 cm) were layered with sandy-loam soil to a depth of 4 cm and filled with two liters of filtered tap water. Initial pH was adjusted to 6-7 (lime addition, 1.5 g/tank) and spontaneously decreased to 3-4 in the bacterial phase, then rose to 7-9 during mat development. Calcium phosphate (2.3 g/tank) and potash (0.3 g/tank) were added to the water column. Silaged grass clippings (0.9 g/tank dry wt) were added to the surface of the water together with the microbial inocula. Control tanks contained all elements except the silage enrichment and microbial inocula, therefore did not produce the microbial mats. Experiments were performed as triplicate sets of test and controls tanks. Illumination was provided on a day/night cycle by incandescent 60-watt bulbs placed 25 cm from the pond surface.

Development of selenium-tolerant microbial strains.

Environmental populations of bacteria and cyanobacteria were selected from selenium-contaminated sediments in the San Luis Drain in the San Joaquin Valley of California. These microbes were exposed (as mixed populations) to step-wise increases (5 mg/L increments) of selenate, infused in a growth medium simulating the pond water column. Exposure was discontinued when final tolerances of 150 mg/L in bacteria and 70 mg/L cyanobacteria were reached. Populations of tolerant bacteria and cyanobacteria (10 mL of log-phase culture) were inoculated into the water column at the beginning of the experiment.

Inoculation of Se-tolerant bacteria into a mature mat system

In separate experiments the mat was cultured without the tolerant bacteria. After maturation of the mats (8 days), a culture of Se-tolerant bacteria was inoculated into the water column under the mat. Bacteria were separated from the water column, before and after the inoculation, by filtration through a 0.45 μm membrane filter. The filtrates and bacterial residues were analyzed for selenium content every 2 days for an eighteen-day period. These experiments were designed to examine the function of the Se-tolerant bacteria in the system and to compare the levels of bacterial-associated selenium with quantities remaining in aqueous form without cellular attachment.

Silage preparation and application.

Anaerobic processing of grass clipping (silaging) was used to prepare the organic feedstock for microbial mat production. Earlier research has demonstrated that the organic acids (products of silaging) act as chemotaxins for motile, slime-producing bacteria (Bender, in press). Fresh-cut grass clippings (mixed wild grasses of Georgia) were packed in 1-liter jars, excluding air pockets, and allowed to process anaerobically for 20 days at room temperature (Herman and Thompson, 1974). Finished silage was remained stable at room temperature for the duration of the research.

Sampling and analyses

The water column was sampled at a depth of 2 cm below the water surface and the mat biomass was harvested by carefully raking it from the pond. Random core samples were taken from pond soil bed and divided into sediment (top 1.5 cm) and soil (remaining core). All biomass and soil samples were acid hydrolyzed according to standard methods (APHA, 1985) and analyzed for selenium content by flame atomic absorption (Varian instrument: Spectra AA-20BQ double beam). The limit of detection for selenium with this method is 5 mg/L.

Oxygen concentrations, redox potentials and pH were measured around the mat and through the water column with microprobes (Microprobes, Inc., Phoenix, AZ), fitted to a pH/mV meter (Orion Model SA 250).

-70

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Selenium speciation experiments with excised mats.

Small sections of mat (0.5 cm^2) were excised and placed in 250 mL flasks with SS medium and 40 mg/L sodium selenate. Two controls contained selenate medium with separate cultures of bacteria and cyanobacteria (component populations that make up the mat, but without the laminated macro-structure of the silage-microbe mat). After 5 days, a red deposit, which developed in the water column with the mat sections, was separated from solution by filtration (Whatman #2 filter) and analyzed by electron microscopy to determine cellular association. The identity of the precipitate was determined by x-ray fluorescence (Atlanta University Center Electron Microscopy Center) and elemental analysis performed by a commercial analytical laboratory (Galbraith Laboratories, Inc., Knoxville, TN).

