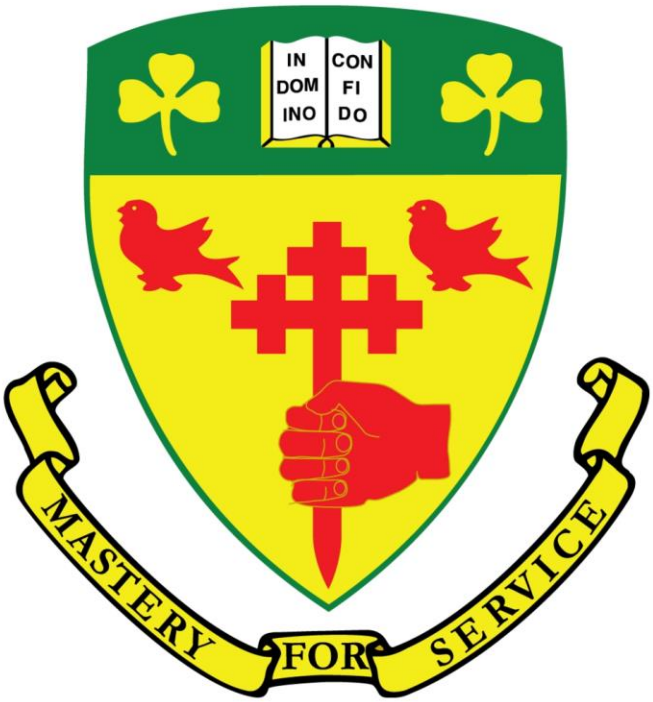
Design 3 Project Report: Hydroponics Fertilizer from Finished Vermicompost

April 10, 2018



Term Paper

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Abstract

The global hydroponics industry is projected to surpass 27 billion USD by 2022, with a cumulative annual growth rate of 9.1% in North America. The Canadian cannabis industry is expected to be valued in excess of 22 billion CAD, with a low-end consumption estimate of 600 tonnes annually in Canada. The company Phytospec wishes to produce an inexpensive biological hydroponics fertilizer to be used at a greenhouse in northern Quebec. Phytospec is primarily interested in phytohormone production, as recent literature suggests phytohormones dramatically alter the alkaloid and terpenoid profiles of cannabis. This report details the design of a bioreactor which ferments the desired fertilizer product from finished vermicompost. Various mixing and flow patterns were empirically tested to achieve optimal homogenization and aeration within the bioreactor. Aeration rates for the bioreactor are estimated at 155.6 cm^3/s, using 1D mathematical vortex flow models. Heat removal by natural convection is demonstrated to be ineffective using the Churchill-Chu correlation, and a heat jacketing system is sized for heat dissipation. Materials and design are selected to comply with industry standard food safety and equipment cleaning practises. Methodology is introduced for demonstrating fertilizer efficacy by empirical trails adhering to NCR101 controlled environment guidelines and by laboratory testing. A user guide is provided for bioreactor operation and maintenance. Finally, observed design flaws and potential improvements are noted and discussed.

Vision Statement

Our team aims to design optimized, economical products for the agricultural industry. Agriculture has the potential to heal the earth and provide humanity with abundant food, fiber, fuel, and industrial feedstocks. This will only occur if agricultural engineers work to create sustainable and environmentally conscious products, practises, and systems. We wish to provide our client with a quality product which meets all specifications, while also considering sustainability.

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List of Acronyms and Abbreviations

1.	ABA	Abscisic acid
2.	CBD	Cannabidiol
3.	CCW	Counter clockwise
4.	CFIA	Canadian Food Inspection Agency
5.	CIP	Clean-in-place
6.	CW	Clockwise
7.	DMAPP	Dimethylallyl Diphosphate
8.	$GA_{1,3 \text{ and } 4}$	Gibberellic acid
9.	$GA_{9, 19 \text{ and } 20}$	Gibberellic acid precursors
10.	IAA	Indole-3-acetic acid
11.	IAAld	Indole-3-acetaldehyde
12.	IAM	Indole-3-acetamide
13.	ICCEG	International Committee for Controlled Environment Guideline
14.	iP	Isopentenyladenine
15.	iPMP	Isopentenyladenosine-5'-monophosphate
16.	IPyA	Indole-3-pyruvic acid
17.	NADH	Nicotinamide Adenine Dinucleotide
18.	NCR101	North Central Region 101
19.	NOP	National Organic Program
20.	NPK	Nitrogen, Phosphorus, Potassium
21.	PPM	Parts Per Million
22.	PVC	Polyvinyl Chloride
23.	THC	Δ^9-tetrahydrocannabinol
24.	TRP	L-tryptophan
25.	USDA	United States Department of Agriculture

1.0 Introduction

This report presents the research, findings, and work conducted in fulfillment of our engineering design project. The project's incentive and our client's goals are introduced to inform and justify design criteria. Current literature, patents, standards, and legislation are reviewed and analyzed for applicability to our project. The main portion examines the prototyping process specific to our project. The final portion details efficacy testing methodology and outlines future work.

Our project was introduced and funded by Phytospec, a hydroponics technology consultant startup based in Laval, Quebec. Phytospec is currently assembling a suite of integrated technologies to be employed in a greenhouse in northern Quebec. Phytospec wishes to construct a bioreactor to produce hydroponics fertilizer from a feedstock of finished vermicompost. Specifically, our client would like to economically produce a solution containing phytohormones, nutrients, and symbiotic microbes. Our goal in this project is to address our client's wishes by providing a functional prototype fulfilling Phytospec's requirements.

Biological waste has been utilized as a soil amendment since the advent of agriculture. Stone pits dated to 4000 BC have been discovered in Neolithic Sumerian cities, used for composting "organic urban waste... for eventual application on agricultural fields" (Diaz, De Bertoldi, & Bidlingmaier, 2007). Compost promotes "the growth and development of plants… such as rooting, time of flowering, leaf area development and lengthening of internodes" (Warman & AngLopez, 2010). Some of these benefits are applicable to field crops only: organic matter additives improve soil texture and field capacity and prevent compaction. Essential plant nutrients and beneficial symbiotic microbial populations may be extracted into a liquid fertilizer by brewing "compost tea", a method developed for spray application over grass lawns. Compost tea is conventionally produced by mixing finished compost and water for 24-48 hours. Liquid chromatography and mass spectrometry have recently shown vermicompost to contain concentrations of the phytohormones indole-3-acetic acid, cytokinins (trans-zeatin, kinetin, N^6-isopentenyladenine, and N^6-isopentenyladenosine), 18 gibberellins, and 6 brassinosteroids (Aremu et al., 2015; Zhang et al., 2014). As a consequence, compost teas are now often aerated and may use additives to promote microbial growth and fermentation of phytohormones.

	Substances	Control	1 μM ABA	10 μM ABA
Monoterpenoids	α-pinene	5.91 ± 0.22	0	0
	β-pinene	2.78 ± 0.15	0	0
	myrcene	2.03 ± 0.09 a	0.78 ± 0.18 b	0
	limonene	2.23 ± 0.18	0	0
	α-campholenal	1.00 ± 0.25 a	0.52 ± 0.02 b	0
	verbenol	10.79 ± 1.25a	6.54 ± 0.74 b	4.23 ± 0.70 c
	pinocarvone	1.17 ± 0.08	0	0
	α-phellanderene	1.00 ± 0.11	0	0
	myrtenal	3.32 ± 0.35 a	1.01 ± 0.25 b	1.13 ± 0.15 b
	verbenone	2.22 ± 0.16 a	1.85 ± 0.14 b	1.16 ± 0.09 c
	citronellol	2.86 ± 0.76 a	2.54 ± 0.54 a	1.98 ± 0.64 a
	carvone	0.76 ± 0.25 a	0.47 ± 0.09 a	0.35 ± 0.02 b
Sesquiterpenoids	caryophyllene	3.70 ± 0.19 a	2.84 ± 0.45 b	1.02 ± 0.27 c
	β-farnesene	2.18 ± 0.64 a	0.63 ± 0.05 b	0
	α-humulene	1.23 ± 0.07	0	0
	germacrene d	1.72 ± 0.24 a	1.00 ± 0.34 b	0.54 ± 0.07 c
	β-bisabolene	1.08 ± 0.23	0	0
	Δ-amorphene	1.32 ± 0.39	0	0
	spathulenol	2.46 ± 0.42	0	0
	caryophyllene oxide	2.76 ± 0.05 b	2.56 ± 0.21 b	3.05 ± 0.15 a
	γ- eudesmol	0.79 ± 0.06 b	0.95 ± 0.22 b	1.35 ± 0.15 a
	valerianol	0.81 ± 0.09 b	1.28 ± 0.25 a	1.33 ± 0.22 a
	β-eudesmol	0.86 ± 0.09 c	1.54 ± 0.29 b	2.06 ± 0.54 a
	bulnesol	0	0.89 ± 0.11 b	1.67 ± 0.34 a
	α- bisabolol	0.53 ± 0.08 c	0.85 ± 0.09 b	1.59 ± 0.25 a
	cryptomerione	0	0	0.48 ± 0.11
Cannabinoids	cannabichrome	2.50 ± 0.40 c	4.67 ± 0.50b	14.22 ± 3.57a
	cannabinol	1.05 ± 0.35 c	1.99 ± 0.31b	2.87 ± 0.23 a

Table 1. Impact of abscisic acid on terpenoid concentration in essential oil of *Cannabis sativa* (Mansouri & Asrar, 2012)

Recent literature suggests that phytohormones affect secondary metabolite production in cannabis (table 1). Mansouri, H., Asrar, Z., & Amarowicz, R (2011) found that "applying of 100 μM GA_3 increased THC and CBD content in comparison with control plants". The authors believe that this is not a direct effect of GA_3 alone, but reflects an interaction between several phytohormones present in the plant. This is partially because earlier work done by Mansouri, Asrar, & Mehrabani (2009) showed that in the flowering stage, GA_3 could also slightly reduce cannabinoid concentrations. GA_3 and abscisic acid have likewise been shown to alter terpene profiles of cannabis (Mansouri & Asrar, 2012). Both increased the production of the cannabinoids while increasing the concentrations of some terpenoids and lowering the concentrations of others.

2.0 Project design criteria

To determine design criteria for the project, we met with our client and discussed the scope and goals of the project. Our client outlined the project incentives and how the project would be implemented. Using this information, we worked together with our client to outline the design criteria for our project (fig. 1).

Specifications

- Food-grade materials
- Heat resistance
- Heat removal
- Durability
- Rust resilience
- Aeration
- Homogenization
- Particle containment/separation

Constraints

- Cost
- Pathogen Control
- Cleaning/maintenance

Figure 1. Design criteria: specifications and constraints

There are three main classes of criteria: materials, functionality, and safety. Materials must be selected for economy, functionality, heat tolerance, cleanability, and compliance with health and safety codes. Functionality entails optimized mixing, aeration, heat removal, and filtration. Safety specifications and constraints include food safety compliance, CIP design compliance, and pathogen control. Ultimately there is overlap between criteria as the listed criteria are all facets of our end goal: economically producing a quality product.

3.0 Review of literature

3.1 Phytohormone biosynthesis

There are two main sources for the production of plant hormones: endogenous sources (those within the plant tissue) and exogenous sources (soil microorganisms and fungi) (Muhammad et al., 1991). The ability to produce auxins, gibberellins, and cytokinins is very common among plant-associated bacteria responsible for plant growth promotion, symbiotic association, and pathogenesis (Muhammad et al., 1991). Many distinct species of bacteria produce these beneficial plant hormones. Different bacteria produce a specific type of phytohormone that has a unique effect on the plant growth.

Auxins, produced by *Azospirillum*, *Rhizobium* and *Bradyrhizobium* show decrease of root lengths on plants, increase of root hair and development. Indole-3-acetic acid (IAA) is a potent physiologically active auxin. It is commonly derived from L-tryptophan (TRP). IAA is produced in two unique metabolic pathways. In the first, TRP is converted by deamination of indole-3-pyruvic acid (IPyA) followed by decarboxylation to indole-3-acetaldehyde (IAAld), then oxidized to IAA. In the second, TRP is decarboxylated into indole-3-acetamide (IAM) which is then hydrolyzed to IAA. TRP is a common metabolite of microbes and fungi with a final product of IAA (Muhammad et al, 1991).

Cytokinins are adenine derivatives, which are classified as either isoprenoid or aromatic. An isoprenoid cytokinin is either an isopentenyladenine (iP)-type cytokinin, which carries an isopentenyl N^6 side chain, or a zeatin-type cytokinin, which carries hydroxylated isopentenyl N^6 side chain. Cytokinins are also commonly produced by *Azospirillum* (Muhammad et al., 1991).
Dictyostelium discoideum enzymes catalyze the transfer of isopentenyl moiety from dimethylallyl diphosphate (DMAPP) to the N^6 position of adenosine 5'-monophosphate (AMP). This process is referred to as AMP: DMAPP isopentenyltransferase activity. The reaction product, isopentenyladenosine-5'-monophosphate (iPMP), is then converted to iP (cytokinin) (Tatsuo Kakimoto, 2003).

Agrobacterium tumefaciens also infect plants and introduce the T-DNA region of the Ti-plasmid into the plant chromosome. T-DNA carries genes that are expressed in a plant cell and are responsible for the production of both auxins and cytokinin. The tmr locus (a fixed position of the tmr gene) consists of a gene responsible for the production of cytokinin. This gene enables plants to catalyze the production of iPMP from DMAPP and AMP. Nopaline-producing (a chemical compound derived from glutamic acid and arginine) strains of *A. tumefaciens* also possess another gene for DMAPP: AMP isopentenyltransferase -the tzs gene-which is present in the Ti-plasmid outside the T-DNA. Genes that also resemble tmr and tzs are present in *Pseudomonas syringae pv savastanoi* (Tatsuo Kakimoto, 2003).

Gibberellins are a class of phytohormones responsible for germination, shoot elongation, and flowering. GA_1, GA_3, and GA_4 are the most commonly known gibberellins that promote plant shoot elongation. Gibberellins are produced by *Azospirillum brasilense* and *Azospirillum lipoferum.* There are two pathways of gibberellin biosynthesis in *Azospirillum* sp.: early 13-hydroxylation involving the metabolism of GA_{19} (and its metabolite, GA_{20}) to GA_1, and an early non-hydroxylation branch where GA_9 is (presumably) the precursor of GA_3. Additionally, *A. lipoferum* hydrolyzed both ether- and ester- glycosides of GA_{20}, which can then be further hydroxylated into GA_1 (Rubén et al, 2004).

Ethylene is a plant hormone responsible for fruit ripening. In addition it promotes leaf and flower senescence and abscission. It is produced by the bacteria *Penicillium digitatum, Pseudomonas amygdali pv. sesami, Pseudomonas savastanoi pv. glycinea, Pseudomonas savastanoi pv. Phaseolicola, Cryptococcus albidus, Escherichia coli, Penicillium digitatum* and *Ralstonia solanacearum* (SRII, 2018a; SRII, 2018b). There are two pathways responsible for the biosynthesis of ethylene. The first pathway involves the dioxygenation of 2-oxoglutarate to form a molecule of ethylene and three molecules of carbon dioxide by the enzyme 2 oxoglutarate-oxygenase as part of the first reaction. The second reaction involves the monooxygenation of 2-oxoglutarate and L-arginine to produce succinate, carbon dioxide and L-hydroxyarginine, catalyzed by 2-oxoglutarate and L-arginine dioxygenase. L-hydroxyarginine is then transformed to (in the third reaction) guanidinium and 1-pyrroline-5-carboxylate by the action of L-hydroxy-arginine lyase (eq. 1). The enzymes used in this reaction are all present in the species of *Penicillium*

digitatum, Pseudomonas amygdali pv. sesami, Pseudomonas savastanoi pv. glycinea and *Pseudomonas savastanoi pv. Phaseolicola* (SRII, 2018a).

