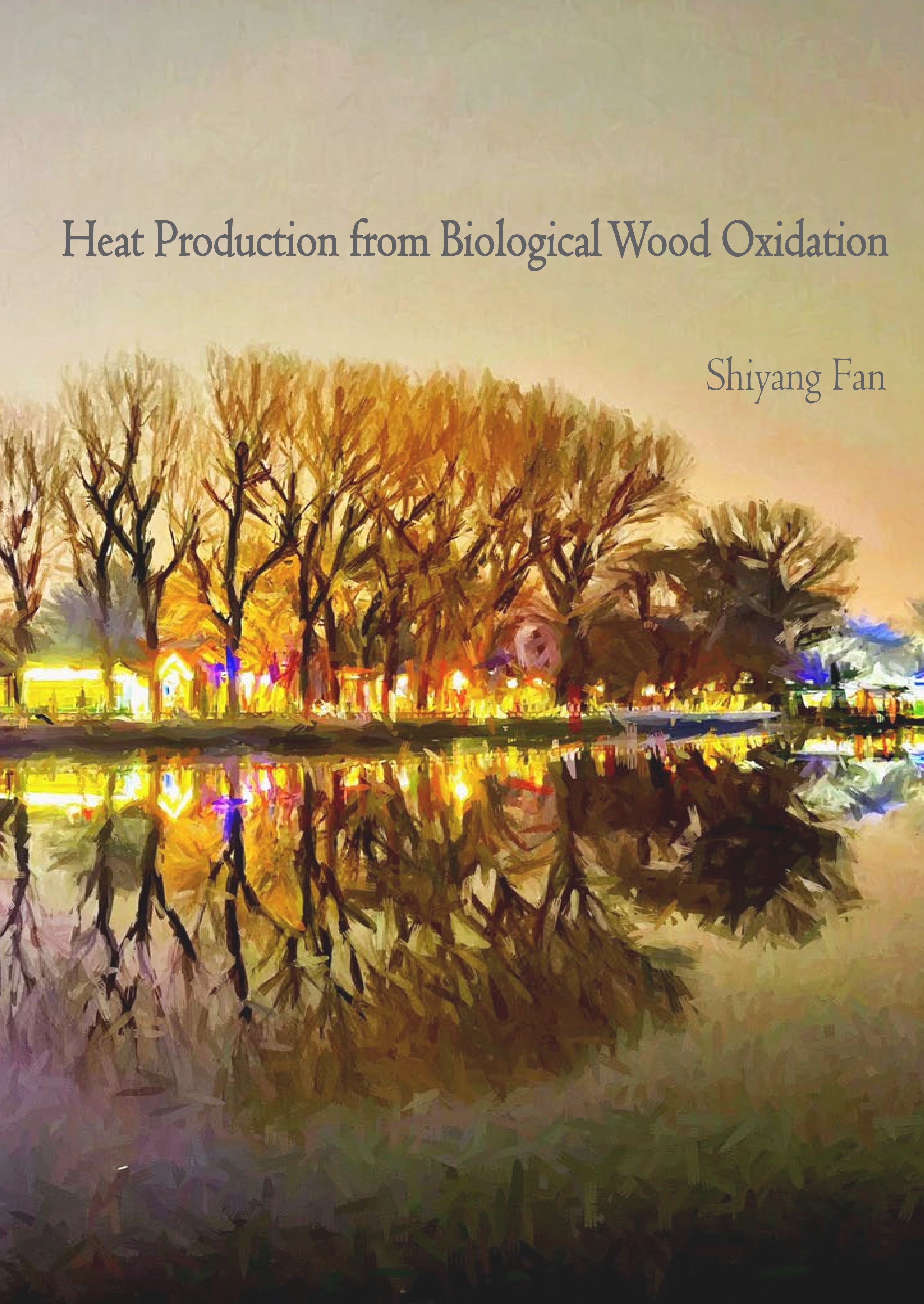
Heat Production from Biological Wood Oxidation

Shiyang Fan



Propositions

1. Human urine is priceless in biological wood waste management.
   (this thesis)

2. The conditions for commercial interesting heat production rates are fastidious.
   (this thesis)

3. AI technology can never take the place of human beings in doing environmental research.

4. In the nearby future, the price to buy waste will increase due to the efficient management of waste.

5. A research question that cannot be solved at the moment is still a good research question.

6. Money solves technical issues more effectively than scientists.

Propositions belonging to the thesis, entitled

Heat production from biological wood oxidation

Shiyang Fan
Wageningen, 29 June 2021

Heat Production from Biological Wood Oxidation

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Thesis committee

Promotor
Prof. Dr. Cees. J.N. Buisman
Professor of Environmental Technology
Wageningen University & Research

Co-promotors
Dr. Annemiek ter Heijne
Associate professor, Department of Environmental Technology
Wageningen University & Research

Dr. Wei-Shan Chen
Assistant professor, Department of Environmental Technology
Wageningen University & Research

Other members
Prof. Dr. JH Bitter, Wageningen University & Research
Dr. AHM Veeken, Attero B.V., Haelen
Prof. Dr. P Zhang, Beijing Forestry University
Dr. A Schievano, University of Milan, European Commission

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Heat Production from Biological Wood Oxidation

Shiyang Fan

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To my beloved family and Fruan

谨以此书献给我最爱的家人和软软

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Chapter 1

General Introduction

1.1 Renewable Energy and Lignocellulosic Biomass

With the boosting of industrialization and growth of population, the global energy consumption has rapidly increased from 13.6 billion tons of oil equivalent (toe) in 2010 to 14. 4 billion toe in 2019 (Enerdata, 2020). It is expected that about 44.6 billion toe energy will be consumed by 2050 (Bilgen, 2014). Fossil fuels, such as natural gas, oil, and coal, are the main energy supplier, accounting for more than 80% of total energy consumption (IEA, 2020a). However, combustion of fossil fuels can lead to environmental issues like global warming and air pollution. For example, it is reported that about 33.2 billion tons energy-related carbon dioxide (CO_2) was emitted in 2019 (IEA, 2020a). These energy-related emissions are the largest source of greenhouse gas emission.

The rising consumption of fossil fuels, and the increasing environmental concerns have led to the necessity of alternative energy sources that are cleaner and more renewable. Renewable energy technologies provide clean, abundant energy derived from self-renewing resources such as the solar, wind, hydro, geothermal, and biomass (Lozano et al., 2018). In 2017, renewable energy contributed to about 17.3 % of total energy consumption, which has a great potential for larger proportion (IEA, 2020c).

