

A CONTEXT BASED APPROACH USING GREEN CHEMISTRY/BIO-REMEDIATION
PRINCIPLES TO ENHANCE INTEREST AND LEARNING OF ORGANIC CHEMISTRY IN
A HIGH SCHOOL AP CHEMISTRY CLASSROOM

By

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ABSTRACT

By

Tricia Miller

The ability of our planet to sustain life and heal itself is not as predictable as it used to be. Our need for educated future scientists who know what our planet needs, and can passionately apply that knowledge to find solutions should be at the heart of science education today. This study of learning organic chemistry through the lens of the environmental problem “What should be done with our food scraps?” explores student interest, and mastery of certain concepts in organic chemistry.

This Green Chemistry/ Bio-remediation context-based teaching approach utilizes the Nature Mill®, which is an indoor food waste composting machine, to learn about organic chemistry, and how this relates to landfill reduction possibilities, and resource production. During this unit students collected food waste from their cafeteria, and used the Nature Mill® to convert food waste into compost. The use of these hands on activities, and group discussions in a context-based environment enhanced their interest in organic chemistry, and paper chromatography. According to a one-tailed paired T-test, the result show that this context-based approach is a significant way to increase both student interest and mastery of the content.

DEDICATION

This thesis is dedicated to my children Jensen and Branden. Their love and patience has been a source of strength and peace in the face of the challenges. I love you both dearly.

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I am deeply thankful to many people for the journey and completion of my Masters in Science degree from Michigan State University.

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My professors at MSU, Merle Heideman, and Ken Nadler guided me in my endeavors to learn and develop as a scientist and educator. Their zeal for understanding, and the pursuit of knowledge aimed at the purpose of developing their students into well-versed scientists, educators, and citizens on the whole is exemplary. At Kellogg Biological station, Chuck Elzinga's class was an inspirational journey into the heart of science and how the processes of science can be used to unfurl the mysteries that make our amazing state of Michigan what it is today. This class sparked a passion in me about my planet that was deeper than I had originally realized. My love for my state, my students, and science converged that fateful summer in a way that altered me in entirety. For that enlightenment, thank you Chuck!

To my dear friends and colleagues, Wendy Johnson, Amanda Stegnick, Cyndi Gibson, Heather Dejonge, Becky Smiggen, Katie Hatchett, and Kevin Gravitt. Their mentorship and friendship have truly helped me as a teacher and a person.

In finality I would like to offer my gratitude to Russ Cohn, the inventor of the Nature Mill®. This thesis would not have been possible with out his clear devotion to fostering good earth stewardship and educating today's young scientists and engineers.

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INTRODUCTION AND THEORETICAL FRAMEWORK

Chemistry is a difficult subject for students (Grove & Bretz, 2011). This is due to the fact that in the abstraction of the classroom students often cannot integrate their experiences with nature with their scientific knowledge in order to develop an overall big picture of why this knowledge is both relevant and necessary. The use of context to teach content is a possible solution to help students make this connection (Karpudewan, Zurida & Wolff 2012, Cartrette, 2011, King, Bellochi & Richie, 2008, King, 2008). According to Queensland Studies Authority, an educational context is defined as “ a group of learning experiences that encourages students to transfer their understanding of key concepts to situations that mirror real life”

(www.qsa.qld.edu.au) The context-based trial pilot Chemistry syllabus was published by Queensland Studies Authority in Australia (2004, p.11). These authors believe that the use of context helps students make connections between their everyday life and the content. Shouldn't all educators be using context-based units?

In an article titled, “Making Connections”, King aptly addresses the need for context to help our students learn content, “In general, when students cannot connect a concept, or skill development to their everyday lives, engagement and motivation to learn, and perform decreases” (King, 2008). It would seem that contexts are an important tool that can be used to build interest when teaching content, but what about Chemistry specifically?

As previously stated, Chemistry is difficult for most students, so the need for making these connections between everyday life and the content is even more critical. In an investigation into the use of context-based instruction in a college level introductory chemistry course Cartrette (2011) states that, “students struggle with connecting organic chemistry to their everyday lives, and thus their interest and performance, measured by problem solving interviews,

may be hindered". The problem solving interviews that Cartrette completed with students used the 'think aloud' technique to collect data about student understanding of chemistry both before and after the context based units were taught. This technique is a process where the examiner instructs the subject to express verbally all thoughts that come to mind while performing a task. Cartrette's results clearly indicate that the context did help students make those important connections between their everyday experiences and their scientific learning, thus enhancing their interest in chemistry. According to Cartrette context-based instruction does enhance interest, but does it increase content mastery directly?

Further into the article "*Making Connections*", King warns that one should not assume that the context will increase content mastery and performance directly. "The results from mostly quasi experimental studies have shown that context based science- technology- society (STS) teaching develops a level of understanding comparable to that of conventional courses". This means that educators should use context to gain the interest of the students in the hopes of increasing motivation to learn, not necessarily to increase performance. This investigation intends to use this context-based method to help students make those important connections thus becoming more motivated to learn due to their increased interest thereby indirectly increasing content mastery in organic chemistry.

Hence, chemistry teachers should provide relevant contexts to explore chemistry concepts to help students develop interest and motivation to learn. Perhaps, students will connect these concepts to the world problems facing every human being on the planet. The question then is, which contexts are considered relevant to students?

One of most widely discussed relevant contexts in the literature is Green Chemistry. The use of Green Chemistry contexts, like Bio-remediation and Education for Sustainable

Development (ESD), are beginning to be at the forefront of how we think about relevant context-based chemistry education. A recent theme issue of *Chemistry Education and Research and Practice* (CERP, Volume 13, Issue 2, 2012) an international journal, was wholly devoted to “Sustainable development and Green Chemistry in Chemistry education.” These papers and editorial (Eilks & Rauch, 2012, Cartrette, 2011; Mageswary, Zurida & Wollf, 2012, Eissen, 2012, Mandler, Mamlock-Naamen, Blonder, Yayon & Hofstien, 2012) chronicle the use of Green Chemistry principles in chemistry curriculum to build interest, motivation, and enhance performance. Eilks and Rauch argue that “school chemistry education should promote competencies of the young generation to become scientifically literate. This means chemistry education has to contribute to making students capable of actively participating in society” (2012). More specifically, Eilk & Rauch point to “future chemists and chemical engineers need to learn what a more resource efficient and environmentally friendly chemistry for the future might look like. That means the ideas of Green Chemistry should be part of their training from the very start” (ibid). There is an abundance of articles in the literature (Eissen, 2012; Karpudewan, 2012; Mandler, 2012) supporting Green Chemistry as a relevant context for teaching chemistry. This is because Green Chemistry knowledge and applications will lead us to a more secure future through sustainable practices, such as Bio-remediation. This is most aptly exemplified in the article by Marco Eissen (2012) who argues that “Chemistry is one of the most important branches of science that can contribute to a sustainable development, because it represents the starting point for important mass flows.” Mass flows here refer to the rate at which microbial communities can transfer nutrients to the cell from the waste. This research points to the need to do more Green Chemistry in schools. Another supporter, Magesway (2011) best summarizes the case for this call to action by arguing that we can “Ensure sustainability of

tomorrow through Green Chemistry integrated with sustainable development concepts (SDC)”. In summary, Mandler, Mamlock, Naamen, Blonder, Yayan, and Hofstien (2012) argue that “ The overall interpretation based on this study, is that students who are exposed to an environmental context in Chemistry find it more related to their everyday lives, and thus find it more attractive”.

According to the authors cited here the goal is clear; chemistry educators should use Green Chemistry contexts to develop our units. How to implement this however, and to what extent is not specified.

The state of Michigan is just beginning to embrace a new set of national science standards, called the Common Core standards (NRC, 2012). The desire to enrich, enhance, and utilize context, inquiry based approaches is clearly evident in the language, but the objectives are still very content based, leaving much to interpretation as to how or what to use as a premise for context. Even if the context choice is clear the sheer volume of content to cover can seem daunting and may hinder some educators from tackling the goal, but there are some emerging leaders.

From personal communication this author learned that there are some universities and classrooms (University of Scranton, Cornell University, and Cornell Elementary in Okemos, Michigan) in our nation using compost and Green Chemistry/Bio-remediation in science education to help students make those important connections to enhance learning.

These sites are using either an outdoor compost pile or a small homemade compost bottle/bucket indoors to teach composting in the classroom. This investigation seeks to build on these predecessors’ work by using a new piece of technology, an automated indoor composting machine called the Nature Mill®. The Nature Mill® speeds up the decomposition process to two

weeks instead of six months by utilizing a controlled temperature and air flow reactor with a mechanized wand that rotates the compost in set intervals. This combination of novel technology and context-based instruction seeks to enhance student learning and interest. The purposeful use of a green chemistry context specifically, engineered bio-remediation, gives the students the ‘opportunity to discuss the interface between science, society, and their personal lives’ (Trautman & Krasney, 1997) and thus facilitates this enhancement.

Although interest will be enhanced, the increased content mastery is also a goal. The overall findings from the theme issue of CERP (2012) are that students like the Green Chemistry context-based approach better, because they relate to it. Meaning connects then to understanding, which fosters the motivation to achieve content mastery. These emotional and cognitive pathways are the goal of this investigation. How to determine IF these pathways are met is a concern for science educators.

In “*Making Connections*”, King advises educators that “ An important challenge for many teachers in implementing a context-based program will be to find new ways for students to demonstrate their conceptual understanding and whether or not they tie this understanding to a specific context” (King, 2000). This transferability, as King calls, it can be addressed by building several units/activities that revisit the same context while adding additional content. The question then arises, what forms of assessment are good to determine if students are tying their understanding to a specific context? In the book ‘*Systems for State Science Assessments*’ Wilson and Berenthal point out that good science assessment recognizes that “ students need to understand science as a way of knowing and to develop skills necessary to both understand and appropriately apply the strategies of scientific inquiry”(Wilson & Bertenthal, 2006). This investigation utilizes a Green Chemistry context, specifically Bio-remediation, to help students

tie their understanding of chemistry to a context that they can relate to. The unique forms of assessment completed in this investigation show that the students are making a connection between the context and the content while developing those skills necessary to understand and appropriately apply strategies of scientific inquiry.

Green Chemistry can be defined as “*Chemistry that prevents pollution*”. (Magesway, 2011). According to the Environmental Protection Agency, pollution is defined as; *the presence of matter or energy whose nature, location, or quantity produces undesired environmental effects.* (<http://www.epa.gov>)

In this investigation food waste is the pollutant. Food waste is a pollutant because it takes up valuable space in a landfill, and produces methane and carbon dioxide, dangerous greenhouse gases, which are clearly undesired environmental effects.

According to the book by Anastas and Warner (Anastas and Warner, 1998) there are twelve principles of Green Chemistry to help us in determining how ‘Green’ a chemical, process, or a reaction is. (Appendix A11) These principles are for Industrial applications. For this study the ideas of the twelve industrial principles were adapted into one Green Chemistry principle with Bio-remediation as the focus:

Food Waste pollution can be prevented using Bio-remediation. Food Waste prevention can be processing a waste product i.e. Food waste, with the end result being a viable resource, compost.

Bio-remediation is defined in the book “*In situ Bioremediation, When does it work?*” (1993) as the “construction of an engineered system to supply microbe stimulating materials by accelerating the desired biodegradation reactions by encouraging the growth of more organisms as well as by optimizing the environment”. The conversion of what could be a

waste product, leftover students' meals, into a valuable organic resource of compost via microorganism facilitated chemical reactions, was the working definition of bio-remediation in this study. The Nature Mill® was this constructed engineered system used to bio-remediate students' waste.

This investigation used the Nature Mill®, and the Green Chemistry/Bio-remediation adapted principle to achieve two goals; (1) more interest in chemistry which indirectly leads to increased organic chemistry content mastery; and (2) raise social awareness of our future scientists, engineers, and citizens about their impact on their planet, and what can be done to safeguard their present and future lives.

Initially, these two goals were to be met through guided questions surrounding a big idea: the world problem of landfill growth is due in part to food waste and the misappropriation of this potentially viable resource. This set the stage for posing questions to students such as, "Why are landfills places that add more pollutants to our environment, while utilizing precious land which could instead be relegated to more useful purposes like food growth for our population at large?" "How much of our landfill is food waste?" "What is food waste, and why is can it be considered a pollutant?" "What can be done to decrease the amount of food waste?" and "Why is Bio-remediation a necessary goal in terms of waste prevention?" These questions were predicted to build an interest in the relevance of this world problem to organic chemistry, and the chemical process of decomposition and its major players. The students saw that Green Chemistry/Bio-remediation can be a relevant context for solving a real world problem using their scientific knowledge.

Therefore, this investigation explored using a Green Chemistry context, Bio-remediation via the Nature Mill® to enhance motivation, and content mastery of organic chemistry in an AP Chemistry classroom.

