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Device for on-site Production of Sterile Water for Injection in a Disaster Zone

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Water For Injection Generation Device

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TITLE: DEVICE FOR ON-SITE PRODUCTION OF STERILE WATER FOR INJECTION IN A DISASTER ZONE

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Abstract:

This project sought to design and produce a device for the on-site manufacture of sterile water to be subsequently used to produce IV fluid in disaster zone. In order to accomplish this, the water produced must be pure, sterile, non-pyrogenic, and satisfy the United States Pharmacopeia (USP) standard for water for injection (WFI). Ideally, the device should be low powered, low cost, robust yet portable and deliver at least 10 liters per hour. Our design incorporated the purification methods of carbon filtration, reverse osmosis and Ultra-violet treatment. However, due to power and cost constraints our device was neither able to produce 10 liters per hour nor produce fluid sterile enough to satisfy USP 24 standards. Further improvements should include a more powerful pump for double pass reverse osmosis, more robust frame structure, portable power source and improved equipment sterilization techniques.

TABLE OF CONTENTS　　　　　　　　　　　　　　　　　　　　　　　　Page

I.   INTRODUCTION

- Motivation:

Disasters happen around the world on a frequent basis. Numerous teams of responders have been formed to provide medical care to victims at disaster zones. However, logistical constraints limit the quantities of essential supplies that can be carried. These supplies include blood, fuel, compressed gases (oxygen) and sterile intravenous fluids. Blood is impossible to manufacture, fuel requires a highly technical process, and devices to concentrate oxygen, thus allowing for greater quantities to be transported, already exist on the market. [2]

Hence, a device for on-site preparation of sterile intravenous fluid from potable water and electrolyte would greatly enhance emergency care. Usually, there is no electric power at a disaster site for a long period of time so developing a non-electric or low powered fluid system would be ideal. These could be low-cost, single patient, gravity-powered ultra-filtration systems (similar to filtered drinking straws used for hiking) or a bulk fluid purification system using non-electric power (mechanical energy) and involving several stages of reverse osmosis, ultrafiltration, and sterilization to produce sterile water for injection. In designing this system cost and weight are major criteria. The ideal device can be built by developing nations using indigenous technology and should be capable of being transported by two healthy adults. The end product of this system should be sterile, pure, non-pyrogenic water, meeting the United States Pharmacopeia (USP) standards, to which electrolyte can be added to produce intravenous fluids. [2]

- Advantages:

This device would greatly decrease cost of emergency relief, as there would be less need to transport IV fluid to disaster zones. It would also increase the level of care in disasters. Moreover, this device could have applications in the military.

- Applications:

Potential for use in anesthesia, emergency disaster medical relief, rural medical clinics or hospitals or even simply produce clean drinking water in developing countries.

RESEARCH

- USP Standards:

The United States Pharmacopeia defines several types of water and specifies the qualifications for sterility and packaging methods. These include Purified Water, Water for Injection, Sterile Purified Water, Sterile Water for Injection, Sterile Bacteriostatic Water for Injection, Sterile Water for Inhalation, and Sterile Water for Irrigation. However there are two basic forms of water preparation, Water for Injection and Purified Water. For our purposes we will need to follow the analytical standards required for the former. [3]

The analytical standards for USP water have been significantly streamlined. In the current USP 24, water for injection must satisfy the following analyses: conductivity, total organic carbon (TOC) and bacteria. As a result, several categories of treatment warrant examination. These include ion reduction, filtration, dechlorination, bacterial control and removal of specific impurities. [3]

WATER PURIFICATION STANDARDS

Pharmacopeia Grade Water			
	USP 24 - Purified	EP - Purified	USP 24 -WFI*
Conductivity (MicroSiemens)	<1.3 uS/cm at 25 C	<4.3 uS/cm at 20 C	<1.3 uS/cm at 25 C
Bacteria	< 100 CFU/ml	<100 CFU/ml	<100 CFU/L
Endotoxin(EU)	-	<0.25 EU/ml	<0.25 EU/ml
TOC	< 500 ug/L	< 500 ug/L	< 500 ug/L
Nitrates	-	<0.2 ppm	-
Heavy Metals	-	<0.1 ppm	-

* - Prepared by distillation or reverse osmosis

Figure 1 – Figure illustrating USP 24 standards required for water for injection (WFI).

