

## **Development of an Environmental Microbiology Laboratory Exercise**

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### Abstract

Environmental microbiology integrates the system boundaries of the various environmental compartments (e.g. soil, water, air, particulate) with the occurrence and proliferation of microorganisms. A laboratory exercise has been developed for this introductory course and encompasses two steps: an introduction to microbial techniques, and an 'independent' team-based project. The purpose of the first laboratory section is to gain familiarity with basic microbiological techniques and skills, which will later be applied in the independent project. The topics covered in this first section include microbial culturing and plating techniques, morphology, metabolic potential, kinetic and growth studies. This experience will highlight both limitations of current techniques and possibilities of novel approaches. The independent lab project is structured around the skills learned earlier. Students can choose to grow and study bacteria from various environmental systems. The team-based project will be presented at the end of the term before the class and will highlight the essential findings and challenges encountered. The practical experience gained in this laboratory will enhance the student's understanding of microbiology in a manner not readily gained through lectures or textbooks. Students will gain appreciation of the intrinsic and external variables impacting microbial growth, proliferation, and adaptability to changing environmental system boundaries.

### Introduction to Microbial Techniques

A series of laboratory exercises was designed to provide the students with basic skills and techniques needed to study and characterize microbial cultures. These exercises use either pure or mixed non-pathogenic cultures.

#### 1. Microbial Culturing and Morphology

Microbiological media used in the isolation and cultivation of microorganisms provide the components needed to support microbial growth and function. In this laboratory, the

student becomes familiar with the preparation of microbiological media and the isolation of bacterial strain from an environmental sample using the streak plate method. In addition, common methods of determining microbial numbers and mass, based on serial dilution and the spread plate techniques are explored.

Students prepare heated agar solution according to several recipes, and pour it into sterile glass or plastic Petri dishes. Agar “slants” are also prepared by pouring agar into sterile test tubes. Since slight variations in media composition can dramatically affect patterns of microbial growth, the concept of selective media is introduced and preparation of media containing either inhibitors against nontarget organisms or single carbon source is used to promote growth of specific bacterium. In this laboratory exercise, the student’s task is to culture and isolate target bacteria from an engineered pool of microorganisms. The challenge encountered here is the isolation of pure microorganisms where all colonies appear to be identical. The morphology of the isolated colonies is further characterized as far as size, color, surface, texture, and density.

## 2. Cell Enumeration and Cell Mass

The number of viable cells in a population can be measured by counting the colony forming units. This method known as plate count assumes that one bacterial cell or clump of cells gives rise to one colony and that the number of colonies formed on an agar plate corresponds to the original bacterial count.

The plate count is performed by the spread plate method after a serial dilution is performed. In this case, students spread a volume of an appropriately diluted culture over the surface of an agar plate, using a sterile glass spreader. The plate is then incubated until the colonies appear, and the number of colonies is counted. The volume spread should be less than 0.1 ml, since larger volumes may cause the colonies to coalesce as they form, making them difficult to count.

With this plate count method, it is critical that the number of colonies developing on the plates is not too large, since on crowded plates some cells may not form colonies and the count will thus be erroneous. It is also essential that the number of colonies is not too small, because the results of the plate count may then be statistically insignificant. For these reasons, only those plates that have between 30 and 300 colonies are considered.

To obtain the appropriate colony number, the sample to be counted must thus usually be diluted. Since one rarely knows the approximate viable count ahead of time, it is usually necessary to make more than one dilution. Several ten-fold dilutions of the sample are commonly performed. Students learn in this laboratory exercise that the number of colonies formed on the agar plate is dependent not only on the inoculum size but also on the suitability of the culture media and the incubation conditions used. In addition, the length of incubation plays a critical role. Too long incubation time may result in indistinguishable colonies, whereas a short incubation time would result in a less than representative count. Therefore, it is usual to determine the optimum incubation conditions (medium, temperature,

time) for colony forming. These observations would come in handy when students have to count their samples in the independent term project.

For many studies it is desirable to estimate the cell masses rather than the cell number. The two methods introduced in this lab are (i) the wet weight method where the cells are centrifuged and the pellet weight represents the cell biomass, and (ii) turbidity measurements which assume that the cells in a liquid medium scatter light in proportion to their total mass in the culture.

