



Abstract

eCYBERMISSION Team Name	Crabyotics
Team Grade	9
Project Start Date	August 10, 2013
Project Finish Date	January 27, 2014
Describe your project and explain how you used STEM (Science, Technology, Engineering and Mathematics) to improve your community (250 words or less)	
<p>Antibiotic resistance is caused by over-prescription of antibiotics and under metabolism of these synthetic drugs. Eventually through sewer and water processing, antibiotics find their way into our drinking water and food, because they cannot be filtered or neutralized through traditional processing methods.</p> <p>Chitosan is a polymer produced after deacetylation of crustacean shells. It has microscopic pores that can be adjusted during the deacetylation process that can absorb molecules of a certain size, including antibiotics. Deacetylation occurs after boiling crab shells in 3N sodium hydroxide, soaking the shells in 3N hydrochloric acid to deproteinize and demineralize the shells, then controlling boiling time in 3N sodium hydroxide at temperatures above 80°C. The resulting product was placed in methylcellulose and within a filter in a syringe filtration device, and then exposed to water with one dosage of ampicillin and amoxicillin. The filtrate was then plated onto nutrient agar plates with E. coli St.K-12. An increase in bacterial growth on the plate indicated neutralization or filtration of the antibiotics. The filtrate was also passed through a spectrophotometer which indicated clarity of the solution.</p> <p>The lab-made chitosan was tested against lab-made chitin, commercially produced chitosan, crab shells, filter, cotton and chitosan laced beads. The beads, lab-made chitosan, chitin and crab filtered out approximately 40% of the antibiotics. The methylcellulose mixed with the filtrate but also allowed bacterial growth. Repeat filtration may increase the amount of antibiotic filtration. Chitosan could be used to filter antibiotics from water, reducing antibiotic over-exposure.</p>	
Tips for writing your abstract: <ul style="list-style-type: none">- Do not go into too much detail about one certain area - be brief!- Include a problem statement and/or your hypothesis- Summarize procedures and the important steps you took to solve your problem- Briefly discuss your observations and results- Summarize conclusions and/or next steps- Do not go over 250 words! <p>*Please e-mail completed abstracts to whitsett@ecybermission.com or fax to 703-243-7177 by April 15.</p>	

State	New Mexico
Grade	9th
Mission Challenge	Environment
Method	Scientific Inquiry using Scientific Practices
Students	grasshoppa99 (Submitted on: 3/4/2014 10:29:05 AM) gingerbread (Submitted on: 3/4/2014 10:31:12 AM) pennymaster (Submitted on: 3/4/2014 10:30:45 AM) twerkmaster (Submitted on: 3/4/2014 10:30:18 AM)

Team Collaboration

(1) Describe the plan your team used to [complete](#) your Mission Folder. Be sure to explain the role of each team member and how you shared and assigned responsibilities. Describe your team's process to ensure that assignments were completed on time and deadlines were met.

To complete this mission folder, each team member was assigned a certain portion of the scientific inquiry [method](#), while all members contributed to the experimentation.

Pennymaster was the team leader, who developed the idea, formulated the problem and experiment design.

grasshoppa99 is the bacterial specialist, who was in charge of overseeing the conducting of the experiment along with assuring that all safety protocols were met.

gingerbread is the research specialist, who was in charge of collating the research as well as determining assays that could be performed to support the data, she worked directly with twerkmaster.

twerkmaster formulated the hypotheses and theories and tied them into the research.

During experimentation:

pennymaster received the data produced by the project, and recorded it, as well as producing the homemade chitin and chitosan.

grasshoppa99 ran the bacterial trials, as well as some of the filtration.

gingerbread did many of the filtration trials, and the spectrophotometer readings.

twerkmaster read the bacterial trials, and double checked the data readings.

Team was in control of their data analysis and conclusion production, as well as community compliance. The team established a timeline, and worked on each of their portions of the experiment and research at the same time, meeting together in a series of meetings to collate their data.

Scientific Inquiry

Problem Statement

(1) What problem in your community did your team try to solve? Why is this problem important to your community?

The problem that this team attempted to solve was to produce a bio-filter from crab shells that would remove antibiotics ampicillin and amoxicillin from water. Antibiotics have been overprescribed since at least the 1970s, but the main problem with this is the fact that many antibiotics do not completely metabolize in the body, and are expelled into the sewer system. Removing them is nearly impossible, and these antibiotics are finding their way into the drinking water, increasing illnesses such as antibiotic resistant strep, staph and other 'superbugs' that are emerging presently. Communities that have long relied on leach fields, open septic processing, and sewer treatment plants where the water is returned back to the environment after being processed have moderately high levels of antibiotics passing through their systems, and into the aquifer where it is returned back to civilization as drinking water. Rural communities such as ours have a high prevalence of antibiotics in the system, due to over-prescription and agricultural applications. This problem is becoming of increasing concern to our community and other communities around the world.

(2) List at least 10 resources you used to complete your research (e.g., websites, professional journals, periodicals, subject matter experts).

See attached.

(3) Describe what you learned in your research.