3 2-oxoglutarate + L-arginine + 3 oxygen + 3 H^+ → 2 ethene + 7 CO_2 + succinate + guanidinium + 1-pyrroline-5-carboxylate + 3 H_2O

Equation 1. Biosynthesis of ethylene (SRII, 2018a)

The second pathway for ethylene biosynthesis uses ferric-chelate reductase present in *Cryptococcus albidus, Escherichia coli, Penicillium digitatum* and *Ralstonia solanacearum* to produce ferrous iron that reacts with oxygen to form superoxide radicals. These radicals are converted to hydrogen peroxide by superoxide dismutase. The hydrogen peroxide then further reacts with the ferrous iron to produce a hydroxyl radical. The last part of the synthesis involves the reaction of the hydroxyl radical with 4-(methylsulfanyl)-2-oxobutanoate, generating ethene along with methanethiol and carbon dioxide (Fukuda et al., 1989).

Abscisic acid (ABA) plays a major role in promotion of embryo growth and prevention of seed abortion (Cheng et al., 2002). ABA also controls plant response to environmental stresses and pathogens. This includes the development of desiccation tolerance, suppression of vivipary, and the closure of stomata in water-heavy environments (Qin et al., 2002; Gazzarrini et al, 2004; Tan et al., 2003). The pathway taken for the biosynthesis of ABAs involves the enzyme neoxanthin synthase. This enzyme catalyzes the conversion of violaxanthin to trans-neoxanthin which is then converted to 9'-cis-neoxanthin. This is oxidized to produce 2-cis,4-trans-xanthoxin and 25-allenic-apo-aldehyde. 2-cis,4-trans-xanthoxin is further oxidized to form NADH + H^+ and cis-abscisic-aldehyde. Finally, Cis-abscisic-aldehyde reacts with water and oxygen to produce 2-cis-abscisate (abscisic acid), hydrogen peroxide and H^+ (SRII, 2018c).

3.2 Bioreactors

A bioreactor is an environment optimized to facilitate a biological process. Bioreactors are utilized in research and industry for the production of cellular biomass, primary or secondary metabolites,

and enzymes. Bioreactors offer exacting control over factors such as substrate, temperature, pH, aeration, and mixing. Bioreactors can operate as batch processes or in steady state flow.

3.2.1 Mixing

Bioreactors may be agitated by impellers or by "airlifts", which inject of gases into the fluid media. Mixing ensures homogeneity of cells, gases, and feedstocks throughout the bioreactor. Mixing also prevents flocculation, sedimentation, and thermal homogeneity. Proper mixing requires a combination of radial flow with axial flow (Mirro & Voll, 2009).

The blades of marine-blade impellers have one flat or concave face and one convex face. This combination promotes smooth axial flow. Marine-blade impellers are used for mammalian cells or other fermentation applications which require gentle mixing to prevent cell lysis. Due to the unidirectional flow, the oxygen mass transfer rate of marine-blade impellers tends to be slightly lower than those of impellers that produce both axial and radial mixing (Mirro & Voll, 2009).

Rushton impellers are often used in applications involving bacteria and fungi; these organisms have a stronger cell structure and are not considered shear sensitive. The blades of the rushton impeller are flat and set up vertically along the agitation shaft which causes flow in a unidirectional radial flow (clockwise or counter clockwise). Rushton impellers spin at much higher number rotations per minute than other impellers, and are thus generally used for aerobic processes to prevent air from leaving the fluid (Mirro & Voll, 2009).

The blades of a pitched-blade impeller are flat and set at 45° angles, causing both radial and axial flow. The combination of the two flow patterns offers optimum mixing and creates a very high transfer rate of oxygen throughout the system, much higher than the marine blade impeller systems. Pitched-blade impellers are low shear impellers that are designed to gently mix the contents of the bioreactor. Therefore they are the preferred impeller for mammalian, insect, or other shear stress-sensitive cells (Mirro & Voll, 2009). Pitched-blade impellers are often used in batch bioreactors and are widely used in fermentation processes that must work with very viscous substances, such as fungi and biofuels (Mirro & Voll, 2009).

Airlift columns induce mixing by “injecting gas at the bottom of the riser, thus creating a density difference” between fluid at the top and bottom of the reactor (Camarasa et al., 2001). Airlift columns have difficulty mixing fluids with large proportion of particulate matter, but provide good “fluid circulation”, oscillation, and increased area for mass transfer at a low energy cost (Al-Mashhadani, Wilkinson, & Zimmerman, 2015). Furthermore, air-lift reactors offer “simple construction, absence of moving parts, [and] well defined fluid flow patterns” (Drandev, Penev, & Karamanev, 2016).

3.2.2 Aeration

A sintered sparger passes air into the bioreactor by using a long tube with a sintered tip at the end (appendix G1). The pores at the end of the tip are 15 mm wide offering a low flow rate of oxygen into the system. Oxygen flows through the system once every ten minutes; this low flow rate offers an even and gentle mixing for the bioreactor. The sintered tip ensures gas enters the liquid phase in a very fine dispersion, offering excellent mass transfer (Dumont, 2018).

The air inlet sparger is a simple type of aeration device, where air is injected through a porous tube at the bottom of the bioreactor (appendix G2). This device offers even spreading of oxygen throughout the system at an even mixing rate. It is recommended when using this type of aeration device that the gas should be filtered prior to entry into the reactor to reduce outside contamination (Dumont, 2018). Although the simple design of an air inlet sparger is appealing, the fact that it only has oxygen coming through the bottom did not work for our design.

3.2.3 Additives

Additives and antifoaming agents are used in bioreactors to improve, expedite, or increase yield efficiency of fermentation. To achieve best results when brewing compost tea, additives may be applied to improve tea fermentation. Bacteria prefer sugar rich additives, while fungi prefer complex carbohydrates and bulking agents (Appendix G). Compost tea brewed with a bacteria rich culture is more beneficial for vegetable plants while compost tea rich in fungi is more beneficial

for trees; thus additives can be used to control microbial populations to optimize tea for use with certain plants (CJ, 2011). Compost additives are not only used for producing specific compost teas according to the plant's needs, they can also be used as a means of accelerating the composting process and overall increasing the efficiency of the system to produce a better feedstock, because the quality of the compost tea is greatly determined by the quality of the compost used. Gabhane et al. (2012) studied green waste compost comprised of grass cuttings and fallen leaves in which different additives were added to the compost during process. These additives were jaggery, polyethylene glycol, phosphogypsum, lime and fly ash. The additives have shown to promote many qualities that enhance the composting process including increasing of biomass and enzymatic activity stimulation (Gabhane et al., 2012).

In aerobic fermentation, agitation, the amount of air entering the system to meet the required dissolved oxygen levels, and the metabolic processes of microbes can lead to foaming. Foam is mainly comprised of air bubbles and can be a nuisance in the bioreactor as it can lead to clogging of pipes (Dumont, 2018). Bioreactors often utilize anti-foaming to prevent or dissipate foaming. Antifoaming agents require the following characteristics: rapid dispersal within the vessel, capacity to quickly reduce foaming, and non-toxicity to cells. Oils act as good antifoaming agents as they are very effective at breaking up foam and are usually safe (Dumont, 2018).

3.2.4 Instrumentation

The environmental conditions within a bioreactor are monitored using instrumentation. Instruments can also collect data which a microcontroller or computer can use to control and maintain the system under specified environmental conditions. A homogeneous fluid is important to ensure that instruments are providing sufficiently representative readings. The pH of a substance is a measure of the balance between H^+ and OH^- ions in the substance. During the aerobic fermentation process occurring inside of the bioreactor, the pH will often shift due to products produced during the metabolic pathways of the microorganisms, such as lactic acid (Dumont, 2018). If the product being made is of high value, it may be worthwhile to purchase a pH probe or pH strips, as having the bioreactor operate at the optimum pH may be important for your product.

The pH can be controlled by the addition of CO_2 gas to raise acidity, and bases can be added to raise alkalinity (Dumont, 2018).

Dissolved oxygen is the amount of oxygen a fluid can hold in parts per million. The amount of oxygen is critical to the aerobic bacteria in the bioreactor as these bacteria use oxygen in their cellular respiration. The standard dissolved oxygen in water at 37 ^{o}C is only 7 ppm; this is a very small amount and aerobic microbes will quickly consume any oxygen entering the system for their metabolic processes (Dumont, 2018). A dissolved oxygen probe can be used to signal the operation or cease the operation of a sparger.

Although our product has an easy to open lid to monitor the foaming, it can still be favorable to purchase an antifoaming probe if an operator is not present to visually monitor the system. An example of this is when the product is fermenting compost over the weekend when no workers are available. When foam comes into contact with the antifoam probe, a pump is activated to release antifoaming agent into the system.

3.3 Patents

A compost tea system designed by Michael et al. (2003) involved a large fluid holding tank supported by an external frame and a lid covering the top of the tank. The lid suspends a basket of compost within the fluid (water) in the tank. The lid contains air vents to ensure contact of the contents of the basket with the atmosphere air. The fluid is also aerated by an air pump. Pleural membrane disk diffuser modules are spaced around the interior of the tank. These modules are connected to an air pump through an airline. This introduces aeration to the liquid in the tank resulting in proper circulation of liquid and constant dissolving of oxygen in the water. This ensures high concentration of oxygen in the liquid providing conditions which are favored by the aerobic bacteria in the system. Once the process has ended the liquid in the tank can be easily drained out through a drain at the bottom of the tank. The basket tray can be easily removed from the tank for convenience of cleaning and maintenance. The basket is made up of an inner layer of mesh and an outer layer of mesh both with relatively large-sized holes. To prevent small particulates from leaking out however there is an inner filter media. The filter media can be made

from plastic or stainless steel. This basket-filter formation ensures proper and continuous mixing of water and basket contents and proper aeration without any leakage of any particles.

Another design done by Stephen et al. (2007) involved an apparatus where an aqueous solution of compost is subjected to circulation, aeration and vortex motion. The system is comprised of a large tank that may be covered or not. The tank's capacity ranges from 0.19 to 0.95 m^3. The tank has a conical bottom with a discharge opening at the end. The material used for the tank may be plastic or stainless steel. At the discharge point at the bottom of the tank there is a plurality of conduits (pipes) that extend from the bottom of the tank to the top. These move water from the bottom of the tank to the top. The conduits then have extended nozzles at the top of the tank. The nozzles are at an angle to the nozzle axis either clockwise or anticlockwise. This results in a swirling movement of the water discharged into the tank from the nozzles. Within the conduits vortices may also be created by forcing the water to swirl as it moves through the conduits. There is an air supply provided by air compressors which are connected via air lines to the conduits. This air supply results in many bubbles above the water in the conduits. The air bubbles oxygenate the water and push the water upwards through the nozzles into the tank. This discharge of water exposes all the microorganisms in the tank to oxygenation promoting the action of aerobic bacteria. The conical bottom allows heavy particulate sediments to settle without using up volume or being sucked back into the tank by the vortex. Once the compost mixture has been processed and the concentration desired has been reached the compost tea is separated from the mixture.

3.4 Standards and legislation

3.4.1 Food grade safety compliance

The Canadian Food Inspection Agency (2010) states that any surfaces that come in contact with food should be designed, constructed and maintained to facilitate hygienic operation. This can be achieved by using surfaces that are non-corrosive, non-absorbent, non-toxic, and free from pitting, cracks and crevices. All products should also be built to withstand maintenance and sanitization. If chemicals are used for cleaning, they should be appropriate for the intended use and should be used according to the instructions. Water used in the system should be protected from

contamination. If any water source is used, it should be ensured that there is no backflow to the source. Water storage units should be covered to prevent contamination. Storage units should also be made from food grade material. Any filters used should be cleaned and maintained effectively and regularly. Chemical treatments used should be monitored and controlled to deliver the desired concentration and to prevent chemical contamination. All water treatment chemicals used should be appropriate for the intended use and used according to the instructions. Water re-circulated for reuse should be treated, analyzed, monitored and maintained for the intended purpose and in accordance with applicable provincial, territorial or municipal requirements. Re-circulated water should have a clearly identified separate distribution system (CFIA, 2010).

3.4.2 Sanitation compliance

The current industry standard bioreactor sanitization practice is use of clean-in-place (CIP) technology. The World Health Organization (2015) suggests that "equipment used during handling of live organisms and cells... should be designed to prevent any contamination during processing. Wherever possible, the use of 'clean in place' and 'sterilization in place' systems… should be used for aseptic connections avoid exposure to the environment and to human intervention, thus reducing the contamination risk". CIP practises entail onsite cleaning and sanitization of a bioreactor without disassembly. CIP design principles maximize ease and effectiveness of cleanability by considering "drainage, elimination of crevices and stagnant areas, minimization of internals, arrangement of valves and pumps, piping welds, sanitary couplings, instrumentation, and instrument ports" (Chisti & Moo-Young, 1994). The system design should also include a "splash-resistant exterior of clean design which is easily washable by hosing or wiping" (Chisti & Moo-Young, 1994). System CIP procedures should include physical removal of particles, chemical residue removal by detergent, and biological contaminant disinfection by sanitizer.

3.4.3 Organic compliance

A secondary incentive for producing a vermicompost based fertilizer is to market an organic product. Organic products are produced without use of synthetic fertilizers and pesticides. In industrialized countries, many consumers are willing to pay premiums for organics because of

sustainability and health benefits (WEC, 2010). As a result, organic farmland has tripled since the end of the last century, and supermarkets report that up to 30% of produce sales are organic (WEC, 2010). Subject to USDA National Organic Program NOP 5021 Compost and Vermicompost (Rev01072211), crops grown with our products processed compost may be certified as organic. The US Department of Agriculture (2011) has determined that "vermicompost made without animal materials as feedstock are not restricted in use, in accordance with the provision for uncomposted plant materials at § 205.203(c)(3), provided all feedstocks are allowed materials (either non synthetic substances not prohibited at § 205.602, or synthetics approved for use as plant or soil amendments)"; "Vermicomposts containing animal materials that do not meet the requirements at 4.3 of this policy may be permitted subject to restrictions of §205.203(c)(1), similar to raw animal manure" (USDA, 2011).

4.0 Bioreactor design

4.1.1 Mixing and aeration

To fulfil the design specification of homogenization and the design constraint of pathogen control, it was important to ensure proper mixing in our system. Pathogen control was achieved by attaining sufficient aeration to inhibit anaerobic pathogens and allow beneficial aerobic microbes in vermicompost to attain dominance. Mixing can be achieved by a combination of turbulence, vortex flow, and/or convection. We therefore first considered common bioreactor mixing methods, and selected a method of aeration to proliferate aerobic bacteria in the system.