GOALS AND OBJECTIVES

This problem was chosen because to date there is no record of the use of the indoor food waste composting machine, Nature Mill®, to teach a Context-based Green chemistry unit with a special emphasis on some of the underlying organic chemistry that occurs in decomposition. There has been minimal instruction and even less experimental based classroom materials developed to study the problem of the huge volumes of food that are wasted everyday. These issues directly affect students' everyday life, as well as our planet.

The majority of my research experience in the summer of 2011 at MSU was spent learning about the biochemical processes at work in the conversion of food waste to compost via the Nature Mill®, and then developing some Bio-remediation/Green Chemistry context based lessons and activities. By taking daily data with Vernier® probes such as pH, carbon dioxide, and temperature. I was able to monitor pH, temperature, and the release of carbon dioxide. These data allowed me to develop a novel lab using the compost as a basis for paper chromatography and the separation of the free amino acids that the bacteria were producing from the breakdown of the protein in the food waste. The goal for this lab was to connect the organic chemistry HSCE's (High School Content Expectations, Appendix A1) to the decomposition biochemical process using a novel piece of technology, the Nature Mill® to answer an everyday problem; what should be done with our food waste?

The development of the notes, lab, and data collection were accompanied by a literature search seeking examples of what some may have already done in the classroom with an indoor composting machine, these specific organic chemistry objectives (HSCE, Appendix A1) within the context of Green Chemistry/ Bio-remediation.

Based on this work the following goals were developed to compliment the organic chemistry objectives (HSCE, Appendix A1). These goals and objectives were interwoven into the context of Bio-remediation.

By then end of this unit students should be able to:

1. Explain what a landfill is and why a landfill that has a large amount of organic waste is undesirable. Describe what an indoor food waste-composting machine does. Discuss the classroom-adapted principle of Green Chemistry, specifically bio-remediation and relate that to the Nature Mill®, and the chemical process of decomposition.
2. Experience Food waste composting by using the Nature Mill® to remediate the waste into a useable form of organic material for agriculture.
3. Explain that Compost is an end product of food waste decomposition. This is a chemical process with matter and energy transformations completed by many microorganisms at work in a functioning compost pile.
4. Explain the process of protein disassembly into free amino acids, lipids into fatty acids, carbohydrates and starches into simple sugars, as well as the overall energy and matter transformations that occur during food waste decomposition.
5. Process Goal: complete a long duration lab aimed at comparing known free amino acids to a compost sample via paper chromatography.

Students completed these goals in a two-week unit that was embedded in the Organic Chemistry unit.

DEMOGRAPHICS

Lowell is a suburban-rural community situated eighteen miles east of Grand Rapids, Michigan. The school district is in Kent County.

Lowell has an old-fashioned downtown complete with many small family owned businesses. The feel of the community is that of a hard-working small town.

According to DTMB (Michigan Department of Technology, Management & Budget). The 2010 Census Data for Lowell Area schools report the population to be 20,366.

(<http://www.michigan.gov/cgi/0,4548,7-158-54534-252541--,00.html>) Ten percent of the population is 55 years or older, 18% are 45-55, 16% are 35-45, and 10 % are 20-24,16% is 10-19, and 14% are 9 and younger. Median age is 38.4 years old. 50.3% are female and 49.7% are male. Ethnic breakdown is predominately White at 95.5%. 1.8% Asian, 2.1 other nationalities, 0.8% African American, 0.3% American Indian.

The Lowell economy does not have any large industrial influence. People who are not farmers tend to either own their own businesses or commute to an industry job nearer to the city of Grand Rapids. Education is an important factor in the economy of any small thriving town. The largest percentage of workers in Lowell, 37.4%, has a high school diploma. 21.2% have had some college education, but no degree, 14% have a college degree, 12.5% have not graduated high school, 8.1% have an associates degree, and only 6.5% have a Graduate or Professional degree.

Lowell High school has 359 (http://www.michigan.gov/cepi/0,1607,7-113-21423_30451_36965---,00.html) students eligible for free and reduced lunches in our ninth through twelfth grade high school building. That is 26.2% of our 1,370 high school students.

Lowell school district counts for free and reduced lunches are 1,227 students, this is 30% of our district wide student population. This is above the State average of 21.1% of the district wide student population, but well below the national average of 66.6%.

The district maintains eight buildings, four of which are elementary schools, one middle school, one early childhood learning center, one alternative high school, and the traditional high school.

Lowell High school is ranked by *US News and World Reports* as one of the top 250 high schools in the nation. At Lowell High there are 75 certified teachers, five counselors, one principal and two assistant principals. Lowell High School has a trimester schedule, which allows students to complete up to sixty different courses before graduation.

Twelve students out of sixteen in the class participated in this study by submitting a signed the Parent Consent and Student Assent form (Appendix A2). The course was an Advanced Placement Chemistry class that met everyday during fifth hour for 180 days. This population is a set of the top-performing students at Lowell high school. Many of the students had multiple AP courses in several different disciplines. Out of the twelve, eleven had already completed college prep Chemistry and eight had completed AP Biology. There were eight females, and four males. All students were white, including one exchange student from Slovakia, and no special education students were involved. Six seniors and six juniors participated in this study. There were no other classrooms or teachers involved in this study.

IMPLEMENTATION

At the beginning of the unit students were asked to complete the student assent Parent consent form and return them (Appendix A2). Students completed the Compost Pre-test (Appendix A3). They were told to try their best to answer the questions. The students expressed interest in the idea of food waste and about “those machines in the back of the room”. They were very curious about how the compost machine worked and why it could produce a smell.

This context based Green Chemistry/ Bio-remediation unit was embedded in the organic chemistry unit in December of the 2011-2012 school year. This unit came after the Thermodynamic unit, and before the Electromagnetic/Quantum Chemistry unit. The entire unit consisted of three weeks. The first week was spent on the High school content expectations for Organic chemistry (Appendix A1) with emphasis on the free amino acids and the combination of many amino acids into proteins. There was a content-based quiz over organic chemistry fundamentals at the end of the first week. The following week began this context-based Green Chemistry/Bio-remediation unit.

This table outlines the activities, discussion, and experiment completed in this two-week unit.

Table One: Two-week plan for Green Chemistry/Bio-Remediation Unit

Day	Main Idea	Activity/ Discussion	Goal
1	What is a Landfill? What is the Nature Mill®?	<ul style="list-style-type: none"> - Brainstorm on Board - Video Nature Mill® on History Channel (Appendix A14) 	Goal 1. Explain what a Landfill is.
2	Handout <i>Greening through the Curriculum: History of Green Chemistry, from University of Scranton.</i> (Appendix A11)	<ul style="list-style-type: none"> - Discuss and define Green Chemistry, Bio-remediation, and adapted principle. 	Goal 1. Explain what a landfill is
3	Draw diagram of Nature Mill® (Appendix A10) Why is compost beneficial?	<ul style="list-style-type: none"> - Students identify parts and function relating to decomposition process. - Discuss compost use. - Bring Lunch scraps to put in Nature Mill and Read Compost Microbiology and Soil food web article. (Appendix A8) 	Goal 2. Food waste can be composted.
4	Using the assigned previous night's article: Discuss the Chemistry of Food waste decomposition, including four main groups of organic macromolecules in food waste, and their end products. List major decomposers and focus on ones related to Protein breakdown.	<ul style="list-style-type: none"> - Put their food scraps in Nature Mill®. - Take summary notes about article and discuss the Chemistry of food waste Decomposition. 	Goal 3. Summarize Chemistry of food waste decomposition

5	Table One cont'd Hand out Lab on Compost Chromatography (Appendix A5)	<ul style="list-style-type: none"> - Students collect compost data and maintain class log of smell, appearance, and temperature on board. - Discuss and complete Pre-Lab as groups of Three 	Goal 5. Students will complete compost chromatography lab.
6	Begin Lab with Precautions noted	<ul style="list-style-type: none"> - Begin lab - Collect Compost data for board 	Goal 5.
7		<ul style="list-style-type: none"> - Lab - Collect Compost data for the board 	Goal 5
8		<ul style="list-style-type: none"> - Lab and end discussion on Post lab questions. - Collect Compost data for the board 	Goal 5
9	Complete Compost Post-Test (Appendix A3)	<ul style="list-style-type: none"> - Post – Test and turn in lab. - Collect Compost data for the board. 	End summary of everything learned
10	End board summary of connections from Goal 1 through 5	<ul style="list-style-type: none"> - Analyze Compost Data on Board, and discuss trends, connect data to five goals. 	End summary of everything learned.

ACTIVITIES SUMMARIZED

(See Table one for related goals)

Upon completion of the Pre-test on the compost the students were directed to the board to address the two guiding questions. (Table one, day one)

Goal 1- two days: Some important responses to the discussion from some students were “ A landfill is where our trash goes, and it smells bad”. No one could answer the second question.

When asked; “is all trash the same?” students said no, but many were not sure how to categorize trash.

The discussion was then directed to the board drawing of the Landfill composition. (Landfill Waste Composition Image courtesy of

<http://www.waitakere.govt.nz/abtci/ec/clnprod/images/demolitiongraph2.jpg>)

The discussion focused in on the 36% that was organic in nature. Students were informed that the organic waste was mostly due to food waste. One student said “Man, that’s ridiculous, that’s a lot of wasted food.” Many concurred. It usually takes up to six months for a compost pile to transform food waste in to useable compost. The class then discussed what composting was, and how it happened. Many students came up with words like breakdown, decomposition, bacteria, smelly, dirt, soil, use for gardens, farms use it, organic. These words were put up on the board. The question was posited again, What is the Nature Mill®? Students then watched the Nature Mill® video on Youtube from the History channel (Appendix A13), and were introduced to the Nature Mill®. Next the students received the *Introduction to Greening through the curriculum: History of Green Chemistry* (Appendix A11). On the board the classroom adapted definition of Green Chemistry and Bio-remediation was discussed.

Goal 2- Day 3: A key question was discussed in small groups; why is compost beneficial? This led to three ideas: 1. Compost reduces landfill space. 2. Compost remediates a waste product into an agricultural resource. 3. Compost is an organic fertilizer far superior to synthetic fertilizers for use in gardens and farms. Students discussed types of food waste that can and cannot be put into the compost machine and why.

Goal 3- Day 4. This goal is closely tied to some of the organic chemistry HSCE's (Appendix A1) that students learned the week before, such as the disassembly of proteins into amino acids. Students were given the web article: *Compost Microbiology and Soil food web* (Appendix A8) as the evening reading assignment for next day discussion. Students put food scraps into Nature Mill® and began taking measurements from the compost in the machine. A daily log of real time data was completed during the two-week unit. The log was kept on the board with temperature, appearance, and smell observations. Students observed the change in temperature each day and the change in appearance from discernable food pieces to amorphous soft, deep brown soil in two weeks.

Goal 4- Day 5. Students summarized the general organic molecules and their decomposed counterparts via a class discussion.

Goal 5- Day 6 through 8. Students completed the Chromatography lab (Appendix A5) and continued with compost data collection, discussing changes they were observing in the compost and postulated reasons why.

Day 9 through 10. Summary of activities completed and concepts learned. Students discussed Post lab questions in Lab groups and analyzed data. They connected the lab data taken from the compost of the Nature Mill® to the compost data on the board. Questions to guide the discussion were: What large changes did we see in the compost, what products were we testing for in the lab? Can those be easily seen in the compost pile? The Compost post-test (Appendix A3) was administered, followed by a wrap up discussion about the unit and the five goals.

RESULTS AND EVALUATION

Before the unit began students were given the Compost Pre-Test. This provided a baseline for their pre-existing knowledge about the five learning goals. The Compost test targeted their content knowledge regarding the chemical processes of food waste decomposition, the function of certain parts of the Nature Mill®, and also probed their personal opinions about some aspects of food waste disposal. The rubric for the Compost Pre/Post test (Appendix A4) assigned points to some questions for fact-based answers, and points to other questions for thoughtful, opinionated answers.

Upon completion of the unit students were given the Compost Post-test (Appendix A3) which was the same as the Pre-test. According to a one tailed T-test (<http://www.changbioscience.com/stat/ttest.html>) the p value was less than .05, where $p=0.00000183$. This indicates the null hypothesis that there is no difference between my two sets of data can be rejected.

Figures One and Two display student performance data on the Compost Pre and Post test. Figure two compares the Pre-test overall performance to the Post-test overall performance of the twelve students. Figure three more specifically compares the Pre and Post-test performance of the twelve students on each of the fourteen questions.

Pre-test scores were high with a mean of 17.4 out of 25 (69.6%). This can be attributed to the large degree of background knowledge the students already had from their previous coursework of college prep Chemistry and AP Biology. The Standard deviation is 3.01 with a

standard error of 0.77. The Post-Test Mean was 23.13 out of 25 (92.5%), with a standard deviation of 2.4 and standard error of 0.62. The fact that the Pre test scores were so high pointed also to the fact that even though many of them said or wrote comments to the effect that they did not know this information, many of them clearly had some accurate pre-existing knowledge. These data clearly support this claim.