Antibiotic resistance is on the rise; it manifests itself in both 'superflu' forms such as resistant strep and staph as well as genetic anomalies such as fish with the same sex, and children with lower immune systems. Antibiotics have been prescribed for numerous ailments in excess since at least the 1970s both preventative and after disease to humans and livestock alike. Some of the prescription of antibiotics is unnecessary, especially

since antibiotics do not fight viral illnesses like the common cold. Antibiotics do not break down completely in the system and are not absorbed by bodily tissue, or metabolized. These chemicals go through the body and exit primarily whole through waste products. The antibiotics then pass through the waste neutralization process from sewers and leach fields into the environment and eventually aquifers, where it can get into the drinking water supply. There are no known neutralizers of antibiotics and they generally can make it through most filters. These antibiotics then re-enter the body through inadvertent exposure, where the immune system becomes used to the presence of these antibiotics, as do other free radicals that cause disease, and therefore produce a resistance.

Chitin is a biopolymer produced in the shells of shellfish such as crabs, shrimp and lobsters. It can be converted to chitosan which is a biopolymer with a flexible porosity that can be adapted to absorb compounds of a certain molecular size. Chitosan is available commercially on the market as a fat blocker, and in aquarium beads that control the bacterial environment of most aquariums. Methylcellulose is another biopolymer used for gelling products including food products, produced from wood fibers that is antibacterial and antifungal, and can act as a preserver.

Hypothesis

(4) State your hypothesis. Describe how your hypothesis could help solve your problem.

Hypothetically, commercial chitosan followed by extracted, 'homemade' chitosan will be the most effective at extracting both amoxicillin and ampicillin antibiotics out of the water, allowing for full bacterial growth.

It is also hypothesized that adding methyl cellulose and filter paper as carriers for the chitosan will enhance the filtration of the amoxicillin and ampicillin, evidenced by increased bacterial survival.

These hypotheses describe the potential outcome of the experimentation, which will use crab shells, chitin, chitosan, commercial chitosan, chitosan based beads, the above with methylcellulose and cotton as filters against two antibiotics; ampicillin and amoxicillin. The focus is to filter solutions through the different substances and determine how much of the antibiotics are neutralized or withheld to produce a filter for antibiotics.

(5) Identify the independent variables and the dependent variables in your hypothesis.

The primary independent variables were to establish an extraction assay for chitin and chitosan from snow crab shells that was safe and not expensive, and to determine if integrating chitosan into a filter for water would extract the antibiotics as indicated by increased bacterial growth and spectrophotometer readings. The dependent variables were two different types of antibiotics, one more soluble than the other, and the different forms of chitosan ranging from the crab shells to commercially obtained chitosan. Adding methylcellulose was also a dependent variable to enhance the filtration.

(6) How did you measure the validity of your hypothesis?

The validity of the hypotheses were tested by exposing the chitosan as well as commercial chitosan in two forms, filter paper, methylcellulose and the snow crab shells to antibiotic laden water, then exposing the resulting water to bacterial impregnated plates and through the spectrophotometer to determine clarity and presence of chemicals. If the bacteria died, then the presence of antibiotics could still be noted, but if the bacteria survived and potentially thrived, then the antibiotics could be determined as eliminated. The clarity of the water also indicates the lack of presence of the antibiotics after filtration.

Experimental Design

(7) List the materials you used in your experiment. Include technologies you used (e.g., scientific equipment, internet resources, computer programs, multimedia, etc.).

See attached.

(8) Identify the control group and the constants in your experiment.

The control group was the commercial chitosan, crab shells, cotton ball methylcellulose and filter paper run independently in the experiment, along with the amount of water (20 mL) run through in each trial. Plain distilled water was also run through each substance as a control. Distilled water was used throughout, as were the amount of antibiotics included in the solutions. The same methodologies were used to extract the chitin and chitosan, to filter the water through, to test the bacterial mortality and to determine the clarity of the water. The filters were replaced after each trial set of three, and the tube was washed out after each trial. Sterile pipettes were used to extract samples each time. The temperature and environmental conditions were kept the same for all assays.

(9) What was your experimental process? Include each of the steps in your experiment.

See attached.

Data Collection and Analysis

(10) Describe the data you collected and observed in your experiment. The use of data tables, charts, and/or graphs are encouraged.

The data collected and observed in the experiment included the color change and general drying process of the homemade chitin/chitosan, then the filtration process. The filtration process was then collected and observed spectrographically, then applied to agar plates pre-inoculated with E.coli Strain K-12 and incubated. The mortality of the bacteria indicated how much of the antibiotic remained in the water, as did the clarity of the water. The was tested with the straight items and a methylcellulose carrier to determine how much more antibiotic could be filtered out.

See attached.

(11) Analyze the data you collected and observed in your experiment. Does your data support or refute your hypothesis? Do not answer with a yes or no. Explain your answer using one of the following prompts: 'Our data supports/refutes the hypothesis because...'

The data collected and observed in this experiment includes spectrophotometric analysis of the filtered solutions to indicate how much of the antibiotic made it through the filters. The time of filtration for 20 mL samples were also noted, as was the amount of filtrate that made it through the filter. Finally, the filtered solutions were then placed on nutrient agar plates pre-inoculated with E. coli strain K-12 and spread. The resulting plate was incubated for 24 hours at 37 degrees Celsius and then the plates were counted for colony development and spread. A lack of colonies indicated that the antibiotics still made it through, existence of colonies indicated that the antibiotics were filtered out. The data primarily supports the hypotheses because the chitosan did extract most of the antibiotics as indicated by bacterial growth and the clarity of the water, therefore supporting the methodology of testing chitosan in filtering antibiotic laden water, and demonstrating an effective exercise of the hypotheses. The addition of methylcellulose did increase filtration, but also dramatically increased the time of filtration and also went through the filter into the water. The methylcellulose moderated the bacterial growth.

