DISCLAIMER

This project was conducted with financial assistance from a grant from the Metropolitan Water District of Southern California (Metropolitan), the U.S. Bureau of Reclamation, the Environmental Protection Agency, the Central Arizona Project, the Southern Nevada Water Authority, the Southern California Gas Company, and the Western Resource Advocates through Metropolitan's Innovative Conservation Program (ICP). The ICP provides funding for research to help document water savings and reliability of innovative water savings devices, technologies, and strategies. The findings of this project, summarized in this report, are solely from the project proponent.

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ICP Final Report

Treatment of Poultry Wastewater and Municipal Secondary Waste Water Using the Advanced Oxidation System (AOS)

Agreement #167584

January 31st, 2018

BioLargo Water, Inc.

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Project Duration: 12 months

Samples:

- 1st source of wastewater: Chill tank wastewater from an Alberta Poultry Producer
- 2nd source of wastewater: Secondary municipal effluent from a California wastewater treatment plant
- 3rd source of wastewater: Downstream poultry wastewater (chill tank, scalding and clean in place streams) from a California Poultry Producer

Project Deliverables:

- **Deliverable 1 (April 30th, 2017):** Procurement, Shipping and Pre-treatment of the 1st and 2nd water sources
- **Deliverable 2 (June 30th,2017):** Testing and analysis of the 1st and 2nd water sources using the AOS reactor
- **Deliverable 3 (July 31st. 2017):** Procurement, Shipping and Pre-treatment of a 3rd water source
- **Deliverable (September 30th, 2017):** Testing and analysis of the 3rd water source using the AOS reactor
- **Deliverable 5 (January 31st, 2018):** Final project report

Executive Summary

BioLargo Water (BioLargo) is developing the Advanced Oxidation System (AOS), a water and wastewater treatment technology that can inactivate bacteria and oxidize organic compounds more effectively and inexpensively than other comparable systems. BioLargo has recently prototyped and demonstrated the AOS concept into a continuous flow-through reactor designed for deployment across multiple industries including oil & gas, food processing, agriculture and domestic water/wastewater. The primary goal of this project was to develop and optimize the AOS technology for the poultry industry's wastewater needs. Poultry waste water from Alberta and California based producers was successfully treated to meet the USDA guidelines for chiller tank water conditioning (recycling). Successful implementation of the AOS treatment train in the California market, is forecasted to lead to water savings in the range of 4 billion liters per annum.

Moreover, another project goal was to also assess the AOS technology for the treatment of municipal secondary wastewater effluents. Under that task, the feasibility of the AOS as a tertiary treatment technology was demonstrated. Based our results and some operating and scale up assumptions, the AOS technology is forecasted to be 50% more efficient on energy consumption per a metric cube of water in comparison to other incumbent technologies.

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Introduction

Broiler chicken production is one of the most water intensive downstream food processes. In the past 30 years, the US has seen a doubling in chicken consumption per capita, leading to the enforcement of stringent production practices to ensure the safety and quality of the product. Due to increasingly strict regulations, high volumes of water are now required during production to ensure pathogen reduction levels are maintained and avoid outbreaks. According to a 2001 survey conducted by the US Poultry and Egg Association (USPEA), facilities use on average 26.5 L of water to process a 2.3 Kg bird. This leads to a daily consumption of 5 million liters of fresh water in each of these facilities. This water is uniquely characteristic in its high biological and chemical oxygen demand content (BOD & COD), fat oil and grease (FOG) content, high levels of total suspended solids (TSS), inorganic contaminants from cleaning detergents, as well as a high pathogenic bacterial load. Disposal of the wastewater into the sewer system usually subjects these facilities to large daily fines. Many facilities have opted to implement some form of wastewater treatment to decrease their BOD and TSS loads before discharge and subsequently lower their fines. However, much greater water and monetary savings can be achieved if the water is reconditioned to a level that would allow recycling. According to USDA guidelines, the reconditioned water can be mixed with fresh water at 90% ratio and recycled back into the process if it meets the water quality detailed in Table 1.

Table 1: Water Reconditioning guidelines for chiller water, USDA

Total Plate Count (cfu/mL)	**Total Coliforms (cfu/mL)**	**Fecal Coliforms (cfu/mL)**	**Turbidity (NTU)**	**Free Chlorine (ppm)**
<500	0	0	<5	>1

