Antibiotic Removal from Waste Streams

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Abstract:

Animal husbandry leaves antibiotics in ppm levels in its discharged wastewater. Bacteria exposed to these low level antibiotics may cause horizantal gene transfer of the antibiotic-resistant gene, and therefor contamination of crops irrigated by the wastewater will occur. To counter this issue, a novel electrochemical approach is used, utilizing the generation of hydrogen peroxide at the cathode. When the medium is supplemented with ferric and ferrous (also from the cathode), Fenton's agents created in the MFC medium generate radicals (HO and HOO) which show satisfactory effects on antibiotics removal such as Chlortetracycline.[i] [ii] Implementing these mechanisms, electrochemical cells where built and the effectiveness of the removal of antibiotics was measured.

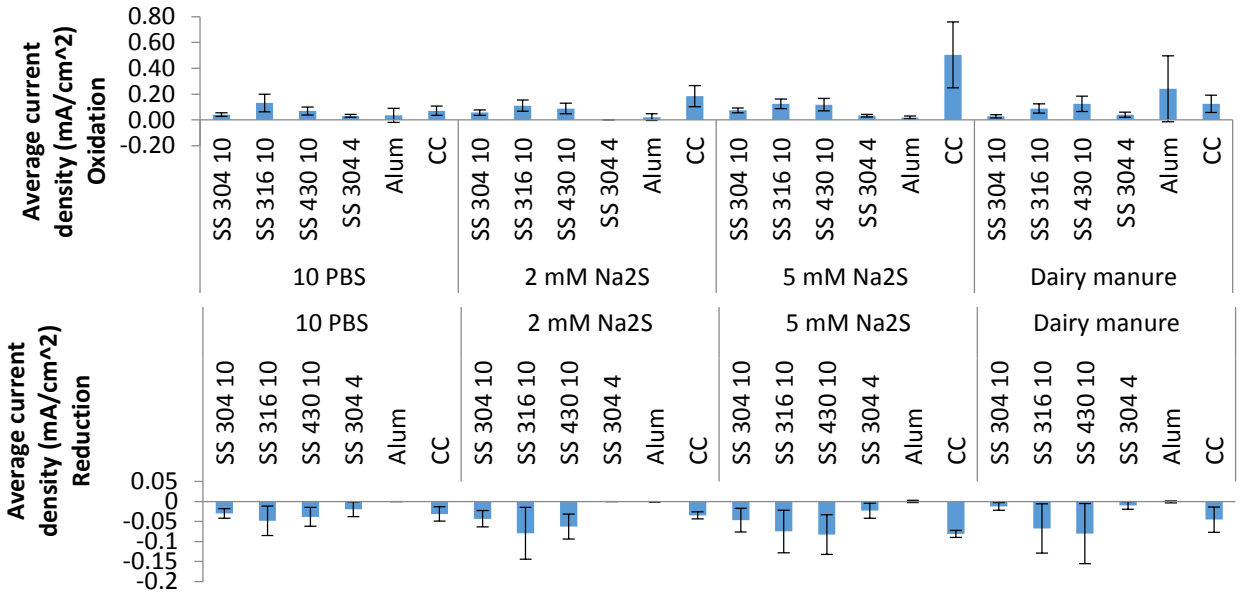
Methods/Results:

Table 1: Results of Cyclic Voltammetry

The first step was determining the electrodes to be used in the reactor. Several materials where tested for their oxidation and reduction potentials in several different mediums, including four types of stainless steel, aluminum and carbon cloth. These tests where done using cyclic voltammetry(Reference 600, Gamry Instrument, Inc., Warminster, PA),, which test the current density at different voltages ranging between -1 and 1.5 V. Each material was tested with dimensions of roughly 1.5cm x 4.5cm. Above are the results of the tests using four different medium: 10mM PBS (a phosphate buffer), 2 mM Na_2S with 10mM PBS, 5 mM Na_2S with 10mM PBS, and Dairy manure.

Figure 1: Results of Cyclic Voltammetry

From the figure above, the best electrode overall would be made with carbon cloth, because it performed in the top two for all of the oxidations and within the top three for reduction.



