BTEXterminator

A GMO Product for Aid in Produced Water Treatment

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Executive Summary (Christine)

BTEXterminator is a co-culture of two *Pseudomonas putida* strains that have been genetically modified to degrade benzene, toluene, ethylbenzene, and xylene (BTEX), as well as their respective isomers. Produced water is classified as any water that is returned to the surface due to the operations and production phases of oil wells, because it is preexisting in the formation. This water is high in hydrocarbons, which includes BTEX, as well as, metal and salt. Currently, there are few remediation processes available for produced water as it is normally injected into salt water disposal wells (SWD), or simply left in an open reservoir or tank where the BTEX will evaporate into the atmosphere before it is transported to disposal, both of which are costly and time consuming. Currently, there are no regulations on BTEX evaporating, and anticipated regulations on BTEX are the need for this project. Also, the option of an SWD is becoming less popular due to the increase in seismic activity near these wells. Produced water disposal is generally one of the highest lease operating expenses operators face. There are opportunities to repurpose this water if it can be treated in a cost and energy efficient manner. As recently as 2009, it was anticipated that there are between 15 and 20 billion barrels of produced water generated in the United States annually.

Each individual *P. putida* strain naturally contains either the TOL or TOD pathway, which in combination, are responsible for the degradation of the components of BTEX. A plasmid is inserted into each strain in order to force both organisms to express both pathways actively. After successful modification is achieved, the co-culture will be established and cultivated up to scale via free cellular suspension. The product may then be transplanted into a reactor where it will be ready to treat produced water.

C&C has designed BTEXterminator with the potential to be as robust as possible; it can handle a wide range of potential water volumes based on the clients' demand, handle multiple sets of water conditions, and the product has room for further adaptation as seen necessary.

However, this is not a system that can be applied to every situation in the field. BTEXterminator will only handle one step of the clients' water remediation process, which is the removal of BTEX. Produced water will flow from a tank, reservoir, or well pad to C&C's reactor(s). The water will then run through a various amount of reactors depending on the water composition and flowrate. BTEXterminator will remove all detectable amounts of BTEX so that the operator can handle the water as seen fit.

An economic analysis was done on a per skid basis to determine the feasibility of this project. A per skid cost basis was generated with nine reactors assumed per skid. The IRR was set to 40% to back calculate a yearly revenue of $6.12 million per skid in order to attain the desired return rate. This project is not feasible when compared to the current prices of produced water disposal. However, if the anticipated regulations are put into place, then this project becomes increasingly favorable in the market.

Scope of Work (Juliet)

Design problem statement

Produced water makes up the biggest waste stream associated with conventional oil and gas production, as well as, unconventional petroleum resources. In a report drafted in 2011, it was estimated that roughly 7 to 10 barrels of water are produced for every barrel of crude oil[18]. This produced water is usually considered a waste byproduct from oil and gas production. The oil and gas industry typically disposes of produced water by three main means: land application or discharge, subsurface injection, and offsite trucking. Land discharge is fairly inexpensive, but requires the water to be of higher quality. Produced water to be discharged to the surface must follow strict regulations set forth by Department of Environmental Quality (DEQ). These regulations ensure that the receiving waters and surrounding areas do not get contaminated. Re-injecting the produced water into the ground is a good alternative, but it can lead to an increase in earthquakes if the subsurface formation does not have the capacity to receive the water. Therefore, the produced water can be trucked offsite to be re-injected into a formation that can accept it. Off-site trucking is very expensive for an operator[18]. Since large volumes of produced water are generated during oil and gas production, it should be investigated for its potential use as an alternative water source.

The characteristics of the produced water directly corresponds to the geographical location from which it originated. Furthermore, the quality of the produced water is affected by the type of hydrocarbon produced, as well as, the geochemistry of the producing formation. An analysis of produced water will find both inorganic and organic constituents. The most commonly found organic constituents in produced water are benzene, toluene, ethylbenzene and xylene compounds (BTEX). C&C can create its own niche in the water treatment industry by focusing on removing certain contaminants in the produced water, specifically the BTEX compounds. BTEXterminator is a co-culture of two *Pseudomonas putida* strains that have been genetically altered to completely degrade BTEX and its respective isomers. Removing BTEX could be the first step necessary in converting the produced water from a waste product into a viable alternative water source for numerous applications.

Constraints on the design of BTEXterminator

In the early design of BTEXterminator, C&C considered the following constraints:

- Economic
  - C&C will only remove the BTEX compounds in produced water. Therefore, the entire process needs to be as inexpensive as possible since the water will require further treatment in order to be considered an alternative water source. If the price per barrel of treated water is much lower for C&C's process, it would attract customers and make this an economically viable project.
- Environmental
  - The water exiting C&C’s process needs to meet the state regulations on BTEX levels which varies depending on how the water will be used. C&C will focus on meeting the state regulations for BTEX concentrations in drinking water. Since we are only focusing on removing BTEX from the produced water, the water will need further treatment to be safe for drinking.
  - C&C will need to consider the potential for genetic transfer between the genetically modified *P. putida* strains and any microorganisms found in the produced water. The reactor residence time is one hour, so any genetic transfer that could occur should be very slow. If C&C finds that the microorganisms found in the produced water can be altered by the *P. putida* strains, then a front-side ultrafiltration membrane and UV disinfection system would be added to the process. This front-side ultrafiltration membrane and UV disinfection system will prevent any microorganisms in the produced water from reaching the reactor housing the co-culture of *P. putida*. The back-side ultrafiltration membrane and UV disinfection system will ensure that the genetically modified microorganisms do not leave the process or enter the environment.
- Sustainability

- The goal of BTEXterminator is to remove all BTEX compounds in the produced water fed to the reactor. Although the co-culture of *P. putida* uses BTEX as a carbon source, it will need other nutrients to perform efficiently. There is a basal mineral salts medium that C&C will use during the research phase, but an alternative nutrient will need to be investigated during scale-up.
- The two *P. putida* strains aerobically degrade BTEX, therefore, C&C will need to look into a safe oxygen source for the organisms on the skid. The current design uses hydrogen peroxide as the oxygen source for the microorganisms based on lab-scale studies done by Shim[11] and his colleagues.
- C&C will need to figure out how long the *P. putida* strains can live in the harsh environments of produced water. *P. putida* was found living in an environment with high metal content; it also has a certain level of halotolerance.

- Manufacturability
  - C&C plans to be able to move all the equipment necessary to treat the produced water on a skid. Therefore, all the process equipment needs to be reasonably sized to allow for easy transportation with minimal costs.
    - The preliminary design shows that a skid-able 5000L reactor will work.
  - The materials chosen for the process equipment should minimize capital costs as well as costs associated with maintenance.
- Ethics
  - The use of genetically modified organisms is a very controversial topic in this country. As a result, C&C will need to take extra precautions to make sure the genetically modified *P. putida* strains are not released in the treated produced water. The co-culture of *P. putida* will be immobilized on the fibrous bed reactor so that a high percent of the cells remain in the reactor. The ultrafiltration membrane and UV disinfection at the end of the process will prevent the release of any genetically modified *P. putida* strains. Educating industry and community

members about the safety of our product will be important when implementation of the project begins.

- Health and Safety
  - BTEXterminator needs to remove all detectable BTEX compounds in the produced water.
  - Transporting oxygen safely unless a different oxygen source is found.

Introduction (Christine)

The operation of oil and gas companies has affected not only the world economy, but also the world's water supply. This significant effect of the petroleum industry is felt all around the world. As recently as 2009, it was anticipated that there are between 15 and 20 billion barrels of produced water generated in the United States annually.[12] Produced water is water that has been returned to surface because of drilling, completing, or producing a well. This is not water that has been injected into the reservoir, but water that was already existing in the earth. The composition of produced water is difficult to quantify, however, there are usually salts, metals, hydrocarbons, and reservoir bacteria present. Some of these hydrocarbons are harmful and there are not currently specific regulations on the components of BTEX (benzene, toluene, ethylbenzene, and xylene). That is, there are not regulations deterring operators from disposing of produced water that contain high levels of BTEX.

In the United States, it is up to the state government to set and adhere to regulations on the acceptable levels of BTEX in waters. The states of Colorado and Wyoming classify the acceptable levels of BTEX based on the use of water, and as a result the regulations for both states are very similar. C&C has set out to provide an organism capable of efficiently removing BTEX from this water so that it may cheaply be treated further to meet different specifications depending on desired use of the water.

The cost to dispose of produced water is very high for operators. In fact, it is about a third of the leasing costs associated with operations. There are anticipated regulations on the disposal of BTEX in produced water, which would make BTEXterminator a competitive product. C&C sees a lack of this technology in the petroleum industry, and the potential of BTEXterminator in that market share.

C&C has created a new product, BTEXterminator, to meet this need. Many bacteria are naturally able to degrade one or many of the components of BTEX. C&C has investigated *Psuedomonas putida*, which is the bacterium used for BTEXterminator. This bacterium was chosen because it has been isolated from the discharge of a petroleum refinery. While other bacteria could be used in BTEXterminator, C&C is confident that *P. putida* is the best choice. Through genetic modification, BTEXterminator is an effective method of removing BTEX in large volumes of produced water. BTEXterminator is a co-culture, meaning that there are two strains of *P. putida* in the product. An advantage of using a co-culture is that one strain has the natural capacity of degrading about half of the components and the other strain has the capacity to degrade the other half. Such complementary strains are BTE1 and TX1.

C&C will attempt to make BTEXterminator as robust as possible armed with the information gleaned from the formations in the region. Genetic modification will be necessary in order for the organism to effectively degrade all components of BTEX, and survive in the harsh conditions of produced water. Hydrocarbons need to be the first components removed from the water in order to undergo further purification processes because of the damaging effects of hydrocarbons on this equipment.

Once the produced water has had BTEX removed there are many possibilities for further treatment of the water in order to reach a specific purity specification. C&C has determined that the best business strategy is to make the microorganism capable of removing BTEX completely, so that it is undetectable. Regardless of the consumers' use of the water they can undergo any further processing to meet their specific needs. Environmental discharge is a possibility if the salt concentration is reduced to ground water regulations. Since Wyoming and Colorado are agrarian states, the water could be used for agriculture, which requires large quantities of water. The choice of the end use of the water is in the hands of the client.

Currently, there are some ways to bioremediate produced water, but there is not justification for the current open system processes due to the lack of regulations. Thus, BTEXterminator is a revolutionary product in a market that is anticipated to arise. C&C realizes that once there are regulations, operators will need this product to meet the required specifications of produced water before disposal. So, the current options of salt water disposal wells or leaving the water in an earthen pit or tank will not be available until the water is rid of BTEX.

Design of Base Case/Product Design (Cody)

Project Definition

C&C has created a new product, BTEXterminator, which can meet the need of ridding produced water of BTEX. The composition of produced water is difficult to quantify; however, it is known that high concentrations of salts and some heavy metals can be present. Also, there are other hydrocarbons in the water, which BTEXterminator is also capable of consuming. This information can be determined from studying the formations in which operators will be fracking. Once the produced water is characterized by region and formation, the composition will be predictable. Genetic modification will be necessary in order for the organism to effectively degrade all components of BTEX, and survive in the harsh conditions of produced water. C&C is focused on only removing BTEX from produced water. As a result of our product being specialized to this task, we really only have two process sections that we need to focus on, one of them being the production and design of BTEXterminator. The second section is the ability for BTEXterminator to perform within a reactor and remove BTEX in an industry setting.

Product Description

There are many bacteria that naturally are able to degrade one or many of the components of BTEX. In order to capitalize on this natural ability, C&C has investigated *Pseudomonas putida* and plans to use its natural abilities through genetic modification in order to produce an effective method of removing BTEX in large volumes of produced water. Some of the strains of this bacterium are naturally capable of using pathways for the degradation of components of BTEX, and thus give a great starting point. BTEXterminator is a co-culture, meaning that there are two strains of *P. putida* in the product. There are many advantages of using a co-culture. The main advantage is that strain A has the natural capacity of degrading about half of the components and strain B has the capacity to degrade the other half. Since these strains are complementary, it is very beneficial. In literature, it was found that the two strains that complement each other in this way were BTE1 and TX1.

BTEXterminator will start out as two separate strains of *Pseudomonas Putida*, TX1 and BTE1. Each strain naturally contains one of the pathways necessary to degrade particular components of BTEX within produced water. Natural expression of this pathway means that it is a chromosomally expressed trait. TX1 contains the TOL pathway, which makes it possible for the strain to consume toluene and xylene, as well as all possible isomers. BTE1 contains the TOD pathway, which is complementary to the TOL pathway by giving this strain the capability to consume toluene, benzene, ethylbenzene, and all of their respective isomers. *Figure 1* can be seen below which gives a more organized representation of each organism's ability to degrade BTEX[6].

	Growth	
Compound	*Pseudomonas putida* TX1	*Pseudomonas putida* BTE1
Benzoate	+	+
Protocatechuate	+	+
Benzene	−	+
Ethylbenzene	−	+
Toluene	+	+
o-Xylene	−	−
p-Xylene	+	−
m-Xylene	+	−
o-Toluate	−	−
m-Toluate	+	−
p-Toluate	+	−
1,2,4-Trimethylbenzene	+	−

Figure 1. Strain Pathways: It is worth noting that o-xylene is not known to be consumed by either pathway but has such a low selectivity that it is usually not present in detectable amounts, as is true with o-toluate. [6]

(Christine Chemistry Section)

The degradation of aromatic compounds is very complicated, as *Figure 2* shows. However, the pathways can be simplified because each component of BTEX is funneled into the same intermediates and eventually are degraded by the same pathways. However, the way the ring is broken and cleaved is specific to either the TOL or TOD pathway.