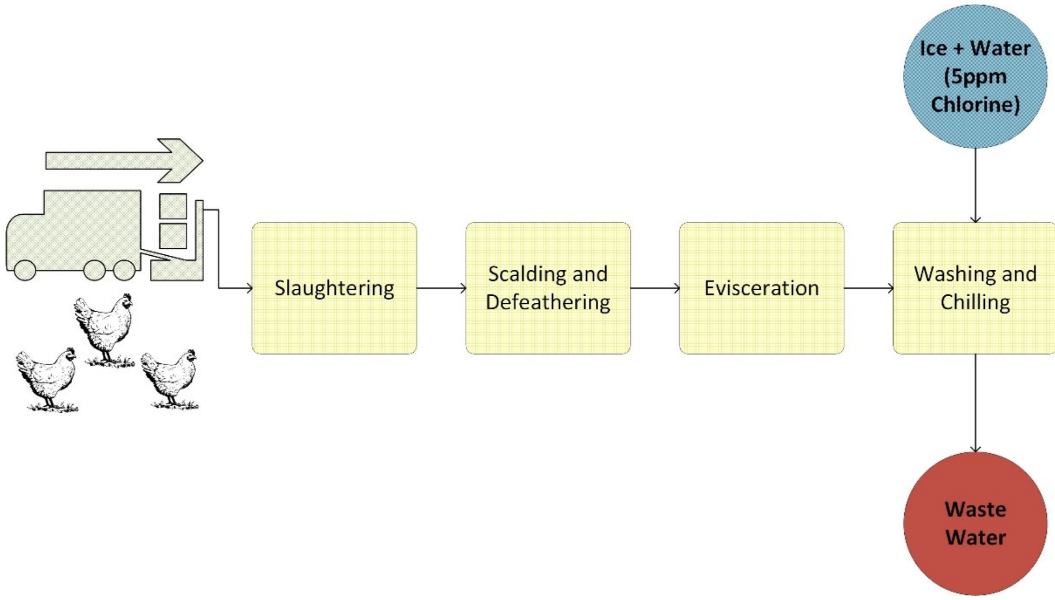

Figure 1: General broiler chicken process

Conventional treatment trains used for the reconditioning of poultry waste water include three major types of technologies:

1. Screening and filtration technologies to reduce solid content;
2. Coagulation, flocculation & aeration technologies to reduce BOD and TSS content; and
3. Disinfection technologies to reduce bacterial loads.

The AOS technology is capable of effectively disinfecting and decontaminating various matrices of waste water, and subsequently leads to a reduction in both bacterial load and organic content at lower cost due to its low energy consumption. The disinfection capabilities of the AOS with regard to total coliforms made poultry wastewater an attractive contender to demonstrate the AOS' ability to achieve great energy and water savings.

Another market that was identified where the AOS could potentially lead to significant energy savings was tertiary treatment of municipal wastewater's secondary effluent. In current practice most, wastewater treatment plants opt to further disinfect their secondary effluents by using one, or a combination of these three technologies: UV, Ozone, and Chlorination. In some cases, the energy requirements to operate these tertiary treatment technologies can be up to 30% of the total plant energy consumption. Accordingly, given the AOS' small energy requirements for comparable disinfection, it presents a potentially new and attractive solution that can match the disinfection performance desired for discharge with lower energy cost.

Project Goals:

1. **Treat poultry wastewater using the AOS to USDA reuse water quality standards, and advise on potential water and energy savings for the state of California.**
2. **Treat a secondary municipal effluent using the AOS and forecast potential energy savings in comparison to incumbent technologies.**

What is the AOS

The AOS technology functions by utilizing iodine electrochemistry in a closed graphite matrix and has been shown to exhibit great capabilities in water disinfection and decontamination.

The AOS reactor can:

1- Completely remove poly-aromatic hydrocarbons,
2- Inactivate 6 logs of bacterial pathogens (Salmonella and *Escherichia. coli*),
3- Inactivate 4 logs of viruses, and
4- Partially remove naphthenic acids (up to 80%).

The system uses low levels of inputted electrical energy and potassium iodide (10 -30ppm), which serves as a biocide.

The principle by which the AOS reactor functions is both simple and long understood. Water passes through a chamber acting as an electrochemical cell, containing an anode at the proximal end and a cathode at the distal end. When the halide salt (KI) is fed into the system in-line, it is oxidized at the anode into its elemental form, iodine, then reduced back to its salt form at the cathode. Iodine, a potent chemical antiseptic, acts as the primary disinfectant in the system. As a corollary to this, we found evidence of formation of higher oxidative state iodine species such as IO_3^- at the anodic interface of the reactor, which may account for some of the disinfection achieved by the AOS reactor. Until now, the limiting factor in such systems has been the surface area of the electrodes. The AOS Reactor solves this by filling both the anodic and cathodic chambers with a novel graphitic material with both high conductivity and high surface area. The two chambers are then separated by a proprietary spacer, which acts as an insulator while still allowing sufficient transfer of ions from one electrode to the other. Altogether, iodine is formed in abundance in an AOS reactor with even minimal applied current. The rate at which iodine is formed is dependent mostly on the current passing through the system, however our results have shown that even modest levels of current are sufficient to generate high rates of bacterial disinfection. This is the basis for the low cost of power needed by the AOS Reactor.

As with any electrochemical system, the biggest challenge for the AOS Reactor is water quality fluctuation in the influent stream. Varying salt, metal, and organic content can impact the efficacy of the iodine-generating reaction, and accordingly trials using field generated samples as opposed to synthetically produced ones are essential to optimize operating conditions for varying water sources. For this project, 4 different reactor geometries/sizes were used in treating the water: the 2” and 6”, 6-chamber AOS Reactors, as well as the spiral and 12-chamber AOS Reactors, all pictured below.