		Performance ranking	
Medium	***Electrode***	***Oxi***	***Red***
10 PBS	SS 304 10	4	4
	SS 316 10	1	1
	SS 430 10	3	2
	SS 304 4	6	5
	Alum	5	6
	CC	2	3
2 mM Na2S	SS 304 10	4	3
	SS 316 10	2	1
	SS 430 10	3	2
	SS 304 4	-	-
	Alum	5	5
	CC	1	4
5 mM Na2S	SS 304 10	4	4
	SS 316 10	2	3
	SS 430 10	3	1
	SS 304 4	5	5
	Alum	6	6
	CC	1	2
Dairy manure	SS 304 10	6	4
	SS 316 10	4	2
	SS 430 10	3	1
	SS 304 4	5	5
	Alum	1	6
	CC	2	3

There was a few issues detecting hydrogen peroxide generation in the reactor, which after a few tests resulted in the conclusion that our test kit was contaminated. We then substituted the reagents in the iodine clock reaction with our own (specifically the molybdate), and the problem resolved itself.

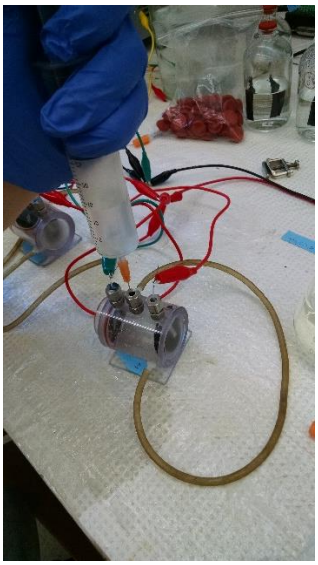
After the electrode material was determined, the construction of the reactor took place. The reactors where designed as small batch reactors (intended for use in other projects by this group as well as for this UROP project), and were constructed in several steps. First, the reactors vessels were made from polycarbonate tubes with a total volume of 30 mL. Three ports were drilled on the vessels in order to place current collectors which connect to electrode inside the vessels, and the middle port can be used for sampling or inserting reference electrode if needed. The second step was making the electrodes. The electrodes had to be circular with a diameter of 2.5cm, as well as making sure that the cathode did not allow the fluid to leave the reactor. The cathode was coated with Fenton's reagents using a reaction between 0.54g $FeCl_3$ in 100ml of distilled water, and a solution composed of 1.8g of $NaBH_4$ in 40ml of distilled water. To prevent the liquid medium from leaking out through the cathode, four thin layers of 60% polytetraflouraethene (PTFE) was painted on and cured, as shown in the picture below *(Picture 1*). Three cathodes where prepared, so multiple reactors could be run. To make sure that the cathodes where symmetrical, a circular punch was used, and then finished with scissors (*Picture 2*). The anodes where prepared with the same methods from plain carbon cloth but without the Fenton's reagents or the PTFE.

Picture 1

Picture 2

The third step was to assemble reactor vessels with electrodes. Titanium wire (40 gage) was bonded to the back of the electrodes with a silver epoxy, and then a non-conductive plastic tube was placed over the wire to prevent any interference from any other conductive surface. The cathode was glued to the o-ring of the reactor using a silicon glue (*Picture 3)* while the anodes where placed in the back. After letting the glue set, the reactors where filled with a synthetic media of antibiotics (at a concentration of 10mg/L with a total volume of 33ml) as shown in Picture 4 and run at 2 volts and room temperature for two hours.