(12) Explain any sources of error and how these could have affected your results.

- 1) Initial tests were indicated to be performed at a 5% 1N solution of both sodium hydroxide and hydrochloric acid; these were too weak to initiate the separation, and the concentration had to be increased. 100% solutions could not be used because this would produce too violent a reaction, which would eventually destroy the chitin.
- 2) The methylcellulose plates were left too long in their petri dishes to dry, and the dishes had to be destroyed to get the plate filters out. These filters were also too brittle, and broke apart before being placed in the filter mount.
- 3) The commercial chitosan would turn into a concrete-like substance when left out in the air and when moisture was extracted, reducing the ability to pass liquid through the filter. These last two sources of error impacted the filtration time of the antibiotic-laden water through the filter system.

Drawing Conclusions

(13) Interpret and evaluate your results and write a conclusion statement that includes the following: Describe what you would do if you wanted to retest or further test your hypothesis. Evaluate the usefulness of the data your team collected. What changes would you make to your hypothesis and/or experimental design in the future, if any?

Upon conducting the experiment and analyzing the data it can be concluded that chitosan, chitin and crab all extracted antibiotics from the water, increasing its clarity and reducing the antibiotic pollution. Commercial chitosan wasn't as effective at the antibiotic removal as the homemade chitosan was, and extended the filtration time. This was primarily due to the commercial chitosan turning into a rock-like substance when it lost moisture and was too compact. The chitosan was more effective at removing amoxicillin over ampicillin as indicated by the color and clarity of the water after filtration. The methylcellulose addition added time to the filtration but in the case of homemade chitosan and crab, also increased the amount of filtration for both antibiotics, and allowed for the moderation of bacterial growth, giving the bacteria nutrients that the antibiotics would have stripped. The data mostly supports the hypotheses as stated. To further this experiment, different concentrations of chitosan along with the full development of a filter would be explored, as well as running the samples through the filter again to determine if multiple filtrations can reduce antibiotic potency.

Uploaded Files:

- [[View](#) **Crabyotics Procedure** (By: pennymaster, 02/27/2014, .docx)
]

Description of experimental procedures.

- [[View](#) **Bibliography** (By: gingerbread, 02/28/2014, .docx)
]

Sources of research and citation for Crabyotics.

- [[View](#) **Crabyotics Materials** (By: grasshoppa99, 02/28/2014, .docx)
]

Materials used to conduct the experiments.

- [[View](#) **Results** (By: grasshoppa99, 03/04/2014, .docx)
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Results in graphical and picture form for Crabyotics.

Community Benefit

(1) How could your experiments and data help solve your problem and benefit your community? Describe next steps for further research/experimentation and how you have or how you could implement your solution in the future.

These experiments provided evidence that chitin, chitosan, fine pore filter paper and methylcellulose can neutralize antibiotics in water by 75-85%. The problem was to produce a filter that could neutralize and/or filter out antibiotics from water so that un-metabolized antibiotics do not reach the aquifer, contributing to antibiotic resistance. Further research/experimentation would incorporate the chitosan into a gel-like suspension and determining if the filtration quality would be the same, as well as testing with higher caliber antibiotics. Fine tuning the filter to a higher percentage of filtration, then implementing it to homes where antibiotics have been used or producing a large enough filter to install at water treatment plants and in sewage outputs is a viable option, and would help our community as well as communities all over who have un-metabolized antibiotic discharge accumulating in their water sources. This filter also removes bacteria and other particulate matter from the water source, providing a nearly clear water. Another focus would be to remove any remaining allergens from the chitin source so that people who have shellfish reactions do not run the risk of allergic reactions.

Uploaded Files:

- [[View](#) **Crabyotics Chitosan Filtration** (By: twerkmaster, 03/02/2014, .pptx)
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Presentation on the filtration abilities of chitosan.

Procedures

1. Chitin Extraction

- a) Clean and dry crab shells.
- b) Break down and crush crab shells as much as possible.
- c) Make 100 mL 3N solution of Sodium Hydroxide and Hydrochloric acid*.
- d) Place 2g shells into 100 mL 25% NaOH solution, place on hot plate with thermometer.
- e) Turn on heat, and increase temperature to a sustained 85-90°C.
- f) Heat at 85°C for 5 minutes.
- g) Remove from heat, pour shells into strainer, wash shells off with regular water.
- h) Place shells in 100 mL 50% Hydrochloric Acid at room temperature
- i) Wait until bubbling reaction stops. Remove shell/substance from HCl, wash off with regular water.
- j) Place chitin in 100 mL 50% NaOH solution, place on hot plate.
- k) Heat chitin to 120°C until chemical reaction ceases, remove from heat
- l) Wash and strain chitosan from solution, allow to cool and set.
- m) Repeat with further batches of crab shells in 2g increments**.

*Sodium Hydroxide is to deproteinize the shells, while the Hydrochloric Acid is to demineralize the shells leaving the chitin behind.

**2g increments are used to guarantee that all of the shell will be properly coated by the chemicals during the process, increasing the amount of chitin and chitosan produced.

2. Nutrient Agar Procedure

- a) Sterilize work area with isopropyl alcohol.
- b) Weigh out 11.5 g of Nutrient Agar powder using electronic scale.
- c) Measure 500 mL of distilled water.
- d) Pour distilled water into 1000 mL beaker.
- e) Place magnetic stirrer in beaker.
- f) Place beaker with distilled water and magnetic stirrer on hot plate.
- g) Turn heat up to setting of 10, turn stirrer up to setting of 5.
- h) Add Nutrient Agar powder to distilled water.
- i) Heat until liquid gently boils and turns slightly clear. (approx. 10 minutes)
- j) Remove from heat using heat resistant glove.
- k) Equally distribute Nutrient Agar solution into three screw cap Erlenmeyer flasks and place in autoclave.