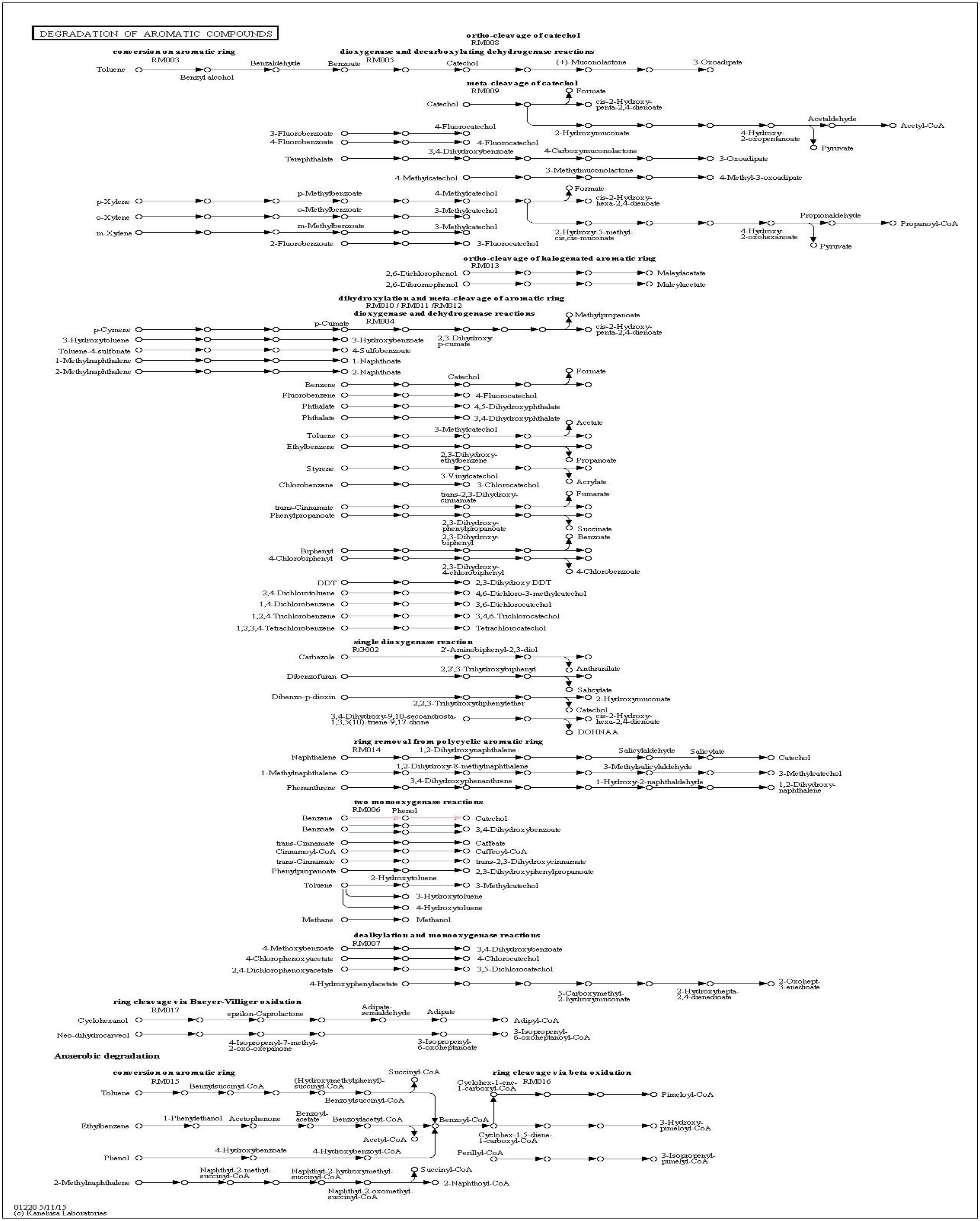

Figure 2. Aromatic Degradation: Common pathway of aromatic degradation in bacteria, large and difficult to see it manages to visualize to the reader the complexity of the biological pathway.[30]

The degradation of BTEX is done aerobically. The easiest component of BTEX to degrade is toluene. In order to facilitate degradation dissolved oxygen must be present to be used for ring activation and cleavage. Also, oxygen is the electron acceptor to allow for complete degradation. The TOL pathway is facilitated by an enzyme, monooxygenase, and the TOD pathway uses the enzyme dioxygenase. The TOL pathway attacks the constituents of an aromatic ring, such as the methyl and ethyl. These substituents are transformed by oxidations; methyl to pyrocatechol and ethyl to phenyl glyoxal. The TOD pathway attacks the aromatic ring by forming 2-hydroxy-substitued compounds. The benzene oxidation is catalyzed by the dioxygenase and is a hydroxylation. Both of these pathways converge in the formation of catechol and its intermediates. The catechol intermediates are then degraded into compounds such as pyruvate and acetaldehyde. These metabolites can then go into the Krebs' Cycle, eventually flowing into the Electron Transport Chain and creating energy for the cell. The Krebs' Cycle, Electron Transport Chain, and energy creation are a part of aerobic respiration that is done in all aerobic bacteria.

Figure 3 shows the simplified version of the pathways. The dotted lines represent the TOD pathway and the solid lines represent the TOL pathway. As *Figure 3* shows, each of the pathways funnel into the TCA cycle.

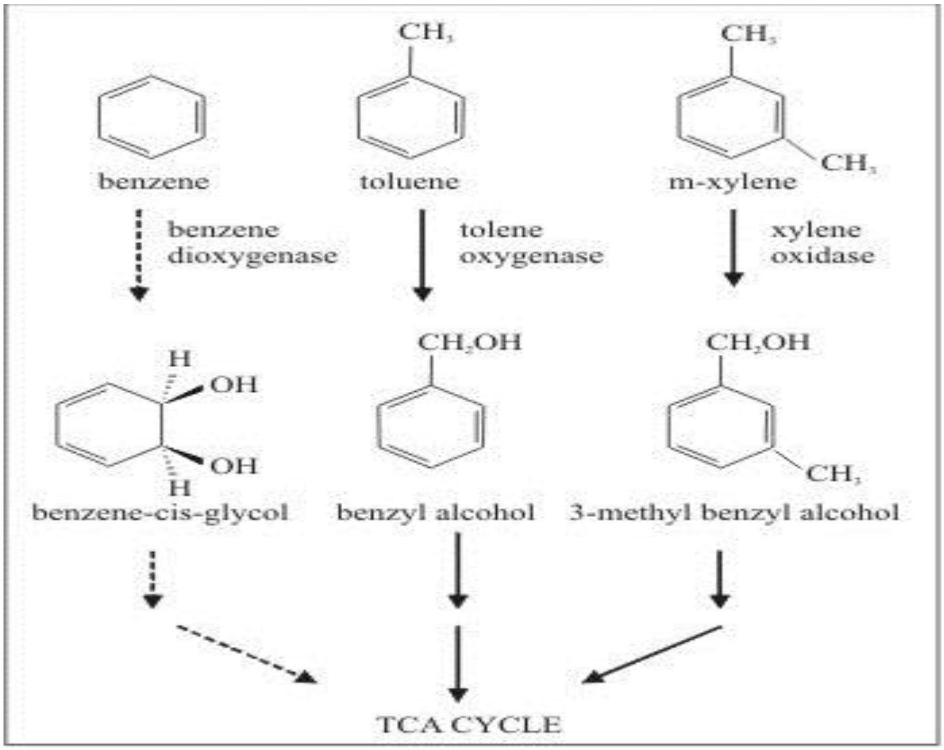

Figure 3. TOL & TOD Mechanism.[29]

(End Christine Chemistry Section)

Genetic modification will then be undergone by each strain separately in order to ensure proper modification. Whichever pathway the strain does not have naturally available to it will be inserted into the organism via plasmid genetic modification. Since both our organisms only differ by strains, and not family or species, they both will require similar conditions to uptake a TOL or TOD plasmid. The TOL plasmid was first characterized in *Pseudomonas aeruginosa* in 1978, and was found to be highly thermo sensitive. It was found to become unstable at 42^0C[7]. In a similar study, the mechanism of the TOL plasmid uptake was explained. This plasmid was introduced to a strain of *Pseudomonas putida* at 30^0C[8]. In both of these studies, the pressure was not indicated, so it is assumed that atmospheric pressure was the condition at which the strains of bacteria were modified. The TOD plasmid is very similar to the TOL plasmid as it is comprised of the same types of pathways, but degrades different components of BTEX than the TOL pathway. Thus, it can be assumed that the TOD plasmid uptake is similar to that of the TOL plasmid.

After modification is complete, each strain will be able to express one of the pathways on a chromosomal level and the complementary pathway will be expressed via plasmid expression.

This dual expression will be beneficial to the organisms in co-culture because regardless of any lateral gene transfer (gene transfer amongst members of the same culture) we have ensured that BTEXterminator will always have both pathways expressed chromosomally and plasmoidally. Vertical gene transfer (gene transfer to offspring) will occur as well as part of the organism's life cycle and both forms of expression should be retained in the long term. This means once we have modified one culture we should simply be able to grow that culture up into quantities necessary to provide our clients with the proper amounts of BTEXterminator and will not need to go back to lab scale operations and undergo the genetic modification again with the original strains.

C&C will then need to cultivate the strains up to larger and larger scales until ready to be introduced to a reactor environment and one another. Pure strains post modification will be streaked and kept in deep-freeze as a reserve. The organism may be modified further after initial trials and research has begun, so it would be beneficial to have each version of our organisms available in reserves. These two separately modified strains will then be introduced to each other in the same environment, again only differing by strains and not species is incredibly opportunistic for us because both organisms require the same nutrients and minerals necessary for their survival, these will be listed later in the material balances section. Equal amounts of each strain will be introduced in the same tank, which will be C&C's stock and reserve for BTEXterminator. The size of this tank or tanks will depend on the companies stage in its timeline, but it will be large enough to supply clients as well as maintain an active and ready to go reserve. This ends the production of BTEXterminator, the lab scale operations should only be done with an incredibly small amount of the organisms (aside from research), and then they should be self sustaining with the right media to grow and cultivate on their own to the proper portions for C&C's client demand.

General Process Description

Once BTEXterminator has been produced, it completely depends on the client's needs how much of the product they will need. C&C has made the process as robust as possible with the hopes of meeting as many situations as possible. The process design is really to test the feasibility of the product; it was the only way C&C could rationalize evaluating the progress made but is not the scope of this project. The organism will be able to survive in a wide range of salt and metal concentrations, as well as, BTEX. Reactor scheme will vary greatly depending on the amount of BTEX and the amount of water a client wants processed, this will change the number of reactors, and perhaps even the number of passes necessary. Design of the reactor will not vary however, and C&C plans on sizing a single type of reactor and making the technology skid-able. So rather than change the reactor size, the amount of BTEXterminator in a reactor, or even residence time of water in the reactor, we will be simply adding more reactors or more passes to meet demand. C&C will be using a fibrous bioreactor in which BTEXterminator is immobilized on the fibrous material; this will make the remediation process more efficient as well as help ensure that BTEXterminator does not get flushed down stream.

With that being said, C&C envisions being hired by a client with a specific desired flowrate for treated water and it will be C&C's job to utilize BTEXterminator to deliver the client that flowrate of BTEX free water. Produced water is returned to the surface in very large volumes, we have found estimates from industry sources such as, Don Whisonant who is an Engineering Advisor for Devon Energy, that tell us over a single oil well's lifetime, it may produce anywhere from 100k to 2000k barrels of water[9]. Clients will very likely have several wells close to each other and it is unlikely they would have a need for such a high volume of water instantly. Thus, C&C anticipates that our clients will store this water in a reservoir; they may then send their desired flowrate of produced water to BTEXterminator to treat the water and meet their water demand. If storage is not an option adding/removing reactors to handle a varying flowrate is no issue as long as the water is characterized.

From a reservoir or formation, produced water will be pumped to C&C's reactor scheme at a constant flowrate. The flowrate from a single well will vary and constantly decrease over its life, however, water will be delivered from multiple wells. C&C will also only run water for the first three months of the well's life; typically, 90% of produced water is forced to surface in the first three months[9]. It would be more economically realistic for the operator to ship the small amount of water, the other 10% over the rest of its life. These wells can have a production lifetime of a few years up to periods of 30 or 60 years, it really varies, but the first three months always produces a majority of the water. As seen in *Figure 4*, the majority of the produced water is forced to surface in the first three months.

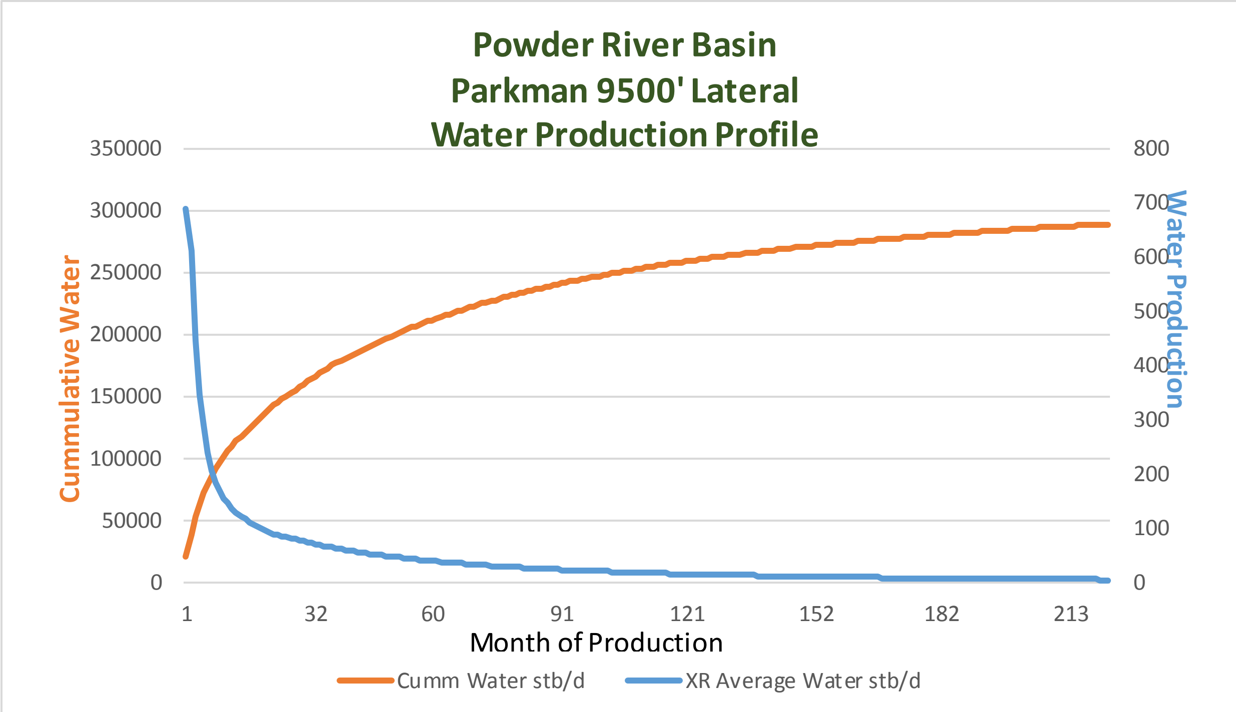

Figure 4. PRB Type Curve. Type curve of a typical well in the Powder River Basin.[9]

Enough reactors and BTEXterminator will be provided in order to ensure complete degradation of BTEX to untraceable amounts, these reactors in parallel will be scaled back as water production decreases over those three months. The water will then exit the reactor scheme and run through a filtration system so no GMO is released downstream. This BTEX free water will then be turned over to the client and be treated further depending on their desired use for the repurposed water. This may include environmental discharge, agricultural use, or even drinking quality.

Base Case Design Assumptions

In order to find an appropriate estimated size for our reactor and the amount of BTEXterminator to treat a certain amount of water, C&C ran some very rough estimates. These estimates are based off of a target 5000 bbl/day of produced water treatment. This volume would treat two sections typical of the Powder River Basin according to Don Whisonant[9]. Sections in the oil and gas industry are defined as a 1 square-mile area. Typically, in the Powder River Basin there are two well pads that each run to two wells within that square-mile. A series of backwards calculations were done based off of approximated literature results. C&C took an ideal target degradation rate we would like to achieve in BTEXterminator, and tried to match the cell concentration necessary to acquire that rate of degradation in our reactors. Based off numbers from Devon Energy, C&C sized the base case to treat 25,000 L/hr, a decent expectation for that square-mile area. The concentration of BTEX within that water was assumed to be constant. These calculations led to an estimated reactor size, BTEXterminator per reactor, and a single reactors production ability per day as displayed in *Table 1*[10]. It was then clear that multiple reactors would be necessary for the base case scenario.

Table 1. Key Design Criteria: Single reactor size and production ability.