- Filtration Techniques:

We will begin our process with source water that complies with drinking water standards as defined by the United States Environmental Protection Agency in the National Primary Drinking Water Regulations. Since U.S. cities subject their city water to these standards, any potable water in the United States will comply with the standards previously mentioned. Although the water is safe to drink, there is still a range of bacteria

and other contaminates in the water that must be removed before the water can be safely used for injection. The primary required treatment for potable water is the reduction of ion content in the water, since virtually no water source will ever meet the conduction standards by default. Fortunately, we will be using membrane technology in our system, and therefore reduction of TOC will occur during the same processes that will reduce conductivity. [3]

Carbon filtration can be used for dechlorination of water and thus reduce conductivity and TOC levels. Activated carbon possesses an extremely high level of surface area to bond with free chlorine found in water via a chemical reaction. This reaction will yield hydrochloric acid, and carbon monoxide or dioxide. A small drawback to using carbon for filtration is that over time, bacteria colonize within the carbon, and reduce the level of filtration. The cleaning process involves using steam or hot water to sanitize the carbon, which is fairly simple to do however it requires time and the availability of hot water. An easy, but slightly more expensive, solution to this problem is to incorporate UV light before and after the carbon filter. This process will help prolong the amount of time between cleanings. [3]

UV light can also be used for dechlorination. UV light at a very specific 254 nm wavelength has been tested to destroy free chlorine. UV light is also capable of destroying chloramine, but it needs to be adjusted to a much higher dosage. Due to the high levels of UV light needed to destroy chloramine, it has been shown that adding an oxidant to the UV treatment can be beneficial for chloramine removal. An advantage to using UV light is it destroys the bacterial colonization ground, which is vital to keeping the system as clean and sterile as possible. [3]

The final method is relatively inexpensive and uses the injection of reducing agents to dechlorinate the water. This method is beneficial because the only cost is purchasing the reducing agent, and nothing else. However this cost is spent each time dechlorination occurs. This method also produces harmful gases and certain organisms, which thrive in the reduced environment. It is extremely important that the reducing agent levels are kept as low as possible to help avoid the promotion of growth of these organisms. [3]

The next important step in our process is ion removal to address the conductivity criterion. There are two main ways in which to reduce the ion levels in water. The first involves reverse osmosis in which the water will be passed through a semi-permeable membrane. This process is such a key contributor in the water purification process, since the reverse osmosis will remove: ions, particulates, organic compounds, and organisms. The best part about using a semi-permeable membrane is that it rejects a certain percentage of ions regardless of the amount found in the water. This is an advantage over the other technique used of ion exchange where each ion it removes in the process must be exchanged. Membranes vary greatly in pore size, molecular weight cut off and ion removal. The membrane we will be using will be on the tight end of the spectrum, meaning pore size will be minimal and ion reduction will be at a premium. We have found that double pass reverse osmosis will almost always achieve the USP standards for conductivity. Occasional failure to reach the standards occurs when gas content is elevated. Carbon dioxide will primarily be the cause of a slightly elevated conductivity reading. However, raising the pH level of the water for the second pass, the carbon dioxide would be converted to bicarbonate ions, which will be rejected by the membrane. A quick way to raise the pH level of water is to add baking soda to the water, thus making it more basic. [3]

Ion exchange could also improve conductivity in the system. However there are still problems which make ion exchange a less attractive component to our system. Ion exchange beds will virtually always end up being colonized by bacteria, and unlike activated charcoal, which is easy to clean, ion exchange beds need to be cleaned using harmful chemicals. Adding this ion exchange step seemingly adds more of a headache to the process than a great solution. [3]

