AC 2012-4704: CITRUS WASTE BIOREFINERY: EFFECTS OF TEMPERATURE, PARTICLE SIZE REDUCTION AND LIME PRETREATMENTS ON GRAPEFRUIT PROCESSING WASTE (GPW) BIOMASS

Miss Nicole Lynn Sears
Mr. Jeffrey L. Beynon, Flour Bluff ISD

Jeff Beynon is a teacher at Flour Bluff High School in the Flour Bluff ISD. He has been teaching Physics AP and Physics Pre AP-B and C for the last five years at this school. He has been teaching for nine years in the science field and has taught biology, chemistry, integrated physics and chemistry (IPC), principles of technology, physics, Physics Pre AP, Physics AP-B, and Physics AP-C. He has an A.S. in biology, B.S. in marine biology, B.S in marine geology, and more than 30 hours in graduate studies in environmental sciences and environmental engineering. He has retired from the city of Corpus Christi, Texas, where he was the Director of Animal Control as part of the City/County Health Department. His professional career has also included being a Golf Course Superintendent, as well as performing marine biology research, environmental science research, mariculture research, and consulting in environmental concerns. He is also a published author of original scientific research involving bird predation on shrimp mariculture ponds with a resulting grant to present the paper in Venice, Italy. He is married and has three adult children.

Mr. Raul C. Rivas, Texas A&M University, Kingsville

Raul Rivas is a Ph.D. candidate in the Department of Environmental Engineering at the Texas A&M University, Kingsville.

Prof. Mohamed Abdelrahman, Texas A&M University, Kingsville

Mohamed Abdelrahman received the B.S. and M.S. degrees in electrical engineering and engineering physics from Cairo University, Egypt in 1988 and 1992, respectively. He received an M.S. and a Ph.D. in measurement and control and nuclear engineering from Idaho State University in 1994 and 1996, respectively. He is currently the Associate Dean of Engineering at Texas A&M University, Kingsville. Abdelrahman's research focus is industrial applications of sensing and control with major research funding from the U.S. Department of Energy, National Science Foundation, and industry. He has also focused on collaborative and innovative educational research. Abdelrahman is passionate about outreach activities for popularizing engineering research and education. His activities in that arena included NSF funded sites for research experience for undergraduates and research experience for teachers. He has published his research results in more than 90 papers in refereed journals and conference proceedings and 30+ technical reports.

Prof. Patrick L. Mills, Texas A&M University, Kingsville

Patrick Mills is the Frank H. Dotterweich Chair and Professor in the Department of Chemical and Natural Gas Engineering at Texas A&M University, Kingsville. He is also President of Catalytic Reaction Engineering Consulting Services, LLC, where he provides consulting in catalytic kinetics, reaction engineering, experimental reaction systems engineering, and process development. Before being appointed to his academic position in Jan. 2006, he was a Senior Research Associate in the DuPont Company's Central Research and Development Department in Wilmington, Del. During his 15+ years at DuPont, he used his expertise in reaction kinetics, multiphase reaction engineering, transport phenomena, and experimental systems engineering to impact many technology areas in various DuPont businesses, such as Dacron, Nylon, Lycra, White Pigments, Fluoroproducts, and Nonwovens. He was awarded an Engineering Excellence Award from DuPont in 1996 for development of the MARS system, which is an automated catalyst-testing productivity device. Prior to joining DuPont, Mills worked for nearly 10 years at the Monsanto Corporate Research Center in St. Louis and the GE Corporate R&D Center in Schenectady, N.Y. His research utilized reaction engineering principles for the discovery and development of new molecules and processes in various technologies, such as agricultural and rubber chemicals, detergent hydrophobes, aromatic polycarbonates, and functionalized olefinic polymers. He also served as a Research Associate Professor at Washington University in St. Louis from 1988 to 1990 and was a member of the technology and business team with Dr. John T. Gleaves that commercialized the TAP (Temporal Analysis of

Products) reactor system. He also held appointments as Adjunct Professor of chemical engineering at the University of Delaware and Washington University in St. Louis where he taught graduate courses in reaction engineering, multiphase reaction engineering, and applied mathematics. Mills is the author or co-author of more than 150 publications in chemical engineering and applied mathematics, has presented more than 150 papers at professional society meetings, and is named co-inventor on several patents. He is a member of the AIChE, Sigma Xi, and the Society for Industrial and Applied Mathematics. He has chaired or co-chaired numerous sessions in reaction engineering at the AIChE annual meetings, served as guest editor for special journal issues and edited volumes, and was elected to serve as Chair of the AIChE Catalysis and Reaction Engineering Division from 2005 to 2006. Mills was also a lecturer for the AIChE Continuing Education series in multiphase reaction engineering. He also serves on the editorial board for Reviews in Chemical Engineering and Applied Petrochemical Research, and is a member of the AIChE Books Publication Committee.