Key Design Criteria Results (reactor and organism estimation)	QTY	UNITS
Reactors=	1	-
Reactor size=	5000	L
BTEXterminator/reactor=	170	lbs
Theoretical Reactor Production=	571.4286	bbl/day
Estimated Residence Time	1	hr

Process Diagrams

Figure 5 displays the big picture of C&C's envisioned role for BTEXterminator. It shows unaltered produced water flowing into our remediation process. Our product then treats the water in order to remove BTEX where the water is then returned to the hands of the client. C&C has split its responsibilities up into two key sections, the modification and cultivation of BTEXterminator, and the products ability to perform within a reactor and actually remove BTEX, both of which can be seen below.

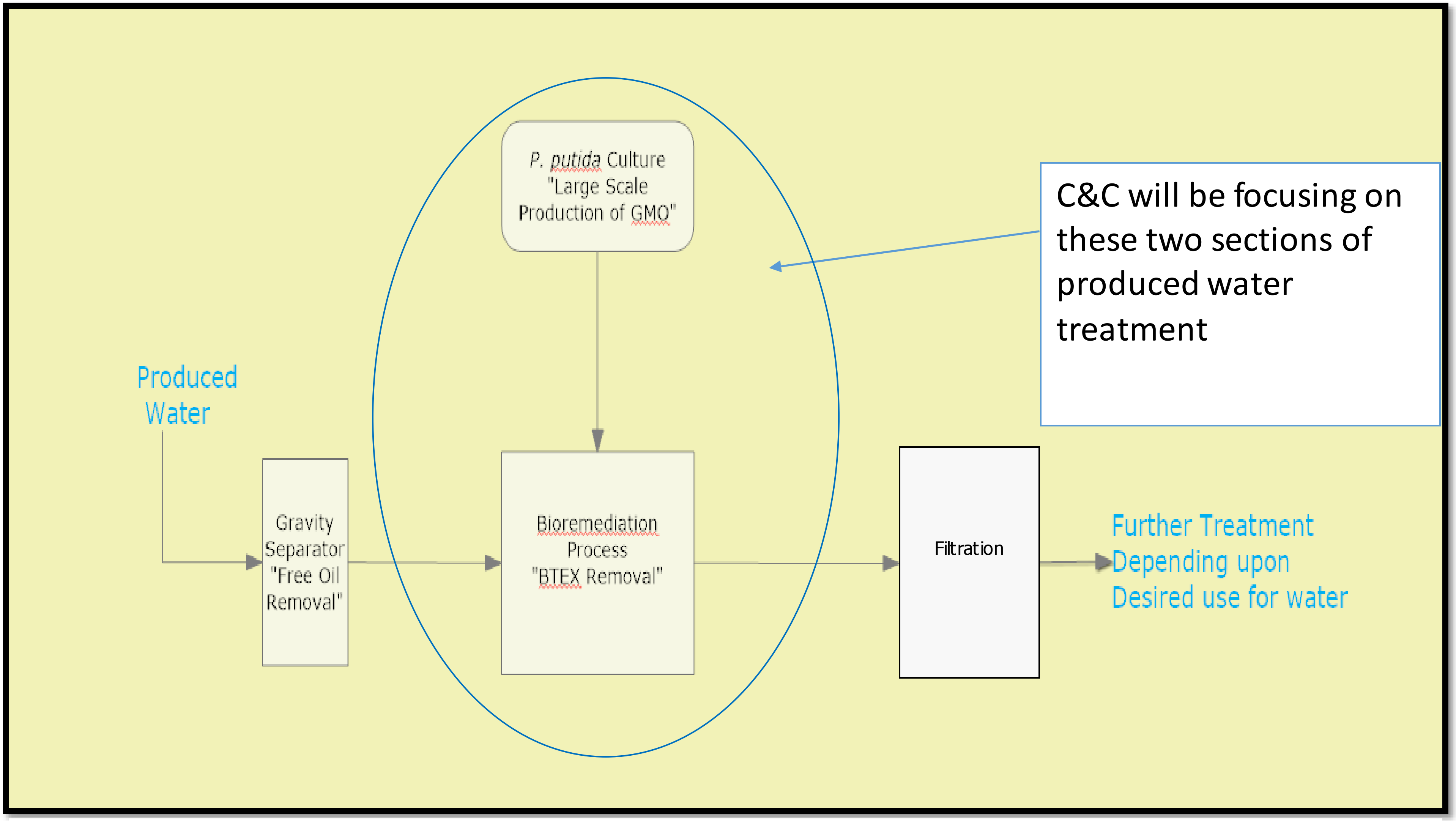

Figure 5. <u>Process & Production Diagram</u>: The overall process and production diagram shows BTEXterminator's role in the water treatment process.

The first step is for BTEXterminator to undergo its genetic modification and be cultivated to a point where introduction to the reactor is appropriate. *Figure 6* displays the process that each individual strain of *P. putida* goes through before they are introduced as a co-culture and transplanted to the reactor shortly thereafter.

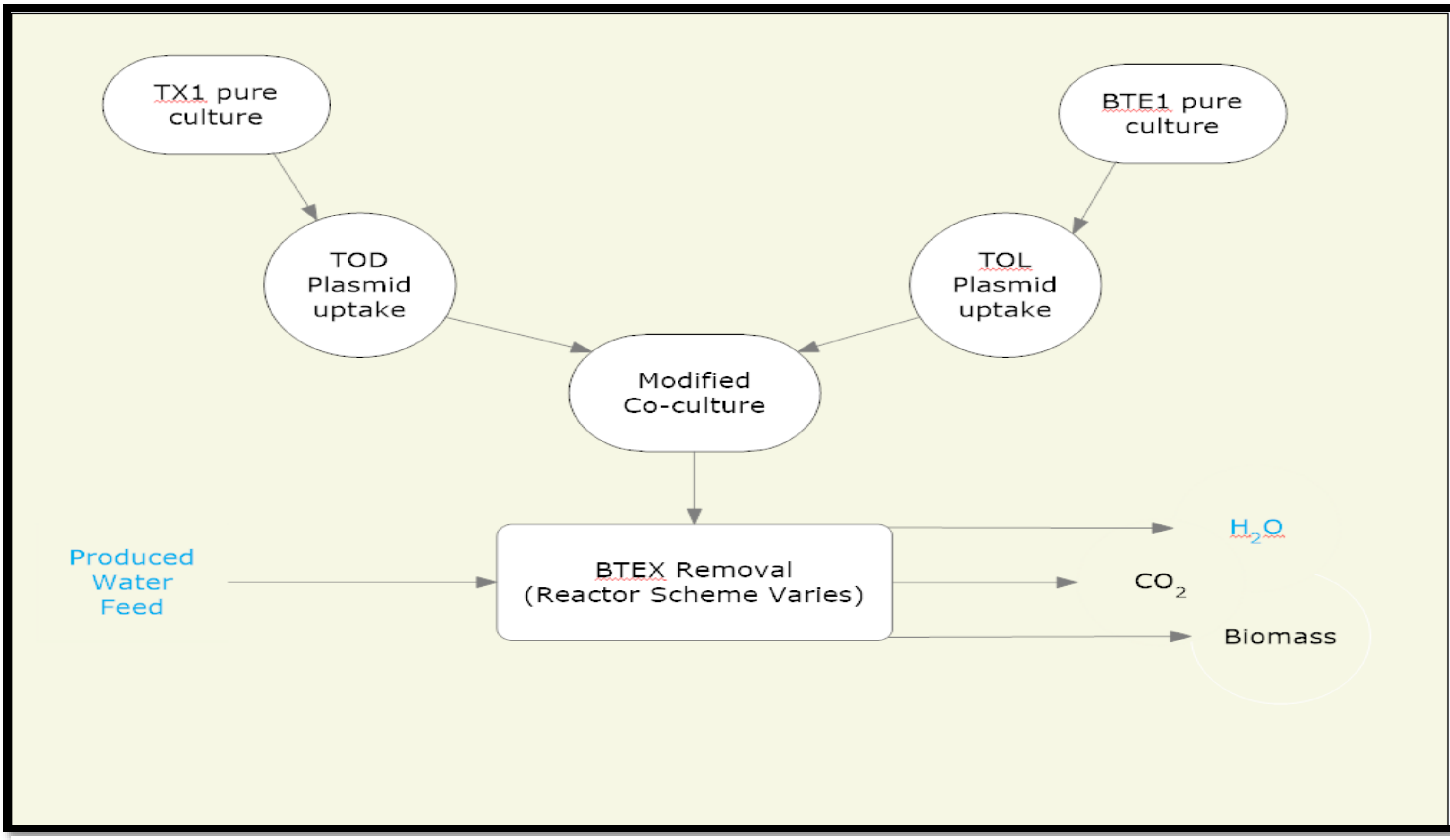

Figure 6. *BTEXterminator Production: This figure expands on the GMO production section C&C will be focusing on, displaying a hierarchical order each strain will go through to create BTEXterminator.*

After BTEXterminator is produced and cultivated to scale within a reactor, it becomes the key part of the BTEX removal process of produced water. *Figure 7* displays this process flowsheet for a single reactor being fed produced water from a reservoir at a constant flowrate. The additional nutrients and supplements feed consists of the basal mineral salts and the oxygen source. This process is as simple as depicted in this process diagram; the real design work is put into BTEXterminator. C&C has designed a single reactor size as well as estimated amount of organism necessary to treat an estimated BTEX concentration. Based off these assumptions and those made previously, a single reactor could treat approximately 570 bbl/day of produced water for BTEX if operated over the 24hr period. Though this is the max treatment rate for a single

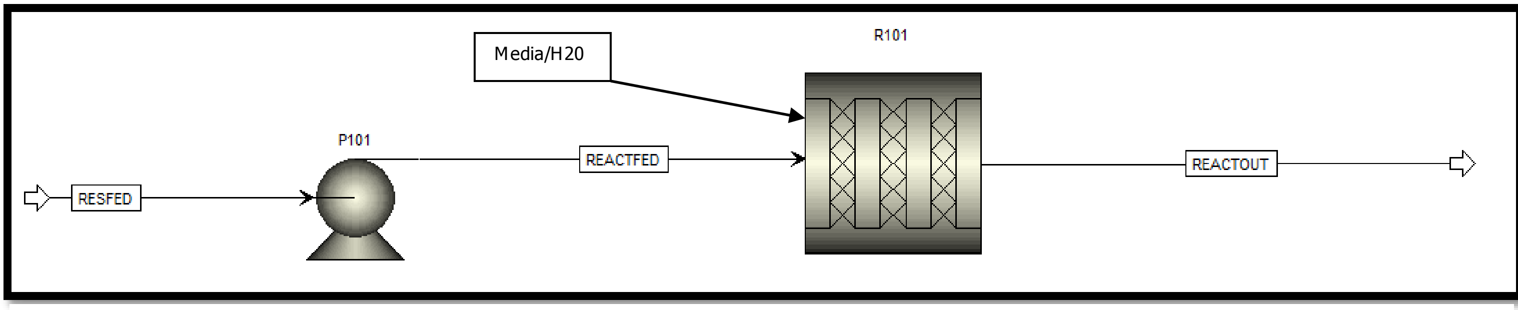

Figure 7. <u>Single Reactor PD</u>: The bioreactor where BTEXterminator is housed is shown being fed a constant amount of produced water from a reservoir.

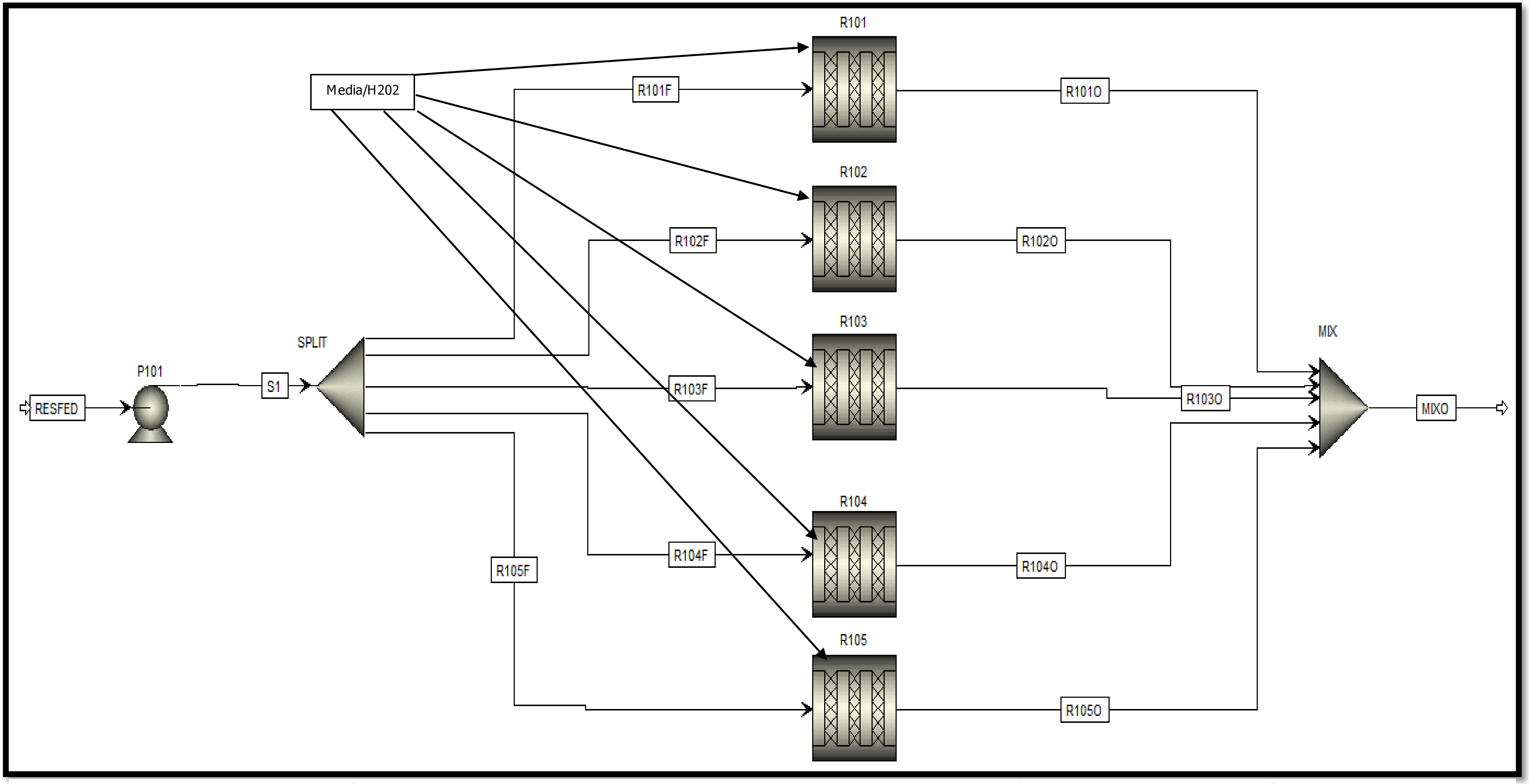

Figure 8. <u>Parallel Reactors PD</u>: Proposal for reactor scheme, each reactor would be the same size and treat the same amount of produced water. The number of reactors would be scaled with the desired production. A membrane and UV filtration would likely be the last step in order to inhibit any GMO or biomass from moving downstream.

