

Algae Involved Generation of Composts

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ABSTRACT

This research investigated the potential application of blue-green algae to improve the quality of composts generated from multiple waste streams including the fly ash, fish waste, and sludge from a local wastewater treatment plant. The algae strain used is *Anabaena* 387, which was obtained from the Canadian Phycological Culture. Different mixture ratio of the fly ash, fish waste compost, sludge and algae were tested and different reaction periods were selected to generate multiple compost products. The parameters including the trace element concentrations, carbon to nitrogen ratio, pH level, moisture content, organic matter, and germination index were monitored to evaluate the quality of composts. Three levels of compost products were generated, which are Type AA, A, and B. The algae treated composts could be used as amendments for agriculture applications.

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LIST OF ABBREVIATIONS AND SYMBOLS

AAFC -- Agriculture and Agri-Food Canada
BaP -- Benzo[a]Purene
BNQ -- Bureau de normalisation du Québec
CCC -- Composting Council of Canada
CCME -- Canadian Council of Ministers of the Environment
C: N -- Carbon to nitrogen ratio
CFIA -- Canadian Food Inspection Agency
dH₂O -- distilled water
FAR -- Fertilizers Act and Regulations
GI -- Germination Index
MSW -- municipal solid waste
NL -- Newfoundland and Labrador
PCDD -- Polychlorinated dibenzodioxins
PCDF -- Polychlorinated dibenzofurans
PPB -- Polyphosphate bodies
RHWTF -- Riverhead Wastewater Treatment Facility
SCC -- Standards Council of Canada
TCDD -- Tetrachlorodibenzodioxin

CHAPTER 1 INTRODUCTION

1.1 Background

The compost as a soil amendment is widely used in agricultural activities. The conventional composting processes have been well developed and studied for many years. However, the algae involved generation of compost by mixing multiple waste sources is rarely studied. Three potential waste streams (i.e. sludge, fly ash, and the fish waste compost after extraction for other purposes) are discussed in the thesis.

The *sludge* generated from the wastewater treatment plants is always a serious environmental concern. Usually after the dewatering process, the remaining solids are compressed and disposed into the landfill directly. According to the official website of the City of St. John's, around 65 tons of solid waste are produced and end up in landfill disposal every day. Complaints come from the nearby community about the odor from this dewatered sludge. These wastes also account for a large space in the landfill site. However, basically these dried sludges are bio-solids or organic materials, which can be further biodegraded and then used as a potential compost amendment for agricultural purposes.

The *Fly ash* is another environmental problem in Newfoundland and Labrador (NL). Fly ash is the main by-product generated by thermal power plants, which use fuel oil as combustion resource (Ahmaruzzaman, 2009; Scheetz and Earle, 1998; Zacco et al., 2014). Newfoundland and Labrador Hydro's Holyrood Thermal Generating Station is an essential part of the province's

generating system, which generates 28,000 tones fly ash annually (Weir, 2013). At this moment, no recycling treatment is applied for the generated fly ash and these fly ash are dumped into the landfill. Fly ash consists of fine particles ranging in size from 2 μm to 10 μm . Because of its physical properties, the improper management of fly ash can lead to air pollution and human respiratory disease (Scheetz and Earle, 1998; Wang and Wu, 2006). One important characteristic of fly ash that can be taken advantage of is the high organic matter content, which is up to 95%. If the fly ash can be utilized as an amendment for the compost products, it will not only provide an environmentally friendly method for recycling fly ash but also generate a useful soil ameliorant.

The *fish waste* produced by fishing companies has caused many environmental problems, such as noxious odors, water pollution, fouling beaches and contaminating ocean. Nowadays, fish waste is considered a superior composting material (Lopez-Mosquera, 2011). The fishery in Newfoundland has a long history and it plays a very important role in the economic development in NL. Still to this day a lot of companies rely on the fishery and its related industries. One of the extended industries is the fish waste composting industry. The fish wastes generated from the seafood manufacturer firms are enriched in nutrients, especially nitrogen and phosphorus, and the wastes can be decomposed quickly. These properties make fish waste composting very quick and producing very rich compost products, which can be used as fertilizers for agricultural application.

In summary, the sludge, fish waste and fly ash are three main solid wastes generated in NL.

All the three waste streams have their specific environmental concerns. Another environmental problem in NL is the algae bloom. One of the popular local algae strain is *Anabaena* strain 387. The *Anabaena* strain caused blooms, such as the one in several Northeast Avalon ponds in 2007 (Government of Newfoundland and Labrador, 2007). However, the ability of blue-green algal biodegradation makes *Anabaena* a potential candidate to improve the chemical qualities of waste-mixed composts (Derbalah et al., 2008; Mohammad et al., 2013; Seal et al., 2012; Sivakumar et al., 2012).

The compost has many benefits for agricultural activities. It can improve soil quality by adding more nutrients and organic matters to the soil, as well as adjusting the carbon to nitrogen ratio of the soil. Because of the harsh environment and barren soil conditions in NL, the generation and application of good quality composting products are highly deseired.

1.2 Objectives

Developing a promising waste-based methodology for compost generation is the objective of this study. This research attempts to provide an innovative method to generate qualified compost products with different levels and also figure out how the compost quality could be affected by algal biodegradation. Two main benefits can be obtained from this research. First of all, it creates an environmentally friendly option for the waste stream management. Secondly, it can generate different levels of qualified compost products, which are significantly in demand in NL. Six parameters are used to evaluate the quality of generated composts and to illustrate the functions

of algae in this process. These objectives entail the following major research tasks:

- 1) To collect raw materials (i.e., sludge, fly ash, and fish waste composts) from different resources and learn how to culture the *Anabaena* strain under laboratory conditions;
- 2) To characterize each raw material quantitatively and qualitatively;
- 3) To mix three waste streams in different ratios and identify the reasonable mixing ratios based on sample characterization;
- 4) To biodegrade the mixers by adding different doses of algae to determine the appropriate dose; and
- 5) To draw biodegradation curves along the process to determine the appropriate treatment duration.

All the results generated by the experiments will be used to clarify the function of the *Anabaena* strain and the function of algae in the process. This research will contribute to the existing needs for waste management in NL and can help with the set-up of the composting program at Robin Hood Bay Facility in the near future. Meanwhile, it will lead to decreased health risks due to air-borne pollutants caused by the fly ash.

1.3 Organization

The Chapter 2 reviews literature regarding the introduction of three waste streams, current algae applications, and the evaluation of compost products. For each waste stream, the properties, generation, management and reuse are provided in details. The current studies about algae

applications and biodegradation potentials are followed. The last part of this chapter is mainly focused on the compost evaluation parameters and the classification criteria provided by three different national organizations. In Chapter 3, the Anabaena culturing technology is presented firstly with the comparisons of two different culturing mediums. The Chapter 3 then presents the detailed experimental design and standard methods regarding the 6 evaluation parameters used in this research, which are the germination index (GI), carbon to nitrogen ratio (C: N), pH value, organic matter content, trace element concentrations and moisture content. The Chapter 4 includes all the experimental results and detailed discussions regarding the characterization of raw materials and mixers, determination of mixing ratios of the three waste streams, identification of appropriate algae dose and reaction duration. The algae biodegradation analysis is also covered in this chapter. The last part of the Chapter 4 classifies the compost products in different levels based on the standards. The conclusions and recommendations for future work are indicated in the Chapter 5.

CHAPTER 2 LITERATURE REVIEW

In this chapter, literature review of three waste streams - fly ash, sludge and fish wastes - was carried out in order to have a good understanding about their properties, generation, management, reuse and relative environmental concerns. The previous studies about algae applications and biodegradation potentials were also introduced. Followed by these, parameters for evaluating the quality of composts and the criteria for compost classification were discussed. The national organizations, which provide requirements, guidelines and or standards, were also briefly introduced.

2.1 Utilization of Waste Streams

2.1.1 Fly Ash

The fly ash is the main by-product generated by coal-burned power plants (Ahmaruzzaman, 2009; Scheetz and Earle, 1998; Zacco et al., 2014). Zacco (2014) divided the fly ash into three types: coal fly ash, flue gas desulphurization fly ash and biomass fly ash. Coal fly ash is the main residue after the coal was burned at 1200-1700°C. The flue gas desulphurization fly ash can be collected by the air pollution control system, which is used to decrease the emission of the sulfur dioxide, in power plants (Zacco et al., 2014). The biomass fly ash can be obtained by the scrubber equipment, which is used to produce power by incinerating and converting the biomass or municipal solid wastes in thermal power plants (Zacco et al., 2014). The properties of fly ash

vary considerably depending upon the coal source (Ahmaruzzaman, 2009; Scheetz and Earle, 1998; Zacco et al., 2014).

According to Ahmaruzzaman's research in 2009, the total amount of coal ash generated around world is about 600 million tones, with fly ash accounting for 83%. So far, many environmental problems are triggered by improper disposal of fly ash. Because the fly ash contains many heavy metals like arsenic, barium, beryllium, boron, cadmium, selenium, and mercury, the rainwater pass through the dumped fly ash might leach the heavy metals and contaminate the ground water (Wang and Wu, 2006). The fly ash, when contacting skin or being taken in by inhalation and drinking water, may also have adverse impacts on human health (Scheetz and Earle, 1998; Wang and Wu, 2006). How to dispose and manage the large amount of fly ash appropriately is a big challenge for environmental engineers.

Research on the potential applications and reuse of fly ash are widely carried out. Because of its special physical properties and chemical content, fly ash is being used as a very popular additive in cement and concrete production. (Scheetz and Earle, 1998). Based on the Ahmaruzzaman's research (2009), fly ash is also a great absorbent used for cleaning flue gas and wastewater. Fly ash has a very high efficiency in removal of NO_x , mercury and organic particles in flus gas. For wastewater, fly ash can help with eliminating both inorganic compounds (like boron, fluoride and phosphate) and organic matters (such as phenolic compounds) (Ahmaruzzaman, 2009; Wang and Wu, 2006).

Another important application of fly ash is as the fertilizer or a soil amendment (Ferreira,

2002; Scheetz, 1998; Zacco, 2014). Fly ash is rich in two elements, phosphorous and potassium, which are essential for plant growth (Ferreira, 2002). Ferreira (2002) also mentioned that the length of plants grew in soil with fly ash was 2 times longer than that grew without fly ash, which indicated that fly ash had the potential to improve soil quality. The research also found that the plants enriched with the two element nutrients were greater in size than those treated with only one nutrient (either phosphorous or potassium). Another study using a crop test was conducted to show fly ash's ability to improve low soil nutrient levels (Zacco, 2014). Besides as a nutrient provider, fly ash can also be treated as a "liming agent" in agriculture and its effectiveness was confirmed by practice (Ferreira, 2002; Zacco, 2014). Usually when fly ash is dissolved in water, it leads to a high pH value. This characteristic makes fly ash being able to reduce soil acidity in some cases (Ferreira, 2002).

From the environmental point of view, however, the application of fly ash as a fertilizer or soil amendment has been questioned by many researchers due to the reasons including the concentrations of heavy metals in fly ash (Ferreira, 2002; Scheetz, 1998; Zacco, 2014). Some of the elements are important for plant growth, such as Cu, Mo, and Zn, when they are in an appropriate concentration. Some of the elements are not good for the agriculture, such as As, Cd and Hg, but they are can still exist in the fertilizer or soil amendment within the "acceptable amount", which referred to the standards. If the concentration of certain element in fly ash is above the relevant environmental standard, it becomes a phytotoxin to the plants (Ferreira, 2002; Epstein, 1997). Therefore, special attention on the concentration of heavy metals should be paid

when considering fly ash as a potential raw material for generating composts.

2.1.2 Sludge

The sludge is the main by-product in wastewater treatment process. It contains the solid wastes from municipal, agricultural, commercial, industrial and surface water (Werther and Ogada, 1999). Werther (1999) subdivided the wastewater treatment process into three main stages: physical sedimentation stage, biological digestion phase and removal of nitrogen and phosphorous. In the physical sedimentation stage, racks and screens can filter coarse solids; and then gravel, sand and other smaller size particles can be eliminated by grit chambers. After this clarification, 50-70% of suspended solids can be separated (Werther and Ogada, 1999). The following stage is biological digestion phase. Microorganisms, especially bacteria play a dominant role in this process. They can convert the water dissolved organic matters into gases and use the energy for self-multiplication (Werther and Ogada, 1999). The last stage is elimination of nitrogen and phosphorous. For nitrogen, ammonia is oxidized to nitrate in nitrification process first and then is further oxidized to nitrogen, which returns the nitrogen back into the atmosphere. For phosphorous, chemical additives are needed for its precipitation (Werther and Ogada, 1999).

So far, abundant sludge has been produced and needs to go through stabilization and dewatering processes (Werther and Ogada, 1999). According to Werther and Ogada (1999), three approaches are usually used for stabilization: biological digestion, lime stabilization and heat

treatment. Biological digestion is widely used for municipal sludge treatment. The main idea of lime stabilization is to provide a very basic environment (pH usually up to 12 or even higher) by adding lime. Under this situation, it is difficult for sludge to be putrefied and emit odors (Werther and Ogada, 1999). During the heat treatment, the sludge is heated at 75-190°C right after heating at 30-75°C shortly under the 26 bar pressure (Werther and Ogada, 1999). After this process, the water content in the sludge is decreased dramatically. Dewatering is also very necessary to make the transporting and disposal much easier (Chen et al., 2012; Werther and Ogada, 1999). After the treatment, sludge can be used for other purposes.

There are three widely applied ways to manage and apply sludge: landfilling, incineration, and fertilizer or soil supplement (Chen et al., 2012; Werther and Ogada, 1999). Other than these, in many developing countries, the sludge was dumped into the sea directly after treatment (Werther and Ogada, 1999); and in China, sludge can also be used as a material to produce cement (Chen et al., 2012).

Landfilling is one of the most popular ways to manage sludge because of its low requirement for high technology and low cost (Chen et al., 2012; Werther and Ogada, 1999). Sludge can be dumped directly (mono-disposal) or can be combined with other municipal solid waste (co-disposal) (Werther and Ogada, 1999). However, the landfilling leads to many environmental issues, such as occupying big space in the landfill sites, contaminating soil and ground water, and emitting odors (Chen et al., 2012; Werther and Ogada, 1999; Yoshida, 2013).

Incineration can reduce the sludge volume dramatically, up to 90% of dewatered sludge

(Werther and Ogada, 1999), by converting it to ash. In addition, incineration can help with minimizing the odor released from the dewater sludge. Furthermore, the energy obtained from the incineration can be reused for municipal power generation (Chen et al., 2012; Werther and Ogada, 1999; Yoshida, 2013). However, the combustion can also break down the organic contaminant and convert it to carbon dioxide emitted into atmosphere, which leads to a concern of releasing greenhouse gases. In addition, incineration can produce lots of carcinogens (i.e. TCDD, PCDD and PCDF), which can cause human cancers (Schetter, 1989).

Because of the high concentrations of nitrogen and phosphorus in sludge, it also has the potential to be used as a good fertilizer or soil supplement after appropriate treatment (Chen et al., 2012; Werther and Ogada, 1999). Based on previous research, sludge based composts can improve the soil properties physically, such as enhancing the soil ability to hold water, keeping soil particles together and improving soil porosity (Chen et al., 2012). Sludge based fertilizer can also help to return the organic matters back into the biological cycle. The microorganisms in the soil can biodegrade and digest many organic pollutants. If the artificial fertilizer can be replaced by the sludge based compost, a lot of energy and resources can be saved (Chen et al., 2012; Werther and Ogada, 1999).

However, the use of sludge in agriculture still presents many challenges to environmental engineers and the high concentration of trace elements or heavy metals is one of them. Previous studies show that the cumulative content of heavy metals in sludge might be over the maximum concentrations for farming purposes (Chen et al., 2012; Werther and Ogada, 1999; Yoshida,

2013). According to the regulations and standards in Canada, the trace element content is one of the main criteria for the guiding classification of composts for both Canadian Council of Ministers of the Environment (CCME) and Bureau de normalisation du Québec (BNQ) (CAN/BNQ/CCME/AAFC, 2005; CCME, 1996).

2.1.3 Fish Waste

Cod fishing in Newfoundland has a long history dating back to 1492, when European arrived to the North American continent. The fishing industry promotes economic development in NL and lots of companies rely on the fishing industry or its offshoots. However, the fishing industry also presents many environmental concerns to the local government, especially when it comes to fish waste treatment.

Fish waste produced by individual fishers and fishing companies has caused an adverse impact on human health and on the environment because of improper storage, handling and/or disposal practices. Some common problems include noxious odors, water pollution, fouled beaches, contaminated ocean, and fish infestations. In addition, the public and domestic water supplies were threatened by the contaminated liquids leaking from the pile of fish wastes and /or containers on transport vehicles (Department of Environment and Conservation, Government of Newfoundland and Labrador, 2004).

According to the Newfoundland's Pollution Prevention Division, Department of Environment and Conservation (2004), *offal* is defined as "all parts of animals which are

removed from the carcass when it is dressed for food, e.g. entrails, heart, liver, head, tail.” Based on this definition, fish waste like cod heart and cod head can definitely be classified as offal and therefore needs appropriate treatment before dumping into the landfill. According to the documents provided by the Department of Environment and Conservation in NL (2004), four disposal options for fish wastes are available:

Option 1: Delivery to a fish waste/ meal processing plant;

Option 2: Disposal as a compost or fertilizer;

Option 3: Ocean disposal; and

Option 4: Disposal at a land based waste disposal site (dumping on a beach is not allowed).

From these four options, options 3 and 4 are not very environmentally friendly and have the potentials to pollute the ocean and cause an adverse effect on human health. For option 1, fish waste generated from the fishing industry would need be transported to the processing plant every day between May and October, and at least every other day between November and April. The transportation for this option is very costly and if the fish waste goes bad and is not qualified for food, option one cannot be selected. Option 2, disposal as compost or fertilizer, cannot only reduce the amount of biodegradable waste being dumped, but also provide commercial products (Colon, 2010).

Fish waste composting has been well developed and advanced techniques can be found in literature (Colon, 2010; Lopez-Mosquera, 2011; Neklyudov, 2005). Because the fish waste is rich in nutrients, especially nitrogen and phosphorus, and it can be decomposed quickly; therefore,

fish waste is considered as the perfect composting material (Lopez-Mosquera, 2011). In addition, the organic matter in the fish waste compost can improve the soil conditions for farming purposes (Colon, 2010; Neklyudov, 2005).

Compost generated by fish waste cannot only directly be used as a fertilizer and soil amendment; it can also be a great source of nutrients for generating valuable products such as biosurfactants. Kazemi et al. (2014) extracted nutrients from fish waste compost by enzyme hydrolysis, and used the extract as substrate to grow biosurfactant producing bacteria. After the extraction, the components in the fish waste composts have been changed. Therefore, the possibility of using such composts as the soil fertilizer needs to be investigated, which has never been reported in literature.

2.2 Algae Application

According to the definition provide by Alexander (1994), biodegradation is a bio-chemical process, during which the complexity of chemicals is reduced biologically. Usually the organic compounds involved in this process can be converted into inorganic products. In other words, the organic C, N, P, S, and other elements in the original compounds can be converted into CO₂ or inorganic forms of N, P, S by the organisms in the system and then released into the surrounding environment (Alexander, 1994).