Impeller mixing was not selected as a mixing method, because of safety and maintenance concerns. Impellers required the use electrical equipment in water providing a potential source of equipment failure and a hazard to the user. Impellers also provided a non-ideal choice between submerging an electric motor, which would cause compliance issues with CIP requirements, and boring a hole through the bottom of the bioreactor to fit a shaft connecting an external motor with the impeller; this latter design option seemed difficult to waterproof, as the spinning impeller shaft could not be tightly sealed with silicone or other waterproofing sealants. We therefore concluded that for our system it would be best to driving mixing with injected gas. This had the advantage of allowing

the mixing system and aeration system to be combined. Strong mixing and a high oxygen transfer rate were achieved with a high flow volume pump, using a manifold to partition total air flow over multiple outlets.

4.1.2 Assessment of flow patterns

Our design criteria mandated mixing, aeration, and homogenization of the working fluid.
We also wished to maintain turbulent conditions to ensure thermal uniformity and prevent stagnation at the bottom. Therefore we assessed four major components of the fluids behavior in the bioreactor: total turbulence, vorticity, mixing at bottom, and convection.

Total turbulence within the bioreactor was gauged by visually observing the shaking of the bioreactor caused by internal forces of the fluid motion. We require turbulence to prevent compost from accumulating and compacting along the inner face of the filter. We are not concerned about the impact of shear stress on microbial populations within the bioreactor because turbulence within a bioreactor is not sufficient to harm them (Dumont, 2018). Vorticity in this scenario corresponds to creation of clockwise circumferential flow and formation of a central vortex. Waitz et al. (1997) claim “It is widely recognized that a powerful mechanism for enhancing mixing is the introduction of strong streamwise vortices”. Vorticity is important as it ensures mixing along the sides. Strong vortex flow at the center of the bioreactor ensures good flow through the filter and helps prevent compost from accumulating and compacting along the inner face of the filter. A 1D model of vortex flow is used below to estimate aeration in our system. This model estimates aeration by considering how the augmentation of a flow pattern by strain (leading to a vortex) increases the range at which particles are capable of interacting, thus increasing mixing (Waitz et al., 1997).

Mixing at the bottom of the bioreactor prevents stagnant anaerobic pockets from forming. Biofilms produced by bacteria may accumulate along the inside and outside faces of tubing and pipes and at the bottom of the bioreactor. These biofilms may cause obstructions and overall deterioration of the system. Mixing helps keep biofilms from aggregating and keeps biological material dissolved in solution. Biofilm deposits may provide an anaerobic environment, increasing risk of contamination by pathogenic microorganisms (Henderson, 2015). Stratification is prevented by

promoting vertical flow (Henderson, 2015). We mitigated stratification in our design by recirculating water from top to bottom.

Convection describes how heat flows through a bulk. From a macroscopic perspective it is movement of matter from a hot region to a cool region (Kobes & Kunstatter, 1999). The way heat is transferred through the system is important to us as the microorganisms in the bioreactor are mesophilic and thrive in a temperature range of 15 – 40 °C (GGI, 2003). As such it is important the temperature is evenly distributed throughout the system. We ensured thermal homogenization throughout the system by actively pumping water from the bottom of the bioreactor to its surface, and promoting vertical flow.

	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Trial 8	Trial 9
Total turbulence	good	low	very good	very good	low	ok	ok	ok	good
Central Vorticity	none	none	none	none	low	good	ok	good	good
Mixing at bottom	ok	good	very good	good	low	low	low	low	low
Convection	ok	low	low	good	low	ok	low	ok	good
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Trial 8	Trial 9
Total turbulence	3	1	4	4	1	2	2	2	3
Central Vorticity	0	0	0	0	1	3	2	3	3
Mixing at bottom	2	3	4	3	1	1	1	1	1
Convection	2	1	1	3	1	2	1	2	3
Sum	**7**	**5**	**9**	**10**	**4**	**8**	**6**	**8**	**10**

Table 2. Pugh chart

To determine an optimal flow pattern which addressed these four elements of mixing, we used a Pugh chart to compare possible flow patterns (table 2). Each flow pattern used either three or four air lines from the pump, as subsequent air lines decreased the air pressure to an unacceptable

degree (fig.2). To rate each element for a given flow pattern, we observed, took video recordings, and analyzed how well the flow pattern accomplished the criteria of that element.

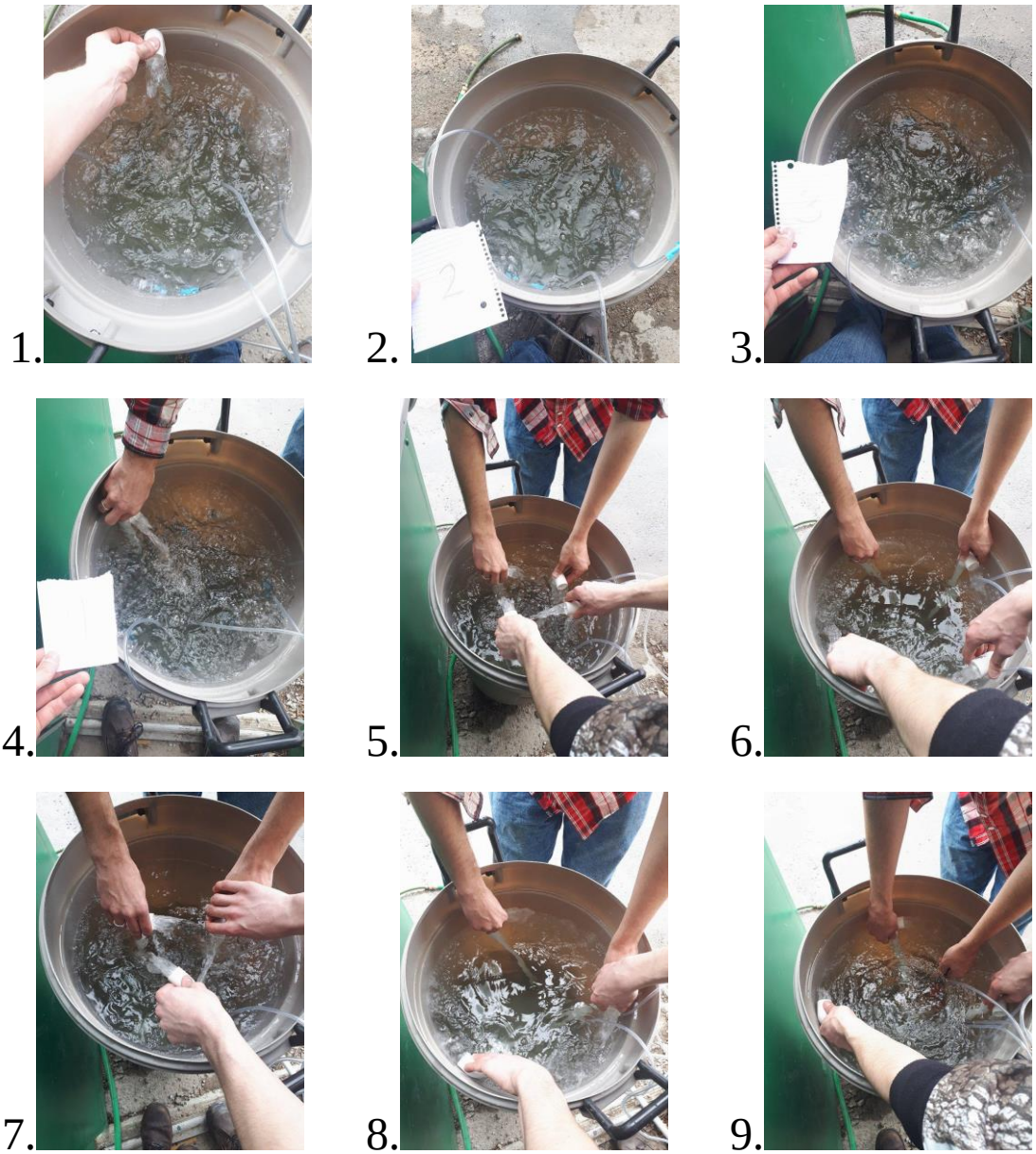

Figure 2. Evaluation of flow patterns within the bioreactor

Trial 1 was performed by setting three aeration tubes at increasing 15 cm intervals from the bottom of the bioreactor facing CW, and one airlift (a PVC pipe pumping water from bottom of the vessel to the top) (fig. 2). This provided “good” overall turbulence, recirculated the water from top to bottom, and the intervals of heights allowed for some mixing at the bottom also. However, there

was no central vorticity and the overall turbulence of the system was not enough to use this as our final design.

Trial 2 used three CW-facing aeration tubes with 15 cm increasing height intervals (fig. 2). An airlift was eliminated because this improved pressure and volumetric air flow rate in the other pipes. The extra flow from the pipes improved flow at the bottom of the reactor, however, this configuration gave very poor overall turbulence, and no central vorticity.

In trial 4, three tubes were fixed at the bottom of the bioreactor (all in a CW orientation) with an airlift depositing water into the compost filter (fig. 2). The submerged tubes gave the system very good overall turbulence, and rising bubbles generated air lift convection streams. The turbulence was so great in this design, the entire bin shook. With the use of the air lift, the bubbles rising from the bottom provided good mixing at the bottom and good overall convection of the heat through the system. There was no central vortex in the filter, however the overall turbulence of the system was sufficient to make up for this. We ran this flow arrangement with dirt in the micron filter and observed that color and liquid seeped from the dirt and were quickly mixed homogeneously throughout the system, while clumps within the filter were adequately agitated to prevent clumping and clogging.

Trial 6 used four airlifts (all CW direction), placed on the perimeter of the bioreactor (fig. 2). This system gave “ok” turbulence as the bioreactor was mixing evenly. The four airlifts at the perimeter offered “good” central vorticity as it was evident that a vortex was forming in the center, indicating that the compost would be easily broken up. Four airlifts also provided good thermal convection throughout the system as the water was spread evenly from top to bottom. The main issue with this flow pattern was low mixing observed at the bottom. It was evident that their needed to be a tube positioned directly at the base of the reactor.

Trial 9 used three CW facing airlifts along the circumference of the bioreactor and one aeration tube at the bottom in a CCW direction (fig. 2). This system had good turbulence as the bioreactor was shaking, and there was an even dispersion of flow throughout the system. There was also a good vortex in the center and the airlifts provided good convection throughout the system as well.

Pugh chart analysis indicated two flow patterns, trial 4 and trial 9, with the most optimal flow patterns. To select a flow pattern between these designs, we gave priority to bottom mixing. Any build up of pathogenic organisms at the bottom or in pipes could jeopardize the quality of our product. We believed the turbulence in trial 4 was sufficient to preclude the need for vorticity, in preventing the clogging of our filter, preventing stratification, and ensuring homogenization.

When inserting surgical tube into the air lift we had a choice of how far we inserted the tubing; we were uncertain in pulling the tubing farther up the pipe would yield better flow. We originally hoped to improve pressure in the pipes by releasing air nearer to the top of the fluid. However, this proved to be an incorrect assumption, and produced the opposite of the desired effect. Inserting aquarium tubing only a short distance into the PVC piping resulted in observably larger flow rates. This was actually a very common-sense result, as the air was able to more fully diffuse and accelerate the water over a longer distance.

4.1.2 Calculated aeration rate

A vortex forms when two fluids of different properties (e.g. chemical composition, momentum or energy) come into contact with each other and form an interface. The convective velocity fields of these two fluids form a vortex, and cause stretching of the interface which causes two interrelated effects (Waitz et al., 1997). The first is an increase in the interfacial surface area and the second is an increase of the magnitude of the gradients normal to the interface; both of these effects combine together to increase the mixing (Waitz et al., 1997). This is important for our client because a high amount of central vorticity means that there will be an increase in the mixing capabilities of our bioreactor and the flow will be more effective at breaking up the compost in the center. As such, high vorticity is one of the requirements that our bioreactors design must comply to. This section will illustrate the importance of vortices and how they impact the mixing in a closed environment. It is important to demonstrate this mathematically because our design is heavily based on vortex formation from fluids with different momentums meeting each other.

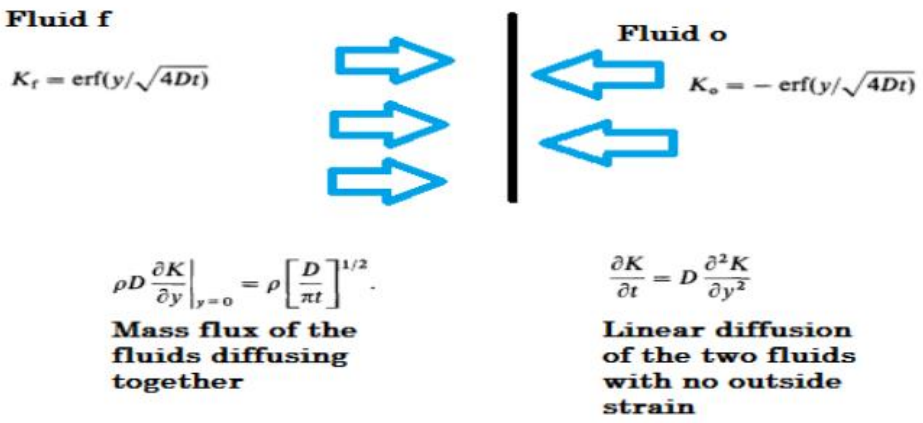

Figure 3. 1D model of mass flow with no strain

For vortices and mixing we must consider two semi-infinite regions on a single axis (e.g. the x axis). The kinetics of individual molecules are not considered for this reaction; this simplifies the problem to a diffusion reaction which is stoichiometrically controlled (fig. 3). The reaction zone is assumed to be an infinite plane, yielding equation 2 (Waitz et al., 1997).

$$\frac{\partial K}{\partial t} = D\frac{\partial^2 K}{\partial y^2} \qquad (2)$$

Equation 2. Diffusion of reactants (Waitz et al., 1997).

K is the concentration of a fluid property per unit of fluid (e.g. momentum). In our bioreactor, K of each respective reactant is the amount of water on each side of the 1D axis (eq. 3). D is the binary diffusion coefficient, a measure of how well each reactant diffuses into the other (Waitz et al., 1997).

$$K_f = \text{erf}(y/\sqrt{4Dt}) \quad \text{and} \quad K_o = -\text{erf}(y/\sqrt{4Dt}) \qquad (3)$$

Equation 3. Concentration of reactants with no strain (Waitz et al., 1997).

In this equation y is the coordinate normal to the interface of the two fluids, and t is time in seconds. The thickness of the diffusion zone grows with $(Dt)^{1/2}$, where Dt is the molecular mixing rate (Waitz et al., 1997). The reactant consumption rate, a direct measurement of molecular mixing, is expressed as the mass flux of either fluid species (eq. 4). In the case of pure diffusion as t-->∞ the

rate of mixing will be zero (Waitz et al.,1997). Density is assumed to be constant throughout the system, as we hope to maintain nutrient and thermal homogeneity.

$$\rho D \left.\frac{\partial K}{\partial y}\right|_{y=0} = \rho \left[\frac{D}{\pi t}\right]^{1/2}. \tag{4}$$

Equation 4. Mass flux with no strain (Waitz et al., 1997).