Picture 3



Picture 4

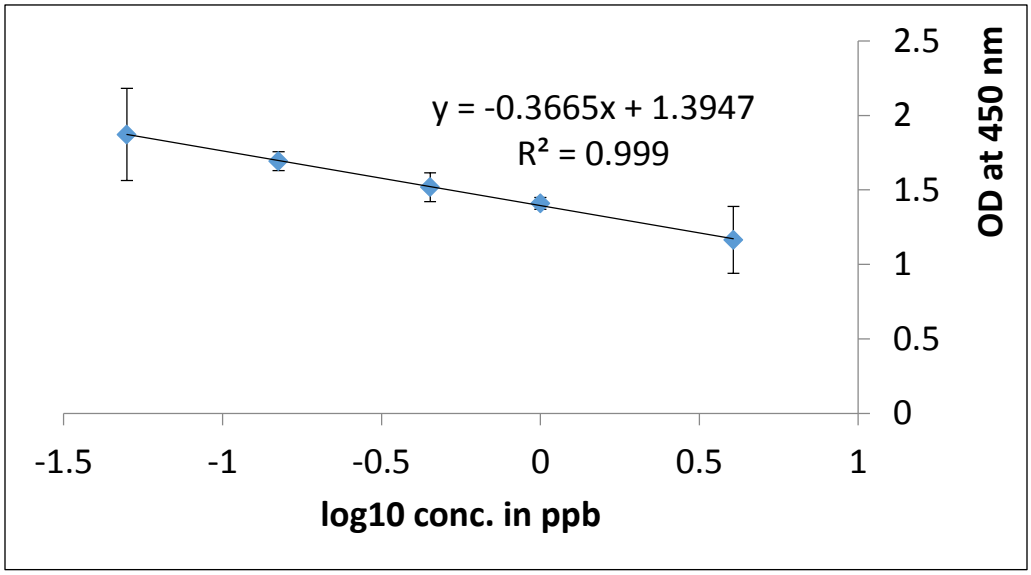


Figure 2: ELISA calibration curve

	Reading	Log Concentration	concentration	Real Conc (µg/L)
Reactor 1	t=0	0.81	1.595362	39.388
	t=0.5	0.915	1.308868	20.364
	t=2	0.938	1.246112	17.624
Control	t=0	0.93	1.26794	18.533
	t=0.5	1.478	-0.22729	0.5925
	t=2	1.38	0.040109	1.0968
Water	blank	1.202	0.525784	3.3557
	blank	0.949	1.216098	16.447

Table 2: Results of Reactor

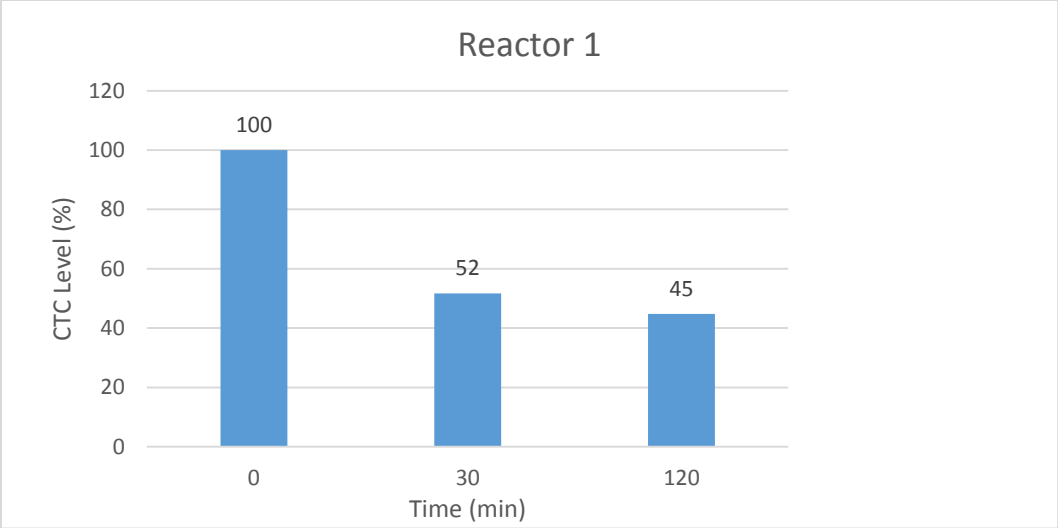


Figure 3: Reactor 1 results

The results of the reactor test where performed using ELISA Antibiotic test kit from Green Spring, and where compared to a standard curve provided by Hongjian Lin. A higher result on the test corresponds to less antibiotics present. Only two of the reactors where tested due to time and resource restrictions.

From these results, it indicates that there is degradation of the antibiotics taking place, however the data is not vary reliable, and careful consideration should be taken when using this data.

It is apparent that there are errors somewhere in the results, indicating some probable contaminations during the analysis of the reactor media or in the reactor themselves. The water blanks should not have any antibiotics in them at all and the control reactor was not attached to the voltage source so there shouldn't be such fluctuations. A possible source of error was during the analysis of the effluent, the test requires that the wells containing the sample be shaken and emptied, and it possible that there was some contamination of the wells during that step. Another source of error would be found in the reagents, which could have settled and needed to be shaken before use. During the construction of the reactors, the control reactor was leaking, and had to be sealed with an epoxy, which could have effects on the results from that reactor. Further analysis and repetition would need to be performed to verify the findings.

