INCREASING STUDENT MOTIVATION AND COMPREHENSION IN MICROBIOLOGY BY EMPLOYING AN OVERARCHING THEME

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ABSTRACT

INCREASING STUDENT MOTIVATION AND COMPREHENSION IN MICROBIOLOGY BY EMPLOYING AN OVERARCHING THEME

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This paper outlines an inquiry-based project in which students adopt a microbial "pet." The student then puts this unknown bacterium through a variety of tests to determine its identity. This three-week project takes place in a high school microbiology classroom. According to the results of this research, students become engaged in their experiments and more interested in the information than in a traditional lecture-based classroom setting. These results were measured using a pretest and posttest, as well as a final lab report. Motivation was measured based on student response and retention. Students become skilled at new techniques while working independently to solve the mystery of the pet's characteristics. They engage in rigorous, meaningful learning leading to intrinsic motivation and knowledge retention.

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INTRODUCTION

Apathy is the arch nemesis of motivation. If a person simply does not care, how could they possibly be motivated to learn the content put before them? In classrooms across America teachers are fighting this battle, waging a war on apathy and using every strategy in their teacher's toolbox to interest, engage, and enlighten students.

Educators point to the lack of student motivation, apathy, as a major factor involved in low student success (Hootstein, 1996). In fact, success is so low that current statistics across the nation show only about one in five high school seniors is proficient in science (Borsuk, 2011). These statistics were based on the ACT results, a standardized test taken by college-bound students across US, including all students in the state of Michigan, where this research takes place. Sadly, apathy is not an emerging social epidemic. It has been a concern of teachers for some time. A Time Magazine article from 1978 cites apathy as an "insidious malady…infecting high schools everywhere."

Reports also show students are not meeting curricular and state goals, possibly because they are unable to apply critical thinking skills and transfer knowledge to new applications (Griffin & Malone, 1995). Is this because they lack the ability to learn? Have we raised the bar too high? According to one former teacher, "It is easier to be passively controlled, than it is to take initiative" (Solmitz, 2000). Students today are accustomed to having information at the touch of a keypad or click of the remote. This has allowed them to become very passive learners. That is, they wait for the information to come to them, instead of seeking it out. Students are "losing sight of their own responsibility as part of the learning process… [They] come to classes merely for the points of attendance or mandatory in-class quizzes…[and] tend to

be easily distracted from classroom discussions and engage in their own activities (e.g., texting to friends). These passive learners are more likely to miss the important components of learning and subsequently lose interest in the course (Mo, 2011). Another author suggests, "Quite frankly, they [students] don't see any value in what they are studying" (Kranendonk, as quoted in Borsuk, 2011). Some would disagree. It is not that students see no value in what they are studying, but they have not been given the opportunity to take ownership of their learning. The question is, how do we empower students to take an active, *not* passive, role in learning and care about the topic?

Perlman & Webster (2011) believe that allowing autonomy in the classroom more fully engages students in their work. Moreover, students are more likely to follow teacher instructions if the instructions resonate with the student's internalized values. Though allowing student autonomy may be an overwhelming idea to some teachers, is important to understand that, within a structured environment, the classroom can provide opportunities for choice, initiative, and problem solving, thus, fostering intrinsic motivation (Reeve, 2006). Intrinsic motivation is the student's desire to learn or achieve simply for the satisfaction of doing so. Deci, Koestner and Ryan (2001) found that intrisic motivation was more successful in promoting achievement and learning than extrinsic motivators. Extrinsic motivation involves completing a task for a tangible, expected reward such as a grade or extra credit.

One way intrinsic motivation can be addressed is through Gardner's Multiple Intelligences (Gardner, 1983). Gardner cites three ways students learn: visual, auditory, and kinesthetic. He insists classrooms that engage students in all of these learning styles achieve higher learning than those that do not. Today, we may refer to the Multiple Intelligences as

different learning styles. Many people have devoted their research to using different learning styles as an intrisically-motivating tool in working with students. The term "learning styles" has been used interchangeably with similar terms, such as Multiple Intellegences and cognitive styles. According to Curry (1983), there are 21 models for learning styles. As a result, a true definition has never been agreed upon (Kulturel-Konak, et al., 2011).

Robert Marzano, a leader in the educational pedagogy reform, lists generating and testing hypotheses among his nine instructional strategies that work. When students form hypotheses, they are making connections between current knowledge and future events (Marzano, et al. 2004). By making these connections, students incorporate the new information into their knowledge set. Forming hypotheses involves a higher level of thinking using decision making, problem solving and investigation skills (Marzano, 1990). At the conclusion of an experiment, writing lab reports "allows them to formulate hypotheses, make inferences based on findings, and develop scientific arguments—cornerstones of scientific literacy" (Ferzli, et al. 2005). Engaging students in this type of scientific thinking can help students in their future careers as well. Problem solving can be engaged in any type of career.

Another way to connect with students is through the rigor, relevance and relationships. "High schools that successfully engage students in learning have many things in common. They set high academic standards and provide rigorous, meaningful instruction and support so that all students can meet them. Teachers also show students the connections between success in school and long-term career plans" (National Academies, 2003). One writer asserts, "schools should encourage and empower students to become independent thinkers" (Solmitz, 2000). Others agree if we are to motivate students, we must do so in an environment that is meaningful to them.

In this way, they will be motivated to create meaning out of whatever is presented to them (Schifter & Fosnot, 1993). Kohn also agrees, saying "teachers must first maximize the opportunity for students to make choices, to discover and learn for themselves" (1998).

Allowing students to be autonomous and learn information for themselves are two key parts to inquiry based learning. "In recent years, the importance of inquiry-based learning has been recognized in helping students to understand and retain material better as well as to improve their performance on tests" (Anjur,2011). Employing inquiry-based learning also engages students in the skills they will need in the future. Describing a classroom in which inquiry-based learning is used, Jansen (2011) states, "In this environment, students use a wide range of resources to collaborate with others to solve authentic problems by thinking critically, actively create content, and communicate with a wide audience."

Our nation has long been facing the problem of apathy in education. This lack of concern affects a student's ability to retain information, think critically and score well on national tests. In order to address this problem, rigorous inquiry-based learning can be employed. This allows students autonomy and ownership of their learning, and increases the intrinsic motivation needed to succeed in both the classroom and the workplace.

School Demographics

This research took place at Carman-Ainsworth High School, an urban school in Flint Township, Michigan. The high school is comprised of approximately 1500 students in ninth through twelfth grade. Caucasians comprise 60% of the population and African Americans make up about 30%.The population of students eligible for free or reduced lunch at the high school is nearly 50%. More than 60% of the population of students considered "at-risk." Students are

classified as “at-risk” if they qualify for two or more of the following factors: (1) Free or reduced lunch, (2) having been the child of or being themselves a teen parent, (3) a family history of a parent not graduating high school, incarceration, or substance abuse, (4) being abused, being below grade level, or (5) behavior and attendance factors.

Microbiology, the course during which this research took place, is an elective. It is a semester long, and offered to juniors and seniors with a C+ or higher in their Biology course, which is a prerequisite. During this study, two classes participated. A total of 34 consent forms were turned in out of 43 (Appendix). The data and subsequent analysis are based on these 34 students. This unit falls near the end of the semester. At this point in the course, students had learned the history and fundamentals of microbiology. They had also completed several labs including: microscopy, simple staining, gram staining, an open-ended hand washing lab, and protist observations. Following this unit, students have an assignment on infectious disease and the final exam.

The hypothesis for this study is that requiring students to determine the identity of a “pet” microbe will engage them in learning laboratory techniques, encourage the knowledge retention related to their pet and increase overall interest in their microbiology course. The problems of apathy and inability use higher-level critical thinking skill are addressed here by increasing students’ intrinsic motivation, through inquiry-based learning and relevant, rigorous coursework.

The rationale is that students love a mystery. They watch *House, CSI,* and *Law & Order*. They solve Sudoku puzzles, every level on their video games, and the Impossible Quiz. This research hinges on the idea that for these students, solving a mystery involves understanding the

pieces to the puzzle. In this case, the pieces to the puzzle are laboratory techniques and the puzzle itself is to identify an unknown microbe.

Objectives

The objectives for this unit were based on the Michigan High School Curriculum Content Expectations (Appendix E). Overall students should be able to describe the structure of bacteria and how bacteria obtain and use energy. Students will also be able to conduct scientific investigations using the appropriate tools, draw conclusions based on evidence, and design an experiment that tests their hypotheses.