- l) Place 250 mL of tap water in autoclave.
 - m) Place 3 screw cap Erlenmeyer flasks in autoclave and seal.
 - n) Place pressure cooker onto hotplate, heat for 15 minutes after autoclave has attained 15 psi pressure and 121° C. Remove autoclave from heat and allow to cool.
 - o) With heat resistant glove, pull Nutrient Agar solution bottles from autoclave, and pour nutrient agar into Petri dishes. Allow Nutrient Agar to gel, invert and refrigerate until time of use.
 - p) Re-sterilize work area with isopropyl alcohol.
3. Nutrient Broth and Bacterial Base Solution Inoculation
- a) Weigh out 11.5 grams of Nutrient Broth powder using electronic scale.
 - b) Measure 500 mL of distilled water.
 - c) Repeat steps 4-14 of NAP procedure with Nutrient Broth solution.
 - d) Pour Nutrient Broth Solution into sterile Erlenmeyer flask and refrigerate until time of use.
 - e) Using a sterilized inoculation loop, place one drop of *E. coli Strain K-12* obtained from Carolina Biological Supply into pre-sterilized screw-cap test tube with 20 mL of Nutrient Broth. Gently agitate.
 - f) Sterilize inoculation loop using isopropyl alcohol and flame, and place bacteria into incubator at 37°C.
4. Filtration Procedure
- a) Set up drip apparatus of a 60 mL syringe, with stopcock suspended on a ring stand with ring clamp with the spigot at 12 cm from the base.
 - b) Place 5g chitin, chitosan, commercial chitosan, biopolymer beads, cotton into syringe with stopcock closed.
 - With powdered substances, place within a folded low pore filter paper on the top of the syringe.
 - Mix powdered substances with 50 mL methyl cellulose to produce a dissolvable filter.
 - *Dissolve 1 g methylcellulose powder to 100 mL of distilled water over low heat until fully dissolved, then add additives once the solution has cooled to room temperature.
 - c) Place 1_mL Amoxicillin or Ampicillin into 50 mL water, stir gently until fully dissolved.
 - d) Plate *E. coli Strain k-12* onto nutrient agar plates (2 drops, spread with sterile hockey stick, re-sterilize hockey stick with isopropyl alcohol and flame.)

- e) Run 20 mL antibiotic water through syringe, allow to set in syringe for 5 minutes, then release.
- f) Remove 2 drops of filtered sample, and place on plate, spread with sterile hockey stick.
- g) Incubate in incubator for 24 hours at 37°C.
- h) Count and observe colonies, observe colony morphology. Increased bacterial counts will indicate the neutralization of the antibiotics. Lower bacterial counts indicate the presence of antibiotics.
- i) Observe filtered water through spectrophotometer to determine general amount of particulate/contaminant via absorbance.

*Control runs will consist of distilled water filtered through each test media.

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Materials**Equipment:**

Chitin/Chitosan Extraction	+Nutrient Agar/Bacteria	+Experimentation
Electronic Scale	Petri Dishes	Glass Hockey Stick
Hammer	Magnetic Stirrer	Glass Petri Dish
Plastic Baggies	3 – 250 mL Erlenmeyer Flasks	Incubator
Strainer	Autoclave/Pressure Cooker	Vernier Probeware/Datalogger
Hot Plate w/magnetic stirrer	1000 mL Beaker	Spectrophotometer
Fume Hood	Inoculation Loop	Cuvettes w/Caps
Thermometer	Bunsen Burner	Lens Paper
Ring Stand and Clamp	Screw cap test tube	Cotton Balls
4- 250 mL Beakers		60 cc syringe with petcock
2 – 150 mL Erlenmeyer Flasks		180 pore filter paper
Rubber Stoppers		Stopwatch
Glass Stirring Rods		
Pipettes		
Spatula		
Paper Towel		
Mortar and Pestle		
Heat Resistant Gloves		

