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Techno-Economic Analysis of Domestic Industrial Scale Plant-Based Production of Resveratrol for Novel Biopolymer Application

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Techno-Economic Analysis of Domestic Industrial Scale Plant-Based Production of Resveratrol for Novel Biopolymer Application

By

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THESIS

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Brandon Pizarro Carbajal
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Techno-Economic Analysis of Industrial Scale Plant-Based Production of Resveratrol for Novel Biopolymer Application

Abstract

A large-scale facility was designed to analyze the techno-economics of current resveratrol manufacturing procedures to provide information for domestic production using a plant-based approach. The present model utilizes the plant Japanese knotweed as a source of resveratrol for production. All manufacturing steps and conditions were tuned to match manufacturing processes listed in publicly available literature and patents on resveratrol production. The model is designed to produce 100 metric tons of resveratrol at 98% purity. This simulated biomanufacturing facility operates for 330 days a year, 24 hours per day, performing 1,295 batches yielding 77.3 kg resveratrol per batch. This simulated manufacturing facility was designed in SuperPro Designer® allowing for initialization of bioprocessing conditions and pricing of materials and equipment utilized in the model. The analysis conducted here provided key insight into the capital expenditures (CAPEX), operating expenditures (OPEX), and Cost of Goods Sold (COGS) expected for such a process. The results from the base case model are as follows: CAPEX of $44.7 million, OPEX of $15.0 million per year, and a COGS of $150/kg resveratrol. A sensitivity and scenario analysis were conducted to assess the relationship between certain operating conditions to both the CAPEX and COGS. The implementation of additional equipment for an ethanol recovery unit was shown to reduce OPEX to about $14.1 million and COGS to $141.4//kg but increase CAPEX to $51.5 million. The simulated facility was assessed for its efficiency, and it was determined that the model has a process mass intensity (PMI) of 529 kg/kg resveratrol. The environment impact factor (EI) of the facility was also analyzed. Here, the EIs were determined to be 26 and 31.6 kg/kg resveratrol for input and outputs, respectively, suggesting a very safe and environmentally-friendly process.

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Chapter 1 : Introduction

Free resveratrol (trans-3,5,4'-trihydroxystilbene) is a naturally occurring plant-made stilbene compound produced in over 70 plant species[1], including but not limited to grapes (*Vitis vinifera*)[2], peanuts (*Arachis hypogaea*)[3], and blueberries (*Vaccinium sp*.)[4]. Resveratrol, an isomer where the trans-form of the double bond separates two phenyl groups (shown in **Figure 1.1**), is a phytoalexin capable of both antifungal and antimicrobial properties[5,6]. This defensive property has prompted researchers to investigate the compound further in search for any potential benefits. In recent years, over 1,900 studies have been published reporting the numerous therapeutic benefits and applications which resveratrol contains (PubMed, 2022). In particular, resveratrol is widely recognized for its beneficial pharmacological effects which include anti-tumor[8], anti-inflammatory[9,10], prevention of cardiovascular disorders[11,12], and anti-aging[13]. For this reason, the utilization of resveratrol is predominantly seen as a supplement in food or in the form of pill capsules in the nutraceutical industry (i.e., Mega Resveratrol®). However, the use of resveratrol is also seen present within the pharmaceutical and cosmetic industries[14].

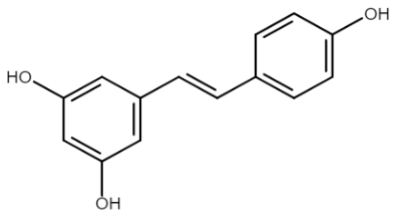

Figure 1.1 Chemical structure of resveratrol

Resveratrol has been gaining a lot of attention recently since chemical research literature has demonstrated that it can be utilized as an active ingredient in synthesizing bio-based polymers and resins[15,16]. Notably, using biorenewable compounds like resveratrol to produce biopolymers is not a novel subject but interest in using biobased compounds for polymer development is growing. In fact, an emerging topic in the field of green chemistry is the investigation of biobased

compounds for polymer development. An example of a biobased compound used for biopolymer applications is lactic acid. Lactic acid, which is mainly derived from corn, has been shown to form polylactic acid (PLA)[17] typically used in 3D printing applications. Another example of a biobased compound used for polymer applications is vanillin. Vanillin, which can be derived from vanilla orchid extract, has been shown to be a precursor for a variety of polymers ranging from polyethylene terephthalate (PET) to polybenzoxazines[18]. The attractiveness of these compounds emerges from a variety of reasons, ranging from their unique chemical structure to their biodegradability[19]. One reason resveratrol is highly sought after in the development of thermoset polymers is the number of hydroxyl groups located on the backbone of the aryl compound. It is extrapolated that these functional groups act as proton donors which can catalyze the curing reaction, allowing for curing to occur at low temperatures while also increasing reactivity of the polymer[20]. Another reason why using resveratrol as a raw material for polymer synthesis is of interest is its sustainability. Typical epoxy resins are designed using environmentally unfriendly petrochemicals which must be chemically synthesized, such as bisphenol A (BPA), which is harmful towards the human immune system[20]. Using alternative, naturally produced, less toxic compounds like resveratrol offers scientists the ability to use renewable materials at large quantity in a safe environmental workplace. An example of a resveratrol-based biopolymer resin and its chemical synthesis pathway can be seen in **Figure 1.2.**

Interestingly, literature focused on resveratrol-based biopolymers have demonstrated that resveratrol epoxy resins have been characterized to hold high flame resistance properties. Specifically, these resins can hold high thermal stability at temperatures of 350°C and higher, causing them to become a viable alternative to environmentally harmful petroleum-based epoxy resins currently being used [21,22]. Additionally, researchers from Hubei University reports epoxy

resins fabricated using bio-based resveratrol can achieve char yields up to 62% at 800°C [23]. Such thermal stability performance makes using resveratrol as a raw material desirable. However, the utilization of resveratrol as a precursor for biopolymers may require a much higher purity than what is currently existing for nutraceutical applications. Unlike the stringent regulations placed on the pharmaceutical industry, the nutraceutical industry remains an unregulated market which receives little to no oversight from the U.S. Food and Drug Administration (FDA). Thus, domestically produced resveratrol capsules can be expected to vary in resveratrol concentrations. A study conducted by Rossi et al., demonstrated that only 5 out of 14 nutraceutical resveratrol products tested contained concentrations of resveratrol in similar values to that branded on the bottle's labels. Further investigation from the same authors allowed them to discover that 2 out of the 14 nutraceutical resveratrol products sampled had resveratrol content below the limit of detection[24]. Such variation prompts the need for an efficient manufacturing process which can produce concentrated amounts of resveratrol reliably.

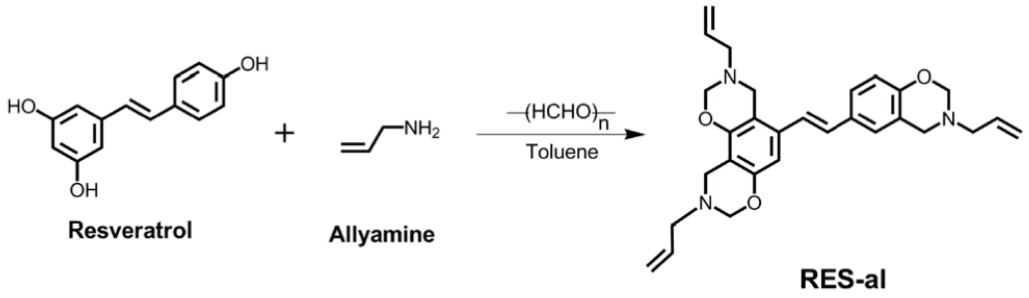

Figure 1.2 Chemical synthesis pathway of benzoxazine monomer (Res-al) (Image provided by [15]).

Literature featuring different production processes capable of producing resveratrol at 98% purity and higher is available but is often only described for small quantities (mg to g) in laboratory-based environments, limiting its potential for large scale production. It has also been demonstrated that highly pure resveratrol can be produced via different expression systems, either through a plant-based, microbial, or chemical synthesis approach. The most common method

utilized for commercial production of resveratrol is the plant-based approach, demonstrated by the dozen companies offering natural plants as their source for production[25]. While alternative production routes such as microbially and chemical synthesis are commercialized, two individual companies, Evolva and DSM, dominate the manufacturing of resveratrol using those methods globally[25]. Japanese knotweed (*Reynoutria japonica*, also known as *Polygonum cuspidatum* and *Fallopia japonica*) is typically used as the plant source since it naturally produces resveratrol at high concentrations within its rhizomes, concentrations ranging from 0.5 – 12 mg/g fresh weight (FW) [26]. Other plant sources such as grapes, peanuts, and seeds of melinjo (*Gnetum gnemon* L.) endosperms only reach a fraction of the resveratrol present in knotweed, yielding values as high as 0.01 to 84.63 µg resveratrol/ g grape leaves FW, 0.03 to 0.14 µg of resveratrol/g peanuts Dry weight (DW) and .223 mg resveratrol/g dried melinjo endosperms[27–29]. Ironically, an additional advantage to using Japanese knotweed is that resveratrol is not the leading compound found within the plant. The resveratrol precursor polydatin, a resveratrol glucoside found in Japanese knotweed, is found in higher concentrations, even up to 7-fold higher compared to resveratrol under certain growing conditions[9]. A deglycosylation and hydrolysis step can convert polydatin to resveratrol, thus increasing the total concentration of resveratrol present in knotweed. The chemical structure of polydatin is shown in **Figure 1.3**.

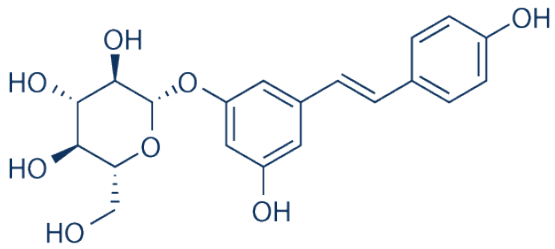

Figure 1.3 Chemical structure of polydatin

The global market for resveratrol is increasing, with current analysts estimating that the global resveratrol market to be within the range of $71.9 to $97.7 million in 2020 alone with

anticipated growth in the coming years[14,30]. A discussion with a resveratrol manufacturer in China led to the claim that the global production volume was roughly 300 MT per year. Despite being able to recover resveratrol from a variety of expression systems, no biotechnological large-scale production facility has been established. It is to the best of our knowledge, the production capacity for a single manufacturer producing 98% pure resveratrol is well below 100 MT per year. Implementation of such biomanufacturing facility presents a viable solution to provide a reliable supply of resveratrol in high purity suitable for biopolymer applications domestically. Nonetheless, launching a new facility for such production scale requires extensive planning, market analysis, process development, and product development. Certain tools such as process simulation tools (PSTs) are beneficial in providing decision making guidance for the design of a new facility as it can provide clarity on unknown processes or technologies. In fact, the utilization of PSTs during different stages of the product life cycle (idea screening, procurement, process development, facility design and manufacturing) can be used to assess and eliminate impractical methods before the actual design and implementation. PSTs are a feasible tool used extensively when designing new manufacturing facilities as estimates for capital investment, equipment size, costs and scheduling, annual operating costs, and cost of goods can be determined. This information is invaluable as it can guide investors and companies on the economics earlier in the design process.

In the following chapters, a techno-economic analysis (TEA), including process design, scale-up simulation, and scenario and sensitivity analyses, is presented for the large-scale plant-based production of resveratrol. A base case production facility will be modeled for an annual production target of 100 MT of 98% pure resveratrol. In Chapter 2, the economic analysis performed for the upstream processing of the field-grown and field harvested Japanese knotweed plants will be discussed. Next, Chapter 3 will address the downstream bioprocessing steps needed

for extraction and purification of resveratrol from the cultivated Japanese knotweed roots. In Chapter 4, a series of sensitivity and scenario analysis will be performed to assess the relationship between certain bioprocessing parameters to economic factors such as capital expenditures and cost of goods sold, along with discussion of certain advantages and disadvantage of the different scenarios performed. Chapter 5 summarizes the environmental impact of the base case model. Chapter 6 will summarize the work performed within this analysis and offer further work and modifications which can be applied to the model.

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Chapter 2 : The Cultivation and Harvesting of Japanese Knotweed

2.1 Introduction

Large scale, open-field agriculture is common practice utilized for the production and cultivation of several thousand metric tons of crops in the United States (U.S.). In a report distributed by the United States' Department of Agriculture (USDA), it was announced that in 2019 the U.S. harvested over 284 million acres of farmland for principal crops including but not limited to corn, oats, wheat, and potatoes[1]. Outdoor agriculture is a technique which has been gaining traction for molecular farming applications, or the production of recombinant proteins or metabolites in plants. Transgenic, or genetically modified, plants have been shown to grow at agricultural scale, reaching protein yields as high as 1000 kg per year[2]. While literature has demonstrated that transgenic plants can produce resveratrol[3], the use of modified plants are not needed for large scale production of resveratrol since it can already be produced in native plants at high concentrations. An additional advantage of using a natural plant source for resveratrol production outdoors over transgenic plants is the exclusion of adhering to regulations for field grown, genetically modified crops[4]. Furthermore, no transformation step is needed when establishing the plant sources, thus reducing upstream costs.

For outdoor production, Japanese knotweed is chosen as the host best suitable to reach the market's need for resveratrol. Japanese knotweed has been shown to grow in open-field plantations by numerous oversea companies, thus demonstrating its usefulness for bulk resveratrol production. In addition to the high concentration of resveratrol found within Japanese knotweed rhizomes and its ability to grow at large scale, there are several other advantages to utilizing it over other natural plant sources. First, and most importantly, Japanese knotweed grows rapidly in a variety of habitats and environments; it classified is an invasive species. In fact, Japanese knotweed has been named

one of the 100 worst invasive species in the world by the Invasive Species Specialist Group (ISSG) and World Conservation Union (IUCN) [5]. Due to its invasive nature, Japanese knotweed has been able to persistently grow across different terrains. According to data retrieved from the University of Georgia's Center for Invasive Species and Ecosystem Health, Japanese knotweed has been detected in 43 out of 50 U.S. states[6]. In addition to Japanese knotweed's ability to grow across different topographies, it has been demonstrated to grow under harsh conditions which other plants might not. Specifically, a group of researchers conducting Japanese knotweed growth conditions studies describe how they expect the plant to continue growing and producing plant metabolites while placed in low-fertile soil with no irrigation[7].

Currently, there exists numerous published techno-economic analysis studies of plant-based production focusing on biofuels[8], recombinant therapeutic proteins[8–10], industrial enzymes[11], and antimicrobial proteins for food safety[12]. Here, this thesis will describe a process simulation model for the techno-economic analysis performed of the plant-based production of Japanese knotweed and for the extraction and purification of the biopolymer precursor, resveratrol, which has not been demonstrated before. This study establishes a framework to help inform decisions on the development of a domestic production route for such polymer precursors.

2.2 Process Design Parameters and Calculations

Due to Japanese knotweed's classification as an invasive species and predominant cultivation in China, literature focusing on optimizing its large-scale growth conditions and economics are limited. Luckily, literature surrounding the production and harvesting of potatoes is vast. Potatoes are a subterranean root vegetables native to the Americas, making them a suitable candidate for modeling Japanese knotweed rhizomes after. Thus, a techno-economic analysis for the upstream portion of the base case model was performed using data retrieved from UC Davis

Agriculture and Resource Economics on potato harvesting. This model considered the different equipment needed, labor costs, land rent, the Capital Expenditure (CAPEX), and Operating Expenditures (OPEX).

The base case scenario assumes an annual production capacity of 100 MT resveratrol. To reach the proposed target, upstream production was modeled using an open-field, staggered plantation of Japanese knotweed plants of about 1,847 acres per batch. Each batch was assumed to have a duration of 2 years (including land turnaround). Key assumptions for the proposed farmland in this chapter are listed in **Table 2.1** A list of process assumptions used to define certain parameters within the model; FW, fresh weight, N/A, not applicable.

Table 2.1 A list of process assumptions used to define certain parameters within the model; FW, fresh weight, N/A, not applicable.

Upstream Processing			
Bioprocess parameter	**Information used**	**Source value**	**Source and Link**
Mass (FW) Knotweed Rhizomes per acre (after 2 years growth)	4,047 kg/acre	10 tons/hectare	Kovářová et al. (2010)
Growth conditions and harvesting practices	Japanese Knotweed growth and harvesting	Potato harvesting	UC Davis Agriculture and Resource Economics
Farming equipment and scaling	Equipment scaled with cultivation acreage (1,847/250); Inflation/cost adjustment of 1.125 from 2015 values	N/A	Federal Reserve Bank of St. Louis
Stilbene concentration in Japanese	Resveratrol: 0.5 mg/g FW Polydatin: 1.5 mg/g FW	Resveratrol: 0.05 -12.07 mg/g Polydatin: 0.43 – 13.68 mg/g	Chen et al. (2013)

knotweed rhizome			
Containment cost	$0	N/A	N/A
Irrigation Cost	$0	$0	Kovářová et al. (2010)
Pesticide Cost	$0	N/A	N/A
Plant Moisture Content	69.4%	69.4%	Beerling et al. (1994)

In efforts to accurately model the upstream portion of resveratrol production, the cost of land rent for non-irrigated crop land in the U.S. was investigated. According to the USDA, the average rent paid for non-irrigated land in the U.S. is $128.00 per acre[14]. It determined that non-irrigated cropland for rent was available for $29.00 per acre in the southwest region of South Dakota[15]. Notably, the cost for non-irrigated land available in South Dakota was less than 77% of the national average. Due to the availability and affordability of farmland, South Dakota was chosen as the state best fit to model knotweed rhizome production in. Using research from the University of Georgia - Center for Invasive Species and Ecosystem Health, it was confirmed that Japanese knotweed can grow in west South Dakota (Error! Reference source not found.). Next, South Dakota's farm operations were assessed to determine whether the state would be able to handle the demand (in acres) needed for resveratrol production. Using the mass of knotweed rhizomes grown per acre (after 2 years growth) per the mass of knotweed rhizomes needed for 100 MT production of resveratrol, our estimates yield a total of 3,695 acres of non-irrigated land needed for suitable growth of Japanese knotweed needed to meet our target production level. In 2021, the USDA reported South Dakota operating 43.2 million acres of land for harvesting of

crops such as corn, wheat, soybeans, and sunflower[16]. Once the presence of Japanese knotweed in the state and available acres for farm operation was confirmed, South Dakota remained a suitable option for domestic production of Japanese knotweed.