In addition to the state's standards, Carman Ainsworth High School, where this research takes place, focuses on the ACT's college readiness standards. If the students are to be measured against their peers nationwide, it is important to have a common scoring system (the ACT) and objectives (the college readiness standards). Students who can perform the following are predicted to score in the 24 to 27 range on the ACT. This is an acceptable range for most university applications.

- Understand the methods and tools used in a complex experiment
- Understand a complex experimental design
- Predict the results of an additional trial or measurement in an experiment
- Determine the experimental conditions that would produce specified results

These objectives are basic skills that students will develop and use throughout this unit.

EXPERIMENTAL DESIGN AND IMPLEMENTATION

Experimental Design: In order to identify an unknown microbe, a series of experiments will be employed by the student and observations recorded. Following these procedures, students will use books and the Internet to identify their microbial pet based on the outcomes of the labs. From this type of authentic, kinesthetic learning, students will retain the information and be intrinsically motivated to complete class work and participate. This approach to microbiology that incorporates all of Gardner's Multiple Intellegences (1983): auditory, visual and kinesthetic types of learning. The students will hear a brief lecture (auditory), read the information from the book and lab protocol (visual), and participate in the lab itself (kinesthetic). They will engage in critical thinking skills as they work through the steps of the labs and attempt to identify their unknown microbe. Higher level thinking like this is one of Marzano's nine strategies for success (2004). As students work independently to determine their microbe's identity, they will be engaging in authentic lab technique, a rigorous curriculum, and building relationships with not only their peers, but also their teacher and their pet. Those characteristics of this project make it inquiry based (Jansen, 2011).

The "Pets"

Nine microbes were chosen by this researcher to be the unknown "pet" microbes on which the students would experiment. The line up included: *Micrococcus luteus, Serratia marcescens, Pseudomonas fluorenscens, Staphylococcus epidermidis, Escherichia coli, Bacillus megaterium, and Sporosarcina ureae. (Branhamella cattarhalis* and *Rhodospirillium. rubrum* unfortunately did not survive to be adopted.) These bacteria were chosen because they are relatively safe to use in the high school classroom and they grow very quickly under normal classroom conditions. Their different characteristics made them ideal for this type of work

because no two microbes shared the exact same results for the eight observations students made (Appendix B).

Micrococcus luteus is a gram-positive, catalase positive aerobe. It produces round, yellow colonies on nutrient agar and has a coccus (sphere-shaped) morphology.

Staphylococcus epidermidis has nearly the same characteristics; however, it produces cream colored, circular colonies on nutrient agar.

Sporosarcina ureae is a coccus-shaped Gram-positive aerobe. It is catalase positive and produces cream to orange colored colonies on nutrient agar. Its defining characteristic is the ability to produce round spores. These cause the colonies to appear black after a short growth period.

Bacillus megaterium is also a spore-producing, bacillus (rod-shaped) bacterium. It is named for the comparatively enormous size of its cells, about 4μm long. It is Gram positive, catalase positive and aerobic. It produces very large cream to yellow colored colonies on nutrient agar. In nature, this microbe is found in soil. It is used industrially to produce vitamin B12 and penicillin, among other things.

Escherichia coli is a bacillus-shaped, gram negative, catalase positive, facultative anaerobe. It will grow on McConkey agar and ferment lactose. It produces smooth, round, cream colored colonies on nutrient agar. This is an intestinal bacterium is important in the production of vitamin K in warm-blooded animals, and is generally harmless. Some pathogenic strains can cause food poisoning.

Serratia marcescens is similar to *E. coli*. It is also a bacillus shaped, facultative anaerobe. It is gram negative and catalase positive. However, it produces red, circular colonies on nutrient agar.

Pseudomonas fluorenscens is a bacillus shaped, gram-negative aerobe. It prefers to grow at room temperature and is catalase positive. Under low iron conditions on nutrient agar, it will produce circular colonies that secrete a green pigment making it appear to fluoresce.

The strains of bacteria were purchased from Carolina Biological Supply and streaked for isolation on complex nutrient agar plates. When isolated cultures were obtained, these bacteria were streaked once again for isolation into randomly numbered agar plates and placed in the incubator. These became available for students to 'adopt' the following day.

This thesis was implemented over the course of three weeks. The groundwork was laid early on with inquiry-based labs that engaged students in the techniques that would be necessary for identifying their pet microbes in the future. Students learned how to simple stain, gram stain, identify colony and bacterial morphology, and use the microscopes before this unit began. They also designed experiments to test effective hand washing techniques and determine bacterial content in milk products. In 55 minutes each day, these labs were sometimes difficult to complete. Because students work at different paces as well, some time had to be built in for those students who were lagging behind to catch up. In addition to this time, students also had the option to work with their pets during lunches, before and after school. Students restreaked pets onto fresh agar plates as needed. A significant amount of teacher prep time was involved in streaking and labeling the individual pets, tracking the information and making additional agar plates for student use. The schedule of student labs and activities follows:

Day #	Activity	Appendix
1	Assign “pet” microbes, observe, restreak onto new plates	
2	Simple stain, morphology identification	C6
3	Gram Stain, confirm bacterial morphology	C7
4	Streaking for isolation	C5
5	Colony morphology, Catalase Test	C9, C8
6	Set up temperature preference tubes	C4
7	Temperature/oxygen preference analysis, Steak on selective media	C4
8	Analyze selective media plates (McConkey Agar/Lactose Fermentation)	
9	Research/Write	
10	Research /Write	
11	Research/Write & Lab books due	
12	Formal Lab Report Due	A3

Table 1: Activity list for microbial pet unit. Class periods were 55 minutes.

Numbered petri dishes of “pet microbes” were assigned to individual students. Some students chose theirs based on colony color or the random number on the petri dish. Some students were placed in groups of two for the project due to an insufficient amount of pets. Out of the 61 bacterial plates prepared by this researcher, 26 failed to thrive. Ten of these were *R. rubrum* and *B. catarrhalis*, two strains that died off entirely and were not recoverable. Unprepared for this failure, and up against a time deadline, winter break, pairing the students seemed a valid solution. Students in one class worked together (n=17) and in the other class they worked individually (n=17). The tasks were developed so that each class period began with a brief lecture on the lab, explaining its importance and what type of information it should reveal

before the students set to work. In an effort to reduce errors and double-check one another's work, students were required to perform the simple stain, Gram stain and the catalase test on both their pet and someone else's pet. This way, if they had conflicting results, the students would repeat their tests to reduce the possibility of misidentifying their pet. When they worked diligently, with few errors, most labs could be finished and observations made before time ran out. A discussion of each lab follows. Instructions for these are found in Appendix C.

Analysis of Activities

Students were required to keep a lab book for this class. Each day, students made observations about their pet. Lab protocols were written into this book as well, in an attempt to make students read the protocol before they set to work on it. Observations about each lab, sketches or pictures, results, conclusions and reflections were also recorded in these books.

Using the oil immersion microscope (Appendix C1) is a basic technique that students must master in order to identify bacterial morphology and Gram reaction. This was the first lab of the semester, and these techniques were important throughout the microbiology semester.

Like using the microscope, sterile technique (Appendix C2), making agar plates (Appendix C3) and making nutrient broth (Appendix C4) are laboratory techniques that are used throughout the course.

Simple staining (Appendix C6) is a method used to observe the bacterial morphology, or shape, under oil immersion. The bacteria used for the experiment were either coccus or bacillus shapes. The stain used was Methylene Blue. This stain penetrates the cell wall and allows visualization of the shape. This lab went very well, because all students had performed this task multiple times and were very comfortable with it.

Gram staining (Appendix C7) uses two simple stains, a mordant, and alcohol. The results, gram positive or gram negative, indicate the presence of peptidoglycan in the cell wall of the bacterium. This is commonly the second step in identifying an unknown bacterium. Again, students had performed this test previously in class, and were fairly comfortable following the protocol. Conveniently, when observing their microbe under oil immersion for this test, they were able to confirm their previous observation about morphology. In a few cases, students found different results between their original observations and this one. In those cases, students started over with both simple and gram staining.