RESULTS AND DISCUSSION

Environmental conditions within the mat system

Figure 2 illustrates the locations of the mats in the pond and the general mat structure (as indicated by electron microscopy). Environmental conditions, assessed in the sediment and surface mats during the light period, show that both oxic and highly reducing conditions exist within the system. Environmental conditions, generated by the mats, are as follows: in the bottom mat - oxygen = 0.1 mg/L, pH = 8.8, E_c = -83 mV to -186 mV; in the top mat - oxygen = 20 mg/L, pH = 8.9 and E_c = +97 mV to +118 mV. The E_c conditions varied somewhat along the mat, probably as a function of mat thickness, specific microbial colonizations of mat zones and localized levels of bacterially available substrates and respiratory activity.

Selenium management in the mat system

Figure 3 shows that in 9 days 80% of the selenium had left the water column of the pond. A red deposit accumulated on the surface of the top mat as the selenium exited from the aqueous phase. This phenomenon was further studied in excised mat experiments described below.

After 27 days, the water columns in the experimental ponds contained undetectable quantities of selenium (within the limits of this method), and the hydrolysates of the mat, sediment and soil samples contained the following percents of selenium with respect to the initial concentrations added: mat = 5.5%, sediment = 67.6%, soil = no detectable quantities. The remaining 26.9% of selenium was presumed to have been released in volatile form. Selenium transport in the control ponds showed great variability. In one pond 25% remained in the water column (balance in the sediment), while the other two tanks retained 60% and 74% of the selenium in the water column. No red deposits were observed in the control tanks and none of the deeper soil samples contained detectable quantities of selenium.

Figure 4 shows that in mat systems structured without tolerant bacteria, the extents of cellular and non-cellular association with selenium were approximately equal (day 0-8). After inoculation of selenium-tolerant bacteria into the system on day 8, the selenium-bacteria association increased significantly, while non-associated component decreased to less than 10%. Migration of the selenium-laden bacteria from the water column by day 18 was indicated by the decrease of total selenium in the water column (associated and non-associated), while the overall bacterial count in the water column decreased at the same time. These data give evidence that the selenium-tolerant bacterial populations were involved in the efficient transport of selenium from the water column to other regions in the ecosystem. General bacterial populations may also to be capable of such transport, but with lower efficiency.

Excised mat experiments.

Small sections of mat, removed from the pond and placed in flasks with 200 mL medium with 40 mg/L selenate, produced the same type of red deposit that appeared on the surface of the mats within the pond system. Heavy, opaque deposits became dispersed throughout the water column and deposited at the bottom of the flask within eight hours. Figure 5 (1) shows that the

ıtors (2) and (3) of Figure 5 show
: the red precipitate by Galbraith
ıately 90% of the inorganic fraction
ırprising, since its formation in this
While the kinetics of this process
favorable. It should be noted here
on of organoselenium compounds
:nium compounds were conducted,
:h is characteristic of these species.
rmed in abundance in these studies
selenium in the mixed microbial

mponent microbes of the mat, but
d precipitate was deposited. These
were mediated by the community-
norphous microbial populations.

f bacteria and cyanobacteria, have
, 1989). Because harsh conditions
nts, these microbial systems may
decontamination. The potential
tegrating the desired microbes into
ıigh physiological resistance to the
mat ecosystem, thereby presenting
:ffectively detoxify the environment

rmation of the contaminant.

ı demonstrates important potential as a low-cost bioremediation
n. The only system inputs are silaged grass clippings, water, and
ıd in soil. In addition, this solar-driven system, organizes
.ined over long periods of time. Although microbial mats are
: diversity of microbes, community organization and mechanisms
highly reproducible and predictable ecosystem.

ACKNOWLEDGEMENTS

ed by the U.S. Bureau of Mines, Grant No. G0192008 and the U.
)AAA15-89-K-0003 and by the Research Centers in Minority
; through National Institutes of Health Grant No. RR03062. The
ın Microscopy Center and R. Taylor are acknowledged for the and
ıicroscopy contributions to this project.