Teaching Topics and Modules based on Biofuels Technology from the Project Effects of Pretreatments for Grapefruit Processing Waste (GPW) Biomass on the rates of enzymatic hydrolysis for conversion to sugars

Abstract

The objective of this work was to develop and test an aspect of the utilization of citrus waste to energy and bring this new knowledge into the classroom for secondary science education and discussion. A citrus waste based biorefinery has the potential to make the disposal process both economically and environmentally beneficial. This potential can be achieved by developing a process that is rapid, inexpensive, and productive. The pretreatment process was the focus of this research project because it makes up "18% of the projected cost… more than any other single step" in the bio-refinery of citrus waste. This step occurs in preparation for the enzymatic hydrolysis. Particle size reduction, the addition of lime (calcium hydroxide), and a change in temperature during the pretreatment can increase surface area and therefore increase the rate of enzymatic hydrolysis. Three teachers participated in various aspects of this research project over the summer. Results from their research are presented in this paper.

Although the three teachers worked on the same research project, each had his/her own research objective. Each of the teachers developed a different learning module based on their research experience. The learning modules focused on AP Physics, Pre-AP Physics, Physics and Algebra. This shows the versatility of using the legacy cycle based on a similar research experience, but different perspectives, in introducing K -12 concepts.

Research Overview

The United States produced 12.5 million tons of citrus juice processing waste (peel, seeds, and pulp) during the 2006/08 seasonal period [9]. The current disposal of this waste is through the manufacturing of low-value feedstock, burning it, or placing the waste in a landfill [7,4,8]. These means of disposal are not economically or environmentally beneficial. This has lead to an incentive for the development of technology that will produce a more environmentally responsible way of dealing with the waste while trying to capture all potential economic value of the waste [8].

Research has revealed citrus waste contains a complex blend of soluble sugars, cellulose, hemicelluloses, and pectin[10]. These compounds can be converted into bioethanol but first it is necessary to develop a way to improve the accessibility of the cellulose, thus helping to optimize the enzymatic hydrolysis step [11]. This can be achieved through a pretreatment process which can lead to the breakdown of "a resistant carbohydrate-lignin shield that limits the accessibility of enzymes" [13]. The addition of both acids and alkali during pretreatment have been used to try and achieve this goal but the alkaline based processes have less sugar degradation and most of the alkaline materials can be recovered[5]. Studies of lime (calcium hydroxide) pretreatment on various kinds of lignocellulosic biomass have shown there is an increase in sugar yield after enzymatic hydrolysis[1,2,3,6,12]. The idea is to find the right combination of particle size distribution and lime concentration to be used in the pretreatment process of GPW, which would decrease the amount of time needed to yield the largest amount of fermentable sugar yields upon enzymatic hydrolysis.

The research team of teachers started with previously sampled and frozen at -20 °C grapefruit processing waste (GPW) from Texas Citrus Exchange Facility in Mission, Texas. It was slowly thawed at 8-10 °C and maintained under refrigeration to be used in the following experiments. The thawed GPW and solid lime (calcium hydroxide) were milled using a knife grinder at conditions shown in table 1 and table 2. The particles size distribution was analyzed using a RO-Tap sieve shaker with a sieve stack ranging from 1mm to 25µm. Each particle size fraction was evaluated for the total dry solids. A GC-MS was used to calculate the amount of glucose released during milling. The visual surface morphology of the crushed dried sample was analyzed using a field emission scanning electron microscope (SEM).

Table 1: Grapefruit Processing Waste

Lime %wt.	Exp. 1	Exp. 2	Exp. 3	Exp. 4
0.0	2000 rpm / 15 sec	3000 rpm / 30 sec	9000 rpm / 45 sec	10000 rpm / 2 min
0.4	2000 rpm / 15 sec	3000 rpm / 30 sec	9000 rpm / 45 sec	10000 rpm / 2 min
0.6	2000 rpm / 15 sec	3000 rpm / 30 sec	9000 rpm / 45 sec	10000 rpm / 2 min
0.8	2000 rpm / 15 sec	3000 rpm / 30 sec	9000 rpm / 45 sec	10000 rpm / 2 min
1.0	2000 rpm / 15 sec	3000 rpm / 30 sec	9000 rpm / 45 sec	10000 rpm / 2 min

Table 2: Grapefruit Processing Waste with Lime Concentration of wt% 0.0 and 0.6

Temperature ^{0}C	Exp. 1	Exp. 2	Exp. 3	Exp. 4
20	2000 rpm / 15 sec	3000 rpm / 30 sec	9000 rpm / 45 sec	10000 rpm / 2 min
30	2000 rpm / 15 sec	3000 rpm / 30 sec	9000 rpm / 45 sec	10000 rpm / 2 min
45	2000 rpm / 15 sec	3000 rpm / 30 sec	9000 rpm / 45 sec	10000 rpm / 2 min

Once the pre-treatment process was complete for the GPW at varying lime concentrations, it was added to a baffled flask and combined with enzymes, water, and antibiotics diluting the solution to 2 wt % of GPW. The enzymes used in the solution included a blend of pectinase, cellulose and several others. The solution was preheated to 45˚C in a shaker bath and left to react for 24 hours. A 200 µL sample was taken every half hour for the first two hours and every hour after up to 12 hours and one final sample at 24 hours. As the samples were taken, they were heated up to stop the reaction and then dried under a nitrogen stream. The samples were then analyzed by the gas chromatograph-mass spectrometer (GCMS) to find the amount of glucose in the sample at the time that the sample was taken.