Figure 2: 6"- 6 chamber AOS - Alpha Prototype

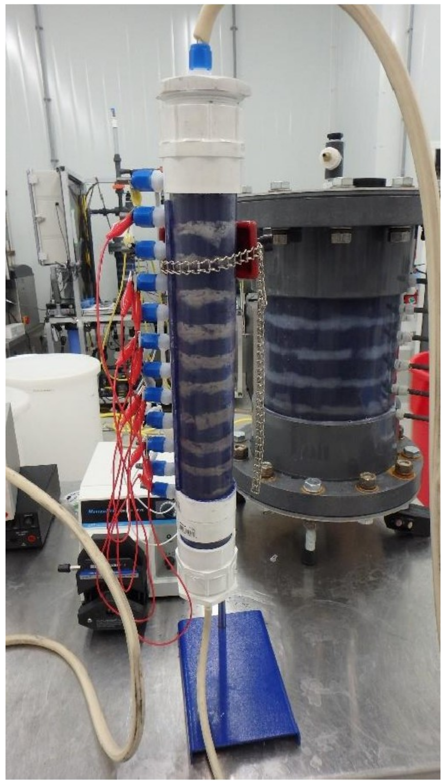

Figure 3: 2"- 12 Chamber Reactor

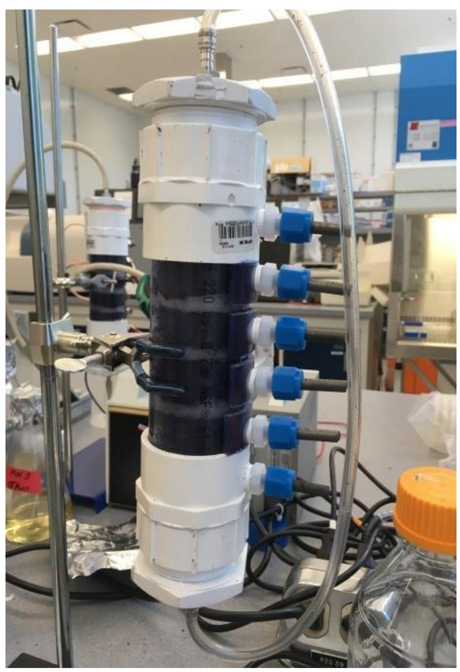

Figure 4: 2"- 6 Chamber Reactor

Treatment of Poultry Wastewater Using the AOS

Treating chill tank wastewater (Alberta)

The first wastewater stream was sourced from a poultry producer in Alberta, Canada. The stream evaluated contained only chill tank water. BioLargo received a total of 2120L of water in three installments between March and July of 2017. The totes were shipped to BioLargo Water's laboratories at the University of Alberta's Agri-Food Discovery Place for treatment. The wastewater did not require any significant pre-treatment, where only screening and coarse filters were used to remove big particulates such as skin and feathers before passing it through the AOS Reactor.

Experimental Design:

For the first two water installments, a series of 18 experiments were conducted in randomized order using two of the 2-inch diameter PVC - AOS reactors (Figure 5). This method was followed to factor in variances that can be due to packing or reactor regeneration/cleaning cycles. The investigated conditions focused on three key control parameters: flowrate, potassium iodide concentration, and electrical power (also referred to as current density) supplied to the system. The chosen values for these parameters were based on lessons learned from previous bench scale tests performed using synthetic water. A list of these experiments can be seen in Table 2:

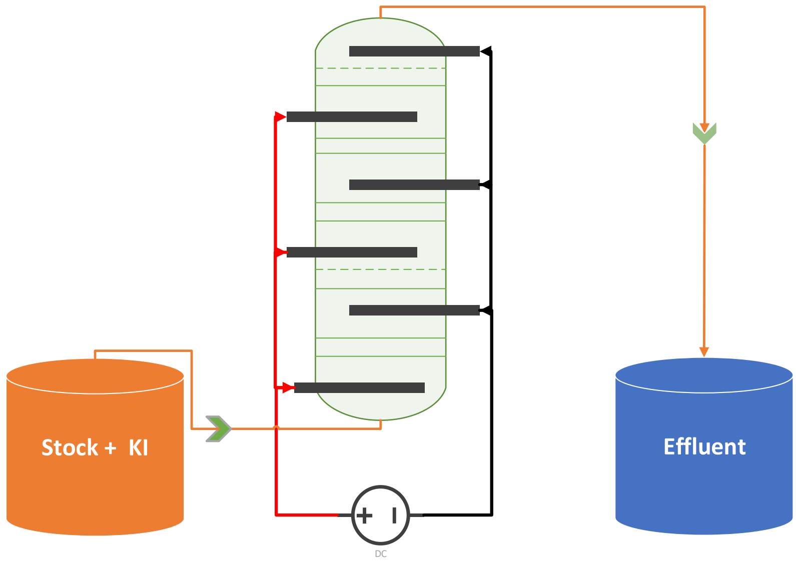

Figure 5: 2"- 6 chamber reactor experimental setup.

Table 2: Experimental conditions for chill tank water treatment using the AOS 2"-6 chamber reactor in the first and second installments.

2" Chill Tank Water Experimental Design				
Flowrate (Q) [mL/min]	**KI [ppm]**	**Power [V]**	**Experiment (randomized order)**	**Reactor used**
First Installment				
80	-	-	2	#1
80	-	8	4	#2
80	10	8	1	#1
80	10	12 (mA)	3	#1
20	-	8	6	#2
20	10	8	5	#2
Second Installment				
80	-	-	7	#1
80	-	8	5	#2
80	10	8	1	#1
20	10	8	10	#2
40	20	8	8	#1
40	30	8	3	#1
80	20	8	12	#2
80	30	8	6	#2
40	20	8	2	#1
40	30	8	11	#2
80	20	8	4	#2
80	30	8	9	#1

During the second installment a series of trials were conducted on the 6”- AOS prototype – Reactor A (Figure 6) following the conditions in Table 3:

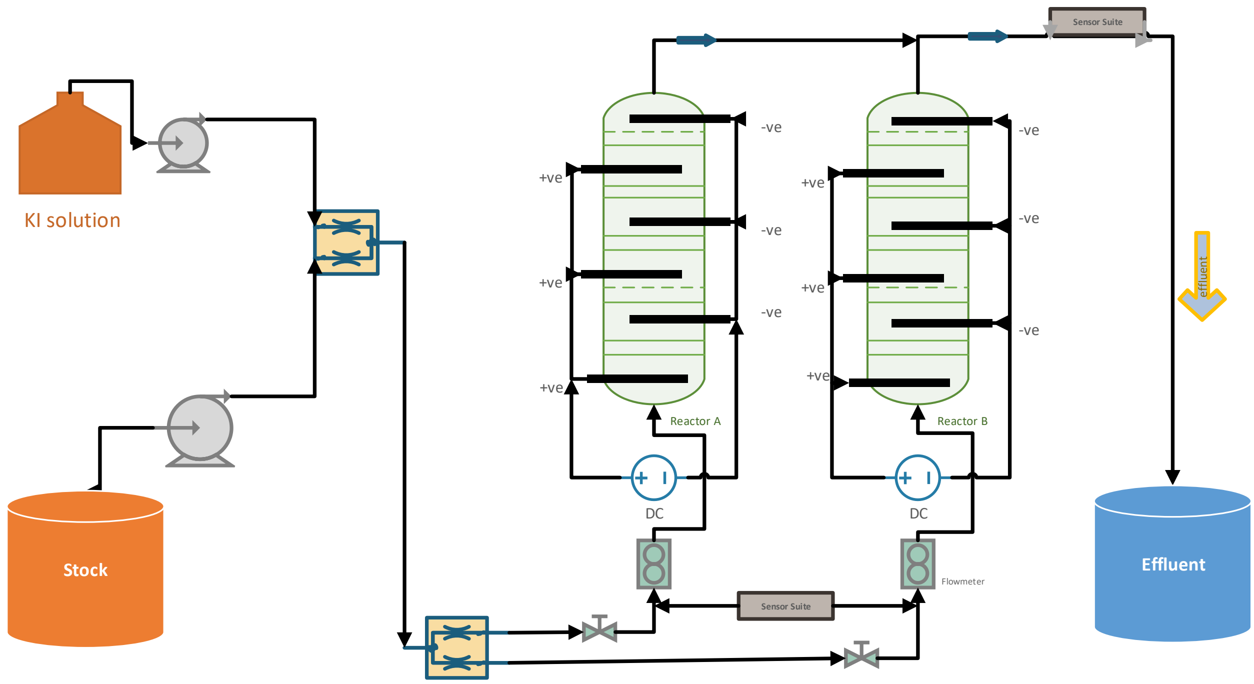

Figure 6: 2"- 6 chamber reactor experimental setup.

Table 3: Experimental conditions for poultry chill tank water on the 6" AOS prototype.

6" Chill Tank Experimental Design		
Flowrate (Q) [mL/min]	**KI [ppm]**	**Power [V]**
2L/min	10	8
2L/min	30	8
4L/min	30	8

As for the third water installment, the goal for this set of experiments on the 2” reactor was to determine: 1) if the performance suffers over an extended period of time, and 2) improvements in BOD removal when water is passed through using a second reactor. The wastewater was treated using two AOS reactors in series, with continuous flow over a 12-hour period. The optimal conditions from previous runs were selected for this phase and then ran in duplicates, along with its control, resulting in 4 experiments in total (Table 4).

Table 4: Experimental conditions for chiller tank water treatment using two AOS 2"-6 chamber reactors in series for the third water installment.

2" Chill Tank Water Experimental Design				
Flowrate (Q) [mL/min]	**KI [ppm]**	**Power [V]**	**Randomized order**	**Setup used**
80	20	8	1	#1
80	0	8	3	#1
80	0	8	2	#2
80	20	8	4	#2

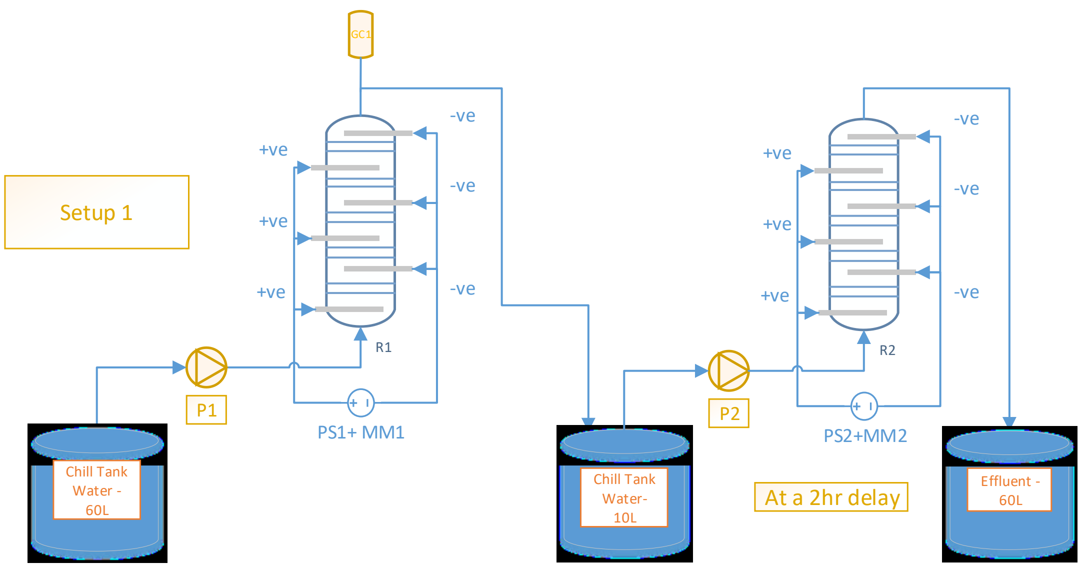

Figure 7: 2"- 6 chamber reactor in series experimental setup.

These trials were assessed using two primary metrics: reduction in total heterotrophic counts as well as reduction in BOD levels. Microbial analysis was performed in house by spiral plating 100 μL samples on LB media and then incubating them at 37 degrees Celsius. BOD samples, were analyzed by a third-party laboratory (Maxxam Analytics), using the BOD5 test protocol.