Among all these renewable energy sources, solar energy is the most abundant renewable resource on earth. Solar energy can be converted into electricity by using a photovoltaic (PV) panel. However, solar energy is unstable and highly dependent on the weather conditions and geographic location (Angèle Reinders et al., 2016; Vaderobli et al., 2020). Wind energy can be converted to electricity by using a wind turbine which converts the kinetic energy (motion) of wind into mechanical energy that is used to generate electricity. However, the construction and maintaining cost of wind energy is high because the turbines are usually located far from population cities. Wind turbines also generate sound pollution and may interfere with animals like birds (Smallwood, 2007; Zimmerling et al., 2013). Moreover, wind energy is also highly dependent on the climate and geographic conditions. Hydro energy, also consisting of tide and wave energy, can be converted to electricity by using a water turbine. Water turbine converts the mechanical energy of flowing water to electricity by forcing it through a penstock (Mohtasham, 2015). It is a proven technology with high efficiency and low operating and maintenance costs. However, the hydro power plants have very high initial costs of facilities, and dependence on rain precipitation. Biomass is the largest renewable energy supplier in the world because it is ubiquitous and cheap (EIA, 2010). It is estimated that all growing biomass on land can supply an energy equivalent of 70 billion toe (Strehler, 2000). Biomass is unique because it is the only renewable energy source of carbon (Demirbaş, 2001). Biomass consists of plants, bacteria, fungi, archaea, protists, and animals, which can be converted to different types of energies, such as heat, electricity, and biofuels (Bar-On et al., 2018).

Plants are the largest part of biomass, supplying 80% of total biomass amount (Bar-On et al., 2018). Lignocellulosic biomass is plant biomass that consists of cellulose, hemicelluloses, and lignin (Brautaset & Ellingsen, 2011; Li & Jiang, 2017). Lignocellulosic biomass has high availability and relative low cost. Besides, it does not compete with food production or animal feed (Sindhu et al., 2016). These advantages make lignocellulosic biomass a promising source of renewable energy. Lignocellulosic biomass can be converted into different forms of energy via thermochemical and biological processes (Fig. 1-1).

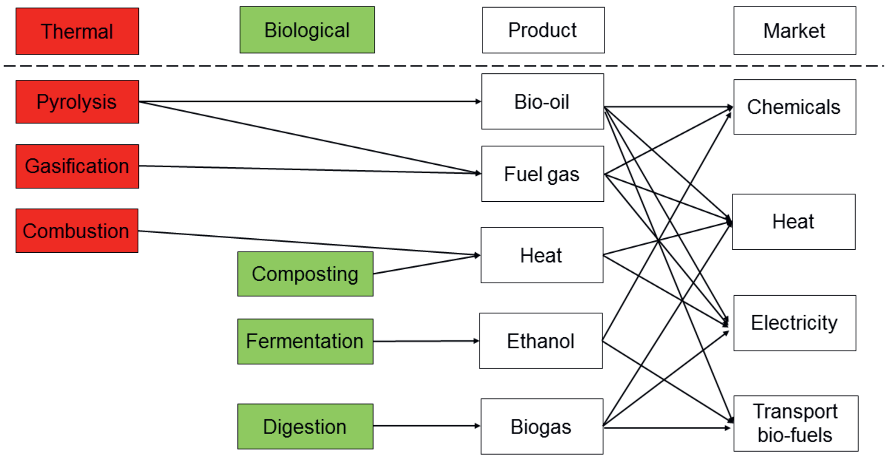

Fig. 1-1 Conversion processes, products and applications for biomass (Bilgen et al., 2015).

Thermochemical processes (pyrolysis, gasification, and combustion) give multiple and often complex products, with catalysts often used to improve the product quality and efficiency (Puig-Arnavat et al., 2019). Pyrolysis is a thermal decomposition process of lignocellulosic biomass in the absence of air/oxygen (Dhyani & Bhaskar, 2018). The product of pyrolysis is bio-oil that can be a source of fuels or chemicals (Pramod & Seshan, 2016). Gasification process converts lignocellulosic biomass to gaseous mixture (syngas or synthesis gas) in the absence of oxygen/air or with insufficient oxygen supply (Akhtar et al., 2018). The syngas, consisting of CO, H_2, CO_2, CH_4, H_2O, H_2S, NH_3, and tar, can be used for energy production (heat and electricity) (Alauddin et al., 2010; Molino et al., 2018). Combustion of lignocellulosic biomass is the easiest way to produce heat. The generated heat can be used for heating and electricity generation (Demirbas, 2000).

Biological conversions (fermentation, digestion, and composting) utilize multiple enzymes for converting biomass to different products such as ethanol, biogas and heat (Bilgen et al., 2015). The high levels of hemicellulose and cellulose make lignocellulosic biomass a potential energy source produced by biological conversion

(Sindhu et al., 2016). During fermentation, the microbes decompose lignocellulosic biomass into ethanol (Althuri et al., 2018). The generated ethanol has many domestic and industrial uses (Wyman et al., 2017). Digestion is a naturally occurring, biological treatment of lignocellulosic biomass, which is carried out by mixed culture microbial communities in the absence of oxygen (Sawatdeenarunat et al., 2015). The main production of digestion is methane, which can be used for generating electricity and heating. However, lignocellulosic biomass has a low rate of fermentation and digestion due to its inert structure (Chang & Holtzapple, 2000). Thus, lignocellulosic biomass usually needs pretreatment, such as thermal treatment and / or acid treatment, to enhance its digestibility for increasing the efficient conversion to methane or ethanol (Hendriks & Zeeman, 2009). Composting is a relatively faster biological process with the presence of oxygen. During composting process, microorganisms decompose lignocellulosic biomass into CO_2, H_2O, humus-like end-product and release considerable amounts of heat (Białobrzewski et al., 2015; MacGregor et al., 1981). The humus-like end-product can be used as soil improver (Boldrin et al., 2009). Compared with other techniques, composting has lower technical complexity and capital investment. Besides, composting causes little environment burdens (Rai et al., 2021).

1.2 Wood and Biological Wood Degradation

Lignocellulosic biomass mainly includes wood, corn stover, straw, wheat stover, algae, etc. (McMillan, 1994; Zhou et al., 2014). Wood mainly consists of cellulose (40%), hemicellulose (20-30%), and lignin (20-30%) (Sjöström, 1993). Cellulose is a fibrous, tough, water-insoluble substance which is a linear polymer consisted of glucose units linked by β-1,4 bonds (Béguin & Aubert, 1994). Hemicellulose is a group of cell wall polysaccharides. The most relevant hemicelluloses are xylans and mannans (Saha, 2003; Wyman et al., 2005). Lignin is a polymer consisting of three kinds of phenyl propionic alcohols as monomers: conifer alcohol, p-coumaryl alcohol and sinapyl alcohol (Fan et al., 2016; Vanholme et al., 2008). The cellulose and lignin are closely interpenetrated by hemicellulose (Alam et al., 2014).

Wood is the largest part of lignocellulosic biomass (Bilgen et al., 2015; Eriksson & Bermek, 2009; Ruppert et al., 2013). It is reported that the annual wood production, which refers to the harvesting of roundwood both from softwood and hardwood, is about 4 billion m^3 (IEA, 2002). The harvested wood is mainly used for wood fuel and industrial roundwood. The industrial roundwood accounts for about half of total global roundwood production (Brack, 2018; FAO, 2018). Industrial roundwood is all roundwood used for any purpose other than energy, it includes pulpwood, sawlogs, veneer logs, and other industrial roundwood (e.g. roundwood used for fence posts or electricity poles). The wood fuel, including charcoal, accounts for the other half of total roundwood production (55%) (Brack, 2018). It was reported that the global use of wood for energy was about 30 EJ in 2010 (Bilgen et al., 2015).