After the water had left the reactor system it would be sent through a membrane filtration unit. A membrane should be capable of stopping any GMO from moving through but still allow for the water to flow with little to no restriction. Since public perception of GMO's is so negative, along with the unknown consequences of releasing BTEXterminator into the environment, C&C will also include a UV filtration step to kill any remaining organisms prior to the water being released to further downstream operations. It is important to keep in mind this system represents the concept of the design BTEXterminator will work in, but will include 9 not 5 reactors, and will include the filtration equipment.

Material Balances

Creating BTEXterminator is all done on small lab scale operations; the genetic modification is done on this scale so that we ensure the colonies that are selected uptake the plasmids, and that we do not have separate colonies, one of which would be resistant to the transformation likely. To successfully do this in lab a material balance is done around the production of BTEXterminator shown in *Table 2*. The components listed in the media would simply be scaled up along with the organism's volume. TX1 & BTE1, along with the plasmids used in the modification, would be used in an incredibly small amount as shown, again to ensure plasmid uptake and expression.

Table 2. BTEXterminator Raw Materials: A materials list/balance that represents inputs required for laboratory operations which can be scaled up to larger cultures.

Materials List:	Qty	Units	Source
TX1	1	mL	based off research article, for initial modification
BTE1	1	mL	
TOL Plasmid	1	mL	Estimate based on 1:1 ratio w/organism
TOD Plasmid	1	mL	
BTEX	0.6	gm/mL-hr	Calculated
Basal Mineral Salts Media	-	-	-
K2HPO4	5	gm/L	(Hubert, Tandem Degradation), for a 20mL suspension culture scale up depending on expected growth rate and volume
NaH2PO4	2.5	gm/L	
(NH4)2SO4	1	gm/L	
disodium nitrilotriacetate	15	mg/L	
MgSO4-7 H2O	30	mg/L	
CaCl2	10	mg/L	
MnSO4-H2O	5	mg/L	
FeSO4-7 H2O	1	mg/L	
CoCl2	1	mg/L	
ZnSO4-7 H2O	1	mg/L	
CuSO4-5 H2O	0.1	mg/L	
H3BO3	0.1	mg/L	
Na2MoO4-H2O	0.1	mg/L	
pH adjuster (NaOH, desired pH 7 for lab)	varies on sample	-	-
aeration chemical (H2O2 if o2 needed for reactor)	varies on dependence	-	-

This provides C&C the ability to scale the required materials for BTEXterminator's survival so that it may operate at optimal conditions. Since C&C also is focused on the performance within produced water a simple mass balance was done around the inputs and outputs of the reactors. *Table 3* displays a simple mass balance, as well as a list of additional key components within produced water that are of importance to our organism's abilities to perform.

Table 3. Simple Mass Balance: A simple mass balance around the reactors is done with concentrations included for other components of interest that will not be consumed.

Mass Balance & Produced Water Concentration Estimates			
Mass Balance	**IN**	**OUT**	**Units**
Produced Water (-BTEX)	599400	599400	kg/day
BTEX (all isomers)	600	0	
CO2 & Biomass	0	600	
Components of Interest	**Anticipated Range**	**Our Water Sample**	**Units**
Salt Content	1k-400k	36300	mg/L
Metal Content	100-2000	200	

Although very simplistic this mass balance serves its purpose when trying to understand our products aside from water, by only removing BTEX the only byproducts we would get would be as a result of the organism's metabolism, even with significant literature it is difficult to anticipate exactly what biomass includes. BTEX may also not be completely removed, and likely would not be, but BTEXterminator's purpose is to remove detectable limits so complete degradation was assumed for this balance. Additionally, salt and metal content ranges were established from the US Department of Energy, these were then compared to the produced water sample we based our calculations off of which were provided by research done at the University of Wyoming[11, 12]. Without lab research it will be impossible to really utilize these numbers and understand if our product will be comfortable with our concentrations, let alone the entire ranges of those concentrations.

Equipment Lists

C&C is also able to split up our equipment lists based off the section we look at, whether GMO production or water treatment sections. For the production of BTEXterminator some specific lab equipment will be needed in order to run experiments and collect answers necessary to scale up to large operations, or even simply create the product. A simplified list can be seen in *Table 4*.

Table 4. Research/Lab Equipment: A preliminary list of equipment necessary for accomplishing the production of BTEXterminator.

Lab Equipment
Gas Chromatographer and Mass Spectometer
Bioreactors
Spectrophotometer
High Performance Liquid Chromatographer
Misc. (mineals/nutrients/glassware/misc. included)
BTE1 P. putida strain
TX1 *P. putida* strain
TOL plasmid
TOD plasmid

This lab equipment would be a great start to producing a sort of prototype of BTEXterminator, which then could be researched to progress closer to becoming a viable industry ready product. A separate equipment list is seen in *Table 5* where the equipment that is anticipated to be required to actually treat the water is listed.

Table 5. Process Equipment: Anticipated equipment requirements to treat produced water with BTEXterminator are listed.

Process Equipment
Pumps
Reactors
Vessels (spare suspenion culture)
Filtration Equipment (biomass/GMO removal)
Gravity Separator (free oil removal)
Mixers/Splitters
Piping/Hoses

These two sets of equipment lists are preliminary by the fact they are merely anticipations, some categories listed may include more than one component or piece of equipment but these lists will be used for a preliminary economic analysis.

Reactor Design

Only a single type of reactor will be designed and used for BTEXterminator to perform. It will be modeled after the design that uses a fibrous bioreactor, which C&C found to be the most promising[10]. This reactor will be suitable as a healthy environment for BTEXterminator to thrive and remove BTEX efficiently. The reactor layout is simply a 5000 L (app. 32 bbl) tank lined and coiled with a fibrous material, which will serve as a means to immobilize BTEXterminator. It is assumed the cotton innards can operate well over a year before too much wear and tear takes plus, but other materials such as nylon or industrial carpet may be considered. Immobilizing the organism ensures that the fibers will be saturated with the product throughout the reactor, and the rate of BTEX degradation in the reactor will not vary based on location within the reactor. *Figure 9* is a very simple layout of the reactor construction.

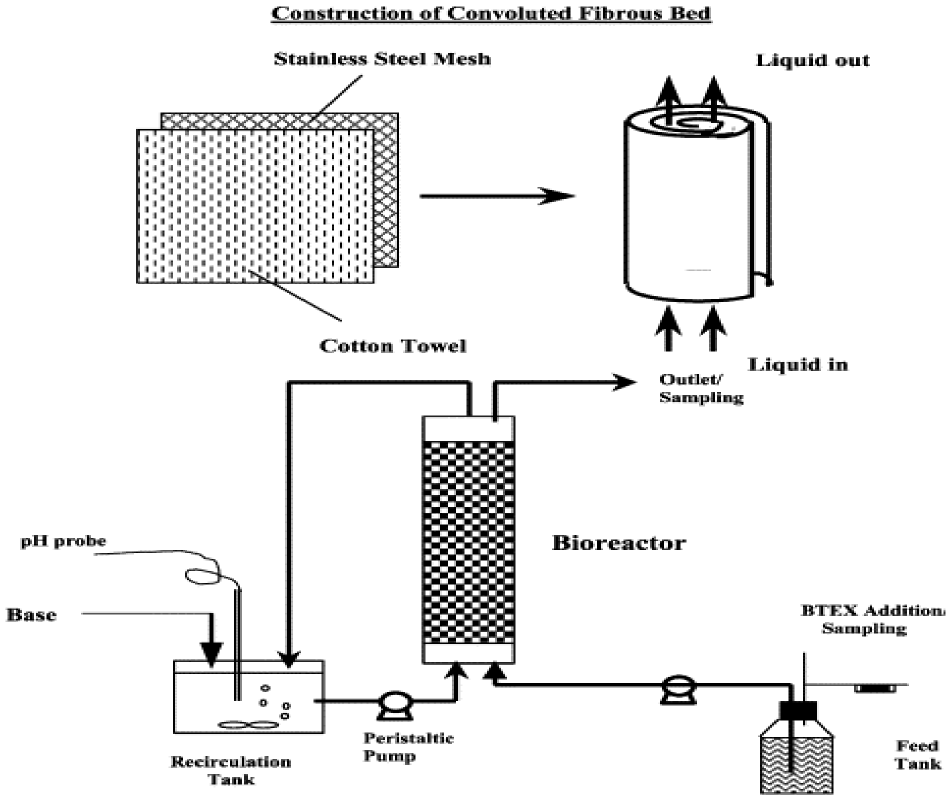

Figure 9. Fibrous Reactor Scheme.[6]

Through experimentation, the optimal temperature for toluene degradation was found to be 35^0C. However, the optimal temperature for degradation of both toluene and benzene was found to be in the range of 15-35^0C. The optimum temperature to degrade all components was found to be 33^0C[13]. The optimum pH was found to be within the range of 6-8, which is not surprising. Specifically, degradation rates increased the most in the range of 7.5-8. These measurements are assumed to be taken at atmospheric pressure. Produced water is not typically outside of this pH range, unless large amounts of acid producing bacteria or acid formations are being dealt with. This is not common in the Powder River Basin, treating any large pH change with this much water would be impractical for BTEXterminator at this time so the pH monitoring is really to watch organism viability. If it changes too much then correcting 25,000 L/hr would not be ideal, other routes would need to be considered at this time (unless BTEXterminator is modified to handle a wider pH range).

This reactor serves only as a housing unit for BTEXterminator and does not have operating specifications outside the conditions necessary for BTEXterminator to survive and perform. However, there are a few important design criteria that we hope to match on a large scale reactor and not just a lab scale. We expect this design to lead to greater than 90% of the cellular mass being immobilized within the reactor. If this is accomplished, then it would be expected to have a very high cell viability. Lab scale studies suggest that the reactor should be able to perform well over a year if these conditions are met. This is a huge estimation and would likely change under produced water conditions and scale up. Upon start-up, C&C would likely resupply BTEXterminator, as well as, the fibrous material within a reactor every three months, then slowly increase turnaround frequency as the system became better understood.

Further Considerations for Reactor Design

Though the reactor design is fairly simplistic the fact that it is housing a biological environment produces many challenges that need to be addressed. There are three immediately noticeable conditions that are detrimental to our system; temperature, pH, and oxygen availability. The optimal ranges and targets for these conditions have been discussed but the solutions to maintaining them have not. Materials necessary for the design are a key design feature as well. C&C needs to minimize the cost while ensuring the material is appropriate for the organism's survival, and does not allow the release of BTEX into the atmosphere via diffusion through improper materials.

Material selection will be the first area of interest discussed. In the original design of this reactor on a lab scale operation a glass tank was used in conjunction with stainless steel and cotton serving as the fibrous material. While this is fine for lab scale a glass reactor on site is not an option and the stainless steel assumption made last semester is simply more expensive than the materials need to be. Rather C&C will be using fiberglass as the material of choice for both the construction of the reactor tank and the infrastructure to support the fibrous cotton innards.

This will not only serve appropriately as a material choice, but it will reduce the capital costs of building a reactor as well as the reactor weight. The main concern for moving away from stainless steel was ensuring that the BTEX components would not diffuse through the system and into the surrounding atmosphere, defeating the purpose of BTEXterminator. With only a one-hour residence time in the reactor, C&C is confident that none of the constituents will be able to diffuse through a fiberglass shell. This can be verified very easily during laboratory testing before any pilot scale up process is considered.

Temperature is one of the most important operating conditions that needs to be met in order for BTEXterminator to degrade efficiently. Being a biological system the temperature range is very small, either meet that range, or fail to degrade BTEX and possibly kill the organisms. C&C has identified two possible ways that the temperature in the reactor could deviate from our range; ambient influence and feed temperature. Since our reactor is a skid design the system will be operating in the oil field directly. Ambient temperature will vary greatly depending on region and season but it is easy to see how running in the subzero temperatures of Wyoming could cause a problem. Now not all reactors will need to have a temperature control system if they are not operating in these widely varying ambient temperature areas but the possibility still needs addressed. Another possibility is for the temperature of the produced water in the feed to the reactor being drastically different from what the organism would like. The temperature of produced water directly from the formation will likely vary greatly depending on formation type. Unfortunately, there is not a lot of readily available information on the temperature of produced water so C&C is required to speculate at this time. The water could be as cool as the ambient temperature at the time or nearly as hot as the ambient temperature; so 5^0 C up to 75^0 C would not be unreasonable. C&C's solution to these temperature concerns is to utilize our short one-hour residence time. The issue of ambient temperature would be an easy design fix, simply adding insulation to the system in combination with the one-hour residence time would be enough to negate ambient temperature concerns. In theory our ideal operating temperature is 33°C, we do not know how quickly efficiency and viability decrease as that temperature is deviated from so specifics on the insulation and heating needed for the system cannot be determined, but instead discussed. In terms of insulation there are single layer and multilayer configurations available, it varies depending on the material selected along with the size of the bioreactor[8]. Insulation need will also change depending on the rest of the temperature control system; this could include the water coming in heated or cooled, constant heat being delivered to the bioreactor to maintain a temperature range, or a combination of both. Either way a cost analysis would be done; this would determine whether less efficient insulation and more utilities, or expensive insulation and lesser utilities is more cost effective. Heating the feed water out of formation would be the easiest direct solution to temperature concerns; simply adding a natural gas combustion heater prior to the water being sent to the reactor would do the trick, and one would think utilities would be reasonable since natural gas would likely be available on site (if not consider propane or diesel). Cooling the water, however, would be a large challenge. It would take time to cool the water 20^0 C-40^0 C without a large refrigeration system which would mean the process could not be done immediately and the water would pile up and sit. It is not economical to cool such large amounts of water at this time; this system may not work for all produced water scenarios. At this time C&C would probably only be able to handle water at or cooler than our desired range; the cost, size, and time of a system to cool water down to our range would be far too much for a skid system. A central remediation facility where the water would be shipped too would be a better option at that time. If it is determined in lab our organisms need a very small defined temperature range to operate then the cost of the solution to our temperature control issue becomes much greater, this costs would be assumptions on assumptions at this point in the design, and will not be accounted for in detail. Instead, when determining the cost C&C would charge to the operator a 40% increased IRR will be used to account for risks and uncertainties such as these.

Another important operating condition for a biological system to maintain is the pH. For our system to run efficiently the organisms need to remain healthy and viable, too acidic or basic of conditions would lead to the death of our product and the inability to remove BTEX. With a typical pH range of 6.5-8 produced water is not too extreme in this category[8]. It is likely that BTEXterminator would be able to operate within this entire range of pH values, it just may be more efficient closer to a neutral 7. If the pH happened to fall outside of that range C&C would recommend shipping the water to a fixed facility where batches of produced water could have pH corrected before potentially harming BTEXterminator. Attempting to change pH by even a little bit with such large amounts of water on site, where the operation would like to be as close to continuous as possible would be infeasible. During startup years, which is all that is accounted for in this analysis, building a skid/system with a pH correction system that would need to be used less than 1% of future scenarios would not be advisable. Maybe as the company scaled up, and if pH issues were ever actually an issue, C&C could incorporate a larger pH range into the organism or add a correction system.

