SOLID WASTE COMPOSTING

AND THE APPLICATION OF COMPOST

FOR BIOSURFACTANT PRODUCTION

by

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ABSTRACT

In Canada, about 9 million tonnes of residential waste with over 40% of organic waste was disposed every year. Another major source of organic waste in Canada is from the seafood processing industry. For effective organic waste management, composting serves as a sound, cost-efficient and environmental friendly measure.

The selection of bulking agents is of primary importance to adjust the moisture and carbon/nitrogen (C/N) ratio of organic waste during composting. Therefore, initially, the performance of locally available bulking agents (i.e., sawdust and peat in Newfoundland and Labrador (NL)) during organic municipal solid waste (MSW) composting was evaluated. Results indicated that to generate a high temperature and a longer duration of high temperature to kill pathogens and sterilize the compost, peat was considerably more effective.

A design of experiment (DOE) based methodology was then adopted to investigate the effects of multiple factors including C/N ratio, moisture content (MC), type of bulking agent (BA) and aeration rate (AR) and their interactions on the maturity, stability and toxicity of compost product. For the first time, enzyme activities were used as indices of maturity and stability during the course of a DOE based composting. The results provided guidance to optimize a MSW composting system that will lead to increased decomposition rate and the production of more stable and mature compost.

Thirdly, the feasibility of using enzyme activities for indicating the state of marine fish waste composting was also examined. A good correlation among enzyme activities and

different physiochemical parameters including oxygen uptake rate (OUR), C/N ratio, and germination index (GI) led to the conclusion that enzyme activities could be feasible indicators of the state and evolution of the composting process.

Raw materials contribute about 30% of the biosurfactant production cost. Evaluation of the feasibility of using fish waste compost (FWC) extract as an unconventional substrate for biosurfactant production was highly desirable to refine the utilization of FWC and achieve the economical biosurfactant production. In this study, the nutrient extraction from FWC was achieved by enzyme hydrolysis and optimized using response surface methodology (RSM). The extract was used to produce biosurfactants by Rhodococcus erythropolis sp. P6-4P and *bacillus* sp. N3-1P strains. FWC extract showed a good potential as an unconventional source of nutrient for microbial growth. The obtained biosurfactants showed excellent properties with high surface tension reduction, high emulsification activity, and exhibited a high level of stability.

The research outputs can contribute to the technical and scientific knowledge to design and operate composting system to manage the organic MSW and fish waste by achieving a double benefit of waste reduction while producing marketable products. Additionally, the products and the bioprocess can be of great value to both scientific understanding and industrial applications.

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LIST OF SYMBOLS AND ABBREVIATIONS

ANN	artificial neural network
ANOVA	analysis of variance
AR	aeration rate
BA	bulking agent
BGH	β-glucosidase activity
BOD	biological oxygen demand
BVS	biodegradable volatile solids
C/N	carbon/ nitrogen
CCD	central composite design
CMC	critical micelle concentration
CMD	critical micelle dilution
COD	chemical oxegn demand
DDW	double distilled water
DGH	dehydrogenase activity
DH	degree of hydrolysis
DM	dry matter
DOC	dissolved organic carbon
DOE	design of experiment
DW	dry weight
E/S	enzyme-substrate ratio
EC	electrical conductivity
EI_{24}	emulsification index

FA	fulvic acid
FP	food waste + peat
FS	food waste + sawdust
FTIR	fourier transform infrared spectroscopy
FWC	fish waste compost
FWCC	fish waste compost ectract as carbon source
FWCN	fish waste compost extract as nitrogen source
GA	genetic algorithm
GC-MS	gas chromatography coupled with mass spectroscopy
GC–FID	gas chromatography-flame ionization detector
GI	germination index
GP	grape pomace
HA	humic acid
HS-GC	head-space gas chromatography
MC	moisture content
MSW	municipal solid waste
NL	Newfoundland and Labrador
NRPOP	Northern Region Persistent Pollution Control
OD	optical density
OFAT	one-factor-at-a-time
OM	organic matter
OP	olive pomace
OUR	oxygen uptake rate
PAWS	passively aerated windrow system

PDE	phosphodiesterase activity
ROM	resistant organic matter
RSM	response surface methodology
SN	soluble nitrogen
SOUR	specific oxygen uptake rate
ST	surface tension
TCA	trichloroacetic acid
TCH	total carbohydrate
TLC	thin layer chromatography
TOC	total organic carbon
TOM	total organic matter
TW	tobacco waste
TTC	5-triphenyl-tetrazolium chloride
VFAs	volatile fatty acids
VOCs	volatile organic compounds
VS	volatile solids
WEOC	water extractable organic carbon
WSC	water soluble carbon
WSN	water soluble nitrogen

CHAPTER 1

INTRODUCTION

1.1 Background

Canada is one of the largest nonhazardous municipal solid waste (MSW) producers in the world (Bruce et al. 2016). The large amount of MSW has led increasing environmental, social and economic problems (Adhikari et al. 2008; Asase et al. 2009). For example, in 2008, daily MSW generation rate in Canada was about 1.2 kg/capita (Bruce et al. 2016). After Alberta and Saskatchewan, in Canada, Newfoundland and Labrador (NL) has the highest quantity of waste disposal per person, and the lowest proportion of waste (48%) from non-residential sources, i.e., 429 kg of residential waste per capita and 382 kg of non-residential waste per capita as it is shown in Table 1.1 (NL 2002).

The increase rate of Canadian MSW generation is alarming. From 1996 to 2010, the per capita generation rate increased by more than 26% (Bruce et al. 2016). NL, Nova Scotia and Saskatchewan had the highest increase in total waste disposed from 2008 to 2010, at 4% each (Statistics Canada 2013). The most common final disposal option utilized in Canada is landfill, where waste is buried in the ground (or sometimes above ground, especially in areas with bedrock) (SWMC 2014). Approximately 97% of the residual MSW waste after diversion (recycling and composting), and recovery (energy-from waste) is landfilled (or about 24,111,546 tonnes per year) (Statistics Canada 2013). The two primarily environmental concerns related to landfills are leachate generation and gas emission (Kjeldsen et al. 2002; Spokas et al. 2006). Stringent environmental regulations for waste disposal and landfills make finding new sites for waste disposal and management a growing challenge (Adhikari et al. 2008; Asase et al. 2009). In addition, disposal sites produce noise, dust and odour which make the surrounding area

undesirable for habitation (Ponsá 2010). Successful waste policy requires effective strategies for proper waste diversion from landfills. Minimizing waste generation and recycling have become the focus of governmental agencies in many countries to reduce human impact on the environment. Biodegradable material such as food waste constitutes approximately 40% of the residential waste stream. The environmental benefits of diverting organic materials from landfill include reduced methane emissions and decreased leachate quantities from landfills and production of renewable energy (David and Canada 2013). With this focus, composting has received a high ranking in the hierarchy of recycling methods and continues to gain importance throughout the world for the conversion of organic MSW to new resources and products (Keener 2010).

Another source of organic waste generation in NL is from seafood processing industry. In 2011, total world fishery production amounted to approximately 91.3 million tonnes form capturing and 158 million tonnes from aquaculture (FAO 2012). In Canada, commercial marine and freshwater landings yielded 832,767 metric tonnes valued at $2.2 billion and aquaculture production was 174,057 metric tonnes, valued at $825 million (FOC 2012). The Atlantic Region accounted for 703,905 metric tonnes (82.76%) for total landing from sea fisheries with a value of $1,828,714 (Ghaly et al. 2013). NL, with 17,450 Km of coastline and with widespread fish processing plants in its coast, currently has one of the most valuable commercial fishing industries in Canada and fish industry is considered as an important economic pillar in the province. The portion of NL from fish production in Canada amounted 256,093 tonnes in 2012 (DFA 2012). Amount of fish waste produced in Atlantic Provinces in 2001 is presented in Table 1.2 (Ghaly AE 2013).

It is estimated that 43% of total fish and shellfish ends up as products for human consumption and the remainder is classed as waste (Ghaly AE 2013). Recent estimates revealed that current discards from the world's fisheries exceed 20 million tons, equivalent to 25% of the total production of marine capture fisheries (Arvanitoyannis 2010). The majority of waste is produced in the on-shore processing sector (35% of the resource) whereas discards and processing waste at sea produce smaller quantities (17% and 5% of the resource respectively) (Brinton 1994; Jayasinghe and Hawboldt 2012; Seafish 2001). For example, amount of waste produced from white fish processing is 27-32% as is shown in Table 1.3 (Arvanitoyannis and Kassaveti 2008). If the fishery waste is not properly be treated, it represents a lost resource and a potential pollution problem (Illera-Vives et al. 2015).

Table 1.1 Residential and non-residential waste disposed in NL

	Residential sources	Non-Residential sources	All sources
Canada	9,350,354	16,557,113	25,907,467
NL	200,918	179,257	380,176

Table 1.2 Fish waste amount by province in 2001

Province	Landing		Product		Waste	
	Tonnes	(%)	Tonnes	(%)	Tonnes	(%)
New Brunswick	113588	13.95	89012	78.36	24576	21.36
Newfoundland and Labrador	267959	32.92	120999	45.15	146960	54.84
Nova Scotia	366381	45.01	146708	40.04	219673	59.95
Prince Edward Island	66046	8.11	39000	59.04	27046	40.95
Total	813974	100	395719	48.61	418255	51.38

Table 1.3 Inputs and outputs of fish production processing

Process	Input	Output			
	Fish (kg)	Wastewater(m^3)	BOD (kg)	COD(kg)	Solid waste (kg)
White fish filleting	1000	5-11	35	50	Skin:40-50 Heads:210-250 Bones: 240-340
De-icing and washing	1000		1	0.7-4.9	0-20
Grinding	1000	0.3-0.4	-	0.4-1.7	0-20
Deheading	1000	1	-	2-4	Head and debris:270-320
Filleting of deaheded fish	1000	1-3	-	4-12	Frames and off cuts:200-300
Skinning	1000	0.2-0.6	-	1.7-5	Skin: 40

1.2 Statement of Problems

1) Insufficient composting system evaluation and optimization

The high organic fraction in MSW makes it easy to be converted to the energy sources through composting (Jolanun and Towprayoon 2010; Ponsá 2010). Therefore, composting has become an increasingly important strategy for the treatment of organic MSW and investments in composting provide opportunities to significantly increase diversion of waste. Composting of food and yard waste has seen a 125% increase in diversion Canada-wide from 2000-2010 (SWMC 2014). It is an inexpensive, simple and environmental-friendly alternative for the treatment of organic MSW (Jolanun and Towprayoon 2010). It is also a useful method to produce a stabilized material from organic MSW that can be used as a source of nutrients and soil conditioner in fields and can improve the physical and chemical properties of amended soils (Brown et al. 1998; Castaldi et al. 2008). However, a composting program was not developed in NL (Table 1.4).

Previously, many studies investigated the physiochemical changes during composting of MSW (Ahn et al. 2008; Canet and Pomares 1995; Castaldi et al. 2008; Chang and Chen 2010; Ciavatta et al. 1993; Eklind and Kirchmann 2000; Garcia et al. 1993; Iqbal et al. 2010; Jolanun and Towprayoon 2010; Kayikçioğlu and Okur 2011; Mote and Griffis 1979; Strom 1985; Xiao et al. 2009). In addition, many studies have been conducted to evaluate the influence of different factors such as temperature (Suler and Finstein 1977), moisture (Suler and Finstein 1977), aeration rate, and bulking agents (Adhikari et al. 2008; Chang and Chen 2010; Eklind and Kirchmann 2000) on composting of MSW.

The formula required to successfully compost organic MSW depends significantly on the selection of bulking agent. There are currently limited studies on the effect of locally available bulking agents (i.e., peat and sawdust) on the maturity and stability indices such as enzyme activities and germination index (GI) in NL. In addition, evaluation and optimization of the MSW composting to increase the decomposition rate and to produce more stable and mature product is still confronted with many challenges. Most of the studies just focused on the effect of one factor on the composting process, with no comprehensive consideration of the interaction among the factors during composting. The function of enzyme activity during composting was not well illustrated. In addition, DOE methodology was not well applied for system optimization of composting.

2) Lack of effective fish waste management technologies

The common methods for disposing fishery waste is wherever possible, directly used for land applications, sent to fishmeal processing plants, to landfill in the absence of suitable facilities and disposed at sea in remote parts where waste cannot feasibly be sent for reprocessing or landfill (AMEC 2003). Direct use of fishery wastes for land manuring, or land spreading, is deterred by the uniquely obnoxious odours of putrefying fish (Mathur S. I. 1988). Since many processors are no longer allowed to discard their offal, it leads to high cost of refining the material before it is discarded (Environment Canada 2005). Environmental concerns and regulations have made it costly to dispose fishery wastes by landfilling due to the nuisance of malodours and scavengers, and the delayed pollution it causes. The slow rate of decomposition of fishery waste in ocean results in the subsequent increase in biological oxygen demand (BOD), release of dissolved

phosphorus and dissolved nitrogen, and the formation of black zone (Arvanitoyannis and Kassaveti 2008; Schaub and Leonard 1996). It can reduce the phytoplankton growth and increase alga growth, attract marine birds and lead to local increases in their populations (Environment Canada 2005; IECS 2005).

Table 1.4 Composting programs in Canada in 2008

Province	Composting Program	Population Served	Total Population
British Columbia	28	2,471,982	3,907,738
Alberta	9	1,005,619	2,974,807
Saskatchewan	2	18,400	978,807
Manitoba	3	82,400	1,119,583
Ontario	57	10,003,304	11,410,046
Quebec	12	2,561,630	7,237,479
New Brunswick	2	138,180	729,498
Nova Scotia	20	750,534	908,007
Prince Edward Island	1	135,294	135,294
Newfoundland	0	0	512,930
Total	134	17,167,343	29,914,315

An important waste reduction strategy for the fishery industry is the recovery of marketable by-products from fishery wastes by treating the waste (Arvanitoyannis and Kassaveti 2008). The goals of solid treatment systems in fishery production are volume reduction (e.g. thickening and dewatering) as well as stabilization. Stabilization of solid waste reduces pathogens (both human and animal) and eliminates offensive odors and the potential for putrefaction (Seymour et al. 2001). Additionally, the stabilization of this types of materials prior to its use can prevent problems associated with the appearance of phytotoxic substances and to diminish their water contents and transportation costs (Adler and Sikora 2004).

Composting is a simple and inexpensive method to achieve the volume reduction, stabilization and valuable soil conditioner production from organic waste like fishery waste (Laos et al. 1998). Composting is a sustainable option, and if done properly can potentially reduce pressure on already overburdened landfills (Miller and Semmens 2002; Seymour et al. 2001). It is one of the lowest cost approaches to nutrient stabilisation, which is the aerobic decomposition of organic material by successive microbial communities (Cole et al. 2015). Also, fishery waste compost is of great potential use in agriculture. Several studies have evaluated the fertilizer effects of composts and have suggested composting as one of the most appropriate techniques for producing organic fertilizers (Shen et al. 2011).

Various enzymes have shown the potential for controlling the biodegradation rate during composting. Enzyme activities have been widely used to evaluate the performance of composting for sewage sludge (Benitez et al. 1999; Vargas-Garcia et al. 2010), MSW

(Castaldi et al. 2008; Raut et al. 2008) and animal manure (Godden et al. 1983; Tiquia 2002), but they have been never applied to the marine fish waste composting process for evaluating its state and evolution.

3) Lack of promising options for fish waste compost usage

Compost made from fish manure and mortalities, or processing waste could provide an effective source of nutrient-rich organic matter. Therefore, composting from organic materials can be used to create a useful and potentially marketable product (Benhabiles et al. 2012).

Biosurfactants are biologically produced surfactants. They are less toxic, more effective and stable at extreme pH, temperature and salinity, and better at enhancing biodegradation are promising substitutes for surfactants with significant toxic effects and persistency in the environment (Muthusamy et al. 2008). Currently, their main application is for enhancement of oil recovery and hydrocarbon bioremediation. The use of biosurfactants has also been proposed for various industrial applications, such as in food additives, cosmetics, detergent formulations (Reis et al. 2013). The global biosurfactants market has grown incrementally. According to a new market report, global biosurfactant market was USD 1,735.5 million in 2011 and is expected to reach USD 2,210.5 million in 2018, with a production of 476,512.2 tonnes by then (Kosaric and Sukan 2014). However, existing production of biosurfactants has suffered from low yields and high cost. The raw materials contribute 30% of the total production cost, so that the utilization of waste streams such as agro based industrial wastes as substrate can help develop economically viable biosurfactants and could be a promising strategy for the

industry to increase its profitability and competitiveness (Mukherjee et al. 2006; Mulligan and Gibbs 1993). As a cheap substrate, fish waste compost (FWC) extract has not been used to produce biosurfactants.

1.3 Research Objectives

The goal of this research, therefore, is to fill knowledge and technical gaps identified above through in-depth investigation of MSW and fish waste composting and utilization of FWC for biosurfactant production. The major research tasks include:1) to design a composting system to achieve a successful composting treatment; 2) to investigate the performance of locally available bulking agents in NL during bench-scale MSW composting; 3) to conduct a design of experiment (DOE) based optimization of the operation parameters of MSW composting using enzyme activities as responses; 4) to evaluate the state and evolution of marine fish waste composting by enzyme activities; 5) to utilize FWC extract as low-cost substrate for biosrufactant production using 2 bacteria isolated from Atlantic Canada.

1.4 Structure of the Thesis

Chapter 2 presents comprehensive literature review including MSW composting, bulking agents for composting, composting system optimization, fish waste composting, system characterization and parameter evaluation, and use of fish waste compost for biosurfactant production. Chapter 3 tackles task 2 and describes methods of experiments and presents the result of the experiments to evaluate the performance of locally available

bulking agents in NL during bench-scale MSW compost and displays the results of monitoring of physiochemical parameters such as temperature, pH, electrical conductivity (EC), GI, OUR and C/N ratio and assessing of enzyme activities during MSW composting with 2 bulking agents. Chapter 4 tackles task 3 and presented a DOE method which has been used to screen the significant factors and their interactions on MSW composting. The importance of each model parameter is evaluated through analysis of variance (ANOVA) by using final C/N ratio, final moisture content, and cumulative enzyme activities as responses. The optimum condition was proposed based on the developed model. Chapter 5 tackles task 4 and describes experimental methods for marine fish waste composting and monitoring of physicochemical parameters and enzyme activities. The data of monitoring parameters and enzyme activities during fish waste composting are shown in the chapter. Chapter 6 and chapter 7 tackle task 5 which describes experimental methods to extract nutrients from fish waste compost through enzyme hydrolysis. The optimization of the enzyme hydrolysis process parameters is presented using response surface methodology (RSM). The extracted nutrient under optimum condition was used as substrate for *bacillus* (N3-1P) strain to produce biosrufactant. The production condition is optimized to increase the efficiency of the process. FWC extract as a novel substrate is also used to produce biosurfactnat by *Rhodococcus* (P6-4P) strain and the production condition using RSM is optimized. Chapter 8 concludes this study with summarized contribution and recommendations for future research.

CHAPTER 2

LITERATURE REVIEW[1]

[1] *This chapter is based on and expanded from the following paper:*

Kazemi, K., Zhang, B.Y., and Lye, L., (2016). Composting of fishery waste: a review (Ready for submission).

Role: Khoshrooz kazemi solely worked on this study and acted as the first author of this manuscript under the guidance of two supervisors, Dr. Baiyu Zhang and Dr. Leonard Lye. Most contents of this paper was written by her and further edited by the other co-authors.

2.1 Composting

Composting is a biological process in which easily degradable organic matter is stabilized and converted by the action of microorganisms into a humus-rich product (Eiland et al. 2001). During composting, compounds such as protein, cellulose, and hemicellulose are utilized by microorganisms as carbon and nitrogen sources. The residual plant organic matter, along with compounds of microbial origin, is transformed by microorganisms to form humic-like substances of increasing complexity (Mondini et al., 2004). Tchobanoglous et al. (1994) suggested the following diagram to describe the composting process.

The objectives of composting are 1) diverting organic matter from landfills and reducing the pressure on landfills, leachate content of and odour potential of landfills; 2) converting organic matter to stabilized forms; 3) decreasing the odour potential of the organic matter; 4) decreasing the moisture content of municipal and industrial sludge; 5) reducing the subsequent cost of transportation; and 5) producing a soil amendment to increase the soil fertility, raise the quality of crops, and improve plant resistance to disease (Haug 1993; Zorpas et al. 2000).

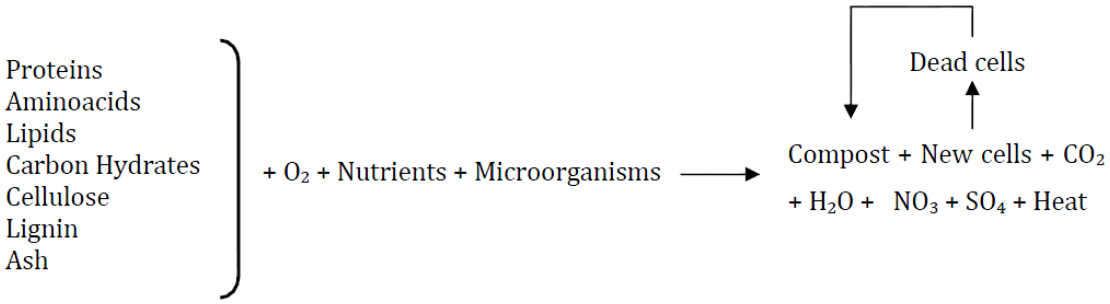


Figure 2.1 Diagram of the composting process

Composting can be divided into four stages which include pre-processing, high-rate phase, curing phase, and post processing. Depending on the raw material (feedstock) and the required quality of final products, pre- and post-processing may be required. The pre-processing includes removing unwanted material and reducing size, adjusting moisture content, adding bulking agents, and mixing feed components to provide the optimum composting conditions. In the high- rate phase, microorganisms reduce biodegradable volatile solids and decompose complex organic matter into the simple organic matter. The high-rate phase proceeds in two steps and each step is characterized by a different set of microorganisms. In the first step, mesophilic microorganisms consume carbon sources and temperature rises to 45 °C. The degradation will then increase the system temperature to 70 °C in the second step and the thermophilic microorganisms start to dominate. The high temperature in the thermophilic phase is important to inactivate pathogens and plant seeds. After the high-rate phase, due to the decreasing of microbial activities, the temperature drops under 40°C so that the curing phase starts and stabilization and maturation of organic matter take part. The final products of a composting treatment will be H_2O, CO_2, and stabilized matter (Figure 2.2 and Figure 2.3) (Haug 1993; van der Wurff et al. 2016).

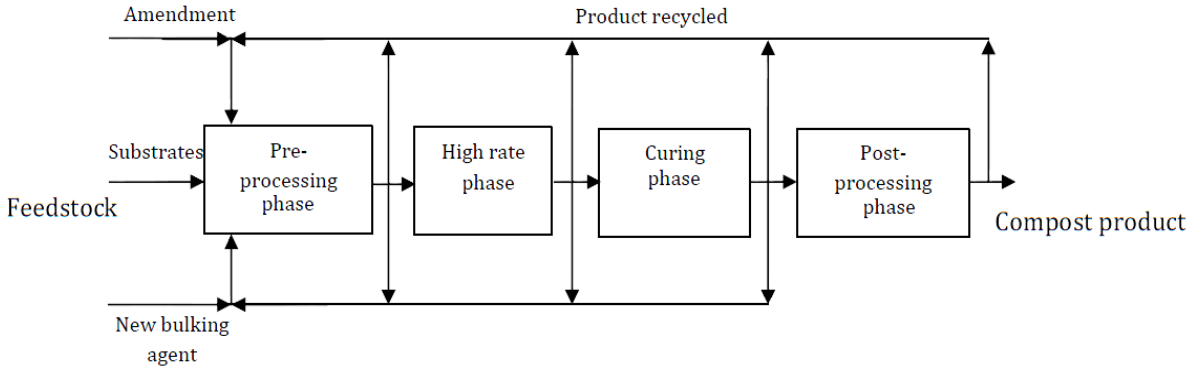


Figure 2.2 Generalized process diagram for composting (Haug 1993)

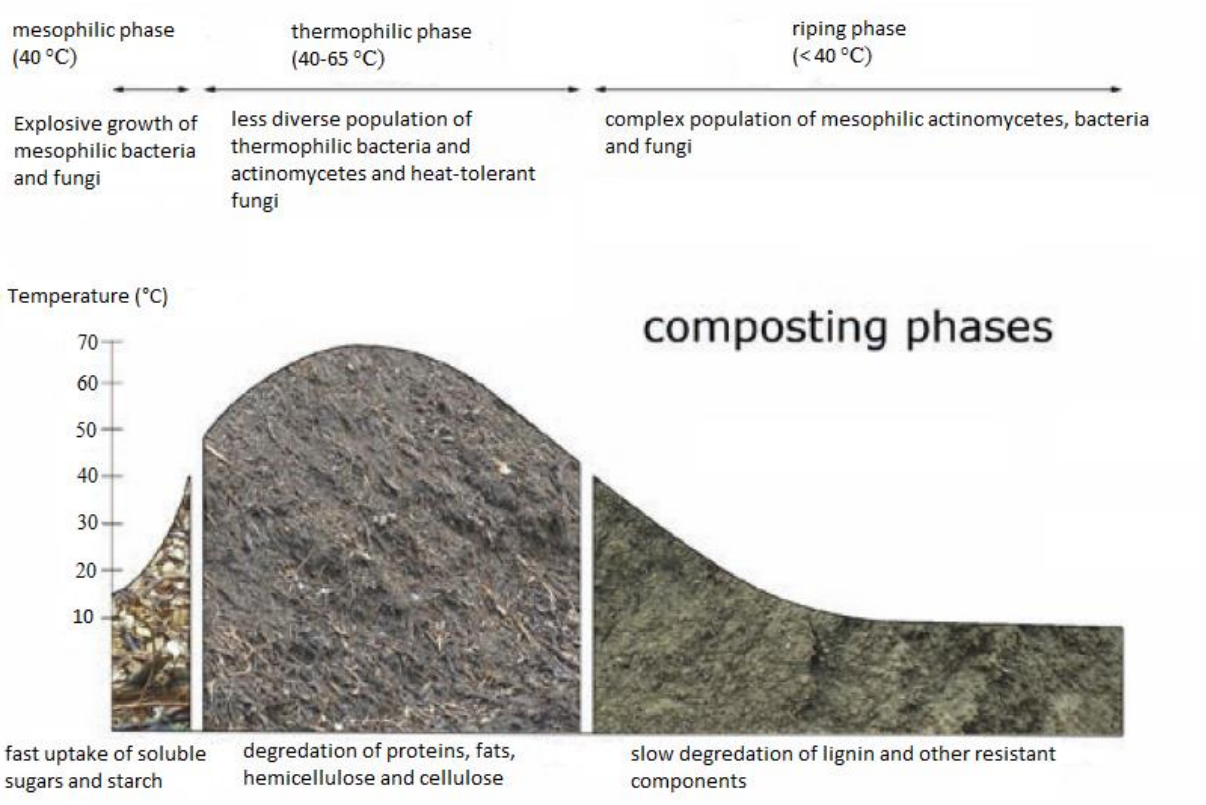


Figure 2.3 General overview of three composting phases and the degradation processes taking place (van der Wurff et al. 2016)

Composting first received attention because it is an inexpensive, simple and environmental friendly process (Magalhaes et al. 1993). It reduces the mass, bulk volume, and water content of organic matter and it returns nutrients to the soil (Arslan et al. 2011; Cronje et al. 2003). In addition, pathogens become inactivated due to the thermophilic stage (Cronje et al., 2003). Physiochemical, microbiological and thermodynamic phenomena and their interaction are involved in the composting process, making the composting very complicated (Petiot and De Guardia 2004). Decomposition of organic matter produces heat. The energy and mass transfer are indicated by temperature, moisture content, and oxygen concentration. To produce a high quality end product from composting, water content, oxygen, and the composition and quantity of raw material play important roles (Magalhaes et al. 1993). Oxygen deficiency increases odour production because it creates anaerobic situation and reduces the growth of aerobic microorganism; however, excessive aeration can increase costs and slows down the composting process via heat, water, and ammonia losses (Guo et al. 2012). High moisture content enhances the anaerobic condition and produces more leachate. On the other hand, low moisture content decreases the microbial activity (VanderGheynst et al. 1997). Due to these concerns, more studies are needed to understand the interactions between the process degradation kinetics and the mechanisms of heat and mass transport as well as the process optimization (Petiot and De Guardia 2004; VanderGheynst et al. 1997). In addition, further studies will allow to reduce the time, energy and cost of the process, and produce a pathogen free, stable, and mature product (Mason and Milke 2005).

2.2 Characteristics of MSW as Raw Material for Composting

The term MSW describes the stream of solid waste generated by households, commercial establishments, industries and institutions (Farrell and Jones 2009). MSW is made up of different organic and inorganic fractions like food, vegetables, paper, wood, plastics, glass, metal and other inert materials. In cities, it is collected by the municipalities and transported to designated disposal sites (Mor et al. 2006). The production and composition of MSW vary from place to place and from season to season. Which is influenced by various factors such as geographical location, population's standard of living, energy source, weather, food habits, urbanization, tradition and culture (Adhikari 2005). The characterization of MSW before composting is of primary importance to balance the recipe in terms of moisture content for aeration, pH for a proper microbial environment, and carbon and nitrogen for proper microbial development (Adhikari et al. 2008).

Fathi et al. (2014) conducted a research to characterize MSW for composting in Zanjan city, Iran. Amount of biodegradable materials in MSW of Zanjan is 75.2 percent, which comes to 225.6 tons per day. C/N ratio of Zanjan ranges from 14.22 to 19.53 and the average is 17.6. Relative Humidity interval in MSW of Zanjan city is 67.94 to 70.3% with a mean of 69.2%. Most organic materials in MSW of Zanjan are in range of 8-40 mm (Fathi et al. 2014). Adhikari et al. (2008) characterize food waste in downtown Montreal a prerequisite for compost recipes from May to August. The C/N ratio was found to decrease from 29.1 in May to 23.1, 18.4 and 17.9 in June, July and August, respectively. Similarly, the food waste pH was found to be the highest in May (4.6 ±

0.25) and to drop in June, July and August. The food waste produced in May had a dry matter (DM) of 13.7% (±2.47%), in June, July and August, the DM dropped to 12.2% (±2.05%), 10.0% (±1.01%) and 10.3% (±0.83%), respectively. In May, the wet bulk density was 269 kg m^{-3} (±84) while it increased to 410 kg m^{-3} (±92), 510 kg m^{-3} (±72) and 552 kg m^{-3} (±80), in June, July and August, respectively.

The percentage of organic waste in MSW characterization in Pakistan has not shown any significant difference in summer (71.79) and winter (72.45) season, where as its average share was 72.12% of the total average waste. The organic waste contains average C (30.2) in summer and (11.06) in winter on dry weight basis, whereas the N (dry weight base) was (1.2) in summer and (0.57) in winter. pH of winter MSW (4.91) was significantly lower than that of summer (5.25) (Iqbal et al. 2010). Table 2.1 summarize the characteristic of MSW reported in previous studies.

Table 2.1 Physical and chemical properties of MSW used for composting

	MSW	Food waste	Food waste	Food waste	Synthesised food waste	MSW	Organic waste (winter)	MSW	Organic fraction of MSW	MSW	Kitchen waste	MSW	Kitchen waste
Moisture content (%)	69.21		70-80	80	80.5	-	-	-	58.0	36	66.9	57.3	65-80
Organic matter (%)	61.38		-	-		690.6 g/kg	-	36	62.9	45	-		-
Ash content (%)	-		-	-	1		79.81	-			-		3-5
pH	5.45	4.1	3.8-6.5	4.4		5.95	4.91	6.5	6.9	7.8	5.75	6.4	
EC	-			2.5 (ds/m)		8.29 ds/m	-	-	3 (ms/cm)		-		
C	44.05	2	47.35	53	44.5		11.06	17.84	34	32	40	39.9	50-52
N	2.61	47.4	5.35	2.2	3.3	21.29 g/kg	0.57	1.84	2	0.05	11.4	2.28	
C/N	17.66	24	8.85	25	13.3	17.1	19.19	9.7	17	40	-	17.7	13-18
	Zanjan, Iran	Montreal, canada	Hsinchu City, Taiwan	Republic of Korea	Kaohsiung, Taiwan	Co´rdoba's (Spain)	Lahore, Punjab, Pakistan	Spain	Barcelona, Spain	Jabalpur, India	Beijing, China	Spain	Kaohsiung, Taiwan
References	(Fathi et al. 2014)	(Adhikari et al. 2009)	(Kumar et al. 2010)	(Kim et al. 2008)	(Chang and Chen 2010)	(Delgado-Rodríguez et al. 2012)	(Iqbal et al. 2010)	(Mato et al. 1994)	(Barrena et al. 2008)	(Gautam et al. 2010)	(Yang et al. 2013)	(Tejada et al. 2009)	(Chang and Chen 2010)

2.3 Characteristics of Fishery Waste as Raw Material for Composting

The composition of the fishery waste varies according to the type of species, sex, age, nutritional status, time of year and health. Most of the fish contains 15-30% protein, 0-25% fat and 50-80% moisture (Ghaly AE 2013). For example, Alaska pollock contains 25% protein in skins, 15.2% in heads, in comparison, pink salmon heads contain 13.9% protein (Tarnai 2009). Frederick (1989) study showed that the fish waste contained 58% crude protein, 22% ash, 19% ether extract, 1% crude fiber, 22% monosaturated acids, palmitic acid and oleic acid. Compared with other waste streams, fishery waste contains a large amount of readily digestible protein and, thus, has a high content of nitrogen and a low C/N ratio. These characteristics of fishery waste may result in a special composting process and compost features that are different from other waste materials as well (Frederick 1989). The physical and chemical characteristics of fishery waste are stabilised by composting are summarized in Table 2.2.

Table 2.2 Physical and chemical properties of fishery waste used for composting

Parameter	Fish waste	Fish waste	Dogfish Gurry	Filleted fish scrap	Crab scrap	Lobester scrap	Fish waste	Shrimp waste	Clam wastes	Herring	Flounder	Fish waste	Scallop viscera	Crab Scrap	mackerel, sardine, tuna, squid waste
Moisture content (%)	44.34 ±1.58		73.0	50	35	45	-	65%	56.5 ± 1.2	70	73	53.7	80	65	69.75 ± 9.01
Organic matter (%)			-	65.5	56.3	-	-		800.0 ± 17.2 g/kg DM				81.65	55.47	
Crude protein (%)							57.92± 5.26							-	
Fat (%)							19.10± 6.06							-	
Crude fiber (%)							1.19± 1.21							-	
Ash (%)							21.79± 3.52		200.0 ± 17.2 g/kg DM					-	
pH	5.70± 0.02		-	-	-			6-7	6.21 ± 0.15	6.7	6.7	-		7.65	5.89 ± 0.48
EC (ds/m)	2.37± 0.07		-	-	-	-			26.5 ± 2.4 mS/cm			-	1.5	6.2	4.81 ± 3.19
C (%)	45.98 ±0.06	39.2	-	32.7	27.7	-			432.0 ± 9.5 g/kg DM			45.2	46.8	-	46.22 ± 2.80
N (%)	9.39± 0.06	1.5	5.49	8.2	8.2	4.6			102.1 ± 3.5 g/kg DM	13.4	14.2	5.0	14.2	8.84	10.17 ± 2.29
C/N	4.90± 0.03		-	3.9	3.3	-		6.59	4.2	3.3	3	9.0	3.3	3.6	4.79 ± 1.24
P (%)	2.05±	0.3	0.92	6.1	6.1	3.5	2.04 ±		3.96 ±			19136		2.25	1.80 ± 0.90

Parameter	Fish waste	Fish waste	Dogfish Gurry	Filleted fish scrap	Crab scrap	Lobester scrap	Fish waste	Shrimp waste	Clam wastes	Herring	Flounder	Fish waste	Scallop viscera	Crab Scrap	mackerel, sardine, tuna, squid waste
	0.03						0.64		0.11 g/kg DM			mg/kg DM			
K (%)	1.25± 0.02	0.2	0.53	0.37	0.32	0.5	0.68± 0.11		0.48 ± 0.04 g/kg DM			3565 mg/kg DM		0.35	0.79 ± 0.46
Ca (%)	1.63± 0.01	1.1	1.40	10.19	14.91	-	5.80 ± 1.35					465 mg/kg DM		14.2	1.86 ± 1.85
Mg (%)	0.06± 0.01	0.2	0.14	0.27	0.9	-	0.17± 0.04					538 mg/kg DM		-	0.15 ± 0.04
Na (%)	1.79± 0.05		0.66	<0.1	<0.1		0.61± 0.08			0.9	0.6	4569 mg/kg DM			0.64 ± 0.16
Cd	0.08± 0.03 mg /kg	0.7 mg /kg	<1 µg/g	ND	ND	-	-					-			
Cr (mg /kg)	<0.06 ±0.00 mg /kg	22 mg /kg	-	-	-	-	-					-			
Pb	0.33± 0.29 mg /kg	mg /kg	2 µg/g	8.7 µg/g	2.8 µg/g	-	-					-			
Ni	2.78± 0.2 mg /kg	6 mg /kg	<0.1 µg/g	3.8 µg/g	3.4 µg/g	-	-					-			
Reference	(Illera-Vives et al. 2013)	(Shelton et al. 1998)	(Mathur S. I. 1988)	(Mathur et al. 1986)	(Mathur S. I. 1988)	(Mathur S. I. 1988)	(Dubois et al. 1956)	(Bicca et al. 1999)	(Hu et al. 2009)	(Brinton and Seekins 1988)	(Brinton and Seekins 1988)	(Tarnai 2009)	(Brinton and Gregory 1992)	(Brinton and Gregory 1992)	(López-Mosquera et al. 2011)

2.4 Composting Technologies

Thermophilic aerobic composting of MSW on a commercial scale uses systems of varying complexity, of which can be classified based on their aeration methods: turned or forced aeration systems. Turned systems are commonly based upon the windrow system, which entails the feedstocks being piled in elongated heaps up to 2 m high and 50 m in length. In contrast to turned systems, actively aerated systems are often more complex with computer controlled aeration regimes, and generally offer greater control over the process conditions. Having greater process control is often desirable with highly heterogeneous wastes such as MSW as this aids the operator in adapting the process to suit the chemical and physical makeup of the feedstock (Farrell and Jones 2009).

Figure 2.4 shows the main systems of centralized composting, that is, aerated static-pile system, enclosed system and the windrow system. In aerated static-pile system, piles of organic waste are formed, which are sometimes covered with screened compost to reduce odours and to maintain a high temperature inside the pile. Aeration is provided through blowers and air diffusers. The enclosed system is either a silo type or an agitated bed type. The enclosed container ensures control of temperature, oxygen concentration, and odours. Windrow is simply a pile of waste material subjected to decomposition. In cross section, the shape of a windrow varies from rectangular to trapezoidal to triangular, depending largely on characteristics of the composting material and equipment used for turning. Aeration of the composting mass is achieved by frequent turning (Kumar 2011). Table 2.3 summarized esign parameters of MSW composting systems.

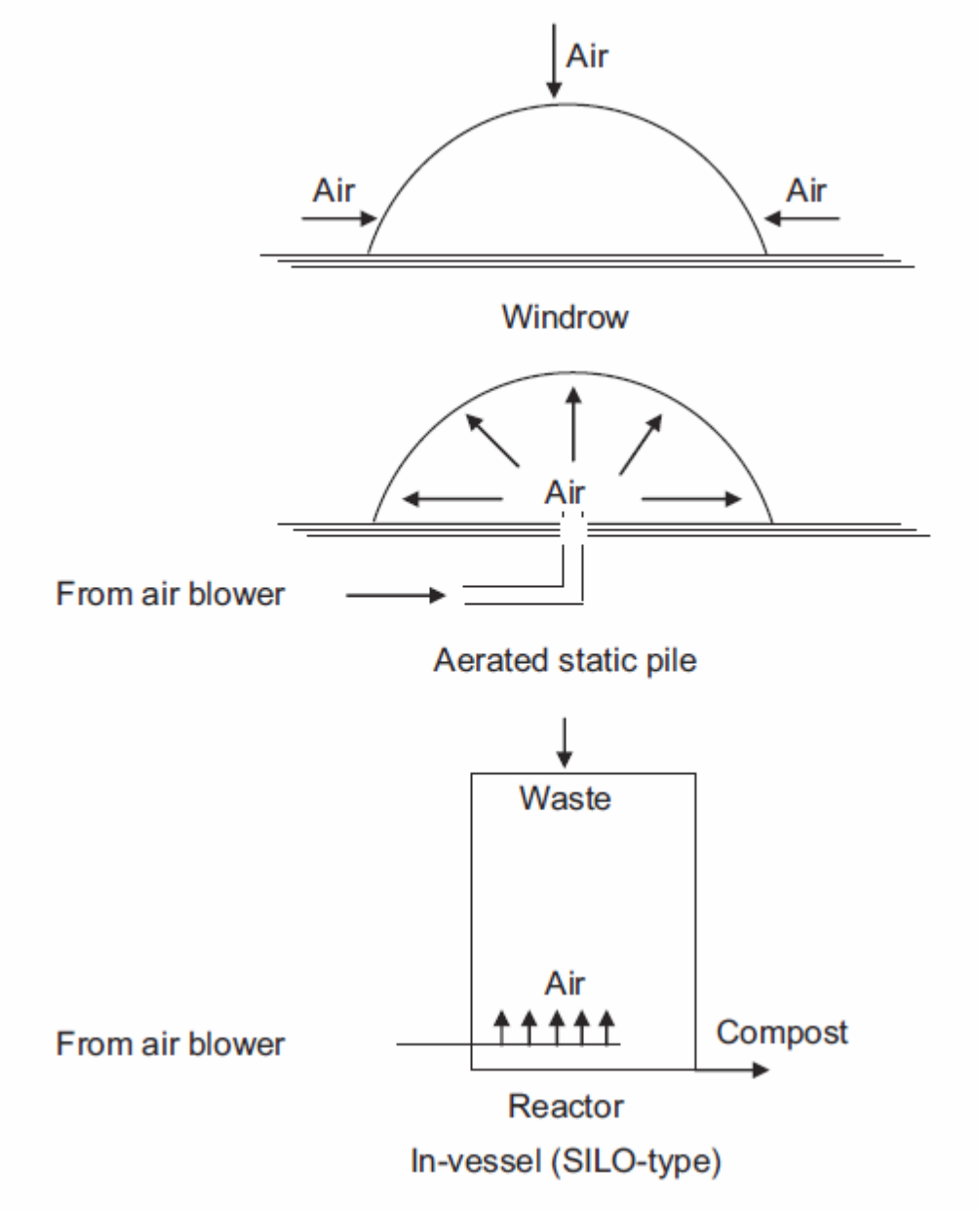


Figure 2.4 Typical composting systems(Kumar 2011)

Table 2.3 Design parameters of MSW composting systems

System	Waste	Source	Size	Scale	Operating for aeration	Bulkign agent/Mixing ratio	Moisture content adjustment	Ref
Windrow	MSW	Jabalpur city	4' high, 8'long	Full scale	Turned manually every 3-5 days	Vegetable, fruit and kitchen waste	Sprinkling water to maintain moisture of 40-60% for 6 weeks	(Gautam et al. 2010)
In-Vessel	Food waste	Hsinchu City Taiwan	120-L stainless steel cylindrical reactor (60 cm length and 50 cm diameter)	Lab-scale	Air pump at a rate of 10 L/min	Food and green wastes	-	(Kumar et al. 2010)
Windrow	MSW	Spain	3x3x1.25 m pile	Full scale	Centrifugal blower	<100 mm fraction of MSW	-	(Mato et al. 1994)
Static piles	Municipal organic waste (MOW)	Patagonia Argentina	Four static piles (8.5 m^3)	Full scale	Turning at 30, 50, 70, and 130 days	Shredded MOW, unshredded MOW, shredded MOW + woodshaving (1:1v/v) and unshredded MOW+ woodshaving (1:1v/v)	Adding water at 50 days	(Tognetti et al. 2007)
In-Vessel	Food waste	Chuncheon, Korea	Compost bay with 45m length, 6m width, and 1.2m depth (a total volume of 324m3)	Pilot scale	Forced aeration at a rate of 0.15m^3/m^3 min	Continuous horizontal flows (food waste/ wood chips 3:4 w/w)	-	(Kim et al. 2008)
Windrow	MSW	Canary island, Spain	Heaps with 3 m in length, 2 m in width and 0.75 m in height.	Full scale	Six to eight turnings for 6-8 weeks	810 kg waste	Adding water to maintain moisture of 40-60%	(Iglesias Jiménez and Perez Garcia 1989)
Windrow	MSW	Carpi, Italy	8 m^3 trapezoidal pile	Full scale	Aerated (turned	MSW and plant	Adding water to	(Castal

System	Waste	Source	Size	Scale	Operating for aeration	Bulkign agent/Mixing ratio	Moisture content adjustment	Ref
					and mixed every 3 days) for 30 days and static 70 days	waste 1:1 v/v	maintain moisture of 40–50%	di et al. 2008)

In order to compost fishery waste, different systems and sizes of reactors have been reported in the literature. Composting was conducted in the full-scale, pilot scale and bench scale reactors for different purposes. The composting methods used by the fish processing industry include passively aerated static piles, actively aerated static piles, turned windrows and in-vessel systems. Selection of the most appropriate method will depend on the nature of the waste, the location of the site (e.g. proximity to urban or rural areas) and the capital and operating funds available (Schaub and Leonard 1996). The windrow and aerated static-pile methods are the most appropriate for on farm fish composting.The windrow method requires turning periodically, while the aerated static-pile method does not require turning. With this method, aeration is generally achieved by piling the mixed raw materials onto a base containing perforated pipes (Liao et al. 1994). Forcing air and/or turning of composts aerates waste and retains the whole mass at a high temperature for a long period. Turned composts or force-aerated static pile composts without a colder envelope therefore tend to lose ammonia that causes odour problems and decreases the fertilizer value of the product (Hayes et al. 1994). Although the composting process works well with windrows in remote locations, composting of seafood wastes can create odor problems for unsympathetic neighbors. Optimal process control of in-vessel systems can control and perhaps accelerate the composting process. Other advantages of in-vessel composting compared to open windrow composting are odor control and prevention of vermin and vector problems (Schaub and Leonard 1996). Actively aerated static pile method was reported to minimize the losses of ammonia while in-vessel systems have the advantage of being immune to climate restrictions. Such systems result

in material composting more quickly and efficiently, and allow increased rates of decomposition. A rough qualitative comparison of these methods is shown in Figure 2.5. In general, higher technology systems require higher capital investment but result in better control of the process and higher waste processing rates. Higher waste processing rates generally result in less area being required for the total system (Schaub and Leonard 1996).

The windrow composting system generally composed of three layers as it is shown in Figure 2.6, the base layer made of bulking agent to drain the composting system and intercept the leachate, composting layer composed of fish waste and bulking agent to generate the high temperature and sustain the aerobic microbial process and the cover layer composed of bulking agent or recycled compost to intercept the ammonia and odor, retard heat loss and retard the fly and animal entry into composting layer (Frederick 1989).

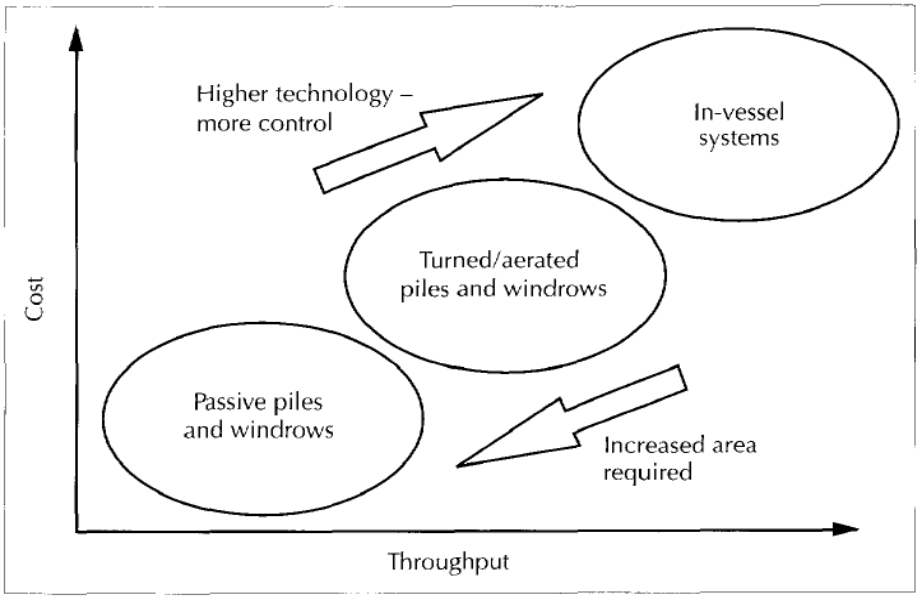


Figure 2.5 Schematic comparison of composting methods (Schaub and Leonard 1996)

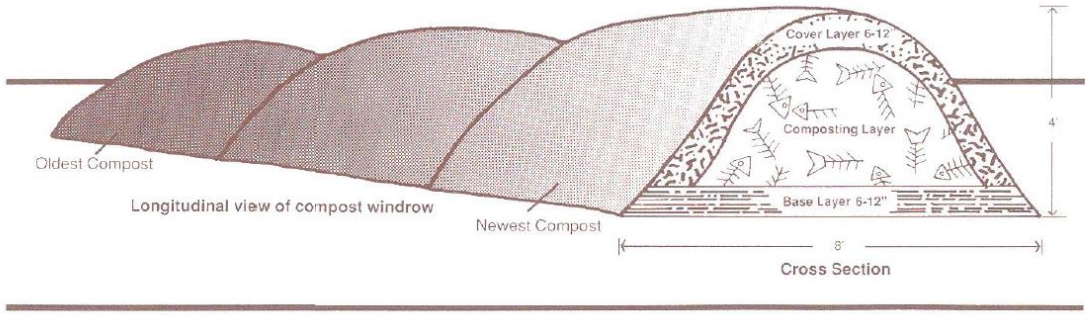


Figure 2.6 Fish waste composting process (Frederick 1989)

Vizcarra et al. (1993) developed a modified version of the static pile method specifically for the composting of whole fish mortalities (Figure 2.7). This two-stage, layered static pile method is particularly suitable for any compostable materials which do not readily lend themselves to mixing. Whole fish and bulking agent were arranged in alternate layers within a reactor located on a platform over an air chamber. This method is more economical, since it entails less labor and equipment. The basic facilities are portable and require minimal space, making the method especially appropriate for small and medium sized fish farming for their composting purposes. With this method, composting could even be done on floating offshore structures where there would be little danger of it giving offence. Evaluating the effect of the height of the modified static pile on both the process and on the quality of the compost produced suggested that the heavier and thicker layers of fishery waste must have slowed down decomposition and also the taller the pile, the longer it takes for the temperature to rise to thermophilic levels and the longer it takes for the temperature to cool down to ambient temperatures. However, after 4 months pile heights had no significant effect on the quality and maturity of the fishery compost (Vizcarra et al. 1993). Liao et al. (1994) studied layered static pile method and their results indicated that thinner layers of fishery waste can improve the temperature profiles and higher mixing ratio (3:1) with thinner layers allows more fishery waste to be composted per unit area.

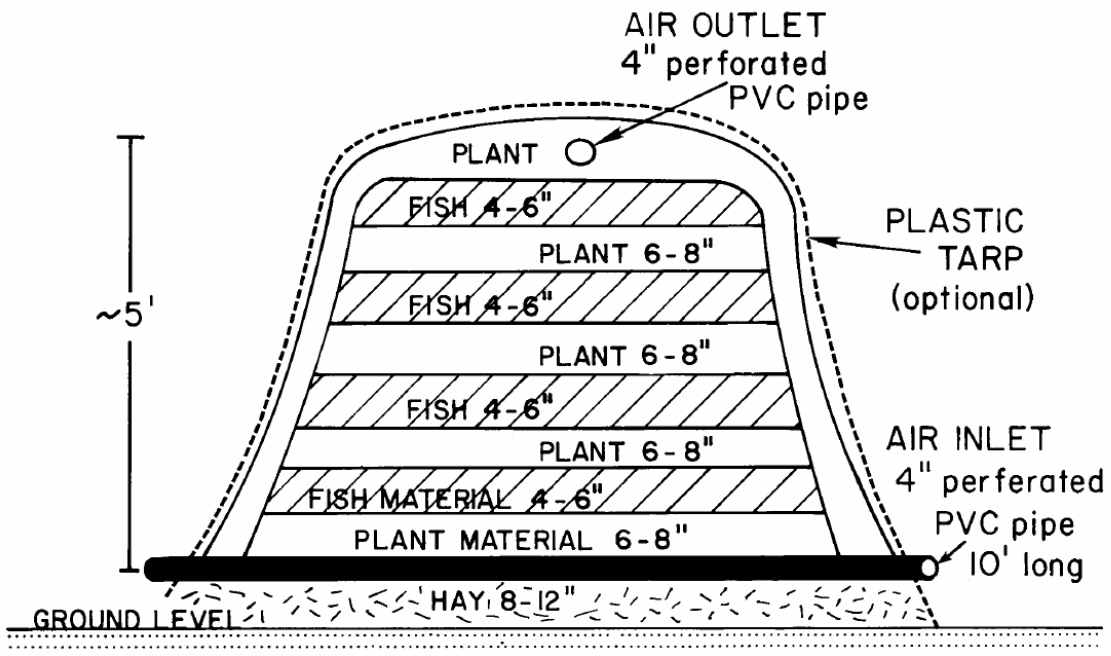


Figure 2.7 Cross section of layered static pile (Vizcarra et al. 1993)

Passively aerated windrow system (PAWS) is a system was developed for fishery waste composting which has two essential features, passive aeration and envelopment. PAWS eliminates needs for turning by placing open-ended air intake pipes at the base, with holes only on the top side so that the heat generated in the compost mass itself energizes the movement of fresh oxygen-rich air into the mix and by enveloping the decomposing mass in already sanitary (hygienic, weed seed and pathogen-free) peat or mature compost. The PAWS technology has been proven to be effective for composting wastes from seafood processing (Hayes et al. 1994). A summary of composting technologies have been used for fishery waste is presented in Table 2.4.