Heat is important because heating is the largest energy end-use. It is reported that providing heating for homes, industry and other applications accounts for about 50% of total energy consumption, followed by transport and electricity (IEA, 2020b). Wood is suitable for generating heat due to its high energy content (Ince, 1977). Considering the fossil fuels are the main energy suppliers in the world, wood is an important alternative to fossil fuels for heat production, because wood is abundant and renewable. Most wood fuel is used in its country of production, particularly in rural areas and in developing countries, for heating and cooking, usually on open fires or in simple cookstoves (Brack, 2018). However, open burn of wood is not optimal because it cause problems such as ash deposition, corrosion, harmful emissions such as NOx and SOx, and too much particulate matter (van Lith et al., 2008). Moreover, the moisture content in wood negatively affects its heating value because water vapor can take up a large part of generated heat (Demirbas, 2007; Tillman, 2012). Wood drying can reduce the moisture content, however, the drying process can consume a large amount of extra energy, which is not an optimal strategy (Minea, 2012).

Besides combustion, wood can also generate heat via composting (Fig. 1-1). Compared to wood combustion, wood composting is a clean technology without any harmful emissions. The final product of wood composting is CO_2, H_2O, and humus, which can be used as soil improver. Moreover, moisture is not an inhibitor during composting because water is necessary to support the metabolic processes of the microbes. Water provides the medium for chemical reactions, transport nutrients, and allows the microorganisms to move about (Agnew & Leonard, 2003). In addition, wood composting is an essential part of the global carbon cycle because the decomposition of wood generates CO_2 to the atmosphere and provides fixed carbon to the soil (Fig. 1-2) (Singer & Woyke, 2013).

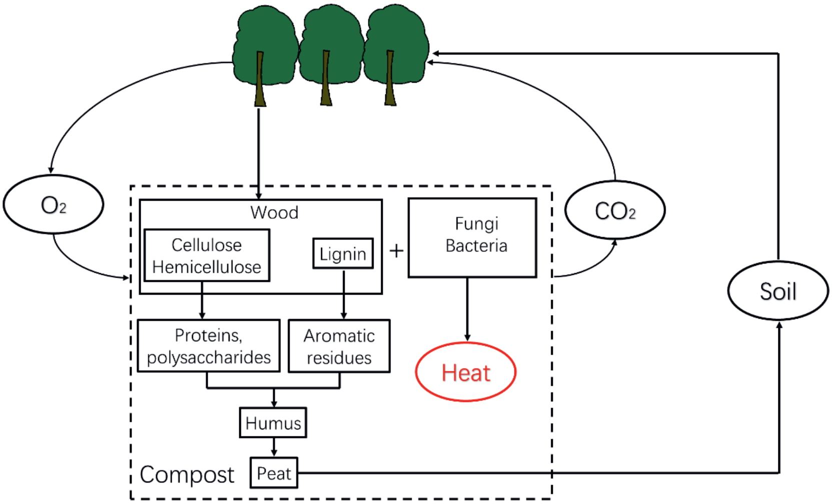

Fig. 1-2 Carbon circle of wood composting (Tuomela et al., 2000).

During the composting process, however, wood usually degrades slowly in low-temperature phases and relatively faster in high-temperature phases (Huang et al., 2010; Shin & Jeong, 1996; Wei et al., 2019; Zhao et al., 2016). Given this context, continuous thermophilic composting is proposed to enhance the degradation process of organic matter (Li et al., 2019a). Continuous thermophilic composting maintains the composting at relatively high temperatures, ranging between 40 and 70 °C (Chang et al., 2019; Hosseini & Aziz, 2013; Schulze, 1962; Xiao et al., 2011; Xiao et al., 2009).

Although CTC has been applied to substrates like municipal solid waste (Xiao et al., 2009), dairy manure (Li et al., 2019a; Qian et al., 2016), food waste (Jiang et al., 2015; Waqas et al., 2018), and mixture of these streams (Li et al., 2019b; Suler & Finstein, 1977; Xiao et al., 2011), it has not been applied to wood waste yet. Moreover, the heat produced in the degradation process of CTC is often overlooked, which is a potential source for renewable heat production.

Biological wood oxidation (BWO) is a continuous thermophilic composting method applied to wood, and it is an alternative to wood combustion for heat production (Caizán Juanarena et al., 2016; Hamelers & Buisman, 2009). BWO maintains wood degradation at a temperature range of 40-55 °C for fast decomposition. During BWO process, microorganisms (fungi and bacteria) decompose woody biomass to CO_2, H_2O, peat and release a large amount of heat. The complete oxidation of wood via BWO can be simplified and described as equation (1) (Caizán Juanarena et al., 2016):

$$CH_{1.5}O_{0.7} + 1.03\ O_2 \rightarrow CO_2 + 0.734\ H_2O + \text{heat} \qquad (1)$$

1.3 Factors Affecting Biological Wood Oxidation

Biological wood degradation is the result of simultaneous action of a variety of biological agents, including fungi and bacteria. Wood decaying fungi can be separated into three groups, depending on the type of decay produced: white-, brown-, and soft-rot fungi (Blanchette et al., 1989). White-rot fungi may degrade all cell wall components (cellulose, hemicellulose and lignin). Brown-rot fungi do not degrade lignin but degrade primarily the polysaccharide components of wood. Soft-rot fungi typically attack higher moisture, and lower lignin content wood. Soft-rot fungi can cause erosion of the cell wall of wood (Goodell et al., 2008). Although fungi play a dominant role in wood decay, certain groups of bacteria can also degrade lignin-containing cell walls. These wood decaying bacteria are named tunneling bacteria and erosion bacteria, which cause strikingly different decay patterns than fungi (Singh et al., 2016). Tunneling bacteria produce tunnels as they degrade the cell walls (Singh et al., 2016). Erosion bacteria can erode cell walls of wood during degradation process in terrestrial and aquatic environments (Kim & Singh, 2000). Together with wood decaying fungi, bacteria can create interactions that are important for wood degradation (Cornelissen et al., 2012; de Boer & van der Wal, 2008).

BWO involves microbes, and therefore factors like temperature, nutrients, oxygen, moisture content, and aeration affect the rate of BWO process. These factors have been extensively studied in room temperature or traditional composting (Brischke & Rapp, 2008a; Brischke & Rapp, 2008b; Humphrey & Siggers, 1933; Toljander et al., 2006). However, there are very limited studies emphasizing the effect of these factors on BWO process. Herein we discuss the current knowledges on these factors.

1.3.1 Temperature

Temperature is an important environmental variable affecting the microbial activity and population (Joshua et al., 1998; Namkoong & Hwang, 1997). Temperatures lower than 20 °C can dramatically slow the composting process while temperatures in excess of 60 °C also remarkably reduce or even stop the microbial activity (Liang et al., 2003). It is extensively reported that the optimal temperature for composting is between 30-65 °C (Beffa et al., 1998; Hassen et al., 2001; Miller et al., 1989; Palmisano et al., 1993). However, the optimal temperatures between BWO and traditional composting are different. In BWO process, the main wood decaying microorganisms are fungi, which tolerate a relatively narrower temperature range (40-50 °C, maximum 55 °C) compared to the dominant bacteria in traditional composting (Tuomela et al., 2000). The proper temperatures of BWO should theoretically fit the optimal temperature of wood decaying fungi that are playing an important role in BWO (de Bertoldi et al., 1983). Furthermore, temperature affects the quantity and quality of the generated heat. Heat with higher temperature can be more easily recovered by using a heat exchanger (Fakheri, 2003). Thus, the effect of temperature on heat production from BWO demands further research.