Streaking for isolation (Appendix C5) was a new skill for students. The purpose is to isolate colonies of the unknown bacterium in order to observe colony morphology. Colony color, elevation, and margins can often be key factors in determining the identity of a microbe (Appendix C9). For students working with *Serratia marcescens*, *Miccrococcus luteus*, and *Psudomonas fluorescens*, this was the most important observation. *S. marcescens* is pink, *M. luteus* is yellow, and *P. fluorescens* is green under low iron conditions. Students often broke or ripped the agar while streaking their new plates, and many failed to sterilize the inoculating loop between each section on the plate. In a few cases, the student started over with a new agar plate, but most were able to work through this small setback.

Testing for the presence of the catalase enzyme is a simple procedure using hydrogen peroxide (Appendix C8). Bubbles indicate the presence of catalase and that the microbe in question is able to decompose hydrogen peroxide into water and oxygen. Few students had errors during this lab, but using a plastic- not metal- inoculating loop and a young colony was important because older colonies and nichrome wire loops can give false results.

Temperature and oxygen preference experiments (Appendix C4) involved inoculating 3 tubes of nutrient broth with the unknown microbe and placing them in the incubator at 37°C, the refrigerator at 15° C, and at room temperature 25° C. Allowing these to grow overnight, observations were made the next day regarding the turbulence of the liquid and where in the tube this cloudiness appeared. Based on this, students could classify their microbes as psychrophiles, mesophiles, or thermophiles. If the cloudiness was concentrated on the top of the nutrient broth, the microbe needed oxygen to function, and it was classified as an aerobe. If it was only found at the bottom of the tube, it was classified as an anaerobe because it required no oxygen. This experiment allowed students to find two results out of a single protocol. They also had the opportunity to use pipettes to measure the broth into the tubes, which was new to them, and to employ sterile technique.

Selective and differential media like McConkey agar allows for multiple observations as well. McConkey agar selects for the growth of only gram-negative bacteria. On this media, lactose fermenters are able to use the lactose present in the plate and produce an acid, lowering the pH and turning the agar pink. Non-lactose fermenters must use the peptone present in the plate instead of the lactose. This causes the production of ammonia, raises the pH and turns the agar white. This test allowed students to confirm or reject their earlier gram stain results. For this test, students were given a petri dish containing McConkey agar and asked to develop their own protocol and conclusion based on their knowledge of differential and selective media.

At this point, students had enough information to research their “pet” online and in the books provided to determine identity. From this information, they wrote a formal lab report that identified their “pet” based in the lab results and research and included a section on the

importance of this microbe to society (Appendix A3). In the interest of this thesis, there was also an optional section in the report for their personal opinions of the overall project.

Analysis of Assessments

Students were given a pretest at the beginning of the class, on the first day of school to assess their prior knowledge about microbiology (Appendix A1). This test was graded using a rubric (Appendix A2). The test contained 10 open-ended questions and had a wide range of possible answers. The rubric shows a variety of possible correct and incorrect answers. If the answer given by students contained at least one wrong answer, it was counted incorrect. All parts to their answer must be correct to receive credit. Each question was worth one point. There were ten questions.

On the last day of class, students took a post-test, which was identical to the pretest (Appendix A1). Both tests were graded using the same rubric (Appendix A2).

At the conclusion of their experiments, students wrote their lab report (Appendix A3) identifying the microbe they had been studying. This report was graded based on a rubric (Appendix A4) and worth a total of 50 points.

RESULTS

Figure 1 shows the breakdown of pretest and posttest data for each student (n=31). Using a paired t-test, the results were determined to be statistically significant (p=0.0001). The mean of pretest was 3.68 out of ten possible points, while the mean of the posttest was 6.02 out of ten points. Students one, two, and three had scores that actually decreased. In all of these cases, the student failed to turn the posttest over and finish it. Because it was the last day of school for most of these students, there may have been some absent-mindedness that resulted in several students (n=8) not turning the page over to finish the posttest. Only 31 students took both the pretest and the posttest. Two students from the original 34 transferred into the class and did not take the pretest. One did not take the posttest because she finished the class one week early.

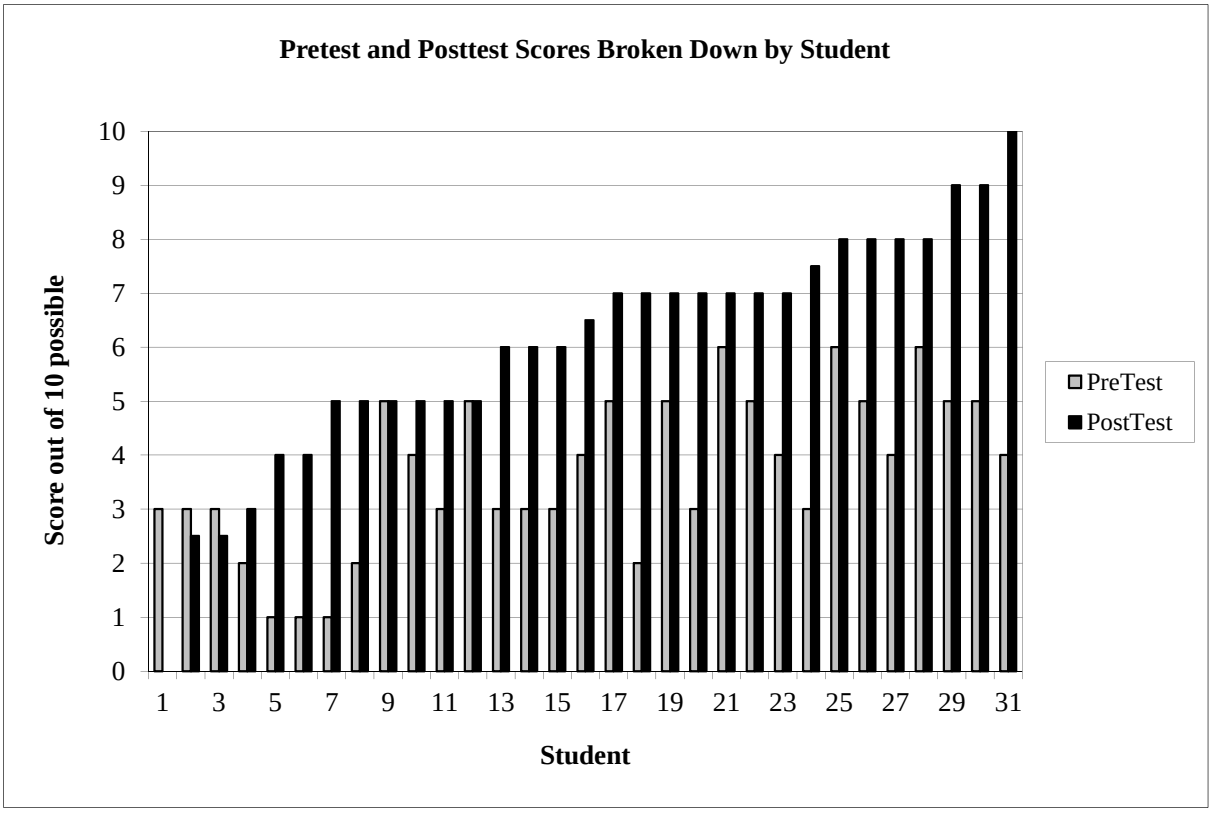

Figure 1: Pretest and Posttest comparison by student (n=31). Using a paired t-test p=0.0001.

In most classrooms, and certainly on standardized tests such as the ACT, omitted questions are considered incorrect. However, this research did not test the students' willingness to answer the question, but instead their ability to recall the information correctly. Calculations omitting students who did not attempt an answer are shown in Figure 2. Figure 2 shows the percent of correct answers for each question on both the pretest and the posttest. All scores show improvement.