Table 2.4 Design parameters of fishery waste composting systems

System	Waste	Source	Size	Scale	Operating for aeration	Bulkign agent/Mixing ratio	Moisture content adjustment	Ref
In-vessel (self heating)	Clam waste	Norfolk, Virginia, USA	20.5 cm 15.5 cm 17.0 cm (length width height), insulation wall (7–8 cm thickness)	Bench-scale	passive aeration	Clam wastes and woodchips1:0.5, 1:1, 1:1.5, 1:2, and 1:3 (w/w)	Sprinkling water	(Hu et al. 2009)
In-vessel (self heating)	Clam waste	Norfolk, Virginia, USA	drums (75 cm height with 40 cm inner diameter) wrapped with glass-wool (8 cm thickness)	Pilot scale	natural aeration/ manually shaken 2–3 min every day	Clam waste to woodchips 1:1 (w/w)	Maintained moisture at 55–65%.	(Hu et al. 2009)
Windrow	Fish gurry	Waldoboro, Maine, USA	30 m3- 178 m3) 5 piles)	Full scale	Turning (keep O2 level over 5%)/ first 3 week (twice per day) after 3 week (once per day)	Sawdust : horse litter: fish gurry (20-90m3) : (7.5-30m3) : (2.8-19 m3) (keep fish waste at 13-24%)	Natural precipitation (rainfall approximately 1 month)	(Brinton and Seekins 1988)
Windrow	Fish waste	Coast of Lugo (NW Spain,	Conical piles (6×2×1.5 m)	Full scale	Open air	Seaweed, fish waste, and pine bark at a volumetric ratio of 1:1:3	Piles were constructed on an impermeable base and covered with geotextile fabric (TopTex®) to avoid moisture loss	(Illera-Vives et al. 2013)

System	Waste	Source	Size	Scale	Operating for aeration	Bulkign agent/Mixing ratio	Moisture content adjustment	Ref
Windrow	Rrock fish head, halibut head, some salmon fish head, and some fish bones	Homer, Alaska	14 feet (4.3 m) long, 12 feet (3.7 m) wide and about 8 feet (2.4 m) in height.	Full scale	Weekly turning	12 totes of fish waste (363 to 454 kg/tote) and 50 cubic yards (38.5 m3) of sphagnum peat moss	-	(Tarnai 2009)
Windrow	Fish waste generated by salmonid fishery	New York's great lake	4 feet high, 5 feet wide and 16 feet long	Full scale	Aeration through perforated pipes	Base of gravel covered with wood chip, mixture of fish waste and peat moss and final layer of peat moss 3000-5000 lb fish waste	Add water to maintain moisture 40-60 percent	(White et al. 1989)
In-vessel	Crab scalp	Maryland's blue crab processing plants, USA	0.2m^3 chamber, covered with polyethylene and wrapped in 9 cm of fiberglass	Bench scale	Controlled aeration based on the signal form timer/ thermometer	Crab scrap, straw ,water and ferrous sulfate to control pH at different ratio	Adding water to the raw material	(Cathcart et al. 1983)
In-vessel	Fish waste, chicken manure and Queen crab shells	Trinity and Foxtrap, Newfoundland, Canada	Bin was 2.5 m long and 1.25 m high and wide, cm plastic foam insulation	Pilot scale	Natural vertical convective flow of air through the bin	25% fish offal, 75% sawdust 25% fish offal, 37-5% sawdust, 37.5% peat 25% fish offal, 75% peat 20% chicken manure, 10% crab waste, 70%	-	(Martin et al. 1993)

System	Waste	Source	Size	Scale	Operating for aeration	Bulkign agent/Mixing ratio	Moisture content adjustment	Ref
						sawdust 20% chicken manure, 10% crab waste, 35% sawdust, 35% peat 20% chicken manure, 10% crab waste, 70% peat		
Layered static pile	Fish mortalities	Vancouver, Canada	Square cross-section of 0.9 m x 0.9 m	Full scale	6 h daily at a rate of 0.2 1/min for every kg of volatile matter.	300 kg of fish morts and 100 kg of sawdust in a 3: 1 ratio by weight.	-	(Vizcarra et al. 1993)
Static pile	Salmon and dogfish waste	East Anglia, United Kingdom (UK)	7.5 m3,2 by 2.5 m and 1.5 m high,	Full scale	Enhanced passive aeration system, loosely turned on average twice	Five piles (unshredded and shredded straw mixed with ground fish wastes and fish slurry	-	(Brinton 1994)
An agitated in-vessel	Offal from fish processors and salmon farm mortalities	University of British Columbia's Research Farm	50 m x 2.5 m x 1.25 m (length × width × height).	Full scale	Aeration not provided	Two batch 1.3705 kg of sawdust and 2850 kg of fisheries wastes, 2. 3960 kg of sawdust and 3960 kg of fisheries wastes	Sprinkle water to maintain a consistent moisture	(Liao et al. 1995)

System	Waste	Source	Size	Scale	Operating for aeration	Bulkign agent/Mixing ratio	Moisture content adjustment	Ref
Windrow	Fish waste (mackerel, sardine, tuna, squid)from the Pescados Rubén, S.L Company	Foz, NW Spain	10 m3 1 m high and 6 m long	Full scale	Aeration not provided, turned weekly during the first two months and every 15 days during the last two months	Fish, seaweed and pine bark 1:1:3	-	(López-Mosquera et al. 2011)
Agitated in-vessel system	Fish waste	Vancouver BC, Canada	20m× 61m	Full scale	Aeration through perforated pipes	Fish waste and wood waste	-	(Holbek and Egan 1992)
Aerated static pile	Salmon-farm mortalities	Vancouver, British Columbia	Square cross-section of 0.9 m x 0.9 m, Height varying from 0.6 to 0.85 m	Full scale	Aeration at a rate of 0.2 liter/ min kg volatile matter for 6h daily	100, 200, and 300 kg of fish molts in each reactor, respectively, with 100 kg of sawdust as bulking agent and 20 kg of cow manure	-	(Liao et al. 1994)
Windrow	Seastar (Asterias amurensis) waste	Hobart, Australia	Windrow with Heaps built to a height of 1.5 m	Full scale	Turning at approximately 10 day intervals over a two month composting period	Eucalypt sawdust: seastar waste in a ratio of 4.'1 (v/v) and of bark waste: seastar waste in respective ratios of 3 : 1 and 5 : 1 (v/v).	-	(Line 1994)

System	Waste	Source	Size	Scale	Operating for aeration	Bulkign agent/Mixing ratio	Moisture content adjustment	Ref
Passively aerated	Head and viscera of chum salmon	Vancouver, British Columbia	76 litre PVC Pail	Small scale	Passing air form air chamber at the bottom to the top of the reactor through pipe	27.25 kg Fish waste+ 5.45 kg bulking agent (sawdust, peat and wood shaving) 5:1 (w/w), covered with fiberglass layers		(Liao et al. 1995a)
Static pile	Fish processing waste	Sea Grant institute Wisconsin,USA	8 feet wide, 4 to 6 feet height	Full scale	Turning every 3 weeks	One volume of fish waste (100,000 lb) to three volume of wood chips, covered with layer of wood chips and mature compost	-	(Frederick 1991)
Windrow	Riach, perch, and Amerch waste	Partala research station, Juva, finland	Six cubic meter	Full scale	Turning the pile	50 or 100 kg/m^3 fish waste mixed with pear, sawdust and reed over 10 cm layer of peat	5 months compost was frozen	(Roinila 1997)
In-Vessel	Crawfish	Louisiana, USA	0.3 m 3 Commercial reactors (Barclay Recycling Inc., Ontario, Canada).	Small scale	Opening in the reactor for aeration	Crawfish residuals mixed 1:5 v /v with pine wood chips and rice hulls and 1:6.5 v /v with bagasse and bark	Adding water to maintain moisture contents between 40-60 percent	(Minkara et al. 1998)

System	Waste	Source	Size	Scale	Operating for aeration	Bulkign agent/Mixing ratio	Moisture content adjustment	Ref
Passively aerated in-vessel	Salmonid waste	National University of Comahue, Bariloche, Argentina	220-Liter PVC container	Pilot sale	A perforated pipe from the air chamber at the bottom to the top of each reactor was installed to facilitate aeration	Rainbow trout offal: 16.2 kg of heads and skeletons and 31.2 kg of viscera; 2) bulking agent: 16.0 kg of sawdust +wood shavings.	Adding water to maintain moisture contents between 40-60 percent	(Laos et al. 2002)
In-Vessel	Crab processing waste	Maine, USA	1 cubic meter (internal box dimensions are 1 m X 1 m X 1 m)	Pilot scale	Intermittent aeration	Crab processing waste to wood shaving (1:2 v/v)	Water was added	(Seymour et al. 2001)
Layered mesophilic compost system	Arctic char iSalvelinus alpinus) waste	Shepherdstown, West Virginia, USA	3 plot of 144 ft2	Full scale	Passive oxygen transfer from base through a six-inch layer of course wood chips	58.3 N, 18.8 P, and 902 Arctic char manure, and 1,089 wheat straw or 1,545 oak sawdust (pounds/acre/day)		(Adler and Sikora 2004)

2.5 Composting Process Variable

In order to control and optimize the composting process toward achieving a product of desired quality, it is necessary to understand the factors that influence the process in one way or the other. By providing a favorable environment for the growth and activities of the desired biota in the system, good quality compost can be produced (Gajalakshmi and Abbasi 2008). It has been demonstrated that physical properties of the composting feedstock significantly affect the composting process (Chang and Chen 2010). There is a range of parameters which can affect composting process and the quality of the end product such as the C/N ratio, moisture content, aeration and temperature. The effect of these factors are discussed below.

2.5.1 C/N Ratio

The C/N ratio is one of the most important parameters to control the composting process and to determine the feedstock recipe and the degree of maturity of the end product of compost (Iglesias Jiménez and Pérez García, 1992; Doublet et al., 2010; Puyuelo et al., 2011, Guo et al., 2012). C/N ratio can influence microbial activity in composting processes (Zhu 2007). Nitrogen has received the most attention in composting systems since it is the most needed element for plant nutrition. Moreover, it has often been recognized as a limiting factor for microbial growth and activity during the decomposition of plant residues especially in materials with a high C/N ratio. Carbon that provides energy for the degradation process is an element that is also most likely to be lost during the composting process (Eklind and Kirchmann, 2000; Tiquia and Tam, 2000;

Dresbøll and Thorup-Kristensen, 2005). Nitrogen content increases through the mineralization of organic matter and consequent loss of CO_2, H_2O and decreases through ammonia volatilization. At the later stage, the activity of nitrogen-fixing bacteria compensates the nitrogen loose partially. High temperature of composting can affect adversely the nitrification and nitrogen balance (De Bertoldi et al., 1983). The C/N ratio of the initial composting material has also been reported to affect N loss during composting. A very low C/N ratio can lead to loss of N through NH_3volatilization (De Bertoldi et al., 1983; Tiquia and Tam, 2000). When C/N ratio is low, the excess of N can be lost from the composting mass through leaching or volatilization as ammonia and lead to potential odor problem. An extremely high C/N ratio makes the composting process very slow as there is an excess of degradable substrate and lack of N for the microorganisms (Gao et al., 2010; Christensen, 2011). Haug, (1993) proposes an optimum C/N ratio value as 15- 30 (Haug 1993). If the initial C/N ratio is greater than 35, microorganisms must oxidize the excess carbon, until a more convenient C/N ratio for their metabolism is reached (De Bertoldi et al., 1983). Composting of a nitrogen/protein-rich (a low C/N ratio) may result in a rapid increase of temperature to thermophilic phase, while in the composting a lignocellulose-rich (high C/N ratio) material, the process may exhibit longer initial mesophilic period before reaching thermophilic phase (Alburquerque et al., 2006). A decreasing trend in the ratio of C/N with eventual stabilization, can generally be observed as composting progresses due to the release of CO_2 as organic substrates are decomposed, resulting in the loss of carbon from the system (Wichuk and McCartney 2010). For C/N ratio as maturity and stability index,

different thresholds ranging from <10:1 to <20:1 has been recommended in the literature (Mathur et al. 1993; Sullivan and Miller 2001),

Food waste has high moisture contents and low C/N ratios for efficient composting (Iqbal et al. 2010; Kumar et al. 2010; Mato et al. 1994). To control the moisture contents and to optimize the C/N ratio, bulking agents are added in composting process for an effective disposal of MSW. Bulking agents also affect ammonia emission and others volatiles during the composting process (Iqbal et al. 2010). Bulking agents serve two purposes in composting; it allows air to enter the pile so bacteria and fungi can work, and it provides a proper C/N ratio so the material will compost efficiently without offensive odor (Frederick 1989; Laos et al. 1998). The required quantity of bulking agents depends on the structure and moisture content of the waste and on the properties of the bulking agents (Schaub and Leonard 1996). Cost and availability are further consideration when choosing bulking agent (Minkara et al. 1998). The weight ratio of bulking agent and waste also affects the total throughput of a composing facility. That is, using less bulking agent in the composting mix means that more wastes can be composted in a given time period (Liao et al. 1995). The amount of bulking agent needed may range from less than 1:1 (parts by volume) to more than 5 bulking agent to 1 part waste (Liao et al. 1995a). Chang and Chen (2010) mixed food waste with three bulking agent including rice husk, sawdust and rice barn to investigate the effects of bulking agents on the composting process of food waste. Their results showed that the water absorption capacity of the composting mixture was the dominant physical property that affected the composting rate. More sawdust in the composting mixture resulted in the increases of the water

absorption capacity and the composting rate, shorter composting and acidification times, and lower final pH value (Chang and Chen 2010). Adhikari et al. (2008) evaluated available bulking agents in the region for food waste composting: chopped hay , chopped wheat straw, pine wood shaving, rough cardboard without a glossy finish, medium rough cardboard with a medium glossy finish, smooth cardboard with a glossy finish, wheat pellets and wasted animal feed. In other study, Adhikari et al. (2009) tested three bulking agents (e.g., chopped wheat straw, chopped hay and wood shavings) at three moisture levels for the composting of food waste. With a food waste ratio resulting in a 20% DM content, the chopped wheat straw (1:1.3) and chopped hay (1.5:1) formulas met an acceptable level of nutrients (total nitrogen, total phosphorous and total potassium). Iqbal et al. (2010) investigated the effect of regionally available bulking agents (bagass, paper, peanut shell, sawdust) for moisture reduction during MSW composting. They found the effect of 40% addition of sawdust was best to optimize the moisture up to 60% in composting. Table 2.5 summarizes the physical and chemical properties of some commonly used bulking agents for MSW composting.

Table 2.5 The physical and chemical properties of some commonly used bulking agents for MSW composting

Material	C (%)	N (%)	pH	Ash content(%)	C/N ratio	Moisture (%)	Ref
Rice husk	37-40	0.6-0.7	-	18.21	60-70	9-11	(Chang and Chen 2010)
Sawdust	43-46	0.2-0.4	-	2-3	140-160	10-12	(Chang and Chen 2010)
Rice barn	48-52	1.6-2	-	8-11	25-30	10-14	(Chang and Chen 2010)
Rice husk	41.56	1.22	7.1-7.3	-	34.17	8-11	(Kumar et al. 2010)
Chopped wheat straw	50.4	0.5	7	-	103	-	(Adhikari et al. 2009)
Chopped hay	51.7	0.9	6.6		59	-	(Adhikari et al. 2009)
Wood shavings	54.5	0.08	5.6	-	676	-	(Adhikari et al. 2009)
Sawdust	45.56	0.49	6.38	17.98	92.36	-	(Iqbal et al. 2010)
Bagass	41.73	0.76	5.92	23.88	54.68	-	(Iqbal et al. 2010)
Peanut shell	36.58	6.0	6.32	34.17	6.06	-	(Iqbal et al. 2010)
Rice husk	21.0	0.83	4.89	62.21	25.15	-	(Iqbal et al. 2010)
Corn pith	32.47	0.87	5.11	41.55	37.25	-	(Iqbal et al. 2010)
Paper	18.97	0.39	6.32	65.86	48.25	-	(Iqbal et al. 2010)
cornstalks	50	10.5	7.43	-	-	48	(Yang et al. 2013)
sawdust	53	2.3	7.24	-	-	6.1	(Yang et al. 2013)
Spent mushroom substrate	33	23.8	6.72	-	-	11.6	(Yang et al. 2013)

Generally, low C/N ratio for fishery waste ranging from 2.6 to 9 was reported in the previous studies (Cathcart et al. 1983; Hu et al. 2009; Laos et al. 2002; Liao et al. 1995). To achieve the C/N ratios between 26 and 35 which have been observed to produce an efficient and rapid composting process, a careful blending should be prepared form fishery origin waste which are more nitrogenous (meaning proteinaceous) materials, can be as high as 12% N, with other carbonaceous bulking agents as a source of carbon to effect transformation of potentially highly malodours protein decomposition products into organic composition (Brinton and Seekins 1988). Since most of the nitrogen in fish wastes is readily available and are generally received and handled as wet slurry; therefore, such wastes require a readily available carbon additive and a relatively dry, high carbon bulking agent. Without it, the composting process will tend to generate an objectionable smell due to the release of ammonia gas (Liao et al. 1997; Liao et al. 1995). In addition, when fish material is not mixed in well with bulking agent, wet pockets remain and can quickly give rise to odor problems (Frederick 1989). Mixing fishery waste with bulking agent (a) has a wide C/N ratio; (b) is acidic and hydrophilic enough to trap NH_3 in solution; (c) has high capacities for adsorbing and complexing cations like NH_4^+ and CA^{++}; (d) is fluffy enough to be well aerated so that malodours of anaerobic decomposition are not created but acidic SO_4^{--}; and NO_3^-, ions that help dissolve bone phosphates are generated (oxidatively); (e) deodorizes any malodours produced even transiently; (f) provides heat insulation; and (g) will, though biodegradable, not sustain thermophilic by itself so that the composts can mature early, would improve composting performance and shorten the composting period (Mathur S. I. 1988).

Bulking agents including peat moss, sawdust, peat, straw (wheat and oil seed rape), bark, bagasse, rice hull and sawmill waste have been mixed with fishery waste to produce desirable mix for composting (Brinton and Seekins 1988; Illera-Vives et al. 2013; Liao et al. 1995; López-Mosquera et al. 2011; Seymour et al. 2001). Wood by products wastes are the most common bulking agents mixed with fishery waste because they contain hemicelluloses and celluloses that degrade easily, and the recalcitrant lignin that contribute heavily to humus formation, also they are more widely available (Hayes et al. 1994). Due to the importance of the choosing of bulking agent and finding optimum mixing ratio, many studies have been focused on investigation of suitable bulking agents to be mixed with fishery waste and generate mature and stable product (Liao et al. 1995a; Liao et al. 1997; Line 1994; Martin et al. 1993; Minkara et al. 1998). Liao et al. (1997) composted fish waste with four bulking agents including fir, Alder, peat moss and vermiculite for 20 days in a full scale composting system. They concluded that all mixes composted well and ammonia emission from composting piles was reduced by the addition of peat moss, vermiculite and alder as bulking agents. Peat moss was more effective than vermiculite in retaining ammonia. Seeing that peat moss contains acidic carboxyl and phenolic hydroxyl compounds, therefore, the peat moss mix had the lowest pH among the different treatments. As a result, more nitrogen was retained as ammonium in the peat moss mix and less ammonia was volatilized. Since peat moss is also an essential ingredient in potting mixes used for plant production, its use would probably be preferred (Liao et al. 1997). Liao et al. (1995a) concluded that composting with peat moss were better able to retain nitrogen so it produced superior compost in comparison to

sawdust and wood shaving. The results of their study indicate that peat moss, sawdust and wood shaving are all potentially good bulking materials for the composting of fish offal (Liao et al. 1995a). In addition peat moss has excellent water absorption capacity which rendering them as a suitable bulking agent for fishery waste with high moisture content (Martin et al. 1993). Frederick (1989) recommended using three volumes of peat moss, one volume of fishery waste, and one volume of covering material (compost or peat moss) to compost fishery waste in a windrow in Wisconsin (Frederick 1989). Adler and Sikora (2004) studied the fishery waste composting with wheat straw and oak sawdust and they found the structure of carbon source affected both the potential for runoff and the oxygen content. The wheat straw's open structure made it possible for it to absorb waste liquid without runoff during both the summer and winter season. The oak sawdust compost mixture did not reach stable stage until sometime during the 118 to 167 day period of composting. However, the wheat straw compost mixture reached stability during the 63 to 118 day period. Mineralization and nitrification rates were higher with wheat straw as indicated by the higher levels of both ammonium and nitrate probably due to the higher rates of decomposition (Adler and Sikora 2004). Wheat straw needs careful consideration before being incorporated into mix recipes. Fresh wheat straw has hydrophobic surface characteristics as well as a high lignin content, both of which can lengthen its breakdown time. As with other types of straw, wheat straw is a bulky material with low density. Thus a very large quantity of straw is needed in proportion to fishery waste, when considering C/N ratios, with the ratios increased to compensate for the ligneous nature of the material (Brinton 1994).

Significant losses of N and other essential nutrients can occur during composting. Such N losses are attributed to NH_3 volatilization as well as subsequent leaching of NH_4 and $N0_3$. Composting crawfish residuals with rice hulls, bark and bagasse resulted in average losses of total N of 47.2 percent, 42.6 percent and 20.2 percent (ash basis), respectively, after 50 days. Because bagasse was the most degradable of the bulking agents, a greater portion of its N may have been retained as organic N thus reducing volatile losses of NH_3. NH_4 and $N0_3$ concentrations in bagasse mixture were consistently low, which support the hypothesis that greater immobilization of N may account for the lower losses of N from bagasse mixture. Bagasse appeared to be able to assimilate mineralized N more readily than bark, rice hulls, or wood chips (Minkara et al. 1998).

Martin et al. (1993) employed composting system to compost fish offal, chicken manure and crap processing waste to evaluate the effect of peat and sawdust as bulking agents on composting process. Relationships were observed between the type of bulk material employed and the composition of the composted material. The acidic peat fibers by their adsorption properties, were able to retain NH_3, which otherwise would escape from the composting process (Martin et al. 1993). The lower pH and longer thermophilic process were observed for fishery waste compost containing peat in comparison to the compost containing straw and reed as bulking agents (Roinila 1997). In composting of blue crap scrap in Florida, fresh cypress sawdust, aged cypress sawdust, pine bark and yard trimming were used as bulking agents. Fresh cypress sawdust sustained longest period of active heating but both composting piles with sawdust completed active composting by about 60th days. When pine bark was used, the heating period was very short-lived. All

piles meet the requirement of 15 days or more temperature above 55ºC for pathogen reduction (Brinton and Gregory 1992). All studies agreed that the choice of bulking agents is important for fish waste composting performance and the quality of the end product. The physical and chemical properties of some commonly used bulking agents for fishery waste composting are summarized in Table 2.66.

Table 2.6 The physical and chemical properties of some commonly used bulking agents for fish waste composting

Material	C (%)	N (%)	pH	EC (mS/cm_1)	Ash content	C/N ratio	Organic matter (%)	Moisture (%)	Ref
Sawdust	50	0.24	-	-	-	208	-	-	Eyras et al.,1998
Sawdust	40	0.08	-	-		500	-	30	(Laos et al. 1998)
Wood chips	483.5 ± 3.8 (g/kg DM)	40.0 ± 1.4 (g/kg DM)	7.75 ± 0.05	2.5 ± 0.3 (mS/cm)	9.1 ± 2.5 (g/kg DM)	12	990.9 ± 2.5 (g/kg DM)	43.2 ± 0.9	(Hu et al. 2009)
Wood chips	50.3	0.1				719	98	5.5	(White et al. 1989)
Straw	47.02	1.26				37.32	81.04	41.5	(Cathcart et al. 1986)
Peat	50	1				50	98.5	65	Mathur S. I., 1988
Pine Bark	49.89	0.4				121.8	-	47.03	Illera-Vives et al., 2013
Aged Bark						83		52	(Brinton and Seekins 1988)
Peat moss	24	1.1	3.6	1.2 (mS/cm)		22.0	-	6.5	(Tarnai 2009)
Vermiculite	0 (%DW)	0.02 (%DW)	-	-	0	-	-	1.0	(Liao et al. 1997)
Pine bark	50.41 ±	0.16 ±	5.63 ±	0.86 ±		304.24	-	38.08 ±	(López-

Material	C (%)	N (%)	pH	EC (mS/cm_1)	Ash content	C/N ratio	Organic matter (%)	Moisture (%)	Ref
	0.02	0.01	0.04	0.02		± 11.84		0.51	Mosquera et al. 2011)
Wood chips	535.9 ± 1.4 (g/kg DM)	18.1 ± 1.2 (g/kg DM)	7.75 ± 0.05	2.5 ± 0.3	9.1 ± 2.5 (g/kg DM)	29.6	990.9 ± 2.5 (g/kg DM)	43.2 ± 0.9	(Hu et al. 2009)
Lameque peat	43.70	0.8	3.00	-	-	53	96.0	50	(Hayes et al. 1994)
Shigawake peat	54.84	1.03	4.4	-	-	53.24	98.72	75.00	(Hayes et al. 1994)
Bagasse	436.3(g/kg DM)	9.3 (g/kg DM)	-	-	-	47	-	53.4	(Minkara et al. 1998)
Rice hulls	372 (g/kg DM)	4.8	-	-	-	76.9	-	9.5	(Minkara et al. 1998)
Bagasse	41.5	0.36	4.56±0.12	0.28±0.03	16.51±2.62	115.2	83.49±2.53	30	(Cole et al. 2015)

2.5.2 Moisture Content

Microbial activity and the physical structure in the composting process can be affected by moisture content, also it has a central influence on the biodegradation of organic materials (Ahn et al. 2008). Moisture content is one of the critical design and operating parameters used in compost engineering systems. It is important to transport dissolved nutrients required for the physiological and metabolic activities of microorganisms (McCartney and Tingley 1998). Moisture works as a medium to transfer dissolved gas and nutrients absorbed through the cell membrane of microorganisms (Christensen 2011; Haug 1993). Moisture content has also significant effects on enzyme activities and microbial respiration of the composting process (Mondini et al. 2004). The moisture content during the active phase of composting is a function of temperature and rate of aeration. Positive aeration, temperature elevation and turning can reduce the moisture content in composting matrix (Said-Pullicino et al. 2007). Over the period of composting, the moisture content drops through leaching and evaporation and inversely, the volume of air within the pile increases until there is sufficient air to meet the needs of the aerobes. At that time, the process of active composting begins and the temperature in the pile starts to rapidly rise (Vizcarra et al. 1993). Monitoring the changes in moisture content over time is considered useful for assessing the progress of composting process, since the heat generation which accompanies decomposition drives vaporization, moisture loss is therefore indicative of the decomposition rate (Liao et al. 1995). In general, a 50% moisture was the minimum requirement for maintaining high microbial activity (Liang et al. 2003). Optimum moisture content for most materials composting is in range from 50%

to 70%, while some other materials can be effectively composted outside this range (about 25–80% on a wet basis) (Cronje et al. 2003; Haug 1993).

CO_2 evolution was used to examine the effect of temperature, aeration, and moisture on composting of food waste by Suler and Finstein (1977). Carbon dioxide formation was maximal at the intermediate moisture content (60%). They stated moisture content was a convenient and useful process control parameter in composting but it was a poor means of comparing the water status of dissimilar organic materials as this relates to microbial activity (Suler and Finstein 1977).

For fish waste, moisture content from 35% to 75% has been reported in the previous studies (Brinton 1990; Hayes et al. 1994; Mathur et al. 1986) . Haug (1993) found most of the bacteria halted their activity at very low moisture content. In some cases, to prevent moisture drop, water was added to the fishery waste composting system to sustain moisture content above 40% (Cathcart et al. 1983; Laos et al. 2002; Minkara et al. 1998; White et al. 1989). Cathcart et al. (1986) stated carbon dioxide generation is a function of moisture content and temperature during crab scrap composting process. Maximum predicted carbon dioxide generation occurred at 55-56 °C and at a moisture content of 55 % (Cathcart et al. 1986).

2.5.3 Aeration

The main purposes of air supply to composting is to provide oxygen for biological degradation, dry up the wet materials and remove excess moisture, and to carry off exhaust gas and generated heat (Haug 1993). Air flow influences spatial distribution of gases, moisture, temperature, and the decomposition rate of the organic matter (Cronje et

al., 2003). Lack of aeration can lead to anaerobic conditions and excess aeration will increase the cost the heat, as well as the loss of moisture and ammonia (Guo et al. 2012). Shen et al. (2011) found that composting never reached the thermophilic phase at low rate aeration. Also at the low aeration rate, the production of organic acids due to anaerobic conditions led to the relatively low pH, large CH_4 production, high N_2O emissions, higher loss of total nitrogen , low total organic carbon (TOC) reduction (Paradelo et al.) and low GI (Shen et al. 2011). Rasapoor et al. (2009) stated that a lower aeration rate had a significant effect on the ammonium and nitrate formation. (Haug 1993) recommended the aeration rate with a value ranging from 1.2 to 2.0 g O_2/g biodegredable volatile solids (BVS) for most composting substrates and a higher value such as 4.0 g O_2/G BVS for saturated substrates (Rasapoor et al. 2009).

The influence of the industrial control composting conditions (aeration 0.05–0.3 L $kg.min^{-1}$ and moisture 40–70 %) of MSW compost on emissions of volatile organic compounds (VOCs) was studied by Delgado-Rodríguez et al. (2012). They reported the highest emissions of VOCs were in the early stages of the MSW composting process (initial and thermophilic phases). Aeration rate had a strong effect on VOCs emissions. High aeration rates (0.3 $L.kg.min^{-1}$) caused normally high emissions of all selected compounds in the early stages of the composting process. A medium moisture value (55%) could be a suitable balance to control compound emissions.

Arslan et al. (2011) determined the effect of various aeration rates (0.37, 0.49, 0.62, 0.74, 0.86, and 0.99 L/min kg volatile solids (VS) on composting to supply the optimum aeration rate for a successful and economic composting of vegetable and fruit waste. The

highest C/N reduction was observed at airflow rate of 0.62 L/min kg VS. They recommended 0.62 L/min kg VS as the optimum aeration rate for forced aerobic composting of vegetable and fruit waste.

The effects of different aeration rates (0.4, 0.6 and 0.9 L/min.kg), and aeration patterns on the composting of MSW was investigated by (Rasapoor et al. 2009). The result of the study showed the lower and medium aeration rates had a significant impact on nitrogen, C/N ratio and temperature profile, while higher aeration rates led to higher EC and values starting at a rate of 0.6 L/ min. kg during first 2 month of the process and continuing at a rate of 0.4 L/min. kg until the end of composting process would result in lower energy consumption (Rasapoor et al. 2009).

Seymour et al. (2001) used the two aeration-control methods including 1) Constant frequency automatic aeration and 2) temperature dependent aeration to determine the comparative efficacy of the different aeration strategies on the crap waste processing composting process. Statistical analysis showed no significant difference for any aeration treatment in maximum temperatures, the duration of maximum temperatures or the slopes of temperature changes while additional air may have increased the drying effect of aeration which in turn could reduce microbial activity. They concluded that measured oxygen content was the best predictor of temperature changes (Seymour et al. 2001) .

2.5.4 Temperature

Temperature is one of the important factors for evaluating composting efficiency (Miyatake and Iwabuchi 2006). It can affect microbial metabolism, population dynamics (e.g., composition and density) of microbes and diversity of microorganisms (Arslan et

al. 2011; Suler and Finstein 1977) and thus can be considered as a promising index of microbial activities and biooxidative stages (Godden et al. 1983). Indeed, composting temperature is the valuable data for assessing the progress of decomposition (Minkara et al. 1998). Temperature increase within composting materials is a function of initial temperature, metabolic heat evolution and heat conservation (Liang et al. 2003). Heat generated during composting can be harnessed to kill human, plant, or animal pathogens (Adler and Sikora 2004). To assure pathogens were effectively reduced after composting, the temperature of the compost should remain higher than 55 °C for more than two weeks. It is also used as a standard measure of success (EPA 1995). It is necessary to consider that, in the self-heating ecosystem; temperature is both effect and cause. The temperature is a function of the accumulation of heat generated metabolically, and simultaneously the temperature is a determinant of metabolic activity (MacGregor et al. 1981). Therefore, it can be considered both as a controlling and monitoring parameter.

Some of the process factors identified as influencing both the maximum temperature attainable in the compost as well as the time taken to attain it are the composition of the organic wastes, the availability of nutrients, moisture content, particle size, and the degree of aeration and agitation (Vizcarra et al. 1993). The temperature of composting layer is also related to the size and shape of the composting pile. Low temperature can be observed in very flat pile which results in odor and leachate generation. Very wide and high pile can sustain high temperature for long time which can lead to rapid drop in moisture and killing beneficial bacterial and fungi required for compost maturation (Frederick 1989).

Many studies showed that nitrogen transformation is influenced by the temperature. (Grigatti et al. 2011) found an intense NH_4–N accumulation up to 650 mg kg^{-1} in correspondence with the temperature peak. Zorpas et al. (2000) reported the total nitrogen is affected by the action of proteolytic bacteria and temperature. At high temperatures, nitrogen is lost in the atmosphere (Zorpas et al. 2000).

Chang and Hsu (2008) stated during food waste composting each temperature peak could be interpreted as the decomposition of a certain material or a composite of decompositions of several materials. They observed the first sharp temperature peak as a result of the aerobic biodegradation of the fast decomposing sugars in the substrate in the first 9–12 h and the second broader temperature peak occurred between 18 and 96 h which was a composite of biodegradations of slowly decomposed materials and were composition-dependent.

For fishery waste composting, the rapid establishment of high temperature in the composting layer is particularly important for stabilizing the fishery waste by dehydration and for encouraging the development of non-purifying fungi (Frederick 1989). It has been reported that in fishery waste composting, in which a high ratio of fishery waste to bulking agent attained a higher temperature since fishery waste contained high levels of easily digestible nitrogenous components such as proteins; when mixed with bulking agent with a high portion of carbonaceous material generated more heat and longer thermophilic period (Hu et al. 2009; Laos et al. 2002). Cathcart et al. (1986) showed temperature, moisture content and C/N ratio are all significant in the unshredded crab scrap composting model and suggested 67% and 63 °C as optimum moisture content and

temperature for composting of unshredded crab scrap, respectively (Cathcart et al. 1986). During clam waste composting, clam waste mixed with wood chips clearly showed the four compost phases: mesophilic, thermophilic, cooling, and maturation phases. The high ratio of clam waste to woodchips resulted in a higher temperature and longer thermophilic phase and, thus, an improved composting performance. Clam waste contained high levels of easily digestible components such as proteins; when mixed with woodchips, the mixes with a high portion of clam waste generated more heat. Sharp increase in temperature was observed during clam waste compost as an index of high microbial activity, which induces the rapid decomposition of the readily digestible components contained in the clam waste at the earlier stages of composting (Hu et al. 2009). Liao et al. (1995) found that during fishery waste composting, at temperatures lower than 30°C, a linear relationship existed between an increase in the efficiency of the process and an increase in temperature. At temperatures in excess of 55°C, efficiency began to drop abruptly, becoming negligible at temperatures higher than 70°C. At temperatures in the region of 65°C, spore formers grow rapidly and most of the non-spore-formers simply died off (Liao et al. 1995).

Secondary peaks in the temperature profiles of fish waste compost pile have been explained in the literature as possibly due to (a) the re-invasion of the center by thermophilic fungi from the cooler outsides of the pile, (b) by mesophilic organisms recommencing activity or (c) excessive presence of ammonia and phenols, which inhibit bacterial growth and activity. Once most of the ammonia and phenols are released to the air, the bacterial population can resume growth, thus causing the minor peaks in

temperature later (Vizcarra et al. 1993). The volatility of ammonia is known to be temperature dependent, and higher levels of ammonia above the composting pile should occur as the temperature of the composting pile increases. As composting proceeded, the release of ammonia was further facilitated by increased pH of the compost piles (Liao et al. 1997). Temperature were sustained over 55 °C long enough for most of fish waste and MSW composting in the literature to meet the EPA requirement to reduce the pathogens (Illera-Vives et al. 2013; Laos et al. 2002; Liao et al. 1994; López-Mosquera et al. 2011). The temperature decrease after active phase of composting is considered a good indicator of compost maturity since it reflects the decrease in microbial activity and, consequently, the depletion of easily biodegradable organic compounds (Illera-Vives et al. 2013).

2.6 Maturity and Stability Assessment of Compost

2.6.1 Compost Maturity and Stability

Maturity and stability are important indices for compost quality assessment and practical use of composted materials in agriculture (Mondini et al. 2004). Stability and maturity are both commonly used to define the degree of decomposition of organic matter during the composting process even if they are conceptually different (Benito et al. 2003). They are helpful to monitor the effectiveness of the biological degradation and process performance and compare different composting systems (Cossu and Raga 2008; Xiao et al. 2009).

Stability is related to the microbial activity and can be expressed by biological indicators like respiration index (OUR or CO_2 evolution rate), heat release as a result of microbial

activity, enzyme activity and total microorganisms count (Benito et al. 2003; Wu et al. 2000). In stable compost, readily biodegradable material was decomposed and converted to humic-like substances so the matter cannot sustain the microbial activity anymore (Xiao et al. 2009). Thus the oxygen consumption reduced and odor cannot be produced. The rate of energy release due to microbial degradation of the organic matter equals the rate of energy loss to the environment, and temperature of the compost thus equals that of the ambient temperature (Zmora-Nahum et al. 2005). The stability of given compost can determine the potential impact of the material on nitrogen availability in soil or growth media and maintain consistent volume and porosity in container growth media (Grigatti et al. 2011).

Maturity refers to the decomposition degree where compost does not pose any adverse effects on plants and growth of various crops (Castaldi et al. 2008; Zmora-Nahum et al. 2005). It is commonly associated with plant-growth potential or phytotoxicity. Mature compost contains negligible or acceptable concentrations of phytotoxic compounds such as NH_3 or short-chain organic acids and a high proportion of humic substances. Maturity has been evaluated based on chemical parameters correlated with plant response (Bernal et al., 2009, Xiao et al., 2009).

Immature and poorly stabilized composts may cause a number of problems during storage, marketing and use. During storage of unstable compost, anaerobic conditions can result in odor, fire, or toxic compounds (CCQC 2001). During the usage of immature and unstable compost, due to the ongoing microbial activities and decomposition, a competition between plants and the microbial biomass for oxygen exist (Benito et al.

2003; Chukwujindu et al. 2006). This may constrain the availability of oxygen to roots, suppress plant growth and produce H_2S and NO_3 (Chukwujindu et al. 2006; Grigatti et al. 2011). Decomposition of unstable composting also produces phytotoxic substrate like phenolic compounds, ethylene oxide, low-molecular weight organic acids, ammonia and toxic nitrogen compounds which could inhibit root growth (Zucconi et al. 1981).

To characterize compost maturity and stability, several factors have been studied including microbial respiration activity (CO_2 evolution) (Benito et al. 2003; Hellebrand and Kalk 2001; Wu et al. 2000), specific oxygen uptake rate (SOUR) (Cabañas-Vargas et al. 2005; Lasaridi and Stentiford 1998; Scaglia et al. 2007), dissolved organic carbon concentration (Grigatti et al. 2011; Mondini et al. 2004; Wu et al. 2000; Zmora-Nahum et al. 2005), seed germination tests (Ghaly et al. 2013; Komilis et al. 2011; Said-Pullicino et al. 2007; Zmora-Nahum et al. 2005; Zucconi et al. 1981), NH_4^+-N and NO_3^-- N concentration (Benito et al. 2003; Chikae et al. 2006; Francou et al. 2005; Gao et al. 2010; Gregory et al. 2011), neutral degradable fiber and lignin (Hutchinson and Griffin 2008), enzyme activity including protease (Benitez et al. 1999; Castaldi et al. 2008; Goyal et al. 2005; Kayikçioğlu and Okur 2011), urease (Benitez et al. 1999; Castaldi et al. 2008; Godden et al. 1983), cellulose (Castaldi et al. 2008; Godden et al. 1983), β-glucosidase (Benitez et al. 1999; Kayikçioğlu and Okur 2011; Mondini et al. 2004), dehydrogenase activities (Barrena et al. 2008; Benito et al. 2003; Castaldi et al. 2008; Tiquia 2005), and phosphatase (Godden et al. 1983; Kayikçioğlu and Okur 2011; Mondini et al. 2004), cation exchange capacity (Gao et al. 2010; Iglesias Jiménez and Perez Garcia 1989), humification parameters (humic acid (HA), fulvic acid (FA))

(Francou et al. 2005; Tiquia 2005; Wu et al. 2000),total organic carbon (Francou et al. 2005; Gao et al. 2010), microbial biomass (Mondini et al. 2004), BOD and COD (Spellman 2012), non-cellulosic polysaccharides, phenolic compounds (Said-Pullicino et al. 2007), and water-soluble organic matter (Said-Pullicino et al. 2007). Threshold for maturity indices have been defined by several studies and summarized in Table 2.7.

The major parameters considered in Canadian compost quality standards are maturity, trace element (heavy metal), time temperature requirements and microbial pathogens (indicator organisms). Based on the Canadian Council of Ministers of the Environment (CCME) requirements, compost must conform two of the three tests including 1) carbon/nitrogen ratio (C/N) $\leq$ 25, 2) oxygen uptake rate < 150 mg O_2 kg^{-1} OM (VS) h^{-1}, 3) germination of cress or radish seeds in compost $\geq$ 90% that of control sample, and plant growth rate in soil–compost mix $\geq$ 50% that of control sample. Additionally, compost must be cured for a minimum of 21 days and must not reheat upon standing to greater than 20 ◦C above ambient temperature (Ge et al. 2006).

Table 2.7 Threshold for maturity and stability parameters

Index	Threshold	Units	Reference
respiration rates	<2	mg CO- C g compost C^{-1} d^{-1}	(Brewer and Sullivan 2003)
Water-soluble organic matter	<2.2	g/litre	(Garcia et al. 1993)
water soluble carbon (WSC)/ water soluble nitrogen (WSN)	<2	-	(García et al. 1991)
WSC/ORG.N	<5	-	(Laos et al. 2002)
index of biodegradability	<2.4	-	(Laos et al. 2002)
C/N ratio	20	-	(Iglesias Jiménez and Perez Garcia 1989))
WSC/N	<0.5	-	(Iglesias Jiménez and Pérez García 1992)
NH4+-N	0,04%		(Seafish 2001)
NH_4^+-N/NO_3^--N	<0.16		(Benito et al. 2003)
NH_4^+-N/NO_3^--N	1.9		(Benito et al. 2003)
dehydrogenase activity	800	mg TPF $kg^{-1}d^{-1}$	(Tiquia et al. 2002)
GI (high phytotoxicity)	<50%		(Zucconi et al. 1981)
GI(no phytotoxicity)	50%-80%		(Zucconi et al. 1981)
dynamic respiration index	500	mg O2 kg^{-1} (VS) h^{-1}	(Adani et al. 2004)
DOC	<17	g kg-1	(Bernal et al. 2009)
DOC	≤10	g kg^{-1}	(Hue and Liu 1995)
WSC	<0.5%		(Laos et al. 2002)
WSC	<1%		(Hue and Liu 1995)
WSC	<1.7%		(Bernal et al. 2009)
C/N ratio	12		(Bernal et al. 2009); (Iglesias Jiménez and Pérez García 1992)
Water extractable organic carbon(WEOC)	<0.4	mg mL^{-1}	(Zmora-Nahum et al. 2005)
dissolved oxygen content	4	g kg^{-1}	(Zmora-Nahum et al. 2005)
C/N ratio	<15		(Saidi et al. 2009)
NH4+-N	< 400	mg/kg	(Saidi et al. 2009)
CO_2-C	< 2000	mg CO_2-C/kg	(Saidi et al. 2009)
dehydrogenase activity	< 1	mg TPF/g DM	(Saidi et al. 2009)
GI	> 80%		(Saidi et al. 2009)

2.6.2 Carbon and Nitrogen Related Maturity and Stability Indices

A number of parameters related to determination of organic matter especially different forms of carbon and nitrogen such as WSC, total carbon, TOC, WSN, $NH_4^+ - N$, and $NO_3^- - N$ have been proposed for testing compost stability and maturity.

WSC is an indication of water-soluble fraction of organic matter of compost. It is the most accessible organic nutrient to microorganisms because it consists of sugars, hemicellulose, and phenolic substances, amino acids, peptides, and other easily biodegradable substances during composting. It has been frequently used as maturity index in the literature (Gajalakshmi and Abbasi 2008; Paradelo et al. 2010).

Ammonium and nitrate are the forms of N, which could be changed during composting. Poor aeration during composting resulted in excessive ammonium (Environment Canada 2005). The $NH_4^+/\ NO_3^-$ ratio has also been proposed to estimate the compost stability. At the end of the composting process, the content of NO_3^- should be higher than that of NH_4^+, indicating that the process has been performed under adequate aeration conditions (Grigatti et al. 2011).

Laos et al. (2002) measured the content of WSC, TOC, total nitrogen content, nitrate nitrogen, ammoniacal nitrogen in the water extract of the compost. They noticed that WSC and WSN decreased significantly with time in all the samples during composting. Also the WSC/WSN showed a decline by proceeding composting. The WSC, WSN, and WSC/WSN were suggested as suitable parameters to reflect maturity of compost. It was recommended that the value of WSC/WSN should be less than 2 in the final matured product of composting.

Said-Pullicino et al. (2007) found that the TOC/N ratio decreased with composting time. The variation in the water-extractable organic carbon to soluble organic nitrogen (WEOC: ON) ratio during the process showed a similar trend to that observed for the TOC/N ratio. The WEOC/ON ratio is generally lower than TOC/N ratio due to the faster degradation of the soluble C with respect to soluble organic N. It could be derived that when the concentration of organic C in the germination media is 1.85 mg mL^{-1}, the phytotoxicity disappeared. Also the ratio of hydrophobic to hydrophilic water extractable organic C could represent the solubilisation and mineralization, that are responsible for the attainment of stability.

2.6.3 Enzyme Activities as Maturity and Stability Indices

Enzyme is a biocatalyst which controls the rate of substrates degradation or accelerates the rate of biological reactions. In degradative processes, enzymes act as the main mediators (Castaldi et al. 2008; Kayikçioğlu and Okur 2011; Valsange et al. 2012). They are responsible for the breakdown of several organic compounds characterised by a complex structure, finally leading to the solubilisation of simple water-soluble compounds (Castaldi et al. 2008). Due to the role played by enzymes in the biological and biochemical processes during composting, enzyme activity can indicate the ability of microbes to degrade a wide range of common organic substrates (Castaldi et al. 2008; Mondini et al. 2004; Tiquia 2002). The presence of a high content of degradable organic compounds in the initial mixture might stimulate microbial growth and enzyme synthesis (Castaldi et al. 2008).

Characterizing and quantifying specific enzyme activities during composting could provide information of dynamics of the composting process. They can reflect the rate of transformation of organic residues and nitrogen, as well as the stability and maturity of end products (Mondini et al. 2004; Raut et al. 2008). Moreover, the determination of enzyme activity, in contrast to other analytical techniques used for compost stability evaluation is easy, fast, and relatively inexpensive (Mondini et al. 2004). Important enzymes involved in the composting process include dehydrogenase for substrate oxidization by a reduction reaction, β-glucosidases for glucoside and amide hydrolysis, as well as phosphatases and arylsulphatase for phosphate and sulphate removal from organic compounds. Other enzymes in composting process are celluloses for cellulose depolymerisation, proteases and urease involved in N mineralization (Mondini et al. 2004).

Dehydrogenases are enzymes belonging to the oxido-reductase group which catalyse the oxidation of organic substances (Kayikçioğlu and Okur 2011). They participate in the metabolic reactions producing energy in the form of ATP through the oxidation of organic matter (Barrena et al. 2008). Dehydrogenases involve in the detachment of electrons from the substrate and their binding with protons (Kayikçioğlu and Okur 2011). The microbial activity during composting, when defining by the dehydrogenase activity, reflects the role of enzymes on the oxidative phosphorylation process and their involvement in the respiratory chain of all organisms (Castaldi et al. 2008; Kayikçioğlu and Okur 2011; Vargas-Garcia et al. 2010). Barrena et al. (2008) used dehydrogenase activity to monitor the composting process. Temperature and dehydrogenase profiles

were very similar during the thermophilic stage; both showed a rapid increase in the first days of composting. However, maximum values of dehydrogenase (0.54 mg TPF g $DM^{-1}h^{-1}$) were observed at the end of thermophilic stage or at the beginning of mesophilic stage. They concluded that dehydrogenase is a useful parameter to follow the evolution of the biological activity of the composting process, since it correlates well with the temperature profile in the reactor.

Phosphatase is group of enzymes that catalysis the hydrolysis both esters and anhydrides of H_3PO_4 (Page 1982). Phosphatase has agronomic value because it hydrolyses compounds of organic phosphorus and transforms them into different forms of inorganic phosphorus assimilable by plants. The phosphatase activity is due to the presence of phosphorylated compounds, a substrate for the microorganisms to synthesize phosphatase. It is considered as a general microbial indicator. Phosphatase is an enzyme for the characterization of microbial activities during composting, since it can only be synthesized by microorganisms but is not originated from plant residues (Raut et al. 2008). Phosphatase includes phosphomonoesterases. phosphomonoesterases or phosphoric monoester hydrolases include acid and alkaline phosphomonoesterase (which hydrolyse monoester bonds including mononucleotides and sugar phosphates). Acid and alkaline phosphomonoesterases do not hydrolyse phosphates of phytic acid (myo-inositol hexaphosphates) but they can hydrolyse lower-order inositol phosphates (Nannipieri et al. 2011).

β-Glucosidase is one of the key enzymes governing the C-cycle. It hydrolyses reducing terminations of b-D-glucose chains and form b-glucose. Its activity is therefore indicative

of the presence of these terminations, which come from the labile organic matter (Kayikçioğlu and Okur 2011; Vargas-Garcia et al. 2010).

Garcia et al. (1993) characterized biochemically three groups of urban wastes used in agriculture, (fresh MSW, fresh sewage sludge, and the composted products of both). Five hydrolase activities in the cycles of C (β-glucosidase), N (urease and protease) and P (phosphatase) were determined. Total urease activity was found to be the highest in the sewage sludge, with variable values being observed in the fresh MSW and low values in the compost. Protease showed quite low values in all cases. They confirmed that the hydrolytic enzymes were biomarkers of the state and evolution of the organic matter.

Kayikçioğlu and Okur (2011) evaluated the enzyme activities during composting of tobacco waste (TW), a mixture of TW and grape pomace (GP), and a mixture of TW and olive pomace (OP). They found that the maximum values of dehydrogenases activity probably corresponded to the end of the thermophilic stage or the beginning of mesophilic stage, as the highest temperature in the composts was determined at the second week of composting processes. β-Glucosidase activity increased during the first 5 weeks and then the activity in TW and TW+GP composts decreased until the 17th week. Results indicated that this enzyme activity was related to the type of humic compounds and humic enzyme complexes which are resistant to microbial attack accumulated.

2.6.4 Maturity and Stability Assessment for MSW Composting

To evaluate the maturity and stability of compost effectively, easy, rapid and reliable testing methodologies for all kind of composts should be developed and applied (Castaldi et al. 2008). Since the origins of compost are different, maturity and stability are not

described by a single property and the combination of multiple parameters is desired for a comprehensive evaluation (Wong 1985).

It is found that SOUR increased with age of compost and presents a consistent trends and highly significant correlations with processing time, thus respiration was suggested as a suitable indicator for compost stability (Hellebrand and Schade 2008). Wu et al. (2000) indicated that, total nitrogen, total phosphorus, total volatile solid, C/N ratio, and HA /FA cannot be considered as a promising indication of compost maturity and stability, because they did not show a consistent trend for different waste feedstock. They found that the respiration test and bioassay test represent different properties of compost, and both CO_2 evolution and seed germination test are needed to be able to assess the compost stability and maturity.

Saha et al. (2010) measured compost stability and maturity by CO_2 evolution for MSW compost produced in 29 cities in India. They concluded that Compost respiration did not have significant correlation with C/N ratio. As C/N ratio is more related to feedstock composition than maturity. Heavy metal content is also considered another important quality parameter necessary in their study for protecting the soil and water resource from pollution.

The HA/FA ratio was also used to describe the relative speed of HA and FA transformation as well as the maturity of the final compost of food waste. Fourier transform infrared spectroscopy (FTIR) which is one of the most important and efficient techniques for monitoring the changes in the functional groups during the humification process of composting has been used by Zhou et al. (2014). The absorption peaks that

express the chemical bonds stretch and bending vibration after energy level transition, explain the changes of chemical structures. HA/FA ratio was high at the end of the composting and that the reduction of aliphatic and carboxylic groups and the increase of aromatic groups demonstrated by FTIR results indicated the stability and maturity of the compost and an efficient humification (Zhou et al. 2014). Kim et al. (2008) also used E4/E6 ratio, which shows the ratio of the humic acid and fulvic acid in the compost, as an assessment index for the molecular weight of humic substance for evaluating maturity of compost during the composting of MSW. EC as a salt content index and heavy metal content of the final compost were measured for final compost quality evaluation. They found the concentrations of heavy metals increased after composting. This observation might be due to the concurrent decrease in the organic matter content of the composting materials (Kim et al. 2008). Iglesias Jiménez and Perez Garcia (1989) stated humic acid-like carbon to fulvic acid-like carbon (C_{ha}/C_{fa}) ratio might constitute a valid parameter to establish the evolutional grade of the organic matter during city refuse composting and therefore of the degree of compost maturity.

Zmora-Nahum et al. (2005) used dissolved organic carbon (DOC) concentration as a maturity index to predict plant response upon compost application. In the MSW compost the plant biomass increased dramatically as the DOC decreased, but it reached its maximum weight long after the DOC concentration reached it constant level.

2.6.5 Maturity and Stability Assessment for Fishery Waste Composting

Numerous methods or indicators were applied to determine the maturity and stability of fishery waste compost including moisture content (Minkara et al. 1998), pH (Line 1994; Minkara et al. 1998), temperature, EC, ash content (Martin et al. 1993), mineral N (NH_4-N and NO_3-N) (Mathur et al. 1986; Roy et al. 1997), total N and total C (Liao et al. 1995a; Minkara et al. 1998; Vizcarra et al. 1993), extractable phosphorus (P) and potassium (K) (Tarnai 2009), Ca, Mg and Na (Mathur et al. 1986), total phosphate (Laos et al. 1998), total carbohydrate (TCH) (Martin et al. 1993), total lipids (Martin et al. 1993), total organic carbon (TOC) (Cathcart et al. 1986; Liao et al. 1994; Martin et al. 1993; Vizcarra et al. 1993), heavy metal content (Line 1994), residual toxicity (Line 1994), WSC, volatile fatty acids (VFAs) (Laos et al. 1998; Liao et al. 1995a; Liao et al. 1994; Vizcarra et al. 1993), phytoptpcixity (Line 1994; Mathur et al. 1986; Roy et al. 1997), oxygen concentration (Minkara et al. 1998), Carbon dioxide generation rate (Cathcart et al. 1986), concentration of H_2S and NH_3 in exhaust gas (Mathur et al. 1986), total microbial numbers (Roy et al. 1997), and self-heating test (Illera-Vives et al. 2013; Minkara et al. 1998).

The parameters are measured to evaluate the maturity and stability depend on the final use of the compost. For example in order to use compost as ecological organic amendment following parameters were measured; particle density, bulk density, total pore space, air capacity, easily available water, water buffering capacity, unavailable water, cation exchange capacity, micronutrients (Cu, Fe, Mn, and Zn), potentially toxic metals (Cr, Hg, Ni, and Pb), sanitization (the levels of Escherichia coli and Salmonella in

the compost) and self-heating test (Illera-Vives et al. 2013) and if the hydrolysate of fishery waste compost is intended to be employed for biotechnological applications TCH measurement is useful (Martin et al. 1993).

Laos et al. (1998) found during the fishery waste composting, values of total nitrogen (TN), TOC/TN and pH did not show a clear trend while in most cases EC, NH_4-N and WSC decreased significantly. They suggested that one of the more adequate parameters to predict compost maturity is WSC/TN $\leq$ 0.7. Fishery waste compost, in their study, met this requirement during summer but WSC/TN of composting in winter was 1.2 which needed additional days to achieve maturity. They concluded that fish offal composting with wood shaving by the in-vessel system was adequate to endure pathogen reduction and organic matter stability. The VFAs also decreased to non-detectable values in the case of butyric, isobutyric and propionic acids, but the acetic acid trend was erratic. Fish waste compost showed higher release of available N at a constant rate of 12% and less soil retention of bioavailable P (Laos et al. 1998).

The presence of VFAs was determined by a head-space gas chromatography (HS-GC) analysis technique (Liao et al. 1997; Vizcarra et al. 1993). It has been argued that the best indicator of compost maturity is the absence of bioinhibitory aliphatic acids and phenolics. Vizcarra et al. (1993) suggested VFAs are a reliable indicator of compost maturity since VFAs are the intermediate products of the biodegradation which occurs in the composting process. An increase in these compounds should therefore reflect the degree of microbial activity and the progress of decomposition (Liao et al. 1997; Vizcarra et al. 1993). In contrast to traditional indications of maturity, the results from HS-GC by

Vizcarra et al. (1993) indicated that the 4 month old composts had not yet reached biomaturity, whilst Liao et al. (1995a) foundlings showed after 102 days of salmon waste composting, the level of VFAs and phenols were low enough that compost reaches maturity which is substantially indicated by traditional indicators of maturity (e.g: temperature, C/N ratio, pH). Liao et al. (1994) used HS-GC method also to determine the organic acid content. After 60 days, the composts still contained very high concentrations of organic acids which are considered to be phytotoxic. Level of acetic, propionic, isobutyric, butyric and isovaleric acids were very high, so it can be concluded the composting pile was still very active after 60 days. In the active stage of composting, the concentrations of VFAs and ammonia were increasing, whereas during the maturing stage of the process their concentrations were declined (Liao et al. 1995; Liao et al. 1995a; Liao et al. 1994).

In Liao et al. (1995) set of experiments, the ammonia levels increased rapidly in the first 3 days of composting, stayed at that level for a period of 6-9 days, then finally declined. During this period, the growing concentrations of ammonia in the composting piles appeared to coincide with greater microbial activity and therefore with more efficient composting. At the same time that the ammonia levels were increased rapidly, there was a rapid increase in temperature. Above 45°C, this began to level out. When temperatures eventually exceeded 60°C, the ammonia concentrations started to decline. This pattern offers support to the assumption that ammonia production is a good indicator of the effectiveness of the composting process in the active stage. That is, the period in which

greater concentrations of ammonia were detected in the composting pile with more microbial activity and more efficient composting (Liao et al. 1995).

Laos et al. (1998) used degree of decomposition which is ratio of mass of CO_2 generated to initial compost dry mass variable for compost quality evaluation since the quantity of carbon dioxide generated by the composting microorganisms is directly proportional to their activity and to the amount of organic material consumed (Laos et al. 1998).

Line (1994) found sodium, calcium and magnesium levels were significantly higher in the seastar compost than in either of the other materials (sewage sludge and dairy manure) while seastar compost mix (seastar compost, 60%; peat, 10%; sand, 30%; lime, 2 g $litre^{-1}$) would be well suited for use as an organic mulch for application to agricultural soils and it did not show toxicity to earthworms (Line 1994).

Eggen and Vethe (2001) used pH, total solids, total carbon, total nitrogen, NH_4-N and NO_3-N, TOC, respiration rate, WSC, WSN and fractionation of humic substances to compare as the stability indices for paper mill sludge compost, fish waste compost treated in static reactor, biowaste compost from windrow system and biowaste compost from small scale composting systems. They found that fish waste compost contained significantly more inorganic WSN than the other composts. Water-soluble TOC was the only parameter that correlated significantly with respiration rate for all samples. Two of the fish waste compost samples at different stages of composting showed low respiration rate even though water soluble TOC values were very high (Eggen and Vethe 2001).

Stability was tested indirectly by the autoheating method through estimation of resistant organic matter present in the sample. The result can be expressed as the percentage ratio

of resistant organic matter (ROM) to total organic matter (TOM), %DS = (ROMx100/TOM). Auto-heating showed the difference between the inside and outside Dewar flask to be less than 10ºC, which indicates maturity of compost (Cathcart et al. 1986).

Volumetric shrinkage results from the loss of moisture and of biodegradable organic matter, and from compaction due to the overlying weight of composting materials was also measured as an important parameter for indicating the progress of composting (Liao et al. 1994). Weight loss during clam waste composting has been used to evaluate composting process; the total weight loss was significantly affected by the ratio of clam waste to woodchips. There were more than 30% weight losses for the ratios of 1:0.5, 1:1 and 1:1.5; while the weight loss for the ratio of 1:2 was about 21%, and the ratio of 1:3 was only 13%. The weight loss is due to the decomposition of easily degradable organic matter and leachate loss.

The matured clam waste compost had high EC value and such ‘‘salty” characteristics should be considered when applying the compost for plant growth (Hu et al. 2009). Mathur S. I. (1988) tested the phytotoxicity. They concluded the GI in the compost was higher than 95 % in all samples, which according to Zucconi et al. (1981) indicates the absence of phytotoxic substances or the presence of only very low levels.

Numerous methods or indicators could be applied for determining the maturity of a compost, but none work equally well for a variety of composts (Martin et al. 1993). (Eggen and Vethe 2001) concluded that different types of compost yield different chemical relationships with respiration rate. Fish waste compost, for example, showed

quite different – and occasionally opposite– index value trends compared with the other types of compost. Therefore, it appears that specific stability indices and index reference levels are required for specific types of compost (Eggen and Vethe 2001). Final fish waste compost properties are presented in Table 2.8.