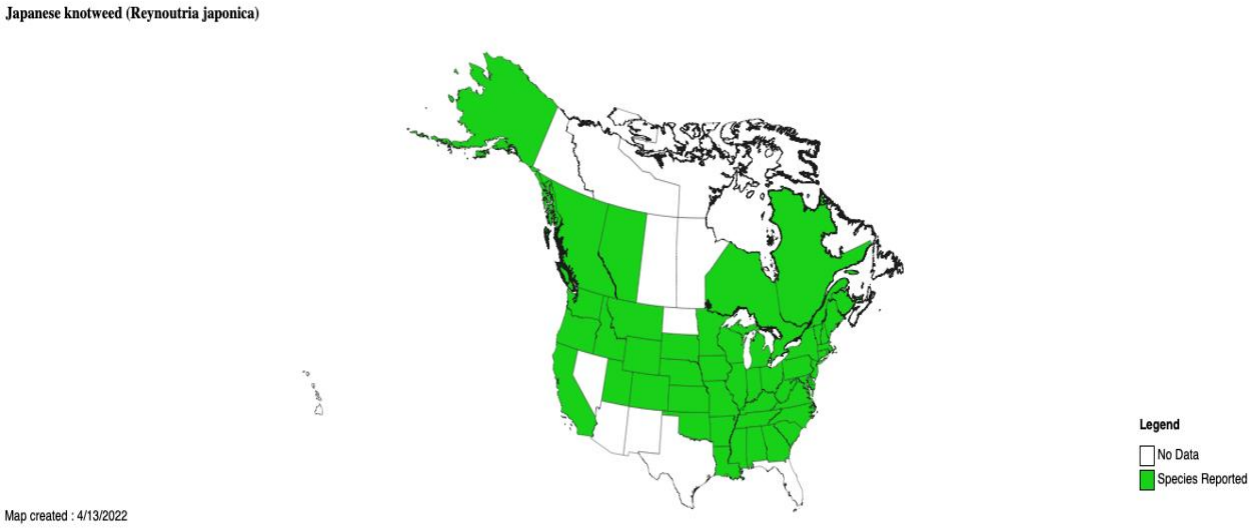

Figure 2.1: A map detailing which states report cases of Japanese knotweed present in the United States and southern Canadian border[6]

As mentioned above, certain assumptions were made during the design of the upstream production process model. The first assumption made was the concentration of free resveratrol (resveratrol without the glucoside) present in Japanese knotweed rhizomes cultivated within North America. While the growth of Japanese knotweed in North America has been previously reported in scientific literature[17], the concentration of resveratrol and its glucoside, polydatin, are seen to differ between samples, ranging between two to three orders of magnitude. Further analysis demonstrated the impacts of seasonal variations[17], available nitrogen in the soil[7], and other environmental factors such as the presence of insects and fungus[18] on the concentration of stilbenes present in Japanese knotweed plants. A variety of sources, shown in **Table 2.1**, describe an average free resveratrol concentration near 1.4 mg/g FW knotweed rhizome. When data on the total resveratrol, both polydatin and resveratrol, concentration were analyzed (**Table 2.2**), the total

resveratrol concentrations in knotweed was determined to reach an average of 9.8 mg/g FW knotweed rhizome, 7-fold higher than for just free resveratrol. Using this information, the natural field grown Japanese knotweed rhizomes were assumed to contain free resveratrol and polydatin at a 1:3 ratio, specifically in concentrations of 0.5 mg/g FW and 1.5 mg/g FW, respectively. These concentrations and ratios fall towards the conservative range values but still align with the range present in Japanese knotweed.

Table 2.2: Free resveratrol concentrations (mg/g FW) found in knotweed rhizomes listed in literature

#	Plant Family	Natural source	Plant Location	Free Resveratrol content (mg/g)	Source
1	*Polygonaceae*	*Polygonum cuspidatum*	Root	0.50	[19]
2	*Polygonaceae*	*Polygonum cuspidatum*	Root	0.52	[20]
3	*Polygonaceae*	*Polygonum cuspidatum*	Root	2.58	[21]
4	*Polygonaceae*	*Polygonum cuspidatum*	Root	0.67	[18]
5	*Polygonaceae*	*Polygonum cuspidatum*	Root	0.52	[22]
6	*Polygonaceae*	*Polygonum cuspidatum*	Root	3.50	[23]
Average concentration of free resveratrol (mg/g)				1.38	

Table 2.3: Total resveratrol concentrations (mg/g FW) found in knotweed rhizomes listed in literature

#	Plant Family	Natural source	Plant Location	Total Resveratrol content (mg/g)	Source

1	*Polygonaceae*	*Polygonum cuspidatum*	Root	10.39	[24]
3	*Polygonaceae*	*Polygonum cuspidatum*	Root	10.90	[21]
4	*Polygonaceae*	*Polygonum cuspidatum*	Root	8.22	[18]
Average concentration of total resveratrol				9.84	

Another assumption made in the upstream process model was that there are no costs attributed to the biological containment of the knotweed within the field. Notably, it is pertinent to mention here that knotweed has the ability of regenerating itself (germinate an entire plant again) from small pieces of pre-existing rhizomes, as small as half an inch (½ in) in length[25]. Data provided by New Hampshire's Department of Agriculture suggests it contains allelopathic properties causing it to release chemicals to eliminate native vegetation[25]. Reports on Japanese knotweed growth have reported its ability to spread vertically for 10 ft and horizontally about 40 ft[25]. Michigan's Department of Natural Resources published an article mentioning knotweed rhizomes' capability to penetrate depths of 7ft in certain soils (www.michigan.gov). A bulletin written by researchers at Montana State University reported cases of Japanese knotweed creating monotypic stands while disrupting infrastructure like concrete in the process[26]. No preventative measures (i.e., concrete protection wall, slabs, pillars, or synthetic liners) were modeled although these may be necessary to fully contain the Japanese knotweed from spreading outward from the dedicated land for growing it. The model can instead be interpreted to have the fallow land surrounded by a deep and wide trench along its perimeter at minimum additional cost.

As stated above, the practices required for upstream production of naturally grown Japanese knotweed followed potato harvesting practices listed by UC Davis Agriculture and

Resource Economics Center. It is imperative to mention that the data provided on potato cultivation were grown in the intermountain region of California, along the Klamath Basin and not Southwest South Dakota as this model emphasizes. Nevertheless, the practices, equipment, labor costs, and investments are assumed to analogous for such production. The breakdown of the equipment, production practices, and costs are described per practice in **Table 2.4**.

First, the model assumed the acres of land needed for Japanese knotweed production had to undergo some preparation prior to planting. Here, 80% of the fresh acreage is assumed to be chopped using a heavy stubble disc and then any residual crops remaining after the initial cutting is mixed in with the soil using a ring roller. Once the mixing is complete, 50% of the acreage is assumed to undergo deep ripping in efforts to alleviate any harden soil. The Japanese knotweed plants are allowed to grow for two years before pre-harvesting steps are taken. The first pre-harvesting procedure to be taken is the spread of a desiccant over some Japanese knotweed plants. The use of the desiccant is to prevent any further growing of the plant's tops. Notably, this step is a method used for potatoes and may not be suitable for Japanese knotweed production. While this step may not be required, it is applied in efforts to dry out the invasive Japanese knotweed found above ground. Here, the desiccant is only applied to 50% of the acreage using an aircraft. Once the desiccant has been added, the beds and vines of the Japanese knotweed plants are rolled and cut. The next step the model incorporates is the harvesting step. The Japanese knotweeds are dug up, harvested, and field cleaned in one step using a single tractor attached to a power take off (PTO) driven four-row digger. The knotweed rhizomes which are harvested are then assumed to be placed in a 15-ton bottom-conveyor truck designated for transporting the rhizomes to a storage facility. Here, the truck is stationed and moved besides the harvester in the open-field to capture rhizomes as they are harvested. The transportation of the knotweed rhizomes is assumed to only

be a 10-mile roundtrip from the field to storage facility. The transportation costs are shown below in Table 2.4. Once the trucks hauling the knotweed rhizomes arrive at the storage facility, the rhizomes are moved via a conveyor located on the back of the trucks onto a large holding tub where they are washed to remove any soil present. While downstream processing may only require the rhizomes stay in storage for a short time, storage fees were still included within our estimates. The total operational cost for the upstream portion was calculated to be slightly under $1.1 million dollars per year. A breakdown of the economics for each category is as follows. The labor rates used in the model were matched with the values used by the UC Davis Agriculture and Resource Economics Center. Specifically, the wage for a machine operator at $20.00 and $14.00 for general labor, including an overage charge of 37%. These values were understood to be the average industry rate as of January 2015 and were not updated for 2022 values. Within the model, the fuel, lube, and repair cost for each practice was estimated by multiplying the hourly operating cost for each piece of equipment for the selected practice by the hours per acres deemed necessary for potato harvesting. The hours needed per practice, cost of fuel (diesel and gasoline), and repair cost used for this model was retrieved by the provided by UC Davis Agriculture and Resource Economics Center. The value used within their report is described as coming from calculations from the American Society of Agricultural Engineers (ASAE) and data from the Energy Information Administration, Department of Energy. The only material cost set when modeling the upstream portion was the cost of the desiccant. Cost for a desiccant for an acre of knotweed were aligned with the cost per acre to produce potato-chippers. Only two practices incorporated any custom costs, the pre- and post-harvest steps. The cost was attributed to the cost to operate the aircraft to spread desiccant and any cost which may be incurred when storing knotweed rhizomes.

Table 2.4 A list of cultural practices and their cost used to estimate the upstream knotweed rhizome production costs. Current costs values were retrieved using UC Davis Agriculture and Resource Economics Center. Ac=acre

	Labor Cost/ acre	Fuel/ acre	Lube& Repair/ acre	Material Cost/ acre	Custom /Rent per acre	Total Cost/ acre	Total Cost
Pre-planting							
Chop Residue, 80%Ac	$3	$3	$2	$0	$0	$8	$14,779.81
Stubbe Disc & Roll	$3	$6	$3	$0	$0	$12	$22,169.72
Sub-Soil, 50% Ac	$4	$7	$4	$0	$0	$15	$27,712.15
Pre-Harvest							
Desiccant Application/ Air 50%Ac	$0	$0	$0	$18	$10	$28	$51,729.35
Cut Vines/Roll Beds	$4	$4	$1	$0	$0	$9	$16,627.29
Harvest							
Dig/Harvest Knotweed Rhizomes - 4 rows	$16	$30	$22	$0	$0	$68	$125,628.42
Bulk Knotweed Rhizomes	$28	$0	$0	$0	$0	$28	$51,729.35
Haul Knotweed Rhizomes to Storage	$48	$18	$4	$0	$0	$70	$129,323.38
Post-Harvest							
Elevate/Holding Tub/Remove Dirt	$24	$10	$10	$0	$0	$44	$81,288.98
Shed/Store Knotweed Rhizomes	$0	$0	$0	$0	$300	$300	$554,243.04
Total Operating Costs							$1,075,231.50
Cash Overhead							
Field Sanitation						$2	$3,694.95

Field Supervisor Salary						$57	$105,306.18
Land Rent - South Dakota						$29	$107,153.65
Liability Insurance						$1	$1,847.48
Office Expenses						$52	$96,068.79
GPS-AutoTrack Activation Fee						$7	$12,932.34
Property Insurance						$1	$1,847.48
Investment Repairs						$4	$7,389.91
Total Annual Operating Costs						$735	$1,411,472.28

In addition to costs of each practice, a breakdown of the cash overhead was also shown in Table 2.4. Field sanitations described within the table refers to any sanitation services provided to laborers in the fields, such as portable bathrooms and hand washing areas. A single field supervisor is assumed to be managing the operations within the model. The wage was set to $57 per acre. Land rent values for unirrigated land in South Dakota were used as mentioned above. Liability insurance, the standard policy which is designated to help manage any expenses which may arise if an individual may sustain any bodily injuries while on the property, was set to $1 per acre. Notably, crop insurance is also an additional standard insurance provided to open-field growers which may provide coverage in the case of an unavoidable loss of crops. No crop insurance was estimated or used within the modeling of the upstream production of knotweed rhizomes. The next expense is attributed to any office expenses. Here, office expenses refer to any office supplies, telephones, road maintenance, booking and accounting and legal fees which may be incurred during production. The value was also aligned with the values listed by the UC Davis Agriculture and Resource Economics Center. Property insurance is an additional expense which was included

in the cash overhead cost. Simply, property insurance accounts for any property loss and is charged at $1 per acre. The last expense is equipment investment repairs. Here, the repairs cost is associated with the annual preventative maintenance, set to $4 an acre. Once the cash overhead cost was calculated, the total annual operating cost or OPEX for the upstream production was estimated to be $1.4 million. Using the OPEX value calculated and the mass (FW) of knotweed rhizomes required each year for 100 MT production of resveratrol, the cost of knotweed rhizome is determined to be $0.19 per kg.

To give a better assessment of the CAPEX required for the upstream production, farm equipment cost was also calculated alongside the annual operating cost. **Table 2.5** list the equipment deemed necessary for harvesting Japanese knotweed rhizomes. Values for the equipment cost were again retrieved from the UC Davis Agriculture and Resource Economics Center. Since the report published by the UC Davis Agriculture and Resource Economics Center was released in 2015, an inflation/cost adjustment of 1.125 was used to estimate the cost of the same farming equipment in 2021. Here, the total farming equipment cost was multiplied by a factor of 0.6 since some equipment purchased may be used rather than new equipment. Additionally, it is assumed that the equipment requirements scaled with cultivation acreage, thus estimating the number of each equipment required was multiplied by 7.4 to account for the larger cultivation acreage than use in the report. Using all this information, the total cost of equipment was to be $3.6 million.

Table 2.5 A list of equipment and their purchase price and total knotweed production cost.

Knotweed Farm Equipment Costs				
Description	**2015 Price**	**2021 Price**	**Number Required**	**Total Cost**
125 HP 4WD Tractor	$115,412	$129,839		
Flail Mower 15'	$13,203	$14,853		
225 HP 4WD Tractor	$245,388	$276,062		
Stubble Disc 16'	$45,000	$50,625		

Ring Roller 16'	$18,000	$20,250		
Subsoiler 16' 9 Shank	$42,400	$47,700		
Cultivator Sled 5 row	$11,000	$12,375		
Potato Digger-Harvester 4 row	$120,000	$135,000		
Potato Truck 15 Tons	$20,000	$22,500		
Elevator	$55,000	$61,875		
Holding Tub	$70,000	$78,750		
Total	$755,403	$849,828	7.388	**$3,569,279**

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Chapter 3 : Recovery and Purification of Resveratrol from Japanese Knotweed Rhizomes

3.1 Introduction

The utilization of natural plants as a source for chemical compounds is an ever-growing field. To date, there have been a variety of compounds which have been extracted from plants, including but not limited to, tetrahydrocannabinol from hemp (*Cannabis sativa L.*)[1], phenolic acids from purple corn (*Zea Mays L.*)[2], and flavonoids from chamomile (*Matricaria chamomilla*) flowers[2]. Although natural plants offer a reduction of complexity in upstream bioprocessing in comparison to using genetically engineered microbes, the downstream and purification methods remain just as rigorous. A standardized process for the extraction of resveratrol from Japanese knotweed remains unestablished as novel technologies and methods are continuously being developed and researched. The most common unit operations seen utilized in current patent and scientific journal articles are shown in **Figure 3.1** as a block flow diagram.

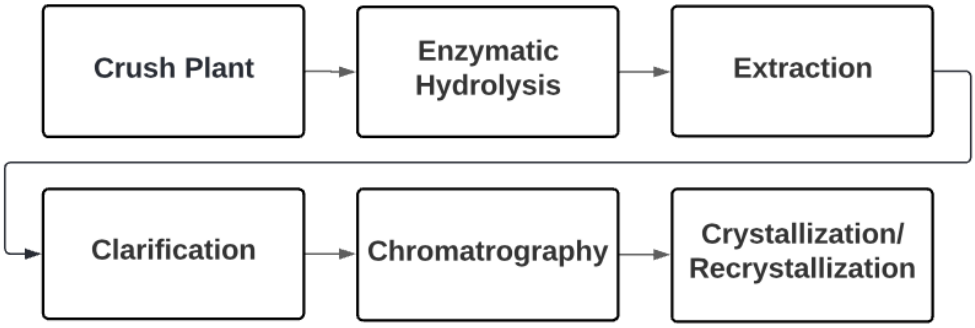


Figure 3.1: A block flow diagram demonstrating the overall plant process for purifying resveratrol from Japanese knotweed

The procedure needed for purification of resveratrol is understood, however, there is no consensus on what the best methods to use are since there remain numerous options for each step, each consisting with their own advantages and disadvantages. Notably, a crucial step during the downstream process is the deglycosylation and hydrolysis of polydatin performed after crushing and shredding the rhizome. Two methods can be employed to hydrolyze the polydatin compound

found beside resveratrol, either using strong acids or enzymes[3]. Performing either method has demonstrated conversion yields of polydatin to resveratrol above 90% [4,5]. However, the use of acid hydrolysis may produce additional adducts in the reaction, thus decreasing the content of resveratrol present and prompting concerns for additional purification procedures. Furthermore, acid hydrolysis has been reported to require harsh processing conditions, often causing pollution as a result[5]. Nevertheless, the large quantity and high cost of industrial cellulase enzymes ($0.68 – $1.47 per gal) serves as the biggest deterrent to the latter approach[6].

Currently, there exists numerous published techno-economic analysis studies of plant-based production focusing on biofuels[7], recombinant therapeutic proteins[7–9], industrial enzymes[10], and antimicrobial proteins for food safety[11]. Here, this chapter will describe the techno-economic analysis performed on the downstream processing required for the plant-based production of Japanese knotweed for the extraction of the biopolymer precursor, resveratrol, which has not been demonstrated before. This study will aim to establish a framework for more informed decisions on the development of a domestic production route for such polymer precursors.

3.2 Process Design Parameters and Model Description

The simulation model and economic analysis was performed using a computer-aided process modeling and design software, SuperPro Designer® ("SuperPro") Version 12, Build 3 Special Build 1600, (Intelligen, Inc., Scotch Plains, New Jersey, USA; http://www.intelligen.com). SuperPro was used to determine equipment sizing, specify equipment process parameters, and determine operations scheduling and raw material requirements. The economic analysis was used to determine the total capital expenditure (CAPEX, $), the total annual operating expenditure (OPEX, $/year), and the cost of goods sold (COGS, $/kg resveratrol). A further detailed analysis provides a breakdown of all costs, e.g., raw materials, consumables, utilities, labor, and waste

disposal- with the goal being to identify the materials and process steps which contribute most significantly to the total cost of production. Pricing for raw materials, consumables, and equipment within the model were calculated using publicly available commercial prices, unpublished personal communications with manufacturers of large-scale bioprocessing equipment, previous SuperPro design files, and in some cases, SuperPro default values. A free trial version of SuperPro found here: https://www.intelligen.com/thankyou-downloads/ can be used to view the model simulation.

The base case model was developed to process 100 metric tons (MT) of resveratrol per year, with the facility operating 330 days a year. A single batch duration is estimated to be 45.6 hours, totaling 1,295 batches per year with a cycle time (the amount of time between consecutive batches) of about 6 hours. Due to the short cycle time relative to the annual operating time, the process may be assumed to be operating under pseudo-continuous conditions. To attain the quantity of 100 MT of resveratrol, roughly 7.3 million kg of knotweed rhizomes a year are required for downstream processing. The model was scaled using publicly available patent literature, scientific journal articles and working process knowledge. Bioprocessing operations and conditions (i.e., percent recovery, ethanol concentrations, type of resins used) for certain unit operations within the simulation were adopted from scientific literature focusing on resveratrol production from various plant sources (i.e., knotweed, peanuts, grapes, pomace, etc.). **Table 3.1** and **Table 3.2** list the various processing techniques utilized for resveratrol purification from Japanese knotweed in patents and scientific journals, respectively.