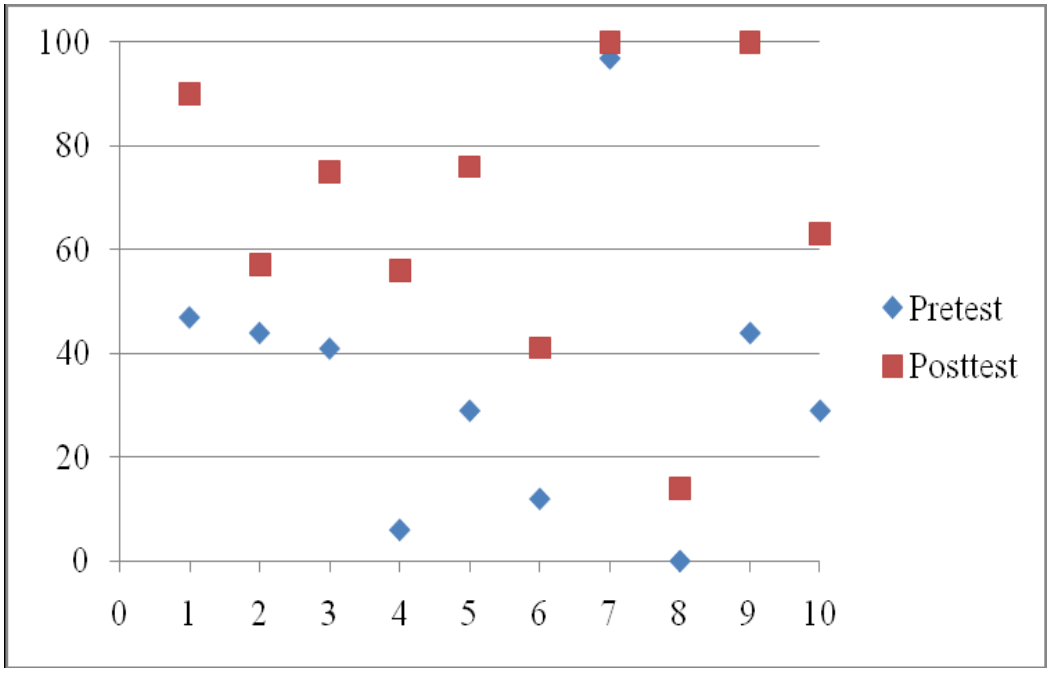

Figure 2: Pretest and Posttest data broken down by question number and percent correct. This scale includes only students who attempted to answer the question. All results show significant improvement. T-test results in a p-value=0.022. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis.

Using a T-test, the p-value calculated (based on a null hypothesis of p=0.05) was p=0.022. This indicates that the improvement is statistically significant. In addition to this, the test average improved from 35 (pretest) to 65 (posttest) percent. A 30 percent improvement is very good.

All answers showed improvement from the pretest to posttest. However, question two, regarding DNA of a parent colony compared to subsequent colonies of E. coli, showed only a small amount of improvement. Most commonly, the incorrect answers on the pretest were that the E. coli DNA would all be different. On the posttest, the common incorrect answer was the

DNA of the E. coli would all be the same. This misconception probably stems from the students' understanding of binary fission, but they failed to account for random mutations in the DNA.

The common wrong answer for number six, which involved sketching a bacterium and labeling the parts, was the inclusion of a nucleus. Most students were able to identify both the cell wall and flagella, but many included a membrane bound nucleus or other organelles. This is a misconception that seems to prevail. Biology and microbiology students alike struggle with this difference between prokaryotes and eukaryotes.

Most students came in with the correct prior knowledge for question seven, regarding how bacteria get into the body. This left little room for improvement on the post-test, and all students answered this question correctly on the posttest.

While they knew how bacteria enter the body, on both the pretest and posttest, students struggled with question eight which asked what bacteria do when they get inside the body. The most common answers were, "attack", "spread", and "make you sick". While, this is true, their responses were so vague that they received no credit. Specific answers involving the method by which bacteria attach, invade cells or produce toxins were considered correct answers.

On the pretest, less than half of the students could identify ways in which bacteria are beneficial to humans. However, on the posttest all students were able to correctly answer this question. Answers ranged from helping to digest food, specific examples of biotechnology, and microbial antagonism by the normal flora on and in the body.

In addition to these test questions, students completed a formal lab report that identified their microbe based on the laboratory tests completed in class (Appendix A3). This report was graded based on a rubric (Appendix A4). The mean score of these lab reports (n=30) was 94

percent. Students' score ranged from 36 to 55 out of a total of 50 points. Some students received more than 100 percent because bonus points were awarded to papers that answered any part of the optional questions regarding the project. Those questions and answers are discussed below. Based on these results, students clearly retained the information necessary to complete the report. They did a stellar job of following the rubric and including all of the experimental data necessary to correctly identify their "pet" microbe. Not one student failed to identify their pet.

Although these scores indicate comprehension and retention of information, they do not address the motivation of the students. One of the goals of this project was to improve motivation through hands-on, authentic lab activities. In an optional response section of their final lab report (Appendix A3), students had the opportunity to speak their mind about the project. Specifically, they were asked: 1. How did they feel about the project in general? 2. How can this researcher improve the project for subsequent years? 3. The best and worst things about the project. Not every student responded to this section, but many did (n=25).

In response to the first question, students described the project as fun, enjoyable, and challenging. One student called it, "frustratingly exciting. Every day there was a battle and a victory." She went on to say she was jealous of the students who would get to do the improved upon project for next year. This same student told me on a Monday morning her pet was the only reason she had come to school that day. Knowing she needed to restreak the microbe onto a fresh agar plate in order to sustain it, she came to class.

Some improvements students suggested in response to the second question were giving students a list of possible microbes, having individual teacher-student conferences to make sure they are on track, and, believe it or not, offering more lab tests. These suggestions appeared in multiple responses.

In response to the third question, the worst aspects of the project were time constraints and the difficulty navigating the Internet in search of their "pet's" identity. One student wrote, "What I didn't like about it was that I had no idea what I was looking for, but what scientist does know?"

Students gave many answers regarding the best aspects of the project for the third question. Many students liked that they must be self-reliant and self-paced. Because they were self-paced, some students came in during lunch or after school to finish labs they had begun, make observations, and discuss their projects. Others commented on the interconnectedness of some of the experiments, like Gram staining and the use of McConkey agar. Several commented this was a real-life lab experience. One student wrote, "I made sure to do things correctly the first time around because I had no one else's results to fall back on." This speaks to the writing of Ellen Glanz, "Students must experience responsibility where others are really depending on them" (Time, 1978). Perhaps even having the responsibility of sustaining a tiny culture of microbes is enough to ward off apathy in this course.

DISCUSSION AND CONCLUSION

The data in Figures 1 and 2 indicate that students learned the information. Students writing and comments on the formal lab report reveal that they enjoyed the method by which they learned it. As an observer in the classroom, this researcher found that everyone, teacher and students alike, benefitted from this addition to the curriculum. This is in keeping with the work of Partin, et al. (2011). They wrote that student attitudes about a class have a major affect on their intrinsic motivation. That is, if you enjoy the work, you are more apt to complete the work. In addition to that, Partin found that students who were more confident scored better on tests. The results from this experiment seem to agree.

Not only did the scores improve on the posttest, the students' lab books were more complete for this project compared to previous labs they had done. Based on the comments discussed previously (results section), students were more eager to come to class. Reese (2001) and Deci (2001) might agree that the intrinsic motivation fostered by autonomy and inquiry led to this shift in attitudes. At the lab tables, their conversations focused more often on the task at hand than other off-topic conversations. Their questions evolved from looking for the "right answer" to reflection on lab technique and time management. These are skills they will carry into the workplace. Because high school is the testing ground for the "real-world," teachers are always looking for opportunities that will prepare students for what is to come. One of the most important traits students should leave high school with is curiosity, and the ability to find an answer to their question. Eager employees can be taught to do nearly anything. This eagerness to learn can be seen in the comments on students' lab reports. These comments show they were motivated to learn the information and they found it both exciting and interesting.

As for my classroom, this style of teaching has infiltrated my other classes as well. Putting the onus on students for reading the lab protocols, making them responsible for the information, as well as the lab set up, has eased the burden of lab days for me. Before implementing this project and seeing its results with Microbiology students, I reviewed all directions with students, set up their lab stations for them, and gave them expected results. It was exhausting. After this experience, I now give students the protocol, have them copy it to their lab books overnight, ask questions the next day and send them to work. If this work is not completed, students may not do the experiment that day. If it is completed, they must set up their own lab stations and determine their own results. I feel this is more authentic and does not allow for as much data falsification as when they knew the expected results. I agree with the author who said, "As often as possible, school should stress learning that is experiential….[C]arrying out real-world projects that involve collecting data, estimating, calculating, drawing conclusions, and making decisions (Daniels et al. 1993). The outcome in both Microbiology and Biology is that students must form their own hypothesis for open-ended labs, perform the lab without knowing the results, and verify their data with other lab groups or sources in books and on the Internet. This aligns with Marzano and others' ideas about engaging students through generating and testing hypotheses (2004).