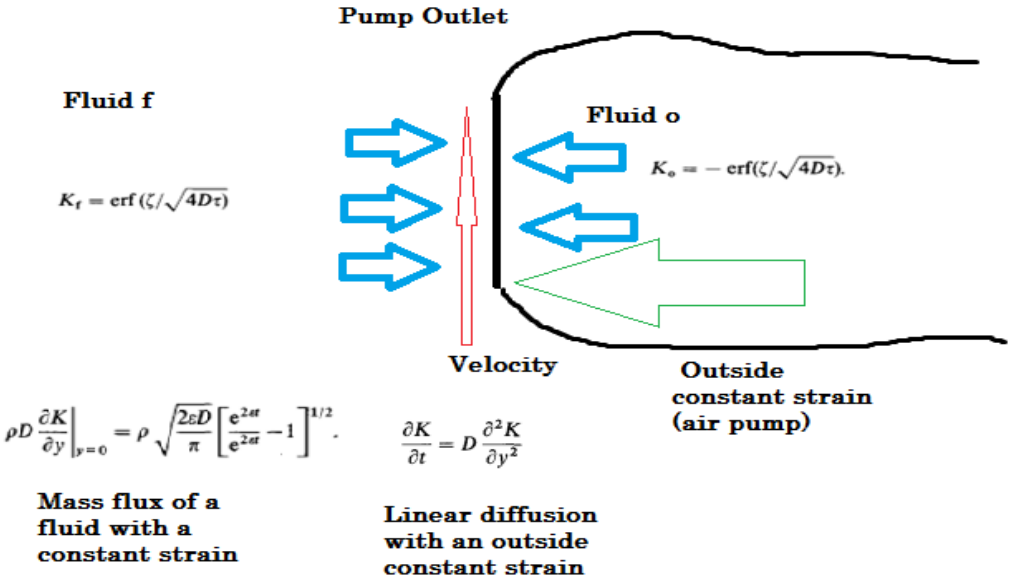

Fig 4. 1D model of mass flow in bioreactor with constant strain

Inside of our bioreactor there will not just be diffusion occurring but also forces applied by our air compressor pump to the fluid causing it to move. A simplified mathematical model will be used to determine total aeration in our bioreactor (fig. 4). A normal strain rate of $\varepsilon = \partial u_x/\partial x = -\partial u_y/\partial y$, will be used instead of individually assessing all forces in the system. This normal strain generates a velocity normal to the plain of interface between the two fluids. The velocity is $u_y = -\varepsilon y$, in m/s (Waitz et al 1997). The equation for mixing becomes equation 5.

$$\frac{\partial K}{\partial t} - \varepsilon(t) y \frac{\partial K}{\partial y} = D \frac{\partial^2 K}{\partial y^2}. \tag{5}$$

Equation 5. Mixing augmentation with constant strain (Waitz et al., 1997).

For solving the above equation two constants are substituted into the formula (eq. 6).

$$\zeta = y \exp\left[\int_0^t \varepsilon \, dt\right], \quad \tau = \int_0^t \left[\exp\left(\int_0^{t_1} 2\varepsilon \, dt_1\right)\right] dt_2 \tag{6}$$

Equation 6. Integration of reactants with constant strain (Waitz et al., 1997).

The solutions for the two reactant concentrations then become equation 7.

$$K_f = \operatorname{erf}(\zeta/\sqrt{4D\tau}) \quad \text{and} \quad K_o = -\operatorname{erf}(\zeta/\sqrt{4D\tau}). \tag{7}$$

Equation 7. Concentrations of reactants with constant strain (Waitz et al., 1997).

Assuming constant strain, strain can be substituted into the equation, yielding the consumption rate of the reaction (Eq.8).

$$\rho D \left.\frac{\partial K}{\partial y}\right|_{y=0} = \rho \sqrt{\frac{2\varepsilon D}{\pi}} \left[\frac{e^{2\varepsilon t}}{e^{2\varepsilon t}} - 1\right]^{1/2}. \tag{8}$$

Equation 8. Mass flux with constant strain (Waitz et al., 1997).

In our bioreactor, the consumption rate can be related to the degree of mixing between air and water. Equation 8 indicates that for mixing to be significantly better than diffusion, the time of mixing, t, must satisfy the criteria $t >> 1/\varepsilon$. The velocity of air in each pipe was calculated at 1.20 m/s (appendix F). Assuming the bioreactor is filled with water to 0.635 m, strain is calculated to be - 1.89 (appendix F); the negative sign implies the water is undergoing a compressive strain. Assuming constant strain, total aeration is calculated at 155.6 cm^3/s (appendix F). The minimum time required to run our pump to achieve better mixing than diffusion is 0.529 seconds. A small number makes sense because turning on the pump for a half of a second will give the approximate results as if the fluids were simply let to diffuse. Total mixing duration within the bioreactor is 86400 seconds, implying that mixing will be superior to natural diffusion. In our bioreactor, we will be diffusing oxygen in water (simplified mathematical model) and thus our interdiffusion coefficient is 0.219 cm^2 /s at temperature of 20 ^oC (assume temperature is constant). This formula can be used because we will be using a very large time $t >>> 1/\varepsilon$ (Waitz et al 1997). Substituting

$\varepsilon = 1.89$ and $D = 0.219$ cm^2/s, the intermolecular mixing rate is found to be 0.513 cm^2/s (appendix F) (Mostinsky, 2011).

The important thing to understand from the mathematical model above is that the strain enhances the reaction rate (mixing) by increasing the interfacial surface area of the fluids in contact and increasing the gradients in the diffusion zone. “Mixing augmentation in a vortex flow field is due to the same physical effects, but in a geometrically more complex situation” (Waitz et al 1997). This is what allows us to use a 1D model and then make certain simplifying assumption such as a constant rate of strain and make the 1D model give us a ballpark range.

4.1.3 Pumps

There are three main types of air compressors: reciprocating, rotary screw, and rotary centrifugal. These types can be further categorized by number of compression stages, cooling method, drive method, and lubrication. Reciprocating air compressors are known as positive displacement machines which means that air pressure is increased by a corresponding reduction in volume. This is accomplished using a piston with a cylinder as the compressing and displacing unit. Depending on the pressure required a single-stage or a two-stage compressor can be used. Single-stage compressors are used to provide pressures in the range of 545 kPa to 690 kPa while two-stage compressors are used to provide pressures in the range of 690 kPa to 1720 kPa. The compressor is considered single acting when only one side of the piston is used and double acting if both sides are used. reciprocating air compressors can be either water cooled or air cooled, in lubricated and non-lubricated configurations (ET, 2018).

Rotary screw compressors are positive displacement compressors. The most common type involves a single stage helical or spiral lobe screw. These types of compressors consist of two rotors within a casting where the rotors compress the air internally. In this design of compressors there are no valves. Cooling is usually accomplished by lubricating internal clearances with oil to prevent frictional heating. These compressors are easy to both maintain and operate, and capacity control is achieved by variable speed and variable compressor displacement. The advantages of these compressors include smooth air output, high output per size, and longevity (ET, 2018).

A centrifugal compressor is a dynamic compressor which relies on the energy transfer from a rotating impeller to the air. These types of compressors produce high pressure discharge by converting angular momentum produced by the rotating impeller. To do this efficiently however, these compressors rotate at a higher speed than the other types of compressors. They are also designed to produce higher capacities because of the continuous flow through the compressors. The flow capacity of these pumps is controlled by adjusting the inlet guide. By closing the guide vanes, the volumetric flows and capacity are reduced (ET, 2018).

We selected a reciprocating compressor for our design. We chose this compressor as it provides a very high aeration rate at an economical price, and uses very little energy.

4.2 Cooling

4.2.1 Cooling incentive

Fermentation releases heat previously trapped as energy in molecular bonds. To grow and reproduce, microbes must catabolize feedstock to anabolize their own biomass. For this biological process to occur, it is thermodynamically necessary that the energy released by catabolism must be greater than the energy required for anabolism. Thus, thermal energy is dissipated to the environment. Vermicomposting is non-thermophilic, so many high energy molecules are still present in finished vermicompost, and available for microbes to utilize.

It is important to prevent our bioreactor from overheating. Excessive temperatures may kill or make dormant mesophilic or thermophilic bacteria that are unable to tolerate conditions suitable only for extreme thermophilic microorganisms. In our plastic prototype, we must also avoid overheating due to materials safety. Our materials are rated for use up to 60 - 70 ^{o}C, and beyond this point will lose structural integrity, performance efficiency, and may no longer be food grade. To determine the need for a jacketing system, we found whether natural convection was sufficient to dissipate excess heat. Heat transfer by external natural convection was calculated using the Churchill-Chu correlation (Eq. 9). The Churchill-Chu correlation provides an acceptable

approximation according to the size of the bioreactor, the properties of the working fluid, and the temperature differential between the external fluid and the bioreactor (Eq. 10-11).

$$q'' = A(T_w-T_b)*k/L*(.68+[.670*(Ra^{1/4}*(T_w-T_b))]/[1+(0.492/Pr)^{9/16}]^{4/9})^2 \quad (9)$$

Equation 9. Churchill-Chu correlation for external natural convection (Baliga, 2017).

$$D/L \geq 35/(Gr)^{0.25} \quad (10)$$

$$Gr = [gB(T_w-T_b)D^3] / (v)^2 \quad (11)$$

Equations 10-11. Criteria for applicability of Churchill-Chu correlation (Baliga, 2017).

By solving equations 10 and 11, this correlation is shown to be a valid approximation for cooling experienced by our bioreactor, and it is valid for all temperatures of the bioreactor and ambient bulk flow air (Eq. 12-13). Assuming properties of room temperature air, Equation 1 can then be simplified and solved to find the magnitude of heat transfer caused by natural external convection (Eq. 14). The resultant heat transfer rate is negligible, indicating that additional cooling jacketing is required to dissipate heat produced by fermentation.

$$Gr = 2.603*10^7*(Tw-Tb) \quad (12)$$

$$0.56/0.6 \geq 35/[2.603*10^7*(Tw-Tb)]^2 \quad (13)$$

$$q'' = 0.0000107661*(Tw-Tb)*[0.0015265*(Tw-Tb)^{5/4} + 0.68]^2 \quad (14)$$

Equations 12-14. Cooling by natural convection (Baliga, 2017).

4.2.2 Heat generation assessment

The heat generated by our bioreactor may be determined stoichiometrically or empirically. In the stoichiometric method, the formation energy of all molecules in each reaction must be evaluated to determine the differences in linked catabolic and anabolic biological processes; the relative rates of these reactions are then weighed to determine total energy released. It is difficult to obtain an accurate estimate using the stoichiometric method, not because it involves many multi-step series of reactions that vary between microorganisms, but because determining metabolic routes requires examining produced primary and secondary metabolite profiles of the bacteria. It is therefore more

accurate to empirically determine the heat released by fermentation. This is accomplished by measuring the rate of change in temperature over time and using the specific heat equation to calculate the total change in energy over time; however, the rate of heat production increases as the population of microbes increases (fig. 5). Therefore, a temperature probe must be used to log temperature change over time, and heat generation should be calculated at the point of greatest rate of change in temperature.

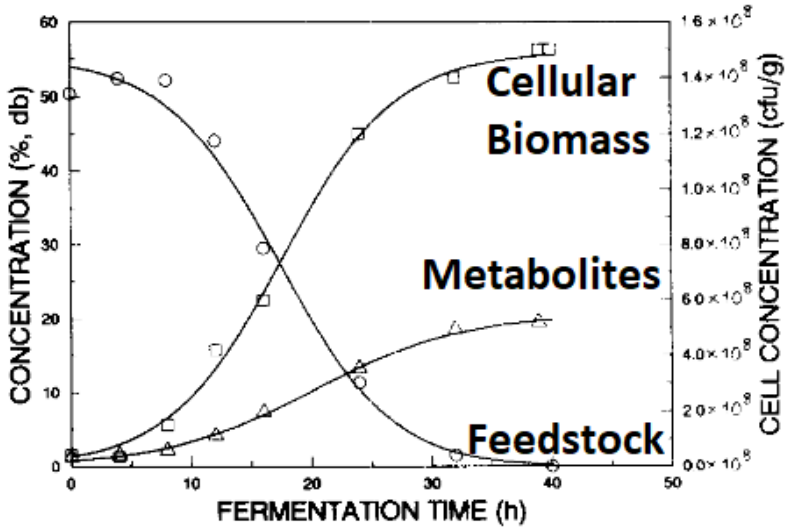

Figure 5. Concentrations of cell biomass, metabolites, and feedstock during fermentation (Ngadi & Correia, 1992)

4.2.3 Jacket sizing

It was impossible to model the cooling system as a heat exchanger because thermal homogenization due to mixing resulted in an undefined log mean temperature difference. The bioreactor cooling jacket instead must be sized by setting up an internal forced convection pipe flow problem. This problem consists of two parts: heat transfer by conduction through the tubing (eq. 15) and internal forced convection between the tubing and fluid (eq. 16-21). Reynolds number (eq. 16) indicates laminar flow within the pipe, necessitating the use of Nusselt's number for laminar flow (eq. 18). Equation 21 is found by substitution in values for the properties of water, hydraulic diameter, and flow rate through the tubing. This system of equations can be solved for two unknowns: the required length of pipe and the temperature along the inner surface of the tubing.

$$Q = 2\pi * L * k * (T_o - T_i) / \ln(r_i/r_o) \quad (15)$$

Equation 15. Conductive heat transfer through tubing (Baliga, 2017)

$$Re = p * u_{av} * D_H / \mu \quad (16)$$

$$Pr = c_p * \mu / k \quad (17)$$

$$Nu = 3.66 + [0.0668 * D_H/L * Re * Pr] / [1+ 0.04(D_H/L * Re * Pr)^{2/3}] \quad (18)$$

$$h = Nu * k_{fluid} / D_H \quad (19)$$

$$Q = h * (2\pi D^2/4) * (T_i - T_b) \quad (20)$$

$$Q = 0.010578 * (3.66 + 0.0181769 / ((0.0000403173 L + 1) L) * (T_i - T_b) \quad (21)$$

Equations 16-21. Forced convection heat transfer through tubing (Baliga, 2017)

Heat transfer in the jacket may be improved by using a more conductive material (table 3). Copper piping is the best material for jacketing, but is expensive and can oxidize. In our final prototype we would like to use stainless steel instead of plastic tubing, as stainless steel is corrosion resistant and has a higher thermal conductivity than plastic tubing.

Piping	Material	W/mK
Steel	Carbon Steel	54
Copper	Copper	401
PEX	Cross-linked High-density Polyethylene	0.51
CPVC	Chlorinated Polyvinyl Chloride	0.14
PE	Polyethylene	0.38
PVC	Polyvinyl Chloride	0.19

Table 3. Thermal conductivity (k) of pipe materials (Vlachopoulos & Strutt, 2002).

4.3 Materials

When assessing materials to use in our prototype, we considered factors such as cost, food grade, heat tolerance, load bearing capabilities, cleaning, corrosion, and method of assembly. After further prototyping, our client Phytospec has requested a final design made of stainless steel. Stainless steel is expensive and must be welded. Therefore, our initial prototype is made of very

inexpensive and easily assembled materials, and is intended to demonstrate the efficacy our our design.

The body of the bioreactor is a mobile waste container. The waste container is made out of polyethylene, a food safe polymer, and is resistant to heat and corrosion. This container provides durability and strength as our bioreactor will be filled with approximately 100 kg of fluids, which will be violently gyrating within the system. The fermentation process is also highly exothermic (60 ^{o}C -70 ^{o}C) and as such the container needs to withstand thermal strain to prevent structural deformation. The waste container also comes with wheels that can be used to easily transport the bioreactor, enabling users to transport the reactor. The price of the waste container is $27.95 from McMaster-Carr, making it a very affordable choice as body of the bioreactor (MCSC, 2018).