Lastly, another large concern of C&C is oxygen availability to our organisms. These organisms operate under aerobic conditions meaning they need oxygen available to them as an electron acceptor in order to perform many of their biological metabolic pathways. In aerobic systems aeration is performed in order to continuously supply the system with oxygen. Normally atmospheric air is introduced into the system and circulated. The purpose of BTEXterminator is to degrade BTEX without releasing any of it into the atmosphere. If air were to be bubbled into the system it would cause an increase in pressure and nitrogen buildup that would need to be properly vented, to avoid pressure buildup. This could be done if the vent contained a filtration system for BTEX. Filters for constituents such as benzene do exist and are used in large scale industrial applications and even individual personal pieces of protective equipment, one that is commonly used on small individual gas masks is an activated carbon and potassium permanganate filter which could easily be fitted to one of C&C's bioreactors. Another route to completely maintain a closed system, no ventilation or anything like that, would be to use a chemical additive as an oxygen source. Hydrogen peroxide is commonly used in lab scale techniques but would likely be too pricey for our final skid design. Most other chemical that could be selected would also be considered additional pollutants, overall C&C will still use hydrogen peroxide at this time but will continue to look into optimizing this cost.

Base Case Assumptions

Below is a list of the many assumptions made throughout the design of the base case.

- Base Case Assumptions
    - Target Degradation of 600 gm/L-hr at the concentration of 15.4 gm/L
    - 25,000 L/hr produced water flowrate to base case
    - 3 Month operation (90% of the well's lifetime water achieved)
    - Produced water pH is not found outside the operable range of our organisms
    - Hydrogen Peroxide would serve as an acceptable oxygen source
    - Cotton innards operable for 4 jobs without replacement, consider nylon
    - 1-hour residence time allows for complete degradation (Below Detectable Limits)
    - Fibrous scheme, membranes, and UV filtration completely reduce risk of GMO release downstream
    - Single reactor can treat a maximum of 570 bbl/day as design but may treat lower levels if nutrients/supplements are still fed (reactor does not have to be full, handles lower rates)
    - Fiberglass reactor materials in combination with 1-hour residence time will not allow volatile hydrocarbons to diffuse out of the reactor and into the atmosphere
    - Present design may not be a one size fits all solution
        - Large temperature corrections may not be possible and minor ones are assumed to be included with our high risk 40% IRR

Lab Work (Christine)

C&C had access to the specific strains of *P. putida* discussed in the literature. Characterizing the viability of the organisms in produced water is paramount to the understanding of how effective BTEXterminator can be. There are many tests to be completed, but in the time restraint, it is unrealistic to complete all of these experiments. Thus, only a few of the experiments were chosen to complete.

In order to complete experiments in the lab, employees must go through specific training. These were offered through the University of Wyoming Environmental and Health Safety Department. The class names were Chemical Hygiene, Hazardous Waste Generator, and Chemical Safety Handling-Benzene. These classes trained employees to work safely and effectively in the laboratory environment. Once these classes were taken, employees were then able to work in the lab.

Before experimentation could be completed, the bacteria needed to be grown using rich media. Specifically, Lysogeny broth was used for this purpose. The media is sold in powder form, which can then be mixed with water to make either liquid media or media with agar. Both of these options were chosen because the different experiments needed either both or either the liquid or solid media. Once this media was mixed, it was then autoclaved to achieve sterility. *Figure 10* shows what was done in the lab this semester.

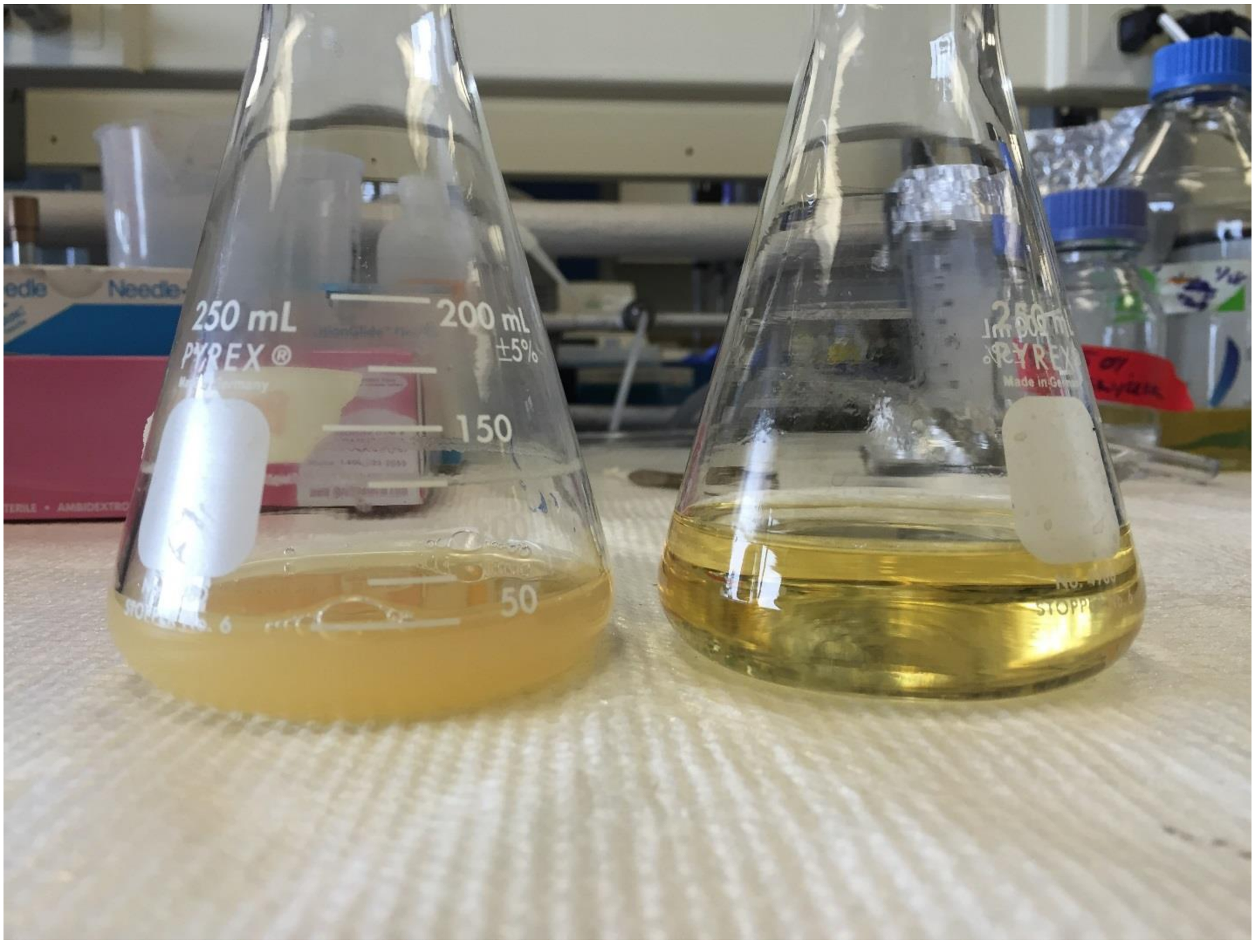

Figure 10. Media Test: On the left, inoculated media with P. putida. *On the right, sterile media for comparison.*

Sterile technique was used in all of the experiments. Then, the liquid media was inoculated with either BTE1 or TX1 to be grown for approximately forty-eight hours. This inoculated liquid media can be used to make reserve stocks of the bacteria in case of contamination or the need for more bacteria during experimentation. Each strain was also streaked onto plates so that individual colonies could be identified. This is important for several reasons. Namely, possible contamination can be visually spotted on the plates and single colonies can be used for further experimentation.

The other experiments to be completed are testing salt tolerance and understanding how the bacteria grow on a carbon source that is a component of BTEX. In order to test the salt tolerance, a carbon source of benzoate will be used. This makes it so that the experiments don't have to be done in the hood, and can instead be done on the bench table. Since the bacteria is also to be tested with a carbon source of BTEX, the understanding of how the bacteria behave with that carbon source will be known.

In the future, the genetic modification of each strain of *P. putida* must be done. There are many ways to force the uptake of a plasmid in bacteria, and the method depends on the bacterium. Typically, *Pseudomondas* are not difficult to modify. Typical research into modifying *P. putida* with the TOL and TOD vectors involves the simple transfer of vectors through conjugation. This means that the vectors are transferred through contact of the donor and recipient cells.[31]

To measure the degradation rate of the components of BTEX from each strain and the co-culture, media containing all necessary nutrients except carbon would be inoculated with the strain of *P. putida*. Then, a component of BTEX in vapor form would be introduced to the bacteria in a glass tube. Then, optical activity can be measured to determine how much of the BTEX component was degraded. This data could be used to compare the degradation rate in literature with the degradation rate of the genetically modified co-culture.

The rate in which the components of BTEX diffuse through the fiberglass shell of the reactor is concerning. The regulations of volatile carbon emissions must be met during the use of BTEXterminator. Thus, the understanding of whether the residence time of one hour is acceptable to assume negligible emissions must be known in order to determine the best material for the reactor. One way of measuring this in the lab is by placing the vapor of a component in a vessel of the material to be tested to see whether the one-hour residence time is acceptable in meeting the emissions regulations. This will be determined by measuring how much of the component is left in the vessel after one hour. Then, the diffusion rate of the components of BTEX at the typical levels of produced water can also be tested. That way, if the tank can handle a high concentration of BTEX in that residence time, it can be assumed that the tank can handle the typical levels of BTEX in produced water. This analysis will determine the reactor material.

Design Alternatives (Juliet)

Hollow Fiber Membrane bioreactor

Membrane bioreactor (MBR) technology has seen widespread use due to a few advantages it holds over conventional treatment processes. MBR systems have a smaller footprint, and they provide better effluent quality, and better process control[16]. Initially, MBR systems were designed using cross flow micro- or ultrafiltration membrane systems, which limited their use due to the high energy demand. In the late 80s, a new approach was developed where the membrane modules were submerged in a tank, aeration was used to induce cross-flow and the effluent was removed using a vacuum as shown in *Figure 11*[16]. C&C can investigate the use of a hollow fiber MBR in its process as an alternative to the fibrous bed bioreactor utilized in the base case. Even though it has several advantages, further research would be needed to ensure that a hollow fiber MBR is a viable alternative to house the *P. putida* co-culture.

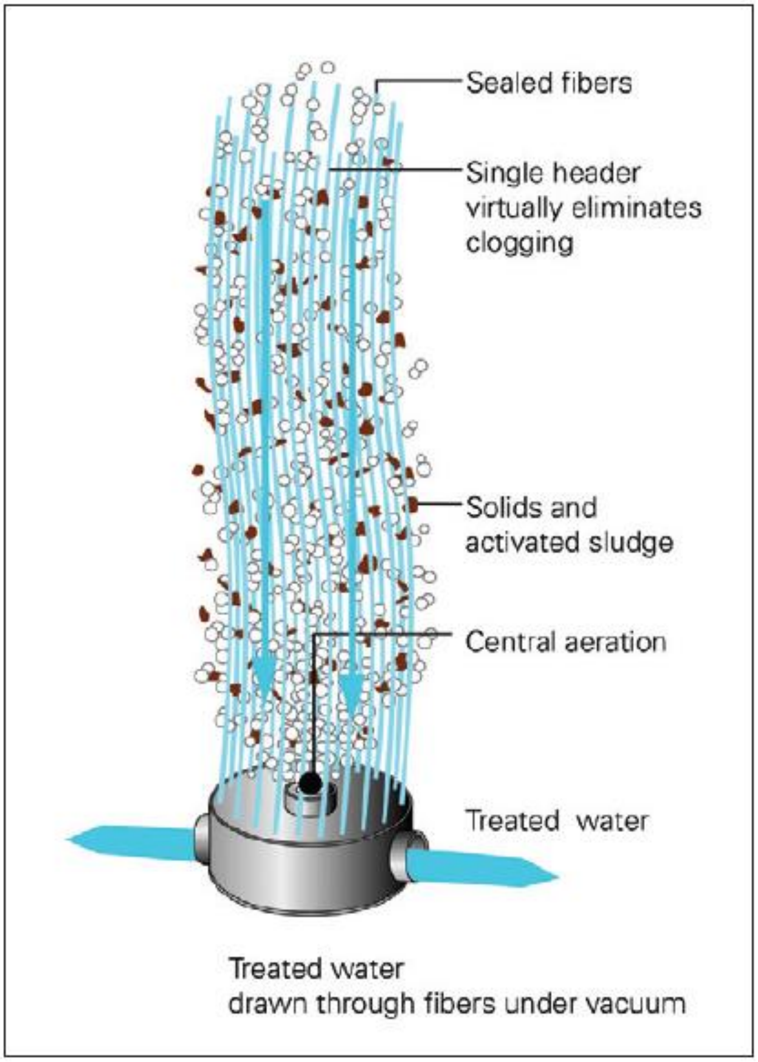

Figure 11. MBR Module.[15]

Figure 12 shows the patented PURON module, a system C&C could implement in the process[15]. In this PURON module, the hollow fibers are sealed at the upper end and allowed to float freely since they are only fixed at the bottom. An MBR module with hollow fibers that are fixed both at the top and the bottom usually experiences a build-up of fibrous materials that can clog the upper ends of the fibers[15]. As shown in *Figure 12*, bacteria, and any solids in the produced water, remain on the outside of the hollow fiber membrane while the treated water is pulled inside the hollow membrane with a vacuum. A high flow-rate is achieved with the outside-to-inside flow pattern as shown in *Figure 12*[15].

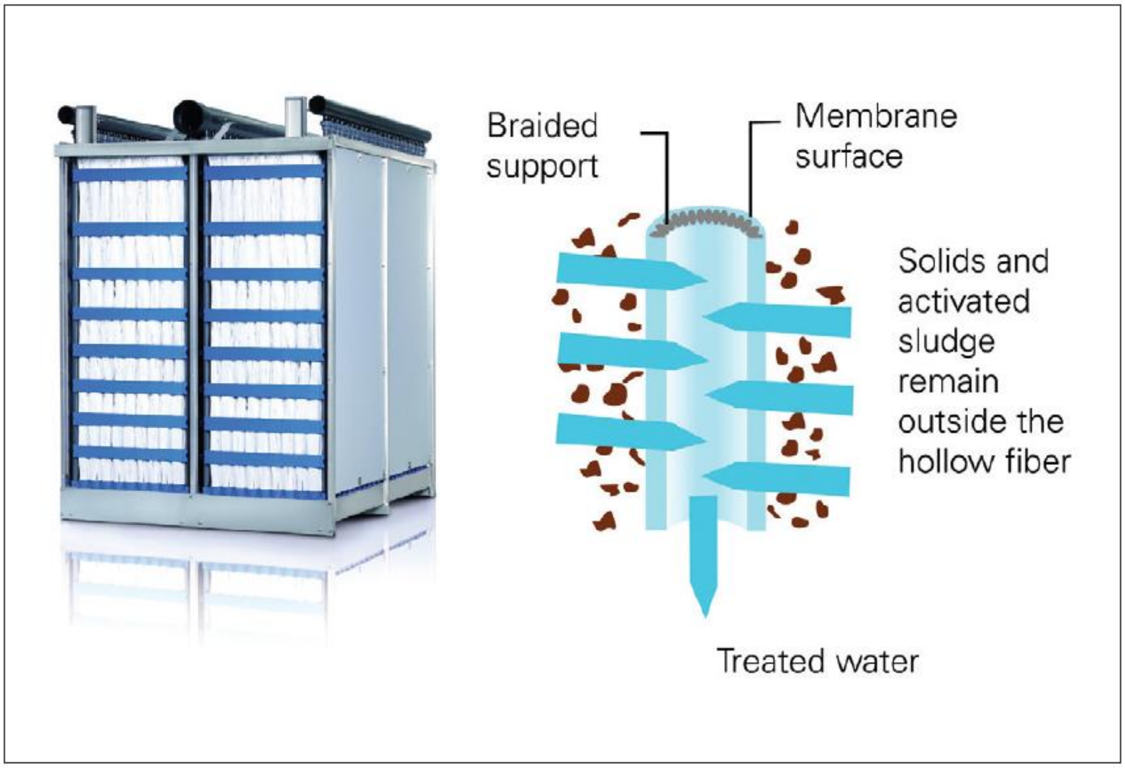

Figure 12. MBR Demo: MBR module and cassette; the cassette as shown on the left would be installed on the skid.[15]

A big disadvantage of using hollow fiber MBR systems is poor cell observation and harvesting[17]. The *P. putida* strains in this MBR would be freely suspended, while the cells growing in the fibrous bed reactor would be immobilized. Immobilized cell cultures hold the following advantages over suspension cultures as outlined by Shuler and Kargi[17]:

1. Immobilization allows for high cell concentrations.
2. Immobilization allows the cells to be reused, thus eliminating the potentially costly process of cell recovery.
3. When dilution rates are high, immobilization eliminates cell washout problems.
4. Immobilization yields high volumetric productivities due to the combination of high cell concentrations and high flow rates.
5. Immobilization may result in higher product yields and rates since it creates favorable micro environmental conditions for the cells such as cell-cell contact, nutrient-product gradients and pH gradients.
6. Immobilization also provides cells with protection against shear damage.