A second test of the reactor was run, with the same control and active reactor as well as a control media in a beaker instead of in the reactor. The time run for the experiment was one hour with collection taking place every half hour as well as the initial start time, in which approximately two milliliters was extracted using a syringe and placed in 2 milliliter microfuge tubes and stored at -5^{o} C. The same analysis kit (ELISA Antibiotic Test Kit from Green Spring) was used, taking care to not contaminate the wells.

Results are not conclusive, and the same sources of error from the first experiment are also present in this experiment. This data suggests that the ELISA test kit is not an accurate method to measure the concentration of the CTC (Chlortetracycline), and as such is not reliable for the determination of the electrochemical effects on the removal of antibiotics. An alternative method that

could be used would be a HPLC (high performance liquid chromatography) system, which has the potential to track not only the CTC more accurately, but the hydrogen peroxide and Fenton's reagents as well.

Appendix:

6. ELISA procedures

Instructions

1. Bring all reagents and micro-well strips to the room temperature (20-25 ℃).
2. Return all reagents to 2-8 ℃ immediately after use.
3. The reproducibility of the ELISA analysis, to a large degree, depends on the consistency of plate washing. The correct operation of plate washing is the key point in the procedures of ELISA.
4. For the incubation at constant temperatures, all the samples and reagents must avoid light exposure, and each microplate should be sealed by the cover membrane.

Operation procedures

1. Take out all the necessary reagents from the kit and place at the room temperature (20 to 25 ℃) for at least 30 minutes. Note that each liquid reagent must be shaken to mix evenly before use.
2. Dilute the six standard solution separately: take 50 μL Standard solution 1(2, 3, 4, 5, 6) to 450μL the diluted redissolving solution, shaken to mix evenly. The 6× standard solution will be: 0 ppb, 0.05 ppb, 0.15 ppb, 0.45 ppb, 1.35 ppb, 4.05 ppb
3. Take the required micro-well strips and plate frames. Re-sealed the unused microplate, stored at 2-8 ℃, not frozen.
4. Solution preparation: dilute 40 mL of the 20× concentrated washing buffer with the distilled or deionized water to 800 mL (or just to the required volume) for use.
5. Numbering: number the micro-wells according to samples and standard solution; each sample and standard solution should be performed in duplicate; record their positions.
6. Add 50μL of the sample or standard solution to separate duplicate wells, and add 50 μL of the antibody solution into each well. Mix gently by shaking the plate manually, seal the microplate with the cover membrane, and **incubate at 37 ℃ for 30 minutes**.
7. Pour liquid out of microwell, add 250 μL/well of washing buffer for 15-30 seconds, repeat four to five times, then flap to dry (if there are the bubbles after flapping, cut them with the clean tips).
8. Enzyme conjugate: Add enzyme conjugate, 100 μL each well. Mix gently by shaking the plate manually, seal the microplate with the cover membrane, and **incubate at 37 ℃ for 30 minutes.** Repeat the last step(step 7).
9. Coloration: add 50 μL of the substrate A solution and then 50 μL of the B solution into each well. Mix gently by shaking the plate manually, and **incubate at 37 ℃ for 15 minutes at dark for coloration.**
10. Determination: add 50 μL of the stop solution into each well. Mix gently by shaking the plate manually. Set the wavelength of the microplate reader at 450 nm to determine the OD value (Recommend to read the OD value at the dual-wavelength 450/630 nm within 5 minutes).

References:

[i] Pignatello, Joseph, Esther Oliveros, and Allison MacKay. "Advanced Oxidation Processes for Organic Contaminant Destruction Based on the Fenton Reaction and Related Chemistry." *Critical Reviews in Environmental Science and Technology*. (2007): n. page. Print.

[ii] Feng, Chun-Hua, Fang-Bai Li, Hong-Jian Mai, and Xiang-Zhong Li. "Bio-Electro-Fenton Process Driven by Microbial Fuel Cell for Wastewater Treatment." *Environmental Science & Technology* 44.5 (2010): 1875-880. Print.