Table 3.1: Publicly available patents focused on the production of resveratrol from Japanese knotweed

Process	Company/Institution publisher	Patent Code
Hydrolysis		

Acid	-	CN1321961C
Acid	Anhui Agricultural University AHAU	CN101519343B
Enzyme	Yangxian County Qin Long Pharmaceutical Co Ltd	CN107721825A
Enzyme	Yangxian Qinlong Pharmaceutical Co Ltd	CN107721825B
Enzyme	Anhui Lonking Biotechnology Co Ltd	CN107353183A
Extraction		
UAE	Yangxian Qinlong Pharmaceutical Co Ltd	CN107721825B
UAE	Anhui Lonking Biotechnology Co Ltd	CN107353183A
UAE	Nanjing Biaoke Biotechnology Co Ltd	CN104263763A
UAE	-	WO 2008/092221
Microwave	Liaoning University of Traditional Chinese Medicine	CN104800299A
Microwave	-	CN103641691A
Purification		
Macroporous Resins	-	CN1321961C
Macroporous Resins	Nanjing Biaoke Biotechnology Co Ltd	CN104263763A
Macroporous Resins	Inner Mongolia Changhui Biological Technology Co, Ltd	CN104341277B
Macroporous Resins	Hubei Zhongxin Biotech Co Ltd	CN107162888A
Alumina Resin	Nanjing Zelang Medical Technology Co Ltd	CN101760483A
Alumina Resin + Silica gel	Hunan Huacheng Biotech Inc	CN101338327A
Silica gel	-	CN107721825A
Silica gel	Enshi Qingjiang Bioengineering Co Ltd	CN102925497A

Dextran-based resin	Sanyuan Runhe Biological Technology Co. Ltd.	CN101698634B

Table 3.2: Different operations employed to produce Rsv from Japanese knotweed

Operations	Source	Doi/Link
Hydrolysis		
Enzymatic	Chen et al. (2014)	10.1007/s00449-013-1113-1
	Wang et al. (2007)	10.1007/s00253-007-0874-3
	Kuo et al. (2015)	10.3390/catal6030032
	Wang et al. (2019)	10.3389/fmicb.2019.00445
Acid	Wang et al. (2012)	10.1016/j.jpha.2012.12.001
Extraction		
Ultrasonic Assisted	Zhou et al. (2019)	10.1016/j.fbio.2019.100442
	Kuo et al. (2013)	10.3390/molecules19010067
	Chukwumah et al. (2008)	10.1016/j.ultsonch.2008.07.007
	Lin et al. (2016)	10.1016/j.ultsonch.2016.03.018
	Chen et al. (2012)	10.1021/np300392n
Supercritical CO_2	Wenli et al. (2005)	10.1002/jsfa.2007
	B. Beňová et al. (2010)	10.1016/j.supflu.2009.10.009
Maceration	Gambuti et al. (2012)	10.1021/jf0354895
Soxhlet	Liu et al. (2018)	10.3389/fphar.2018.00347
Enzyme Assisted	Wang et al. (2019)	10.3389/fmicb.2019.00445

Purification		
Macroporous resin column chromatography	Jia et al. (2020)	10.1080/01496395.2019.1604755
Reverse-Phase High-Performance Liquid Chromatography	Omar et al. (2014)	10.1021/jf5001277
High-speed counter-current chromatography	Chen et al. (2001)	10.1016/s0021-9673(00)00960-2
	Chu et al. (2005)	10.1016/j.chroma.2005.08.008
Silica Gel Column Chromatography	Vastano et al. (2000)	10.1021/jf9909196
Adsorption Chromatography	Gu et al. (2006)	10.1365/s10337-006-0081-x
Macroporous resin adsorption and reversed phase liquid chromatography	Zhang et al. (2008)	10.1016/j.seppur.2008.12.013

In the design of the downstream production process model, certain key bioprocessing parameters were extrapolated from publicly available information on resveratrol production. In particular, the quantity of enzymes per batch required to hydrolyze polydatin to resveratrol was tuned to match production methods found in patents. Patent literature detailing the extraction of resveratrol have suggested amounts of 2 – 4 weight % of enzymes per knotweed per batch should hydrolyze polydatin to resveratrol effectively for processing[12]. However, scientific literature focused on optimizing the enzymatic hydrolysis process for resveratrol in knotweed have reported using enzyme concentrations closer to 10% of the total processed knotweed[13]. An average of these

values resulted in 6.5 weight % of enzymes per the total knotweed rhizomes per batch; this percentage was used in the process simulation.

Another parameter which was adjusted in the model to match resveratrol production methods described in patents was the percent recovery of resveratrol after undergoing the extraction process. Multiple sources have denoted the use of the ultrasonic technology and Ultrasonic Assisted Extractions (UAEs) to remove resveratrol from knotweed rhizomes but fall short by failing to provide key parameters such as percent recovery[12,14,15]. UAEs are becoming a common operation for extracting polyphenols from plant biomass[16]. Its application has already been utilized to extract resveratrol from other plant sources such as grape stems[17] and grape leaves[18]. These two studies demonstrate the capability of UAE technology in extraction of resveratrol while noting a percent recovery of 78.8% and 80%, respectively. A conservative approach was taken, and the former of these values was used to define the resveratrol recovery in the UAE operation used in the process model. In an effort to accurately model the UAE operation in SuperPro, the other parameters associated with UAE such as power, temperature, and duration of agitation were aligned to Japanese knotweed roots processing conditions [19].

Traditional methods of purification involve using silica gel resins in chromatography columns[20,21]. However, silica gel resins are predominantly used for smaller scale production[22] and there is often complication scaling up for large scale manufacturing[23]. More recently, macroporous resins have been utilized to serve as a replacement as they hold several advantages over their silica gel counterparts[12,24]. First, silica gel resins utilize mixtures of acids, such as chloroform and methyl alcohol, to serve as their eluents[22], whereas macroporous resins only require mixtures of ethanol and water[12]. Additionally, the cost of using silica gel resins is higher compared to using macroporous resins while the recovery of using silica gel remains lower[25]. In attempt to accurately

model an industrial chromatography unit in SuperPro, an unpressurized vessel filled with macroporous resin (NKA-II) was initialized to operate as an adsorption mixing tank. The resin is exchanged with fresh resin every 100 batches. All process flow specifications and assumptions used in the development of the downstream facility model is shown in **Table 3.3** along with its source.

Table 3.3: A list of process assumptions used to define certain parameters within the model

Downstream Processing			
Enzyme Hydrolysis			
Bioprocess parameter	**Information used**	**Source value**	**Link**
Type of enzymes	β-glucosidase, cellulase	1) Cellulase, β-glucosidase 2) β-glucosidase, beta glucan enzyme cellulase and hemicellulose, zymase	1) CN101698634B 2) CN101338327A
Cost of enzymes	$0.85 per gal	$0.68 – $1.47 per gal	Klein-Marcuschamer et al. (2011)
Ratio of enzymes used (w/w FW of knotweed)	80% for cellulase and 20% for β-glucosidase	80% cellulase and 20% polygalacturonate	CN104263763A
Amount of enzymes used	6.5% of knotweed used per batch	1) 2 - 4 % FW knotweed 2) 10% of dissolved Crude powder	1) CN104263763A 2) M. Chen et al. (2014)
Buffer	Citric acid	Citric acid	CN101338327A

pH	5.0	4.0 - 5.0	CN101338327A
Reaction temperature	55.0	45 - 55	CN104263763A
Temperature for enzyme deactivation	85℃	85℃	CN101338327A
Percent conversion of polydatin to resveratrol	90	90-100%	Wang et al. (2007)
Ultrasonic Assisted Extraction			
Bioprocess parameter	**Information used**	**Source value(s)**	**Link**
Substrate used	Knotweed Rhizomes	1) Grape Stems 2) Knotweed Rhizomes	Piñeiro et al. (2013)
Solvent used	Ethanol/water 80:20% EtOH (v/v)	1) Ethanol/water 80:20-90:10% (v/v) 2) Ethanol/water 60:40-80:20% (v/v)	1) CN104263763A 2) Piñeiro et al. (2013)
Power	150 W	1) 150 – 250 W 2) 150 W	1) CN104263763A 2) B. Y. Chen et al., (2012)
Agitation Duration	60 mins	1) 60 - 90 mins 2) 20 - 60 mins	1) CN104263763A 2) B. Y. Chen et al., (2012)
Temperature	55℃	55℃	Lin et al. (2016)
Frequency	Not Applicable	40 kHz	Lin et al. (2016)
Percent Recovery	78.80%	78.80 - 96.70%	Piñeiro et al. (2013)
Filtration			
Bioprocess parameter	**Information used**	**Source value**	**Link**

Belt Press Filter	95%	95%	Working Process Knowledge
Plate and Frame Filter Percent Recovery	95%	95%	Internal SuperPro Model and working process knowledge
Chromatography			
Bioprocess parameter	**Information used**	**Source value**	**Link**
Type of adsorption vessel	Batch macroporous adsorption vessel with resin flowing in liquid suspension	1-2) Macroporous resin column 3) Advance alumina 4-5) Silica gel	1) CN101698634B 2) CN104263763A 3) CN101338327A 4) Kato et al. (2009) 5) Tang et al. (2011)
Type of Resin used	NKA II	ADS-5 resin	Xiong et al. (2014)
Substrate Used	Japanese knotweed rhizomes	Extract of peanut sprouts (*Arachis hypogea*)	Xiong et al. (2014)
Washing buffer	Water	1-2) Water 3) Chloroform:methanol 10:1 (v/v)	1) CN101338327A 2) CN104263763A 3) Tang et al. (2011)
Elution Buffer	Ethanol/water 70:30% EtOH:water (v/v)	1-3) 60-90% EtOH/water	1) Xiong et al. (2014) 2) Sun et al. (2018) 3) Tian et al. (2008)
Amount of Elution buffer used	2 BV	2-3 BV	Extrepure Resin (Shanghai) Co., Ltd. specifications (NKA-II Supplier)

Percent Recovery	87%	88.33%	Xiong et al. (2014)
Crystallization			
Bioprocess parameter	**Information used**	**Source value**	**Link**
Pressure	.780 atm	Reduced pressure	1) CN101698634B 2) Zhang et al. (2009)
Solvent used	Ethanol	Methanol	CN101338327A
Resuspension unit	Mixing stream	1) Rotary vacuum evaporator 2) Centrifuge or plate and frame filter	1) Zhang et al. (2009) 2) CN101698634B
Recrystallization step needed?	Yes	1-4) Yes	1) CN101338327A 2) CN101698634B 3) CN104263763A 4) Zhang et al. (2009)
Crystallization Temperature	60 ℃	60 ℃	Zhang et al. (2009)
Percent Recovery	91.20%	91.20%	Zhang et al. (2009)

A detailed downstream process flow sheet depicting the purification of resveratrol from Japanese knotweed is shown in **Figure 3.2**. Each batch begins with the harvested knotweed rhizomes being transported from a designated storage warehouse (BGBX-103) to a silo bin (SL-101) using a conveyor belt (BC-101). Approximately 5,635 kg of knotweed rhizomes are retrieved from the silo bin and transferred to a washer (WSH-101) where they are washed with water at a 1:1 w/w ratio. Next, the knotweed rhizomes are mixed with water (MX-103) at a 1:1 w/w ratio and

are grounded into a slurry solution using an industrial grinder (GR-101) operating at a throughput of 11,327 kg/hr. The slurry is pumped down the process line where 100 kg of citric acid is mixed (MX-104) with the solution to adjust the pH value down to 5.0. Following the pH adjustment step, a stream of enzymes (366 kg, or 6.5 weight % of knotweed rhizomes required per batch) consisting of cellulase and ß-glucosidase at an 80:20 ratio enters the process line where it is mixed (MX-104) with the solution before being pumped into a batch vessel reactor (R-101). Within the reactor (13,382 L), the solution is agitated for fifteen minutes and heated to 55 ℃ to allow the enzymatic deglycosylation and hydrolysis of polydatin to occur, converting 90% of existing polydatin to resveratrol. To deactivate the enzymes and halt the reaction, the reactor is heated to 85 ℃ using steam. Once the deactivation step is complete, the slurry is transferred to the ten ultrasonic assisted extractor units running in parallel (BGBX-102, 1300 L). The ultrasonic assisted extraction units are first charged with ethanol (80% w/w) at a 1:1 mass ratio with the plant biomass where it is then agitated for sixty minutes, allowing for efficient polyphenol extraction from the plant biomass (78.8% recovery). The slurry solution is then sent to a belt press filtration unit (BF-101) where the plant biomass can be separated from the liquid solution and disposed of properly (95%, recovery, although this biomass could potentially be used for energy generation through anerobic digestion and/or as a fertilizer). An additional separation unit in the form of a plate and frame filter (PFF-101, 54.5 m^2) is used to capture and separate any residual plant biomass within the solution before being sent for further processing. The filtrate is then pumped into a batch adsorption vessel (V-101, 5815L) containing NKA-II, a macroporous adsorption resin. The solution is then agitated with the resin for one hour to allow the binding of resveratrol. After the binding step occurs, the resin is first rinsed with water (1000 L) to remove any impurities which may have been captured. Following the washing step, the adsorption vessel is set to charge in a stream of ethanol (1,800L,

70% w/w) to elute the resveratrol from the macroporous resin (87% recovery). After the elution step, the eluate is then pumped into a crystallization column (CR-101, 263L) for further processing. The crystallizer operates under reduced pressure, an evaporation temperature of 98 ℃, and a crystallization temperature of 60 ℃, removing most of the residual ethanol and water present from before and yielding solids crystals (91.2% recovery). These crystals undergo a mixing step (MX-101) where they are resuspended in ethanol (100L, 95% w/w). This liquid-solid mixture is then sent to another crystallizer unit (CR-102, 105 L) operating under similar conditions as before, but this time yielding 77.3 kg of resveratrol at 99% purity. A Gantt chart detailing the equipment occupancy for a single batch and multiple batches can be seen in **Figure 3.3**.

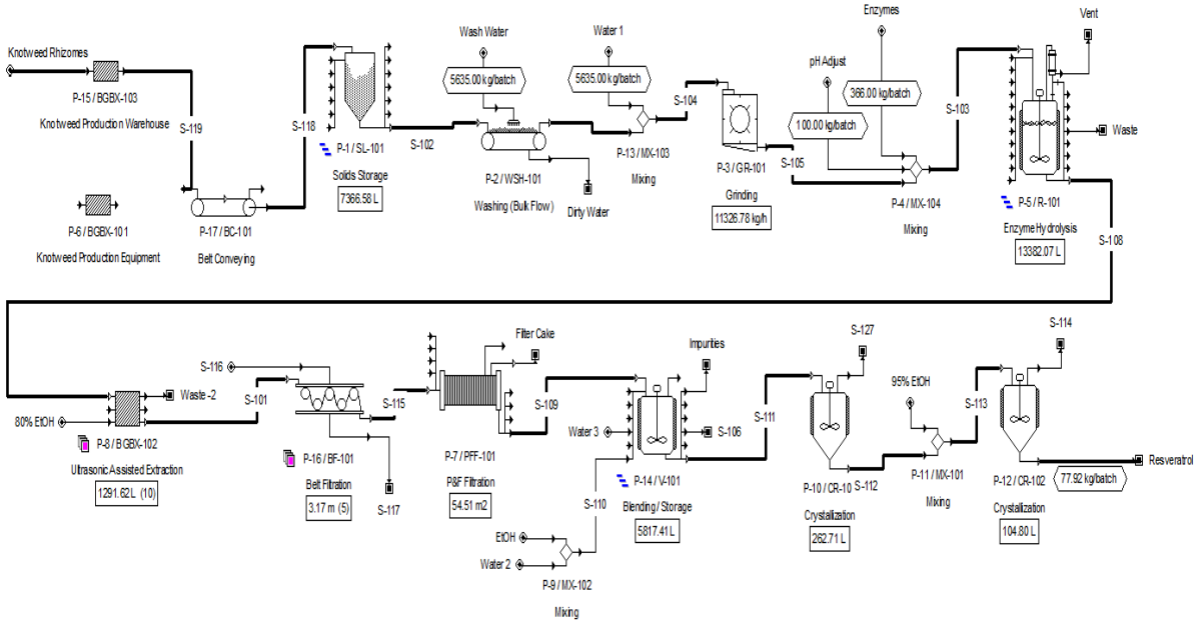
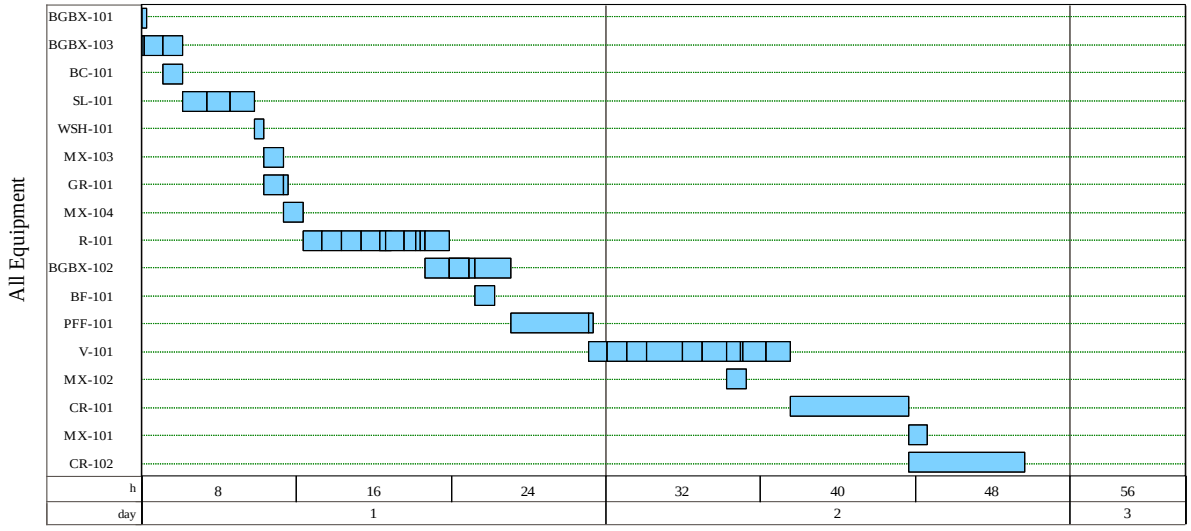


Figure 3.2 SuperPro Designer model flow sheet of the downstream facility

a



b

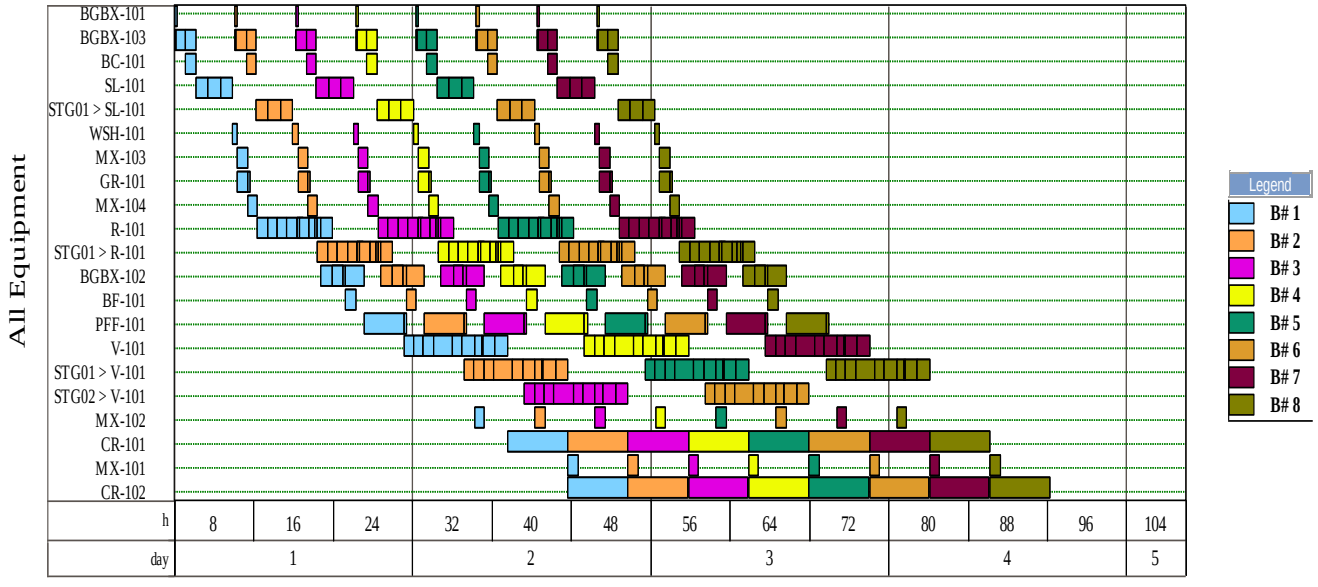


Figure 3.3: Equipment occupancy chart demonstrating the utilization of the equipment in **a)** a single batch **b)** 8 concurrent batches

3.3 Economic Analysis

A summary of the economic analysis results from the base case model set to produce 100 MT resveratrol is presented in **Table 3.4**. The CAPEX is the sum of the direct fixed capital (DFC) (the fixed assets of the total project investment), working capital (the funds required to be available when first operating the process), and startup and validation cost (a one-time expenditure incurred to prepare the process for operation). The DFC for the facility was calculated by using a distributed set of purchase cost factors shown in **Table 3.5** to estimate the facility direct costs (DC), indirect costs (IC), and other costs (OC). Here, the purchase cost (PC) is the sum of all major equipment purchase costs shown in the SP flowsheet and the unlisted equipment purchase cost (UEPC) is assumed to be 20% of the PC. A list of all the major equipment, their composition, and purchase prices can be found in **Table 3.6**. The price of unlisted equipment was also included in Table 6 for reference. The working capital was estimated to two percent of the total DFC. The startup and validation cost combined were estimated to be about 1% of the DFC. Most of the equipment purchase costs were estimated using SuperPro default values. The composition of a majority the equipment (i.e., vessels, filters, and crystallizers) are carbon steel (CS) except for the UAE, which is composed of Stainless steel 316L and the silo bin which is composed of concrete. The justification for using equipment fabricated using carbon steel instead of more costly stainless steel is recognizing that the process is being designed for polymer applications and not for a more regulated pharmaceutical application. The total cost of the all the UAEs, depicted by the Batch Generic Box (BGBX-102) in SuperPro, was the most expensive ($3.3 million), followed by the belt press filters ($1.7 million). For better cost estimates of the UAEs used in the model, a quote was obtained from an industrial manufacturer named REUS® (etsreus.com, France). Pricing for

this equipment was reduced by about 5%, assuming a conversative approach on the expected discount for bulk purchase of equipment (i.e., the 10 used within the model).