Table 2.8 Final fish waste compost properties

Waste mixing ratio	C (%)	N (%)	C/N (%)	pH	EC (mS /cm)	OM	References
Fish waste: seaweed: pine bark 1:1:3 (v/v)	46.28±2	2.11±0.03	21.9±0.78	6.68±0.14	2.47±0.23	79.88±3.45	(Illera-Vives et al. 2013)
Bark: seastar waste 3:1 (v/v)		1.56	-	7.1	2.56	-	(Line 1994)
Salmon : sawdust (1:2)	37.7	3.63	10	5.9	-	79.6	(Eggen and Vethe 2001)
Fish waste: wood debris (3:1)	48.8	0.8	61	5.06	2.06	-	(Sen 2010)
Fish, seaweed and pine bark (1:1:3).	47.97 ± 0.15	2.13 ± 0.11	22.56 ± 1.24	7.08 ± 0.07	1.45 ± 0.01	-	(López-Mosquera et al. 2011)
Clam waste : woodchips 1:1 (w/w, wet weight).	449.8 ± 7.8	30.8 ± 2.2	14.6	6.54 ± 0.11	14.5 ± 1.3	833.0 ± 14.1	(Hu et al. 2009)
Blue crab scrap : wood by-products	23.5	0.97	24.3	7.64	3.2	33.69	(Brinton and Gregory 1992)
Scollap viscera:sawdust:red algae:tree trimming 2:0.5:1:1	-	0.31	78.4	-	2.4	45.4	(Brinton 1990)
Salmo-morts + sawdust 2:1	52.5-52.8	2.87-3.2	16.4-18.4	8.3-8.8	-	-	(Liao et al. 1994)
Crap scrap +peat	31.7	2.61	12.14	7.75	-	63.4	(AOAC 1995)
Various fish waste :peat	35.7	1.35	29.9	6.9	-	68.7	(Bimbo 2012)
Atlantic Marine Compost	13.63	0.63	21.98	7.9	-	-	(Bimbo 2012)
Fish offal:sawdust:yard trimming	477 -480 g/kg	27 -30 g/kg	16-18	6.3-6.8	2.4 -3.5	-	(Laos et al. 2002)

2.7 Design of Experiments in Composting

Many factors can influence composting process and the quality of the end product. Design of experiments is an effective tool to research the effect of these factors and their interaction. Although in the literature the effect of one factor at time has pronominally been used to conduct the experiments, some literature used different methods of experimental design to perform the experiments (Antony, 2003). Liang et al. (2003) investigated the influence of temperature and moisture contents on the aerobic microbial activity of bio-solid (municipal wastewater treatment sludge) composting using 2 factorial design method with six temperatures and five moisture contents. They concluded that the moisture content can affect microbial activity so that a higher microbial activity accrues at higher moisture content.

The effect of bulking agent/sludge ratio, bulking agent particle size and composting volume on the compostibility of the municipal wastewater sludge has been studied by a full factorial design (Leiva et al., 2003). The mixture 1:1 of sludge and wood chips was indicated as the optimum value for laboratory scale sludge composting. It was concluded that the experimental design is a valid tool to determine the initial operation condition for the composting of raw sludge.

Paradelo et al. (2010a) used 3^3 fractional factorial design to study the optimal condition for the composting of the hydrolyzed Grape marc and vinification Lees, in which three dependent variables (temperature, addition of vinification lees and addition of $CaCO_3$) were assayed at three levels. The proportion of vinification lees in the mixtures was the

factor with the main influence in the final nutritive properties of the composs. The result of the DOE suggested 1:1 mixture of hydrolyzed grape marc and vinification lees, amended with no more than 5 g of $CaCO_3$ per 100 g of hydrolyzed as the optimum value. Central composite design (CCD) was used by Bueno et al. (2008) to study the influence of moisture, aeration, particle size and time on the nitrogen conservation during legume trimming residues composting.

It was concluded that the experimental design methods are valid tools to evaluate the effect of composting process variable and to determine the initial operation condition for the composting (Leiva et al. 2003).

A few studies have applied DOE (e.g., factorial designs) to study the effects of a few factors such as temperature and moisture (Liang et al. 2003); temperature, aeration and moisture (Suler and Finstein 1977); operation volume, bulking agent particle size and bulking agent/sludge volume ratio (Leiva et al. 2003) on the performance of composting system. The investigated factors were not comprehensive enough to illustrate their effects and interactions on the performance of a composting process. Enzymatic activities could apparently give interesting information on the rate of decomposition of organic matter and, therefore, on the produced compost stability (Jurado et al. 2014), however, they have never been reported as responses for optimizing MSW composting based on DOE methods as it is reported in Table 2.9.

Table 2.9 Summary of DOE, factors and responses for composting

Raw material	Factors and their levels	Responses	Method	Reference
Hydrolyzed grape marc (HGM)	Temperature(20-50 °C), concentration of vinification lees (5-100 g/100 g HGM), concentration of $CaCO_3$ (g of $CaCO_3$/100 g of HGM)	OM, total organic C (TOC), total N (TKN), EC and pH, water-soluble ammonium and nitrate, water-soluble carbon (WSC), water-soluble Mg, K, P, Na and GI	3^3 Factorial design	(Paradelo et al. 2010)
Sludge	Temperature (22, 29, 36, 43, 50, and 57 °C), MC (30, 40, 50, 60, and 70%)	OUR, cumulative OUR, maximum OUR	2-factor factorial design	(Liang et al. 2003)
Legume trimming residues	MC (40, 55, 60%), AR (0.2 l, 0.41, 0.61 air/ min kg), particle size (1, 3, 5 mm) and time (78 days)	TKN, N-NO3, N-NH4 , C/N, P2O, N-losses, OM	Central composite experimental design	(Bueno et al. 2008)
Sludge	BA: sludge ratio (1:1 to 1:4 (w/w), BA particle size(0.5 to 2.5 mm), composting volume (1.5 to 25 L)	Relative heat generation	Factorial composite experimental design	(Leiva et al. 2003)
Pig feces and corn stalks	AR (0.24, 0.48, 0.72 L kg^{-1} DM min^{-1}), C/N ratios (15, 18, 21), and MC (MC: 65%, 70%, 75%)	GI, TN, TOC, NH_4-N, NO_3 -N and NO_2 –N. oxygen content , CO_2-C	3^3 Factorial design	(Guo et al. 2012
Pulp and paper mill sludge (PMS)	Incubation temperature (35°C and 55°C), nutrient addition (mineral nitrogen, phosphorus and	cumulative dry weight loss as $C0_2$, cumulative nitrogen loss as NH_3 and final C:N	2x2 factorial design	(Jackson and Line 1997)

Raw material	Factors and their levels	Responses	Method	Reference
	potassium) (full nutrient addition (FNA) and an incremental nutrient addition (INA))			
MSW (mixed paper waste food waste and yard waste)	Absence or presents of a component or the seed (compost from MSW composting facility) from the digester	CO_2-C and NH_3- N yields and production rates	Factorial design and mixture experiment al design	(Komilis and Ham 2006)
Paper mill sludge with broiler litter	Temperature (35, 45, 55, 60, and 65°C) and Initial MC (30, 35, 40, 45 and 50%)	Rates of decomposition based on captured CO_2, final ash contents, MC, oxygen concentration	5x5x2 factorial design	(Ekinci et al. 2004)
Mixture of dewatered sludge, food waste, mixed paper waste, sawdust and branches	Absence or presents of a component in such a manner, so as to maintain initial moisture contents of the resulting mixtures between 56% and 65% (w/w) and initial C/N between 19 and 30	MC, OM, total C and total N, CO_2, O_2 and CH_4 concentrations, NH_4 concentrations	Mixture experimental design	(Komilis et al. 2011)
Barley dregs and sewage sludge	Mix ratio of barley dregs/sewage sludge and MC	Carbon decomposition rate (CDR) and total volatile solids (TVS) loss rate, CO2 evolution rate (CER), MC, and pH	central composite design (CCD)	(Lu et al. 2008)
MSW	AR (0.005–0.300 L/ kg) and MC (40–70 %)	Volatile organic compounds (VOCs) (limonene, b-pinene, 2-butanone, undecane, phenol, toluene, dimethyl sulfide, dimethyl disulfide),	Two level, full factorial design	(Delgado-Rodríguez et al. 2012)

Raw material	Factors and their levels	Responses	Method	Reference
		temperature		
MSW – Legume trimming residue	MSW– Legume trimming residue mixing ratio (1:0, 1:1, 1:2 (w/w)), AR (0.05, 0.175, 0.3 L/Kg.min), MC (40, 55,70%), C/N ratio (21,60, 77),	Chemical oxygen demand (COD), nitrate, Nitrogen-losses (%) and biodegradability, coefficient	Factorial design	(Cabeza et al. 2013)
Vinasse/cotton waste mixtures	Vinasse added (11 to 69 % (w/w) and time of operation (7 to 45 days)	pH, TKN, C/N ratio, biodegradability, Kjeldahl-N losses, and GI	Central composite experimental design	(Dıaz et al. 2003)
Wastewater sludge	Initial MC (20–70%) and BA particle size distribution (8–>40 mm)	OM, total carbon, COD, OUR, airflow dispersion coefficient	Central composite factor design	(Trémier et al. 2009)
Urban sludge	Pile depth (-2.4 to -2m), MC (50 to 65%), particle size (<20 mm to >20 mm), and type of BA (recycled and fresh wooden pallets)	Bulk density, free air space, air permeability, and thermal conductivity	Factorial design	(Huet et al. 2012)
MSW	Effects of protein and fat	CO_2 and O_2 concentrations, temperatures, pH, MC, ash, TKN, and total carbon	Mixture experimental design	(Chang and Hsu 2008)
MSW and green waste	MC (45–75%) and C/N ratios (13.9–19.6)	MC, pH, temperature, volatile solids, water soluble total organic carbon (TOCW), and water soluble total Kjeldahl nitrogen (TKNW), germination index, relative root growth	Central composite design	(Kumar et al. 2010)
Food waste	Effects of rice husk, sawdust and rice bran	Composting and acidification times, lowest and final pH	Mixture experimental	(Chang and Chen 2010)

Raw material	Factors and their levels	Responses	Method	Reference
		values, highest temperature, water-soluble organic carbon to water soluble organic nitrogen (COW/NOW ratio), water soluble organic carbon to total organic nitrogen (COW/NOT) ratio, CO_2 and O_2 concentrations	design	
MSW	AR (0.4, 0.6 and 0.9) L $min^{-1}kg^{-1}$	C/N ratio, NO_3–N, nitrogen, potassium and phosphorous	Nested design method	(Rasapoor et al. 2009)
MSW	AR (0.3 and 0.5 L/min.kg), MC (55 and 70%), BA (sawdust and peat), C/N ratio (12 and 17)	Cumulative dehydrogenase activity, cumulative β-glucosidase activity, cumulative phosphodiesterase activity, final C/N ratio, final GI, and final moisture content	Factorial design	Present work

2.8 Compost Usage

Compost is used generally as soil conditioners to promote soil aggregation, improve the soil water retention capacity and encourage the more extensive development of root system. It also improves soil structure by adding organic matter and the breakdown of organic matter results in slow release of phosphorus and nitrogen in a rate that can be utilized by the plants. Compost also is used as a mulch to conserve moisture, improve soil structure and hold down weeds in soil. In summer it will keep soil temperature down and in winter it will prevent frost around the plants. The performance of fishery waste compost produced at Lake Michigan from fish offal as a fertilizer was comparable to commercial composts. The concentration of heavy metal was acceptable to be used in land and their concentration was lower than sewage sludge concentration (Frederick 1989). Illera-Vives et al. (2015) found compost from fish waste and seaweed is suitable soil amendment for horticultural crops grown, as indicated by the tomato and lettuce yields. The application of compost at a rate of 66 t/ha significantly increased the tomato yield and was associated with increased fruit weight and larger fruit diameter compared to crops receiving mineral fertilization or no fertilization (Illera-Vives et al. 2015).

Roy et al. (1997) developed a novel composting process characterized by two thermophilic phases to produce chitin waste based compost. Chitin has been used as a soil amendment to control fungal diseases and root parasitic nematodes. Shrimp waste was added after the first thermophilic phase to compost from cow manure, peat moss and pine sawdust (in a 1 :1 :1 proportion). Compost obtained potentially has phytoprotective

properties and sufficient concentration of oligomeric chitin to elicit successfully the defense reactions in plants. The disease suppressive properties of such composts will be very attractive for applications in horticulture and in biological agriculture (Roy et al. 1997).

Although compost has mainly been used in agriculture as a fertilizing agent and soil amendment, its extracts is increasingly could be employed as an inexpensive nutrient source for fermentation processes and support the mycelial growth. It could also be employed in environmental applications, such as in packed columns for the biofiltration of liquid and gas effluents (Martin 1999). Also new uses such as bioremediation and pollution prevention are being suggested for compost which help save money, and conserves natural resources (Keener 2010).

2.9 Biosurfactant Production

2.9.1 Biosurfactant Definication and Characteristics

Biosurfactants are surface-active metabolites produced by microorganisms (Heyd et al. 2008). They possess both hydrophilic and hydrophobic moieties and are able to display a variety of surface activities that, among other roles, help solubilize hydrophobic substrates and lead to the reduction of surface tension and interfacial tension (Satpute et al. 2010). Microorganisms synthesize a wide variety of high- and low molecular- mass biosurfactant. The low-molecular-mass biosurfactant lower surface and interfacial tensions, whereas the higher-molecular-mass biosurfactant are more effective at stabilizing oil-in-water emulsions (Rosenberg et al. 2013). Biosurfactants are classified

based on their chemical structure and the organisms that produce them (Makkar et al. 2011). The hydrophilic parts of biosurfactants can be polysaccharides, phosphates, amino acids, carbohydrates, polyhydroxy structures, andcyclic peptide, while the hydrophobic parts are usually made up of aliphatic hydrocarbons (Mao et al. 2015). Diverse functional properties namely, emulsification, wetting, foaming, cleansing, phase separation, surface activity and reduction in viscosity of crude oil, makes it feasible to utilize them for many application purposes (Satpute et al. 2010). Biosurfactants can potentially replace virtually any synthetic surfactant and, moreover, introduce some unique physico-chemical properties (Reis et al. 2013). Biosurfactants have countless advantages in comparison with chemical surfactants, especially regarding biodegradability, compatibility with the environment, low toxicity, high selectivity and their activity even in extreme temperature, pH and salinity conditions (Souza et al. 2014).

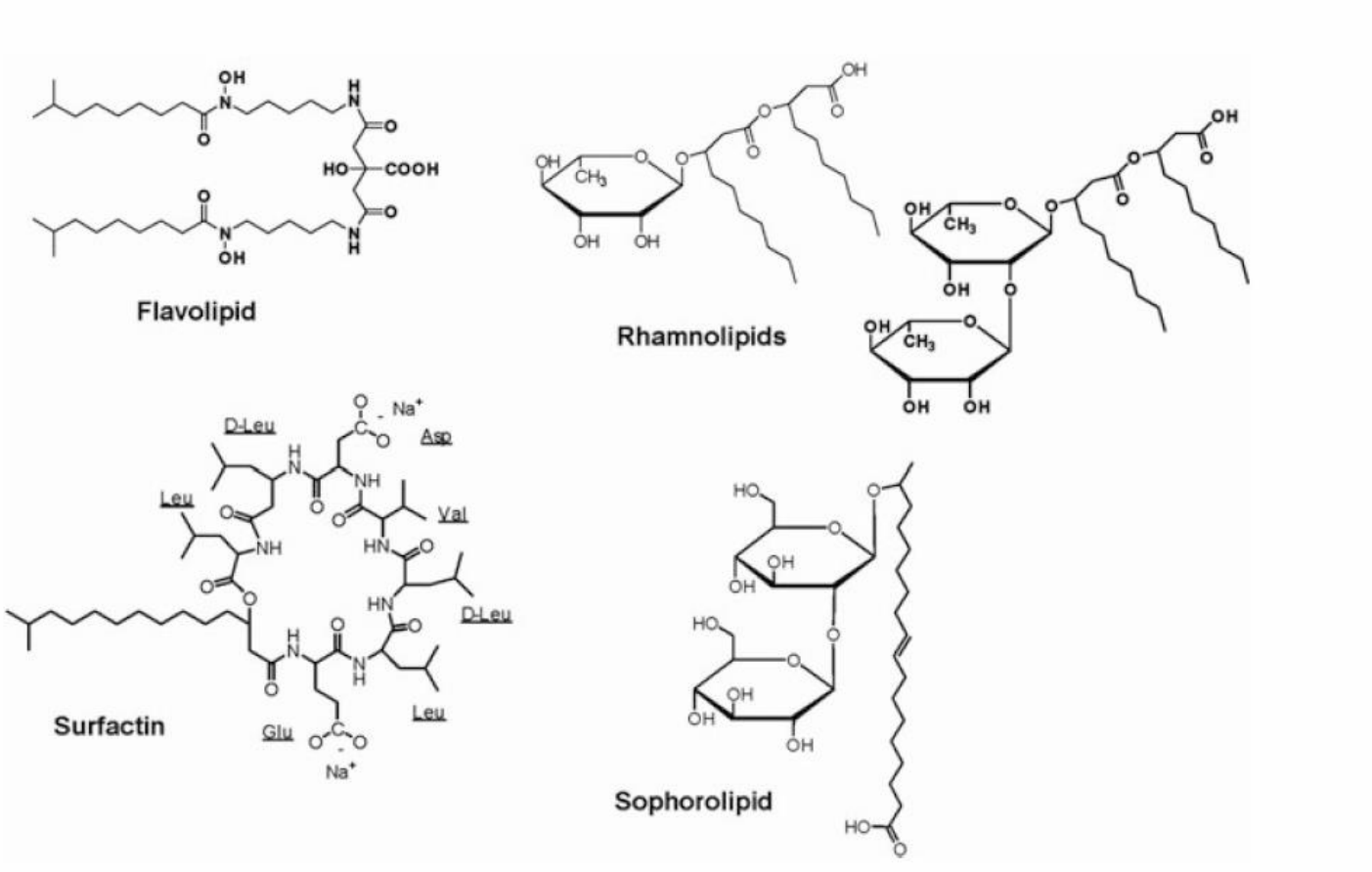


Figure 2.8 Representative structures of biosurfactants (Soberón-Chávez 2010)

Three natural roles for biosurfactant have been proposed: (1) increasing the surface area of hydrophobic water-insoluble growth substrates, (2) increasing the bioavailability of hydrophobic substrates by increasing their apparent solubility or desorbing them from surfaces, and (3) regulating the attachment and detachment of microorganisms to and from surfaces (Rosenberg et al. 2013). With these natural roles, biosurfactants have many environmental applications such as bioremediation and dispersion of oil spills, enhanced oil recovery and transfer of crude oil. Other potential applications of biosurfactants relate to food, cosmetic, health care industries and cleaning toxic chemicals of industrial and agricultural origin (Saharan et al. 2011). Biosurfactants have potential for stabilising emulsions between liquid hydrocarbons and water, thus increasing the surface area available for bacterial biodegradation. In addition, above the Critical Micelle Concentration (CMC), a significant fraction of the hydrophobic contaminant partitions in the biosurfactant micelle cores which results in a general increase in the bioavailability of contaminants for degrading microorganisms(Banat et al. 2010). In oil spill responses, the most common role of biosurfactants is to enhance the dispersal of contaminants in the aqueous phase and increase the bioavailability of the hydrophobic substrate to microorganisms, with subsequent removal of such pollutants through biodegradation (Silva et al. 2014).

The production economy of every microbial metabolite is governed by three basic factors: (1) initial raw material costs; (2) availability of suitable and economic production and recovery procedures and (3) the product yield of the producer microorganisms. Despite all advantages, the application of biosurfactants has been significantly restricted

due to their low yields and high production cost as well as the lack of desired producing microorganisms (producers) (Montgomery 2008). It was estimated that biosurfactants would cost 3-10 times of synthetic surfactants (Mulligan and Gibbs 1993). The biosurfactant surfactin (98% purity) available from Sigma Chemical Company costs approximately $153 for a 10 mg vial. In comparison the cost of chemical surfactants is around one dollar/lb (Makkar et al. 2011). Currently there is only a very limited offer of commercially available biosurfactants, e.g., surfactin, sophorolipids and rhamnolipids (Sen 2010).

The production of biosurfactants with the use of renewable substrates and different microbial species as well as the variation in culture parameters (incubation time, stirring speed, pH of the medium and added nutrients) allow the acquisition of compounds with distinct structural characteristics and physical properties. This makes biosurfactants comparable to or even better than synthetic surfactants in terms of efficiency, although production costs do not yet allow greater competitiveness with their petrochemical counterparts (Sobrinho et al. 2013). Three basic strategies were adopted worldwide to make this process cost-competitive: (1) the use of cheaper and waste substrates to lower the initial raw material costs involved in the process; (2) development of efficient bioprocesses, including optimization of the culture conditions and cost-effective separation processes for maximum biosurfactant production and recovery and (3) development and use of overproducing mutant or recombinant strains for enhanced biosurfactant yields (Mukherjee et al. 2006; Saharan et al. 2011).

The use of the alternative substrates such as agro based industrial wastes is one of the attractive strategies for economical biosurfactants production. Another approach involves using raw substrates with negligible or no value. Main problem associated with this approach is the selection of suitable waste material with the right balance of nutrients that permits cell growth and product accumulation (Makkar et al. 2011). Examples of inexpensive raw material for biosurfactant production is reported in Table 2.10 (Montgomery 2008).

Table 2.10 Summary of inexpensive raw materials for biosurfactant production

Raw Material	Biosurfactant type	Producer microbial genus	Maximum Yields(g/l)
Rapeseed oil	Rhamnolipids	Pseudomonas	45
Babassu oil	Sophorolipids	Candida	-
Turkish corn oil	Sophorolipids	Candida	400
Sunflower and Soybean oil	Rhamnolipids	Pseudomonas	4.31
Sunflower oil	Lipopeptide	Serratia	-
Soybean oil	Mannosylerythritol lipid	Candida	95
Waste frying oil	Rhamnolipids	Pseudomonas	2.7
Soybean soapstock waste	Rhamnolipids	Pseudomonas	11.72
Sunflower oil soapstock waste	Rhamnolipids	Pseudomonas	16
Oil refinery waste	Glycolipid	Candida	10.5
Soybean oil refinery wastes	Rhamnolipids	Pseudomonas	9.5
Crude whey and distillery wastes	Rhamnolipids	Pseudomonas	0.92
Potato process effluents	Lipopeptide	Bacillus	-
Cassava flour wastewater	Lipopeptide	Bacillus	2.2-3.0

Moreover, Cell growth and the accumulation of metabolic products are strongly influenced by medium compositions such as carbon sources, nitrogen sources, growth factors, and inorganic salts. Thus, it is difficult to search for the major factors and to optimize them for biotechnological processes as several parameters are involved (Rodrigues et al. 2006). The use of different carbon sources alters the structure of the biosurfactant produced and its properties and can be exploited to get products with desired properties for particular applications (Saharan et al. 2011). Several studies have aimed to optimize the biosurfactant production process by changing the variables that influence the type and amount of biosurfactant produced by a microorganism. Important variables are carbon and nitrogen sources, potential nutrient limitations and other physical and chemical parameters such as oxygen, temperature and pH (Banat et al. 2010; Reis et al. 2013).

Experimental design techniques have been extensively used to optimise biosurfactant production. The use of RSM effectively enhanced the production of biosurfactant by Rhodococcus spp. MTCC 2574 growing on n-hexadecane with yields of biosurfactant increasing from 3.2 to 10.9 g/L (Banat et al. 2010). Rodrigues et al. (2006) optimized the medium for biosurfactants production by probiotic bacteria using RSM to obtain a higher cellular growth and higher cell-bound biosurfactant production yield. (Pal et al. 2009) compared two optimization techniques, (artificial neural network (ANN) coupled with genetic algorithm (GA) and RSM, that were used to enhance the yield of Rhodococcus biosurfactant by media. They observed that use of an organic nitrogen source gave higher cell mass than with inorganic nitrogen. Their results showed that ANN had better

generalization capacity, and ANN-GA was more accurate in predicting the optimum than RSM.

The commercial success of microbial surfactants is currently limited by the high cost of production. Optimised growth/production conditions using cheaper renewable substrates could make biosurfactant production more profitable and economically feasible (Banat et al. 2010).

2.9.2 Biosurfactant Producers

Biosurfactants are produced by a diverse group of microorganisms mainly bacteria, fungi, and yeasts from various substrates including sugars, glycerol, oils, hydrocarbons, and agricultural waste (Shekhar et al. 2015; Soberón-Chávez 2010). The quantity of biosurfactant production mainly depends on the type of microorganisms and their sources. Microorganisms play a major role in biosurfactant production (Shekhar et al. 2015). Microorganisms can carry out biosurfactant production when grown either on insoluble substrates (such as hydrocarbons, oils and waxes) or on soluble ones (carbohydrates) (Carrillo et al. 1996). Several reports have been published on screening and isolation of biosurfactant-producing microorganisms, their growth characteristics and on product type and efficiency (Banat 1993; Cai et al. 2014; George and Jayachandran 2013; Najafi et al. 2010; Rocha e Silva et al. 2014; Youssef et al. 2004).

Cai et al. (2014) collected samples from offshore oil and gas platforms in North Atlantic Canada, crude oil, formation water, drilling mud, treated produced water and seawater to screen for potential biosurfactant producers. They identified 59 biosurfactant producing strains from which 24 strains and 20 strains belonged to genera of *Rhodococcus* and

Bacillus, respectively. The most effective isolates were P6-4P (*Rhodococcus sp*.) and N3-1P (*Bacillus sp*.).

Bacillus sp. is mostly known for the production of lipopeptides, lichenysin, surfactin, lipid protein complexes, and subtilisin from the different marine ecosystems (Shekhar et al. 2015).*Rhodococcus sp*. is known for the production of glycolipid surface-active molecules. Different types of biosurfactants such as glycolipids, polysaccharides, free fatty acids, and trehalose dicorynomycolate are produced by *Rhodococcus erythropolis* (Shekhar et al. 2015). A summary of isolated biosurfactant producers and classification of produced biosurfactant is presented in Table 2.11 (Shekhar et al. 2015).

Table 2.11 Classification of biosurfactants based on the chemical nature and the source microorganisms

SR. No	Biosurfactant	Source
1	Glycolipids	*Rhodococcus erythropolis, Nocardia*
	Trehalolipids	*erythropolis, Arthrobacter* sp., *Mycobacterium* sp.
	Trehalose dimycolates	*Mycobacterium* sp., *Nocardia* sp.
	Trehalose dicorynomycolates	*Arthrobacter* sp., *Corynebacterium* sp.
	Rhamnolipids	*Pseudomonas aeruginosa, Pseudomonas* sp.
	Sophorolipids	*Torulopsis bombicola, Torulopsis apicola, Torulopsis petrophilum Torulopsis* sp.
	Cellobiolipids	*Ustilago zeae, Ustilago maydis*
2	Lipopeptides and lipoproteins	
	Peptide-lipid	*Bacillus licheniformis*
	Serrawettin	*Serratia marcescens*
	Viscosin	*Pseudomonas fluorescens*
	Surfactin	*Bacillus subtilis*
	Fengycin	*Bacillus* sp
	Arthrofactin	*Arthrobacter* sp.
	Subtilisin	*Bacillus subtilis*
	Gramicidins	*Bacillus brevis, Brevibacterium brevis*
	Polymyxins	*Bacillus polymyxa, Brevibacterium polymyxa*
	Lichenysin	*Bacillus licheniformis*
	Ornithine	lipids *Myroides* sp. *SM1, Pseudomonas* sp., *Thiobacillus* sp., *Agrobacterium* sp., *Gluconobacter* sp.
3	Fatty acids, phospholipids, and neutral lipids	
	Neutral lipids	*Nocardia erythropolis*
	Phospholipids	*Thiobacillus thiooxidans*
	Bile salts	*Myroides* sp.
	Fatty acids	*Candida lepus, Acinetobacter* sp., *Pseudomonas* sp., *Micrococcus* sp., *Mycococcus* sp., *Candida* sp., *Penicillium* sp., *Aspergillus* sp.
4	Polymeric surfactants	

SR. No	Biosurfactant	Source
	Emulsan	*Arethrobacter calcoaceticus*
	Biodispersan	*Arethrobacter calcoaceticus*
	Mannan lipid protein	*Candida tropicalis*
	Liposan	*Candida lipolytica*
	Carbohydrate protein lipid	*Pseudomonas fluorescens, Debaryomyces polymorphus*
5	Protein PA	*Pseudomonas aeruginosa*
	Particulate biosurfactants	biosurfactants
	Vesicles and fimbriae whole cells	*Arthrobacter calcoaceticus*

2.9.3 Substrate Extraction and Hydrolysis

Hydrolysis of food proteins has a long history, mainly for vegetable and milk proteins; these proteins are widely used in the food industry. Most work on the hydrolysis of fish proteins was conducted in the 1960s. Hydrolysates can be defined as proteins that are chemically or enzymatically broken down into peptides of varying sizes. Chemical and biological methods are the most widely used for protein hydrolysis with chemical hydrolysis used more commonly in industrial practices. Biological processes using added enzymes are employed more frequently, and enzyme hydrolysis holds the most promise for the future because it results in products of high functionality and nutritive value (Kristinsson and Rasco 2000). Enzymatic hydrolysis of fish protein has been employed as an alternative approach for converting underutilized fish biomass into edible protein products, instead of animal feed or fertilizer (Schaub and Leonard 1996). The raw material for fish waste hydrolysis is the material remaining after fillets are removed, and if viscera is included, this can represent something on the order of 64% of the weight of whitefish, the protein content of this waste being about 10%. Using added enzymes to hydrolyze food proteins is a process of considerable importance used to improve or modify the physicochemical, functional, and sensory properties of the native protein without jeopardizing its nutritive value, and often protein absorption is improved. Figure 2.9 outlines a fairly typical process for producing fish protein hydrolysates (Kristinsson and Rasco 2000). In the process of hydrolyzation, proteolytic enzymes are used to solubilize the fish protein, resulting in two distinguishable fractions, soluble and insoluble. The insoluble fraction may be used as animal feed and the soluble fraction,

which contains the hydrolyzed protein, may be converted into a food ingredient, incorporating into food systems, or used as a nitrogen source for bacterial growth. Dehydration of the soluble hydrolysate results in a more stable powder which is high in protein content. Such a product is known as fish protein hydrolysate (Arvanitoyannis and Kassaveti 2008). A flow sheet for the enzymatic hydrolysis of fish protein to make fish protein hydrolysate is presented in Figure 2.9.

RSM is a useful technique for the investigation of complex processes. It has been successfully applied to optimize seafood processing operations (Arvanitoyannis and Kassaveti 2008; Shahidi et al. 1995). RSM consist of a group of mathematical and statistical procedures that can be used to study relationship between one or more responses (dependent variables) and a number of factors (independent variables). RSM defines the effect of the independent variables alone, and in combinations, in the process. In addition to analyzing the effects of variables, this experimental methodology generates a mathematical model that accurately describes the overall process using a significant estimation (Arvanitoyannis and Kassaveti 2008; Diniz and Martin 1996). CCD is the most popular second-order RSM design. The design involves, F factorial points, 2k axial points and n_c center points (Figure 2.10). The areas of flexibility in CCD resides in the selection of α, the axial distance, and n_c, the number of center distance.

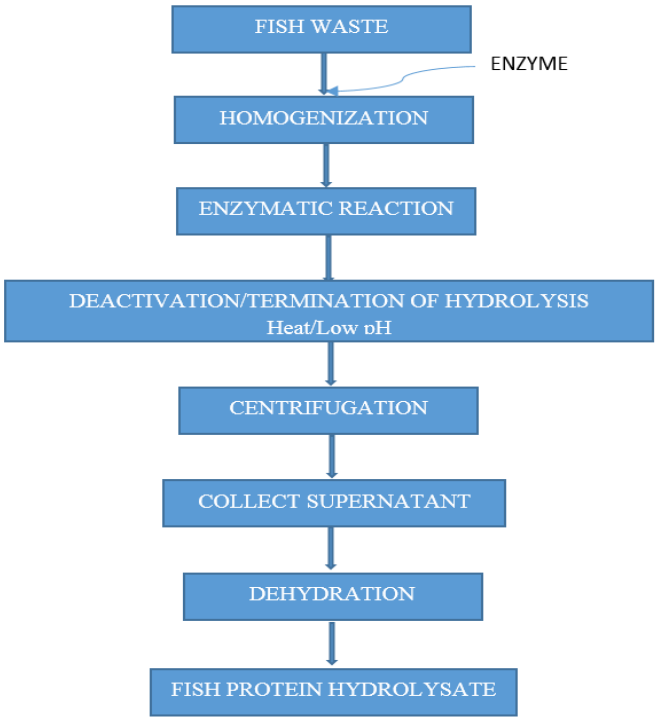


Figure 2.9 A flow sheet for the enzymatic hydrolysis of fish protein to make fish protein hydrolysate

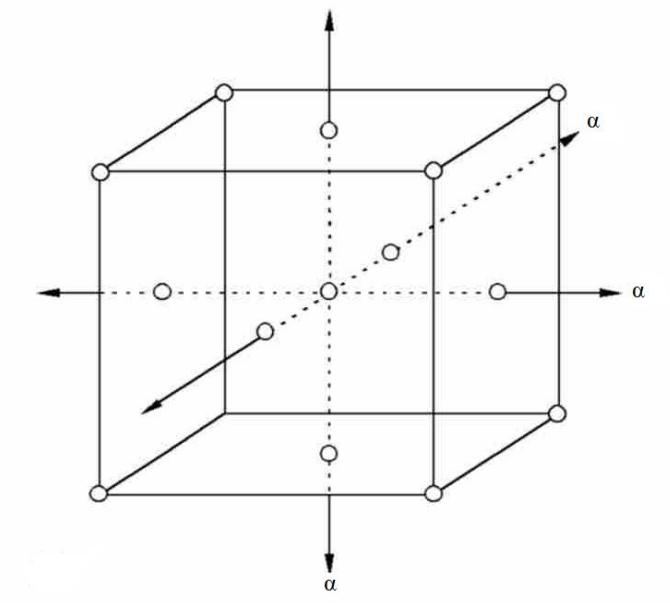


Figure 2.10 Central composite design for k=3

For fish waste hydrolysis, given a particular enzyme any hydrolysis process involves at least five independent variables. These are S (protein substrate concentration: %N × 6.25), E/S (enzyme-substrate ratio in % or in activity units per kg N × 6.25), pH, temperature, and time (Kristinsson and Rasco 2000). Arvanitoyannis and Kassaveti (2008) Optimized hydrolysis conditions (enzyme activity, temperature, and time) to produced hydrolysate from the viscera of yellowfin tuna (Thunnus albacares) using RSM. A factorial design was applied to minimize enzyme utilization and modeling of degree of hydrolysis. The protein efficiency ratio of tuna visceral hydrolysate was 2.85–5.35. The effect of temperature, pH, E/S ratio on degree of hydrolysis (DH) of dogfish muscle protein using RSM has been studied by Bernal et al. (2009). Their results indicate that all three factors markedly influenced the peptide bonds cleavage in the protein substrate.

The preferred commercial enzymes for most researchers are protease preparations of bacterial origin like Alcalase, Neutrase, Protease N and Protamex (Aspmo et al. 2005). Alcalase, an alkaline enzyme produced from Bacillus licheniformis and developed by Novo Nordisk (Bagsvaerd, Denmark) for the detergent industry, has been proven repeatedly by many researchers to be one of the best enzyme used to prepare functional fish protein hydrolysis and other protein hydrolysates (Kristinsson and Rasco 2000). Generally, Alcalase® 2.4-L-assisted reactions have been repeatedly favored for fish hydrolysis, due to the high degree of hydrolysis that can be achieved in a relatively short time under moderate pH conditions, compared with the neutral or acidic enzymes (Aspmo et al. 2005; Kristinsson and Rasco 2000; Rasapoor et al. 2009). Cod viscera were hydrolysed by endogenous enzymes alone or in combination with one of seven different

commercial protease preparations (Alcalase1 2.4L, Neutrase1 0.8L, ProtamexTM, Papain, Bromelain, Actinidin and a plant protease mix). Alcalase was clearly the most efficient enzyme when it comes to degrading the larger proteins of the substrate mixture. The concentration of a-amino groups in the hydrolysate may be used as an indication of the amount of peptide bonds broken in the reaction (Aspmo et al. 2005).

Approximately 15–17% of the weight of the cod is viscera. The cod viscera contained 19.0% DM, including 1.2% ash and 2.2% nitrogen (Aspmo et al. 2005). The effect of enzyme including Alcalase, Neutrase, Papain, and Autolysis has been investigated through RSM under different temperature, reaction pH, inactivation pH and E/S ratio by Liao et al. (1997). Although many factors affect the yield of hydrolysis, the type of enzyme used had a marked effect on the yield and properties of the final product. Neutrase had a much lower activity than Alcalase and was suitable only when a low degree of hydrolysis is preferred. High protein recovery by Alcalase and its low cost may provide an incentive for using it in commercial operations (Liao et al. 1997).

2.10 Summary

This chapter started with introduction of composting process. Some physiochemical properties of MSW and fish waste have been reviewed, followed by the factors that influence composting process including C/N ratio, moisture content, aeration and temperature. The review from this part concluded that composting is a viable mean for MSW and fish waste management. In addition, MSW and fish waste should be mixed with bulking agent to adjust their C/N ratio and moisture content. Subsequently, the

reviews extended to the composting technologies have been used for MSW and fish waste composting. The following section reviewed indices have been applied to evaluate compost maturity and stability. It has been revealed that a few studies have applied DOE (e.g., factorial designs) to study the effects of factors (e.g., moisture content, aeration rate, C/N ratio and bulking agent, temperature) on the performance of composting system, enzyme activities have never been reported as responses for optimizing MSW composting based on DOE methods. Additionally, enzyme activities never have been investigated to evaluate the state and evolution of fish waste composting. In the following section, compost usage was reviewed. One of the usages of compost extract is substrate for fermentation process. In the last following section of this chapter, biosurfactant has been introduced and using the waste material as substrate to reduce the biosurfactant production cost has been discussed. Then it was explored that fish waste compost extract has never been used as substrate for biosurfactant producing bacteria.

CHAPTER 3

PERFORMANCE OF LOCALLY AVAILABLE BULKING AGENTS IN NEWFOUNDLAND AND LABRADOR DURING BENCH-SCALE MUNICIPAL SOLID WASTE COMPOSTING [2]

[2] *This chapter is based on the following paper:*

Kazemi, K., Zhang, B., Lye, L. M., and Lin, W. (2014). Performance of locally available bulking agents in Newfoundland and Labrador during bench-scale municipal solid waste composting. *Environmental Systems Research*, *3*(1), 1.

Role: Khoshrooz Kazemi solely worked on this study and acted as the first author of this manuscript under the guidance of two supervisors, Dr. Baiyu Zhang and Dr. Leonard Lye. Weiyun Lin participated in conducting experiments. Most contents of this paper was written by Kazemi and further polished by the other co-authors.

3.1 Background

Population growth, aggregation of human settlements, higher living standards, and increased development and consumption of less biodegradable products have increased solid waste generation over the last 20 years (Adhikari et al. 2008; Asase et al. 2009). Municipal Solid Waste (MSW) management has thus become one of the biggest environmental concerns in recent decades (Iqbal et al. 2010). MSW contains high moisture content (60-70%) and large organic fraction (70-80%), posing adverse environmental impacts if it is not treated properly. Fortunately, the high organic fraction of MSW can be easily converted to energy sources through composting (Bradford 1976; Jolanun and Towprayoon 2010). Therefore, composting has become an increasingly important strategy for the treatment of MSW. Composting is a biological process in which easily degradable organic matter is stabilized and converted into a humus-rich product by the action of microorganisms (Eiland et al. 2001). The advantages of composting are diverting organic matter from landfills, reducing waste volume, decreasing the potential odour, decreasing the moisture content of MSW, and amending soil/improving soil quality (Arslan et al. 2011; Cronje et al. 2003; Hasan et al. 2012; Haug 1993).

Some environmental conditions (moisture content, aeration rate, pH, and temperature) and substrate characteristics (C/N ratio, particle size, bulking agents, nutrients contents, and free air space) affect the composting process (Iqbal et al. 2010). Selection of a bulking agent which should be inexpensive and readily available in the vicinity of the composting region is very important because bulking agents can affect the condition of

the starting composting mixtures, biodegradation kinetics and composting performance as well as the final compost quality (Blanco and Almendros 1995; Chang and Chen 2010; Jolanun and Towprayoon 2010). Bulking agents have different properties because of their carbon source, physical shape, particle size, water absorption capacity, and their bulking density (Iqbal et al. 2010). Bulking agents are usually fibrous and carbonadoes material with low moisture content; therefore they can absorb part of the leachate produced during decomposition to keep the moisture and sustain the microbial activity (Adhikari et al. 2008; Dias et al. 2010; Iqbal et al. 2010). The bulking agent provides structural support to prevent physical compaction, promotes porosity and air void, and improves the compost aeration and gas exchange (Adhikari et al. 2008; Dias et al. 2010; Doublet et al. 2011; Yañez et al. 2009). It can also act as a buffer against the organic acids in the early stages of composting and help maintain the mixture's pH within a range from 6-8 for proper microbial activity (Haug 1993), and adjust C/N ratio of the feedstock and encourage microbial activity without inhabitation (Jolanun and Towprayoon 2010). Numerous studies have used different bulking agents, which are mostly from agriculture byproducts. They include sawdust (Adhikari et al. 2008; Banegas et al. 2007; Blanco and Almendros 1995; Chang and Chen 2010; Martin et al. 1993; Yang et al. 2013), wheat straw (Banegas et al. 2007; Blanco and Almendros 1995), hay and pine wood shaving (Banegas et al. 2007), bagasse and paper (Adhikari et al. 2008), rice husk and rice barn (Chang and Chen 2010), wooden palette (Huet et al. 2012), cornstalks and spent mushroom substrate (Yang et al. 2013), wheat flour (Silva et al. 2014), peat (Martin et al. 1993; Mathur et al. 1986; Mathur et al. 1990; Nolan et al. 2011; Vuorinen 2000), and barley straw (Vuorinen 2000).

Sawdust is a by-product of cutting, grinding, drilling, and sanding of wood, and it is a very common and easily available bulking agent used in composting to provide the free air space, control moisture, and maintain the C/N ratio (Batham et al. 2013). Banegas et al. (2007) mixed aerobic and anaerobic sludge with sawdust in two ratios (1:1 and 1:3 v: v), and concluded that sawdust is a good bulking agent for sludge composting because of its dilution effect on the nutritional components of the compost. Iqbal et al. (2010) suggested that the effect of 40% addition of sawdust to MSW was best to optimize the moisture content to up to 60% in composting. Chang and Chen (2010) found more sawdust in the composting mixture resulted in the increase of the water absorption capacity and the composting rate, shorter composting and acidification times, and lower final pH value.

Peat is an accumulation of partially decayed vegetation or organic matter which has been used as a bulking agent because it has high water absorption capacity, is rich enough in exchangeable H^+ ions to neutralize the ammonia and the cations released by decomposition prevents the loss of ammonia by remaining slightly acidic environment throughout the composting process. Peat has the capacity for adsorbing anions and retarding the leaching of NO_3^- and PO_4^{-3} when added to soil. It is fluffy to provide thermal insulation and replaceable air to prevent anaerobic production of malodours, and also has an exceptionally high capacity for enhancing soil organic matter (Mahtur et al. 1990).

In Canada, Newfoundland and Labrador (NL) has the highest quantity of waste disposal per capita after Alberta. This amounts to about 429 kg of residential waste per capita

(Pande et al. 1963). NL comprises more than 200 small communities with population between 100 and 600. Most of these small communities are located in remote and isolated areas and cannot access large solid waste disposal sites or central organic processing facilities. Therefore, on-site composting facilities have been considered as a viable means to deal with organic wastes in the small communities. Although a lack of extensive agricultural production in the northern region of NL could limit the selection of bulking agents for composting, NL generally possesses extensive peat resources. In addition, the forestry industry in NL produces wastes organic materials in the form of sawdust, bark, and wood chips, which can be used as the bulking agent for MSW composting (Martin et al. 1993). The food waste constitutes approximately 40% of the MSW and it represents a significant proportion of organic material found in MSW. Diversion of food waste from landfill since it is the biggest organic stream in municipal solid waste is essential to reach high diversion target (Dubois et al. 1956). Therefore, detailed knowledge of the performance of the composting process with locally available bulking agents would allow the improvement of community-scale composts quality in the small communities of NL.

For compost quality assessment and practical use of composted materials in agriculture, maturity and stability indices are important (Mondini et al. 2004). Stability can be expressed by biological indicators such as the respiration index (i.e., oxygen uptake rate (OUR) or CO_2 evolution rate) and enzyme activity (Benito et al. 2003; Bernal et al. 2009; Wu et al. 2000). Important enzymes involved in the composting process include dehydrogenase activity for substrate oxidization by a reduction reaction, β – glucosidase

activity for glucoside and amide hydrolysis, as well as phosphodiesterase activity for phosphate removal from organic compounds (Mondini et al. 2004). Maturity refers to the degree of decomposition where the compost does not pose any adverse effects on plants and growth of various crops (Castaldi et al. 2008; Zmora-Nahum et al. 2005). It is commonly reflected using the germination index (GI). There are currently limited studies on the effect of bulking agents (i.e., peat and sawdust) on the maturity and stability indices such as enzyme activities and GI.

Therefore, in this study, the performance of locally available bulking agents on the bench-scale MSW composting in NL was examined. Meanwhile, a comprehensive investigation of parameters indicating compost maturity and stability and monitoring composting process was conducted. The OUR and enzyme activities were selected to reflect compost stability, and GI was investigated to evaluate compost maturity.

3.2 Methods

3.2.1 Raw Materials and Experimental System

The synthetic MSW (food waste) consists of potato, carrot, meat, rice, cabbage, and soybean. The composition of the composting mixture is presented in Table 3.1. Food material was shredded with food processor to approximately 5 mm in diameter and was then mixed with locally available sawdust or peat (in a ratio of 10:1 by wet weight) with the moisture content adjusted to 70%. Two mixtures including FP (food waste + peat) and FS (food waste + sawdust) were composted in two identical lab-scale reactors for a month. Each composting reactor (50×20×25 cm) was made of acrylic sheets (Figure 3.1).

Six mixers were installed to enable homogenous materials. A perforated plate was installed over the bottom of reactor to distribute the injected air. The aeration rate was monitored by a flowmeter. The exhaust gas was discharged into a flask containing H_2SO_4 solution (1 M) to absorb NH_3, and then primarily monitored by gas monitoring system before released through ventilation system. The leachate outlet was used to collect the outcome leachate. A thermometer was used to monitor the temperature. The reactor was cover by heat insulating material to prevent the heat loss.

Table 3.1 Composition of composting mixtures (unit: kg)

	FP	FS
Meat	0.3	0.3
Rice	2.2	1.9
Carrot	2.2	2
Potato	1.1	1.1
Lettuce	0.2	0.2
Soybean	0.3	0.8
Peat	0.7	-
Sawdust	-	0.7

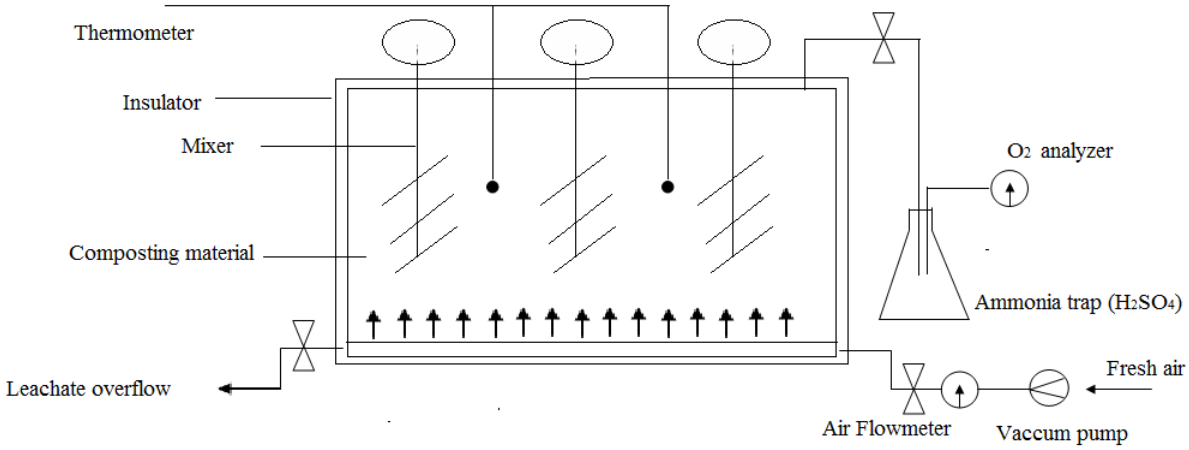


Figure 3.1 Schematic diagram of the composting system

3.2.2 Sampling and Analysis

Samples were collected randomly from 3-4 different points in the reactors after turning material, and then mixed together in a beaker on the 2nd, 4th, 6th, 8th, 10th, 12th, 16th, 20th, and 27th days. The effect of bulking agent on composting was evaluated through measuring pH, EC, C/N ratio, moisture content, ash content, dehydrogenase activity, phosphodiesterase activity, β – glucosidase activity, and GI. Temperature and OUR were recorded every 12 hours and all parameters were measured in duplicate. The average value for each duplicate measurement was used in figures and tables.

Temperature was recorded by bi-metal dial thermometer (H-B Instrument Company, PA). EC and pH were measured in 1:2 (w:v) aqueous extract by using a pH/Ion meter (Metller Toledo. EL20-Educational line pH, EL3-Educational line conductivity). The moisture content was determined by gravimetric loss on-ignition of 10 g sample at 105 °C for 24 h, and the ash content of the dried samples after measuring moisture were determined by burning at 550 °C in a muffle furnace (Blue M Electric Company, Blue Island, USA) for 4 h. The outlet oxygen concentration in the compost exhaust gas was monitored by passing the air through a M40 Multi-Gas Monitor (Industrial Scientific Corp., Oakdale, PA, USA). OUR was calculated through the following equation:

$$OUR = ((O_{2\ out}\ (\%) - O_{2\ in}\ (\%)) \times \text{airflow rate (L/min)} \qquad (3.1)$$

Where $O_{2\ out}$ (%) is the oxygen concentration in compost exhaust gas and $O_{2\ in}$ (%) is the oxygen concentration in the inlet air (20.9%) at airflow rate (0.5 L/min/kg) which is injected to the system.

For seed germination test, water was extracted from the samples by shaking fresh samples with double distilled water (DDW) at solids: DDW = 1:10 (w/v) for 1 h, then suspensions were centrifuged at 3,000 rpm for 20 min before filtering through Whatman No-1 filter paper. A filter paper was placed in the petri dish and almost 10 milliliter of water extract was introduced into the petri dish. Ten cucumber seed were placed on the filter paper. For control experiments, the DDW was used. The dishes were placed in the oven at 25 °C in the darkness for 5 days. Test for each sample was run in triplicate. The GI was calculated according to Zucconi et al. (1981):

GI (%) = (Seed germination x Root length of the treatment x 100) / (Seed germination x Root length of the control) (3.2)

The total carbon and nitrogen contents of the composting sample were determined by the Perkin Elmer 2400 Series II CHNS/O analyzer.

For dehydrogenase activity determination, a 5 g sample was suspended in 5 mL of 3% w/v 2, 3, 5-triphenyl-tetrazolium chloride (TTC) at 37 °C for 24 h in the dark, and then 40 mL acetone was added and incubated at room temperature for 2 h in the dark. The suspension was filtered through a glass fiber filter and absorbance was measured at 546 nm (Alef and Nannipieri 1995; Thalmann 1968). Phosphodiesterase activity was measured using the method of Browman and Tabatabai (1978) and Tabatabai (1994). After the addition of a Tris buffere (pH 8) and Sodium bis-p-nitrophenyl phosphate (Sigma; for phosphodiesterase activity) to 1 g compost, samples were incubated for 1 h at 37 °C. The p-nitrophenol released by phosphodiesterase activity was extracted and coloured with calcium chloride and determined spectrophotometrically at 400 nm. For β

– glucosidase activity measurement, a 1g sample was suspended in 0.25 mL toluene and 4 mL of MUB (Modified Universal Buffer, pH 6.0) plus 1 mL p-nitropenyl-β-D-glucopyranoside (Sigma; for glucosidase). After incubation for 1 h at 37 °C, 1 mL of 0.5 M $CaCl_2$ and 4 mL Tris buffer (0.1M, pH 12) were added and the suspension was filtered through a glass fiber filter. The release of p- nitrophenol was measured spectrophotometrically at 400 nm (Alef and Nannipieri 1995; Eivazi and Tabatabai 1988).

Temperature, OUR, moisture content, ash content, and C/N ratio were measured in duplicate. pH, EC, GI, and enzyme activities are tested in triplicate. The average value for each duplicate measurement was used in figures and tables.

3.3 Results and Discussion

3.3.1 Temperature and OUR

The changes in composting temperature and OUR for FP and FS are shown in Figure 3.2. The temperature of the composting reactor indicates the breakdown of the organic matter and the quality of the compost, since the rise of temperature is the result of decomposition of readily available organic matter and nitrogen compounds by microorganisms (Lee et al. 2009; Ros et al. 2006). Temperature is one of the important indices to evaluate compost efficiency (Lee et al. 2009) because it affects the biological reaction rate, the population dynamic of microbes, and the physiochemical characteristics of the compost (Hue and Liu 1995). (Godden et al. 1983) suggested three distinct stages during composting, including the mesophilic (below 40°C), thermophilic (above 40°C),

and cooling (ambient temperature) stage. As the FP composting proceeded, the temperature of the decomposing waste rose rapidly and reached to a maximum temperature of 68 °C after 2 days. It is known that the highest thermophilic activity in the composting system was maintained at a temperature between 52 and 60°C (Kalamdhad et al. 2009; Liang et al. 2003). The high temperature ensured the elimination of all pathogens; only 3 days at 55°C was sufficient for elimination of pathogens (Rasapoor et al. 2009). Although the temperature of the FS compost showed an increase to 52°C on the third day, the high temperature period on compost was not sufficient to ensure the hygiene safety of the end product. Longer high temperature period was observed in FP composting, indicating effective pathogen removal and sterilization. The microbial activity and the organic matter breakdown rate decreased when the organic matter became more stabilized and consequently the temperature dropped for almost two weeks in both compost to the ambient temperature (Ros et al. 2006). Microbial respiration has been used to measure the microbial activity during composting. It has also been used to assess the evolution of the composting process and maturity of the final product (Ros et al. 2006). High OUR was recorded for FP during the first 5 days of composting and then it decreased sharply. High OUR indicates that organic matter are available for microorganisms to be degraded, and therefore the material is not stabilized yet. Low OUR indicates organic matter are more stabilized and most of the organic matter has been decomposed by microorganisms (Said-Pullicino et al. 2007). Increase of OUR for FS was smoother and reached the highest value at the end of the first week. Although the maximum OUR for FP was almost double the value of that for FS, the duration of the

high OUR was much longer for FS than for FP. The OUR eventually decreased and appear to reach a steady state.

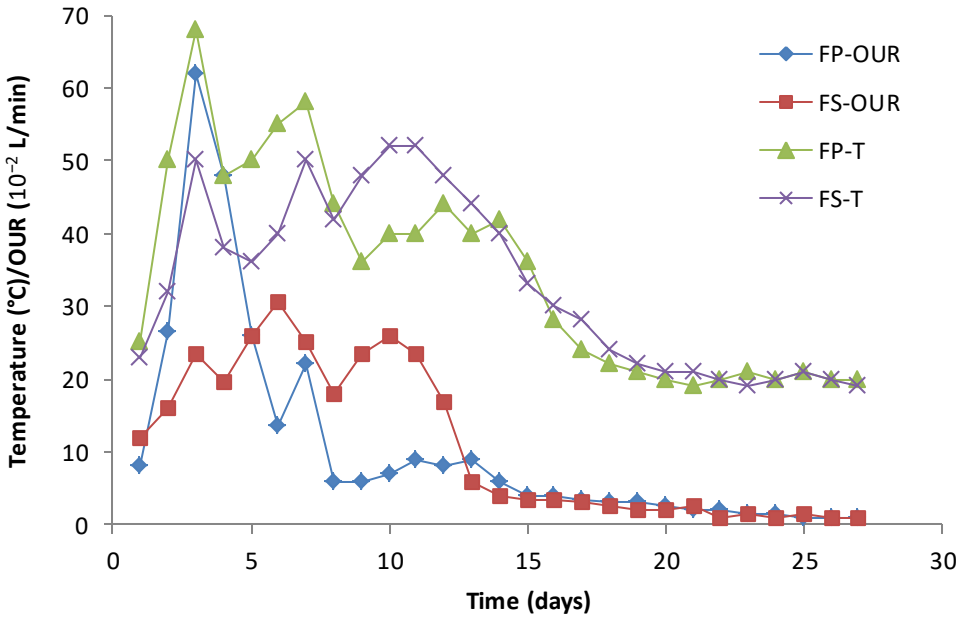


Figure 3.2 Temporal variations of temperature and OUR

3.3.2 pH and EC

The pH values for FS and FP ranged from 4.6 to 8.68 during composting. The pH value of the compost is one of the important factors to evaluate compost stability and maturity due to its influence on the physical-chemical and microbiological reactions in the compost (Banegas et al. 2007). The initial pH and the pH in the first week of FS and FP composting were slightly acidic as a result of organic acids such as acetic acid and butyric acid, partially contained in the food waste and partially produced by microorganism reactions (Adhikari et al. 2009; Eklind and Kirchmann 2000; Smårs et al. 2002). When microorganisms consume organic acids as a substrate, pH started to increase (Adhikari et al. 2009). The highest pH was observed after 8 days for FS and after 16 days for FP compost This delay for FP compost could be due to the loss of ammonium through volatilization and nitrification, and accumulation of organic acid and CO_2 during decomposition of the simple organic matter like carbohydrates (Banegas et al. 2007; Chukwujindu et al. 2006; Kayikçioğlu and Okur 2011). Compost with low pH indicates lack of maturity due to the short composting time or occurrence of the anaerobic process (Iglesias Jiménez and Perez Garcia 1989).The final pH for FS and FP was above 8 and pH levels stayed almost steady by the end of composting.