There is little information about the effect of temperature on BWO. Firstly, the optimal temperature of BWO is still unknown. Second, the effect of temperature on the degradation rate of BWO remains unclear. Third, the knowledge about microbial population at different temperatures is missing.

1.3.2 Nutrients

Wood is inherently rich in carbon (C) and low in macro- and micro- nutrients (Ulyshen, 2016). Among all these nutrients, nitrogen (N) is particularly critical for microbial growth, because it is essential constituent of protein, nuclei, enzyme and amino acids (Tuomela et al., 2000). More importantly, N is a key factor that regulates the lignocellulolytic enzymes production, which therefore determines the microbial wood-degrading capacity (Tuomela et al., 2000). Wood contains very low N content (the C:N ratio of wood is usually between 300-1000:1) (Cowling & Merrill, 1966; Merrill & Cowling, 1966), which makes the addition of N important. Under low C:N ratio (high N content and low C content) condition, the available C is fully utilized without stabilizing all of the N, which can generate excess ammonia and slow down the degradation process (Jiang et al., 2011). Too high C:N ratio (low N content and high C content) will decrease the growth of microorganisms and inhibit the wood degradation process. The optimal C:N ratio of composting is 30-35:1 (Pace et al., 1995). For BWO, it is reported that wood composting is effective at a relatively higher C:N ratio of 40-70:1 (Rao et al., 1995) because wood decaying fungi can effectively assimilate, utilize, and conserve N (Cowling & Merrill, 1966). High level of N might stimulate the growth of other competing bacteria, and inhibit the growth of wood decaying fungi, which both are obstacles to BWO. Besides N, other nutrients such as phosphorus (Nicholas & Militz, 2008), vitamin (Levin et al., 2010), manganese (Hofrichter, 2002; Tekere et al., 2001) are also reported important to wood degradation.

Effect of nutrients on wood degradation has been extensively studied at room temperatures. However, there are few articles relating to BWO at elevated temperatures, which needs more attention. For example, the optimal concentration of macro nutrients (nitrogen and phosphorus) should be figured out. Moreover, the effect of different concentration of macro-nutrients on BWO at 40-55 °C is still not clear. Besides, the mechanisms of micro-nutrients on BWO demands more attention.

1.3.3 Aeration

Since BWO is an aerobic process, oxygen is of primary importance. The oxygen availability affects the growth of microorganisms. BWO consumes large amount of oxygen and generates substantial amount of heat in the early stages (Caizán Juanarena et al., 2016). If the oxygen supply is limited, the BWO may turn anaerobic, which will slow down the BWO process obviously (Pace et al., 1995). Besides, the aeration rate also affects BWO. If the aeration rate is too low, the generated heat will accumulated and the temperature of BWO will be too high (Jiang et al., 2011). While too high aeration rate will cause excessive heat loss, decreasing the temperature and reducing microbial activity (de Bertoldi et al., 1983). The porosity of BWO is considered higher than the porosity of traditional composting because wood is usually used as bulk agent during composting process which absorbs water and a part of leachate to give structure and porosity for proper aeration (Adhikari et al., 2009; Barrington et al., 2002). Also, the aeration may sensibly influence the humidity in the BWO process.

Effect of aeration on traditional composting has been studied (Bari et al., 2000; Gao et al., 2010a; Gao et al., 2010b; Lau et al., 1992). However, there is few articles about BWO, and more information about the aeration should be known before large scale application of BWO. For instance, the optimal aeration rate of BWO is still unclear.

1.3.4 Moisture content

Water is necessary to support the metabolic process of microorganisms by providing the medium for chemical reactions, transporting nutrients, and allowing the microorganisms to move (Agnew & Leonard, 2003). Optimal moisture content varies and depends on the physical stage of the composting and the particles' size (Agnew & Leonard, 2003). Low moisture content can restrict the activity of microorganisms and results in low degradation rate. While high moisture content can displace air from pore spaces, and fill the small pores between particles, which slows down the O_2 transport and results in anaerobic reactions (de Bertoldi et al., 1983; Pace et al., 1995). Furthermore, high moisture contents can decrease the strength of the material matrix and make the substrate more easily compressible (Haug, 1993). In addition, high moisture content and matrix compression will lead to an increase of thermal conductivity, allowing heat to be lost more easily (Agnew & Leonard, 2003).

Effect of moisture on wood degradation at room temperature has been widely studied (A'Bear et al., 2014; Crockatt & Bebber, 2015; Herrmann & Bauhus, 2013; Ringman et al., 2019; Rinne-Garmston et al., 2019). It has been reported that the optimum moisture content of wood degradation at room temperature is ranging from 40% to over 70% (Griffin, 1977; Zelinka et al., 2020). Nevertheless, there is little information on temperatures between 40-55 °C. In the BWO systems, higher temperature leads to higher rate of water evaporation. Thus, the moisture content is likely to decrease quickly during BWO process, which may have different kinetic models of moisture content and result in potential challenges of drying out. Besides, at room temperature, the main wood decaying microorganisms are mesophilic fungi and mesophilic bacteria. However, it is the thermophilic fungi and thermophilic bacteria that are playing important roles in BWO. The optimal moisture content and its mechanisms need further research.

1.3.5 pH

In traditional composting, the acidic pH in the early stage may inhibit the bacterial activity, sometimes even severely hampers composting reaction (Beck-Friis et al., 2001; Sundberg et al., 2004; Yu & Huang, 2009). The addition of alkali (such as lime and NaAc) can enhance the degradation rate of composting (Nakasaki et al., 1993; Yu & Huang, 2009). However, this is not the case in wood degradation process. The pH of wood depends on the presence of volatile acids, the most important of which are acetic and formic acids. Due to these free acid, the pH of fresh wood often is acidic (Highley, 1979). A remarkably pH decrease can be observed during wood degradation process (Humar et al., 2001). This might be due to the formation of organic acids, such as lactic acid, oxalate acids (Goodell, 2003; Sundberg & Jönsson, 2008). It has been reported that wood decaying fungi prefer an acid environment while bacteria prefer a nearly neutral pH (de Bertoldi et al., 1983; Schmidt, 2006). The effect of pH on BWO is complicated because BWO is a high solid-state system. There is little information available about the effect of pH on BWO at temperatures 40-55 °C. The effect of pH on BWO has not been studied and the optimal pH of BWO remains unknown. Moreover, the changes of pH during BWO process have not been studied yet.

1.4 Research Objective, Questions and Thesis Outline

Considering the huge amount of wood that is used for heat production, and the environmental issues generated by wood combustion, BWO is proposed as an alternative to wood combustion with huge potential to release the environment burden. A recent study demonstrated the heat production of BWO and investigated the effect of two fungal inoculum (one thermophilic and one thermotolerant) at 41 °C (Caizán Juanarena et al., 2016). The results showed that the natural biota in wood were as effective in degrading the wood as the artificially added fungi (Caizán Juanarena et al., 2016).