In the future, I will continue to do this project with Microbiology students, adding more tests such as glucose fermentation and starch hydrolysis. Additional strains of bacteria such as *B. cereus* and *B. subtillus* will be added to my repertoire. Others, such as *B. catarrhalis*, will be eliminated based on the difficulty involved in sustaining them. The addition of Bergey's Manual to the classroom library will be an essential part of the future changes as well. Microbiologists identifying unknown bacteria consider this manual the go-to resource for determining expected

results of tests. If possible, in future years, the project will be shifted to the last 2 weeks of the semester. This would allow students to gain lab skills and as much information as possible before the onset of the project. As the final exam, incorporating a lab practical would be interesting. Students would have to identify a certain bacterium based on several lab stations showing results around the classroom. With limited time, students could be provided a dichotomous key by which to identify the microbe. This would be a more rigorous, college-like assessment for students.

Based on these results, I will pursue more inquiry-based units for both Biology and Microbiology students. This research revealed that students are more intrinsically motivated to learn, and more apt to retain the knowledge when there are authentic, rigorous activities involved.

APPENDICES

ASSESSMENTS: APPENDIX A1

Pretest

FACTS: ***Escherichia coli*** (or ***E. coli***) is a species of bacteria commonly found inside portions of the human digestive system. In the proper environment, a single ***E. coli*** bacterium can reproduce in 20 minutes.

1. Imagine that there were 10,000 individual bacteria in a petri dish. If you were to then expose these *E. coli* to a dose of an antibiotic (a chemical that is designed to kill *E. Coli),* what would you say might happen to the bacteria population in the dish over the next week or so? Explain your answer.

2. If you were to grow a colony of 100,000 E. coli from a single individual inside of a Petri dish and then determine the DNA (genetic material) sequence of 100 individuals, how similar or different would you expect to find the DNA of different individual bacteria to be? Explain.

It is often said that all living things must possess certain characteristics that distinguishes them from non-living things. For example, living things must possess the ability to use energy, self-repair, grow, reproduce, move, respond to changes in their environment, etc. Non-living things do not have these abilities.

3. How do bacteria most commonly reproduce? Explain in your own words (but you may also add drawings if you wish).

4. How do individual bacteria obtain the building blocks and energy to get larger? Explain in your own words (but you may also add drawings if you wish).

5. How do bacteria move? Explain in your own words (but you may also add drawings if you wish).

6. In the space below, draw a bacterium and illustrate it with as much detail as you can. Please name or label any structures that you draw inside or on the outside of the bacteria.

Some bacteria are associated with humans or human activities and some of these can cause disease in humans.

7. How do bacteria get inside the body?

8. Once they are inside the body, what do they do?

9. Provide one example of how are bacteria helpful to humans. Explain.

10. There are 1000 bacteria per cubic millimeter in garden soil. What resources do these bacteria use to grow and reproduce?

APPENDIX A2

Pretest Rubric- Expected answers

1. Antibiotic on E.coli question:
    a. Not all bacteria die immediately
    b. Slowly die off
    c. Some survive & reproduce if resistant
    d. Population of resistant bacteria will increase dramatically

    Wrong answers

    e. All die
    f. Nothing happens
    g. IDK (I don't know)
    h. Did not attempt
2. E. coli DNA of colony
    a. Almost exactly the same
    b. Some small differences due to random mutations

    Wrong answers

    c. All different
    d. All the same
    e. IDK
    f. Did not attempt
3. Reproduction
    a. Binary fission
    b. Asexually

    Wrong answers

    c. Sexually
    d. IDK
    e. Did not attempt
4. Energy to grow

a. Surroundings
b. Broken down molecules
c. Cellular Respiration
d. Fermentation
e. ATP

Wrong answers

f. Photosynthesis (kind of)
g. Absorbtive
h. IDK
i. Did not attempt

5. Movement
    a. Axial filament
    b. Flagella
    c. Cilia

    Wrong answers

    d. Feet/legs
    e. Fly
    f. Fimbria
    g. Pili
    h. IDK
    i. Did not attempt
6. Picture of bacteria
    a. Accurate drawing
    b. Labelled: DNA, Cytoplasm, Ribosomes, Cell wall, Cell membrane, peptidoglycan, Flagella or cilia (etc)

    Wrong answers

    c. Included a nucleus
    d. Drew a virus
    e. Included membrane bound organelles
    f. IDK
    g. Did not attempt
7. Get in body by…

a. Contact
   - Direct
   - Indirect
   - droplet

b. Vehicles
   - Air
   - Water

c. Vectors

Wrong answers

d. IDK

e. Did not attempt

8. Infection
   a. Are moved by body to…wherever (intestines, stomach, lungs etc)
   b. Attachment
   c. Penetration
   d. Damage to cells by
      - Using host nutrients
      - Causing direct damage to surroundings
      - Producing toxins
      - Causing hypersensitivity reaction

   Wrong answers

   e. Lytic or lysogenic cycle
   f. IDK
   g. Did not attempt

9. Examples of helpful bacteria:
   a. Lactobacilli
   b. Normal Microbiotia
   c. Decomposers, insect control, etc
   d. Production of insulin, etc

   Wrong answers

   e. IDK
   f. Fungus example

g. Virus example

h. Antibiotics

i. Did not attempt

10. Garden soil

a. Decomposers of material in soil

Wrong answers

b. IDK

c. Did not attempt

APPENDIX A3

Formal Lab Reports: Unknown Bacterial Pet Project

Pet #_____

Student Name_____

Your formal lab report will include a section on each test you conducted. The sections should describe the purpose of each test, and your results. Here, you will briefly describe what happened including any errors. Include pictures where appropriate. Cite ALL sources!

After the description of the analysis of your pet, explain to the reader the identity of your pet, and the way you went about determining it. That is, I might say "my pet was *Staphylococcus aureus*. I determined this based on the previous tests which deemed it a gram negative, catalase positive, lactose fermenting aerobe that prefers warm conditions and grows as purple colonies on nutrient agar." Cite ALL sources that led to your conclusions!

In the final section, you will describe your pet's importance to society in general. What diseases does it cause, where does it flourish, why should we care? Think about how to control the growth of your microbe & if it is helpful to society in anyway.

(As a bonus, describe how you felt about this project. How could I improve it for future years? What were the best/worst things about it, etc…)

This paper will be typed in the standard format. While there is no page limit, a complete report will be several pages long. On the other hand, don't write me a novel—I have to grade these before February!!

Tests/Observations included, but may not have been limited to:

- Day 1 observations
- Streaking for isolation
- Colony Morphology
- Simple stain/Meth Blue stain/Bacteria Morphology
- Gram Stain
- Catalase Test
- Temperature Preference
- McConkey Agar/Lactose Fermentation

If you have questions, be sure to ask! This grade will make or break your marking period!

Kroos@carman.k12.mi.us

APPENDIX A4

Formal Lab Report Rubric: Unknown Bacterial Pet Project

Kid: Pet #:

Test	Reason	Results		Points
☐	☐	☐	Day 1 observations	3
☐	☐	☐	Streaking for isolation	3
☐	☐	☐	Colony morphology	3
☐	☐	☐	Simple Stain/Bacterial Morphology	3
☐	☐	☐	Gram Stain	3
☐	☐	☐	Catalase Test	3
☐	☐	☐	Temperature Preference/Oxygen Preference	3
☐	☐	☐	McConkey Agar/Lactose Fermentation	3

☐	Correct Pet ID	5
☐	How did you identify pet?	5
☐	Diseases caused by pet	2
☐	Where does it flourish?	2

☐	Why should we care?	2
☐	Growth control?	2
☐	Benefits to society	2
☐	Format/Grammar/Effort/Subjective points	6
☐	Bonus Points	0

Comments: Total Score: /50

APPENDIX B

Expected Results of Labs

Name	Bacteria Morph.	Colony Morph.	Gram Rxn.	Cat. Rxn.	Oxygen Pref.	Temp. Pref.	Grows on McC	Lac. Ferm
Micrococcus luteus	coccus	Yellow, round	+	+	Aerobe	37C	No	N/A
Serratia marcescens	Bacillus	Red, Circular	-	+	Fac anaerobe	37C	Yes	No
Pseudomonas fluorenscens	Bacillus	Circular maybe green	-	+	Aerobe	25-30C	Yes	No
Staphylococcus epidermidis	Coccus	Cream, circular	+	+	Aerobe	37C	No	N/A
Escherichia coli	Bacillus	Round, cream	-	+	Aerobe	37C	Yes	Yes
Bacillus megaterium	Bacillus	Large, Yellow?	+	+	Aerobe	Var.	No	N/A
S. ureae	coccus	Cream/ orange	+	+	Aerobe	Var.	no	N/A

Table 2: Expected results of experiments for each bacterium. This table was not provided to students but used as a teacher resource only.

