Rearing Black Soldier Fly to Convert Spent Grain to High Value Biomass

Kin Erick Somera

Student Number: 11006375

Massey University

School of Engineering and Advanced Technology

Abstract

The main objective of the study was to provide the engineering expertise to upscale the BSF production of Prescient Nutrition. This study was novel as no published work has been found that attempted designing a full-scale plant to produce BSF biomass was done before. However, some facilities may have tried designing and running a full-scale plant. The project focused on rearing BSF larvae to BSF prepupae and was undertaken in partnership with Plant and Food Research (PFR) and Prescient Nutrition. Values of key parameters were obtained from literature and used in the mass and energy balance of the system. The sensitivity of the balances from each parameter was tested and it was deduced that the larvae, prepupae and spent grain must be characterized nutritionally and the time requirement of the larvae to reach the prepupae stage must be confirmed. Several factors were also optimized for the design.

The feed load was optimized to 18 g dry weight of spent grain per 1 g dry weight of 1-week-old larvae introduced in the system. The ideal feeding regime for the tested conditions was found to be weekly feeding without frass removal. This regime was selected for the design since this feeding regime resulted in comparable results to the regime with frass removal and is more practical. The feed height of 18 cm was found to not have an adverse effect on the growing patterns of the BSF larvae and hence was used in the design of the system. Using this feeding regime (18:1 ratio, 18 cm depth, weekly feeding without frass removal), the biomass yield was estimated to 3.33 kg dry BSF output per 1 kg dry larvae input. Methods for harvesting larvae from the feed were also explored. It was found that force harvesting through heat (32°C to 39°C) is the most cost effective and simplest way of harvesting larvae from the feed.

A pilot study was initially planned with a pilot cabinet that can handle 5 kg wet weight of 1-week-old (1.08 kg dry) larvae and a total of 80 kg wet weight of spent grain (19.84 kg dry, yielding a feeding ratio of approx. 18:1 kg/kg); this pilot unit was fabricated. The larvae were to be grown over 2 weeks to produce 13 kg wet weight of BSF prepupae. However, PFR was not able to provide the necessary larvae for the pilot and hence it was not done. The initial design for the full-scale production was a modular unit that can be placed in the customers' premises. However, the economics and logistics of this design is expensive, with the transportation costs alone reaching \$62 000 per year, and hence a centralized plant operated by 1 operator that can produce a weekly biomass output of 1200 kg dry weight was chosen. This plant will require a land area of about 2000 m² and 26 wet tonnes of spent grain weekly at a feeding ratio of 18:1. The design will have an operational cost of \$270 000 a year and a gross

revenue of $670 000 a year. The capital for the plant was estimated to be $1 540 000 and the NPV of the plant was estimated to be $2 100 000. The internal rate of return (IRR) for the project was calculated to be 31% which is above the average minimum acceptable rate of return (MARR) for new plants which is 15% (Bizfluent, 2018).

Table of Contents

Table of Tables

Table of Figures

Introduction

The world population is projected to increase to 10 billion by 2050 (United Nations Department of Economic and Social Affairs, 2015). This population surge results in the demand for food, such as beef, chicken, pork and fish to significantly increase (Baker, 2015) which would result in higher food demands.

Waste conversion into high value resources involves using the waste generated by an industry or process as a raw product for a different process. The products of these processes can then be used for profit. This type of process provides a way to minimize the waste generation and increase profit creation. The use of Black Soldier Fly (BSF) to convert organic waste from different sectors is an example of this type of process. Organic wastes produced by animals, household, commercial and institutional waste are usually generated in large quantities and can be environmental and human health hazards if not handled properly (Li, et al., 2016). These wastes contain a lot of residual nutrients and energy that would be lost if they were to be placed in landfills (Matufela R. , 2015).The use of BSF in order to convert waste have many advantages over other ways of waste conversion such as odour, biomass, and pollution reduction. Since BSF competes with houseflies, the use of BSF was also beneficial for housefly control (Li, et al., 2016).

The main commercial waste that was studied in this project was brewer's waste or spent grain. This was the waste generated after the brewing of beer. The brewing facility of Garage Project in Wellington, New Zealand would be the supply of the spent grain for the project. The facility initially produced 2 tonnes (wet) of spent grain per day. This amount increased over the course of the study. This type of waste generally contains protein, carbohydrate and ash contents of 20, 70, and 5%, of the dry weight (FAO, 2016), the remaining fraction (5%) was assumed to be mainly composed of lignin. The moisture content and average bulk density of spent grain were set to be 70% (FAO, 2016) and 432 kg per m^3 (Aqua-Calc, 2016), respectively. The BSF can transform the spent grain nutrients in to a biomass which is rich in protein and fat content. The protein, fat, and ash content of the larvae and prepupae were 41, 30, and 21% of the dry weight, respectively, on average (FAO, 2015); the remaining fraction (8%) was assumed to be mainly composed of carbohydrates.

Black Soldier Fly Larvae (BSFL) is high in protien and fat, approximately 40% and 30%, respectively (Sheppard C. , Newton, Thompson, & Savage, 1994). This protien rich substance can be used as a feed for livestock in replacement to the established feed made up of fish meal.

Maize is currently the predominant ingredient (up to 65%) in chicken feed in New Zealand (Poultry Association of New Zealand and Egg Producers Federation of New Zealand, 2011). This results in a competition for fish use as human food and poultry feed. An alternative type of poultry feed providing the necessary protein at a competitive price is therefore needed. BSFL may provide this alternative source of poultry feed (Cullere, et al., 2016) while also having the potential to serve as food for fish farming (Li, et al., 2016; Webster, et al., 2016). Due to these reasons, many studies are currently being conducted on BSFL.

The focus of this study was to provide the engineering expertise to design a growing unit in order to industrialize the production of BSFL. The idea of producing Black Soldier Fly Pre-pupae (BSFP) instead of BSFL was also studied depending on the nutrient content of the BSFP. Many experiments were conducted to ascertain important parameters that were not readily available in literature and prior studies. Many literature values were confirmed using experiments to determine its reliability in a different setting of New Zealand.

This study would aim to determine the optimum type of processing (modular or centralized) for the system. Base lines will also be set for the nutrient content and growing period of the BSF to ensure the accuracy of the balances and the design. Parameters would have to be researched and confirmed to ensure that the design of the plant can be conducted. The life of the BSF will have to be studied through research to determine the necessary parameters for the optimum growth of the larvae and to understand as to which life cycle will require focus.

The growing requirements of the BSF were also researched. This included researching the optimum conditions for each stages of the life cycle and the range of feed that the BSF can thrive in. This was extremely important as the rearing of BSF would be greatly impacted by the conditions that the BSF were placed in. A research on the advantages of using BSF in the waste conversion industry as well as research on patents on this technology was also conducted. This was done to better confirm the suitability of the technology for the purpose it was being built. The patent search was helpful in finding initial points of design as well as providing designs that cannot be used due to patent breach. A research in to the suitability of the spent grain or brewer's waste as a feed was also conducted.

Objectives.

The objectives of the study were:

1) To determine:
   a) Biomass conversion rate.
   b) Residence time to reach maturity.
   c) Optimum feeding regime and feeding ratio.
   d) Protein, fat, ash and moisture content of the feed, 1-week old BSFL, 3-week old BSFL, BSFP and the waste of the system (frass).
   e) Optimum conditions for the growth of BSF.
2) Formulate a design of industrial BSF rearing unit.
3) Run a pilot test to check the validity of the design.
4) Study, propose and describe the possible scale up of the design.

Limitations and Challenges.

There are several challenges that were encountered during the undertaking of this project. This project assumed that 1-week old BSFL would be supplied to the system. The project did not look at the breeding of BSF and at the rearing of the BSF from egg to 1-week old larvae. The project would be limited with regards to the technical and scientific aspects of the life stages of BSF on this part.

The project focused on the technical and engineering aspect of the technology. Hence, the legal requirement and implications of the technology were not fully studied. More work would be required to ascertain all the permits and legality of building the plant and setting up a major production of the product.

The project encountered a challenge on the supply of 1-week old larvae for the pilot study. Plant and Food Research (PFR) was not able to provide the required 5 kg of 1-week old larvae for the pilot study. Due to this, no pilot study was conducted. However, all necessary equipment for the pilot study was completed and stored.

Literature review

Black Soldier Fly

The potential of Black Soldier Flies (BSF) as an effective bio converter of organic waste has been observed since 1916 when L. H. Dunn came across a decaying corpse being voraciously fed on by BSF larvae (Diener, 2010). In 1973, Hale raised the idea of using the common soldier fly to convert waste material into usable, high quality nutrient supplements, stating that the biological degradation and recycling of organic waste materials by the BSF could be one way of alleviating some of the problems of waste disposal worldwide (Hale, 1973).

Description

The Black Soldier Fly (BSF), scientifically known as *Hermetia Illucens*, is a large fly, ranging from 13 mm to 20 mm, belonging to the Diptera order and the Stratiomyidae family (Tomberlin, Sheppard, & Joyce, 2002). The BSF was often mistaken for a wasp in the early days of its discovery. The main differences between the two species are that the wasp has four wings whereas the BSF has only two functional wings and a pair of modified wings called halteres, and that the wasp possesses a proboscis whereas the BSF does not (Diclaro II & Kaufman, 2009).

Figure 1. Adult Black Soldier Fly (BSF)

Barry (2004) reported that the BSF is native of the tropical, subtropical and warm temperature zones of America. Banks (2014) and Diener et al. (2009) reported that the BSF can now be found in different parts of the world especially in tropical and warm regions.

Life cycle

There are five main stages in the life cycle of BSF – egg, larval, prepupal, pupal and adult stages (Banks, 2014). The larval and pupal stages are the longest part of the BSF life cycle (Barros- Cordeiro et al., 2004; Popa & Green, 2012). According to Diclaro II & Kaufman (2009), the adult BSF gains the ability to mate two days after emerging from its pupal case. This is important because the adult BSF lives for 5-8 days after emerging from the pupal case (Barry, 2004). BSFs initiate mating with a process known as lekking, whereby the males engage in a competitive display that entices the visiting female surveying for copulation partners (Institute for the Environment, 2013). Once mating is completed, the females lay their eggs on crevices and cracks located near the food source. The proximity to the food source is necessary to ensure that the offspring thrive (Institute for the Environment, 2013). Each female adult can lay egg clusters of about 500 to 900 eggs (Banks, 2014; Diclaro II & Kaufman, 2009). The eggs normally start hatching 102 to 105 hours after being laid (Booth & Sheppard, 1984).

After hatching, the larvae crawl to the food source (Banks, 2014). The larvae have a translucent body with a pair of black eye spots (Barry, 2004). This stage of the life cycle is the only time when the BSF consumes food (Tomberlin et al., 2002). This makes this stage critical for waste management and conversion. The larvae consume food until necessary levels of fat are stored in its body. This fat is later used by the adult BSFs, since the adults do not feed anymore. The larvae consume food for about two weeks before moving to the next life cycle stage – prepupae (Tomberlin et al., 2002). The larval stage can be prolonged up to four months by reducing the amount of food available (Sheppard et al., 1994).

Once enough fat is stored, the BSF larvae moves into the prepupal stage. This stage is characterized by three important features: i) a darkening of the cuticle from white to dark brown; ii) migration away from the food source to a place of pupation, which ideally should be dry and dark, and iii) the loss of the feeding organ (Banks, 2014; Barry, 2004; Tomberlin et al., 2002). The migration of the prepupae away from the food source allows the remaining larvae to gain better access to the food, as less BSFs are present in the space. The migration also allows the prepupae to find a place of little disturbance, as it is far away from the feeding ground (Institute for the Environment, 2013). This trait is a great advantage for waste conversion because it allows for self-harvesting, which segregates the BSF prepupae from the feed and waste. The prepupae can climb inclines of up to 40° to reach a suitable pupation place (Banks, 2014; Barry, 2004).

Once the prepupae reaches a suitable place, it starts to pupate. Two weeks after the BSF turns into a prepupae, an adult BSF emerges, thus completing the life cycle. The time frames mentioned above vary depending on the environmental conditions present at each stage and the type of food consumed by the larvae (Sheppard et al., 1994). The life cycle of the BSF is shown further in Figure 2 below.

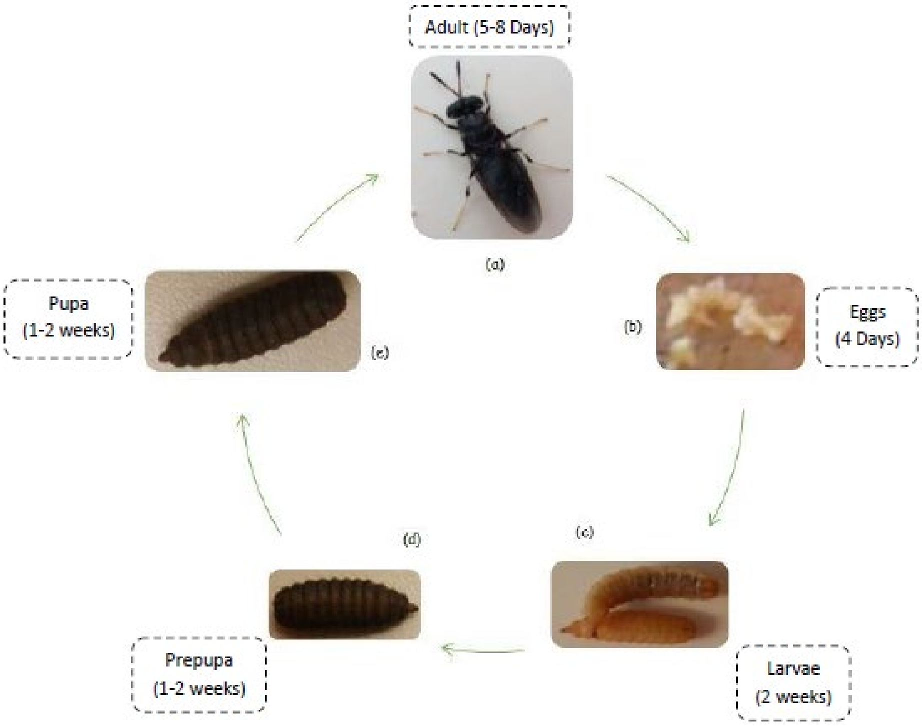


Figure 2. Life cycle of BSF

Advantages of using BSF technology

BSFs advantageously enable to convert organic waste into valuable biomass (Huis, et al., 2013). As discussed above, BSF larvae can feed on different types of feed. Several studies have been conducted to determine the waste reduction potential of the BSF. Barry (2004) reported a 50% of waste reduction when BSF larvae were fed with high fat and protein diet. The diet was added five days a week over 13 days on an incremental schedule because the larger larvae requires more food than young larvae. The moisture of the diet was kept at 72%, the temperatures ranged between 27.90°C to 29.10°C, the relative humidity ranged from 50% to 75% and the light period was kept at 12-hour light to 12-hour dark. Diener et al. (2011) reported 65-75% organic waste reduction depending on the feeding amount and feeding regime used.The highest waste reduction was recorded on larvae with less food but the effective daily waste material reduction was significantly higher in larvae with high feeding rate. Diener et al.

(2011) also reported that daily waste reduction was higher on experiments were extra feed were added on top (780 g/day) of the residue of the previous feeding compared to when the residue and new feed were mixed (740 g/day). The most recent paper reported a 78%waste reduction when BSF larvae were fed with dairy manure (Li, et al., 2011). This high reduction rate was achieved on a controlled environment where the temperature was a constant 27°C and the humidity was 60 to 75%. The larvae, which were placed on the feed a day after hatching, crawled out of the feed 21 days after being placed ready to pupate.Aside from waste reduction, BSF larvae also convert the waste into high value biomass. The fat and protein content of the BSF were found to be 100 to 200 times higher than the value of typical waste (Tomberlin & Sheppard, 2001).

Due to the voracious appetite of BSF larvae, feed processing happens fast. Also, the wrigling and crawling of BSF larvae which enable them to reach all the feed, results in the aeration and drying of the organic waste. This results in the suppresion of bacterial growth and to some extent, of odour reduction. (Diener et al., 2011; Huis, et al., 2013; Li, et al., 2011).

Because BSFs do not have a mouth, they do not interact with human food and do not bite. In addition, the BSF does not engage in pest-like behaviour because its adult life is not associated with human dwellings and is predominantly spent on trying to breed (Institute for the Environment, 2013). Consequently, the adult BSF is not considered as a vector of diseases (Mutafela, 2015). As established earlier, lauric acid is found at high concentration in BSF larvae. This allows BSF larvae to feed on many types of feed and reduce the number of pathogens present in the feed. BSF larvae were shown to reduce the number of *Salmonella* spp and of gram positive bacteria, such as *E. faecalis*, on inoculated feeds made up of a mixture of pig manure, dog food and human feces (Lalander et al., 2015; Park, et al., 2014). The experiment set up was a continous process and the first prepupae were recorded 3 weeks after the start.

BSF larvae were also found to be suitable as fish food. For example, substituting a portion of fish meal, which is the common feed for tilapia, with a meal made up of BSF larvae fed brewer's waste, do not have any significant effect on the growth response of the tilapia (Webster, et al., 2016). The amino acid profiles of fish fed BSF larvae meal were not significantly different than the profile of fish fed with fish meal (Li, et al., 2016; Webster, et al., 2016). A similar study on juvenile Jian Carp showed that carp growth and nutrient utilization was not affected by using a food pellet made from BSF larvae (Li, et al., 2016). The fatty acid composition of the carp was not significantly influenced by the feed (fish meal or BSF larvae) but the Omega 3 levels of carps fed with BSF larvae were lower than those of carp fed fish meal (Li, et al., 2016).

Meal made from BSFL was also found to be suitable for broiler quail – it was indeed reported that there was no significant difference between BSF larvae and fish meals for apparent digestibility and feed choice by quail. The starch and energy content did not differ drastically between the two types of feed. Microbial composition of the excreta of each feed type were comparable. The quail fed BSF larvae produced the toughest and least red meat. These parameters were still within commercial limits (Cullere, et al., 2016).

It was finally reported that complete substitution of fish meal with BSF larvae meal resulted in decreased feed consumption, but this reduction was believed to be due to the high energy content of the BSF larvae meal and the fact that quails stopped eating once their energy requirement were met. BSF larvae meal could be used up to 50% of the total feed without affecting feed consumption. The quails fed on BSFL meal produced more eggs that had stronger shells compared to quails fed fishmeal (Widjastuti et al., 2014).

BSF larvae can also be used to produce biodiesel. The carbon number of BSF larvae fat ranges from 10 to 22 and the fat is composed of 58.2% saturated fat and 39.8% unsaturated fat (Uemura et al., 2017). The biodiesel produced from BSF larvae grease has density, flash point, and kinematic viscosity within the standard EN 14214, which is the biodiesel standard used in most countries. BSF larvae based biodiesel however has a higher acid value (Li, et al., 2011). Residues from anaerobic fermentation of corncob, which produce biogas, can be converted by BSF larvae into biomass; then the larvae grease can be used to produce biodiesel (Li, et al., 2015).

Limitations and Drawbacks

Despite all the advantages of the BSF technology, it still suffers from many drawbacks and limitations. Insect producers, around the globe have reported that operations were not as smooth as advertised (Mutafela, 2015). The main drawback of the technology has been observed mainly on the use of insects as livestock feed ingredients. The issue of insects as human or pet food have been explored during recent years. Nevertheless, the standards and regulations that acknowledge the use of insects as flood ingredients are rare at the international or national levels (Huis, et al., 2013). Despite some legislation touching up on the use of insects as feed ingredients, there is still a lack of legal framework specific to this. This acts as a hindrance for the technology as some investors do not feel comfortable without the legislations to base the technology on (Mutafela, 2015). In New Zealand, strict sanitary regulations for setting up farms, as well as lack of guidelines on the mass rearing of insects could be factors that can drive away potential investors.

Europe have made promising steps in this regard with Regulation (EC) No. 1069/2009 which categorized insect meal as a Processed Animal Protein (PAP) that would be suitable as animal feed if a strict standard of processing was met. Regulation (EC) No. 999/2001, however, categorically prohibited the feeding of farm animals with PAPs (Huis, et al., 2013). The EU also states that insects must not enter the human food chain unless in negligible, unavoidable amounts, and hence provide a barrier on the use of insects as livestock feed (Mutafela, 2015).

Another drawback on insects as feed ingredients is the perception of insects. Generally, insects are perceived as inherently unsanitary (Huis, et al., 2013), especially if the term “fly” is associated to it. Due to the number of sickness and health risks associated with house flies over the course of history, a perception of a harmless fly would seem impossible for some. The unsanitary perception becomes more pronounced when the insect is suggested as a feed ingredient, especially for human consumption (Mutafela, 2015). This perception is a priority area of PROteINSECT, a company that tries to alleviate world hunger using BSF larvae as livestock feed. PROteINSECT focus on the development of Pro-insect platforms, and the dissemination of the platforms’ activities (PROteINSECT, 2017).

Design requirements

A general process for the system would be like Figure 3. This system was to be a modular system to be left in a client's premises. Fresh feed and larvae would be placed in the system periodically.

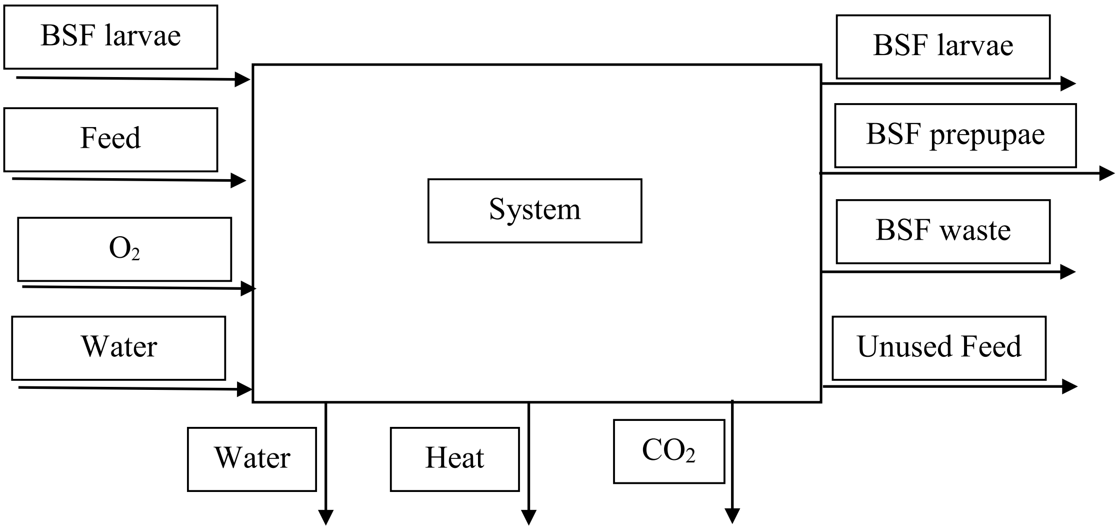


Figure 3. Schematic drawing of the system

For this system to be fully designed, the following parameters must be quantified:

- Feed requirement of the BSF larvae.
- Feed conversion rate of the BSF to biomass.
- O_2 requirement and CO_2 production rate.
- Heat generation of the system.
- Optimum feeding regime.
- Optimum environmental conditions for BSF larvae to prepupae growth.
- Harvesting methods of BSF larvae from the waste.
- Possible restriction on feed bed height in the system.

Growth Requirements

Despite the robustness and resilience of the BSFs different environmental conditions are required at different stages of the life cycle (Institute for the Environment, 2013). This section of the review explores the possible optimum environmental conditions (temperature, relative humidity and feed moisture) needed to raise BSF larvae as this stage is used for waste conversion. Feed requirement, possible feed supply and optimum feeding regimes were also explored.

Larvae activity has been shown to decrease significantly at temperatures below 10°C, while temperatures above 40°C cause larvae to crawl out of the system (Harnden & Tomberlin, 2016). Lipids account for 45% of the weight of the BSFL, lauric acid being the most prominent at 38.4 dry wt% of all lipids (Ushakova, et al., 2016). In humans, lauric acid is converted into monolaurin, which itself serves as an antiviral, antibacterial and antiprotozoal glyceride. Ushakova, et al. (2016) hypothesized that lauric acid serves the same purpose in BSF, thus allowing the BSF larvae to eat and thrive even in pathogenic conditions. Lauric acid has a melting point of 43°C, which may explain why BSFs do not like temperatures exceeding 40°C. Temperature, therefore, needs to be kept between 27°C and 30°C to ensure optimum larvae activity and biomass yield (Mutafela, 2015; Tomberlin, Adler, & Myers, 2009; Zhou, Tomberlin, Zheng, Yu, & Zhang, 2013). For example, Harnden and Tomberlin (2016) reported that larvae reared at 27.6°C were 30% heavier and 5% longer than those reared at 24.9°C. Tomberlin et al. (2009) also reported that female BSF larvae weighed 17-19% more at 27°C compared to larvae reared at 32°C. Other studies have shown that larvae crawl out of feeds that are too moist and that a dry feed results in low biomass conversion (Zhou et al., 2013). A feed moisture content of 70% was observed to support optimum biomass growth (Zhou et al., 2013). Holmes (2012), reported that larvae reared at 70% relative humidity (RH) have the highest adult emergence rate (93%) and longevity of adult BSF (8 days) in comparison to RH 40% (59% and 7 days) and RH 25% (16% and 5 days).

BSF larvae have been reported to thrive on different types of feed ranging from, but not limited to plant and animal based feed up to feed devoid of any solid materials (Tomberlin et al., 2002; Popa & Green, 2012; Zhou et al., 2013). Higher activities of leucine arylamidase, α-galactosidase, β-galactosidase, α-mannosidase and α-fucosidase were observed from the gut extracts of the BSF larvae compared with those of house fly larvae. These findings may explain previous reports that the BSF larvae can digest food wastes and organic materials more efficiently than any other known species of fly (Kim, et al., 2011). Nguyen et al. (2015) indeed reported that BSF larvae can thrive in different diets of 1) poultry feed, 2) pig liver, 3) pig manure, 4) kitchen waste, 5) fruits and vegetables. Kitchen waste, which has the highest fat content, produced the heaviest and longest larvae. BSF larvae were also found to thrive in feedrich in organic solutes but devoid of any particulate matter such as sewage water, a whey solution, compost leachate and milk. Relative to its initial wet weight, the average BSF larval weekly mass gain was 15.2% in sewage water, 39.5% in whey, 70.7% in compost leachate, and 173.7% in milk (Popa & Green, 2012). The larvae were also found to thrive in animal wastes (Banks, 2014; Diener, 2010; Li, et al., 2011).