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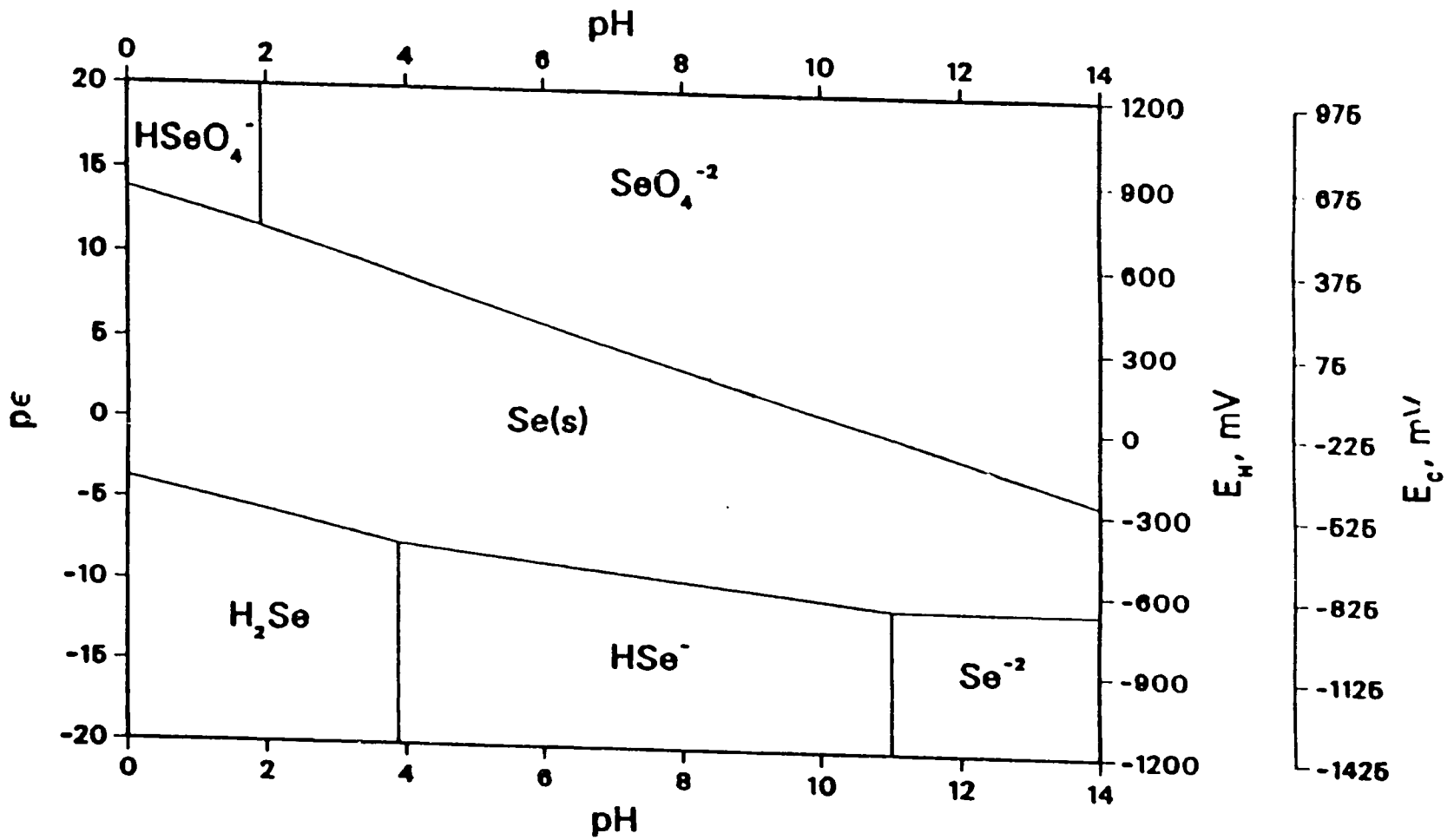
Figure 1. pH-pε distribution diagram for the system $SeO_4^{2-}/Se(s)/Se^{2-}$ at a selenium pC_t value of 4.

Figure 2. Schematic diagram of vertical cross section of silage-microbe mat.

Figure 3. Comparative removals of selenium from water column in presence and absence(control) of the silage-microbe mat.

Figure 4. Levels of soluble and solid phase selenium in water column prior to and following inoculation of simulated pond with selenium tolerant bacteria on day 8.