A fastener was required to fasten PVC piping to the inside of the body of the bioreactor. The fastener must be rugged, resistant to corrosion, and cheap. The plastic routing clamp is made out of polypropylene a strong corrosion resistant plastic polymer. Routing clamps will maintain shape under extreme temperatures of -34.4 °C to 170 °C. At 10$ for a bag of 50 of these clamps, they are very affordable (MCSC, 2018). More information can be found in Appendix C.

PVC (polyvinyl chloride) is one of the most important types of pipe used in the engineering world; PVC pipes are found in wastewater treatment plants, agricultural irrigation, and land drainage systems (AWWA, 2002). PVC is part of a large group of plastics being constantly developed to meet new demands of cheap and effective piping. PVC piping can exhibit a range of properties such as being more rigid or soft depending on the intended use. PVC pipes and connectors are highly modular allowing easy configuration to fit any intended design. This makes our design easier to build as the PVC parts can be directly bought and combined saving costs on machining processes. This further implies that any design will be easy to replicate. PVC is also ideal for our design as it is leak free and corrosion resistant. PVC has some advantages over steel, as PVC has a lower installation cost, is easier to machine, and has a longer service life (Whitfield & Associates, 2008). PVC is also hydraulically smoother than some other metal pipes (e.g. Iron), this means that it is far less prone to deposit builds up, which is important as these buildups can contain harmful bacteria that may affect the outcome of our product. The corrosion resistance of PVC also means

that it does not form “scaly” deposits that can constrict water flow causing an irregularity in pressure: 3 mm of “scaly” deposit can double the pressure drop and pumping power required in the pipes (Whitfield & Associates, 2008). Scaly buildup also provides a surface which can harbor microbes and that is difficult to sanitize, violating compliance with CIP design practises. Finally, PVC is a good material to choose for our design as it is food safe, PVC has multiple uses in the food industry one of these is storage (ex. Tupperware). So long as temperatures remain below 200 ^{o}C, there will be no migration of dioctyl adipate (a plasticizer in PVC) to the food product (Badeka & Kontominas, 1996). As temperatures in our fermentation process will be nowhere near this temperature, PVC can safely be used as a material none of the plasticizing agent will enter. Unfortunately, PVC will deform if left under UV radiation, so PVC components within the system should remain shaded (PPP, 2018). Appendix C contains further information on PVC components.

The micron filter, nuts, bolts, and washers are made of stainless steel. Stainless steel is an alloy containing a minimum of 10.5% chromium by mass (SSF, 2018). Stainless steel is widely recognized for its excellent corrosion resistance as well as its strength and low maintenance. Stainless steel is commonly used in the food industry and as a material for constructing bioreactors. It is therefore stainless steel is a common choice where strength and corrosion resistance are required. In our design, the cylindrical micron filter containing vermicompost is made of a 300 micron stainless steel filter to prevent any particles from leaking out. Stainless steel is also not particularly difficult to make modifications to as we have easy access to welding at the shop. Stainless steel is quite durable, and can last up to decades if properly used and maintained. Bacterial growth on stainless steel surfaces is negligible (FRC, 2018). Stainless steel is also 100% recyclable, allowing the filter to be ecologically disposed of after it has been exhausted (Johnson et al., 2008). Stainless steel does not produce toxic gases when burnt as compared to PVC, can withstand much higher temperatures and is far stronger than PVC (IP, 2018). Further information on the filter can be found in Appendix C.

4.5 Cost

Item	Quantity	Cost
Waste bin	1	$27.79
6.35 mm flexible tubing	12 m	$11.76
19.05 mm PVC pipes	3 m	$5.50
PVC connectors	4	$1.40
Conduit clamps	Bag of 50	$9.86
Bolts, Nuts, & Washers	8 (each)	$4.80
Air pump	1	$87.95
Micron Filter	1	$49.99
Subtotal		**$223.26**
Tax @ 15%		$33.49
Total		**$256.75**

Table 3. Project expenses

5.0 Testing methodology

To demonstrate the efficacy of our product, an empirical test will be conducted. Wheatgrass seedlings will be raised under controlled growth conditions, and fertilized with trials of uninoculated product, inoculated product, commercial nutrient fertilizer, and water (control). Plant growth will be measured. Wheatgrass was selected in this experiment because of its rapid germination and growth time, which are traits suitable for our needs. Further studies should be conducted using the hops plant *Humulus lupulus* as this plant produces many interesting secondary metabolites and is the closest plant relative to cannabis.

Wheatgrass will be planted in soil samples. 10 repetitions of each trial will be performed, using water, commercial nutrient solution, uninoculated bioreactor product, and inoculated bioreactor products as fertilizers. One tablespoon of each fertilizer will be added to each seed daily. Days until germination will be recorded for each seed. Height of each plant will be recorded daily following germination (Appendix E1). NPK and H_2O_2 levels will be measured in each fertilizer using commercially testing kits. H_2O_2 is tested for to indicate successful lactobacillus microbial activity in the fermenter, and because it is a desirable antibacterial product against microbes such as E. Coli. Phytohormones may be indicated to be present if bioreactor fertilizer product outperforms commercially available hydroponics fertilizer on germination time and growth rate despite testing lower for nutrient concentrations. Plant height will hopefully indicate the presence of phytohormones in the fertilizer, especially if seedlings raised under such trials outcompete seedlings raised on commercially available hydroponics fertilizer. Required materials for this experiment are design 3 bioreactor, wheatgrass seeds, soil, plastic seedling trays, growth lights, finished vermicompost, hydrogen peroxide testing kit, NPK testing kit

Factor	Control
Substrate	Commercially purchased soil of known brand and pH in plastic seedling trays
Light	Fluorescent lights of known brand and µmol-light / m^2-s intensity
Temperature	Room maintained at 15 ^oC +/- 3 ^oC
Ventilation	No special ventilation control
Air	N_2, O_2, CO_2, etc levels were not measured

Table 4. Experiment environmental factors and controls

NCR101 is a committee of the United States Department of Agriculture organized to help scientists understand how to take proper recordings in scientific experiment and to effectively and efficiently use technologies in a controlled environment. In compliance with 2016 *Guidelines for Measuring and Reporting Environmental Parameters for Experiments in Greenhouses Facilities* we have listed specifics on factors we will be controlling in our experiment (ICCEG, 2016). These

guidelines ensure replicability and help reconcile results that might otherwise seem contradictory due to differences in growth environment (table 4).

Nutrient concentrations in the vermicompost must be adjusted to meet the demands of the client for proper application. This process is done by taking samples of the leachate that has been processed by our bioreactor to a laboratory for nutrient concentration analysis. The leachate may then be diluted depending on the final desired nutrient concentration. If needed nutrient additives will be placed in the processed vermicompost to meet the demands of the client. Microbial cultures should also be taken at this time to determine the magnitude of microorganismal activity present in the fertilizer product**.** A sample of unprocessed vermicompost leachate was tested in the lab using commercial aquaponics nutrient testing kits, to obtain a rough idea of nutrient concentrations that could be expected for a baseline for our product (appendix E2). The analysis showed high ammonia concentrations of about 8 ppm. The calcium levels were found to be more than what could be measured (greater than 450 ppm). Moderate magnesium levels of approximately 1080 ppm were determined to be in the leachate, and a non-zero nitrate concentration was found. We experienced technical issues in determining nitrite and phosphate levels.

6.0 User information

6.1 Usage directions

The user should first ensure all pieces of the bioreactor are attached and undamaged. The bioreactor should be stored and operated in a well-ventilated, weather protected location with level surfaces and easy access to a power source. The bioreactor should first be filled to the fill mark with water. Air tubing should now be unbundled and attached to the manifold. The user should check all valves on the manifold to ensure that each valve connected to a tube is open and all superfluous valves are closed. The user should check to ensure the aeration pump is elevated above the bioreactor. The aeration pump may now be plugged in. Chlorine is present in many sources, it can act as a microbial inhibitor even in small quantities: Before fermentation, chlorine levels must first be reduced before using the bioreactor. The chlorine can be dissipated in the tank by aerating the water for 15 - 20 min before adding compost into the system (MLL, 2018). The aeration pump

should now be unplugged. Vermicompost and additives should be mixed together. Compost should smell sweet, rich and earthy; a sour smell may indicate pathogenic bacterial contamination. The filter should be packed loosely with compost and not compacted. The user should now secure the micron filter in place. The aeration pump may now be plugged back in. The pump feeding the cooling jacket should also now be plugged in. The bioreactor should be covered with its lid and left for 24 hours. Pumps should then be turned off and finished vermicompost sample removed.

Proper cleaning is very important for maintaining the bioreactor and ensuring a quality product. Biologically active systems typically produce biofilms. Biofilm can be identified as a thin, slick film that adheres to the surface of the bioreactor (MLL, 2018). Routine cleaning after every use of the system is required to prevent the accumulation of biofilm throughout the system. If soaps, bleach, or casuistic solutions are used for cleaning, the bioreactor must be thoroughly rinsed three times to remove chemical residues which will interfere with proper microbial production in the bioreactor. To clean the bioreactor, the lid, and filter holder, and filter should be removed. The inside of the bioreactor should be rinsed with a garden hose to remove all deposits. The water should run clear before this process is finished. Next, all PVC pipes and tubing should be rinsed. A non-abrasive sponge should be used wipe away any biofilm or residue that has accumulated. Allow time for the aeration pump to clear out any residual water within the tubing before the next operation of the system. Finally, the filter should be cleaned with a non-abrasive sponge and hot water to remove all compost residues.

6.2 User safety

Safety has already been maximized from a design perspective. Protruding bolts have been cut short and blunted to prevent puncture wounds. Equipment has been protected by fixing the aeration tubes at a position elevated above the water level to prevent backflow into the pump. However, additional actions should be practised by the user to mitigate risks to the user and protect equipment. This product should be used only for the production of fertilizer and operated according the provided instructions. Do not operate the bioreactor if it has a damaged electrical cord or plug. No exterior forces should be applied to the bioreactor when it is in operation. Always unplug the bioreactor prior to cleaning or maintenance. The Electricity at Work Regulations 1989 require that

any electrical equipment that has the potential to cause injury is maintained in a safe condition (HSE, 1989): Grasp plug to remove power cord from electrical outlet. Do not unplug by pulling on the power cord as this may damage the electrical wire. Only use the bioreactor with a standard electrical supply feed (120V) to prevent risk of electrical shock or equipment damage (MLL, 2018). The air pump should be oriented in either a "vertical or horizontal position" (SSI, 2014). If the [pump is situated "below the water level", a one way valve must be added to the line to prevent backflow into the pump in the event of electrical failure (SSI, 2014).

Fertilizer is not intended for human consumption. If consumption occurs, please contact poison control; do not induce vomiting unless directed to do so by medical personnel. Our product should not be applied to crops less than seven days before harvesting for edible consumption, to mitigate residual bacteria on the crop (MLL, 2018). Fertilizer should be used within 24 hours of extraction; a longer duration may allow beneficial microbes to die and potentially harmful organisms may begin to colonize the fertilizer if it exposed to the open air. Do not store fertilizer in another container at an attempt to extend shelf life as the container may be contaminated (MLL, 2018). Unused compost can be stored it in a cold place as to slow microbial metabolic processes, extending the life of the aerated fertilizer to 48 hours.

6.2 Environmental factors

The bioreactor vessel is watertight to prevent unwanted leakage. Besides presenting an economic loss in product yield, compost nutrients can enter groundwater and cause eutrophication in surrounding bodies of water (National Oceanic and Atmospheric Administration, 2009). Hydroponic growers must ensure that their vermicompost bin has not been contaminated with E.coli, as this pathogen may be conveyed to plants via compost tea fertilizer (Grant, 2015). Our fertilizer should eliminate E. coli as the bacteria cannot undergo sustained growth in an aerobic environment, and will quickly die. Meat based compost feedstocks should be avoided. Although worms can digest meat into a quality feedstock, meat can attract rodents, insects and other pests (Grant, 2015). Some of the most prevalent urban pests that can pose significant health risks to people in urban areas are cockroaches, mice, wasps and flies (Bonnefoy, Kampen, & Sweeney, 2008). The fecal matter of the pests also poses a threat to the respiratory health of small children, causing asthmatic symptoms from an allergenic mouse antigen (Bonnefoy, Kampen, & Sweeney,

2008). If the compost temperatures are not high enough (≥ 50°C), flies, particularly stable flies, will use these piles as massive breeding grounds (Hogsette, 1981).

Our fertilizer is intended for use in hydroponics systems. If applied to lawns or other outdoor aras, it is important to avoid applying before storms as nutrients can "run off" and effect the local environment (National Oceanic and Atmospheric Administration, 2009). Worm tea is potentially acidic in nature, especially if wood biomass was used as a feedstock for compost: acidic fertilizers can mobilize metals within the soil and lower dissolved oxygen levels in water. The dissolved oxygen levels are very important for fish survival, and the overall balance of the marine ecosystem (PHDC, 1992).

7.0 Recommendations

We hope to work on further prototypes of our bioreactor before finalizing it in stainless steel. We would like to test vortex flow using a single combined inlet for all air lifts as we believe this will increase flow pressure and lead to better mixing, homogenization, and aeration. We have ordered parts to begin building a prototype along these lines and would like to compare it to our current prototype.

A possible contamination point behind tubing in pipe, must be tested for and addressed. This problem is caused by a pipe area not subject to constant flow, thus offering a stagnation and buildup point. This violates CIP design practises. Pipes routing air tubes to the bottom of the bioreactor should be capped, with holes bored through the caps to allow the tubing to pass through. This is a minor issue to fix, and will be eliminated from the design in our next prototype.

In future projects we will order all parts from the McMaster-Carr catalog at once instead of purchasing them piecemeal at local retailers. This will save costs and time. The one exception to this is that we should have ordered our pump last, after empirically sizing pressure and flow rates at the shop using the compressed air tank. We initially purchased a pump with very high airflow rate and low pressure. Later, during testing, we consulted Mr. Scott Manktelow at the machine shop, and concluded that we should have first measured the required pressure before ordering our

pump. He suggested we use the compressed air tank at the machine shop with a pressure regulator to introduce an air flow into the aquarium tubing at the bottom of the PVC water lifting/aeration pipes and then gradually increase the pressure setting on the pressure regulator until we reached the pressure we required to force the water up the tube and out. Despite this planning error, our pump proved to be sufficient for our project; under marginally different conditions however, this would have represented an expensive purchasing error.

We would like to purchase a fermentation vessel with a spigot at the bottom to allow easier drainage. This poses a potential issue however, because we must keep our pump elevated above the reactor to prevent backflow; when using a spigot, we would want to elevate the bioreactor to allow better drainage, which would necessitate the pump being fixed at an unreasonable height.

For future prototypes we plan on installing a heat jacket system, as the fermentation process of the microorganisms (explained earlier) is exothermic. The microorganisms of importance are mesophiles and thrive in a temperature range of 15 – 40 °C (GGI, 2003); however, expected temperatures of our system are (60 – 70 °C). If the system is not sufficiently cooled, bacterial populations may perish. As such it is recommended that this heating jacket be added, and it should run around the entirety of the body of the bioreactor for optimum cooling.

Constructing our final bioreactor out of stainless steel allows for several design possibilities that are otherwise unfeasible. Welding allows internal components of the reactor such as the micron filter to be positioned in any location within the reactor, and pipes can likewise be welded to the perimeter of the reactor without requiring holes to be bored for bolts. An impeller system would also be possible to implement in stainless steel, without causing leakage issues. Finally, a copper or stainless steel pipe jacketing system would give a higher heat transfer rate than plastic tubing jacketing.