Results:

Out of the first 18 experimental conditions, we were able to determine that the optimal operating parameters for the AOS 2” reactor were: 80 mL/min, 20 ppm KI at 8V. Under these conditions we can achieve inactivation of the bacterial load to below detection limit (LoD), while still sustaining a 40% reduction in BOD. Even though lowering the flowrate to 20 mL/min achieved higher BOD removal (50 – 67%), this flowrate is not feasible for scale up engineering design where large flowrates need to be tackled in a small window of time. Moreover, we further confirmed that adsorption and electrooxidation without the addition of a potassium iodide was not effective in achieving full disinfection.

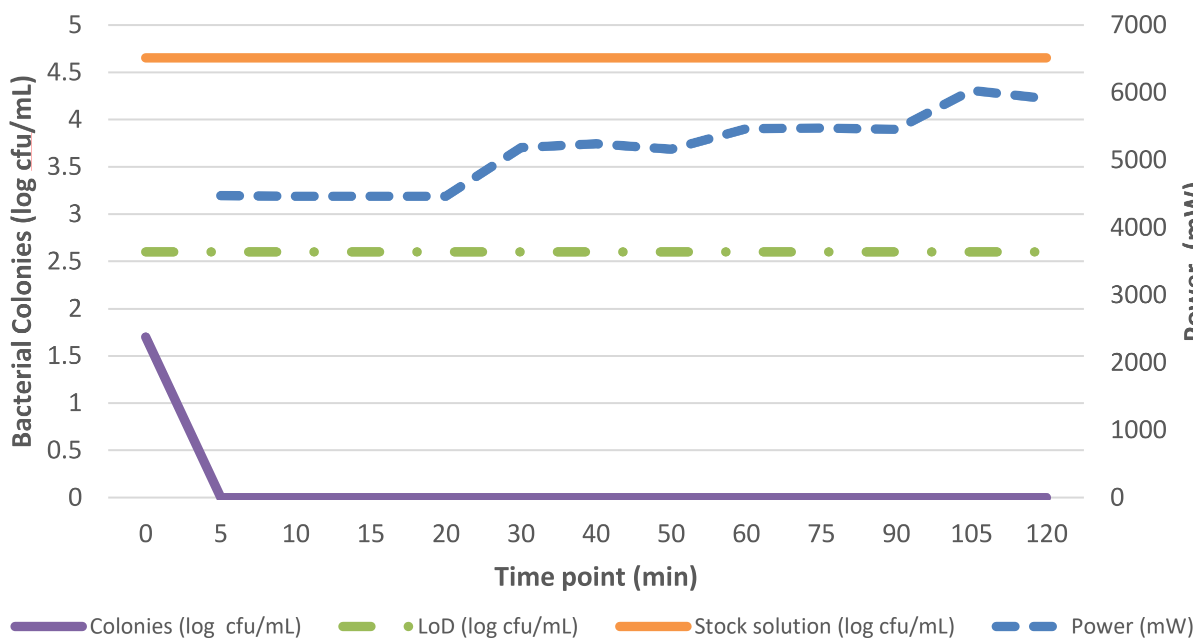

Figure 8: Bacterial inactivation in chill tank water using the AOS 2" reactor at 80 mL/min, 8V and 20 ppm KI.

The 6” reactor runs were also successful, where we found that we were able to achieve disinfection along with 50% BOD removal up to 4L/min. These results suggest that we can push the flowrate higher and maintain desired performance. Logistically however, we were unable to set up and run higher flowrates, and these trials will be reserved for field pilot tests.

Trials conducted on reactors in series, showed that the additional recycling step allowed us to enhance BOD removal from 28% to 41% on average.