Bacterial control poses one of the greater challenges of our process. Since many of our parts are at risk of accumulating colonies of bacteria, we will need to sterilize many of our parts often. For this reason, we would like for the parts to our system to be easily cleaned, easily removable, or easily replaced. These parts should be easily cleaned using boiling water, or using a variety of suitable chemical compounds. We are less inclined to use chemical compounds because once we use them, they must be removed using a different method, which would only be adding more steps to the process. [3]

Other than our problem of limiting bacteria build up, we will also need to address bacteria that already exist in the water. Our main technique for bacteria removal will be UV treatment. We found that "Ultraviolet light at a wavelength of 254 nm and a dosage of 30,000 microwatt seconds per square centimeter will provide an approximate 6 log kill rate of most bacteria" [3]. This kill rate would be more than suitable for our system. A great advantage to using UV light treatment for the water is that cleaning will occur without ever imparting any supplemental chemical changes to the water.

- <u>Quality Control:</u>

To maintain quality and acknowledge that our system is performing as effectively as it should we would need to use sterile material and parts as well as test the product to obtain adequate feedback that the system is functioning.

To achieve this, we sterilized our tubing, parts and storage container just before construction using boiling water and dilute bleach water. This will certainly destroy any unwanted bacteria and remove any possible chemical residue.

In the manufacturing industry, another factor of quality control involves the production of sterile IV fluid in a "clean room". This refers to the air particle composition and concentration of the production and packaging room. In our situation, we hope to have our device produce sterile water for injection at a possible disaster zone. Hence acquiring a "clean room" would be quite difficult and we're aiming to have our system perform optimally without this quality control requirement. As a result we must take extra care of the parts in our system and clean them on a regular basis to ensure that the air particles are not giving us unwelcomed bacteria throughout our system. Also any parts that are exposed to the air in our system must be covered at all times, and disinfected accordingly.

II.   DESIGN AND IMPLEMENTATION

- <u>Design Considerations:</u>
    o *Straight Single Stream w/ Repeating RO Loop:*

Activated Carbon → UV Treatment → Pump → Reverse Osmosis → Storage

Considering our research, a design concept involving a constant loop of reverse osmosis treatment was developed. However, this introduced the problem of designing a differential equation to determine when equilibrium is achieved since single pass reverse osmosis treated water would eventually be mixed with double pass reverse osmosis treated water. A reverse osmosis loop needs a pump that can generate enough negative pressure to pull the water back into the system once it has passed through the RO once. A slightly more expensive alternative would be to have two separate pumps, one before the reverse osmosis, and one after to send the water back into the system.

    o *UV followed by Double RO via Double Storage:*

Activated Carbon → Pump → UV Treatment → Reverse Osmosis → Storage 1 → Pump → Reverse Osmosis → Storage 2