Figure 5. X-ray fluorescence pattern of precipitated red selenium.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pε
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SeO4-2
Se(s)
H2Se
HSe-
Se-2
1200
900
600
300
0
-300
-600
-900
-1200
EH, mV
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675
375
75
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-525
-825
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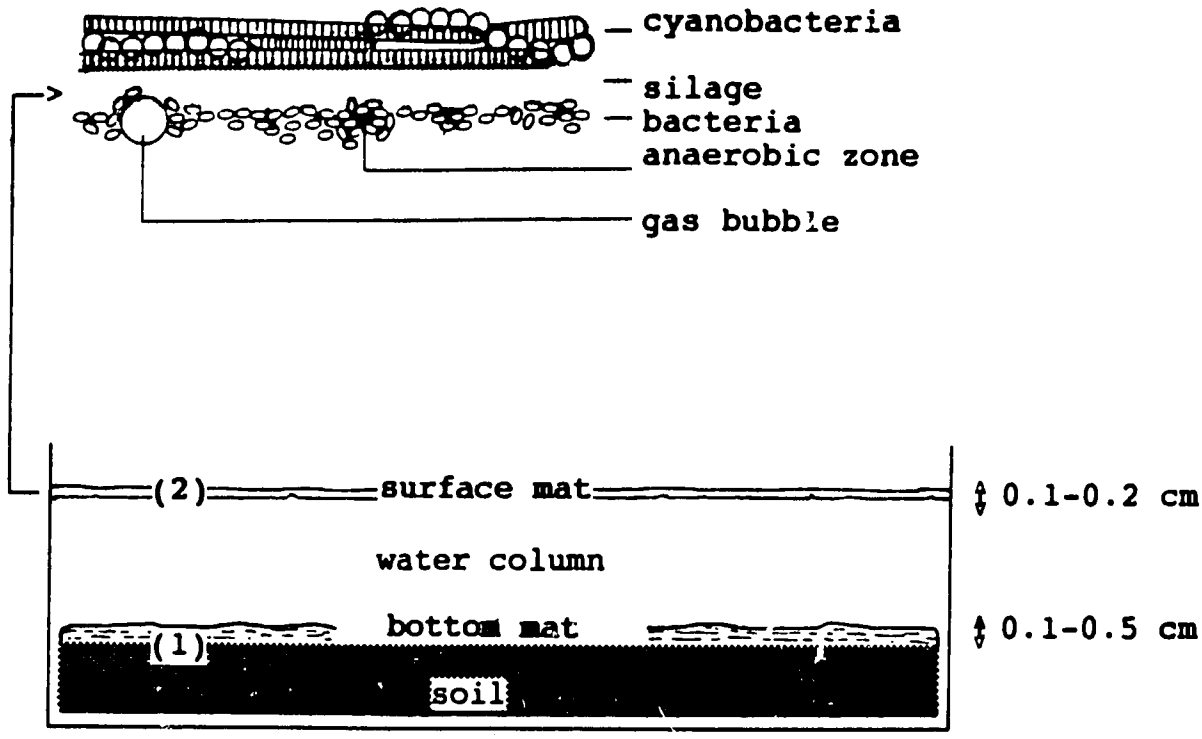
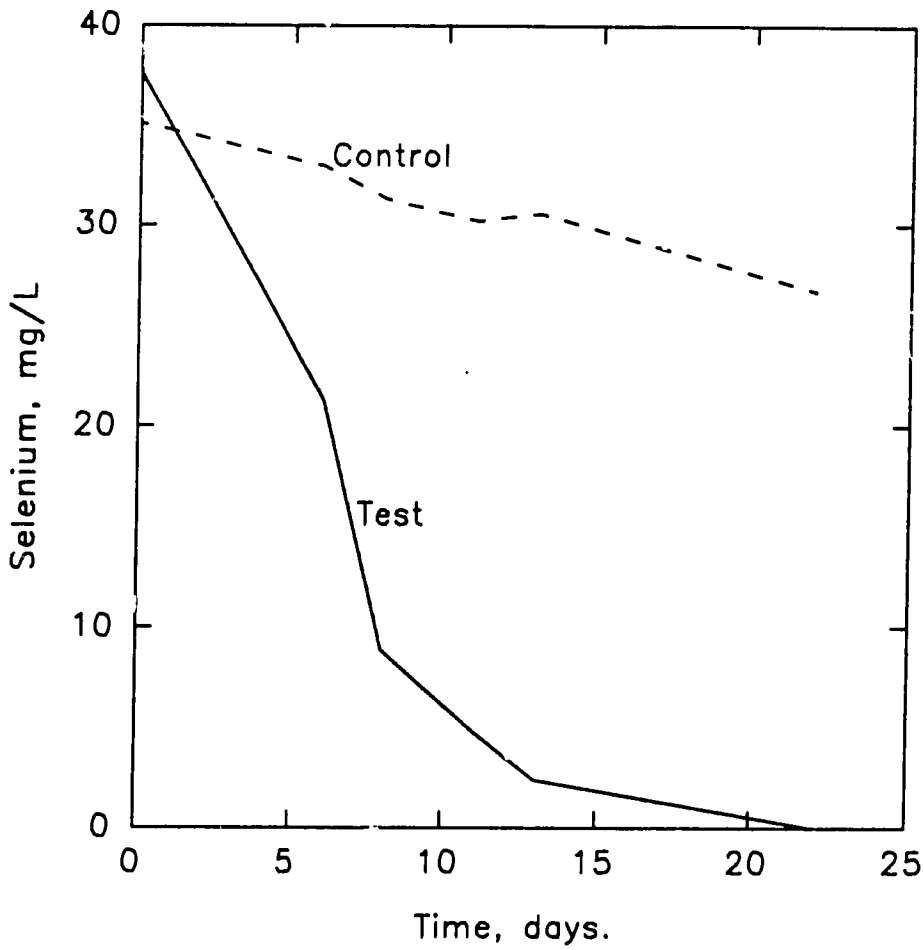
Ec, mV