However, there are still many unknowns about the BWO process. For example, BWO, as a continuous thermophilic composting using wood as the sole substrates, has low degradation rate because its physical barrier formed by strong bonds between the molecules that inhibit the microbial degradation significantly (Kamimura et al., 2019; Zhang et al., 2014). Thus, it is important to increase the rate of BWO for a higher heat production. Moreover, the effects of other factors, such as temperature, nutrients, aeration, pH, and moisture content, have not been studied yet. Insight in these factors is crucial to further improve the degradation rate and heat production performance of BWO.

The general objective of this PhD project is to explore the possibility of BWO as a promising technology for sustainable heat production. To understand the mechanisms of BWO better, we choose nutrients and temperature as the main studying factors of this dissertation. Linked to the research background, the objectives of each chapters are:

1) Give a complete review on heat potential, production, recovery and utilization from composting (**Chapter 2**);
2) Understand the effect of different nutrient supplements (nitrogen and phosphorus) on BWO under different pH (**Chapter 3**);
3) Investigate the effect of synthesis human urine, fresh human urine, and nutrients re-addition on BWO (**Chapter 4**);
4) Understand the effect of different temperatures (30, 40, and 50 °C) on BWO in a bottle-scale experiment (**Chapter 5**);
5) Investigate the effect of temperatures (40 and 50 °C) on BWO in a continuously bench-scale experiment for a long-term running (**Chapter 6**).

Table 1-1 summarizes the individual research questions of each research chapter.

Table 1-1 Overview of the research questions in each chapter

Chapter	Research questions
2	What are the major sources of organic solid waste in composting used for heat production? How to calculate the heat potential of different organic solid waste? How to estimate the actual heat production from composting? How to recover and reuse the generated heat?
3	What is the optimal pH and optimal concentration of nitrogen and phosphorus in BWO process? What is the effect of nitrogen, phosphorus and pH on BWO?
4	Can human urine be used as an alternative to chemical nutrients? What is the best dilution time of synthetic human urine? Can real human urine have a better BWO performance than synthetic human urine at the same nitrogen level? How does the concentration of nutrients change during BWO process? Will nutrients re-addition enhance the BWO rate?
5	What is the optimal temperature of BWO? What is the response of BWO towards temperature change? What is the microbial gene density at different temperature? What is the response of microbial gene density towards temperature change?
6	What is the preferred temperature of BWO at reactor scale under the condition that moisture is continuously supplied? Will BWO at reactor scale have the similar performance compared to batch scale? What is the change of microbial gene density in the course of incubation time?

In this PhD thesis, we first review the literatures connecting heat and composting and propose proper methods to calculate the heat production of BWO (Chapter 2). The methods are used in the following experiments. Second, we explore the effect of nutrients and temperature on BWO. For the nutrients, we do experiment to explore the optimal concentration of nitrogen, phosphorus under different pH condition (Chapter

3); to reduce the cost and environmental pressure, we do experiment to explore the possibility of using human urine as an alternative to chemically synthesized nutrients (Chapter 4). For the temperature, we do experiment to find out the preferred temperature of BWO at batch scale and its response to temperature change (Chapter 5). Based on the results of Chapter 5, we do experiment at a larger scale to figure out if BWO at larger scale can get the similar BWO performance as at batch scale. The schematic outline of this thesis is shown in Fig. 1-3. The detailed outline of each chapter is:

In Chapter 2, we firstly point out the types of organic solids wastes that are amenable to compositing. Next, we summarize the methods to estimate the potential heating value of organic solid wastes. Furthermore, we discuss the advantage and disadvantages of different methods that are used for calculating the actual heat production from composting. In addition, we summarize and evaluate the different heat recovery methods that have been used for composting. Moreover, we list the potential applications to the generated heat. Finally, we give an outlook about studies relating to composting heat.

Chapter 3 and Chapter 4 of this thesis present an investigation into the effect of nutrients addition on BWO. In Chapter 3, we investigate the optimal addition amount of NH_4Cl addition and KH_2PO_4 addition under different pH values on BWO. We evaluate their performance by monitoring the oxygen consumption and weight loss of dry wood. To reduce the cost and environmental impacts of chemical nutrients, we therefore choose urine as an alternative source of nutrients.

The effect of urine addition on BWO will be discussed in Chapter 4. In Chapter 4, we investigate the possibility of human urine as nutrients of BWO by determining the oxygen consumption and weight loss of dry wood. We first determine the optimal dilution ratio of synthetic human urine by assessing the oxygen consumption and weight loss of dry wood. Thereafter, we investigate the effect of fresh human urine addition on BWO. To explore the relationship between nutrient availability and degradation rate, we re-add the synthesis human urine to the BWO system and continue the incubation process.

Chapter 5 and Chapter 6 of this thesis present the effect of temperature on BWO. In Chapter 5, we investigate the effect of different temperatures (30, 40, and 50 °C) on BWO in a bottle-scale experiment. We also change the temperature when the degradation rate becomes stable to explore if temperature can stimulate the degradation rate. Moreover, we also track the ratio between fungi and bacteria during the BWO process. In Chapter 6, we investigate effect of two temperatures (40 and 50 °C) on BWO by running a continuous bench-scale reactor for 150 days. We evaluate the BWO process by measuring the oxygen consumption and weight loss of dry wood during incubation. Besides, we also monitor the ratio between fungi and bacteria.

In Chapter 7, we address the research questions and discuss the bottlenecks of BWO for sustainable heat production based on the results of this PhD thesis. First of all, we calculate how much heat can be recovered from a scaling-up BWO system. Secondly, we analyze the potential challenges to the scaling-up application and come up with possible solutions based on this PhD dissertation. In the last, we discuss the future perspectives on research and application of BWO.