Nguyen et al. (2013) reported that daily handling of the BSF larvae, to mix fresh feed to the system and weigh the larvae, resulted in prepupae that were 12-15% lighter and shorter compared to prepupae from larvae that were undisturbed. Mutafela (2015) also reported that batch feeding results in heavier prepupa in shorter time than prepupa from larvae fed and disturbed daily. The optimum feeding requirement depends on the type of feed used. Diener et al. (2009) reported that 100 mg per larvae per day was the optimum feeding rate for chicken feed while Nguyen et al. (2013) reported that 30 mg of kitchen wastes per larvae per day provided the necessary nutrients for the larvae to prepupate. Lalander et al. (2015) reported about 750 mg per larvae per day of pig and human feces were required to provide the BSF larvae enough nutrition to prepupate within 3 weeks.

Spent grain/Brewer's waste

As established earlier, the feed requirement of the BSF larvae rely on the type of feed used. To narrow the scope of the study, spent grain or brewer's waste was used as the feed for the larvae. This was selected as it was readily available for the study and due to its homogeneity and non-volatile nature.

Spent grain is generated during the mashing process and is removed from the brewing process from the mash before the boiling step of brewing. Spent grain is an abundant brewery by-product that is high in protein (more than 20% dry wt.) and fibre and can be used as animal feed or, in some instances, for human consumption (Kerby & Vriesekoop, 2017). Aside from the high protein content, spent grain provides the essential amino acid containing nutrients that animals require (Mussatto, 2014). Craft brewers typically sell wet spent grain in the form of cake for ruminant feeds.

Spent grain can also be used for composting although, the high moisture content could cause issues (Thomas & Rahman, 2006). A different waste streams must therefore often be added to properly compost spent grain (Stocks et al., 2002). Some large breweries also use spent grain to produce biogas, using anaerobic digesters located either onsite or at a third-party energy producer. The biogas was produced using anaerobic digesters which produces mostly methane gas (Deublein & Steinhauser, 2011). Using spent grain for biogas production however requires a large quantity of grain to be economically viable (Kerby & Vriesekoop, 2017).

Intellectual Property

Due to the commercial nature of the project, a preliminary patent search was conducted to ensure that the proposed design would not result in any patent breach. The patent search was limited to approved patent. Terms larvae, black soldier fly, *Hermetia Illucens*, rearing, insects, attractant, dried distiller grain, and collection were used in the search.

One such invention that was granted a patent for includes a method and apparatus for rearing insect larvae (United States of America Patent No. US5351643A, 2004). This apparatuses made of an enclosed rearing unit with three sections: 1) a diet space, 2) a larval space, and 3) a frass space. The diet space is large and filled with the feed. The larval space is made of perpendicular tubing spaced out evenly along the diet space. The larvae are placed in the tubing reach the feed via holes perforated in the tubing. The frass space is situated below the larval space and is used to collect the frass generated by the larvae (United States of America Patent No. US5351643A, 2004). This invention provides an interesting set-up for introducing larvae into the feed but still requires the prepupae to be separated by hand once feeding is completed.

United States of America Patent No. US8733284B2 (2014) describes a device that can handle the whole life cycle of the BSF. This device is made of an opaque circular tubing for pupation attached to an opaque box for larval feeding and a circular mesh tube that allows flies to mate. This technology also uses self-harvesting to separate larvae and prepupae. Using this technology, BSFs can be left alone to develop as long as the condition permits. The weakness is however deemed to be the lack of controls and suitability under cold climate. There is also, no technique for seperating larvae from feed.

One of the technical challenges of the project was to separate the larvae from the feed. One of the technology that was investigated is based on the use of light to repel BSF larvae away from the feed. Another way of attracting the larvae away from the feed is through smell. A patent for a controlled release of a carrier gas that has a targeted scent for an insect larvae was filed and granted (United States of America Patent No. US4563344A, 2007). This technique could be utilized for this project however, a target scent would need to be identified and isolated.

Knowledge gaps

Despite the numerous advances of using BSF in the conversion of waste, there are still some gaps on the knowledge of the technology. Many key parameters required by the project have not been studied. The O_2 requirement and CO_2 production rate of the BSF larvae have not yet been quantified and studied. Several studies have looked at the nutritional content of the different life stages of the BSF but the heat generation of the rearing of the larvae have not been fully studied. The self-harvesting ability of the BSF prepupae have been taken advantage on multiple studies but this also resulted in the lack of research into other forms of separation technique for the larvae and waste.

The ability of the BSF larvae to thrive in a wide range of feed resulted in a lack of study on the maximum bed height of the feed. The maximum feed bed height is a crucial parameter as the feed bed could suffer from compacting issues which could prohibit the BSF larvae growth. However, the different types of feed would result in different feed bed height due to the varying feed characteristics. Despite the number of studies that indicates that BSF larvae waste conversion can be industrialized, no studies have been conducted yet to explore the economic feasibility and design a potential system for this.

This study would try to quantify and explore these gaps in the literature.

Methods

System Scheme

The focus of this project is to design a pseudo-modular system which would allow the production of BSFL on fish and poultry farm. This project would focus on rearing black soldier fly until the larvae matures into a prepupa. The prepupa can then be used directly as feed or can be processed to pellets.

In order to provide a better direction for the project, some restrictions in the form of assumptions were created. The general assumptions with regard the system and the projects were:

- The average age of the larvae that would be introduced in the system is 1 week old.
- The larvae would stay in the system for 2 weeks.
- All of the tasks that the operator would need to do on the system would only be 8 hours per week.
- The temperature, moisture and oxygen concentration inside the system would have to be controlled.
- Water and leachate can be drained from the system.
- The prepupae would be allowed to self-harvest into a collection box.
- The prepupae can be fed directly to other animals.
- The primary feed to be used would be spent grain or spent grain.

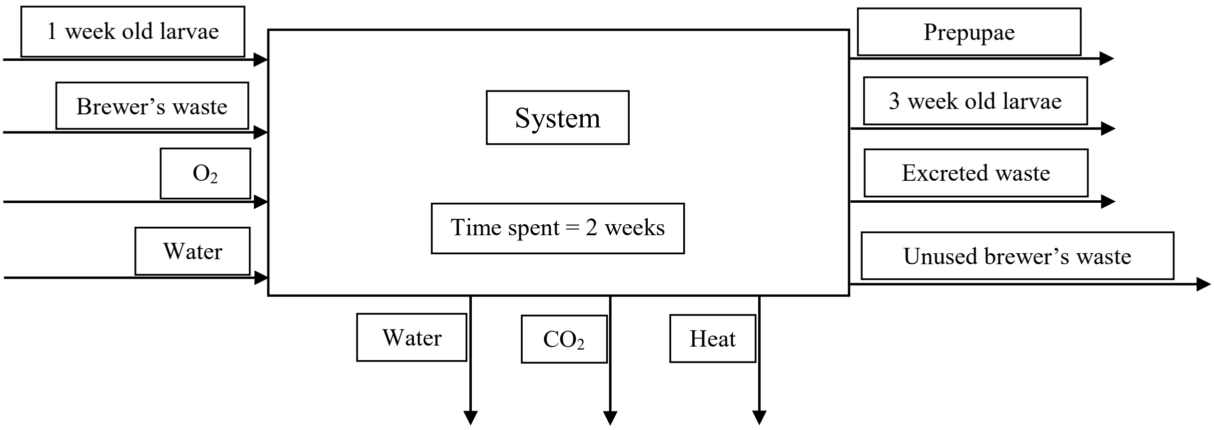


Figure 4. Schematic drawing of the system's incoming and outgoing mass and energy flow

The schematic diagram of the system can be seen on Figure 4. This scheme shows the flows of mass and energy. Based from the assumptions outlined previously, 1-week old larvae would be introduced in the system. Aside from the larvae, a specified amount of spent grain would also be placed in the system. The moisture content of the feed was to be controlled at 70%, as this was the ideal concentration as revealed in the literature survey. The oxygen content and moisture content of the system would be closely monitored and controlled.

The temperature and moisture control were deemed essential for the system. Therefore, these parameters need to be kept at an optimum range of 25°C to 30°C to ensure the optimization of the larvae activity and biomass conversion.

During the two weeks that the larvae would spend in the system, many of the larvae would reach the prepupae stage. This prepupae would be allowed to self-harvest. The other larvae that would not turn into prepupae would be harvested after 2 weeks in the system. The excreted waste as well as the unused spent grain would be collected. These two "wastes" would be collected as one as these two would form a mixture.

Initial Mass and Energy Balance

Mass and energy balances were conducted for the scheme shown in Figure 1 in order to quantify the engineering parameters governing it and to assess the relationship of these parameters. The values used for the initial mass and energy balance were gathered from journal articles, experiment conducted in Plant and Food Research (PFR) and research conducted by organizations outside the traditional academic publishing or distribution channels.

The nutritional content of the spent grain that would be used as the feed for the black soldier fly larvae were shown in Table 1. These nutritional contents were taken from Food and Agriculture Organization of the United Nations (2016). These nutritional contents were taken as the percentage of the dry weight of the feed with exception to the water content which was on a wet weight basis. According to Aqua-Calc (2016), the average density of spent grain is 432 kilogram per cubic meters. The "other" nutritional content was assumed to be primarily lignin for the spent grain.

Table 1. Nutritional content of the Spent grain (Food and Agriculture Organization of the United Nations, 2016).

Protein	20%
Carbohydrates	70%
Ash	5%
Other	5%
Water	70%

The breakdown of the fate, or usage, of feed can be seen on Table 2. This breakdown was obtained from early experiments conducted by PFR prior to the present study.

Table 2. Breakdown of fate feed. Values were assumed to be percentage of dry weight of feed.

Biomass conversion	5%
Feed lost within the system	35%
Undigested + waste	60%

The nutritional content of the larvae and prepupae were assumed to be similar. This nutritional content was obtained from Food and Agriculture Organization of the United Nations (2015) and tabulated on Table 3. These nutritional content were taken as percentage of the dry wiegh of the black soldier fly except for the water content which was taken as the percentage of wet weight. The "other" nutritional content for the black soldier fly was assumed to be carbohydrates.

Table 3. Black soldier flies nutritional content (Food and Agriculture Organization of the United Nations, 2015).

Protein	41%
Fat	30%
Ash	21%
Other	8%
Water	63%

The full mass and energy balance calculation were outlined and explained in Appendix 1.

The dry weight mass balance was calculated by using the breakdown of feed shown in Table 2 to ascertain the necessary feed required per larvae. Using a set parameter for the output dry biomass, the amount of Black Soldier Fly required was worked out. Once the amount of feed and number of Black Soldier Fly were known, the nutritional content shown in Table 1 and 3 was used to provide the breakdown of the weights, Table 44 in Appendix VI show the results.

In order to quantify the oxygen requirement for the system, a chemical oxygen demand (COD) balance was conducted. The oxygen demand of each component was worked out and the difference on the oxygen demand of the outputs and the inputs was deemed as the oxygen used within the system. Using the COD balance, the oxygen requirement for the system was calculated. This value was used to calculate the amount of air required by the system. The air requirement calculated by the COD balance would result in oxygen depletion as all the oxygen would be used for the oxygen demand. It was deemed that the total oxygen concentration in the system should not fall below 18% by volume in the gas phase. Oxygen levels lower than this could result in extra stress for the Black Soldier Fly and very low oxygen levels would result in fatality. Due to this, the total oxygen demand would be six folds than the calculated COD. This was done to ensure that once all the COD oxygen was used up, the total concentration of oxygen in the air will not fall below 18%.

The amount of carbon dioxide that would be produced by the system was calculated using a total organic carbon (TOC) balance. The total organic carbon was calculated for each stream of the system. The difference of the carbon content was then used to work out the weight of CO_2 produced by assuming that the carbon lost within the system could only be "lost" as CO_2.

In order for the energy balance to be worked out, the energy content of the different components were assumed. The energy content of the different components of the spent grain were shown in Table 4 (Winnie, Arjen, & Theo, 2008). The energy content of the similar components of the Black Soldier Fly were assumed to be of the same value.

Table 4. Energy content of the different component. Taken as gram of dry weight (Winnie, Arjen, & Theo, 2008).

Component	High Heating Value (kJ/g)
Carbohydrates	17.3
Fats	37.7
Proteins	22.7
Lignin	29.9

The energy balance was calculated simply as the difference between the energy content of the output streams and the energy content of the input streams. The difference would be the heat consumed or generated by the system.

Balance sensitivity analysis

The mass and energy balance conducted from the previous section relied heavily on many assumed values. These values have a great impact on the designing of the final product. The impact of these values would vary but there would be some key elements that needs to be fully quantified. Therefore, to assess which parameters would be required to determine experimentally, a sensitivity analysis was conducted.

The values obtained from literature and PFR were considered as the reference values. These values were then increased and the changes on the resulting parameters were recorded. The BSF larvae weight and prepupae weight were increased by 50%, individually, and the changes to the feed required, number of larvae required, CO_2 produced, and COD were recorded. The fates of the feed were increased by 10% individually and the effect on feed required, CO_2 produced, and COD were recorded. The added 10% to the biomass conversion, and metabolized and excreted fates were taken from the non-digested percentage. The added 10% to the non-digested percentage was taken from the metabolized and excreted fates during the respective tests. Similarly, the energy contents of the component of the spent grain and prepupae, such as the fat, protein and carbohydrates, were increased by 50%, individually, and the effect on heat generation was recorded.

The relative difference of each change was calculated as the ratio of the difference of the reference and new values and the maximum of the two values. This will give an insight on how sensitive the system is on the changes in the parameter.

Setting baselines

The nutritional content of the spent grain dictates how much biomass would be available for the Black Soldier Fly to feed on. This is an important aspect as the rate of biomass growth would rely heavily on how much available nutrients are accessible to the BSFL. Similarly, for the mass balance conducted previously, the waste and unused biomass were assumed to have the same nutritional content as the initial feed. This could possibly not be correct as the waste biomass have already been digested by the BSFL. Therefore, it would be important to know the actual nutritional component of this waste. The knowledge of the nutritional content of the waste would be beneficial in correctly calculating how much energy the system would release as breaking down of chemicals would likely result in heat.

The mass and energy balance relied heavily on the feed use experiment conducted by PFR shown in Table 2. This breakdown of how the spent grain would be used was essential in identifying how much feed would be needed and how much waste would be produced. This key element of the mass balance must be confirmed through an experiment.

The average weight of the initial larvae and final prepupae was directly responsible for the amount of BSFL that was required to obtain the necessary throughput of 100 kilogram of dry biomass. The difference between these two weights give a constraint as to how much biomass can be produced by a single larva. Therefore, this needs to be confirmed in a laboratory instead of being assumed.

Similarly, the nutritional components of the Black Soldier Fly at the different stages of its life cycle has to be confirmed. The nutritional components have an indirect impact on the amount of energy produced. The nutritional content of the Black Soldier Fly was also assumed to be constant for all stages. This have to be confirmed in order to fully understand the amount of nutrients that can be derived from the bugs.

The average time required by the larvae to reach pupation was also assumed to be 2 weeks for this mass balance. This time requirement must be confirmed as this dictates how much time would be required for the BSFL to stay within the system.

The densities of both the spent grain and the Black Soldier Fly at the different stages play an important role on the designing of the product. These two densities would directly dictate the amount of volume required for a certain amount of dry biomass requirement. This space requirement relies heavily on these two densities.

The energy content of the different streams must be confirmed. For the sake of this mass balance, the energy content of each individual stream was calculated using an assumed heating value. The actual heating value can be different to the assumed value as there would be different chemicals present on the spent grain, Black Soldier Fly and waste stream. Therefore, this must be tested.

The respiration rate of the bugs can also be easily experimented on to confirm whether the COD agrees to this. Similarly, a total organic carbon can be calculated indirectly and confirmed.

Feed characterization.

Two different batches of spent grain were compared – a frozen sample was obtained from PFR and a fresh sample was collected from the Garage Project on January 13th 2017. For the purpose of this experiment, the sample obtained from PFR was referred to as "*frozen*" while the sample obtained from the Garage Project was referred to as "*fresh*". Two 500 mL beakers were weighed and filled with one type of feed until the 400 mL of each feed. The filled beakers were then weighed again and then filled with water until the spent grain was submerged. The densities of the two feeds collected were obtained by dividing the spent grain weight by the volume of the spent grain. The amount of water gained at saturation was calculated as the difference between the saturated weight and the initial weight of the spent grain. The samples were thus left for 20 minutes to allow the spent grain to become saturated.

The beakers were finally drained of water using a strainer and weighed again to calculate the change in mass due to water sorption. These samples were then incubated at 30°C for a week to assess the stability through pH levels.

An additional experiment was conducted where two 100 mL beakers, were supplied with 10 grams of each feed. The beakers were then filled with water until the 70-mL mark and the beakers were stirred with a glass stirrer for 20 minutes. The water was finally drained and used in order to measure the pH of the spent grain using a CyberScan pH 510 meter.

The moisture and ash contents of the feeds were measured by subsequently drying and incinerating known amounts of feeds at 105°C (2 days) and 550° (2 hours), based on standard methods (Reeb & Milota, 1999) and (Sluiter, et al., 2005).

Feed sample were sent to the Nutrition Laboratory of Massey University for a fat, protein and energy content analysis. Left over "fresh" feed was then frozen for later use.

Respiration

A total of 51 larvae (approx. 1 week old) were obtained from PFR and counted into 120 mL Wheaton flasks. These flasks were thus supplied either 0 (control), 1, 5, 10, 15 or 20 larvae, sealed using a septum and a metal cap to prevent gas exchange and incubated for 24 hrs at room temperature. Gas samples (6 mL) were then withdrawn from each flask using a syringe and analysed using gas chromatography to quantify the CO_2 content.

An additional test was conducted to assess the impact of feed supply on O_2 used by the larvae. Three Wheaton flasks were prepared with one flask with neither feed nor larvae, one flask supplied with 5 larvae and 2 grams of fresh spent grain, and one flask supplied 5 larvae but no feed. All flasks were then sealed as described before and incubated for 24 hrs (25°C, 75% moisture content and complete darkness) before 6 mL gas samples were collected and analysed.

Another test was conducted to quantify the hourly O_2 consumption and CO_2 production of 1 week old larvae. A total of 6 Wheaton flasks were prepared: 5 flasks were supplied with 5 larvae and 2 g of fresh spent grain and the remaining flask was supplied 2 g of fresh spent grain but no larvae to serve as control. The flasks were then sealed and incubated at 25°C. Every hour, a 6 mL gas sample was withdrawn from a new bottle and used to quantify the O_2 and CO_2 content.

Once the experiment was completed, the septa were replaced with pierced aluminium foil to allow gas exchange and the flasks were thus incubated for one week with 2 g of feed at 25°C and 75% moisture content. After 1 week, the flasks were sealed and rates of O_2 consumption and CO_2 production were quantified again. After the test, the septa were again replaced with aluminium foil and incubated at the same conditions to allow the larvae to reach the prepupae stage. Once this was achieved, the respiration rates were quantified again.

Another set of similar experiments were conducted on 20 1-week-old larvae with food, 20 mature larvae with food, 20 prepupae with food, and 20 prepupae without food.

Feed use and larvae characterization

Two hundred 1-week-old larvae were obtained from PFR. These larvae were washed with water and then separated into two groups of 100 larvae each and labelled Group A and Group B. A 50-mL beaker was weighed, and Group A placed in the beaker. The beaker was weighed again to get the collective weight of Group A. From this, the average weight of 1 larvae was calculated.

Group A was then placed in a freezer for 24 hours to deactivate them. Once deactivated, Group A was placed in a clean and weighed 50 mL beaker. The level that the group has reached in the beaker was marked using a white board marker. The group was then separated into two groups – Group A1 and Group A2. The beaker was then filled with water until the mark made by the level of the larvae were reached. The beaker containing the water was weighed again. The weight of the water in the same volume as the larvae was used to determine the bulk density of the larvae.

Group A1 was placed in a small sample bag and was sent to Nutrition Laboratory for analysis. Group A2 was placed in a container. This group was then placed in the 105°C oven for 24 hours to dry. Once the drying was complete, the container with the dry larvae were weighed again to calculate the moisture content. The dried larvae were then crushed and placed in a weighed crucible as seen in Figure below. This cuvette was then placed in a 550°C furnace for 2 hours. After this treatment, the crucible with the ash was then weighed to obtain the ash content of the larvae. These characteristics were deemed to be uniform to both Group A and Group B.

Figure 5. Crushed larvae

Group B was placed in a 300-mL beaker. According to the initial mass balance performed, shown in Appendix 1, each larva would need a total of 1.3 g of food to pupate. This amount of feed would be introduced in a weekly feeding in a span of two weeks. Hence the total feed placed in the beaker was calculated by multiplying the feed requirement by 100 larvae and dividing it by two feeding. The feed obtained from the Garage Project was used as the feed for this experiment. This beaker was then covered by aluminium foil with holes and placed on top of a large container. This was done to catch any prepupae that might leave the beaker. This set-up was placed in a temperature and moisture controlled environment with the parameters of 25°C and 75% moisture. This set-up was left undisturbed for 1 week.

After 1 week, the large container was weighed, and the contents of the beaker were tipped in the container. The larvae and prepupae were separated into two clean and weighed sample bags. Once all the larvae and prepupae have been segregated to the respective sample bags, the container was weighed again. The beaker that was used for the experiment was also weighed to calculate the waste and unused grain that was left behind. The bags containing the larvae and prepupae were then weighed. The larvae and prepupae were then cleaned using a water wash and placed on two new sample bags. The sample bags with the clean larvae and prepupae were then weighed. This allowed for all the unused and wasted spent grain to be accounted for.

The prepupae were placed in a freezer for further analysis later into the experiment. The remaining larvae were placed on a clean and weighed 300 mL beaker. The amount of feed added to this beaker was adjusted based on the remaining number of larvae present in the experiment. This was again sealed by an aluminium foil cover with holes and placed on a temperature and moisture controlled environment.

Two weeks after the start of the experiment, the same process of separating the prepupae and larvae and weighing of containers and beakers were conducted. The remaining prepupae and larvae were then placed in the freezer for 24 hours to deactivate them. Once this was done, the prepupae from the 1st week and the prepupae from the end of the experiment were placed in a 100-mL beaker. The similar way of calculating the bulk density as in Group A was done to determine the bulk density of the prepupae. Half of the total prepupae was then used to obtain the moisture and ash content, like how it was done for Group A. A picture of the dried prepupae can be seen in Figure 6 below.

The remaining half was then sent to the Massey University Nutrition Laboratory for fat, protein and energy content analysis. Due to the low number of larvae left after 2 weeks in the experiment, all the remaining larvae were used to determine the ash and moisture. None of the remaining larvae were sent for the fat, protein and energy content.

Figure 6. Dried prepupae

Optimization Experiments

Once the baselines for important parameters were known, optimization experiments were conducted to provide the most efficient biomass conversion.

Feeding ratio

Experiments were conducted to reduce the amount of feed introduced in the system. 3 replicates of group B for the feed use experiment were conducted. These experiments were to serve as replicates for group B as well as controls for the feed optimization experiments. Based on the values used for the feed use experiment, it was calculated that a total of 12 grams of dry weight of feed per 1 gram of dry weight of 1-week-old larvae were placed on the system at the first feeding and a ratio of 6 grams of dry weight of feed per 1 gram of dry weight of mature larvae for the second feeding.

Two replicates were made for a feeding ratio of 6 grams of dry weight of feed per 1 gram of dry weight of the larvae at each feeding. This was conducted by firstly randomizing a group of 1-week-old larvae to obtain 100 larvae for each replicate. Each replicate was placed in a special container that was purpose built to have a feed bed height of 3-3.5 cm. The diameter of the container was adjusted to obtain the necessary height using known averages of the larvae weight and the density of the feed. Each group of larvae were cleaned, dried and weighed before being placed in the container. Using the moisture content calculated on the previous experiments, the dry weights of each group were then found. The necessary dry weights of the feed were then calculated and using the moisture content of the feed, the wet weights of the required feed were calculated and placed on the respective group. After one week, the system was weighed and the larvae and prepupae were separated from the frass, cleaned, and dried. The prepupae were then separated from the larvae and placed in the freezer to be deactivated. The weights of the larvae were then collected to calculate the required feed for the next feeding. After the second week, all prepupae and larvae were collected, separated, and frozen. The frass from each feeding were also collected and frozen. The moisture content and ash content of the larvae, prepupae and frass were calculated using the same protocols outlined before. Two sets of replicates for the feeding ratios of 3:1, 4:1 and 5:1 were also made using the same method outlined before.

The biomass conversion yield, and biomass output were calculated from each replicate. Two types of biomass conversion yield were calculated. One was calculated as the ratio of sum of all output BSF biomass subtracted by the amount of initial BSF biomass and the sum of the initial feed subtracted by the amount of frass formed. The other one was calculated as the ratio of sum of all BSF output biomass subtracted by the amount of initial BSF biomass and the total weight of the initial feed. The biomass output was calculated as the ratio of the sum of all output BSF biomass and the total weight of the initial feed.

Feeding regime.

Three types of feeding regime were looked at: 1) Partial feeding at week 1, clean and harvest, and then partial feeding at week 2 (control), 2) Full feeding at week 1, 3) Partial feeding at week 1 then partial feeding at week 2.

One replicate for 2 and 3 were created. The larvae were randomized, cleaned, weighed, and placed in a container like the ones on the previous experiments. For both experiments, the total amount of required feed was calculated based on the initial larvae weight of each group. The feeding ratio used was 12:1 and then 6:1. For 2, the whole amount of feed was placed at the start and the experiment was untouched for 2 weeks. For 3, a third of the feed were placed at the first week, and then the remaining feed were placed on the start of the second week. For both feeding regimes the ash and moisture content of the larvae, prepupae and frass were measured. The biomass conversion yield, and biomass output were calculated from each group in the same manner that was done before.

<u>Bed depth and feed type experiment</u>

The maximum allowable height of the bed depth had a great impact on the area requirement of the design. Due to this, experiments were conducted to find out the maximum bed depth height of the feed without sacrificing too much in terms of biomass gained.

The feeding ratio of 6:1 was used for this experiment. It was calculated that this feeding ratio would amount to a total of 18 gram of dry weight of feed per 1 gram of initial 1-week-old larvae. Two types of feed were used for this experiment – minced brewer’s waste and not minced brewer’s waste. A total of 1200 larvae, collected from PFR, were cleaned, dried, randomized and placed into 8 groups. Groups 1 to 4 had 100 larvae each and were labelled A1, A2, B1, and B2. Groups 5-8 had 200 larvae each and were labelled C1, C2, D1, and D2. The required feed for each group were separated in to three parts. One part of the feed was placed on the first week and two parts of the feed were placed on the second week. This would mean that for the second week, the height of the feed would be triple than the height for the first week.