The data collected from sieve stack (not shown) demonstrated the effect of varying pretreatment conditions on the particle size distribution. The increase in milling speed and milling time shifted the particle size distribution from the larger particle sizes to the smaller particle sizes. The GC-MS analysis revealed the amount of glucose released from the GPW after milling and lime treatment. The analysis showed for each particle size distribution there is an optimal amount of lime that should be used to release the most glucose during the milling process. There is a point where the combination of smaller particle sizes and the concentration of lime start to work against each other decreasing the overall release of glucose due to the release of pectin which forms gels with the soluble sugars and solids. The SEM micrographs showed the increased of lime concentration correlated with the increases of micro and nano pores on the material's surface. Consequently, this increased the surface area, which will help to facilitate the attack of the enzymes on the cellulosic fractions.

The data collected from the GPW pretreated with varying temperature with 0% and 0.6% lime pretreatment conditions on the particle size distribution illustrated that temperature does have an affect on the particle distribution, especially at the higher speeds.

The GC-MS revealed the concentration of glucose found in GPW samples taken during the enzymatic hydrolysis. After 6 hours, the glucose concentration almost always reached its maximum of only about 1 mg/mL, half of the maximum reached by the lower concentrations. However without the lime treatment under the same milling speed, it took twice as long to reach the maximum glucose concentration. At the higher millings speeds (smaller particle size distribution), the maximum glucose concentration reached by both 0.0% and 0.4% took 12 hours. A sample of the results collected through the experimental work is presented in Figures 1 through 6.