Figure One: Miller AP Chemistry Pre and Post test Data Percentage scores as a percentage (n= 12). *For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis.

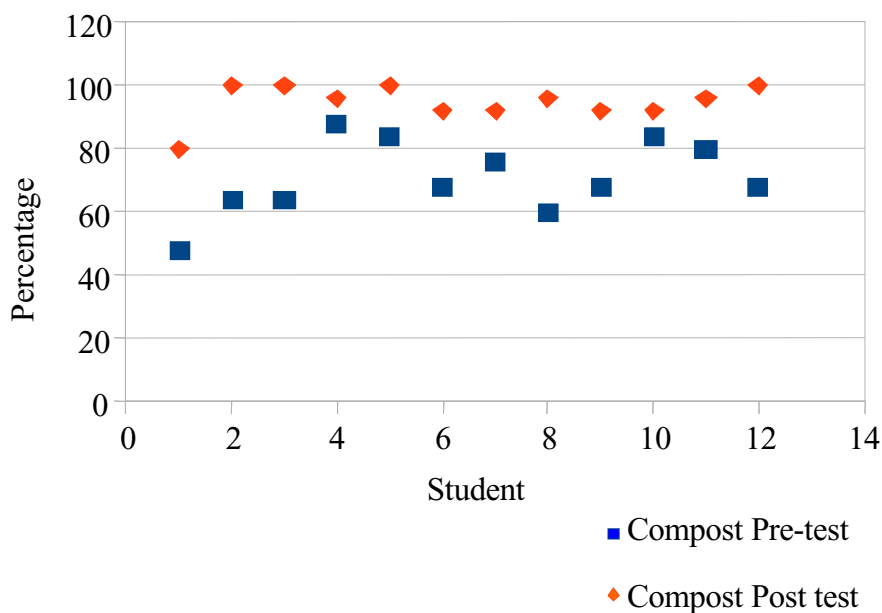
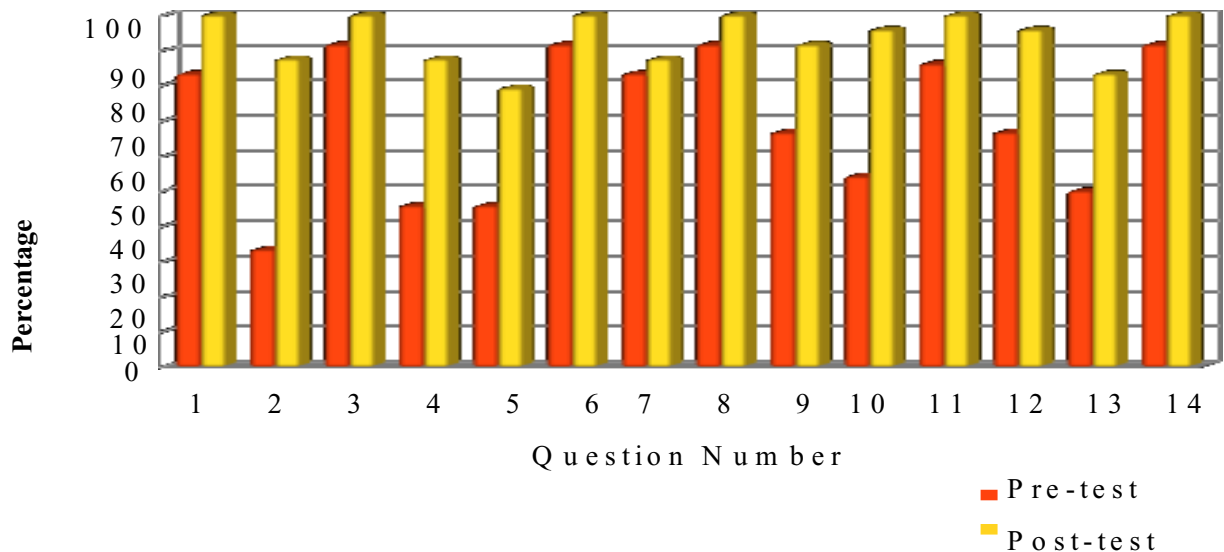


Figure Two: The percentages correct for each 14 questions on the Compost Pre- and Post-Test.



Analysis of individual Question responses from Figure two, Compost Pre/Post:

Comparison of Individual Question responses (see Figure Two).

Question one (# 1 on Compost test) was aimed at determining whether students thought that compost has living things in it. Two students on the Pre-test thought that Compost has no living things in it. These two students pointed to the fact that compost should have “dead things in it, and living things could cause disease”. This shows that they may not realize that many different types of microorganisms are involved in decomposition.

The fact that most of the students claimed the statement was false and pointed to the bacteria, fungus, and mold shows that for the most part this class was clear that compost has living things in it.

Question 2 (#2 on compost test) asked students why dirt would be added to the compost machine initially. Sixty percent of the students answered incorrectly on the Pre-test. They

thought the ‘dirt’ was added to ‘absorb’ the water or moisture from the food waste. Little connection was made to the dirt they were adding that was actually serving as the starter populations of microorganisms. This is a common misconception amongst students. They think dirt is just an abstract solid that serves as some sort of background for all other processes to occur ‘on’, but not ‘within’. Many class discussions followed regarding the fact that dirt is alive and should be seen as more of a matrix of living and non-living material. One student said with great enthusiasm, “Hey is that why they say, God made dirt and dirt don’t hurt?” Laughter ensued and many other students chimed in with great anecdotes about how ‘it’s good to play in the dirt, it exposes you to things and builds up your immunities.” It seemed as though they were not connecting the dirt ‘outside’ in their playgrounds, gardens, and likewise, with the dirt we had brought in to add the compost machines. The Post-test scores (86%) reveal that a significant portion of the students correctly connected the living micro-organisms in the dirt to the need for the initial dirt sample addition.

The next three questions were designed to probe students’ knowledge about the energy and matter transformations that were taking place in the composting material.

Question three (#3 on compost test): has a high Pre and Post test average of 90%/100%, indicating that most of the students knew that there were microbes in the food waste that release heat and water gas and carbon dioxide.

Question 4 and 5(# 4a and 4b on compost test): The Energy and Matter transformation questions were clearly the most difficult for students. Both questions showed a little more than 40% mastery on the Pre-test. On Question Four many of the students pointed to the compost changing, but they were uncertain how to categorize that change into energy or matter

transformations. Some pointed to heat but failed to connect this as thermal energy that must be coming from some biochemical processes.

On the post-test answers the students listed the transfer of the chemical energy in the food waste to the bacteria. They explained that the bacteria used this energy to drive their life processes thus further aiding in the decomposition process and releasing thermal energy as a by product.

The matter transformations in Question five seemed even more difficult for them. Most of the pre-test responses indicated that food changes to compost, but the idea that there are smaller components in food that must be broken down by many specialized processes and organisms was foreign to them.

Post test responses for this question had more specific answers (85%) detailing the breakdown of the proteins, carbohydrates, and fats, into amino acids, and the like. Students pointed to the liquid water turning into steam and the other gases, ammonia, methane, or carbon dioxide, as products in the decomposition process.

Questions 6 and 7(#5 and 6 on compost test): These were easier questions that most students got correct (Pre and Post-test averages above 80%), about the breaking of the bonds in the food waste, and their sources.

Question 8 (# 7 on compost test): Students were asked if they thought that it should be illegal to put food waste into a landfill, and to explain their answer. Although there is not much change in the performance (90-100%), the importance of this question lies in the changing opinions of the students and their more informed ideas for solutions of food waste placement on the Post-test.

On the Pre-Test there was one undecided student, seven students said 'Yes' and four students said 'no'. This showed that the majority of students already thought that food waste should not go into the landfill. Interestingly, many students who said they thought it should be illegal on the

Pre-test, pointed to possible restrictions on what food could be thrown away. In the pre-test one student said “I think that ideally putting large amounts of compostable food into a landfill should be illegal, but there has to be places for the food waste to go. Preventing every food scrap from entering the landfills would be impossible, but prevention of large scale dumping could be averted.” Clearly, many of them realized that just saying it’s illegal is not a good solution. Another student said “Yes, but only if there are a number of safe, environment-friendly, relatively cheap cost-saving (to both the individual and community) alternatives readily available. How could we decide that? Pointing fingers? Maybe there should be some incentives instead?” From her answer one can see that she was thinking deeply about the logistics behind some legislative action like this. She realized that benefits and costs occur to not only the individual but to the community at large. One student said, “No, even though this would be extremely beneficial, people should not be forced to do certain things with their food, only encouraged.”

In the Post-test responses the undecided student changed her mind to yes it should be illegal, because, “The energy can be renewed, and the compost can be used elsewhere”. One of the original ‘yes’ students changed her mind to ‘No’ stating that incentives would be more practical, “but, it should be personal choice”.

Only further questioning, and no interjection of the instructor’s opinion facilitated the dialogs that occurred between the Pre- and Post-test time frame. The debates in class, during the lab, or while collecting compost data between the students were some times heated, blossoming into statements from one “the government already regulates too much!” and another “This whole thing is related to global warming, and I don’t believe in global warming”. Yet another student chimed in with “ yeah, but just tossing all your food waste into a landfill is a waste of valuable

resources, and it's damaging our planet". The students were learning from each other that a simple act like throwing away your food in the trash could become a scientific and political statement. This question from the compost Pre/Post test was a wonderful insight into the ways our students see their natural world and how they think about the possible solutions to solve planetary problems such as landfill size reduction, food waste allocation, and use.

Question 9 (# 8 on compost test): Responses on both the pre-and post test showed that students seemed to understand that the some of the gases produced via decomposition were carbon dioxide, methane and water gas. There was a gain of 25% on the post-test scores for this question.

Question 10 (# 9 on compost test): This question put students in the role of a compost expert and presented them with the problem of wet and foul smelling compost.

There was a large variation of answers. Pre-test data (average of 50%) indicated that the students weren't sure of the cause or fix for the wetness or the smell. One student on the pre-test said, "Uh.....add a carbon source? Baking soda? Pine shavings?" This student knows that you have to add something, but it is clear she was not certain what or why.

As a class we talked in great detail about this many times while standing over the open bins, and they brought up this question. They made statements like "Gross, you need to add something". What that 'something' was became more apparent as we talked about the science behind the causes of the wetness. The Post-test answers were much more specific and detailed (95%) to include the cause of the problem and the solution. The same student on the post-test said, "The foul smell is due to the bacteria producing NH_3 , or NH_4 . The wetness is due to the breakdown of green food, which produces the water, add a carbon source like pine shavings, and some baking

soda.” The student connected the bacteria and specific by products of their biochemical processes to the problem and understands what will fix the problem and why.

Question 11(# 10 on compost test): Students were asked to explain in terms of the law of conservation of mass, why the mass of the resulting compost is less at the end of the decomposition process. Many students, above 80% on the Pre-test, pointed to the production of gases and the subsequent loss of those gases upon opening the compost machine. On the Post-test scores were near 100%.

Question 12,13 and 14(# 11a, b, and c on compost test): These questions were based on a diagram of the Nature Mill®. The objective of this set of questions was to explain the machine’s interior workings. It was not expected that students would know any of this before the unit. The goal was to enlighten students about this new invention, while developing a clear understanding of the machines inner workings and tie that to ways to accelerate organic decomposition. Furthermore, this would avoid the misconception that the Nature Mill® is some sort of black box with little connection to their learning. Students would see it as a new device with scientifically purposeful parts to accomplish the decomposition of food at a faster rate.

The Pre-test data indicated that students struggled with the function of a heated mixing bar and air pump in the compost bin. Subsequently, the students worked daily with the compost bins for two weeks. They came to realize that the heat, question 12, is needed because it speeds up the rate of reaction for the bacteria to complete the process of decomposition. The air pump in question13 adds the necessary oxygen for the aerobic bacteria. The drip tray in question 14 was an easy connection for the students, they all understood that there would be excess liquids, and there would be a need to collect it separately.

The post-test data (averages above 90%) for questions 12, 13, and 14, indicated that the students understood the purpose of these components and the science behind why the inventor put them in the machine.

Overall, the data from questions one through eleven were most beneficial in assessing students' content knowledge in the science of decomposition, and opinions about landfill reduction, food waste allocation. Questions 12 through 14 were focused on the Nature Mill® function.

Analysis of Student performance on Chromatography lab (Appendix A5): The goal of this lab was to use chromatography skills to investigate free amino acid production in a compost sample. Comparison to nine amino acid standards on the chromatogram made it possible to determine which free amino acids were produced in the compost.

This lab required three days. The lab report consisted of the completed answers to both the pre- and post- lab questions in the packet, as well as a typed report containing the purpose, list of materials, procedure, results with attached chromatogram, and conclusion section. See grading rubric in Appendix A7.