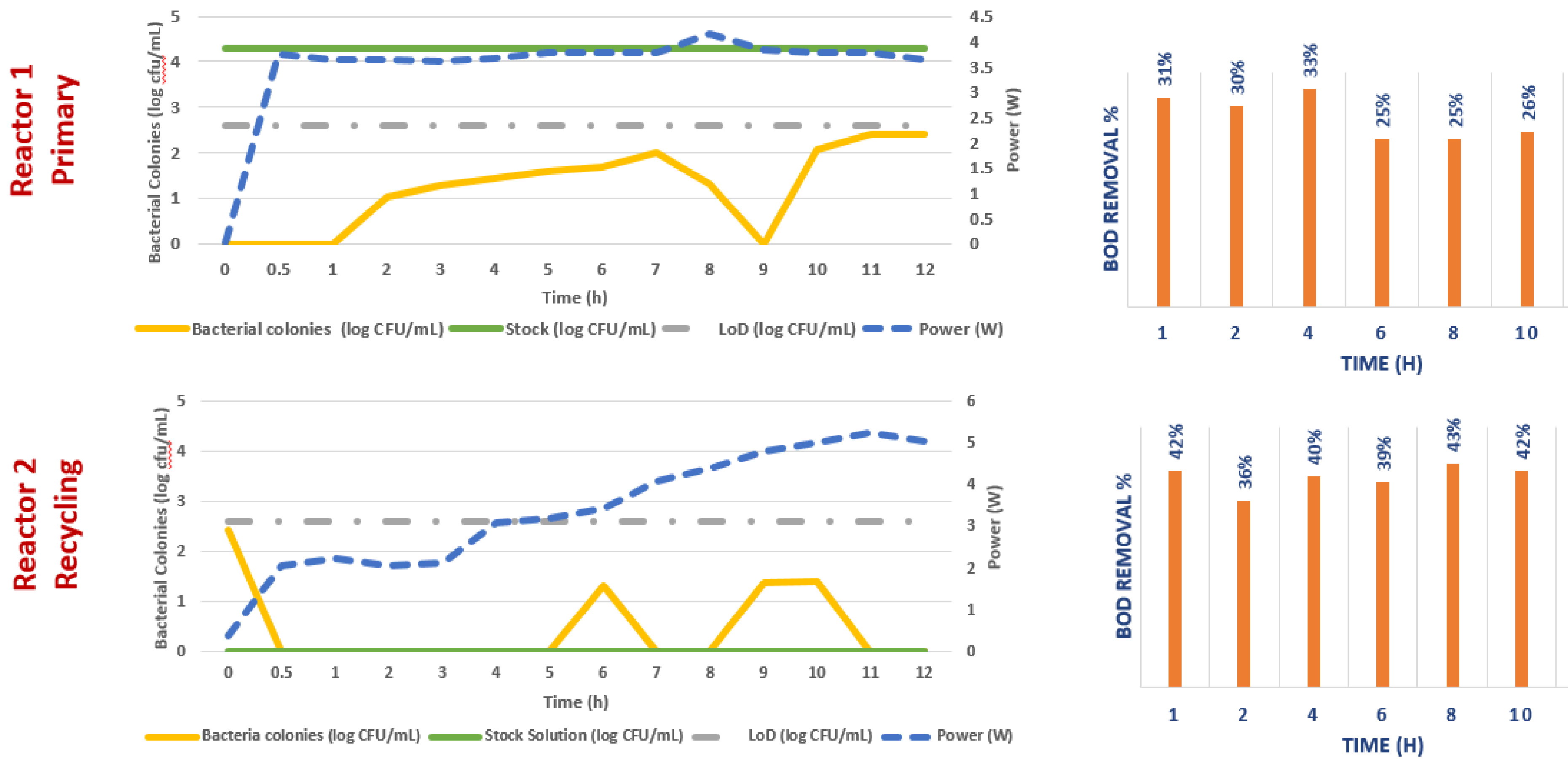

Figure 9: Bacterial inactivation and BOD removal in chill tank water using two 2" reactors in series at 80 mL/min, 8V and 20 ppm KI.

Figure 10: Chill tank stock (left) and post AOS treatment (right).

Treating a poultry mixed wastewater stream (CA)

Following the success of the chill tank water trials, a new poultry waste stream was sourced out from a California based producer. This time the source was a combination of multiple waste streams including chill tank water, scalding water and clean in place water. BioLargo received the first instalment of 30L in July of 2017, for preliminary assessment and chemical tests. The purpose of this preliminary run was to ensure that the same operating parameters used in previous phases of the project were still applicable for this source of water (i.e. flowrates, KI concentrations, and current densities). Moreover, we used this trial to assess what pre-treatment steps would be needed for the second larger shipmen of wastewater. In September of 2017, we received a second installment of 1250L of wastewater.

Experimental Design:

The first 30 L of water received was filtered using a 50-micron sedimentation cartridge to reduce suspended solids, then it was passed through the AOS 2"-6chamber reactor at 8V, 20ppm KI and 80 mL/min. For the subsequent 1250L, the wastewater was first filtered through two sediment filters in series: 50 microns to 5 microns, it was further treated with a coagulation/flocculation step using $AlCl_3$, then passed through a 1-micron filter. The goals of the pre-treatment steps were to reduce the high TSS and BOD loads and to prevent clogging of the AOS Reactor. Moreover, a small carbon filter was added after the AOS as a polishing step. Figure 11 shows the general treatment train that was implemented. A series of tests were conducted to assess the quality of water before and after the pre-treatment, AOS reactor, and post filtration. Four different AOS reactors with varying designs were tested in this phase. Table 5 lists the series of conditions that were tested.

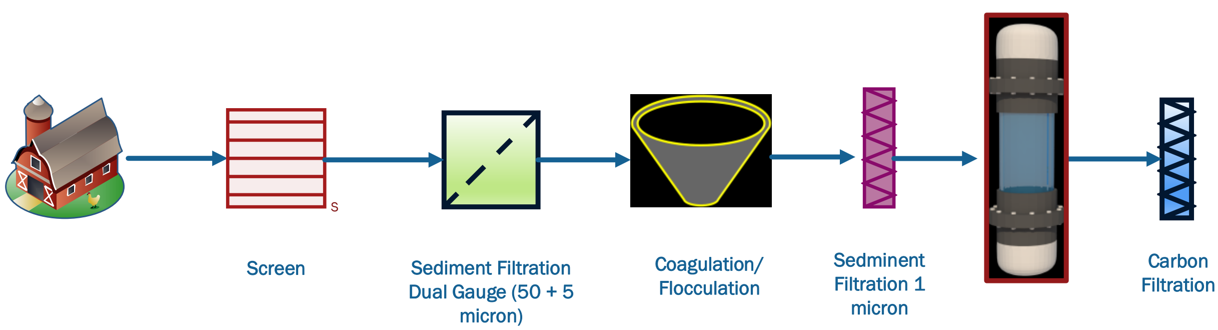

Figure 11: Proposed Treatment Train for Poultry Waste Water.

Table 5: poultry wastewater experimental conditions.

Reactor Used	Flowrate [mL/min]	KI [ppm]	Power [V]
Spiral reactor	80	30	8
2" - 12 Chamber	600	20	8
2" – 6 Chamber	80	20	8
Alpha 6" Reactor	4000	30	8

Figure 12: 1250L of poultry wastewater.

For these trials, parameters tested in house were: TSS, Turbidity, fecal coliform, total coliforms (membrane filtration) and total heterotrophic plate counts (spiral plating). BOD was again sourced out to a third-party laboratory (Maxxam Analytics) for analysis.