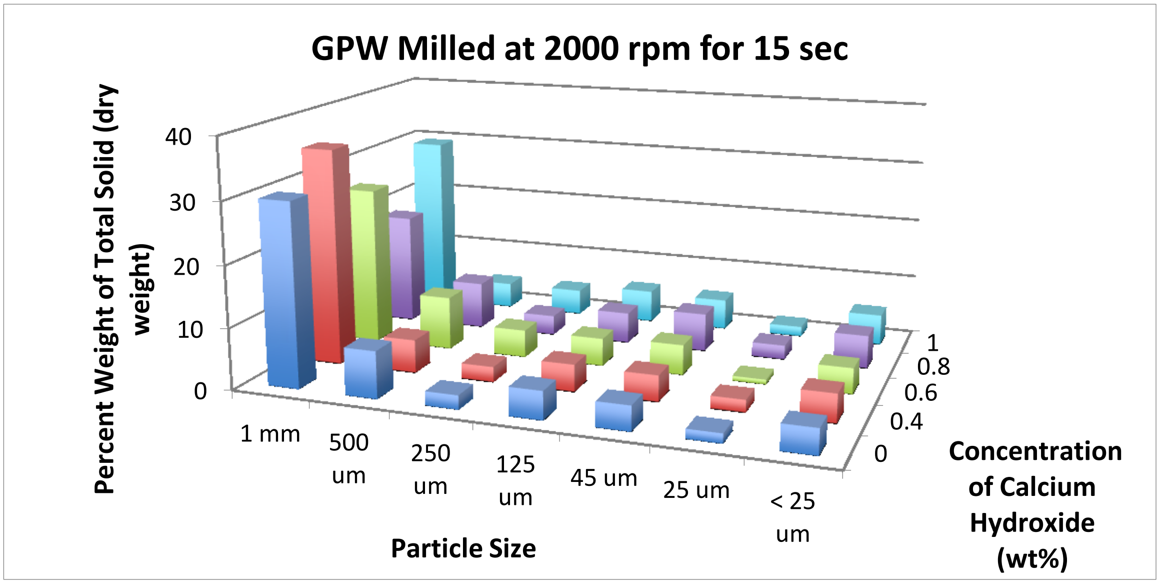

Figure 1: Particle Size Distribution of GPW milled at 2000 rpm for 15 seconds

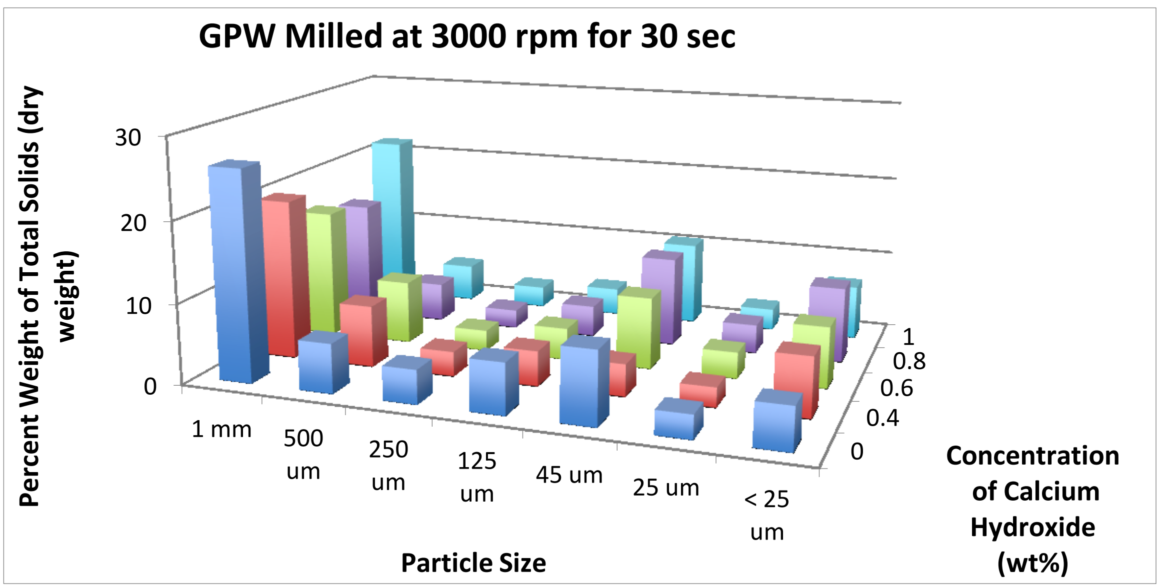

Figure 2: Particle Size Distribution of GPW milled at 3000 rpm for 30 seconds

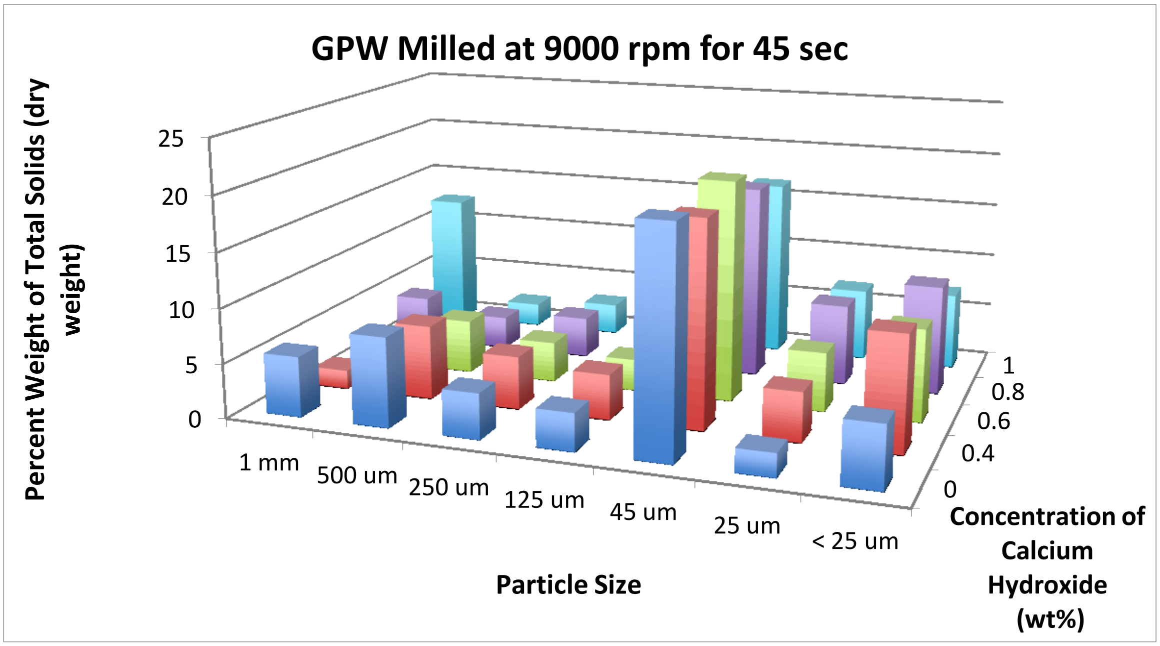

Figure 3: Particle Size Distribution of GPW milled at 9000 rpm for 45 seconds