Many contaminants that exist in the wastewater or fly ash are directly toxic or become more hazardous because of the bio-magnification along the biologic chain. Since biodegradation can

break down the original organic compounds and convert them into much less toxic or not toxic inorganic products, the organisms involved in the process play a very important role in the biotransformation of pollutants. According to the literature, biotransformation can be applied in many different types of environments, such as sewage-treatment systems, contaminated soil, groundwater, surface water, and oceans, and bacteria are the most popular microorganisms carrying out the biotransformation process (Alexander, 1994). Recently, algae have been used more and more in the applications because of their biodegradation ability and rapid growth characteristic. Aside from these benefits, from the experimental point of view, algae are much easier to be cultured in the lab compared to the bacteria.

Many features of algae make them good candidates to carry out the bioremediation/ biotransformation process or to be used in agricultural applications. Main characteristics of algae are summarized as follow:

(1) Stabilization of phosphorus as biomass: Water soluble phosphate in the fertilizer is very easy to lose from soil. Only 20 % of the phosphate in the fertilizer can be absorbed by the plants and almost 80 % of phosphorus ends up with entering the oceans from river runoff (Sivakumar, 2012). However, in the algal cells, polyphosphates can be stabilized as polyphosphate bodies (PPB) and stored inside an organelle called acidocalcisome (Sivakumar, 2012). This biological phenomenon can happen whenever phosphorus becomes available to the algae cells, no matter if the cells are starved for phosphorus or not. Meanwhile, algae can grow rapidly in the phosphorus rich environment and then they can capture and fix phosphorus. Later on, algae can return the

phosphorus back into the terrestrial environment when used as agricultural fertilizer additives (Sivakumar, 2012).

(2) *Stabilization of nitrogen as biomass:* Algae can absorb large amounts of nitrogen from the atmosphere and fix them as biomass inside the cells (Sivakumar, 2012). Even though the detailed mechanism is still not clear, this phenomenon was demonstrated by Sivakumar in 2012.

(3) *Biodegradation of contaminants:* Algae can use the organic contaminants as the carbon source and convert them into CO₂ and releasing it into the atmosphere by the end of process. According to the research conducted by Takacova (2014), algae *Ch. Kessleri* has the ability to biodegrade and remove Benzo[a]Purene (BaP) from waste water. BaP is one of the polycyclic aromatic hydrocarbons (PAHs), which are widely present in the environment. By monitoring the respiration gases during the biodegradation process, Takacova concluded that the highest biodegradation rate happened after 48 hours and oxidation of carbohydrates including PAHs to CO₂ and H₂O. Takacova (2014) also summarized possible factors which led to the decreasing biodegradation rate, including continual decrease of moisture content, selective decomposition of better decomposable substances, decrease of nutrient content, and accumulation of toxins. There were 30% of BaP removed by the end of the biodegradation process. Warshawsky (1994) also found a freshwater green alga, *Selenastrum capricornutum*, could metabolize BaP to cis-dihydrodiols by using dioxygenase enzyme system.

(4) *Other characteristics including productions of micro- algal derivatives:* Algae can absorb the energy from the sun by photosynthesis process and can accumulate biomass which

contains lots of hydrocarbons, proteins, pigments and small molecules during their rapid growth (Sivakumar, 2012). Some of these pigments or small molecules can be extracted and then used for food additives, cosmetics, food and fertilizer purposes (Sivakumar, 2012).

2.3 Evaluation of Compost Quality

In this part, the national organizations, which provide requirements, guidelines and/ or standards for evaluating the quality of composts are introduced. The evaluation parameters and classification criteria are then summarized.

2.3.1 National Organizations

In Canada, based on CCME (The Canada Council of Ministers of the Environment) Guidelines (2005), composting is considered to be an environmental friendly waste management solution and also an important supplement. During the past few years, with the rapid increasing demand of composting products, the necessity of establishing evaluation criteria for compost is becoming more and more important. Many organizations focused on establishing a series of evaluation guidelines. The CCME, BNQ and Agriculture and Agri-Food Canada (AAFC) are three main national participants (CAN/BNQ/CCME/AAFC, 2005). Different organizations have their own particular responsibilities.

Bureau de normalization du Quebec (BNQ)

In Canada, five standard writing organizations form the Standards Council of Canada (SCC), and BNQ is an important member of this council (CAN/BNQ/CCME/AAFC, 2005). The main responsibility of BNQ is developing and amending the national regulations and standards related to human health and safety, environmental issues and public works (CAN/BNQ/CCME /AAFC, 2005; CCME, 1996; OCQS, 2012). Many areas are covered, such as treatment and management of municipal solid waste, wastewater treatment, and application of municipal sludge, fertilization, and soil improvement (CAN/BNQ/CCME/AAFC, 2005). Management of composting and the evaluation of composts are under its responsibility, because compost products can be regarded as fertilizer and/ or soil amendment depending on the end use. By 1992, BNQ established national standards for evaluating fertilizer and soil amendment products (CAN/BNQ/CCME/AAFC, 2005).

BNQ has its own standards, different from CCME, for classifying compost products and also provides two analysis methods for evaluating the quality of the composts (CAN/BNQ/CCME/AAFC, 2005; Ge, 2006). Meanwhile, BNQ has the right to give the certification to the compost companies if their products meet BNQ standards. The cost of the certification process is provided by the compost companies (Ge, 2006). After the certification program, qualified composts are allowed to use the BNQ labels to show their quality (Ge, 2006). BNQ divides the compost products into three different levels - Type AA, Type A and Type B. Type AA has the best quality (CAN/BNQ/CCME/AAFC, 2005; Ge, 2006). This action can not only help with regulating compost production, but can also improve the competitiveness of the

qualified compost companies. All compost producers within Canada can apply for these certification labels (Ge, 2006). However, the compost product, which has the BNQ label, does not mean it is also identified to be a qualified product by CCME and AAFC organizations (CCME, 1996).

Canadian Council of Ministers of the Environment (CCME)

The CCME is consisted of 14 governmental members and it motives the cooperation of intergovernmental actions about environmental issues (CAN/BNQ/CCME/AAFC, 2005; Ge, 2006). The main responsibility of CCME is developing and promoting the national “guidelines” which can be applied to all provinces across Canada. Human health and environmental protection are always their concerns (CCME, 1996). Based on this objective, CCME promotes the classification and separation of municipal solid waste and also encourages the composting industry using the municipal solid waste as resources or raw materials to products high quality composts (CAN/BNQ/CCME/AAFC, 2005; CCME, 1996).

In the early 1990s, CCME, BNQ along with Canadian Food Inspection Agency (CFIA) worked together to draft consistent but flexible standards and guidelines to regulate the composting industry and compost evaluation (CAN/BNQ/CCME/AAFC, 2005; CCME, 1996). In order to make sure the guidelines and standards can be used for all the provinces and situations, CCME usually keeps the lower requirements, especially for trace element concentration in the compost product. Different from BNQ, CCME classifies the compost

products into two levels, Category A and Category B (in decreasing quality order) based on four criteria: maturity, trace element concentration in compost product, foreign matter and pathogenic organisms (CCME, 1996). CCME, BNQ, and CFIA guidelines for compost classification are very similar and will be presented later in the thesis (referring to pages 42-51).

AAFC, CFA and CFIA

AAFC, which was established in 1997, is another organization responsible for the evaluation of composts and that helps with the government regulating compost market. When CCME and BNQ work together to discuss and draft guidelines and standards, AAFC has to attend and observe the meeting in order to make sure the new policies are consistent with the requirements of the Fertilizers Act and Regulations (FAR) (CAN/BNQ/CCME/AAFC, 2005; CCME, 1996; Ge, 2006).

Canadian Food Inspection Agency (CFIA) is responsible for sampling the compost products and fertilizers sold in the market to make sure their quality and safety follow the standards provided by Canadian Federation of Agriculture (CFA) (Ge, 2006). Based on the standards of CFA, fertilizer and soil supplements are two different products (CAN/BNQ/CCME/AAFC, 2005). Fertilizer is supposed to be a nutrition provider, which is rich in nitrogen (N), phosphorous (P), and potassium (K). Supplement, however, is regarded as any product, which can help with improving the plant yield or growth, or promoting the soil conditions (CAN/BNQ/CCME/AAFC, 2005; Ge, 2006). The compost can be classified as either a fertilizer

or a supplement or both, depends on its end use and its quality. No matter which category the compost belongs to, it should not be harmful to humans, plants, animals or the environment (CAN/BNQ/CCME/AAFC, 2005; Ge, 2006).

Safety is always the priority for AAFC and no classification system was developed by AAFC (CAN/BNQ/CCME/AAFC, 2005). For the trace element concentrations in compost, AAFC follows the standards provided by Trade Memorandum T-93, which is discussed in the last section of this chapter.

2.3.2 Evaluation Parameters

“Composting” and “Compost” are two different subjects. Composting refers to the managed process, which involves the biological decomposition of organic matter (CAN/BNQ/CCME/AAFC, 2005; Epstein, 1997). Compost is regarded as the solid, mature and stabilized products from the composting process (CAN/BNQ/CCME/AAFC, 2005; Epstein, 1997; Wichuk, 2010). This section thus focuses on the parameters for evaluating quality of compost products rather than the composting process.

As discussed previously, organizations have different compost evaluation and classification standards; however, they all use five quality evaluation criteria including maturity, trace elements, foreign matters, pathogenic organisms, and organic contaminants (CAN/BNQ/CCME/AAFC, 2005). The terms “maturity” and “stability” are often used for evaluating the quality of composts (Wichuk, 2010). “Stability” represents the completion of organic matter decomposition. It is

usually reflected by the content of total organic matter (TOM) in the compost (CAN/BNQ/CCME/AAFC, 2005; Wichuk, 2010). “Maturity” means that a qualified compost has no disadvantageous impacts on the plants for a particular end use (Ge, 2006; Wichuk, 2010). Stability and maturity of a compost are generally determined by parameters including germination index (GI), and carbon to nitrogen ratio (C: N) (CAN/BNQ/CCME/AAFC, 2005). Besides the two parameters, pH, Organic Matter (OM), trace element concentrations and foreign matters in composts, as well as moisture or water content of a final compost product are also widely applied and thus indicated below.

Germination Index (GI)

Seed GI Test is a very popular method for Phytotoxicity analysis (Alexander, 1999).

According to California Compost Quality Council (CCQC), *Germination* is defined as:

“The extent of sprouting of a test seed as in cress or lettuce in a sample or extract of compost. The test results are dependent on preparation of the media especially in regards to concentration. Any test of germination should report the plant method and concentration of compost or extract used.”

GI is an important parameter indicating whether the fertilizer or compost has any harmful impact on the plants. An immature compost product, which might have water-soluble organic acids or high concentration of free ammonia, would inhibit the plant growth; even have hazardous impacts on humans and animals (Seal, 2012). Wichuk (2010) considered the seed GI

test could be treated as the “ultimate” treatment for evaluating compost maturity, especially when compost products were applied in soil supplements or used as fertilizers. When multiple resources of raw materials were used to generate a product, which was further applied as a compost or soil supplement, it is very necessary to know whether any toxic containments in the multiple resources exist in the compost which hinder the seeds germination or roots growth (Alexander, 1999; Seal, 2012).

For the GI test, cress and radish seeds were widely used because of their sensitivity to toxic substances and their quick germination speeds (Ge, 2006). Either a compost product or its extracts can be used for the seed GI test (Wichuk, 2010).

Carbon to Nitrogen Ratio (C: N)

Carbon and nitrogen are the two most important nutrients for the biodegradation process (Epstein, 1997). Carbon is used for microorganism cellular multiplication. By breaking down the organic matters, the microorganism can obtain energy and also return the carbon to the atmosphere in the form of carbon dioxide (Alexander, 1999; Epstein, 1997; Seal, 2012). As the biodegradation process goes on, less carbon dioxide is emitted because of the less available carbon in the reaction media. In other words, the concentration of organic contaminants in the compost decreases dramatically (Epstein, 1997). Epstein (1997) mentioned that the surface of the particles is the place where the microbial degradation occurs; therefore, the bigger the areas of surfaces are, the more rapid biodegradation would occur.

In the biodegradation process, the C: N continues a decreasing trend till it gets a stable stage (Epstein, 1997; Ge, 2006; Wichuk, 2010). The decreasing trend occurs because of two main reasons. One is the decomposition of the organic compounds, which results in the total carbon loss in the system. Another reason is the increasing concentration of nitrogen (Seal, 2012). Seal (2012) and Alexander (1994) indicated that many microorganisms can fix the atmospheric nitrogen into the soil system during the biodegradation process. The concentration of nitrogen is also an important value for a compost product, because the microorganisms need nitrogen for protein synthesis. However, if the concentration of nitrogen in soil is too high, it might lead to nitrogen immobilization, which is a microbial process consuming lots of oxygen in the soil. As a result, there is not enough oxygen for seed germination and root growth (Alexander, 1994; Epstein, 1997; Seal, 2012). The nitrogen content in a compost product varies from 1% to 2% (Alexander, 1994).

However, Wichuk (2010) indicated that the C: N couldn't provide a good indication as a sole test of maturity because of its limitations. First of all, in some cases, the change of the C: N is because of the different pH value of a compost rather than the change of carbon or nitrogen content in the compost (Wichuk, 2010). For example, when the pH is higher than 7.5, the carbon in the compost might be transformed to carbon dioxide, meanwhile nitrogen might be converted into NH_3 and escape from the system (Epstein, 1997; Khan et al., 2009; Seal, 2012). It is thus hard to expect the trend and determine whether the compost product is mature or not. Secondly, sometimes the compost is also mature with a very high C: N. When the temperature is high in

summer time, especially combined with a basic environment, the nitrogen might be changed to NH_3 and volatilized from the compost (Epstein, 1997; Khan et al., 2009; Seal, 2012). Thirdly, the C: N varies, depending on its raw material (Epstein, 1997).

Overall, because of the C: N uncertainty stated above, it is hard to set up an absolute value for C: N and apply it to all types of compost products. The ratio less than 25 is recommended by both BNQ and CCME. Other tests should be combined together with C: N to indicate the maturity of compost (CAN/BNQ/CCME/AAFC, 2005; CCME, 1996).

pH

The pH value indicates the alkalinity or acidity of the compost (Epstein, 1997). Wichuk (2010) illustrated that during the microbial decomposition of organic compounds, ammonium (NH_4^+) was generated and led to the increasing pH, usually to above 8. As the biodegradation continues, NH_4^+ was emitted from the medium as NH_3 , meanwhile some organic acid was produced to neutralize the compost, which caused the decrease of pH again. Finally, the pH value of the compost was supposed to be stable (Epstein, 1997; Wichuk, 2010). Some researchers such as Alexander (1997) and Ge (2006) indicated the final pH should be a neutral value for a matured compost product, while others like Wichuk (2010) and Epstein (1997) stated that any stable value obtained was acceptable.

Usually compost is used as a soil supplement or fertilizer, and mixed with the soil to improve the medium nutrient content. Rarely is it used alone. Therefore the final medium pH

also depends on background pH value of the original soil. From this point of view, if the original soil is acidic, a compost with a higher pH would be preferred and vice versa. In addition, because the application of a pure compost product without soil is not suggested to support plant growth, it is not necessary to set up a fixed value of pH for compost products (CAN/BNQ/CCME/AAFC, 2005; CCME, 1996). No limitations were required by CCME, BNQ and AAFC about this parameter.

Organic Matter (OM)

Organic matter is another important parameter for evaluating compost quality. During the composting process, due to the microbial activities, the content of organic matters decreases gradually till reaching a stable stage, which indicates the high maturity and stability of the compost products (Epstein, 1997; Ge, 2006; Wichuk, 2010).

In Canada, according to BNQ standards, the minimum content of organic matters for Type AA, Type A and Type B of composts are 50%, 40% and 30%, respectively (CAN/BNQ/CCME/AAFC, 2005). For BNQ, organic matter is an essential parameter for evaluating and ranking compost levels.

Organic matters in composts also play an important role in improving the nutrient content in the soil. Mazza et al.,(2014) summarized the roles of organic matters in improving the soil quality physically and bio-chemically:

First of all, organic matter can promote the soil structure. In some cases, the soil is either too sandy or too clayey. By adding the composts, the organic matter can help with the “neutralization” of the soil. For the very sandy soil, organic matter can hold the soil particles together. This can help with prevention of the wind erosion; especially in Newfoundland, where strong wind is always a concern. For the very clayey soil, organic matter can improve the water penetration and ability of holding water and nutrients in the soil by a chemical process called “cation exchange capacity”. This can help with prevention of run-off water on the soil surface and improvement of the nutrient content in the soil.

Secondly, organic matter can be treated as nutrients for growing microorganisms in the soil. These microorganisms can biodegrade and convert organic matter into “readily available” (Mazza, 2014) nutrients to be absorbed by the plant roots. In addition, Mazza (2014) also mentioned the existence of the organic matter can increase the availability of other nutrients for the plants.

Trace elements (heavy metals)

According to BNQ, a *Trace Element* is defined as: “ a chemical element present in the compost at a very low concentration.” Most of the trace elements are heavy metals; therefore, in the literature, trace elements and heavy metals are equally treated. In Canada, eleven heavy metals are considered in the standards: arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), mercury (Hg), molybdenum (Mo), nickel (Ni), lead (Pb), selenium (Se), and zinc

(Zn) (CAN/BNQ/CCME/AAFC, 2005; CCME, 1996). Some of the trace metals are important elements for plant growth, such as, Cu, Mo, or Zn in appropriate concentrations. However, they will become harmful to the plants if the concentrations are too high (CAN/BNQ/CCME/AAFC, 2005; CCME, 1996; Epstein, 1997).

In Canada, three popular ways are used for the calculation of the maximum acceptable trace elements: “no net degradation”, “no observable adverse effects level” and “best achievable approach” (CAN/BNQ/CCME/AAFC, 2005). According to the Supporting Document of Compost Quality Criteria (2005), “No net degradation” means the application of the compost product has no effects on the original “background” concentration of each heavy metal in the environment. “Background” is a key word in this definition and is explained by Environment Canada (1991) as “the concentration of chemical substances found in an environment removed from any source of industrial activity for a specific area and for a region considered to be relatively uninfluenced by industrial activity.”

“No observable adverse effects level” is defined as enough information can be predicted to testify to no harmful impacts on the environment after the application of a compost product; while the “best achievable approach” is defined as the maximum acceptable concentration of each heavy metal and depends on the best available technology used to produce composts (CAN/BNQ/CCME/AAFC, 2005). Based on the definitions of these three approaches, “No net degradation” is the most restricted one and thus used by the BNQ in its standard. The Tables 2.1-2.3 listed the maximum concentrations of heavy metals in standards of BNQ, CCME and

AAFC organizations, respectively. When comparing the data in the three tables, BNQ covered all the eleven heavy metals, while Zn was not listed by CCME and Cr and Cu were not considered by AAFC.

Foreign matter

Foreign matter is an important factor for evaluating the physical quality of a compost. It is used by BNQ to divide the composts into three levels. According to BNQ (2005), *foreign matter* is defined as:

"Any matter over a 2 mm dimension that results from human intervention and having organic or inorganic constituents such as metal, glass and synthetic polymers (e. g., plastic and rubber) that may be present in the compost but excluding mineral soils, woody material and rocks."