Pre-lab-Students were asked to list the main macromolecules in food waste, and the microorganisms involved.

The next two questions were more chemistry oriented. Students were given a set of paragraphs related to these questions that explained the details necessary for answering these two questions. Students were to determine if protein decomposition was a chemical process, then draw the reactants and products including following the flow of energy, and one of the organisms involved.

The last pre-lab question asked why chromatography could be used to analyze the compost and free amino acid solutions. Since this lab was done at the end of this unit and the class had time to discuss the pre-lab answers, the answers to the pre-lab were excellent and showed a level of mastery before beginning this lab.

Completion of Lab-The lab involved the use of the compost four days after students had begun putting their food waste from their lunches into the Nature Mill®. Skills utilized were as follows: centrifuging, decanting of a supernatant, chromatogram paper preparation, spot sample deposits, chromatography chamber development and capillary action, Ninhydrin fixing, and Rf (Retention factor) value determination and analysis.

The students expressed their enjoyment this lab. One student said, “Finally! A lab that’s not cookie cutter style”, and “working with dangerous chemicals make me feel like a big boy”.

This lab supplemented the context-based approach. Students connected the compost solution used during the chromatography lab to the compost inside the machine. They were able to show that the proteins from the food waste were being broken down into amino acids.

Most of the Pre and Post-lab questions were built to mirror and supplement the Compost Pre and Post-Test. The excellent performance on the Lab and on the report mean score of 195.5 out of 200 points indicated that students mastered the content as well as the advanced skills they so much enjoyed doing.

Post-lab- Many of the students’ chromatograms had two different types of free amino acids in their compost samples, while there were nine different known free amino acid standards. They were able to match up the Rf value of their compost sample to the possible known free amino acids standards. By ‘eye’ one could often see the matches. The fact that some of the students

wanted to keep their chromatogram is a clear indicator they were proud of their experimental data.

Post-Lab question number seven asked students if there were amino acids present in their sample, and if so why. All twelve participants were able to answer yes, and support the claim with their chromatograms. The explanation regarding why there would be amino acids in the compost solution was a 100% clear answer from all twelve participants: the compost contained proteins in the food waste and the decomposition process via the microorganisms had digested the proteins into amino acids.

In the lab report conclusion, eight of the twelve students mentioned how much more involved the decomposition process was than they had originally thought. One student said, “Composting is a more complex process than I initially anticipated. It is also one of the most valuable processes to biological organisms on this planet, and absolutely essential to life as we know it.” All twelve stated that their compost solution had free amino acids in them, and they connected this back to the protein in the food waste. Discussions also followed about how we could use the compost to help the greenhouse plants grow better and thus accomplish bio-remediation on a small scale in our school.

DISCUSSION AND CONCLUSION

The use of Nature Mill® and this context based teaching method increased the interest, laboratory skills, and content mastery of the common organic chemistry principles (HSCE, Appendix A1) required in AP Chemistry. The conversations, both oral and written, depict a level of interest rarely seen in my experience in the organic chemistry unit, as well as measurable gains in content mastery on the post-test, and reflected by the Chromatography lab scores. The context of Green Chemistry/Bio-remediation presented an everyday problem with testable ideas and materials. The students were moving every day, discussing in structured large and small groups, adding food waste and collecting data for the board from the Nature Mill®, and completing the compost chromatography lab. These experiences enriched the curriculum and taught them to look at chemical and biological processes as a united meshwork.

Through many discussions and the exploration of these data reported here, it is clear the students see food waste as a renewable resource. They also seemed to have a deeper understanding and appreciation for the complex biochemical process of food waste decomposition.

Upon completion of day one it was clear that the students were very interested. The students walked back to the Nature Mill® excitedly, but many noted the smell and were reluctant to get too close to the Nature Mill®. This exemplifies why our society discredits composting food waste. To overcome this students were asked if they have ever visited a landfill. No one had. One of them made the argument in good humor that this was why. Next year, plans to visit one will be added to the unit if time allows.

Judging by the many conversations & questions during class, and in the laboratory, it was clear that the article that was handed out; *Compost Microbiology and the Soil Food Web* (Appendix

A8) was not enough to support learning of the complex details of protein decomposition, the relevant organisms, chemicals, and energy. The learning of microbial decomposition alone is difficult. Compound that with trying to follow the energy and the organisms through the steps and one can get a little overwhelmed. Future notes will be developed with fill in the blank spots, and diagrams with flow charts depicting the process, the organism and the chemicals involved in the stages of decomposition. This will be helpful because the notes will integrate the difficult vocabulary with visual organizers and pictures that foster the connections between the organisms, and the matter/ energy flow. This will build a clear framework for the connections between the context, and all the detailed content, while developing smooth transitions between the decomposition process, the energy flow, and the matter transfers/transformations.

Follow up questions about the article will be included on the Pre- and Post-test as well. A clearer connection needs to be made between the Green Chemistry/Bio-remediation ideas on the Compost Pre and Post-test, and the Chromatography lab. To address this concern of making clear connections between the Green Chemistry context of Bio-remediation, the use of the Nature Mill®, and the Compost test/Chromatography lab, the test and lab will be reformatted to include content based questions such as

- What is Green Chemistry?
- What is Bio-remediation?
- How does the Nature Mill® apply these principles?
- What organic chemistry is going on in the Nature Mill®?

Student comments were encouraging regarding their interest in the biochemical process of decomposition that goes on in the ‘dirt’. At the end of the unit a student said, “I will never look

at food waste, or dirt the same!” The goal of using a Green Chemistry/Bio-remediation context to teach organic chemistry seems to not only have had a positive effect on their content mastery, noted by their performance on the Compost Post-test, but also has changed the way they see their food waste.

From an educator’s perspective, the benefits of the unit are clear. The passionate discussions about our planet and our role as scientists and citizens developed students’ awareness by introducing them to a real world problem they rarely think about, or consider may be easily solved. The interwoven science and politics of landfill growth due to food waste misallocation became evident as the students delved deeper into the question of the legality of food waste in a landfill. The nature of food waste and why not all food waste should be put in a landfill are not just a problem of space misuse. This allowed for discussions and notes about the chemical processes of food waste decomposition.

The amount of time for the pre- and post-lab discussions was too short. It felt as if the students needed more explanation, and there seemed to be a general underlying current of slight confusion at times, judging by the questions students were asking. We had enough time for the lab, and the three days of the lab were full of great discussions about capillary action in paper chromatography, with questions like what are adhesion, cohesion, and an eluent? The students enjoyed the complexity of the lab. At times it seemed as though students were confused about exactly how to do certain things, such as where exactly to draw the line through the migrated spot for Rf calculations, and many other small details involved in the lab.

Overall the lab, discussions, and notes were effective at gaining students interest, and developing content mastery as measured by the post-test scores, and lab performance.

The students were well engaged and very interested in the discussions, the labs, and the constant data collection everyday from their food waste in the Nature Mill®. The lectures and note taking were well received with many comments about the how cool it was to ‘tie everything together’ about our environment and chemistry. Some students expressed dismay at the length of the lab. One of the females communicated that the use of Ninhydrin in the fume hood, ‘made her nervous’. For many of the students this was the first time wearing full lab gear, an apron, goggles, and a facial mask.

The lab from a teacher’s perspective was both enjoyable and high maintenance. I will do the lab again but there are several things I will change.

- Give students a list of the nine free amino acids categorized by polarity, as well as an explanation about the predicted movement of the individual amino acids through the #4 Whatman Chromatography paper, and why. This will aid in the clarification of which nine standards they are using, what they look like, and how their structure plays a role in the separation technique.

-Do the lab with different timed samples from the compost. (Day 2, day 4, day 6, day 8) The teacher can prepare the compost solutions in advance, store them in the freezer and then complete the lab all at once in three days with four different groups doing each different sample. This will alleviate the concern that there may be no free amino acids present due to the possible re-incorporation of the free amino acids into the bacteria cell walls. It is difficult to predict when this will happen when you are adding food waste everyday to the already existent pile in the

compost machine. The four different chromatograms may provide interesting comparative analysis, and explanations for the differences in the compost solution spot presence, or migration behavior.

- Make sure students know to roll up the chromatography paper so the sample spots are on the outside of the cylinder, so they can watch the movement and easily see when to stop the reaction by pulling out the paper. This prevents the spot moving off the top of the paper.
- Have a camera available for pictures of compost the day students take samples, and pictures of the chromatogram before students fix it with the Ninhydrin, and then the following day upon drying. The students can then share the picture and print ones for their report, while their partner hands in the original. The pictures of the compost the day of sampling can then be compared to later days of processing to show the degree of decomposition.
- Clearly define where on the eluted spot to measure from for Rf values, and state that Rf can be written as a decimal number with two sig-figs. This should alleviate any confusion about where on the migrated spot to mark so that Rf calculation is more uniform throughout class data. The sig-fig requirement will also render more trustworthy Rf values.
- Locate in advance a source for the #4 Whatman chromatography paper. It is expensive to buy from Whatman because they only sell it in such large volumes. Locating a local university or community college and asking them for some sheets should solve this problem.

I enjoyed teaching this unit, and will do it again. Adding an outdoor compost pile for comparison of data will be an excellent addition to the board data we were taking daily.

The notes for the unit need to be redone and organized. More pictures with labeling will aid in the ability to follow the chemical pathways, along with the microorganisms involved. A couple of short quizzes in clicker or web format will aid for more formative assessment and help in tracking student's progress.

I would like to include several required journal responses to important questions that will require more student research:

- 1a) Which landfill services Lowell High schools trash? Aside from space what are some of the other drawbacks to putting food waste in the landfill? Call your landfill and ask them.
- 1b) Find a city or town that has a food waste or yard waste collection program. How does it work? What are some of the problems? Do they make use of the food/yard waste? If so, how?
- 2) How much of our daily trash is food waste at Lowell High school? Of that, how much could be composted?
- 3) What does it cost our district monthly for trash removal?
- 4) What are some potential roadblocks and costs to packaging and selling the compost from our school?
- 5) What is 'bad' compost? What would 'bad' compost look and smell like? What might make it dangerous?
- 6) What laws exist already to protect gardeners and farmers from 'bad' compost?
- 7) If you think it should be illegal to put certain food into the trash headed for a landfill what solutions do you have for the average consumer who cannot purchase their own compost machine?
- 8) Would you be willing to build an outdoor compost pile at your house? Explain.

The work in the future will also focus on writing a grant to the Sustainable Agriculture Research and Education (S.A.R.E.) program, or Exxon Mobil for money to purchase many compost machines or build one or two large one for our cafeteria collaboratively with our engineering and industrial arts students. These agencies have an expressed interest in science education, with an emphasis on 'Green' curriculum development.

The need for a student to manage the proposed bins after school may become a need if the goal is zero food wastes thrown into the trash. The students could then package and sell, or donate the compost to local gardeners and farmers. This would require some intensive research and training on the teacher(s) part, as well as discussions with school administrators/board of education.

The more involved the students are with decisions at every level the better. The students can design and build the compost machines, champion the use of the composters, manage them, develop safety protocols for compost sales, determine the type of packaging for the compost and market the sales.

It is my hope with the aid, vision, and passion of my students we can do our part to stop adding to the volumes of food waste that go into our landfill. It would be great to eventually have our students present to our Board of Education using data, charts, and graphs produced from their data quantitatively showing the reduction in volume of trash, and subsequent reduction in trash removal cost, as well as any profits made by compost sales.

Continuing in the future, students will hopefully walk away with the scientifically sound vision that the natural world is their laboratory, and the planetary problems are their muse.

APPENDICES

APPENDIX A1

MICHIGAN HIGH SCHOOL SCIENCE CONTENT EXPECTATIONS FOR THIS UNIT

(HSCE):

C1.1D Identify patterns in data and relate them to theoretical models.

C1.1E Describe a reason for a given conclusion using evidence from an investigation.

C1.1f Predict what would happen if the variables, methods, or timing of an investigation were changed.

C1.1h Design and conduct a systematic scientific investigation that tests a hypothesis. Draw conclusions from data presented in charts or tables.

C4.4b Identify if a molecule is polar or non-polar given a structural formula for the compound.

C5.2A Balance simple chemical equations applying the Conservation of matter.

C5.8C Recognize that proteins, starches, and other large biological molecules are polymers.

C2.1b Describe energy changes associated with chemical reactions in terms of bonds broken and formed (including intermolecular forces).

C2.1a Explain the changes in potential energy (due to electrostatic interactions) as a chemical bond forms and use this to explain why bond breaking always requires energy.

C1.2f Critique solutions to problems, given criteria and scientific constraints.

C3.2a Describe the energy changes in the combustion of sugar (or some other food component) in terms of bond breaking and bond making.

APPENDIX A2

PARENTAL CONSENT/STUDENT ASSENT LETTER developed by Tricia Miller

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, **Using Compost to teach Chemistry in an everyday context**, that I will conduct as part of AP Chemistry and Chemistry 9 this year. My name is Tricia Miller. I am your science teacher this year and 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 researchers any questions you may have.