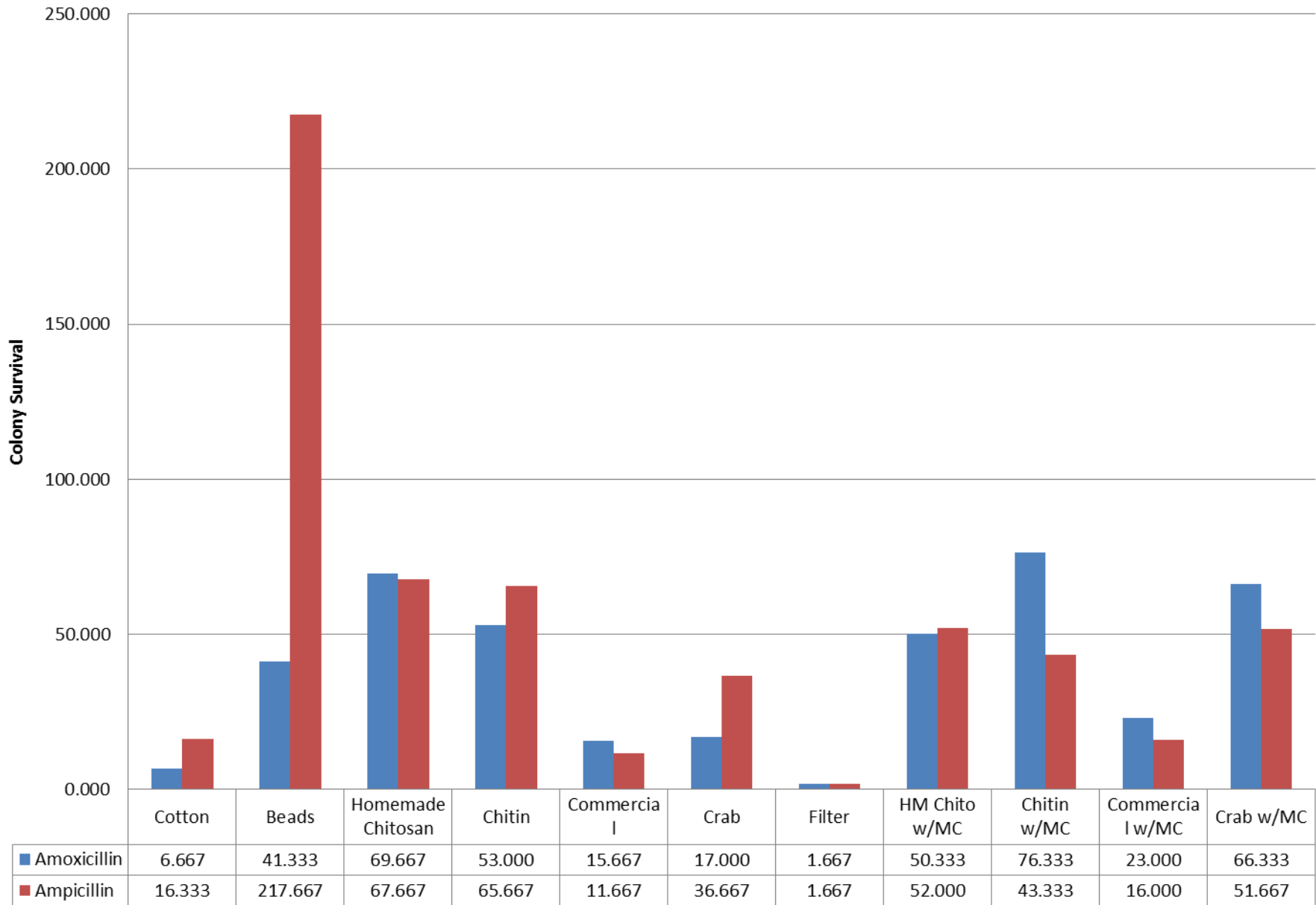
Chemicals:

Chitin/Chitosan Extraction	+Nutrient Agar/Bacteria	+Experimentation
3N Hydrochloric Acid	Isopropyl Alcohol	Commercial Chitosan
1N Sodium Hydroxide	Matches	Chitosan Beads
3N Sodium Hydroxide		Methylcellulose
Distilled Water		
Tap Water		

Biological:

Chitin/Chitosan Extraction	+Nutrient Agar/Bacteria	+Experimentation
Snow Crab Shells	Nutrient Agar	Amoxicillin
	Escherichia coli Strain K-12	Ampicillin
	Nutrient Broth	