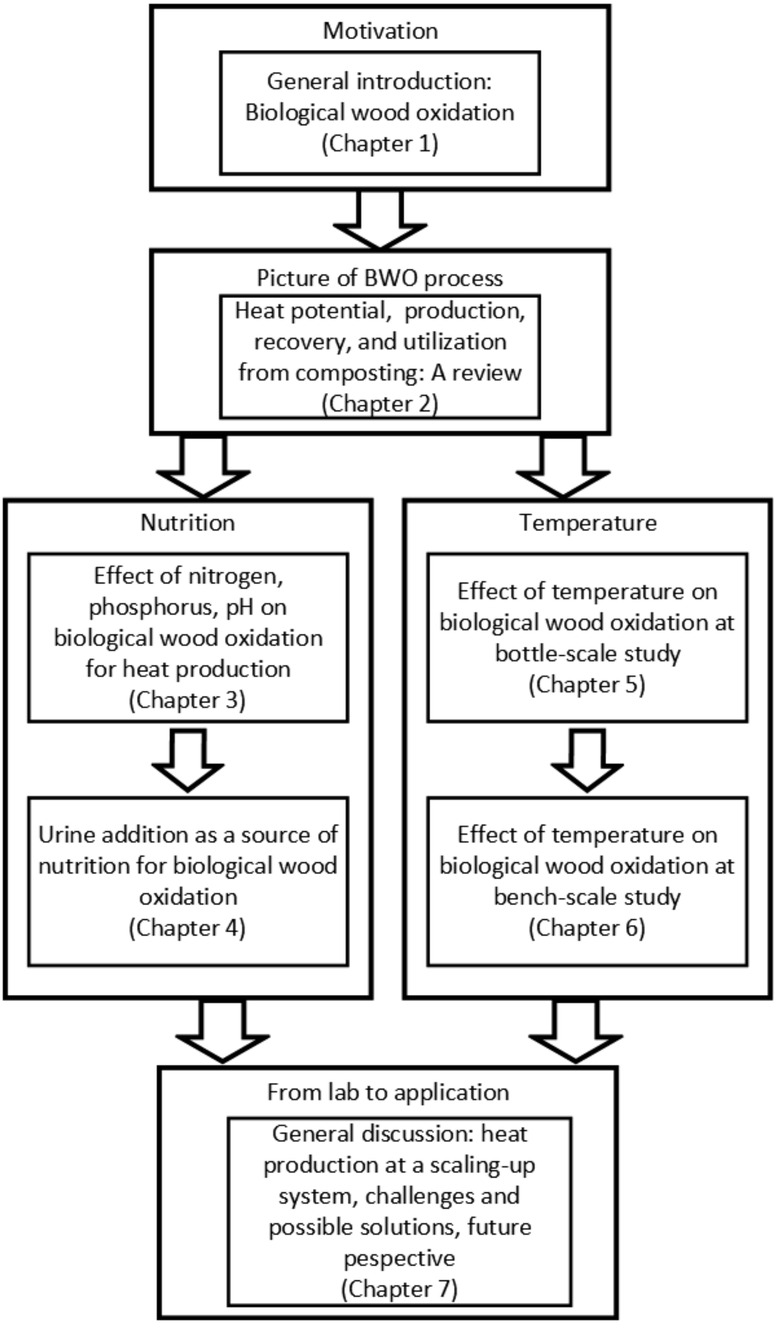

Fig. 1-3 Schematic outline of this dissertation.

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Chapter 2

Heat Potential, Production, Recovery and Utilization from Composting: A Comprehensive Review

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Shiyang Fan, Annemiek ter Heijne, Cees J.N. Buisman, Wei-Shan Chen,

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Abstract

With the rapid population growth and industrial development, there is a significant increase in the production of organic solid waste. Management of the organic solid waste is a global challenge. Composting is an efficient and effective way to dispose the organic solid waste, which aims to degrade organic solid waste and generate a product that can be used for improving soil fertility. Composting is also a process which releases a large amount of heat. However, the heat generated during composting has not been reviewed systematically. In this review, we bring together the current knowledge on heat generated from composting, based on decades of literature. This review includes 1) an analysis of heat potential of different organic solid waste; 2) an in-depth discussion on the methods estimating specific heat production generated from composting; 3) an analysis of heat recovery methods; 4) a summary of application of heat generated from composting; and 5) an outlook for future study.

Keywords: *composting, heat, energy recovery, renewable energy, biomass, organic solid waste*

2.1 Introduction

In recent years, the amount of solid waste has increased rapidly with growing population, urbanization, and industrialization (Singh et al., 2014). It is reported that about 11 million tons of solid waste will be produced every day in the world by the end of 21st century (Hoornweg et al., 2013). Organic solid waste (OSW) is the largest part of the solid waste, which accounts for 46% of the total amount of solid waste (Hoornweg & Bhada-Tata, 2012). There are several main components of OSW: sewage sludge, kitchen waste, lignocellulosic waste, and manure waste (Chen et al., 2020). The huge amount and the different components of OSW makes its management a global challenge (Potdar et al., 2016).

Currently, the management of OSW includes landfilling, incineration, and biological treatment (composting and anaerobic digestion), which is shown in Fig. 2-1. The landfill competes with living space for human beings (Slater & Frederickson, 2001) and may pollute the soil and ground water (Ančić et al., 2020; Mor et al., 2006; Yang et al., 2013). Incineration reduces the volume of OSW quickly; however, it may generate harmful emissions, like NO_x, CO, and fine particles (Wang et al., 2012). Moreover, the high moisture content of OSW reduces its heating value and prolongs the drying process, which is not optimal for incineration (Lin et al., 2015). To the opposite, biological treatment of OSW is an environmental-friendly management method demanding high moisture content for microbial growth. The biological treatment of OSW mainly includes anaerobic digestion and aerobic composting. Anaerobic digestion of OSW is carried out in the absence of O_2. In anaerobic digestion, microorganisms degrade OSW into gaseous products like CH_4 and CO_2 as well as solid compounds like digestate (Kumar & Samadder, 2020). While aerobic composting is under the presence of O_2 and yields compost as the final product. Fig. 2-1 summarizes the current management methods of OSW.

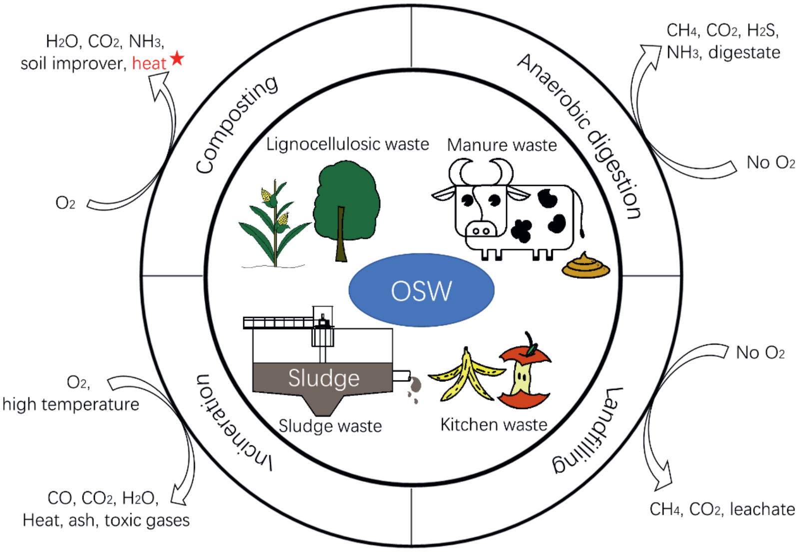

Fig. 2-1 Graphic outline of current organic solid waste management.