Given the above stated design problem, a system incorporating two storage containers and shut off valves was considered. The shut off valves would allow us to fill storage container 1 with the single pass reverse osmosis treated fluid. Then via the pump and adjusting the shut off valves accordingly, we would be able to pump the fluid in storage 1 through the reverse osmosis membrane a second time and subsequently into storage 2. Unfortunately we were unable to implement this design because we did not receive sufficient flow out of our reverse osmosis membrane. As a result we would be unable to accumulate enough water to send back through the system, and this technique would have taken far too long to reach an adequate amount of product water.
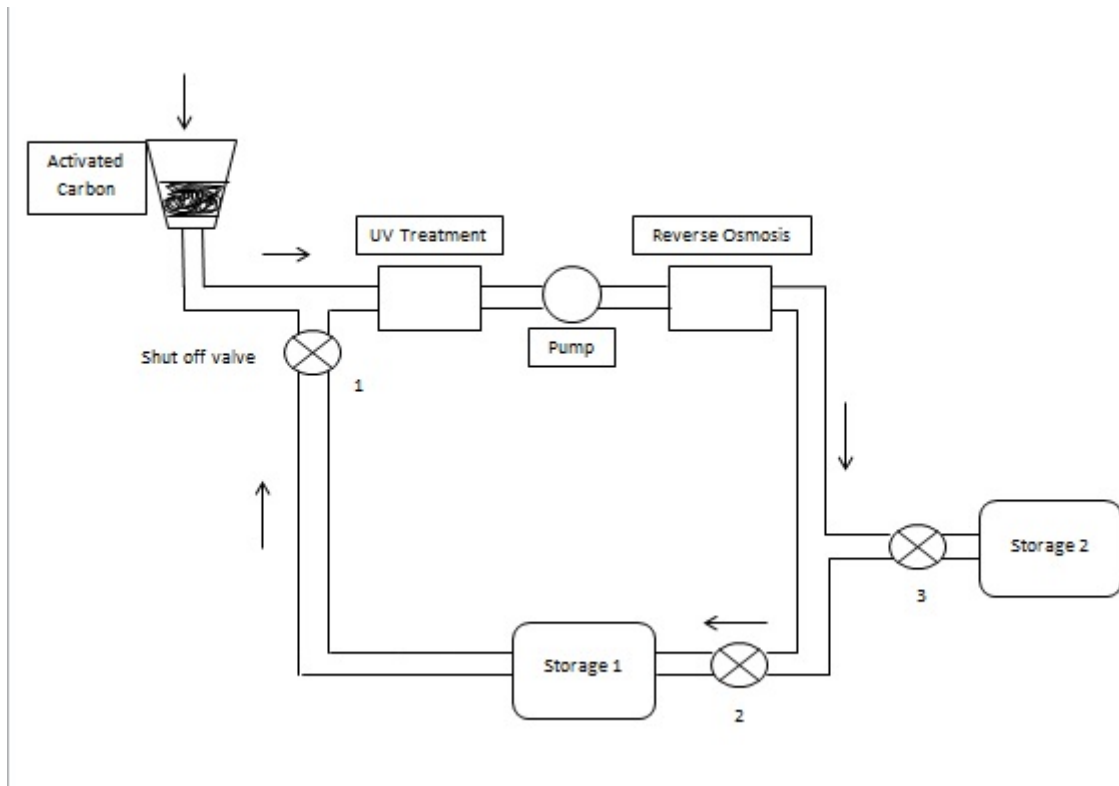
○ *Double RO and Double UV:*

Figure 2 (a) – Illustration of one of our intermediary schematic designs.

Activated Carbon → UV Treatment → Pump → Reverse Osmosis → Storage 1 → UV Treatment → Pump → Reverse Osmosis → Storage 2

The previous design had to be refined because in our attempts to minimize cost it turned out the pump we purchased was not powerful enough to apply a large enough negative pressure to Storage 1 and pull the water back into the system. Furthermore, it was not powerful enough to pull through the UV treatment and push through reverse osmosis membrane hence, we had to connect it directly to the membrane and have product water simply flow through the UV treatment system.