### 3. Metabolic Potential

To determine the functional diversity of the microbial community, rapid and easy methods of fingerprinting the metabolic potential of bacterial communities have been shown to be very valuable. One application of this methodology is the BIOLOG<sup>®</sup> system (BIOLOG<sup>®</sup> Inc., CA) that consists of a 96-well microtitre plate with various carbon sources and a negative control. The carbon substrates can be classified by categories that include carbohydrates, carboxylic acids, polymers, amines, amides, amino-acids, and other carbon sources. Each well contains nutrients, salts, the redox dye tetrazolium violet, plus a test carbon source for the cells to oxidize. Addition of a cell suspension activates the chemical reaction by reducing the dye during respiratory activity to insoluble Formazan (violet color). After incubation at a suitable temperature for a period of time, the microplate yields a pattern of clear and purple wells in an 8-by-12 matrix which can be quickly read and analyzed.

Because this pattern is a result of differences in respiration of the different carbon sources, it can be called a metabolic "breathprint". The availability of a reference data base enables a rapid best-fit analysis, by comparing the metabolic "breathprints" of an unknown strain with those of the reference strains. BIOLOG<sup>®</sup> profiles have been utilized to compare the metabolic roles and functional diversity among microbial communities. Differences in BIOLOG<sup>®</sup> utilization patterns have been explained by environmental variables imposed upon the communities<sup>1</sup>, and discrimination in the oxidation profiles between closely related habitats as a function of soil types has been observed<sup>2</sup>.

In this laboratory exercise, students are asked to inoculate BIOLOG<sup>®</sup> plates with microbial suspension, incubate them, and read them over 48-hour period using a plate reader. BIOLOG<sup>®</sup> profiles will then be compared. The outcome will be a difference in metabolic potential among the various microbial culture.

### 4. Growth Kinetic

The objectives of this laboratory exercise are to measure the growth of a bacterial population on different substrate concentrations, to calculate the Monod growth kinetic variables, and to compare the growth of two different species on the same complex medium.

Microbial growth is studied by preparing growth curves and calculating the kinetic variables. Each lab group determines the specific growth rate for two bacterial cultures: *Escherichia coli* and *Pseudomonas putida* mt-2. The substrate concentration in both cultures for each group will vary so that collectively the lab sections can generate a Monod curve showing the effect of substrate concentration on the growth of these two species

The outcome of this lab is the development of a Monod curve for each microorganism. The maximum specific growth rate and the half-saturation constant are both determined by a Lineweaver-Burke plot and nonlinear regression approaches.

### Team-based Project

The objective of the term project is to apply the skills learned throughout the teaching laboratory exercises. The ultimate goal of the project is to motivate the students and engage them equally in a journey of scientific discoveries and excitement. The students should choose an environmental sample, culture and characterize the microbial communities, study their metabolic potential and growth kinetic variables.

A suggested timeline is given to the groups at the beginning of the project and the members are highly encouraged to follow it. A progress report is required on a weekly basis. The report should be very concise and should describe the group and individual accomplishment during the week. These reports are to be signed by all members of the group.

At the end of the project, the team should summarize their results in a paper that follows the format of a scientific article for a technical journal with a minimum of 5 references included. A 15-minute oral presentation is required from each team and all members are recommended to present their findings, challenges, and observations.

### Conclusion

Course lectures provide access to the information students need to solve their assignments and pass their exams. Laboratory exercises are essential for motivating, engaging, and teaming of students. The goal in designing and developing this laboratory exercise is to stimulate student interest to become life long learner and hopefully perform the research necessary to move the field forward with new discoveries.

### Bibliography

1. Bossio, D. A., and K. M. Scow. 1995. *Impact of carbon and flooding on the metabolic diversity of microbial communities in soils*. Appl. Environ. Microbiol. 61: 4043-4050.

2. Winding, A. 1994. *Fingerprinting bacterial soil communities using Biolog microtitre plates*. In K. Ritz, J. Dighton, and K. E. Giller, Eds., *Beyond the biomass: compositional and functional analysis of soil microbial communities*. John Wiley & Sons Ltd., U.K., pp. 85-94.

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