Compared with other management methods of OSW, composting has many advantages. First of all, composting has lower technical complexity and capital investment (Cadena et al., 2009). Secondly, composting causes little environment burdens as pathogens are usually killed during the high temperature phases (Rai et al., 2021). Moreover, the residue of composting is humus (soil improver) and can be used in agricultural soils (Boldrin et al., 2009; Weber et al., 2014). OSW is amenable to composting as it consists of heterogeneous organic matter, including sugars, fats, proteins, hemicelluloses, celluloses, and lignin, which are important energy sources for involved microorganisms (Cheung et al., 2010). During composting process, microorganisms decompose OSW into CO_2, H_2O, NH_3 and release considerable amounts of thermal energy (Bell, 1970; Ghaly et al., 2006; Haug, 1993; Mudhoo & Mohee, 2008). This process is shown in equation (1)

$$Organic\ matter + O_2 \rightarrow CO_2 + H_2O + NH_3 + humus + energy \qquad (1)$$

Part of the generated energy is used for sustaining microbial metabolism, and the rest is normally lost to the surrounding environment as heat (Tuomela et al., 2000; Zhao et al., 2017). However, the generated heat is usually ignored as the main aim of composting is the safe disposal of OSW and the production of soil improver (Boldrin et al., 2009; Bollen et al., 1989; Haug, 1993; Lu et al., 2001). With the rapid increase of worldwide energy demand, the generated heat gains more and more interests, as it can be seen as a sustainable alternative to fossil fuels and one of the primary possibilities for preventing global warming (Benjamin et al., 2020).

Over the past decades, many research papers about heat generated from composting have been published (Allain, 2007; Ge et al., 2020; Hess et al., 2004; Liang et al., 2003; Sokolovs et al., 2015; Themelis, 2005; Wu et al., 2020; Xu et al., 2020; Yu et al., 2020; Yu et al., 2019). There are only several review papers relating to heat (Ajmal et al., 2020; Mason, 2006; Smith et al., 2017; Walling et al., 2020; Zhao et al., 2017). These reviews have studied: 1) the mathematical models of composting process, including models of energy balance and mass balance (Ajmal et al., 2020; Mason, 2006; Walling et al., 2020); 2) different heat recovery data and different heat recovery methods. For example, Smith et al. (2017) summarized 45 composting heat recovery systems and reported their heat recovery data chronologically. Zhao et al. (2017) reviewed the current heat recovery methods from composting, and simply analyzed the heat recovery aspects such as optimal heat recovery temperature, potential energy. However, there is no review article yet that discusses heat from composting in-depth, including heat potential, heat generation, heat recovery and heat utilization.

Therefore, in this review paper, we firstly report the heat potential of different components of OSW. Thereafter, we introduce the different methods to estimate the specific heat production during composting and discuss the advantages and disadvantages of these methods. Next, we summarize the different heat recovery methods that have been used for composting and evaluate both traditional recovery technologies and new low-temperature heat recovery technologies. Finally, we summarize the current heat utilization cases and give an outlook for the future study.

2.2 Heat Potential of Composting and its Estimation

Generally, composting consists of four stages according to its temperature regimes: mesophilic stage, thermophilic stage, cooling stage, and maturation stage (Partanen et al., 2010). Fig. 2-2 displays the changes in the temperature and heat production rate during these four stages. In the mesophilic stage and thermophilic stage, the heat production rate is high and maintains high for a long period, because the involved microbes quickly degrade the energy-rich and easily degradable compounds (starches, sugar and fats), and some more resistant substances (proteins, hemicellulose and cellulose). Also, high temperature can help accelerate the breakdown of proteins, fats and complex carbohydrates like cellulose and hemicellulose. These two phases can last for several weeks (in food waste composting) to several months (in lignocellulosic waste composting) under proper insulation (Insam & De Bertoldi, 2007; Tuomela et al., 2000). In the cooling stage and maturation stage, the microbial activity declines due to the depletion of the easily degradable materials. As a result, the heat production rate decreases, and the temperature of the compost pile declines (Epstein, 2011). The cooling stage and maturation stage may last for several weeks to several months (Tuomela et al., 2000).

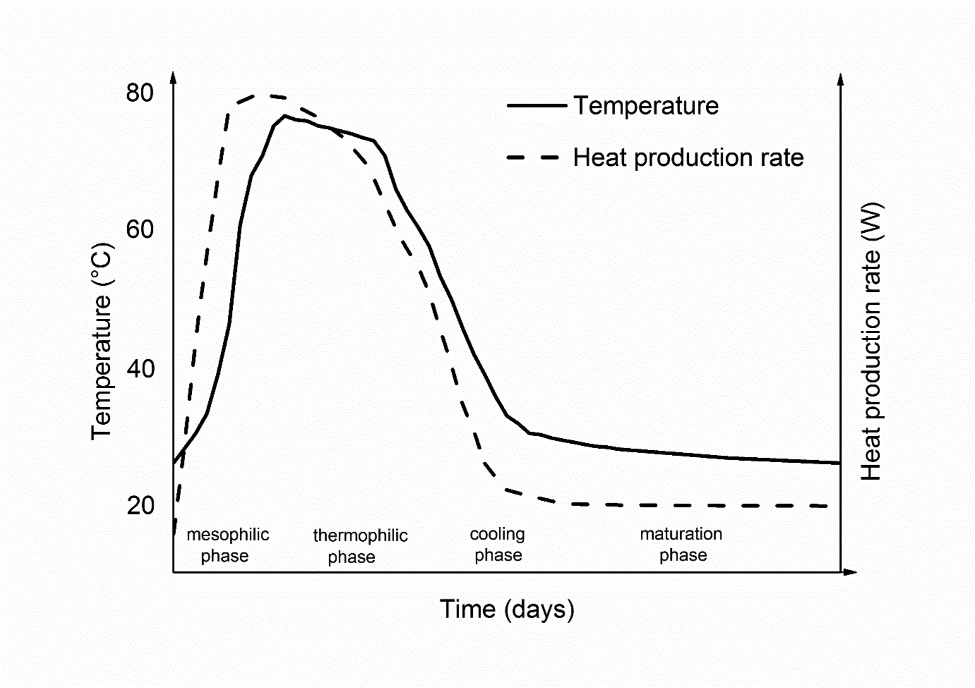

Fig. 2-2 General schematic figure of the composting process.

Composting process can release a large amount of heat, especially in the first two stages. The heat production in composting originates from the chemical energy stored in the organic matter (Haug, 1993). The ultimate energy from composting is the same as that from combustion of the substrates as both are aerobic processes if all substrates are fully oxidized (Smith et al., 2017; Sobel & Muck, 1983). Heating value (HV, in the unit of MJ/kg), namely calorific value, heat of combustion, or higher heating value, is defined as the “amount of energy released from combustion per kg of the mass”. Sobel and Much (1983) estimated about 12.8 MJ/kg heat could be produced from poultry droppings composting. Themelis (2005) reported the HV of yard and food waste was 17.8 MJ/kg. Walling et al. (2020) found that the HV ranges between 0.3-22 MJ/kg depending on different substrates. The properties of different OSW components used in composting are shown in Table 2-1.

Table 2-1 The properties of four different organic solid waste components.