Colony Count After Filtration

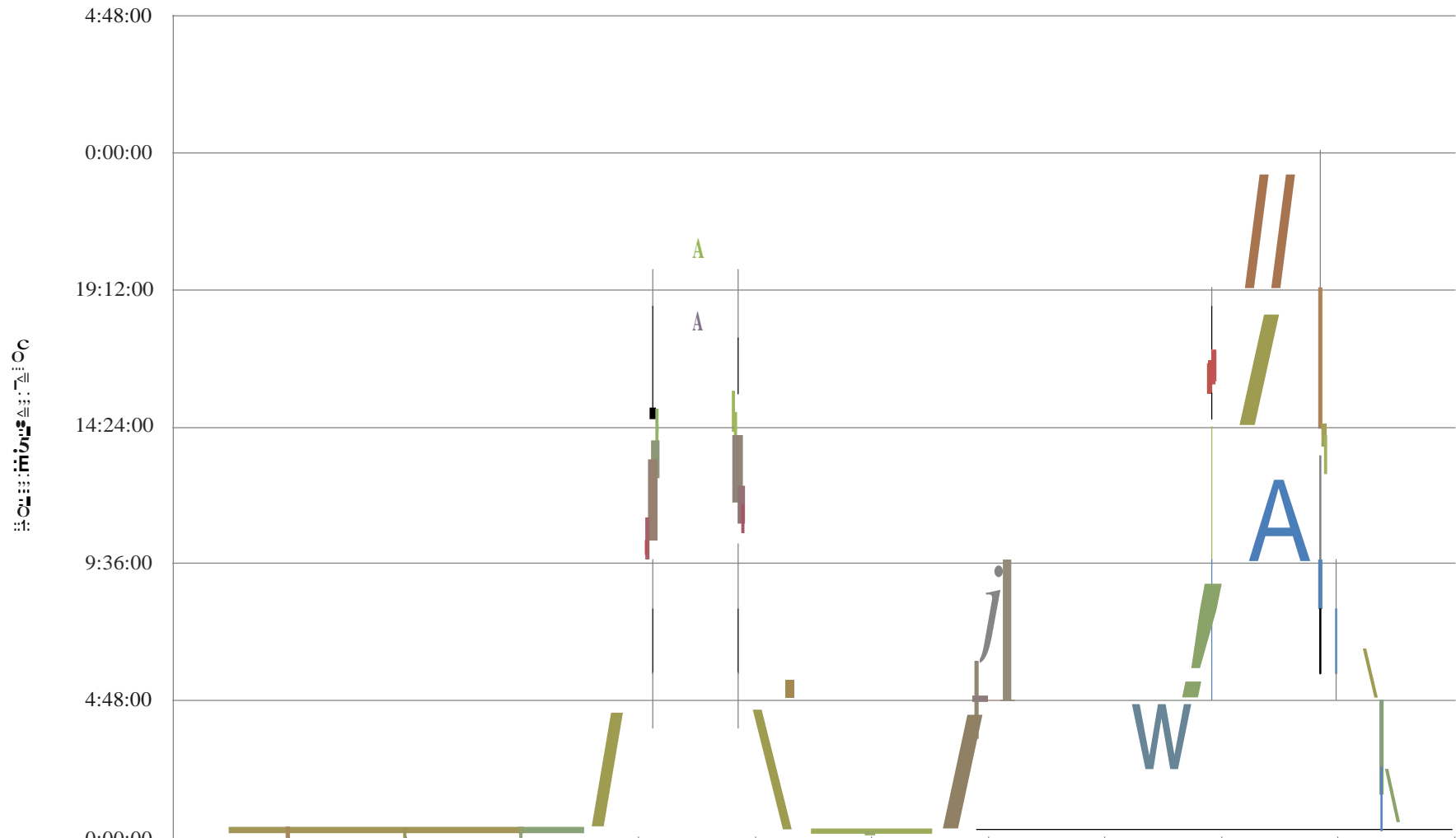


Absorbance/Reflection (Spectrophotometer)



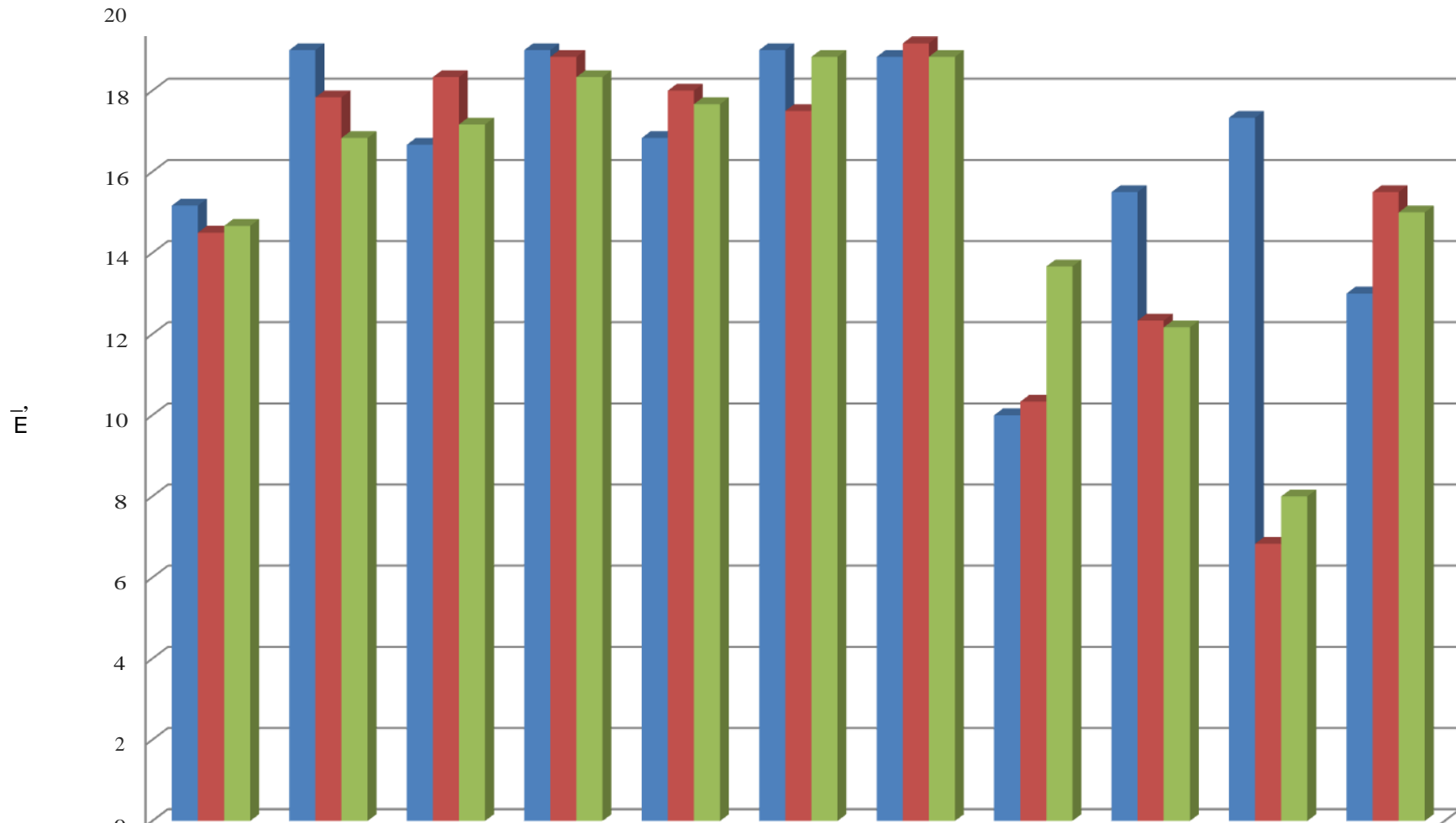
		Cotton	Beads	Homemad e Chitosan	Chitin	Commercia l	Crab	Filter	HM Chito w/MC	Chitin w/MC	Commercia l w/MC	Crab w/MC
—	control	0.00232	0.00142	0.00270	0.00048	0.11253	-0.00068	0.00015	-0.00012	0.00003	-0.00195	-0.00054
—	Amoxicillin	1.31530	2.03200	-0.21516	-0.14629	0.02394	-0.01869	0.00305	-0.00596	-0.03497	0.03331	0.03630
—	Ampicillin	0.97583	1.77100	0.12750	0.03387	0.23141	0.10220	0.20773	0.09761	0.06251	0.22293	0.05846