What is the purpose of this research? I have been working on effective ways to combine teaching Chemistry concepts like Chromatography, and Acids/Bases, using Food waste Composting, 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 teachers' understandings about the best way to teach about science topics. 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).

What will students do? You will participate in the instructional unit about the Chemistry of Compost, and the Analytical Chemistry methods that can be used to learn about the nature of the compost ecosystem. You will complete the usual assignments, laboratory experiments and

activities, computer simulations, class demonstrations, and pretests/posttests just as you do for any other unit of instruction. There are no unique research activities – 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 2011 until August 2012. 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. This project will continue from September 2011 until August 2012.

What are the potential 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.

What are the potential risks? There are no foreseeable risks associated with completing course assignments, laboratory experiments and activities, computer simulations, class demonstrations, and pretests/posttest. In fact, completing course work should be very beneficial to students. Another person will 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.

How will privacy and confidentiality be protected? 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 myself, 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 in Dr. Heidemann's locked office at MSU (after the study) for at least three years after the completion of the study.

What are your rights to participate, say no, or withdraw? 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.

Who can you contact with questions and concerns? If you have concerns or questions about this study, such as scientific issues, how to do any part of it, or to report an injury, please contact the researcher [Tricia Miller at tmiller@lowellschools.com](mailto:tmiller@lowellschools.com), (616)-987-2916 at [Lowell High school](#) and /or Dr. Merle Heidemann: 118 North Kedzie Lab, Michigan State University, East Lansing, MI 48824; heidema2@msu.edu; 517-432-2152 x 107].

If you have questions or concerns 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? If you agree to participate in this study, please complete the attached form. Both the student and parent/guardian must sign the form. Return the form to [designated person] by [date].

Name of science course:

Teacher:

School:

Parents/guardians should complete this following consent information:

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

Please check all that apply:

Data:

_____ I give Tricia Miller 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.

Photography, audiotaping, or videotaping:

_____ I give Tricia Miller permission to use photos, audiotapes, or videotapes of my child in the class room doing work related to this thesis project. I understand that my child will not be identified.

_____ I do not wish to have my child's images used at any time during this thesis project.

Signatures:

(Parent/Guardian Signature)

(Date)

I voluntarily agree to participate in this thesis project.

(Student Signature)

(Date)

*****Important*****

Return this form to [where will be deposited](#).

APPENDIX A3

COMPOST PRE and POST-TEST developed by Tricia Miller

Compost Chemistry Pre-Post Test

1. True or false: Compost has no living things in it. Explain your answer

2. You are a scientist and you have invented an indoor food waste-composting machine.

You have to add some soil first before you add any food waste. Why would you do this?

3. When you open the lid of the compost machine you notice matter steam (gases) and heat that were not initially present. Why are these coming out of the compost?
- a. The chemicals in the food are breaking down all on their own producing steam and heat.
 - b. There are some processes that microbes (bacteria and fungus) in the food waste are doing that produce heat and steam (gases)
 - c. The machine is adding heat and water only to the compost.
 - d. The photosynthesis done by the plants in the machine is producing the heat and steam

4. A. What energy transfers/ transformations are taking place in a composting material?

B. What matter transformations are taking place in a composting material?

5. There are proteins, carbohydrates and fats in the food composter, where did they come from?

6. Proteins, fats, and carbohydrates are large molecules with many bonds between their atoms. Why does this make this an important source of chemical energy?

7. Should it be illegal to put food waste in a landfill? Explain

8. Which gases are being **produced** by the aerobic (well-oxygenated) compost process?

- a. Oxygen
- b. Carbon dioxide
- c. Methane
- d. Steam (water vapor)
- e. a. b. and d.
- f. b. and d.
- g. b. c. and d.

9. You are a compost expert hired by a school to manage an indoor food waste composting machine. You arrive at the school and open the lid on the machine and notice the compost looks very wet and has a foul smell coming from it. What has happened to the compost, how would you fix it, and why?

10. The mass of the compost at the beginning of the process weighs more than the mass at the end. Explain what has happened in terms of the law of conservation of mass.

11. Refer to the Figure 3 in appendix (A10) to answer the following question(s)

Please explain why the inventor of the Nature Mill; Russ Cohn added the following features?

a. An air pump and mixing bar?

b. A heater?

c. A drip tray?

APPENDIX A4

RUBRIC FOR COMPOST PRE AND POSTTEST

Developed by Tricia Miller

Table two:

Question	Correct answer	Incorrect answer	Point value
1.	False. Compost has microorganisms in it.	-True -No attempt	2 points
2.	Soil provides base microorganism population for starting compost	-soil is used to absorb moisture -No attempt	2 points
3.	b. There are some processes that microbes (bacteria and fungus) in the food waste are doing that produce heat and steam (gases)	a. The chemicals in the food are breaking down all on their own producing heat and steam. c. The machine is adding heat and water only to the compost	1 point
4a.	Chemical energy from the food is being transformed into thermal energy, and mechanical energy for the bacteria to do work.	-food to heat -no attempt	2 points
4b.	The macromolecules like proteins, lipids, and carbohydrates are broken down into amino acids, fatty acids, simple sugars, and gases like steam, Nitrates, methane, and carbon dioxide.	-Food to compost -No attempt	2 points
5.	The food waste	-Any other source -No attempt	2 points
6.	There is stored potential	- No mention of	2 points

Table Two cont'd	energy in chemical bonds. When they are broken that energy can be transferred or used for other processes.	stored energy in bonds - No attempt	
7.	Opinion, Yes or No, and reason	No attempt	2 points
8.	g. b, c, and d. CO ₂ , Methane, Steam	a. Oxygen b. Carbon dioxide c. Methane d. Steam (water vapor) e. A, b, and d f. B. and d.	One point
9.	The compost has become wet due to too many leafy greens. It smells due to ammonia and carbonic acid production due to more anaerobic processes. Fix it with Baking soda and pine shavings.	- Any other reason - No attempt	2 points
10.	There is no mass lost due to law of conservation of mass. The decomposition processes produce many gases that can escape and decrease mass of solid compost.	- any explanation related to losing the mass due to some undefined reason. - No attempt	2 points
11a.	Air pump- to add oxygen for microorganisms. Mixing bar to evenly aerate the food waste.	-any other reason -no attempt	2 points
11b.	Heater to increase rates of reaction for the enzymes the microorganisms' uses. Speeds up compost production	- keep dirt warm - no attempt	One point
11c.	To catch liquid waste from leafy green food waste.	-any other reason -no attempt	1 point

APPENDIX A5

CHROMATOGRAPHY LAB STUDENT VERSION developed by Tricia Miller

Using Chromatography to determine free Amino Acids in Compost

Pre- Lab: Introduction to Chromatography using Food Waste Compost Chemistry

The compost we are working with is made from food waste. In the foods we eat there are four main large macromolecules.

- 1.) Working with your partner please list these macromolecules-

_____ Teacher initial

2. Compost is the breaking down of food through a process known as decomposition. The process of decomposition is a complex set of many reactions and processes that happen in succession aided by many organisms. Please list at least three microscopic organisms that you think are involved in the decomposition process.

_____Teacher initial and group discussion

These microorganisms are doing some Chemistry that is very important to our lives. They breakdown these large macromolecules which liberates an immense amount of thermal energy, as well as producing simpler polar/non-polar molecules with stores of chemical energy that the same micro-organisms themselves can eat and use to build their biomass.

One of these processes is the breakdown of the protein in our food. A protein is large molecule (polymer) made by a string of amino acids bonded together. It is these bonds that contain chemical energy and as these bonds are broken bacteria use some of this energy for their life processes. The protein is broken down into groups of free amino acids. These amino acids are food to the microorganism and they re-uptake these amino acids into their structures to build new proteins. This is a constant large-scale cycle happening all the time in the compost. At any given time the concentration of amino acids in the compost can fluctuate widely. Our goal in this lab is to extract a sample of compost in solution, and analyze and compare using chromatography with eight standard amino acids to see if we can determine if there are any free amino acids in our sample.

3. Is the decomposition of a protein a chemical process? If you think it is, please draw the reactants and the products including the energy (is this process Endo- or exothermic?) and the one of the groups of living things involved in the process.

_____Teacher initial and discussion

4. Why do you think we can use chromatography to analyze our compost and our eight amino acid solution samples? Relate this to your knowledge of forces of attraction and polarity.

_____ Teacher initials

AP Chemistry: Separation of Amino Acids in Compost by Paper Chromatography

This part of the lab has been adapted from:

www.macalaster.edu.com

I. Discussion:

Chromatography is a common technique for separating chemical substances. The prefix “chroma,” which means, “color,” comes from the fact that some of the earliest applications of chromatography were to separate components of the green pigment, chlorophyll. You may have already used this method to separate the colored components in ink.

In this experiment you will use chromatography to separate and identify amino acids, the building blocks of proteins, will be investigating whether our compost has any amino acids in it, and concluding where they may have come from and what process in the compost is responsible for their presence. The proteins of all living things are composed of 20 different amino acids, some of which are described below.

Chromatography is partially characterized by the medium on which the separation occurs. This medium is commonly identified as the “stationary phase”. Stationary phases that are typically used include paper (as in this experiment), thin plates coated with silica gel or alumina, or columns packed with the same substances. The “mobile phase” is the medium that accompanies the analyzed substance as it moves through the stationary phase. Both liquids and gases can be used as mobile phases depending on the type of separation desired. To refer to gas or liquid chromatography, chemists often use the abbreviations GC or LC, respectively. These abbreviations explicitly identify the phase of matter of the mobile phase. The term “paper chromatography” used in this experiment’s title identifies the composition of the stationary phase.

The compositions of the stationary and mobile phases define a specific chromatographic method. Indeed, many different combinations are possible. However, all of the methods are based on the rate at which the analyzed substances migrate while in simultaneous contact with the stationary and mobile phases. The relative affinity of a substance for each phase depends on properties such as molecular weight, structure and shape of the molecule, and the polarity of the molecule. The relationship between molecular shape and polarity will have been discussed already.

In this experiment, very small volumes of solutions containing individual amino acids, compost, and mixtures of amino acids will be applied (this process is sometimes called “spotting”) at the bottom of a rectangular piece of chromatography paper. For ready comparison of each trial, it is vital that each solution be applied on the same starting line. After the solutions have been applied, the paper will be rolled into a cylinder stapled at each end of the cylinder, and placed in a 1000 ml beaker that contains a 55 ml of the solvent (the liquid mobile phase). For this separation, a solution containing butanol, water, glacial acetic acid and Ninhydrin is the optimum mobile phase. As soon as the paper is placed in the mobile phase, the solution (sometimes called the eluting solvent) will begin to rise up the paper. This phenomenon is called capillary action, the spontaneous rising of a fluid up a surface due to adhesive and cohesive forces.

As the mobile phase rises on the paper it will eventually encounter the “spots” of individual amino acids, the compost, and the amino acid mixture. The fate of each spot in the mixture now depends on the affinity of each substance for the mobile and stationary phases. If an amino acid has a higher affinity for the mobile phase than the stationary phase, it will tend to travel with the solvent front and be relatively unimpeded by the chromatography paper. In contrast, if the spot

has a higher affinity for the paper than the solvent, it will tend to “stick” to the paper and travel more slowly than the solvent front. It is these differences in the amino acid affinities that lead to their separation on the paper. The affinities of these amino acids for the mobile phase can be correlated to the polarity and solubility of the different amino acids in the solvent (i.e., an amino acid that is highly soluble in the eluting solvent will have a higher affinity for the mobile phase than an amino acid that is less soluble in the solvent.).

When the solvent front comes near the top of the filter paper, the paper is removed from the beaker and allowed to dry. At this point, the various amino acids are invisible. The acids can be visualized by spraying the paper with a compound called Ninhydrin. Ninhydrin reacts with amino acids to form a blue-violet compound. Therefore, the sprayed filter paper should show a number of spots, each one corresponding to an amino acid, or the combination of amino acids. The further the spot from the starting line the higher the affinity of the amino acid for the mobile phase and the further its migration.

The relative extent to which solute molecules move in a chromatography experiment is indicated by R_f values. The R_f value for a component is defined as the ratio of the distance moved by the sample spot ($D_{m \text{ spot}}$) divided by the distance moved by the solvent ($D_{m \text{ total}}$).

Measurements are made from the line on which the original samples were applied to the center of the migrated spot. In the figure, $D_{m \text{ spot}}$ is the distance traveled by one of your sample spots
 $D_{m \text{ total}}$ is the distance traveled by the eluting solution (the solvent).

Note that R_f values can range from 0 to 1. Paper chromatography is most effective for the identification of unknown substances when known samples are run on the same paper chromatograph with unknowns.

To best understand why different amino acids have unique R_f values, it is important to understand the structural features of these molecules. As the name suggests, each amino acid contains an amino group, $-NH_2$, and a carboxylic acid group, $-COOH$.