cyanobacteria
silage
bacteria
anaerobic zone
gas bubble
(2)
surface mat
0.1-0.2 cm
water column
bottom mat
0.1-0.5 cm
(1)
soil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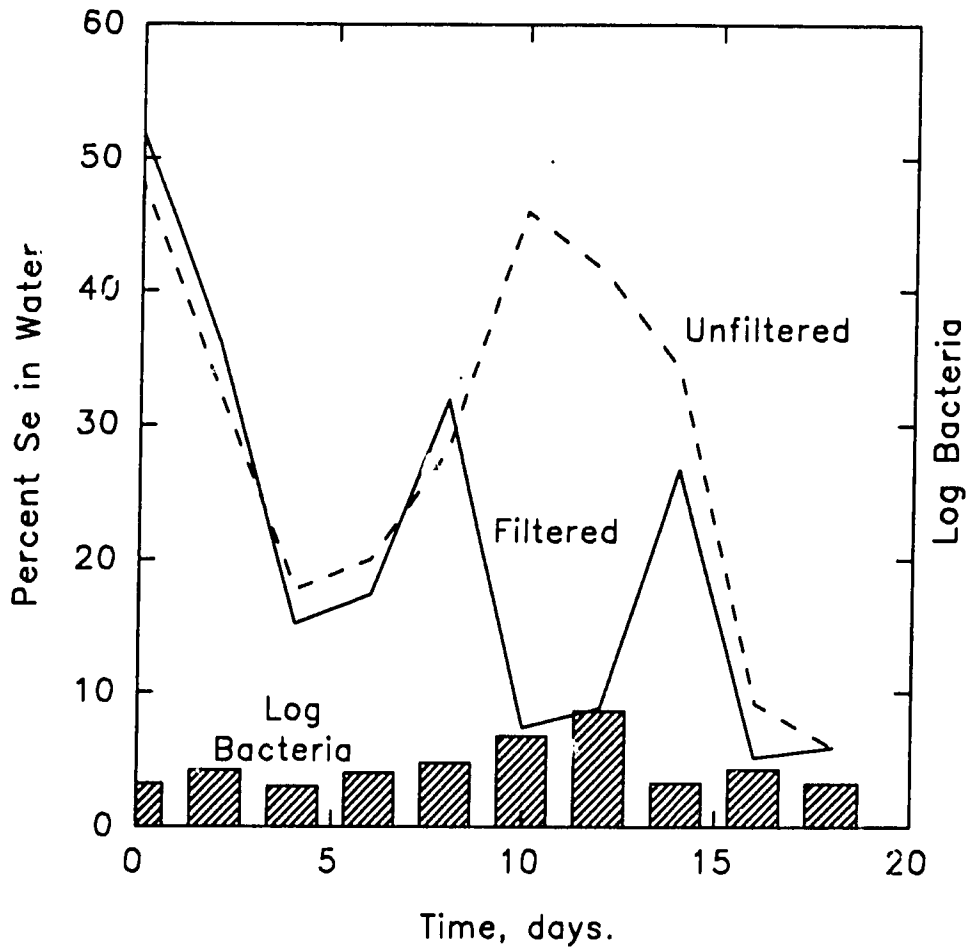
30
20
10
0
Selenium, mg/L
Control
Test
0
5
10
15
20
25
Time, days.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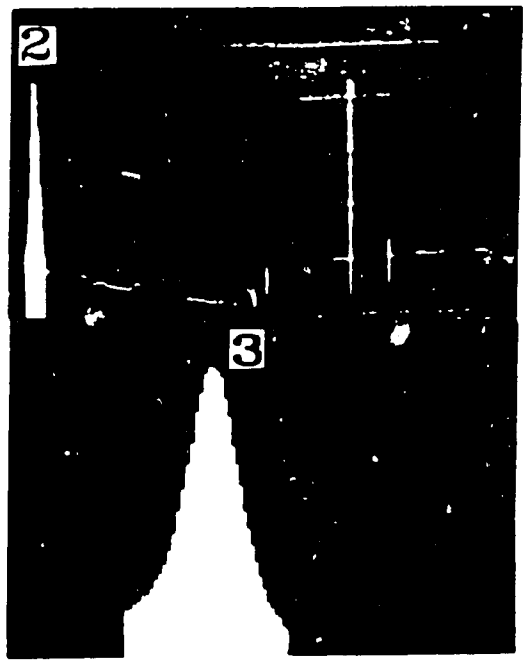
50
40
30
20
10
0
Percent Se in Water
Log Bacteria
Unfiltered
Filtered
Log
Bacteria
0
5
10
15
20
Time, days.