Table 3.4 Capital expenditure (CAPEX), operating expenditure (OPEX), and cost of goods sold (COGS) to produce resveratrol from Japanese knotweed

CAPEX	$44.7 million
OPEX	$15.0 million per year
COGS	$150 per kg
OPEX without depreciation	$11.1 million per year
COGS without depreciation	$111 per kg
OPEX without depreciation, insurance, factory expenses or local taxes	$7.94 million per year
COGS without depreciation, insurance, factory expenses or local taxes	$79.4 per kg
Direct Fixed Cost	$43.6 million
Equipment Purchase Cost	$13.6 million
Working Capital	$665 thousand
Start-up and Validation Cost	$401 thousand

The OPEX, otherwise referred to as annual operating cost, and COGS are shown with and without depreciation, insurance, and local taxes. The annual operating cost and COGS without including depreciation are more representative of the expected cost for production since depreciation is typically spread over years to effort to expense cost over time while simultaneously lowering the value of the asset (Investopedia.com/depreciation). Also depreciation is not a cash outlay so does not have a negative impact on profitability (in fact has a positive impact since it reduces taxes). Here, depreciation was calculated using the straight-line method with a depreciation period of 10 years and salvage value of 5% of the DFC. Insurance was estimated to be 1% of the DFC and local taxes were estimated to be 2% of the DFC. As expected, evaluation

of the facility's annual operating costs and COGS including depreciation, insurance and local taxes are higher compared to without.

Table 3.5: Total fixed capital cost factors based on the purchase cost (PC) of major equipment items shown on the SP process flowsheet as well as Unlisted Equipment Purchase Costs (assumed to be 20% of the PC). Item specific equipment installation costs are not shown.

Direct Cost (DC)	
Piping	0.25 x PC
Instrumentation	0.15 x PC
Insulation	0.03 x PC
Electrical Facilities	0.1 x PC
Building	0.1 x PC
Yard Improvement	0.15 PC
Auxiliary Facilities	0.1 x PC
UE Installation	0.5 x UEPC
Indirect Cost (IC) Multiplicative Factor	
Engineering	0.25 x DC
Construction	0.35 x DC
Other Cost (OC)	
Contractors Fee	0.05 x (DC + IC)
Contingency	0.10 x (DC + IC)
Lang Factor	2.63

Table 3.6: A list of the equipment used in unit operations used specifically for downstream processing, their size or processing capacity, material of construction, and purchase cost per unit. The cost of the warehouse to store knotweed roots is shown as a generic box.

Name	**Type**	**Number of Unit Operations**	**Standby/ Staggered**	**Size (Capacity)**	**Units**	**Material of Construction**	**Purchase Cost ($/Unit)**
BGBX-103 : P-15	Generic Box	1	0/0	-	1	N/A	500,000
BC-101: P-17	Belt Conveyor	1	0/0	5,631	kg/h	Carbon Steel	278,000
SL-101 : P-1	Silo/Bin	1	0/1	7360.9	L	Concrete	146,000

WSH-101 : P-2	Washer	1	0/0	11262.2	kg/h	Carbon Steel	0
MX-103 : P-13	Mixer	1	0/0	11318.4	kg/h	N/A	0
GR-101 : P-3	Grinder	1	0/0	11318.4	kg/h	Carbon Steel	124,000
MX-101 : P-4	Mixer	1	0/0	11784.4	kg/h	N/A	0
R-101 : P-5	Stirred Reactor	1	0/1	13621.43	L	Carbon Steel	303,000
BGBX-102 : P-8	Generic Box	10	0/0	1290.77	L	Stainless steel 316L	330,000
PFF-101 : P-7	Plate & Frame Filter	3	0/0	54.48	m^2	Carbon Steel	187,000
MX-102 : P-9	Mixer	1	0/0	1508.57	kg/h	N/A	0
V-101 : P-14	Blending Tank	1	0/0	5814.23	L	Carbon Steel	97,000
CR-101 : P-10	Crystallizer	1	0/0	262.69	L	Carbon Steel	459,000
Mx-101 : P-11	Mixer	1	0/0	164.4	kg/h	Carbon Steel	0
CR-102 : P-12	Crystallizer	1	0/0	104.77	L	Carbon Steel	425,000
Unlisted Equipment							2,004,000
Total Cost							13,590,000

An analysis was performed to investigate the effect of factors such as depreciation, insurance, factory expenses and local taxes on the economics of the model. A breakdown of the annual operating for each case are shown in **Figure 3.4 through 3.6**. In all cases, the annual operating costs are composed of the following: raw materials, labor dependence, facility dependence, Laboratory (Lab), Quality Assurance (QA), Quality Control (QC), consumables, waste treatment and utilities. When the first of the three cases were analyzed, it was determined

that the largest contributor to the annual operating costs was the facility dependent cost. Here, facility dependent costs include maintenance, depreciation, insurance, taxes, and factory expenses. Maintenance of each equipment was determined using equipment-specific multipliers, default values provided by SuperPro. No pre-existing depreciation of equipment was assumed in the model. Local taxes were assumed to be 2% of the DFC, in alignment with values from municipal tax charts for South Dakota (South Dakota Department of Revenue). Percentages for insurance and factory expenses were estimated using a previous SuperPro model performed internally which collaborated with a plant based biomanufacturing facility for pricing. The second largest contributor to the annual operating cost was the raw materials. A breakdown of the raw materials and their costs can be seen in **Figure 3.7**. Here, raw material costs amounted to a value of $5.7 million per year. A list of the raw materials used, the quantity used annually, and their total cost can be seen in **Table 3.7**. Ethanol, or ethyl alcohol as defined in SuperPro, had the biggest yearly expense out of all the raw materials, totaling over $4.1 million. The model estimates 6.1 million kg of ethanol needed to produce 100 MT of resveratrol. It was determined that the cost of ethanol is over two thirds of the total raw material cost, accounting for 71% of the raw material cost. The price used in the model was $2.00 per gallon of ethanol. This value was retrieved using data by Market Insider which tracks the price of commodities like ethanol daily (Ethanol Indicator). For the second case, which only excludes depreciation as a factor contributing to facility dependent costs, the largest contributor was raw materials. Raw materials costs are the same in all three cases but now account for 50.9% of the annual operating cost, with the cost of ethanol remaining the largest contributor. The second largest raw material cost is the knotweed rhizomes used in the process. Roughly 7.3 million kg of knotweed rhizomes are needed in the process simulation to produce 100 MT of resveratrol. The cost for producing a kg of knotweed rhizomes was estimated

to be about $0.19, totaling $1.4 million or 24.3% of the raw materials cost. Estimates for a kg of knotweed were calculated to include costs associated with pre-planting, harvesting, post-harvesting, farm equipment operating costs, and cash overhead (i.e., repairs, office expenses, land rent in South Dakota, etc.). A breakdown of these calculations is shown in Chapter 2. Following the second case, which provides a clearer estimate of annual operating cost because depreciation is not included, labor was the third highest contributor to the annual operating cost.

Table 3.7: A list of the raw materials, their purchase cost, the annual amount used, and total annual cost for each raw material

Bulk Material	Unit Cost ($)	Annual Amount	Unit	Annual Cost ($)	% Of Total Cost
Acid Rinse	0.02	1,756,300	kg	29,815.09	0.53
B-Gal	0.00	97,050	kg	0	0.00
Caustic Water	0.01	1,757,173	kg	18,936.13	0.33
Cellulase	0.19	379,176	kg	71,436	1.26
Citric Acid	0.90	129,500	kg	116,550	2.05
Ethyl Alcohol	0.75	6,115,296	kg	4,036,095.52	71.09
Japanese knotweed Rhizomes (*Polygonum cuspidatum*)	0.19	7,292,249	kg	1,413,237	24.27
Water	0.001	26,789,873	kg	26,682.71	.47
TOTAL	**-**	**-**		**5,677,750**	**100.00**

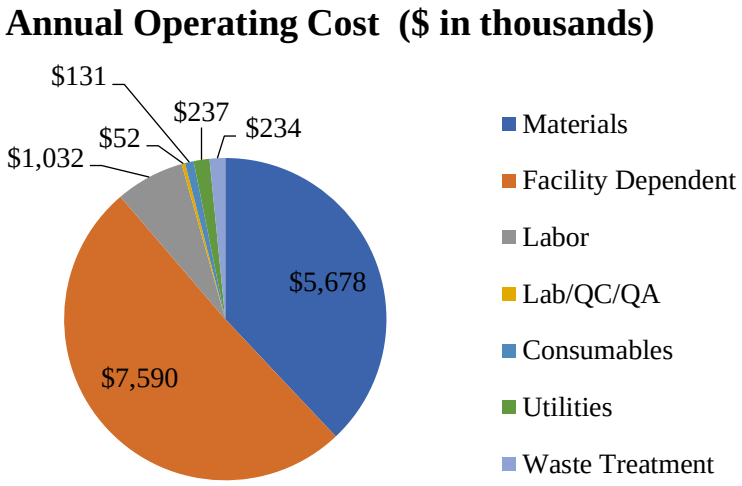


Figure 3.4: Annual operating cost including facility dependent cost consisting of maintenance, depreciation, insurance, taxes, and factory expenses

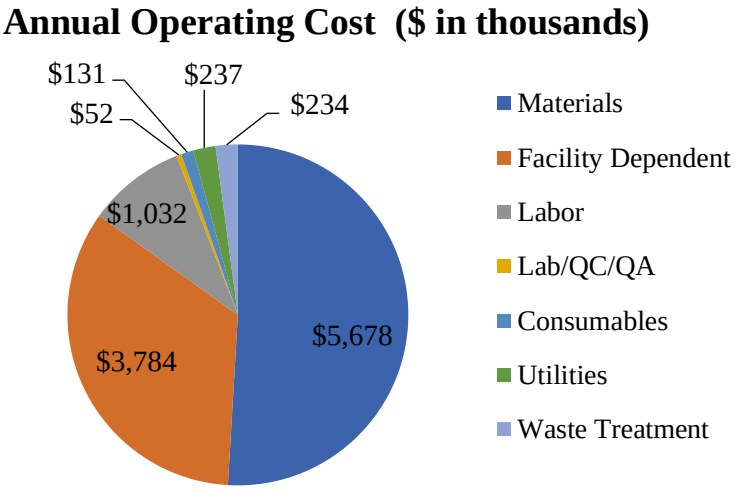


Figure 3.5: Annual operating cost including facility dependent costs consisting only of maintenance, insurance, taxes, and factory expenses related costs (i.e., excluding depreciation)

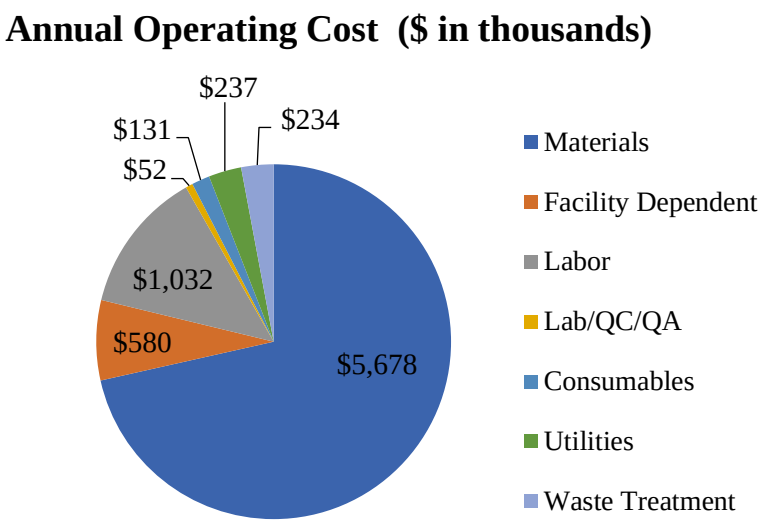


Figure 3.6: Annual operating cost including facility dependent costs consisting only of maintenance related costs

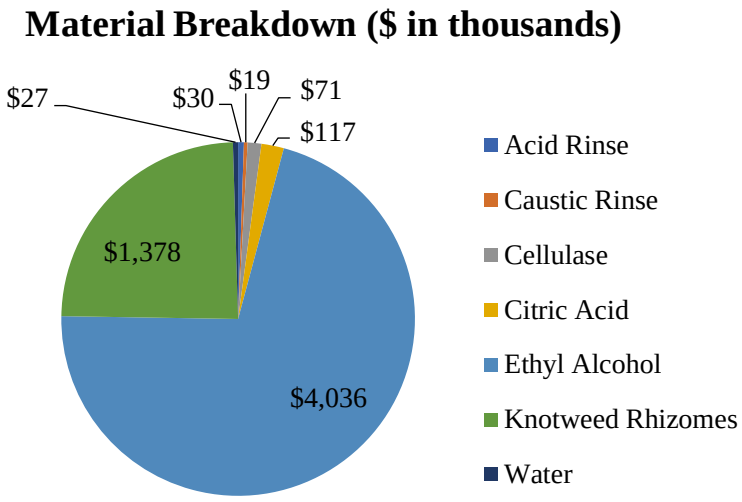


Figure 3.7: Cost of material in thousands of dollars per year

Here, labor dependent costs refer to the costs associated with labor hours required to effectively operate the downstream processing section. Labor cost for the upstream production of Japanese knotweed rhizome was already included in the overall unit cost for a kg of knotweed. In

this model, the downstream operators earn a wage of $22 per hour. The downstream labor cost includes 40% benefits factor, 10% operating supplies factor, 20% supervision factor, and 60% administration factor. An example of this distribution is as follows: for every $20 paid to an operator for an hour of work, there is an additional cost of $8 for benefits, $2 for supplies, $4 for supervision and $12 for administration. Total labor hours amount to 20,398 hours per year with operators devoting most of their time to the blending tank (V-101) used as an adsorption vessel. This was in alignment with our estimates since the blending tank is one of the two equipment with the highest number of operations needed compared to every other unit in the model. The next factor contributing to the annual operating cost was the Lab, QA, and QC group, which accounted for less than 1 percent. No funds were allocated to on-going research and development whereas, QA and QC were estimated to be 5% of the total labor cost. Only one consumable was defined within the process, as listed in **Table 3.8**. This consumable was the macroporous resin, NKA-11, which is used within the batch adsorption vessel (V-101). The annual cost of the resin amounted to $130,536. Pricing for this resin was estimated using commercial values found online by a large scale supplier (Extrepure Resin (Shanghai) Co., Ltd.). Waste treatment costs were also incorporated into the annual operating cost calculations. This waste treatment category incorporates the price to safely dispose of different waste streams generated in the facility. Exiting waste streams are classified under one of four groups: organic waste, aqueous waste, solid waste, or gaseous emissions. Here, the cost of emissions was negligible due to low concentration of nitrogen, oxygen, ethanol, and water vapor being released into the atmosphere. The cost to dispose of each group is as follows: organic waste is $0.01/kg, aqueous waste is $0.001/kg and solid waste is $0.01/kg. The annual amount of waste produced by the process is about 44 million kg, estimated at about $234,000 per year in waste treatment costs. While the cost for waste treatment of each

group remained similar, the largest contributor to the cost is attributed to organic waste, which include the disposal of the biomass. Resin disposal costs were negligible. These results are in alignment with the cost and use of raw materials throughout the process. A breakdown of the annual waste treatment contributors is shown in **Figure 3.8**.

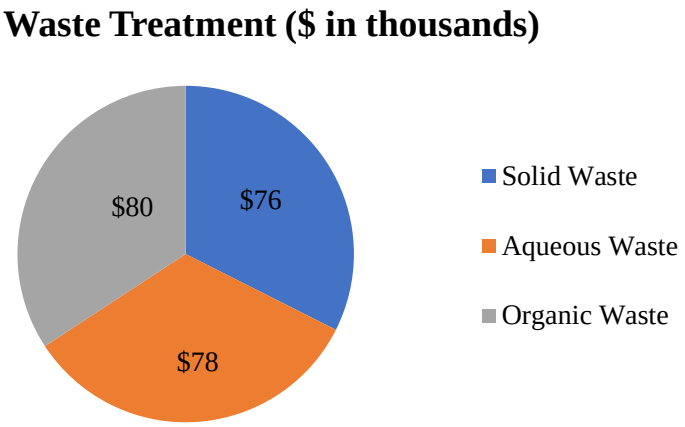


Figure 3.8: Cost of each waste treatment group in thousands

Table 3.8: A list of the consumables, their purchase cost, the annual amount used, and total annual cost for each consumable

Bulk Material	Unit Cost ($)	Annual Amount	Unit	Annual Cost ($)
NKA-II Resin	3.36	38,850	L	130,536

Utilities are the last factor which contribute to the annual operating cost. A detailed breakdown by utility type used in the process model annually can be seen in **Figure 3.9**. Three utility types were used in the model: standard (Std) power, steam, and chilled water. Pricing for each utility were set as, $0.10 per kW-h of power, $12.00 per MT of steam, and $0.40 per MT of chilled water. The cost of std power was the highest of all three utilities. Std power was used to operate all major equipment including but not limited to the stirred reactor acting as the enzymatic

hydrolysis unit, the blending vessel serving as the adsorption vessel, the ultrasonic assisted extractor unit, and the grinder. Surprisingly, the grinder responsible for crushing and homogenizing the plant material into powder required the largest amount of power to operate. Total utilities costs only amounted to a 1% of the annual operating cost. The COGS was calculated by dividing the annual operating cost by the annual production. In this model, the annual operating cost for each case described above were used and divided by 100 MT, resulting in a COGS of $150/kg, $111/kg, and $79.4/kg. A summary of both the process parameters and economic analyses from the base case model is shown in **Table 3.9**.