For this experiment, 4 bed heights were looked at – 3 cm, 6 cm, 9 cm and 18 cm. Special containers were created from plastic containers to achieve these heights. Using the average weight of the larvae, the expected weight of 100 larvae were calculated to be 2.71 g which would require 66.2 g of minced brewer's waste. Using the density of the minced brewer's waste and the larvae, the volume requirement of the containers was calculated. Using a design parameter of 9 cm required height, the diameter of the container was calculated to be 4.6 cm. This implied that once all minced brewer's waste requirement was placed on the container, the height inside of the container should be 9 cm. Hence, for the first week, where only a third of the feed was placed, the height should be 3 cm. The same calculation was conducted for the not minced brewer's waste to calculate the diameter of the container. These containers should give the 3 cm and 9 cm experiments for both the minced and not minced feed. To obtain the required diameters for the 6 cm and 18 cm, the number of larvae were doubled to 200. This would result in the bed height to be 6 cm and 18 cm for week 1 and 2, respectively.

Two replicates of each type of feed for each bed height were made. A1 and A2 were replicates of 3 cm and 9 cm height for the not minced waste. B1 and B2 were replicates of 3 cm and 9 cm height for the minced waste. C1 and C2 were replicates of 6 cm and 18 cm height for the not minced waste. D1 and D2 were replicates of 6 cm and 18 cm height for the minced waste.

The moisture content, ash content, and mortality rate of each replicate were calculated.

Harvesting methods.

The BSF have a self-harvesting method once it reached the prepupae stage. However, there were no established method of separating the larvae from the feed. Several experiments were conducted to try and achieve this separation.

Sedimentation method

Two 100 mL beakers were filled by 50 larvae each. One beaker was filled with 30 g of frass from minced brewer's waste while the other beaker was filled with 30 g of frass from not minced brewer's waste. After 30 minutes, the contents of the beakers were tipped in separate 300 mL beakers filled 3/4ths of the way with water. The beakers were then left and checked every 30 minutes to check whether the larvae would have floated, or separate layers of frass and larvae occurred. After 3 hours, the contents of the beaker were emptied.

Screening/sieving method

Two 100 mL beakers were filled by 50 larvae each. One beaker was filled with 30 g of frass from minced brewer's waste, and labelled A, while the other beaker was filled with 30 g of frass from not minced brewer's waste, labelled B. Four screen sizes were used – 2.4 mm, 2.8 mm, 3.6 mm, and 4.8 mm. After 30 mins, the contents of beaker A were placed in the 2.4 mm sized sieve. The contents were spread along the sieve and the sieve was shaken. The amount of biomass that passed through the sieve were recorded. This was done for beaker B as well before moving to the next sieve size.

Washing method

This was done similar to the screening method. However, once the frass and larvae were placed on the sieve, a low-pressure tap running water was allowed to pass through the frass and sieve. This was done on the same sieve sizes that were used previously.

Food attraction method A

40 1-week-old larvae were collected from PFR and then separated to 2 groups of 20. Halfway lines were drawn on two filter papers. One side of each filter paper was labelled A while the other half was labelled B. 50 g of sugar was placed on side A of each filter paper and the larvae were placed in the middle of each filter paper. This was done to check whether the larvae would be attracted to the smell of a potential food source.

Food attraction method B

A 1 litre ice cream tub container was divided into two by placing a 2-cm high divider in the middle. 20 1-week-old larvae were placed on one side while 50 g of not mince brewer's waste was placed on the other side. The container was closed, and some holes were punctured on the top to allow air exchange. The container was checked every hour for 5 hours to count how many larvae moved to the area with food.

Forced harvesting through heat

Two 0.5 cm diameter holes on opposite walls of the container were created in a 12 cm long by 8 cm wide by 10 cm high Styrofoam container. The inlet and outlet tubing of a water bath, with a temperature of 40°C, were inserted on the holes. A heater was created by connecting three 7-cm long metal pipe with flexible plastic. The heater was placed at the bottom of the container and connected to the inlet and outlet tubing of the water bath. 20 1-week-old larvae and 30 g of frass from not minced brewer's waste were placed on top of the heater. The water from the water bath could pass through the heater and hence heat up the frass and larvae. Three holes that were 3 cm higher than the tubing holes were created to allow exits for larvae. The temperatures looked at for this experiment were 25°C, 28°C, 30°C, 35°C, and 38°C. During each temperature, the system was untouched for 2 hours to check for any crawling out of the larvae. The time for each crawling out was recorded.

Pilot study and Full-scale production

A pilot-scale cultivation cabinet containing a rearing tray was then sized to accommodate 5 kg wet weight of 1-week-old larvae over 2 weeks at 27°C to 30°C. Based on the larvae characterization and feed ratio experiments, the amount of feed to be used was estimated to be 20 kg dry weight, or 80 kg wet weight, and will produce 4.4 kg dry weight, or 13 kg wet weight, of BSF prepupae. During fabrication, PFR announced that it would not be able to supply the 1-week-old larvae required for the pilot study. Due to this, the pilot cabinet was stored in Prescient space and would wait until the required 1-week-old larvae can be supplied before it can be used.

A full-scale production unit was designed for the project and the economics was studied.

Results and Discussion

The results were outlined by first identifying the initial mass and energy balances and then providing the sensitivity analysis to the values used for the balances. The key parameters that the balances are sensitive to were then determined experimentally. Optimization experiments were then conducted to optimize certain aspects of the design. A pilot study was then attempted, and a full-scale design was conducted.

System Scheme

A schematic representation of the production system can be seen in Figure 4. In this system, 1-week old larvae are introduced together with a specified amount of spent grain. The moisture content of the feed is controlled at 70 % (Zhou, et al., 2013) (Matufela R. N., 2015). The oxygen content of the system is monitored and controlled.

Temperature and moisture control are deemed essential: Larval activity has been shown to decrease significantly below 10°C while temperatures above 40°C results in premature crawl out of the larvae (Harnden & Tomberlin, 2016). Further studies also showed that the larvae crawled out of feed that were too moist while having dry feed results in low biomass conversion (Zhou, et al., 2013). Therefore, temperature need to be kept at an optimum range of 25°C to 30°C to ensure the optimization of the larvae activity and biomass conversion (Zhou, et al., 2013) (Matufela R. N., 2015) (Harnden & Tomberlin, 2016).

During the two weeks spent in the system, many larvae are expected to reach the prepupae stage. These prepupae will be allowed to self-harvest. The remaining larvae will be harvested. The excreted waste and unused spent grain will be collected together.

Water may exit the system as leachate and as moisture in the outgoing gas. Oxygenated air will be pumped to the system to remove the CO_2 produced by respiration and to replenish the O_2 consumed by the larvae. The larvae and prepupae activity will also cause the generation of heat that needs to be extracted to keep the temperature constant.

Initial Mass and Energy Balance

The balances for this part of the report were based on the assumptions outlined previously and the data gathered from literature and PFR. A partial dry weight mass balance is summarized on Figure 7 (mass is lost via gases such CO_2 but an exact mass balance cannot be provided as mass is also gained and lost via H_2O). As can be seen, and based on the data used, 1142 kg of spent grain is required to produce 100 kg of prepupae, thereby generating 685 kg of unused feed and wastes.

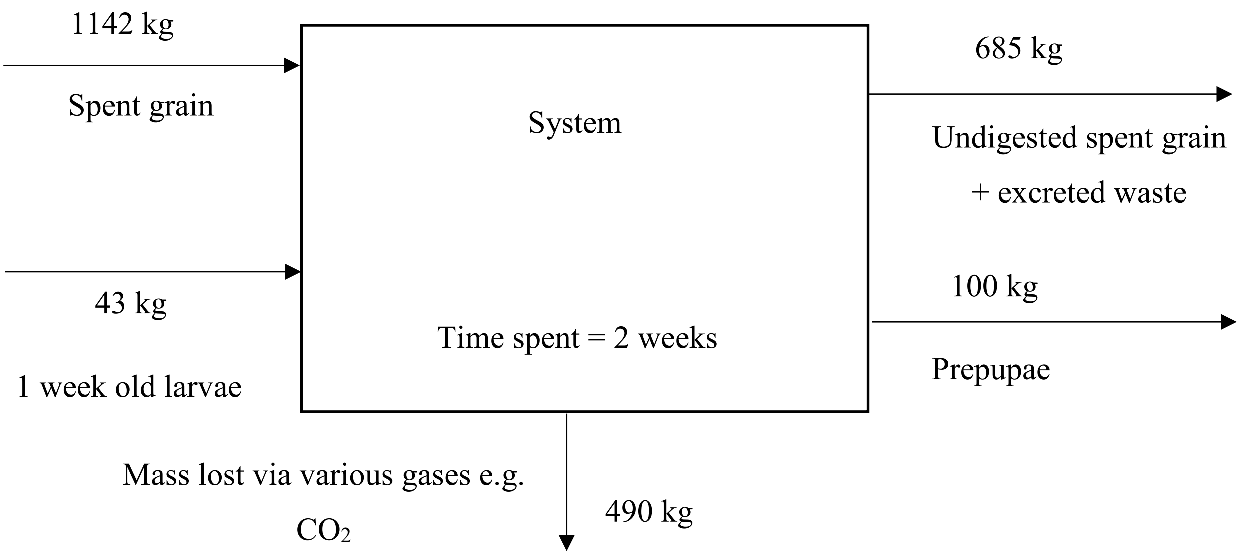


Figure 7. Dry weight partial mass balance

The results for the COD balance are summarized on Figure 8 (see Appendix I for detailed calculations). The oxygen requirement for the system was thus estimated to 449 kg of O_2 (14 k moles), or 1604 m^3 of air over two weeks (assuming O_2 molar content of 21% and air density of 24 L/mol at 25°C).

To ensure aerobic conditions at all times, the actual total air flow was increased 6 folds to 9621 m^3 of air for the course of two weeks (approx. 8 L/min).

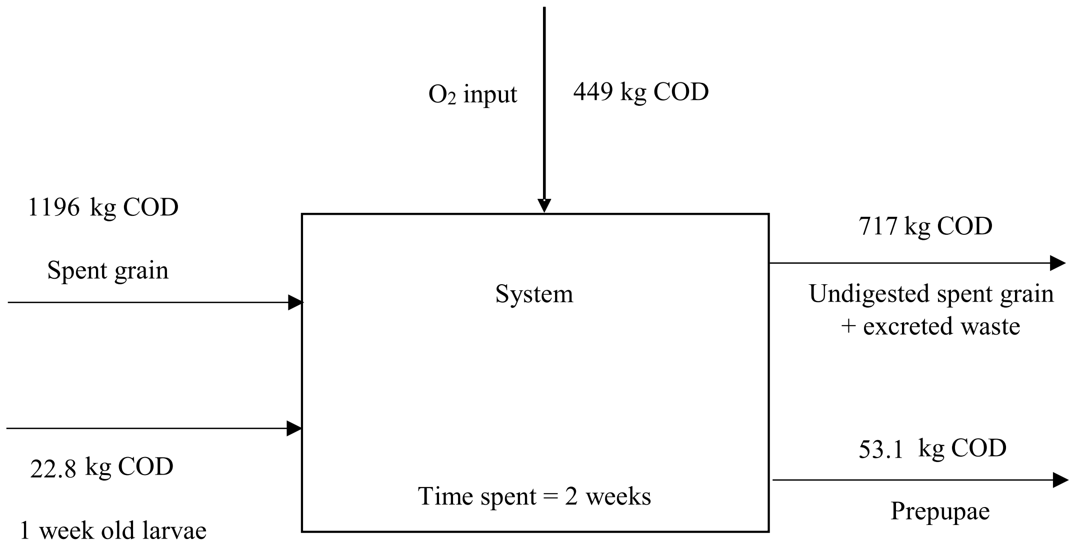


Figure 8. COD balance of the system. Weight shown signifies kg of O_2 required.

The amount of carbon dioxide produced by the system was calculated using a total carbon balance (Figure 9, see Appendix I for detailed calculations). The total organic carbon "lost" from the system was thus estimated to be 166 kg of C, or 611 kg of CO_2.

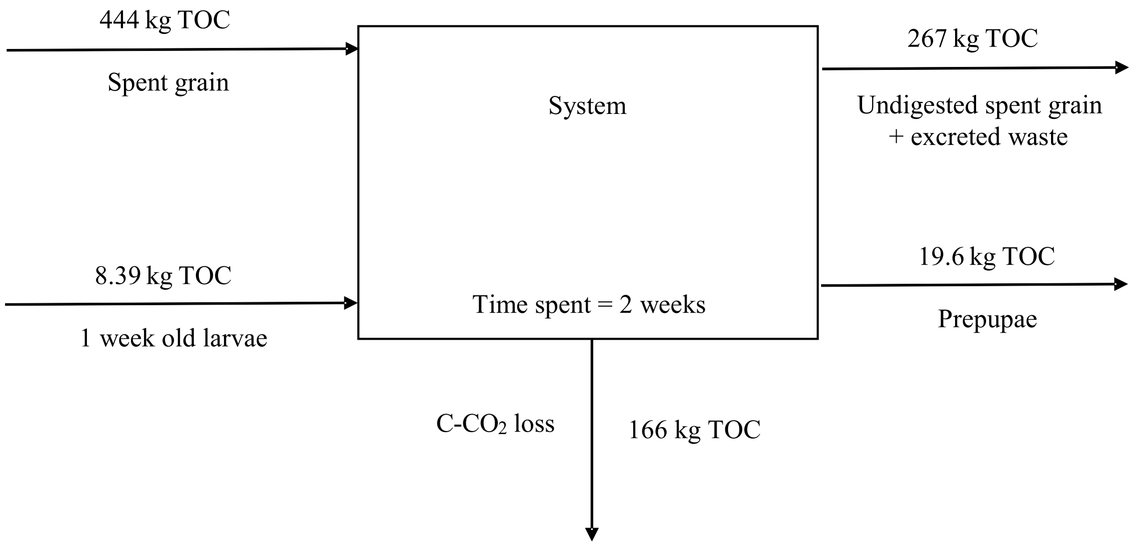


Figure 9. TOC balance. Weight shown was expressed as kg of C.

In order to compute the energy balance, the energy content (as High Heating Values) of the different components were assumed. The energy content of the different components of the spent grain ware shown in Table 4 (Winnie, Arjen, & Theo, 2008). The energy content of the similar components of the Black Soldier Fly were assumed to be of the same value. The energy balance was calculated by assuming that the difference between the energy content of the output streams and the energy content of the input streams represents the heat consumed or generated by the system. The energy balance is illustrated in Figure 10.

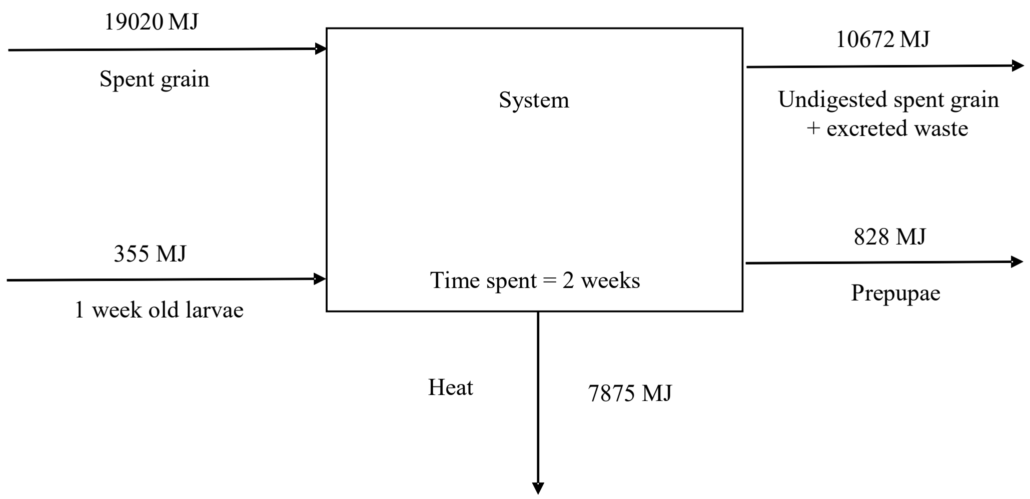


Figure 10. Heat balance of the system.

Based on the energy balance, 7875 MJ of energy will be lost in to the environment as heat over 2 weeks, which is equivalent to an average of 6.51 kW. This is considerable even considering some of this heat will be used to generate water vapour. Experimental trials are therefore need to assess if heat accumulation could become a problem.

Balances sensitivity analysis

In order to assess the sensitivity of the system on each parameter, a sensitivity analysis was made. The effects of the weight of the larvae and the prepupae are shown in Table 5.

Table 5. Sensitivity analysis of the BSF weight.

Parameter	Change	Relative difference			
		Total feed required	Number of larvae required	CO_2 produced	COD
Initial larvae weight	increase by 50%	-37.5%	0%	-37.5%	-37.5%
Final prepupae weight	increase by 50%	20.0%	-33.3%	20.0%	20.0%

The larvae and prepupae weights have great impact on the feed and larvae requirement, and the amount of CO_2 produced by the system. An increase of 50% on the larvae weight signifies a 25 mg increase from 50 mg reported by PFR. Larvae weight higher than 75 mg has been recorded in literature (Matufela R. N., 2015) hence the larvae weight would have to be experimentally determined to ensure the accuracy of the design. The larvae weight is directly affected by the initial diet, temperature, humidity and feed bed height. Since the larvae would be obtained from PFR where the conditions would be reasonably constant, the larvae weight will only be calculated once. Similarly, the prepupae weight will be directly affected by the environmental conditions and type of feed used during the larvae stage. Therefore, tests would need to be conducted to provide more accurate prepupae and larvae weights.

Table 6. Sensitivity analysis of the feed use.

Parameter	Change	Relative difference			
		Total feed required	Frass Produced	CO_2 produced	COD
Biomass conversion	Added 10%	-66.7%	-72.2	-66.7%	-68.9%
Metabolized & Excreted	Added 10%	0%	-16.7	22.2%	22.8%
Non-digested	Added 10%	0%	14.3	-28.6%	-0.54%

As expected, increasing the amount of the feed used for biomass conversion results in less feed requirement, frass production, and CO_2 production. Consequently, increasing the amount of feed utilized on “metabolized and excreted” resulted in more CO_2 production as more feed was used for respiratory purposes. Increasing the non-digested feed resulted in lesser CO_2 production since lesser feed have been used by the BSF larvae. The percentage used for biomass conversion of 5% is considerably smaller in comparison to other studies in literature. Diener et al. (2011) reported a 12% biomass conversion while Banks et al. (2014) reported a 22% conversion. Due to this, testing is required to determine the actual feed use and to maximize the feed used for biomass conversion.

Table 7. Sensitivity analysis of the energy contents.

Parameter (energy content)	Change	Relative difference		
		Energy in	Energy out	Heat produced
Carbohydrates	increase by 50%	12.0%	2.24%	31.9%
Fat	increase by 50%	0.97%	3.52%	31.9%
Protein	increase by 50%	25.6%	33.2%	36.6%

The energy content of the spent grain depends significantly on the parameters used on brewing as well as the type of grain used. Therefore, the energy content of the spent grain varies significantly on a case by case basis. For the mass balance, the energy content of the spent grain was calculated based on the breakdown of its main components – carbohydrates, fat and protein. As seen on Table 7 increasing the energy content of each component by 50% would result in more heat production that has to be controlled. Hence, it is essential to experimentally determine the energy content of the spent grain used as feed. The fat and protein energy content vary depending on the type of fat and protein. A better representation of the system will be to determine the actual energy content of the larvae and the waste stream instead of approximating its respective fat and protein content. Also, the prepupae energy content will depend on the type of feed used during the larvae stage. Hence, experimental requirement is needed to determine the energy content of the spent grain, larvae, prepupae and waste streams. Due to the sensitivity of the system to the changes in these parameters, experiments will be required to experimentally determine these.

Setting baselines

Feed characterization.

The results for the feed characterization are shown on Table 8.

Table 8. Results for the feed characterization experiment.

Parameter	Fresh	Frozen
Bulk Density (kg/m³)	445	360
pH	5.30	4.21
Moisture content wet basis	75.2%	70.4%
Moisture content wet basis @ saturation	93.8%	97.5%
Ash content (% dry mass)	4	7
Parameter	Fresh	Frozen
Ash content (% wet mass)	1	2
Fat (% wet mass)	5.38	
Protein (% wet mass)	2.78	
Energy (kJ/g wet mass)	5.15	

Fungal development was observed after 1 week (Figure 11). The top part of the feeds had also dried out and the pH had increased from 5.30 to 5.90 for the fresh feed and from 4.21 to 6.92 for the frozen feed.

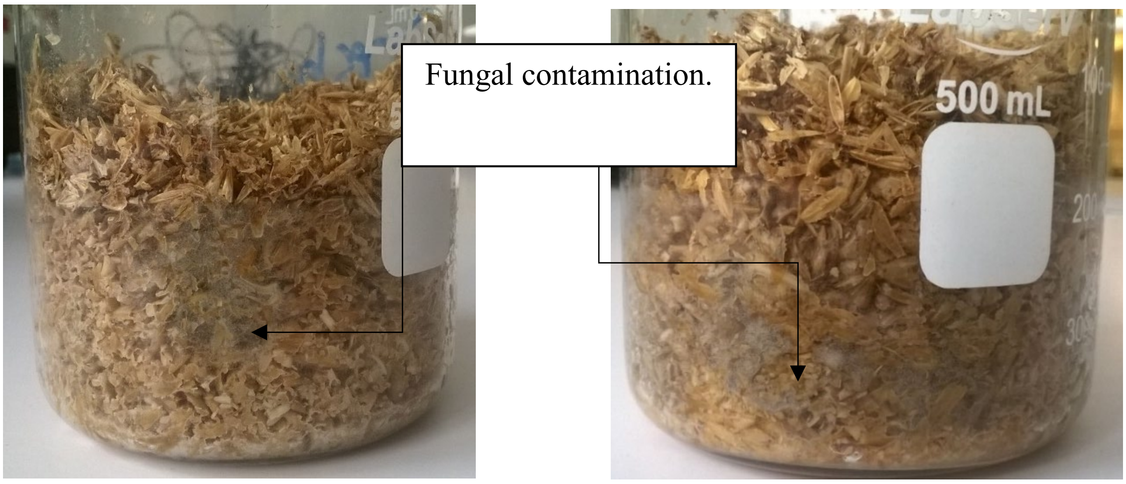


Figure 11. Fresh (left) and frozen (right) spent grain after 1 week.

The fat, protein and energy contents of the spent grain were calculated using the Dumas method, Mojonnier method, and bomb calorimeter, respectively. The fat, protein and energy contents of the spent grain were calculated to be 5.38%, 2.78% and 5.15 kJ/g, respectively. The densities calculated for the fresh and frozen feed agree within reasonable room for error to the published density data of 432 kg per m^3 (Aqua-Calc, 2016). The *"fresh"* feed was found to be 19% denser than the *"frozen"* sample (Table 2) and this difference could be due to differences during brewing and/or the type of grain used as the samples were obtained from different batches. Density is an important parameter as it directly impacts the size and area requirement of the final product. Due to this, the production will be designed to accommodate the fresh sample density with an added 10-15% capacity to act as a buffer for other possible type of feeds to be used.

Frozen feed was found to be more acidic than the fresh sample. This could also be due to the variation in brewery parameters as previously explained. Another possible reason for this difference could be that microbial activity during thawing of the frozen sample could have resulted in further fermentation of the grain. Black Soldier Fly are believed to be resilient to the acidity of the environment (Zhou, Tomberlin, Zheng, Yu, & Zhang, 2013). Hence, the acidity of the feed has insignificant effect on the conversion of biomass.

The moisture content of the two feeds were within the optimum feed moisture content range of 65% to 80% (Matufela R. N., 2015). This means that no preparation should be required for spent grain to be used as a feed source for BSFL.

The results of the nutritional testing showed that the actual protein content was significantly smaller than the assumed value used in the mass and energy balance. It was also determined that the actual energy content of the spent grain used as feed (5.20 kJ/g) was significantly lower than the value calculated using the mass balance which was 19 kJ/g. The literature used for these values used the average of the tabulated values from 17 conducted studies. The lowest values of these studies for crude protein and energy content were 6.2% and 7.4 kJ/g which were closer to the values calculated. This can be, in part, due to the lower amount of protein available than assumed. This can also be due to a different chemical composition present on the spent grain. Nevertheless, this information will be used to improve the accuracy of the mass and energy balances.

Overall, the two types of spent grain had similar properties. The homogeneity of the spent grain is a vital piece of information for the system. The uniformity of the spent grain could reduce the temporal variability within the system.

Respiration experiments.

This part of the study attempted to quantify the O_2 requirement and CO_2 production of the BSF to provide a better understanding on the air system required by the production system. Based on preliminary experiments shown in Appendix II, a respiratory assay was conducted using 5 larvae.

The effect of feed in the respiration of the BSF larvae were observed. An empty flask was set as the control. A flask with only larvae and a flask with larvae and feed were set up and left for 24 hours. The impact of feed supply on the oxygen consumption is shown in Figure 9.

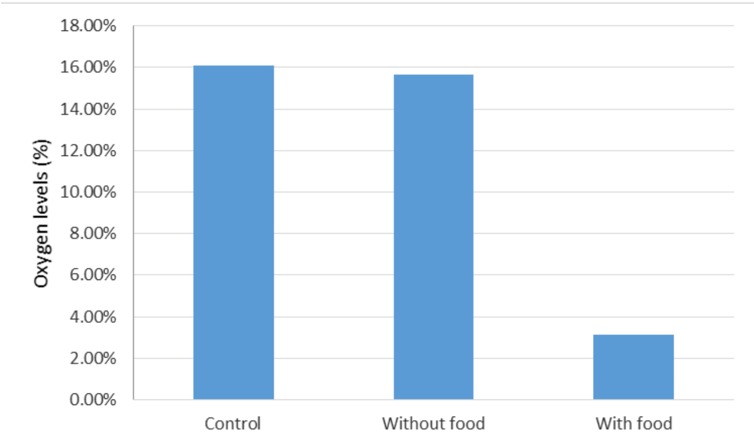


Figure 12. Effect of Food on O_2 consumption in sealed flasks supplied with five 1-week old larvae and 2 g of fresh spent grain.

The effect of food supply also played an important role on the usage of oxygen as can be seen on Figure 12. Recorded O_2 consumption was higher by 68% in the flask supplied with food. Feed supply results in more activity as the BSF larvae move to reach the feed, respire and consume the feed. This test also showed that on the absence of feed supply, the larvae are almost dormant.

Oxygen usage and carbon dioxide production at the different life stages of the BSF were calculated by dividing the hourly reading of each life cycle by the time elapsed on the experiment. These values are shown in Table 9.