1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3

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FINLAND

Kuopio

No
Ref.

1 August 1990

Dr. Judith A. Bender
Department of Biology
Clark Atlanta University
Atlanta, Georgia 30314
USA

Dear Dr. Bender:

I would like to obtain one or more copies of your paper:

Bender, Vatcharapijarn, & Russell. 1989. Fish Feeds from Grass Clippings. Aquacultural Engineering 8:407-419.

I work primarily with freshwater crayfishes which are polytrophic like tilapias. Most species are cultivated by establishing detrital food chains from vegetation grown in ponds during a dry period when the crayfish remain in burrows. That is why I am especially interested in your paper because it is useful in describing the trophic dynamics of our crayfish systems. I work regularly in Louisiana and am a visiting scientist on the staff here coming most summers. Please forward reprints/correspondence to the following address:

Jay Huner, Director
Crawfish Center
P.O. Box 44650
University of Southwestern Louisiana
Lafayette, Louisiana 70504 USA

Our Louisiana crayfish, the red swamp crayfish, Procambarus clarkii, was introduced into the Domincan Republic some years ago. We have very little information about the utilization of the species, its distribution, and its life cycle there, and so forth. I thought that you might be able to provide some information about this crayfish as you have worked there and my guess is that it has invaded tilapia ponds in the DR. Can you send any information or contact your colleague Dr. Russell about this matter?

We have an International Association of Astacology - study of crayfish biology. I would be pleased to send you information about it in the event that you or your colleagues might be working or have interest in working with crayfishes. I will also be pleased to send you copies of review publications about crayfish aquaculture.

I look forward to receiving your reply upon my return to Louisiana.

Sincerely yours,

Jay V. Huner

Jay V. Huner, Docent
Applied Zoology

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