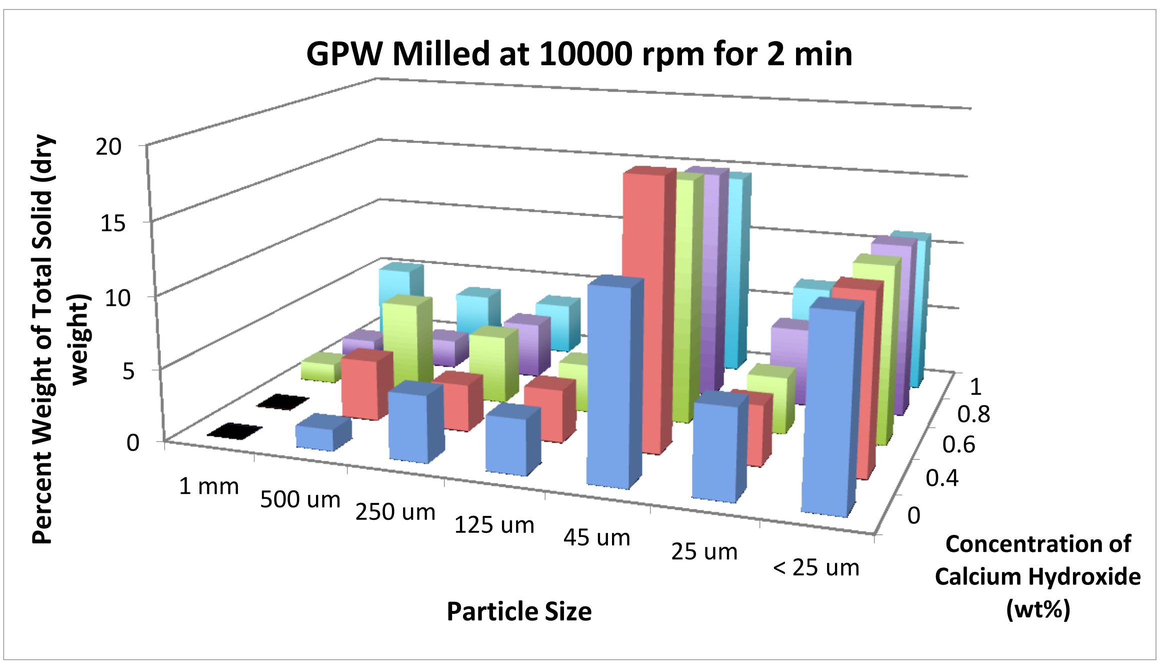

Figure 4: Particle Size Distribution of GPW milled at 10000 rpm for 2 minutes

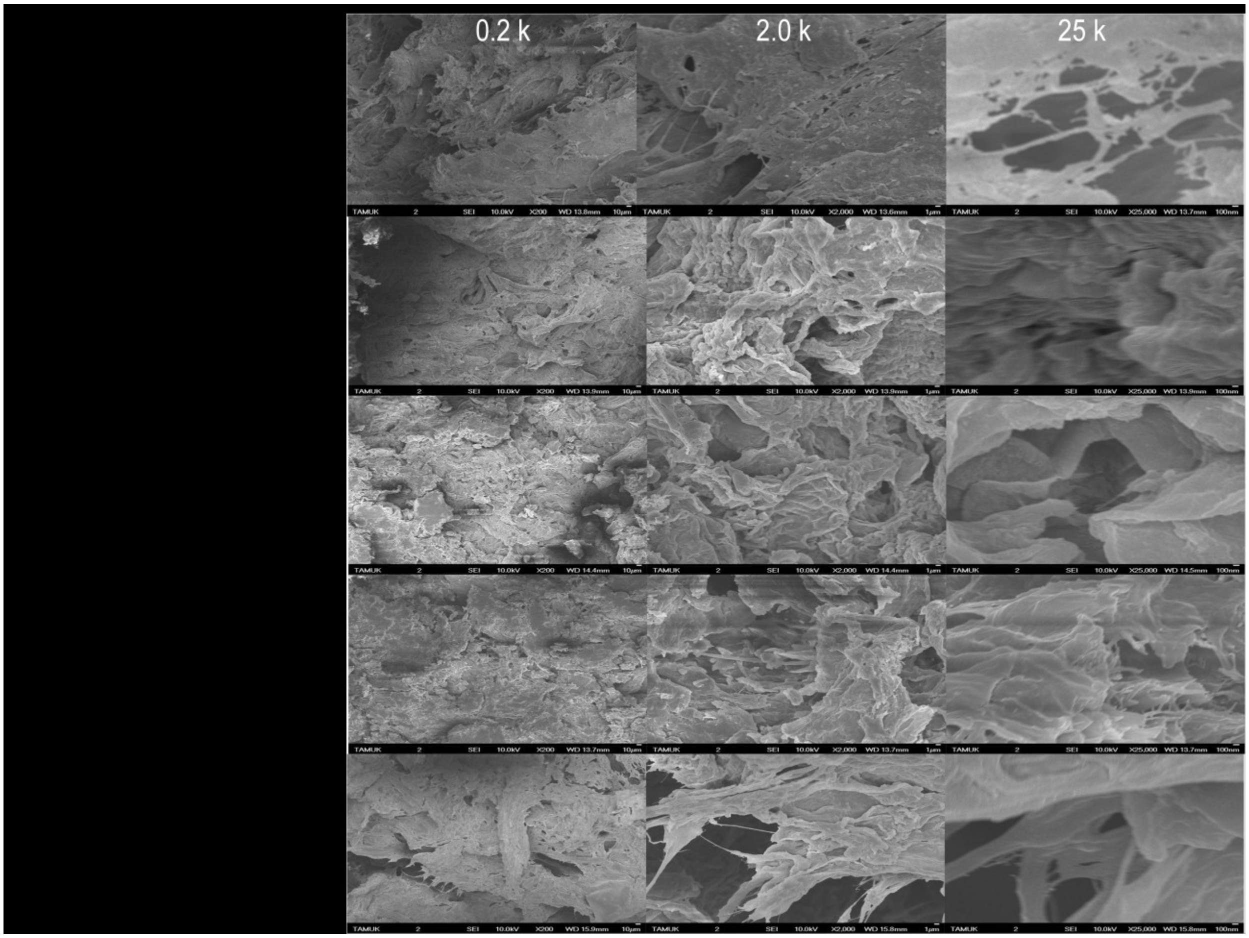

Figure 5: SEM photographs of GPW milled at 2000 rpm for 15 seconds with various lime concentration.

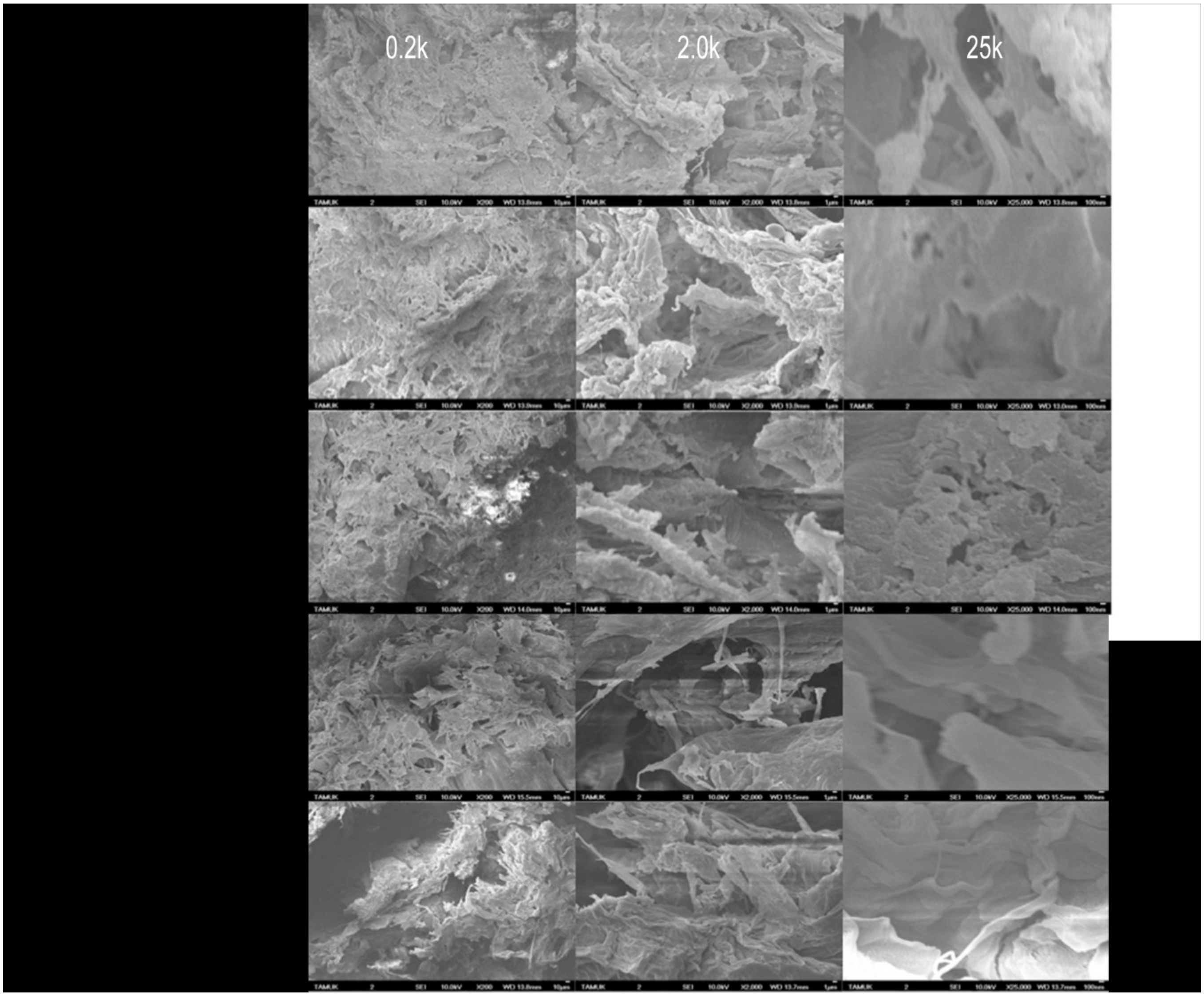

Figure 6: SEM photographs of GPW milled at 10000 rpm for 2 minutes with various lime concentration

It was concluded in the research that particle size distribution was affected by the speed and time of milling. Temperature does have an effect on the particle distribution and at the higher temperature additional washing of smaller particles is needed to help with soluble solid removal at higher temperatures to relieve water tension and viscosity.