Results:

The preliminary run was successful, where we observed a complete inactivation of total coliforms present in the water. We also found that we could achieve a 70% reduction in turbidity and 23% reduction in BOD. The results from this phase were very encouraging and highlighted the need to implement further pre-treatment steps for the subsequent runs.

Table 6.: 2"- 6 chamber AOS performance results

	TSS [ppm]	Turbidity [NTU]	Total Coliforms [cfu/mL]	BOD [ppm]
Stock	1063	268	8.6E+06	710
After AOS	133	82	0	546

Trials using the treatment train depicted in Figure 10 were also successful, and we were able to achieve the USDA water quality goals for chill tank water reconditioning. Operating conditions were successfully scaled up from the bench scale at 80mL/min to the alpha prototype at 4L/min. Moreover, the water was treated to a 99% reduction in both microorganisms and turbidity. As for other reactor designs, it should be noted that their aim is to make the reactor more efficient and reduce the system's foot-print. The 12 Chamber reactor is the same diameter as the original design, at 2", but it's double in height. This design allowed for an exponential increase in flowrate from 80mL/min to 600 mL/min. The spiral reactor on the other hand, is only 1/10th the size of the original 2" reactor and successfully matched its performance at 80 mL/min. Results from these trials can be found in Table 7:

Table 7: AOS treatment train for poultry wastewater performance results.

	TSS [ppm]	Turbidity [NTU]	Total Coliforms [cfu/mL]	Fecal Coliforms [cfu/mL]	Heterotrophic Plate Count	BOD [ppm]
0.Stock	1090	647	3.30E+04	2.90E+04	3.30E+05	1300
After Pre-Treatmen	135	111	5.30E+03	4.20E+03	4.16E+04	960
After 12C	37	16	0	0	0	750
After Spiral	48	22	0	0	0	760
After Alpha	7	5	67	96	750	770
After Post Filtration	12	2.81	0	0	5	540

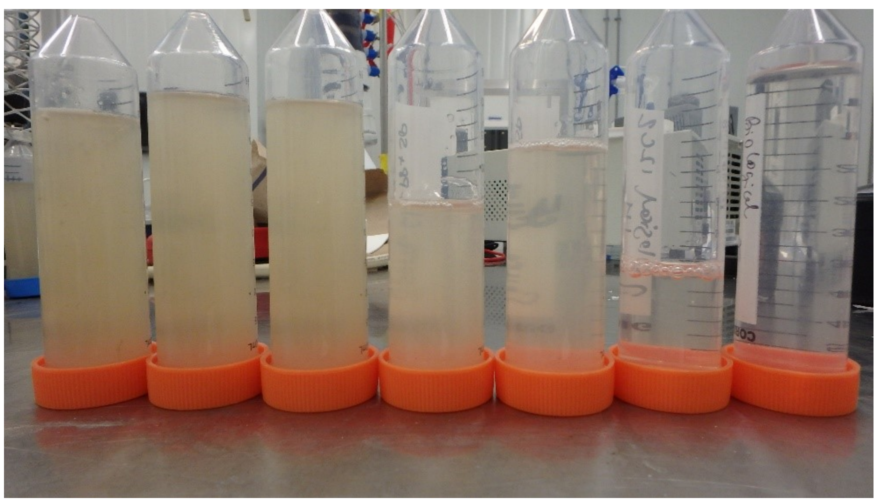

Figure 13: Poultry Wastewater samples after each step of the treatment train

Figure 14: 50-micron filter post treatment (left) and 5-micron filer post treatment (right)

Potential Water and Energy Savings in the California Poultry Industry:

In the United States, an average sized broiler producer processes around 200,000 birds per day for 250 days of the year. In 2014, California poultry processors made up 3% of all poultry in the US, slaughtering 244 million broiler chickens that year. The minimum water limit required by HACCP in poultry production is 9.5L per bird, however the USPEA found in their survey that average water consumption is around 26.5L per bird. This means that the California poultry industry uses on average 6.5 billion liters of fresh water per year on average. With California's growing population and mounting droughts, significant pressure is put on industrial operators to reduce their water spending by implementing wastewater treatment processes and reuse measures. Consequently, a large economic burden is placed on producers, from buying fresh water to paying discharge fees on the heavily contaminated wastewater stream.

Assuming a case study with an average sized poultry producer operating at the minimum water spending limit, with a chill tank operation accounting for 6% of their water use as listed below.

Birds/day	200,000
L/birds	9.5
Total water spending [L]	1,892,705
Chill tank water spending [L]	115,200
AOS Flowrate [L/min]	16
Shift length [hr]	8
Number of AOS Reactors	15

If the AOS reactors were implemented to treat and reuse the chill tank water effluent, this would require 15, 6" AOS reactors operating each at 16L/min. Assuming the AOS delivers water quality commensurate with USDA standards, then 1L of fresh water is needed for every 1.1L of reconditioned water to be recycled back into the chill tank. This means treating and recycling 90% of the chill tank water volume, or in our case 104,727L of reconditioned water daily. This leads to water savings of around 26 million liters for this facility per year and projected savings of 360 million liters per annum for California producers in general. It should be noted that if the whole process waste stream is treated and not only the chill tank water, much greater water savings can be achieved around the state, in the range of 4 billion liters per annum.

In addition to these water savings, significant monetary savings would also be made upon implementation of AOS for poultry water reuse. Assuming the national price of fresh water in the US is $1.77/1000 gallon (though this figure is certainly much larger in California) and discharge fees are \$2.81/1000 gallons, every 1000 gallons of water used by a producer are costing the them \$4.58. The cost of operating 15 AOS reactors, considering KI and electrical power, would be 1 cents/1000 gallons. This leads to potential savings of \$4.8 million for the state of California through simple recycling and reuse of poultry's chill tank water using our AOS water treatment platform.