The requirements for regulating the three levels of composts are summarized in Table 2.4.

Moisture/ Water Content

The water content in composts cannot be too high (Epstein, 1997). If the water content is too much in the compost, the water will squeeze out the air between the particles, and lead to the deficiency of the oxygen in the compost (Alexander, 1994; Epstein, 1997).

Both BNQ and CCME have regulated the water content in compost. In the composts of Type AA, Type A, Type B (classified by NBQ) and Category A, Category B (classified by CCME), the maximum acceptable water content must NOT be higher than 60% (CAN/BNQ/CCME/AAFC, 2005; CCME, 1996; Ge, 2006).

Table 2. 1 Maximum Trace Element Concentrations in the Three Types of Composts Based on BNQ Standards (Revised from Support Document for Compost Quality Criteria, 2006)

	Types AA and A	Type B
Trace Elements	Maximum trace element concentrations in compost (mg/kg, air-dried mass)	Maximum trace element concentrations in compost (mg/kg, air-dried mass)
As	13	75
Cd	3	20
Co	34	150
Cr	210	1060
Cu	100	757
Hg	0.8	5
Mo	5	20
Ni	62	180
Pb	150	500
Se	2	14
Zn	500	1850

Table 2. 2 Maximum Trace Element Concentrations in the Two Categories of Composts Based on CCME Standards (Revised from Support Document for Compost Quality Criteria, 2006)

Trace Elements	Category A	Category B	
	Maximum trace element concentrations in compost (mg/kg dry weight)	Maximum trace element concentrations in compost (mg/kg dry weight)	Acceptable cumulative metal additions to soil (kg/ha)
As	13	75	15
Cd	3	20	4
Co	34	150	30
Cr	210	NA	NA
Cu	100	NA	NA
Hg	0.8	5	1
Mo	5	20	4
Ni	62	180	36
Pb	150	500	100
Se	2	14	2.8

Table 2. 3 Maximum Trace Element Concentrations in the Composts Based on AAFC Standards (Revised from Support Document for Compost Quality Criteria, 2006)

Trace Elements	Maximum acceptable concentration within product (mg/kg)	Acceptable cumulative metal additions (mg/kg)
As	75	15
Cd	20	4
Co	150	30
Hg	5	1
Mo	20	4
Ni	180	36
Pb	500	100
Se	14	2.8
Zn	1850	370

Table 2. 4 Requirements of Foreign Matter in the Three Types of Composts Based on BNQ Standards (Revised from Support Document for Compost Quality Criteria, 2006)

	Type AA	Type A	Type B
Foreign matter content as a percentage of oven-dried mass	<0.01	<0.5	<1.5
Foreign matter, maximum dimensions, in mm	12.5	12.5	25

2.3.3 Classification of Composts

As discussed previously, different standards are provided by CCME, BNQ and AAFC based on multiple criteria, leading to the classification of composts. Two categories have been established by CCME: Category A and Category B, which Category A has a higher quality. BNQ however, divided the composts into three levels with a decreasing order of quality (i.e. Type AA, Type A, and Type B). Only one class of compost is recognized by AAFC (CAN/BNQ/CCME/AAFC, 2005). Depending on the status of the composting industry and particular environmental conditions in each province, different classification standards are followed to label their compost products. Most of the provinces prefer to use the CCME and BNQ standards for compost classification (e.g., Ontario and NL follow CCME). More details about the classification of composts are stated in below.

CCME Classification

The end use of composts is the main criteria generated by CCME to divide compost products into two categories: A and B (CCME, 1996). Category A compost can be used in any application without limits, such as community gardening, agriculture, horticultural business, and others (CAN/BNQ/CCME/AAFC, 2005; CCME, 1996). Category B has to be subjected to restricted uses (CCME, 1996). CCME evaluates the composts by using two parameters, trace element (heavy metals) concentrations and foreign matter content in a compost (CAN/BNQ/CCME/AAFC, 2005; CCME, 1996). Table 2.5 shows the maximum concentrations

of heavy metals in Category A and B composts. Table 2.6 shows the levels of composts judged based on foreign matter content, respectively in the CCME standards. Category A compost has a better quality than Category B compost.

The standards of parameters for evaluating the stability and maturity of composts including organic matter, pH and moisture/ water content were the same in both Category A and B composts. After at least 21 days of curing, one of the three requirements in below should be met for a category A or B compost (CAN/BNQ/CCME/AAFC, 2005; CCME, 1996):

- (1) The temperature of the compost should not rise up 20°C (8°C based on CCME, 1996) above the ambient temperature;
- (2) the respiration rate should be less than or equal to 400 mg O₂/ kg volatile solids(or organic matter)/ hour; or
- (3) the CO₂ evolution rate should be less than or equal to 4 mg CO₂/ g organic matter/ day

OR

Two of the following three requirements should be met (CAN/BNQ/CCME/AAFC, 2005):

- (1) C: N should be less than 25;
- (2) a GI by using radish seeds and cress seeds to show no phytotoxic impact; and
- (3) an O₂ uptake should be less than 150mg O₂/ kg volatile solids/hour;

Table 2. 5 Maximum Trace Element Concentrations Based on CCME Standards (Revised from Support Document for Compost Quality Criteria, 2006)

Trace Elements	Category A	Category B	
	Maximum trace element concentrations in compost (mg/kg dry weight)	Maximum trace element concentrations in compost (mg/kg dry weight)	Acceptable cumulative metal additions to soil (kg/ha)
As	13	75	15
Cd	3	20	4
Co	34	150	30
Cr	210	NA	NA
Cu	100	NA	NA
Hg	0.8	5	1
Mo	5	20	4
Ni	62	180	36
Pb	150	500	100
Se	2	14	2.8
Zn	500	1850	370

Table 2. 6 Requirements of Foreign Matter Content in the Composts Based on CCME Standards (Revised from Support Document for Compost Quality Criteria, 2006)

	Category A	Category B
Sharp foreign matters dimension (mm/500mL)	≤ 3	≤ 12.5 (≤ 3 pieces of sharp foreign matters) *
Other foreign matters (mm/500mL)	≤ 25 (≤ 1 pieces of foreign matters)	≤ 25 (≤ 2 pieces of foreign matters)

*This type cannot be used in parks or residential application (CCME 1996).

According to the CCME standards, no specific values or requirements were defined for the organic matter and the pH value in a compost. To become a Category A or B compost, the maximum acceptable water content (calculated as the percentage of humid mass in the compost samples) must NOT be higher than 60% (CAN/BNQ/CCME/AAFC, 2005; CCME, 1996; Ge, 2006).

BNQ Classification

Different with the classification criteria for CCME, for BNQ, the levels of compost “safety and quality” are considered significantly (CAN/BNQ/CCME/AAFC, 2005). BNQ classified the composts in three levels: Type AA, Type A and Type B, among which the Type AA compost has the best quality and highest safety level (CAN/BNQ/CCME/AAFC, 2005). BNQ evaluated the composts by using three parameters for classification: trace element (heavy metals) concentrations, foreign matter content and the total organic matter in a compost (CAN/BNQ/CCME/AAFC, 2005). Table 2.7 shows the maximum concentrations of heavy metals in Types AA, A and B composts. Type AA and Type A have more restricted limits on the maximum heavy metal concentrations than Type B. Therefore, to use Type B compost, appropriate application instruction should be followed due to its limited quality and safety (CAN/BNQ/CCME/AAFC, 2005). Table 2.8 shows the levels of composts judged based on the foreign matter content; Table 2.9 shows the different requirements of organic matter in multiple types of composts, respectively (CAN/BNQ/CCME/AAFC, 2005).

Table 2. 7 Maximum Trace Element Concentrations in the Three Types of Composts Established by BNQ (Revised from Support Document for Compost Quality Criteria, 2006)

	Types AA and A	Type B
Trace Elements	Maximum trace element concentrations in compost (mg/kg, air-dried mass)	Maximum trace element concentrations in compost (mg/kg, air-dried mass)
As	13	75
Cd	3	20
Co	34	150
Cr	210	1060
Cu	100	757
Hg	0.8	5
Mo	5	20
Ni	62	180
Pb	150	500
Se	2	14
Zn	500	1850

Table 2. 8 Foreign Matter Contents in the Three Types of Composts Established by BNQ*
(Revised from Support Document for Compost Quality Criteria, 2006)

	Type AA	Type A	Type B
Foreign matter content as a percentage of oven-dried mass	<0.01	<0.5	<1.5
Foreign matter, maximum dimensions, in mm	12.5	12.5	25

*According to BNQ standards, soil, sand, rocks and pebbles are not treated as foreign matters; however, metals, glass, rubber, and plastic matters are.

Table 2. 9 Organic Matter Contents in the Three Types of Composts Established by BNQ
(Revised from Support Document for Compost Quality Criteria, 2006)

	Type AA	Type A	Type B
Organic Matters (%)	≥ 50	≥ 40	≥ 30

The standards of parameters for evaluating the stability and maturity of composts including pH and moisture/ water content were the same in Type AA, A and B composts. After at least 21 days of curing, two of the three requirements in below should be met for a Type AA, A or B compost (CAN/BNQ/CCME/AAFC, 2005):

- (1) C: N should be less than 25;
- (2) a GI by using radish seeds and cress seeds to show no phytotoxic impact; and
- (3) an O₂ uptake should be less than 150mg O₂/ kg volatile solids/hour.

According to the BNQ standards, there are no specific values or requirements were defined for pH value in a compost. To become a Type AA, Type A or Type B compost, the maximum acceptable water content (calculated as the percentage of humid mass in the compost samples) must NOT be higher than 60%, the same as the CCME requirement (CAN/BNQ/CCME/AAFC, 2005; Ge, 2006).

AAFC Classification and Criteria

Safety was considered as the most important factor for AAFC. In Canada, AAFC identified the minimum safety requirement for the entire compost products sold in the market (CAN/BNQ/CCME/AAFC, 2005; Ge, 2006; Wichuk, 2010). The compost was recognized as acceptable one or not (CAN/BNQ/CCME/AAFC, 2005). For evaluating the stability/ maturity of a compost, AAFC followed the same standards as BNQ (CAN/BNQ/CCME/AAFC, 2005). In addition, the same as BNQ and CCME, to become an acceptable compost by AAFC, the

maximum acceptable water content (calculated as the percentage of humid mass in the compost samples) must NOT be higher than 60% and no specific values or requirements were defined for pH value in a compost (CAN/BNQ/CCME/AAFC, 2005; Ge, 2006). For the trace element concentrations in a compost, AAFC followed the standards provided by Trade Memorandum T-93, which are listed in Table 2.10 (CAN/BNQ/CCME/AAFC, 2005; CCME, 1996).

Table 2. 10 Maximum Trace Element Concentrations in Composts Established by AAFC
(Revised from Support Document for Compost Quality Criteria, 2006)

Trace Elements	Maximum trace element concentrations within compost (mg/kg dry weight)	Acceptable cumulative metal additions to soil (kg/ha)
As	75	15
Cd	20	4
Co	150	30
Cr	NA	NA
Cu	NA	NA
Hg	5	1
Mo	20	4
Ni	180	36
Pb	500	100
Se	14	2.8
Zn	1850	370

2.4 Summary

In this section, three solid wastes (i.e., fly ash, sludge and residue of fish waste composts after extraction) were introduced and identified to be potentially used as the nutrient sources to produce composts. Each solid waste has its limitations on compost generation, thus needed to be carefully considered. For example, special attentions should be paid on the concentrations of heavy metals in both fly ash and sludge. For fish waste compost, its characteristics (especially the nutrient content) have been changed after extraction. Its availability for being a soil fertilizer thus needs to be further investigated.

Many features of algae make them good candidates to carry out biodegradation processes and generate compost products with good qualities. In Canada, three important national organizations (i.e., CCME, BNQ and AAFC) provide the standards and criteria for qualification and classification of compost products. Each organization has its own particular responsibilities; however, many indexes or parameters used for evaluation of compost quality are the same. These indexes or parameters include trace element concentrations, C: N, pH level, moisture content, organic matter, and germination index. In addition, the classifications of compost products by the three organizations were also stated.

CHAPTER 3 METHODOLOGY

3.1 Algal Culturing

The blue-green algae used in this study are *Anabaena* strain 387, which caused the blue-green algae bloom in NL in 2007 (Government of Newfoundland and Labrador, 2007). *Anabaena* strain 387 was obtained from the Canadian Phycological Culture Centre (CPCC) (CPCC, 2011) and then further cultured in the lab.

Two *Anabaena* culturing mediums were selected, which are Bold's Basal Medium (BBM) and Modified Bold's Basal Medium (3NBBM) (Andersen, 2005). The receipts of 3NBBM and BBM are listed in Table 3.1 and Table 3.2 respectively. Comparing the performance of these two mediums was conducted first to generate the appropriate environment for growing *Anabaena* 387.

Table 3. 1 Components of Modified Bold's Basal Medium (3NBBM)

Autoclave. The final pH should be 6.6. Final volume is 1000mL.				
Item	Component	Stock Solution (g/L dH ₂ O)	Quantity Used	Concentration in Final Medium (M)
1	NaNO ₃	75.00	10mL	8.82×10^{-3}
2	CaCl ₂ -2H ₂ O	2.50		1.70×10^{-4}
3	MgSO ₄ -7H ₂ O	7.50		3.04×10^{-4}
4	K ₂ HPO ₄	7.50		4.31×10^{-4}
5	KH ₂ PO ₄	17.50		1.29×10^{-3}
6	NaCl	2.50		4.28×10^{-4}
	Alkaline EDTA Solution			
7	EDTA	5.00		1.71×10^{-4}
8	KOH	3.10		5.53×10^{-4}
	Acidified Iron Solution			
9	FeSO ₄ -7H ₂ O	0.498	1.79×10^{-5}	
10	H ₂ SO ₄		0.1mL=100uL	
	Trace Metal Solution			
11	H ₃ BO ₃	11.42	1 mL	1.85×10^{-4}
12	ZnSO ₄ -7H ₂ O	8.82		3.07×10^{-5}
13	MnCl ₂ -4H ₂ O	1.44		7.28×10^{-6}
14	MoO ₃	0.71		4.93×10^{-6}
15	CuSO ₄ -5H ₂ O	1.57		6.29×10^{-6}
16	Co(NO ₃) ₂ -6H ₂ O	0.49		1.68×10^{-6}

Table 3. 2 Components of Bold's Basal Medium (BBM)

Autoclave. The final pH should be 6.6. Final volume is 1000mL.				
Item	Component	Stock Solution (g/L dH ₂ O)	Quantity Used	Concentration in Final Medium (M)
1	NaNO ₃	25.00	10mL	2.94×10^{-3}
2	CaCl ₂ -2H ₂ O	2.50		1.70×10^{-4}
3	MgSO ₄ -7H ₂ O	7.50		3.04×10^{-4}
4	K ₂ HPO ₄	7.50		4.31×10^{-4}
5	KH ₂ PO ₄	17.50		1.29×10^{-3}
6	NaCl	2.50		4.28×10^{-4}
	Alkaline EDTA Solution			
7	EDTA	5.00		1.71×10^{-4}
8	KOH	3.10		5.53×10^{-4}
	Acidified Iron Solution			
9	FeSO ₄ -7H ₂ O	0.498		1.79×10^{-5}
10	H ₂ SO ₄		1mL	
	Trace Metal Solution			
11	H ₃ BO ₃	11.42	1 mL	1.85×10^{-4}
12	ZnSO ₄ -7H ₂ O	8.82		3.07×10^{-5}
13	MnCl ₂ -4H ₂ O	1.44		7.28×10^{-6}
14	MoO ₃	0.71		4.93×10^{-6}
15	CuSO ₄ -5H ₂ O	1.57		6.29×10^{-6}
16	Co(NO ₃) ₂ -6H ₂ O	0.49		1.68×10^{-6}

The *Anabaena* 387 was cultured in these two mediums separately with all operation conditions being the same. Biomass was calculated at the first day and after 5 days and 10 days of growth respectively, following the procedures in below.

- (1) To ignite a crucible at 550°C for one hour in a muffle furnace (FD 1535M) and cool down it in a desiccator to room temperature;
- (2) To weight the crucible to obtain the tare weight, recorded as W_0 ;
- (3) To pipet 10 mL of algae solution to the crucible, and then place it in a drying oven at 103 to 105°C for 24 hours;
- (4) To cool down the crucible in a desiccator to room temperature and weighs it, recorded as W_1 .
- (5) To calculate the algae biomass using Equation (3-1):

$$\text{Algae Biomass} = \frac{W_1 - W_0}{10 \text{ mL}} \quad \text{Equation (3-1)}$$

Where: W_0 = initial tare weight of crucible in mg

W_1 = weight of algae biomass + crucible after drying in mg

For each sample, algae biomass was tested three times in parallel. The data were shown with the format of average \pm standard derivation for analyzing the algae biomass. Table 3.3 indicated the biomass growth in these two mediums.

Table 3. 3 Biomass of Anabaena 387 using 3NBBM or BBM medium (mg/ml).

Medium	Biomass	Day 1	Day 5	Day 10
BBM	Biomass-1	1.651	1.874	2.067
	Biomass-2	1.650	1.870	2.070
	Biomass-3	1.653	1.870	2.069
	Average	1.652	1.871	2.069
3NBBM	Biomass-1	1.650	1.943	2.182
	Biomass-2	1.647	1.942	2.182
	Biomass-3	1.648	1.943	2.185
	Average	1.648	1.943	2.183

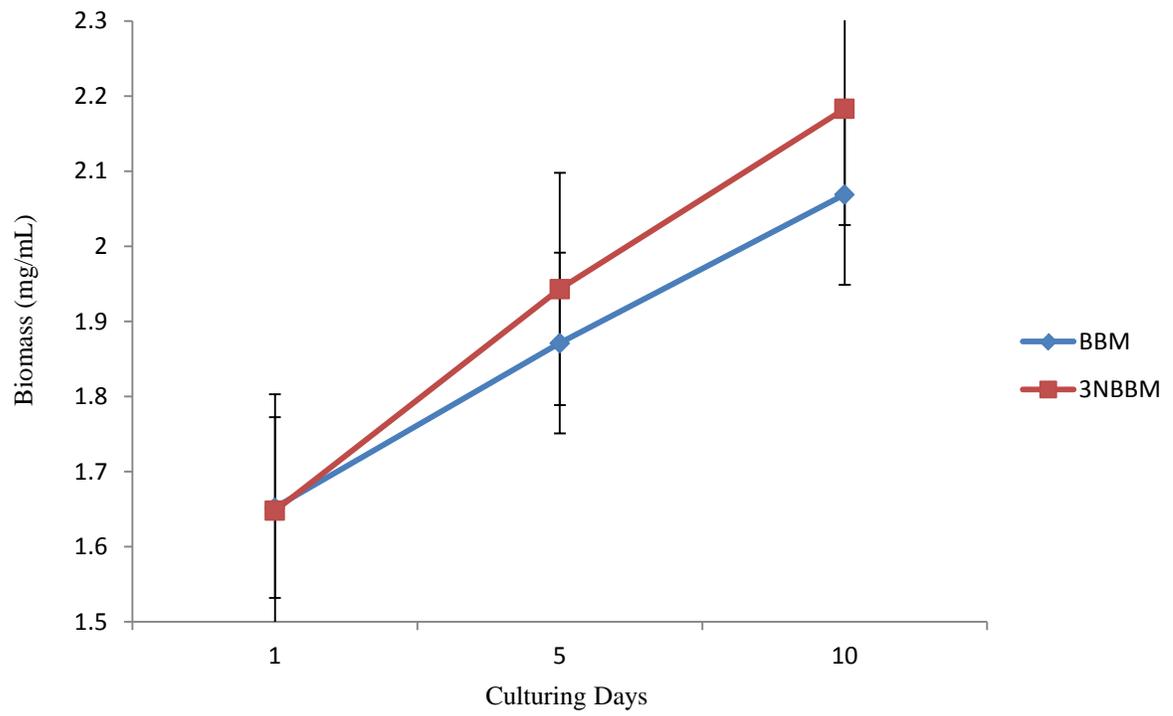


Figure 3. 1 Anabaena Biomass Growth Curves in 3NBBM and BBM Medium

As shown in Figure 3.1, the biomass in BBM increased 13 % and 25 % after 5 days and 10 days of growth, respectively; the biomass in 3NBBM increased 18% and 32% after 5 days and 10 days of growth, respectively. As a result, 3NBBM was selected as the medium for supporting the growth of *Anabaena* 387. Figure 3.2 shows *Anabaena* growing in 3NBBM solution.