LABORATORY PROTOCOLS: APPENDIX C1

Using the Oil Immersion Microscope

Successful operation of the microscope depends upon proper maintenance and correct use. Acquaint yourself with the mechanical construction and names of the various parts of the microscope. Thoroughly read the following points on the handling and care of the instrument.

1. When lifting the microscope, grasp it by the arm with one hand and place the other hand underneath the microscope. DO NOT lift it by the head, the adjustment knobs or any other parts.

2. The microscope should be placed on the desk with the stage facing away from you. The stage should be kept horizontal to prevent immersion oil or other fluids from running off the slide. (Do not tilt the microscope up to you! You move to it!)

3. Wipe the lenses of the microscope before and after class use. The exterior lens of the eyepiece should always be carefully cleaned before use by wiping with lens paper. Being exposed, this lens easily accumulates dust from the air. Do not use alcohol as a cleaning fluid. Objective lenses should be kept clean at all times. They are best cleaned by wiping with lens paper. Under no conditions should objectives be removed from the revolving nosepiece.

The oil immersion objective must be wiped with lens tissue at the end of each period.

4. Always be certain that a low-power objective (4X or 10X) is in the working position, i.e., in the line with the body tube, before putting the microscope away. Be sure to wrap the cord (not super-tight) around the holder and cover the microscope with the dust cover before returning it to the appropriate cupboard.

5. When observing wet mounts, it is best to minimize the amount of light by lowering the condenser and partially closing the diaphragm.

6. When observing Gram stains or other dry stained slides, the condenser should be all the way up and the diaphragm wide open.

7. A general rule when looking at bacteria is to first focus on a slide with the low-power (10X) objective, move to high dry objective (40X), and then switch to the oil objective (100X with oil).

8. In focusing the low-power objectives (4X & 10X), first place the slide securely into the spring-loaded lever on the stage of the microscope. Swing the low-power objective under the body tube and, using the coarse adjustment, raise the stage until the front lens is about 1/4 inch from the object. Then, while looking through the eyepiece, slowly lower the stage by the coarse adjustment until the image is distinct. Focus sharply by means of the fine adjustment.

9. In focusing the high-power objective (40X), first swing the lens under the body tube. Then, while looking between the objective and the slide, slowly raise the stage with the

coarse adjustment until the lens of the objective almost touches the slide. Focus downward by the fine adjustment until the image is in sharp focus.

10. In focusing the oil-immersion objective, first place a drop of oil over the object on the slide. Raise the stage very carefully with the coarse adjustment until contact is made with the oil. Do not raise the stage too rapidly; otherwise, there will be danger of cracking the glass slide with possible injury to the objective lens. Then focus downward with the fine adjustment until the image is in sharp focus.

11. The immersion oil should have as near as possible the same refractive index as glass. The objective must be wiped with lens paper at the close of each period. If this is not done, an accumulation of oil will occur, making focus impossible.

12. Illumination of the object is optimized by adjusting the rheostat. Adjust the light intensity to give a good degree of contrast. Be sure this rheostat is turned all the way off when you are finished with the microscope before you unplug the cord.

13. The sub-stage iris diaphragm serves several purposes. It may be used to control the intensity of the illumination, but it is not recommended for this purpose; neutral filters should be used instead. Too much light may cause an uncomfortable glare; too little may cause undue exertion. Both should be avoided. The main functions of the diaphragm are to increase contrast and to improve definition. It happens sometimes that different diaphragm openings are required for different types of work within the same slide preparation.

TROUBLE-SHOOTING - If you are unable to focus on the smear, there are several things you should check before calling the instructor.

1. Did you perform the staining procedure correctly? Did you heat fix?
2. Be sure that you can see something on the slide either with the unaided eye or with the low power objectives [DO NOT go back to these lenses if you have already placed immersion oil on the slide.]
3. Is the slide placed appropriately on the stage? Is it upside-down?

APPENDIX C2

Sterile Technique

Good sterile technique is the first and most important step in ensuring consistent results. Sterile technique refers to procedures by which cultures may be manipulated without infecting the worker or contaminating the cultures or the laboratory environment.

Because contaminating bacteria are ubiquitous and are found on fingertips, bench tops, etc., it is important to minimize contact with these contaminating surfaces. When working with the inoculation loops and agar plates, the round circle at the end of the loop, the tip of the pipetter, and the surface of the agar plate should not be touched or placed onto contaminating surfaces.

The flaming of lips of tubes and flasks must ALWAYS be done whenever culture liquid is to be poured from a container (e.g., pouring plates). Flaming should be routinely done when caps are removed from tubes during transfer of cultures. The purpose of flaming is not to sterilize, but to warm the tube and create warm air convection currents up and away from the opening. This "umbrella" of warm, rising air will help to prevent the entrance of dust particles upon which contaminating bacteria reside.

Petri dish lids prevent dust from falling directly onto plates but allow diffusion of air around the edges. There are no direct air currents into the plate, and to enter, dust particles would have to rise vertically more than a centimeter. This does not often occur because of the density of the particles. Whenever the lid is removed, it should be held over the plate as a shield. Do not place the lid on the bench top. Do not leave plates uncovered. Do not walk around the room with an

open plate.

When working with cultures in test tubes, work as rapidly as is consistent with careful technique. Keep the tubes open a minimum amount of time. While the tubes are open, hold them at a 45 degree angle so that dust cannot fall into the open tube. Hold the tubes away from your face while transferring.

Test tubes are handled in the following manner:

- The test tube is held in the left hand (for a right-handed person).
- The instrument (loop, pipet, or needle) is held in the right hand.
- The test tube cap is grasped by the little finger of the right hand, and removed.
- While continuing to hold the cap with the little finger, the tube is lightly flamed and the instrument is manipulated appropriately, and withdrawn.
- The cap is replaced on the test tube and the test tube is put back into the rack.

Label all cultures with the name or number of the organism, and your name.

Always clean all work areas (your bench, balance area, sink area, gel area, etc.) thoroughly before leaving the laboratory! The last step before leaving the lab is to wash your hands thoroughly.

APPENDIX C3

Making Agar Plates

Materials

- 4 petri dishes
- 200+ mL Flask
- 1 magnetic stirrer
- 1 hot plate
- 1 oven/hot mitt
- 2.5g Nutrient Agar
- 100mL distilled water

Procedure

1. Measure out 2.5g Nutrient agar- put into flask
2. Add 100 mL of water
3. Insert magnetic stirrer
4. Place on a hot plate on **med-high** heat and **low** stir
5. Bring to a boil for 1 minute
6. Distribute evenly over 4 agar plates
7. Replace lid and let plates cool for 20 minutes.

APPENDIX C4

Making a Nutrient Broth

The only difference between broth and agar media is that broths do not contain an agar component. We use broth tubes primarily for specific assays, or (rarely) for bacteria that will not form colonies on a solid surface. In broth a species may display motility and/or a characteristic pattern of association among individual cells, such as chains or clusters, that is not as obvious in agar cultures. To prepare broth a dry medium is layered onto the surface of a measured volume of water as with agar media, mixed, and distributed into individual loosely capped or vented capped tubes in racks. Heating to dissolve components is sometimes required, but not always. Racks are steam sterilized and then allowed to cool, and caps tightened to prevent evaporation. Unlike preparation of agar plates, tubes are prepared with media already in the incubation vessel. A large volume syringe can facilitate distribution of media into individual tubes.

Analyzing growth in broth cultures

Rank the broth cultures as to which has the most growth (most turbid) to least growth (most clear). Describe the broth beneath the surface as turbid if it is cloudy, flocculent if large flaky masses are floating in the medium. If there is sediment, note that fact, agitate the tube to bring it into suspension, and describe the sediment by size of particle and whether or not it is viscous (use a sterile loop to determine whether or not it is "gooey."

The surface may be characterized by a coating consisting of a thin membrane or a thick pellicle. There may also be a ring of material (like a pellicle or membrane, but only at the edges), or flocculent material at the top. Note that conditions in an undisturbed broth tube become

anaerobic below the top 0.5 cm or so of liquid. Presence/absence of growth in deeper layers may confirm the relationship to oxygen.