Compost EC affects microbial population and organic matter transformation. High EC values of compost may have phytoxicity effects on the plant and negatively influence the plant growth and seed germination (Arslan et al. 2011; Banegas et al. 2007; Kalamdhad et al. 2009). Experimental results showed that EC values of FS compost increased earlier than FP compost (Figure 3.3). This increase could be due to the release of mineral cation

concentration such as ammonium ions and phosphate which did not bind to the stable organic complex or went out of the system through leachate (Francou et al. 2005; Kalamdhad et al. 2009).

3.3.3 Moisture and Ash Content

Moisture and ash content variations are shown in Figure 3.4. As shown in the figure, moisture content showed descending trends in both compost. The combination of evaporation because of high temperature and aeration lead to the decrease of moisture content during composting, especially at high temperatures (Lashermes et al. 2012; Said-Pullicino et al. 2007). Moisture content for FS compost showed a slow declining trend by 10 days, which is an indication of decomposition of organic matter (Arslan et al. 2011; Kalamdhad et al. 2009). The temporary increasing trend observed for FS and FP compost was because temperature was not high enough to evaporate the water produced through microbial activity. The amount of ash increased consistently. The ash content increasing trend had a large slope at the thermophilic stage, and then the slope became smoother when the temperature dropped. During composting the organic matter was decomposed into volatile compounds, and consequently the final compost has lower organic matter and higher ash content (Kalamdhad et al. 2009).

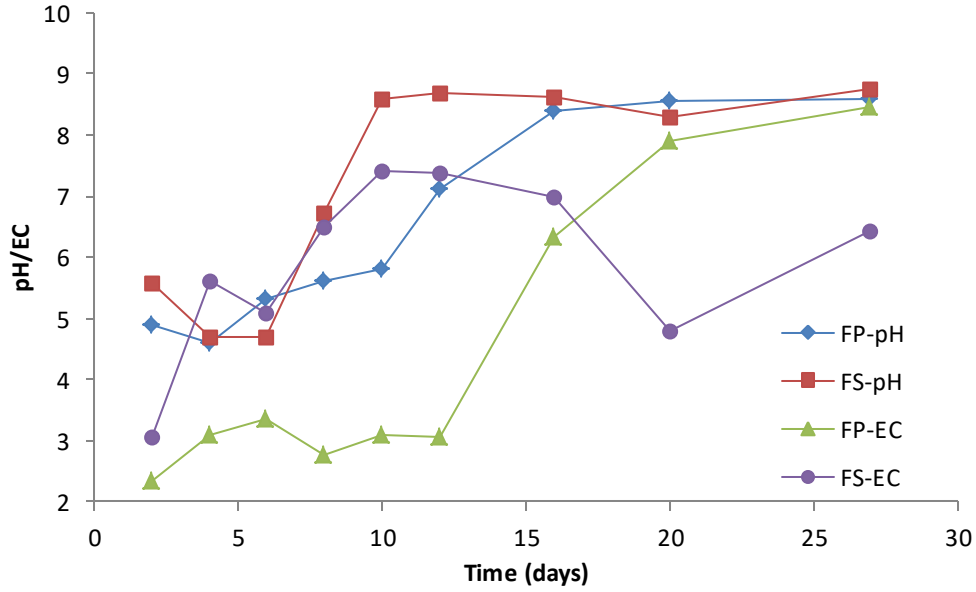


Figure 3.3 Temporal variations of pH and EC

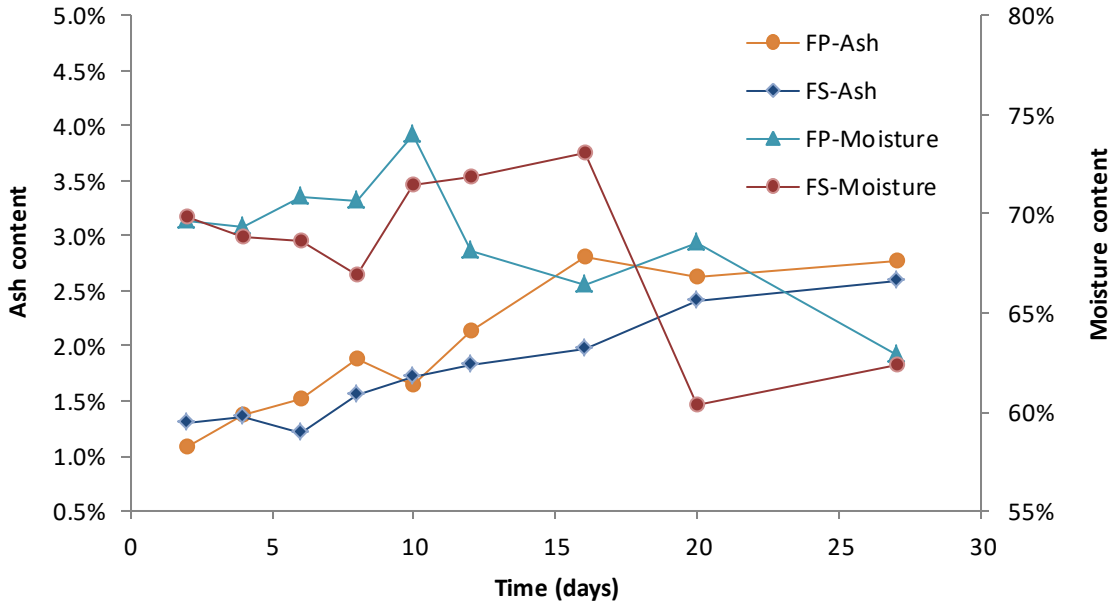


Figure 3.4 Temporal variations of moisture and ash content

3.3.4 C/N Ratio

Figure 3.5 shows the C/N ratio variation for both FS and FP compost. Both composts have close C/N ratio in the beginning (17). The initial C/N ratio has a strong influence on the performance of the composting process and the quality of the end product (Gao et al. 2010). An extremely high C/N ratio makes the composting process very slow as there is an excess of degradable substrate and lack of N for the microorganisms. On the other hand, a very low C/N ratio can lead to loss of N through NH_3 volatilization and generate potential odour problem (Gao et al. 2010; George and Jayachandran 2013; Seafish 2001). For FS and FP composting, the initial C/N ratio were lower than the optimum value recommended for composting, i.e., 25 to 30 (Haug 1993). C/N ratio decreased for both composts during thermophilic phase. Decrease was very fast for FP whereas after the first week, the C/N ratio for FP dropped to 9 while it was 13 for FS. High microbial activity and high decomposition of organic matter after two weeks led to a C/N ratio decrease in both treatments. The C/N ratio stayed steady after two weeks by the end of the experiments for both FS and FP composts. The final value of C/N ratio of FP was low than that of FS.

3.3.5 GI

The maturity of the compost has been evaluated based on chemical parameters correlated with plant response (Bernal et al. 2009; Xiao et al. 2009). Seed germination test helps to evaluate the efficiency of the composting process for plant growth and seed germination (Banegas et al. 2007). As it is shown in Figure 3.6, GI is high at the beginning since the raw material is synthetic and non-toxic food waste. GI decreased as a result of formation

of toxic compounds such as alcohols, phenolic compound, and organic acids during the thermophilic phase as a result of the composting process. This decrease was sharp for FS compost by the end of the first week and after that it started to increase quickly. It has been suggested that a GI over 80% indicates the absence of phytotoxicities in compost (Tiquia and Tam 1998; Zucconi et al. 1981). At the end of the composting, GI for FS was higher than 80%; but for FP, GI did not reach 40%, which can be associated with the stage of the composting. Higher degree of maturity was found for the FS compost.

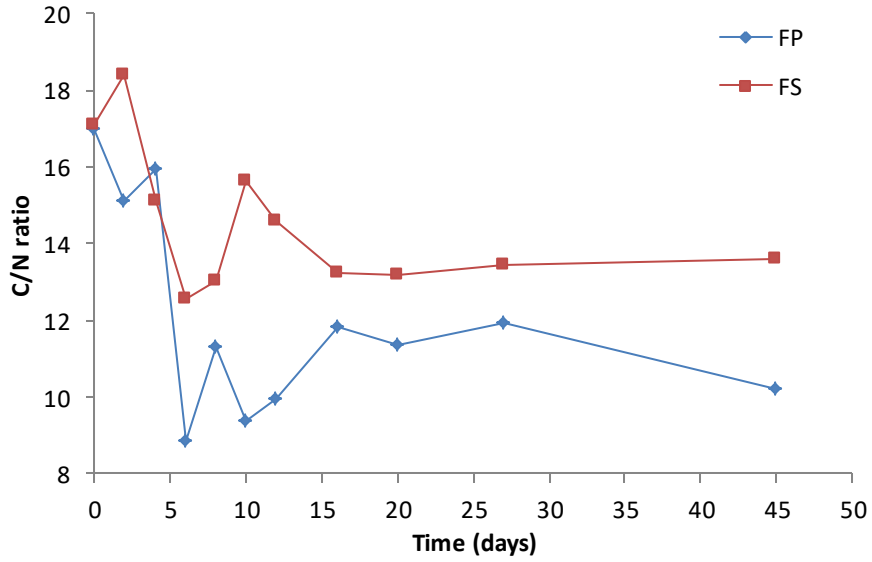


Figure 3.5 Temporal variations of C/N ratio

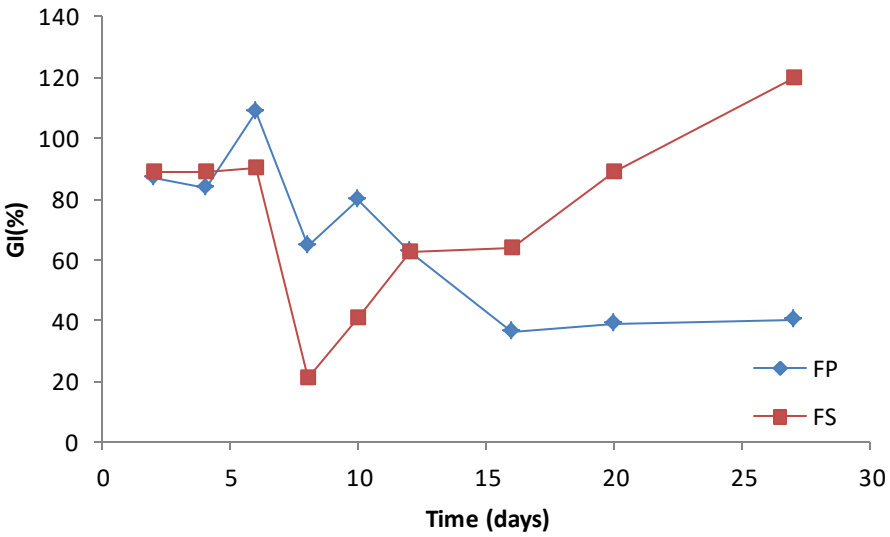


Figure 3.6 Temporal variations of GI

3.3.6 Enzyme Activities

Enzymes are responsible for the breakdown of several organic compounds characterised by complex structures, finally generating simple water-soluble compounds (Castaldi et al. 2008). Characterising and quantifying specific enzyme activities during composting could provide information of dynamics of the composting process. Enzyme activities can reflect the rate of transformation of organic residues and nitrogen, as well as the stability and maturity of end products (Mondini et al. 2004; Raut et al. 2008). Moreover, the determination of enzyme activity, in contrast to other analytical techniques used for compost stability evaluation, is easy, fast, and relatively inexpensive (Mondini et al. 2004). Garcia et al. (1993) confirmed that the hydrolytic enzymes were biomarkers of the state of the composting and evolution of the organic matter.

Dehydrogenase activity was 1,959 and 837 (µg TPF g DM $^{-1}$) on the second day for FP and FS, respectively. Dehydrogenase is an enzyme belonging to the oxido-reductase group which catalyzes the oxidation of organic substances (Kayikçioğlu and Okur 2011). Bernal et al. (2009) used dehydrogenase activity to monitor the composting process. They concluded that dehydrogenase is a useful parameter to follow the evolution of the biological activity of the composting process, since it correlates well with the temperature profile in the reactor. Dehydrogenase activity increases for FS and FP. FS reached the maximum value, 19,106 (µg TPF g DM $^{-1}$) after 10 days corresponding to the peaks of temperature and OUR. The maximum value, 18,815 (µg TPF g DM $^{-1}$) for FP observed at 16 days at the end of the thermophilic phase or the beginning of the mesophilic stage is similar to the results of Kayikçioğlu and Okur (2011) and Bernal et al. (2009). Vargas-

Garcia et al. (2010) stated that the higher dehydrogenase activity values were related to the higher microbial activity and large account of mesophilic and thermophilic bacteria and lower dehydrogenase activity values associated with the maturation phase. The longer period of high dehydrogenase activity was observed for FP compost. As shown in Figure 3.7, after 20 days the dehydrogenase activity decreased, which means that most of the organic matter has been degraded by the microorganism and converted to stable materials and consequently the respiratory process slowed down (Benitez et al. 1999; Benito et al. 2003; Kayikçioğlu and Okur 2011; Ros et al. 2006; Tiquia 2005; Vargas-Garcia et al. 2010). The cumulative dehydrogenase activity for FP (94, 899 µg TPF g DM $^{-1}$) was much higher than the cumulative dehydrogenase activity for FS (67, 924 µg TPF g DM $^{-1}$).

β-glucosidase is one of the key enzymes governing the C-cycle. It hydrolyses reducing terminations of b-D-glucose chains and form b-glucose. Its activity is therefore indicative of the presence of these terminations, which come from the labile organic matter (Kayikçioğlu and Okur 2011; Vargas-Garcia et al. 2010). The temporal variation of the β – glucosidase activity is shown in Figure 3.8. β – glucosidase activity was high at the beginning for both composts. At the end of the first week, β – glucosidase activity showed a peak, 11,980 (µg PNP g $DM^{-1}h^{-1}$), and then dropped. The peak of β – glucosidase activity for FS was observed later than for FP after the second week but with almost the same value. β – glucosidase activity for both of the composts decreased by the end of composting and it was lower for the FS compost.

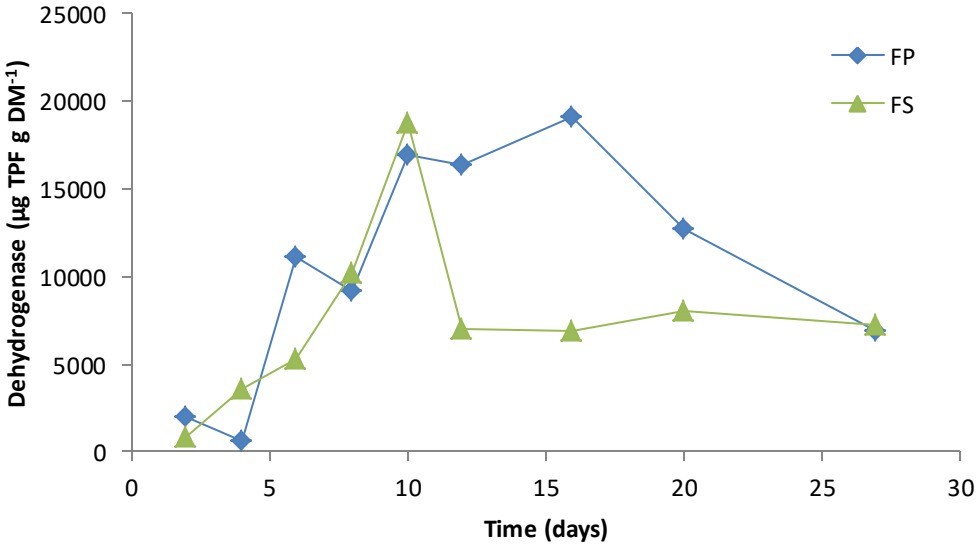


Figure 3.7 Temporal variations of dehydrogenase activity

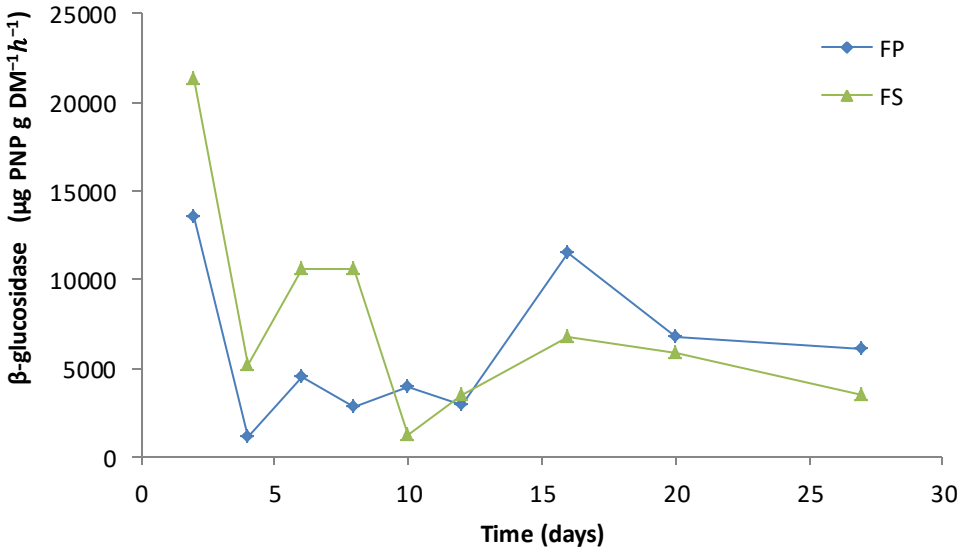


Figure 3.8 Temporal variations of β – glucosidase activity

Phosphodiesterase (phosphoric diester hydrolases) hydrolyse one or two ester bonds in phosphodiester compounds including nucleases, which catalyze the hydrolysis of phosphodiester bonds of nucleic acids to produce nucleotide units or mononucleotides but not inorganic phosphates. Phosphodiesterase catalyzes phospholipids and nucleic acids degradation which are among the major sources of fresh organic P inputs (Nannipieri et al. 2011). In the beginning, phosphodiesterase activities were high in both composts. Phosphodiesterase activities showed the same trend for FS and FP in the first two weeks. The peak values observed at 8 days, 25,366 and 21,032 (µg PNP g $DM^{-1}h^{-1}$) for FP and FS, respectively. After 2 weeks, the phosphodiesterase activity dropped dramatically for FS compost and reached zero by the end of the experiment, whereas for FP compost, phosphodiesterase activity was 9,401 (µg PNP g $DM^{-1}h^{-1}$) at the end of experiment (Figure 3.9).

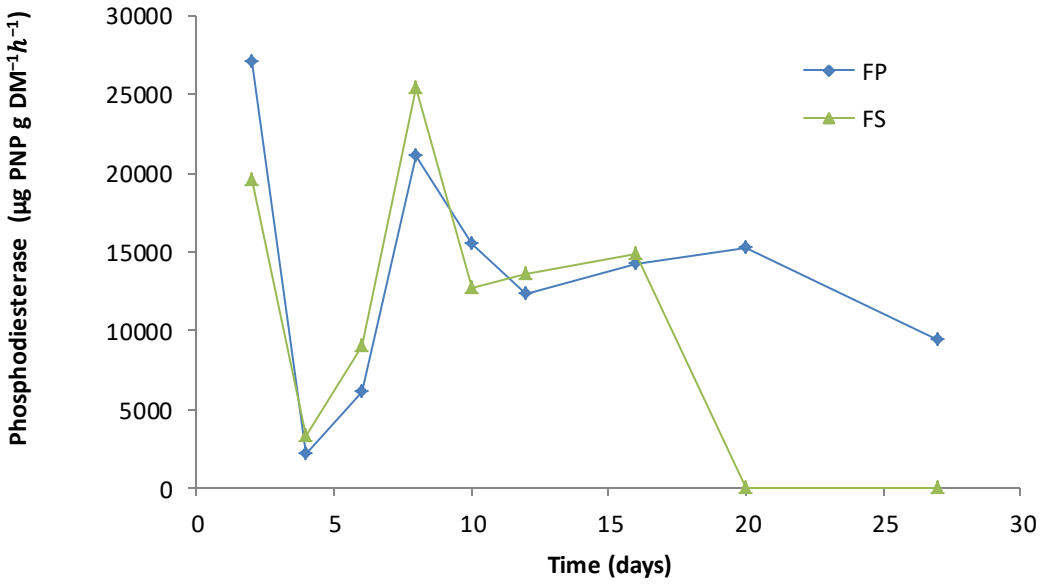


Figure 3.9 Temporal variations of phosphodiesterase activity

3.4 Summary

The results of different maturity and stability indices indicated the choice of bulking agents is important for composting performance and the quality of the end product. Applying different bulking agents in composting influence temperature, OUR, GI, dehydrogenase activity and β-glucosidase activity. The final GI values for food waste composting with sawdust as a bulking agent was found to generate more mature compost with less phytotoxicity. The choice of bulking agent did not affect dehydrogenase and β – glucosidase activities values at the end of the composting for both treatments, but the final value for phosphodiesterase activity for FS was much lower than that for FP. High dehydrogenase and β-glucosidase activities during the third week of composting for FP indicate high microbial activities. To generate a high temperature and a longer duration of high temperature to kill pathogens and sterilize the compost, peat was considerably more effective. Both sawdust and peat are effective bulking agents for bench-scale composting. The choice depends on the availability of the bulking agent and land in the target community, the price of the bulking agent and its transportation, and the desired quality (e.g., higher maturity or stability) of the end compost.

CHAPTER 4

DESIGN OF EXPERIMENT (DOE) BASED SCREENING OF FACTORS AFFECTING MUNICIPAL SOLID WASTE (MSW) COMPOSTING[3]

This chapter is based on the following paper:

Kazemi, K., Zhang, B., Lye, L. M., Cai, Q., and Cao, T., (2016). Design of Experiment (DOE) based Screening of Factors Affecting Municipal Solid Waste (MSW) Composting, Waste Management.

Role: Khoshrooz Kazemi solely worked on this study and acted as the first author of this manuscript under the guidance of two supervisors, Dr. Baiyu Zhang and Dr. Leonard Lye. Qinghong Cai *and* Tong Cao *participated in conducting experiments. Most contents of this paper was written by Kazemi and further polished by the other co-authors.*

4.1 Background

Municipal solid waste (MSW) is one of the major environmental concerns in recent decades due to the aggregation of human settlements and increased individual solid waste generation rate (Iqbal et al. 2010). In North America, 0.75 tonnes of garbage is produced per capita per year (Adhikari et al. 2008; Asase et al. 2009). In Canada, around 75% of total MSW go to landfills (Statistics Canada 2008) which may have negative environmental impacts on groundwater and surface water due to the toxic and polluting components (e.g. halogenated organics and heavy metals) generated from the landfill leachate (Kjeldsen et al. 2002). Besides, landfill gas emission is another environmental concern as the emitted gases are explosive and toxic and require treatment (Spokas et al. 2006). Moreover, noise, dust and odour from the disposal sites make the surrounding area undesirable for habitation (Garrod and Willis 1998). In Canada, the strict environmental regulations render finding new sites for MSW disposal and management a growing challenge (Adhikari et al., 2008).

Composting has become an inexpensive, simple and environmental-friendly alternative for the treatment of MSW as easily degradable organic matter (OM) in MSW is prone to be stabilized and converted to the humus-rich compost by the action of microorganisms (Jolanun and Towprayoon 2010; Ponsá et al. 2010). The mature compost can then serve as soil conditioner for agriculture and gardening with high economic values (Wong et al. 1996). The maturity of the compost is evaluated by diverse parameters such as pH value, electrical conductivity (EC), final carbon/nitrogen (C/N) ratio, germination index (GI) and ash content (Laos et al. 2002; Zucconi et al. 1981). In addition, temperature

dynamics, oxygen uptake rate (OUR) and diverse enzyme activities are also important parameters to determine the overall performance of the composting process and the quality of the compost (Ros et al. 2006; Said-Pullicino et al. 2007).

The optimization of the above mentioned parameters entails appropriate set-up design factors such as C/N ratio (C/N), moisture content (MC), aeration rate (AR) and type of bulking agent (BA), to allow the sufficient development of the microbial population with robust enzymatic activities, which control the OM degradation and thus the maturity of the compost (Garcıa-Gómez et al. 2003) Specifically, C/N of 25–30 are considered ideal for composting (Min and Wong 1999) although the C/N of MSW generally is lower than the ideal value. Inappropriate C/N limit the microbial activity slowing down composting and generating immature products (Christensen 2011; de Bertoldi et al. 1983; Gao et al. 2010; Tiquia and Tam 2000). MC, when too low halts microbial activity (Haug 1993) and limits oxygen flux if it is too high (Liang et al. 2003; Sundberg and Jönsson 2008). The AR determines the oxygen amount, while affecting the MC and temperature of the system (Kuter et al. 1985). BA adjusts water content, porosity and provides free air space (Haug 1993). Different BA provide diverse adjustments to the above parameters which lead to the different maturities of the compost (Leiva et al. 2003).

Although many studies have investigated the effects of these design factors on the composting performance and maturity of the compost, most studies only worked with one or two parameters using one-factor-at-a-time approach (OFAT) (Guo et al., 2012). However, OFAT generally requires more experimental runs, has less accuracy and is not able to estimate factor interactions when compared with designed experiments (Czitrom

1999). A few studies have applied design of experiments (DOE) (e.g., factorial designs) to study the effects of a few factors such as temperature and moisture (Liang et al. 2003); temperature, aeration and moisture (Suler and Finstein 1977); operation volume, bulking agent particle size and bulking agent/sludge volume ratio (Leiva et al. 2003) on the performance of composting system. The investigated factors were not comprehensive enough to illustrate their effects and interactions on the performance of a composting process. Enzymatic activities could apparently give interesting information on the rate of decomposition of organic matter and, therefore, on the produced compost stability (Jurado et al. 2014), however, they have never been reported as responses for optimizing MSW composting based on DOE methods.

In this study, statistically significant factors that affect the performance of a composting process were screened based on the DOE technique. A two-level (or 2^k) factorial design was applied to study the effects of four factors, AR, MC, BA, and C/N on the maturity, stability and toxicity of compost product by evaluating temperature, OUR, pH, EC, and ash content of the compost. In addition, final C/N ratio, GI and enzyme activities were also used as responses to evaluate system performance.

4.2 Material and Methods

4.2.1 Experimental Design and Statistical Analysis

Factorial design was employed to screen factors that may have significant effects on response(s) because it is the most efficient available method for conducting multifactor experiments. The significant factors can then be used to develop a model to optimize and

predict the response (Montgomery 2008; Montgomery et al. 1997), if needed. The most common factorial design is the two-level (or 2^k) design. Based on the analysis of variance (ANOVA), the significant factors are determined and used to produce the regression prediction model. The regression model representation of a 2^4 factorial experiment can be written as:

$$\hat{Y} = \hat{\beta}_0 + \hat{\beta}_i x_i + \hat{\beta}_{ij} A_i B_j + \hat{\beta}_{ijk} A_i B_j C_k + \hat{\beta}_{ijkl} A_i B_j C_k D_l + \varepsilon, \qquad (4.1)$$

$$i=1,2,\ldots,4,\ j=1,2,\ldots,4,\ k=1,2,\ldots,4,\ l=1,2,\ldots,4$$

Where $\hat{Y}$ is the response, $\hat{\beta}_0$ is the mean of all treatment combinations, $\hat{\beta}_i$, $\hat{\beta}_{ij}$,$\hat{\beta}_{ijk}$, and $\hat{\beta}_{ijkl}$ are half of the effect estimated corresponding to significant effects, A_i, B_j, C_k, and D_l are coded variables that represent significant effects and take on values between -1 and +1, and ε is a random error term. The random error terms are assumed to have a normal distribution, a constant variance, and are independent. The four factors investigated in this study include AR, MC, BA, and C/N. The high and low levels of variables are presented in Table 4.1. The high/low levels of the variables were selected based on the values reported in the previous studies (Delgado-Rodríguez et al. 2012; Gao et al. 2010; Guo et al. 2012; Kumar et al. 2010; Rasapoor et al. 2009). For four factors, the design requires 16 runs which are shown in Table 4.2.

Table 4.1 Design factors and their high and low levels

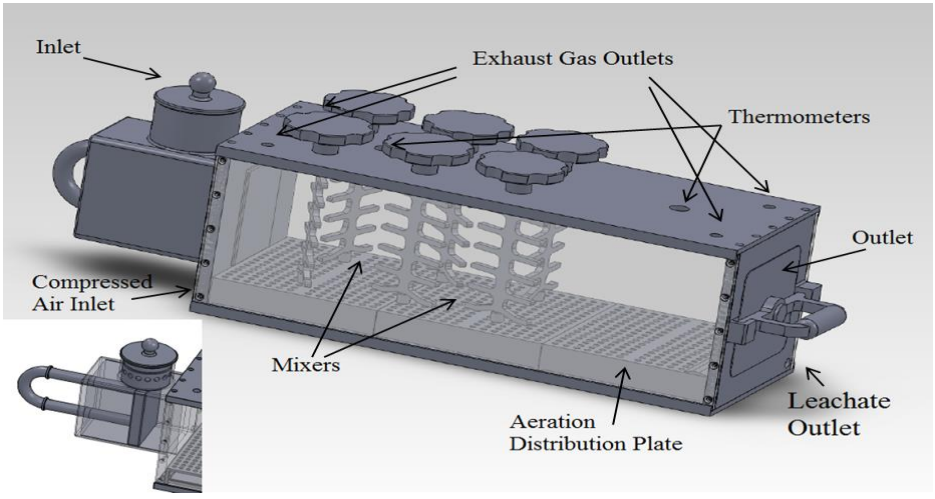
Factor	High level	Low level
A-AR (L/min.kg)	0.5	0.3
B-MC (%)	70	55
C-BA	Peat	sawdust
D-C/N	17	12

Table 4.2 Experimental design for MSW composting

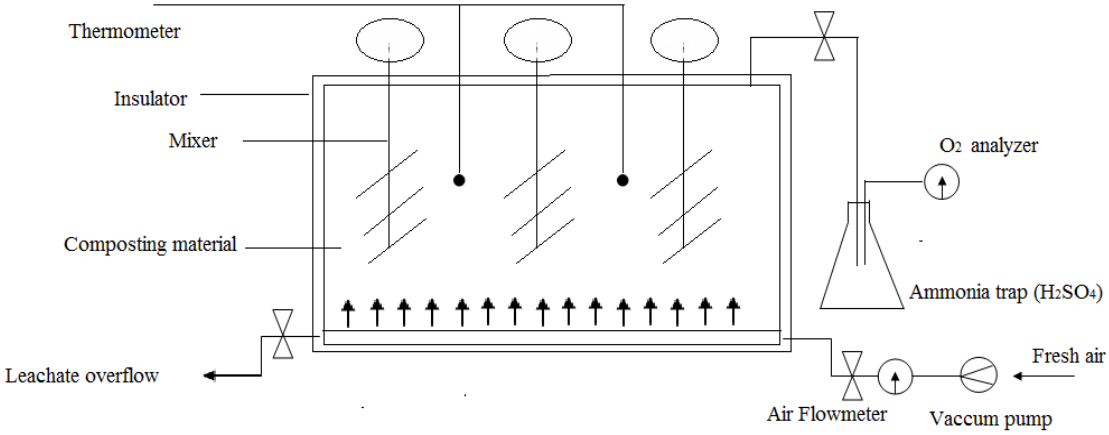
Std	Run	A:AR	B:MC	C:BA	C/N
1	1	0.3	55	Sawdust	12
4	2	0.5	70	Sawdust	12
8	3	0.5	70	Peat	12
14	4	0.5	55	Peat	17
11	5	0.3	70	Sawdust	17
7	6	0.3	70	Peat	12
15	7	0.3	70	Peat	17
10	8	0.5	55	Sawdust	17
16	9	0.5	70	Peat	17
2	10	0.5	55	Sawdust	12
12	11	0.5	70	Sawdust	17
3	12	0.3	70	Sawdust	12
13	13	0.3	55	Peat	17
6	14	0.5	55	Peat	12
5	15	0.3	55	Peat	12
9	16	0.3	55	Sawdust	17

4.2.2 Composting Process

Six identical composting reactors (50×20×25 cm) made of acrylic sheets (Figure 4.1) were designed and manufactured for the experiments. To facilitate the technology transfer and system scale-up, in this study, the bench-scale system was designed as rectangular. Mixers were installed to enable homogenous mixing of materials. A perforated plate was installed over the bottom of the reactor to distribute the injected air. The aeration rate was monitored by a flowmeter (Acrylic block flowmeter, FR2000, VWR). The exhaust gas was discharged into a flask containing H_2SO_4 solution (1 M) to absorb NH_3, and monitored by a gas monitoring system, then treated and released through a ventilation system. The leachate outlet was used to collect the outcome leachate. The top of the reactor can be opened for feeding and after feeding, the arm in the feeding part can push the waste forward along the tunnel. A thermometer was used to monitor the temperature. The reactor was covered with a layer of aluminum foil and then two layers of foil insulation "reflectix bubble pack" which is filled with a 3.5 inch thick fibreglass layer to prevent heat loss through the reactor walls.



(a)



(b)

Figure 4.1 (a) 3D view of the designed composting system; (b) Schematic diagram of the composting system

Synthetic MSW (food waste) consisting of potato, carrot, meat, rice, cabbage, soybean, and different bulking agents (i.e., sawdust and peat) was used for composting. The food material was shredded with a food processor to approximately 5 mm in diameter and then mixed in different ratio to adjust the C/N based on the experimental design. Sawdust or peats as BA (in a ratio of 1:9 by wet weight) were added to the material. The initial moisture content was adjusted by natural drying to 55% or 70% according to the experimental design. The composition of the composting mixture is presented in Table 4.3.

Table 4.3 Composition of composting mixture (unit: kg)

	Run (3,6,14,15)	Run (4,7,9,13)	Run (1,2,10,12)	Run (5,8,11,16)
Meat	0.5	0.3	0.5	0.3
Rice	1.4	2.2	1.3	1.9
Carrot	1.5	2.2	1.2	2
Potato	1.4	1.1	1.4	1.1
Lettuce	0.1	0.2	0.1	0.2
Soybean	1.4	0.3	1.8	0.8
Peat	0.7	0.7	-	-
Sawdust	-	-	0.7	0.7

The composting material was turned with mixers twice a day in order to get homogenized samples. After turning, approximately 100 g compost was collected randomly from 3-4 different points in the reactor, and was then mixed in a beaker on the 2nd, 4th, 6th, 8th, 10th, 12th, 16th, 20th, and 27th day. The collected samples was divided into different sub-samples to measure pH, EC, C/N, MC, ash content, enzyme activities, and GI. Temperature and OUR were recorded every 12 hours. MC, ash content, pH and EC were measured in duplicate and the presented data are the average values. Measurements for enzyme activities and tests for GI were carried out in triplicate.

4.2.3 Analytical Methods

Temperature was recorded by a bi-metal dial thermometer (H-B Instrument Company, PA). EC and pH were measured in 1:2 (w:v) aqueous extract by using a pH/Ion meter (Metller Toledo. EL20-Educational line pH, EL3-Educational line conductivity). The MC was determined by gravimetric loss on-ignition of 10 g sample at 105 °C for 24 h, and the ash content of the dried samples after measuring moisture, were determined by burning at 550 °C in a muffle furnace (Thermo Scientific, Type FD1500M) for 4 h. The outlet oxygen concentration in the compost exhaust gas was monitored by passing the air through a multi-Gas Monitor (M40 Industrial Scientific Corp., Oakdale, PA, USA). OUR was calculated through the following equation:

$$OUR = ((O_{2\ out}\ (\%) - O_{2\ in}\ (\%)) \times \text{airflow rate (L/min)} \qquad (4.2)$$

Where $O_{2\ out}$ (%) is the oxygen concentration in compost exhaust gas and $O_{2\ in}$ (%) is the oxygen concentration in the inlet air (20.9%) at airflow rate (0.3 or 0.5 L/min/kg) which is injected to the system.

For seed germination test, water was extracted from the samples by shaking fresh samples with double distilled water (DDW) at solids: DDW = 1:10 (w/v) for 1 h, then suspensions were centrifuged at 3,000 rpm for 20 min before filtering through Whatman No-1 filter paper. A filter paper was placed in the petri dish and almost 10 milliliter of water extract was introduced into the petri dish. Ten cucumber seeds were placed on the filter paper. For control experiments, the DDW was used. The dishes were placed in the oven at 25 °C in darkness for 5 days. Test for each sample was run in triplicate. The GI was calculated according to Zucconi et al. (1981):

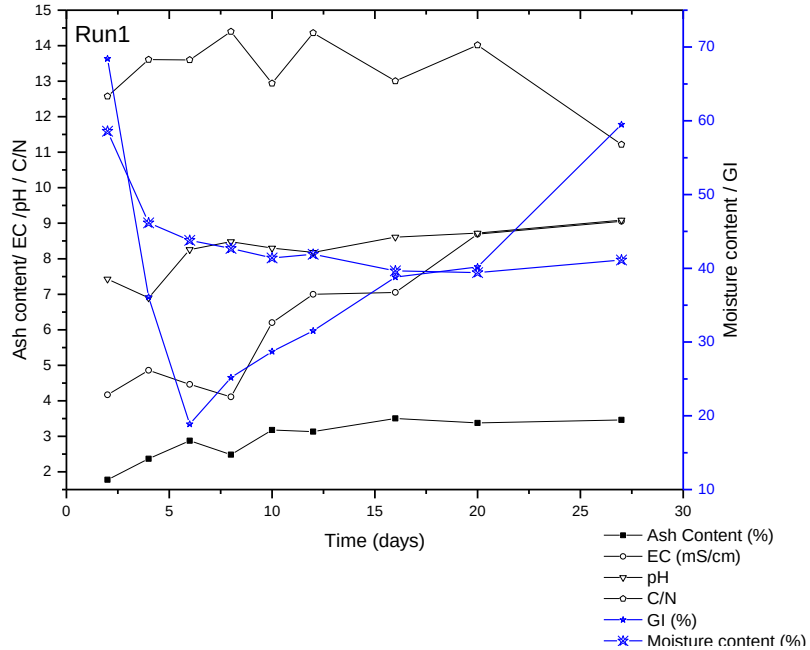
GI (%) = (Seed germination x Root length of the treatment x 100) / (Seed germination x Root length of the control) (4.3)

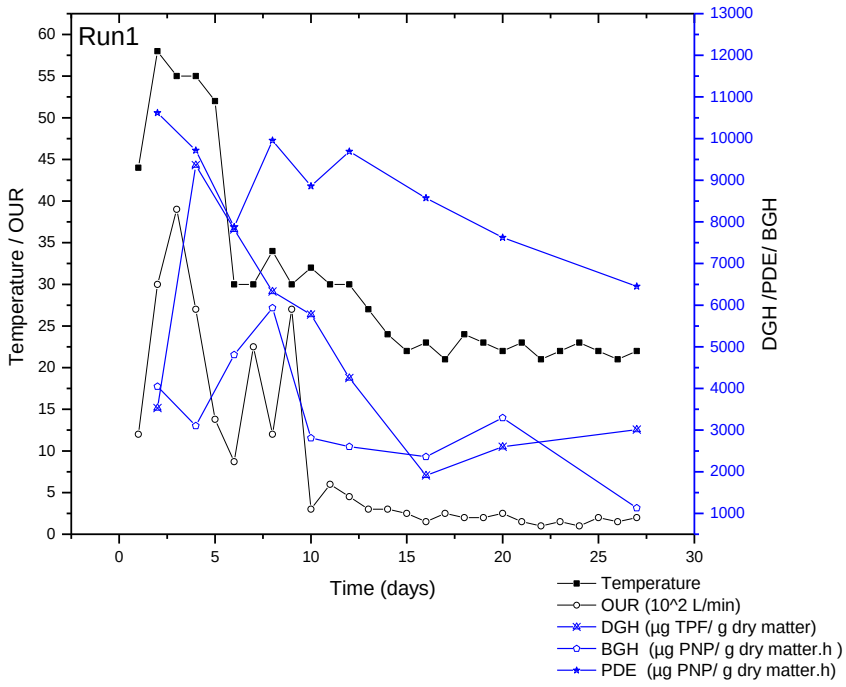
For dehydrogenase activity (DGH) determination, a 5 g sample was suspended in 5 mL of 3% w/v 2, 3, 5-triphenyl-tetrazolium chloride (TTC) at 37 °C for 24 h in the dark, and then 40 mL acetone was added and incubated at room temperature for 2 h in the dark. The suspension was filtered through a glass fiber filter and absorbance was measured at 546 nm (Alef and Nannipieri 1995; Thalmann 1968). Phosphodiesterase activity (PDE) was measured using the method of (Browman and Tabatabai 1978) and (Tabatabai 1994). After the addition of a Tris buffer (pH 8) and Sodium bis-p-nitrophenyl phosphate (Sigma; for PDE) to 1 g compost, samples were incubated for 1 h at 37 °C. The p-nitrophenol released by PDE was extracted and coloured with calcium chloride and determined spectrophotometrically at 400 nm. For β – glucosidase activity (BGH) measurement, a 1g sample was suspended in 0.25 mL toluene and 4 mL of MUB (Modified Universal Buffer, pH 6.0) plus 1 mL p-nitropenyl-β-D-glucopyranoside

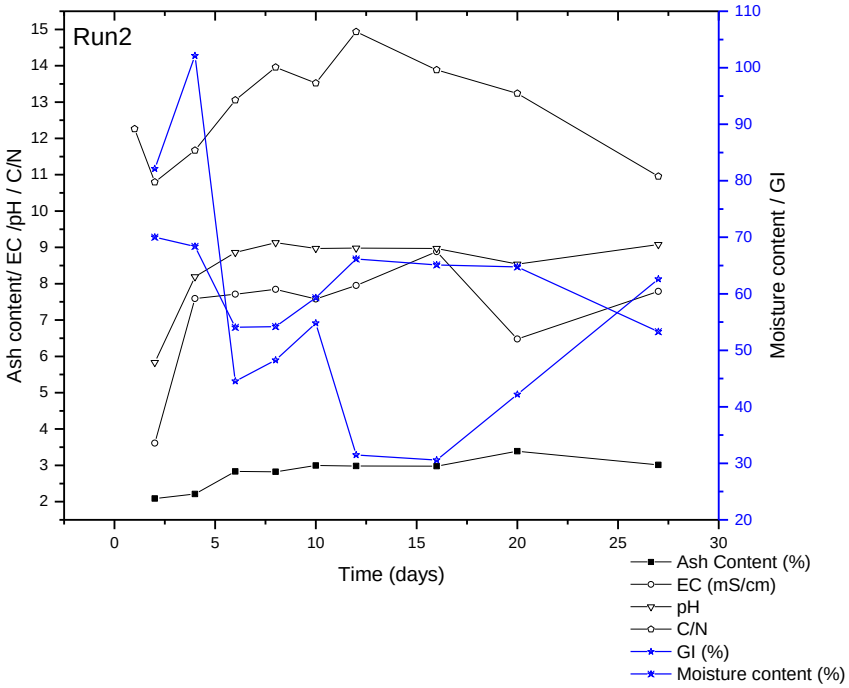
(Sigma; for glucosidase). After incubation for 1 h at 37 °C, 1 mL of 0.5 M $CaCl_2$ and 4 mL Tris buffer (0.1M, pH 12) were added and the suspension was filtered through a glass fiber filter. The release of p- nitrophenol was measured spectrophotometrically at 400 nm (Alef and Nannipieri 1995; Eivazi and Tabatabai 1988).The total carbon and nitrogen contents of the composting sample were determined by the PerkinElmer 2400 Series II CHNS/O analyzer.

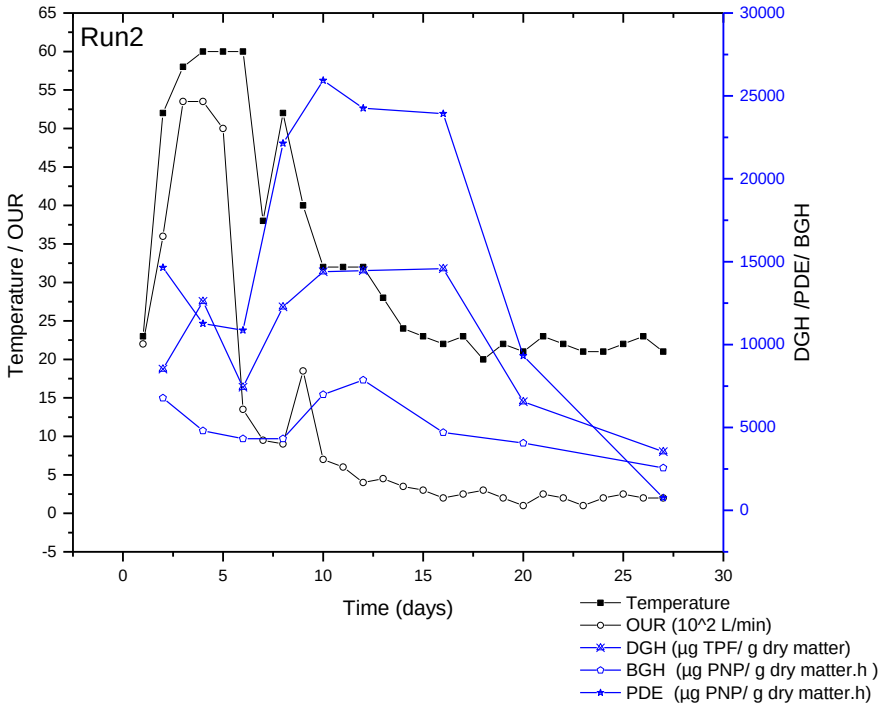
4.3 Results and Discussion

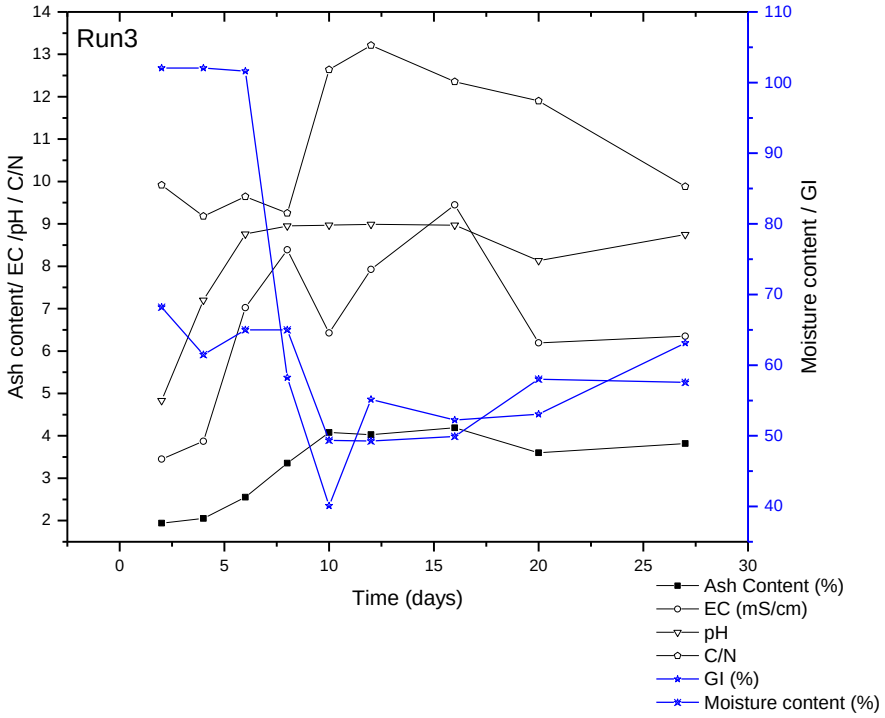
The temporal variation of the temperature, OUR, pH, EC, MC, ash content, C/N, GI, and enzyme activities are presented in Figure 4.2 .Temperature is one of the important indices to evaluate compost efficiency (Lee et al. 2009) due to its effect on the biological reaction rate, the population dynamic of microbes, and the physiochemical characteristics of the compost (Hue and Liu 1995). Decomposition of readily available OM and nitrogen compounds by microorganisms led to the rise of temperature. Composting temperature can indicate the breakdown of the OM and the quality of the compost (Lee et al. 2009; Ros et al. 2006). The microbial activity and the OM breakdown rate decrease when the OM becomes more stabilized and consequently the temperature drops to the ambient temperature (Ros et al., 2006).

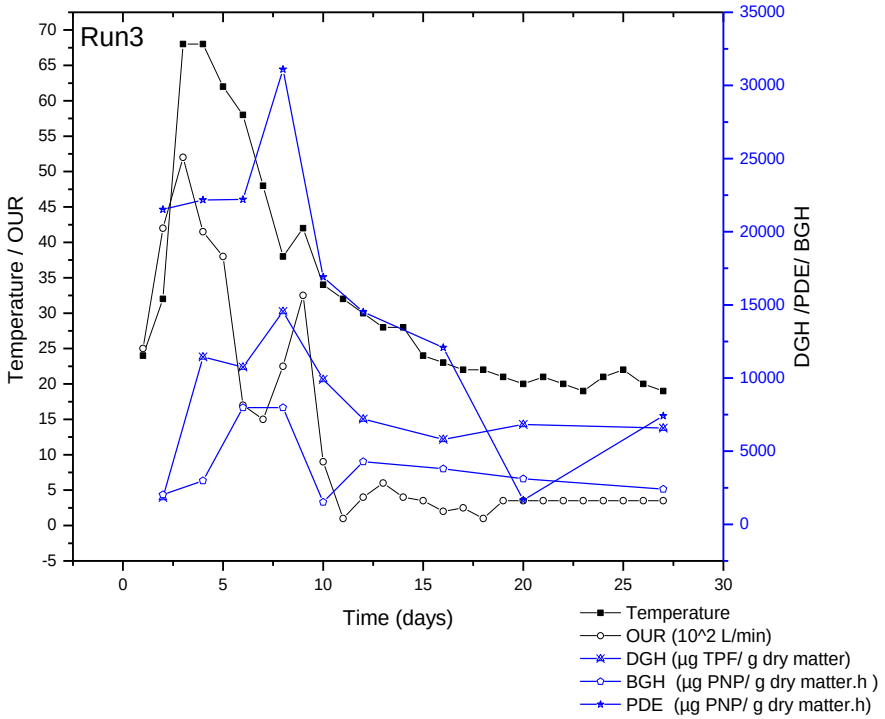

Run1
Ash content/ EC /pH / C/N
Moisture content / GI
Time (days)
Ash Content (%)
EC (mS/cm)
pH
C/N
GI (%)
Moisture content (%)

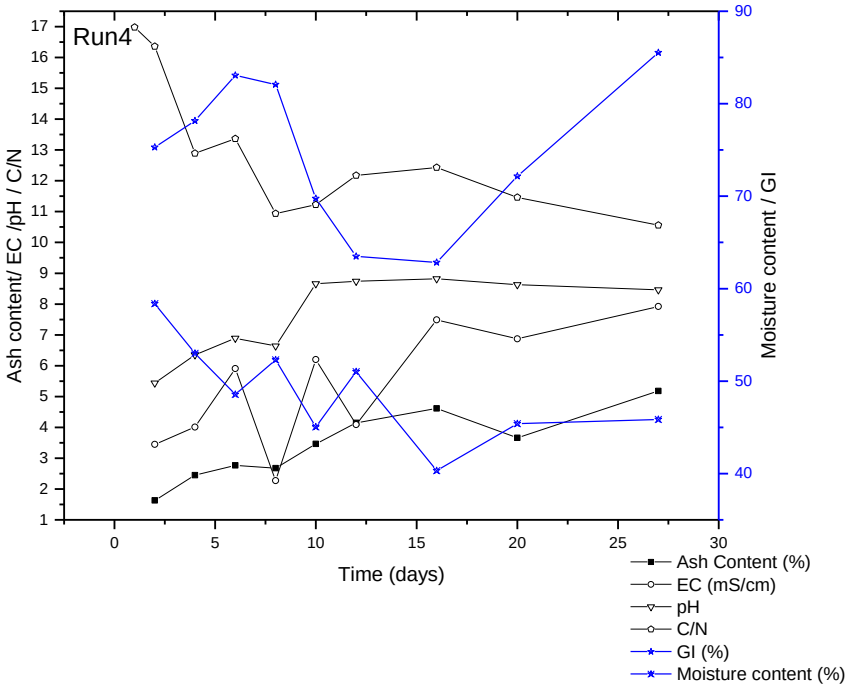

Run1
Temperature / OUR
DGH /PDE/ BGH
Time (days)
Temperature
OUR (10^2 L/min)
DGH (µg TPF/ g dry matter)
BGH (µg PNP/ g dry matter.h)
PDE (µg PNP/ g dry matter.h)

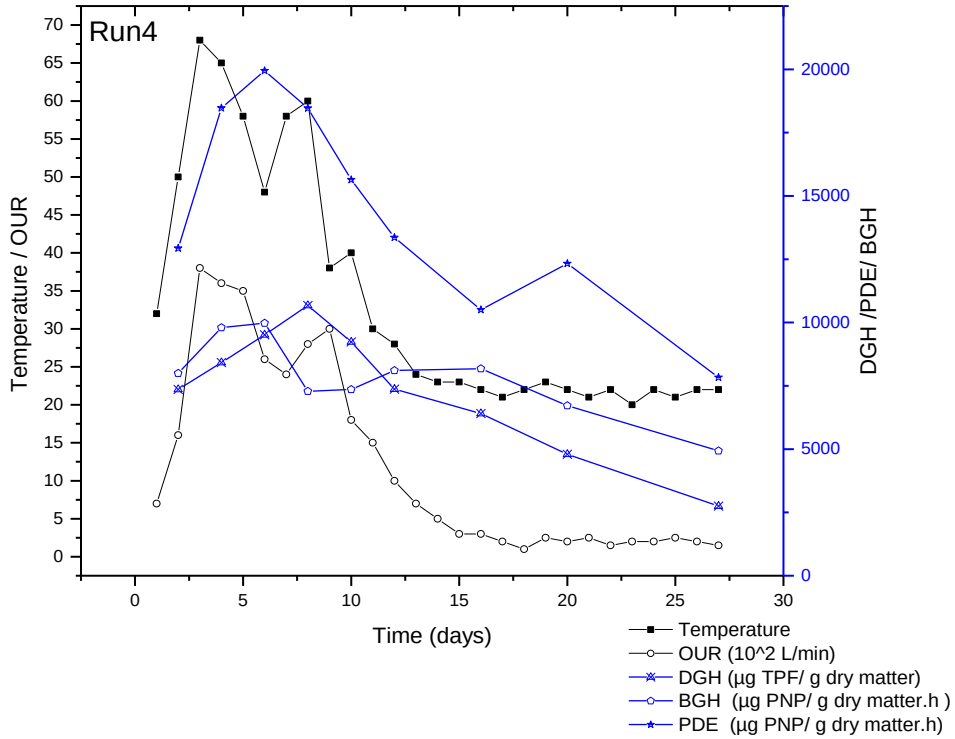

Run2
Ash content/ EC /pH / C/N
Moisture content / GI
Time (days)
Ash Content (%)
EC (mS/cm)
pH
C/N
GI (%)
Moisture content (%)

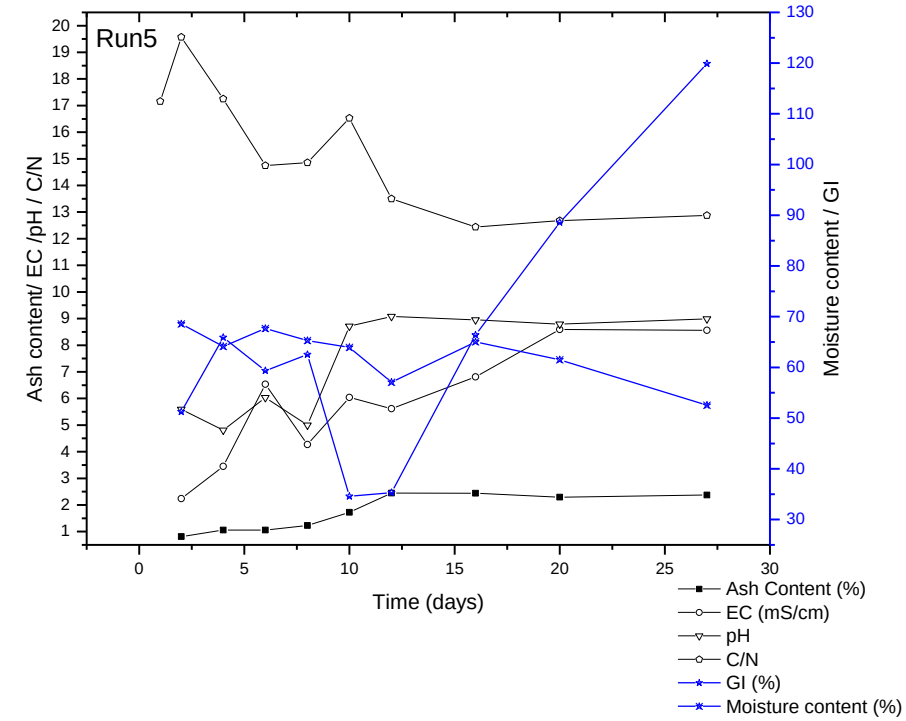

Run2
Temperature / OUR
DGH /PDE/ BGH
Time (days)
Temperature
OUR (10^2 L/min)
DGH (µg TPF/ g dry matter)
BGH (µg PNP/ g dry matter.h)
PDE (µg PNP/ g dry matter.h)