If C&C were to truly consider the use of a hollow fiber MBR as an alternative design for water treatment, there are several design requirements that need to be addressed during the research phase. When designing a MBR system, there needs to be a way to control or reduce the formation of biological foam in the reactor. Filamentous bacteria that float can be trapped inside the MBR, causing biological foaming which "results in undesired loss of biomass and increased membrane fouling rates"[15]. These filamentous bacteria may be found in the produced water that enters C&C's water treatment process. A solution to this problem could be installing a front-side ultrafiltration and UV disinfection system as shown in *Figure 15*. The front-side ultrafiltration and UV disinfection system would ensure that any bacteria that is present in the feed produced water does not enter the hollow fiber MBR. Additionally, the produced water may contain high enough mineral or metal concentrations that will contribute to membrane fouling. Membrane fouling will increase C&C's operation and maintenance costs in the form of additional chemical cleans necessary for optimum membrane operation. C&C will need to select membrane materials that are reliable and energy efficient.

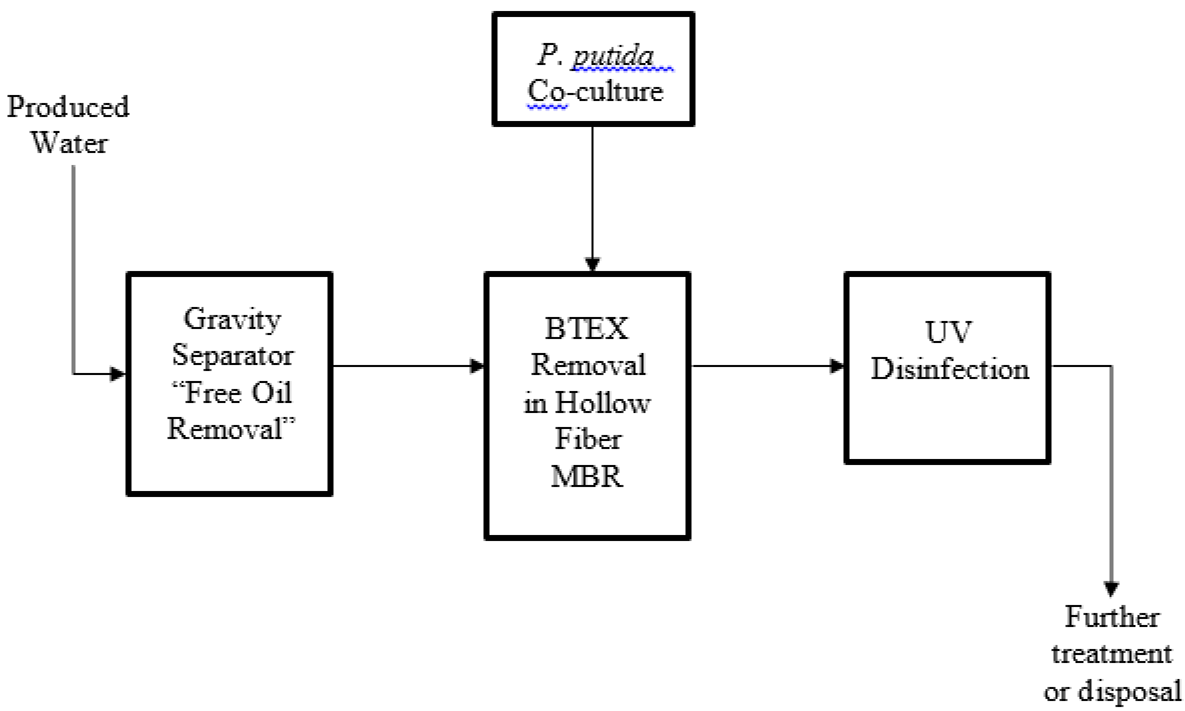

Figure 13. Hollow Fiber Reactor PD: Overall Process and production diagram using a hollow fiber membrane bioreactor.

Figure 13 shows the alternative process diagram where C&C uses a hollow fiber MBR. The hollow fiber MBR is set up to keep the *P. putida* cells outside and the treated water inside the fibers as shown in *Figure 12*. If a hollow fiber MBR is used, C&C will eliminate the need for an ultrafiltration membrane unit after the bioreactor. C&C would just need to install a UV disinfection to kill any bacteria that manages to escape with the effluent.

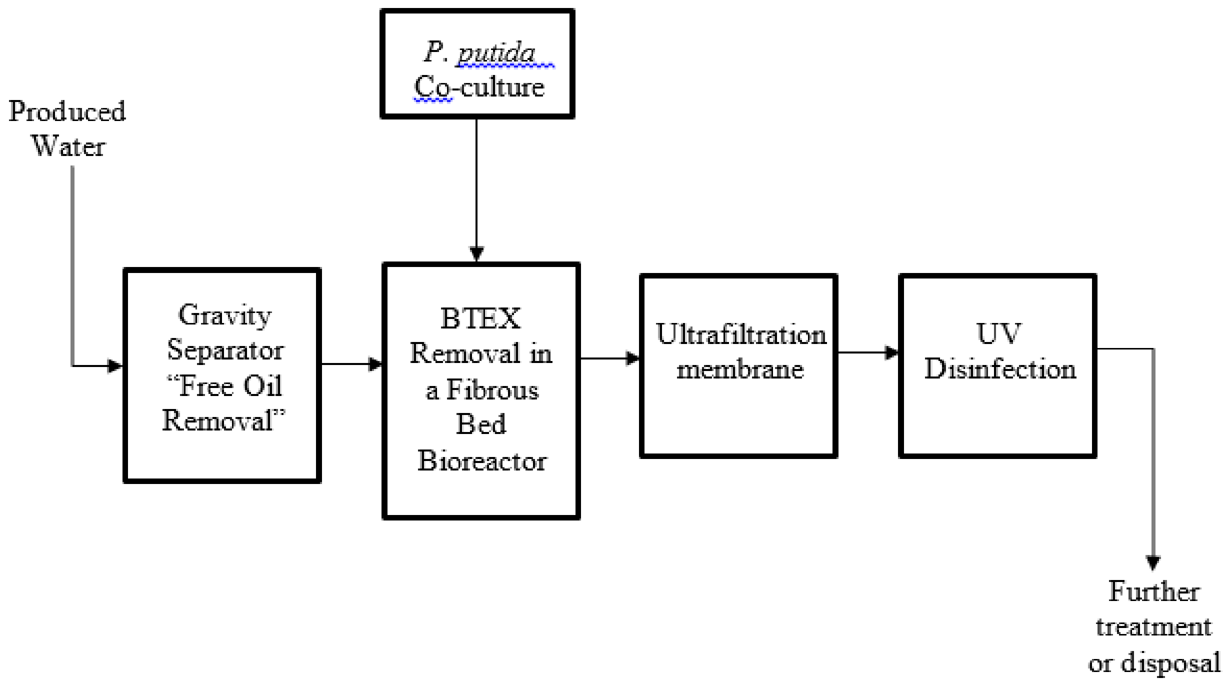

Figure 14. Fibrous Reactor PD: Overall process and production diagram using a fibrous bed bioreactor.

Figure 14 shows the base case description of C&C's BTEXterminator using ultrafiltration membrane and UV disinfection to prevent the discharge of the genetically modified *P. putida* strains in the effluent. In this base case process diagram, C&C is using a fibrous bed bioreactor operating under the conditions necessary for BTEXterminator to survive. Using the fibrous bed bioreactor will require C&C to install an ultrafiltration membrane. In the alternative process diagram where C&C uses a hollow fiber MBR, the ultrafiltration membrane is eliminated. The effluent is already filtered through the membrane modules in the MBR system so that the *P. putida* cells remain in the reactor. In both situations, a UV disinfection system is needed to kill any microorganisms that manage to escape.

Front-side ultrafiltration membrane and UV disinfection

During the research and development phase, C&C will need to investigate the effect the microorganisms present in the produced water have on the two *P. putida* strains ability to degrade BTEX and its constituents. If the microorganisms coming in through the produced water fed to C&C's system do negatively affect the *P. putida* strains ability to degrade BTEX, then a front-side ultrafiltration membrane and UV disinfection system will need to be installed as shown in *Figure 15*. The two units will ensure that no foreign microorganisms are present in the bioreactor. In the alternative design using a hollow fiber membrane, the front-side ultrafiltration membrane and UV disinfection system will be necessary to remove filamentous bacteria that can contribute to biological foaming.

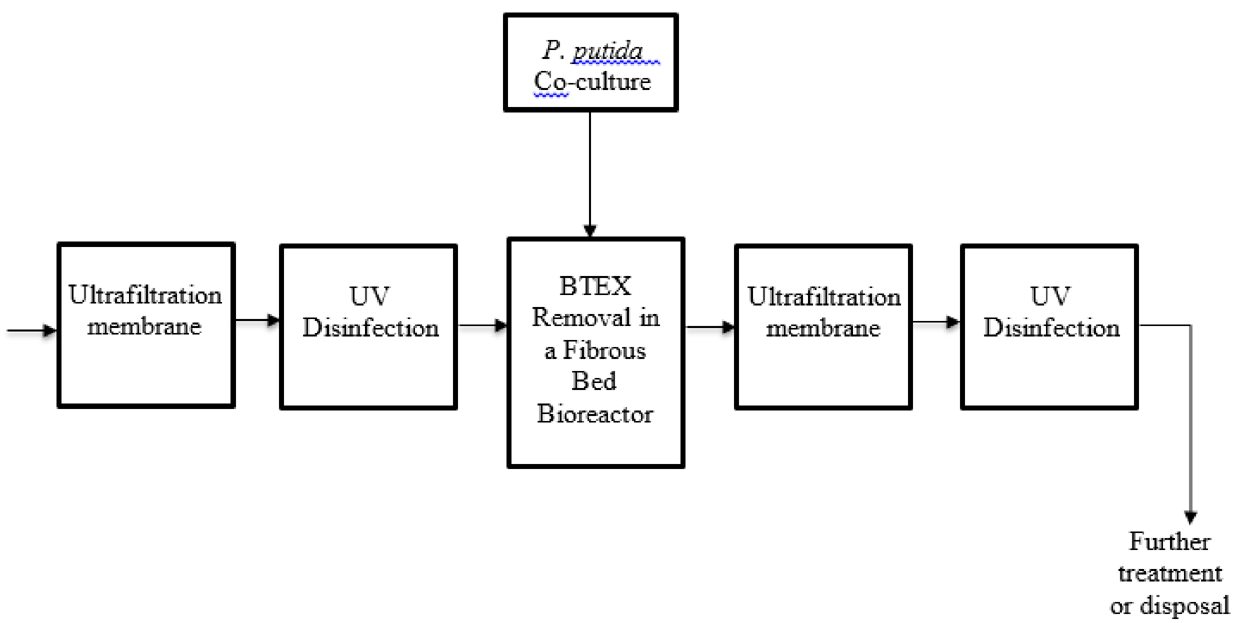

Figure 15. Front Side Filtration: Base case process diagram with the addition of a front-side ultrafiltration membrane and UV disinfection system.

Figure 16 shows how C&C would set up the process to incorporate a front-side ultrafiltration membrane and UV disinfection system to remove microorganisms present in the produced water. To maintain the most effective UV disinfection system, all the surfaces between the UV radiation and the target organisms will need to be frequently cleaned.

The biggest disadvantage to adding a front-side ultrafiltration membrane and UV disinfection system is its associated costs as shown in *Figures 16* and *17*. UV disinfection systems rely on UV radiation that is “either the low-pressure or medium-pressure mercury arc lamp with low or high intensities”[19]. The medium-pressure lamps have about 15 to 20 times the germicidal UV intensity of low-pressure lamps so they can inactivate microorganisms faster. Since medium-pressure lamps operate at high temperatures, they consume more energy, increasing operational costs of C&C’s process. Some microorganisms can use a repair mechanism known as photo-reactivation to repair and effectively reverse the destructive effects of UV[19]. If the microorganisms present in the process water are capable of doing this, then some would be able to pass through and get to the bioreactor housing the *P. putida* strains. The tubes in the UV system are subject to fouling, so the UV system will add to maintenance costs. “The lamps in the UV systems will also need to be replaced every so often. The average lamp life ranges from 8760 to 14000 working hours, and the lamps are usually replaced after 12000 hours of use”[19]. C&C will need to research the amount of UV radiation needed to negate the effects of the total suspended solids (TSS) in the produced water; TSS can shield embedded bacteria. TSS in the water determines “how much UV radiation reaches the target organism; the higher these concentrations, the lower the UV radiation absorbed by the organisms”[19]. If the TSS concentration in the produced water exceeds 30mg/L, C&C will only be able to use UV disinfection systems with medium-pressure lamps[19].

Item	Range*	Typical*
UV lamps	($/lamp)	($/lamp)
1-5 mgd	397-1,365	575
5-10 mgd	343-594	475
19-100 mgd	274-588	400
Construction cost for physical facilities	(% of UV lamp cost) 75-200	(% of UV lamp cost) 150

* Costs are based on a 1993 Engineering News Record Construction Cost Index of 5,210.
Source: Adapted from: Darby et al. (1995) with permission from the Water Environment Research Foundation.