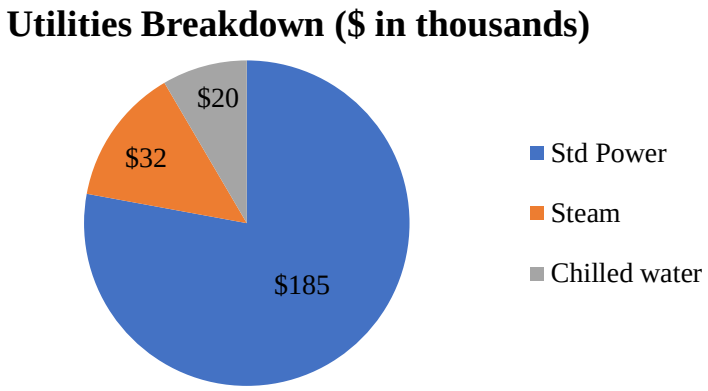


Figure 3.9: Cost of each utility type in thousands per year

Table 3.9: A simplified list of the process parameters and economic analyses

Knotweed Processed	5,631 kg/batch	7.3 million kg/year
Resveratrol Produced	77.2 kg/batch	100,000 kg/year
Yield	1.4%	
Batch Duration	45.63 hours	
Cycle Time	6.08 hours	
Batches per year	1,295	
Knotweed Production Cost	0.19 $/kg	
Enzymes used (Cellulase)	366 kg/batch	379,176 kg/year
Industrial Ethanol Purchase Price	2.00 $/gal	
Amount of Ethanol used	7.5 million liters per year	
Resin Replacement Frequency	100 batches	

CAPEX	$44.7 million
OPEX	$15.0 million per year
COGS	$150 per kg
OPEX without depreciation	$11.1 million per year
COGS without depreciation	$111 per kg
OPEX without depreciation, insurance, or local taxes	$7.94 million per year
COGS without depreciation, insurance, or local taxes	$79.4 per kg

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Chapter 4 : Sensitivity and Scenario Analysis

4.1 Introduction

Throughout the design of the simulation model, certain parameters were initialized to match resveratrol production practices described in both patents and scientific literature. When extrapolating bioprocessing parameters from these sources, a conservative approach was taken so that the lower values from a range of data was used in the model in effort to portray realistic results expected during large scale production. An example of this is including a loss of 5-10% material (90-95% recovery) during a filtration step, instead of expecting an 100% recovery every batch. As acknowledged in Chapter 3, certain bioprocessing parameters used in the model were assumptions, thus leading to some uncertainty whether the process outputs were reliable. For this reason, a sensitivity analysis was performed to assess how a certain variation effects the economics of the model, specifically the CAPEX, OPEX, and COGS. Similarly, it is acknowledged that there are multiple designs which can be implemented to produce 100 MT of resveratrol and this model simply serves as an example of one method. Therefore, certain scenario analysis were performed to assess the effect of design and production amount on key economic parameters.

4.2 Sensitivity Analysis

4.2.1 Resveratrol Content

To assess the sensitivity of the base case model, we varied certain process parameters to investigate their impact on the CAPEX, OPEX, and ultimately the COGS. One parameter which was defined in the SuperPro model using a conservative approach was the concentration of resveratrol present in the Japanese knotweed rhizome used for processing (i.e., 0.5 mg resveratrol/g rhizome). **Figure 4.1** demonstrates the relationship between COGS and when the concentration of resveratrol per knotweed rhizome is increased up to 3 mg/g FW. The concentration of polydatin

was also increased proportionally along with resveratrol. Each facility simulation is redesigned for each concentration tested while still reaching 100 MT resveratrol annually.

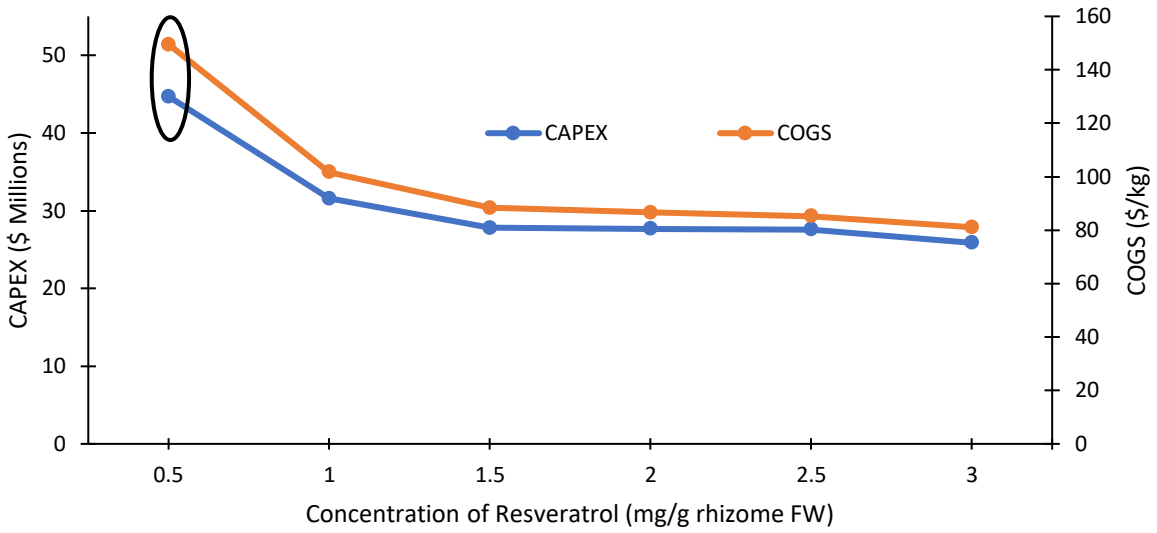

Figure 4.1: Cost of goods sold (COGS) and Capital Expenditures (CAPEX) vs Concentration of resveratrol. Here, depreciation is included in COGS calculations. The base case model results are circled in black.

As expected, when a larger concentration of resveratrol was modeled to be present within the Japanese knotweed rhizome entering the process, the model resulted in a decrease in both the CAPEX and COGS. The largest drop in CAPEX occurs directly when the concentration of resveratrol is increased an order of magnitude to 1.0 mg/g. The decrease in CAPEX from 0.5 mg/g to 1.0 mg/g is $13 million, over 3-fold larger than the average drop between increments. Expectedly, the COGS also decreases the largest amount between the first two concentrations. The COGS drops a value of $48/kg to a value of $102/kg, a 32% drop. Values for both economic parameters begin to plateau around a concentration of at 1.5 mg resveratrol/g FW, approximately at values of $27.8 million and $88/kg for CAPEX and COGS (including depreciation), respectively. It was discovered that resveratrol is found in a wide range of concentrations in Japanese knotweed rhizomes. A table listing different resveratrol concentrations found in Japanese

knotweed is shown in Chapter **2.** As mentioned, an average value of the total resveratrol concentration in knotweed, including polydatin, was about 7 folds higher than just free resveratrol. Using information on resveratrol concentrations for Japanese knotweed rhizomes grown specifically in North America[1], we calculated an average concentration value of 2.6 mg resveratrol/g FW. If a future process used rhizomes under similar conditions (2.5 mg resveratrol/g), our simulation suggests a cost decrease of about one third (38%) for CAPEX and 43% for COGS compared to our base case model operating at 0.5 mg Rsv/g. Notably, the same authors that describe an average concentration of 2.6 mg Rsv/g Japanese knotweed roots also mention certain samples contain concentrations as high as 12 mg Rsv/g FW and 12 mg Polydatin/g FW. Using this information, a simulation operating at the same conditions as the base cased was modeled using concentrations of resveratrol and polydatin at a 1:1 ratio at 12mg/g for each stilbene compounds. The same economic analysis was performed on the 12mg/g case, the resulting CAPEX, OPEX, and COGS values are shown in **Table 4.1**.

Table 4.1: Capital expenditure (CAPEX), annual operating expenditure (OPEX), and cost of goods sold (COGS) to produce resveratrol from Japanese knotweed at 12 mg/g

CAPEX	$22 million
OPEX	$6.5 million per year
COGS	$65 per kg
OPEX without depreciation	$4.8 million per year
COGS without depreciation	$48 per kg

4.2.2 Consumption of Ethanol

In the previous chapter, the cost of ethanol was identified to be the major contributor to the annual operating cost and the largest bottleneck. Due to the low concentration of resveratrol specified within the Japanese knotweed rhizome used to simulate the base case model, approximately 6,000 kg of Japanese knotweed rhizomes is needed per batch. While ethanol is widely used across the simulated facility, a large quantity of ethanol at an approximate 1:1 ratio

(L ethanol: kg knotweed rhizome mass) is required during the extraction process, largely attributing to the high cost for ethanol. To demonstrate the relationship between resveratrol concentration and the quantity and cost of ethanol in the process, we calculated ethanol consumption as we increased the concentration. Here, **Figure 4.2** illustrates the change in the total cost of ethanol ($) and amount of ethanol used (kg) when the concentration of resveratrol per knotweed rhizome is increased up to 3 mg/g.

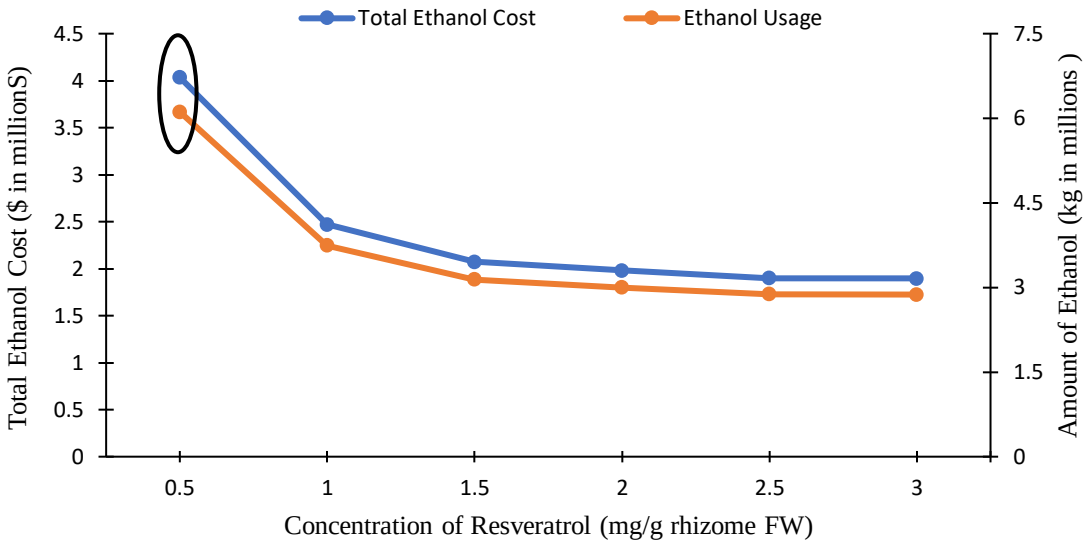

Figure 4.2: Relation between increasing resveratrol concentration (mg/g rhizome FW) and the amount of ethanol used (kg) and total cost of ethanol ($). Results from the base case are circled in black.

The increase of resveratrol found within the rhizomes led to an exponential decrease in the quantity of ethanol needed for processing. In comparison to the base case model, there's a reduction of $1.6 million (39%) to the overall cost of ethanol when the concentration of resveratrol is increased an order of magnitude to 1mg/g. When resveratrol concentration is as high as 3 mg/g, ethanol cost decrease by $2.1 million, a 53% decrease from the base case model results. When the cost of total ethanol used is compared between 1 mg/g and 3 mg/g models, the difference is only

about $575 thousand or a 23% decrease. A simple method to evaluate the effect of the concentration in rhizomes, we measured the mass of knotweed rhizomes needed for processing as concentration was varied. As expected, an increase in knotweed concentration results in fewer knotweed rhizomes needed for processing. **Figure 4.3** shows the change in mass of knotweed rhizomes needed and ethanol consumption per increasing concentration of resveratrol within the knotweed.

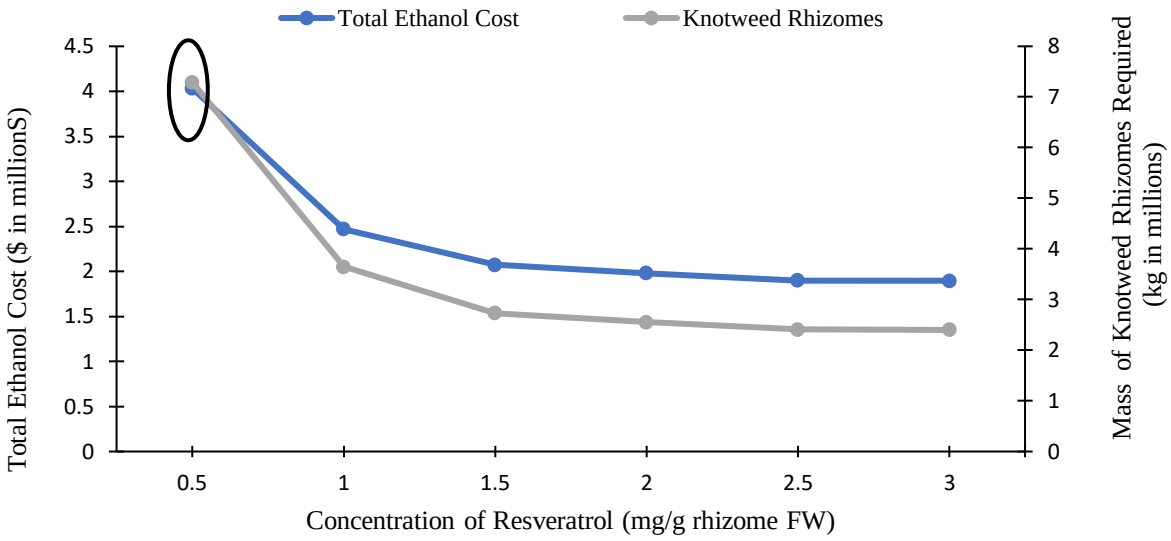

Figure 4.3: Relation between increasing resveratrol concentrations (mg/g rhizome FW) and the total ethanol cost ($ in millions) and mass of Japanese knotweed roots needed for 100 MT production. Results from the base case are circled in black.

4.2.3 Cost of Ethanol

Notably, ethanol is a commodity which has experienced some volatility in price during the last few years. A commodity tracker provided by tradingeconomics.com tracks the cost of ethanol per gallon in USD daily (https://tradingeconomics.com/commodity/ethanol). *Trading Economics* demonstrates that the price of ethanol per gallon reached a low of about $0.95 in April of 2020 and a high of $3.43 on November of 2021, with a current price of about $2.65 per gallon during the

time this report was written (April 2022)[2]. The initial jump in ethanol price was over 260% in only a time span of one year and seven months. The price has since dropped about 23% since its peak in a span of 4 months with financial analysts at *Trading Economics* forecasting a further reduction in price in the near future. This fluctuation in price ultimately limits the ability to effectively assess the effect that the cost of ethanol has towards resveratrol production and similar biomanufacturing facilities utilizing large quantities of ethanol. In effort to assess the effect the ethanol price has on the OPEX, the price of ethanol was varied by increments of $0.50 from $1.00 to $3.00. The corresponding OPEX values (including and not including depreciation) for each scenario is demonstrated in **Figure 4.4** below.

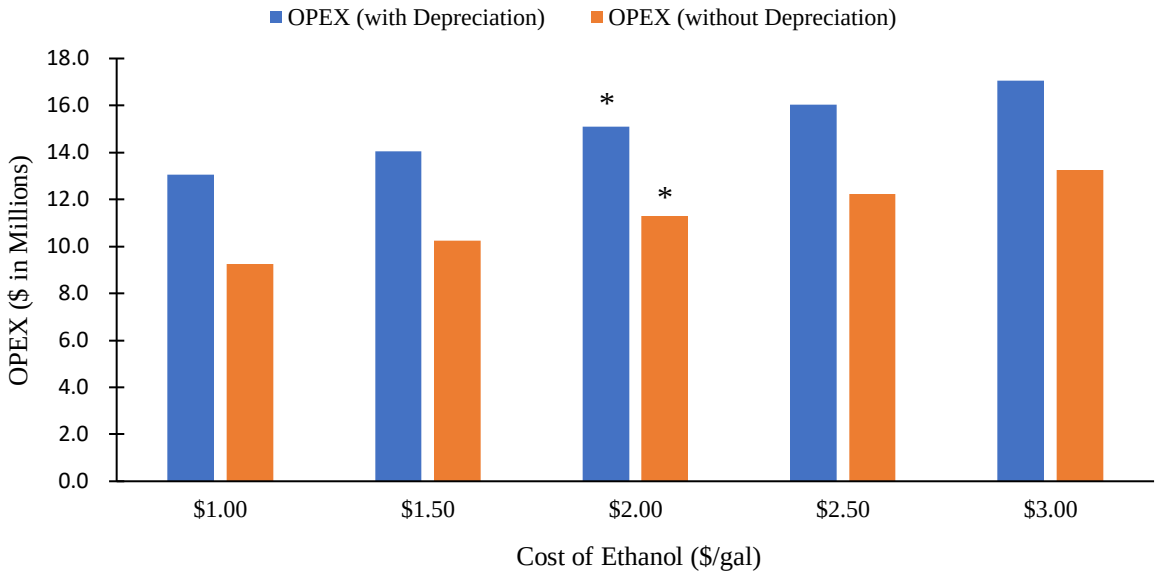

Figure 4.4: Relation between increasing ethanol cost ($/gal) and the annual operating costs ($ in millions). Results from the base case are indicated by the black asterisks (*).

In the scenario where ethanol was priced towards its low price of $1.00 per gallon, the annual operating cost to produce 100 MT of resveratrol is reported to be $13.2 and $9.2 million, with and without depreciation, respectively. In the same simulation file, where ethanol is now priced at $3.0 per gallon, the annual operating cost increases to $17.0 and $13.2 million, with and

without depreciation, respectively. The base case scenario was performed using a price of $2.00 per gallon of ethanol. The annual operating cost for the base case model is $15.0 and $11.2 million, with and without depreciation, respectively. The difference in annual operating cost is roughly 16% when compared to the $1.00 per gallon scenario and 11.5% when compared to the $3.00. With each increment of $0.50, the annual operating costs steadily increases an average of 6.8% from the last. No immediate outlier was determined during the variation of the ethanol prices. To assess the cost of ethanol to the COGS, the COGS values (including and not including depreciation) was plotted against the change in ethanol price, shown below in **Figure 4.5**.