Time of Filtration

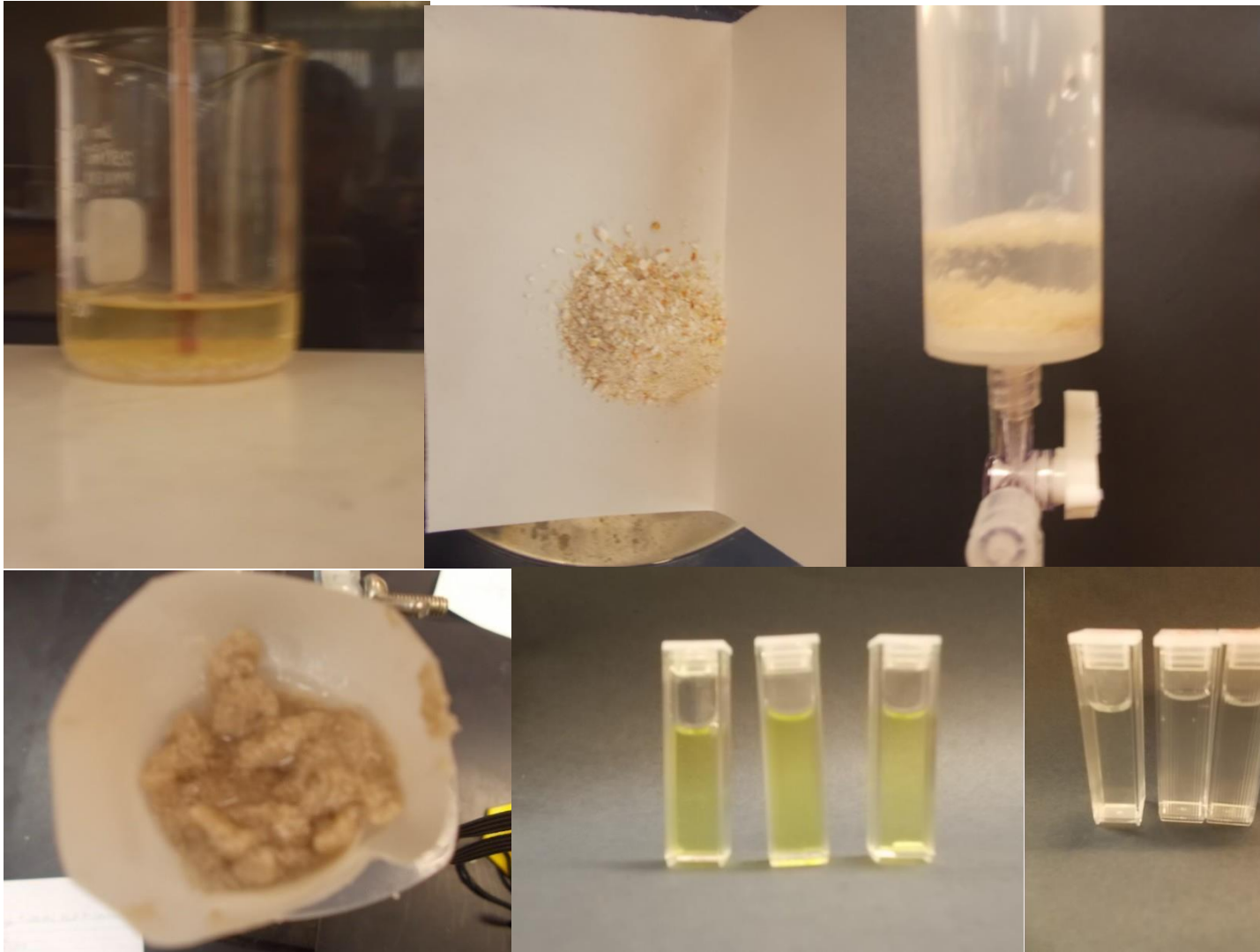


	Cotton	Beads	Homemade Chitosan	Chitin	Commercial I	Crab	Filter	HM Chito w/MC	Chitin w/MC	Commercial I w/MC	Crab w/MC
control	0:20:33	2:53:37	0:13:48	0:10:57	19:11:58	0:16:33	0:09:15	10:56:22	2:28:23	12:32:15	0:44:05
Amoxicillin	0:10:59	0:13:16	0:14:33	0:17:40	18:26:13	0:17:07	0:10:48	10:02:53	10:52:45	23:59:59	0:35:57
Ampicillin	0:21:22	0:18:26	0:15:23	0:16:35	20:58:16	0:14:13	0:12:14	13:24:32	3:42:36	22:08:07	0:47:51