Figure 16. UV Filtration Pricing: Lamp costs for UV disinfection system.[19]

Cost Item	UV System Cost ($)
Capital Costs	
Equipment	120,000
Structural modifications	64,000
Electrical	20,000
Miscellaneous	40,000
Total:	**244,000**
Annual operating and maintenance costs	
Energy	3300
Lamps and chemicals	2840
Cleaning	1180
Maintenance	1440
Process control	6240
Testing	4160
Total	**19,190**

Figure 17. UV Filtration Operating Costs: Capital costs and annual operating and maintenance costs.[19]

Back-side ultrafiltration membrane and UV disinfection

Originally, the base case process employed a sand filter at the end to remove microorganisms. Sand filters are not as effective at removing microorganisms as ultrafiltration membranes. Membranes are also more compact than conventional sand filtration systems.[20] The new design that C&C has adopted will use ultrafiltration membrane and UV disinfection at the end of the process instead of a sand filter previously designed. Ideally, it would be the same equipment as the one for the front-side ultrafiltration and UV disinfection, so the same considerations will apply in the design.

Permitting and Environmental Concerns (Juliet)

Environmental issues associated with C&C's design

If produced water treated by C&C's is to be disposed of, the water will need to be discharged in a way that minimizes erosion and damage to stream banks, beds and ditches. "The most commonly reported environmental concerns are as follows: degradation of soils, ground water, surface water, and ecosystems they support"[18]. Produced water contains high levels of salts, hydrocarbons, and trace elements harmful to the environment. Even after C&C removes the BTEX compounds in the produced water, salts, hydrocarbons, and trace elements will remain. As a result, the water leaving C&C's process will need further treatment, such as a desalination step, before it can be discharged. The idea behind C&C's process is to become one part of the produced water treatment industry. C&C's will only remove BTEX found in produced water. Trace elements in produced water include boron, lithium, bromine, fluorine, and radium. They can be poisonous to plants and are adsorbed in the soil; therefore, they can remain in the soil long after the saline water has been washed away in the stream[18]. The compounds remaining in the water cannot exceed the concentrations set forth in the permits received from the state. If the concentrations of certain compounds are high, aquatic and plant life around the point of discharge could be adversely affected. Furthermore, the water at the point of discharge could be upstream of the water supply to the public, or agricultural and industrial use.

Produced water contains volatile organic compounds (VOCs) such as benzene, which contribute to the air pollution. When VOCs are released into the atmosphere, they can react with nitrogen oxides to form ozone. Treating produced water can result in the release of VOCs into the atmosphere. For example, in their study, Field et al.[21] found that the produced water treatment facility in the Upper Green River Basin emitted significant levels of VOCs into the atmosphere.

C&C will also need to consider any potential for genetic transfer between the genetically modified *P. putida* strains and any microorganisms found in the produced water. Any genetic transfer that occurs in the naturally occurring microorganisms will bring environmental issues. Opponents of genetically modified organisms will protest this issue. Additionally, environmental agencies will be concerned with how the naturally occurring microorganisms will interact once released into the environment. Permit limitations and regulations will dictate whether or not microorganisms can be released from C&C's process.

Permits that must be obtained

- Wyoming pollutant discharge elimination system application for permit to surface discharge produced water from oil and gas production unit discharges.
    - Need to know information about the discharge point, immediate receiving stream, mainstream (nearest perennial water), exact location of the discharge water including latitude and longitude.
    - This permit contains information that shows the limitations and conditions necessary to keep Wyoming's surface water quality standards protected.
    - Permit holders will need to perform routine sampling at the discharge point to show the state that they are complying with the permit limitations and conditions. Note: A blank permit application is attached in the appendix.

BACT analysis for air emissions (still a work in progress)

Fibrous Bed Bioreactor

Step 1 – Identify all control technologies

- Reactor is designed to be a completely enclosed system so that no BTEX constituents escape

Step 2 – Eliminate technically infeasible options

- No technically infeasible option
  - If an aeration system is installed, then volatilization of the BTEX compounds will need to be addressed. Current design uses hydrogen peroxide as the oxygen source.

Step 3 – Rank remaining control technologies by control effectiveness

- The only option is a totally enclosed system

Step 4 – Evaluate most effective controls and document results

- An enclosed system is the only control option so an evaluation step is not necessary

Step 5 – Select BACT

- C&C will use an enclosed bioreactor

Safety and Risk Management (Cody)

The process BTEXterminator undergoes in order to remove BTEX from water is fairly safe when compared to typical chemical engineering processes. BTEXterminator being a biological system means that the process is to be ran at moderate temperatures ($20^{o}C$-$35^{o}C$) and pressures (atmospheric). With no real risk of extreme operating conditions, the operating safety of the process itself is more of a potential environmental risk (as previously mentioned) than it is an immediate human safety risk. When operating the risk of equipment failure is largest when discussing the potential release of GMO into the environment along with BTEX concentrated water. Neither of these concerns are immediately noticeable or lethal but the exact consequences are unknown. During normal operations C&C has designed the reactor with multiple barriers so that the GMOs are unable to go downstream with the remediated water, these safety measures should be sufficient seeing as how there is really no risk for releasing these concerns any other way. To visually display the consequences of failure to operate within designed guidelines C&C has created a HAZOP table, as seen in *Table 6*. This table outlines particular pieces of equipment from the base case, the parameter investigated, and the consequences of deviating from designed value of that parameter.

While the risks of coming into contact with BTEX is self-explanatory at this point the real human safety risk lies within the laboratory as opposed to normal full operations. During the design and testing of this GMO product C&C will have to come into contact with BTEX, as well as, the modified organisms themselves. To minimize this risk members working with these compounds in the lab will follow a specific procedure that includes proper safety equipment. The particulars on that are discussed in detail in the *Experiments* section of this report.

Table 6. HAZOP: Summary HAZOP for BTEXterminator.

HAZOP				
Equipment/Operation	**Parameter/Variable**	**Deviation**	**Possible Causation**	**Consequences**
Bioreactor (R101)	**Temperature**	Less	1. Influent temperatures are lower than normal	Microorganisms present in the reactor system decrease activity & die
			2. Reactor pressure has decreased	
		More	1. Influent temperatures are higher than normal	Microorganisms present in the reactor system decrease activity & die
			2. Reactor pressure has increased	
	Pressure	NA		
	Composition	As well as	1. Contamination from incoming organisms	Unknown
			2. Metals, salts, hydrocarbons fall outside suitable range for BTEXterminator	Microorganisms present in the reactor system decrease activity & die
			3. Nutrients necessary are not present in proper amount	Microorganisms present in the reactor system die

		Other than	1. The experimentally determined cell concentration	BTEXterminator insufficiently degrades BTEX or perhaps fails to do so
<u>Pump (P101)</u>	**Flow**	Less	1. Valve/operations to pump are faulty or off	Damage to pump
				Lack of hydrocarbon sources available for organisms
		More	1. Valve/operations to pump are faulty or improperly operated	Damage to pump
				Too much water delivered to system, probable pressure increase leading to leakage of water and organisms into environment
		No	1. Reactor is not in operation	Organisms still need a carbon source and additional

				nutrients to grow and live
	Temperature	More	1. Water/upstream causes warmer water than usual	Possibly pump damage or lines
				Reactor influent will be unnecessarily warm and possibly kill organisms
		Less	1. Water/upstream causes cooler water than usual	Possibly pump damage or lines
				Reactor influent will be unnecessarily warm and possibly kill organisms
Pump (P102)	Same conditions and hazards as for the influent pump (P101) but geared towards downstream operations			
Membrane Filter (F101)	**Temperature**	More	Not for our system	

		Less	1. Condensate forms on membrane	Inefficient membrane filtration
	Flow	More	1. Damage Filter	Allows GMO to move downstream

Project Economics (Cody)

BTEXterminator is an innovative technology in produced water treatment because the genetic modification and design of the co-culture to treat produced water is unique. This made running economic estimates incredibly difficult and the numbers in many categories are very rough since a working prototype does not exist at this time. All pricing was estimated with either a direct price online or factors commonly used in CHE 3070. C&C split costs up into two sections, research costs and process costs, similar to the key focus of two categories within the overall water treatment diagram. The research and lab equipment cost section was used as a startup cost; this was the money required to research over the anticipated five years of research time. *Table 7* shows a majority of the physical lab equipment, but further costs were anticipated and estimated since research is so expensive. This cost was negated by outside funding in the cash flow statement, assuming that this would be provided for by investors and/or grants. Since it is difficult to estimate operation ranges for BTEXterminator so early into its design, no utilities will be included in the economics specifically, but will be accounted for by increasing the required IRR to be 40%. In this analysis, equipment costs, common economic factors used from CHE 3070, and some sourced prices are used.

Table 7. Research Costs: Depicts the large capital expenses anticipated for research of BTEXterminator, high risk is associated with the assumption of unforeseen engineering costs.

Lab Equipment/Research Costs	QTY	Price $	EQUIP ISBL $
Gas Chromatographer and Mass Spectometer	1	95000	95000
Bioreactors	2	50000	100000
Spectrophotometer	1	15000	15000
High Performance Liquid Chromatographer	1	50000	50000
Misc. (mineals/nutrients/glassware/misc. included)	1	20000	20000
BTE1 P. putida strain	0.1 mL	FREE	0
TX1 *P. putida* strain	0.1 mL	FREE	0
TOL plasmid	2 mL	500	500
TOD plasmid	2 mL	500	500
Engineering Costs	-	10000000	10000000

Process equipment was a list of the equipment required to treat produced water, this was based on an assumption that nine reactors would be used for a single job in the base case, listed in *Table 8*. These prices are purchase prices and do not include installation, an installation factor is later multiplied with the total ISBL to compute the installed FCI. Miscellaneous process equipment such as pumps, mixers, piping, etc. is assumed to be a fraction of the ISBL. The reactor prices were reduced drastically in the spring semester due to a change in material, from stainless steel to fiberglass. Both the UV and membrane filtration units were priced as individual units for a single reactor, rather than for the entire system because it allows for C&C to continue the scalability of the design. Different clients will need a different number of reactors so each reactor cost will be incurred in the economic analysis, including their own filtration units.

Table 8. Process Equipment Costs: Economics of process equipment used to treat produced water, referred to equipment name and indication given to process diagram equipment number, ISBL cost is the purchase price.

Process Equipment (Process Diagram Nomenclature)	QTY	Size	Price $	EQUIP ISBL $
Pumps (P101/102)	NA	NA	10% Process ISBL	
Reactors (R101-R109)	9	5000L	51400	462600
Spare Cellular Suspension Tank (NOT IN PD)	1	5000L	25000	25000
UV Filtration Unit (NOT IN PD)	9	10 gpm	750	6750
Membrane Filtration (NOT IN PD)	9	5000 L/hr	20000	180000
Gravity Separator (NOT IN PD)	1	25000 L/hr	20000	20000
Mixers/Splitters (Misc.)	NA	NA	10% Process ISBL	
Piping/Hoses (Misc.)	NA	NA	10% Process ISBL	

The variable costs are a bit different for the process as well. C&C's variable costs consider the amount of additional nutrients and chemicals required to help the product perform per reactor over a year of operation time as shown in *Table 9*. This variable cost would scale based on the number of jobs and number of reactors C&C was operating under, but for this analysis a weekly amount of the basal mineral salts media was priced. During the research phase C&C would define the own media so this will likely change quite a bit, plus the consumption rate of these media components will not be known until then.

Table 9. Variable Costs: Variable cost per reactor. Based on the nutrient needs and environmental controlling chemicals BTEXterminator needs to thrive in a reactor for approximately one week.

BTEXterminator Costs for a single reactor	Amount	Units	Price $$$/yr
Co-Culture	77180	gm	0
Basal Mineral Salts Media	-	-	170 $/kg
K2HPO4	25000	gm	mass based (required per week)
NaH2PO4	12500	gm	
(NH4)2SO4	5000	mg	
disodium nitrilotriacetate	75000	mg	
MgSO4-7 H2O	150000	mg	
CaCl2	50000	mg	
MnSO4-H2O	25000	mg	
FeSO4-7 H2O	5000	mg	
CoCl2	5000	mg	
ZnSO4-7 H2O	5000	mg	
CuSO4-5 H2O	500	mg	
H3BO3	500	mg	
Na2MoO4-H2O	500	mg	
pH adjuster (NaOH, HCL desired pH 7.5-8)	NA	NA	NA
aeration chemical (H2O2 if o2 needed for reactor)	231540	gm	18298.8
BTEX	3000000	gm	0

A simple cash flow statement was then created and analyzed, it was assumed that C&C would scale up operations until nine skids had been built (enough for the base case). Depreciation was taken into account using the MACRS5 model and its factors. The capacity factor and the number of skids in operation was included into the cash flow analysis based on a ramp up C&C feels is reasonable. Start up (research costs) were included but negated with the assumption that C&C would acquire additional funding to cover those costs. Fixed costs per skid were included regardless of the capacity factor. Variable costs per skid scaled with the capacity factor for the given year. A tax rate of 35% was assumed and taken into account on all projected taxable income. Cash flows for a 0% and 12% discount rate were done to take into account the time value of money during the anticipated project life. *Table 10* shows the template and the categories calculated in the cash flow statement, the statement itself is over a 15-year project life and too large to read in a Word document.

Table 10. Cash Flow Statement Template.

Year (for discounting)(year end)
FCI (for one skid)
WC (FCI*0.89/5.04)
Funding (private/grants/etc)
MACRS5 Factors
Depr Amount
Depr Credit
Nameplate Revenue per skid annually
Capacity factor
Skids Operating
Revenue
StartUp (Lab Equipment/research years)
Variable Costs (MM$ per skid annually, at 4 jobs a yr)
Fixed Costs (salaries, maintenance, %FCI)
Total Expenses (including SU)
Revenue - Expenses (including SU expenses)
Tax Liability on Above Item (35%)
Cash Flow
CumCF (PV0)
DF12
PV12
CumPV12

The FCI along with fixed and variable costs were put into a per skid cost basis, C&C assumed a skid would operate four separate jobs a year (three months of peak water production). These are the cost to build a single skid with one reactor, own that skid for the year, and effectively operate that skid during the year. The number of skids was scaled up over the course of the first few years rather than just building nine reactors in year 1, which would not be reasonable. C&C then back calculated a required revenue to achieve an IRR of 40%, a reasonably high estimation because of all the risk and uncertainty with this project. This method was done because a price has not been established for this new technology and product as compared to more traditional chemical engineering designs. A payback period of approximately five years is estimated based on the cash flow analysis; this yields a net present value at a 12% discount rate of nearly $81 million dollars. A summary of the results can be seen in *Table 11*.

Table 11. Economic Summary

Annual Economic Summary		
FCI (per skid)	6.01	$MM/yr
Fixed Costs (per skid)	2.00	$MM/yr
Variable Costs (per skid)	1.19	$MM/yr
IRR	40.00	%
NPV0	264.20	$MM
NPV12	80.47	$MM
PBP	4.50	yrs
MARR	30.00	%

Once a required revenue was calculated to generate the desired IRR, C&C then used that revenue to calculate the approximate cost per barrel to run the process for the operator. This was done by taking the profit margin per skid (Profit=revenue-fixed costs-variable costs) and dividing that by the number of barrels of produced water treated in three months from the base case. Operators are not going to run this process if it costs them more than salt water disposal wells (SWD), unless forced with regulations. C&C wanted to know the price of the process to the operator per barrel so it could be compared to SWD. The pricing summary shown in *Table 12* displays these prices and compares them to the price Don Whisonant[4] reported for the operator to use the SWD route.