Figure 3. 2 Anabaena strain 387 in Modified Bold's Basal Medium Solution

3.2 Raw Materials

Fly ash

Fly ash used in this study was from a Power Plant. The fly ash was produced by the scrubber equipment, which was used for power generation by incinerating the municipal solid.

Fish waste compost

The fish waste compost was previously generated in the lab through composting (Kazemi, 2014). The carbon and nitrogen were then extracted from the compost for another research purpose (Kazemi, 2014). The extraction process is explained in detail below: 5g of fish waste compost with peat were mixed with distilled water in a ratio of 1:10 (w/w). The enzyme, *Alcalase*, was then added into the solution in a range from 0.5% to 5% (v/v). The optimum enzyme activity occurred at pH value between 6 and 10, and temperature between 50° and 70°C. The reaction was performed in water bath to keep the temperature stable. After 3 hours of reaction, the solution was taken out from the water bath and cooled down to the room temperature. The residue of the solution was collected after centrifuged at 10,000 rpm for 10 min (Kazemi, 2014). The residue of compost after extraction was used as a candidate nutritious material in this study.

Sludge

The sludge used in this study was obtained from the Riverhead Wastewater Treatment Facility (RHWTF). It is located in St. John's, NL. It is a liquid - solid mixture after physical sedimentation and biological digestion process.

3.3 Experimental Design

3.3.1 Characterization of Raw Material

All three raw materials (i.e., fly ash, sludge, and residue of fish waste compost after extraction) were characterized using six parameters including C: N, GI, moisture content, organic matter content, pH and trace element concentrations. The standard method for testing each parameter is described in section 3.4.

3.3.2 Different Mixing Ratio

Based on the characterization results, eight mixture ratios – R1, R2, R3, R4, R5, R6, R7 and R8 were established. After mixing, all parameters (C: N, GI, moisture content, organic matter content, pH and trace elements concentration in the eight mixtures) were tested. According to the classification criteria provided by BNQ (2005), some of these eight mixtures are qualified for Type B composts but other ones are not qualified to be any levels of composts. Algae have the potential to biodegrade components in the mixtures. Therefore, the original mixtures, which are not qualified to be composts, might have the potential to become Type A or Type AA composts after algal treatment. Mixtures of Ratio 7 and Ratio 8 were selected for the following experiments based on the characterization results.

Table 3. 4 Mixing Ratio (% in a 20g mixture)

Mixture	Residue of Fish Waste Compost (%)	Sludge (%)	Fly ash (%)
R1	88	10	2
R2	80	10	10
R3	70	20	10
R4	80	14	6
R5	76	16	8
R6	50	40	10
R7	90	6	4
R8	79	14	7

3.3.3 Different Dose of Algae

Anabaena 387 was used to improve the chemical characteristics of the selected mixtures. C: N and organic matter content were mainly focused on at this stage. In order to find the appropriate dose of Anabaena solution to add in each mixture, three doses (5ml, 10ml, and 20ml) with every 20g mixture sample were tested. Six runs were established and their representations were listed in below:

Run 1: 20 grams Ratio 7 mixture samples with 5ml algal solution;

Run 2: 20 grams Ratio 7 mixture samples with 10ml algal solution;

Run 3: 20 grams Ratio 7 mixture samples with 20ml algal solution;

Run 4: 20 grams Ratio 8 mixture samples with 5ml algal solution;

Run 5: 20 grams Ratio 8 mixture samples with 10ml algal solution; and

Run 6: 20 grams Ratio 8 mixture samples with 20ml algal solution.

3.3.4 Biodegradation Curve

The mixtures of solid wastes and algal solution were treated through a biodegradation process. The biodegradation behavior was then investigated. Day 1 (D1) represents the first day algal solution and solid waste samples were mixed. Along the biodegradation process moving on, six parameters were tested every six days until some parameters, such as C: N and organic matter content, reached the stable values. At the end of this biodegradation process, the appropriate reaction days were identified. Meanwhile, in order to verify the appropriate dose of algae

solution determined from section 3.3.3, three doses - 5ml, 10ml, and 20ml with 20g samples were used.

3.3.5 Classification of composts

After the biodegradation process, the compost products were generated. According to the classification criteria provided by BNQ (2005) and CCME (1996), qualified compost products were classified into Type AA, Type A and Type B OR group A and group B, respectively. While some samples were probably still not qualified for either.

3.4 Sample Analysis

3.4.1 Determination of GI

The phytotoxicity was determined based on GI by using cucumber seeds on compost extract (Mohammad, 2013; Tiquia and Tam, 1998). Five grams (dry weight) of a compost sample was weighed and mixed with 50 ml of distilled water in a tube with a screw cap. The extract solution was then placed in an electric shaker (SHKA 4000, Model 4320) at 220 rpm for 30 minutes. After shaking, the compost extract was centrifuged (Sigma 2-16) at 5000 rpm for 15 minutes. The supernatant was then filtered through a filter paper (Fisherbrand Q5, Dia 11.0 cm). The extract was sprayed over a Petri dish with one layer of filter paper. Ten cucumber seeds were planted per plate and allowed to germinate. All plates were incubated at 20°C on a tray placed in

an Isotemp Waterbath. Seedling germination was recorded after five days of incubation. Control treatment was conducted using the same amount of distilled water and following the same procedures. The number of germinated seeds and root growth were recorded. The GI test for each compost extract and control were conducted three times. The average \pm standard derivation was used to represent the relative root growth. The relative seed germination, relative root growth and GI were calculated using the equations in below:

$$\text{Relative seed germination (\%)} = \frac{\text{NO.of seeds germinated in extract}}{\text{NO.of seeds germinated in control}} \times 100\% \quad \text{Equation (3-2)}$$

$$\text{Relative root growth (\%)} = \frac{\text{Mean root length in extract}}{\text{Mean root length in control}} \times 100\% \quad \text{Equation (3-3)}$$

$$\text{GI (\%)} = \frac{(\% \text{ Seed germination}) \times (\% \text{ Root growth})}{100} \quad \text{Equation (3-4)}$$

3.4.2 Determination of C: N

Perkin Elmer 2400 Series II CHN analyzer was used for the determination of the nitrogen and total organic carbon in the samples (Mohammad, 2013; Seal, 2012). Samples were dried for about 24 hours at 80°C then exposed to an HCl acid environment for another 24 hours to remove the inorganic carbon. They were then dried for a further 24 hours. Each subsample was then weighed to obtain 2 ± 0.1 mg, and loaded into a tin capsules, before being placed into the CHN

analyzer. The carbon and nitrogen values were given separately and the C: N was calculated using the Equation (3-5):

$$\text{Carbon to nitrogen ratio (\%)} = \frac{\text{Total organic carbon}}{\text{Total organic nitrogen}} \times 100\% \quad \text{Equation (3-5)}$$

For each sample, C: N was tested three times in parallel. The average \pm standard derivation was used for data analysis.

3.4.3 Determination of pH Level

A glass electrode microprocessor pH meter (Mettler Toledo AG, 8603 Schwerz bach) was used for the measurement of the pH value of each sample extract. One gram (dry weight) of a compost sample was weighed mixed with 10 ml of distilled water in a tube with a screw cap and placed in an electric shaker (SHKA 4000, Model 4320) at 220 rpm for 30 minutes. After shaking, the compost extract was centrifuged (Sigma 2-16) at 5000 rpm for 15 minutes. The supernatant was then filtered through a filter paper (Fisherbrand Q5, Dia 11.0 cm) before its pH was measured. For each sample, pH level was tested three times in parallel. The average \pm standard derivation was used for data analysis.

3.4.4 Determination of Organic Matter

The organic matter content in the compost was estimated by calculating the volatile solids

content (Wichuk, 2010). To clean all the organic matters in the crucible, a crucible was placed at 550°C for one hour in a muffle furnace (FD 1535M). The crucible was cooled down in a desiccator to room temperature and then weighed to obtain the tare weight, and the tare weight was recorded as W_0 . A 2 to 3 grams of sample was weighed in the pre-weighted crucible, and its weight was recorded as W_1 ; and then placed in a drying oven at 103°C to 105°C for 24 hours. The crucible with sample was cooled down in a desiccator to room temperature and weighed, and the weight was recorded as W_2 . Then the dried sample with crucible was placed into muffle furnace at 550°C for 30 minutes. The crucible with sample was weighed after it cooled down to room temperature, recorded as W_3 . The organic matter content (%) was calculated using the Equation (3-6):

$$\text{Organic matter content (\%)} = \frac{W_2 - W_3}{W_1 - W_0} \times 100\% \quad \text{Equation (3-6)}$$

Where: W_0 = initial tare weight of crucible in mg

W_1 = weight of compost samples + crucible before drying in mg

W_2 = weight of compost samples + crucible after drying in mg

W_3 = weight of compost samples + crucible after ignition in mg

For each samples, organic matter content was tested three times in parallel. The results were presented using the format of average \pm standard derivation.

3.4.5 Determination of Trace Elements Concentration

A 0.1000g of sample was put into a clean dry Teflon screw cap jar and its weight was recorded. Two milliliter of 8N HNO₃ was added into the jar and the solution was swirled, and then 1ml HF was added into the jar and the solution was swirled again. The sample was heated on a hot plate (around 70°C) for two days. The Teflon jar was cooled down to room temperature and then the cap was opened to add 3ml of Aqua Regia (3:1 conc. HCl : HNO₃). The cover was replaced and the sample was heated on a hot plate for another two days. After the sample was cooled down to room temperature, 2ml of 8N HNO₃ was added into the jar and the sample was heated on a hot plate for 4-5 hours. After the sample was cooled down to room temperature, 1ml H₂O₂ was added into the jar. Then the jar was put back on the hot plate until the bubbling stopped. The jar was removed from the hot plate and cooled down, another 1 ml H₂O₂ was added into the jar and the sample was heated for another 4-5 hours. The jar was removed from the hot plate and the content was transferred into a 45 ml snap seal container. The Teflon jar was washed using nano pure water and then the water was poured into the snap seal container as well. Additional Nano pure water was added into the container until the weight of solution was 45g. The solution was filtered into another 45ml snap seal container using a funnel and a NO.1 Whatmann filter paper. A 0.5g of the filtered solution was added into a 11ml clean test tube and its weight was recorded. Then the solution was tested by Elan DRC II ICP-MS analyzer. For each sample, trace element concentration was tested three times in parallel. The results were presented using the format of average \pm standard derivation (Mohammad, 2013; Seal, 2012).

3.4.6 Determination of Moisture Content

Ignited a crucible at 550°C for one hour in a muffle furnace (FD 1535M) and cooled down in a desiccator to room temperature. Then weighed to obtain the tare weight, recorded as W_0 . A 2 to 3 grams of compost sample was weighed in the pre-weighted crucible, recorded as W_1 ; and placed in a drying oven at 103 to 105°C for 24 hours. Cooled down the crucible in a desiccator to room temperature and weighed, recorded as W_2 (Wichuk, 2010). The moisture content (%) was calculated using Equation (3-7) (Wichuk, 2010):

$$\text{Moisture content (\%)} = \frac{W_1 - W_2}{W_1 - W_0} \times 100\% \quad \text{Equation (3-7)}$$

Where: W_0 = initial tare weight of crucible in mg

W_1 = weight of compost samples + crucible before drying in mg

W_2 = weight of compost samples + crucible after drying in mg

For each samples, moisture content was tested three times in parallel. The results were presented using the format of average \pm standard derivation.

CHAPTER 4 RESULTS AND DISCUSSION

4.1 Characterization of Raw Materials

Each raw material including fly ash, sludge, and residue of fish waste compost after the hydrolysis treatment was characterized, respectively. Six parameters including C: N, GI, moisture content, organic matter content, pH and trace elements concentrations were used for the characterization. The standard methods for testing the six parameters were stated in section 3.4. Results are listed in Tables 4.1 and 4.2.

For the residue of fish waste compost after extraction, its GI was not as high as 90% and the moisture content was higher than the standard. The organic matter in the residue of fish waste compost was 27%, which was much lower than the standard, thus this raw material was not qualified to be a compost, even a Type B one. The concentrations of all the heavy metals in the residue of fish waste compost were much lower than the standard values. Therefore, the residue of fish waste compost was free of trouble caused by heavy metals, making it a great candidate for compost generation throughout the algae treatment.

For the sludge sample, the GI was 25 %, much lower than the standard. The moisture content was 92.45 %, which is much higher than the standard. The organic matter in sludge was 5.19%, which was much lower than the standard, which meant the sludge was not qualified to be a compost product, even a Type B one. Meanwhile, the concentration of Cu was 110.6 mg/kg and the concentration of Se was 10.097 mg/kg, which were higher than the standards. The C: N was

13.32, lower than 25 (the standard value). The results indicated that the sludge had a potential to be a component in the compost.

For the fly ash, the C: N was 126.54, which was much higher than standard. The GI and moisture content were very low, which were 10.11% and 0.15%, respectively. The concentrations of Mo and Ni were 14.698 mg/kg and 876.698 mg/kg, respectively, which were higher than the standards. The organic matter content in fly ash was high enough, even based on the standard for a type AA level of compost. The organic matter content made the fly ash a good candidate for compost generation throughout the algae treatment.

In summary, each raw material was not qualified to be a compost product directly as alone. However, all the raw materials had their special characteristics and potentials to be part of the compost products throughout the algae treatment.

Table 4. 1 Results of Raw Material Characterizations

Raw Materials	C:N	GI (%)	Moisture (%)	Organic Matters (%)	pH	Trace Elements
Standards	<25	>90	<60	Type AA >50; Type A >40; Type B >30;	NA	See Table 4.2
Residue of Fish Waste Compost	12.03	61.62	66.73	26.87	8.93	
Sludge	13.32	24.73	92.45	5.19	5.15	
Fly Ash	126.54	10.11	0.15	96.26	3.1	

Table 4. 2 Trace Element Concentrations in Raw Materials (mg/kg)

Trace Elements	Type AA and A	Type B	Fly ash	Sludge	Fish waste compost
As	13	75	0.897	1.888	6.988
Co	34	150	0.815	1.79	0.831
Cr	210	1060	7.835	17.369	3.672
Cu	100	757	<LD*	110.6	7.95
Mo	5	20	14.698	3.119	0.307
Ni	62	180	876.698	8.87	3.436
Se	2	14	<LD*	10.097	<LD*
Zn	500	1850	13.74	162.83	78.855
Cd	3	20	<LD*	<LD*	0.698
Hg	0.8	5	<LD*	0.018	<LD*
Pb	150	500	2.531	19.295	3.864

Note: <LD* means the concentration is lower than limit detection.

4.2 Determination of Mixing Ratio

4.2.1 Mixing Ratio

Based on the characterization results, the three materials were mixed together using 8 ratios for further treatment by algae. Table 3.4 in page 63 shows the corresponding mixing ratios in percentage and 20g was set as the final sample mass for each treatment run.

As shown in Table 3.4, residue of fish waste compost after extraction is the major component and fly ash accounts for the lowest percent in each mixture. The high content of organic matter and C: N in fly ash can help to improve the overall organic matter content and C: N values in the mixtures. Special attention was paid to the high concentration of heavy metals in fly ash and sludge. Since heavy metals cannot be biodegraded by algae, to generate qualified composts, the percentages of fly ash and sludge thus had to be limited to certain levels in the mixture. The mixture with ratio 6 contains the highest percentage of both sludge and fly ash, which might lead to the generation of compost with the lowest quality. The mixture with ratio 7 contains the highest percentage of residue of fish waste compost and less sludge and fly ash, making it a good candidate to generate composts with high quality.

4.2.2 Characterization of Mixtures with Eight Ratios

After mixing the fly ash, sludge and residue of fish waste compost in eight different ratios, the characterization of each mixture was conducted. The results are listed in Tables 4.3 to 4.10.

As shown in Table 4.3, for the mixture with Ratio 1, the GI, C: N, and moisture content of the mixture meet the BNQ standards. The organic matter content in Ratio 1 mixture was 61.40%, which was even higher than the standard values for a type AA compost. However, the concentration of Se was 8.846 mg/kg, which was higher than 2 mg/kg (standard value for type AA and A levels of compost) but still lower than 14 ppm (standard value for type B level of compost). Therefore, the mixture with Ratio1 can be classified as a compost at the type B level, even though the organic matter was higher than 50% (standard value for type AA level of compost).

As shown in Table 4.4, for the mixture with Ratio 2, the GI, C: N, and moisture content of the mixture meet the BNQ standards. However, the concentration of Ni was 107.764 mg/kg, which was higher than 62 mg/kg (standard value for type AA and A levels of compost) but still lower than 180 mg/kg (standard value for type B level of compost). The concentration of Se was 4.45 mg/kg, which was higher than 2 mg/kg (standard value for type AA and A levels of compost) but lower than 14 mg/kg (standard value for type B level of compost). Therefore, the mixture with Ratio 2 can also be classified as a compost at the type B level, even though the organic matter was 60.36%, which was higher than the standard value for a type AA compost.

As shown in Table 4.6, for the mixture with Ratio 4, the concentration of Se was 2.004

mg/kg, which was a little bit higher than 2 mg/kg (standard value for type AA and A levels of compost) and was lower than 14 ppm (standard value for type B level of compost). The Ratio 4 mixture thus can be classified as a compost at the type B level. All other parameters meet the BNQ standards.

According to the CCME (1996) and BNQ (2005) standards, the C: N of a compost product should be lower than 25. As shown in Tables 4.5, 4.7 and 4.8, for the mixtures with Ratio 3, Ratio 5 and Ratio 6, the C: N value was 28.14, 42.65 and 35.27, respectively. According to the standard of concentrations of heavy metals, the maximum acceptable concentration of Ni was 180 ppm. For the mixtures with Ratio 3, Ratio 5 and Ratio 6, the concentration of Ni was 182.507 mg/kg, 237.831 mg/kg, and 302.525 mg/kg, respectively. Since heavy metals were not biodegradable, the algae treatment cannot help to decrease the heavy metal concentrations in the mixtures. These three mixtures were thus not qualified to be compost products because of their high C: N and high concentration of Ni.