Procedure:

1. Weigh out 8 grams of nutrient broth powder.
2. Add to 1.0 Liter of distilled water in a flask
3. Dissolve the powder with heat and stirring on a hot plate.
4. dispense into 250ml flasks for sterilization in autoclave.
5. Sterilize at 121deg. C for 20-25 minutes.
6. Using sterile technique, dispense into individual culture tubes with screw caps.
7. Inoculate 3 tubes of broth with your culture.
8. Place 1 tube in each of 3 temperatures: warm, room temperature, and cold.
9. Observe results after 24 hours.

APPENDIX C5

Streaking for Isolation

In order to identify bacteria, it is necessary to obtain a pure culture. This is done by using the streak-plate method. Bacterial cells are spread over the surface of an agar plate in a continuous dilution, so the cells will be separated from each other. When the plate is incubated, those individual cells will grow into colonies that originated from a single cell.

Tips for Success

- Obtaining well isolated colonies takes practice.
- Keep the plate covered as much as possible to avoid contamination.
- While streaking a section of the plate, keep the inoculating loop in contact with the surface of the agar at all times.

Procedure:

1. Heat sterilize an inoculating loop.
2. For a liquid culture, dip the loop into the broth, or for solid media, lightly touch a colony with the loop.
3. Using the loop, spread the culture over the surface of one quadrant of the plate as shown in the figure below.
4. Sterilize the inoculating loop again.
5. Continue the streak into the next quadrant.
6. Repeat steps 3-5 until the pattern is complete.
7. Incubate the plate. Then, observe for the presence of well isolated colonies.

APPENDIX C6

Simple Staining/Morphology Identification

Preparing a smear

A properly prepared smear accomplishes two things. It causes bacteria to adhere to a slide so they can be stained and observed. It also kills them, rendering pathogenic bacteria safe to handle. An objective in preparing smears is to learn to recognize the correct density of bacteria to place on the slide. Too many, and they overlap each other giving false positives or crowding each other to make a mess. Too few, and they cannot be located on the slide.

- A circle should be marked on the under side of a slide with a glass etching tool. Several circles can be located on the same slide.
- To prepare a smear from a dry culture, a very small drop of distilled water should be placed over the circled area. After aseptically removing material from a culture it is then mixed with the drop or placed directly on the slide if it is a dilute broth culture. It takes very little material to produce a successful smear.
- The drop is air-dried completely, which takes a short time if a small drop is prepared.
- While holding the slide with a clothespin it is quickly passed it through a flame. Three quick passes are usually sufficient to kill the bacteria and cause them to adhere.
- After cooling the slide, the smear is stained.

Tips for success

- Clean the slide
- Don’t use too much material - suspension should be just barely cloudy

- Don’t use too much liquid- takes a very long time to dry
- Don’t heat the smear before letting it air dry completely, it will boil the bacteria instead of attaching them
- Don’t overheat the smear, it may result in melting cell walls and possibly breaking the slide

Methylene Blue Staining

1. Place 1 drop of methylene blue stain on your sample.
2. Allow this to sit for 30 seconds
3. Rinse slide with water bottle until the water runs clear from the slide.
    - Be careful not to spray the smear directly! Rinse with the water hitting above the smear and your slide tipped down.
4. Blot (DO NOT WIPE) dry.
5. Observe under the microscope.

Adapted from: http://www.ruf.rice.edu/~bioslabs/bios318/staining.htm#smear

APPENDIX C7

Gram Stain Reaction

The gram stain is one of the most useful methods in identifying bacteria. In this procedure, the bacteria is stained and examined under the microscope. Organisms are judged to be gram positive if they retain the crystal violet after decolorization and will appear purple. Gram negative organisms are decolorized and appear pink to red because they take up the safranin counter stain.

Tips for Success

Only perform gram stains on fresh cultures (24hr incubation). Using older cultures often yields unexpected results. Be sure to stop the application of decolorizer immediately after it runs clear to avoid over-decolorizing. Always run positive and negative controls from known cultures.

Procedure:

1. Place a loopful of sterile distilled water onto a microscope slide.
2. Touch an isolated colony with inoculating loop and swirl it in the drop of water on the slide.
3. Let the smear air dry at room temperature
4. Heat fix the smear by waving the slide over a flame, being careful not to overheat.
5. Flood the slide with crystal violet, and let stand for one minute.
6. Wash the slide briefly with cold water.

7. Flood the slide with gram's iodine; let stand for one minute; wash off with water.
8. Decolorize until the solvent flows colorlessly from the slide.
9. Flood the slide with safranine; let stand for 30 seconds; wash off with water.
10. Blot the slide dry with bilbous paper.
11. Examine the slide under the microscope for gram reaction (100x oil-immersion objective)
12. Gram positive organisms will appear purple, while gram negative organisms will appear pink.

APPENDIX C8

Catalase Test

The catalase test is a test for the presence of the catalase enzyme. Most organisms possess this enzyme capable of breaking down hydrogen peroxide. Organisms containing the catalase enzyme will form oxygen bubbles when exposed to hydrogen peroxide.

Tips for Success

Do not use a nichrome loop as this will give a false-positive reaction. Because hydrogen peroxide is unstable, always check the expiration date on the solution. Do not perform the catalase test on blood agar, because blood cells in the agar contain the catalase enzyme. Always run positive and negative controls from known cultures.

Procedure:

1. Place a drop of 3% hydrogen peroxide onto a clean microscope slide.
2. Touch an isolated colony with an inoculating loop
3. Place the loop, carrying some of the isolate, into the drop of hydrogen peroxide.
4. Observe the slide for the evolution of bubbles
5. The reaction is positive if oxygen bubbles form rapidly.

APPENDIX C9

Colony Morphologies

Bacteria grow tremendously fast when supplied with an abundance of nutrients. Different types of bacteria will produce different-looking colonies, some colonies may be colored, some colonies are circular in shape, and others are irregular. The characteristics of a colony (shape, size, pigmentation, etc.) are termed the colony morphology. Colony morphology is a way scientists can identify bacteria. In fact there is a book called Bergey's Manual of Determinative Bacteriology (commonly termed Bergey's Manual) that describes the majority of bacterial species identified by scientists so far. This manual provides descriptions for the colony morphologies of each bacterial species.

Although bacterial and fungi colonies have many characteristics and some can be rare, there are a few basic elements that you can identify for all colonies

- Form - What is the basic shape of the colony? For example, circular, filamentous, etc.
- Elevation - What is the cross sectional shape of the colony? Turn the Petri dish on end.
- Margin - What is the magnified shape of the edge of the colony?
- Surface - How does the surface of the colony appear? For example, smooth, glistening, rough, dull (opposite of glistening), rugose (wrinkled), etc.
- Opacity - For example, transparent (clear), opaque, translucent (almost clear, but distorted vision, like looking through frosted glass), iridescent (changing colors in reflected light), etc.

- Chromogenesis (pigmentation) - For example, white, buff, red, purple, etc.

Refer to the figure below for illustrated examples of form, elevation, and margin:

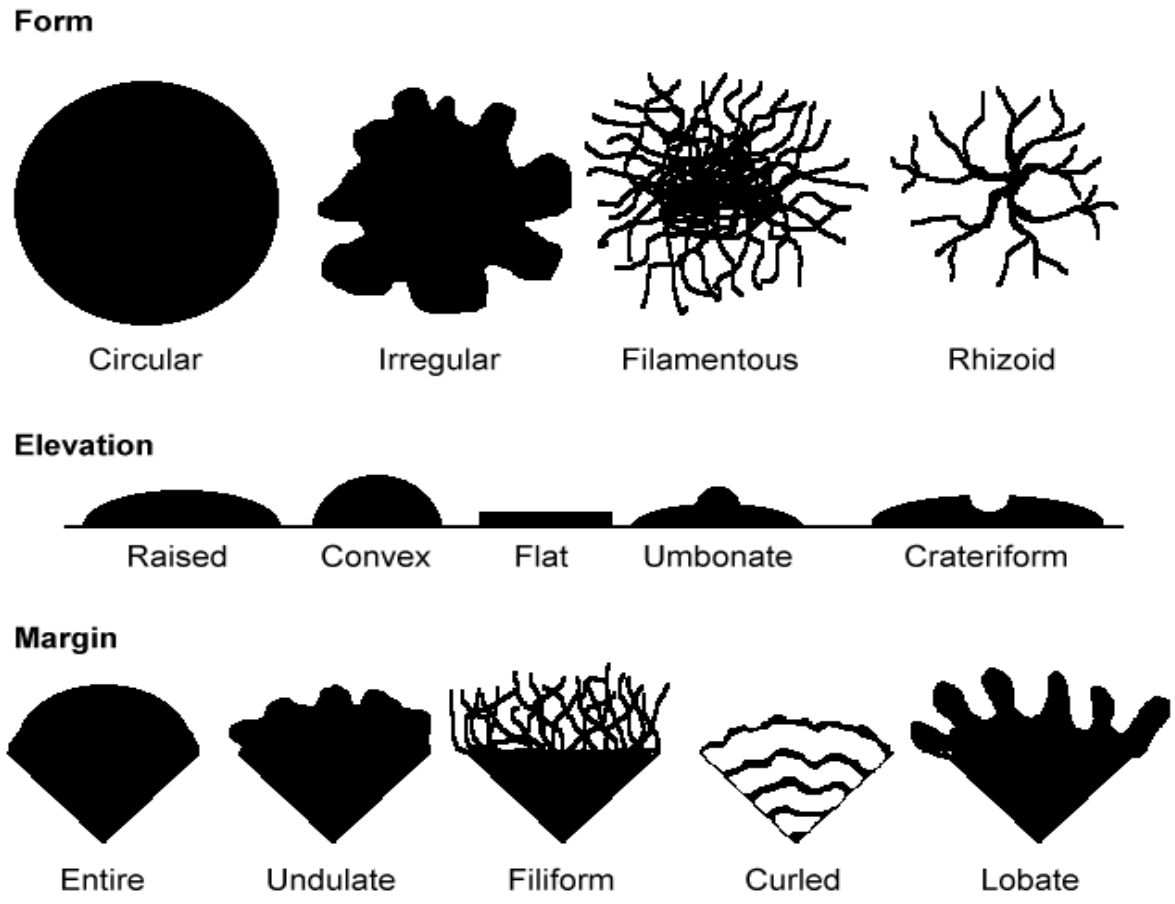

Figure 3: Colony Morphological Characteristics

APPENDIX D

Increasing Student Motivation and Comprehension in Microbiology By Employing an Overarching Theme

PARENTAL CONSENT AND STUDENT ASSENT FORM

Dear Students and Parents/Guardians:

I would like to take this opportunity to welcome you back to school and invite you to participate in a research project, **Increasing Student Motivation and Comprehension in Microbiology By Employing an Overarching Theme,** that I will conduct as part of microbiology this semester. Not only am I your science teacher, I am also a master's degree student at Michigan State University. Researchers are required to provide a consent form like this to inform you about the study, to convey that participation is voluntary, to explain risks and benefits of participation, and to empower you to make an informed decision. You should feel free to ask the researcher (me) any questions you may have.

Purpose: I have been working on effective ways to teach microbiology experiments and I plan to study the results of this teaching approach on student comprehension and retention of the material. The results of this research will contribute to other teachers' understanding about the best way to teach about microbiology. Completion of this research project will also help me to earn my master's degree in Michigan State University's Division of Math and Science Education (DSME).

Students will: participate in the instructional unit about bacterial identification. You will complete the usual assignments, laboratory experiments and activities, class demonstrations, and pretests/posttests just as you do for any other unit of instruction. There are no unique research activities and participation in this study will not increase or decrease the amount of work that students do. I will simply make copies of students' work for my research purposes. This project will continue from September 2010 to August 2011. I am asking for permission from both students and parents/guardians (one parent/guardian is sufficient) to use copies of student work for my research purposes.

Benefits: My reason for doing this research is to learn more about improving the quality of science instruction. I won't know about the effectiveness of my teaching methods until I analyze my research results. If the results are positive, I can apply the same teaching methods to other science topics taught in this course, and you will benefit by better learning and remembering of course content. I will report the results in my master's thesis so that other teachers and their students can benefit from my research.

Potential risks: There are no foreseeable risks associated with completing course assignments, laboratory experiments and activities, computer simulations, class demonstrations, and pretests/posttests. In fact, completing course work should be very beneficial to students. I will have another person store the consent forms (where you say "yes" or "no") in a locked file cabinet that will not be opened until after I have assigned the grades for this unit of instruction. That way I will not know who agrees to participate in the research until after grades are issued. In the meantime, I will save all of your written work. Later, I will analyze the written work only

for students who have agreed to participate in the study and whose parents/guardians have consented.

Privacy and confidentiality: Information about you will be protected to the maximum extent allowable by law. Students' names will not be reported in my master's thesis or in any other dissemination of the results of this research. Instead, the data will consist of class averages and samples of student work that do not include names. After I analyze the data to determine class averages and choose samples of student work for presentation in the thesis, I will destroy the copies of student's original assignments, tests, etc. The only people who will have access to the data are me, my thesis committee at MSU, and the Institutional Review Board at MSU. The data will be stored on password-protected computers (during the study) and in a locked file cabinet at MSU for at least three years after the completion of the study.

Participation in this research is completely voluntary. You have the right to say "no". You may change your mind at any time and withdraw. If either the student or parent/guardian requests to withdraw, the student's information will not be used in this study. There are no penalties for saying "no" or choosing to withdraw.

Questions and concerns: If you have questions or concerns, such as scientific issues, how to do any part of it, or to report an injury, please contact the researcher (Krystal Roos 810-591-5450 kroos@carman.k12.mi.us 1300 N. Linden Road, Flint, Mi 48532 or the Primary Investigator: Dr. Heidemann: Michigan State University, 118 North Kedzie Lab, East Lansing MI 48824, heidema2@msu.edu.) about your role and rights as a research participant, would like to obtain information or offer input, or would like to register a complaint about this study, you may contact, anonymously if you wish, the Michigan State University's Human Research Protection

Program at 517-355-2180, Fax 517-432-4503, or e-mail irb@msu.edu or regular mail at 207 Olds Hall, MSU, East Lansing, MI 48824.

How should I submit this consent form? Please complete the attached form with your consent or dissent. Both the student and parent/guardian must sign the form. Return the form to Room 311 (Mrs. Lawrence) by 9/13/10.

To summarize:

- My research project is designed to improve student motivation & comprehension.
- You are not obligated to participate and your grade will not be affected whether you do or not.
- Sign the form below and check whether I can use your work (anonymously) or not.
- Turn it in to 311 by Monday, 9/13/10. **EVERYONE must turn in a form regardless of participation in the study.**

Thank you,

Krystal Roos

Microbiology Teacher

Carman-Ainsworth High School

Microbiology- bacterial ID unit

Krystal Roos

Carman Ainsworth High School

Parents/guardians should complete this following consent information:

I voluntarily agree to allow _____ to participate in this study. (print student name)

Please check all that apply:

Parent/Guardian:

_____ I give Krystal Roos permission to use data generated from my child's work in this class for her thesis project. All data from my child shall remain confidential.

_____ I do not wish to have my child's work used in this thesis project. I acknowledge that my child's work will be graded in the same manner regardless of their participation in this research.

Student:

_____ I give Krystal Roos permission to use data generated from my work in this class for her thesis project. All of my data shall remain confidential.

_____ I do not wish to have my work used in this thesis project. I acknowledge that my work will be graded in the same manner regardless of my participation in this research.

Signatures:

_____ _____

(Parent/Guardian Signature) (Date)

Student: sign one below

I voluntarily agree to participate in this thesis project.

_____

_____

(Student Signature) (Date)

I **do not** wish to participate in this thesis project.

_____

_____

(Student Signature) (Date)

*****Important*** Return this form to Room 311- Mrs. Lawrence.**

APPENDIX E

The objectives for this unit were based the following Michigan High School Curriculum Content Expectations.

B2.5D- Describe how individual cells break down energy rich molecules to provide energy for cell functions.

B2.4h- Describe the structures of…bacteria.

B2.2f- Explain the role of enzymes & other proteins in biochemical functions

B1.1C Conduct scientific investigations using appropriate tools and techniques (e.g., selecting an instrument that measures the desired quantity—length, volume, weight, time interval, temperature—with the appropriate level of precision).

B1.1E Describe a reason for a given conclusion using evidence from an investigation.

B1.1h Design and conduct a systematic scientific investigation that tests a hypothesis. Draw conclusions from data presented in charts or tables.

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