Table 9. Respiration rates.

Age of BSF	O_2 Consumption (mg hr^{-1})	CO_2 Production (mg hr^{-1})
1-week-old larvae	0.062-0.078	0.104-0.214
2-week-old larvae	0.153-0.200	0.264-0.368
Prepupae (with food)	0.162-0.212	0.274-0.392
Prepupae (no food)	0.160-0.209	0.272-0.389

The maximum oxygen consumption was 0.212 mg per hour. According to a study conducted by Heatwole, Done, & Cameron (1981), the mean oxygen consumption of the family Stratiomyidae, which includes the Black Soldier Fly, was 1.73 mL of O_2 per g of dry weight per hour. This equates to 0.0751 mg per hour of O_2. To normalize the two values, the O_2 consumption per dry weight was calculated by dividing the O_2 consumption by the mean dry weight recorded. Based from the experiment, the prepupae consumed 6.4×10^{-3} mg of O_2 per mg of dry weight per hour. Based on the literature study, the average recorded weight for the species of the Stratiomyidae family was 30 mg dry weight. Using the same procedure as before, the O_2 consumption per dry weight was calculated to be 2.5×10^{-3} mg of O_2 per mg of dry weight per hour. Literature suggest that the observed prepupae consumption is higher than the average species in the same family. However, these values are within the range of each other and the difference could be due to the prepupae being heavier. The larvae O_2 consumption using the same procedure was calculated to be 3.7×10^{-3} mg of O_2 per mg of dry weight per hour which was closer to the literature value.

Based from the experiment, the 0.212 mg per hour O_2 usage seemed to be reasonable. This relates to about 71 mg of O_2 per larvae for two weeks. Assuming that the same number of larvae were present as in the mass and energy balance shown on the previous part, this equates to 165 kg of O_2 over 2 weeks. This amount of O_2 was lower than the value of 449 kg calculated using the COD balance. The difference can be due to errors in the assumptions used in the balances (e.g. nutritional contents).

Feed use and larvae characterization experiments.

This part of the study aimed to quantify the wet and dry weights, and the ash content of the larvae and prepupae. The breakdown of the feed use was also observed and compared to the study conducted by PFR.

The 1-week-old larvae had more moisture content than previously thought in the initial mass and energy balance, which was 63%, implying that the larvae have less dry weight. Since the system will be designed on a dry weight basis, this was an important parameter. The value of the ash content was found to be smaller than the 21% used from the initial balances.

A total of 100 1-week old larvae was initially used in this experiment. After 1 week of incubation, 40 larvae had reached the prepupae stage and 3 larvae were lost. These larvae were likely to have died and be eaten by the other BSF larvae. Loss of 3% to the population was deemed to be acceptable for the context of this study.

Table 10. Result of feed use and larvae characterization. Experiment lasted for 2 weeks.

Parameter	Start	End
Total wet weight of larvae (g)	6.20	1.64
Total wet weight of prepupae (g)	0.00	8.54
Total dry weight of larvae (g)	1.34	0.547
Total dry weight of prepupae (g)	0.00	2.93
Number of larvae (g)	100	9
Number of prepupae (g)	0.00	88
Total number of Black Soldier Fly on the system (g)	100	97
Average wet weight of 1 larvae (g)	0.0620	0.182
Average wet weight of 1 prepupae (g)	0.00	0.097
Average dry weight of 1 larvae (g)	0.0134	0.0607
Average dry weight of 1 prepupae (g)	0.00	0.0333
Ash content (% dry mass)	11.1%	5.88%
Moisture content (% wet mass)	78.4%	65.7%
Total wet mass (g)	6.20	10.18
Total dry mass (g)	1.34	3.49

The individual dry weight of the larvae increased throughout the experiment as evidenced by the results shown in Table 10. Interestingly, the average individual dry weight of the prepupae was lower than the average individual dry weight of the final larvae. This can be explained by the fact that prepupae do not consume food and, hence, used stored fat for pupation. Data from Table 9 suggests that the prepupae were metabolically active due to the O_2 consumption and CO_2 production. Since prepupae do not consume food, this activity is evidence of the use of intracellular resources. Based on this finding, a potential for the larvae to be used as a product was raised to Prescient. Evidenced by the results of the experiments, the larvae stage has the most biomass and hence harvesting while BSF larvae have not reached pupation might be the best way to maximize biomass production. This can also be explained by the fact that the prepupae have lost most of its gut contents in comparison to the larvae. However, if it was deemed that only the prepupae should be considered as an end product, the biomass lost due to pupation should be reduced. Allowing the prepupae to harvest in a cold environment to slow down the metabolic activity of the BSF can be a way to achieve this. Similarly, killing the prepupae as soon as it self-harvests could be a way to reduce the amount of biomass lost to pupation.

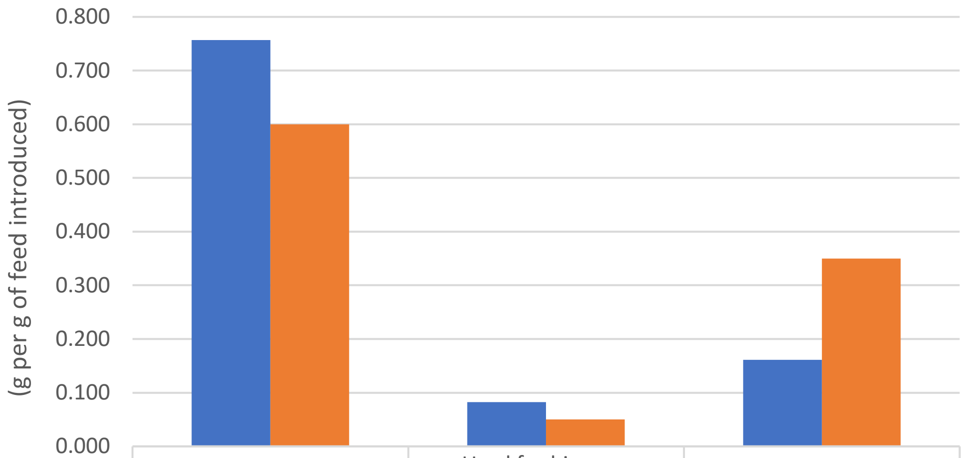
The breakdown of how the feed was used is listed in Table 11.

Table 11. Breakdown of feed distribution.

Total spent grain placed in the system (g dry basis)	26.2
Waste + unused grain left in the system (g dry basis)	19.8
Total mass of grain converted by the system (g dry basis)	6.37
Total initial dry mass of the Black Soldier Fly colony (g)	1.34
Total final dry mass of the Black Soldier Fly colony (g)	3.49
Total dry mass converted into Black Soldier Fly biomass (g)	2.15
Dry mass lost within the system (g)	4.22
Weight of biomass produced per weight of spent grain converted	0.338 $g_{biomass\ produced}/g_{spent\ grain\ converted}$
Weight of spent grain lost per weight of spent grain converted	0.662 $g_{spent\ grain\ lost}/g_{spent\ grain\ converted}$

Based on the amount of waste and unused grain recovered, approx. 24.3% of the initial amount of feed was 'converted' into biomass and carbon dioxide (assuming no other form of C losses). The weight difference between the amount of feed initially added and the amount of feed and waste ultimately recovered is henceforth referred to as 'spent grain converted' as it is not possible to isolate waste materials from unspent grain.

The spent grain converted within the system was calculated to be 6.37 g as dry mass. This spent grain was either used to make biomass within the Black Soldier Fly or converted into other carbon forms that were not identified. About 34% of this used or converted spent grain was used for biomass conversion. The use of the feed obtained in this experiment was compared to the similar experiment conducted by PFR. The feed use comparison of two experiments are shown in Figure 13.



	As waste and unused	Used for biomass production	Lost within the system
Conducted experiment	0.757	0.082	0.161
PFR experiment	0.600	0.050	0.350

Figure 13. Feed use comparison between 2 experiments.

As can be seen, the majority (76% as dry mass) of the feed initially introduced was recovered as a mixture of excreted waste and/or unused feed and only 8.2%, as dry mass, was used for biomass production. The amount of feed lost within the system was 16.1% as dry mass. This part of the feed was used mainly for respiration of the larvae and prepupae and hence in the production of CO_2. Using the TOC procedure as before, the difference between the TOC of the inputs and the outputs were calculated to be 16.06 dry g or 61.4% of the dry mass of the initial feed. This was higher than the 16.1% feed lost within the system recorded. The difference could be from the errors on how the TOC was calculated. To calculate the TOC, the chemical formula of the different contents of each inputs and outputs were assumed. An error on the formula of the fats and proteins present in the spent grain and the excreted waste and/or unused feed would have significant impact on the TOC difference calculated. However, the majority of the lost biomass in the system was still attributed to the carbon lost due to respiration. Also, BSF larvae used part of the feed that was already consumed as energy to consume more spent grain. The larvae required moving around to reach food which in turn would use energy and hence add to the feed that was not measured within the system. Aside from this, as established before, biomass was lost while the BSF pupates as it requires metabolic activity for energy whilst not consuming food.

The results suggest that the system is inefficient and that a large fraction of the feed was not consumed. This imply that a large fraction of the cultivation system will be used for feed storage rather than biomass conversion. This meant that a large fraction of the feed will be wasted, and that, per amount of larvae biomass produced, the system will waste expenses in dealing with unnecessary feed and wastes. Similarly, maximizing the amount of feed used in the system will optimize the size of the system. However, this could mean that the metabolizable part of the spent grain might be a small portion and might need further processing to access more material.

Updated Mass and Energy Balance.

The results from the testing conducted by the Nutrition's Laboratory of Massey University was tabulated in Table 12.

Table 12. Nutritional content. Expressed in dry basis. Carbohydrate % was estimated by subtracting the sum of other components to 100%.

Sample Name	Protein %	Fat %	Ash %	Carbohydrate %	Gross Energy (kJ/ g)
Prepupae	51.2	34.4	5.9	8.5	26.5
Larvae	54.6	17.7	11.1	16.6	36.3
Spent grain	17.6	11.2	3.77	67.4	20.8
Unused feed + waste	21.6	8.00	3.77	66.6	20.0

The assumed parameters for the previous mass and energy balances were replaced with the data gathered from the experiments conducted. Using these new set of data, the mass and energy balances were completed again.

Using the data gathered for the moisture content as well as the weights of the larvae and prepupae, the dry weight mass balance was recalculated. The updated partial dry weight mass balance is illustrated in Figure 14.

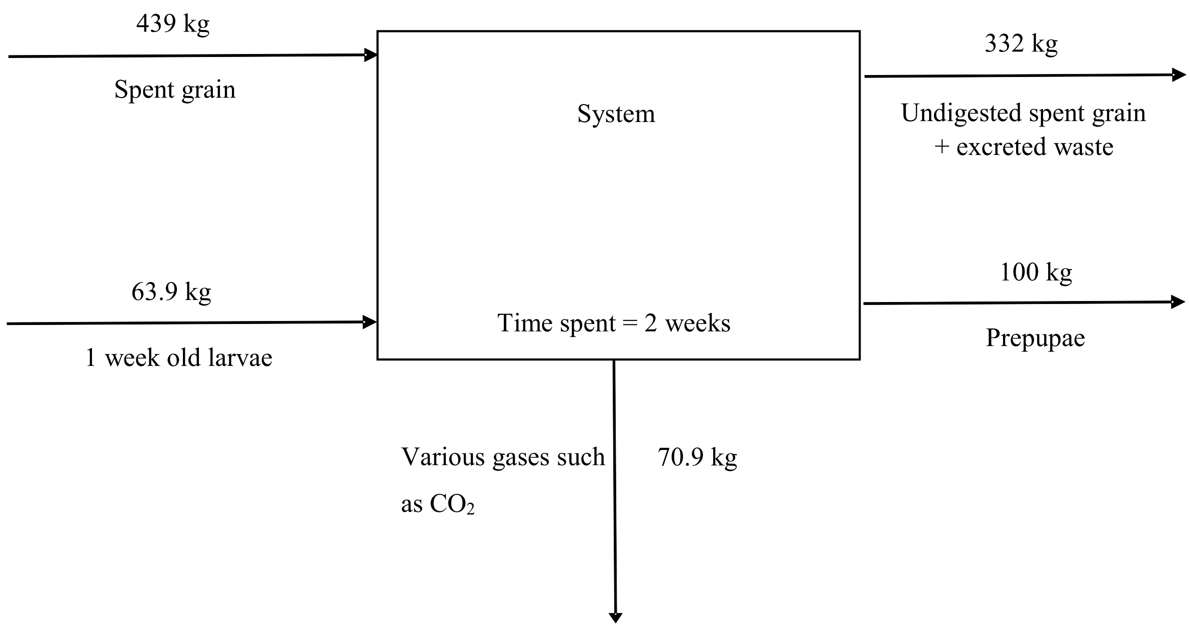


Figure 14. Updated partial dry weight mass balance.

The updated COD balance was calculated using the nutritional content obtained through the experiments. The updated COD is illustrated in Figure 15.

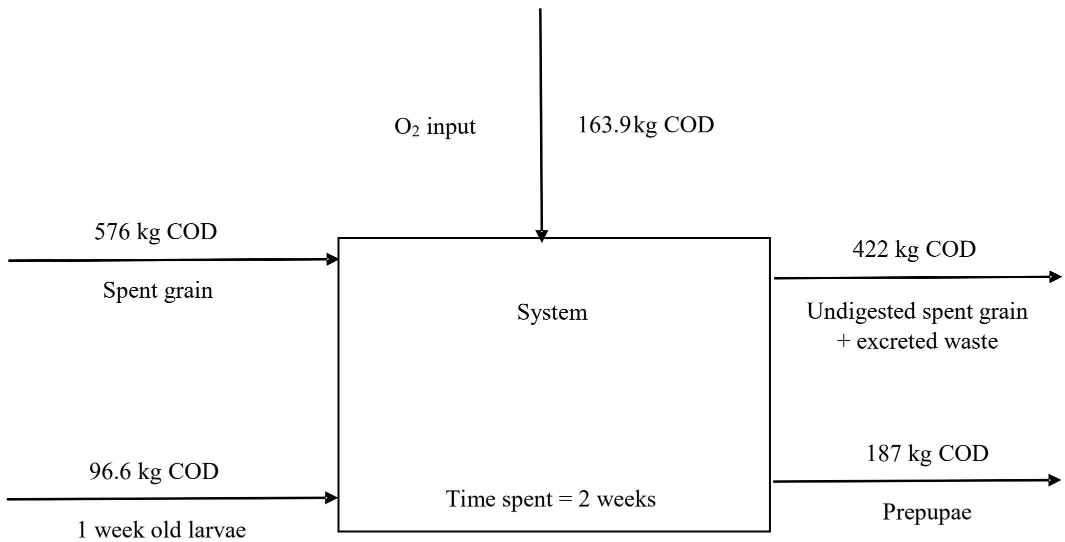


Figure 15. Updated COD balance. Weight expressed as kg of O_2 required.

Based from the new COD balance, the new O_2 requirement was worked out to be 63.9 kg of O_2 to produce 100 kg of dry BSF biomass. This was much less than the initial calculated value of 449 kg of O_2 and closer to the value predicted by respirometry (165 kg). This was mainly due to the assumed nutritional composition being incorrect which results in the inaccurate values of COD. This smaller O_2 requirement equates to 1370 m^3 of air supplied over 2 weeks which was significantly less than the flow initially calculated. This calculation was also affected by the over estimation outlined on the COD calculation on the initial mass balance. The updated total organic carbon balance is illustrated on Figure 16.

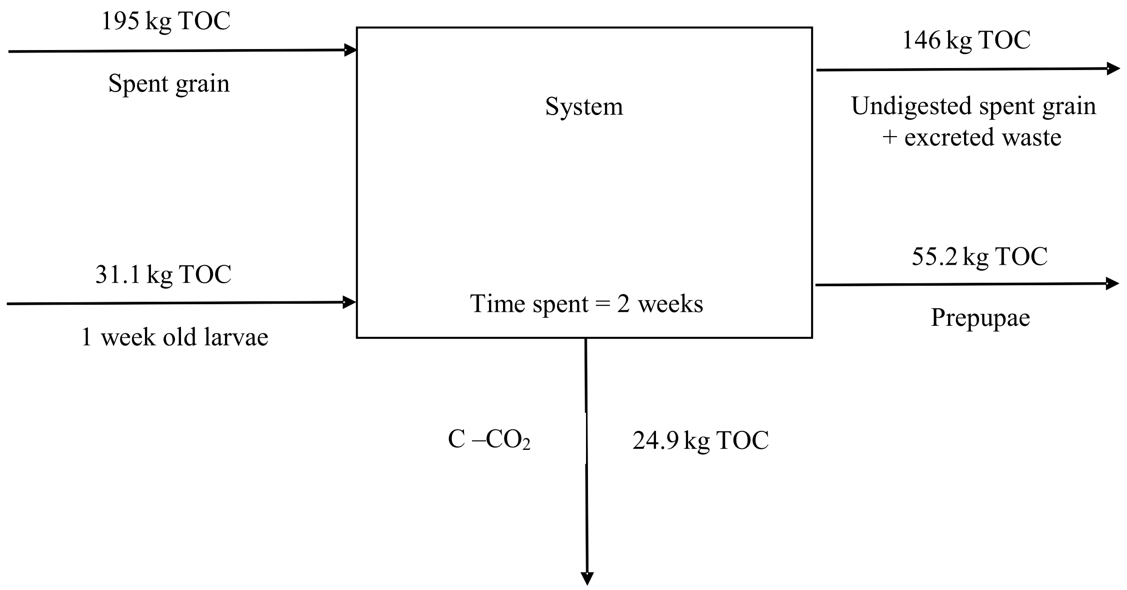


Figure 16. Updated TOC balance. Weights expressed as kg of C.

Based from the new TOC, the total carbon "lost" within the system was estimated to 24.5 kg. This equates to 89.8 kg of CO_2 against 609 kg of CO_2 initially calculated. The COD and TOC balance of the BSF have not been studied before. Therefore, the findings of this part of the study were novel and has no precedence.

The energy balance was recalculated using the energy content obtained from the experiments and is shown in Figure 17. Based from the new energy balance, the amount of heat produced was estimated to 491 MJ over two weeks, which averages as 0.406 kW of power. This was lower than the initial value calculated which was 6.5 kW over 2 weeks. The inaccuracy of the energy contents of the larvae, prepupae and spent obtained from literature values was deemed as the main factor of the discrepancy. As established previously, the energy flows of the system were sensitive to changes in the energy content of each component.

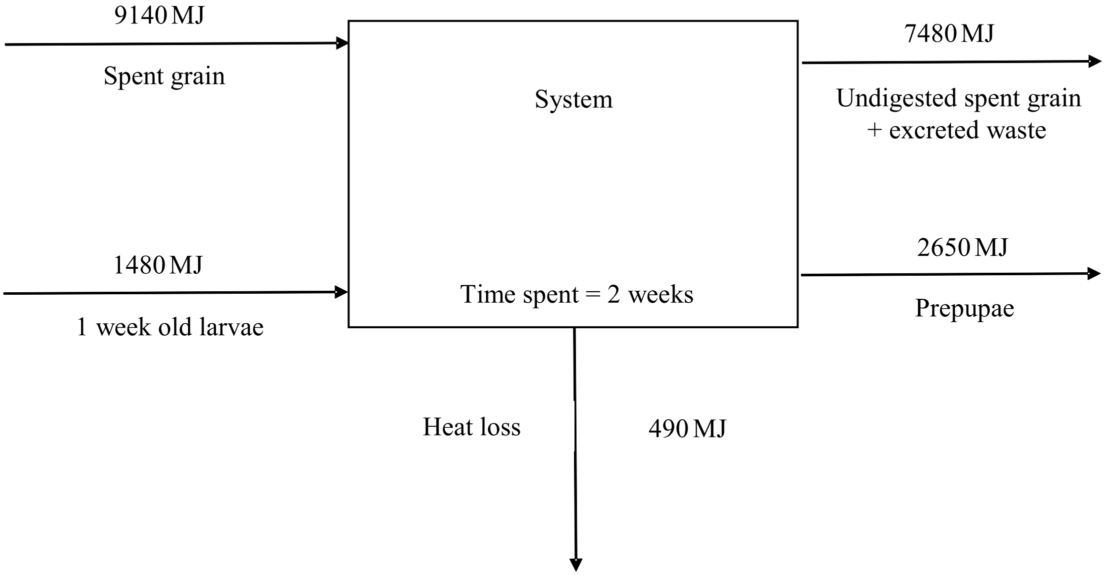


Figure 17. Updated energy balance.

Optimization Experiments

Feeding ratio.

This part of the study explored the optimum feed ratio for the BSF larvae. As established earlier, most of the feed were left unused by the BSF larvae and, hence, the amount of feed introduced in the system can be optimized. The parameter that was used to study the feed amount was the ratio of the dry weights of the feed and the larvae. This was used to accommodate the changing weight of the larvae. This parameter would also be robust in that it can be used in other studies and applications. For ease, the undigested feed + excreted waste was collectively called "frass" in the consecutive parts.

The biomass conversion yields for different feeding ratios were calculated and compared. This parameter and the biomass output indicates the efficiency of the BSF larvae to convert the biomass from the feed to BSF biomass. The efficiency of the BSF larvae in converting the feed directly affect the size and cost of production since the amount of feed will be a main source of operational cost and the area requirement of the plant.

The feed load observed the total amount of feed per larva that was placed on the system. The mortality rate and pupation rate were calculated as the amount of BSF that died and prepupae, respectively, divided by the initial population. These were important parameters as the survival of the larvae is key in lowering the cost of production, since the production of 1-week-old larvae will be a cost intensive process, and the prepupae is deemed as the sellable product.

Based on the challenges faced by this study, the production of 1-week-old larvae will be the bottleneck of the process. Due to this, effective use of the larvae will be required hence, the survival of the larvae will be critical for the process.

Table 13. Feed ratio experiment results.

Feed ratio	**12:1**	**12:1**	**12:1**	**6:1**	**6:1**	**5:1**	**5:1**	**4:1**	**4:1**	**3:1**	**3:1**
Feed in (g dry)	30.2	27.8	24.1	16.1	15.3	13.5	13.3	10.6	11.1	5.50	5.53
Frass out (g dry)	16.6	15.8	13.5	8.35	8.31	7.14	7.04	5.60	5.78	2.76	3.14
Feed used (g dry)	13.6	12.1	10.6	7.72	6.98	6.33	6.25	4.96	5.33	2.74	2.39
Larvae in (g dry)	1.24	1.11	0.93	0.90	0.90	0.92	0.92	0.91	0.91	0.73	0.73
Larvae + Prepupae out (g dry)	8.48	5.84	5.05	4.24	3.87	3.95	3.8	3.45	3.24	2.81	3.08
Biomass gained (g)	7.24	4.73	4.12	3.34	2.97	3.03	2.88	2.54	2.33	2.08	2.35
BSF alive	86	90	88	97	99	95	98	90	89	82	86
Ave. weight gain (g dry per insect)	0.084	0.053	0.047	0.034	0.030	0.032	0.029	0.028	0.026	0.025	0.027
Mortality rate (%)	14%	10%	12%	3%	1%	5%	2%	10%	11%	18%	14%
Pupation rate (%)	80%	86%	82%	92%	97%	85%	89%	76%	72%	62%	71%
Biomass Conv Yield (g DW per g used)	0.53	0.39	0.39	0.43	0.42	0.52	0.53	0.69	0.69	0.86	0.90
Biomass Conv Yield (g DW formed per g DW fed)	0.24	0.17	0.17	0.21	0.19	0.25	0.24	0.31	0.32	0.43	0.39
Biomass Output (g DW out per g DW input)	0.28	0.21	0.21	0.26	0.25	0.33	0.32	0.42	0.41	0.56	0.51
Feed load (feed DW per larvae DW)	24.3	25.1	25.9	17.9	17.0	14.7	14.5	11.5	12.1	7.54	8.25
Feed conversion rate (g DW frass per g DW feed)	0.55	0.57	0.56	0.52	0.54	0.53	0.53	0.53	0.52	0.50	0.57

The results for the feed ratio experiment is listed in Table 13. The most efficient biomass conversion yield was at a feeding ratio of 3:1. The high conversion yield, while beneficial with regards to minimizing the amount of feed needed, may however evidence food deprivation with detrimental impact on insect quality. Food deprivation can have two potential effects on the larvae: 1) it may slow down the larvae growth, resulting in lower pupation rates; and/or 2) it may result in increased mortality. Both phenomena were reported as the highest mortality rate and the lowest pupation rate recorded were indeed achieved at the lowest feed ratio tested. The lowest average weight gain was also recorded in the 3:1 feeding ratio giving further evidencing of food deprivation.

Of all the feeding ratios studied, the 6:1 ratio was found to be the best of the conditions tested. The 6:1 ratio have, on average, higher biomass conversion yield than the controls of 12:1 which indicated that the feed were more efficiently used in the 6:1 ratio than in the 12:1 ratio. The control of 12:1 ratio have the highest average weight gain but the 6:1 ratio have the highest pupation rate and the lowest mortality rate. This implied that the larvae found the environment of the 6:1 feeding ratio more favourable for pupation. The ratios were taken before the weekly feeding regime was introduced (see below). For practicality, the feed load based on the initial amount of larvae will be used as the parameter since weekly weighing the actual amount of larvae will be an inefficient use of the operator’s time in the actual design. For the second week of feeding, the amount of feed required was assumed to be twice (12:1) of the first week feed. Due to this, a feed load of 18 times the initial larvae weight will be used to design the system.

This feed load equates to 46 mg wet per larvae per day which is comparable to the report of Nguyen et al. (2013) that 30 mg of kitchen wastes per larvae per day and within limits to the 100 mg per larvae per day of chicken feed reported by Diener et al (2009). The spent grain used as feed for the system has a different nutrition profile than the 2 other type of feed which may be the reason for the difference.

Feeding regime.

Based from the literature review, different feeding regimes have different effects on the growth of BSF larvae. The aim of this part was to observe and study these effects and to provide the optimum feeding regime for the system. Feeding once will be the ideal feeding regime to minimize the operational cost of the system. However, problems like feed spoilage could occur. Due to this, three feeding regimes were tested: 1) feeding the larvae twice, once a week, with frass removal before the introduction of new feed, 2) feeding the larvae twice, once a week, without frass removal before the introduction of new feed, and 3) feeding the larvae once and left alone for two weeks.

Table 14. Feed regime experiment results.