As shown in Tables 4.9 and 4.10, for the mixtures with Ratio 7 and Ratio 8, all the parameters meet the BNQ standards (2005). In addition, according to the standards of concentrations of heavy metals and organic matter content, these two mixtures can be classified as type A level compost products.

Table 4. 3 Characterization of the mixture with Ratio 1

	BNQ Standards			Ratio1
Organic carbon	NA			37.64
Nitrogen	NA			2.82
C: N	<25			13.35
GI (%)	>90			158.08
Moisture (%)	<60			32.46
Organic Matter (%)	Type AA >50	Type A >40	Type B >30	61.40
pH	NA			8.14
Trace Elements (mg/kg)	Type AA	Type A	Type B	R1
As	13		75	7.547
Co	34		150	0.844
Cr	210		1060	6.580
Cu	100		757	20.718
Mo	5		20	1.525
Ni	62		180	28.220
Se	2		14	8.846
Zn	500		1850	92.525
Cd	3		20	0.561
Hg	0.8		5	<LD*
Pb	150		500	4.768

Table 4. 4 Characterization of the mixture with Ratio 2

	BNQ Standards			Ratio 2
Organic carbon	NA			52.98
Nitrogen	NA			2.77
C: N	<25			19.12
GI (%)	>90			153.11
Moisture (%)	<60			31.67
Organic Matter (%)	Type AA >50	Type A >40	Type B >30	60.36
pH	NA			8.17
Trace Elements (mg/kg)	Type AA	Type A	Type B	R2
As	13		75	6.080
Co	34		150	0.576
Cr	210		1060	6.810
Cu	100		757	15.280
Mo	5		20	3.374
Ni	62		180	107.764
Se	2		14	4.450
Zn	500		1850	71.210
Cd	3		20	0.399
Hg	0.8		5	<LD*
Pb	150		500	3.371

Table 4. 5 Characterization of the mixture with Ratio 3

	BNQ Standards			Ratio 3
Organic carbon	NA			51.21
Nitrogen	NA			1.82
C: N	<25			28.14
GI (%)	>90			139.21
Moisture (%)	<60			37.15
Organic Matter (%)	Type AA >50	Type A >40	Type B >30	53.26
pH	NA			8.11
Trace Elements (mg/kg)	Type AA	Type A	Type B	R3
As	13		75	5.991
Co	34		150	0.655
Cr	210		1060	8.422
Cu	100		757	15.523
Mo	5		20	5.227
Ni	62		180	182.507
Se	2		14	0.158
Zn	500		1850	75.423
Cd	3		20	0.601
Hg	0.8		5	0.039
Pb	150		500	2.649

Table 4. 6 Characterization of the mixture with Ratio 4

	BNQ Standards			Ratio 4
Organic carbon	NA			43.29
Nitrogen	NA			2.17
C: N	<25			19.95
GI (%)	>90			146.88
Moisture (%)	<60			35.57
Organic Matter (%)	Type AA >50	Type A >40	Type B >30	54.72
pH	NA			8.15
Trace Elements (mg/kg)	Type AA	Type A	Type B	R4
As	13		75	8.615
Co	34		150	<LD*
Cr	210		1060	6.970
Cu	100		757	21.204
Mo	5		20	1.929
Ni	62		180	49.253
Se	2		14	2.004
Zn	500		1850	103.475
Cd	3		20	0.691
Hg	0.8		5	<LD*
Pb	150		500	4.718

Table 4. 7 Characterization of the mixture with Ratio 5

	BNQ Standards			Ratio 5
Organic carbon	NA			46.91
Nitrogen	NA			1.10
C: N	<25			42.65
GI (%)	>90			152.06
Moisture (%)	<60			34.84
Organic Matter (%)	Type AA >50	Type A >40	Type B >30	60.45
pH	NA			8.12
Trace Elements (mg/kg)	Type AA	Type A	Type B	R5
As	13		75	3.456
Co	34		150	0.454
Cr	210		1060	7.371
Cu	100		757	7.616
Mo	5		20	6.723
Ni	62		180	237.831
Se	2		14	<LD*
Zn	500		1850	56.768
Cd	3		20	1.059
Hg	0.8		5	<LD*
Pb	150		500	2.649

Table 4. 8 Characterization of the mixture with Ratio 6

	BNQ Standards			Ratio 6
Organic carbon	NA			49.02
Nitrogen	NA			1.39
C: N	<25			35.27
GI (%)	>90			143.05
Moisture (%)	<60			52.05
Organic Matter (%)	Type AA >50	Type A >40	Type B >30	43.64
pH	NA			8.09
Trace Elements (mg/kg)	Type AA	Type A	Type B	R6
As	13		75	3.664
Co	34		150	0.649
Cr	210		1060	8.653
Cu	100		757	8.465
Mo	5		20	6.859
Ni	62		180	302.525
Se	2		14	<LD*
Zn	500		1850	47.466
Cd	3		20	0.229
Hg	0.8		5	<LD*
Pb	150		500	2.117

Table 4. 9 Characterization of the mixture with Ratio 7

	BNQ Standards			Ratio 7
Organic carbon	NA			39.30
Nitrogen	NA			3.49
C: N	<25			11.26
GI (%)	>90			160.24
Moisture (%)	<60			15.8
Organic Matter (%)	Type AA >50	Type A >40	Type B >30	44.02
pH	NA			8.01
Trace Elements (mg/kg)	Type AA	Type A	Type B	R7
As	13		75	7.400
Co	34		150	0.759
Cr	210		1060	4.582
Cu	100		757	13.171
Mo	5		20	1.249
Ni	62		180	52.662
Se	2		14	1.033
Zn	500		1850	86.038
Cd	3		20	0.930
Hg	0.8		5	<LD*
Pb	150		500	3.985

Table 4. 10 Characterization of the mixture with Ratio 8

	BNQ Standards			Ratio 8
Organic carbon	NA			45.32
Nitrogen	NA			2.89
C: N	<25			15.68
GI (%)	>90			150.44
Moisture (%)	<60			21.34
Organic Matter (%)	Type AA >50	Type A >40	Type B >30	45.78
pH	NA			8.23
Trace Elements (mg/kg)	Type AA	Type A	Type B	R8
As	13		75	4.805
Co	34		150	0.387
Cr	210		1060	2.864
Cu	100		757	6.750
Mo	5		20	1.484
Ni	62		180	57.849
Se	2		14	<LD*
Zn	500		1850	63.033
Cd	3		20	0.502
Hg	0.8		5	<LD*
Pb	150		500	2.776

Two mixing ratios (i.e., Ratio 7 and Ratio 8) were selected for further treatment by algae. The operation conditions of algae treatment were determined and the quality improvements of composts after the biodegradation treatment are evaluated in sections 4.3 to 4.4.

4.3 Determination of Treatment Period

4.3.1 Results of 6 Runs Based on A 24-Day Algal Treatment

Along with the algal biodegradation process, all the six parameters (i.e., C: N, GI, the concentration of heavy metals, pH, moisture content and organic matter) were tested every 6 days until a stable stage was reached. The algal treatment was conducted using the mixtures with Ratio 7 and Ratio 8, respectively. For each ratio, three doses (i.e., 5ml, 10ml and 20 ml) of algal solution were selected. The solution was added to a 20g mixture sample before the algae treatment. Six runs were established and their representations are listed below:

Run 1: 20 grams mixtures in Ratio 7 with 5 mL algal solution;

Run 2: 20 grams mixtures in Ratio 7 with 10 mL algal solution;

Run 3: 20 grams mixtures in Ratio 7 with 20 mL algal solution;

Run 4: 20 grams mixtures in Ratio 8 with 5 mL algal solution;

Run 5: 20 grams mixtures in Ratio 8 with 10 mL algal solution; and

Run 6: 20 grams mixtures in Ratio 8 with 20 mL algal solution.

All six runs were conducted at the same time and under the same operation conditions. The amount of algal solution added into the mixture was the only variable. The results are listed in

the Tables 4.11 to 4.22.

For the C: N in Run 1, no obvious difference was observed in the first 6 days. In the following 6 days, the C: N was decreased gradually from 25 to 20. Eventually it reached 16.39 after 18 days. For the nitrogen content in Run 1, no obvious difference was observed as well in the first 6 days; however, it was increased significantly from 1.57% to 2.27% in the next 12 days and then kept relatively stable for the rest of the process. In Run 1, the organic matter content was increased dramatically from 44.61% to 47.60% in the first 6 days and then only increased to 49.06% in the remaining 12 days. The content of organic carbon was decreased gradually from 39.26% to 36.13% throughout the treatment. Overall, a stable stage was achieved after 18 days, which indicated that the algal biodegradation process was completed. The algae treatment period was thus determined to be 18 days.

According to the BNQ standards (2005), after 24 days of algal biodegradation treatment, the Run 1 product was qualified to be a type A level compost. Even though it was still at the same level as before the algal treatment, its qualities improved in many ways, such as the C: N value was decreased from 25.33 to 15.21, which was lower than 25 (the standard value); the nitrogen content was increased from 1.55% to 2.31%; the GI was improved from 155.78% to 160.74%; and the organic matter content was changed from 44.61% to 49.06%, which almost reached 50% (standard value for type AA level of compost). The concentrations of heavy metals are qualified as type A level compost.

Table 4. 11 Results of Run 1 for Determination of the Algae Treatment Period

Days	C:N	Organic Carbon (%)	Nitrogen (%)	GI (%)	OM (%)	Moisture (%)	pH	Trace Elements
Standards	<25	NA	NA	>90	Type AA>50; Type A>40; Type B>30	<60	NA	See Table 4.13
D1	25.33	39.26	1.55	155.78	44.61	34.66	8.01	
D6	24.56	38.44	1.57	157.44	47.60	28.47	8.12	
D12	19.82	37.47	1.89	158.20	48.96	27.41	8.06	
D18	16.39	37.21	2.27	158.71	49.03	25.21	8.04	
D24	15.21	36.13	2.31	160.74	49.06	24.87	7.99	

Table 4. 12 Variations of Trace Elements in Run 1 during the 24-Day Algae Treatment Process

Trace Elements (mg/kg)	Type AA	Type A	Type B	D1	D6	D12	D18	D24
As	13	75	7.318	7.319	7.403	7.289	7.311	
Co	34	150	0.760	0.755	0.760	0.756	0.761	
Cr	210	1060	4.579	4.580	4.577	4.567	4.584	
Cu	100	757	13.168	13.170	13.165	13.169	13.171	
Mo	5	20	1.243	1.239	1.245	1.244	1.248	
Ni	62	180	52.666	52.659	52.660	52.655	52.664	
Se	2	14	1.024	1.034	1.029	1.031	1.033	
Zn	500	1850	85.978	86.042	86.040	86.036	86.033	
Cd	3	20	0.926	0.935	0.925	0.933	0.914	
Hg	0.8	5	<LD*	<LD*	<LD*	<LD*	<LD*	
Pb	150	500	3.978	4.001	3.998	3.980	3.988	

Note: <LD* means the concentration is lower than limit detection.

For Run 2, the C: N was decreased gradually in the first 18 days from 29.06 to 18.06, then it was decreased slowly to 16.15 in the last 6 days. For the nitrogen content in Run 2, no obvious difference was observed in the first 6 days; it was then increased significantly from 2.19% to 2.78% in the following 12 days and ended up with 2.84% after 24 days. In Run 2, the organic matter content was increased obviously from 45.45% to 56.77% in the first 12 days and then increased slowly in the next 12 days. Then content of organic carbon was decreased gradually from 58.41% to 45.88% through the 24 days process. Overall, a stable stage was achieved after 18 days, indicating that the algal treatment process was completed. Therefore the appropriate treatment period was determined to be 18 days.

After the 18 days treatment, the C: N in Run 2 was decreased from 29.06 to 16.15, which was lower than 25 (the standard value). The nitrogen content was increased from 2.01% to 2.84%. The GI was improved from 156.35% to 161.26% and organic matter content was increased from 45.45% to 58.00%. The concentrations of heavy metals are qualified as type AA level compost. Based on the BNQ standards (2005), after 24 days of the algal biodegradation treatment, the Run 2 product was changed from a type A level compost to a type AA level compost.

Table 4. 13 Results of Run 2 for Determination of the Algae Treatment Period

Days	C: N	Organic Carbon (%)	Nitrogen (%)	GI (%)	OM (%)	Moisture (%)	pH	Trace Elements
Standards	<25	NA	NA	>90	Type AA>50; Type A>40; Type B>30	<60	NA	See Table 4.15
D1	29.06	58.41	2.01	156.35	45.45	42.32	8.14	
D6	26.58	58.23	2.19	157.46	52.34	34.22	8.23	
D12	22.58	56.70	2.51	158.91	56.77	29.31	8.18	
D18	18.06	50.21	2.78	159.23	57.20	27.77	8.10	
D24	16.15	45.88	2.84	161.26	58.00	25.81	8.21	

Table 4. 14 Variations of Trace Elements in Run 2 during the 24-Day Algae Treatment Process

Trace Elements (mg/kg)	Type AA	Type A	Type B	D1	D6	D12	D18	D24
As	13	75	7.512	7.334	7.489	7.501	7.524	
Co	34	150	0.811	0.768	0.902	0.801	0.799	
Cr	210	1060	4.823	4.997	4.874	4.698	4.820	
Cu	100	757	12.998	12.670	13.014	12.876	13.112	
Mo	5	20	1.340	1.278	1.401	1.387	1.299	
Ni	62	180	51.784	52.045	50.956	50.887	51.689	
Se	2	14	0.994	1.031	1.110	0.897	1.004	
Zn	500	1850	85.887	86.029	85.994	86.078	84.764	
Cd	3	20	1.033	0.940	0.892	1.100	1.021	
Hg	0.8	5	<LD*	<LD*	<LD*	<LD*	<LD*	
Pb	150	500	4.100	3.945	3.228	4.111	3.598	

Note: <LD* means the concentration is lower than limit detection.

In Run 3, for the C: N, no obvious difference was observed in the first 6 days. In the following 12 days, the C: N was dropped from 27.66 to 20.33. Eventually the C: N reached 18.14 after the treatment for 24 days. For the nitrogen content in Run 3, an obvious increase was observed between 6 days and 18 days with a change from 2.30% to 2.79%. For the organic matter content, it was increased from 46.77% to 49.01% in the first 12 days and followed by a slow increase in the last 12 days. All the parameters met the BNQ standards after 24 days of the algal treatment except the moisture content. When adding the liquid phase algal solution to the mixtures, additional water was introduced to the samples as well. Even though the moisture content was decreased from 87.22% to 61.09% after 24 days of treatment, the final content was still higher than 60% (the standard value). Therefore, the Run 3 product cannot be used as a compost because of its high moisture content, even though the concentrations of heavy metals are qualified as type A level compost.

In summary, for the mixtures with Ratio 7 after 24 days of the algal treatment, the Run 1 product can be used as a type A compost. The Run 2 product can be classified as a type AA compost. The Run 3 product was not qualified to be a compost, even a type B one.

For the mixtures with Ratio 8, three doses (i.e., 5mL, 10mL and 20mL) of algal solution were selected as well. The solution was added to a 20g mixture before the algae treatment. The results of Run 4, 5, and 6 are shown in the following Tables 4.17 to 4.22.

Table 4. 15 Results of Run 3 for Determination of the Algae Treatment Period

Days	C: N	Organic Carbon (%)	Nitrogen (%)	GI (%)	OM (%)	Moisture (%)	pH	Trace Elements
Standards	<25	NA	NA	>90	Type AA>50; Type A>40; Type B>30	<60	NA	See Table 4.17
D1	29.36	65.47	2.23	150.37	46.77	87.22	8.07	
D6	27.66	63.62	2.30	150.99	47.22	80.13	8.07	
D12	22.44	59.02	2.63	152.03	49.01	72.99	8.14	
D18	20.33	56.31	2.79	152.98	49.68	64.25	8.09	
D24	18.41	52.65	2.88	154.44	49.70	61.09	8.08	

Table 4. 16 Variations of Trace Elements in Run 3 during the 24-Day Algae Treatment Process

Trace Elements (mg/kg)	Type AA	Type A	Type B	D1	D6	D12	D18	D24
As	13	75	7.689	7.778	7.214	7.819	7.683	
Co	34	150	0.633	0.645	0.629	0.700	0.648	
Cr	210	1060	4.824	4.339	4.584	4.799	4.612	
Cu	100	757	14.001	13.569	13.278	13.560	14.120	
Mo	5	20	1.289	1.387	1.199	1.350	1.394	
Ni	62	180	52.557	51.874	51.294	52.395	52.583	
Se	2	14	0.994	0.819	1.033	1.098	0.978	
Zn	500	1850	85.208	85.101	84.921	86.120	84.298	
O	3	20	0.980	0.938	1.001	0.863	0.995	
Hg	0.8	5	<LD*	<LD*	<LD*	<LD*	<LD*	
Pb	150	500	3.879	3.987	3.590	4.001	3.733	

Note: <LD* means the concentration is lower than limit detection.

For the C: N in Run 4, a slight decrease was observed in the first 6 days. In the following 12 days, the C: N was decreased from 24.58 to 14.77. Eventually the C: N reached 13.73 after 24 days of treatment. For the nitrogen content in Run 4, a sharp increase was observed between 6 days and 18 days with the change from 1.82% to 2.59%. For the organic matter content, it was increased from 45.98% to 47.53% in the first 12 days and followed by a slow increase in the last 12 days. The organic carbon content was decreased gradually from 45.38% to 35.98% throughout the 24 days of treatment. Overall, a stable stage was achieved after 18 days, indicating that the algal treatment process was completed. Therefore, the appropriate treatment period was determined to be 18 days.

Based on the BNQ standards (2005), after 24 days of the algal biodegradation treatment, the Run 4 product was qualified to be a type A level compost. Even though Run 4 was still classified as type A level compost, which was the same as before the algal treatment, its quality was improved in many ways, such as the C: N in Run 4 was decreased from 26.54 to 13.73, which was much lower than 25 (the standard value); the nitrogen content was increased from 1.71% to 2.62%; the GI was improved from 140.55% to 147.06% and organic matter content was increased from 45.98% to 48.07%. The concentrations of heavy metals are qualified as type A level compost.

Table 4. 17 Results of Run 4 for Determination of the Algae Treatment Period

Days	C: N	Organic Carbon (%)	Nitrogen (%)	GI (%)	OM (%)	Moisture (%)	pH	Trace Elements
Standards	<25	NA	NA	>90	Type AA>50; Type A>40; Type B>30	<60	NA	See Table 4.19
D1	26.54	45.38	1.71	140.55	45.98	36.78	7.91	
D6	24.58	44.74	1.82	142.43	46.44	30.78	7.89	
D12	19.01	42.01	2.21	145.21	47.53	28.66	7.92	
D18	14.77	38.25	2.59	146.88	47.91	27.73	7.88	
D24	13.73	35.98	2.62	147.06	48.07	26.54	7.88	

Table 4. 18 Variations of Trace Elements in Run 4 during the 24-Day Algae Treatment Process

Trace Elements (mg/kg)	Type AA	Type A	Type B	D1	D6	D12	D18	D24
As	13	75	4.789	4.801	4.729	4.635	4.294	
Co	34	150	0.401	0.399	0.381	0.385	0.391	
Cr	210	1060	2.888	2.490	2.496	2.503	2.623	
Cu	100	757	6.690	6.396	6.597	6.792	6.422	
Mo	5	20	1.198	1.270	1.423	0.997	1.255	
Ni	62	180	57.779	57.395	56.930	56.244	57.396	
Se	2	14	<LD*	<LD*	<LD*	<LD*	<LD*	
Zn	500	1850	63.290	62.979	62.489	61.396	63.594	
Cd	3	20	0.498	0.482	0.500	0.429	0.511	
Hg	0.8	5	<LD*	<LD*	<LD*	<LD*	<LD*	
Pb	150	500	2.679	2.492	3.011	2.496	2.557	

Note: <LD* means the concentration is lower than limit detection.