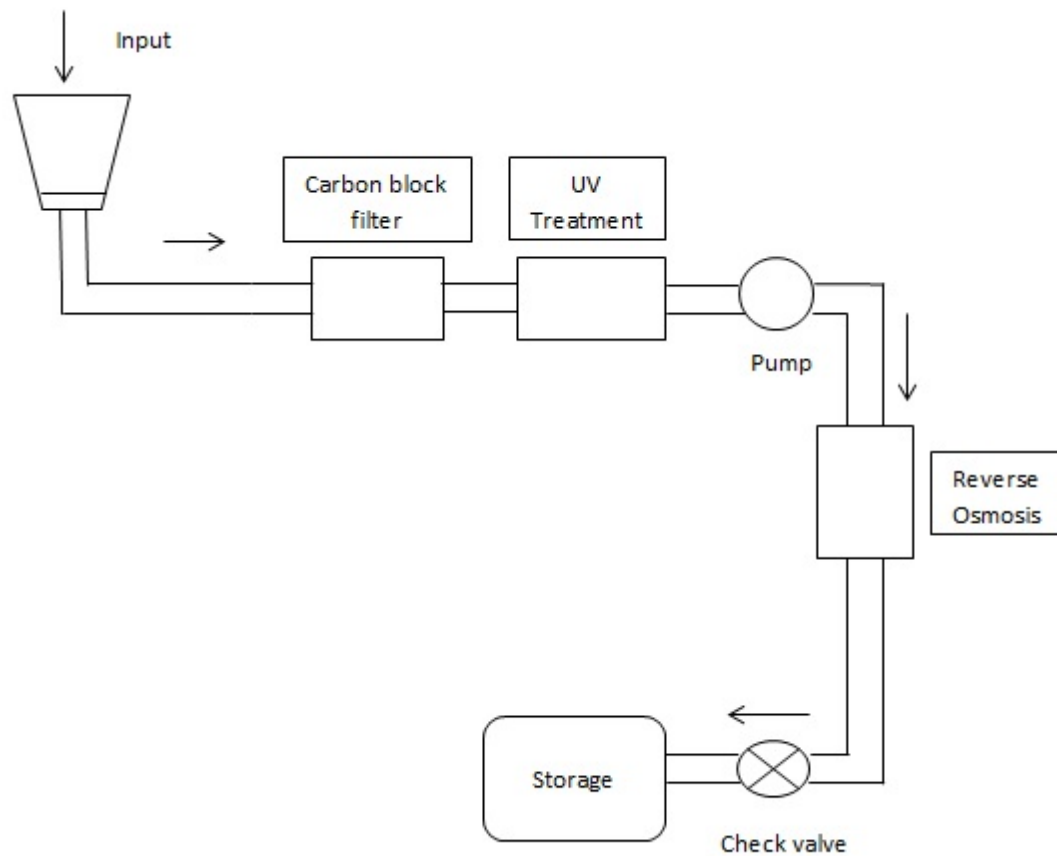- <u>Final Conceptual Hardware Design:</u>

Figure 2 (b) – Illustration of final conceptual hardware design.

Figure 2 (b) shows our final conceptual hardware design. Based on cost analysis and overall efficiency of each filtration technique we decided to combine the above techniques in this order. Before finalizing the installation of these parts, each part except the membrane was thoroughly sanitized using water with a couple caps of bleach. A quick description of the components used is as follows:

Figure 2 (c) – Photo of constructed final design.

Carbon Block Filter:

The carbon block filter as the first treatment stage dechlorinates the input water by chemical reaction with the free chlorine in water. This is necessary as the first step given our reverse osmosis membrane is a Thin Film Composite membrane (TFC) which operates ideally with non-chlorinated water. Carbon filters are also effective for total organic carbon (TOC) reduction which is one of the criteria for producing water for injection (WFI) [3]. Initially we designed this treatment stage to include a water jug, granulated activated carbon and a sieve. Carbon filtration is directly proportional to contact time; hence, the use of a large water jug

would've allowed the water to maintain contact with the activated carbon for a long period of time. However, when we attempted to run water through this stage, carbon particles were able to sneak through the sieve and clog later stages of our system. As a result, we replaced this design with a carbon block filter manufactured and donated by Culligan Water.

UV Treatment:

Our second treatment step was designated as UV Treatment. As stated in our design considerations, the pump would not have been powerful enough to push through both the UV Treatment and Reverse Osmosis stages. As a result we decided to install this stage before the pump and use gravitational energy to propel the input water through this treatment stage. This system is low cost, low powered and produces 1 gallon per minute (3.79L) thus, satisfying our major design criteria. By using UV Treatment we're able to disinfect the input water and reduce TOC. The UV light eliminates bacterial colonization ground and kills the bacteria thus preventing further reproduction [3].

Reverse Osmosis:

To complete the system, reverse osmosis was incorporated after the pump as our third treatment stage. This was done to ensure maximum pressure was supplied to the membrane to achieve maximum permeate flow. This stage possessed the double role of reducing conductivity in addition to further filtering out the shells and bodies of dead bacteria. Designing a semi-permeable membrane would be quite difficult and a senior capstone project on its own, as a result we purchased a residential reverse osmosis system and incorporated it into our device system. However, due to the extremely low flow (approximately 0.12L/hour) it was difficult to run the product water through the system again to achieve double pass reverse osmosis.