Feed regime	Feeding twice with frass weekly removal	Feeding twice without frass weekly removal	Feeding once
Biomass Conv Yield (g DW per g used)	0.44	0.48	0.17
Biomass Conv Yield (g DW formed per g DW fed)	0.19	0.22	0.15
Biomass Output (g DW out per g DW input)	0.23	0.27	0.2
Feed load (g feed DW per g larvae DW)	25.1	20.5	21.2
Mortality rate (%)	12%	0.00%	33%
Ave. Weight Gain (g dry per insect)	0.131	0.099	0.072
Feed conversion rate (g DW frass per g DW feed)	0.56	0.54	0.12
Pupation rate (%)	83%	88%	47%

Larvae fed only once over two weeks had the lowest pupation rate, highest mortality rate, and the lowest average weight gained. This poor performance was suspected to have been caused by feed spoilage midway through the experiment. After 9 days since the beginning of the experiment, a spoiled odour was observed for this feeding regime. This means that the spent grain stability will need improvement should this feeding regime be used at full scale.

Feeding twice over 2 weeks without frass removal was found to be the best regime under the conditions tested. This feeding regime yielded a comparable conversion yield and biomass output than that of the feeding twice over 2 weeks with frass removal. The regime without frass removal however yielded lower average weight gain than the regime with frass removal because waste removal improved accessibility to fresh feed. The larvae were observed to crawl up to reach the fresh feed on top of the frass and then crawl back down to the bottom of the container. This could potentially mean that the larvae must spent more energy to reach the fresh feed in the regime without frass removal and hence could result in lower biomass gain. This negative effect would be offset by the reduced frass production in the feeding regime without frass removal. Since the frass of week 1 was not removed after 1 week, the larvae have more time to process it and, therefore, lesser frass would be produced as was evidenced by the feed conversion rate. The feed conversion rate indicates the weight of frass produced per weight of feed introduced. These results agreed with Mutafela (2015) who reported batch feeding as the optimum feeding regime for the larvae growth and with Nguyen et al. (2013) that mixing the fresh feed to the frass in the system resulted in lighter and shorter prepupae.

Feed height and feed type experiment.

Table 15. Bed depth and feed type experiment results. Bed depth was tripled for the 2nd week of the experiment by adding twice the amount of feed in comparison to the 1st feeding.

Experiment	Biomass gained (g dry)	Mortality rate (%)	Pupation rate (%)	Moisture (%ww)	Ash (%dw)
3 cm + 6 cm raw	2.47	0%	100%	63.6%	4.69%
3 cm + 6 cm raw	2.89	1%	90%	61.8%	5.88%
3 cm + 6 cm minced	2.93	5%	93%	64.9%	5.56%
3 cm + 6 cm minced	2.68	2%	98%	65.0%	3.51%
6 cm + 12 cm raw	5.14	2%	93%	64.8%	4.81%
6 cm + 12 cm raw	4.66	2%	94%	67.2%	4.82%
6 cm + 12 cm minced	4.07	14%	85%	67.8%	4.82%
6 cm + 12 cm minced	4.44	13%	86%	66.9%	4.81%

The height of the feed has been controlled to 3 cm for the majority of the experiments done previously. This was done due to the consultation of PFR that 3 cm will offer the optimum bed height for the larvae to grow. However, the feed height affects the area requirement of the plant as increasing the feed height reduces the area requirement of the plant. This will be an important parameter on the design as this directly affect the capital cost of the full-scale production and hence, four bed heights were tested – 3 cm, 6 cm, 9 cm, and 18 cm. The main problem with increasing the bed height will be compacting issues in the bed and the lack of O_2 within. The effect of these issues will be increased as the feed becomes finer. Due to this, minced spent grain and raw spent grain were used as feed for each bed height, separately, to see the effect of mincing.

The BSF larvae could indeed survive in feed heights up to 18 cm when fed raw spent grain without any significant effects on mortality and pupation rates. In contrast, the larvae could only survive at 9 cm bed height when fed minced spent grain without having an adverse effect. Larvae reared on 18 cm of minced spent grain had the highest mortality and pupation rates among the feed heights tested. Mincing the spent grain resulted in a density increase from 450 kg per m^3 to 875 kg per m^3 as the smaller particle size of the minced spent grain resulted in compacting of the feed bed. This likely resulted in a shortage of air pockets inside the bed as University of Alaska Fairbanks (2015) reported that air cannot move through a pile made from grounded spent grain and hence bulking agents will be required to ensure air pockets within the spent grain. The lack of air pockets will result in a lack of oxygen available for the larvae. Bed compacting also likely impaired accessibility to fresh feed. Due to this, raw spent grain was deemed to be the best substrate and it can be supplied up to a bed height of 18 cm. As established earlier, no prior study about optimum feed height was conducted due to the varying nature of the feed used for growing BSF larvae. This finding can not be compared to literature and hence is a novel find.

Harvesting Methods

The harvesting of larvae from the frass was a main challenge for this study as the larvae need to be separated from the frass with minimal operator time. There were no prior studies in to how this can be achieved. Five harvesting methods were tried to achieve this separation – 1) sedimentation, 2) screening, 3) washing, 4) food attraction, and 5) force harvesting.

The sedimentation method as a means of harvesting did not show any promise. The prepupae floated on the top while the larvae and the frass settled at the bottom of the beaker. This did not provide any feasible method as the necessary separation required was between the frass and the larvae. The sieves used for the sieving method, 2.4 mm, 2.8 mm, and 3.6 mm sieves, were unable to separate the frass from the larvae due to the mesh getting clogged by the frass and the vibration of the screen did not help in unclogging.

The washing method worked best for minced spent grain as running water was able to wash the feed away from the larvae on the 3.6 mm sieve and the pressure of the water was able to unclog the mesh of the frass. This method, however, has two major disadvantages – 1) process water is usually expensive, and 2) the minced spent grain cannot support the required bed height. For 30 g of frass to be removed from 50 larvae, 1 to 1.5 litre of tap water was used, therefore, this method has a potential to be expensive. Even if the amount of water requirement is to be reduced, it could still be a major operating expense. Hence, other methods could be more suitable.

The two "food attraction" methods worked to some extent for harvesting larvae. For the method that used sugar solution in a filter paper, 50% of the larvae found the sugar solution and stayed to feed. It was interesting to note that the larger larvae were able to find the sugar solution which could mean that larger larvae are better equipped to sense the direction of food. Similar results were observed for the food attraction method in a container with a divider. The largest 20% of the larvae were able to detect the food on the other side of the divider and crawl there.

The forced harvesting through heat was deemed to be the cheapest and easiest method among the other methods tested as it only requires the change in ambient temperature. It was therefore selected for the design of the final system. Larvae crawl out started at 32 °C and was fastest (1 hour and 15 minutes) at 38°C to 39°C.

Using heat to force harvest larvae have not been studied before but it has been observed that larvae crawl out on temperatures of 40°C and above by several studies (Harnden & Tomberlin, 2016) (Ushakova, et al., 2016). The effect of heating in the larvae physiological functions have not been studied in this study. Ushakova et al (2016) reported that lauric acid, which as a boiling point of 40°C, accounts for majority of the lipids present in the BSF. Therefore, excessive heat could potentially negatively affect the BSF larvae.

Pilot study

Experiments conducted prior to this part were laboratory scale and would need to be tested on a larger scale to increase confidence. A pilot study will be undertaken to ensure that the parameters and phenomena observed previously will be observed in a larger scale. The O_2 demand and CO_2 production were estimated using COD and TOC calculations and would require testing in a larger scale. The feed height and the effects of feed area in the ability of the BSF larvae and prepupae to harvest will also be tested better on a pilot scale.

The parameters to be used for the pilot and full scale study were the results of the optimization experiments. The feeding ratio is 18 g dry weight feed to 1 g dry weight larvae using the feeding regime of twice feeding without frass removal as this reduced the load on the operator. The feed height selected was 3 cm for the 1st week and an extra 6 cm of feed for the 2nd week to ensure minimal mortality rate. The harvesting method is by forced harvesting by increasing the temperature as this is the most effective and least labour intensive method.

Based on discussion with the project partners, delivery of 1-week old larvae of up to 5 kg wet weight can be used for the pilot study. A pilot-scale cultivation cabinet containing a rearing tray was then sized to accommodate 5 kg wet weight of 1-week-old larvae (1.08 kg dry, based on larvae moisture content of 78.4%) over 2 weeks at 27°C to 30°C. Based on the larvae characterization (Table 10) and feed ratio experiments (Table 13), the amount of feed to be used was estimated to 19.4 kg dry weight, or 78 kg wet weight, and will produce 5.4 kg dry weight, or 13 kg wet weight, of BSF prepupae (based on a yield of 0.27 kg dry larvae biomass output per kg of dry fee input, Table 14). The rearing tray was sized to 150 cm length by 100 cm width so that the initial feed height, of raw spent grain, will be 6 cm which will increase to 18 cm in the second week, based on the findings of the feed height experiment. The height of the tray was sized to be 30 cm to accommodate ramps on each side to allow self-harvesting.

Prescient later suggested that a first test could be made using 2.5 kg wet weight of 1-week-old larvae. Due to this, the design was altered to include 2 handling 2.5 kg of 1-week-old larvae each. This resulted in a single tray with a size of 120 cm width and 100 cm length including a 80 cm wide rearing compartment equipped with 40 cm wide ramps on each side (see picture below). The ramps are set at an incline of 15° and have 50 cm long hole with a radius of 18 cm to allow the prepupae to fall in the collection cabinet underneath. A schematic drawing of the tray is shown in Figure 18.

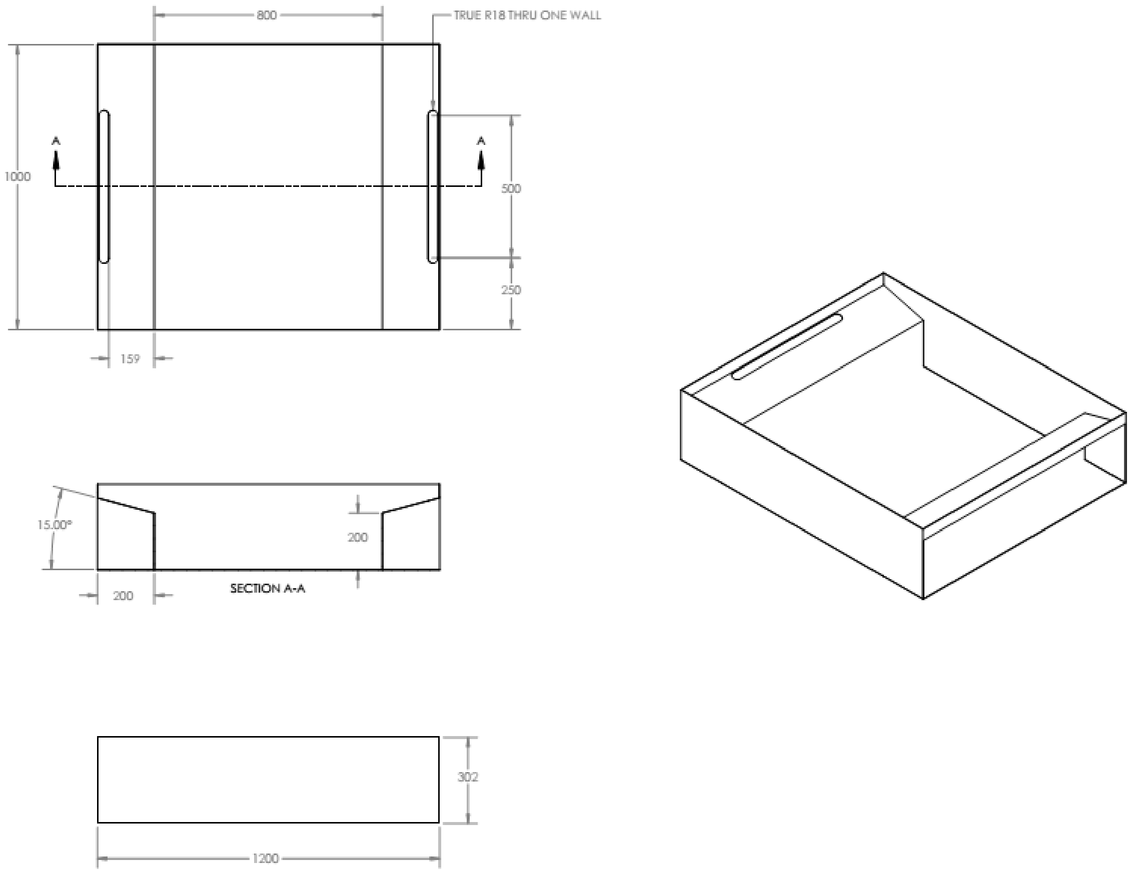


Figure 18. Tray design.

The trays are placed in a support frame inside the cabinet. This support frame can be removed from the cabinet and is fabricated from ISO 4 cm x 4 cm x 0.4 cm square tube. The support has a gap with a size of 150 cm wide by 100 cm long by 50 cm height to accommodate the rearing trays. The extra width was placed for ease of sliding the trays. The drawings of the support frame are shown in Figure 19.

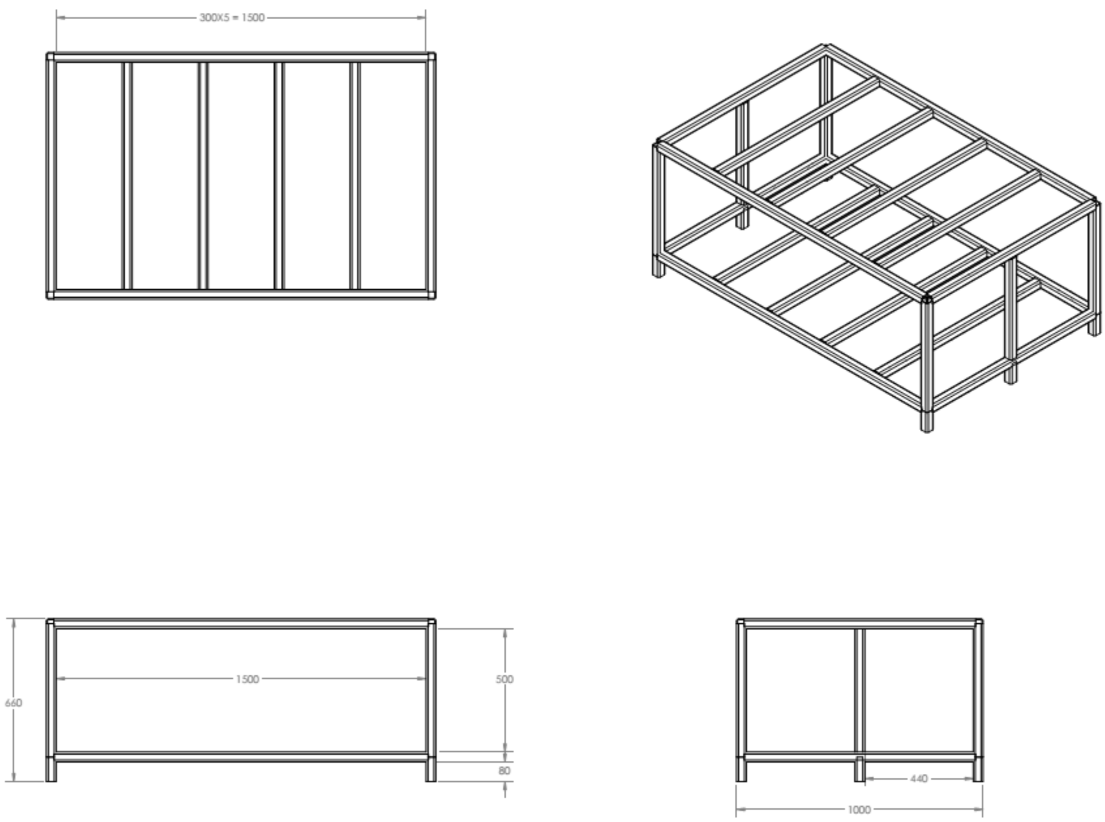


Figure 19. Support frame drawing.

The inside dimensions of the cabinet were set to 160 cm wide by 110 cm high by 120 cm long. An 8-cm diameter hole was placed on the roof of the cabinet to allow for air exchange. Four air holes were placed in the side of the cabinet which would be attached to the air pump to ventilate air inside the cabinet. A door was placed on the cabinet which would be closed using friction latches. The cabinet and the trays are fabricated with 2.0 mm T304 2B stainless steel to prevent corrosion. A schematic drawing of the container is shown in Figure 20.

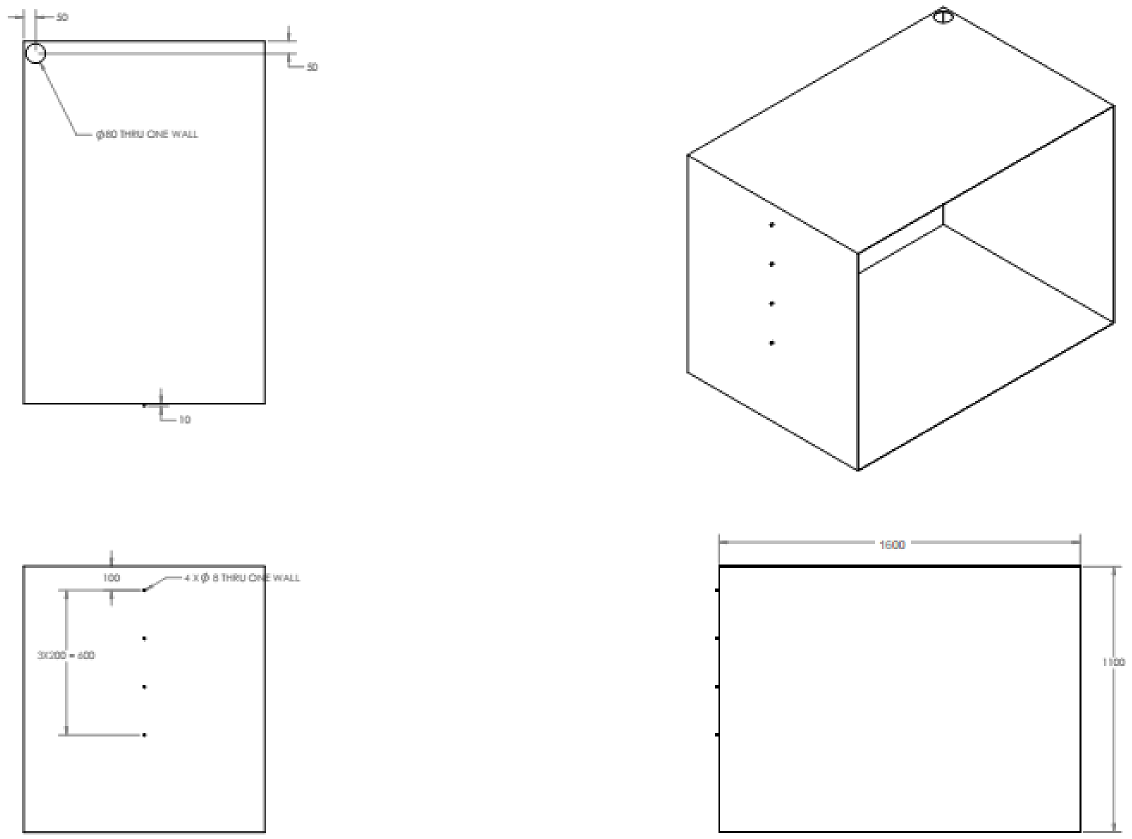


Figure 20. Container drawings.

All components of the pilot cabinet were fabricated and stored in a container rented by Prescient. The pilot was not tested as Plant and Food Research was not able to supply the necessary larvae for the pilot study. Testing would be conducted once supply can be secured.

For the pilot study, 2.5 kg of larvae and 13 kg of feed would be placed on each tray. Collection boxes would then be placed underneath each ramp and the trays would be placed on the cabinet. The air pump would be connected to the air holes and started. The entire cabinet would be placed in a temperature controlled environment at 27°C to 30°C and a relative humidity of 75%. If relative humidity control is not available, water would need to be introduced every week. After 1 week, 26 kg of feed would be added onto each tray. After another week, the temperature of the cabinet would be increased to 38°C to 39°C. The cabinet would be left at this temperature for a day. After this, the trays would be lifted by two operators out of the cabinet. Then, the larvae and prepupae in the collection boxes would be collected and frozen while the frass can either be bagged or thrown away.

Full scale plant.

A modular mainly automated design for the production system was initially planned. The operation of this design was assumed to happen 1 day per week to remove frass, larvae and prepupae, and to add new feed. For this design, a small production throughput of 100 kg dry weight of BSF biomass per week should be viable. This will need 1800 kg dry weight of feed and produce 900 kg dry weight of frass. Two scenarios were investigated for this design: 1) Placing the system on breweries or places which have continuous supply of food for the larvae, and 2) Placing the system on farms or premises of end-users were the larvae were to be grown and directly fed to animals. This approach meant that capital used for feeding and/or harvesting is essentially only used once a week. The main problem with this design is in the logistics of the feed and the larvae. For the first scenario, prepupae will be collected on a weekly basis from places were the system was placed while on the second scenario, feed will have to be regularly supplied to farmers. According to Prescient, the cost of feed and 1-week old larvae production were \$0.05 per kg and \$1 per kg, respectively. In order to obtain the larvae and the feed required will already cost about \$20 000 per year without delivery cost. A quote from a trucking company estimated that a 1000 kg wet weight crate with a volume of 2 m^3 delivered from Wellington to Palmerston North costs about \$300 per trip. Using the wet weights of the larvae, prepupae, feed and frass, it was estimated that each trip will have an average of 2000 kg wet weight and 4 m^3 hence doubling the quoted delivery cost per trip. Due to the weekly nature of the design, the collection of the frass and prepupae, and delivery of the larvae and the feed will amount to 104 trips which amounts to \$62 000 per year of delivery cost per site. For \$5 per kg of BSF biomass selling price, the amount of revenue from the BSF biomass collected from this system do not justify the cost of transporting the larvae, feed, frass, and prepupae. Hence the costs of delivering food and/or larvae and collecting prepupae do not make this approach economically viable for a small amount of BSF biomass.

System description

A centralized system where the larvae will be reared, with a fixed amount of spent grain, in a controlled environment was chosen over the modular system. This system will have a nursery as part of it to ensure the constant supply of larvae. The delivery of the feed and the collection of prepupae will be centralized to one location hence reducing the cost. According to Lowder et al. (2016) an agricultural farm of up to 2 hectares, on average, can be run by a family of 5-6 people.

Using this scale, a single operator should be able to handle a 0.1 hectare, or 1000 m^2, plant with the correct equipment. This land area should be able to produce 1200 kg dry weight of prepupae weekly. Due to the 2 weekly incubation period of the larvae to reach harvesting stages, two sets of rearing pits, each with a capacity to supply 1200 kg dry weight, will be constructed to ensure a weekly supply. Each set will be comprised of 5 pits, with each pit being 14 meter long 5 meter wide and 0.5 m deep, and corresponding to a certain day of the week. A schematic drawing of a singular pit is shown in Figure 21.

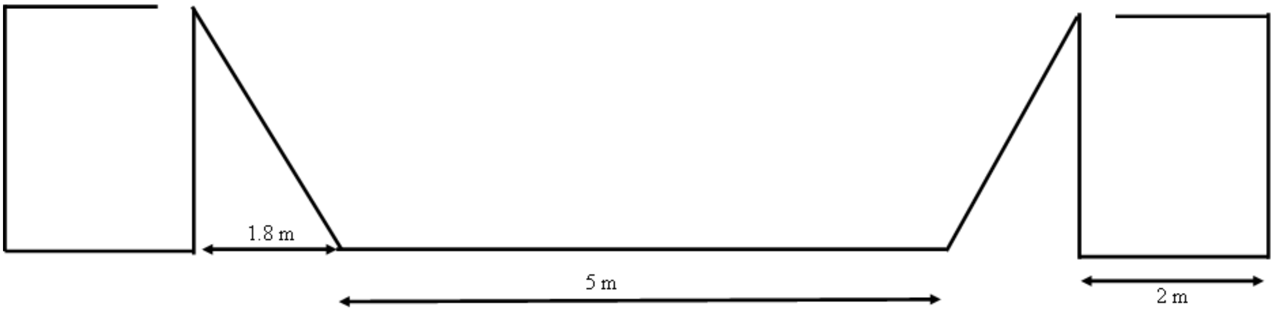


Figure 21. Schematic drawing of a pit.

Each pit will be dug out of the ground and cemented. A 15° inclined wall will be used instead of straight walls. These inclines will start from the bottom of the pit and will be 1.0 meter long. At the end of the inclines, will be the opening to harvesting container which will be 14 meter long by 2 meter wide and temperature controlled at 20°C. These containers will be covered except within 40 cm after the incline.

The pits will be kept aerobic and temperature controlled using air ventilation. The total O_2 demand of the system was calculated to be 767 kg O_2 used over 2 weeks. This was done using the findings of updated mass balance in Figure 15 – 63.9 kg O_2 per 100 kg of dry biomass output. This O_2 flowrate was then converted to kilograms per hour using the total number of hours in 14 days. The resulting value of 2.28 kg O_2 per hour was then multiplied by 6 to ensure aerobicity as per the findings of the respiration expirement. The O_2 flowrate was then converted to air flowrate by dividing it with the weight percent of oxygen in air which is 21%. The volumetric flow rate was then calculated by dividing the mass flowrate with the density of air which is 2.2249 kg per m^3. The volumetric flowrate was then converted to m^3 per min by dividing it by 60 minutes. The resulting volumetric flow of 0.488 m^3 per min was converted to liters per min by multiplying it by a factor of 1000. The total air requirement of the system was calculated to be 488 liters per minute. The system will be designed for 500 liters per min to account for pressure drop and air losses. Each pit will be supplied 50 litres of air per minute. The air will be introduced using 3 pipes with 20 cm diameter on each pit. The pipes will be secured in the concrete and the air holes present on the base of the pits. The air holes will have mesh to prevent the larvae from escaping. A singular pump system will supply air for the whole plant but a redundant pump will be attached to the system with a bypass valve to allow for maintenance and repair should the pump require it. The air compressors may need to be located in a separate house to reduce noise. To control the moisture content and temperature of the growth beds, the air will be moisturized and thermoregulated. From the previous experiments, it was noted that drying only occurs on the top 10% of the height. Due to this, humidifier nozzles will be placed on the walls of the pits close to the top to add moisture as required. The temperature will be regulated in each stream using an industrial heater should the metabolic heating not be enough to retain the temperature. Each pit will also be covered by a tarpaulin type material to reduce evaporation and heat losses. This cover can also be removed in seasons where evaporation is to be encouraged.