For Run 5, the C: N was decreased gradually in the first 18 days from 27.46 to 18.14, then it was decreased slowly in the last 6 days. For the nitrogen content in Run 5, no obvious difference was observed in the first 6 days; it was then increased significantly from 2.46% to 2.88% in the following 12 days and ended up with 2.94% after 24 days. For the organic matter content in Run 5, it was increased gradually from 46.01% to 55.78% throughout the algae treatment process and the content of organic carbon was decreased gradually from 62.89% to 50.66% at the end of the process. Overall, a stable stage was achieved after 18 days, indicating that the algal treatment process was completed. Therefore, the appropriate treatment period was determined to be 18 days.

After the 18 days of treatment, the C: N in Run 5 was decreased from 27.46 to 17.23, which was lower than 25 (the standard value). The nitrogen content was increased from 2.29% to 2.94%. The GI was improved from 141.98% to 152.04% and organic matter content was increased from 46.01% to 55.78%. The concentrations of heavy metals are qualified as type AA level compost. Based on the BNQ standards (2005), after 24 days of the algae biodegradation treatment, the Run 5 product was changed from a type A level compost to a type AA level compost.

Table 4. 19 Results of Run 5 for Determination of the Algae Treatment Period

Days	C:N	Organic Carbon (%)	Nitrogen (%)	GI (%)	OM (%)	Moisture (%)	pH	Trace Elements
Standards	<25	NA	NA	>90	Type AA>50; Type A>40; Type B>30	<60	NA	See Table 4.21
D1	27.46	62.89	2.29	141.98	46.01	44.38	7.90	
D6	24.78	60.96	2.46	144.29	48.04	35.76	7.87	
D12	20.88	53.66	2.57	148.66	49.99	29.44	7.91	
D18	18.14	52.24	2.88	151.22	54.86	28.11	7.91	
D24	17.23	50.66	2.94	152.04	55.78	26.02	7.88	

Table 4. 20 Variations of Trace Elements in Run 5 during the 24-Day Algae Treatment Process

Trace Elements (mg/kg)	Type AA	Type A	Type B	D1	D6	D12	D18	D24
As	13	75	4.029	4.708	5.209	3.998	4.258	
Co	34	150	0.320	0.411	0.392	0.457	0.389	
Cr	210	1060	2.739	2.920	2.858	2.144	2.502	
Cu	100	757	6.891	6.295	5.998	6.282	6.487	
Mo	5	20	1.335	1.204	1.400	1.295	1.420	
Ni	62	180	56.294	57.202	57.479	56.153	56.298	
Se	2	14	<LD*	<LD*	<LD*	<LD*	<LD*	
Zn	500	1850	62.995	62.895	63.223	62.485	62.668	
Cd	3	20	0.407	0.498	0.499	0.469	0.472	
Hg	0.8	5	<LD*	<LD*	<LD*	<LD*	<LD*	
Pb	150	500	2.738	2.692	2.587	2.534	2.622	

Note: <LD* means the concentration is lower than limit detection.

For the C: N in Run 6, no obvious difference was observed in the first 6 days. In the following 12 days, the C: N was dropped from 26.81 to 19.98. Eventually the C: N reached 18.01 after 24 days of treatment. For the nitrogen content in Run 6, an obvious increase was observed between 6 days and 18 days with the change from 2.51% to 3.01%. For the organic matter content, it was increased from 46.33% to 47.49% in the first 12 days and followed by a slow increase in the last 12 days. All the parameters met the BNQ standards after 24 days of the algal treatment except the moisture content. Even though the moisture content was decreased from 86.97% to 60.20% after 24 days of treatment, the final content was still higher than 60% (the standard value). Therefore, the Run 6 product cannot be used as a compost because of its high moisture content, even though the concentrations of heavy metals are qualified as type A level compost.

In summary, for the mixtures with Ratio 8 after 24 days of the algal treatment, the Run 4 product can be used as a type A compost. The Run 5 product can be classified as a type AA compost. The Run 6 product was not qualified to be a compost, even as a type B one.

Table 4. 21 Results of Run 6 for Determination of the Algae Treatment Period

Days	C: N	Organic Carbon (%)	Nitrogen (%)	GI (%)	OM (%)	Moisture (%)	pH	Trace Elements
Standards	<25	NA	NA	>90	Type AA>50; Type A>40; Type B>30	<60	NA	See Table 4.23
D1	28.88	69.31	2.40	137.47	46.33	86.97	7.88	
D6	26.81	67.29	2.51	140.24	47.09	80.64	7.84	
D12	22.78	63.10	2.77	143.22	47.49	74.34	7.87	
D18	19.98	60.14	3.01	147.05	48.78	65.32	7.86	
D24	18.01	55.65	3.09	150.17	49.24	60.20	7.86	

Table 4. 22 Variations of Trace Elements in Run 6 during the 24-Day Algae Treatment Process

Trace Elements (mg/kg)	Type AA	Type A	Type B	D1	D6	D12	D18	D24
As	13	75	4.829	4.734	4.556	4.890	4.765	
Co	34	150	0.356	0.322	0.401	0.379	0.367	
Cr	210	1060	2.668	2.592	2.538	2.490	2.612	
Cu	100	757	6.349	6.298	6.267	6.422	6.199	
Mo	5	20	1.256	1.328	1.292	1.198	1.177	
Ni	62	180	57.890	56.287	57.209	56.928	57.110	
Se	2	14	<LD*	<LD*	<LD*	<LD*	<LD*	
Zn	500	1850	62.889	62.492	62.960	61.957	62.184	
Cd	3	20	0.487	0.472	0.398	0.426	0.419	
Hg	0.8	5	<LD*	<LD*	<LD*	<LD*	<LD*	
Pb	150	500	2.665	2.496	2.536	2.470	2.398	

Note: <LD* means the concentration is lower than limit detection.

4.3.2 Determination of Treatment Period

The appropriate biodegradation period depends on the algae strain used in the process and the biodegradable components existing in the system. Generally, the trend of biodegradation rate is similar in most cases, which is a relative slow biodegradation rate observed in the beginning, followed by a higher level biodegradation rate, then ending up with an accomplishment of biodegradation. The decreasing biodegradation rate can be caused by the continual decreasing of moisture and nutrients in the system, accumulation of toxic intermediates and selective decomposition of substances.

Takacova (2014) conducted an experiment by using algae, *Ch. Kessleri*, to biodegrade Benzo[a]Purene (BaP) and it took 5 to 6 days to biodegrade 30% of BaP. In addition, Warshawsky et al. (2014) found that a freshwater green algae, *Selenastrum capricornutum*, was able to metabolize BaP to *cis*-dihydrodiols by using the dioxygenase enzyme system inside the algae.

In this section, monitoring results of four parameters (i.e., C: N, organic carbon, organic matter, and nitrogen content) were discussed in detail to determine the appropriate algal treatment period. Figures 4.2 to 4.5 show the tendency of these 4 parameters for each run along with the 24 days of the algal treatment process.

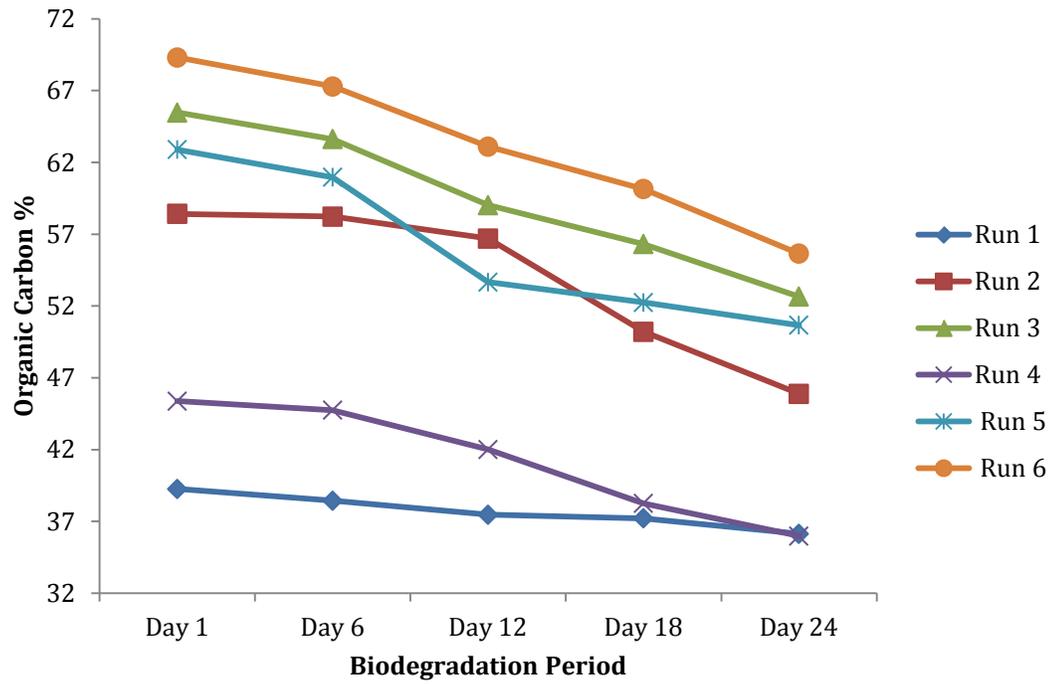


Figure 4. 1 Organic Carbon Content of 6 Runs in the 24-Day Algae Treatment Process

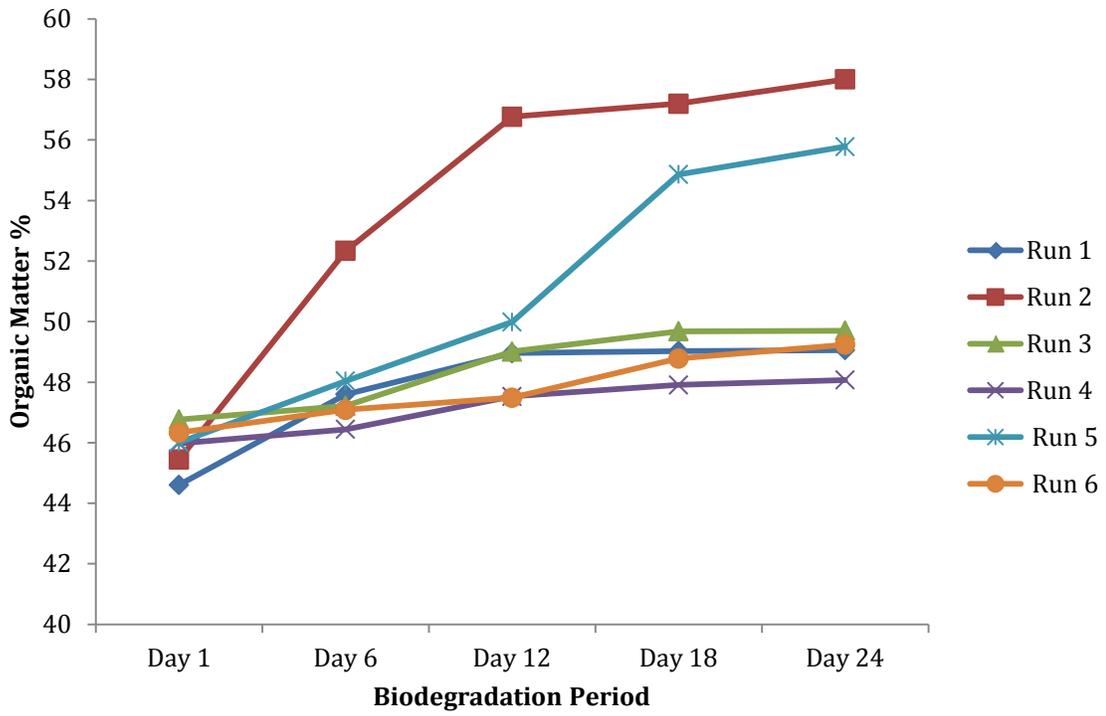


Figure 4. 2 Organic Matter Content of 6 Runs in the 24-Day Algae Treatment Process

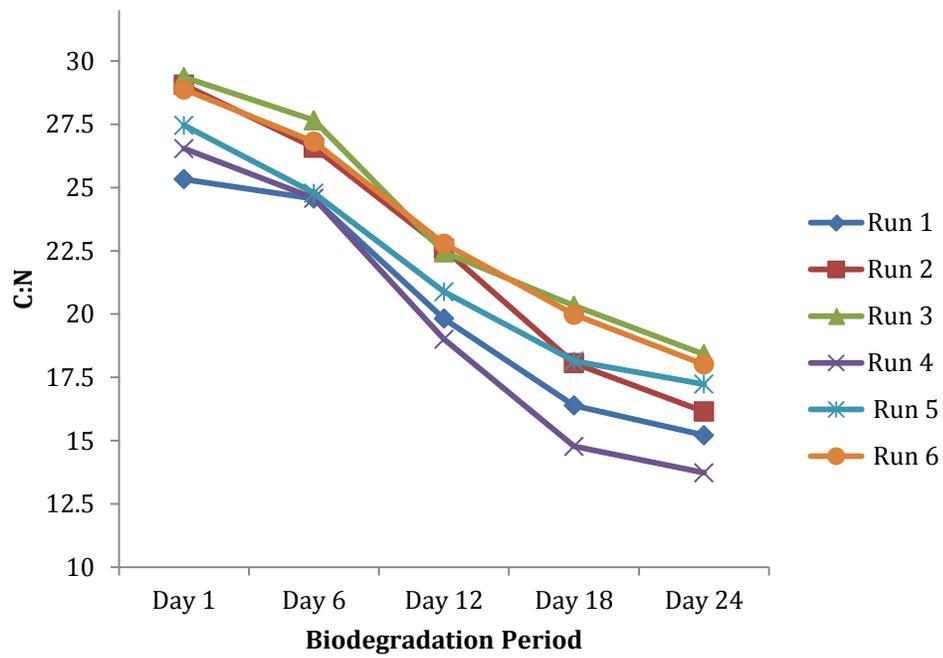


Figure 4. 3 C: N of 6 Runs in the 24-Day Algae Treatment Process

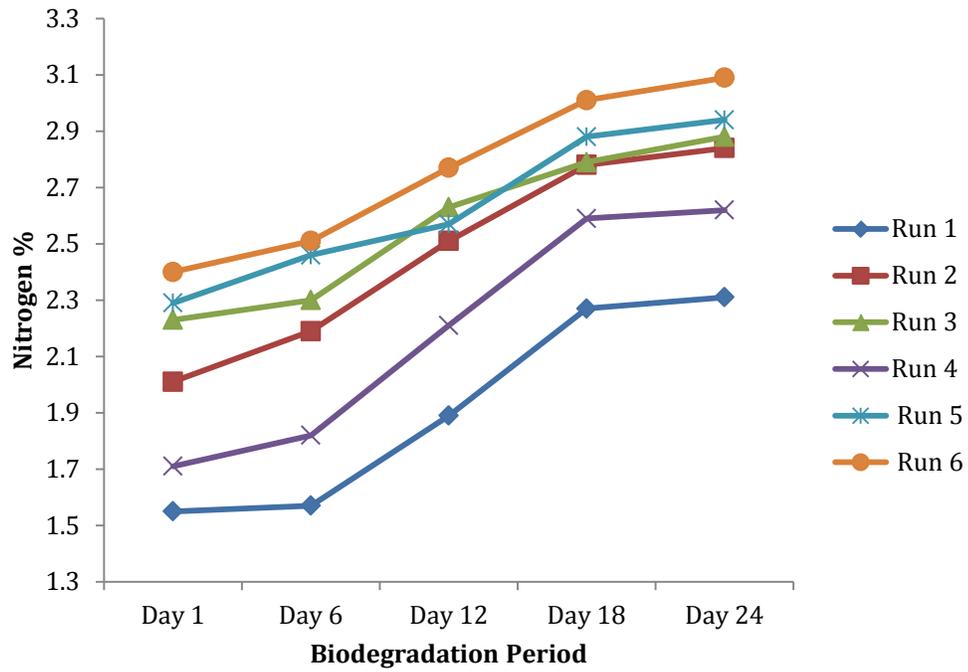


Figure 4. 4 Nitrogen Content of 6 Runs in the 24-Day Algae Treatment Process

According to the curves, the rates of bio-transforming of both organic carbon content and organic matter content slowed down after 12 days of the treatment and the rates of changing C: N and nitrogen content slowed down after 18 days of the treatment. The decreased biodegradation rates reflected the accomplishment of the algal biodegradation process. Therefore, the quality of each 6 run reached to a relative stable level (without big change in organic carbon content, organic matter content and C: N), after 18 days. To decrease the period of treatment and increase the treatment capacity of a facility, the algal treatment period was determined to be 18 days.

After 18 days algal treatment, Run 1 and Run 4 products can be used as type A compost; Run 2 and Run 5 products can be classified as type AA compost; however, Run 3 and Run 6 products were not qualified as compost because of their high moisture contents.

4.4 Determination of Algal Dosage

Three dosages of algae solution (i.e., 5 mL, 10 mL and 20 mL) were added into the mixtures with Ratio 7 and Ratio 8, respectively, to identify the appropriate algal dosage for the mixture. Six runs were conducted and four parameters (i.e., C: N, nitrogen content, organic carbon content and organic matter) were considered. The results are shown in Figures 4.5 to 4.8.