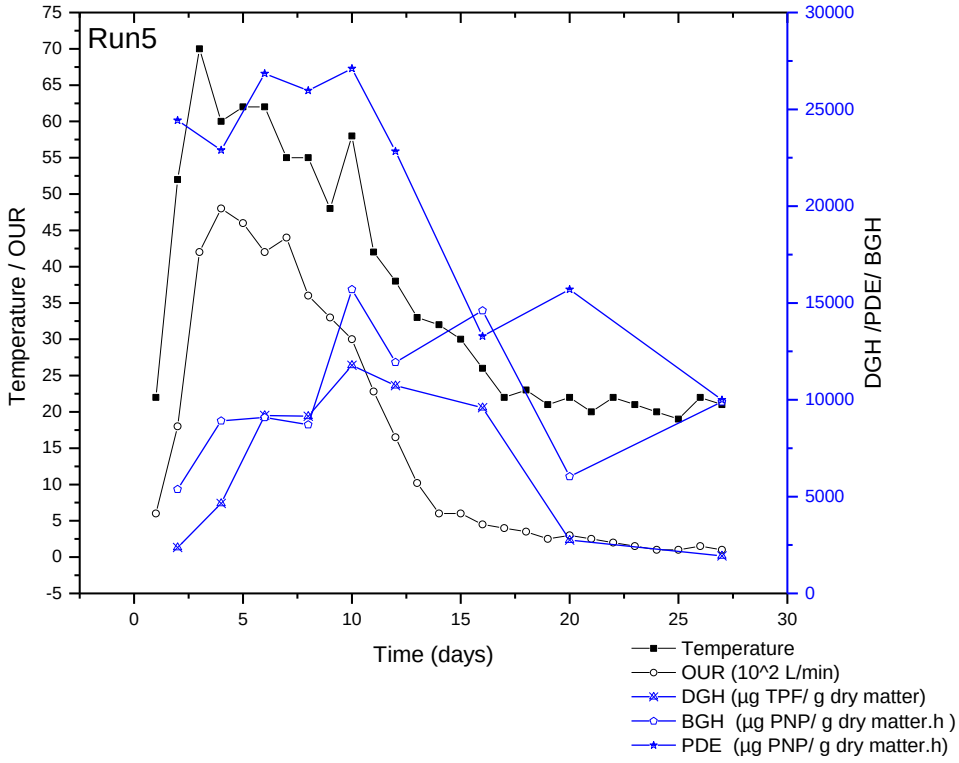

Run3
Ash content/ EC /pH / C/N
Moisture content / GI
Time (days)
Ash Content (%)
EC (mS/cm)
pH
C/N
GI (%)
Moisture content (%)

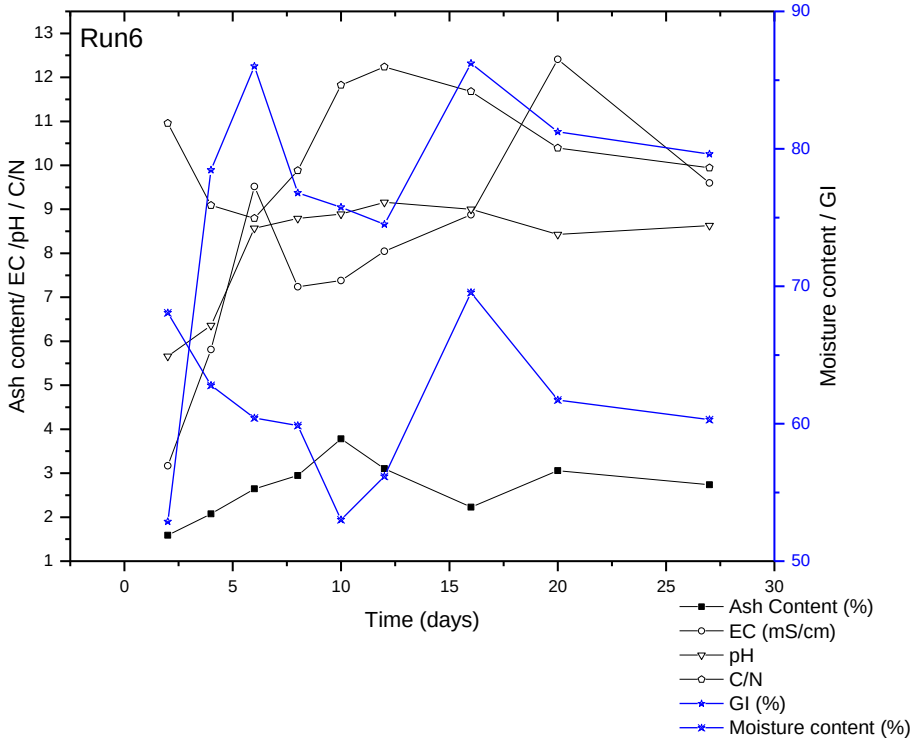

Run3
Temperature / OUR
DGH /PDE/ BGH
Time (days)
Temperature
OUR (10^2 L/min)
DGH (µg TPF/ g dry matter)
BGH (µg PNP/ g dry matter.h)
PDE (µg PNP/ g dry matter.h)

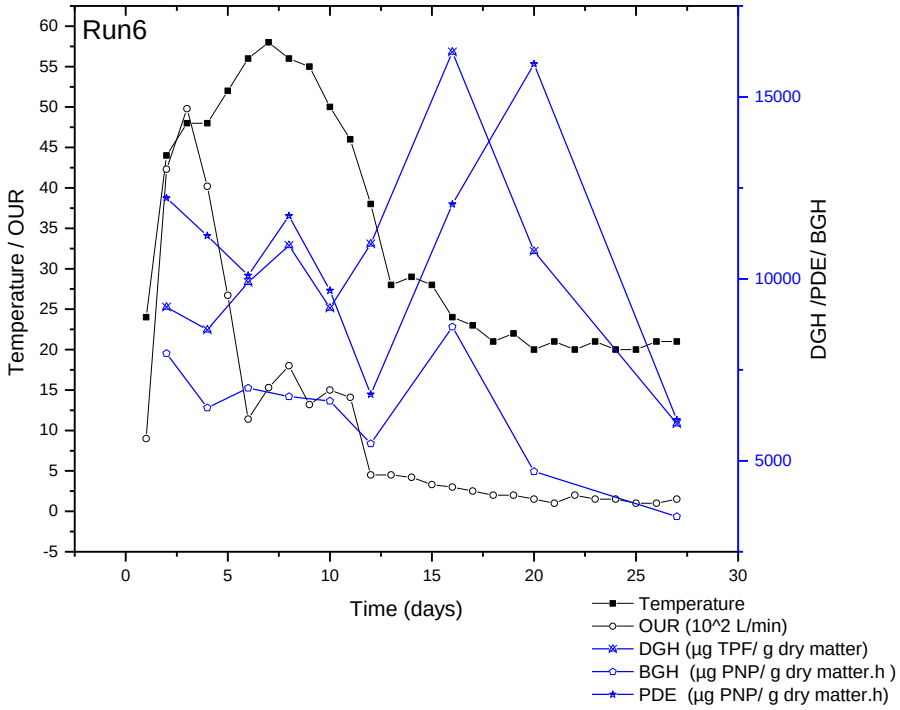

Run4
Ash content/ EC /pH / C/N
Moisture content / GI
Time (days)
Ash Content (%)
EC (mS/cm)
pH
C/N
GI (%)
Moisture content (%)

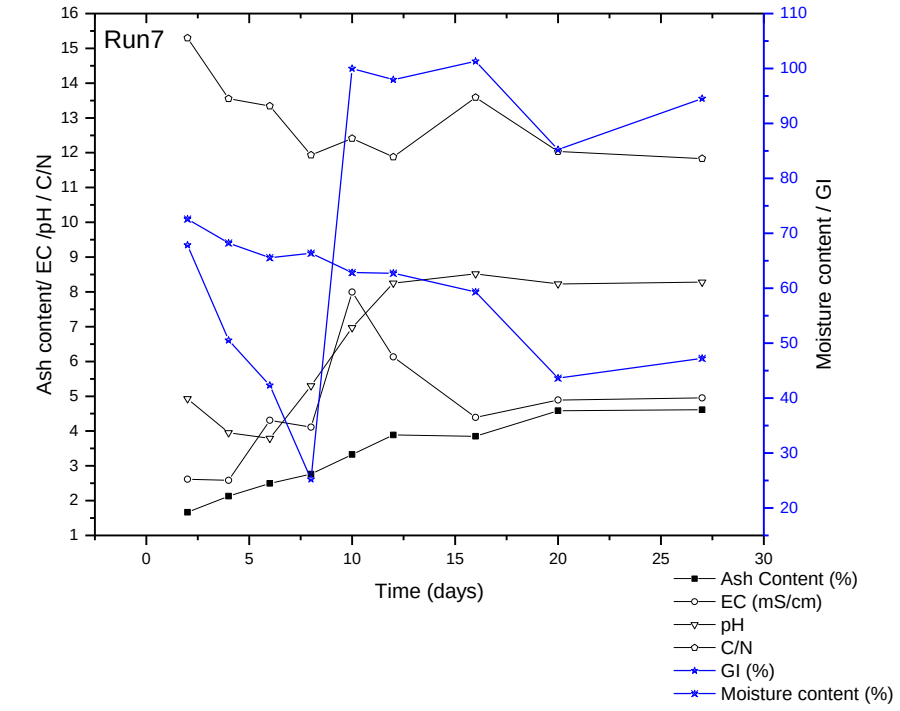

Run4
Temperature / OUR
DGH /PDE/ BGH
Time (days)
Temperature
OUR (10^2 L/min)
DGH (µg TPF/ g dry matter)
BGH (µg PNP/ g dry matter.h)
PDE (µg PNP/ g dry matter.h)

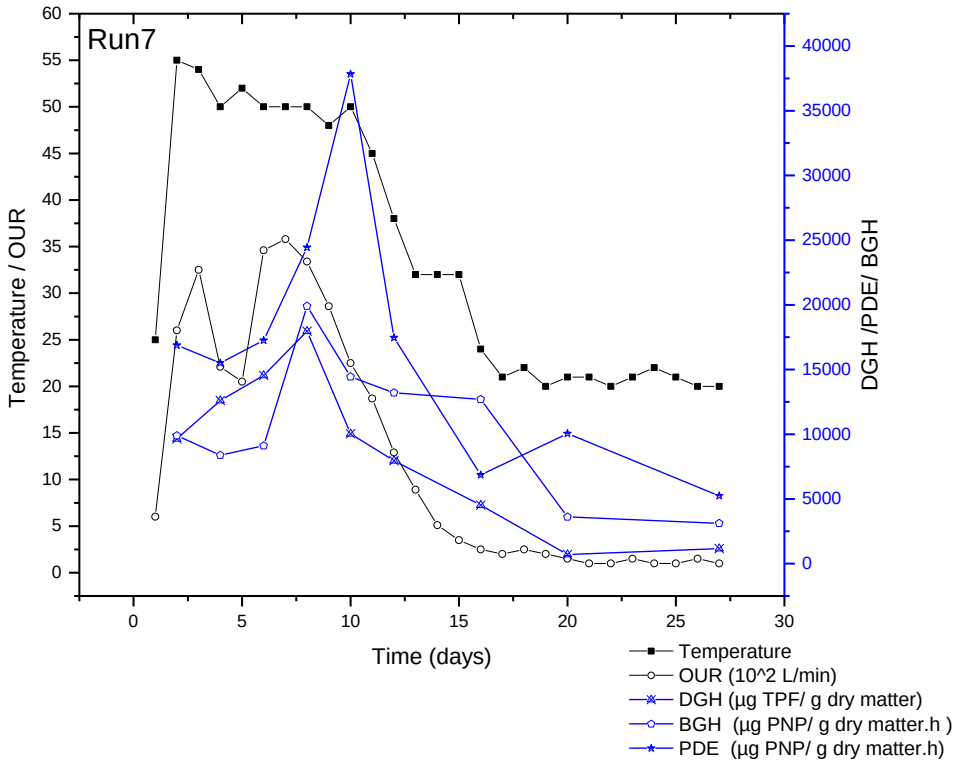

Run5
Ash content/ EC /pH / C/N
Moisture content / GI
Time (days)
Ash Content (%)
EC (mS/cm)
pH
C/N
GI (%)
Moisture content (%)

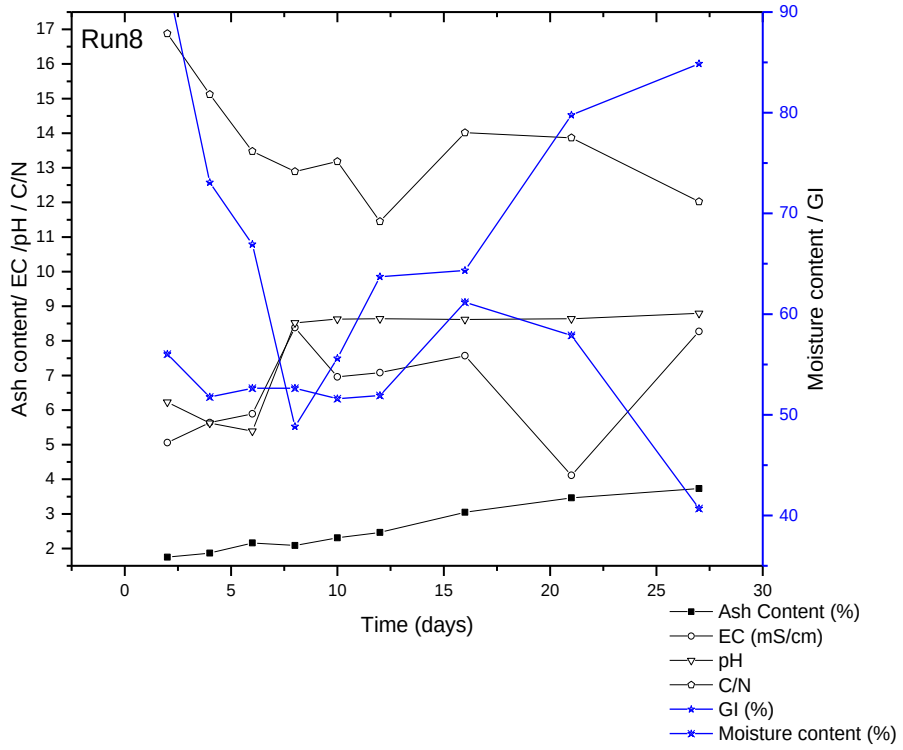

Run5
Temperature / OUR
DGH /PDE/ BGH
Time (days)
Temperature
OUR (10^2 L/min)
DGH (µg TPF/ g dry matter)
BGH (µg PNP/ g dry matter.h)
PDE (µg PNP/ g dry matter.h)

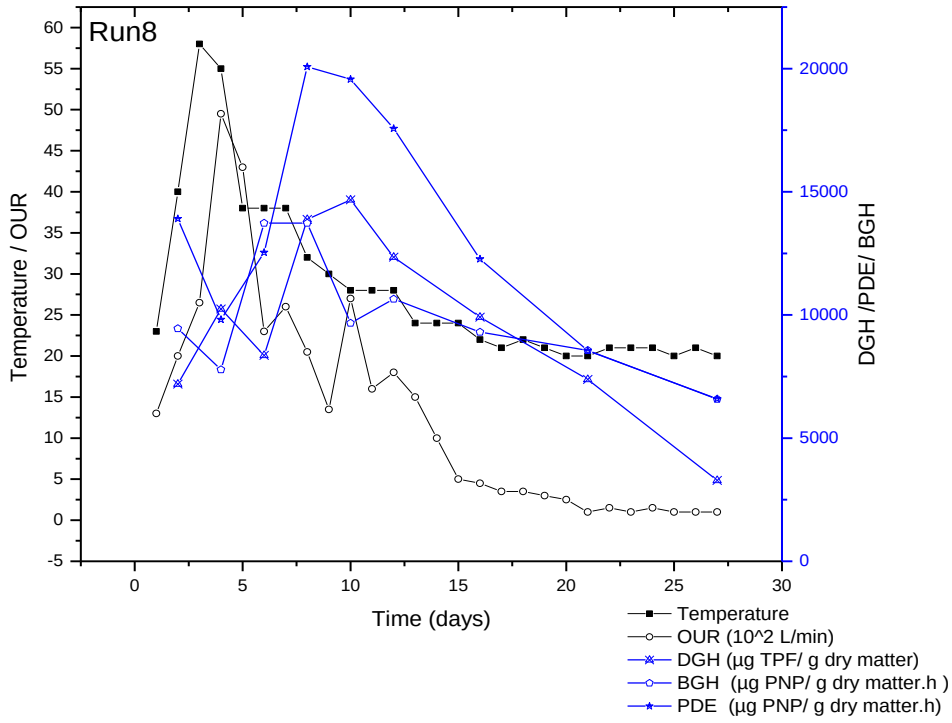

Run6
Ash content/ EC /pH / C/N
Moisture content / GI
Time (days)
Ash Content (%)
EC (mS/cm)
pH
C/N
GI (%)
Moisture content (%)

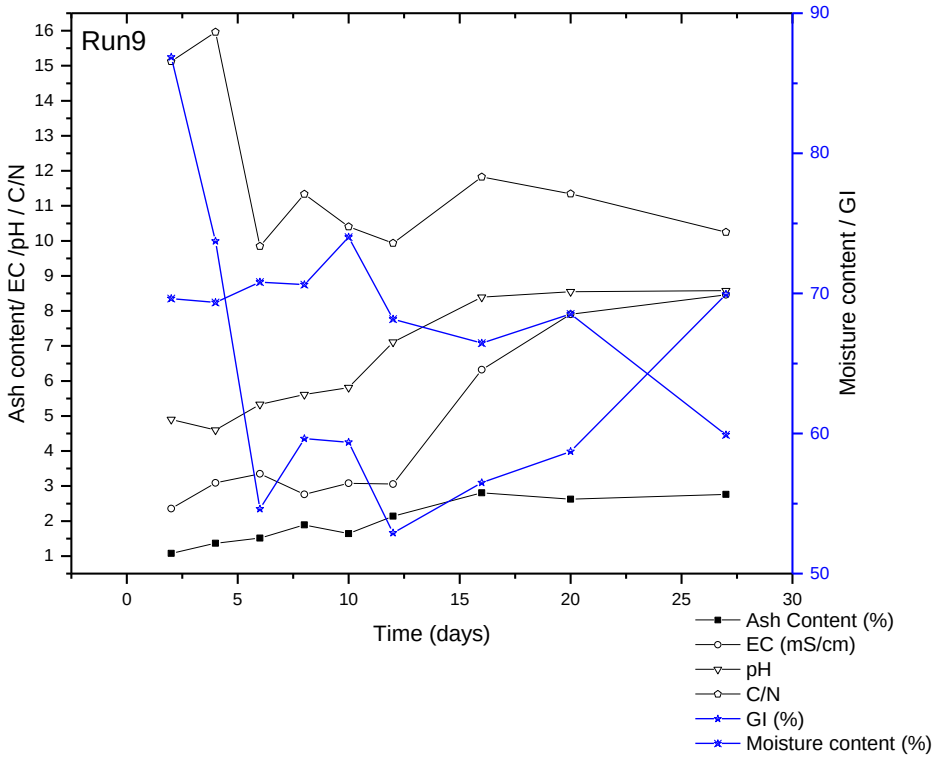

Run6
Temperature / OUR
DGH /PDE/ BGH
Time (days)
Temperature
OUR (10^2 L/min)
DGH (µg TPF/ g dry matter)
BGH (µg PNP/ g dry matter.h)
PDE (µg PNP/ g dry matter.h)

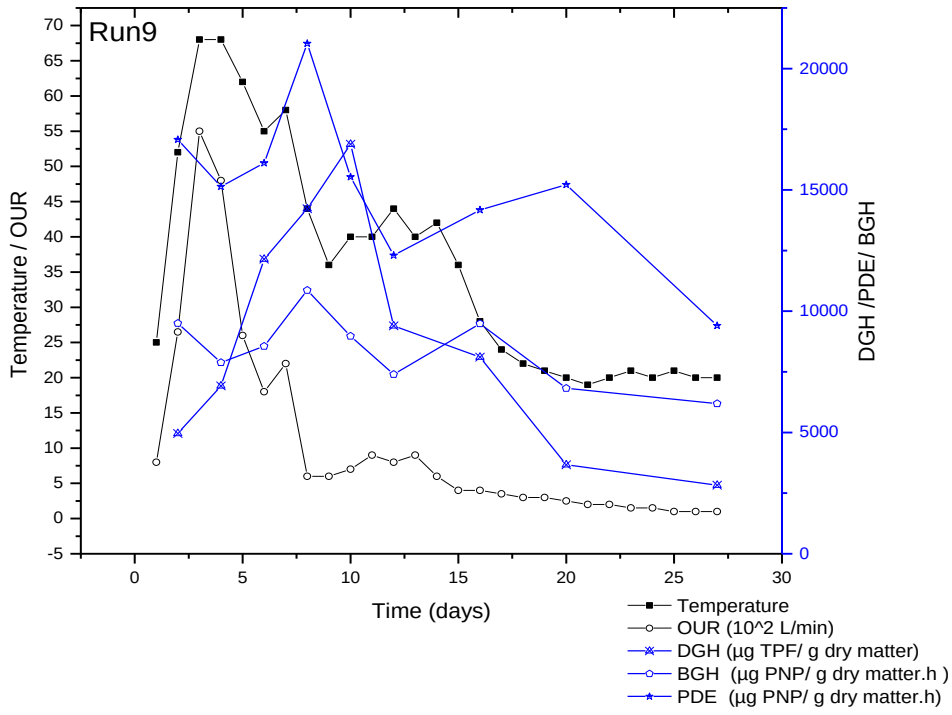

Run7
Ash content/ EC /pH / C/N
Moisture content / GI
Time (days)
Ash Content (%)
EC (mS/cm)
pH
C/N
GI (%)
Moisture content (%)

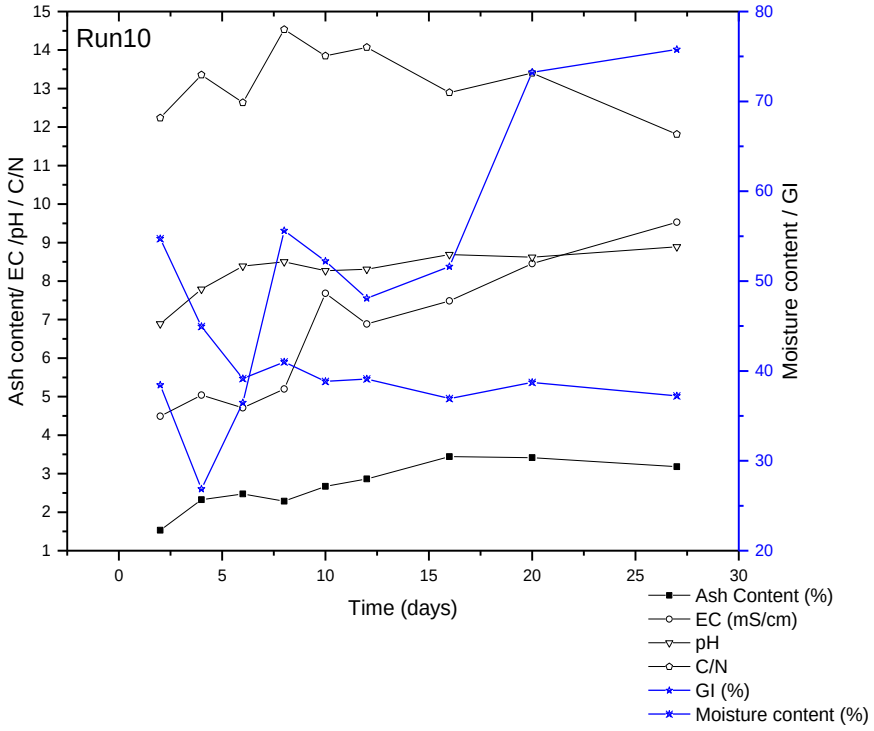

Run7
Temperature / OUR
DGH /PDE/ BGH
Time (days)
Temperature
OUR (10^2 L/min)
DGH (µg TPF/ g dry matter)
BGH (µg PNP/ g dry matter.h)
PDE (µg PNP/ g dry matter.h)

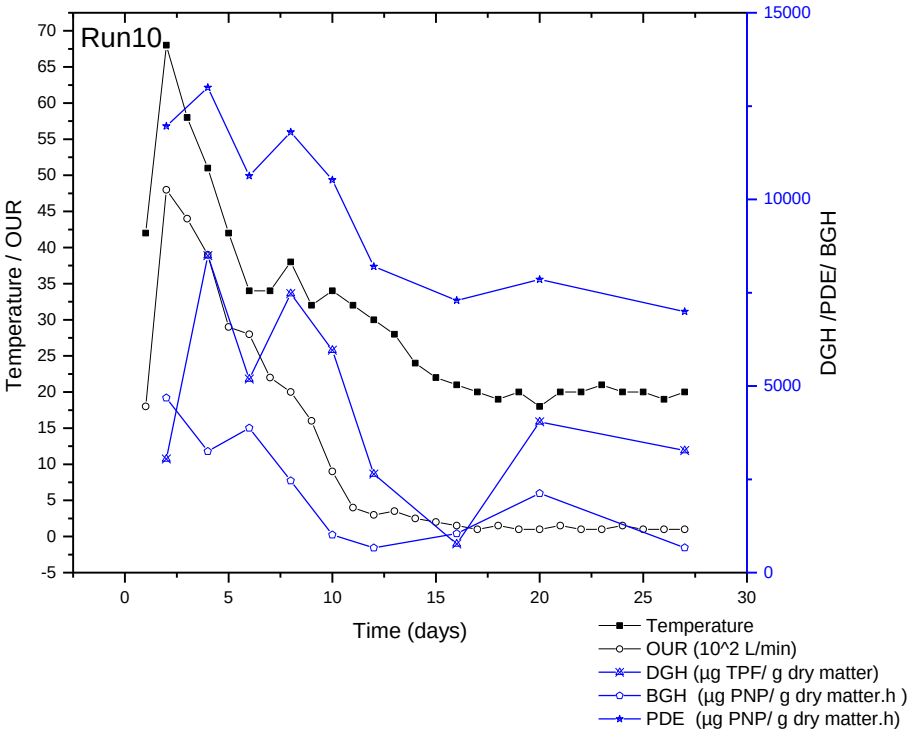

Run8
Ash content/ EC /pH / C/N
Moisture content / GI
Time (days)
Ash Content (%)
EC (mS/cm)
pH
C/N
GI (%)
Moisture content (%)

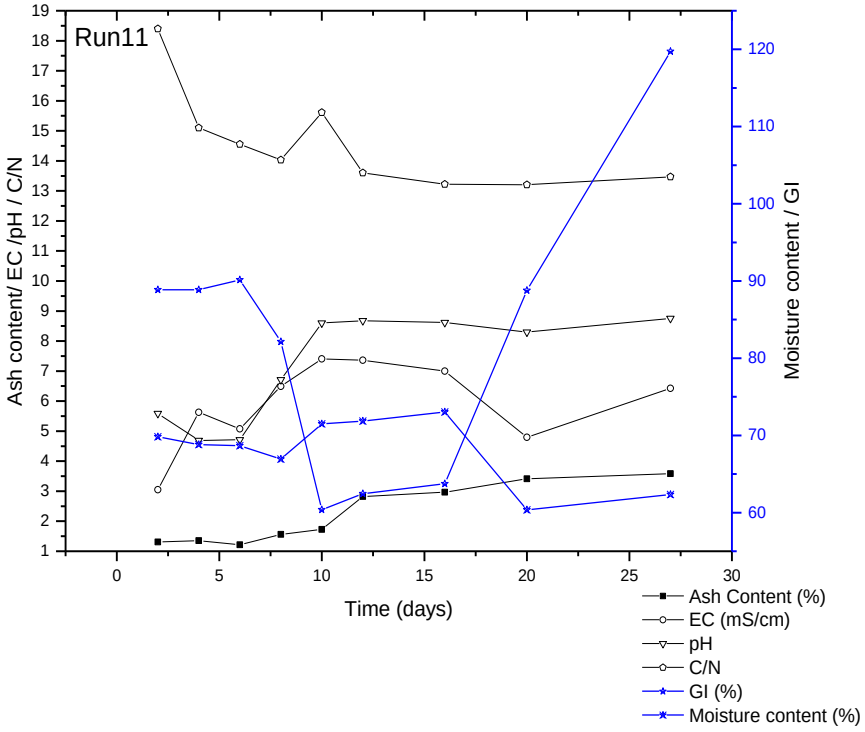

Run8
Temperature / OUR
DGH /PDE/ BGH
Time (days)
Temperature
OUR (10^2 L/min)
DGH (µg TPF/ g dry matter)
BGH (µg PNP/ g dry matter.h)
PDE (µg PNP/ g dry matter.h)

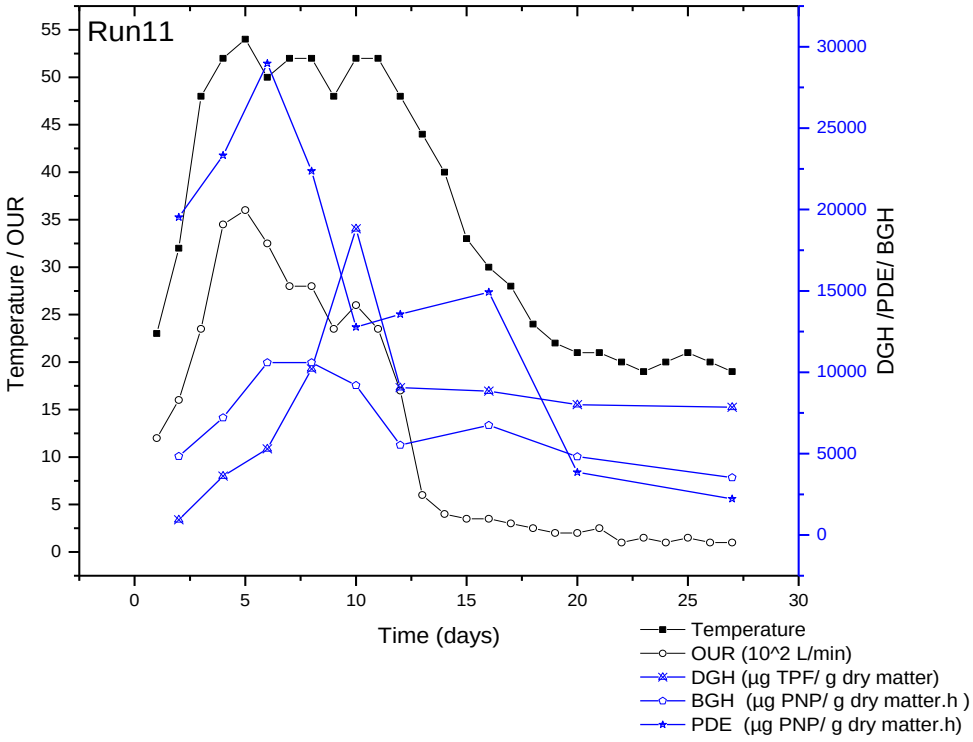

Run9
Ash content/ EC /pH / C/N
Moisture content / GI
Time (days)
Ash Content (%)
EC (mS/cm)
pH
C/N
GI (%)
Moisture content (%)

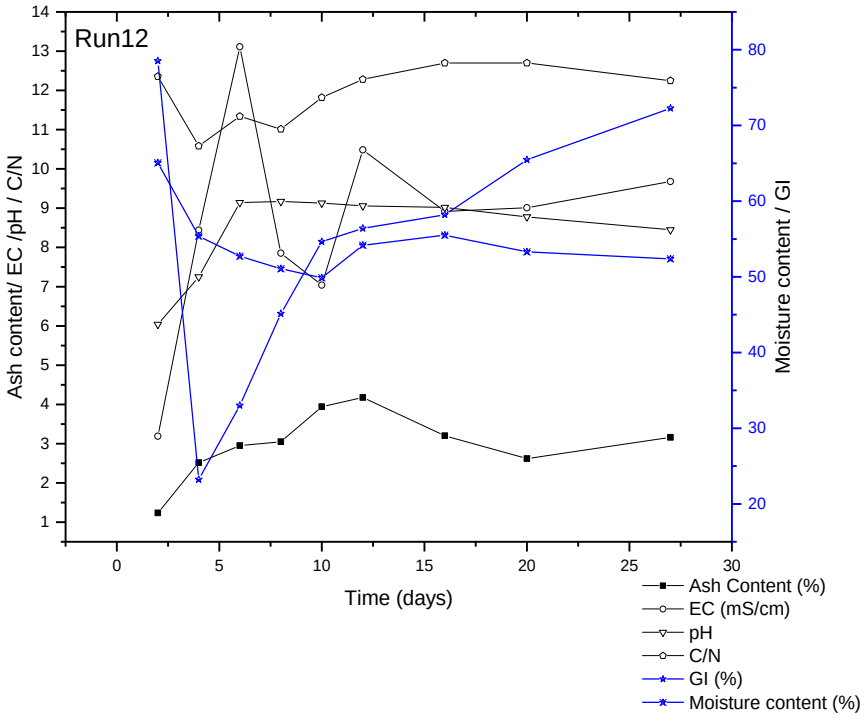

Run9
Temperature / OUR
DGH /PDE/ BGH
Time (days)
Temperature
OUR (10^2 L/min)
DGH (µg TPF/ g dry matter)
BGH (µg PNP/ g dry matter.h)
PDE (µg PNP/ g dry matter.h)

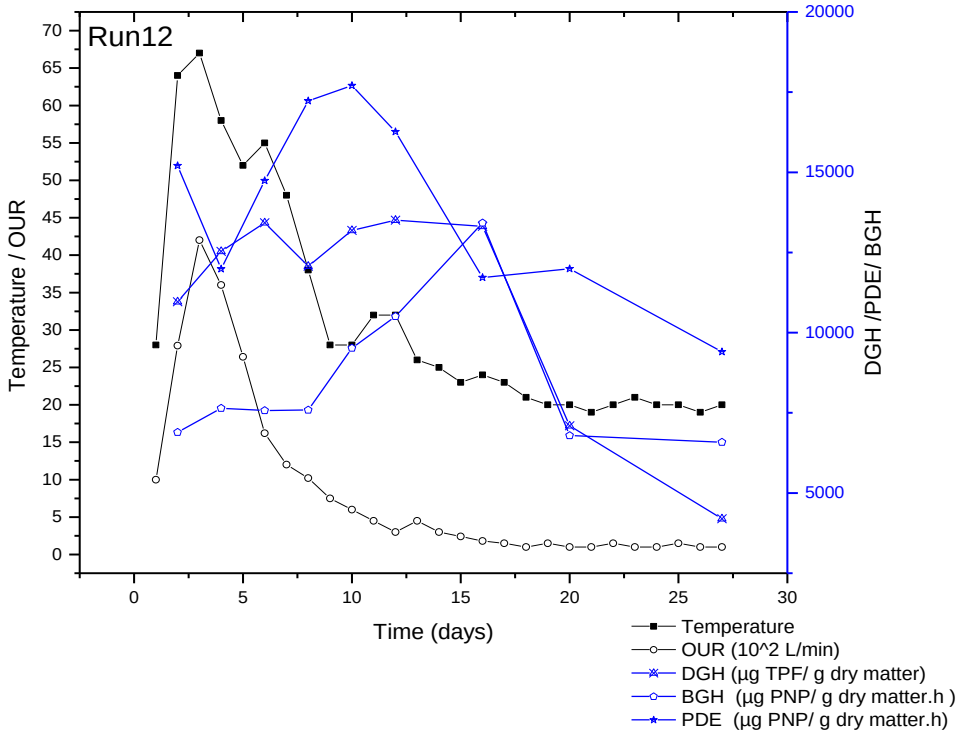

Run10
Ash content/ EC /pH / C/N
Moisture content / GI
Time (days)
Ash Content (%)
EC (mS/cm)
pH
C/N
GI (%)
Moisture content (%)

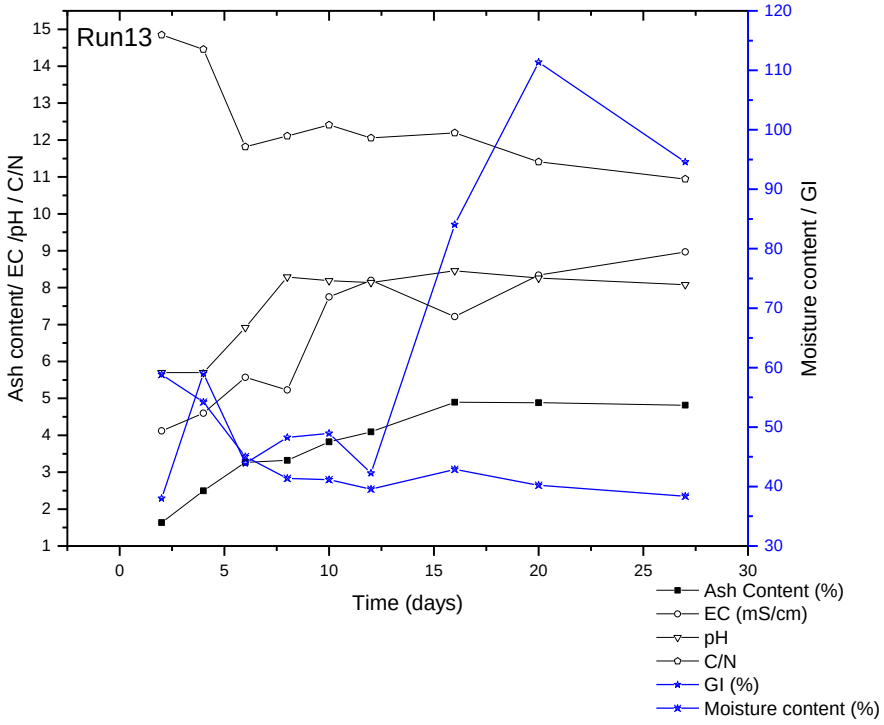

Run10
Temperature / OUR
DGH /PDE/ BGH
Time (days)
Temperature
OUR (10^2 L/min)
DGH (µg TPF/ g dry matter)
BGH (µg PNP/ g dry matter.h)
PDE (µg PNP/ g dry matter.h)

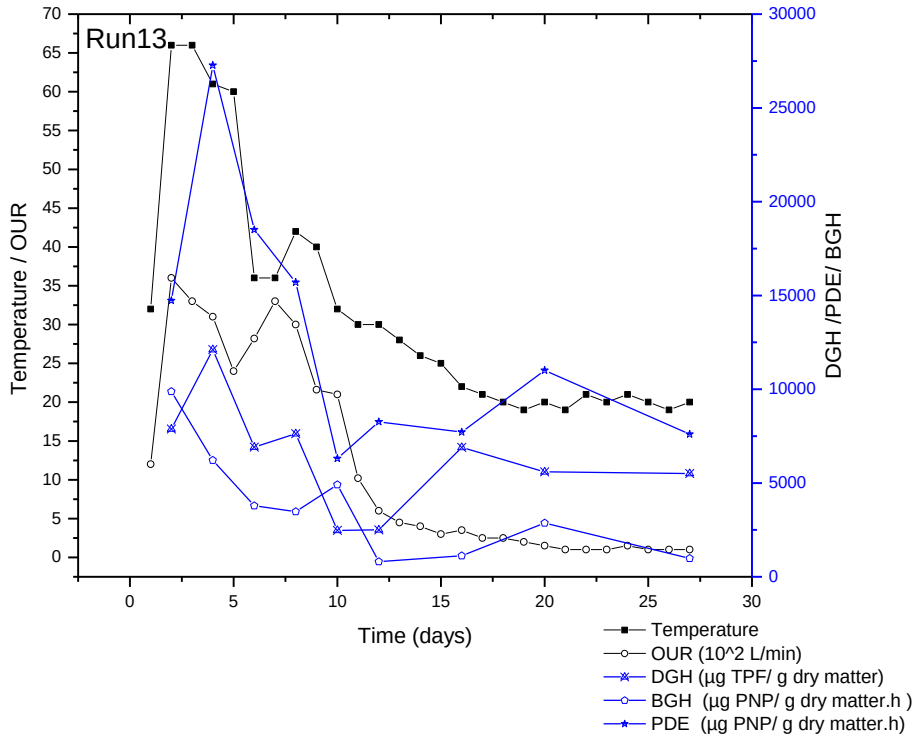

Run11
Ash content/ EC /pH / C/N
Moisture content / GI
Time (days)
Ash Content (%)
EC (mS/cm)
pH
C/N
GI (%)
Moisture content (%)

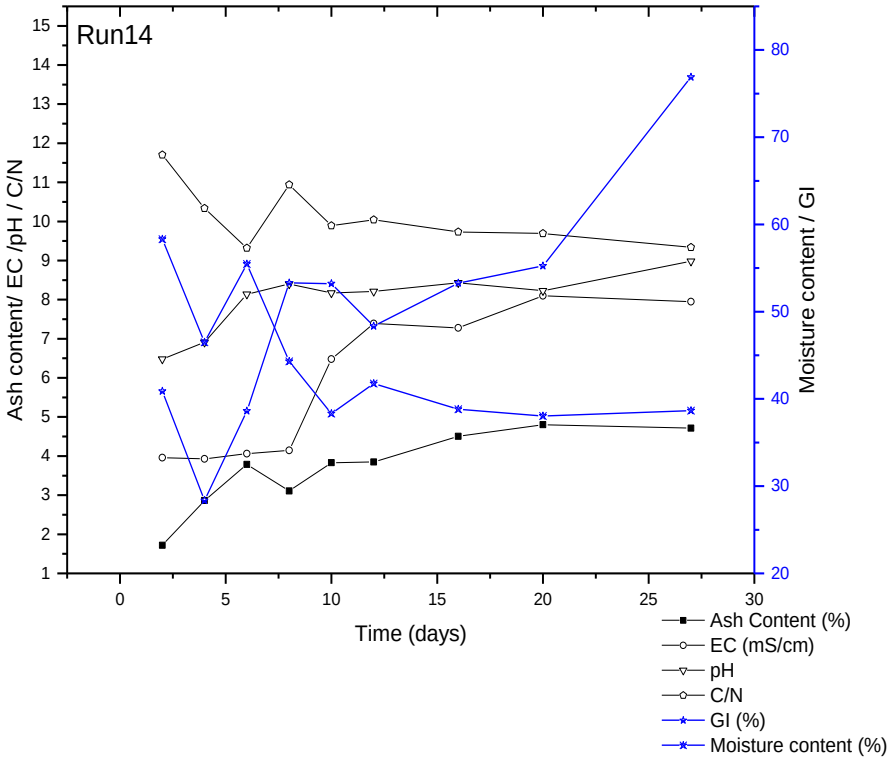

Run11
Temperature / OUR
DGH /PDE/ BGH
Time (days)
Temperature
OUR (10^2 L/min)
DGH (µg TPF/ g dry matter)
BGH (µg PNP/ g dry matter.h)
PDE (µg PNP/ g dry matter.h)

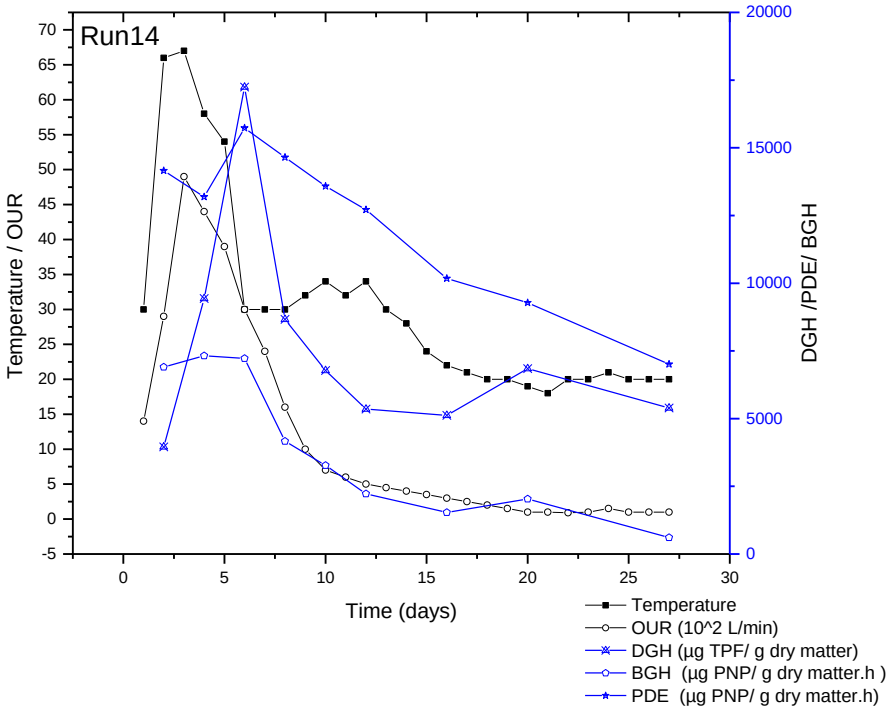

Run12
Ash content/ EC /pH / C/N
Moisture content / GI
Time (days)
Ash Content (%)
EC (mS/cm)
pH
C/N
GI (%)
Moisture content (%)

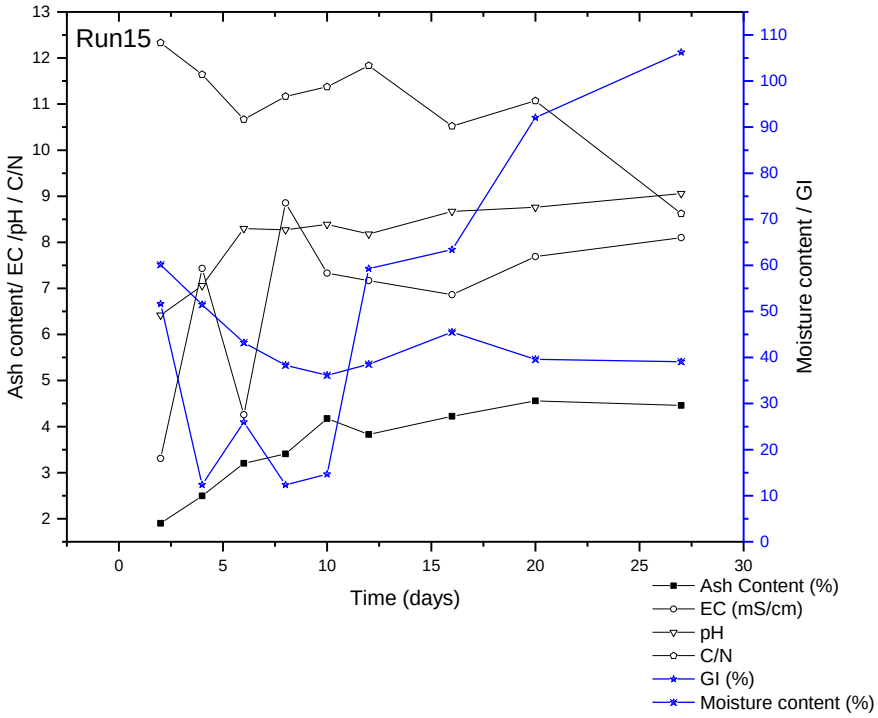

Run12
Temperature / OUR
DGH /PDE/ BGH
Time (days)
Temperature
OUR (10^2 L/min)
DGH (µg TPF/ g dry matter)
BGH (µg PNP/ g dry matter.h)
PDE (µg PNP/ g dry matter.h)

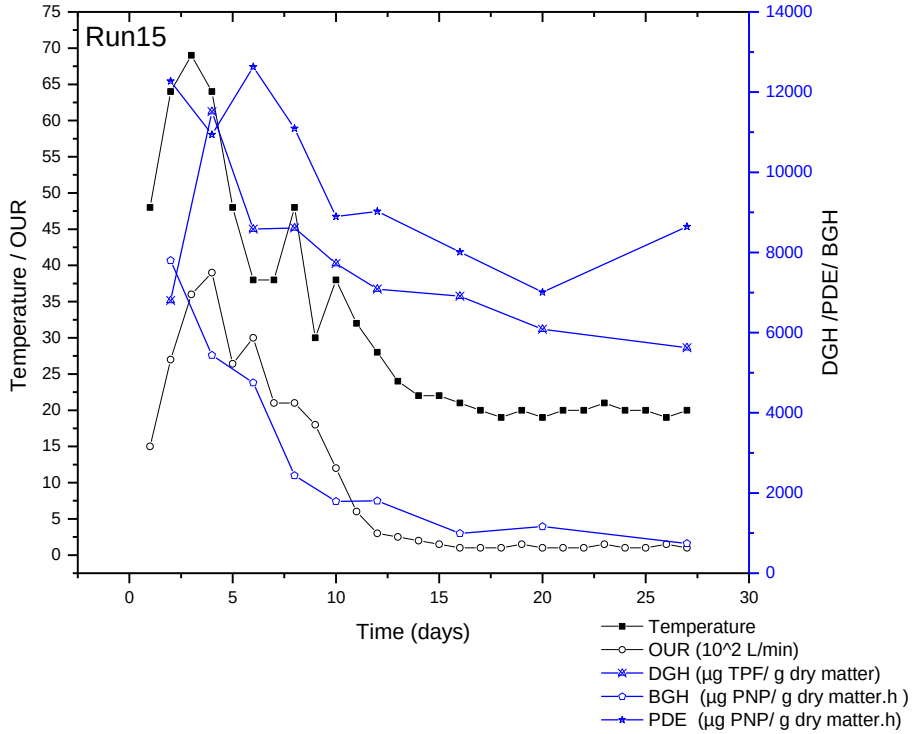

Run13
Ash content/ EC /pH / C/N
Moisture content / GI
Time (days)
Ash Content (%)
EC (mS/cm)
pH
C/N
GI (%)
Moisture content (%)


Run13
Temperature / OUR
DGH /PDE/ BGH
Time (days)
Temperature
OUR (10^2 L/min)
DGH (µg TPF/ g dry matter)
BGH (µg PNP/ g dry matter.h)
PDE (µg PNP/ g dry matter.h)


Run14
Ash content/ EC /pH / C/N
Moisture content / GI
Time (days)
Ash Content (%)
EC (mS/cm)
pH
C/N
GI (%)
Moisture content (%)


Run14
Temperature / OUR
DGH /PDE/ BGH
Time (days)
Temperature
OUR (10^2 L/min)
DGH (µg TPF/ g dry matter)
BGH (µg PNP/ g dry matter.h)
PDE (µg PNP/ g dry matter.h)


Run15
Ash content/ EC /pH / C/N
Moisture content / GI
Time (days)
Ash Content (%)
EC (mS/cm)
pH
C/N
GI (%)
Moisture content (%)


Run15
Temperature / OUR
DGH /PDE/ BGH
Time (days)
Temperature
OUR (10^2 L/min)
DGH (µg TPF/ g dry matter)
BGH (µg PNP/ g dry matter.h)
PDE (µg PNP/ g dry matter.h)

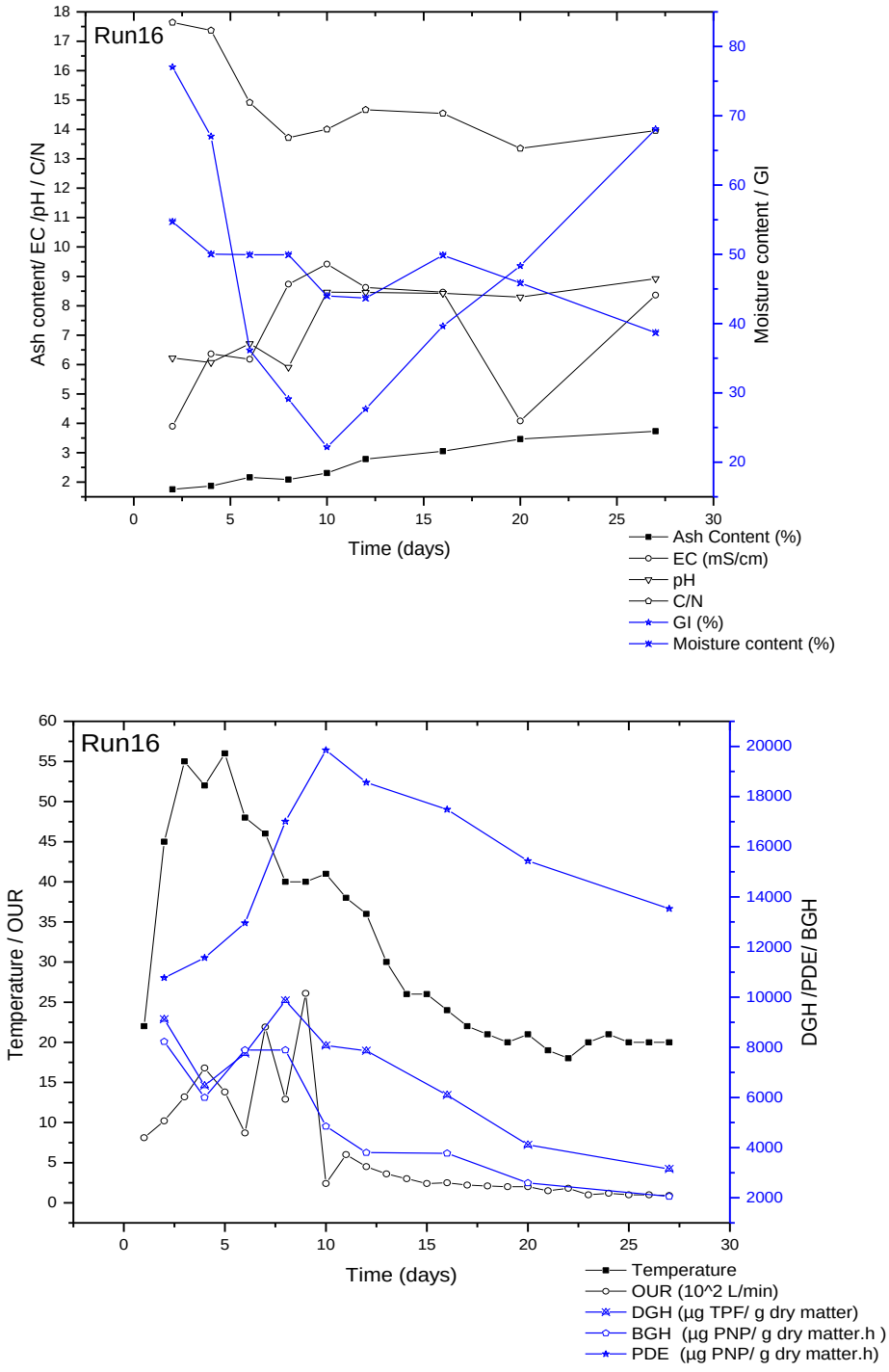


Figure 4.2 Temporal Variations of Temperature, OUR, pH, EC, MC, Ash content, GI, C/N, DGH, BGH, PDE

The temperature pattern corresponded to the typical composting temperature profile at the laboratory scale reactor for most of the runs. Generally, the composting process occurs in 2 stages: the biooxidative stage and the maturing stage. The biooxidative stage can be divided into three phases: (i) during the mesophilic phase, mostly bacterial and fungi degrade simple OM such as sugar, amino acids, and proteins; (ii) during the thermophilic phase, fats, cellulose, and hemicellulose are degraded by themophilic microorganisms and the pathogens are suppressed; and (iii) during the last phase or cooling phase, the deficiency of the biodegradable material leads to reduction of microbial activity and decrease in temperature (Bernal et al. 2009). The appearance of the temperature peak depended on the composition of the waste material which affects the microorganism growth. The first temperature peak is a result of aerobic biodegradation of the fast decomposing OM in the food waste. Some runs including run 2 and 4 showed second peak at the beginning of the second week. The second peak value can be a result of degradation of slowly decomposing material. Except run 7, 8, 10, 11, and 16 all other runs met the requirement of the US Environmental Protection Agency (EPA) regulation; based on the US EPA regulation, aerated static piles and in vessel systems should maintain a minimum operating temperature of 55°C for at least 3 days to inactivate pathogen and destruct seed (EPA 2003). The highest temperature was recorded for run 5 and the longest thermophilic activity occurred also on run 5 which was 10 days. Run 3, 6 and 11 reached the themophilic phase later than the others.

Respiration (CO_2 evolution rate or OUR) has been used to determine biological stability and microbial activity (Xiao et al. 2009). Said-Pullicino et al. (2007) observed the

maximum specific oxygen uptake rate (SOUR) during the active phase in which most of the OM was degraded. It can thus be concluded that OUR max is related to the concentration of immediate carbon sources (i.e., sugars, amino sugars, amino acids, simple organic acids). Microbial respiration has been used to assess microbial activity, the evolution of the composting process and stability of the final product (Ros et al. 2006). The trend of OUR in all runs were very similar to the trend of the temperature. The highest OUR was recorded during the thermophilic phase, when the temperature rises. High OUR indicates high biological activity in the composting system and OM are available for microorganism to be degraded, therefore the material is not stabilized yet (Said-Pullicino et al. 2007). Similar to the temperature, OUR showed several peaks and after the active phase, the OUR decreased and reached a steady state. Low OUR indicates OM are more stabilized and most of the OM has been decomposed by microorganisms (Said-Pullicino et al. 2007). For some runs like run 5, the duration for high OUR was longer whereas for runs 6 and 9 a sharp increase was observed and then it decreased dramatically. The maximum values of OUR was between 26.1 and 62 $(\frac{10^{-2}\mathrm{L}}{\mathrm{min}})$ for run 16 and for run 9, respectively.