The entire production system will be placed inside a 2000 m^2 shed which will be temperature controlled. For health and safety purposes, alarms for the fumes in the pit will be installed and trigger once industry regulated thresholds were reached. Exhaust fans will also be installed in the shed to ensure air quality is kept to safe working conditions. The shed will have a bagging area for the frass to be bagged onsite, a space to house the excavator, operator room, and storage areas for the end products. The excavator will be purchased brand new as hired cost will overcome the capital cost of purchasing brand new over the life of the equipment. The operator room will have instrument reading that will display the moisture, oxygen level and temperature of each pit. The operator can also alter the temperature from the operator room.

Daily, one pit from a set will be harvested and restarted by the introduction of new larvae and feed while another pit from the other set will just be placed with new feed. A total of 330 kg wet weight of 1-week old larvae (71.3 kg dry) and 1.7 tonnes wet weight of feed (422 kg dry) will be placed to the pit being restarted (initial feed ratio of 6:1 during first week) while 3.5 tonnes of feed will be placed on the pit from the other set for that day (equivalent feed ratio of 18:1 over 2 weeks). The larvae will be introduced in the pit by tipping over a 1.5 m^3 tub from the nursery holding the larvae. The feed will be introduced in the pit by a ducting system that moves the feed from storage silos erected outside the shed to the pits operated from the operator room.

Harvesting will be a 2 day process, the remaining larvae will be forced out of the pits by heating air going to the pit to 40°C using a heater. This temperature will be kept for a day to ensure that most of the larvae will self harvest. On the next day, an excavator will be used to to remove the frass from the pit and the larvae and prepupae mix will be harvested from the harvesting containers. The excavator chosen must have enough length to reach the frass from any side of the pit hence it should not reach the Twenty percent of the larvae and prepupae mix will be introduced back to the nursery while the rest will be stored in a low temperature storage area.
The harvestings containers can be opened from the top to expose 4 holes. A crane and chains system will be attached to this holes to lift up the harvesting containers. The containers will then be tipped to a bin that will then be carried by a forklift to the storage area. The crane system will be installed on the roof and should be able to reach all the rearing units. The BSF left in the frass would be de-activated once the frass is stored in a cool storage before being sold. The dead BSF in the frass can serve as further soil nutrient increasing the favourability of the frass to be used as soil fertilizers.
On a weekly basis, 26 tonnes wet weight of spent grain, used as feed, will be delivered on site, 16 tonnes wet weight of frass will be delivered to retailers and/or end consumers, and 2.8 tonnes wet weight of BSF will be delivered to post processing companies or end consumers. The feed will be stored in two silos that have the capacity to store 75% of the required weekly feed each. The silos might need to be temperature controlled to ensure the quality of the feed.

Nursery

This research mainly focused on the growing unit from 1-week-old larvae to prepupae. However, it would be inefficient to detach the nursery and breeding unit from the plant as it would cause many problems. Firstly, the plant would require a daily input of larvae and hence a daily shipment would be required should the nursery be different from the plant. Based from experience, the security of the supply of the 1-week old larvae is the bottleneck of the process hence some sort of design would be required in order to produce the necessary amount required by the system.

The nursery could be a 5 m by 5 m shed situated just outside the growing units. This shed would be the breeding shed which would have light control and moisture control. A percentage of the harvested prepupae would be placed on the shed and be allowed to mature, breed and oviposit. The ovipositing mechanism would be a wooden frame of 2 m by 2 m constructed from 50 cm diameter wood which would be hollow on the middle. The middle would be made up of strips of wood 20 cm high running the length of the frame. Each line of wood would be made up of two strips attached by a magnet at each side with an opening of about 2 cm to allow the BSF to oviposit the eggs in the middle. The whole frame would be covered by these strips of wood equidistant of about 20 cm from each other. A total of 700 wet kg of prepupae or 7800 prepupae will be introduced in the nursery every week. Assuming a 50% split between female and male, this amount of prepupae has the potential to produce about 100 000 eggs. Assuming that only 50% of the mating was successful, this could produce 50 000 eggs. Assuming a fertility rate of 50%, the number of larvae that can be used for production will still exceed the 24 000 required.

The operator would take one frame daily from the shed and place it on top of a 1.5 m^3 tub already containing feed for the fresh larvae. After 1 week, the operator will take the tub and dump the contents of it in the pit that will be restarted.

Costing

The cost of purchase was estimated to be $310 000 based on the value of industrial land in the central north island region of the same size (Realestate.co.nz, 2017). This region was selected as the land cost should be affordable and the site would be connected to major roadways.

The construction cost of building the shed was approximated using the method outlined by Rawlinsons Ltd (2014). This method approximated that in New Zealand, an industrial shed that is made from light steel frame with concrete slab was about $250 to $325 per m^2 depending on the site. The calculation used a value of $300 per m^2 which is the nominal price for sheds built in the north island. The shed floor plan was estimated to be 2000 m^2, the base price of the shed was calculated to be $600 000. The cool store to be placed in the shed was approximated using the same method using the cost parameter of $860 per m^2. The cool store was assumed to have a floor area of 46 m^2 totalling to an amount of $40 000. The office was also costed similarly using a price parameter of $1000 per m^2 and a floor area of 30 m^2 totalling to $30 000. The total cost of the shed, the office and the cool store was $670 000.

The price indicated by Rawlinsons Ltd (2014) was calculated in values in 2014, the year of the publication of the hand book. The cost was adjusted to 2017 values using the historical construction price index of New Zealand (Statistics New Zealand, 2017). The construction price index for 2014 was 1100 while for 2017 was 1200. The price (C) was inflated (C_i) using the formula below and was calculated to be $730 000.

$$C_i = C\frac{1200}{1100}$$

Extra cost was added for engineering ($20 000), consents ($20 000), equipment (30 000), connection to utilities ($10 000) and compliance cost ($50 000). Adding these costs, the total value of the shed was calculated to be $860 000.

The cost of creating the pits which serves as the growing unit for the larvae, were also approximated using the Rawlinsons method. Since the shed will be made out of concrete, only the walls were necessary to create the pit. These walls were to be made out of concrete slabs at a price point of $300 per m^2. The total area of the concrete slabs required was 190 m^2 and hence totalling to $57 000. The rollers and lining was approximated to cost $30 000 and the conveyor belts for the harvesting was costed to be $50 000. This amounts to $137 000 and inflated to 2017 values as $150 000.

The excavator can either be purchased for $100 000 (Gough CAT, 2017) or rented at a daily renting price of $300 (Rawlinsons Ltd, 2014). This equipment would be used for the harvesting of the frass which should happen daily and hireage cost will overcome the purchasing cost after 1 year and 4 months of hireage. Therefore, purchasing of a new excavator was chosen. The forklift can be hired on a monthly basis of $2,000 (ECR Forklifts, 2018) or purchased brand new for $55 000 (Stellar Machinery, 2018). The average economic life of a forklift is 7 years (Hyster, 2018). The hireage cost will overcome the purchasing cost after 2 years and 4 months. However, additional yearly maintenance and repairs on the forklifts can offset this as repairs and maintenance are part of a lease agreement (Hyster, 2018). Forklifts also depreciate aggressively and it would be more economical to hire a new unit after every 5 years.

The air system of the plant was priced by pricing the compressors required by the system. Firstly, the horsepower of the required compressor was calculated. The head of the compressor was first calculated using the formula

$$Head = ZRT\,\frac{\frac{P_2}{P_1}^{\frac{k-1}{k}} - 1}{\frac{k-1}{k}}$$

Z = compressibility, T= inlet temperature in ºR (Rankine), k= Cp/Cv= 1.4 for ideal gas, P1 = inlet pressure, P2 = outlet pressure.

The compressibility of the air was set to be 1 as it was assumed to be ideal gas. Due to this assumption, k was also set as 1.4. The inlet pressure was assumed to be 1 atm and the outlet pressure was set to 2 atm while the inlet temperature was assumed to be 20°C or 528°R. Once the head was calculated, the theoretical horse power was calculated.

$$Theoretical\ HP = Head(ft) \times \frac{2.2\ \times mass\ rate\ \left(\frac{kg}{s}\right)}{550 \times 7.23 \times \frac{kg.m}{s.HP}}$$

Using the theoretical HP, which was calculated to be 20 HP, the actual HP was calculated to be 30 HP by dividing with the efficiency of the compressor which was set to 80%. Having 5 compressors rated at 6 HP should allow for better control of flow and input parameters. A 6 HP compressor is priced at \$7600 per piece (Trademe, 2018). Therefore, the total cost of the compressors totalled to \$38 000. This price has goods and services tax at 15% and removing this results in the price to go down to \$32 000. Piping and equipment necessary to run the compressors was assumed to incur costs at \$10 000 and \$20 000, respectively. These prices totalled to \$62 000.

For the feeding system, the main piece of equipment was determined to be the vessel or silo where the food would be stored. Due to this, the pricing was determined by costing the vessel. Two silos would be used which should be able to handle 45 m³ of feed each. A silo similar to this was priced at \$15 000 on Trademe (2018). The price was adjusted to account for ducting and pipeworks, which was assumed to cost \$30 000, to total \$60 000.

The capital breakdown of the cost was shown in Table 16. Nursery cost is not included in the capital cost calculation as the project focused on the rearing of larvae to prepupae. The nursery will be on site but is not costed for this project.

Table 16. Capital cost breakdown.

Land cost	$	310,000.00
Excavator cost	$	100,000.00
Shed cost	$	860,000.00
Pit cost	$	150,000.00
Feeding system cost	$	60,000.00
Air system cost	$	62,000.00

Total Cap cost	$ 1,540,000.00

Operational costs were calculated based on a number of assumptions. A single operator was assumed to work 40 hours per week on the plant. Due to the specialist training required of the operator such as license for operationg heavy mobile equipment, training on health and safety and other trainings, the salary for the operator was assumed to be $20 per hour. To account for administration work, supervisory work, work on nursery, and cleaning, the labour cost was adjust to 2 full time employee (FTE) equivalent. According to Prescient, the cost of spent grain is only the petrol used in its collection. However, as the process will be scaled up, a more reliable source of pricing estimate is required. Ben-Hamed et al. (2001) reports that in the United Kingdom, wet spent grain is sold to farmers by the breweries at a price of £10.60 per tonnes wet weight in 2011. This price was then adjusted to New Zealand dollars and inflated to 2017 to obtain a price of $24.00 per tonnes wet weight or $0.02 per kg wet weight of spent grain. This price was close to the estimate given by Prescient which was $0.05 per kg wet weight of spent grain. The 1-week-old larvae was estimated by Prescient to cost $2.00 per kg wet weight to produce in the current nursery without transportation cost. However, the cost incurred by the nursery outlined in this report will mainly be for electricity cost and labour hours, the cost to produce was reduced to $1.00 per kg wet weight.

The electricity requirement of the plant was assumed to be mainly incurred by the air supply. The electricity requirement of the air compressors were calculated specified at 4.5kW by the supplier. Assuming that these compressors were used at full capacity everyday, a total of 197 000 kWh of energy. The cost of electricity was set as $0.15 per kWh (Ministry of Business, Innovation, and Employment, 2017) for industrial purposes and hence totalling the electricity cost to $30 000 per year. The fuel consumption of the excavator and the forklift was estimated at 14 L per hour (Gough CAT, 2017). The operator will use the excavator and the forklift for about 2 hours a day for harvesting and hence the total yearly fuel usage was calculated to be 7300 L per year. The price of diesel was assumed to be $1.50 (AA Motoring, 2017). The yearly expenses were then calculated base off these values. The yearly expenses are shown in Table 17.

Table 17. Operational expenses.

Yearly wage	$	83,000.00	$/year
Feed cost	$	27,000.00	$/year
1-week-old larvae production	$	85,000.00	$/year

Electricity requirement	$	30,000.00	$/year
Forklift hireage fee	$	24,000.00	$/year
Fuel usage	$	7,300.00	L/year
	$	13,000.00	$/year
Total Op cost	$	240,000.00	$/year

The frass is comparable to compost in gardening use and can be sold as a replacement product. Prescient indicated that the selling price for the frass is $0.5 per wet kg or about $0.23 per liter, which are comparable to high grade compost retail prices on the market (Bunnings Warehouse, 2017), while the BSF biomass was to be sold at $5 per wet kg. However, it is important to note that retailers will have to take a mark-up price and distribution costs must also be covered. The yearly net revenue of the plant was calculated as the difference between the operational cost and the gross revenue of the sellable products. This was shown in Table 18.

Table 18. Revenues.

Frass income	$	420,000.00	$/year
Biomass income	$	250,000.00	$/year
Gross revenue	$	670,000.00	$/year
Net Revenue	$	401,000.00	$/year

The income from biomass produced was calculated to be 80% of the total produced since 20% of the prepupae produced was reintroduced in the nursery to ensure the constant supply of larvae. The yearly revenue of the system with the current parameters were calculated to about $401,000 per year. The break-even point of the company is to sell all the BSF biomass produced at $4.77 per wet kg and to sell no frass. The selling of frass as compost will have the seasonal trend of sales similar to compost. However, the total sales for the year should be positive despite the seasonality due to the comparable uses to compost. Selling the BSF biomass at $4.77 per wet kg should be attainable as fishmeal can be sold at a comparable price once delivery cost is incured (Quandl, 2017).

Net Present Value (NPV) calculations and Sensitivity analysis

The net present value was calculated to check the economic feasibility of the project. It was assumed that the plant will take 1 year to build, year 0. On the first year of full production, the plant will only produce 75% of the capacity since this year would require some fine tuning and training. The plant would be at full production at the following years.

A discount rate of 10% was assumed for the plant since this rate is often used as a baseline for rate for possible investments (Investopedia, 2017). The life cycle of the plant was assumed to be 20 years. No depreciation and interest rate from the bank were accounted for in this analysis. The cash flow for each period was calculated as the gross profit of the plant for that period. The present value (PV) for each period was then calculated using the formula below.

$$PV = \frac{Cash\ flow\ for\ period}{(1+R)^t}$$

R is the discount rate of 10% and t is the time period or number of years of the plant operation.

The net present value (NPV) was then calculated by summing up all the present value for each period and subtracting the amount of the initial investment. The return on investment, ROI, of the plant was calculated as the proportion of the yearly revenue and the initial investment.

The NPV of the plant was calculated to be $2 100 000 which is a positive value NPV. This is a great indication of the profitability of this investment. This indicates that the inflow of cash outweighs the outflows and hence a profitable investment. The ROI of the plant was calculated to be 35%. The internal rate of return (IRR) for the project was calculated to be 31% which is above the average minimum acceptable rate of return (MARR) for new plants which is 15% (Bizfluent, 2018).

Using this design, the BSF biomass product is produced at $3.81 per kg and the frass is produced at $0.28 per kg. Most of the income from the investment will come from the frass due to the amount that is being produced and the low cost of sourcing its raw ingredient and producing it.

The sensitivity of the NPV in the components of the operational cost were tested by altering the price of each component and recording the change in NPV. The costs were altered by increasing and decreasing it by 25% and recording how the NPV is affected by each change. The effect on the NPV was calculated as a percentage change which was calculated by firstly finding the difference of the initial NPV and the changed NPV and then dividing this by the initial NPV. A tornado graph of the NPV changes is illustrated in Figure 22.

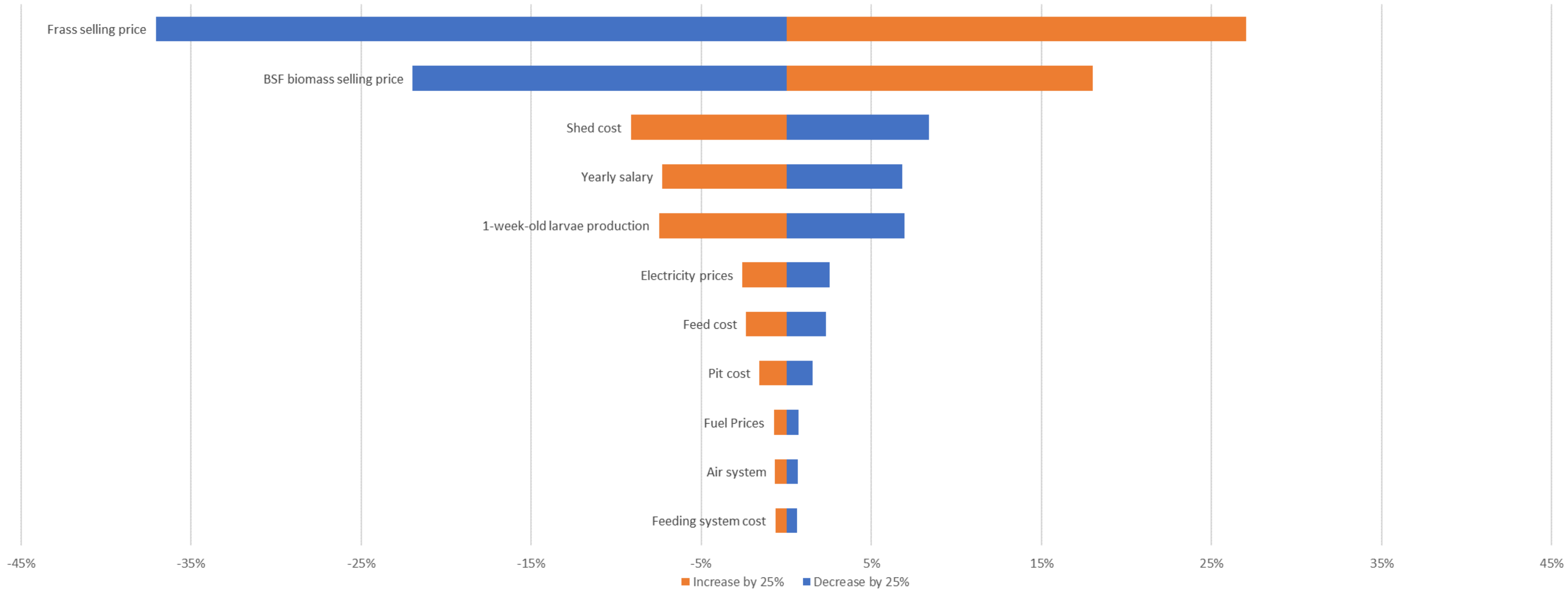


Figure 22. Sensitivity analysis for the NPV

The value of the NPV was very sensitive on the selling price of the products, i.e. frass and biomass. A decrease on the selling price of the frass of only 25% or $0.13 per wet kg have a 37% decrease in the NPV of the plant. The NPV of the project becomes $ -1,400,000 if the frass cannot be sold hence, the venture does not become profitable. This is critical, especially if the frass will be sold as a compost alternative due to the seasonality of compost. The sales of compost are very high during the spring to summer season but drop down during the winter season. Due to this, plans should be in place to ensure that the price of the frass can be kept at a competitive price on winter and fall seasons. This plan can include negotiating with farmers from other countries that have growing season during the winter and fall seasons in New Zealand. The large amount of frass produced by the system will result in a bit more revenue than the BSF biomass. Hence, the price of frass has more effect on the price of the biomass. This gives the company a secondary source of income.

Similarly, a 25% decrease in the BSF biomass selling point will result in a 22% decrease in the NPV of the plant. The use of BSF biomass as a feed source might not be as affected by season as the frass, it is still important to ensure that there will be customers for the product all through out the year. A 25% change in the selling price of BSF biomass equates to a range of $3.75 to $6.25 per wet kg. The $6.25 per wet kg price range seemed to be too expensive for the market while the $3.75 per wet kg can easily be reached by the market value. This price is also close to the break-even point previously calculated. A $5 per wet kg of BSF biomass seemed reasonable but further market study on the selling price of the BSF biomass needs to be conducted to ensure the success of the investment. Also, other possible industries that can use the BSF biomass, such as pet food, should be explored to diversify the customer for the BSF biomass. Contracts for year round supply of the product can be negotiated to ensure that the profitability of the plant remains high.

The production of larvae is also an operational cost that has significant effect on the NPV of the plant. This part of the operational cost was not fully studied in this report as it is outside the scope. However, the cost of $1 per kg wet weight that was placed on the larvae could be reasonable as most of the cost incurred will be for electricity and minor manual labour. This price should further be studied and minimized if possible. The NPV of the plant still remains positive ($230 000) if the price of producing the BSF rise up to $4 per wet kg.

The feed cost changes of 25% equates to spent grain price range of $0.015 to $0.025 per kg wet weight. A 25% change in the spent grain price affect the NPV value with the same percentage. The price used for the simulation of $0.02 per wet kg seemed reasonable since most of the cost of the spent grain is in the collection part as breweries do not charge for this

Conclusion

The main objective of the study was to provide the engineering expertise to upscale the BSF production of Prescient Nutrition. This course of study was novel as no prior study has been published designing a full-scale plant to produce BSF biomass. The project focused on rearing BSF larvae to BSF prepupae and was undertaken in partnership with Plant and Food Research (PFR) and Prescient Nutrition.

A detailed literature review on the current situation of the BSF cultivation was conducted. In this literature review the optimum conditions to rear the BSF larvae to prepupae based on previous studies, were recorded. The advantages and limitations of using BSF was also explored. For the full-scale production system to be designed, key parameters around the mass and energy balances must be quantified.

No previous study was conducted by Prescient Nutrition to determine these key parameters. Hence, values from literature were used for these parameters and a mass and energy balance was calculated. The sensitivity of the mass and energy balances in these key parameters were tested by changing the values of the parameters then the changes in the feed requirement, larvae requirement and energy outputs were recorded. Based from the sensitivity analysis, it was deduced that the larvae, prepupae and spent grain must be characterized nutritionally. The time requirement of the larvae to reach the prepupae stage was also tested. Once these key parameters were found, the mass and energy balances were updated with the new values.

The use of the feed was also explored to confirm the results reported by PFR previously. It was found that majority of the feed (76%) were left in the system as a mixture of unused feed and feed that pass through the digestive system of the larvae. Further tests were conducted to explore the possibility of optimizing the usage of the feed as well as minimizing the feed requirement of the larvae. It found that a feeding load of 18 g dry weight of spent grain per 1 g dry weight of 1-week-old larvae introduced in the system over 2 weeks (1/3 initially and 2/3 after one week, without removing the frass) was the optimum feeding load. This feeding load provided the maximum pupation rate and survival rate and a reasonable dry feed to dry biomass conversion rate of about 27% and dry feed to dry frass conversion rate of 53%. This feeding load was used for the design of the final product.

The feeding regime for the design was also explored. Initially, a weekly feeding with frass removal was proposed for the full-scale plant. This proved to be impractical as the amount of frass produced by the system can potentially be greater than 100 kg. Due to this, three types of feeding regimes were tested – 1) weekly feeding with frass removal, 2) feeding only once, and 3) weekly feeding without frass removal. The feeding regime of feeding only once resulted in a high mortality rate due to the spoilage of the feed midway through the experiment. The weekly feeding without frass removal was selected for the design since this feeding regime resulted in comparable results to the regime with frass removal and was more practical.

The feed height was maximized to reduce the area requirement of the plant. This was conducted by testing feed heights of 6 cm, 9 cm, 12 cm and 18 cm. It was found that the feed height can be as high as 20 cm without any adverse effects on the growth of the larvae if the feed used is not made up of fine particles. For the tests conducted, the minced spent grain was not able to supply the necessary conditions to keep the larvae alive at a height of 18 cm. Fresh spent grain with a feed height of 18 cm was used in designing the full-scale plant.

The optimum harvesting method was also explored during this course of study. On previous study, the self-harvesting quality of the prepupae was greatly relied upon to harvest the BSF. However, for the full-scale plant, it was theorized that a part of the BSF were still at the prepupae stage once harvesting starts. Due to this, several harvesting methods that can separate the larvae from the frass and feed were explored. Harvesting can be forced through heat. Heat forced harvesting was found to be the most effective and cheapest harvesting method. Larvae starts crawling out of the feed at 32°C with the fastest crawl out happening between 38°C and 39°C.

The parameters to be used for the pilot and full scale study were the results of the optimization experiments. The feeding ratio was 18 g dry weight feed to 1 g dry weight larvae using the feeding regime of twice feeding without frass removal as this reduced the load on the operator. The feed height selected was 3 cm for the 1st week and an extra 6 cm of feed for the 2nd week to ensure minimal mortality rate. The harvesting method was by forced harvesting by increasing the temperature as this was the most effective and least labour intensive method.

A pilot study was initially planned to be conducted. A pilot cabinet that can handle 5 kg wet weight of 1-week-old larvae and a total of 80 kg wet weight of spent grain was fabricated. The larvae were to be grown over 2 weeks to produce 13 kg wet weight of BSF prepupae. However, PFR was not able to provide the necessary larvae for the pilot and hence it was not done. The cabinet and its rearing trays were stored in a temperature controlled container waiting to be tested once enough larvae can be secured.

The initial design for the full-scale production was a modular unit that can be placed in the customers' premises. However, the economics and logistics of this design is expensive and hence a centralized plant operated by 1 operator that can produce a weekly dry biomass output of 1200 kg weight was chosen. This plant will require a land area of about 2000 m^2 and 26 tonnes of spent grain weekly. This plant was costed and the NPV of the plant was calculated.

The entire production system will be placed inside a 2000 m^2 shed which will be temperature controlled. The production system will consist of 10 rearing pits which are 14 meter long, 5 meter wide and 0.5 m deep. Each pit will be dug out of the ground and cemented. Harvesting pits are located at the end of a 15° inclined wall on the sides of the pits. To ensure aerobic conditions at all times, 250 litres per minute of fresh air will be introduced to the system.
On a weekly basis, 26 tonnes wet weight of spent grain, used as feed, will be delivered on site, 16 tonnes wet weight of frass will be delivered to retailers and/or end consumers, and 2.8 tonnes wet weight of BSF will be delivered to post processing companies or end consumers. The feed will be stored in two silos that have the capacity to store 75% of the required weekly feed each. The silos might need to be temperature controlled to ensure the quality of the feed.
The project will require a capital investment of $1 540 000. The shed for the project accounts for the biggest capital investment ($860 000) followed by the cost of the land ($310 000). The net present value of the plant was calculated to be 2 100 000 with an internal rate of return of 31%.

References

AA Motoring. (2017, November 9). *November 2017 petrol and diesel prices*. Retrieved from AA motoring: http://www.aa.co.nz/cars/motoring-blog/petrolwatch/november-2017-petrol-and-diesel-prices/

Aqua-Calc. (2016, November 1). *Brewers, grain density in 285 measurement units*. Retrieved from Aqua-calc conversions + calculations: http://www.aqua-calc.com/page/density-table/substance/brewers-coma-and-blank-grain

Banks, I. (2014). *To assess the impact of Black Soldier Fly (Hermetia Illucens) Larvae on faecal reduction in pit latrines.* London: London School of Hygiene and Tropical Medicine.

Barros-Cordeiro, K., Bao, S., & Pujol-Luz, J. (2014). Intra-puparial development of the black soldier-fly, Hermetia illucens. *Journal of Insect Science Vol 14*.