8.0 Conclusions

The engineering design process has been respected in this project. Thus far, we have obtained a client and consulted our client to understand the client's needs. Relevant literature, patents,

standards, and legislation were reviewed for applicability to our project, and considered during design. Potential options for mixing, aeration, and materials were considered, weighed, and selected. An estimated aeration rate for the reactor was analytically calculated at 155.6 cm^3/s with a mathematical model. Natural convection was demonstrated to be insufficient for cooling, and a cooling jacket sized for the system. A first prototype was constructed at the machine shop. However, we have not satisfactorily fulfilled the aim of our project because we have not yet produced a finished product for our client. Furthermore, we have not even begun to test the efficacy of our product, and can only project its merit based on findings in published literature. Kai Rockafield and John Weilenmann will continue working over summer 2018 to complete the project for our client and hopefully be able to show a final prototype by Fall 2018. We plan to refine our presentation to compete in the 2019 Quebec Engineering Competition.

There are several specific skills and lessons we have learned in this course and from our design project. 3D modeling programs (Fusion 360 and Solidworks) have the McMaster-Carr component catalog built into them, including item specifications, models, and model properties; it would have thus been useful to purchase all components from this catalog so that an exact model could have been drawn and used for modeling physics and structural tests. Our model used mostly components drawn by us, as we ordered most components from private distributors. We should have completed our model, ran fluids and structural simulations on it, and then ordered all parts from the McMaster-Carr catalog. Working with private distributors was not useless however, as it improved our ability to professionally interact with manufacturers ranging from Canada, the US, and China. Finally, we learned the value of tools such as the Pugh chart and Gantt chart, which allow an engineer to show how decisions were justified and utilize time effectively.

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Appendices

Appendix A: Test trials

1.

Figure A1. Observed flow patterns in testing

The testing of the flow patterns was performed by evaluating different potential combinations of air sparger and airlift positioning within the bioreactor. Figure A1 depicts pipe orientation #2 as an example of the process that was performed for each of the 9 tests. The first step was to orient the surgical tubing in the desired direction to cause flow. In trial 2, all tubes were oriented in a counterclockwise direction at 15.24 cm increasing height intervals. The three large circles were vorticities appearing to the front and above of where air was injected by surgical tubing. This is caused by the flow patterns of two momentums colliding into each other causing the forming of a vortex (Waitz et al. 1997). The smaller circles show a collection of flows with smaller momentums colliding into each other, thus producing smaller vortices. These smaller momentum flows could be coming from the air flow of water rotating around the base of the bioreactor for a second time. The vortices on the edges are good for the overall flow, however they do not offer good mixing at center. This is because they are forming "boundary layer" of flow as shown by the red triangular shape in the center. Stagnant areas are example of potential issues encountered when working with vortices troublesome. Baffles may be added to disrupt flow patterns and develop a move complex flow pattern (Dumont, 2018). The process described above was used to visually and empirically analyze all the other flow patterns observed in the test trials.

2.

Trial	Arrangement
Trial 1	3 aeration tubes, height interval, 1 lift
Trial 2	3 aeration tubes, height interval
Trial 3	3 tubes at the bottom
Trial 4	3 tubes at bottom, 1 lift
Trial 5	4 lifts, at center
Trial 6	4 lifts, at perimeter
Trial 7	3 lifts, at center
Trial 8	3 lifts, at perimeter
Trial 9	3 lifts, 1 aeration

Figure A2. A table of test trials.

Appendix B: phytohormones and chemical Information

1.

Microbe	Optimum temperatures (^{o}C)	Fermentation product	Reference
Azospirillum lipoferum	35	Gibberellic acid	Kaushik et al. (2001)
Azospirillum brasilense	*35*	Gibberellic acid	Kaushik et al. (2001)
Agrobacterium tumefaciens	*22*	Auxins and cytokinin	Willy et al. (1997)
Dictyostelium discoideum	*22*	Cytokinin	Zada-Hames & Ashworth (1978)
Erwinia herbicola	*22-28*		Pusey et al. (2004)
Pseudomonas syringae pv savastanoi	*22-25*	Auxins and cytokinin	Varvaro (1983)

Figure B1. Optimal temperatures for phytohormone producing bacteria

2.

Functions of the Major Plant Hormones

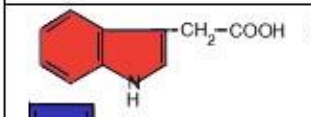
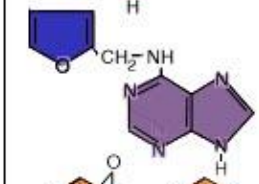
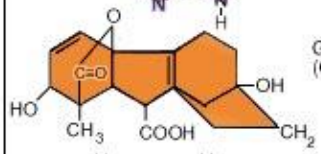
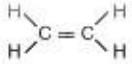
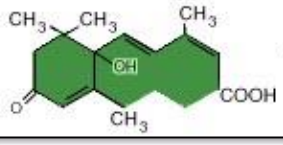
	Hormone	Major Functions	Where Produced or Found in Plant
	Auxin (IAA)	Stem elongation: promotion of vascular tissue growth; suppression of lateral buds	Shoot apical meristems
	Cytokinins (Kinetin)	With auxin, stimulation of cell division and determination of course of differentiation	Produced in roots and transported from there
	Gibberellins (GA_3)	Stem and internode elongation; mobilization of enzymes during seed germination	Apical portions of roots and shoots
	Ethylene	Controls abscission of leaves, flowers, fruits; retardation of lateral bud elongation; hastening of fruit ripening	Leaves, stems, young fruits
	Abscisic acid	Suppression of bud growth; important role in stomatal opening; promotion of leaf senescence	Mature leaves, fruits, root caps

Figure B2. Functions of major plant hormones (BT4Y, 2018).

3.

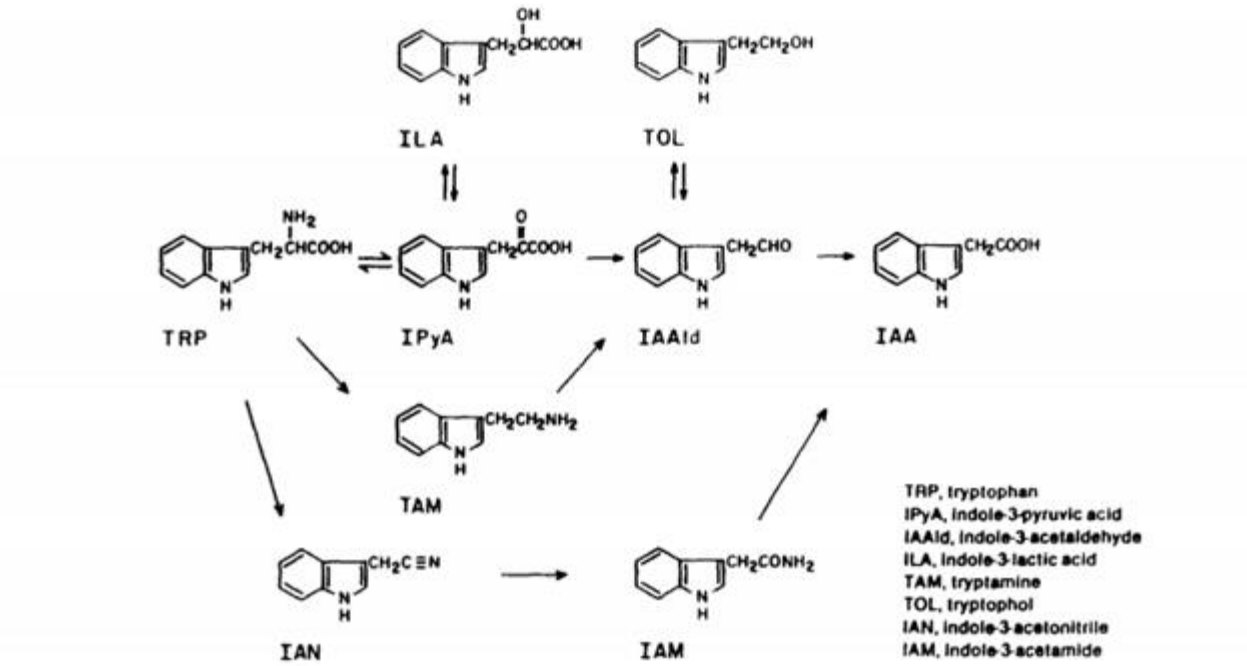


Fig. 1. Proposed pathways for the biosynthesis of indole-3-acetic acid in soil (IAA) (Frankenberger and Brunner, 1983).

Figure B3. Pathway of auxin biosynthesis (Muhammad et al., 1991).

4.

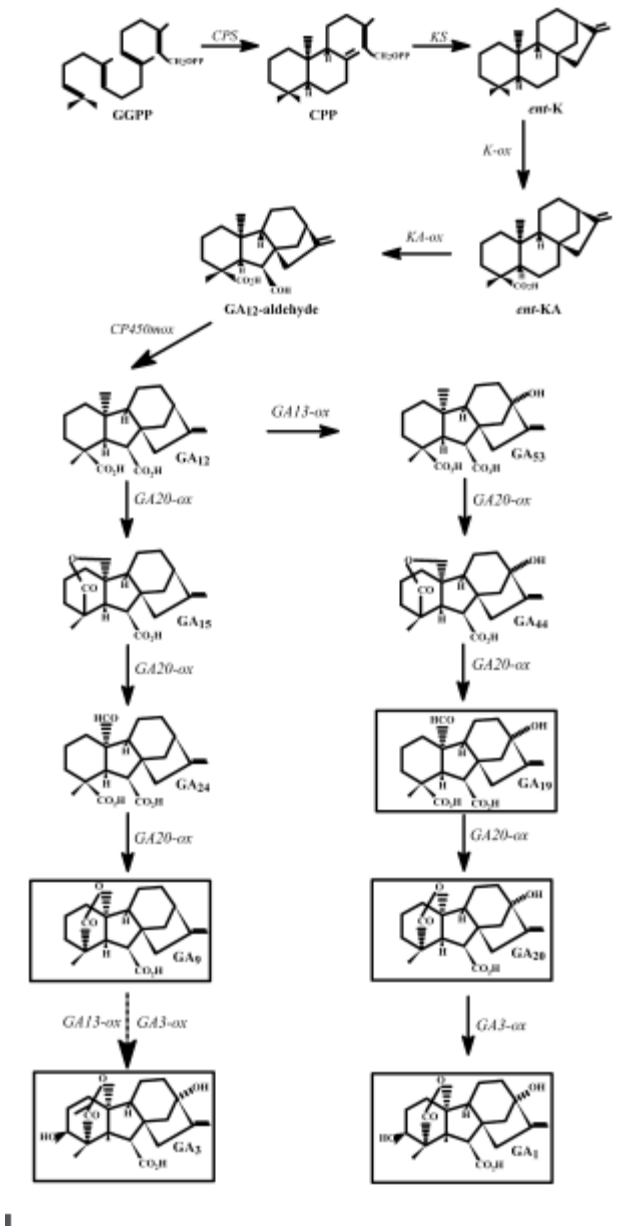
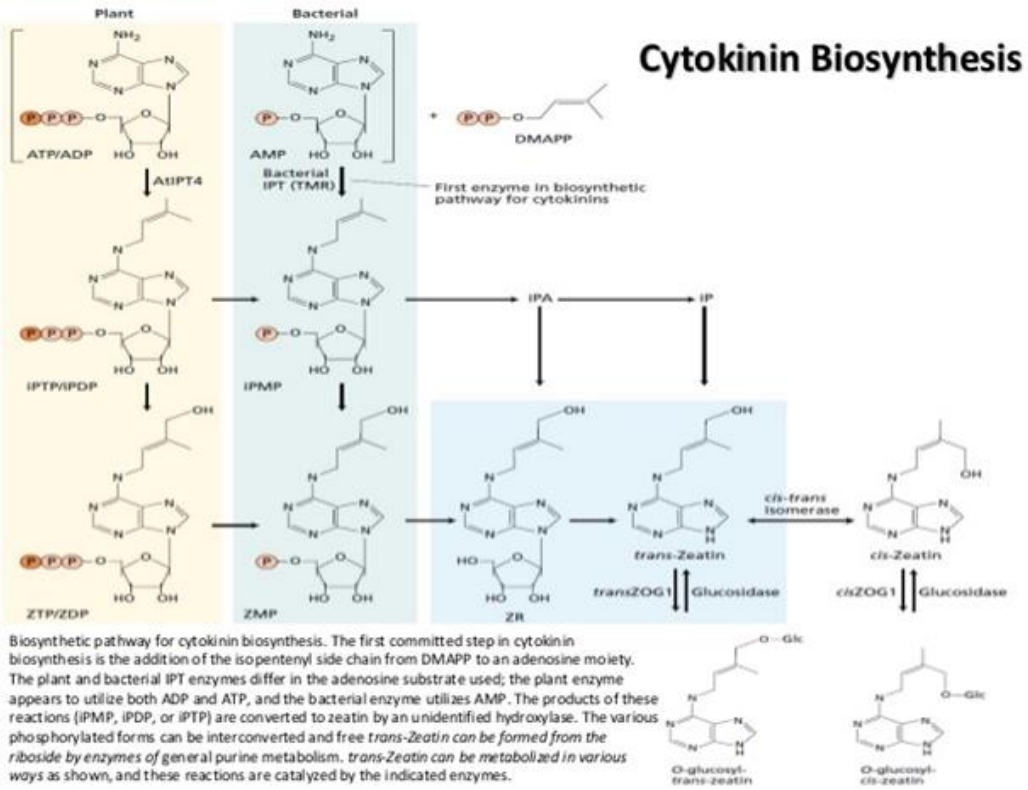


Figure B4. Pathway of gibberellin biosynthesis (Ruben, 2004).

5.



Biosynthetic pathway for cytokinin biosynthesis. The first committed step in cytokinin biosynthesis is the addition of the isopentenyl side chain from DMAPP to an adenosine moiety. The plant and bacterial IPT enzymes differ in the adenosine substrate used; the plant enzyme appears to utilize both ADP and ATP, and the bacterial enzyme utilizes AMP. The products of these reactions (iPMP, iPDP, or iPTP) are converted to zeatin by an unidentified hydroxylase. The various phosphorylated forms can be interconverted and free *trans-Zeatin can be formed from the riboside by enzymes of* general purine metabolism. *trans-Zeatin can be metabolized in various ways* as shown, and these reactions are catalyzed by the indicated enzymes.

Figure B5. Pathway of cytokinin biosynthesis (SS, 2015).

6.