The initial pH of some runs such as 3, 5, 7, 9, 11 were slightly acidic as a result organic acids like acetic acid and butyric acid have been produced by microbial activity during storage and initial phase of composting (Adhikari et al. 2009; Eklind and Kirchmann 2000; Smårs et al. 2002). The pH value of the compost can influence the physical-chemical and microbiological reactions in the compost and it is one of the important factors to evaluate compost stability and maturity (Banegas et al. 2007). When

composting process proceeds, pH increases which can be associated with the accumulation of NH_4^+ due to proton assimilation during ammonification and N mineralization and consumption of organic acids as a substrate by microbial activity (Dresbøll and Thorup-Kristensen 2005; Rasapoor et al. 2009). Loss of ammonium through volatilization and nitrification, and accumulation of organic acid and CO_2 during decomposition of the simple OM like carbohydrates lead to delay in pH increase for some runs including 4, 5, 7, 8, 9, 11, and 16 (Banegas et al. 2007; Kayikçioğlu and Okur 2011). Run 9 had the longest duration before pH increases; it could be due to the rapid increase of temperature during the thermophilic phase. Microorganisms cannot tolerate high temperature and low pH at the same time. Thermophilic bacterial are not acid tolerant so the low pH led to a decline in microbial activity and the low microbial activity resulted in low degradation rate and longer period of the acidic pH (Sundberg and Jönsson 2008). In all runs, after almost 10 days, pH increased above 8 and it stayed almost steady by the end of the experiment.

The EC values of runs on the second day of composting were between 2.02 to 5.05 mS/cm. Generally, EC values increased with time for all runs. This increase could be due to the release of mineral cation concentration such as ammonium ions and phosphate which did not bind to the stable organic complex or went out of the system through leachate (Francou et al. 2005; Kalamdhad et al. 2009). For a short period of time a slight decrease was observed for runs 6 and 9, this decrease may be attributed to the reduction of water soluble substances such as organic acids during the composting process (Arslan et al. 2011). Final values of EC were between 5 and 9.68 mS/cm at the end of the process.

MC showed descending trends in all runs. High temperature and aeration caused evaporation and evaporation led to MC decrease during composting, especially at the thermophilic phase (Lashermes et al. 2012; Said-Pullicino et al. 2007). Runs 7, 10, 12, 13, and 15 showed corresponding trends, as temperature increased, moisture decreased. Decrease of the MC during composting is an indication of decomposition of OM (Arslan et al. 2011; Kalamdhad et al. 2009). On the first 6 days, except run 9, high temperature had positive effect on moisture reduction. Although in general, moisture showed reducing trend in all runs, temperature was not high enough to evaporate the water produced through microbial activity and consequently moisture showed temporary increasing trend for a short period of time for some runs including runs 6, 8, 9, and 11. Moisture content for runs 9 and 11 exceeded 70% at some points, which is undesirable for composting because it is capable of creating anaerobic condition (Tiquia and Tam 1998). The highest reduction was observed for run 7 which could be associated to the long period of high temperature. Haug (1993) suggested 40% as the minimum MC to continue microbial activity. MC at the end of experiments, in all runs with 70% initial MC, was above 40%. In contrast, MC in runs with 55% initial MC, reached under 40% except run 4 and run 8.

The amount of ash increased consistently in all runs. The ash content increasing trend had large slope at the thermophilic stage, and then the slope became smoother when temperature dropped. During composting, the OM was decomposed into volatile compound, and consequently the final compost has lower OM and higher ash content (Kalamdhad et al. 2009). The Ash content of the samples at the beginning of experiment was between 0.8 % and 1.2%. At the end of the experiments, the ash content increased to

2.1% to 5.5%. The highest increase happened in run 7 which could be due to the long thermophilic phase.

As Wichuk and McCartney (2010) mentioned, decomposition of OM releases carbon dioxide and it results in carbon lost from the composting system, therefore a decreasing trend with eventual stabilization were observed for C/N ratio during composting process. The highest declines in C/N ratio were observed during the thermophilic phase and at the end of the first week for most of the runs similar to the results of Brewer and Sullivan (2003) and Bustamante et al. (2008), except run 1, 2, and 10. After the themophilic phase, the C/N ratio stayed almost stable except runs 3, 6, 12, and 15. The biodegradation still is ongoing during this phase because when pH is higher than 7.5, carbon and nitrogen lost are happening through CO_2 and NH_3 release but C/N ratio remains stable (Hao et al. 2004). Although a C/N ratio below 20 is suggested as indicative of acceptable maturity in the final product, and a ratio of 15 or even less is preferable (Iglesias Jiménez and Perez Garcia 1989; Raut et al. 2008), the C/N ratio cannot be used as an indicator of the state of maturation of these composts because of the variation and the low initial C/N of composting material (<20). The periodic monitoring of C/N ratio during composting in conjunction with other parameters such as temperature can be a good indicator for OM degradation and reliable factor for compost stability and maturity evaluation (Iglesias Jiménez and Pérez García 1992; Wichuk and McCartney 2010). An increase of C/N ratio occurred during the thermophilic phase for runs 3, 6, 12 and 15 after the primary decrease which can be attributed to NH_3 volatilization (Hutchinson and Griffin 2008).

Seed germination test helps to evaluate the efficiency of the composting process for plant growth and seed germination (Banegas et al. 2007). In this study, the raw material is synthetic food waste, which does not contain any toxic material for plant growth; consequently, the germination indices in most of the runs at the beginning were very high. In some runs low germination indices at the beginning can be a result of the fast starting of biological activity and the formation of toxic compounds such as alcohols, phenolic compound, and organic acids which inhibits seed germination (Vargas-Garcia et al. 2010). GI decreased during the thermophilic phase and during the transition of thermophilic to mesophilic phase. This decrease attributed to the production of low molecular weight short chain volatile fatty acids (mainly acetic acid) and ammonia (Fang et al. 1999). In the majority of runs GI started to increase after 3 weeks of composting. It has been suggested that a GI over 80% indicates the absence of phytotoxicities in compost (Tiquia and Tam 1998; Zucconi et al. 1981). Only GI of runs 4, 5, 8, 11, 13, and 15 raised over 80%, in other runs the low GI can be associated with the stage of the composting. Runs 1, 2 and 3 showed low GI after 4 weeks. EC can affect adversely seed germination in these runs.

DGH is not related to a specific element cycle. It has been used to evaluate the microbial activity because it belongs to the group of intercellular enzymes which catalyse the oxidation of compost OM (Benito et al. 2003; Saidi et al. 2009; Vargas-Garcia et al. 2010). Due to the relationship between DGH and temperature, Barrena et al. (2008) suggested to use DGH to describe the biological activity during the thermophilic and mesophilic stage. DGH initially increased for all runs. The DGH values at the beginning

of some runs were high, which could be a result of the fast starting of microbial activity or starting microbial activity during storage time. As Kayikçioğlu and Okur (2011) and Barrena et al. (2008) pointed out the maximum values of DGH corresponded to the end of the thermophilic phase or the beginning of the mesophilic stage. In this study, runs 2, 3, 4, 5, 6, 8, 9, 11, 14, and 16 showed the similar patterns. For some runs including 1, 10, 13, and 15 the peak temperatures, OUR, and DGH appear simultaneously. Vargas-Garcia et al. (2010) and Kayikçioğlu and Okur (2011) stated that the higher DGH values are accompanied by higher microbial activity and lower values associated with the maturation phase. After day 20, the DGH decreased, which means that most of the OM has been degraded by microorganisms and converted to the stable material and consequently the respiratory process slowed down.

The BGH was high at the beginning of the composting. High BGH in the initial phases was also observed by Vargas-Garcia et al. (2010). BGH catalyses the hydrolysis of the b-glucoside bonds of the carbohydrates, which contributes to the release of energy for microbial activity. The high BGH is related to the availability of the readily metabolized substrate in initial phase (Ros et al. 2006; Vargas-Garcia et al. 2010). After thermophilic phase, a decline has occurred in BGH in all runs and then it started to rise again for some runs. Mondini et al. (2004) reported the increase of BGH during the composting process. Benitez et al. (1999) also reported decrease of BGH after an increase throughout the composting process. Increase in the later stages may be related to the release of carbon compounds from the cellulytic and hemicellulytic activities and lignin content after

consuming of the easily metabolized carbon (Castaldi et al. 2008; Vargas-Garcia et al. 2010).

Phosphatases are group of enzymes that perform an important function by transforming organic P into inorganic phosphate (HPO_4^{2-} , $H_2PO_4^{-4}$) which is directly available for organisms. Phosphatases are classified into phosphomoesterases, phosphodiesterases and phosphotriesterase base on the number of ester bonds of the respective substrate (Margesin and Schinner 1994) . Although, measurement of acid and alkali phosphomonoesterases are used to assay phosphatase activity in compost in the previous studies, phosphodiesterase (phosphoric diester hydrolases) is catalyzes phospholipids and nucleic acids degradation which are among the major sources of fresh organic P inputs. PDE plays an important role in the P cycle in compost by hydrolysing one or two ester bonds in phosphodiester compounds including nucleases to produce nucleotide units or mononucleotides (Nannipieri et al. 2011). PDE showed a peak at the end of the first week of the experiment for runs including 1, 2, 3, 4, 10, 11, 13, 14, and 15. For runs including 5, 7, 8, 9, 12, and 16 the peak observed at the second week and for run 6 it happened at the third week in which highest temperature observed later than other runs. The decreasing trend happened at the last days of the experiment for all runs. In the set of runs that conventional maturity and stability parameters, OUR, C/N ratio, GI indicated compost maturity, enzyme activities showed consistent results since after bio-oxidative phase enzyme activities were declined. This consistency supports the possibility of using enzyme activities as indicator of compost stability.

4.4 Statistical Analysis

In this work, a factorial design was employed to study the optimal conditions for the composting of MSW, in which four variables were assayed at two levels. Statistical analysis was conducted to screen for statistically significant factors for responses including cumulative DGH (y1), cumulative BGH (y2), cumulative PDE (y3), final C/N ratio (y4), final GI (y5), and final moisture content (y6). The result of the experiments allowed the development of regression models describing the interrelationship between operational variables and responses by equations including linear and interaction terms. The regression coefficients and their statistical significance for the calculated models and statistical parameter (R-Squared) to measure the fit of the model are presented in Table 4.4. To assess the reproducibility of the composting experiment, duplicated experiments with identical initial condition was conducted and C/N ratio and temperature were evaluated. Results indicated that the processes can be developed similarly. The data deviations were less than 10% and the analysis of deviation between the replicate profile of C/N ratio and temperature showed that the composting process was duplicable. As such blocking was deemed unnecessary in this study.

Table 4.4 Regression coefficients, significance level (P), and R-Squared to predict cumulative DGH (y1), cumulative BGH (y2), cumulative PDE (y3), final C/N ratio (y4), final GI (y5), and final moisture content (y6)

Coefficient	y_1	P_{y1}	y_2	Py2	y3	P_{y3}	y_4	P_{y4}	y_5	P_{y5}	y_6	P_{y6}
b_0	72590.5	-	55784.61	-	125258.01	-	11.11		83.30	-	47.76	-
b_1	471.92	0.7771	-1272.64	0.6779	2159.12	0.5738	-	-	-3.54	0.1778	-	-
b_2	9215.63	0.0012	11656.30	0.0024	16349.89	0.0011	-	-	1.87	0.4550	7.89	00001
b_3	749.82	0.6546	-	-	-	-	-0.98	< 0.0001	0.51	0.8361	-	-
b_4	-1733.45	0.3184	14751.44	0.0004	15079.57	0.0019	0.79	< 0.0001	8.87	0.0072	-	-
b_{12}	-	-	-10861.5	0.0039	-	-	-	-	-	-	-	-
b_{13}	-	-	-	-	-	-	-	-	-6.40	0.0303	-	-
b_{23}	-1312.1	0.4418	-	-	-	-	-	-	-8.87	0.0071		
b_{14}	5061.33	0.0192	-	-	-10530.64	0.0164	-	-	-	-	-	-
b_{24}	-6806.5	0.0053	-	-	-	-	-	-	7.01	0.0209	-	-
b_{34}	-1038.72	0.5387	-	-	-	-	-	-	-6.51	0.0283	-	-
b_{123}	-	-	-	-	-	-	-	-	-	-	-	-
b_{124}	-	-	-	-	-	-	-	-	-	-	-	-
b_{234}	7465.13	0.0034	-	-	-	-	-	-	-	-	-	-
R-Squared	0.9350	-	0.8285	-	0.8000	-	0.8508	-	0.8866	-	0.7927	-

The assumptions of ANOVA which are independency of the individual observation, normal distribution of random errors, and homogeneity of variance of random errors were checked and found satisfactory. Design-Expert 9.0 by Statease.com was used for all statistical analysis. Statistical significance is tested at the 5% level. Figure 4.3 shows the cumulative enzyme activity for all 16 runs. Statistical analysis was conducted to investigate the significant factors on cumulative enzyme activities.

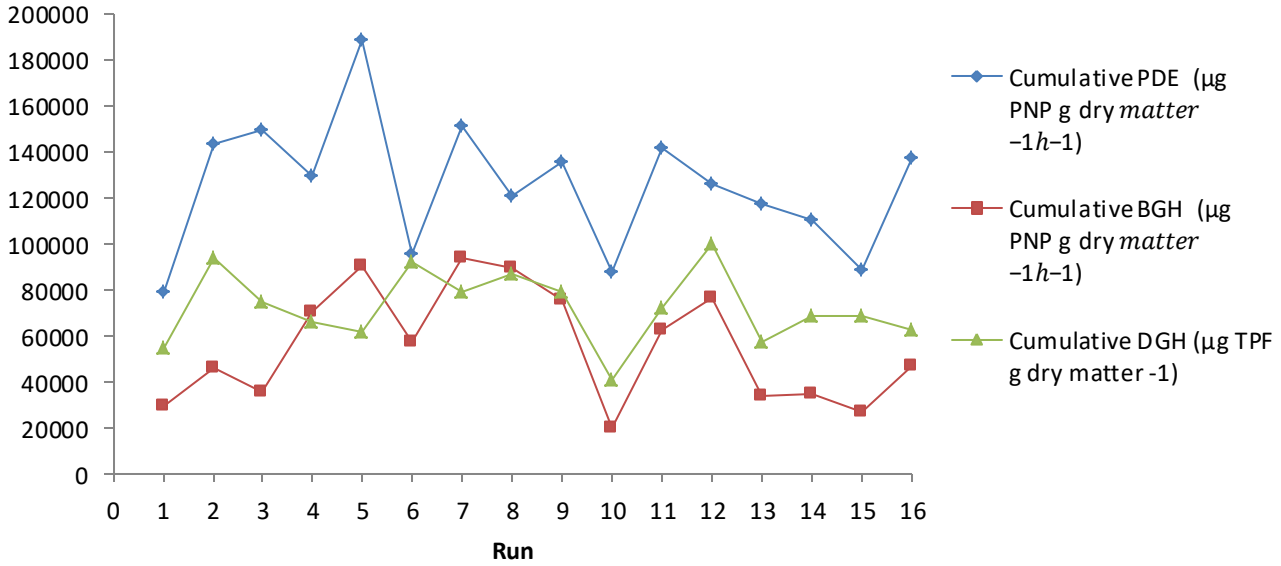
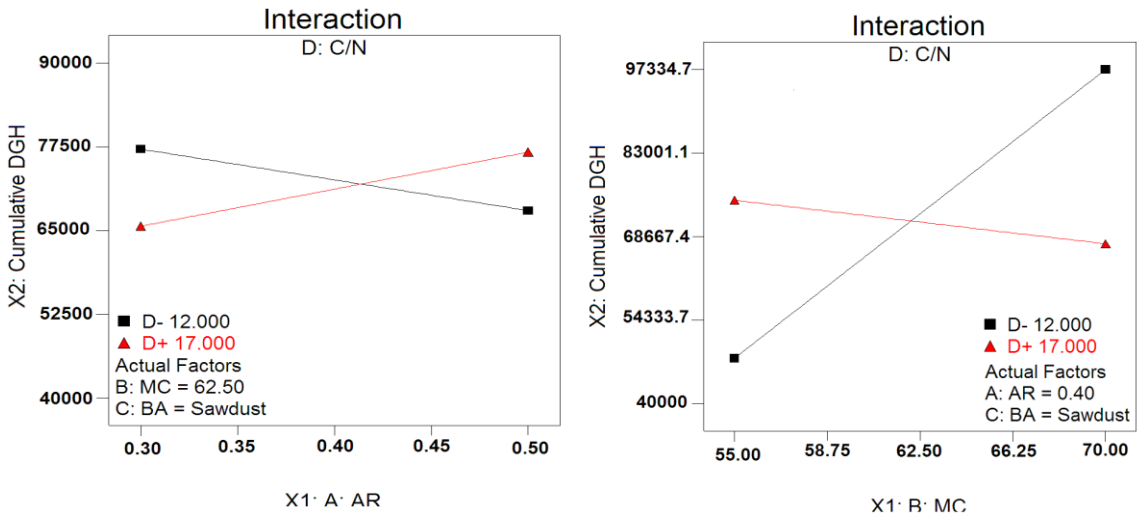
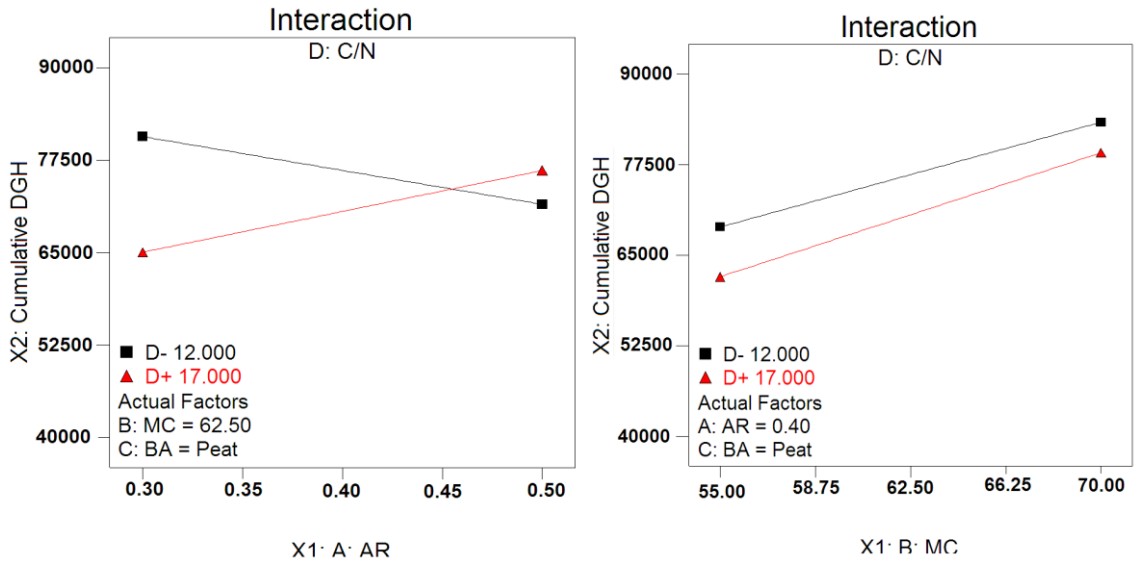


Figure 4.3 Cumulative DGH, BGH, and PDE for 16 runs

Results of the analysis of variance (ANOVA) for the response cumulative DGH verified that MC and interaction between AR and C/N, MC and C/N and three factor interaction of MC, BA and C/N are significant effects at the 5% level. Cumulative DGH increased when MC increased from 55% to 70% for both C/N but it has more positive effect on runs with C/N of 12, as it is shown in Figure 4.4. It also showed different trend for runs with peat and sawdust.



(a)



(b)

Figure 4.4 Interaction effect plot AR and C/N, MC and C/N of cumulative DGH with (a) sawdust (b) peat

Main effects MC, C/N and the interaction between AR and MC are statistical significant factors at the 5% level for cumulative BGH. Increase of AR from low level to high level has positive effect on BGH when MC is 55% and it has negative effect when MC is 70% (Figure 4.5). Increase of C/N would result in increase of cumulative BGH.

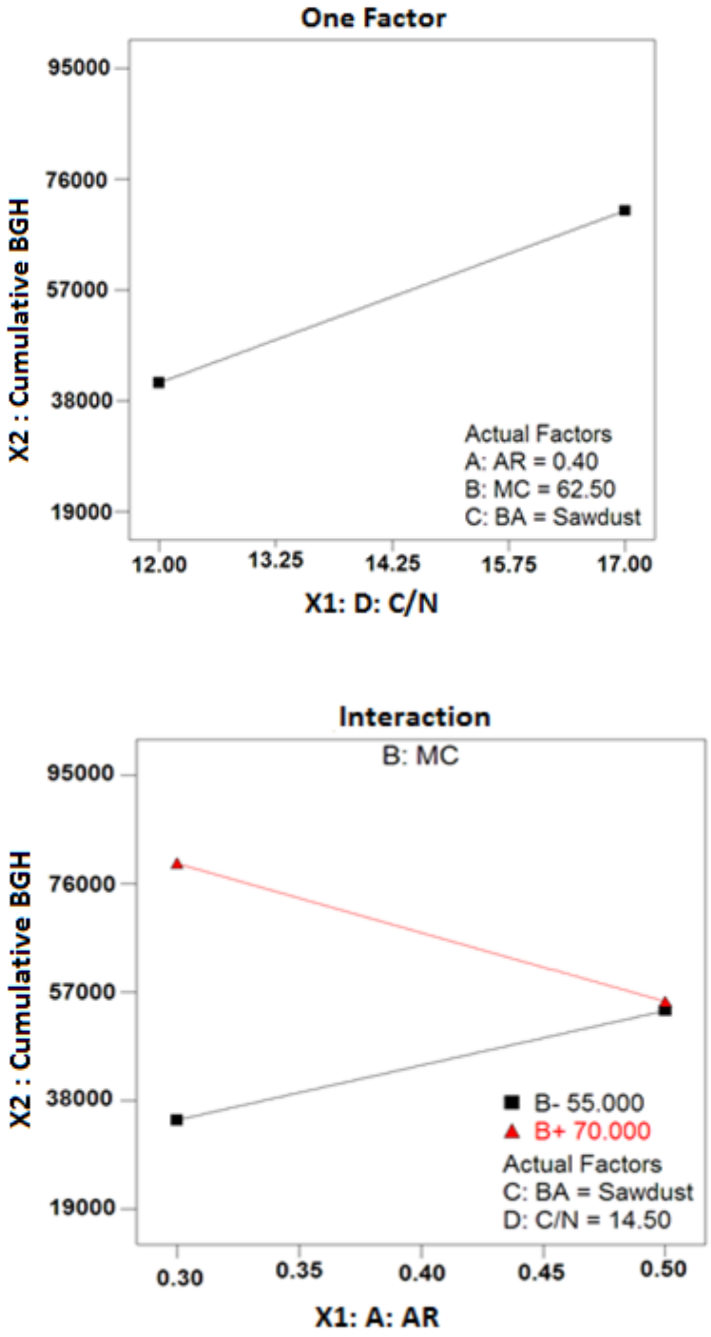


Figure 4.5 Main effect of C/N plot and interaction effect plot AR and MC of cumulative BGH

MC, C/N and interaction between AR and C/N are significant factors for cumulative PDE. Increasing MC and C/N has positive effect on cumulative PDE (Figure 4.6). The highest cumulative PDE was observed at highest C/N and lowest AR.

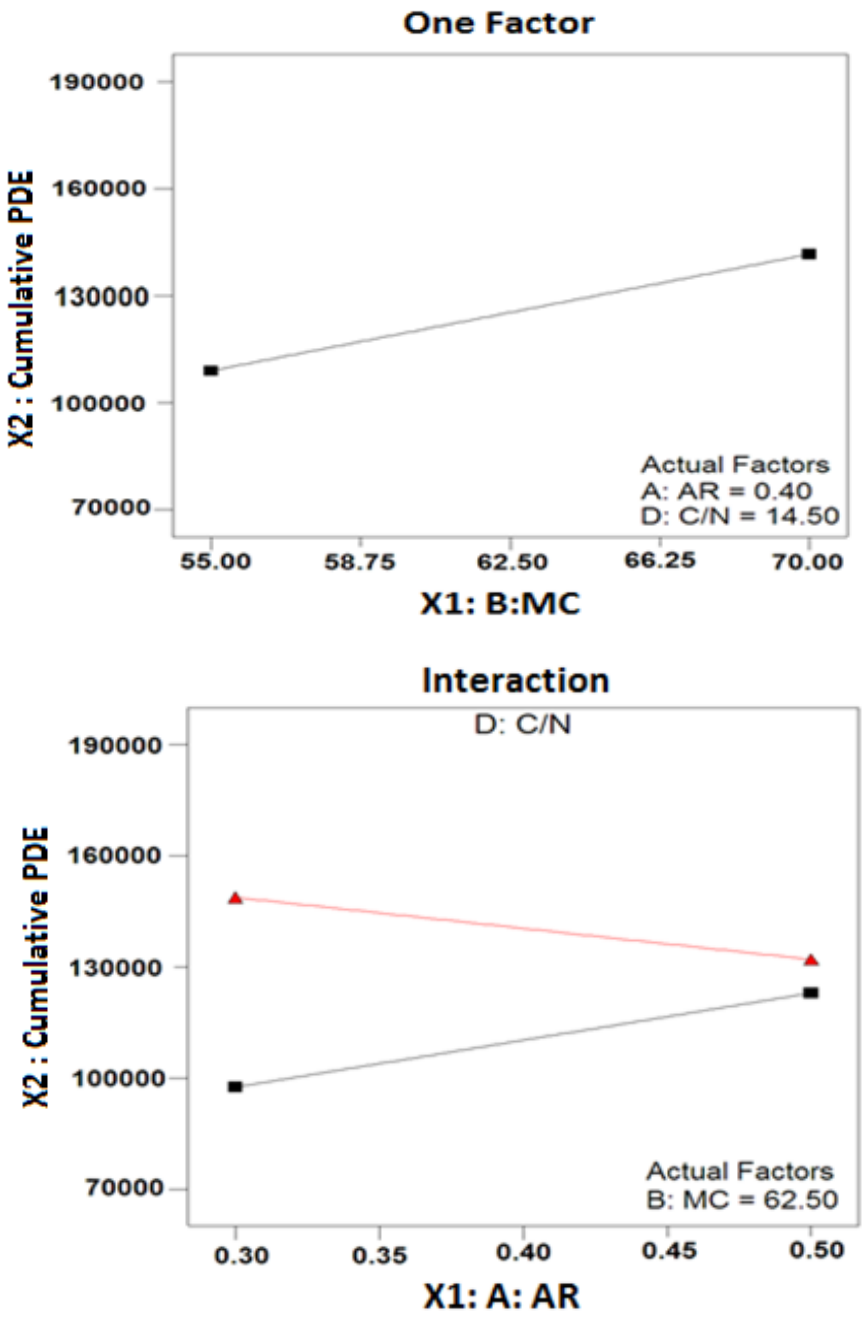


Figure 4.6 Main effect MC and interaction effect plot AR and C/N of cumulative PDE

For the response final C/N ratio main factors including BA and C/N are statistically significant factors at the 5% level. Figure 4.7 presents effect plots of BA and C/N for final C/N ratio. Generally, runs with peat has lower final C/N ratio compare to runs with sawdust as BA. Also, runs with low C/N have lower final C/N ratio compare to the runs with high C/N. Although Bueno et al. (2008) found a decrease on C/N using high aeration level and concluded to obtain lower C/N ratio, medium-to-high aeration levels (0.4-0.6 l air/min kg) can be used, in this study, AR was not significant factor for final C/N ratio.

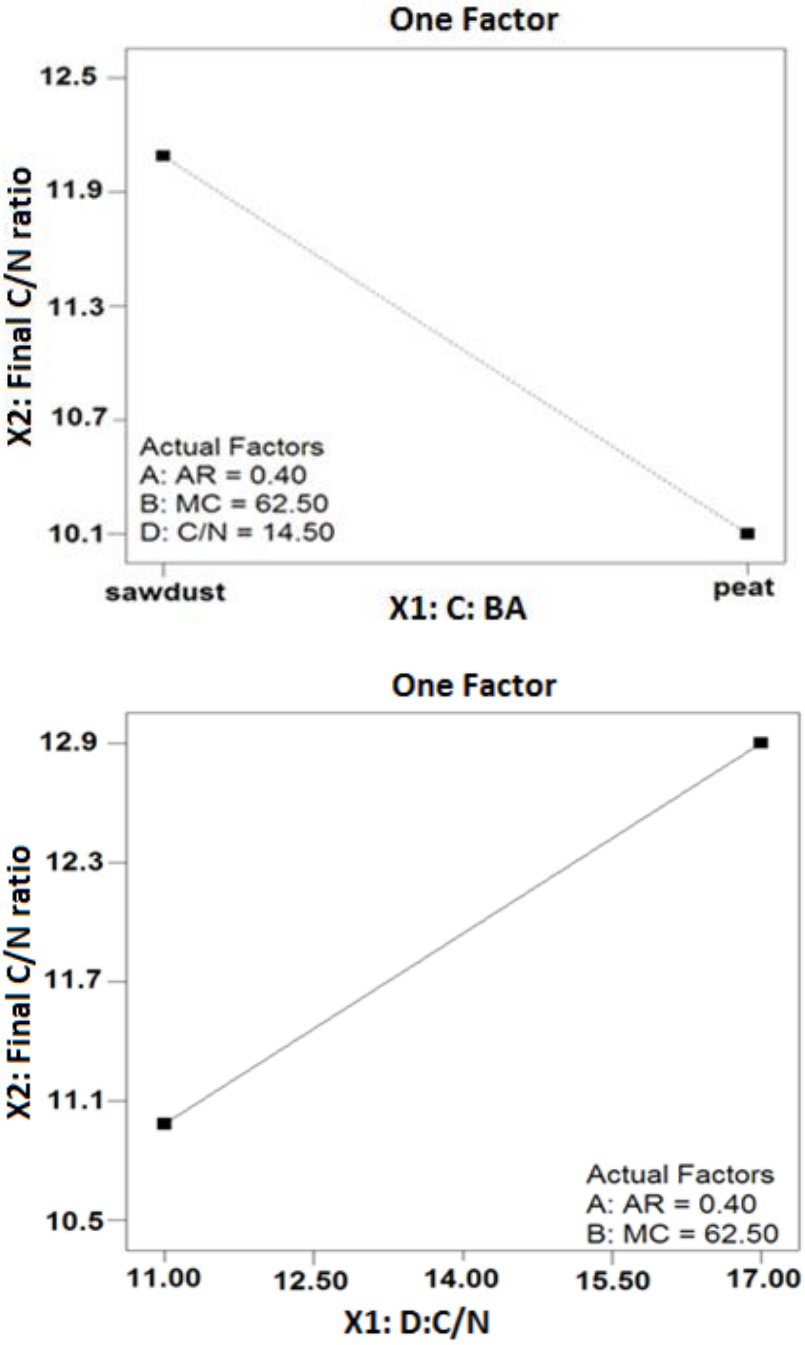


Figure 4.7 Main effect plot of BA and C/N of final C/N ratio

Main effect C/N and the two factor interaction including AR and BA, MC and BA, MC and C/N, and BA and C/N are the statistically significant effects at the 5% level for final GI. As it is seen in the Figure 4.8 when AR increase, GI for runs with peat decreases while it increase for runs with sawdust. Also increase of MC, has positive effect on GI of runs with sawdust and runs with higher C/N and negative effect on runs with peat and runs with low C/N. Generally, runs with high C/N showed higher final GI (86%) whereas for low C/N, lower GI (51%) was recorded. Also at low AR, high GI was observed for runs with peat and at high AR, high GI was observed for runs with sawdust. (Gregory et al. 2011) showed C/N had a significant influence on GI but MC and AR were not important influential factors.

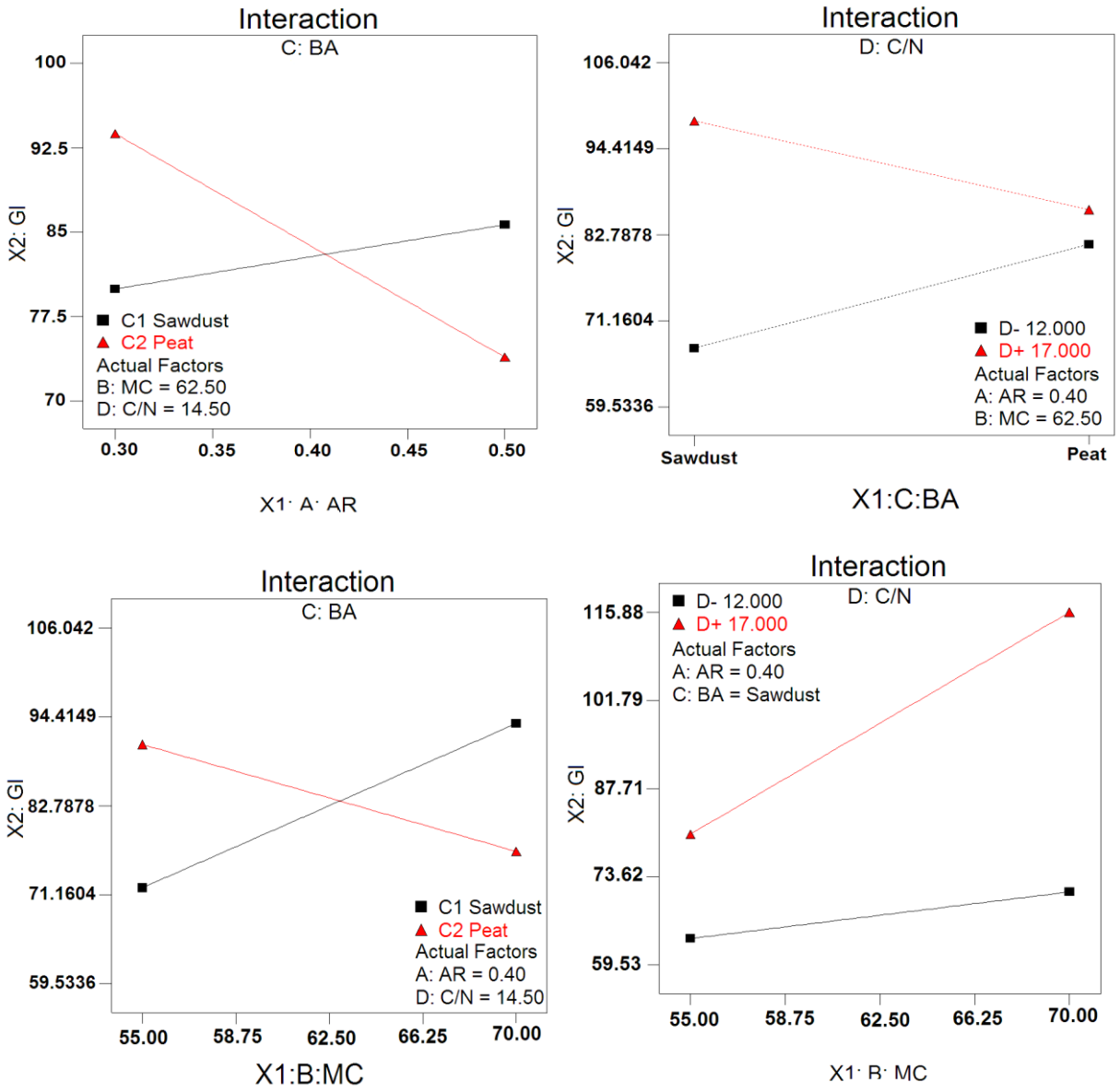


Figure 4.8 Interaction plot AR and BA, BA and C/N, MC and BA, MC and C/N of final GI

The MC was the only statistical significant effect for final moisture content. The runs with the low level MC showed lower moisture content (39%) in comparison to the runs with high level MC (57%). Figure 4.9 presents the main effect plot of MC on the final moisture content.

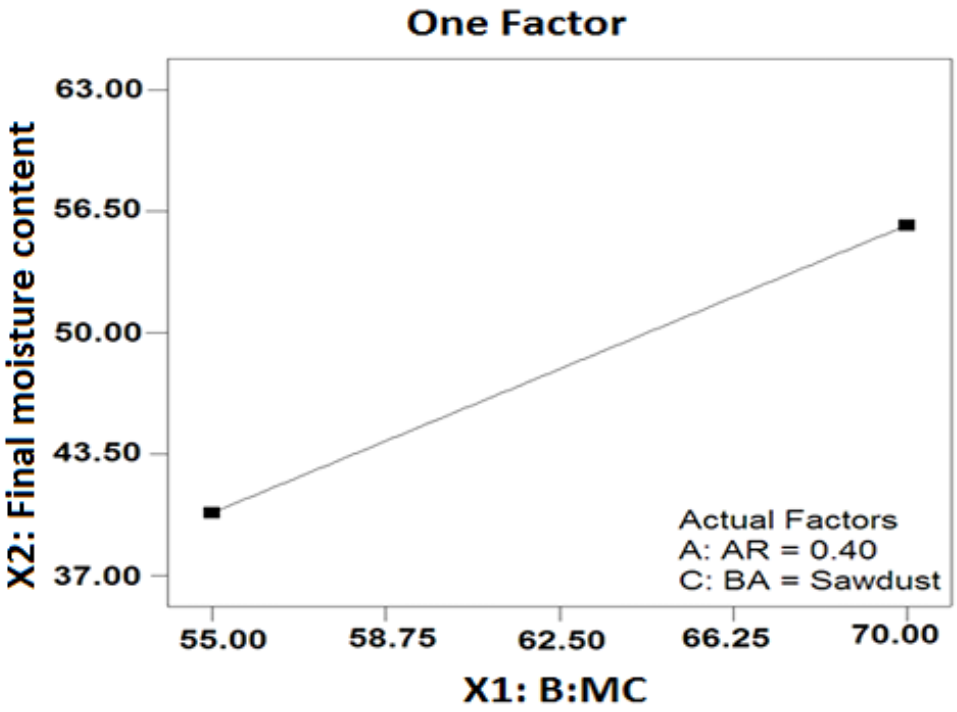


Figure 4.9 Main effect plot of MC of final moisture content

4.5 Summary

In this study, a 2-level four factor factorial design was adopted to evaluate the effect of AR, MC BA, and C/N on the composting process. A laboratory scale MSW composting system was designed and environmental and biochemical parameters including temperature, pH, EC, OUR, C/N ratio, GI and enzyme activities were selected to monitor the composting process. The final C/N ratio, cumulative enzyme activities, final GI and final moisture content were used as responses. Results indicated that C/N was one of the main factors that affect compost maturity and stability. The type of BA has a statistically significant effect on final C/N, final GI and cumulative enzyme activities. AR in the selected range affect statistically compost stability and maturity. The cumulative enzyme activities were influenced by all factors and their interactions. The research results can be applied to optimize initial conditions to start the composting and can help to increase the efficiency of the composting process. Because the relationship between composting factors is very complicated in the dynamic composting process, replicating response data and validating the linearity of the regression models will be of interest and useful in assessing the effects of these factors on the composting process. To economically treat the increasing quantities of MSW and achieve maximum cumulative enzyme activities, maximum GI and low C/N ratio composting with an AR of 0.3 L/min. kg, C/N of 17, MC of 70% with peat as BA is recommended.

CHAPTER 5

EVALUATION OF STATE AND EVOLUTION OF MARINE FISH WASTE COMPOSTING BY ENZYME ACTIVITIES[4]

[4] *This chapter is based on the following paper:*
Kazemi, K., Zhang, B., Lye, L. M., Zhu. Z., (2016). Evaluation of State and Evolution of Marine Fish Waste Composting by Enzyme Activities, Submitted to the Canadian Journal of Civil Engineering

Role: Khoshrooz Kazemi solely worked on this study and acted as the first author of this manuscript under the guidance of two supervisors, Dr. Baiyu Zhang and Dr. Leonard Lye. Zhiwen Zhu *participated in conducting experiments. Most contents of this paper was written by Kazemi and further polished by the other co-authors.*

5.1 Background

The fishery industry is important economically in a number of countries worldwide (Teh and Sumaila 2013). The increasing trend of global seafood production in the past decade due to the increasing consumption of seafood products is expected to continue (Benhabiles et al. 2012). The Province of Newfoundland and Labrador (NL), Canada, currently has one of the most valuable commercial fishing industries in the world, and fish processing plants are widespread in its coastal areas as illustrated in Figure 5.1. However, the fishery industry also generates large volumes of fish waste such as fish offal. These wastes are either disposed off through ocean dumping, or by on land disposal. It is estimated that fish waste accounts for up to 30-45% of its initial weight depending on the type of utilization (Teh and Sumaila 2013). During the period April 2009 to March 2010, around 66,185 tonnes of fish waste was generated in Atlantic Canada of which NL contributed about 40% (Environment Canada 2010).

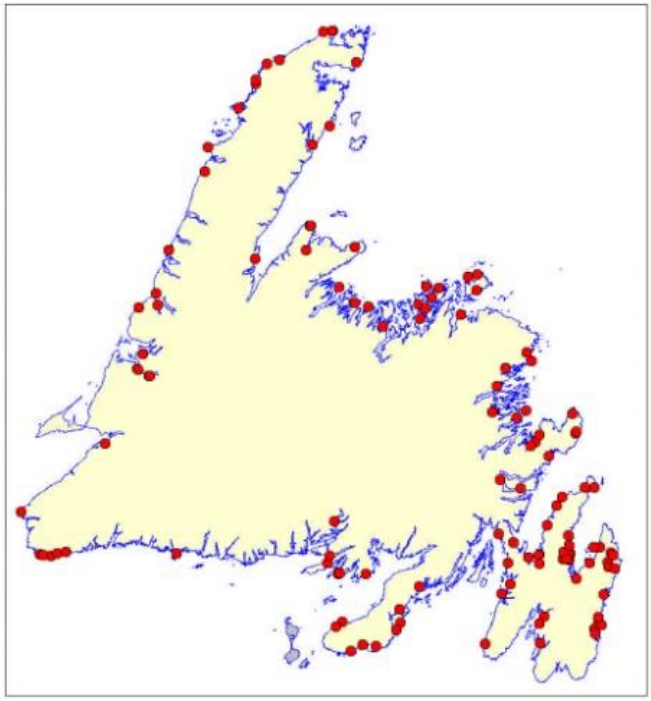


Figure 5.1 Locations of fish processing plants in NL

To appropriately utilize the nutrient rich fish wastes and to reduce their adverse environmental impacts, composting has been considered to be an appealing solution to the problems of waste disposal experienced by the fishery processing industry (Illera-Vives et al. 2013; Liao et al. 1997). Composting is a biological process that can reduce the volume and mass of organic waste in a sustainable manner. There are three main states of composting. In the first state, mesophilic microorganisms consume carbon sources and temperature rises to 45 °C. In the second state thermophilic microorganisms start to dominate and the energy released during this rapid microbial respiration produces heat which typically raises the temperature of a compost to 70 °C. Pathogens will be eradicated due to the heat generated during the thermophilic phase. Finally, in the last state, mesophilic fungi and actinomycetes colonize the compost for the breakdown and transformation of humic substances and lignin (Farrell and Jones 2009). Therefore, the organic composition of waste, in the meantime, can be converted into an odourless, stable, stabilized, and nutrient-enriched soil amendment through the mineralization and humification processes which greatly reduce its volume (Bernal et al. 2009; Jurado et al. 2014). Fish wastes are a rich source of organic materials, containing high levels of nitrogen and phosphorus (Chowdhury et al. 2010). These organic materials in fish wastes can be broken down by various microorganisms and hydrolytic enzymes may accelerate the breakdown of organic materials into small, stable compounds that can be easily assimilated by plants (Benitez et al. 1999). Therefore, composting of fishery by-products can be an appealing alternative to reduce wastes in landfills (Laos et al. 1998; Laos et al. 2002). Furthermore, the generated fish wastes based compost can serve as a good organic

fertilizer and nutrient for both local agricultural and landscaping purposes and for rehabilitation of degraded areas (Liao et al. 1997).

Physiochemical parameters such as temperature, moisture content, C/N ratio and electrical conductivity (EC) have been widely applied to monitor the composting process (Kopcic et al. 2014; Silva et al. 2014). Some biochemical parameters, especially enzyme activities, have attracted increasing attention recently as good indices of the aerobic biotransformation of organic wastes throughout the composting process (Mondini et al. 2004; Raut et al. 2008). Various enzymes have shown the potential for controlling the biodegradation rate during composting, and the most promising enzymes are dehydrogenases, β-glycosidases, and phosphatases (Vargas-Garcia et al. 2010). These enzyme activities could provide correct estimations of the events that take place throughout a composting process. As a group of membrane bound enzymes, dehydrogenases play an important role in the metabolic pathways of microbial activities by acting as a catalyst for the synthesis of ATP through oxidative phosphorylation (Barrena et al. 2008). The dehydrogenase enzyme activities thus are used as an indicator of biological reactions (Saviozzi et al. 2009). β-glucosidases and phosphatases, on the other hand, are employed to represent specific cycles during biotransformation. β-glucosidases are enzymes that can break carbohydrate polymers into small organic compounds through hydrolyzation, and facilitate future enzyme activities while phosphatases could be actively involved in the release of phosphate groups from organic compounds (Albrecht et al. 2010; Sardans et al. 2008). Therefore, tracking the changes of

β-glucosidases and phosphatases activities could contribute to a deeper understanding of carbon and phosphorous cycling during composting (Mondini et al. 2004).

Although enzyme activities have been used previously to evaluate the performance of composting, they have been never applied to the marine fish waste composting process for evaluating its state and evolution. Due to the high complexity of fish wastes, different enzymes worked collaboratively to fully decompose the organic materials into stable compounds (Islam et al. 2004). It is thus meaningful to conduct a detailed monitoring, characterization, and quantification of the enzymatic activities during composting. The results could provide a clearer picture about the dynamics of the composting process in terms of the decomposition of organic matter and the maturity of composted products (Tiquia et al., 2002). It would also advance the understanding of the underlying mechanism of fish waste composting, as well as the development of a better way to control the process and evaluate performance of the composting system accordingly.

In this study, a composting system was thus designed for achieving the effective reduction of fish wastes, and generating mature and high quality compost products. Enzyme activities including dehydrogenase, β- glucosidase, and phosphatase were monitored to gain a better insight into the dynamic process of fish waste composting and establish a relationship between the degradation of organic matter and enzyme activities. The correlation between enzymes activities and previously employed maturity and stability (i.e., C/N ratio, OUR, temperature, pH, and GI) was investigated to evaluate possibility of using enzyme activities as stability and maturity indices.

5.2 Materials and Methods

5.2.1 Composting System

The fish waste composting experiment was performed in a 35L bench scale cylindrical reactor. A schematic diagram of the composting reactor is shown in Figure 5.2. The cylindrical reactor has a removable lid with two holes, one for installation of a temperature sensor and the other for gas collection. Temperature was monitored during the composting process using the sensor located in the middle of the reactor. The exhaust gas mixture was trapped in a flask containing H_2SO_4. Before treating and then emitting through a ventilation system, the remaining discharging gases were further determined for the concentration of O_2. The leachate was collected at the bottom of this reactor. Three layers of aluminum foil and Styrofoam were wrapped around the reactor to achieve the microbial self-heating condition. A vacuum air pump and an airflow meter were equipped at the bottom of the reactor for air supply at a rate Fish waste composting.

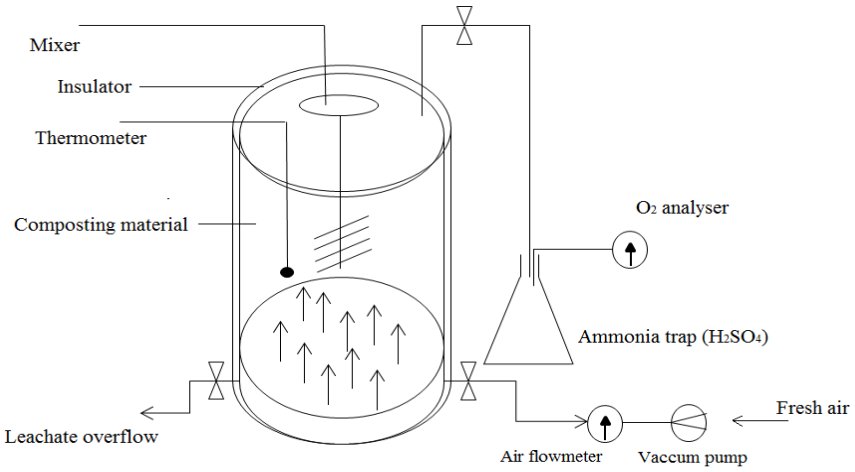


Figure 5.2 Schematic view of the composting system

The fish (cod) wastes comprise a complex mixture of fish heads, bones, and viscera were obtained from a local seafood processing center in NL and transported to the laboratory in a cooler at 4 ºC. The cod wastes were grinded to a size of 1 to 2 cm. Peat was used as the bulking agent and thoroughly mixed with fish wastes at the ratio of 1:10 (w:w). The raw materials were then transferred into the composting reactor.

The raw materials were mixed twice per day by turning the mixer to increase their homogeneity. During composting, the materials were randomly collected from 4 different points in the reactor and then well mixed to create a consistent and representative sample. Samples were obtained on the 2nd, 5th, 9th, 14th, 18th, 26th, 38th, and 50th days. Each collected sample was divided into different sub-samples to measure pH, EC, C/N ratio, moisture content, ash content, enzyme activities, and germination index (GI). Temperature and oxygen uptake rate (OUR) were recorded every 12 hours.

5.2.2 Analytical Methods

Temperatures were recorded by a bi-metal dial thermometer (H-B Instrument Company, PA). EC and pH were measured in 1:2 (w:v) aqueous extract using a pH/Ion meter (Metller Toledo. EL20-Educational line pH, EL3-Educational line conductivity). Moisture content was determined by gravimetric loss on-ignition of 10 g sample at 105 °C for 24 h. The sample was further used for the determination of ash content by continuously burning it at 550 °C in a muffle furnace (Thermo Scientific, Type FD1500M) for 4 h. The concentration of outlet oxygen in the exhaust gas was monitored by passing the air through a M40 multi-gas monitor (Industrial Scientific Corp., Oakdale, PA). OUR was calculated using the following equation:

$$OUR = (O_{2\ out}(\%) - O_{2\ in}(\%)) \times \text{airflow rate (L/min)} \quad (5.1)$$

Where $O_{2\ out}$ (%) is the oxygen concentration in the exhaust gas and $O_{2\ in}$ (%) is the oxygen concentration in the inlet air (20.9%) with an injected airflow rate of 0.3L/min/kg.

For seed germination test, extraction was first conducted through mixing each fresh sample with double distilled water (ddH_2O) at 1:10 (w:v) and shaking the mixer for 1 h. The suspension was then centrifuged at 3,000 rpm for 20 min before filtering the mixer through a Whatman No-1 filter paper. Another filter paper was placed in the petri dish and almost 10 milliliter of water extract was introduced into the petri dish. Ten cucumber seeds were placed on the filter paper. For control experiments, the ddH_2O was used to replace the water extract. The dishes were placed in the oven at 25 °C in the darkness for 5 days. The GI was then calculated:

GI (%) = (Seed germination x Root length of the treatment x 100) / (Seed germination x Root length of the control) (5.2)

For dehydrogenase activity determination, 5 g of sample was suspended in 5 mL of 3% (w:v) 2, 3, 5-triphenyl-tetrazolium chloride (TTC) at 37 °C for 24 h in the dark, and then 40 mL acetone was added. The mixer was incubated at room temperature for 2 h in the dark. The suspension was then filtered through a glass fiber filter. The absorbance of the filtered solution was measured at 546 nm (Pepper et al. 1995).

Phosphatase activity was determined based on *p*-nitrophenol release after cleavage of the synthetic substrate (*p*-nitrophenyl phosphate) with a concentration of 15mM. Acid and alkaline phosphatase assays differed only in the selected modified universal buffer (MUB

with pH of 6.5 and 11, respectively) to examine the activity of acid and alkaline phosphatase. One gram of sample was mixed with 4ml of MUB and 0.25 ml of toluene and then incubated for 1 h at 37 °C. 1 ml of $CaCl_2$ (0.5 M) and 4 ml of NaOH (0.5M) were added after incubation and the absorbance of the filtered suspension was recorded at 400 nm (Guo et al. 2012; Petiot and De Guardia 2004).

β – glucosidase activity was measured by suspending 1g of sample in a solvent with 0.25 mL of toluene, 4 mL of MUB (pH 6.0) and 1 mL of p-nitropenyl-β-D-glucopyranoside (Sigma). After the incubation at 37 °C for 1 h, the sample was well mixed with 1 mL of 0.5 M $CaCl_2$ and 4 mL of Tris buffer (0.1M, pH 12), and the suspension was filtered through a glass fiber filter. The release of p- nitrophenol was measured spectrophotometrically at 400 nm (Tabatabai 1994).

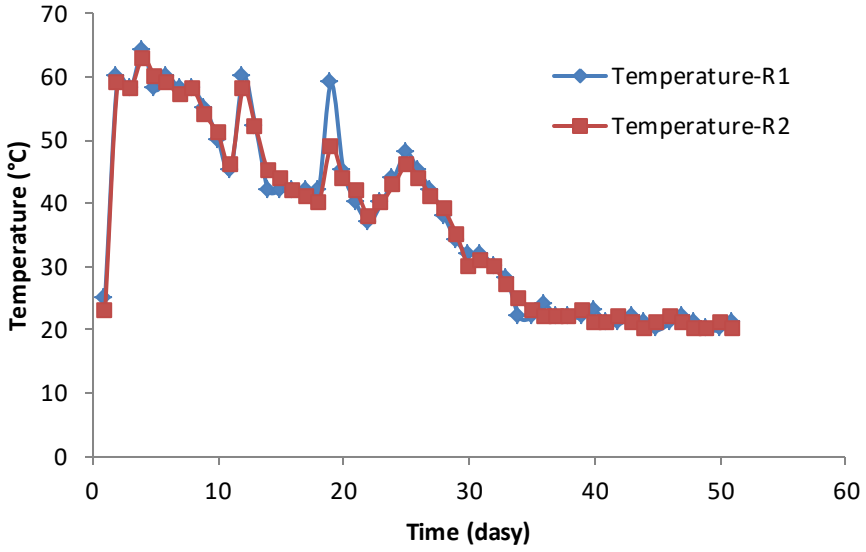
The total carbon and nitrogen contents of the composting samples were determined by the Perkin Elmer 2400 Series II CHNS/O analyzer. Duplicated tests were conducted for measuring moisture content, ash content, pH and EC, respectively. GI and enzyme activities were determined in triplicate. The data presented are the average values.

5.3 Results

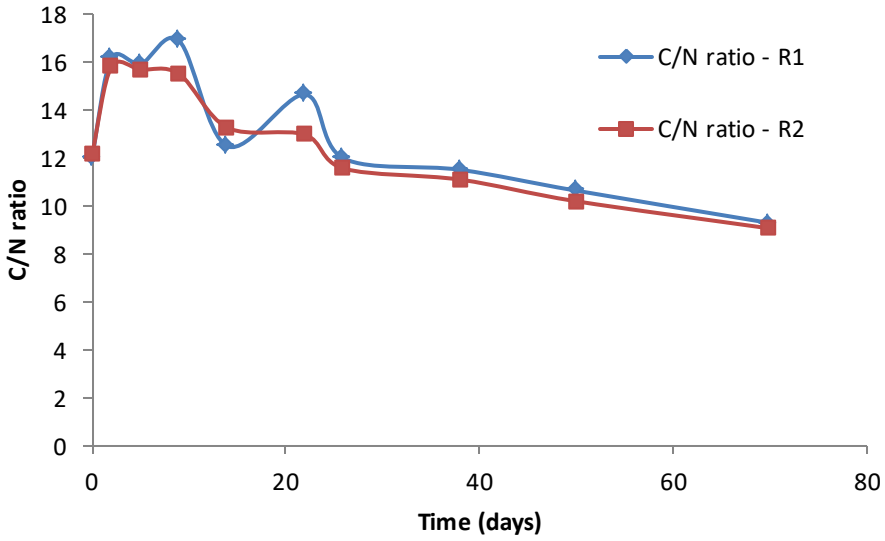
5.3.1 Reproducibility of Reactor Performance

To assess the reproducibility of the composting experiment, duplicated experiments with identical initial condition was conducted and C/N ratio and temperature were evaluated. As shown in Figure 5.3 the processes were developed similarly. The data deviations were less than 10%. During the 50 days of the composting process, the average difference

between the duplicated experiments for C/N ratio and temperature were 4.05% and 3.67%, respectively. The analysis of deviation between the replicate profile of C/N ratio and temperature shows that the composting process was duplicable.



(a)



(b)

Figure 5.3 Temporal variations of (a) C/N ratio and (b) Temperature of duplicated experiments (R1and R2)

5.3.2 Physiochemical Parameters

To establish the relationship of enzyme activities with the physiochemical parameters during the fish waste composting and evaluate the feasibility of using enzyme activities for indicating the state of the process, the profile of parameters including temperature, EC, OUR, pH, moisture content, C/N ratio and GI were monitored.

5.3.2.1Temperature, EC, OUR and pH

A temperature profile can indicate the microbial activity along the entire composting process and determine the stability of the organic material. It can also reflect the composting process evolution (Jurado et al. 2014). Figure 5.4 shows the temporal variations of temperature and OUR, and Figure 5.5 shows the temporal variations of pH and EC during the fish waste composting process. From Figure 5.4, the temperature of the fish waste compost reached 60°C on the second day of composting and stayed over 55°C for more than 8 days. After this period, temperature declined to 50°C but was above 40°C until the twentieth day of the composting. Temperature dropped to the ambient temperature after 33 days due to the decrease of microbial activity and depletion of easily biodegradable organic compounds (Laos et al. 1998). The temperature profile was similar to the result of Shelton et al. (1998) and Illera-Vives et al. (2013), except that in this study, the temperature climbed rapidly and reached maximum value on the second day. This trend indicated the mesophilic period was quickly substituted by the thermophlic phase as a result of aerobic biodegradation of the fast decomposed organic matter. Another temperature peak was observed on the 24^{th} day as a result of the degradation of

slowly decomposed raw materials. The United States Environmental Protection Agency (USEPA) recommends maintaining the temperature of compost piles at above 55 °C for 3 days in in-vessel composting to ensure the hygiene safety of the end product (Scaglia et al. 2007). This work strictly followed the recommendation and the generated fish waste compost met the requirement for a proper disinfection of the waste materials from pathogens.