Barry, T. (2004). *Evaluation of the Economic, Social and Biological Feasibility of Bioconverting Food Wastes with the Black Soldier Fly (Hermetia Illucens).* North Texas: University of North Texas.

Bizfluent. (2018, February 4). *How to Calculate the MARR*. Retrieved from Bizfluent: https://bizfluent.com/how-7864929-calculate-marr.html

Booth, D., & Sheppard, D. (1984). Oviposition of the black soldier fly, Hermetia Illucens (L) (Diptera: Stratiomyidae): Eggs, masses, timing, and site characteristics. *Environ. Entomol. 13*, 421-423.

Bunnings Warehouse. (2017, November 4). *Compost*. Retrieved from Bunnings Warehouse: https://www.bunnings.co.nz/our-range/garden/gardening/garden-maintenance/compost

Clarke, J. L. (2005). *United States of America Patent No. US6112453A.*

Courtright, G. N. (2014). *United States of America Patent No. US8733284B2.*

Cullere, M., Tasoniero, G., Giaccone, V., Miotti-Scapin, R., Claeys, E., & De Smet, S. (2016). Black soldier fly as dietary protein source for broiler quails: apparent digestibility, excreta microbial load, feed choice, performance, carcass and meat traits. *The animal Consortium*, 1923-1930.

Deublein, D., & Steinhauser, A. (2011). *Biogas from waste and renewable resources: An introduction.* Germany: John Wiley and Sons.

Diclaro II, J. W., & Kaufman, P. E. (2009). *Black Soldier Fly Hermetia Illucens Linnaeus (Insecta: Diptera: Stratiomyidae).* Florida: University of Florida.

Diener, S. (2010). *Valorisation of Organic Solid Waste using the Black Soldier Fly, Hermetia Illucens, in Low and Middle-income countries.* Zurich: Swiss Federal Institute of Aquatic Science and Technology.

Diener, S., Solano, N. S., Gutierrez, F. R., & Zurbrugg, C. (2011). Biological treatment of municipal organic waste using Black Soldier Fly Larvae. *Waste and Biomass Valorization* , 357-363.

Diener, S., Zurbrugg, C., & Tockner, K. (2009). *Conversion of organic material by black soldier fly larvae: establishing optimal feeding rates.* Dübendorf, Switzerland: Swiss Federal Institute of Aquatic Science and Technology.

ECR Forklifts. (2018, February 4). *ECR Equipment*. Retrieved from ECR Equipment: http://ecrl.y9.co.nz/index.php/forklifts

Engineering Toolbox. (2014, December 2). *Air - Density and Specific Weight*. Retrieved from The Engineering Toolbox: http://www.engineeringtoolbox.com/air-density-specific-weight-d_600.html

Engineering Toolbox. (2015, September 02). *Air composition*. Retrieved from The Engineering Toolbox: http://www.engineeringtoolbox.com/air-composition-d_212.html

Food and Agriculture Organization of the United Nations. (2015, October 20). *Black Soldier Fly larvae (Hermetia Illucens)*. Retrieved from Feedipedia Animal Feed resources information system: http://www.feedipedia.org/node/16388

Food and Agriculture Organization of the United Nations. (2016, Jun 17). *Brewers grain*. Retrieved from Feedipedia Animal Feed Resources information system: http://www.feedipedia.org/node/74

Gough CAT. (2017, November 6). *303.5E CR Hydraulic Excavator*. Retrieved from Gough CAT: https://www.buycat.co.nz/buildyourown/mini-excavators/303-5e-cr-hydraulic-excavator/

Hale, O. (1973). Dried Hermetia illucens larvae (Diptera: Stratiomyidae) as a feed additive for poultry. *Journal of the Georgia Entomological Society*, 16-20.

Harnden, L. M., & Tomberlin, J. K. (2016). Effects of temperature and diet on black soldier fly, Hermetia Illucens, development. *Forensic Science International*, 109-116.

Holmes, L. (2012). Relative Humidity Effects on the Life History of Hermetia illucens (Diptera: Stratiomyidae). *Environmental Entomology 41*, 971-978.

Hughes, P. R. (2004). *United States of America Patent No. US5351643A.*

Huis, A., Itterbeeck, J., Klunder, H., Mertens, E., Halloran, A., Muir, G., & Vantomme, P. (2013). *Edible insects - Future prospects for food and feed security.* Rome: Food and Agriculture Organization of the United Nations.

Hyster. (2018, February 4). *The Optimal Time to Replace your Forklift*. Retrieved from Logistics and Maintenance Blog: http://www.aalhysterforklifts.com.au/index.php/about/blog-post/the_optimal_time_to_replace_your_forklift

Institute for the Environment. (2013). *The Black Soldier Fly – How to Guide.* North Carolina: Chapel Hill: University of North Carolina.

Investopedia. (2017, May 10). *What is the formula for calculating net present value (NPV)?* Retrieved from Investopedia: https://www.investopedia.com/ask/answers/032615/what-formula-calculating-net-present-value-npv.asp

Kerby, C., & Vriesekoop, F. (2017). *An overview of the utilisation of brewery by-products as generated by British craft breweries.* United Kingdom: Department of Food Science and AgriFood Supply Chain Management, Harper Adams University.

Kim, W., Bae, S., Park, K., Lee, S., Choi, Y., Han, S., & Koh, Y. (2011). Biochemical characterization of digestive enzymes in the black soldier fly, Hermetia illucens (Diptera: Stratiomyidae). *Journal of Asia-Pacific Entomology 14*, 11-14.

Kotz, M. (2007). *United States of America Patent No. US4563344A.*

Lalander, C. H., Fidjeland, J., Diener, S., Eriksson, S., & Vinneras, B. (2015). High waste-to-biomass conversion and efficient Salmonella spp. reduction using black soldier fly for waste recycling. *Agronomy for Sustainable Development*, 261-271.

Li, Q., Zheng, L., Qiu, N., Cai, H., Tomberlin, J., & Yu, Z. (2011). Bioconversion of dairy manure by black soldier fly (Diptera: Stratiomyidae) for biodiesel and sugar production. *Waste Management 31*, 1316-1320.

Li, S., Ji, H., Zhang, B., Tian, J., Zhou, J., & Yu, H. (2016). Influence of black soldier fly (Hermetia illucens) larvae oil on growth performance, body composition, tissue fatty acid and lipid deposition in juvenile Jian carp (Cyprinus carpio var. Jian). *Aquaculture 465*, 43-52.

Li, W., Li, Q., Zheng, L., Wang, Y., Zhang, J., Yu, Z., & Zhang, Y. (2015). Potential biodiesel and biogas production from corncob by anaerobic fermentation and black soldier fly. . *Bioresouce Technology, 194*, 276-282.

Matufela, R. (2015). *High Value Organic Waste Treatment via Black Soldier Fly Bioconversion.* Stockholm: Royal Institute of Technology.

Matufela, R. N. (2015). *High Value Organic Waste Treatment via Black Soldier Fly Bioconversion.* Stockholm: Royal Institute of Technology.

Ministry of Business, Innovation, and Employment. (2017). *Quarterly Survey of Domestic Electricity Prices.* Auckland: Ministry of Business, Innovation, and Employment.

Mussatto, S. (2014). Brewer's spent grain: A valuable feedstock for industrial applications. *J. Sci. Food Agric. 94*, 1264-1275.

Mutafela, R. N. (2015). *High Value Organic Waste Treatment via Black Soldier Fly Bioconversion.* Stockholm: Royal Institute of Technology.

Nguyen, T., Tomberlin, J., & Vanlaerhoven, S. (2013). Influence of resources on Hermetia Illucens (Diptera: Stratiomyidae) larval development. *Journal of Medical Entomology 50 (4)*, 898-906.

Nguyen, T., Tomberlin, J., & Vanlaerhoven, S. (2015). Ability of Black Soldier Fly (Diptera: Stratiomyidae) Larvae to Recycle Food Waste. *Environmental Entomology 44*, 406-410.

Outhen, P. (2014). *A survey of commercial small-scale poultry production systems and nutrient characterisation of local feed ingredients in Laos : a thesis presented in partial fulfilment of the requirements for the degree of Master of AgriScience at Massey University.* Palmerston North: Massey University.

Park, S., Chang, B., & Yoe, S. (2014). Detection of antimicrobial substances from larvae of the black soldier fly, Hermetia illucens (Diptera: Stratiomyidae). *Entomological Research 44*, 58-64.

Popa, R., & Green, T. (2012). Using black soldier fly larvae for processing organic leachates. *Journal of Econ. Entomol. 105*, 374-378.

Poultry Association of New Zealand and Egg Producers Federation of New Zealand. (2011). *Poultry management in New Zealand: production, manure management and emission estimations for the commercial chicken, turkey, duck and layer industries within New Zealand.* Wellington: Ministry of Agriculture and Forestry.

PROteINSECT. (2017, April 27). *About the Project*. Retrieved from PROteINSECT Insects as Sustainable Sources of Protein: http://www.proteinsect.eu/index.php?id=32

Quandl. (2017, November 14). *Fishmeal price*. Retrieved from Quandl: https://www.quandl.com/data/ODA/PFISH_USD-Fishmeal-Price

Rawlinsons Ltd. (2014). *Rawlinsons New Zealand Construction Hand Book.* Auckland: Rawlinsons Publications.

Realestate.co.nz. (2017, November 2). *Crown Road, Hilltop Commercial for sale industrial land*. Retrieved from Realestate.co.nz: https://www.realestate.co.nz/3217110

Reeb, J., & Milota, M. (1999). *Moisture content by the oven-dry method for industrial testing.* Oregon: Oregon State University.

Sheppard, C., Newton, G. L., Thompson, S. A., & Savage, S. (1994). A value added manure management system using the black soldier fly. *Bioresource Technology*, 275-279.

Sheppard, C., Newton, G., Thompson, S., & Savage, S. (1994). A value added manure management system using the black soldier fly. *Bioresource Technology 50*, 275-279.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., & Templeton , D. (2005). *Determination of Ash in Biomass.* Colorado: National Renewable Energy Laboratory.

Statistics New Zealand. (2017). *Price indexes for the construction industry.* Auckland: Statistics New Zealand.

Stellar Machinery. (2018, February 4). *Stellar Forklifts*. Retrieved from Forklifts: https://www.stellarmachinery.co.nz/forklifts/

Stocks, C., Barker, A., & Guy, S. (2002). The composting of brewery sludge. *J. Inst. Brew. 108*, 452-458.

Thomas, K., & Rahman, P. (2006). Brewery wastes. Strategies for sustainability. A review. *Asp. Appl. Biol. 80*, 147-153.

Tomberlin, J. K., Sheppard, C. D., & Joyce, J. A. (2002). Selected Life-History Traits of Black Soldier Flies (Diptera: Stratiomydae) Reared on Three Artificial Diets. *Entomology Society of America 95*, 379-386.

Tomberlin, J., & Sheppard, D. (2001). Lekking behaviour of the black soldier fly (Diptera: Stratiomyidae). *Florida Entomology 84*, 729-730.

Tomberlin, J., Adler, P., & Myers, H. (2009). Development of the Black Soldier Fly (Diptera: Stratiomyidae) in Relation to Temperature. . *Environmental Entomology 38*, 930-934.

Trademe. (2018, 01 19). *6HP compressor*. Retrieved from Trademe: https://www.trademe.co.nz/business-farming-industry/industrial/compressors-pumps/compressors/auction-1513502763.htm?rsqid=ae1c71d18991486e9eebf86fa9546ff0

Trademe. (2018, 01 19). *Grain Silo*. Retrieved from Trademe: https://www.trademe.co.nz/business-farming-industry/farming-forestry/harvest-postharvest/other/auction-1410231309.htm?rsqid=09e9861495ea4e95b4447c912e60f888

Uemura, Y., Lam, M., Ramli, A., Bashir, M., & Tham, L. (2017). Optimization of self-fermented period of waste coconut endosperm destined to feed black soldier fly larvae in enhancing the lipid and protein yields. *Renewable Energy 111*, 646-654.

Ushakova, N. A., Brodskii, E., Kovalenko, A., Bastrakov, A., Kozlova, A., & Pavlov, D. (2016). Characteristics of lipid fractions of larvae of black soldier fly Hermetia Illucens. . *Biochemistry, Biophysics and Molecular Biology Vol 468*, 209-212.

Webster, C., Rawles, S., Koch, J., Thompson, K., Kobayashi, Y., & Gannam, A. (2016). Bio-ag reutilization of distiller's dried grains with solubles (DDGS) as a substrate for black soldier fly larvae, Hermetia Illucens, along with poultry by-product meal and soybean meal, as a total replacement of fish meal in diets for Nile Tilapia. *Aquaculture Nutrition 22*, 976-968.

Widjastuti, T., Wiradimadja, R., & Rusmana, D. (2014). The effect of substitution of fish meal by black soldier fly maggot meal in the diet on production performance of quail. *Scientific Papers. Series D. Animal Science. Vol. LVII*, 125-129.

Winnie, G.-L., Arjen, Y. H., & Theo, H. v. (2008). *The water footprint of energy.* New York: Cornell University.

Zhou, F., Tomberlin, J. K., Zheng, L., Yu, Z., & Zhang, J. (2013). Developmental and Waste Reduction Plasticity of Three Black Soldier Fly Strains (Diptera: Stratiomyidae) Raised on Different Livestock manures. *J. Med. Entomol. 50*, 1224-1230.

Appendices

Appendix I. Mass and Energy Balance Calculations

The total dry biomass output of 100 kilogram per week was used as a design parameter. The average wet weights of a single 1 week old larvae and a single final prepupae were assumed to be 50 milligram and 117 milligram, respectively. These wet weights were taken from the experiment conducted by PFR.

The dry weights of the 1 week old larvae and final prepupae were calculated by firstly calculating the weight of water present in the larvae as shown below. The moisture content of the Black Soldier fly shown in Table 3.

$$Weight_{water} = Weight_{wet} \times Moisture\ content \quad (1)$$

$$Weight_{water,prepupae} = Weight_{wet,prepupae} \times Moisture\ content$$

$$Weight_{water,prepupae} = 117 \times 63\%$$

$$Weight_{water,prepupae} = 73.7\ milligram$$

The weight of the water was then subtracted to the total wet weight to obtain the dry weight.

$$Weight_{dry} = Weight_{wet} - Weight_{water} \quad (2)$$

$$Weight_{dry,prepupae} = 117 - 73.7$$

$$Weight_{dry,prepupae} = 43.1\ milligram$$

Hence, the dry weight of a single prepupae was 43.1 milligram. The dry weight of a single larvae was calculated using the same method outlined and was calculated to be 18.5 milligram.

Assuming that the final prepupae leaving the system would have the same dry weight, the total number of Black Soldier Fly required in order to produce the 100 kilogram of dry weight was calculated.

$$Total\ no.of\ Black\ Soldier\ Fly = \frac{Total\ Dry\ Biomass\ Required}{Weight_{dry,prepupae}} \quad (3)$$

$$Total\ no.of\ Black\ Soldier\ Fly = \frac{100\ kilogram}{43.1\ milligram}$$

$$Total\ no.of\ Black\ Soldier\ Fly = 1142442$$

In order to produce 100 kilogram of dry biomass per week, the system should be able to harvest 1,142,442 bugs in a week.

Using the dry weights calculated, the biomass gained by a single Black Soldier Fly was calculated as the difference between the final prepupae weight and the initial 1 week old larvae. This was calculated as shown below.

$$Biomass\ gained = Weight_{dry,prepupae} - Weight_{dry,larvae} \quad (4)$$

$$Biomass\ gained = 43.1\ milligram - 18.5\ milligram$$

$$Biomass\ gained = 24.6\ milligram$$

This implies that a single Black Soldier Fly would gain 24.6 milligram of biomass inside the system within 2 weeks, on average. Using the breakdown of feed from the experiment conducted by PFR, shown in Table 2, the feed requirement of a single Black Soldier Fly was calculated.

$$Biomass\ gained = \%\ of\ feed\ used\ for\ biomass\ conversion\ \times Feed\ used \quad (5)$$

$$Feed\ used_{dry} = \frac{Biomass\ gained}{\%\ of\ feed\ used\ for\ biomass\ conversion}$$

$$Feed\ used_{dry} = \frac{24.6\ milligram}{5\%}$$

$$Feed\ used_{dry} = \frac{24.6\ milligram}{5\%}$$

$$Feed\ used_{dry} = 0.492\ gram\ of\ feed$$

Therefore, a single Black Soldier Fly would consume 0.492 grams of feed in the system over 2 weeks on average.

The weight of the dry feed required to produce the necessary dry biomass weight can be calculated by multiplying the feed consumption rate of a single Black Soldier Fly by the total number of Black Soldier Fly required.

$$Total\ Feed\ used_{dry} = Feed_{dry,single\ BSFly} \times Total\ number\ of\ BSFly \tag{6}$$

$$Total\ Feed\ used_{dry} = 0.492\ gram \times 2{,}317{,}729$$

$$Total\ Feed\ used_{dry} = 1141531\ grams$$

$$Total\ Feed\ used_{dry} = 1142\ kilograms$$

This indicates that in order to produce 100 kilogram of dry biomass, a total of 1142 kilogram of spent grain would be required.

The weight of the undigested feed and waste, and the feed lost within the system were calculated using the percentage outlined in Table 2 and equation (5). The weight of the undigested feed and waste, the feed lost within the system were 685 kilogram and 400 kilogram, respectively.

The total initial weight of the Black Soldier Fly in the system was calculated by multiplying the weight of a single 1 week old larvae and the total number of Black Soldier Fly required.

$$Total\ Weight_{dry,larvae} = Weight_{dry,1\ larvae} \times Total\ number\ of\ larvae \tag{7}$$

$$Total\ Weight_{dry,larvae} = 18.5\ milligram \times 2{,}317{,}729$$

$$Total\ Weight_{dry,larvae} = 42.9\ kilogram$$

The total weight of the initial larvae in the system was calculated to be 42.9 kilogram. The total weight of the final prepupae in the system was calculated to be 100 kilogram, which was also the design parameter.

The weight of each component of the Black Soldier Fly, the feed and the waste can be calculated using Equation 8.

$$Weight_{component} = \% \, of \, weight_{dry,component} \times Weight_{dry,substance} \tag{8}$$

Hence, the weight of the carbohydrates present on the spent grain was calculated as shown.

$$Weight_{carbohydrate} = \% \, of \, weight_{dry,carbohydrate} \times Weight_{dry,brewer's\,waste}$$

$$Weight_{carbohydrate} = 70\% \times 343 \, kilogram$$

$$Weight_{carbohydrate} = 240 \, kilogram$$

Therefore, the weight of the carbohydrate present on the total feed used was 800 kilogram. The weight of each component of the initial feed, initial larvae, final prepupae and waste were calculated using this method and tabulated in Table 19.

Table 19. Breakdown table of weights expressed in kilograms.

Substance	Total Dry Weight	Protein	Carbohydrates	Fat	Ash	Other
Spent grain	1142.4	228.5	800	-	57.1	190.4
Undigested feed and waste	685.4	137.1	479.8	-	34.3	114.2
Total initial larvae	42.88	8.6	30.0	-	2.1	9.3
Total final prepupae	100.0	20.0	70.0	-	5.0	21.6

The weight of the water was then calculated using equation (1) and the moisture contents outlined. The total wet weight was then calculated as the sum of the water weight and the dry weight. The breakdown of weights was shown in Table 20.

Table 20. Water and total wet weights expressed in kilograms.

Substance	Total Dry Weight	Water Weight	Total Weight
Spent grain	1142.4	799.7	342.7
Undigested feed + waste	685.5	479.8	205.6
Total initial larvae	115.9	73.0	42.9
Total final prepupae	270.3	170.3	100.0

In order to quantify the air requirement of the system, the Chemical Oxygen Demand (COD) was used. The COD is the measure of how much oxygen is required to fully oxidize all oxidisable organic compounds in the system. The COD of all incoming and outgoing streams were calculated. The total COD of the incoming streams were calculated as the sum of the COD of all incoming streams. Similarly, the total COD of the outgoing streams were calculated. The difference of the total COD of the incoming and outgoing streams would be the oxygen requirement of the system.

The electron balanced have to be worked out in order to obtain the COD of a stream. This was done by writing an oxidation reaction for each component and assuming that all nitrogen was released as NH_4^+. Assuming that the protein present on the spent grain and the Black Soldier Fly was generalized on the empirical formula $C_{16}H_{24}O_5N_4$, the oxidation reaction can be written as:

$$C_{16}H_{24}O_5N_4 + 27H_2O \rightarrow 16CO_2 + 4NH_4^+ + 62H^+ + 66e^- \quad (9)$$

According to equation (9), the oxidation reaction needs 66 moles of electron (e^-). Using the fact that one mole of oxygen (O_2) accepts 4 moles of electron, the COD can be calculated as:

$$COD = \frac{Total\ moles\ of\ electron}{Moles\ of\ electron\ that\ can\ be\ accepted} \quad (10)$$

$$COD = \frac{66}{4}$$

$$COD = 16.5$$

This indicates that for every 1 mole of protein to be oxidized, a total of 16.5 moles of oxygen would be used. Using the chemical of the protein, the molecular weight can be determined by adding together the total molecular weight of each element on the formula.

Table 21. Protein Molecular Weight. Weights are expressed as kilogram per kilo mole.

Element	Molecular weight	No. of atoms	Total molecular weight
C	12	16	192
H	1	24	24
N	14	4	56
O	16	5	80

Based from the values on Table 23, the total molecular weight for the protein was calculated to be 352 kilogram per kilo mole. This implies that 1 kilo mole of protein weighs 352 kilogram. The weight of oxygen needed to oxidize this amount of protein, was calculated using the molecular weight of oxygen and the oxygen required, which was 16.5 kilo moles. The weight of the COD was calculated to be 528 kilograms. Using the weights of the protein and the COD, the COD to molecular weight ratio can be obtained.

$$\frac{COD}{MW} = \frac{Weight\ of\ Oxygen\ Demand}{Weight\ of\ 1\ kilo\ mole\ of\ component} \tag{11}$$

$$\frac{COD}{MW} = \frac{528}{352}$$

$$\frac{COD}{MW} = 1.5$$

Hence, for every 1 kilogram of protein that would be oxidized, 1.5 kilogram of oxygen would be used. Using this method, the COD to molecular weight ratio of the components of the spent grain and the Black Soldier Fly was calculated and shown in Table 22.

Table 22. Chemical oxygen demand and molecular weight ratio.

Component	Formula	Molecular weight (kg/kmol)	COD/MW
Carbohydrates	CH_2O	30	1.07
Proteins	$C_{16}H_{24}O_5N_4$	352	1.50
Fats	$C_6H_{16}O$	104	2.92
Lignin	$C_{10}H_{12}O_3$	178	2.07

Based from the value shown in Table 19 the weight of protein present on the spent grain that was used as feed was 228.5 kilogram. Using the COD to molecular weight ratio, the amount of oxygen required to oxidize this protein can be calculated.

$$COD_{component} = \left(\frac{COD}{MW}\right)_{component} \times Weight_{component} \tag{12}$$

$$COD_{protein} = \left(\frac{COD}{MW}\right)_{protein} \times Weight_{protein}$$

$$COD_{protein} = 1.5 \times 228\ kilogram$$

$$COD_{protein} = 343\ kilogram \text{ of } O_2$$

Therefore, the COD of the protein present on the spent grain that was fed to the system was 343 kilogram of Oxygen.

The total organic carbon (TOC) content of each component was also calculated. This was done to be able to calculate the amount of carbon lost within the system. Once the weight of the carbon "lost" from between the incoming and outgoing stream was quantified, the weight of carbon dioxide produced can be deduced.

The TOC was calculated by multiplying the number of elements of carbon, which exist in an organic substance, by the molecular weight of carbon. According to equation (9), carbon elements were present on both $C_{16}H_{24}O_5N_4$ and CO_2 for the oxidation of the protein. However, carbon dioxide, CO_2, is an inorganic substance. Due to this the number of element of carbon used for the calculation of the TOC was 16. This number of element of carbon was multiplied by the molecular weight of carbon, which is 12 kilogram per kilo mole, to obtain the TOC of the protein as 192 kilogram. The ratio of TOC to molecular weight can be obtained similarly to that of the COD to molecular weight ratio.

$$\frac{TOC}{MW} = \frac{Weight\ of\ organic\ carbon\ present}{Weight\ of\ 1\ kilo\ mole\ of\ component} \tag{13}$$

$$\frac{TOC}{MW} = \frac{192}{352}$$

$$\frac{TOC}{MW} = 0.545$$

Hence, there would be 0.545 kilogram of total organic carbon for every 1 kilogram of protein.

The total weight of organic carbon present on the protein of the spent grain used as feed can then be calculated as:

$$TOC_{component} = \left(\frac{TOC}{MW}\right)_{component} \times Weight_{component} \qquad (14)$$

$$TOC_{protein} = \left(\frac{TOC}{MW}\right)_{protein} \times Weight_{protein}$$

$$TOC_{protein} = 0.545 \times 228\ kilogram$$

$$TOC_{protein} = 125\ kilogram$$

Therefore, there was a total of 125 kilogram of organic carbon present on the spent grain that was used as feed.

The oxygen requirement (COD) and organic carbon (TOC) was calculated for every component of all streams. These were tabulated on Table 23.

Table 23. Oxygen demand and organic carbon content.

Incoming stream	Weight (kg)	COD (kg)	TOC (kg)
Spent grain protein	228	343	125
Spent grain carbohydrates	800	853	320
Spent grain other	61.7	128	41.6
1 week old larvae protein	17.6	26.4	9.59
1 week old larvae fat	12.9	37.6	8.91
1 week old larvae other	3.60	3.84	1.44
Total in	1124	1391	506
Outgoing stream	**Weight (kg)**	**COD (kg)**	**TOC (kg)**
Undigested feed + waste protein	137	206	74.8
Undigested feed + waste carbohydrates	480	512	192
Undigested feed + waste 'others'	37.0	76.5	25.0
Prepupae protein	41.0	61.5	22.4

Prepupae fat	30.0	87.7	20.8
Prepupae 'other'	8.40	8.96	3.36
Total out	733	952	338

Based from the values shown on Tables 23, the oxygen requirement of the system can be calculated as the difference to the total COD out and the total COD in. The difference would give the amount of oxygen used within the system and was calculated to be 439 kilogram of oxygen.

Similarly, the difference on the TOC, calculated to be 167 kilogram, would indicate the amount of organic carbon "lost" on the system. Assuming that the carbon could only leave the system as CO_2, the weight of the carbon dioxide production can be calculated.

$$Weight_{CO_2} = Weight_C \times \frac{Molecular\ weight_C}{Molecular\ Weight_{CO_2}} \quad (15)$$

$$Weight_{CO_2} = 167 \times \frac{44}{12}$$

$$Weight_{CO_2} = 615\ kilogram\ of\ CO_2$$

Hence, the system would produce 615 kilogram of CO_2 over the course of 2 weeks which needs to be displaced out of the system.

