AMERICAN
SOCIETY FOR
MICROBIOLOGY

Education Board

August 9, 2006

Sarah Boomer,
Natural Sciences and Mathematics
Western Oregon University
Monmouth, Oregon 97361

Dear Dr Boomer:

Thank you for submitting your laboratory exercise "Exploring and Enriching for Nitrogen Cycling Microbes: Nitrogen Fixation, Ammonia Oxidizing Lithotrophy, and Anaerobic Nitrate Reduction" to ASM's Microbe Library. The Curriculum Resources Committee liked the inclusiveness of this laboratory exercise. It is innovative in it's incorporation of the entire nitrogen cycle into a single exercise. There are some minor modifications that the committee would like to see before it is accepted for publication. I was the primary reviewer and I'd like to give you some feedback from our review conference to help you with the modifications.

Overall, the committee felt that this submission is interesting and will be a valuable addition to the MicrobeLibrary Curriculum Resources. In the words of one reviewer the exercise will "help students make connections to concepts and practice some lab skills." The committee would like to see some changes that will make the exercise more easily adopted by instructors with limited experience working with N-cycling microorganisms. I have elaborated on these below. We would like to encourage you to make the suggested changes quickly and return the edited version to me. It will be re-reviewed quickly with a goal towards publishing it in the MicrobeLibrary in September.

Changes:
List of Themes: add Theme 1: Energetics and Metabolism
Keywords: add energetics and cellular metabolism
Throughout:

- Instructions in many cases indicate incubations should happen on the "countertop." To make the exercise more applicable to other settings, I suggest changing this to say "room temperature". In the instructor version then the author should add a sentence that indicates roughly what temperature is room temperature in her lab – it may be different someplace else (w/air conditioning vs without, for example).
- there are no storage instructions for media (room temperature, refrigerator, etc); these should be added.

Materials: Session 1

- it is never made clear why each student team needs "1 sterile 50 ml beaker and collecting scoop for soil collection" for the Azotobacter and Ammonia and Nitrate Oxidizer enrichments. It should be made clear in Appendix 1, the student version of instructions, how these are supposed to be used.
- Azotobacter N-free Media recipe: to what volume should water be added to the ingredients of each solution? A sentence should follow each solution recipe: "Add distilled water to ? ml."
- Rhizobium agar and Ammonia Oxidizer Liquid Medium: At the end of these should be added the sentence: "Add distilled water to make 1L."

Instructor Version

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- 3rd paragraph, 3rd sentence: The author indicates here that she has no difficulty finding organic and nitrogen-poor soils b/c she knows where to look after trial and error. This should be followed up with a recommendation to users of the exercise one where they might find such soils. What sorts of environments (describe) should they look for when making collections? This is very important so that the lab is more easily adaptable by new users.
- 3rd paragraph, 3rd sentence: It should also be made clear here whether the soil collection needs to be aseptic and how soil should be stored prior to its use in the lab. Also, how long can it be stored before being used in lab?
- 4th paragraph, 9th sentence: change "surface sterilize" to "surface disinfect" to reflect more accurately the kind of control treatment being applied
- 4th paragraph: suggest someplace where clover could be purchased commercially, or where seeds could be purchased. This will help instructors in northern climes who may not have access to live clover outdoors for much of the academic year.
- Session Two, 1st paragraph: following the description of Azotobacter and Rhizobium colonies, list some methods that could be used to confirm their identities (for example they are G+, etc.).

Safety and Handling Issues:

- Add that safety glasses and lab coats should be worn when students are working with chemicals.

Appendix 1, Activities:

- Azotobacter Inoculation and Enrichment section and the Rhizobium Inoculation and Enrichment section: make clear that students should streak plates **for isolation of colonies.** The bolded point is not stated at all, but important to the final evaluation of the results.
- Azotobacter Inoculation and Enrichment section and the Rhizobium Inoculation and Enrichment section: make clear whether soil weighing (of 1g samples) needs to be done aseptically and if so, provide instructions for this (i.e. sterile weighing paper or boats, spatulas, etc).
- Rhizobium Inoculation and Enrichment, 1st sentence: add an "a" between "in" and "sterile"; remove the last two periods from the end of the sentence.
- Nitrification Inoculation and Enrichment, 1st sentence: "9ml" should be replaced with "10 ml" to match the materials list and detailed instructions for this activity.
- Nitrate Reduction Inoculation and Enrichment: the way this is written is very confusing, I would rewrite it the following way:

  Obtain 4 nitrate reduction tubes – LABEL them carefully, according to the following directions:
  Two tubes should each be inoculated with 1 ml organic soil dilution (collected/prepared above in the Nitrification Inoculation and Enrichment)
  Two tubes should each be inoculated with 1 ml N-poor soil dilution. Prepare the dilution by adding 1g of N-poor soil to 10 ml sterile water.
  After 1 week on the countertop, complete all portions of the nitrate reduction test (directions provided below).

- Nitrate Reduction Inoculation and Enrichment, boxed directions for tests: for (2) change to read "drops of nitrate test reagents A and B"; for (3) add the word "powdered" after the word "grains"

Appendix 3:

- the 1st sentence states that Rhizobium medium is **less** selective (than what? I am assuming Azotobacter medium). Does that mean it is not selective at all, or is selective in a different way or to a lesser degree? This is not at all clear.

Possible Modification:

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- addition of confirmatory tests to identify organisms isolated in enrichments would raise the challenge level of the exercise

Spelling/Grammar changes:
Learning Objectives: remove the word "better" from (1)
Background: 2nd paragraph, 3rd sentenced: he word "staining" should not be capitalized.
Instructor Version:

- 1st paragraph, 1st sentence: "nitrate/nitrate testing" should be "nitrate/nitrite testing"
- 2nd paragraph, 2nd sentence: "Methanogenic" should be entirely lowercase; "Archaebacteria" should be replaced with "Archaea"; "Anoxygenic Phototrophs" should be entirely lowercase
- 2nd paragraph, 4th sentence: "Enteric" should be entirely lowercase

Appendix 3:

- 2. add the word "nitrogen" after the word "fix"
- 10. "ANAEROBIC" is spelled incorrectly

Please find attached the review criteria the committee uses in the review process. We hope that you can use these to aid your revision process.

We do ask that you review and sign the attached copyright agreement form, which states that ASM has the non-exclusive right to publish the activity in the MicrobeLibrary. Contact Jean Kayira at ASM with any questions regarding the copyright agreement. She can be reached at <jkayira@asmusa.org> or by phone at 202-942-9299.

Again, thank you for submitting your exercise to MicrobeLibrary. I do hope that you will consider making the modifications necessary for publication. Please contact me if you have any questions regarding the comments above.

Sincerely,

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Attachments