The 20 different amino acids that make up our proteins, and those of most other living things, differ in the identity of the side chain R. In glycine, the simplest amino acid, R is a hydrogen atom. Eight amino acids have R groups that consist of carbon atoms with attached hydrogen atoms. An example is phenylalanine, which contains a benzene ring with R equal to $-CH_2(C_6H_5)$. These non-polar hydrocarbon side chains are hydrophobic or “water-hating.”

Hence, they tend to lower the water solubility of the corresponding amino acids. Six amino acids have polar but neutral R groups that tend to promote water solubility. For example, for serine R is $-CH_2OH$. In two amino acids, glutamic acid and aspartic acid, the side chains carry carboxylic acid groups. For example, in glutamic acid, R is $-CH_2CH_2COOH$. Finally, three amino acids have basic R groups. One of these is lysine, for which R is $-CH_2CH_2CH_2CH_2NH_2$. Both acidic and basic R groups tend to promote water solubility.

In fact, the water solubility of all amino acids varies with the acidity of the solution, i.e. the H^+ ion concentration that is commonly communicated via pH values. This is because all amino

acids, even those with neutral side chains, contain an acidic $-\text{COOH}$ group and a basic $-\text{NH}_2$ group. The most prevalent ionic form of an amino acid in solution therefore depends on the pH of the solution.

In solutions of low pH (high H^+ concentration) (your solvent is a pH = 4), the amino and acid groups are both protonated and this contributes a net plus charge. Near the neutral pH of 7, an H^+ has dissociated from the carboxylic acid group and the positive and negative charges balance each other. In solutions of still higher pH (low H^+ concentration), the amino group is in the $-\text{NH}_2$ form and the net charge is negative because of the $-\text{COO}^-$. This means that the rate of migration of an amino acid will depend on the pH of the mobile phase, and that the details of this dependence will vary from amino acid to amino acid. The presence of an acidic or basic R group further complicates this pH dependence.

The acidic group (COOH) of one amino acid can react with the basic group (NH_3) of another to form what is called a peptide bond, with the elimination of a water molecule. This process can be repeated to form polypeptides or proteins. Many proteins contain well above 100 amino acids.

Please read this lab completely and make a list of materials needed for this lab. You will need your teacher's initials under this list in your lab book before you may begin this lab (10pts)

II. Experimental Procedure you must wear Latex Gloves and Goggles for this entire lab!

-Before you do anything else get the 1000 ml beaker and add 55 ml of the Chromatography solvent to the bottom, cover the beaker with a watch-glass or saran wrap. This is to saturate air in the chamber with solvent. (Let sit for 10 minutes at least)

Obtain a sheet of #4 Chromatography paper cutting out a 13cm by 26 cm rectangle. Draw a faint pencil line 2 cm from one of the long edges and parallel to that edge (refer to figure 1 again if needed). This will be the bottom of the chromatogram. Mark off ten equally spaced (about 2-cm apart) points along this line. Your sample spots will be applied to these spots. The laboratory contains solutions of eight identified amino acids, one solution of compost, and a sample of a mixture of amino acids that will contain two or more of the known amino acids.

The samples can be applied to the paper by using a narrow capillary tube. The procedure is pretty simple, but it is a good idea to practice making sample spots on a separate sheet of filter paper before you start on your chromatographic paper. Dip the open end of a clean capillary into the solution to draw up a small volume of the solution into the tube. Lightly and briefly touch the tube to the paper and allow the sample to transfer. The spot should be about 2-3 mm in diameter. Once you have mastered the technique, place one spot of each of the eight known amino acids, the compost solution, and the mixture on the separate points that you previously marked on the filter paper. Be careful not to contaminate either the solutions or the spots. Label each spot (with pencil and below the starting line) to indicate its identity. Finally, measure 10 cm up from the

pencil line and draw a light line as well. This is your solvent migration maximum you will allow it to move. It's a good idea to avoid getting fingerprints on the chromatographic paper. (This is why you use gloves; the Ninhydrin will react with the oils in your print and give you false data!) When you have finished spotting your paper allow it to dry by hanging from a ring stand with a paper clip unfolded into the shape of an S. Meanwhile, in the hood, pour about 55 ml of the eluting solution (n-Butanol, Acetic acid, Ninhydrin) into a clean, dry 1000 ml beaker and cover the beaker with a watch glass or plastic wrap, allow to sit for 10 minutes to allow the air to become saturated with the solvent.

When the sample spots have dried, roll the paper into a cylinder, with the short sides almost touching (NOT OVERLAPPING). Use a staple along the top and bottom of the paper to hold the cylinder together. Evenly lower the paper cylinder, sample side down, into the beaker. The solvent will wet the paper, but the sample spots should not be immersed. In addition, the paper should not touch the walls of the beaker.

At this point, cover the beaker with a watch glass or plastic wrap and place the beaker in the hood. When the solvent front gets maximum line that you have already drawn on the top (about 1 hr), remove the paper, use a pencil to mark the solvent front at several points IMMEDIATELY! Unroll the cylinder, and let the chromatography paper dry in the hood. When the paper is dry, spray it with Ninhydrin reagent in the spray bottle.

Allow the paper to dry overnight by hanging from the same S shaped paper clip on your ring stand.

When the chromatographic paper has fully dried, outline the spots, mark the centers of each of the spots, and note their colors. (Not all amino acids give the same color with ninhydrin). Measure and record the distances the solvent and each of the amino acids traveled from the origin. Use these distances to calculate R_f values for each sample.

Comparison of the spots should enable you to identify the amino acid(s) present in your unknown mixture.

Completion of lab, list of materials and typed Report (200 points)

Your report should include the following experimental data and conclusions:

1. Your marked chromatograph with all measurements recorded. (20 pts)
2. **A Data Table** with R_f calculations as a whole number ratio, and results (color and shape for each 10 spots, the identification of the amino acid(s) in your group's mixture, and compost if you can identify any of them. (20pts)
3. The pre-lab section (10 pts)
4. One of formulas for the 8 amino acids you chromatographed with the area(s) circled that are interacting with your solvent.(10pts)
5. Explanations for the observed differences in R_f values for these eight amino acids. (10 pts)
6. Why are you advised to mark the paper with pencil and warned not to get fingerprints on it?(5pts)
7. Were there any amino acids in your compost sample? Why would there be any amino acids in the compost solution? If there were not please explain where they may have gone.(Hint timing of sample extraction and what the bacteria use these amino acids for)(20 pts)
8. Where would these free amino acids have come from in the compost? (10 pts)
9. Please discuss if you would change any of your answers to the pre-lab questions and why.(20 pts)

10. Do you still have any questions about the Decomposition of Proteins in the compost?

(20pts)

APPENDIX A6

Teacher Notes for Chromatography Lab: This lab addresses Goal Five in Thesis

Teacher version: This lab is meant to replace the traditional chromatography lab that is usually done in AP Chemistry. This lab was developed to address the following HSCE Standards:

C1.1D Identify patterns in data and relate them to theoretical models.

C1.1E Describe a reason for a given conclusion using evidence from an investigation.

C1.1h Design and conduct a systematic scientific investigation that tests a hypothesis. Draw conclusions from data presented in charts or tables.

C4.4b Identify if a molecule is polar or non-polar given a structural Formula for the compound.

C5.8C Recognize that proteins, starches, and other large biological molecules are polymers.

C2.1b Describe energy changes associated with chemical reactions in terms of bonds broken and formed (including intermolecular forces).

This is an inquiry-based lab designed to integrate Green Chemistry into the classical AP Chemistry classroom. I have a set of indoor food waste composting machines called the Nature

Mill. This machine is novel in its approach because it accelerates the rate of decomposition from 6 months to two weeks due to the heated, air fluctuation controlled reactor chamber. The students can throw away most of their lunch food waste and utilize it to learn about Chemistry while producing a useful resource for growing of plants that could be donated to your greenhouse, life science classes, or used for soil chemistry lessons.

Pre-Lab preparation:

This lab is somewhat intensive on the teacher end.

1. List of Materials:

- Eight standard amino acids powder form: Leucine, Cysteine, Glycine, Lysine, Proline, Phenylalanine, Aspartic acid, Histadine
- Table top centrifuge model 5415 C Eppendorf
- Centrifuge Posi-Click tubes : C-2170
- 10-20 Nine inch Pastuer pipette
- Rulers
- Scissors, Pencils
- Whatman #4 Chromatography paper
- 1-2 gallons Distilled water
- Fume Hood
- Sec-Butyl Alcohol 1L; Merck
- Galcial Acetic acid

- Ninhydrin Sigma grade 100g bottle (Caution! Toxic and Photosensitive) 251658240 
- Digital scale
- 6-8 1000 ml beakers for Chromo chambers
- Large watchglass (6-8) or Saran wrap to cover 1000mL beaker for chromatography chamber
- Small vials with lids
- Paper clips
- pH paper just for you to test your solvent (pH=4)
- Spray bottle for Ninhydrin spray
- Latex gloves
- 6-8 100 ml glass graduated cylinders
- Blender for compost solution
- Facial mask for nose and mouth for teacher
- Zip-Lock Freezer bags
- Goggles
- 40 grams FOOD WASTE Compost sample (from 3 days or LESS if indoor machine for best sampling of possible free amino acids), if using outdoor, 2 weeks or less sample.
Can be a wet sample.

IN FUME HOOD-

Note: You must prepare these solutions the day of the lab. The Ninhydrin is very sensitive and may photodegrade to the point where your solvent and spray do NOT work.

Ninhydrin Spray:

Using gloves, mask, and goggles. Measure out 2 grams of Ninhydrin, and add distilled water up to volume of 100 ml to create a 2% Ninhydrin solution.

Ninhydrin Solution:

20 ml of sec-Butanol, 5 ml of glacial acetic acid, .5g of Ninhydrin. Mix this with 45% Ethanol to bring up to 500 ml.

Chromatography solvent: Make new solvent every other day (add fresh Ninhydrin daily)

1. Solution A:

200 ml sec-butanol (n-butanol may be used also)

200 ml glacial acetic acid

50 ml distilled water

50 ml of above Ninhydrin solution

2. Solution B:

200 ml sec-butanol

100 ml glacial acetic acid

25 ml distilled water

50 ml of Ninhydrin solution

To optimize the Chromatography solution, add 300mL of solution A to 300 ml of solution B. This is the best concentration to pull out your amino acids. (50% A: 50% B)

Amino Acid standards:

- Leucine, Cysteine, Glycine, Lysine, Proline, Phenylalanine, Aspartic acid, Histidine
In separate flasks add 50 ml of distilled water to 0.5g of each amino acid and add Mix well.

For your mixture of unknowns: Proline and aspartic acid make a good mixture because they both produce a easily distinguishable individual spot Please refer to the results for more help.

Compost solution preparation:

- Measure out 40 grams of compost and add 100 ml of distilled water to blender. Blend on high for 2 minutes.
- Add this solution into as many posi-click tubes as your centrifuge can hold.
- Centrifuge on 1200 min^{-1} for 3 minutes
- Pour off the supernatant into a vial. Date this and put in freezer until ready to use. This will slow down sample degradation.

Lab set-up for your students:

The ninhydrin spray should be put into a spray bottle just before the lab. All the solutions MUST stay in the fume hood with the exception of the amino acids, and the compost supernatant. These can be at some common station for use.

The Chromatography paper can be set out in a bag, students must use gloves to measure out the size of the sheet.

They will be putting one drop of each sample on the line they have drawn. You will need to make sure the dots are 2 cm apart as well. The 1000mL beaker makes an excellent chamber, but remind them to watch the migration and stop the process before the hour is up. They must apply the Ninhydrin spray before leaving for the day. About three sprays give you good enough

saturation. Hanging the chromatographs over night from a ring stand will work. The following day the students can analyze their data and complete the lab write-up. The chromatograms should be put in a freezer storage bag and stapled to their assignment.

Disposal: The solvent and spray needs to be disposed have into a hazardous materials container not down the sink. Make sure students know this in advance.

APPENDIX A7

RUBRIC FOR CHROMATOGRAPHY LAB

Developed by Tricia Miller

Table Three:

	Chromatography Pre-Lab		
Q	Correct answer	Incorrect answer	Credit no credit
1	Lipids, Carbohydrates, Proteins, and Nucleic acids	-other substances -no attempt	
2	Bacteria, Actinomycetes, and Fungi/molds	-Other organisms not discussed. -no attempt	
3	Yes. Drawing should have chemical structure of one of the macromolecules with arrow and products being amino acids, fatty acids, simple sugars, and various gases. Process is exothermic. List bacteria, actinomycetes, or fungi/mold.	-No -endothermic -No attempt	
4	We use chromatography because the solvent front movement creates a predictable retention factor, Rf that measures the amino acids attraction to the solvent versus its attraction to the Chromo paper. This works because based on size and polarity (amino acids have carboxylic acids that create charges) different amino acids will migrate up the chromo paper creating a characteristic spot formation. The compost sample may have several different amino acids in it.	-no attempt -no discussion about polarity of the amino acid.	