The results of OUR followed the same pattern as temperature except its first peak appeared on the 4^{th} day. Another increase accrued during the third week and it stayed high for 7 days. After 30 days, it started to decrease and dropped to the minimum value by the end of the experiments. A high OUR value indicated that a high content of organic matter was available for microorganisms to be degraded, and therefore the raw material was not stabilized yet. A low OUR value indicated that the organic matter was more stabilized and had been mostly decomposed by microorganisms (Said-Pullicino et al. 2007).

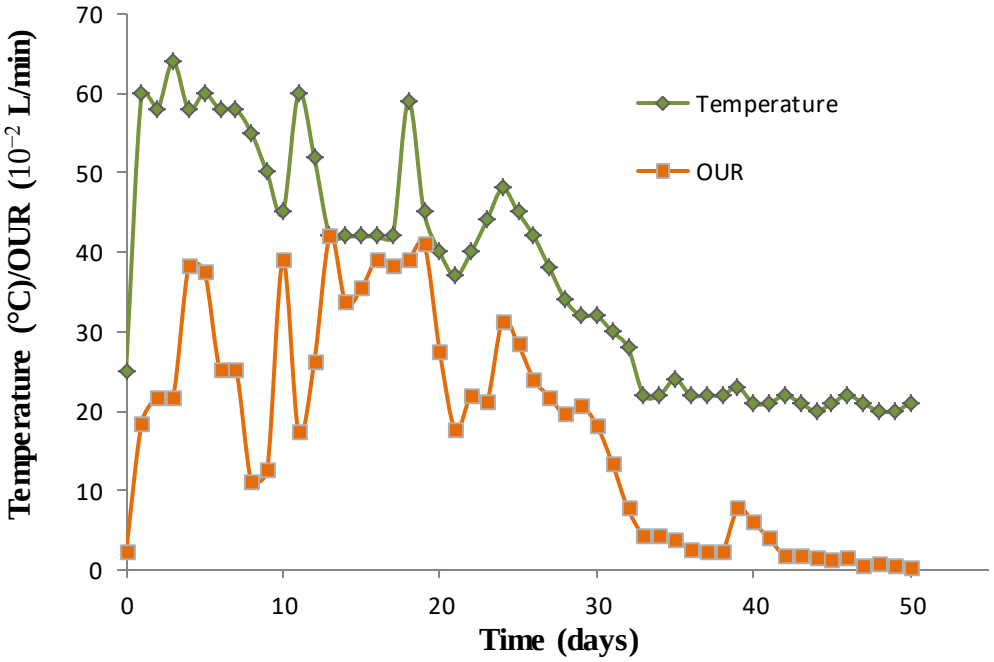


Figure 5.4 Temporal variations of Temperature and OUR during fish waste composting

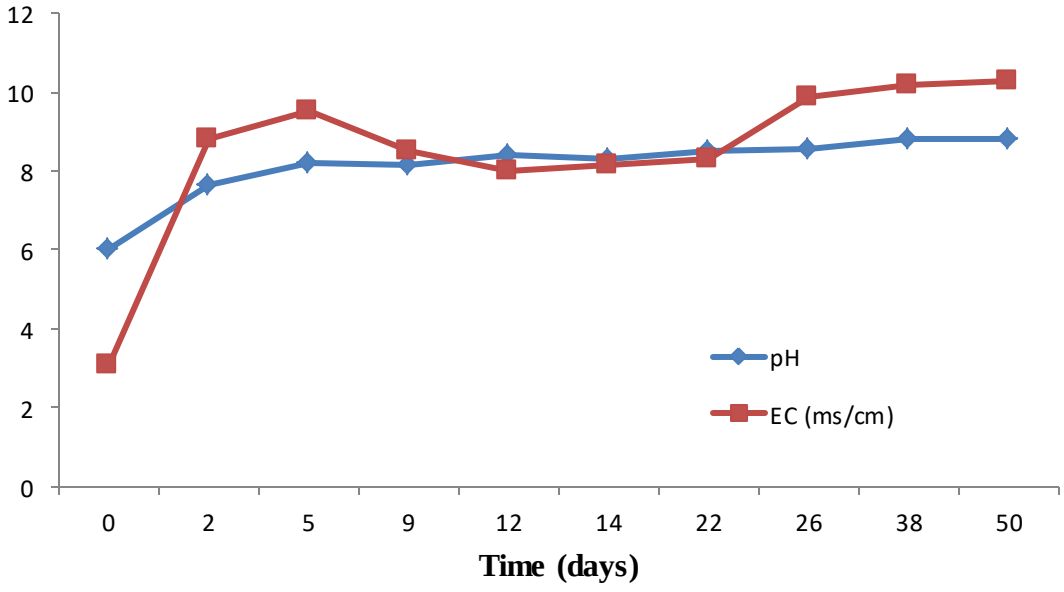


Figure 5.5 Temporal variations of pH and EC during fish waste composting

The characteristic values of pH, EC, C/N ratio and moisture content of fish waste are presented in Table 5.1. The pH values of the fish waste was slightly acidic as a result of the organic acids contained inside (Aspmo et al. 2005; Klute 1986). The pH gradually increased due to the microbial usage of the organic acids as a substrate (Adhikari et al. 2009), and then finally stayed stable. The EC value showed a sharp increase in the beginning of the process as a result of the release of mineral cations such as ammonium ions, phosphate, and accumulation of salts and nutrients (Kalamdhad et al. 2009). Although a slight decrease occurred during the third week which can be attributed to volatilization of ammonia and the participation of mineral salts (Rasapoor et al. 2009), the EC value increased and stayed stable by the end of the experiment.

5.3.2.2Moisture Content

Moisture content could affect microbial activities, physical structure, and the biodegradation of organic materials in a composting process (Ahn et al. 2008). Figure 5.6 showed the moisture content during the composting period. The moisture content of the raw material was 52% initially. After 10 days of composting, the moisture content decreased to 44%. High microbial activity and high rate of organic matter decomposition increased the temperature and evaporation rate. Temperature rise had a positive effect on moisture reduction. However, this temperature was not high enough to evaporate the generated water from organic matter degradation, so moisture content increased after its initial decrease (Haug 1993). After a month, moisture content eventually dropped to 41%. Haug (1993) suggested 40% as the minimum moisture content to continue microbial

activity which means microbial activity cannot be sustained in the system after 50 days of composting. Ash content had an increasing trend during the experiment. During the thermophilic phase the trend was sharper and it had a smoother trend after that. Decomposition of the organic matter produced more volatile compounds and the final product contained more ash content and less organic matter (Kalamdhad et al. 2009).

Table 5.1 Characteristic values of fish waste

Material	pH	EC	C/N ratio	Moisture content
Fish waste	6	3.06 mS/cm	12	52%

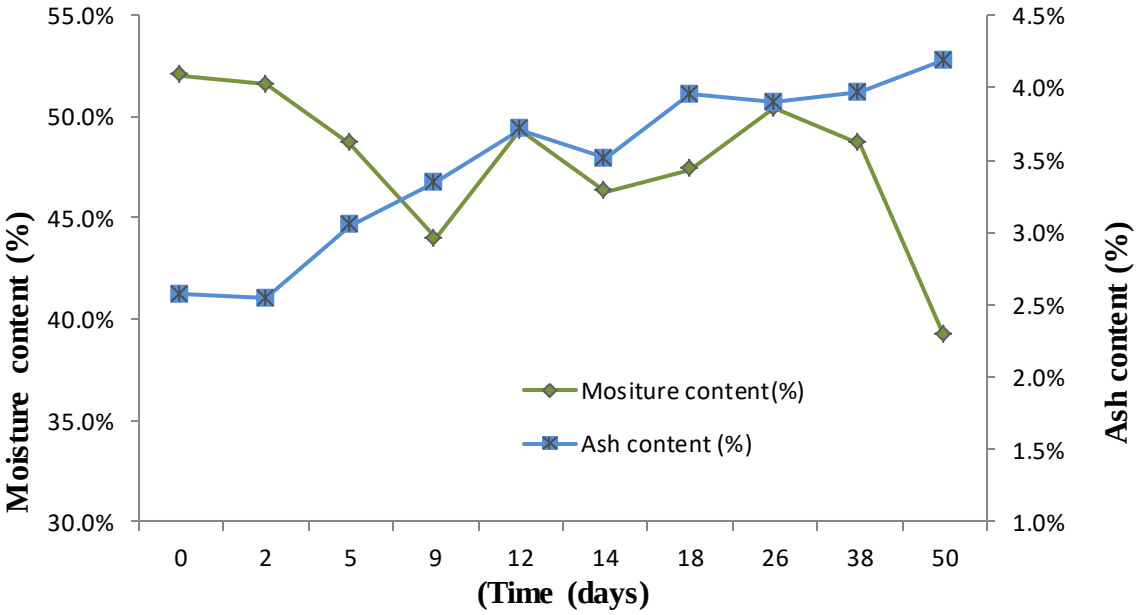


Figure 5.6 Temporal variations of moisture content and ash content during fish waste composting

5.3.2.3C/N Ratio

The fish waste generally contains high amounts of N and P, highly saline, and a low C/N ratio (Illera-Vives et al. 2013). Figure 5.7 showed the variations of C/N ratio and GI. The initial C/N ratio of the fish waste was 12, which was lower than the optimum value recommended for composting, i.e., 25 to 30 (Haug 1993). The C/N ratio increased during the first and second weeks of composting. This increase can be attributed to the intensive nitrogen loss through ammonia emission when pH and temperature were high (Sánchez-Monedero et al. 2001). During twenty days of composting, carbon was decomposed via microbial respiration to carbon dioxide (CO_2) thus a decline in C/N ratio was observed and the declining trend continued by the end of the experiment. Finally C/N ratio of the compost reached 10.6.

5.3.2.4GI

Seed germination test helps to evaluate the efficiency of the composting process for plant growth (Banegas et al. 2007). In the beginning of the composting, the seed germination inhabitation (GI) was observed because of the biological activity and the formation of toxic compounds such as alcohols, phenolic compound, and organic acids. The minimum GI recorded after 10 days and it started to increase smoothly by the end of the experiments. Over 80% of seed germination was obtained in extracts of 7 weeks old compost (Zucconi et al. 1981). According to Tiquia and Tam (1998), this was an indication of the production of a phytotoxic-free and mature compost. The change in GI with time was also shown in Figure 5.7.

5.3.3 Enzyme Activities

Measurement of enzyme activities is helpful in understanding microbial metabolism during composting (Mondini et al. 2004). The biosynthesis of the hydrolytic enzymes began in the initial phase of composting. Those enzymes were responsible for transformations of complex compounds of carbon, nitrogen and organic phosphorus in composts. Hydrolases enzymes, due to their inductive character, were a good indicator of qualitative and quantitative changes in the content of particular organic polymers in the process of composting (Bohacz and Korniłłowicz-Kowalska 2009; Vargas-Garcia et al. 2010).

5.3.3.1 Dehydrogenase

Dehydrogenase activity can be used as an indication of the oxidation of simple organic sources of carbon and overall microbial activity due to its involvement in the respiratory chains of all microorganisms (Bohacz and Korniłłowicz-Kowalska 2009; Castaldi et al. 2008). Dehydrogenase activity as shown in Figure 5.8 increased fast from the beginning of the composting and the highest dehydrogenase activity was recorded after 14 days. Oxidation of simple carbon substrates catalysed by enzymes led to rapid increase in dehydrogenase activity in the initial period of composting (Bohacz and Korniłłowicz-Kowalska 2009). After 30 days, a progressive decrease was observed in dehydrogenase activity, which indicated the depletion of easily available organic matter for microorganisms and end of the active decomposition phase (Benito et al. 2003; Bohacz and Korniłłowicz-Kowalska 2009; Tiquia 2005). The profile of dehydrogenase activity

was very similar to the one reported by Castaldi et al. (2008) for municipal solid waste and plant waste compost, and Tiquia et al. (2002) for yard waste trimming compost.

5.3.3.2 β-Glucosidase

β-glucosidase is related to carbon mineralization and is one of the important hydrolytic enzymes during composting that microorganism-induced degradation of organic matter depends on them (He et al. 2013; Jurado et al. 2014). In this study, β-glucosidase increased from the beginning of the experiments and the highest value (1894 µg PNP g $DM^{-1}h^{-1}$) was recorded at the end of the second week (Figure 5.8). β-glucosidase activity is indicative of the presence of labile organic matter easily usable by the microorganisms (Castaldi et al. 2008) and it hydrolyses reducing terminations of β-D-glucose chains to give β-glucose (Nannipieri et al. 2011). Composting phases characterized by a higher availability of various β-glucosides, mainly cellobiose, are associated to greater β-glucosidase activity (Vargas-Garcia et al. 2010). He et al. (2013) found the activities of dehydrogenase and b-glucosidase during chicken manure composting peaked at the same time. The peaks of dehydrogenase and β-glucosidase activities during fish waste composting were also accrued almost simultaneously. After the third week a sharp decrease was observed in β-glucosidase activity and reached a stable lowest value at the end of the experiments. The same result was reported during tobacco waste and tabacco waste - olive pomace compost for β-glucosidase activity reported by Kayikçioğlu and Okur (2011).

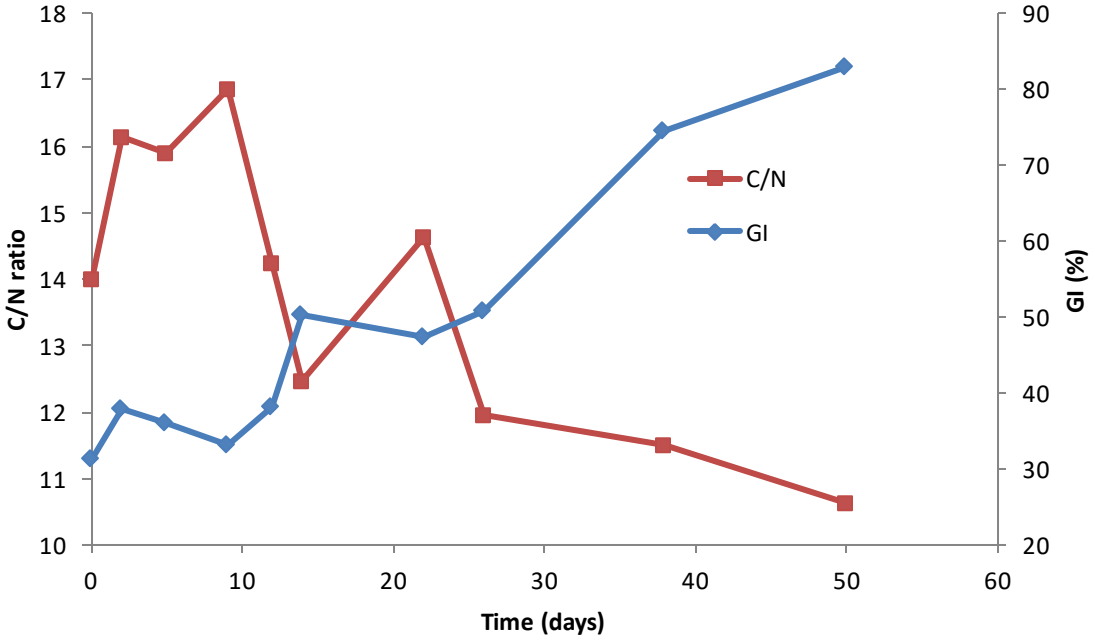


Figure 5.7 Temporal variations of C/N ratio and GI during fish waste composting

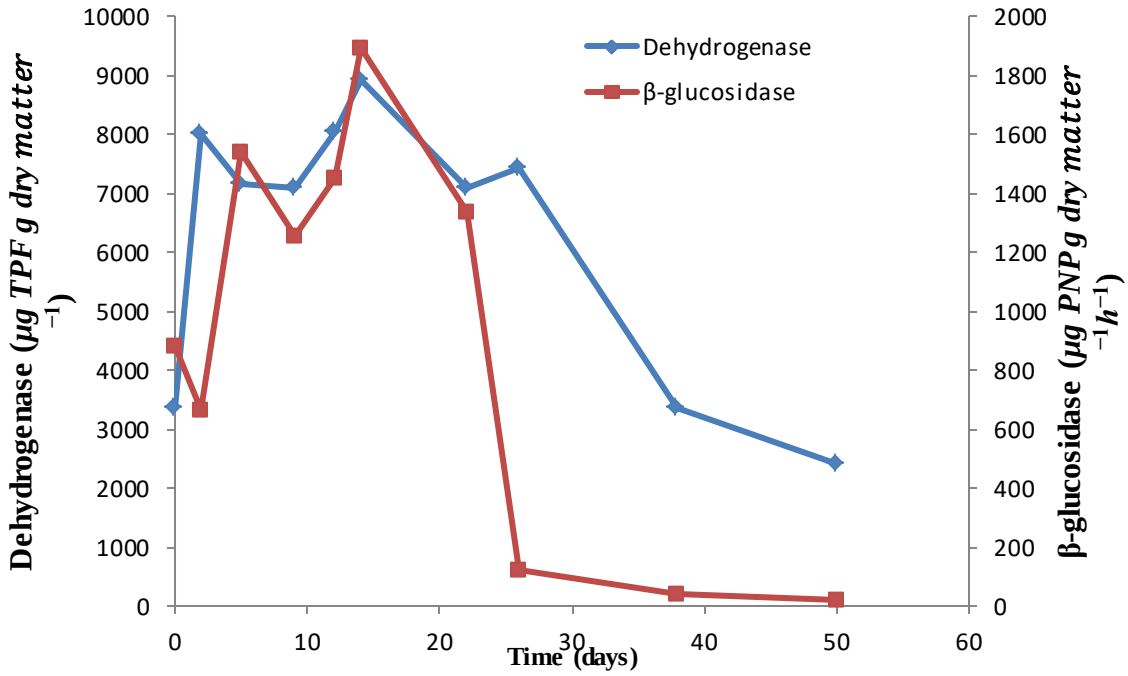


Figure 5.8 Dehydrogenase and β-glucosidase activities during fish waste composting

5.3.3.3 Phosphatase

The term "phosphatases" is used to describe a group of enzymes that catalyse the hydrolysis of esters and anhydrides of orthophosphoric acid (Margesin and Schinner 1994). It catalyses the hydrolysis of organic phosphorus compounds to different inorganic forms which plants can metabolize. Phosphatase is considered as a general microbial indicator because of the critical role it plays in P cycles (Vargas-Garcia et al. 2010). Information about the evolution and behaviour of phosphatases also gives information about the hydrolytic enzymes as a whole in composting, since they are considered to be good representatives of overall hydrolytic activity, at least in organic soils (Vuorinen 2000).

Acid and alkaline phosphatase are subdivision of phosphatases which differ from other phosphatases by their substrate specificity and their optimum pH for activity, namely in the acid range for acid phosphatase or in the alkaline range for alkaline phosphatase (Margesin and Schinner 1994). As seen in Figure 5.9, the initial concentration of both acid and alkaline phosphatase was high in the raw material. Though a sharp drop was observed since the start of the composting process, they stayed at a fairly high value by the end of the experiments. The high level of phosphatase activity may indicate a successive influx of available forms of phosphorus in the course of fish waste composting. It should also be attributed to stronger growth of microorganisms (Bohacz and Korniłłowicz-Kowalska 2009). After 50 days of composting, phosphatase declined slightly. (Godden et al. 1983) found the level of enzyme increased during the early mesophilic period and remained approximately constant during the later periods of the

process. They also reported that when the heaps of composting pile began to cool, the enzyme activities began to decrease. For the first week of composting a decrease was observed in the case of acid phosphatase. This result was similar to the findings of Vuorinen (2000) that had an increasing trend during the later period. The evolution of the alkaline phosphatase during composting also varied greatly on the basis of raw materials and type of composting process (Jurado et al. 2014). The alkaline phosphatase plays a role in the use of alternative phosphorus sources and might be considered as a general index of microbial activity in compost since it is only synthesized by micro-organisms and cannot be originated from plant residues. (Godden et al. 1983; Kayikçioğlu and Okur 2011).

The enzyme activity has been consistent with the temperature and OUR pattern. In general, during the second and third weeks of the process, the presence of the readily available substrates leads to rise in temperature and enzymatic activity. After active phase, when temperature dropped and availability of nutrients reduced, enzymatic activity decreased evidently. Decline of enzyme activities in compost samples observed concurrently with decrease in C/N ratio and increase in GI. Thus, enzyme activities could represent a useful index of state of composting since the conventional maturity and stability parameters, OUR, C/N ratio, GI support the use of enzyme activity as indicator of compost stability in this work. Because enzyme activity is depending on nature and origin of the raw material, it is hard to establish a threshold for enzyme activities as a stability index.

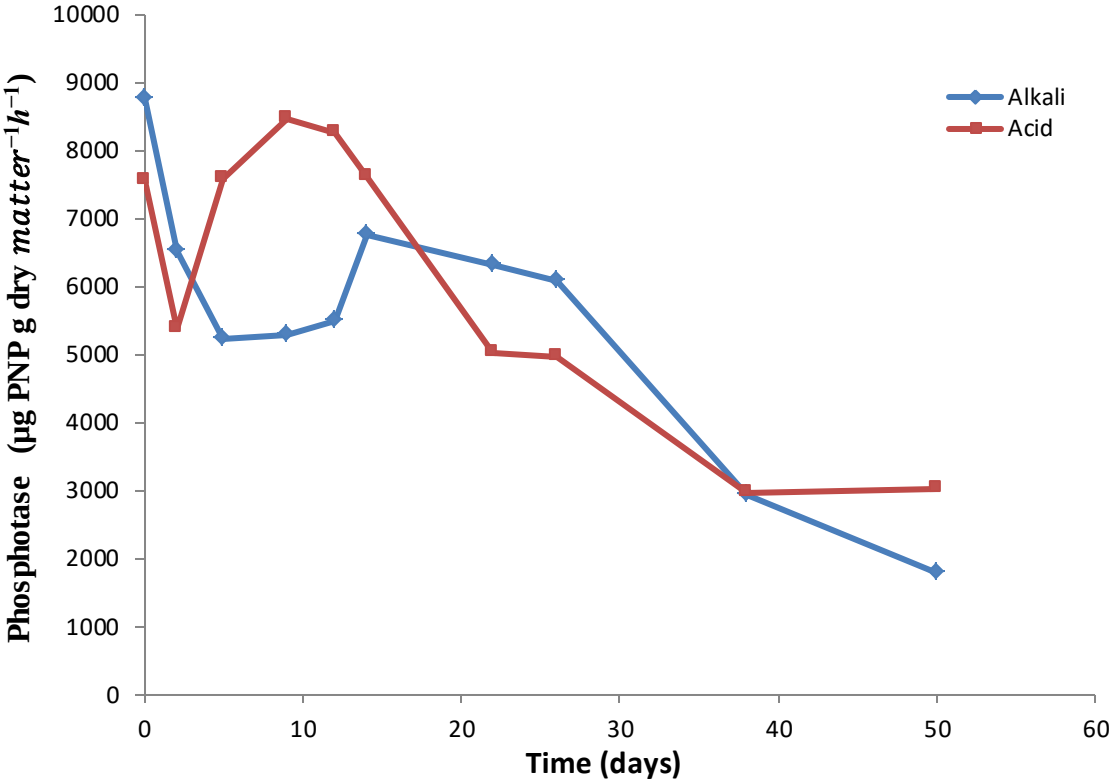


Figure 5.9 Acid and alkali phosphatase activity during fish waste composting

5.4 Correlation Analysis

Moisture content, ash content, pH, and EC tests were conducted in duplicate. GI and enzyme activities were determined in triplicate. The typical error in the measurement was less than ± 5%. The correlation matrix plot of the parameters is presented in Figure 5.10. The relationships among the parameters are mostly not linear as it is seen in Figure 5.10. As such the Spearman's rank correlation coefficient (ρ) is used to indicate correlations among the variables. The Spearman's rank correlations among the enzyme activities and other monitoring parameters are shown in Figure 5.10 with sample size of 8.

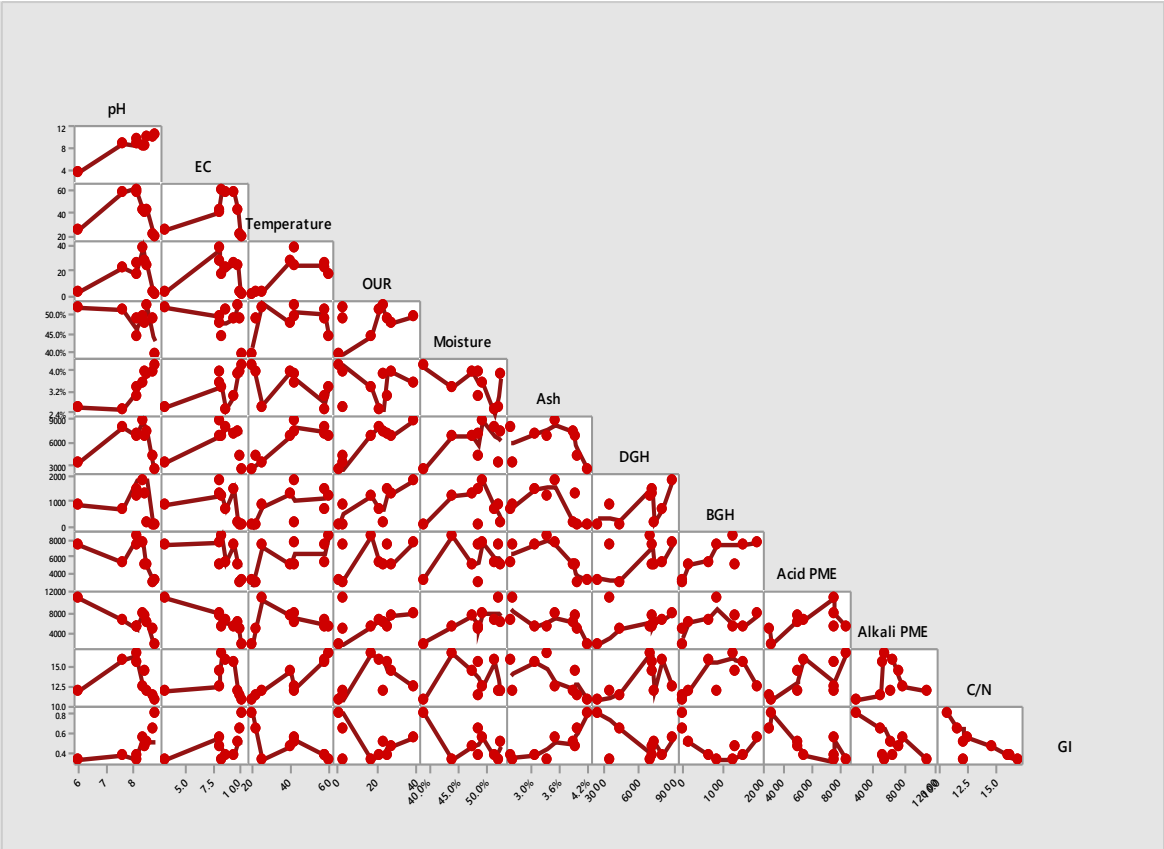


Figure 5.10 Correlation matrix plot of all variables with lowess fit line

A significant correlation was recorded between temperature and dehydrogenase activity (ρ= 0.622, P<0.1). Tiquia (2005) reported that dehydrogenase activity was the most important factor affecting pig manure compost temperature. Barrena et al. (2008) stated that due to the high correlation between dehydrogenase activity and temperature, dehydrogenase activity was a useful parameter to follow the evolution of the biological activity of the composting. Strong correlation was observed between OUR and dehydrogenase activity (ρ = 0.828, p <0.05) as well. Bohacz and Korniłłowicz-Kowalska (2009), and Barrena et al. (2008) also found significant correlation between respiratory activity and dehydrogenase activity. OUR was also significantly correlated with β-glucosidase activity (ρ=0.803, P<0.05) (see Table 5.2).

Castaldi et al. (2008) found significant correlation between β-glucosidase and dehydrogenase activity (r= 0.973, P < 0.001) and Vuorinen (2000) stated a correlation between β-glucosidase activity with dehydrogenase activity in the mature cattle manure composts (r=0.886, P<0.01). The correlation measures used in those studies were based on Pearson correlation r which requires a linear relationship. In this study, the relationship between β-glucosidase and dehydrogenase activities was also positive (ρ=0.536, P < 0.05). The correlation coefficient for β -glucosidase and C/N ratio (ρ=0.682, p <0.1) was higher than the values reported by He et al. (2013) (ρ=0.385). The negative correlation between acid phosphatase with EC and alkaline phosphatase with EC (ρ= -0.683, and ρ= -0.917 P<0.05, respectively) were obtained. Kayikçioğlu and Okur (2011) also reported the higher EC levels of tobacco waste compost could negatively affect alkaline phosphatase. Tripathi et al. (2007) also observed a high salinity that led to

a decrease in the alkaline phosphatase activity. Acid phosphatase activity showed significant negative correlations with ash content and GI (ρ= -0.650 and ρ= -0.667, $p<0.1$, respectively). Furthermore, a strong correlation in this study between acid phosphatase with the C/N ratio (ρ= -0.767, $p<0.05$), was in agreement with results of Vuorinen (2000) who reported a strong correlation between acid phosphatase with the C/N ratio. pH value was negatively correlated with acid phosphatase activity (ρ= -0.717, $P<0.05$) since the pH value of the compost was alkaline for the most period of the experiment. All enzyme activities showed a negative correlation with composting time (dehydrogenase activity ρ=-0.267, β-glucosidase ρ=-0.510, acid phosphatase ρ =-0.683, and alkaline phosphatase ρ=-0.617) which were similarly in sign as those reported by Bohacz and Korniłłowicz-Kowalska (2009), except their results were based on Pearson's r. GI correlated with pH, ash content, and C/N (ρ=0.9, ρ=0.817, ρ=– 0.7, $P<0.05$ respectively).

Table 5.2 Correlation matrix between different parameters and enzymatic activities during fish waste composting process

Parameter	Temp	OUR	pH	EC	M	Ash	C/N	GI	DGH	BGH	Acid PME
OUR	0.489										
pH	-0.588	-0.142									
EC	-0.286	-0.489	0.733**								
M	0.084	0.192	-0.35	-0.283							
Ash	-0.664*	-0.201	0.95**	0.567	-0.467						
C/N	0.9 **	0.452	-0.717**	-0.483	-0.08	-0.7**					
GI	-0.588*	-0.092	0.9**	0.633*	-0.217	0.817**	-0.7**				
DGH	0.622*	0.828 **	-0.233	-0.08	0.35	-0.35	0.46	0.05			
BGH	0.549	0.803**	- 0.477	-0.711**	-0.033	-0.427	-0.628*	-0.444	0.536		
Acid PME	0.748**	0.444	-0.717 **	-0.683**	-0.033	-0.650*	0.767**	-0.667*	0.383	0.812**	
Alkali PME	0.168	0.477	-0.633*	-0.917**	0.567	-0.533	0.3	-0.483	0.4	0.544	0.450

Note: Temp, temperature; OUR, oxygen uptake rate; EC, electrical conductivity; M, Moisture content; Ash, Ash content; C/N, carbon to nitrogen ratio; GI, germination index, DGH, dehydrogenase activity; BGH, β -glucosidase activity; Acid PME, acid phosphatase; Alkaline PME, Alkaline phosphatase. Spearman rank correlation coefficient values with ** and *are significant at $P < 0.05$ and $P<0.1$ respectively.

In summary, inter-correlations among the hydrolytic enzymes and dehydrogenase activity and among enzyme activities with other parameters confirm that enzyme activities are important indicators of the process activity and evolution of the organic matter during fish waste composting. Strong correlation between enzyme activities and maturity and stability indicators also allows using enzyme activities as stability and maturity parameters of a compost sample.

5.5 Summary

This study reported fish waste based composting with peat as the bulking agent. Results indicated that composting could be a feasible method to dispose and utilize fish waste in NL, which led to the production of stable and hygienic compost. Dynamic changes in the enzymatic activities during composting were observed. The maximum enzyme activities were observed in the first 3 weeks, at the active phase of decomposition. The changes of enzyme concentrations served as useful indices to evaluate the effectiveness and progress of the fish waste composting. A number of key physicochemical properties were also monitored and their correlations with the enzyme activities were investigated. The correlation results suggested characterizing compost maturity and stability by each isolating parameter might be not reliable. The combination of multiple parameters is desired for a comprehensive evaluation of fish waste compost maturity and stability such as enzyme activities and GI. The research results provide a way forward for improving fish waste composting and contribute to a better understanding of the biodegradation process in fish waste management.

CHAPTER 6

MANAGEMENT OF FISH WASTE COMPOST: LOW COST SUBSTRATE FOR BIOSURFACTANT PRODUCTION[5]

[5] *This chapter is based on the following paper:*

Kazemi, K., Zhang, B., and Lye, L. M., (2016). Management of Fish Waste Compost: Low Cost Substrate for Biosurfactant Production

Role: Khoshrooz Kazemi solely worked on this study and acted *as the first author of this manuscript under the guidance of two supervisors, Dr. Baiyu Zhang and Dr. Leonard Lye. Most contents of this paper was written by Kazemi and further polished by the other co-authors.*

6.1 Background

Compost generated from fish waste composting is an effective source of nutrient-rich organic matter. Generally it is used as a fertilizer to improve plant production. Many studies have been evaluated their effect on plant growth (Atikpo et al. 2008; Danaher et al. 2009; Laos et al. 1998; Welke 2005). Beside fertilizer, it can be used as a good source of nutrient for production of other valuable products (Shelton et al. 1998). Martin (1999) used fish waste compost (FWC) extract as a fermentation substrate to grow fungus S. acidophilum. Desai and Banat (1997) used compost extract to enhance desorption of α-naphthol and naphthalene from pristine and contaminated soils. Janzen et al. (1995) added compost extract to soil to simulate community-level controls on soil microorganisms which are involved in element cycling. Compost extract was recognized as a solution of chemically-defined growth factors because all the materials in the compost extract are directly or indirectly derived from microbial activity. It also contains other growth factors such as chelated micronutrients.

In most previous studies, the compost nutrient was extracted by mixing the compost with water at different ratios and passing through filters or a centrifuge (Welke 2005; Weltzien 1991). In some studies, $CuSO_4$ was added to collect the flocculate colloids (Janzen et al. 1995; Shelton et al. 1998). Martin (1999) used acid hydrolysis to extract FWC with peat. Enzyme hydrolysis is also widely used in the food industry for protein recovery. Enzymatic modification of proteins uses selected proteolytic enzyme preparations to cleave specific peptide bonds (Ovissipour et al. 2009). Although researches have been dedicated to enzyme hydrolysis of fish waste (Aspmo et al. 2005; Nilsang et al. 2005;

Ovissipour et al. 2012), it has never been reported in the literature for FWC. Enzymatic hydrolysis of proteins is a complex process because of several peptide bonds and their specific accessibility to enzymatic reactions. Beside the specificity of enzymes, there are other environmental factors such as temperature, time and pH which can affect the peptide profile of the final product (Kristinsson and Rasco 2000a). Generally, there is an optimum combination of factors where an enzyme is the most active. Temperature and pH extremes deactivate the enzymes by denaturing them (Kristinsson and Rasco 2000a). The variables with the most important roles in this complex enzymatic reaction have been reported to be enzyme concentration, protease specificity of the enzyme, time, pH and temperature of the reaction, the nature of the protein substrate, and the degree of hydrolysis attained (Kristinsson and Rasco 2000b; Ovissipour et al. 2009). The preferred commercial enzymes for most researchers are protease preparations of bacterial origin such as Alcalase, Neutrase, Protease N and Protamex(Aspmo et al. 2005). Alcalase, an alkaline enzyme produced from *Bacillus licheniformis* has been proven repeatedly by many researchers to be one of the best enzyme used to prepare functional fish protein hydrolysis and other protein hydrolysates (Kristinsson and Rasco 2000a). Generally, Alcalase® 2.4-L-assisted reactions have been favored for fish hydrolysis, due to the high degree of hydrolysis that can be achieved in a relatively short time under moderate pH conditions, compared with the neutral or acidic enzymes (Aspmo et al. 2005; Ovissipour et al. 2012).

For fish waste hydrolysis, given a particular enzyme, any hydrolysis process involves at least five independent variables. These are S (protein substrate concentration: %N ×

6.25)(, E/S (enzyme-substrate ratio in % or in activity units per kg N × 6.25), pH, T (temperature), and t (time) (Kristinsson and Rasco 2000a). Modern statistically based experimental designs are useful techniques for the investigation of complex processes. It has been successfully applied to optimize seafood processing operations (Liao et al. 1997; Ovissipour et al. 2012). Ovissipour et al. (2012) used RSM to optimize hydrolysis conditions (enzyme activity, temperature, and time) to produce hydrolysate from the viscera of yellowfin tuna (Thunnus albacares). A factorial design was applied to minimize enzyme utilization and modeling of degree of hydrolysis. The effect of temperature, pH, enzyme-substrate ratio on degree of hydrolysis(DH) of dogfish muscle protein using RSM has been studied by Bernal et al. (2009). Their results indicated that all three factor markedly influenced the peptide bonds cleavage in the protein substrate. In this study, of the extraction of FWC nutrients through enzyme hydrolysis will also use design of experiment methods (e.g. response surface methodology, factorial design).

Biosurfactants are a diverse group of surface-active chemical compounds mainly produced by hydrocarbon-utilizing microorganisms (Banat 1995). They are environmentally friendly, biodegradable, less toxic and non-hazardous. They have better foaming properties and higher selectivity. They are active at extreme temperatures, pH and salinity as well (Pacwa-Płociniczak et al. 2011). Despite of the above advantages, the application of biosurfactants has been significantly restricted due to their low yields and high production cost as well as the lack of desired producing microorganisms (producers) (Mukherjee et al. 2006). The use of waste streams or cheap substrates to reduce the initial raw material costs which is 10-30% of the final product costs is one of

the attractive strategies for economical biosurfactant production (Makkar et al. 2011; Mukherjee et al. 2006). Peanut oil cake (Canada 2008; Thavasi et al. 2011), molasses and whey (Joshi et al. 2008), canola waste frying oil, soybean waste frying oil, and corn steep liquor (Rocha e Silva et al. 2014) are few examples of alternative substrates that have been used for biosurfactant production. A novel raw material to produce biosurfactant can be FWC extract which is a source of nutrition for microorganism. Use of statistical experimental strategies including factorial design and response surface methodology (RSM) will help in better optimization of production of biosurfactants (Makkar et al. 2011; Min and Wong 1999). Using FWC extract as substrate for biosurfactant production and the relevant methodology has not been previously reported.

This paper describes the extraction of FWC nutrients using enzyme hydrolysis under optimum conditions and then utilize the extract as a novel substrate for biosurfactant production. The production of biosurfactant was compared with other carbon and nitrogen sources. The optimum condition for biosurfactant production was explored and surface tension, emulsification activity, and critical micelle concentration of the produced biosurfactant were measured and tested.

6.2 Materials and Methods

6.2.1 Compost

The fish (cod) wastes were obtained from a local seafood processing center in Newfoundland and Labrador (NL) and mixed with peat as a bulking agent then composted in a 35L bench scale cylindrical reactor at the Northern Region Persistent

Organic Pollution Control (NRPOP) laboratory, Memorial University of Newfoundland, for two months and then it is left to pass the curing phase for 6 months. The mature and stable compost is then ground for further experiments.

6.3 Enzyme Hydrolysis

6.3.1.1Enzyme

Alcalase, a serine bacterial endopeptidase (generic name, Subtilisin Carsberg) prepared from a strain of *Bacillius licheniformis*, was provided by Sigma Aldrich, a food grade enzyme, having a specific activity of 2.4 anson units g^{-1} was stored at 4°C until it is used for hydrolysis experiments. Its optimum activity occurs at a temperature between 50° and 70°C, and at pH values between 6 and 10. Its density is 1.18 g mL-1, and its deactivation temperature is 85°C for 10 min (Bernal et al. 2009).

6.3.1.2Experimental Design

Experiments to investigate the effects of hydrolysis variables in the range given in Table 6.1 were conducted based on a central composite design (CCD) with five centre points (Table 6.2). All reactions were performed in a 250 mL flask in water bath. 5 g of FWC was mixed with distilled water 1:1 (w/w). The pH of the solution was in the optimal range for the enzyme. The enzyme based on the design range from 0.5% to 2.5% (v/v) was added to the flask. The temperature can range from 55 to 65 °C and time can range from 1 to 5 h, were adjusted based on the experimental design and the reaction was allowed to proceed. The reaction was terminated by heating to 90 °C for 20 minutes,

assuring the inactivation of the enzyme. The hydrolysate were then cooled to room temperature and centrifuged at 10,000 rpm for 10 min to collect the supernatant. The SN-TCA method uses trichloroacetic acid (TCA) to precipitate the unhydrolyzed protein that may be present. To the supernatant, one volume of 20% TCA was added, followed by centrifugation at 10,000 rpm, 10 min to collect the 10% TCA-soluble materials. The supernatants were collected for TCA-soluble N determination using the Kjeldahl method (AOAC, 2005). The Kjeldahl digestion was carried out in a Buchi 402 rapid digestion unit (Flaw& CH) and a distillation unit (Buchi 322).

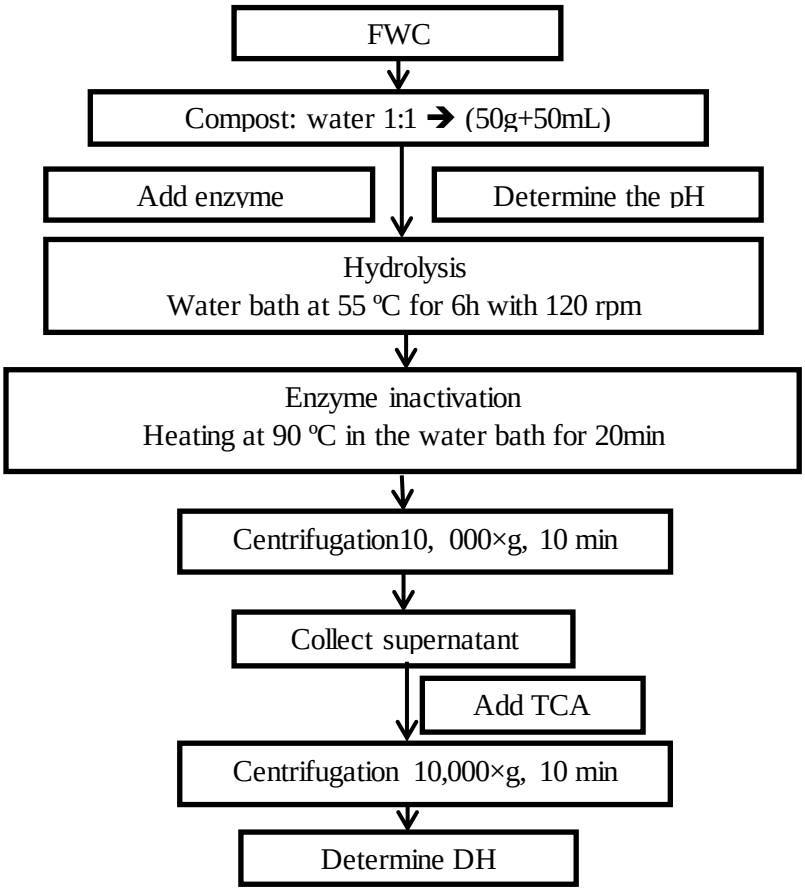


Figure 6.1 Flow sheet for the enzymatic hydrolysis of FWC

The approximate degree of hydrolysis (DH) of FWC samples was determined by the ratio, percent of 10% trichloroacetic acid (TCA)-soluble nitrogen to total nitrogen. The degree of hydrolysis (DH) was calculated as:

$$DH = (\text{10\% TCA-soluble N in sample} / \text{Total N in sample}) \times 100 \quad (6.1)$$

Table 6.1 Hydrolysis variables and their low and high levels

Independent variables	Symbols		Levels	
	Coded	Uncoded	Low	High
Time	A	Time (h)	1	5
Enzyme/ Substrate (E/S)	B	E:S	0.5	2.5
Temperature	C	Temperature (°C)	55	65

Table 6.2 CCD used in the experiment and the response for DH (observed and predicted values)

Run	A: Time	B: (E/S)	C: Temperature.	Y_0 Observed DH (%)	Y Predicted DH (%)
1	3	1.5	60	20.4354	19.168
2	6	1.5	60	47.5893	46.386
3	3	1.5	60	20.3158	19.1392
4	5	0.5	55	26.9727	29.021
5	3	3	60	18.48	22.644
6	1	2.5	65	17.4853	13.377
7	3	1.5	67.5	11.9524	14.192
8	1	0.5	65	24.2748	24.520
9	1	2.5	55	15.2112	13.445
10	3	1.5	52.5	13.2456	14.294
11	3	0	60	25.2453	24.369
12	3	1.5	60	18.2231	19.168
13	3	1.5	60	16.9231	19.168
14	5	2.5	55	38.6111	37.864
15	1	0.5	55	26.9279	24.588
16	5	2.5	65	38.653	37.864
17	0	1.5	60	20.2546	24.746
18	3	1.5	60	22.4104	19.168
19	5	0.5	65	28.8244	28.824

6.3.2 Biosurfactant Production

6.3.2.1 Strain and Culture Condition

Biosurfactant producing microorganism N3-1P (*Bacillus* sp.) was isolated as an effective biosurfactant producing microorganisms from petroleum hydrocarbon contaminated marine sources (Cai et al. 2015). The bacteria colony was then transferred from the agar plate to a 125-ml Erlenmeyer flask containing 50 ml BD 23400 nutrient broth (Fisher scientific company, Ottawa, Canada) to grow the culture on a rotary shaker for 24h at 37 °C and 180rpm to reach the optical density at 600 nm (OD600) of 0.8. For further incubation, this culture was used with a production media as 1% (v/v). The bacteria was transferred from culture to the medium composed of NaCl, 2.2 g; $FeSO_4 \cdot 7H_2O$, 2.8×10^{-4} g; KH_2PO_4, 3.4 g;$K_2HPO_4 \cdot 3H_2O$, 4.4 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; yeast extract, 0.5 g, n-Hexadecane 50 ml/L, $(NH_4)_2SO_4$ 15 g, and 0.5 ml/L trace element solution in 125-ml conical flasks. The trace element solution contained $ZnSO_4$, 0.29 g; $CaCl_2$, 0.24 g; $CuSO_4$, 0.25 g; $MnSO_4$, 0.17 g/L was sterilized separately. The medium was adopted and modified from (Peng et al. 2007). Chemicals used were analytical grade, unless otherwise specified. After 1 day, before inoculation, a purity check was conducted by spreading the medium over nutrition broth agar plate to avoid cross contamination. Nutrition broth composed of peptone, 8g; yeast extract, 3g; NaCl, 6 g; Glucose, 1 g: and agar, 15 g.

6.3.2.2Effect of Carbon and Nitrogen Sources on Biosurfactant Production

Different carbon source including FWC extract (FWCC), glucose, sucrose, n-hexadecane, glycerol, and starch (at 5% concentration in the place of n-hexadecane) and different nitrogen sources such as FWC extract (FWCN), yeast and $NaNO_3$ (at 15% concentration in the place of $((NH_4)_2SO_4)$ were tested for biosurfactant production by the selected strain. Table 6.3 shows the different carbon and nitrogen sources have been used to compare the efficiency of FWC extract as substrate for biosurfactant producing bacteria.

Table 6.3 Carbon and nitrogen sources for biosurfactant production

Carbon source	Nitrogen source
n-Hexadecane	
Sucrose	$(NH_4)_2SO_4$
Starch	
Glucose	
Glycerol	
FWCC	
	Yeast extract
n-Hexadecane	Ammonium nitrate
	FWCN

A 1% bacterial cell suspension from a 24-h culture was used as inoculum.15 ml medium was prepared in a 50 ml conical flask and incubated at 30^{o}C, 200 rpm for 5 days. Cells were removed from the culture by centrifugation at 12, 000 rpm for 20 min. Cell- free culture broth was used for analytical measurements. Three parameters including surface tension, CMD and emulsification index were measured to evaluate the efficacy of the generated biosurfactants.

Surface tension measurements of the culture broth supernatants were performed according to the Ring method with a surface tensiometer (DuNouyTensiometer, Interfacial, CSC Scientific). To increase the accuracy of the surface tension measurements, an average of triplicates was determined. All measurements were performed at room temperature (20 C). Critical micelle concentration (CMD) is the dilution of the culture broth upon reaching the critical micelle concentration (Shavandi et al., 2011). After centrifuging at 12,000 rpm for 20 min and discarding the pellet, the cell free broth were diluted with distilled water, while the surface tension of each dilution was measured. The CMD was determined as the highest dilution with which the surface tension did not significantly increase. As the broth consists of both aqueous and oil phases, each dilution was conducted with sonication to ensure homogeneity. Before each measurement, the sonicated solution was allowed to stand for 15-20 min to achieve equilibrium. The maximum standard deviation observed for surface activity measurements was 0.20.

The emulsification index (EI_{24}) of culture samples was determined according to the methods of (Cooper and Goldenberg 1987), by adding 2 mL of hexadecane to the same

amount of culture, mixing with a vortex at high speed for 2 min, and leaving to stand for 24 h. The emulsification activity was evaluated by EI_{24} using following equation:

$$EI_{24}=H_{EL}/H_S\times100\% \quad (6.2)$$

where H_{EL} is the height of the emulsion layer and H_S is the height of the total solution.

6.3.2.3Biosurfactant Production Optimization

A CCD design with 5 center-points (Table 6.5) was used to analyze the responses and subsequently to optimize biosurfactant production. Incubation time and concentration of FWC extract were considered as independent variables and CMD was used as the response to evaluate biosurfactant production. Table 4 presents the variables and their low and high levels.

Table 6.4 Biosurfactant production variables and their low and high levels

Independent variables	Symbols		Levels	
	Coded	Uncoded	Low	High
Time (days)	A	Time (d)	3	7
FWC extract concentration	B	Concentration (%)	20	80

Table 6.5 CCD for biosurfactant production

Run	A: Time	B: Concentration	Y_0 Observed CMD	Y Predicted CMD
1	7	80	18.55	18.81
2	8	50	12.79	11.11
3	5	5	1.6	0
4	5	50	8.78	9.13
5	2	50	20.83	20.81
6	5	50	8.78	9.3
7	5	50	8.78	7.3
8	5	95	31.58	32.5
9	3	80	32.86	31.87
10	5	50	8.78	12.5
11	5	50	8.78	5.26
12	3	20	4.5	6.26
13	7	20	8.13	11.11

6.3.2.4Biosurfactant Extraction and Assay

To extract the biosurfactant, the cell free culture were mixed with equal volume of chloroform/ methanol (1:2 v/v) and shaken on an orbital shaker (200 rpm) for 24 hours. The solvent was then evaporated by rotary evaporator and kept at 4°C. Biosurfactant production was determined through mixing 10 ml of cell free culture with equal volume of chloroform/ methanol (1:2 v/v) and shaken on an orbital shaker (200 rpm) for 24 hours in a glass tube, the solvent was evaporated. The final and initial weight of the glass tube was recorded to calculate the weight of produced biosurfactant. Characterization of the biosurfactant was done by thin layer chromatography (TLC). TLC was performed on silica gel 60 plates (Sigma Aldrich) with chloroform, methanol, acetic acid and water mixture. Standard spray, Ninhydrin reagent, phenol–sulphuric acid and iodine were used to detect protein, carbohydrate and lipid spots respectively. Total protein content and total carbohydrate of biosurfactant was determined by the Bradford (1976) and phenol–sulphuric acid Dubois et al. (1956) method, respectively. Total lipid was analysed based on the method which is described by Pande et al. (1963).

6.3.2.5Biosurfactant Stability Test

Stability studies were done using cell-free broth obtained after 72h of cultivation. Broth samples were incubated in a water bath at different temperatures including 4, 20, 40, 60, 80 and 100°C and cooled at room temperature. The pH stability was performed by adjusting the broth to different pH (2, 4, 6, 8, 10, 12) values by adding 1N NaOH or 1 N HCl. For studying the effect of salt addition on the biosurfactant, different concentrations

of NaCl comprises 0, 5 10, 15 and 20% (W/V) were added to broth samples and mixed until complete dissolution. The stability of the biosurfactant was monitored for one week. The surface tension values of each treatment were performed as described. The data are presented in terms of averages of at least three replicates.

6.4 Results and Discussion

6.4.1 Hydrolysis

The effect of time, E/S ratio and temperature on degree of hydrolysis of FWC was evaluated using a CCD response surface design. The observed values for DH at different combinations of the independent variables are presented in Table 6.2. Results of the ANOVA for model and significant factors at the 5% level are summarized in Table 6.6. A quadratic model was selected to describe the DH since there is nonlinearity in the responses. As it is suggested by the model, time interaction of time and E/S ratio and temperature in the selected ranges are significant factors. According to other studies, hydrolyzing conditions significantly influence the peptide bond cleavage in the protein substrate (Bhaskar et al. 2008; Diniz and Martin 1996). Response surface graphs from the model are presented in Figure 6.2 to show the interaction of factors. The combined effects of each pair of factors, indicate that an increase of time at low and high E/S ratio has significant effect on DH, it has positive effect at high level of E/S and it has negative effect at low level of E/S ratio. Higher DH was observed at high level of time and high level of E/S ratio. For temperature, increase of temperature causes increment in DH until it reaches to 60°C, after this point increase of temperature leads to a decrease in DH.

Such a decrease in hydrolysis rate over higher enzyme activity values, temperatures, and time may be due to denaturation of the enzyme and reducing its activity (Ovissipour et al. 2012). Therefore high DH can be achieved at a high level of time and at the mid-level of temperature. The assumptions of ANOVA were checked and they met the requirement so the model can be used to predict the optimum condition for DH. The analyses were carried out using Design-Expert Version 8 (Statease Inc., 2013).

Table 6.6 ANOVA table of DH affected by time, E/S ratio and temperature during optimization experiment

Source	Sum of Squares	Degree of freedom	Mean Square	F Value	p-value
Model	1430.35	6	238.3917	21.50428	< 0.0001
A-Time	650.3645	1	650.3645	58.66657	< 0.0001
B-E/S	4.13241	1	4.13241	0.372767	0.5529
C-Temperature	0.014464	1	0.014464	0.001305	0.9718
AB	199.7321	1	199.7321	18.01697	0.0011
A^2	519.2061	1	519.2061	46.83534	< 0.0001
C^2	49.37227	1	49.37227	4.453659	0.0565
Residual	133.0293	12	11.08578		
Lack of Fit	114.878	8	14.35975	3.164455	0.1404
Pure Error	18.15131	4	4.537828		
Cor Total	1563.379	18			
R-Squared 0.9149	Adj R-Squared 0.8724	Pred R-Squared 0.7227		Adeq Precision 17.28	

Goodnessness of fit measures indicated a good fit of the model to the data. There is also no statistically significant lack of fit of the model indicating that the model can be adequately used for predicting the degree of hydrolysis for any combination of experimental independent variables.

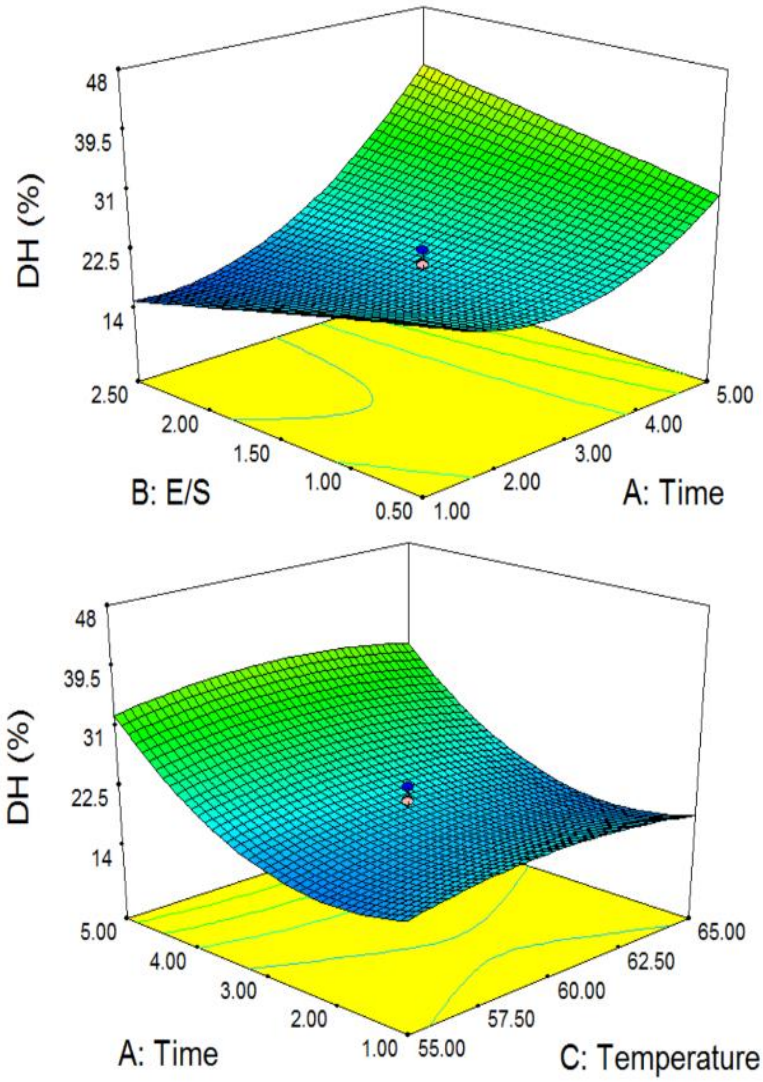


Figure 6.2 Response surface plot for DH of FWC (1) as a function of time and E/S ratio, (2) as a function of temperature and time

The model equation for the response variable (DH (%)) of FWC as a function of the three independent variables (A, B, and C) and their interactions is given by:

$$DH\ (\%) = DH\ (\%) = +20.50 + 7.21*A - 0.57*B - 0.034*C + 5.00*A*B + 7.24*A^2 - 2.23*C^2 \quad (6.3)$$

Where A, B and C represent time, E/S ratio and temperature, respectively.