Treatment of Secondary Municipal Effluents

For the second objective of this project, we started with a preliminary performance assessment of the AOS for the disinfection of municipal wastewater. The goal was to achieve removal of total coliforms, fecal coliforms, and total *Enterococci* to below the levels required by California Reuse Standards (Title 22 2.2: Unrestricted Reuse) of < 2.2 MPN/100ml. Secondary effluent samples were provided by a California wastewater treatment plant in four 30 L installments between March and July of 2017.

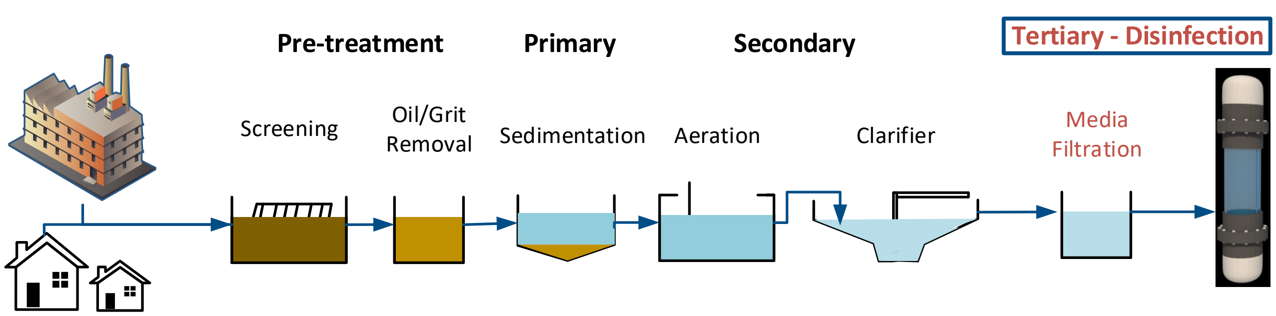

Figure 15: Generic municipal wastewater treatment plant with the AOS as the tertiary treatment step.

Experimental Design:

A 2-inch diameter PVC - AOS reactor was used to run a series of 9 experiments shown in the table below. Analysis for disinfections were performed in-house using membrane filtration and selective media. BOD analysis was completed by a third-party laboratory, Maxxam Analytics.

Table 8: Secondary wastewater 2" AOS experimental conditions.

Flowrate (mL/min)	KI (ppm)	Power (V)	Repeats
100	10	6	1
100	10	3.3	1
100	10	12 mA	1
80	10	6	2
80	0	6	2
80	0	6	2

Results:

The most efficient treatment conditions were at 80 mL/min, 6V and 10 ppm KI (Table 9). These preliminary results were very encouraging and suggest that the AOS would be a competitive option to other tertiary disinfection technologies. Moreover, it suggests that this water matrix should be further scaled up and tested on the AOS prototype to complete the technical assessment.

Table 9: 2" AOS performance with secondary wastewater at 80 mL/min, 10 ppm KI and 6V.

	Total Coliforms [cfu/100 mL]	Fecal Coliforms [cfu/ 100 mL]	Total *Enterococci* [cfu/100 mL]	BOD [ppm]
Stock	3.80 E+04	300	63	11

Post AOS	≤ 1	≤ 1	0	3.2

Potential Energy Savings in Tertiary Municipal Water Treatment:

The AOS reactor is less energy intensive compared to other disinfection technologies, such as UV and ozone-based systems. Additionally, it requires far less maintenance cost than chlorination. A UV/AOP (96% UVT/850mJ/cm^2) unit operation at a wastewater treatment facility usually has a specific energy consumption of 0.056 kWh/m^3. If the energy cost in for the municipal treatment plant was assumed to be $0.09/kWh, the treatment cost due to energy consumption would be $5.1 per million liters of water. Moreover, if chemical and pumping costs were also factored in, the total treatment cost would be $8.7 per million liters using UV/AOP.

As for ozonation, assuming on-site ozone generation and a dose of 15 mg/L, the total specific electricity consumption will be around 0.23 kWh/m^3 or $20.57 for a million liters.

On the other hand, the forecasted numbers for an AOS reactor show a specific oxidation energy, consumption of only 0.019 kwh/m^3 or 1.69$ for a million liters. When chemical and pumping costs are factored into the AOS operation cost, the total treatment price jumps to $7.45 per million liters.

If we take as an example the sanitation districts of Los Angeles county, the wastewater treatment plant treats 624 million liters a day. If we were to treat the water using the estimates provided in the previous paragraphs, we would find that with the implementation of the AOS the facility can increase their savings to $777,450 per annum or in other words reduce their energy spending by 8.6 million kWh per year.

With more than 900 wastewater treatment plants and an average of 4 billion gallons of wastewater generated daily in the state of California, the AOS technology could present an effective and cost-efficient alternative to market-available tertiary treatment technologies, and stands to afford major water and costs savings to the Californian municipal wastewater market.

Conclusions

This project has allowed Biolargo Water to demonstrate and scale up the treatment of poultry wastewater with the AOS treatment train. It has showcased the AOS' ability to produce energy and water savings in the State of California, which can lead to more environmentally-friendly and less costly broiler operations. As well, this work has laid the groundwork for conceptual design of a field pilot that will allow Biolargo Water to complete a full techno-economic assessment of the AOS platform in the poultry market.

As for the treatment of secondary municipal wastewater, the preliminary trials have helped the team identify another potential application where the AOS platform can bring significant energy savings while maintaining the desired level of performance. Next steps include scaling up the bench scale conditions on the AOS pilot prototype, and completing the full energy assessment for this application.

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