Although all three teachers have strong backgrounds in science, this research experience brought upon the realization that engineers don't only work on processes, they develop them. The teachers also realized the groundwork necessary for chemical engineers to find the most lucrative and efficient parameters prior to running a process on a large scale. Every single step of the process must be picked apart to find the most efficient time, temperature, concentration, surface area, particle size, required equipment, materials, etc. The teachers were surprised to find how time consuming and repetitive the research required to develop a process can be. Each step of the way, they were performing the same tasks with minor differences, however, every step of the way was extremely necessary. Chemical engineers are essential in every part of the process, from discovery to development to implementation.

Learning Modules

A component of the RETainUs experience was the development and implementation of a Legacy Cycle. The goal of the Legacy Cycle was to bring aspects of the research experience and findings back into the classroom in a way that connects with the high school curriculum standards. A Legacy Cycle is composed of six different parts. First the students are presented with a "Challenge Question" which introduces them to the task at hand. They are then asked to "Generate ideas", a part of the cycle where they discuss what they already know about the task and what they need to learn. The next section of the cycle is appropriately called "Multiple perspectives" because in this section students gain information from various experts. The "Research and Revise" section of the cycle allows student to test their hypothesis. A formative assessment section of the cycle lets the students access what they have learned thus far and identify any weakness; this section is title "Test your Mettle". Finally the "Go Public" section gives the students an opportunity to show their learning by creating some project that is presented to the class or in another public venue. Each researcher took to the classroom in the form of a Legacy cycle a different aspect of the research and presented it in very different ways.

Alternative Fuel Source Legacy Cycle

The overall objective of the research was to find a way to make the Grapefruit Processing Waste a profitable resource. One profitable resource would be the creation of ethanol from the sugars in the waste. The idea of the legacy cycle is to cover and review the high school physics curriculum standards on energy and electricity while introducing the students to challenges engineers face.

The specific challenge question presented to the students will be:
You have just graduated from Texas A&M University - Kingsville with an engineering degree and have landed your dream job with a Bio-Energy company. This company is placing a major emphasis on developing a non-toxic long-lasting battery-like energy storage device as a clean alternative to alkaline batteries, a major source of groundwater pollution. The company has asked you to design a prototype of a Direct Ethanol Fuel Cell that will power a mobile electronic device.

The intent is for this question to generate ideas and questions about the Bio-Energy, ethanol, ethanol as a fuel source, the operation of fuel cell, and the amount of electricity required to power various devices. The students will gain perspective on this issue and process of research by having a class interview with an engineering student or professor from Texas A&M University-Kingsville. Their research and collection of background information will be done through lectures from the teacher, a Web Quest, and lab research. The lab research will provide an opportunity for the students to physical collect data on how much electricity and energy can be generated from ethanol and other alternative energy resources. The students will demonstrate their knowledge gained by comparing energy resources and providing an explanation on which energy resource they feel has the most potential in future advances. The final project will be presented to the class in media form of the students choosing (i.e. PowerPoint, prezi, video).

The high school that this legacy cycle will be implemented at is 83.4% Hispanic, 10.9% White, 4.1% African American, and 1.6% other with 76.7% of the students economic disadvantaged. 54.0% of the students are classified as at risk and only 4.3% are classified as English Language Learners. This specific legacy cycle will be implemented on approximately 120 students. Of those students, 75 of them will be Pre-AP Physics student while the others are enrolled in regular Physics.

Experimental Design Legacy Cycle

The research question for the effect of temperature on surface area at 0% and 6% lime concentrations by weight puts the emphasis on developing an experimental plan to test for the effects on the surface area. It involves a Physics AP class that must know how to develop an experimental design. The process has been tested on the AP test the last 2+ years in the free response area of the AP test. The student must do research, decide on equipment available for use, and design an experimental plan.

The following is the research question the student will deal with:
Can you develop an experimental process and procedure that will use the 4-5 concepts of solubility to achieve your goal? You will have to research what products are produced from orange processing waste and decide on what products you will emphasize your efforts on. Some of the products may be used in another process you have to reduce your costs. Some of the products may be used to sell to outside vendors. Some of the products will have to be disposed of at a cost to the company.

The student will do original research on current processes of citrus waste disposal. They must decide on what products that will be produced for use, sale, or disposal and the costs associated with these products. They have to decide on an experimental procedure/process to produce these products using the 4-5 concepts of solubility and the equipment provided on the lab tables. They then write an experimental procedure for the solubility portions of their overall experimental design and describe how each piece of equipment will be used and what it will achieve in the process.