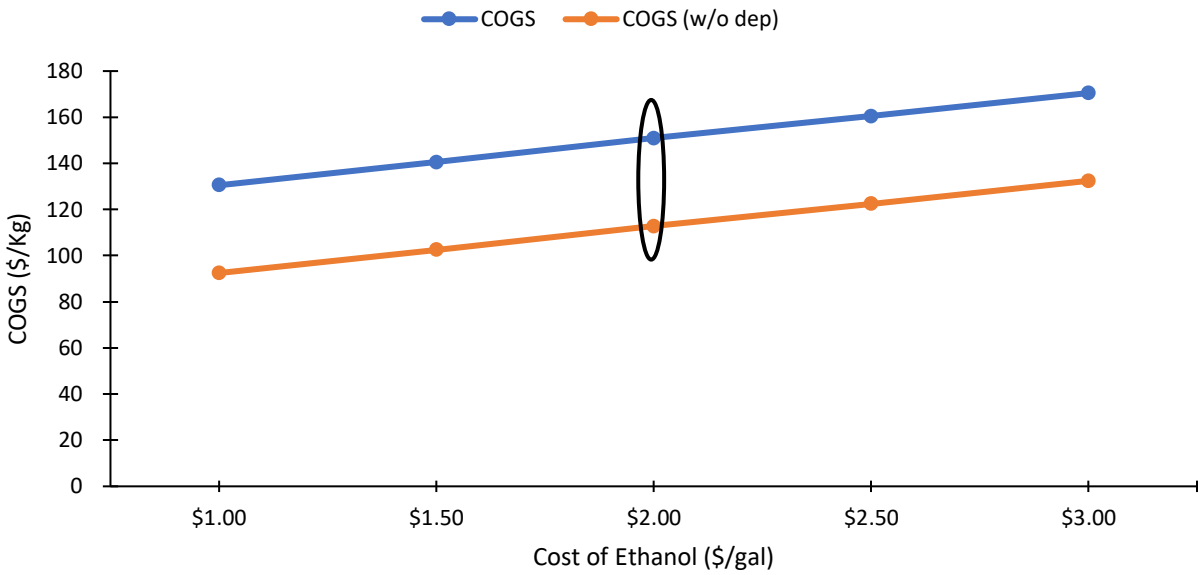

Figure 4.5: Relation between increasing ethanol cost ($/gal) and the COGS ($/kg). Results from the base case are circled.

As expected, the COGS was shown in increase in a linear fashion, in a similar trend to that shown in the relationship between OPEX and ethanol price. An incremental increase of $0.50 from $1.0 to $3.0 per gallon of ethanol increased the COGS (including and not including depreciation) an average value of $10. The COGS value for the optimistic case of $1.0 per gallon case is reported to be $131 and $92 including and not including depreciation, respectively. The variation in price is about $20 for both COGS values and a percentage difference of 13.5% and 18.1%, including

and not including depreciation, respectively. While ethanol is understood to change in price due to many unprecedented factors, it is recommended that the price of ethanol be discussed and be agreed upon for long periods of time (6 – 12 months) with commercial supplies in efforts to hedge against the variation in price in the global market.

4.2.4 Cost of Enzymes

Another commodity deemed essential to produce 100 MT of resveratrol is the cellulase enzymes used for hydrolysis in the process. Patents released by three resveratrol manufacturers in China detail steps on how to utilize ß-glucosidase for hydrolyzing polydatin into resveratrol to increase production (**Table 3.1**). Here, the simulation for the base case model was designed in a similar fashion to incorporate the utilization of ß-glucosidase found in cellulase. As a result, the annual cost of cellulase enzymes was expected to be $71,436 or 1.3% of the annual operating cost. However, the price of cellulase enzymes used within the model was retrieved using literature values derived from a techno-economic analysis on enzymes costs for biofuel production[3]. This value was not discussed or confirmed with a large-scale commercial manufacturer of industrial enzymes. The use of industrial enzymes remains a challenge as prices remain inconsistent due to enzymes being reported in terms of dollars per gallon of biofuels[3,4]. These prices often account for factors beyond the cost of enzymes themselves, such as overall biofuel yield, feedstock choice, and enzyme loading [3]. Consequently, a wide range of prices for industrial cellulase enzymes exists. Notably, an analysis performed by scientists at the United States National Renewable Energy Laboratory (NREL) mention retrieving a Multi-Year Program Plan from the Office of the Biomass Program, Energy Efficiency and Renewable Energy, U.S. Department of Energy (DOE) where the price of cellulase enzymes was anticipated to be within the range of $0.35/gal in 2007 and $0.12/gal by 2012. The same authors at the NREL performed a techno-economic analysis on the

design and economics for conversion of lignocellulosic biomass to ethanol and concluded they were able to retrieve a price of $0.34/gal when using their own on-site enzyme production section, aligning their cost with the expectations of the DOE. Novozymes, an industry leader in industrial enzyme production, released a press release titled “New enzymes turn waste into fuel” in February of 2010 mentioning they can offer cellulase enzyme at a competitive price of $0.50 per gallon of cellulosic ethanol[5]. Using the pricing information retrieved by the techno-economic analysis on cellulase enzymes for industrial applications mentioned above[3], a range of prices for cellulase enzymes per gallon of ethanol can be found to be between $0.68-$1.47. The difference in price is understood to be attributed to using the maximum theoretical yields of sugar consumption and if yields were based on saccharification and fermentation yields found in literature[3]. Since the price of enzymes are another variable cost which attribute to the cost of production, a sensitivity analysis was performed to assess the impact of such a large spread between enzyme cost. A scatter plot demonstrating the relationship between the COGS (including and not including depreciation) and enzymes cost is shown below in **Figure 4.6.**

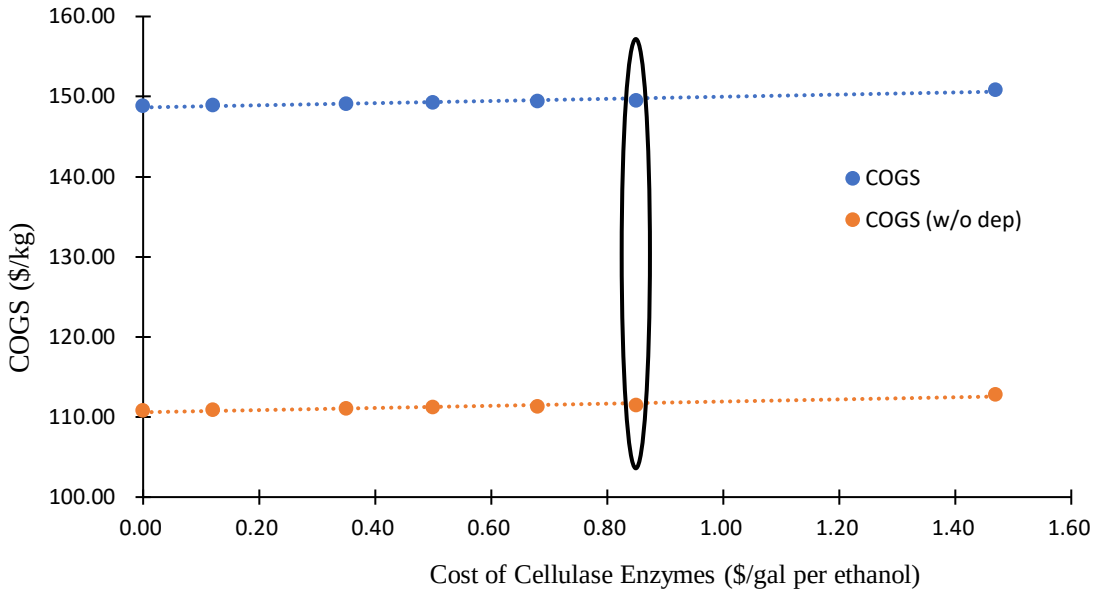

Figure 4.6: Relation between increasing enzyme cost ($/gal) and the COGS ($/kg). Results from the base case are circled.

The range of ethanol used for this analysis were chosen from the values retrieved during our search for enzymes costs and a case where the enzymes are supplied at no additional cost. The list is as follows, $0.00/gal, $0.12/gal, $0.35/gal, $0.50/gal, $0.68/gal, $0.85/gal, and $1.47/gal. A large change in COGS values was not seen. The largest change in COGS when comparing to the base case occurred when the price of enzymes increased an order of magnitude to $1.47/gal. Here, the change in total cost was 188%, or a price increase of $134,000 a year when compared to the base case model. The low cost of enzymes for resveratrol production can be expected since the amount (kg) of enzymes being loaded to the reaction vessel is relatively small compared to the total mass also entering the reactor (~3%). To ensure the model was appropriately modeled, the percentage of enzyme costs was measured as the enzyme cost was varied, shown in **Figure 4.7**. As expected, as the cost of enzymes increase, as did the percentage of total enzymes cost to the total raw material costs.

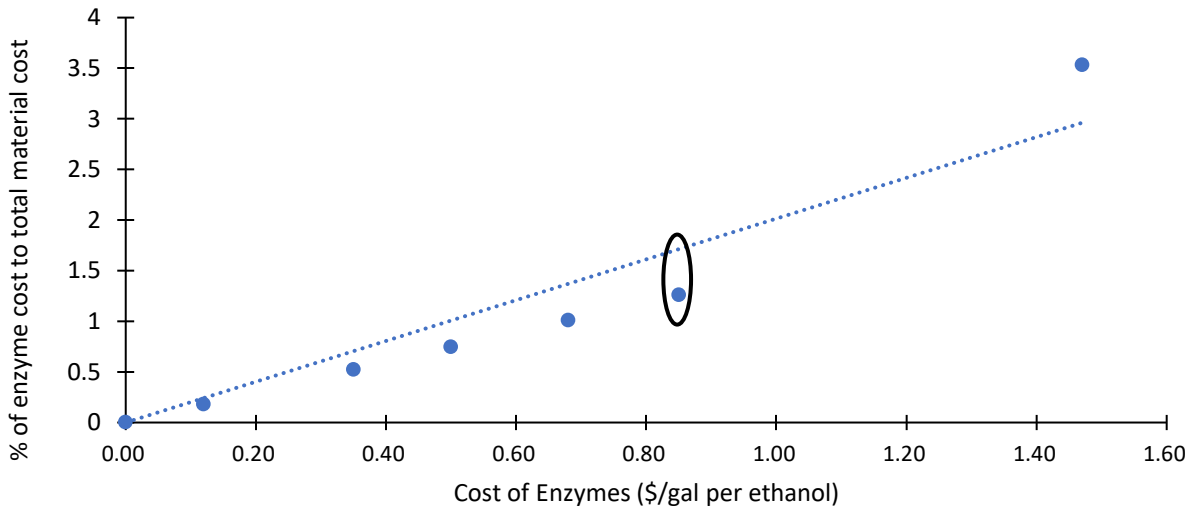

Figure 4.7: Relation between increasing enzyme cost ($/gal) and the percentage of total enzyme cost to total raw material cost (%). Results from the base case are circled.

An alternative to single use enzymes highlighted in other Rsv production patents and scientific literature is the use of fermentation. Rather than purchasing and mixing pure enzymes with plant tissue, this approach mixes plant tissue with microbial cultures, utilizing the enzymes secreted within the solution, ultimately reducing operating costs and raw material cost associated to the addition of water for mixing. This method's feasibility has already been demonstrated by Wang, H. et al., who effectively compares hydrolyzing *P. cuspidatum* herbs using fermented fungi versus using acid hydrolysis was performed and dubbed using fungi as an effective and feasible alternative[6]. Arguably, applying this method for large scale production might not be practical, as one patent reports the time for fermentation ranges from 10 to 15 days[7], significantly reducing processing time and annual production throughput. Additionally, other researchers argue that the activity of the β-glucosidase enzyme responsible for converting polydatin to Rsv does not perform optimally under fermentation conditions (i.e., temperature at 30°C)[8]. Another alternative which can be utilized to address high enzyme costs was demonstrated in the analysis performed by the U.S. NREL. Design a bioprocessing facility with its own on-site enzyme production section[4]. This approach is one that has already been utilized in Rsv literature where the feasibility of fermenting

Aspergillus oryzae and separating the β-glucosidase enzyme from solution to hydrolyze *P. cuspidatum* plant tissue was demonstrated[8]. The use of on-site enzyme production is expected to reduce purchasing costs and provide a consistent supply of enzymes available for industrial use.

4.2.5 Enzymatic Conversion

As described above, the most utilized approach when extracting resveratrol from Japanese knotweed is the use of an enzymatic hydrolysis step to convert any existing polydatin to resveratrol. However, data surrounding large scale processing of Japanese knotweed is limited, therefore, bioprocessing parameters such as percent conversion was retrieved using literature on laboratory scale experiments. It should be noted that literature describes an efficient process where conversions can yield values as high as 100%[9]. Rather than initializing 100% conversion within the simulation, another conservative approach was taken, and 90% conversion was specified within the base case model. To evaluate the impact that the percent conversion had on the CAPEX and COGS, the percentage was varied from 90 to 100 by increments of two, shown in **Figure 4.8.**

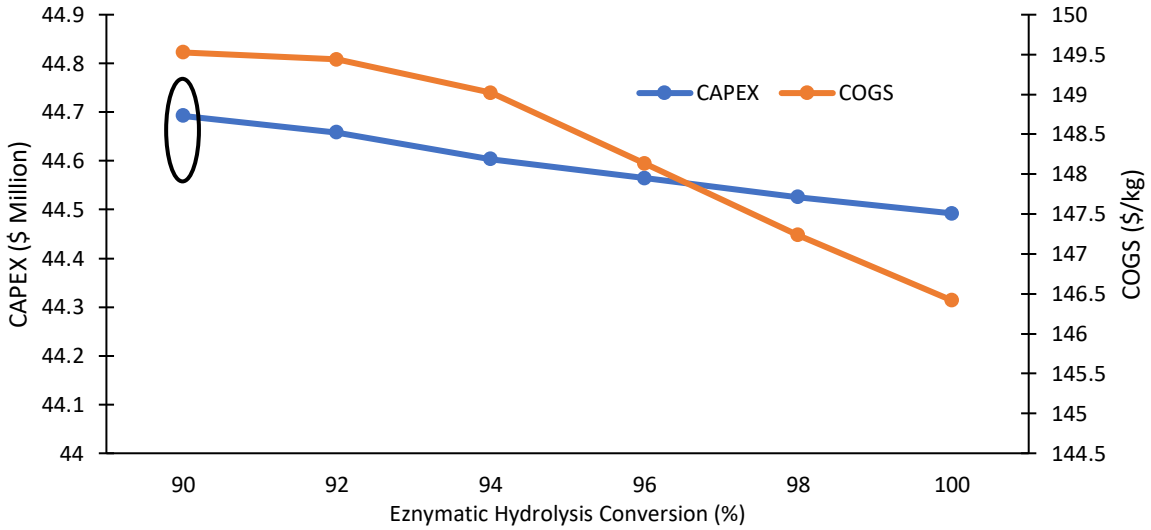

Figure 4.8: Relationship between an increase in enzymatic conversion with CAPEX and COGS. Here, depreciation is included in the COGS calculations. Results from the base case are circled in black.

Increasing the enzymatic conversion led to a reduction in both CAPEX and COGS. Utilizing the 90 and 100 percent conversion results for comparison, the difference in COGS is $3.1 (2.1%) and a $200 thousand (<1%) difference in CAPEX. The largest drop in CAPEX among the different percent conversions occurred between 92 and 94 percent conversion, yielding a decrease in CAPEX of 55 thousand (a .12% difference from the base case). Here, the reduction in price is attributed to 3 factors: the reduction in equipment size, the reduction of units needed for processing knotweed, and lastly, the reduction in raw materials such as water and ethanol entering the process at a 1:1 mass ratio with the mass of knotweed. The relationship between Japanese knotweed and COGS to increasing enzymatic conversion percentages is shown in **Figure 4.9**. One specific example where the reduction in CAPEX is seen is the reduction of reactor size needed to perform the enzymatic hydrolysis. The size of the reactor in the base case model is 13,621 L but the reactor is resized to 12,925 L when the conversion was increased to 94%. While there is a price decrease in both the CAPEX and COGS when 100% conversion is initialized, the author would advise against expecting to replicate similar values as 100 percent conversion may not be practical at large scale. While it is certainly plausible that an industrial hydrolysis step can yield 100% conversion, this small decrease in COGS (a $2.60 difference from 94% to 100%) may not be worth the added difficulty of extending the hydrolysis time or effort. Thus, it is suggested that a 94 percent conversion may a reasonable operating condition to aim for.

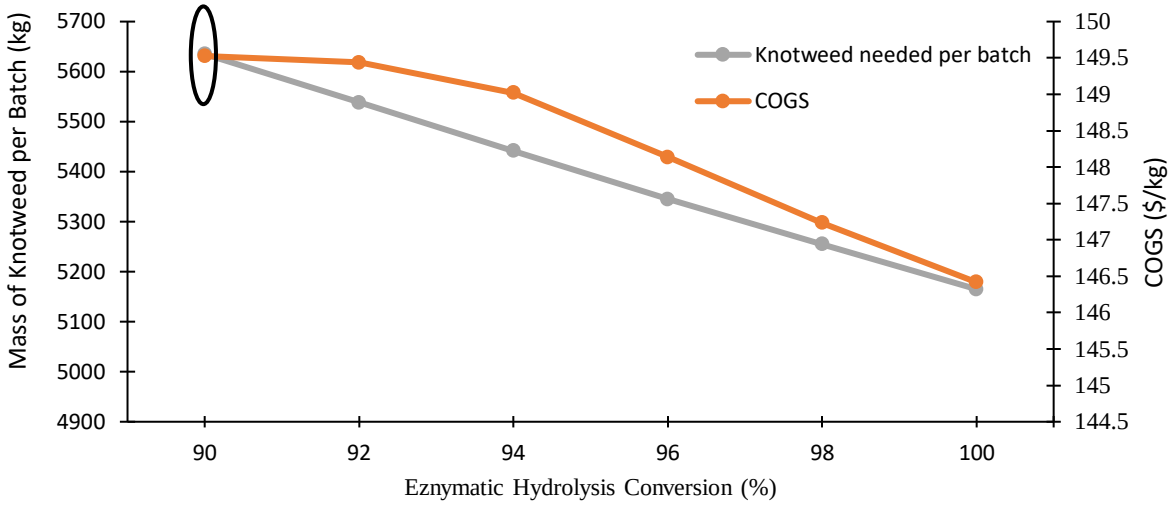

Figure 4.9: Relation between increasing enzymatic hydrolysis conversion (%) and the COGS (including depreciation) and mass of Japanese knotweed roots (kg) needed for 100 MT production. Results from the base case are circled in black.

4.2.6 Extraction Efficiency

Extraction of resveratrol in plants has been demonstrated using variety of techniques, such as UAE and Soxhlet extraction. However, as mentioned above, certain bioprocessing values such as percentage recovery for resveratrol extraction from Japanese knotweed using UAE is not specified in literature. Thus, leading to us using a percent recovery value derived from literature on ultrasonic assisted extraction of resveratrol from grape stems in our base case model, a value of 78.8%. Interestingly, the same authors describe a significant increase in percent recovery when the plant biomass undergoes a subsequent agitation with fresh solvent, increasing the recovery up to 96.7%[10]. In efforts to assess the relationship between extraction recovery and CAPEX and COGS, the extraction process was simulated to undergo two agitation steps with the introduction of fresh ethanol (70% v/v as opposed to the initial 80% v/v ethanol during the first step), while varying the percent recovery by increments of two from 90% to 100%. **Figure 4.10** demonstrates

a graphical representation of the relationship between CAPEX, COGS, and percent recovery during the extraction step.