Table Three cont'd	Chromatography Post- Lab		
Q	Correct	Not correct	Point value
1	Completed marked lab chromatograph	-missing	20 points
2	Rf calculations, either on actual chromatograph or drawn onto a copy of their chromatograph, Identification of any amino acids in compost, and unknown sample.	-Missing any data or calculations	20 points
3	Completion of pre-lab section	-No attempt	10 points
4	Drawing one of the eight amino acids they were given with possible carboxylic acid, or side chain portion circled	-no attempt -no portion, or wrong portion like carbon center circled	10 points
5	The difference in the Rf values is due to the polarity and solubility in the solvent of each amino acid present. The forces of attraction cause the migration, which can be calculated and then compared to some Rf standards.	-answers that are vague and only reference the migration but fail to explain why they migrate. -no attempt	10 points
6	The Ninhydrin fix that we spray on at the end on the chromo paper will react with amino acids in the oils from your fingers producing a false positive.	-no connection between Ninhydrin and oils on fingers -no attempt	5 points
7	Yes or No. Because the proteins from the food waste are being broken down by microorganisms into amino acids. They may have been missing because the bacteria	-no mention of food waste, protein, or bacteria re-incorporation.	20 points

Table three cont'd	may have already re-incorporated them into their cell wall or used them for their life processes.	-No attempt	
8	From the protein in the food waste	-no mention of protein -no attempt	10 points
9	Yes or No, with some reason	-No attempt	20 points
10	Yes or No, with explanation	-No attempt	20 points

APPENDIX A8

Compost Microbiology and the Soil Food Web

Introduction

Compost is the product of an aerobic* process during which microorganisms* decompose organic matter into a stable amendment for improving soil quality and fertility. During composting, microorganisms use the organic matter as a food source, producing heat, carbon dioxide, water vapor, and humus* as a result of their furious growth and activity. When applied to and mixed into the soil, humus can promote good soil structure, improve water- and nutrient-holding capacity, and help to control erosion. Humus makes up approximately 60 percent of finished compost. A wide range of organic materials such as yard trimmings, manure's, and food processing discards go into producing composts. Materials used to feed compost

microorganisms are referred to as compost feedstocks.

Part I of this fact sheet addresses the composting process and associated microorganisms. Part II then addresses how compost contributes to the soil food web and overall plant health.

Part I: The Composting Process and Associated Microorganisms

Compost Microorganisms

Sources. The microorganisms needed for composting are found throughout the natural environment. They are present in compost feedstock as well as in the water, air, soil, and machinery the feedstock and compost are exposed to during processing.

These sources ensure a high diversity of microorganisms, which helps to maintain an

active microbial population during the dynamic chemical and physical processes of composting, such as shifts in pH, temperature, water, organic matter, and nutrient availability. Only on rare occasions will the addition of microorganisms be warranted (see “Inoculating Compost” section).

Microbe Types and Requirements. The microbiological components of compost consist of bacteria and fungi. Because of their unique nature, *actinomycetes* are discussed here as a third microbiological component, though in actuality actinomycetes are a particular kind of bacteria. The majority of microorganisms responsible for the formation of compost are aerobes in that they require or work best in the presence of oxygen.

Many difficulties associated with composting may be traced to insufficient oxygen levels to support the decomposition of compost feedstock. Compost microbes also require a moist environment because they live in the water films surrounding composting organic

matter particles. A 50 to 60 percent moisture content is optimal.

Fungi. Fungi form their individual cells into long filaments called hyphae. Fungal hyphae are larger than actinomycetes and may be more easily seen with the naked eye. They penetrate throughout the composting material, decomposing both chemically and mechanically the more recalcitrant* organic matter fraction such as lignins and cellulose. Fungal hyphae physically stabilize the compost into small aggregates, providing the compost with improved aeration and drainage. Fungi number between 0.01 and 1 million propagules* per gram of soil. About 70,000 different species of fungi have been described worldwide, but an estimated 1 million additional species remain undiscovered and undescribed. Ecologically, fungi play a vital role in breakdown of dead plant materials.

Bacteria. The most numerous biological component of compost is the bacteria. Although they often can exceed 1 billion microorganisms

per gram of soil, bacteria (with the exception of actinomycetes) do not contribute as much to the overall microbiological mass as fungi because of their relatively small size. Although bacteria (with the exception of actinomycetes) exist as individuals and do not form filaments, they also contribute to the stabilization of aggregates through the excretion of organic compounds that bind adjacent organic matter and soil particles together. Bacteria are typically associated with the consumption of easily degraded organic matter. They are the dominant population throughout the entire composting process, whereas the actinomycetes and fungi typically proliferate in the later stages.

Actinomycetes. While actinomycetes are visually similar to fungi in that they have networks of individual cells that form filaments or strands, they are actually a type of bacteria. These filaments allow for a colony* of actinomycetes to spread throughout a compost pile, where they are typically

associated with the degradation of the more recalcitrant compounds.

Actinomycetes number between 0.1 and 10 million propagules per gram of soil. Their filaments contribute to the formation of the stable organic aggregates typical of finished compost. Actinomycetes are tolerant of lower moisture conditions than other bacteria and are responsible for the release of geosmin, a chemical associated with the typically musty, earthy smell of compost.

Composting Process

Composting proceeds in predictable stages. During different stages, temperatures and nutrient availabilities vary and affect the kinds and numbers of microorganisms that develop. Initially, the pile is at approximately the ambient temperature. The composting material warms through the *mesophilic** temperature range (50°–105°F) as the microorganisms become more active. Soon, microbial activity raises the temperature of the pile to *thermophilic**

temperatures (106°–170°F). This is considered the most productive stage of composting.

Mesophiles and thermophiles are microbes adapted to mesophilic and thermophilic conditions, respectively. Composting proceeds at a much faster rate under thermophilic conditions. Eventually, readily available substrates within the feedstock are exhausted, temperatures gradually return to the mesophilic range, and curing begins. The following section expands on the microbiology of each stage.

Initial Stage. The process of transporting and manipulating the feedstock for composting exposes the organic matter to additional sources of microorganisms, all of which may contribute toward initiating the composting process. Initially, mesophiles predominate and proceed to decompose the readily degradable sugars, proteins, starches, and fats typically found in undigested feedstocks.

The availability of easily usable organic substances enables the proliferation of the fastest-growing microorganisms, the bacteria.

Mesophilic bacteria, therefore, dominate initial decomposition. These bacteria release heat from the breakdown of large amounts of easily degraded organic matter. This heat begins to raise the temperature within the pile due to the high insulating capacity of a properly sized compost pile. Within just hours the temperature of the compost pile can rise above the 106°F thermophilic threshold.

Active Stage. As the compost reaches higher temperatures, thermophiles begin to dominate the bacterial community. The active stage is typically the stage where most of the organic matter is converted into carbon dioxide and humus, and the microorganism population grows. The thermophilic population continues generating more heat by decomposing the remaining organic matter.

Due to limitations with isolation techniques, laboratory studies have only been able to isolate a few genera of bacteria from the thermophilic stage (*Bacillus*, *Clostridium*, and *Thermus*), but many microorganisms remain

to be discovered and described. In a properly ventilated composting pile, the temperature will be maintained between approximately 131° and 155 °F. Fortunately, pathogens such as human viruses and infectious bacteria are typically unable to persist in such a hostile environment. The higher temperatures will ensure rapid organic matter processing while simultaneously providing optimal conditions for the destruction of human and plant pathogens as well as weed seeds. Because the composting pile is cooler on its outer surface, periodic mixing of the outer layer into the pile is essential for maximum pathogen and seed kill. Mixing or turning the pile also helps to ventilate it by increasing the size and number of air pores. This is important because in an unventilated compost pile, the temperatures can exceed 160°F, effectively stopping all microbial activity. The air pores also serve as passages for oxygen to enter the pile. Microbes require oxygen to efficiently break down organic matter.

Overheating. If a pile does overheat, surpassing approximately 170°F, most microbes will be destroyed and microbial activity will virtually cease. Surviving microorganisms are typically those able to survive as *spores*.* The spores will germinate when the composting pile returns to a more favorable temperature. These spores are thick-walled structures that are formed by the microorganism under stress such as heat, cold, drought, and low nutrient conditions. After overheating, the composting pile will cool to a mesophilic state, requiring the activity of mesophilic microorganisms to return the pile to thermophilic conditions. If the composting pile is low in readily utilizable organic substrates, the pile may not be able to support the microbial activity needed to return to thermophilic conditions. In such a case, it may be necessary to supplement the composting pile with additional feedstock to ensure maximal degradation and pathogen removal.

An overheated composting pile may return to thermophilic temperatures through the germination and activity of spore-forming microorganisms, and through the infiltration of microorganisms from the outer surface of the composting pile where the temperature was less extreme.

Curing Stage. A properly functioning composting pile will eventually deplete itself of a majority of the easily degradable organic substrates leaving some cellulose, but mainly lignins and humic materials. Bacteria are generally considered less adept at metabolizing these remaining compounds. Consequently, the bacterial population will decline in numbers as compared to fungi and actinomycetes. Because less heat is generated at this point, the temperature of the composting pile will slowly fall to mesophilic temperatures. With the return of mesophilic conditions, the final curing stage of composting begins.

During the curing stage, the fungi and actinomycete populations predominate, while the bacterial population may decline somewhat. Fungi and actinomycetes proliferate on the remaining less degradable organic matter such as chitin, cellulose and lignin. These compounds are more persistent because they are insoluble in water and, due to their size and chemical complexity cannot pass into the bacterial cell. Thus, degradation of these compounds requires the use of extracellular enzymes.*

Once the complex organic compounds are broken down into smaller and more soluble forms, they can enter the cell and become food and energy for the microorganism. Microbes able to produce extracellular enzymes suitable for breaking down recalcitrant materials will have a selective advantage at this point in the composting process.

A novel feature of many of the extracellular enzymes common in fungi is that they are capable of breaking down a wide range of

compounds that would otherwise require several specific enzymes*, a feature not commonly found in a single microorganism. Fungi, though they grow and reproduce more slowly than bacteria when food is readily available, are well suited for exploiting an environment rich in complex recalcitrant organic compounds like those found in the compost during the curing stage. The curing process can vary in duration; a longer curing period provides more assurance that the compost is free of pathogens and phytotoxins.* If the compost is incompletely cured (i.e., not stable), it maintains a higher microbial activity, leading to increased oxygen consumption. When unstable compost is applied in the field, it can thereby decrease the supply of oxygen available to plant roots. In addition, immature compost can contain higher levels of soluble organic matter (i.e., organic acids), which can lead to toxicity problems for certain horticultural applications, such as seed germination. Detailed

information on assessing compost stability and maturity is included in the California Integrated Waste Management Board (CIWMB) publication *Compost: Matching Performance Needs with Product Characteristics* listed at the end of this document.

As the curing stage continues, there is a gradual increase in the humus fraction. Humus is a complex class of chemicals that result from the incomplete degradation of organic matter. Humus is among the most resistant compounds to degradation in nature. It is also one of the major mechanisms for the retention of nutrients (e.g., nitrogen, phosphorus) and micronutrients (e.g., copper, zinc, iron, manganese, calcium) in the soil. Because humic compounds retain micronutrients and water so well, they are often the site for the highest biological activity, including microorganisms, protozoans, invertebrates (e.g., worms, springtails) and plants.

The Microbiology of Cured Compost

Identifying Compost Microbes. Compost microbes are tremendously diverse and their ecologies are extremely complex. Methods used to identify individual species include analysis based upon metabolic activity and/or fatty acid content.

However, because of the great diversity, identification of individual species in cured compost is rarely done and is generally considered impractical and extraordinarily expensive. Laboratories, instead, are more likely to identify and count species by organism group, such as actinomycetes, aerobes, anaerobes, fungi, nitrogen-fixing bacteria, or pseudomonads.

Guidelines for desirable levels of each of these microbe groups are listed in the *Compost Quality Standards* document referenced at the end of this fact sheet. A commercial laboratory that specializes in compost analysis developed these levels, which are based on

numerous samples and observations in various applications.

New techniques of DNA analysis are providing researchers with additional tools to identify compost and soil microbes. However, this method of identification is in its infancy and is not commonly available in commercial soil labs.

Inoculating Compost. Many researchers and companies suggest they can determine the “health” of a compost product and recommend inoculants to improve its quality or performance. However, there is no conclusive evidence that the addition of any specific microorganism to cured compost will improve any characteristic of compost. Native microorganisms may quickly dominate introduced microorganisms. The introduced microorganisms may provide possibly nothing more than additional nutrients to organisms already in the compost. Inoculants, if desired, can be added just prior to application of the compost.

Part II: Contributions of Compost to the Soil Food Web and Plant Health

Many growers think of compost as primarily a source of nutrients to add to the soil.