Amount Filtered



	Cotton	Beads	Homemade Chitosan	Chitin	Commercial	Crab	Filter	HM Chito w/MC	Chitin w/MC	Commercial w/MC	Crab w/MC
Control	15.166667	19	16.666667	19	16.833333	19	18.833333	10	15.5	17.333333	13
Amoxicillin	14.5	17.833333	18.333333	18.833333	18	17.5	19.166667	10.333333	12.333333	6.833333	15.5
Ampicillin	14.666667	16.833333	17.166667	18.333333	17.666667	18.833333	18.833333	13.666667	12.166667	8	15



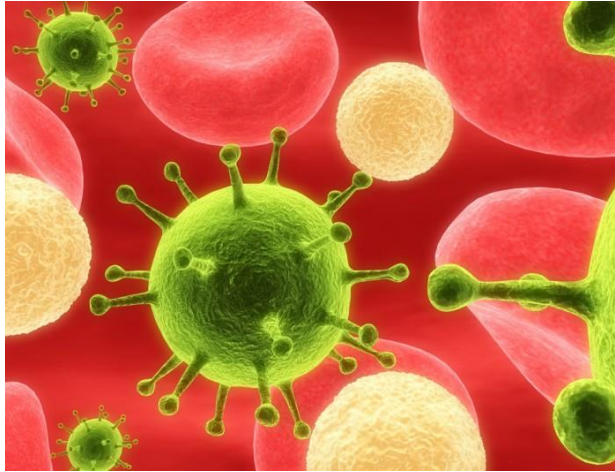
Top row: Chitosan in production during hydrochloric acid boiling process, finished chitin, chitosan in filtration apparatus.

Bottom row: Commercial chitosan in filter, being processed, ampicillin filtered through homemade chitosan; the colorant does not disappear, but the majority of the particulate matter is gone, and ampicillin filtered through homemade chitosan, from a creamy white to clear.

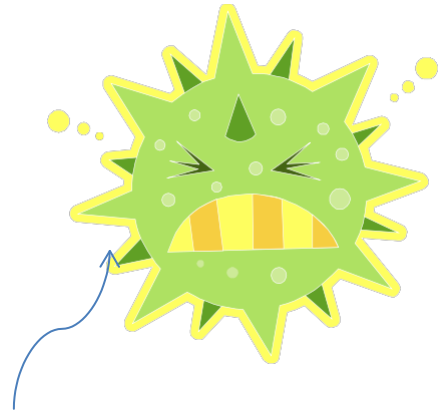
Avoid the Freakshow!



Antibiotics Prescribed for Everything



Antibiotics miss some bacteria



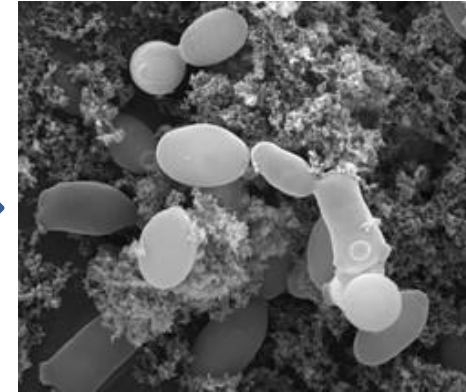
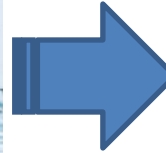
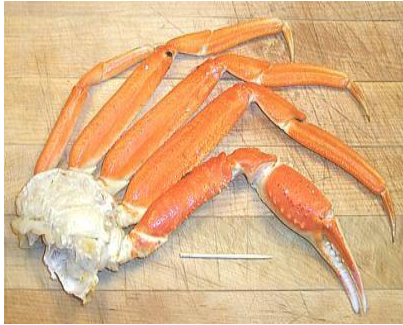
Bacteria become superflu



Antibiotic resistance is produced by antibiotics being consumed in higher quantities by humans and livestock. These chemicals are not completely metabolized in the body and don't get all the bacteria they are supposed to. The result, it gets into our food and water supply and our immune systems build up resistance to the excess antibiotics in our consumption supply = bacterial resistance.

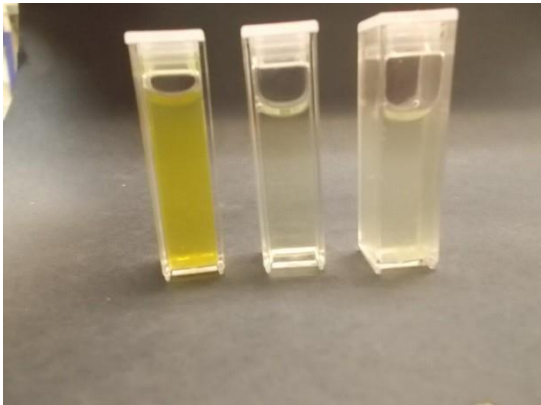
Chitosan Treatment

To reduce antibiotic resistance in already existing water sources, turn to nature and get crabby – shellfish shells which contain chitin that can be turned into chitosan.



Snow crab and other shellfish produce chitin that can be turned into chitosan.

Chitosan has small pores that 'grab' onto molecules of varying sizes, and can be adjusted to have larger or smaller pores.



Antibiotic water before filtration through Chitosan, after Chitosan and with Chitosan with Methylcellulose: the majority of the particulate matter was trapped in the chitosan producing clear water.