Table 12. Price summary

Price Summary		
Revenue (per skid)	6.12	$MM/yr
Fixed Costs (per skid)	2.00	$MM/yr
Variable Costs (per skid)	1.19	$MM/yr
Profit Margin	2.93	$MM/yr
BTEXterminator Operator Cost	3.61	$/bbl
SWD Operator Cost	3.5	$/bbl

This economic analysis was very rough based off of the prices we were working with. There are a ton of assumptions made that can sway these results either way. The major price categories that will change the cash flow analysis for this project include; the research cost, a lot of the major process equipment prices, and the IRR was set high to account for risk but could cause the required revenue to be high. It is unlikely C&C would achieve a 40% in the real world so the fact that this project still cannot undercut SWD prices is worrisome. The costs to build and operate these skids needs to come down but in order to make that happen the degradation rate of the organism needs to be at least equal to the target used in this analysis, preferably better.

A simple 20% variable sensitivity was run to determine their effects on the cost of BTEXterminator to the operator for FCI, fixed costs, and variable costs per skid. Analyzing the cost to the operator is a direct metric to evaluate the feasibility of the project, after changing to a fiberglass reactor it drastically dropped the price per barrel of water being charged to the operator. *Table 13* displays the sensitivity calculations done and their effect on the price of BTEXterminator compared to typical SWD operations in the Powder River Basin.

Table 13. Sensitivity Analysis

Sensitivity Variable Change	Base Case	20% FCI Increase	20% FCI Decrease	20% VC Increase	20% VC Decrease	20% FC Increase	20% FC Decrease	Units
FCI (per skid)	6.01	7.21	4.81	6.01	6.01	6.01	6.01	$MM/yr
Fixed Costs (per skid)	2	2	2	2	2	2.4	1.6	$MM/yr
Variable Costs (per skid)	1.19	1.19	1.19	1.43	0.95	1.19	1.19	$MM/yr
IRR	40%	40%	40%	40%	40%	40%	40%	%
NPV0	264.2	318.2	211.77	264.24	264.79	264.95	265.09	$MM
NPV12	80.47	96.99	64.51	80.47	80.68	80.75	80.8	$MM
Required Revenue (per skid)	6.12	7.12	5.14	6.36	5.89	6.53	5.73	$MM/yr
Profit Margin	2.93	3.53	2.35	2.93	2.94	2.94	2.94	$MM/yr
BTEXterminator Operator Cost	3.61	4.2	3.03	3.75	3.47	3.85	3.38	$/bbl
SWD Operator Cost	3.5	3.5	3.5	3.5	3.5	3.5	3.5	$/bbl
Margin for Operator (SWD to treat water further)	-0.11	-0.7	0.47	-0.25	0.03	-0.35	0.12	$/bbl

The sensitivity analysis was difficult for the economics of this project due to the fact that a revenue was back calculated, feedstock and product prices do not exist for this project. A more detailed sensitivity could be done once the independent variables in the variable cost section were identified (rather than purchasing one media type we could buy fertilizer or components separately and make our own), or perhaps individual pieces of equipment could be checked but this should be modeled within reason by varying the FCI. *Figure 18* shows a tornado diagram visually depicting the most sensitive design variables from greatest to least.

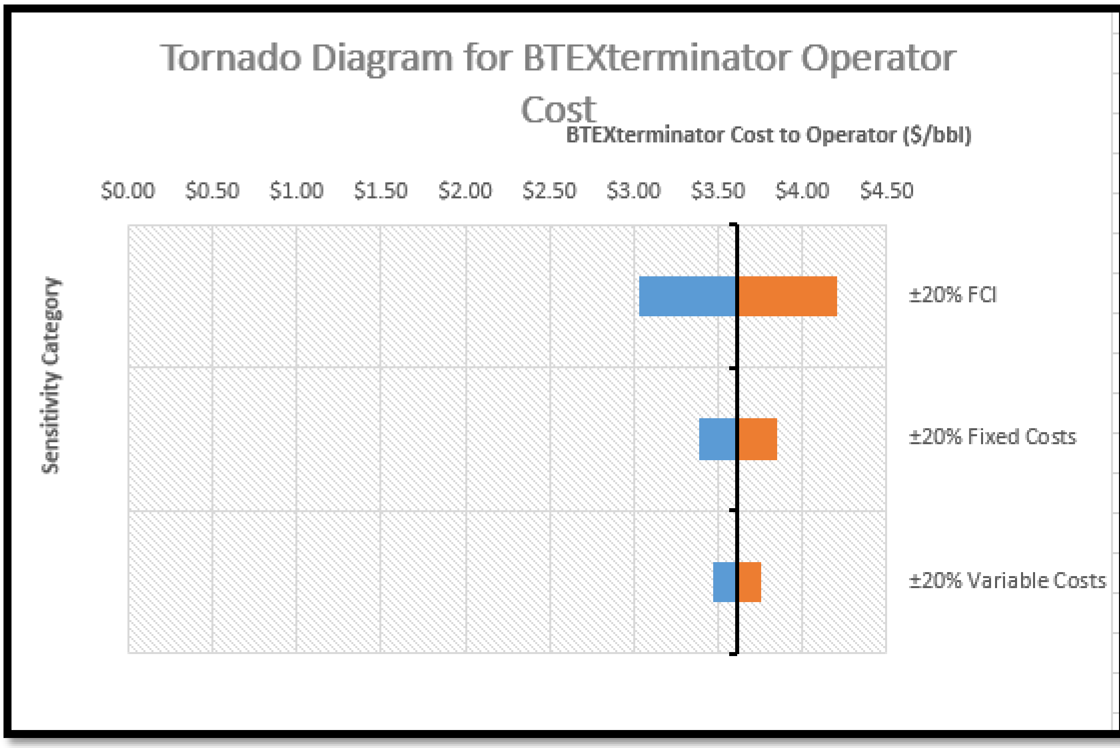

Figure 18. Tornado Diagram: depicting the variables most and least effecting the operator margin with a 20% change.

For the variables manipulated FCI had the highest impact on the operator's margin. This is not surprising when considering what all is included into the FCI from a design standpoint. The immediate costs incurred in the FCI are equipment purchase prices (reactors, pumps, filters, etc.) which will likely drastically change as this project is explored further. Reactor size changes when BTEXterminator's efficiency changes, the degradation rates are still unknown and could change by a factors up to 1000 possibly. Number of reactors would change based on the degradation rate, as well as, the amount of water that needs to be treated and the formation it comes from. Overall, it is far too early in BTEXterminator's business plan to feel confident or deflated over economic results. They are nice to have in place and as more information because available in C&C's research it can be immediately applied to the models we have created here, but as of now has little significance on the feasibility of this project. Below is a list of the assumptions made through the economics section.

- Economic Assumptions
  - Utilities included in high risk assumption made with 40% IRR
  - Startup/research costs would be negated by incoming outside funding
  - Yearly fixed costs to operate a skid are a fraction of the FCI
  - Variable costs scale with the production rate of the skid
  - Installation factor included in the FOB of the skid equipment
  - 12% discount factor included in cash flow analysis for time value of money
  - Revenue generated calculated based on the required revenue to achieve 40% IRR
  - Devon Energy SWD costs well represented expected markets cost for all SWD operations

After a 15-year project life of the base case C&C does not anticipate to have any substantial salvage value for the project. Most of the process equipment will probably need replaced before the end of the project life (filtration system and pumps), and the reactors themselves will have spent 15 years running produced water and growing microorganisms within them. Perhaps the reactor could be cleaned thoroughly and sold, but the shutdown and clean up expenses would likely be greater than the value of the equipment itself at that point.

Global Impacts (Christine)

While there are many regulations already in place for the disposal of produced water, as previously mentioned there is anticipation of further regulation in regard to the components of BTEX in produced water. There has also been a push in major plays across the United States for reuse of the water for agricultural purposes[1]. The same article goes on to state that "…it is anticipated that some of the next steps in the regulatory environment of Texas will include tax incentives for water treatment infrastructure investment." Currently in the state of Colorado, the disposal of flowback and produced water is regulated by the COGCC and UIC programs[2]. C&C hopes to provide a product to meet the anticipated need of water treatment due to further regulation, and the increasing need to use produced water for uses other than reuse in operations. It is important to note that the political climate of both the state governments, and the federal government has a role in the implementation and adherence to regulations pertaining to issues like water.

Water is a valuable resource all over the world. C&C's technology and product BTEXterminator can be used in an effort for oil and gas operators to reuse water for agricultural use and even for drinking water. Better access to water can dramatically change the lives of people all around the world. This access can also make it possible for economies to grow and prosper.

Public perception of genetically modified organisms is a significant portion of the community involvement in the use of BTEXterminator. According to a poll administered by ABC, Americans almost unanimously are against genetically modified foods[3]. While this is not directly applicable to C&C's product, it should be considered. Americans don't like the infiltration of GMOs into food sources, it is not a far step to think about GMOs in the environment and the concerns they may have. It is optimistic that a study showed that "nonfood applications tended to receive higher support levels than genetically modified foods. … Only perceived benefits were significantly related to each biotechnology application." [4] Many companies fear that public perception, and governmental red tape make it unprofitable to use GMOs for bioremedial purposes, but that is simply not true. In fact, the use of GMOs in bioremediation is becoming increasingly popular in many worldwide applicatoins[5].

Conclusions and Recommendations (Christine)

In this design, BTEXterminator will be placed into nine, 5000 L reactors. This process will be made on skids so that mobility is achieved. These reactors are fibrous bed reactors, which allows for the immobilization of the product. Also, these reactors are made of fiberglass. Along with the reactors, different methods of controlling the flow rate of produced water, as well as, sanitizing the produced water will be online. These are precautions that must be taken in order to control the process as much as needed.

The economic analysis was done on a per skid basis, and did yield results that show this project could potentially be feasible. The cost per skid is $6.01MM and the revenue required per skid required to achieve an IRR of 40% is $6.12MM. This makes it so that the cost to the operator per barrel of produced water is $3.62. The current price of the disposal of water is around $3.50/bbl. So, while this technology is not currently cost effective, C&C believes that it will be once new regulations on BTEX in produced water are put into place.

The hope of this project is that the technology will be developed and ready to use once new regulations are passed. That way, C&C can meet the operators' needs first, and can be more efficient than other new technologies that emerge due to the regulations.

Future Work (Christine)

Since this project is mainly theoretical, there are many unknowns to the project. So, a lot of future work must be done in order to truly understand the logistics of the project. The biggest uncertainty in the current design is the degradation rate of BTEX in produced water. The literature sources do not account for a co-culture comprised of both of the P. putida strands used in BTEXterminator. So, C&C assumed a rate greater than that found in the literature in the hopes that the product will be more efficient than current designs. The degradation rate in literature is 600 mg/L/hr, and C&C assumed the rate to be 600 g/L/hr as a result of the co-culture. In the future, C&C needs to calculate the optimum degradation rate of BTEX that results in an acceptable FCI. The sizing of the equipment and the time it takes for the BTEX to be removed is directly related to this rate, which is why this is such a critical number. If the rate is too low, then the feasibility of the design is greatly affected.

C&C also needs to do an analysis on the interactions between the bacteria existing in the produced water and the organisms that make up BTEXterminator. These interactions can hinder the rate of degradation of BTEX, which again could greatly affect the feasibility of the design. So, a core analysis could be done of the typical formations that are in the Powder River Basin, which would show the natural makeup of bacteria in those formations. Then, lab work could be done with those organisms to simulate those interactions between the existing organisms and BTEXterminator.

Understanding the behavior of the bacteria in starvation conditions is an integral aspect of the design for many reasons. First, the bacteria will be without a carbon source when the reactors are transported to the well pads. Second, the concentration of BTEX can be much lower at times than the assumed concentration in the preliminary design. Gaining an understanding of how the bacteria react to these conditions will make it possible to know the limitations of the bacteria during the times of transportation and low BTEX concentrations.

In the current design, C&C uses hydrogen peroxide as the oxygen source for BTEXterminator. On a lab scale, the literature shows that this works well for the organisms. However, more work into aeration should be done in order to know whether this is the best option on a large scale. For example, a different chemical or method of aeration could lower the price of the equipment, which would make it so that the process itself was much less expensive.

Finally, the organisms' viability should be tested with produced water from the Powder River Basin. The water that C&C has access to at the University of Wyoming is not from the Powder River Basin, but from the Front Range, so the content of salts, metals, bacteria, and hydrocarbons could be very different. This information can be used to understand how the pH and temperature need to be monitored during the use of BTEXterminator. This would allow for a more specific design of the process, which would make the economic analysis more accurate.

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Appendices

All supporting documents not directly displayed within this report can be found in our supporting documents database. Aspen, Excel, PDFs, etc. can all be found within this database and will have the same file name as listed below.

Database

- MSDS Sheets
  - (NH4)2SO4 msds.pdf
  - Benzene msds.pdf
  - CaCl2 msds.pdf
  - CoCl2 msds.pdf
  - CuSO4 5 H2O msds.pdf
  - Disodium nitrilotriacetate msds.pdf
  - Ethylbenzene msds.pdf
  - FeSO4 2 H2O msds.pdf
  - H2O2 msds.pdf
  - H3BO3 msds.pdf
  - K2HPO3 msds.pdf
  - MgSO4 7 H2O msds.pdf
  - MnSO4 H2O msds.pdf
  - Na2MoO4 H2O msds.pdf
  - NaH2PO4 msds.pdf
  - NaOH msds.pdf
  - Toluene msds.pdf
  - Xylenes msds.pdf
  - ZnSO4 7 H2O msds.pdf
- Word Documents
  - GMO Fall 2015 Final Report.docx
  - Progress Report 1.docx
  - Progress Report 2.docx
  - Chrissy's Senior Project Questions.docx
- Excel Worksheets
  - Economics Estimates updated.xls
  - Process & GMO material balances.xls
  - Reactor Design.xls
  - PRB Parkman Water Profile 151122.xlsx
- Power Points
  - Progress Report 1.ppt
  - Progress Report 2.ppt
  - GMO Senior Symposium Presentation.ppt
- Aspen Files
  - Simple Process Flowchart parallel.apwz
  - Simple Process Flowchart.apwz
- PDF Files
  - Does Application Matter? An Examination of Public Perception of Agricultural Biotechnology Applications
  - Aerobic biodegradation of BTEX: Progresses and Prospects
  - A continuous fibrous-bed bioreactor for BTEX biodegradation by a co-culture of Pseudomonas putida and Pseudomonas fluorescens

- Isolation and Expansion of the Catabolic Potential of a Pseudomonas putida Strain Able to Grow in the Presence of High Concentrations of Aromatic Hydrocarbons
- Biodegradation of BTEX mixture by Pseudomonas putida YNS1 isolated from oil-contaminated soil
- Strains Degrading/Utilizing Natural or Xenobiotic Compounds
- MBR module design and operation
- Membrane bioreactors: Advanced membranes improve energy efficiency and reduce lifecycle costs
- Wastewater Technology Fact Sheet: Ultraviolet Disinfection
- Oil and Gas Produced Water Management and Beneficial Use in the Western United States
- WYPDES Application for Permit to Surface Discharge Produced Water from Oil and Gas Production Unit Discharges
- Performances of dead-end ultrafiltration of seawater: From the filtration and backwash efficiencies to the membrane fouling mechanisms.