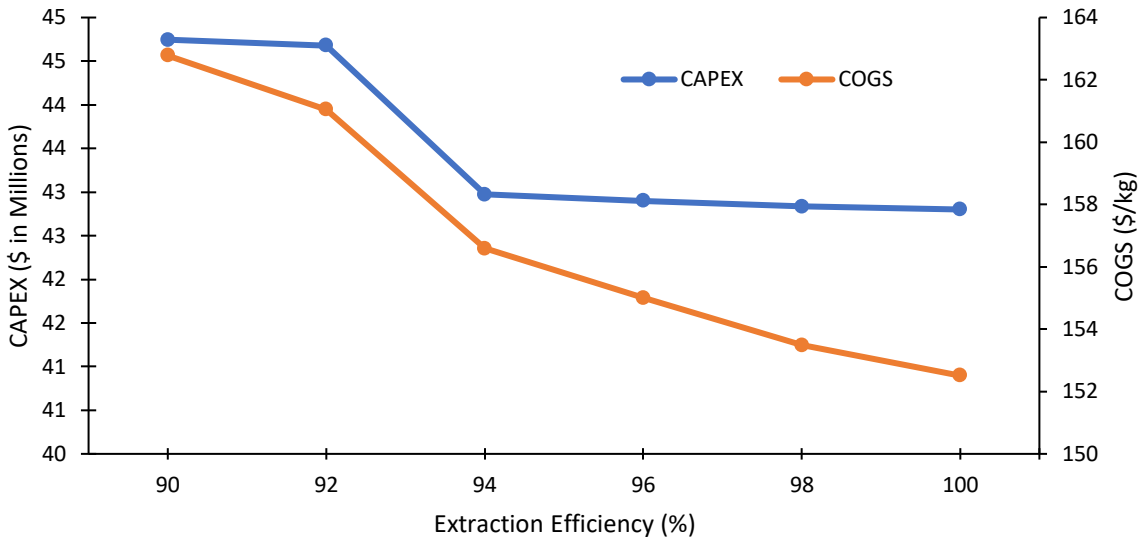

Figure 4.10: Relationship between extraction efficiency and CAPEX and COGS. Here, depreciation is not included in COGS calculations. All simulations have been designed to produce 100 MT of resveratrol. Base case values not shown here.

During the extraction efficiency sensitivity analysis, an immediate outlier was determined. When the extraction efficiency was set to a 94% recovery, the CAPEX saw a reduction in price of $1.7 million when compared to the 92% recovery. Interestingly, the CAPEX only dropped an average of $60 thousand among other increments. Upon further inspection, the drop in CAPEX was attributed to multiple factors which effect the DFC and the reduction in total plant direct costs (i.e., direct physical cost surrounding the facility). First, 19% of the $1.7 million dollar reduction is directly related to the removal of one ultrasonic assisted extraction unit ($330 thousand) needed for processing. With the increase in extraction efficiency, the mass of knotweed required for processing decreases (**Figure 4.11**), subsequently requiring less equipment and piping needed to installation (unlisted equipment). The simulation model was able to recognize that 9 UAEs were enough to process the incoming mass instead of the 10 initially required in the first initial scenarios (Base Case (78.8%), 90% and 92% recovery). Along with the decrease in listed and unlisted

equipment, the simulation model also reduced the costs associated with installation, process piping, instrumentation, insulation, electrical, building, yard improvements, and auxiliary facilities, leading to an additional decrease in price (28%). A large portion of the CAPEX reduction (32%) is credited to the decrease in total plant indirect costs (TPIC), which incorporates the engineering and construction costs associated to the design and construction of the process. The engineering and construction costs are calculated as a factor of the total direct cost, 25% and 35% of the DC respectively. The remaining price decrease is attributed to the reduction in contractors fee (5% of the DC), working capital and startup costs. Overall, there is a downward trend and decrease in the CAPEX and COGS when extraction efficiency was optimized. While an extraction efficiency of 94% seems to be the best percentage to aim for, the efficiency of resveratrol being extracted from Japanese knotweed during a UAE step still requires to be confirmed in a laboratory scale prior to scaling to large scale, as values may vary from those simulated here.

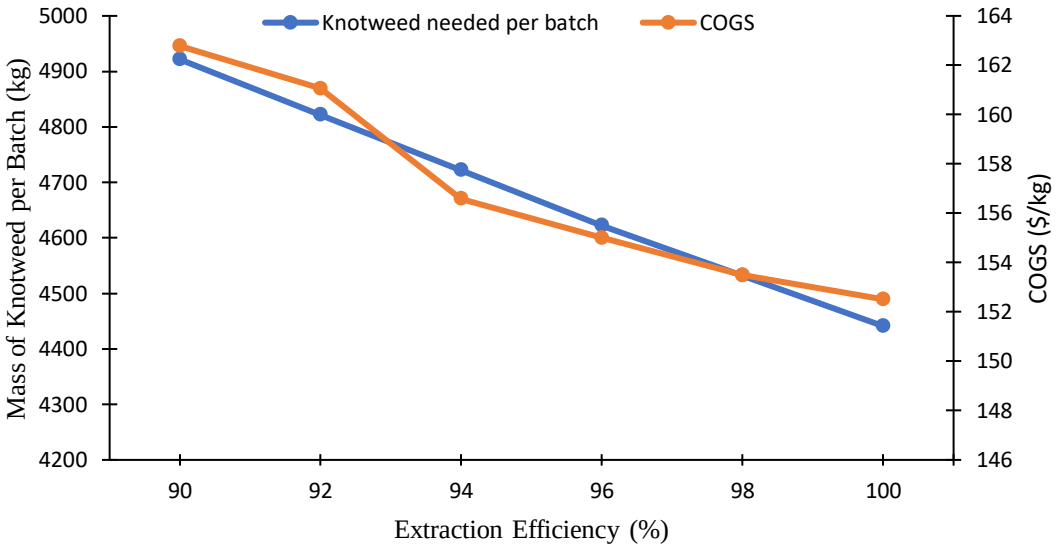

Figure 4.11: Relationship between extraction efficiency, COGS (including depreciation), and mass of Japanese knotweed roots (kg) needed for 100 MT production. Here, depreciation is not included in COGS calculations. Base case values not shown here.

4.2.7 Resin Replacement Frequency

Many factors can influence how often resin must be replaced; thus, we did not have an estimate for the resin replacement frequency. For simplicity, it was assumed that the resin would be replaced every 100 batches. A scenario analysis was performed on the resin replacement frequency to see how the COGS was affected by this assumption. **Figure 12** shows the relationship between COGS and the resin replacement frequency.

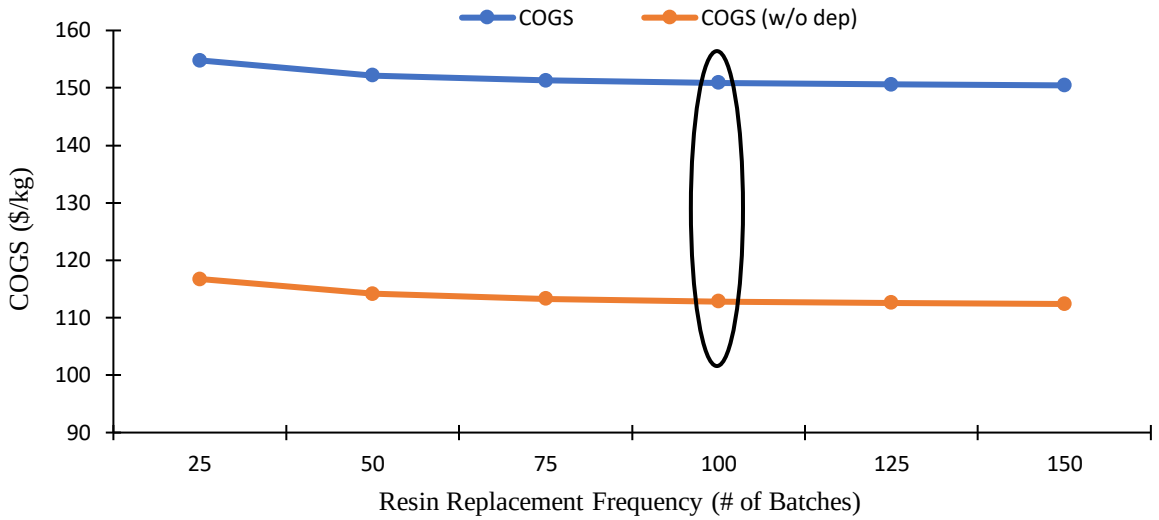

Figure 4.12: Relationship between resin exchange frequency and COGS. COGS was calculated with and without depreciation, shown in blue and orange, respectively. Results from the base case are circled in black

Since the cost of resin accounted for less than 1% of the annual operating costs (1.2% when excluding depreciation), it is no surprise there is a little impact of resin replacement frequency on COGS. Replacing the resin every 25 batches leads to a significant increase in the COGS, an increase of about $4 (2.6%) from the base case (100 batches). Replacing the resin every 150 cycles versus the 100 cycles as specified within the base case only decreases the COGS by $0.43 (<1%). For a better assessment on the effect resin exchange has on COGS, it is recommended that the number of times NKA-II can be reused and still capture resveratrol be defined experimentally before scaling up.

4.3 Scenario Analysis

4.3.1 Ethanol Recycling

As certain technologies improve, and more pilot and production scale data become available, certain bioprocesses parameters like resin exchange frequency, enzymatic hydrolysis conversion, and extraction efficiency can be tuned. An alternative approach to pursuing these technologies might be to redesign current facilities using current technology in attempts to reduce CAPEX and/or COGS. Here, acknowledging that the amount of ethanol needed in our process is the bottle neck, we performed a scenario analysis where we simulate the addition of an ethanol recovery unit to the current base case model. In the base case model, ethanol is not being recycled and is disposed of immediately after being used in the extraction unit (BGBX-102) and the adsorption vessel (V-101). In efforts to reduce the overall cost of ethanol, an additional plate and frame filter (PFF-102) was introduced directly after the initial plate and frame filter (PFF-101) to increase the separation between ethanol and plant biomass during the separation process. The ethanol recovered was sent to a holding vessel for future processing. Additionally, the ethanol used to elute impurities from the NKA-II resin in the adsorption vessel (V-101) was captured and sent to the same holding vessel. The ethanol within the holding vessel was then transferred into a distillation column where ethanol is distilled and separated from any other impurities recovered. The vapor is sent to a condenser where the exiting liquid ethanol is then recycled to the ultrasonic assisted extractors for reprocessing. A comparison of CAPEX and COGS (including depreciation) for a facility with and without the ethanol recovery equipment is shown in **Figure 4.13** and **Figure 4.14**, respectively.

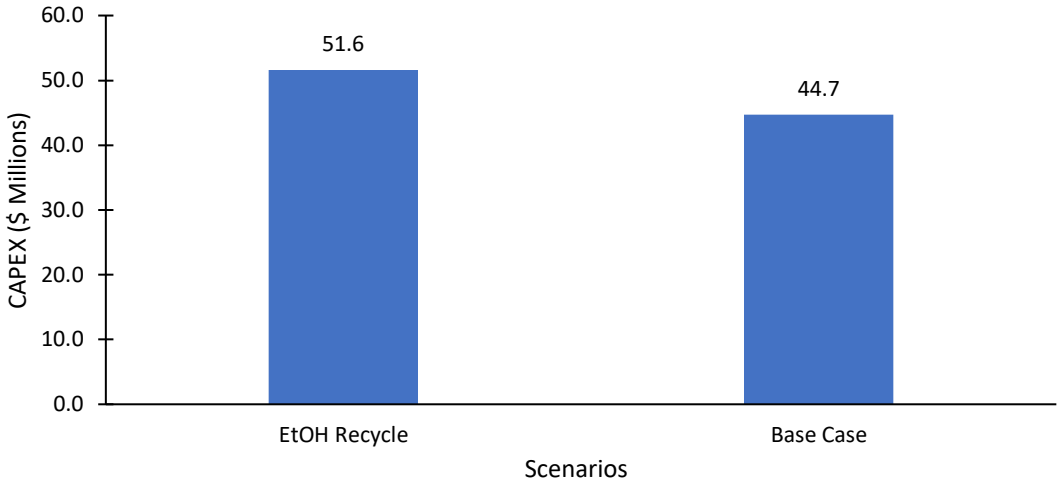

Figure 4.13: CAPEX is shown in USD in millions for two different scenarios. The original model is labeled as Base Case whereas the simulation including the ethanol recovery unit is labeled EtOH Recycle.

When comparing the difference in CAPEX between both scenarios, the model recycling ethanol was determined to be $6.8 million (15%) more costly than the base case, for a total cost of $51.5 million. The justification for this arises from the additional equipment needed to ensure sufficient recovery and processing of ethanol needed for recycling within the process, i.e., a plate and frame filter, a distillation unit, holding vessel, and a condenser. While CAPEX is higher for the new proposed facility, both the cost of ethanol and COGS decrease. The base case model utilizes about over 6 million kg of ethanol to process 100 MT of resveratrol. The proposed facility suggests a reduction of 70% of total ethanol used, only utilizing 2.1 million kg of ethanol per year needed for processing. The cost in ethanol in the base case was determined to be over $4 million whereas when the recovery unit was added, the cost of ethanol was reduced to $1.3 million, a decrease of 67% which aligns with the recovery percentage calculated. This reduction in ethanol is reflected in the COGS of the two facilities, shown graphically in **Figure 4.14**.

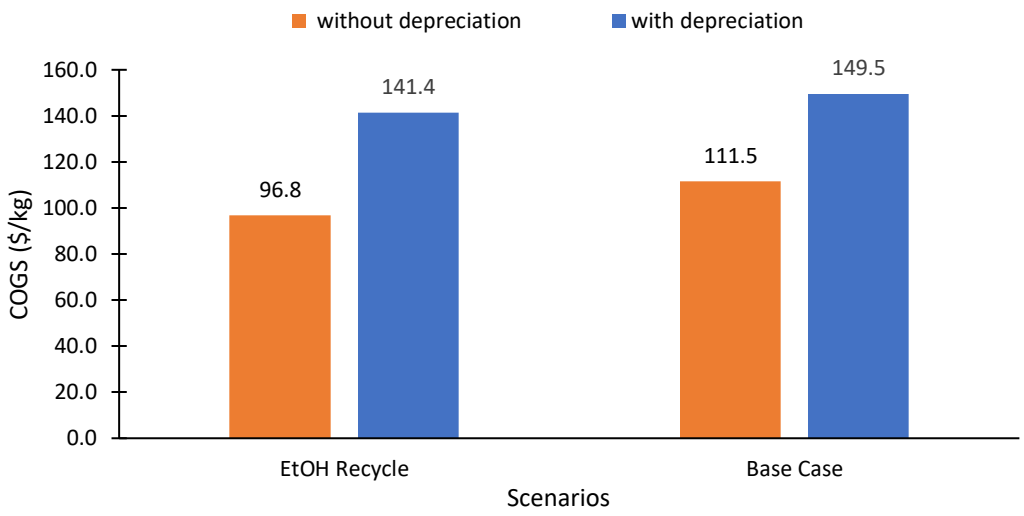

Figure 4.14: COGS is shown with and without deprecation for two different scenarios, shown in blue and orange, respectively. The original model is labeled as Base Case whereas the simulation including the ethanol recovery unit is labeled EtOH Recycle.

The model suggests that the COGS for the base case simulation is about $149.5/kg including depreciation and $111.5/kg Rsv without including depreciation. Here, it is shown that the addition of a recycling stream can reduce the COGS down to $141.4/kg resveratrol (5.4%) including depreciation and $96.8/kg Rsv (15.8%) without including depreciation. Although the reduction in ethanol was significant, the overall COGS did not reduce in a similar fashion. The difference in price is $7.5 (5%) for COGS including depreciation and $ 14.2 ($12.7) for COGS not including depreciation. One reason the scenario model did not reduce COGS further is because of the increase in labor attributed to the new pieces of equipment needed for recycling ethanol. In the scenario simulation, labor costs increased $400 thousand to $1.4 million from $1.0 million, a 26% increase. In this analysis, labor time allocated for certain operations used across the model remained constant (i.e., labor time for transferring of in-process material or labor during agitation steps), however, the additional labor time was allocated to the distillation, condenser, plate and frame filter, and extractor unit. Furthermore, the cost for water increased over $10 thousand/year in the ethanol recovery model since fresh water was being added into the recycling stream to reduce

the concentration of the recycle stream to create optimal conditions for extraction to be performed. One method the COGS can further be reduced is if water was also recovered leaving the distillation unit and recycled. The authors highly recommend implementation of such recycling unit for large scale processing.

4.3.2 Annual Production Amount

One scenario analysis which may be of interest is the relationship between COGS, CAPEX, and annual production amount. The base case model was simulated to produce an annual capacity of 100 MT. Through research and personal communications with resveratrol manufacturers in China, it has been understood that 100 MT may account for 1/3 of the global resveratrol production market, dominating production over current resveratrol manufacturers. To assess the impact the production amount with COGS and CAPEX, we simulated biomanufacturing facilities operating under similar conditions as the base case but now capable of producing amounts ranging from 25 to 200 MT. A new SuperPro file was designed for each annual production model where equipment was resized appropriately. **Figure 4.15** demonstrates the relationship between production amount to CAPEX and COGS.

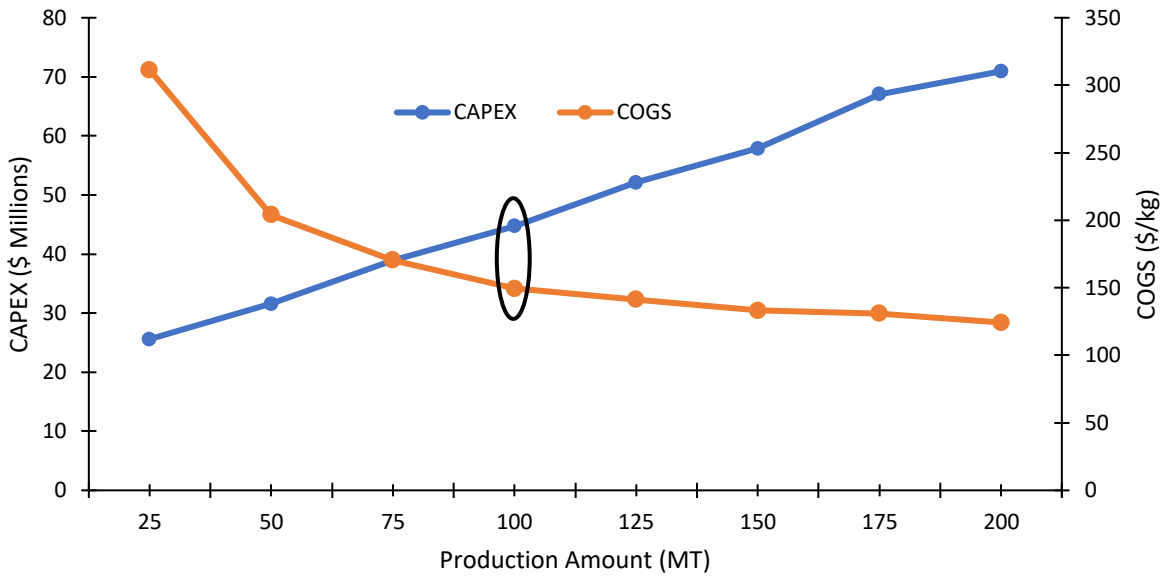

Figure 4.15: Relationship between CAPEX and COGS and annual resveratrol production amount. Here, depreciation is included in the COGS calculations. Results from the base case are circled in black.