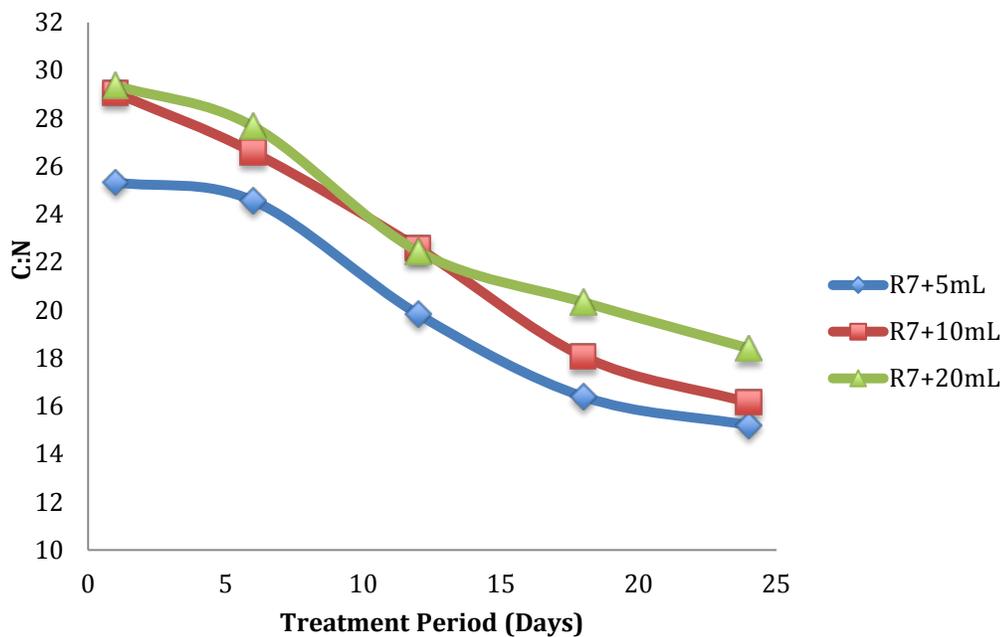


Figure 4. 5 (a) C: N versus Treatment Period for Runs 1, 2, and 3

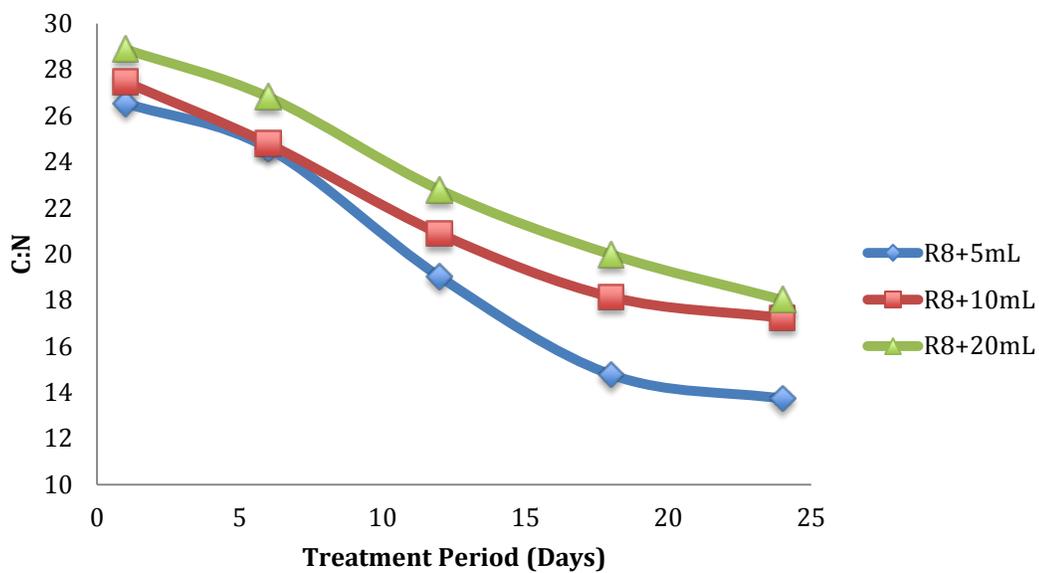


Figure 4. 6 (b) C: N versus Treatment Period for Runs 4, 5, and 6

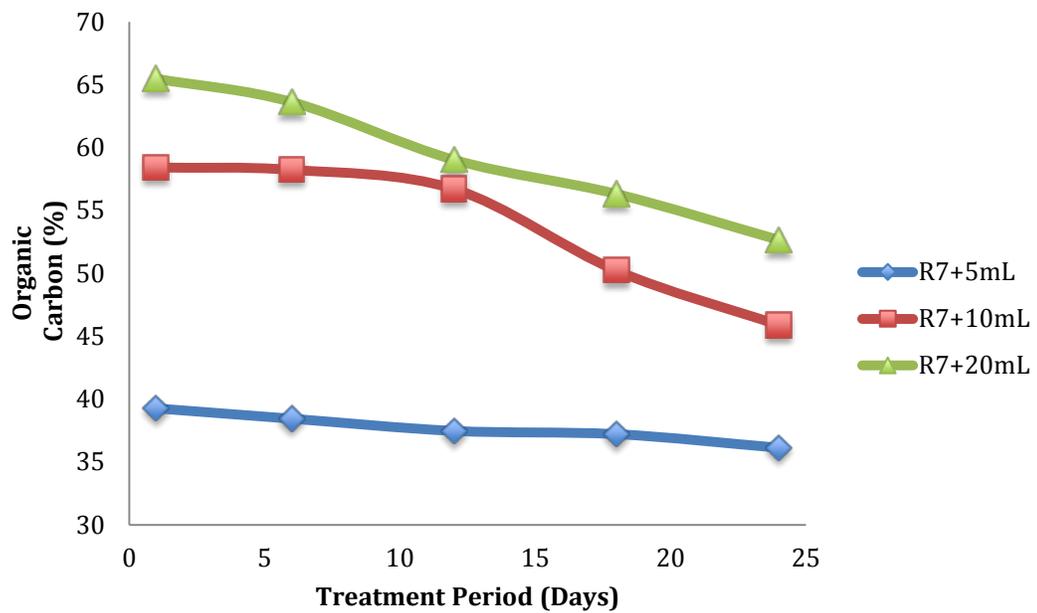


Figure 4. 7 (a) Organic Carbon Content versus Treatment Period for Runs 1, 2, and 3

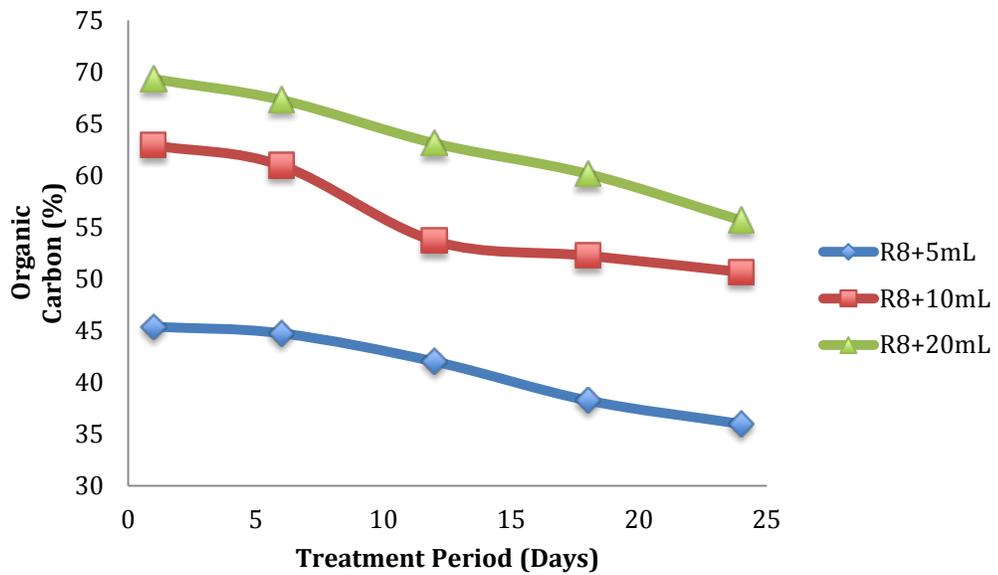


Figure 4. 8 (b) Organic Carbon Content versus Treatment Period for Runs 4, 5, and 6

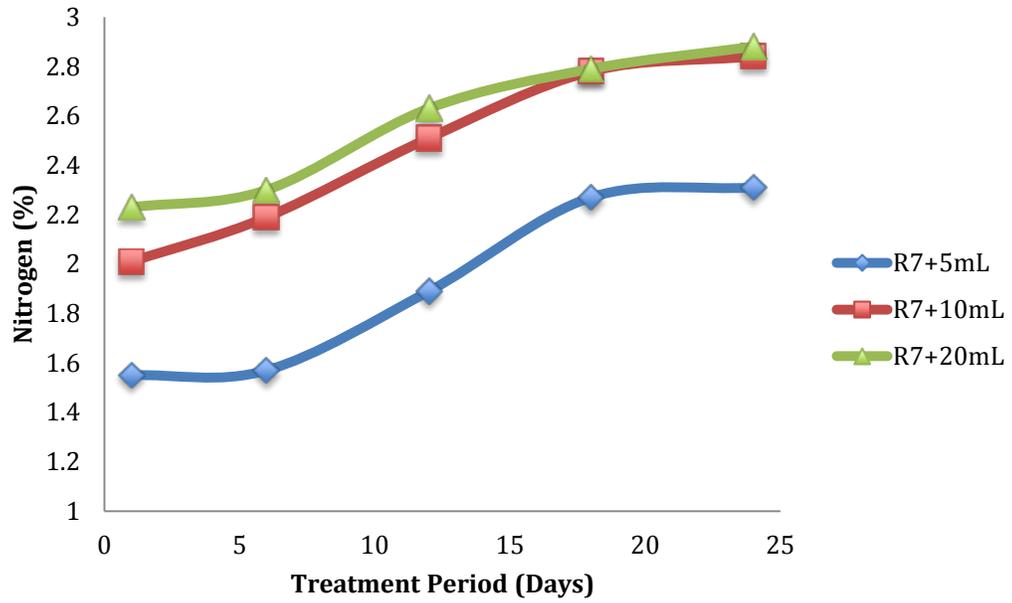


Figure 4. 9 (a) Nitrogen Content versus Treatment Period for Runs 1, 2, and 3

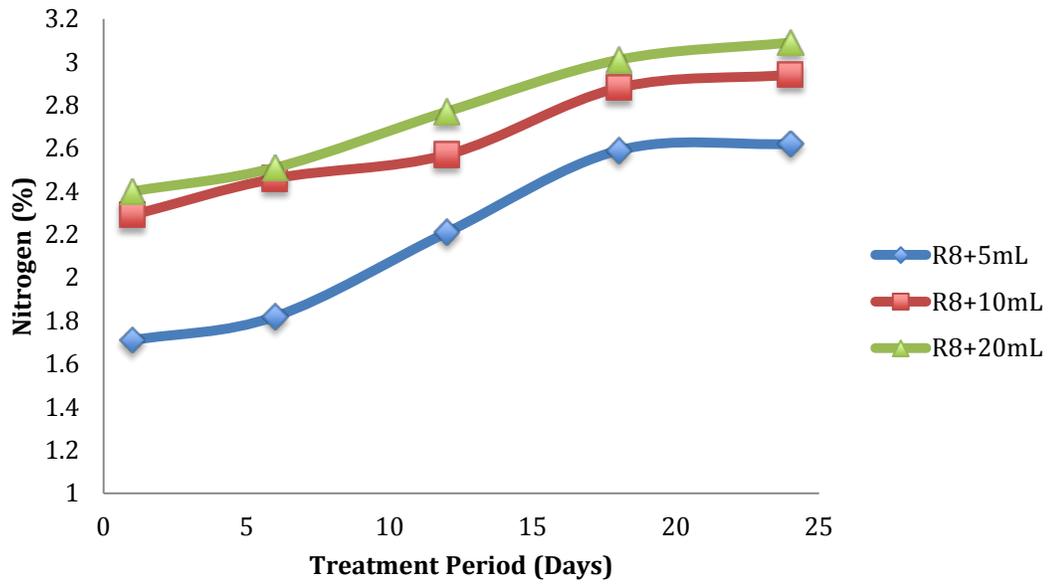


Figure 4. 10 (b) Nitrogen Content versus Treatment Period for Runs 4, 5, and 6

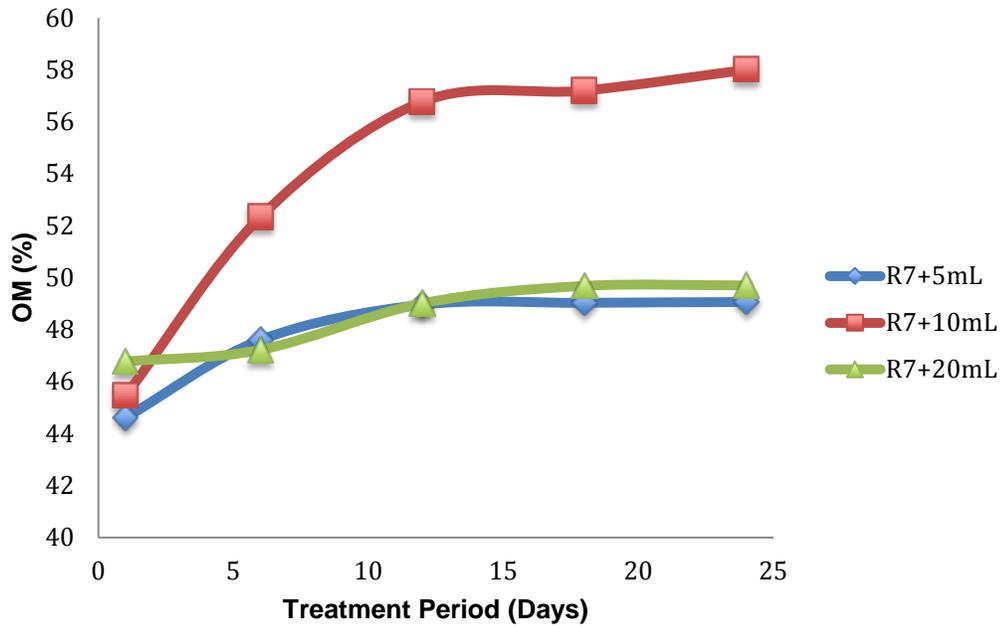


Figure 4. 11 (a) Organic Matter (OM) Content versus Treatment Period for Runs 1, 2, and 3

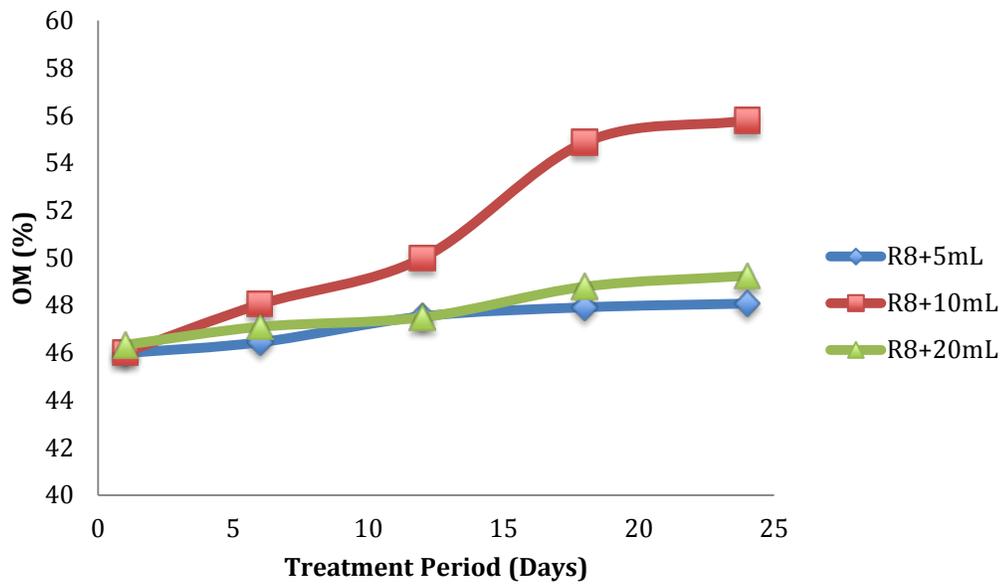


Figure 4. 12 (b) Organic Matter (OM) Content versus Treatment Period for Runs 4, 5, and 6

The potential of *Anabaena* strain 387 for generating compost products with good quality has been confirmed in previous sections. However, it doesn't mean the more algae added into the mixtures, the better compost products could be generated. The 20mL algal solution contained too much water and thus made the mixture with Ratio 7 not qualified as a compost product. Therefore, 20mL was not considered as a practical dosage.

For the mixtures treated by 5mL and 10mL algal solutions, the ones with 10mL solution had shown better qualities than those with 5 mL solution. After 24 days of algal treatment, the nitrogen content in Run 2 (with 10mL algal solution), was much higher than that in Run 1 (with 5mL algal solution). Based on the BNQ standards (2005), the final product in Run 1 was qualified as a type A compost and that in Run 2 was qualified as a type AA compost. In addition, the organic matter content of the final product in Run 2 (with 10mL algal solution) was much higher than that in Run 1 (with 5mL algal solution) and Run 3 (with 20mL algal solution).

One possible explanation of this phenomenon is the difference of the contact surface area between algae and biodegradable components among the three scenarios. In the mixtures with 20 mL algal solution, when the system was stable without turbulence, the majority of the algae existed in the upper liquid phase; while the biodegradable components were in the solid phase in the bottom. Therefore, the biodegradable components that were attached to the surface of solid particles had less chance to be contacted with algae than to be biodegraded. On the contrary, for the mixtures with 5mL and 10 mL algal solution systems, the solid phase was immersed in the liquid phase, thus the algae cells could fully access the biodegradable components inside the

solid phase and carry on the biodegradation reactions. The mixtures in Run 2 with 10mL algae solution has thus led to a better quality of final product than that in Run 1 with 5mL algae solution.

For mixtures with Ratio 8, the addition of 20mL of algal solution made the moisture content in the final product after 24 days of treatment in Run 6 over 60% (the standard value). For the mixtures treated by 5mL or 10mL algal solution, the ones with 10mL solution had led to better qualities of products than those with 5mL solution. After 24 days of the biodegradation treatment, the nitrogen content in Run 5 (with 10mL algal solution) was higher than that in Run 4 (with 5mL algal solution). Additionally, the organic matter content in Run 5 product was much higher than that in Run 4 or Run 6. The GI of Run 4, 5, and 6 products were 147.06, 152.04 and 150.17, respectively. Run 5 product (with 10mL algal solution) had shown the best quality compared with the product in Run 4 or Run 6. Based on the BNQ standards (2005), Run 4 product was classified as a type A compost and Run 5 product was classified as a type AA compost. In summary, based on the qualities of compost products generated with Ratio 7 and Ratio 8, the appropriate algal dosage added into the mixture was selected as 10mL of algae per 20g of a solid mixture.

4.5 Algae Treatment Analysis

4.5.1 Biotransformation of Carbon

Algae can obtain energy and return the carbon to the atmosphere in the form of carbon

dioxide by breaking down the biodegradable components (Alexander, 1999; Epstein, 1997; Seal 2012). According to the research conducted by Takacova (2014), algae *Ch. Kessleri* had the ability to biodegrade and remove BaP from the contaminated water and 30% of BaP was removed by the end of the biodegradation process. Warshawsky (1994) also found a freshwater green alga, *Selenastrum capricornutum*, was able to metabolize BaP to *cis*-dihydrodiols by using the dioxygenase enzyme system inside the algae. Warshawsky (1994) also mentioned that diphenol oxidase, peroxidase and cytochrome P450 were the three most important enzyme systems for detoxification of contaminants. In addition, both lysosomes and mitochondria in the algal cells were more active when the algae was exposed to the organic pollutants (Alexander, 1999; Takacova, 2014; Warshawsky, 1994).

Algae can use the organic pollutants as a carbon source and convert them into CO₂ and release the CO₂ into the atmosphere by the end of biodegradation process (Alexander, 1999; Takacova, 2014; Warshawsky, 1994). According to Takacova's research (2014), this process started with attacking aromatic rings by dioxygenase enzyme system and the *cis*-dihydrodiol was generated as a product. Then the *cis*-dihydrodiol was dehydrogenated into pyrocatechol. Pyrocatechol was the main intermediate product in this splitting process. The aromatic rings were split between hydroxyl groups or between hydroxyls. This process led to the degradation of the molecule structure and then enabled these degraded molecules to enter into the middle metabolic path inside the algae cells. At the end of this degradation process, carbon was returned back into the atmosphere in the form of CO₂ (Takacova, 2014).