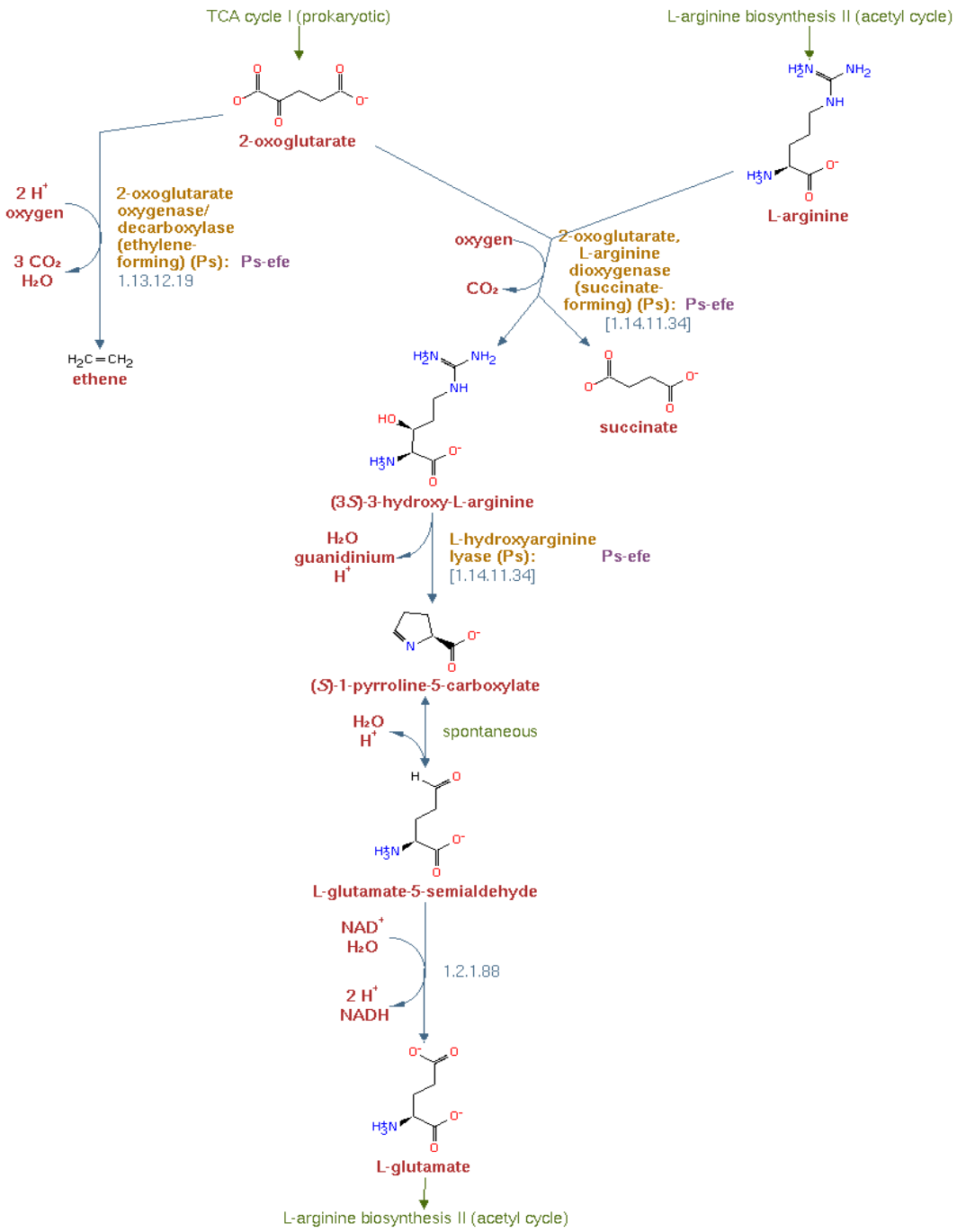


Figure B6. First pathway of ethylene biosynthesis (SRII, 2018a).

7.

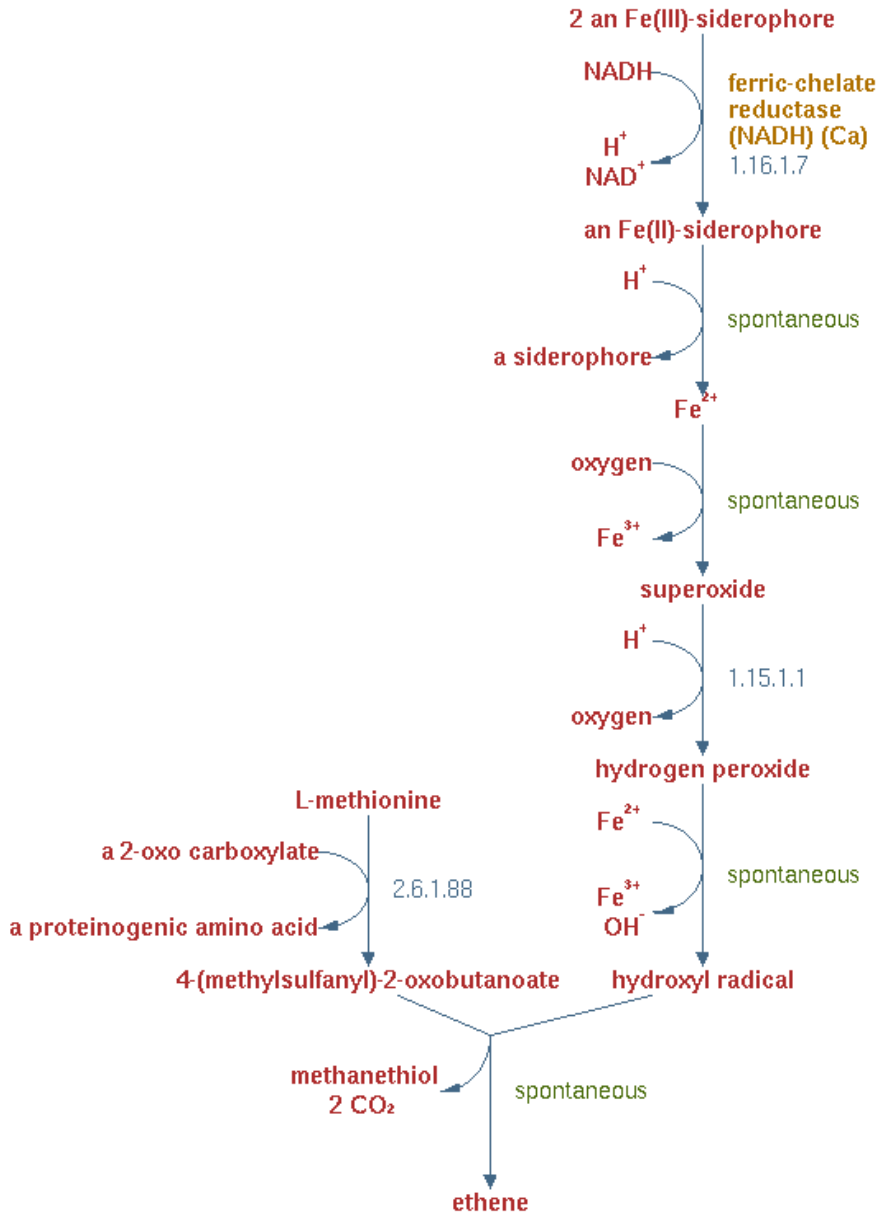


Figure B7. Second pathway of ethylene biosynthesis (SRII, 2018b).

8.

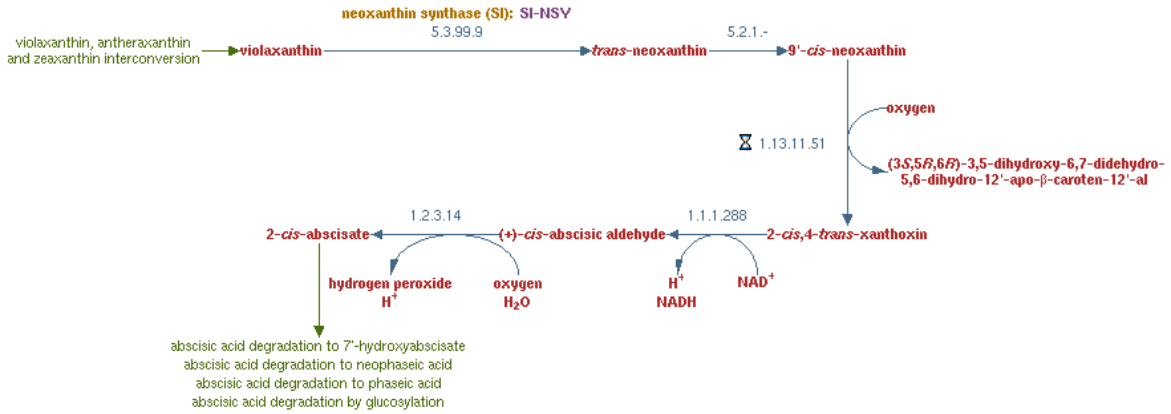


Figure B8. Pathway of abscisic acid biosynthesis (SRII, 2018c).

9.

General guide as to the gas requirements of both microbial and mammalian systems

Gas	Cell culture	Microbial culture
	Sparging	
Air	Approx. 0.1 vvm	1–2 vvm
O_2	10% of 'air'	20–30% of 'air'
CO_2	10–25% of 'air'	20–30% of 'air'
N_2	10–25% of 'air'	20–30% of 'air'

vvm = volume per (working) volume per minute.

Figure B9. Gas requirements of microbial and mammalian systems (Dumont, 2018)

10.

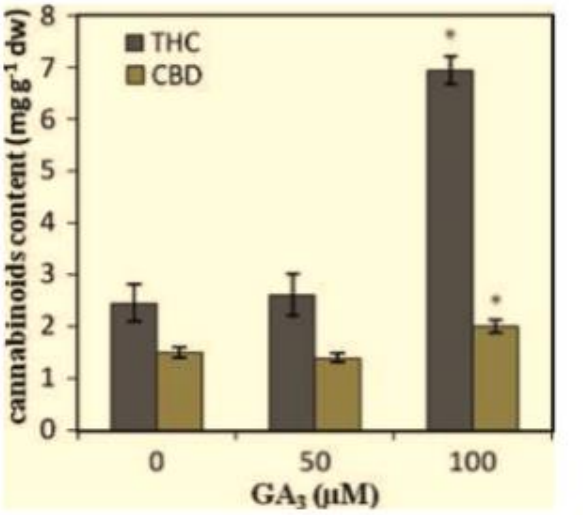
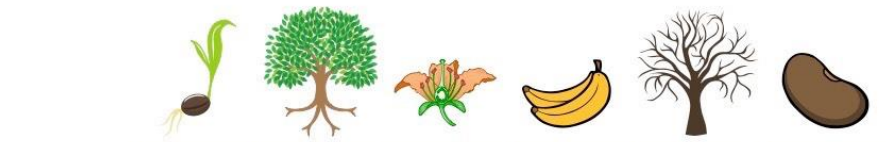


Figure B10. Effect of gibberellic acid on THC and CBD content in the leaves of cannabis (Mansouri, 2012).

11.



	Germination	Growth to Maturity	Flowering	Fruit Development	Abscission	Seed Dormancy
Gibberellin	✓	✓	✓	✓	✗	✗
Auxin	✗	✓	✓	✓	✗	✗
Cytokinins	✗	✓	✓	✓	✗	✗
Ethylene	✗	✗	✓	✓	✓	✗
Abscisic Acid	✗	✗	✗	✗	✓	✓

\

Figure B11. Main classes of phytohormones (BN, 2018).

Appendix C: Materials catalog

1.

Features

6"x14" Kettle Hop Spider

Specifications:

14" Height

6" Diameter

300 Micron Stainless Steel Mesh

Figure C1. Kettle hop spider (OBK, 2018).

2.

728457 - EcoPlus Commercial Air 5 - 80 Watt Single Outlet 1300 GPH	
	EACH
Upc:	870883008390
Quantity:	
Volume (cf):	0.2420
Weight (lb):	6.4
Length (in):	9.9
Width (in):	6.6
Height (in):	6.4

Figure C2. EcoPlus Commercial Air 5 compressor (Amazon, 2018).

3.

Mobile Waste Container

Round, Lift Off Lid, 32 Gallon Capacity

Each

ADD TO ORDER

In stock
$27.79 Each
4504T31

Style	A
For Use With	Waste
Shape	Round
Capacity	32 gal.
Width	20 3/4"
Depth	22 3/4"
Height	35"
Circumference	87"
Weight Capacity	60 lbs.
For Use Outdoors	Yes
Mobility	Mobile
Lid Type	Lift Off
Material	Plastic
Color	Black

Figure C3. Mobile Waste Container (McMaster-Carr, 2018).

4.

Standard-Wall Unthreaded PVC Pipe for Water

3/4 Pipe Size, 10 Feet Long

Each

ADD TO ORDER

In stock
$5.50 Each
48925K12

For Use With	Drinking Water
Shape	Straight
Type	Pipe
Schedule	40
Threading	Unthreaded
Connection Type	Pipe
Pipe Connection Type	Socket Connect
Socket Connect Type	Cement
Gender	Male
Pipe Size	3/4
Length	10 ft.
Maximum Pressure	480 psi @ 72° F
Minimum Temperature	Not Rated
Maximum Temperature	140° F
Material	PVC Plastic
Color	White
For Fitting	
Material	PVC Plastic
Schedule	40
Specifications Met	ASTM D1784, ASTM D1785, NSF/ANSI Standard 61 for Drinking Water
RoHS	Compliant

5.

Standard-Wall PVC Pipe Fitting for Water

45 Degree Elbow Connector, White, 3/4 Socket-Connect Female

Each

ADD TO ORDER

In stock
$0.78 Each
4880K32

For Use With	Drinking Water
Shape	45° Elbow
Type	Connector
Connection Type	Pipe
Connection	Socket-Connect Female
Socket Connect Type	Cement
Pipe Size	3/4
Schedule	40
Material	PVC Plastic
Color	White
Minimum Temperature	Not Rated
Maximum Temperature	140° F
For Pipe	
Material	PVC Plastic
Schedule	40
Specifications Met	ASTM D1784, ASTM D2466, NSF/ANSI Standard 61 for Drinking Water
RoHS	Compliant

6.

Standard-Wall PVC Pipe Fitting for Water

90 Degree Elbow Connector, White, 3/4 Socket-Connect Female

Each

ADD TO ORDER

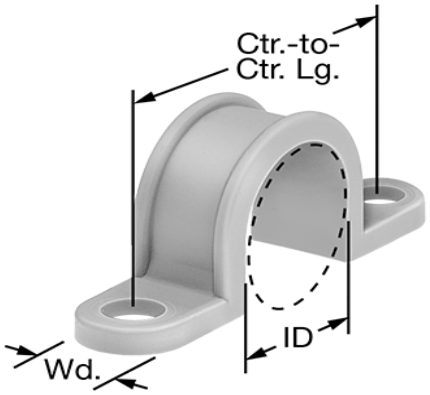
In stock
$0.35 Each
4880K22

For Use With	Drinking Water
Shape	90° Elbow
Type	Connector
Connection Type	Pipe
Connection	Socket-Connect Female
Socket Connect Type	Cement
Pipe Size	3/4
Schedule	40
Material	PVC Plastic
Color	White
Minimum Temperature	Not Rated
Maximum Temperature	140° F
For Pipe	
Material	PVC Plastic
Schedule	40
Specifications Met	ASTM D1784, ASTM D2466, NSF/ANSI Standard 61 for Drinking Water
RoHS	Compliant

Figures C4-6. PVC Piping (McMaster-Carr, 2018).

7.

Plastic Routing Clamp
Polypropylene Plastic, 13/16" ID



Packs of 50

In stock
$9.86 per pack of 50
3192T51

ADD TO ORDER

Material	Polypropylene Plastic
For Number of Lines	1
Number of Mounting Points	2
ID	13/16"
For Pipe Size	1/2
For Rigid Conduit Trade Size	1/2
For EMT Conduit Trade Size	3/4
For IMC Conduit Trade Size	1/2
For Copper Tube Size	3/4
Center-to-Center Length	1 1/2"
Length	2 1/16"
Width	9/16"
Height	1"
Thickness	5/32"
Color	Gray
Temperature Range	-30° to 180° F
For Use Outdoors	No
Mounting Fasteners Included	No
Mounting Hole Diameter	3/16"
Capacity	Not Rated
Mount Type	Screw On
RoHS	Compliant

Figure C7. Plastic Routing Clamp (McMaster-Carr, 2018).