- Testing Procedure

In order to justify the functionality of our system the water produced would have to be tested for bacteria count, conductivity, total organic carbon and endotoxins. Testing for the latter two would be impossible with the equipment we have available to us however, bacteria count and conductivity can be related to endotoxins and total organic carbon respectively so choosing these

tests would be a sufficient start to evaluating our design. Pre-grown E. coli bacteria were used to contaminate the input samples to genuinely test our system.

Plate count agar, which is a rich medium, will be used to plate the product of our system and test for bacteria count. A rich medium was used to ensure that even the smallest concentration of bacteria would grow. The procedure is as follows:

- A glass rod used to spread the product water sample on the agar plate was sterilized using ethanol and a Bunsen burner.
- 1 mL was pipetted from each sample into a small test tube.
- These test tubes were placed in an ultracentrifuge to pellet the bacteria organisms.
- 0.1mL was pipetted from the small test tube onto an agar plate and evenly spread using the glass rod.
- The agar plates were placed in an incubator at 37°C for 48 hours.

The procedure to test for Conductivity is as follows:

- The handheld conductivity probe was calibrated using 10µS/cm standard conductivity solution.
- Each sample was then measured by submerging the probe in the collected product water. This conductivity was then recorded.
- The probe was cleaned using pre-made rinse in between testing each sample.

III.   RESULTS

Table 1 – Table illustrating conductivity measurements of water samples.

Sample	Conductivity (µS/cm) @ 25°C
Tap water (control)	72.6
Tap water w/ e.coli (a)	76.8
Tap water w/ e.coli (b)	77.2
Tap water w/ e.coli (c)	77.0
Product water (a)	101.2
Product water (b)	104.1
Product water (c)	101.5

Table 1 illustrates our conductivity measurements of tap water (control), input water and product water. WFI requires a conductivity of <1.3µS/cm (See Figure 1) however, we noticed an increase in our conductivity measurements from input water to product water. High conductivity does not necessarily imply the water is dirty but rather there is a high concentration of ions in the water thus making it more conductive.

Figure 3 – Picture illustrating agar plate of tap water (control)

The agar plate in Figure 3 acts as a control to our bacteria count test and confirms to us the water we began with possessed very limited bacteria.
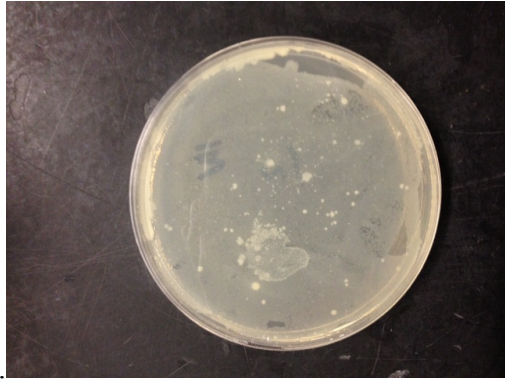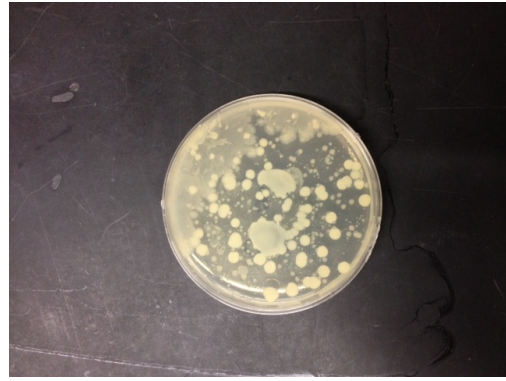
Figure 4 – Input sample 1
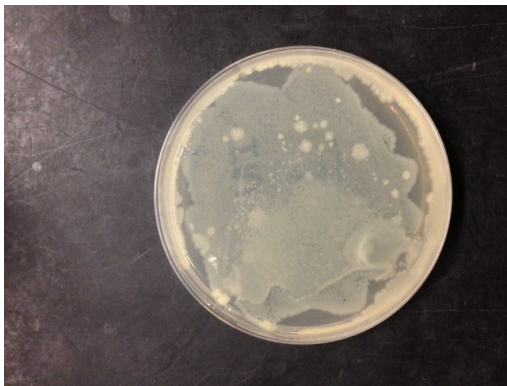