The optimum hydrolysis condition for hydrolyzing FWC based on the quadratic model was obtained using the desirability function approach available within Design-Expert. The optimum condition obtained was: time of 5 h, E/S ratio of 2.5 and temperature of 59.97 for maximum DH (39.97%). To generate the FWC extract to be used in the later stages these conditions were applied. The CHN analysis result for the FWC extract generated under optimum hydrolysis condition gave the amount of total carbon, total organic carbon and total nitrogen in FWC hydrolysis as 366.7, 332.6 and 48.97 mg/g, respectively.

6.4.2 Biosurfactant Production

To evaluate the possibility of using FWC extract as an effective alternate substrate for the production of biosurfactant by a newly isolated *Bacillus* (N3-1P), it was used as carbon (5%) and nitrogen (15%) sources. Surface tension, EI_{24} and biosurfactant productivity rate were determined. The performance of FWC as a nutrient source was compared with the other organic and inorganic carbon and nitrogen sources. Results are presented in Figure 6.3. There are evidences that carbon and nitrogen play important roles in the production of surface-active compounds by microorganisms (Wu et al. 2008). The carbon source and nitrogen source influenced surface tension, EI_{24} and productivity similarly in

this study as well. Surface tension measurements was used as an indirect measure of biosurfactant production and to evaluate the efficiency of the produced biosurfactant (George and Jayachandran 2013).The lowest surface tension was observed for Glucose which is in agreement with other studies for *Bacillus* strain (Abdel-Mawgoud et al. 2008; Joshi et al. 2008). Glucose, sucrose, starch, glycerol, yeast and FWCC decreased the water surface tension to 28.5, 29.1, 31.7, 34.5, 32.9, and 32.43 (mN/m), respectively. FWCN and hexadecane did not decrease the surface tension of water significantly. The inhibitory effect of hydrocarbons (including *n*-hexadecane and paraffin) as the only carbon source on bacteria growth and biosurfactant production with different *Bacillus* strains have been reported in previous studies (Pereira et al. 2013). The emulsification index (% EI_{24}) provides a rapid and reliable measure of the quantity of biosurfactant (Pal et al. 2009). The high emulsification activity was observed for glucose, glycerol, sucrose, starch and FWCC, respectively. Yeast and FWCN did not show remarkable emulsification index. The highest amount of biosurfactant was produced by glucose, then starch and sucrose. Glucose and sucrose have been reported as the best carbon sources for growth using different *Bacillus* isolates (Abdel-Mawgoud et al. 2008; Makkar and Cameotra 1997). While FWCC yielded 1.48 g/L, FWCN yielded very low production rate. Although the production rate of FWCC was lower than glucose and glycerol, it is a sustainable substrate for biosurfactant production, since its cost is low. The CMD values are useful data to indicate the biosurfactant concentration present in medium (Marin et al. 2015). Sucrose showed the highest CMD and then the high CMD was associated to glycerol. FWCC showed CMD equal to 7.30. Results showed that the strain, *Bacilluse*,

N3-1P was able to grow and produce biosurfactant when cultivated in the FWC. Using FWC as a substrate for biosurfactant production will add value to the FWC. FWC demonstrated a promising performance as substrate for biosurfactant producing bacteria. In the next step it is used as the sole carbon and nitrogen source and the production condition was optimized using response surface methodology.

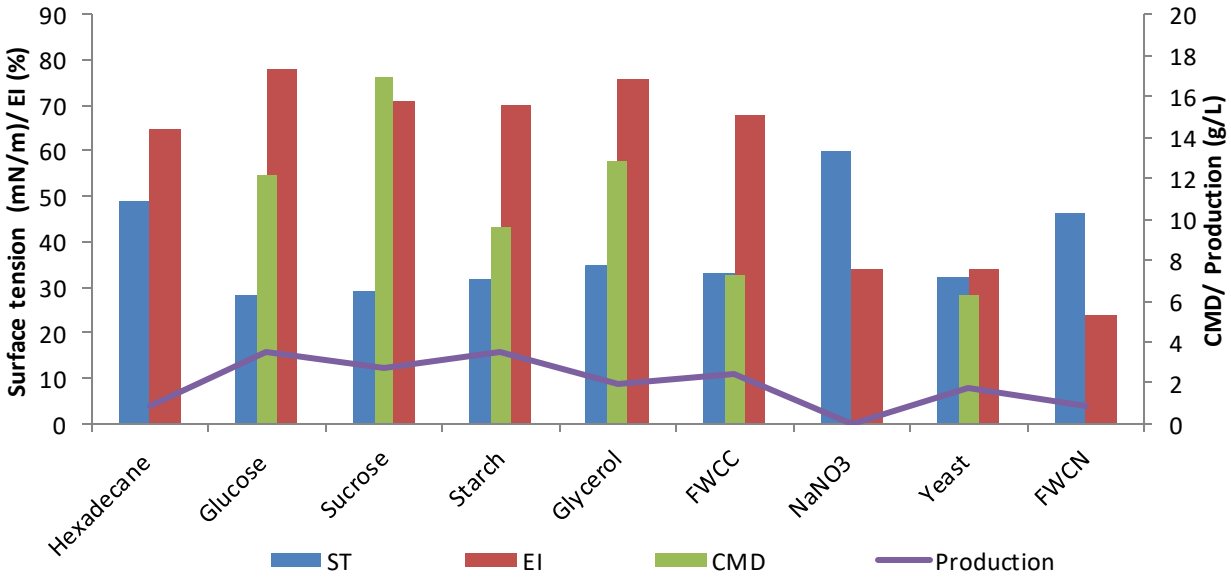


Figure 6.3.Effect of carbon and nitrogen sources on the biosurfactant production

6.4.3 Optimization of Process Parameters for Biosurfactant Production

The media components play a key role in controlling yield and specific productivity in microbial production process which is mostly complex and nonlinear (Pal et al. 2009). Therefore, optimization of the media condition is an effective method to increase the productivity of biosurfactant. To screen the significant factors, design of experiment (DOE) based method was selected to also investigate the effect of each factor and their interactions. To optimize biosurfactant production, the time of inoculation was varied from 3 days to 7 days and the concentration of FWC extract as a sole source of carbon and nitrogen was varied form 20% to 80% of the medium. CMD of the produced biosurfactant was selected as a response. From the ANOVA table (Table 6.6), both time and concentration of FWC are significant factors at the 5% level for biosurfactant production. As shown in Figure 6.4, with an increase in concentration of FWC, biosurfactant production was enhanced dramatically. Most of the biodegradable substances present in the substrates seemed to be consumed by the bacteria after 3 days since longer period of incubation did not show superior biosurfactant production (Partovi et al. 2013). There is also a significant interaction between time and concertation (p-value = 0.0132). The highest CMD was observed after 3 days incubation with 80% of FWC concentration as nutrient source. A second-order polynomial equation was used to relate the independent process variables with biosurfactant production. The fitted regression model and subsequent optimization using the desirability function approach suggested time of 3 days, FWC extract concentration of 80% as optimum condition. The maximum CMD for produced BS obtained by using the above optimized values of the variables is

32. The maximum CMD obtained experimentally was 30.5. This is in close agreement with the model prediction.

Table 6.7 Total carbon, total organic carbon and total nitrogen in FWC extract after hydrolysis

Source	Sum of Squares	Degree of freedom	Mean Square	F Value	p-value
Model	1118.556	5	223.7112	30.29961	0.0001
A-Time	60.94325	1	60.94325	8.254198	0.0239
B-Concentration	792.2169	1	792.2169	107.2984	< 0.0001
AB	80.19203	1	80.19203	10.86127	0.0132
A^2	105.5434	1	105.5434	14.29488	0.0069
B^2	113.3033	1	113.3033	15.34588	0.0058
Residual	51.68312	7	7.383303		
Lack of Fit	22.90464	3	7.634881	1.061193	0.4586
Pure Error	28.77848	4	7.19462		
Cor Total	1170.239	12			
R-Squared 0.9384	Adj R-Squared 0.9242		Pred R-Squared 0.8212		Adeq Precision 16.37

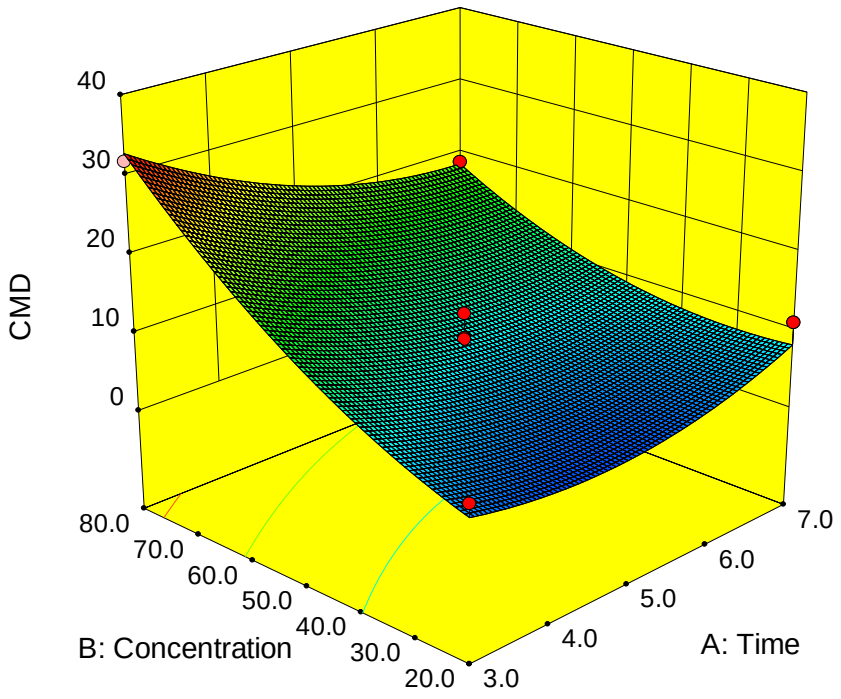


Figure 6.4 Response surface plot showing biosurfactant production as function of FWC concentration and time

$$CMD = +8.78 - 2.68 * A + 9.65 * B - 4.48 * A * B + 3.57 * A^2 + 3.70 * B^2 \quad (6.4)$$

Where A and B are time and concentration of FWC, respectively. The goodness of fit measures showed a reasonable good fit of the quadratic model to the responses with no significant lack of fit.

6.4.4 Biosurfactant Assay

The composition of the medium, as reflected by nutrients balance, is of critical importance for determining product yield and biosurfactant properties (Sheppard and Mulligan 1987), therefore, it is essential to characterize the properties of the biosurfactant obtained from FWC. Minimum concentration necessary to initiate micelle formation is called critical micelle concentration (CMC). In practice, it is the maximum concentration of surfactant monomers in water (Mulligan 2005). The CMC is also the point at which the surface tension abruptly increases (Mulligan et al. 2001). The CMC can be determined by plotting surface tension as a function of biosurfactant (or broth) concentration since the slope of the curve abruptly changes at the CMC. However, the abruptness is a function of both the particular biosurfactant and the presence of impurities in the system (Sheppard and Mulligan 1987). The method of (Sheppard and Mulligan 1987) was followed to determine CMC of the produced biosurfactant. In Figure 6.5 the minimum effective concentration of biosurfactants corresponds to 0.013 g/ml. Structure of biosurfactants, pH, ionic strength, temperature, and the polarity of the solvent influences CMC (Desai and Banat 1997).

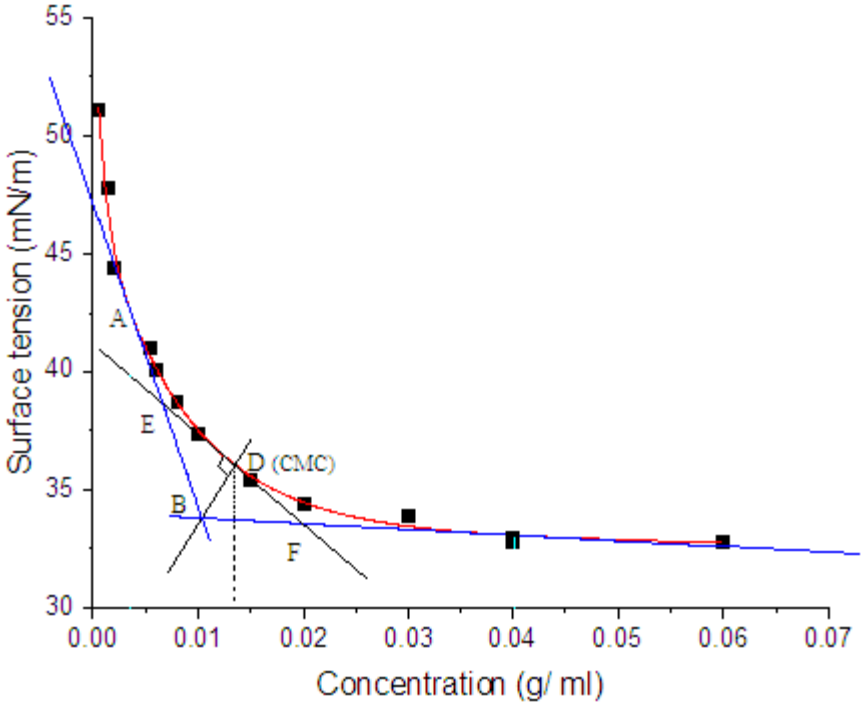


Figure 6.5 CMC determination through measuring surface tension

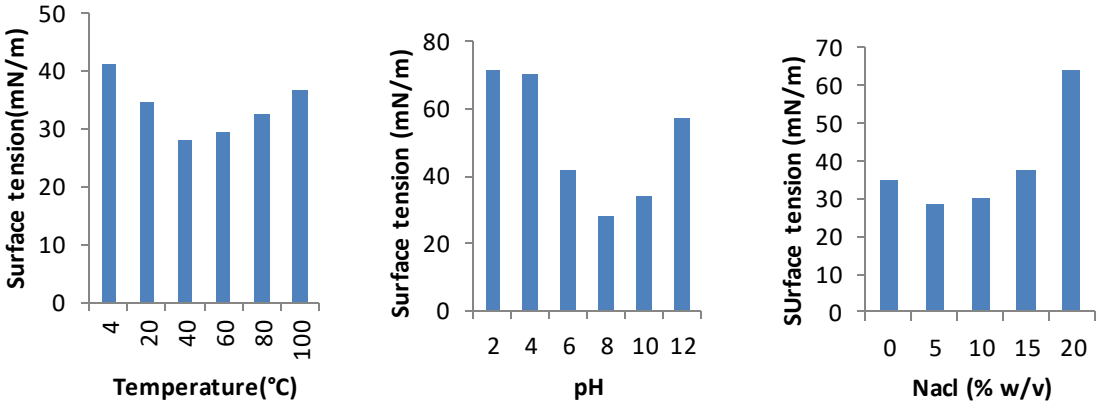
Biosurfactants can be glycolipids, lipopeptides, lipolysaccharides, polysaccharide-protein complexes, fatty acids and lipids (Makkar et al. 2011). Although *Bacillus* species are known to produce exclusively lipopeptide biosurfactants (Thaniyavarn et al. 2003), the biosurfactant produced by B. megaterium was classified as a glycolipid (Thavasi et al. 2008). Cyclic lipopeptides produced by bacilli like surfactin (produced by *B. subtilis*) and lichenysin (produced by *B. licheniformis*) are, the most effective biosurfactant discovered so far (Joshi et al. 2008). Preliminary characterization of the produced biosurfactant showed that it was composed of lipid (58%) and protein (18%) and carbohydrate (32%). After extraction and thin layer chromatography, a single purple spot with Rf = 0.45 was observed after spraying with ninhydrin and a single brown lipid spot with Rf = 0.7 was seen on exposure to iodine vapors, while a spot was detected with phenol–sulphuric acid reagent had Rf = 0.3. From the results of CHN analysis, biosurfactant contains 35% carbon with 33% of organic carbon and 3.6% nitrogen. The biggest portion of the lipid composition of biosurfactant is an acetone mobile polar lipid that represents part of lipid fraction that is acetone extractable. Glycolipid is also an important part of this class (Quigley et al. 1989). The fatty acid composition of the biosurfactant is important for their activity (Youssef et al. 2005). The total fatty acid analysis reveals that C18 has the biggest portion which accounts for about 50%.

Table 6.8 Lipid composition of the biosurfactant (percent of the total amount of lipid)

% Lipid Composition of biosurfactant	
Hydrocarbons	1.81
Free Fatty Acids	1.19
Alcohols	0.52
Sterols	5.17
Acetone Mobile Polar Lipids	59.99
Phospholipids	31.32

6.4.5 Biosurfactant Stability

Salinity is one of the critical factors for controlling the production of biosurfactants especially for those producers isolated from salty environments (Najafi et al. 2010). Biosurfactant reduced surface tension in the temperature range of 20–80 °C and maximum reduction was observed at 40 C. Maximum surface tension reduction was obtained in the presence of 5% (w/v) of NaCl and it retained almost 80% of its activity in presence of 15% (w/v) of NaCl. Biosurfactant stayed stable for 4 days when salt concentration was 5% (w/v) at 80 °C in the pH range of 6.5–11. Stability studies of the produced biosurfactant indicated the biosurfactant to be thermostable and also pH stable from values over 6.0. Biosurfactant showed salt tolerance of up to 15%. These findings revealed that the product obtained could be very useful in situations where extreme conditions of temperature, salinity and alkaline pH are present, such as enhancing oil recovery and bioremediation of soil and marine environments (Nitschke and Pastore 2006).



(a) (b) (c)

Figure 6.6 Effect of (a) temperature, (b) pH, and (c) NaCl concentration on surface tension of the biosurfactant

6.5 Summary

Raw material can account for about 30% of the overall cost of a microbial surfactant production, therefore the economical production of biosurfactant depends on the development of cheaper processes and the use of low-cost raw materials (George and Jayachandran 2013). In this study, the potential utilization of low-cost substrates of FWC as an inexpensive nutrient source for the production of biosurfactants was studied to minimize the waste and produce cost-efficient biosurfactant and improve biosurfactant production economics by incorporation of the waste. To extract the nutrient from FWC, enzyme hydrolysis was optimized and extract was conducted under optimum condition. To the best of our knowledge, enzyme hydrolysis of FWC, optimization of the hydrolyse process, and usage of FWC extract as nutrient source for the production of biosurfactant have not been reported in the available literature.

The biosurfactant obtained from FWC extract showed high surface tension reduction and high emulsification activity, exhibited a high level of stability (thermostable, pH stable and stable in presence of salts) suggesting that further studies can help to achieve practical production, therefore reduce the usage of synthetic surfactant and preserve the environment. In this study, each step in the biosurfactant production including the selection of suitable strains with the desired properties, use of inexpensive alternative substrates and application of experimental design approach for optimizing process parameters have been applied to enhance the production rate of biosurfactant.

CHAPTER 7

PRODUCTION OF BIOSURFACTANT BY *RHODOCOCCUS ERYTHROPOLIS SP.* CULTIVATED IN A NOVEL FISH WASTE COMPOST EXTRACT SUBSTRATE[6]

[6] *This chapter is based on the following paper:*

Kazemi, K., Zhang, B., and Lye, L. M., (2016). Production of biosurfactant by *Rhodococcus erythropolis sp*. cultivated in a novel fish waste compost extract substrate, Proceedings CSCE Annual Conference, June, 1-4, 2016, London, Canada

Role: Khoshrooz *kazemi solely worked on this study and acted as the first author of this manuscript under the guidance of two supervisors, Dr. Baiyu Zhang and Dr. Leonard Lye. Most contents of this paper was written by Kazemi and further polished by the other co-authors.*

7.1 Bachground

Biosurfactants are amphiphilic compounds produced by a wide variety of microorganisms containing both hydrophilic and hydrophobic moieties that allow them to array at the interface of polar and nonpolar media (Sen 2010). They have been identified for several industrial applications in cosmetic, pharmaceutical, food processes, and environmental engineering as emulsifiers, humectants, preservatives, and detergents. Because of their structural diversity (i.e., glycolipids, lipopeptides, fatty acid esters), low toxicity thus ecologically safe, and high biodegradability, biosurfactants have potential for replacing synthetic surfactants in bioremediation and waste treatments (Pal et al. 2009). Despite all advantages that biosurfactant have, low yields and high production cost limit the extension of biosurfactant applications (Makkar et al. 2011). Raw material can count almost 30% of the overall cost of a microbial surfactant production, therefore the economical production of biosurfactant depends on the development of the use of low-cost raw material and optimization of the production processes (George and Jayachandran 2013). The use of the alternative substrates such as industrial and/or municipal wastes is one of the attractive strategies for economical biosurfactants production to minimize the pollutants and produce valuable product (Kosaric 1992). Improvement of efficiency of the production process (e.g., optimization of cultural condition) can also help to overcome the economic constraints associated with biosurfactat production (Mukherjee et al. 2006).

Form 267,959 tonnes of fish landed in Newfoundland and Labrador, 54% was classified as fish waste in 2001(Ghaly AE 2013). Composting is considered to be a viable solution

to the problems of waste disposal experienced by fish processing plants and fish farms (Liao et al. 1995). Compost made from fish waste is rich in nutrients, particularly nitrogen and phosphorous (Benhabiles et al. 2012; Illera-Vives et al. 2013; Laos et al. 2002). It can be used to generate subtract for bacterial growth and production of valuable products such as biosurfactants.

Medium compositions such as carbon sources, nitrogen sources, and inorganic salts strongly influence cell growth and the accumulation of metabolic products (Li et al. 2002). Environmental factors and growth conditions such as pH and time of cultivation also affect biosurfactant production through their effects on cellular growth or activity (Desai and Banat 1997). Through studying the effect of these factors on production process and optimizing media condition, the yield of biosurfactant production can be elevated (Kiran et al. 2009; Mukherjee et al. 2006). Among various statistical methods, response surface methodology (RSM) is the most widely used method in system optimization. Through integrating a collection of statistical tools and techniques, RSM leads to constructing and exploring an approximate functional relationship between a response variable and a set of design variables (Venter 1998).

The present paper investigates the potential usage of fish waste compost (FWC) extract as a novel substrate for biosrufactant production by a strain *Rhodoccocus (P6-4P)* isolated from Atlantic ocean. In order to optimize media conditions to enhance the yield of *Rhodococcus* biosurfactant using FWC extract as an involving factor and develop an empirical model of the process, one of the most important RSM designs methods namely central composite design used in process optimization.

7.2 Material and Methods

7.2.1 Strain and Culture Condition

Biosurfactant producing microorganism P6-4P (*Rhodococcus erythropolis* sp.) isolated as an effective microorganisms from petroleum hydrocarbon contaminated marine sources in the North Atlantic Canada in the NRPOP lab, Memorial University of newfoundland, Canada was selected to produced biosurfactant (Cai et al. 2015). Bacteria colony was transferred from agar plate to 125-ml Erlenmeyer flask containing 50 ml BD 23400 nutrient broth (Fisher scientific company, Ottawa, Canada) to growth the culture on a rotary shaker for 24h at 37 °C and 180rpm to reach the optical density of the culture at 600 nm (OD600) of 0.8. Growth and biosurfactant production by the isolate was evaluated using media which is adopted and modified from (Peng et al. 2007) including NaCl, 2.2 g; $FeSO_4 \cdot 7H_2O$, 2.8×10^{-4} g; KH_2PO_4, 3.4 g;$K_2HPO_4 \cdot 3H_2O$, 4.4 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; yeast extract, 0.5 g, N-hexadecane 30 ml/L, $(NH_4)_2SO_4$ 15 g, and 0.5 mL/L trace element solution in 125 mL conical flasks. The trace element solution contained ZnSO4, 0.29 g; CaCl2, 0.24 g; CuSO4, 0.25 g; MnSO4, 0.17 g L^{-1} and was sterilized separately. The chemicals used were analytical grade, unless otherwise specified. Incubation was conducted at 30^oC, 200 rpm for 2 days. After 2 days, before inoculation, purity check was conducted by spreading the medium over nutrition broth agar plate to avoid cross contamination. Nutrition broth composed of peptone, 8 g; yeast extract, 3 g; NaCl, 6 g; Glucose, 1 g: and agar. 15 g. Difference carbon and nitrogen sources has been

used to compare the efficiency of FWC extract as substrate for biosurfactant producing bacteria.

7.2.2 Effect of Carbon and Nitrogen Sources on Biosurfactant Production

The effect of different carbon sources was studied by replacing the n-hexadecane with sucrose, starch, glucose, and fish waste compost extract (FWCC). The different carbon sources were added to the media at a concentration of 5 g l^{-1}. To evaluate the nitrogen sources, ammonium sulphate was replaced by an equivalent amount of different nitrogen sources, namely yeast, ammonium nitrate and FWC extract (FWCN). The different nitrogen sources were added to the media at a concentration of 15 g l^{-1}. A 1% bacterial cell suspension from a 24-h culture was used as inoculum. 15 ml medium has been prepared in the 50 ml conical flask and incubated at 30^{o}C, 200 rpm for 5 days. Cells were removed from the culture by centrifugation at 12, 000 rpm for 20 min. Cell- free culture broth was used for analytical measurements.

7.2.3 Surface Tension and CMD Measurement

Surface tension measurements of culture broth supernatants were performed according to the Ring method with a surface tensiometer (DuNouyTensiometer, Interfacial, CSC Scientific). To increase the accuracy of the surface tension measurements, an average of triplicates was determined. All measurements were performed at room temperature (20 °C). Critical micelle concentration (CMD) is the dilution of the culture broth upon reaching the critical micelle concentration (Shavandi et al., 2011). After centrifuge at 12,000 rpm for 20 min and discard the pellet, the cell free broth were diluted with

distilled water, while the surface tension of each dilution was measured. The CMD was determined as the highest dilution with which the surface tension did not significantly increase. As the broth consists of both aqueous and oil phase, each dilution was conducted with sonication to ensure homogeneity. Before each measurement, the sonicated solution was allowed to stand for 15-20 min to achieve equilibrium.

7.2.4 Emulsifying Activity

Emulsifying activity was determined by the addition of 2 ml of *n*-hexadecane to the same volume of cell-free culture broth supernatant in glass test tubes. The tubes were mixed with vortex at high speed for 2 min and subsequently incubated at 25 °C for 24 h. The stability of the emulsion was determined after 24 h, and the emulsification index (EI_{24}) was calculated as the percentage of the height of the emulsified layer (mm) divided by the total height of the liquid column (mm). All emulsification indexes were performed in triplicate.

7.2.5 RSM Experimental Design

To examine the combined effect of three different medium conditions and to obtain the functional relationship between incubation component including time, pH and FWC concentration and response namely CMD, a CCD of $2^3 = 8$ plus 6 center points, plus one replicate of star point and one replicate of factorial point leading to a total of 20 experiments was designed. The value of the response (CMD) was the mean of three replications. Trial version of Design Expert software (version 8.0, Stat-Ease, USA) was

used to conduct the statistical analysis. Table 7.1 presents the variables and their high and low levels.

Table 7.1 Biosurfactant production variables and their high and low level

Independent variables	Coded	High	Low
Time (d)	A	3	7
pH	B	6	8
FWC concentration (%)	C	20	80

Table 7.2 CCD for biosurfactant production

Run	Factor A	Factor B	Factor C	Y_0 observed CMD	Y predicted CMD
1	5	8.5	50	10.8	8.75
2	5	7	50	14.2	13.34
3	2	7	50	20.8	21.99
4	5	7	50	12.2	13.34
5	3	8	20	15.5	14.03
6	5	7	50	12.4	13.34
7	7	6	20	2.2	0
8	3	8	80	21.5	25.75
9	7	8	20	0	0
10	7	8	80	8.13	10.03
11	5	7	5	0	4.56
12	5	7	95	23	22.13
13	5	5.5	50	0	2.35
14	5	7	50	17.6	13.34
15	7	6	80	9.13	9.95
16	8	7	50	0	4.7
17	5	7	50	16.2	13.34
18	3	6	20	2.83	5.55
19	3	6	80	20.5	17.26
20	5	7	50	16.7	13.34

7.2.6 Biosurfactant Extraction and Assay

To extract the produced biosurfactant, the cell free culture were mixed with equal volume of chloroform/ methanol (1:2 v/v) and shaken on an orbital shaker (200 rpm) for 24 hours. The solvent then evaporated by rotary evaporator and kept at 4°C. For total lipid (Pande et al. 1963) method and for total carbohydrate (Dubois et al. 1956) method were used. The total carbohydrate in the sample solution was expressed in terms of D-Glucose (g/ 100 mL) and the total lipid in the sample solution was expressed in terms of Palmitic acid (g/ 100 g/mL). The surface tension of 10 mL diluted biosurfactant solution at various concentrations was determined in triplicate with a surface tensiometer at 25°C for CMC estimation. The CMC was determined by plotting the surface tension versus the concentration of biosurfactants in the solution. Total lipid and fatty acids test was conducted at ocean science centre (OSC) Memorial University of Newfoundland. Lipid samples were extracted according to Parrish (1999). Lipid class composition was determined using an Iatroscan Mark VI TLC-FID, silica coated Chromarods following three-step development method (Parrish 1987). The fatty acids composition of surfactant extracts was analysed by GC–FID.

7.2.7 Stability of Biosurfactant

Stability studies were done using cell-free broth obtained after 72h of cultivation. Broth samples were incubated in a water bath at different temperatures including 4, 20, 40, 60, 80 and 100°C and cooled at room temperature. The pH stability was performed by adjusting the broth to different pH (3, 6, 9, and 12) values by adding 1N NaOH or 1 N HCl. Different concentrations of NaCl comprise 0, 5 and 10 % (W/V) were added to

broth samples and mixed until complete dissolution to study the effect of salt addition on biosurfactant.

7.3 Results and Discussion

7.3.1 Effect of Carbon and Nitrogen Sources on Biosurfactant Production

The genus *Rhodococcus* bacteria with a diverse and efficient metabolism is able to transform, biodegrade or utilize as carbon source several hydrophobic compounds such as hydrocarbons, chlorinated phenols, steroids, lignin, coal and crude oil. This capability could be of great commercial and industrial importance (Bicca et al. 1999). Biosurfactants produced by some Rhodococcus species have been reported to be more effective and efficient in reduction of surface and interfacial tensions than many synthetic surfactants (Bell et al. 1998). Therefore, *Rhodococcus erythropolis* sp has been selected for this study. The type of carbon and nitrogen source affected biosurfactant yield which is depicted through ST, EI_{24} and production rate in Figure 7.1. Biosurfactant produced with FWC as carbon source and nitrogen sources showed excellent surface tension reduction activity and they reduce water surface tension to 29.33 and 28.95 mN/m, respectively. The lowest surface tension was recorded for sucrose of 24.61 mN/m. All carbon and nitrogen sources except glycerol reduced water surface tension to under 40 mN/m. highest emulsification activity was observed for n-hexadecane. Also, FWCN showed well emulsification activity while it was less than n-hexadecane and yeast. Yeast yielded the highest production rate, after yeast ammonium nitrate and FWCN produced higher biosurfactant. The lowest production rate belongs to glycerol. According to

surface tension, emulsification activity and biorurfactant production FWC was able to promote the production of biosurfactant as a nitrogen source and carbon source and it can be considered as a promising nutrient source for selected Rhodococcus strain.

7.3.2 Biosurfactant Production Optimization

The ANOVA of a quadratic regression model demonstrates that the model is highly significant as it is shown by the model F-value of 19.02. There is only a 0.01% chance that a model F-value this large could occur due to noise. Noise, which is responsible for most of the variability in the response, arises due to parameters that are hard and expensive to control in process settings (environmental conditions such as temperature and humidity, variations in raw material, accuracy limits of instruments, etc.), and it varies randomly within the process.

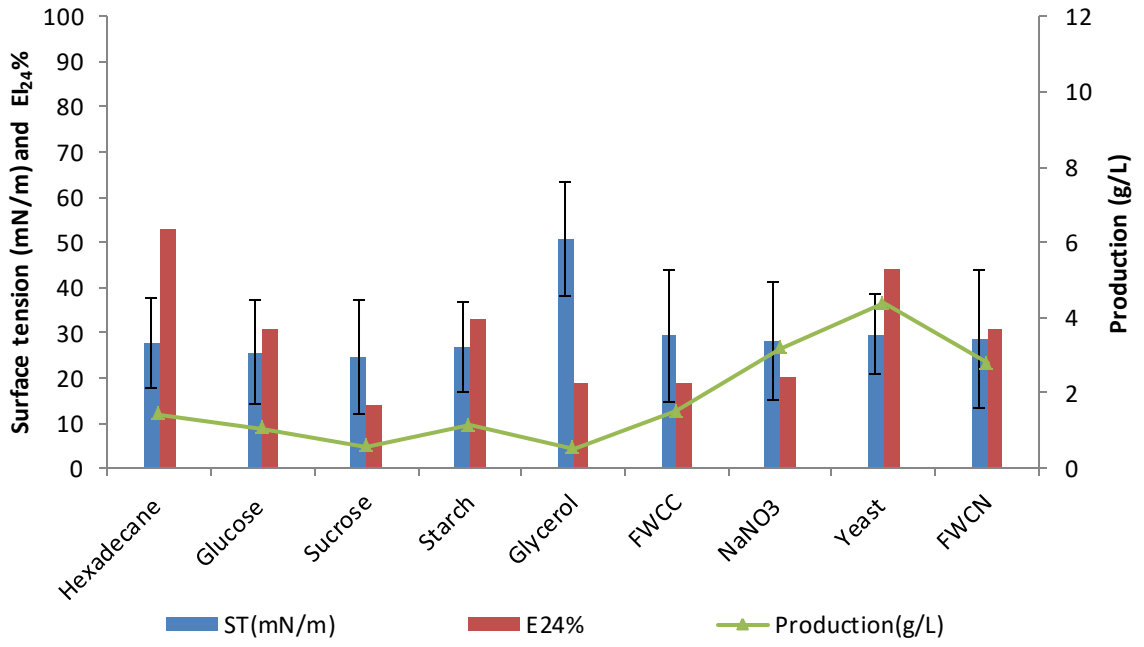


Figure 7.1 Effect of carbon and nitrogen sources on the biosurfactant production by P6-4P

Table 7.3 ANOVA table for biosurfactant production

Source	Sum of Squares	df	Mean Square	F Value	p-value
Model	1060.715	5	212.1429	19.02782	< 0.0001
A-Time	415.5268	1	415.5268	37.27001	< 0.0001
B-pH	56.90311	1	56.90311	5.103833	0.0404
C-FWC (%)	429.0106	1	429.0106	38.47942	< 0.0001
AB	35.57461	1	35.57461	3.190808	0.0957
B^2	123.6994	1	123.6994	11.09502	0.0049
Residual	156.0873	14	11.14909		
Lack of Fit	129.839	9	14.42655	2.748089	0.1390
Pure Error	26.24833	5	5.249667		
Cor Total	1216.802	19			
R-Squared 0.8717	Adj R-Squared 0.8259		Pred R-Squared 0.7074		Adeq Precision 15.045

Values of P less than 0.05 indicate model terms that are significant. The coefficient and the corresponding P values suggest that, among the input variables time, pH , FWC concentration, interaction of time and pH are significant model terms. The lack-of-fit F-value of 2.75 implies the lack of fit is not significant. Relatively lower value of coefficient of variation (CV = 29.%) indicates a better precision and reliability of the experiments carried out. The coefficients of regression equation were calculated using Design Expert and the following regression equation was obtained.

$$Y = +13.35 - 5.77 * A + 2.13 * B + 5.86 * C - 2.11 * A * B - 3.46 * B^2 \quad (7.1)$$

Where Y is the response that is CMD of the produced biosurfactant and A, B and C are coded values of the test variables, time, pH and FWC concentration (%), respectively.

The regression equation and determination coefficient R^2 was used to test the fit of the model. The model presented a high determination coefficient (R^2 = 0.8717) explaining 87% of the variability in the response. An adequate precision of 15.04 indicates an adequate signal for the signal–noise ratio. The value of the adjusted determination coefficient is also very high to indicate a high significance of the model (Khuri and Cornell 1996).

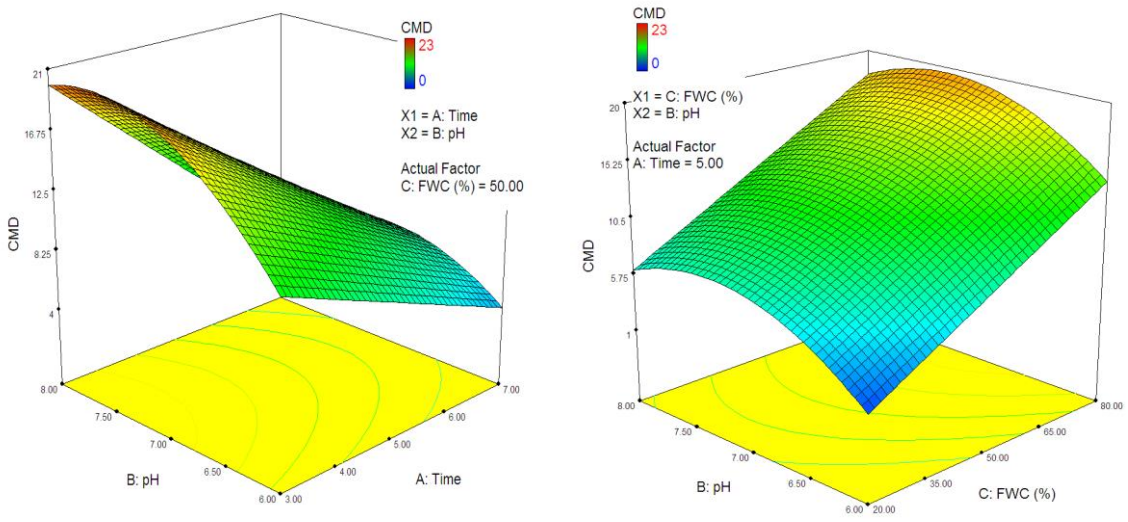


Figure 7.2 Response surface plot for CMD of biosurfactant production (1) as a function of time and pH, (2) pH and FWC concentration

Since linear effect of FWC concentration is significant which means that it can act as limiting nutrient source and variation in its concentration will change the product CMD. Increase of time has a negative effect on CMD. pH has a positive linear effect and negative quadratic effect on CMD of the produced biosurfactant, therefor its changes alter the CMD. To achieve the highest CMD the time of 3 days, pH of 7.2 and FWC concentration of 76.37 were suggested as optimal condition to generate biosurfactant for further tests.

7.3.3 Biosurfactant Production Assay

Biosurfactant production over the course of 72 h was investigated through ST, EI_{24}% and production rate measurement. As expressed by decrease in surface tension, the Rhodococcus (P6-4P) started to produce biosurfactant after 12 hours of cultivation. Dramatic decrease was observed in surface tension until 24 hours and then it reached its minimum values at 31 (mN/m). The emulsification index increased continuously until it reached its maximum value after 30 h. The maximum production rate of biosurfactant has been observed after 60 h of cultivation which was 3.2 g/l.

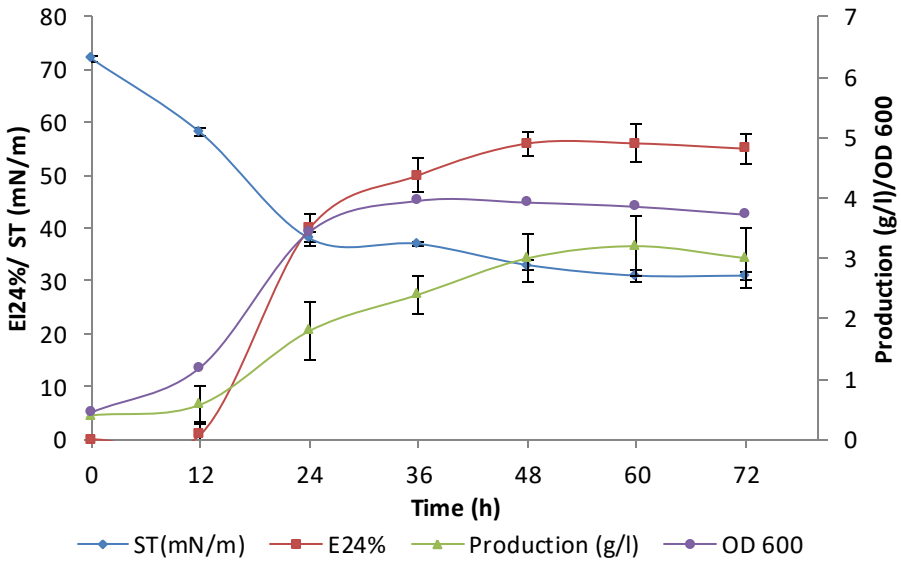


Figure 7.3 Times course of growth and biosurfactant production

A direct relationship between biosurfactant production and cell growth was observed during the biosurfactant production by *Rhodococcus* (P6-4P). The biosurfactant production with FWC started soon after inoculation and increased progressively, then remained constant during stationary phase and reached its maximum value at the end of the stationary phase. Cell growth stayed at stationary phase form 24 h to 60h of cultivation. Although it can be stated that FWC is a promising substrate for bacterial growth and biosurfactant production by the tested microorganism, it cannot be claimed that biosurfactant production by *Rhodococcus* (P6-4P) with FWC is a metabolic process and it is growth-associated, since biosurfactant production continued during the stationary phase.

7.3.4 CMC Determination

When biosurfactants were produced in the water, the surface tension changes with increasing concentration of biosurfactants until it reaches the critical micelle concentration (CMC), at this point surface tension remains constant and biosurfactant molecules start to form aggregates like micelles because of the chemical interactions between the polar head groups and the non-polar tail groups including hydrophobic, Van der Waals' force, and hydrogen bonding (Mulligan 2005; Schramm 2000; Soberón-Chávez and Maier 2011). To evaluate biosurfactant content in the cell free broth,the CMC was determined by measuring the surface tension of the supernatant at various dilutions (Mulligan et al. 2001). The CMC can be determined by plotting surface tension as a function of biosurfactant (or broth) concentration since the slope of the curve abruptly changes at the CMC. However, the abruptness is a function of both the particular

surfactant and the presence of impurities in the system (Sheppard and Mulligan 1987). The method of (Sheppard and Mulligan 1987) has been followed to determine CMC of the produced biosurfactant. In the Figure 7.4 the minimum effective concentration of biosurfactants corresponds to 0.0155 g/ml. CMC varies with the structure of surfactants, pH, ionic strength, temperature, and the polarity of the solvent (Desai and Banat 1997). Improving the downstream process for biosrufactant extraction and reducing the impurities can reduce the CMC.

Biochemical composition of the produced biosurfactant revealed that the total carbohydrate content in 1 g biosurfactant was 18.2 mg in term of D-glucose and the total lipid content was 48.6 mg in term of Palmitic acid in 1 g product. Certain species of *Rhodococcus*, such as *Rhodococcus erythropolis* are important biosurfactant producers. These species produce biosurfactants through the utilization of water-insoluble hydrocarbons. Most of the biosurfactants produced in this way are lipids containing trehalose. Although the diversity of *Rhodococcus* glycolipids has been reported while free fatty acids are rarely reported as major biosurfactant products of *Rhodococcus erythropolis* (Peng et al. 2007), the major component of produced biosurfactant was fatty acid and accounted for 64% of total lipid composition.

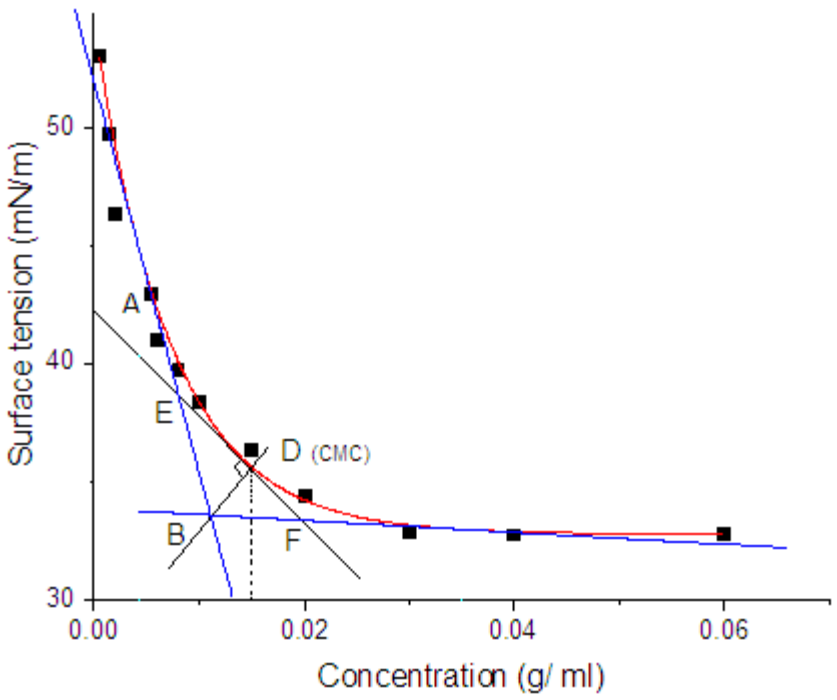


Figure 7.4 CMC determination through measuring surface tension

Table 7.4. Lipid composition of the biosurfactant (percent of the total amount of lipid)

% Lipid Composition in biosurfactant	
Hydrocarbons	2.71
Steryl Esters/Wax Esters	0.00
Ethyl Esters	0.79
Methyl Esters	0.00
Ethyl Ketones	1.99
Methyl Ketones	0.00
Glycerol Ethers	0.00
Triacylglycerols	0.00
Free Fatty Acids	63.84
Alcohols	0.93
Sterols	6.64
Diacylglycerols	0.00
Acetone Mobile Polar Lipids	9.80
Phospholipids	13.31

Table 7.5 Fatty acid composition of the biosurfactant (percent of the total amount of fatty acids)

% ID'ed Fatty acids in biosurfactant	
14:0	1.30
Trimethyltridecanoic acid (TMTD)	1.04
16:0	8.26
16:1w7	1.77
i17:0	1.29
18:0	7.88
18:1w9	5.36
18:1w7	7.30
18:2w4	3.22
20:1w11?	1.67
20:1w9	23.06
20:1w7?	2.71
22:1w11(13)	17.50
22:1w9	4.86
21:5w3?	1.99
24:1	1.72
Sums	100.00

7.3.5 Biosurfactant Stability

Surface tension of the produced biosurfactant was measured under wide range of temperature, pH and salinity to study the stability of the biosurfactant. The biosurfactant showed stability at all temperature ranges and salinity ranges and its surface tension changed slightly. At the low pH, biosurfactant precipitated and surface tension was high. Biosurfactant stayed stable for 4 days when salt concentration was 5% (w/v) at 80 °C in the pH range of 6.5–10.5. Stability studies demonstrated that the biosurfactant is stable under extreme temperature and salinity and wide range of pH.

7.4 Summary

This study aims to contribute to the use of FWC as substrates for the production of biosurfactant. The use of an experimental design to reveal the influence of media condition on biosurfactant production, allowed the screening of experimental significant factors on media conditions for optimization of biosurfactant production. The effort of using waste as a cheap substrate and optimization of the production condition to enhance the biosurfactant production rate and decrease the cost can help to make the microbial surfactant competitive with synthetic surfactants. The significant achievement of the present work lies in the fact that the FWC as cheap and novel source of nutrient can be used to produce biosurfactant and experimental design can be applied to enhance biosurfactant production.

CHAPTER 8

CONCLUSIONS AND RECOMMENDATIONS

8.1 Summary

In Canada, nationally, the amount of non-hazardous waste sent to private and public waste disposal facilities was approximately 25 million tonnes in 2010. Residential waste accounted for 37% of the total waste disposed, with about 9.3 million tonnes in 2010 and organic waste makes up to 40% of the residential waste. Aside environmental concerns related to landfill such as leachate generation and gas emission, landfill post closure and maintenance funds of $93 million in Canada in 2010. In addition, legislative mandates, recycling goals, protection of the water and soil resources, strict and costly landfilling and incineration regulations and other objectives set forth by national, state, and local governments provided the impetus for waste managers to look for alternatives to divert the amounts of waste disposed on the land.

The amount of waste diverted to recycling or organic processing was 8.1 million tonnes and organic materials accounts at 27% (2.2 million tonnes) or 236 kg per person in Canada in 2010. Among all Canadian provinces, Nova Scotia diverted the most MSW from landfills. This province banned organic waste from landfill sites and organized separate curbside collection and recycled 310 kg per capita, which contributed to 45% of its total wastes. NL has the highest quantity of waste disposal per capita after Alberta. This amounts to about 429 kg of residential waste per capita (Pande et al. 1963). Based on the waste management survey by Government of Canada, there has been no allocation of funds to operate an organics processing facilities in NL whereas one of the primary stages of the Provincial Solid Waste Management Strategy (2010) was increasing waste diversion by diverting 50% of materials going to disposal by 2015.

Another source of organic waste in NL is fishery waste since the fishery industry is important economically in the province. Fishery industry generated 115,263 tonnes of waste in 2013. Where there is no opportunity to reuse fish waste, it is disposed in the ocean under permit from Environment Canada at approved locations. The immersion and presence of fish offal could potentially have an impact on fish and marine habitat at the dumping site. The slow rate of decomposition of fish waste results in the subsequent increase in biological oxygen demand (BOD), release of dissolved phosphorus and dissolved nitrogen, and the formation of black zone.

Composting is considered to be a useful method to produce a stabilized material from organic MSW and fish waste and it is a viable solution to the problems of waste disposal. Furthermore, it is a promising mean for NL to achieve the goal of solid waste management plan, with diverting 50% of the organic wastes from landfill.

Selection of a bulking agent which should be inexpensive and readily available in the vicinity of the composting region is very important because bulking agents can affect the condition of the starting composting mixtures, biodegradation kinetics and composting performance as well as the final compost quality. Therefore, in this research, the performance of locally available bulking agents on the bench-scale MSW composting in NL was examined. Meanwhile, a comprehensive investigation of parameters indicating compost maturity and stability and monitoring composting process was conducted. The OUR, enzyme activities and GI were selected to reflect compost stability and maturity. Effective MSW composting was achieved based on the analysis of the results of the physiochemical and biological parameters. A higher temperature for a longer duration

was observed during composting using peat as the bulking agent, leading to more effective pathogen removal and sterilization. A high enzyme activity of dehydrogenase, β-glucosidase, and phosphodiesterase in the third week of composting with peat implies high microbial activity and high decomposition rate. In addition, the low final C/N ratio for compost with peat implies acceptable stability states. Maximum temperature and high OUR for composting using sawdust as the bulking agent were observed in the third week of composting and the peak of dehydrogenase, β-glucosidase, and phosphodiesterase activities occurred in the second week. Composting with sawdust generated a higher germination index, indicating higher maturity.

Physicochemical parameters such as temperature, moisture content, C/N ratio and EC are widely applied to monitor the composting process. Enzyme activities can indicate the ability of microbes to degrade a wide range of common organic substrates due to the role played by enzymes in the biological and biochemical processes during composting. Characterising and quantifying specific enzyme activities during composting could provide information of dynamics of the composting process. Moreover, the determination of enzyme activity, in contrast to other analytical techniques used for compost stability evaluation is fast, and relatively inexpensive. Despite their advantages as stability and maturity indices, enzyme activities have never been used in DOE based composting process optimization. For the first time, cumulative enzyme activities were served as responses for 2-level four factor factorial design to evaluate the effect of AR, MC BA, and C/N on the composting process. Results indicated that C/N was one of the main factors that affect compost maturity and stability. The type of BA has a statistically

significant effect on final C/N, final GI and cumulative enzyme activities. The developed regression models can be used to optimize initial operational conditions of composting and can help to increase the efficiency of the composting process. To economically treat the increasing quantities of MSW and achieve maximum cumulative enzyme activities, maximum GI and low C/N ratio, composting with an AR of 0.3 L/min. kg, C/N of 17, MC of 70% with peat as BA is recommended.

Enzyme activities were applied to evaluate the state and evolution of marine fish waste composting process. A composting system was designed for achieving the effective reduction of fish wastes, and generating mature and high quality compost products. It was meaningful to conduct a detailed monitoring, characterization, and quantification of the enzymatic activities during fish waste composting. Due to the high complexity of fish wastes, different enzymes worked collaboratively to fully decompose the organic materials into stable compounds. The maximum enzyme activities were observed in the first 3 weeks, at the active phase of decomposition. The changes of enzyme concentrations served as useful indices to evaluate the effectiveness and progress of the fish waste composting. Decline of enzyme activities in compost samples observed concurrently with decrease in C/N ratio and increase in GI. Thus, enzyme activities could represent a useful index of state of composting since the conventional maturity and stability parameters, OUR, C/N ratio, GI support the use of enzyme activity as indicator of compost stability in this work. A number of key physicochemical properties were also monitored and their correlations with the enzyme activities were investigated. The

correlation results suggested characterizing compost maturity and stability by each isolating parameter might be not reliable.

Nutrients of generated FWC were further used to produce a valuable product such as the biosurfactant. Biosurfactants are found to be less toxic, more effective and stable at extreme pH, temperature and salinity, and enhancing biodegradation. Using inexpensive raw materials and optimization of cultural condition can help to cope with the economic constraints associated with bulk production of biosurfactats. Thus, in the present research, FWC nutrient was extracted using enzyme hydrolysis. The effect of time, E/S ratio and temperature on DH of FWC was evaluated using a CCD response surface design. Time, interaction of time and E/S ratio and temperature in the selected ranges were significant factors. Higher DH was observed at high level of time and high level of E/S ratio. The optimum condition obtained was: time of 5 h, E/S ratio of 2.5 and temperature of 59.97 for maximum DH. Two newly isolated strains (e.g., *Bacillus* (N3-1P) and *Rhodococcus erythropolis sp*. (P6-4P)) were selected to evaluate the feasibility of using FWC extract as substrate for biosurfactnt production. The performance of FWC extract as a nutrient source was compared with the other organic and inorganic carbon and nitrogen sources by determining surface tension, EI_{24}, CMD and biosurfactants productivity rate. FWC extract demonstrated a promising performance as substrate for biosurfactant producing bacteria. RSM was implied to optimize the production condition. A quadratic model was selected to predict the CMD of the produced biosrufactant. The maximum CMD predicted by model was 32 and obtained experimentally was 30.5 by *Bacillus* (N3-1P). The CMC of the produced biosrufactant was 0.013 g/ml and 0.0155 g/ml for N3-1P and

P6-4P, respectively. The biosurfactant obtained from fish waste compost extract showed high surface tension reduction and high emulsification activity, exhibited a high level of stability. The effort of using waste as a cheap substrate and optimization of the production condition to enhance the biosurfactant production rate and decrease the cost can help to make the microbial surfactant competitive with synthetic surfactants. The significant achievement of the present work lies in the fact that the FWC as a cheap and novel source of nutrient can be used to produce biosurfactant and experimental design can be applied to optimize the process thus enhance biosurfactant production.

8.2 Research Contributions

This research can be summarized and highlighted by the following contributions:

1) The impact of two locally available bulking agents on the performance and quality of the final product of the compost of MSW in NL was investigated for the first time. Both sawdust and peat are effective bulking agents for Bench-scale composting and they generated mature and stable compost. The choice of a bulking agent for a particular community then depends on the availability of the agent and land in the adjacent region.
2) Enzyme activities (dehydrogenase activity, β – glucosidase, and Phosphodiesterase) have never been used as responses for DOE based examination of MSW composting process. Effect of the composting process variables including moisture content, aeration, bulking agent, and C/N ratio was investigated during MSW composting based on the DOE technique. In this

research, the variation of enzyme activities has been assessed to monitor the organic matter decomposition and progress of the composting process. The cumulative enzyme activities also have been used as responses beside GI, OUR, Final C/N ratio and moisture content to develop a model. The research results can be applied to optimize initial operational conditions of composting and can help to increase the efficiency of the composting process to economically treat the increasing quantities of MSW.

3) Composting as a solution was used for growing fish waste problem. Enzyme activities have been innovatively proposed to evaluate the state and evolution of the fish waste composting process. The results of the experiments revealed that enzyme activities could represent a useful index of state of composting since the conventional maturity and stability parameters, OUR, C/N ratio, GI support the use of enzyme activity as indicator of compost stability in this work.
4) Fish waste compost extract was generated through enzyme hydrolysis and the hydrolysis process was optimized based on RSM as an innovative process to extract compost nutrient. The extract was used as novel substrate for newly screened biosurfactant producer strains from northern Atlantic Canada including *Bacillus* (N3-1P) and *Rhodococcus erythropolis sp*. (P6-4P). The generated biosurfactants from fish waste compost extract reduced surface tension and had the ability to stabilize emulsions. The developed methodology can extract nutrient from compost effectively and generate biosurftacnts in economical way by using waste stream as substrate.

8.3 Recommendations for Future Research

1) There are small communities in NL which are located in remote and isolated areas and cannot access large solid waste disposal sites or central organic processing facilities. In addition, mostly fish processing plants are located at the remote costal area. Therefore, in such area both MSW and fish waste should be treated and there are limited studies relevant to the composting of the mix waste. The feasibility of composting of mixture of fish waste and MSW can be investigated to conduct composting in the remote communities.
2) Using enzyme activity to evaluate the state of the composting could be improved by investigating the relationship between other compost maturity and stability indices such as CO_2 evolution rate, WSC, NH_4^+-N and NO_3^--N and enzyme activities to establish a set of thresholds for enzyme activities as maturity and stability indices in relation to other parameters.
3) Further structural characterization of generated biosurfactant can be carried using GS/MS. fast atom bombardment mass spectral (FABMS) and HPLC. Also, the application of the generated biosurfactant in bioremediation and enhance oil recovery can be further investigated.

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