8.

SERVICE TEMPERATURE RANGE	
Service Temperature [Min]	25 °F -4 °C
Service Temperature [Max]	150 °F 65 °C

NOMINAL SPECIFICATION	
Brand	KLEARON™ Kuri Tec®
Series	K010
Size Code	0304
Nominal ID	3/16 in 4.8 mm
Nominal OD	1/4 in 6.4 mm
Nominal Wall	1/32 in 0.8 mm
Working Pressure at 70°F (20°C)[1]	50 psi
Standard Length Ctn/Coils	100 ft
Approx. Wt. Per Pkg.	1.2 lb
Packaging	Carton/Coils
Type	Tubing
Style	Clear Food and Beverage Grade Phthalate Free PVC Tubing
Material	PVC
Industry Standards/Certifications	RoHS Phthalate Free NSF® 3A UL USDA USP FDA
Color	Crystal Clear

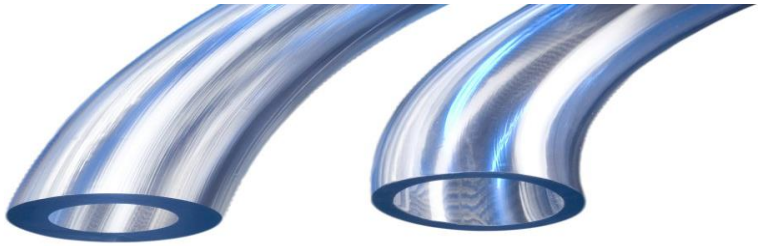


Figure C8. Thermoplastic tubes (KT, 2018).

Appendix D: Unit conversions

Volume

SI unit: Cubic meter (m^3)

1 liter (L) = 1.00 x $10^{-3}m^3$

= 1000. cm^3

= 1.056710 quarts

1 gallon = 4.00 quarts

Pressure

Si unit: Pascal (Pa)

1 pascal = 1 N/m^2

= 1 kg/m * s^2

1 atmosphere = 101.325 kilopascals

Mass

SI unit: Kilogram (kg)

1 kilogram = 1000. grams

1 gram = 1000. milligrams

1 pound = 453.59237 grams

= 16 ounces

1 ton = 2000. Pounds

Temperature

Si unit: Kelvin (K)

K = 273.15 °C

K = °C + 273.15 °C

°C = (5 °C/9 °F)(°F - 32 °F)

°F = (9 °F/5 °C)°C + 32 °F

Length

SI unit: Meter (m)

1 kilometer = 1000. meters

= 0.62137 mile

1 meter = 100. centimeters

1 centimeter = 10. millimeters

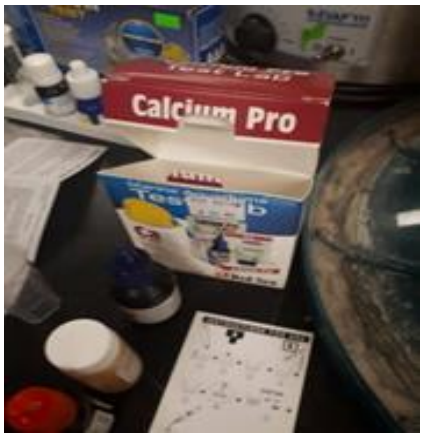
(Univeristy of North Carolina, 2018)

Appendix E: Fertilizer efficacy testing

1.

Test	NPK Start	Germination Time (days)	Plant Height (mm)	Water Input (ml)	H2O2 concentration
Bio Reactor Fertilizer Standard			Day 1 Day 2 Day 3 Day 4 Day 5	Day 1 Day 2 Day 3 Day 4 Day 5	
Bio Reactor Fertilizer Inoculant 1			Day 1 Day 2 Day 3 Day 4 Day 5	Day 1 Day 2 Day 3 Day 4 Day 5	
Bio Reactor Fertilizer Inoculant 2			Day 1 Day 2 Day 3 Day 4 Day 5	Day 1 Day 2 Day 3 Day 4 Day 5	
Bio Reactor Fertilizer Inoculant 3			Day 1 Day 2 Day 3 Day 4 Day 5	Day 1 Day 2 Day 3 Day 4 Day 5	
Hydro store nutrient mix			Day 1 Day 2 Day 3 Day 4 Day 5	Day 1 Day 2 Day 3 Day 4 Day 5	
Water (Control)			Day 1 Day 2 Day 3 Day 4 Day 5	Day 1 Day 2 Day 3 Day 4 Day 5	

Figure E1. Datasheet for fertilizer efficacy testing



Appendix E2. Nutrient concentration testing kits

Appendix F: Aeration assumptions

Using certain assumption to evaluate our design, first we are assuming a time of at least 24 hours of mixing, or 86400 seconds. Our pipe openings are 0.635 cm diameter, indicating a cross sectional area in each pipe of 2.00 cm^2 = 0.0002m^2 .

1) We have a 4920 liter per hour pump, giving a flow rate of 0.00136695m^3/s of air, over 4 tubes is 3.4173*10^{-4} m^3/s in each tube. Airflow rate is therefore found to be 3.4173*$10^{-4}$$m^3$/s / 0.0002$m^2$ or **1.71 m/s** per pipe

2) We will be assuming that at 63.5 cm of the bioreactor will be filled up, 63.5cm.

u_y / y = ε_y: 1.71 /.635 = 2.69

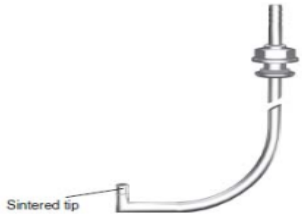
3) $(2De/\pi)^{1/2}$. (2*.219*2.69/Pi)$^{1/2}$ = 0.612 cm^2/s

4) 0.612 cm^2/s * 63.5 cm = 38.9 cm^3/s

38.9 cm^3/s *4 (each of the pipes) = 155.6 cm^3**/s**

Appendix G: Bioreactors

1.



Sintered sparger

Figure G1. Sintered Sparger (Dumont, 2018)

2.

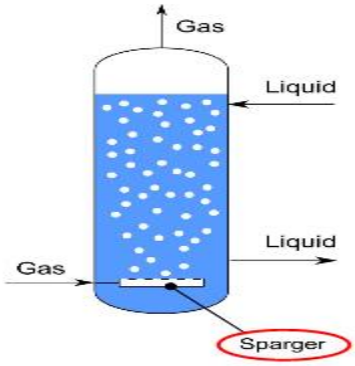


Figure G2. Air Inlet Sparger (Dumont,2018)

3.

Ingredient	Feeds	Ingredient	Feeds
White Sugar	Bacteria	Maple Syrup	Bacteria
Corn Syrup	Bacteria	Cane Sugar	Bacteria
Molasses	Bacteria/Fungi	Fish Emulsion	Bacteria
Fruit Pulp	Bacteria/Fungi	Fish Hydrolysate	Fungi
Kelp	Bacteria/Fungi	Ground Oatmeal	Fungi
Rock Dusts	Bacteria/Fungi	Yucca	Fungi
Humic Acids	Bacteria/Fungi	Soybean Meal	Fungi

Figure G3. A table showing some compost additives and the species they feed (CJ, 2011).

4.

Type of Plant	Type of Tea
Most brassicas	Highly Bacterial
Vegetables, Grasses	Moderately Bacterial
Berries	Balanced Bacteria to Fungi
Deciduous Trees	Moderately Fungal
Coniferous Trees	Highly Fungal

Figure G4. A table listing some common plants and their favourable compost tea solutions for growth (CJ, 2011).

Appendix H: Bioreactor models

1.

Figure H1. Side view of our bioreactor modelled in fusion 360

2.

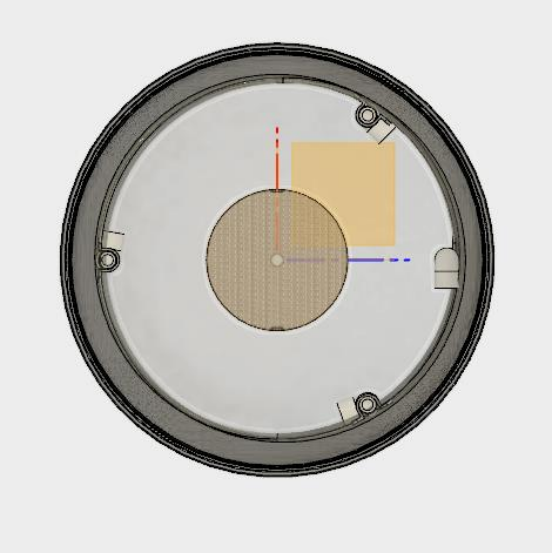


Figure H2. Top view of reactor

3.

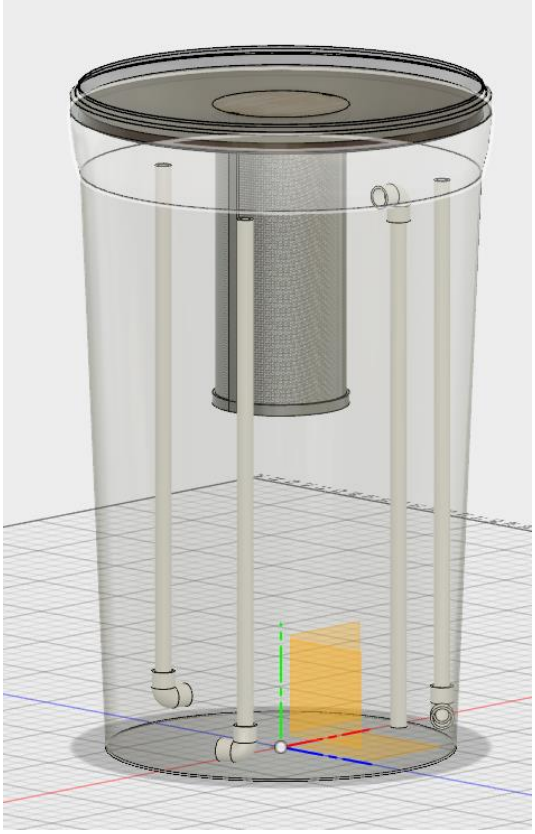


Figure H3. Bioreactor model

4.

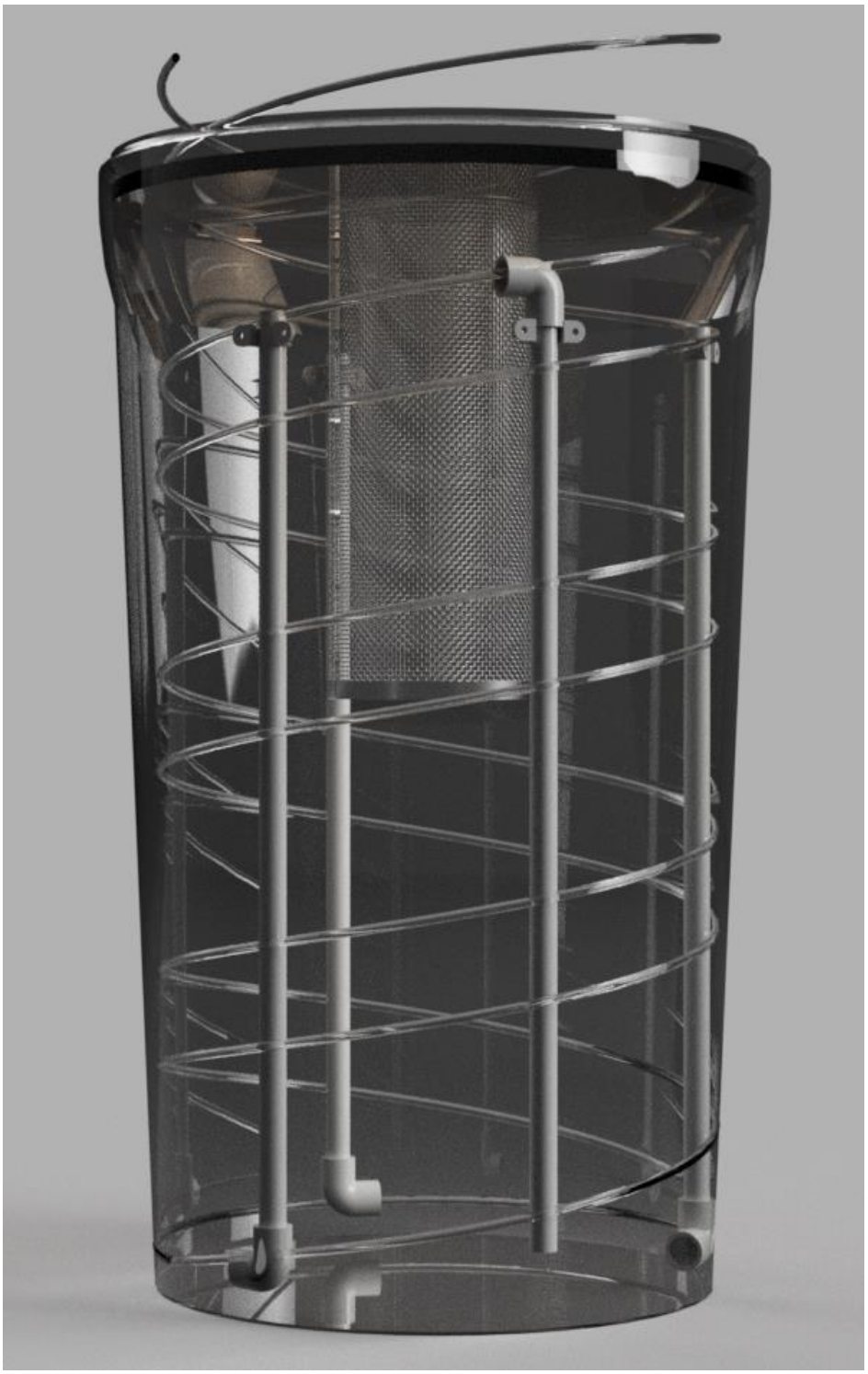


Figure H4. Rendering of Bioreactor

5.

Figure 5. Low resolution rendering of bioreactor

Appendix I: Warranty

LIMITED WARRANTY

Our company guarantees that for the period beginning of the purchase and ending 6 months after that date, this product will be free from defects in material and workmanship. If any defect is discovered during this period of limited warranty, then by our company's judgement we may either provide a new product, send pieces to replace the broken ones, or send over a repairman to fix the customer's product. If the product must be shipped for repair, then the shipping costs shall be paid for by our company. In order for this warranty to apply, the product can only be used for agricultural purposes and the product must not be modified in any ways that is discernable by our company. Our company does not guarantee that this product will suited for the customer's particular growing requirements and nutrient requirements as every agricultural product has different requirements. This warranty is limited only to the repair, replacement of parts and hiring of a repairman, and in no way is our company liable for any monetary sum other than the aforementioned scenarios. Our company is not liable for any damages relating to the use of our product or any damages resulting from the user's discretion (i.e. using a meat-based compost, applying compost tea right before market etc.). There are no warranties extending beyond the situations stated above.

The above mentioned warranty has been created from an example warranty of a product created by Martin Lishman Ltd (2018).