Figure 5 – Product water sample 1

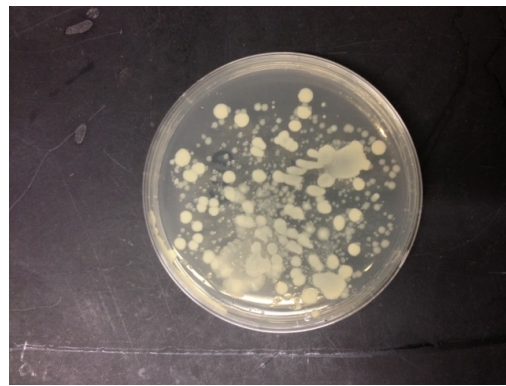**Figure 6 – Input sample 2**

Figure 7 – Product water sample 2
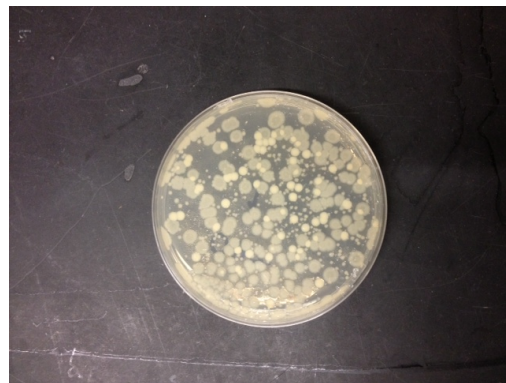

Figure 8 – Input sample 3

Figure 9 – Product water sample 3

Observing the plates of the input samples we can observe a highly noticeable increase in bacteria compared to our tap water control. This proves the contamination of our tap water with E. coli was successful. Comparing the input samples to our product water sample plates we observed a decrease in bacteria count.

IV.   DISCUSSION

USP 24 (See Figure 1) states WFI requires a conductivity less than 1.3µS/cm, a bacteria count < 100 CFU/mL, Endotoxin (EU) < 0.25 EU/mL and finally a TOC content < 500µg/L. With the resources available to us, we sought out to test for conductivity and bacteria count to evaluate the success of our system because equipment to test TOC content costs $3000 – $5000 and Endotoxin is a toxic protein that's released when bacteria ruptures or disintegrates. Reverse osmosis should certainly filter out these proteins. [1]

Observing Figure 1, there were only a few colony forming units (CFU) present indicating our control fluid was suitable for use. Then, upon comparing each input sample to their corresponding output sample there is certainly a decrease in bacteria count. The input sample had such high bacteria counts that it was able to grow as a "lawn" with the bacteria all sharing resources on the plate. But looking at the product water plates we can observe individual bacterium forming colony units on the plate. This is a clear indicator of lower bacteria counts and also indicates that our system was effective at reducing the bacteria count of our input samples. With respect to conductivity, our system's performance was unexpected but can be easily explained. The conductivity of our product water was measured to be higher than our input water. However, this can be attributed to the carbon contamination our reverse osmosis membrane endured. Carbon and salts trapped in a reverse osmosis membrane increases the conductivity of permeate produced.

Altogether, our results did not conform to USP 24 standards for WFI. While our bacteria were drastically reduced, the count was still too high and our conductivity increased. All is not lost though, from this experience there are numerous lessons in problem solving, budget and time management, and design and construction to be learnt. The next group to tackle this problem should invest a greater budget as the standards required are precise and do not allow much room for error.

V. PROBLEMS ENCOUNTERED AND FURTURE IMPROVEMENTS

- Cost:

    With a budget of $250 and limited additional funds from our project advisor, there was a lot of pressure on us to be certain about our decisions before making any purchases. This not only restricted our experimental freedom but also cost us time second guessing our designs and research. We would recommend that any further wok on a project of this magnitude should have a much bigger budget. Without at least one thousand dollars, we do not think that it is worth attempting this project.