As expected, the largest difference in CAPEX and COGS price arose between the base case and the models producing the lowest and highest amount of resveratrol, 25 and 100 MT respectively. The average difference in CAPEX between each model and the base case was about $23 million, with the exact values being $25.6 million (43% less in price) and $71.0 million (58% more in price) for the 25 and 200 MT simulations, respectively. It was determined that increasing the production amount led to an increase in the annual operating costs. However, the larger production amount resulted in a lower COGS value. These results were anticipated as the notion of economy of scales is demonstrated, (i.e., buying in bulk is cheaper). When an economic analysis was performed on the simulation producing 200 MT, the COGS was determined to be $124/kg, a reduction of $25 (16.8%) from the base case. The inverse is accurately represented within our results as well. While the CAPEX and OPEX for a biomanufacturing facility producing 25 MT of resveratrol did decrease, the COGS increased significantly, resulting in a COGS of $311/kg resveratrol, an increase of $162 (108%) from the base case. The COGS for all the production

amount scenarios are shown below in **Figure 4.16**. One assumption made during this scenario analysis was that the price of knotweed rhizomes remains a constant price of $0.19/kg. This value was calculated as the cost for the base case when 7.3 million kg of knotweed rhizomes was needed. This assumption would be valid if the quantity of knotweed being harvested annually remained the same. However, there would be a large difference when altering production amount since the mass of knotweed varied with the production amount. Specifically, when the biomanufacturing facility was modeled to produce 25 MT, only 1,415 kg of knotweed rhizomes was required per batch. In the model where the aim was to produce 200 MT of resveratrol, over 11,000 kg of rhizomes were needed per batch. One justification for not increasing the price of knotweed rhizomes is acknowledging that the concentration of resveratrol per knotweed rhizome varies on environmental factors and a larger batch may be needed to offset the low concentrations per plant. The reverse can also be said for the 200 MT model. If the concentration of resveratrol from the base case were doubled to 1.0 mg/g, the number of plants required to produce 200 MT originally would be halved and the current base case (which uses 5,635 kg) would serve as an appropriate model.

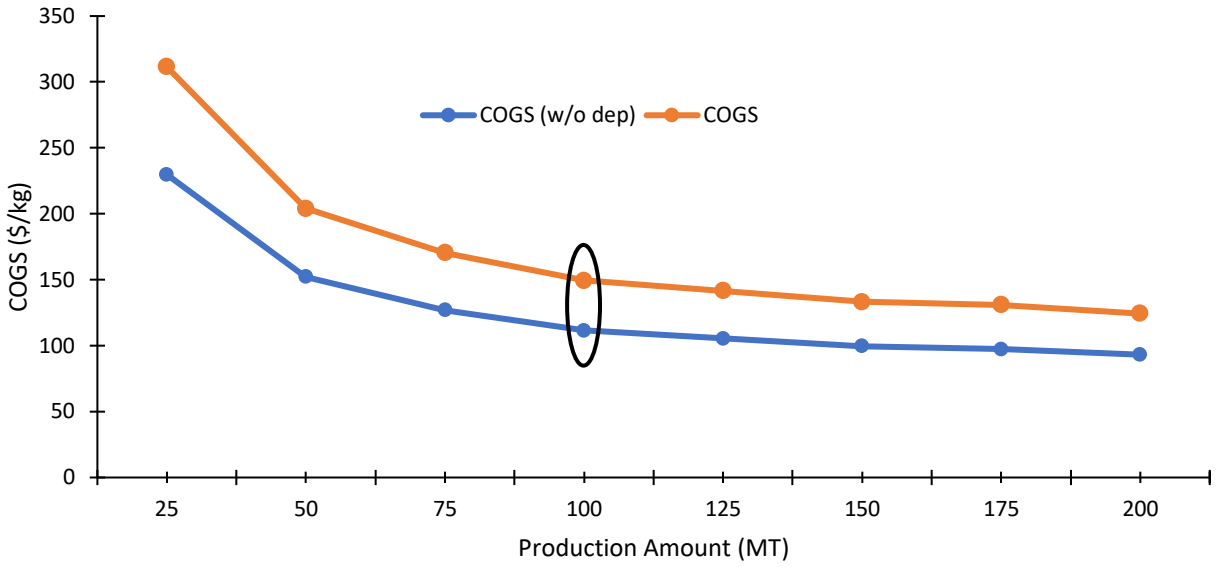

Figure 4.16 Relationship between COGS and annual resveratrol production amount. COGS was calculated with and without depreciation, shown in orange and blue, respectively. Results from the base case are circled in black.

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Chapter 5 : Environment, Health, and Safety Analysis

As an evaluation of the sustainability of our proposed facility, we conducted two environmental assessments on the base case scenario. Following methods listed by Budzinski et al., and by Biwer and Heinzle, the process mass intensity (PMI) and Environmental, Health, and Safety Assessment (EH&S) were calculated, respectively. The PMI compares how much of a certain component of the mass balance is consumed or formed per unit amount of final product, depicting the mass or material efficiency of the process[1]. A completely efficient and ideal process has a PMI value close to 1 indicating an efficient process. A large PMI value indicates a large input is required for small amounts of yield. Budzinski's method of calculating the PMI fails to include inputs used for cleaning during the cleaning-in-place operation. In this report, the calculation of PMI will include cleaning inputs since exclusion of these inputs may result in an inaccurate environmental analysis.

After performing the PMI procedure with the cleaning inputs, the proposed process was found to have an overall PMI of 529 kg inputs/kg resveratrol. A breakdown of the PMI is shown graphically in **Figure 5.1**. As expected for plant bioprocessing, the PMI value is dominated by water consumption, which accounts for 72% of the overall PMI. Water is used throughout the process to wash the incoming plants, create slurry solutions, as a component for extraction, and for rinsing and cleaning certain units before each operation. The next largest contributor is the Japanese knotweed rhizomes. The plant biomass needed to produce 100 MT makes up about 14% of the overall PMI by itself. The only consumable listed within the simulation is the resin used for purification within the adsorption vessel. The percentage of consumable to the overall PMI is less than 1% and was demonstrated as consumables in the breakdown shown in **Figure 5.1**. As the use of plant-based production routes are still considered an emerging technology, literature

surrounding PMI values for plant bioprocessing practices remain limited. Thus, for interpretation of this result, we compare our PMI value to the value found in Budzinski et al., which details their PMI for producing monoclonal antibodies (mAb) in the biopharmaceutical field. The authors describe using a process where an average input of 7,700 kg is required to obtain 1 kg of product, resulting in a PMI of 7,700 kg inputs/kg mAb.

This simple comparison assures us our calculations are within an acceptable range and our processes is not operating at similar conditions as one designed for clinical and pharmaceutical applications. Another interpretation is the plant based biomanufacturing facility detailed here is material and process efficient in producing high concentration of resveratrol at 100 MT. While the PMI value for our process is only an order of magnitude less than a mAb production facility, this may be attributed to the addition of the CIP to the PMI calculation and the limited cleaning procedures initialized within our simulation. Here, the CIP process was simulated to follow the steps needed for an industrial chemical application, thus limiting the cleaning procedure to the following order: water rinse, caustic rinse, water rinse, acid rinse, and water rinse. Certain biopharmaceutical CIP procedures are much more rigorous, including a larger variety of components than listed here. The PMI attributed to the CIP procedure was 163 kg/kg resveratrol, about 30% of the total PMI calculated for the entire facility. Notably, the same authors mention that the water input accounts for a large percentage of the PMI, 90% of the total mass used within their process. This claim is in alignment with our results shown below.

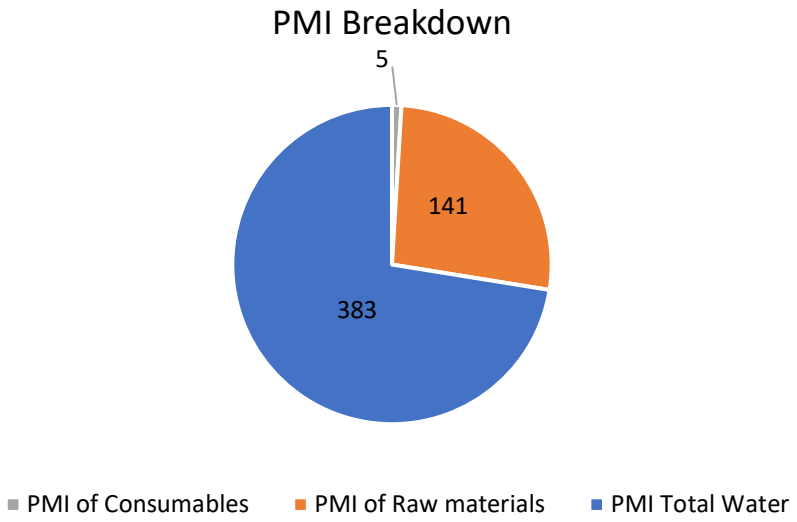

Figure 5.1: A breakdown of the overall PMI for the proposed process

After performing an assessment on the mass intensity of the process, further analysis was conducted using the EH&S method to evaluate how the chemicals implemented within the simulation effected the environment. Here, the materials being utilized were categorized into impact groups, where individual environmental indices (EI) can be calculated[2]. The sum of the individual component EI's gives an overall EI value for the process. This value may be used to assess the facility's effect on the environment. Notably, components with high EI values can also identified here as they represent hazardous materials which can be replaced to provide a safer working environment.

To perform the EH&S method described by Biwer and Heinzle, the MSDS sheets for all components in the input and output streams in the simulation were assessed. Using the information contained in the MSDS for each chemical, ABC classifications for the various impact groups were given to each chemical. These classifications were assigned environmental factor (EF) values of 1 (A), 0.3 (B), and 0 (C). These EF's could be averaged to give a single EF for each input and output, where high values indicate potentially dangerous chemicals and lower factors indicate safe

chemicals. To keep track of the amount of each material used, we calculated mass indices (MI). The calculations for MI's were performed by dividing the total mass of each chemical in or out of the process per batch by the total amount of product produced per batch. Lastly, the EI's are calculated by multiplying each EF by its respective MI for all components. This analysis yields a series of bar graphs where the most impactful components make up the largest portions of either the input or output bars. Results of this analysis are shown in **Figure 5.2a and Figure 5.2b.** As discussed, the MI's presented in Figure 5.2a are rescaled based on each components EF yielding Figure 5.2b highlighting any dangerous components. In Figure 5.2a we see that the proposed process generates input and output MIs of 480 and 487 kg/kg resveratrol, respectively. Both MIs are dominated by water which is absent from the overall EI's presented in Figure 5.2b, because water is nontoxic and generates an EF value of 0. The EI's for the inputs and outputs are 26 and 31.6, respectively. As shown in Figure 5.2a, the MIs are dominated by ethanol and plant biomass. The consistency of ethanol dominating in both breakdowns alludes to reducing the usage of ethanol as one method for operating a more environmentally friendly process.

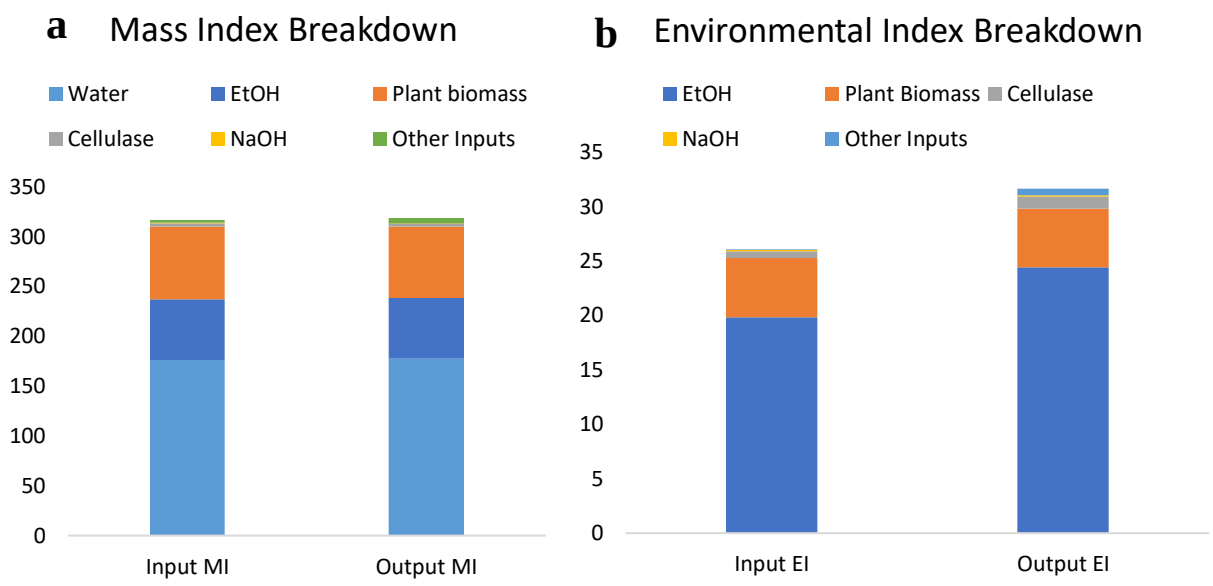

Figure 5.2: Breakdown of the final EH&S analysis results (a) provides a breakdown of the mass index for inputs and outputs of the system (b) provides a breakdown of the environmental index each component has during the inputs and outputs of the process

References

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Chapter 6 : Future Improvements and Conclusion

Here, a techno-economic analysis was performed on a base case production facility modeled for an annual production target of 100 MT of 98% pure resveratrol using Japanese knotweed rhizomes as the source. The economic results for a biomanufacturing facility operating under similar conditions to the base case model are estimated to be as follows: CAPEX of $44.7 million, OPEX of $15.0 million per year, and a COGS of $150/kg resveratrol. As described, the model was built using certain assumptions. Notably, the largest assumption was the limiting the concentration of resveratrol present in Japanese knotweed to 0.5 mg/g FW. As demonstrated by the sensitivity analysis which altered the concentration of resveratrol present in the processed rhizomes, the design of the facility and economic costs are altered significantly when the concentration is varied. When a concentration of 1.5 mg/g FW was used within the model, the CAPEX for such a facility dropped 38% from $45 to $28 million. To control the yield (yield = resveratrol concentration x biomass) grown domestically, the allocation of resources such as additional R&D personnel is required to research and generate data on the factors which largely contribute to the variation between plants. Factors may include treating the soil with fertilizers to replenish any depleted nutrients within the soil, controlling moisture content via irrigation, and the testing of different field sites in search for optimal environmental conditions and low seasonal variation. These are all additional costs to be considered within the annual operating costs but are deemed insignificant, a couple of orders of magnitude less compared to the total cost required. At this time, containment costs were ignored during the design of the model. Due to the limited information available on containment methods for Japanese knotweed grown in plantations, strategy for physical containment of vegetably propagated plants from the USDA are suggested as

an alternative solution until further research or information on this topic presents itself. One improvement might be to include the cost for discing 100 ft of land surrounding the specified cropland and the cost of herbicides required to prevent any rhizomes (or any other vegetation) from propagating outwards. The use of invasive species for manufacturing is a controversial method which may require multiple regulatory oversight from local, state, and federal regulatory agencies. Another improvement would be initiating conversations with these regulatory bodies and aligning production of Japanese knotweed with any regulations outlined. Here, the analysis of the upstream portion of the facility was determined using information provided by UC Davis Center for Agriculture and Resource Economics. Further improvement might be to model the upstream portion using SuperPro and incorporate the production process with the purification steps, so a single simulation file can reflect the entire biomanufacturing process as well as total CAPEX, OPEX and COGS.

Downstream operations available for Japanese knotweed processing are vast. The method deployed to model the base case biomanufacturing facility simulation is acknowledged to only be one strategy used for resveratrol production. Certain techniques and processing equipment can be added or removed to best serve the incorporation of future technology and improved manufacturing practices. Future experimental work might investigate alternative processing methods and compare both the economics and processing capabilities to explore optimal conditions and a more standardized process. An example might be to investigate the use of supercritical CO_2 rather than ultrasonic technology for extraction of phenylpropanoids from plants. During the downstream processing, multiple bioprocessing parameters were initialized using data provided in publicly available literature/patent. Sensitivity and scenario analysis were performed to assess the effects few conditions had towards the economics of the biomanufacturing facility.

Certain parameters such as resveratrol content, cost of ethanol, cost of enzymes, enzymatic conversion, extraction efficiency, and resin exchange frequency were altered. Each parameter was varied using reasonable increments aligned with what can be expected. For example, the cost of ethanol and enzymes were varied within a certain ranged and assessed for their effect towards the annual operating costs. The increase of resveratrol concentration demonstrated a decrease in all economic factors measured. A decrease in CAPEX by $13 million and COGS by $48 was observed during a single incremental concentration change from 0.5mg/g to 1.0 mg/g. The cost of ethanol was varied between $1.00 - $3.00 a gallon to align with volatile costs for the commodity currently being faced within the market. As expected, the annual operating cost is expected to heavily move towards the direction of the cost of ethanol since the solvent remains the largest bottleneck in the process. The cost of enzymes was investigated as enzymes are also a commodity which can be subjected to change. Nonetheless, the low amount of enzymes used within the process allowed total costs for enzymes to remain relatively low compared to the annual operating cost, about 1% of the OPEX. Enzymatic conversion values for polydatin to resveratrol is widely detailed in literature for laboratory scale experiments but is limited for large scale processing. A sensitivity analysis was performed varying different possible conversion values and analyzing its effect on capital required for operation. It was determined that a reduction in equipment size was possible when conversions increased thus resulting in lower CAPEX and COGS values compared to the base case. The extraction efficiency of resveratrol from Japanese knotweed in an ultrasonic assisted extractor is a bioprocessing parameter which requires additional research to be conducted in an effort to model with more accuracy. Modeling the process after other plant sources demonstrated a downward trend and decrease in the CAPEX and COGS when extraction efficiency was optimized. Resins were used within the adsorption vessel used to bind and capture resveratrol

arriving from the filtrate. The frequency these resins were replaced and exchanged with new resins was varied between every 25 cycles to 150 cycles in a sensitivity analysis to determine how much it impacted the COGS. Replacing the resin every 25 batches lead to an increase in COGS of about $4 (2.6%) while replacing the resin every 150 cycles only decreases the COGS by $0.43 (<1%). The implementation of the ethanol recycling stream was explored as one solution to combatting the high estimated cost of ethanol required for processing. Additional processing equipment needed for recycling resulted in a higher CAPEX (from $44.7 to $51.6 million) but decreased the COGS (from $149.5 to $141.4/kg). The economy of scales was also a scenario analysis examined using the simulation. The biomanufacturing facility simulation was modeled to produce annual production amounts ranging from 25 MT to 200 MT of resveratrol. As anticipated, the capital required for equipment and operating the facility steadily increased as the production amount increased but the COGS decreased exponentially. The CAPEX for the facilities producing 25 and 200 MT were $26 million and $71 million, respectively. The COGS for the 25 MT and 200 MT cases were $311/kg and $124/kg, respectively. Further sensitivity and scenario analysis can be explored to examine other variables within the process. The cost of Japanese knotweed rhizomes was limited to $0.19/kg for each scenario analysis as calculated for the base case. As the mass of Japanese knotweed rhizomes required for processing varies across different analysis, the production amount is also expected to vary. Calculating the cost of rhizomes for each sensitivity analysis may reflect a more realistic value for each case and improve the overall economics estimates. Another improvement which can be made is using other parametric uncertainty analysis such as using crystal ball or monte carlo rather than using a define set of values to test[1].

Two methods were performed on the model to demonstrate the environment, health, and safety of the simulated facility. The first method which examined the process mass intensity

determine the facility had a PMI value of 529 kg inputs/kg, well under the 7000 kg inputs/kg value shown for a biopharmaceutical facility producing mAbs[2]. When environmental and mass indices were calculated using the methods described by Biwer and Heinzle, the EI's for the inputs and outputs are 26 and 31.6, respectively and the input and output MIs of 480 and 487 kg/kg, respectively[3]. These calculated values allow this stimulated biomanufacturing facility to be classified as operating a safe and environmentally-friendly process.

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