Guest speakers with research experience on how and when research is done or accomplished will visit with the students to inform them of all processes involved with original research. Students will have to use library and electronic resources (i.e. computer, internet, TV, videos, etc.) for researching citrus processing waste disposal and the experimental design portion of the research project. The student will generate an original idea/thought process to achieve a research goal with reasoning and then follow that with the proving or disproving of the hypothesis as normally done in any type of research. The students will then write an original research report to the criteria of a recognized scientific journal that will be suitable for printing in that journal. They will have to research the types of journals available and then research to format for that particular journal.

The high school that these students attend is 72% White, 15% Hispanic, and 13% Other with 32% of the students economic disadvantaged. 44% of the students are classified as at risk and only 1.5% are classified as LEP. The Physics AP class does not follow most of the areas of the overall school's classification. The class consists of 80% White, 11% Hispanic, and 9% Other with on 22% economic disadvantaged. This is most likely due to the subject matter of the course and that most of the students are in the top 10% of the class. Only 15% of the AP students are considered at risk. All are seniors and have already had Physics or Physics Pre AP as a prerequisite course.

The Fresh Fruit Legacy Cycle

The Algebra teacher involved in this project chose to use the business objectives behind this project to create a legacy cycle in the classroom. In algebra I, students learn about functions and dependency, collect and graph data for the first time. This is also many students' first encounter with varying quantities represented by a letter. Throughout the year, students tend to ask when and where they will benefit from knowing and understanding these concepts. The lesson created for Algebra I is meant to simultaneously cover these objectives and answer these questions.

Students will be asked to create their own juice company by first choosing a fruit and a location where this fruit grows abundantly. The idea of starting a business will have them encounter the idea of initial costs which translate to y-intercepts in Algebra I. To kick off the unit, students will be presented the following challenge question:
You just won $500,000 on a scratch off and you are looking for a reasonable investment. When you turn on the 10 o'clock news, Joe Gazin is talking about the booming fruit juice business. One quick Google search later, you find out that the reason it is a good investment is that you are able to recycle the waste and produce ethanol currently used in the most United States fuel. You decide the fruit juice business it is! How long does it take you to make your first million?

The Algebra I students will be presented with a few different fruits in the classroom (oranges, grapefruits, lemons, limes, etc.) and asked to squeeze the fruit until they have a half quart of juice. The students will design a logo and create a name for their fruit juice business. The students will figure how many of their fruit it takes to make half a quart and use this to figure the amount per gallon. They will create tables and graphs of data to figure what it would cost to produce their juice on a large scale.

The students will then to do some research to find where their chosen fruit grows in abundance, the cost of land in this location, and the cost of labor. They will also look into the different methods used to produce the fruit juice. Once all this is figured, the students will figure the start up cost for their business.

Similar to engineers in the industry, the algebra class will create excel spreadsheets of the dollars that will be spent depending on the number of gallons. There will be a column in the spreadsheet for number of fruit, number of half quarts, number of gallons, cost of labor, cost of bottling, and cost of product transportation. At this point, the students may begin to feel that the first million is far from reach. As part of the legacy cycle, a local business owner will come speak to the class about what it takes to run a lucrative business. The business owner will explain what it took to start up their business and have a question and answer session with the class.

The students will then do some research to find an average price per gallon for their juice and decide on a reasonable price per gallon they will sell their juice at. This information will then be added to their spreadsheet. The students will finally be on their way to their first million.

Students will likely find that making their first million will take much more work than they thought. The idea that their waste can be taken to a bio-refinery and used to make ethanol will then be presented to the class by a local graduate student currently working on the project at the university.

Once students figure their route to their first million, they will write a proposal for other wealthy investors in the area describing the goals of their company. Students will also explain what makes the fruit juice business such a good investment including the idea that their waste can be used to literally fuel the economy. The development of the company and the proposal will then be presented to the class.

To close out the legacy cycle, students will be given a Microsoft Excel utilization test. They will be given a much smaller project that must be solved by creating tables and graphs in an Excel spreadsheet.

Conclusion and Summary

The overall experience of the RETainUS Program was enriching and helped to reestablish the original reason that teaching was chosen as a profession. The ability to take the experience back to the classroom and help encourage/stimulate the students to think about science/math as a career makes this program even more valuable. The RET project did require an extensive commitment of time and effort by the participants and their families for the participants to give the best of their ability. The time and effort involved with this project was well worth it. Participants are now able to challenge students in the classroom even more and be able to respond to questions with better understanding on the student's part. At the home campuses of the participants, other educators have been interested in the program after experiencing the results of the participants. A better understanding of the engineering processes resulted from the experience and will allow the participants to help guide students, teachers, and counselors to better decisions on what courses/studies need to be undertaken to achieve students' and parents' ultimate goals. Decisions on which math and science courses/studies need to be taken for the different areas of engineering will be more informed and will result in a student that is better prepared for the advanced studies required of the college and post graduate work. The dedication to detail and the ability to deal with the required redundancy of the research is probably one of the biggest lessons from the RETainUS program. It allows the participant to be more relaxed with students/family and to have the patience to deal with all types of stress that is present in everyday life and work. Overall the experience will be remembered and used for many years in the future.

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