The results obtained from this research supported Takacova's findings. The organic carbon content in each run is shown in Figures 4.9 (a) and (b). The organic carbon content decreased after 24 days algal treatment for both mixtures with Ratio 7 and Ratio 8. In the 24 days of the algal treatment process, *Anabaena* strain 387 used the organic carbon inside the mixtures as the carbon source for biodegradation and converted it into CO₂, which was released into the atmosphere during the treatment. The released CO₂ caused the decreasing content of organic carbon in the mixtures.

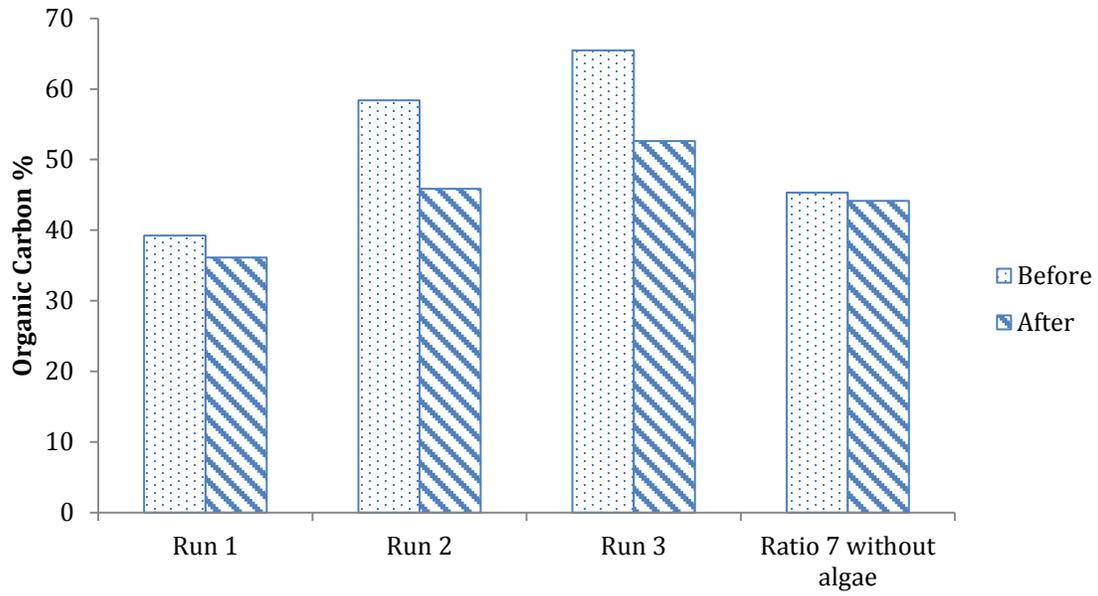


Figure 4. 13 (a) Organic Carbon Content in the mixtures with Ratio 7 Before and After Algae Treatment

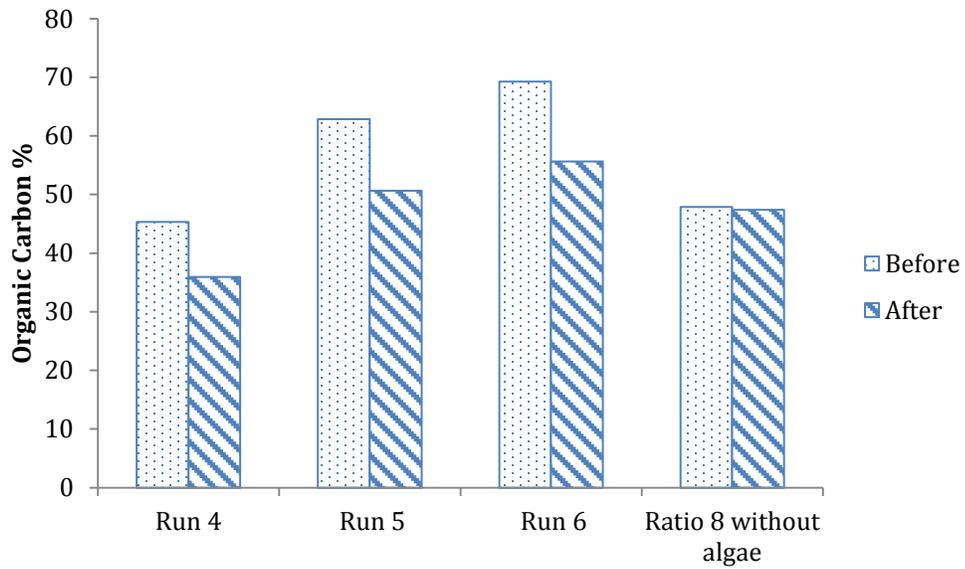


Figure 4. 14 (b) Organic Carbon Content in the mixtures with Ratio 8 Before and After Algae Treatment

4.5.2 Fixation of Nitrogen

Algae have the ability to absorb nitrogen from the atmosphere and fix it as biomass inside their cells (Sivakumar, 2012). Even though the detailed mechanism is still not clear, this phenomenon was confirmed by Sivakumar (2012). The results obtained from this research are another strong support for Sivakumar's findings.

The nitrogen content in each run is shown in Figures 4.10 (a) and (b). The nitrogen content increased significantly after 24 days of the algal treatment for both mixtures with Ratio 7 and Ratio 8. In the 24 days of the treatment process, *Anabaena* strain 387 stabilized the nitrogen from the atmosphere and fixed it as biomass inside the cells. The fixation of nitrogen thus caused the increasing content of nitrogen in the mixtures.

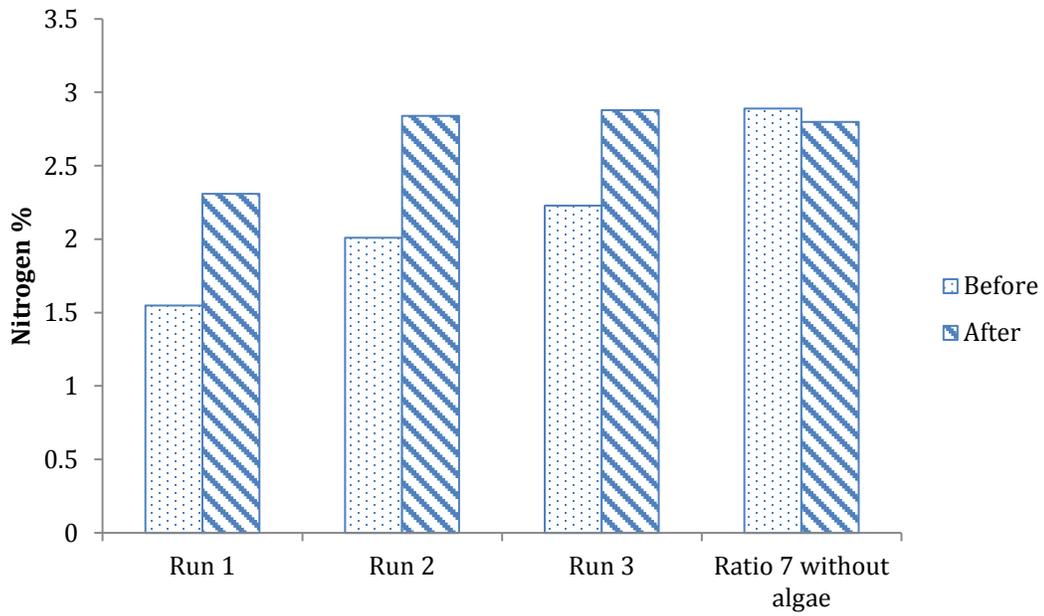


Figure 4. 15 (a) Nitrogen Content in the mixtures with Ratio 7 Before and After Algae Treatment

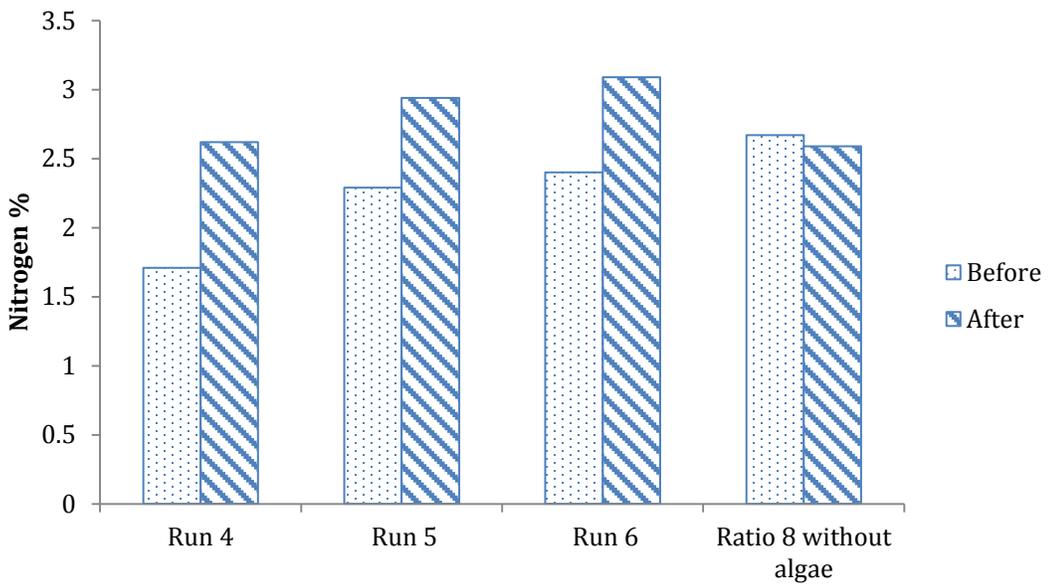


Figure 4. 16 (b) Nitrogen Content in the mixtures with Ratio 8 Before and After Algae Treatment

4.5.3 Adjustment of C: N

The previous discussion confirms that *Anabaena* strain 387 has two abilities, which can be used for the generation of compost products with good qualities. They are biotransformation of carbon and fixation of nitrogen. The two abilities could help with adjusting the C: N of the compost products during the algae treatment. Releasing CO₂ into the atmosphere led to the decreasing content of organic carbon in the compost and fixing nitrogen from atmosphere caused the increasing content of nitrogen in the compost. As a result, the C: N of the compost was decreased after 24 days of algal treatment. This result is reflected in Figures 4.11 (a) and (b).

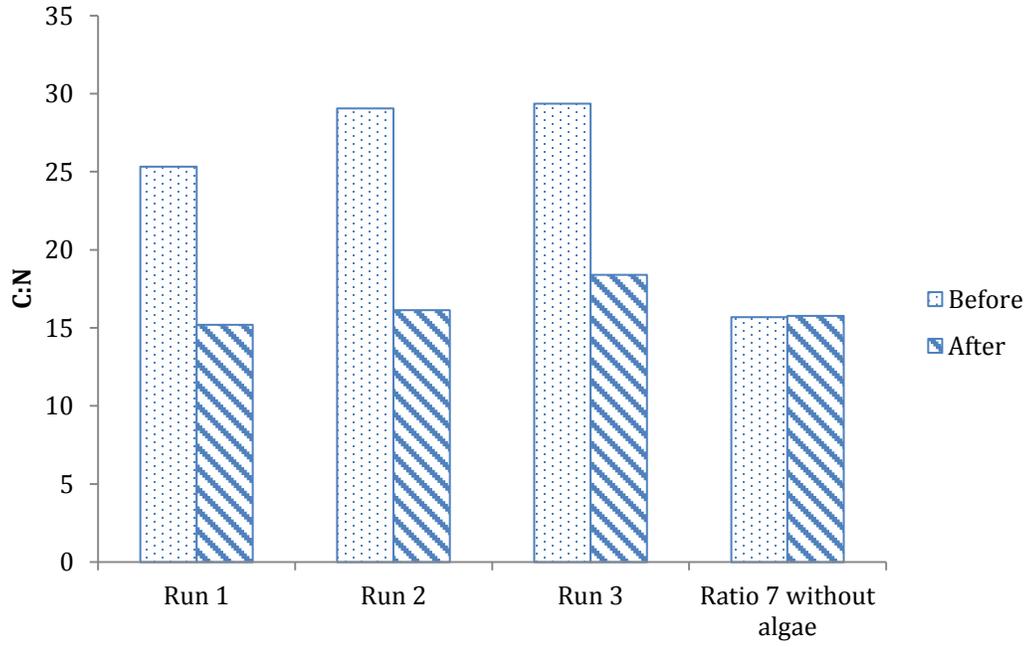


Figure 4. 17 (a) C: N of the mixtures with Ratio 7 Before and After Algae Treatment

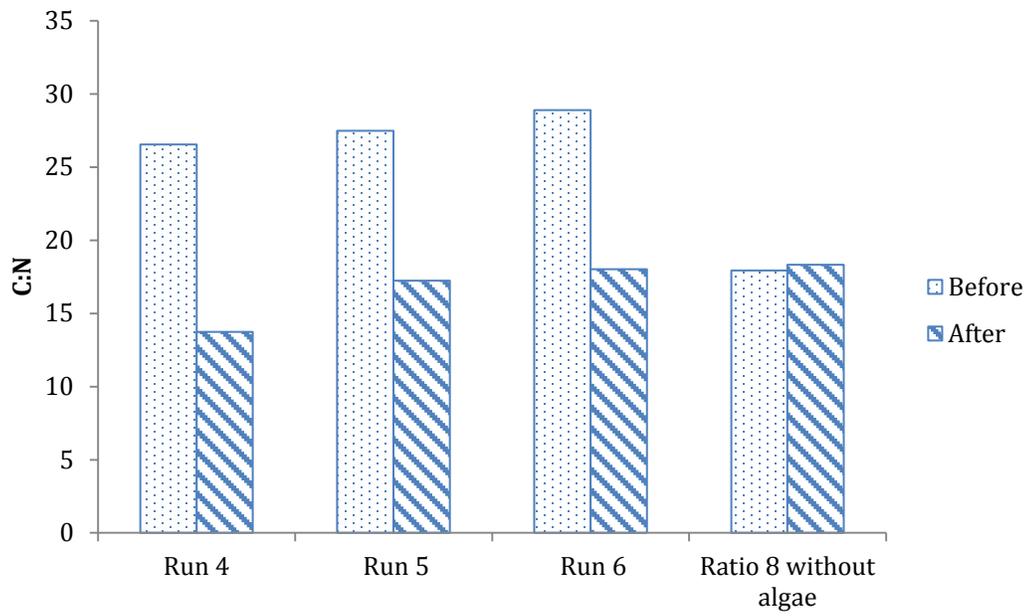


Figure 4. 18 (b) C: N of the mixtures with Ratio 8 Before and After Algae Treatment

4.6 Classification of Compost Products

After mixing three raw materials (i.e., fly ash, sludge and the residues of fish waste compost after extraction) in eight ratios, three mixtures before algae treatment, which were those with Ratios 3, 5, and 6, were not qualified to be compost products. Another three mixtures were qualified as type B level compost products, which were the mixtures with Ratios 1, 2 and 4. Mixtures with Ratio 7 and Ratio 8 were qualified as type A level of compost, and were selected for further algal treatment. Three dosages (i.e., 5mL, 10mL and 20mL) of algal solution were considered respectively. The algae treatment period was determined as 18 days. After 18 days of algal treatment process, two type AA level compost products and two type A level compost products were generated. Two mixtures were not qualified to be compost products after algae treatment. They were Run 3 and Run 6 products. The detailed classification of compost products generated from the 6 runs after the algae treatment is summarized in Table 4.24.

Table 4. 23 Classification of Compost Products

Classification	Index	Compost Generated after Algae Treatment
Type AA Level Composts	OM > 50 % GI > 90 % C: N < 25 Moisture < 60 %	Run 2 product
		Run 5 product
Type A Level Composts	OM > 40 % GI > 90 % C: N < 25 Moisture < 60 %	Run 1 product
		Run 4 product
Not Qualified Composts	Moisture > 60 %	Run 3 product
		Run 6 product

CHAPTER 5 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

In this research, three raw materials (i.e., the residues of fish waste compost after extraction, fly ash and sludge) were selected as the candidates for generating compost products. Each raw material was not qualified to be the compost on its own. However, they all had their special characteristics and potentials leading to a compost product after being mixed together in a certain ratio. Based on the characterizations of mixtures with eight mixing ratios, the ones with Ratios 3, 5 and 6 were not qualified to be compost products because of the high concentrations of certain heavy metals. The mixtures with Ratios 1, 2 and 4 were qualified directly as type B level compost products and mixtures with Ratio 7 and Ratio 8 were qualified as type A level compost products. Algal biodegradation treatment was applied to generate products with better qualities. A treatment period of 18 days was selected based on the monitoring results of four parameters during the process. Three dosages (i.e., 5mL, 10mL, and 20mL) of algal solution were added, respectively, into the mixtures with Ratio 7 and Ratio 8 for algal biodegradation. After the 18 days of the algal treatment process, two type A level compost products with better qualities than those of the original mixtures were generated in Runs 1 and 4, respectively. Another two type AA level compost products were generated in Runs 2 and 5, respectively. The compost product with best quality was generated from 20g mixtures with 10mL algae solution after the algae treatment in Run 2 or 5.

The results confirmed that *Anabaena* strain 387 had the ability to improve the quality of compost products through biodegradation. *Anabaena* strain 387 can bio-transform the organic carbon and fix nitrogen from the atmosphere in the compost, and thus decrease the C: N of the compost products.

5.2 Research Significance

An innovative method for the generation of different levels of compost products from solid wastes was developed in this research. The research contributions include:

- 1) Solid wastes (i.e., fly ash, sludge, and the residues of fish waste compost after extraction) were transferred into valuable compost products, which led to an environmentally friendly way of waste management and recycling, and could directly benefit industries, government, and communities in NL and beyond;
- 2) Algae was involved to generate compost products with a higher nitrogen content and more organic matters through biodegradation, which provided an example of transferring an environmental problem (algae bloom) to a valuable product (compost) using a biotechnology (biodegradation); and
- 3) Multiple levels of compost products were generated in a cost-effective way. Type B level or Type A level composts could be used for gardening and horticulture purposes, while Type AA level composts could be applied for agriculture activities.

5.3 Recommendations for Future Work

Despite the advancement and outputs of this research, there is still considerable work to be done for a better control of algae involved biodegradation processes. Recommendations for future research are stated in below.

This research was conducted at the room temperature (20°C) under a slightly basic condition (pH was 7-8). Factors, such as temperature and pH, could affect the biochemical reactions among algae and the mixtures. Therefore, multiple reaction conditions of temperature and pH need to be considered for achieving a better process control.

In addition, numerical modeling tools, such as COMSOL, can be applied to obtain a better understanding of how the experimental conditions (e.g., pH, pressure, solution volume and temperature) affect the results. Soft computing methods, such as Artificial Neural Networks (ANN) Black Box and Design and Analysis of Experiments (DOE) can be used for factor effect analysis. The system optimization can help generate better compost products with less energy inputs and shorter treatment periods.

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