However, its contribution of a diverse set of microorganisms combined with its high levels of organic matter may offer even more significant benefits.

Soil consists of many organic and inorganic components that interact with each other in a dynamic, living system. From organisms as small as bacteria to larger insects such as earthworms, all of these players help cycle nutrients and contribute to the overall health of the soil food web and surrounding plant life.

A quality compost that has been prepared under aerobic conditions and adequately cured can contribute to the health of plants and the soil food web in several ways. Compost introduces a variety of microorganisms that may assist in the cycling of nutrients and in the control of pathogens. Compost also

contributes organic matter to the soil that may serve as a source of food for the various microbes, among other functions.

Compost Introduces Beneficial Microorganisms

When incorporated into soil, compost introduces a wealth of beneficial microorganisms. As discussed in Part I, plant and human pathogens are destroyed during the composting process. The remaining beneficial microbes assist with a number of functions that assist in soil and plant health.

Nutrient cycling. To be available to plants, nitrogen must be in an inorganic form, such as nitrate (NO_3^-) or ammonium (NH_4^+). Plants are not capable of converting organic nitrogen to these inorganic forms. Fortunately, microorganisms commonly found in soil and compost convert organic nitrogen into inorganic nitrogen, a process called mineralization. Plants may then take up the nutrients released by these.

Soils that have been exposed to harsh agricultural pesticides, such as methyl bromide, may have reduced populations of these beneficial microorganisms. Compost may help to re-inoculate these soils with nutrient-cycling microbes. It is important to note that inadequately cured, unstable compost may immobilize nitrogen in soil. Detailed information on assessing compost stability and maturity is included in the CIWMB publication *Compost: Matching Performance Needs with Product Characteristics* listed at the end of this document.

Disease suppression. Composts contain an astonishing variety of microbes, many of which may be beneficial in controlling pathogens. Beneficial microbes help to control plant pathogens through either specific or general suppression.

General suppression occurs when a beneficial microbe fills an ecological niche that would otherwise be exploited by a pathogen. For

example, a beneficial organism may out-compete a pathogen for energy, nutrients, or “living space,” thereby decreasing the survival of the pathogen.

Specific suppression occurs when a beneficial organism secretes chemicals toxic to a pathogen or when it preys upon the pathogen for food. Many plant pathogens contain cellulose (the principal component of paper) or chitin (commonly found in insects, and fungi), and all contain sugar-polymers (commonly found in all life). Certain compost microorganisms, such as *Gliocladium*, *Pseudomonas*, *Trichoderma*, and *Streptomyces*, produce enzymes capable of breaking these compounds down, killing the pathogens in the process.

Exposure to heat during the thermophilic stage of composting is often responsible for killing plant and human pathogenic microorganisms. This heat also kills those beneficial microorganisms that cannot tolerate the high temperature. Thus for compost to serve as a

means for minimizing plant pathogens in the field, it must be re-colonized by beneficial microorganisms.

Commercial compost producers in California do not routinely inoculate their compost.

Analysis, when performed, commonly shows that this re-inoculation occurs naturally.

However, some studies suggest that controlled inoculation of compost with known biocontrol agents (fungi and bacteria) is necessary for consistent levels of pathogen suppression in the field after application.

Degradation of pollutants. Mature compost has been shown to be an effective tool for reducing organic pollutants in contaminated soils and water. Compost bioremediation has proven effective in degrading or altering many types of contaminants, including chlorinated and nonchlorinated hydrocarbons, solvents, pesticides, and petroleum products. The microorganisms in the compost break down the contaminants into components that pose less of an environmental hazard. The United

States Environmental Protection Agency (U.S. EPA) publication *Innovative Uses of Compost: Bioremediation and Pollution Prevention* discusses bioremediation in detail. It is available on the U.S. EPA's Web site listed at the end of this document.

Compost Provides a Source of Organic Material

Soil organic matter can come from a variety of sources, including crop or plant residues, cover crops, and compost. Compost consists primarily of organic matter, which serves a variety of vital functions in the soil:

- **Provides food for microorganisms.** Bacteria and fungi that release nutrients from soil use organic matter as their food or source of energy. Thus, compost provides a source of both microorganisms and their fuel. Compost also provides an excellent habitat for microorganisms.
- **Holds nutrients and water.** In addition to providing a source of nutrients, organic material can hold onto many nutrients

through its cation exchange capacity.*

Because compost molecules are negatively charged, they attract and hold onto positively charged ions, such as calcium, potassium, ammonium, and magnesium.

- **Forms aggregates and increases porosity.** Organic matter increases the aggregation of soil that results in a crumb-like structure. Changes in porosity can

Cation exchange capacity—The ability of negatively charged particles to hold positively charged ions (cations) through an electrical attraction.

Colony—A microbial population originating from the same cell.

Extracellular enzyme—Complex protein structures that degrade organic compounds outside the cell of the microorganism.

Enzyme—Commonly a protein that speeds up a chemical reaction or reactions. Lactose intolerant people lack the enzyme lactase,

alter water retention properties and the water infiltration rate. Consequently, consistent compost use may improve irrigation efficiency.

Glossary

Aerobic—Requiring oxygen for metabolic processes.

which is used in the chemical reaction of breaking down lactose (a sugar).

Feedstock—Starting materials to be composted.

Humus—Recalcitrant, highly stable byproducts of organic matter decomposition.

Mesophilic—Temperature range of 50–105°F.

Microorganism—Bacterium (including actinomycetes) or fungus.

Phytotoxin—Chemicals harmful to plant health.

Propagule—Any part of a microorganism that can grow and reproduce.

Recalcitrant—Relatively resistant to biological, chemical, and/or photodegradation.

Spore—A dormant and highly resilient microbial state induced by unfavorable environmental conditions.

Thermophilic—Temperature range over 105°F.

Additional Resources

Compost: Matching Performance Needs with Product Characteristics, CIWMB

Publication

#443-00-005. Available from the CIWMB at (916) 341-6300 and also at

www.ciwmb.ca.gov/

Publications/Organics/44300005.doc.

Composting Reduces Growers' Concerns

About Pathogens, CIWMB Publication

#442-00-014. Available from the CIWMB at (916) 341-6300 and also at

www.ciwmb.ca.gov/

Publications/Organics/44200014.doc.

Persistence and Activity of Pesticides in Composting, CIWMB Publication

#442-00-015. Available from the CIWMB at (916) 341-6300 and also at www

www.ciwmb.ca.gov/

Publications/Organics/44200015.doc.

Compost Quality Standards, Organic Ag Advisors and BBC Laboratories, Inc.

Available from the CIWMB at (916) 341-6300. California Integrated Waste

Management Board:

www.ciwmb.ca.gov/Organics/

Soil Quality Institute's Soil Biology Primer,

www.statlab.iastate.edu/survey/SQI/SoilBiologyPrimer/

U.S. EPA's Bioremediation Fact Sheet,

www.epa.gov/epaoswer/non-hw/compost/bioremed.pdf

BBC Laboratories, Inc., www.bbclabs.com,

(480) 967-5931.

Soil Foodweb, Inc., www.soilfoodweb.com/,

(541) 752-5066.

APPENDIX A9

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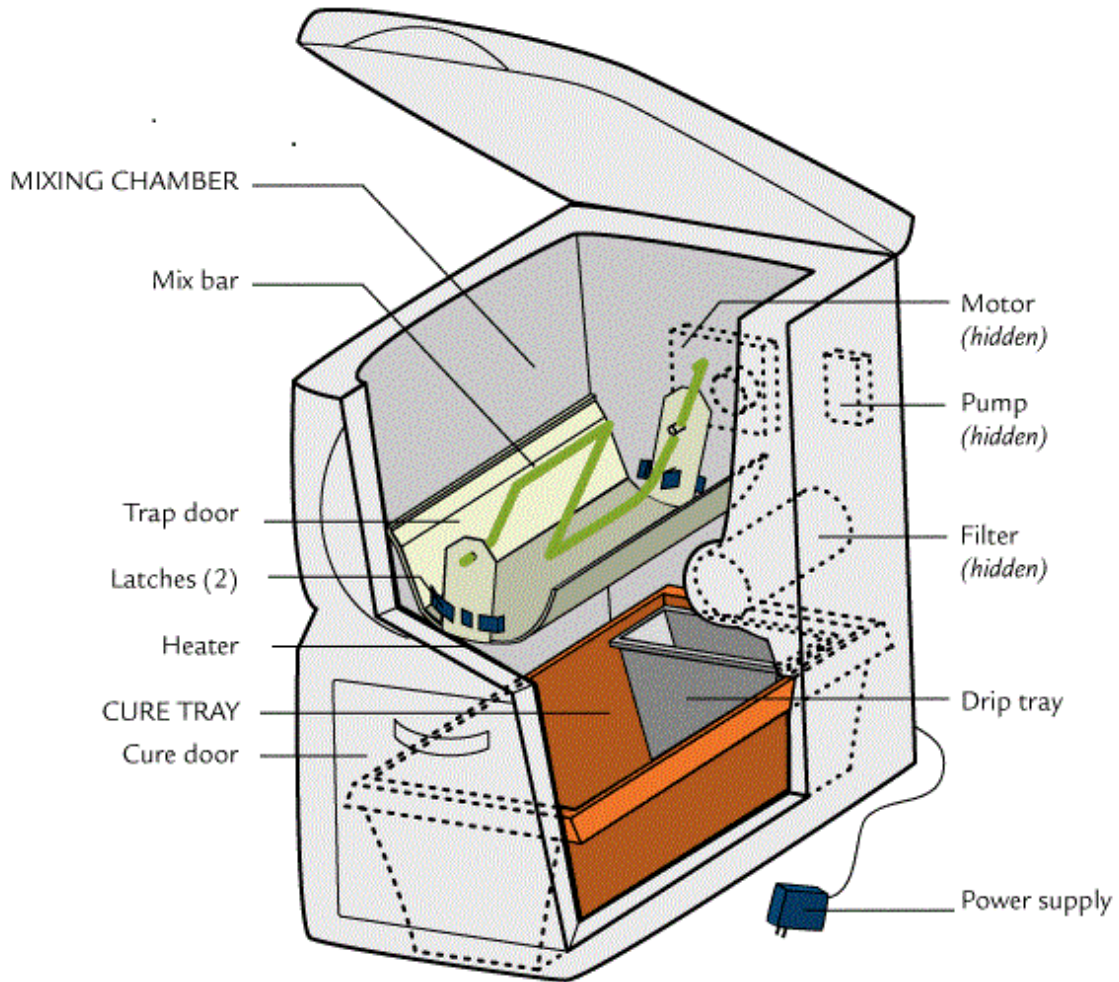
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APPEDIX A10: Nature Mill diagram

Diagram courtesy of www.naturemill.com

Figure Three:



APPENDIX A11

Greening the Curriculum: History of Green Chemistry

Notes downloaded and reprinted for use in the classroom courtesy of University of Scranton, Pennsylvania.

<http://academic.scranton.edu/faculty/canm1/intro.html>

APPENDIX A12

Anastas, Paul T., and John C. Warner. Green Chemistry: Theory and Practice. New York: Oxford University Press, 1998. N. pag. Print.

THE TWELVE PRINCIPLES OF GREEN CHEMISTRY:

Anastas and Warner have developed the Twelve Principles of Green Chemistry to aid one in assessing how green a chemical, a reaction or a process is.

- 1. It is better to prevent waste than to treat or clean up waste after it is formed.*
- 2. Synthetic methods should be designed to maximize the incorporation of all materials used in the process into the final product.*
- 3. Wherever practicable, synthetic methodologies should be designed to use and generate substances that possess little or no toxicity to human health and the environment.*
- 4. Chemical products should be designed to preserve efficacy of function while reducing toxicity .*
- 5. The use of auxiliary substances (e.g. solvents, separation agents, etc.) should be made unnecessary whenever possible and, innocuous when used.*
- 6. Energy requirements should be recognized for their environmental and economic impacts and should be minimized. Synthetic methods should be conducted at ambient temperature and pressure.*
- 7. A raw material feedstock should be renewable rather than depleting whenever technically and economically practical.*

8. *Unnecessary derivatization (blocking group, protection/deprotection, temporary modification of physical/chemical processes) should be avoided whenever possible.*
9. *Catalytic reagents (as selective as possible) are superior to stoichiometric reagents.*
10. *Chemical products should be designed so that at the end of their function they do not persist in the environment and break down into innocuous degradation products.*
11. *Analytical methodologies need to be further developed to allow for real-time in-process monitoring and control prior to the formation of hazardous substances.*
12. *Substances and the form of a substance used in a chemical process should be chosen so as to minimize the potential for chemical accidents, including releases, explosions, and fires.*

APPENDIX A 13

Youtube Video Courtesy of History Channel about Nature Mill®

<http://www.youtube.com/watch?v=Fly1WI6qOUE>

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