- Technology:

    Professional manufacturers have expensive equipment to accomplish the production of intravenous fluids. However, in our case this technology is not readily available as well as we'd hope our device to perform optimally in the absence of this expensive technology. This technology includes clean rooms, in which the air particle concentration is monitored, equipment to measure total organic carbon and sterilization machinery to name a few.

- Pump and Reverse Osmosis Membrane:

    We decided to go with an 80 psi pump from the same company that we bought the Reverse Osmosis membrane from thinking that an RO booster pump would be our best choice for our system. The pump seemed like the best fit because it was made to use the same size fittings and tubing. At first our pump and RO system seemed to be working fine though our flow was still a little less than what was advertised from the pump and RO system. We began having issues with the pump when we combined the pump and RO system with our activated carbon stage. Sieves with very small openings in the mesh are fairly expensive (about 50$) and due to our budget restraint we decided that a household sieve would be sufficient to hold our activated carbon. However, when we first used the sieve it let carbon squeeze though, and we ended up having carbon in our pump and RO system. Since that time our RO system has stopped producing a steady flow or permeate (product water) and has instead began only giving us drips at a time if at all. Furthermore,

immediately after our carbon spill we realized that the pump was no longer giving us steady flow, and it was failing to pump the water at all. We took the pump apart, and cleaned all of the carbon we could find that had made its way into the pump. The pump then returned to a semi-normal state, though we could tell it was no longer pumping as it once had. The negative pressure (which was already small to begin with) was now almost non-existent. Due to our lack of pump pressure, we did not know at first that our reverse osmosis membrane had been compromised. We addressed the pump situation by using a pump generously donated to us by Professor Dressaire in series with our pump to increase the inlet flow, and thus raised the outlet pressure of our RO booster pump. This pump combination worked very well and we returned to having steady flow from our RO booster pump (maybe even better flow than previously had before anything went wrong). By the time we realized that our RO membrane had been drastically affected by the carbon, we had no time or money left to order another membrane. Consequently, we drove to Windsor, CT to meet with a pharmaceutical expert who works at Culligan Water System, and he graciously gave us another membrane to try in our system. Unfortunately the RO membrane he gave us was a different model and was incompatible with our membrane housing. We ultimately used our first reverse osmosis membrane, though our results indicate it was severely compromised.

- Sterilization:

As mentioned previously in the quality control section, pharmaceutical companies produce their IV fluids in clean rooms. Since our device is supposed to be used in a disaster zone, we don't have the luxury of performing tests in a clean room. This caused a small problem for us because we can't be sure if contaminants are entering our system from the air, or if our system itself has contaminants. We cleaned the carbon block, UV light, and our pumps by mixing a bit of bleach into a bucket of water and running the warm bleached water through all of those parts. Unfortunately, our reverse osmosis membrane is fixed in its housing, and we can't run bleached water through it because it would damage our membrane. There exists reverse osmosis membrane cleaners, and we would encourage any future participants in this study to purchase this product. Unfortunately there is no way to know with absolute certainty if the membrane is clean

by merely looking at it, but there are signs to look out for that would suggest that the membrane has been compromised. An increase in product water conductivity, salt passage, feed pressure are all signs that the membrane needs to be cleaned or replaced. Additionally, a decrease in normalized permeate flow is a sign that the membrane needs to be cleaned or replaced. Given these parameters are deteriorated by more than 30%, the membrane is passed saving, and it should be replaced. [4]

- <u>Input samples with proteins and known contaminant concentrations</u>:
  If this device were to be economically viable, it should also be able to remove harmful proteins that may be suspended in the input water. Tests using a suitable stain, to identify proteins before and after treatment should be incorporated. In addition, input samples with known contaminant concentrations should also be tested using mass spectrophotometry.

- <u>Double pass reverse osmosis</u>:
  For future improvements to the system, double pass reverse osmosis should definitely be added into the system. We were only able to do a single pass due to budget constraints, and our results suggest that single pass reverse osmosis is not sufficient to reduce the conductivity to below 1.3μS/cm. But studies have shown that multiple passes of reverse osmosis further increases purity and consequently would further reduce conductivity.

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