Table 24. Air weight component breakdown.

Oxygen	23%
Nitrogen	75%
Carbon Dioxide	0.062%
Other	1.3%

The weight component of air was obtained from Engineering Toolbox (2015) and shown in Table 24. These compositions were percentage of dry weight of air. For this mass and energy balances, the system was assumed to be kept at 27°C which was within the optimum range for the BSFL growth. The air on the system was assumed to be saturated. The saturation concentration of 28 gram of water per kilogram of air was calculated using the calculator outlined on TLV (2016). The density of air was assumed to be 1.2 kilogram per cubic meters (Engineering Toolbox, 2014).

In order for the system to be kept at aerobic conditions, fresh saturated air would be added for the carbon dioxide produced to be displaced. The amount of air to be added would be based on the oxygen demand of the system. Using the oxygen demand, 439 kilogram of oxygen, and the breakdown of the weight components of air, the total air requirement can be calculated. The total amount of air required was calculated to be 1936 kg wet air for the course of two weeks.

However, this air would result in oxygen depletion as all of the oxygen would be used for the oxygen demand. It was deemed that the total oxygen concentration in the system should not fall below 18% by wet weight. Oxygen levels lower than this could result in extra stress for the Black Soldier Fly and very low oxygen levels would result in fatality. Due to this, the total oxygen demand would be six folds than the calculated COD. The breakdown of the incoming and outgoing air, giving enough oxygen to keep the oxygen level within 18%, can be seen in Table 25. This amount of air equates to 30 cubic meters of air per hour.

Table 25. Breakdown of air streams expressed as kilograms. Exit air would have 18% oxygen level to keep optimum conditions. Component weights are dry weights.

Air stream	Total Weight	Water	Total Dry Weight	CO_2	O_2	N_2	Other
Air in	11616	263	11352	7.04	2634	8568	144
Air out	11792	263	11529	622	2195	8568	144

The energy balance can be conducted using the heating value of each component and a similar process of multiplying the weight of each component for each stream. Hence, the energy content of the protein content of the spent grain can be calculated as:

$$E_{component} = HHV_{component} \times Weight_{component} \tag{16}$$

$$E_{protein} = 22.7\ \frac{MJ}{kg} \times 228\ kg$$

$$E_{component} = 5187\ MJ$$

Therefore, the energy content of the protein on the spent grain was 5187 MJ. This was done for every component of each stream. These calculated values were shown in Table 26.

Table 26. Energy contents of each stream.

Inputs	**Energy (MJ)**
Spent grain protein	5187
Spent grain carbohydrates	13835
Spent grain other	1845
1 week old larvae protein	399
1 week old larvae fat	485
1 week old larvae other	62.3
Total in	21813
Outputs	**Energy (MJ)**
Undigested feed + waste protein	2372
Undigested feed + waste carbohydrates	8301
Undigested feed + waste 'others'	1107
Prepupae protein	931
Prepupae fat	1131
Prepupae 'other'	145
Total out	13986

The difference in the energy content of the outgoing and incoming stream would be the amount of energy released by the system as heat.

$$\Delta Heat = Energy_{out} - Energy_{in} \tag{17}$$

$$\Delta Heat = 13986\ MJ - 21813\ MJ$$

$$\Delta Heat = -\ 7826\ MJ$$

This meant that a total of 7826 MJ of energy would be lost. The negative sign indicates that this would be lost in to the environment as heat. This energy would be released in the course of two weeks. Assuming that the rate of energy loss would be constant.

$$\dot{H} = \frac{Heat}{time} \tag{18}$$

$$\dot{H} = \frac{7826\ MJ}{1{,}209{,}600\ sec}$$

$$\dot{H} = 0.0065\ MW$$

$$\dot{H} = 6.5\ kW$$

Therefore, a total of 6.5 kW of energy would be released by the system.

Appendix II. Preliminary experiment for respiration experiments.

A total of 51 larvae (approx. 1 week old) were obtained from PFR and transferred into 120 mL Wheaton flasks. These flasks were thus supplied either 0 (control), 1, 5, 10, 15 or 20 larvae, sealed using a septum and a metal cap to prevent gas exchange and incubated for 24 hrs at room temperature. Gas samples (6 mL) were then withdrawn from each flask using a syringe and analysed using gas chromatography to quantify the CO_2 content.

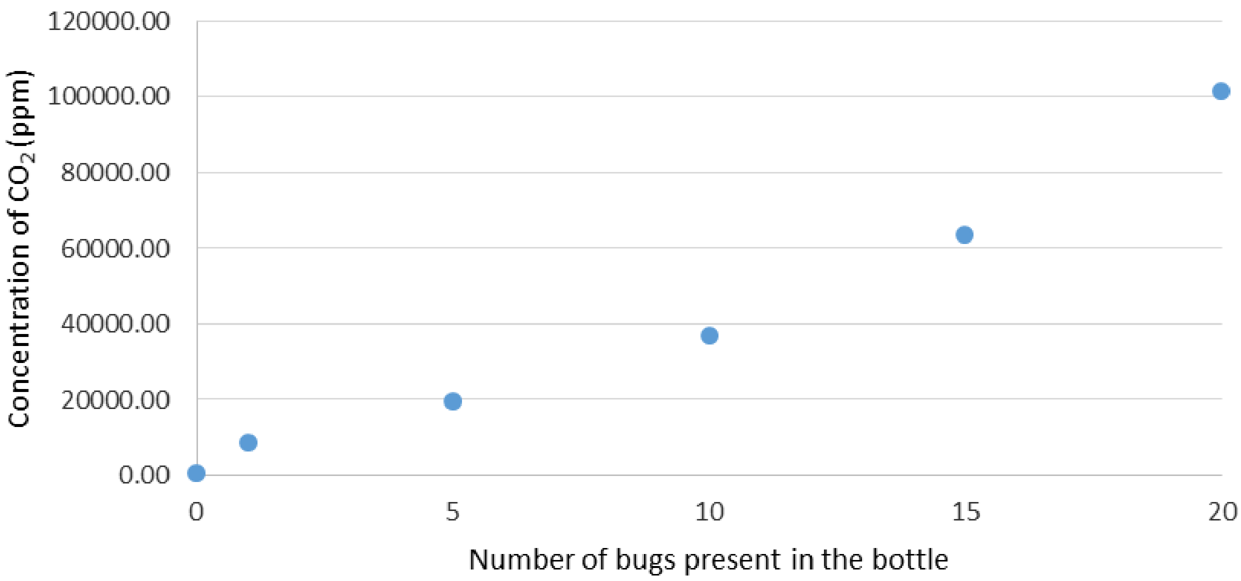


Figure 23. CO_2 concentrations (ppm) after 24 hrs in sealed flasks without feed.

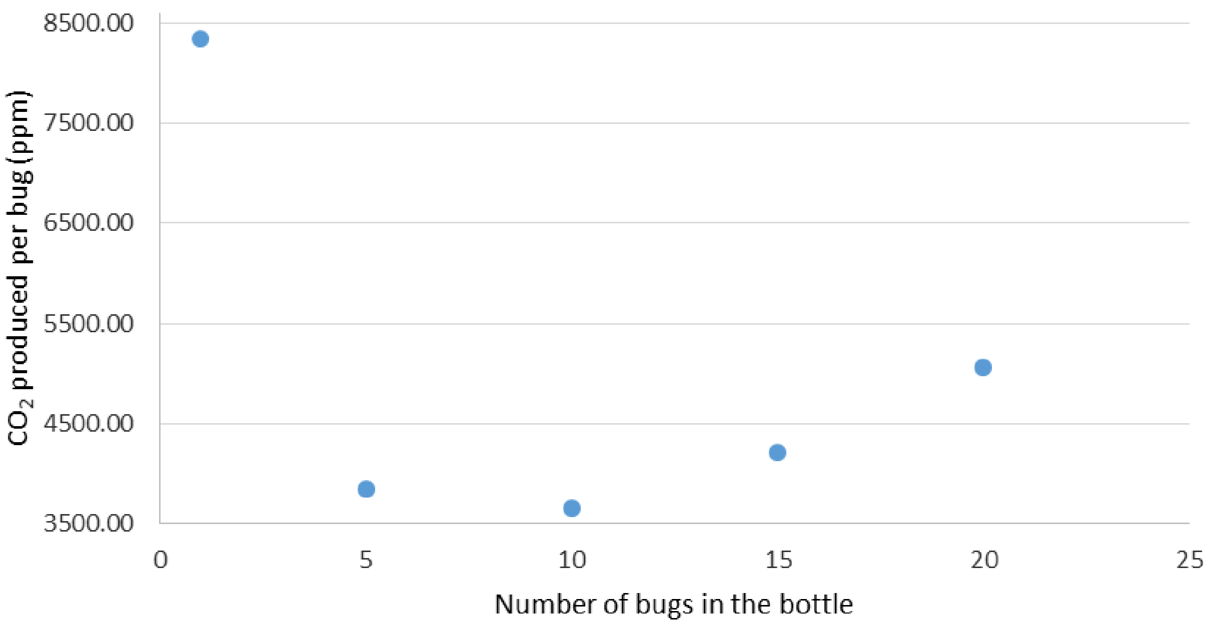


Figure 24. Specific CO_2 production per larvae after 24 hr in sealed flasks (no feed).

Based from the results shown in Figure 23 it can be inferred that as the number of bugs increases, the response to the CO_2 concentration becomes more evident. Therefore, the most number of bugs possible should give a clearer picture of the CO_2 concentration changes. However, based on Figure 12 it was evident that as the number of bugs inside the flask increases, the larger the amount of specific CO_2 was produced by the larvae. This can be due to the lack of space and shortage of oxygen available to each individual larva. Due to this, it was deemed that having 5 larvae per flask should give the necessary sensitivity to register a response while keeping the number of larvae subjected to testing.

Appendix III. Method for finding rate of gas consumption and production.

The calibration curves used for the O_2 and CO_2 were shown below.

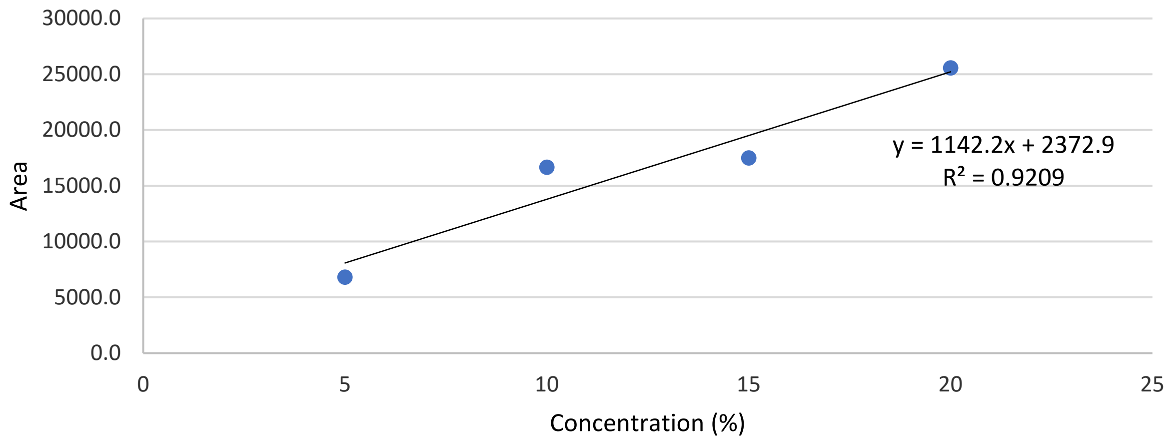


Figure 25. Oxygen calibration curve.

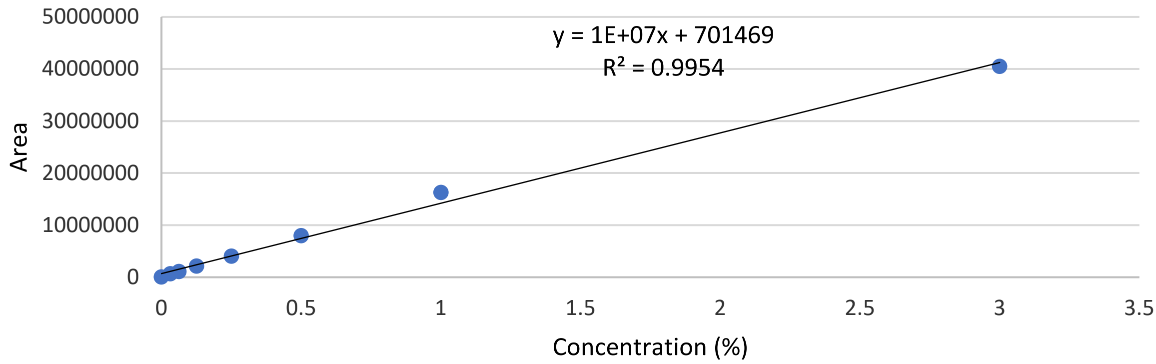


Figure 26. Carbon Dioxide calibration curve.

Using the area reading of the gas chromatograph and the calibration curves, the concentration at each hour reading was calculated. The difference of the reading for that hour and the control was found. This difference was then divided by the total hour spent by the bugs on that bottle before the sample was taken. This was deemed to be amount of oxygen used, in %. This concentration was converted to parts per million by dividing it by 10 000. Using the equation below, the concentration at mg/m³ was found.

$$Concentration\ (mg\ m^{-3}) = Concentration\ (ppm) \times \frac{Molecular\ mass\ (\frac{g}{mol})}{Molar\ volume\ (L)}$$

The molar volume in this equation is the volume that 1 mole of gas occupies at STP which is 22.4 litres. Once the amount of concentration difference in mg m^{-3} was found, it was multiplied by total volume of the Wheaton bottles which is $1.2X10^{-4}$ m³ to find the amount of gas used or produced within 1 hour. This value was then divided by 5, as there were 5 bugs in each bottle, in order to find the gas production or consumption per hour per bug.

Appendix IV. Results from feed and larvae characterization experiments.

The feed characterization results were tabulated in Table 27.

Table 27. Feed characterization result.

Parameter	Fresh	Frozen
Beaker weight (g)	172	186
Beaker + spent grain weight (g)	350	330
Spent grain weight (g)	178	144
Volume (ml)	400	400
Bulk Density (kg/m³)	450	360
Initial spent grain weight (g)	178	144
Beaker + saturated weight (g)	390.	384
Saturated weight (g)	219	198
Water gained (g)	40.5	53.5
Container weight (g)	2.10	2.00
Container + wet mass (g)	44.9	37.5
Initial wet mass (g)	42.8	35.5
Container + dry mass (g)	12.9	12.7
Dry mass (g)	10.6	10.5
Water mass (g)	32.2	25.0
Moisture content wet basis	75%	70%
Total water mass at saturation (g)	205	193
Moisture content wet basis @ saturation	94%	98%

The spent grain ash content determination experiment result was tabulated in Table 28.

Table 28. Ash content of spent grain. Dried at 105°C (2 d) and incinerated at 550° (2 hr)

Initial dry mass (g)	10.6	10.5
Container (g)	77.9	62.7
Container + ash (g)	78.3	63.4
Ash content (g)	0.40	0.70
Ash content (% dry mass)	4%	7%
Ash content (% wet mass)	1%	2%

The results for the bulk density calculation for Group A can be seen on the Table 29 below. The volume of the water that covers the same volume of the grubs was calculated using the density of water, 1 gram per millilitre, and the mass of the water. Using the weight of the larvae and the volume of the larvae, the bulk density was calculated.

Table 29. Density calculation for 1-week-old Larvae.

Beaker weight (g)	30.9
Beaker + 100 grub weight (g)	37.1
Weight of 100 grubs (g)	6.20
Weight of 1 larvae (g)	0.06
Water + beaker weight (g)	48.2
Weight of water at same volume (g)	17.3
Volume of water (mL)	17.3
Density of grub (g/mL)	0.36
Density of grub (kg/m^3)	360

The moisture content and water content calculation results for Group A for the larvae characterization experiment were shown in Table 30.

Table 30. Moisture content results.

Drying container mass (g)	1.5
Container + wet mass (g)	5.2
Dry mass + container (g)	2.3
Wet mass (g)	3.7
Dry mass (g)	0.8
Water content (g)	2.9
Moisture content wet basis	78%

The results of the ash content experiment for Group A for the larvae characterization experiment on Table 31 below.

Table 31. Ash content results.

Container for furnace weight (g)	45.1
Dried sample + container (g)	46
Ash + container (g)	45.2
Dry sample (g)	0.9
Ash content (g)	0.1
Ash % dry basis	11%

The breakdown of the type of Black Soldier Fly present on the system after one week can be seen on Table 32 below.

Table 32. Black Soldier Fly distribution after 1 week.

Initial total number of larvae	100
Number of prepupae recovered	40
Number of larvae recovered	57
Total remaining grubs on system	97

The breakdown of BSF weights after 1 week of experiment can be seen on Table 33.

Table 33. BSF weight analysis after 1 week.

Ave. initial weight of 1 larvae (g)	0.062
Total initial weight of larvae on system (g)	6.20
Total weight of prepupae recovered (g)	2.30
Total weight of larvae recovered (g)	7.42
Ave. weight of 1 larvae after 1 week (g)	0.13
Ave. weight of 1 prepupae after 1 week (g)	0.058
Ave. weight gained by 1 larvae after 1 week (g)	0.068
Ave. weight change by 1 prepupae after 1 week (g)	-0.073

The breakdown on the usage of spent grain on the first week was shown in Table 34.

Table 34. Spent grain usage on the first week.

Initial spent grain on the system (g)	66.80
Total waste recovered (g)	53.56
Total weight gained by the system (g)	3.52
% of wasted grain	80%
% of grain used for biomass conversion	5%

The breakdown of the type of grubs present on the system after 2 weeks can be seen on Table 35.

Table 35. Black Soldier Fly distribution after week 2.

Initial total number of larvae	57
Number of prepupae recovered	48
Number of larvae recovered	9
Total remaining grubs on system	57

The breakdown of weights after 2 weeks can be seen on Table 36.

Table 36. Weight Distribution after week 2.

Ave. initial weight of 1 larvae (g)	0.130
Total initial weight of larvae on system (g)	7.42
Total weight of prepupae recovered (g)	6.24
Total weight of larvae recovered (g)	1.64
Ave. weight of 1 larvae at end of week (g)	0.205
Ave. weight of 1 prepupae at end of week (g)	0.127
Ave. weight gained by 1 larvae after 2nd week (g)	0.075
Ave. weight gained by 1 prepupae after 2nd week (g)	-0.003

The breakdown on the usage of spent grain on the first week was shown in Table 37.

Table 37. Spent Grain usage after week 2.

Initial spent grain on the system (g)	37.9
Total waste recovered (g)	25.7
Total weight gained by the system (g)	0.46
% of wasted grain	68%
% of grain used for biomass conversion	1%

Appendix IV. Feed ratio experiment results

The initial inputs for the feed ratio experiments were tabulated in Table 38.

Table 38. Initial inputs.

Feed ratio	12:1	12:1	12:1	6:1	6:1	5:1	5:1	4:1	4:1	3:1	3:1
No. of Larvae	100	100	100	100	100	100	100	100	100	100	100
Total Larvae DW (g)	1.24	1.11	0.93	0.90	0.9	0.92	0.92	0.91	0.91	0.73	0.73
Ave. larvae DW (g)	0.012	0.011	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.007	0.007
Feed placed DW (g)	14.88	9.27	8.04	5.36	5.10	4.49	4.43	3.52	3.70	1.83	1.84

The results for week 1 for the experiments were tabulated in Table 39. The average weight of the larvae at the start was decreasing as the feed to larvae ration decreases for Table 38 and 39 due to error in methodology. The larvae used were not randomized properly causing a bias to select heavier larvae at the start.

Table 39. Week 1 results.

Feed ratio	12:1	12:1	12:1	6:1	6:1	5:1	5:1	4:1	4:1	3:1	3:1
No. of Larvae	90	90	90	97	99	95	98	92	95	89	90
Total Larvae DW (g)	1.68	1.55	1.34	1.79	1.70	1.80	1.77	1.76	1.85	1.22	1.23
Ave. larvae DW (g)	0.019	0.017	0.015	0.018	0.017	0.019	0.018	0.019	0.019	0.014	0.014
Feed placed DW (g)	20.1	18.6	16.1	10.7	10.2	8.98	8.86	7.04	7.41	3.67	3.69
Frass produced DW (g)	5.53	5.25	4.50	2.78	2.77	2.38	2.35	1.87	1.93	0.92	1.05

The results for week 2 were shown in Table 40.

Table 40. Week 2 results.

Feed ratio	12:1	12:1	12:1	6:1	6:1	5:1	5:1	4:1	4:1	3:1	3:1
No. of Larvae	6	4	6	5	2	10	9	14	17	20	15
Total Larvae DW (g)	0.372	0.248	0.378	0.3	0.116	0.560	0.522	0.490	0.544	0.580	0.480
Ave. larvae DW (g)	0.062	0.062	0.063	0.060	0.058	0.056	0.058	0.035	0.032	0.029	0.032
No. of Prepupae	80	86	82	92	97	85	89	76	72	62	71
Total Prepupae DW (g)	8.11	5.59	4.68	3.94	3.75	3.39	3.28	2.96	2.70	2.23	2.60
Ave. Prepupae DW (g)	0.101	0.065	0.057	0.043	0.039	0.040	0.037	0.039	0.037	0.036	0.037
Frass produced DW (g)	11.1	10.5	9.00	5.57	5.54	4.76	4.70	3.73	3.85	1.84	2.10

Appendix V. Feeding regime results.

The initial inputs for the feed regime experiments were tabulated in Table 41.

Table 41. Initial inputs for feeding regime experiments.

Feed regime	Feeding twice with frass weekly removal	Feeding twice without frass weekly removal	Feeding once
No. of Larvae	100	100	100
Total Larvae DW (g)	0.94	0.91	0.93
Ave. larvae DW (g)	0.009	0.009	0.009
Feed placed DW (g)	11.280	10.920	11.160

The results for week 1 for the experiments were tabulated in Table 42.

Table 42. Week 1 results for feeding regime experiments.

Feed regime	Feeding twice with frass weekly removal	Feeding twice without frass weekly removal	Feeding once
No. of Larvae	95	100	85
Total Larvae DW (g)	2.05	1.29	1.43
Ave. larvae DW (g)	0.022	0.013	0.017
Feed placed DW (g)	12.31	7.74	8.56
Frass produced DW (g)	6.32	5.90	1.34

The results for week 2 were shown in Table 43.

Table 43. Week 2 results for feeding regime experiments.

Feed regime	Feeding twice with frass weekly removal	Feeding twice without frass weekly removal	Feeding once
No. of Larvae	5	12	20
Total Larvae DW (g)	0.32	0.73	0.64
Ave. larvae DW (g)	0.063	0.061	0.032
No. of Prepupae (g)	83	88	47
Total Prepupae DW (g)	4.17	3.37	2.32
Ave. Prepupae DW (g)	0.050	0.038	0.049

Frass produced DW (g)	6.90	4.18	1.03

Appendix VI. Feed height experiment

The inputs for the feed height experiments were tabulated in Table 44. The larvae dry weights were calculated from the wet weights and the moisture content of the larvae. The feed dry weight was then calculated using the feed ratio of 18 g feed DW to 1 g larvae DW

Table 44. Initial inputs for feed height experiment. WW indicates wet weight while DW indicates dry weight.

Experiment	No. of Larvae	Larvae WW (g)	Larvae DW (g)	Ave larvae weight (g)	Total Feed placed DW (g)	Total Feed placed WW (g)
3 cm + 6 cm raw	100	2.80	0.95	0.028	17.1	68.5
3 cm + 6 cm raw	100	2.60	0.88	0.026	15.9	63.7
3 cm + 6 cm minced	100	2.80	0.95	0.028	17.1	68.5
3 cm + 6 cm minced	100	2.68	0.911	0.027	16.4	65.6
6 cm + 12 cm raw	200	5.62	1.91	0.028	34.4	137
6 cm + 12 cm raw	200	5.28	1.80	0.026	32.3	129
6 cm + 12 cm minced	200	5.22	1.77	0.026	32.0	127
6 cm + 12 cm minced	200	4.68	1.59	0.023	28.6	114

The resulting prepupae and larvae characteristics were tabulated in Table 45.

Table 45. Resulting BSF from feed height experiments.

Experiment	No. of larvae	No. of prepupae	Larvae DW (g)	Prepupae DW (g)	Ave larvae weight DW (g)	Ave prepupae weight DW (g)
3 cm + 6 cm raw	0	100	0	3.42	0	0.034
3 cm + 6 cm raw	9	90	0.073	3.16	0.008	0.035
3 cm + 6 cm minced	2	93	0.275	3.37	0.138	0.036
3 cm + 6 cm minced	0	98	0	3.59	0	0.037
6 cm + 12 cm raw	12	185	0.063	6.35	0.005	0.034
6 cm + 12 cm raw	10	187	0.077	5.74	0.008	0.031
6 cm + 12 cm minced	2	170	0.211	5.45	0.106	0.032
6 cm + 12 cm minced	3	172	0.198	5.48	0.066	0.032

Appendix VI. Cash flows

The calculated cash flows and present value for the plant for 20 years were tabulated in Table 46.

Table 46. NPV calculations.

Year	Gross profit	Cash flow	Present value
1	$ 301,202	$ 301,202	$ 273,820
2	$ 401,603	$ 401,603	$ 331,904
3	$ 401,603	$ 401,603	$ 301,730
4	$ 401,603	$ 401,603	$ 274,300
5	$ 401,603	$ 401,603	$ 249,364
6	$ 401,603	$ 401,603	$ 226,695
7	$ 401,603	$ 401,603	$ 206,086
8	$ 401,603	$ 401,603	$ 187,351
9	$ 401,603	$ 401,603	$ 170,319
10	$ 401,603	$ 401,603	$ 154,835
11	$ 401,603	$ 401,603	$ 140,760
12	$ 401,603	$ 401,603	$ 127,963
13	$ 401,603	$ 401,603	$ 116,330
14	$ 401,603	$ 401,603	$ 105,755
15	$ 401,603	$ 401,603	$ 96,141
16	$ 401,603	$ 401,603	$ 87,401
17	$ 401,603	$ 401,603	$ 79,455
18	$ 401,603	$ 401,603	$ 72,232
19	$ 401,603	$ 401,603	$ 65,665
20	$401,603	$ 401,603	$ 59,696