Substrates	Sources	Composition	Heating value (MJ/kg)	Reference
Lignocellulosic waste	Wood, wheat straw, corn straw, grass, bamboo	Cellulose, hemicellulose, lignin, ash	15-34	(Demirbaş & Demirbaş, 2004)
Food waste	Egg, meat, rice, oil, fruit and vegetables	Protein, lipid, fat, carbohydrate, inorganic compound	6-20	(Melikoglu et al., 2013; Menikpura & Basnayake, 2007)
Sludge waste	Domestic sludge, industrial sludge	Microorganism, organic fiber, extracellular polymer substance, inorganic particle	4-7	(Kim et al., 2005)
Manure waste	Chicken manure, horse manure, pig manure, cattle manure, human waste	Bedding materials, feces, urine	11-47	(Sahu et al., 2016)

It is difficult to determine the HV of OSW due to its heterogeneity. Thus, many different empirical correlations have been proposed to estimate or predict the HV. For lignocellulosic waste, predicting models are usually based on ultimate analysis (elements analysis) (Friedl et al., 2005; Huang & Lo, 2020; Maksimuk et al., 2020; Nitsche et al., 2017; Prasityousil & Muenjina, 2013), proximate analysis (fixed carbon and volatile matter analysis) (Cordero et al., 2001; Demirbaş, 1997; Jiménez & González, 1991), and composition analysis (lignocellulosic components analysis) (Acar & Ayanoglu, 2012; Demirbaş, 2001; Jiménez & González, 1991). The HV of sludge waste, manure waste and food waste can also be estimated by using these methods (Abbas et al., 2011; Choi et al., 2014; Qian et al., 2018; Sanli et al., 2014; Thipkhunthod et al., 2005). Models used for estimating the HV of different OSW components are summarized in Table 2-2.

Table 2-2 Models used for estimating heating value of different organic solid waste components.

Models	R^2 or error band	Application	Reference
	Based on ultimate analysis		
$HV = 0.3137C + 0.0318O + 0.7009H - 1.3675$	0.834	Lignocellulosic waste	(Sheng & Azevedo, 2005)
$HV = 0.2949C + 0.8250H$	0.9737	Lignocellulosic waste	(Yin, 2011)
$HV = 0.00355C^2 - 0.232C - 2.23H + 0.0512C * H + 0.131N + 20.6$	0.943	Lignocellulosic waste	(Friedl et al., 2005)
$HV = 0.0053C^2 - 0.5321C - 2.8769H + 0.0608C * H - 0.2401N + 32.7934$	±10%*	Lignocellulosic waste	(Nhuchhen & Afzal, 2017)
$HV = 0.301C + 0.525H + 0.064O - 0.736$	0.830	Lignocellulosic waste	(M. Ebeling & M. Jenkins, 1985)

$HV = 0.3443C + 1.192H - 0.113O - 0.024N + 0.093S$	0.9939	Lignocellulosic waste	(Huang & Lo, 2020)
$HV = 0.350C + 1.01H - 0.0826O$	0.935	Food waste, lignocellulosic waste	(Shi et al., 2016)
$HV = 0.2266C + 0.6544H + 0.1054O + 0.3927N - 1.6402S + 0.7357$	0.729	Manure waste	(Choi et al., 2014)
$HV = 0.4302C - 0.1867H - 0.1274N + 0.1786S + 0.1842O - 2.3799$	0.905	Sludge waste	(Thipkhunthod et al., 2005)
$HV = 0.4328C - 0.29773H + 0.28745N + 0.35608$	>0.9	Lignocellulosic waste	(Huang et al., 2009)
	Based on proximate analysis		
$HV = 0.1905VM + 0.2521FC$	0.9714	Lignocellulosic waste	(Yin, 2011)
$HV = 0.1846VM + 0.3525FC$	±10%*	Lignocellulosic waste	(Nhuchhen & Afzal, 2017)
$HV = -3.0368 + 0.2218 + 0.2601FC$	0.617	Lignocellulosic waste	(Sheng & Azevedo, 2005)

$HV = 35.43 - 0.1835VM - 354.3ASH$	NF	Lignocellulosic waste	(Cordero et al., 2001)
$HV = 0.3543FC + 0.1708VM$	NF	Lignocellulosic waste	(Cordero et al., 2001)
$HV = -10.81408 + 0.3133(VM + FC)$	<10%*	Lignocellulosic waste	(Jiménez & González, 1991)
$HV = 0.1970VM + 0.3955$	0.806	Manure waste	(Choi et al., 2014)
$HV = 0.25575VM + 0.28388FC - 2.38638$	0.899	Sludge waste	(Thipkhunthod et al., 2005)
	Based on composition analysis		
$HV = 0.0889L + 16.8218$	0.9504	Lignocellulosic waste	(Demirbaş, 2001)
$HV = 0.0877L + 16.4915$	0.9302	Lignocellulosic waste	(Demirbaş, 2001)
$HV = 0.112157Fw + 0.183386Pa + -0.288737Pl + 5.064701$	0.779	Food waste, plastic, paper	(Kathiravale et al., 2003)

Note: the amount of element (C,H,N,O,S), volatile matter (VM), fixed carbon (FC) are expressed in weight percentage; NF=not found; Ce, L, H are weight percent of cellulose, lignin and hemicellulose on dry basis; Fw, Pa, Pl are weight of percent of food waste, paper and plastic on dry basis; * the value is the error band.

Composting usually consists of various types of OSW components to ensure a feasible composting conditions (Adamtey et al., 2009). For example, food waste is rich in nutrients while sludge waste has high moisture content. Lignocellulosic waste is often co-composted with food waste or sludge waste to obtain a proper C:N ratio and a proper porosity (Iqbal et al., 2010). The total HV of the mixed OSW can be calculated from the composition and proportion. In addition, calorimetric techniques can also be used for determining the HV of mixed substrates (Ahn et al., 2007; Prasityousil & Muenjina, 2013).

2.3 Actual Heat Production from Composting and its Estimation

OSW has great heat potential; however, decomposition of the organic matter is often not complete at the end of composting process. The actual heat released from composting cannot exceed the potential heat available in the feeding substrates. The actual amount of generated heat is affected by the factors such as substrate mass, substrate degradability, and duration of composting (Smith et al., 2017; Sobel & Muck, 1983). Moreover, the generated heat affects the temperature, pathogen elimination, and the degradation rate of OSW. Thus, it is important to know the actual amount of heat production (Shaw & Stentiford, 1996).

There are several methods for quantifying heat generation from composting, namely degradation method, oxygen consumption method, heat balance method, and some other methods such as CO_2 evolution method, temperature method, and heating value method. Those methods are based on the fact that theoretical heat production in composting is positively correlated with O_2 consumption and degradation rate, which is also associated with the temperature and CO_2 production (Białobrzewski et al., 2015). Table 2-3 summarizes the different studies using these methods to estimate the actual heat production from composting. These methods will be discussed in more detail in the following paragraphs.

Table 2-3 The heat production estimated by different methods in related literature.

Substrate	Mass	Moisture content (%)	Method	Heat production (in rate or quantity)	Duration	Reference
Wood	50 t	60	DD	2.47 kWh/kg BOM	1 y	(Kimman, 2019)
Organic fraction of municipal waste	17.7-19.2 t	34-39	DD	0.75 kWh/kg BOM	50 d	(Robinzon et al., 2000)
Food waste, wood waste	419-697 g	55-65	DD and HB	0.74-1.19 kWh/kg VS	10 d	(Lemus & Lau, 2002)
Paper mill sludge, broiler litter	208 L	38-47	DD	0.22 kWh/kg DM/d*	NF	(Ekinci et al., 2006)
Biosolid, woodchips	208 L	38-47	DD	0.68 kWh/kg DM/d*	NF	(Ekinci et al., 2006)
Poultry manure, wood shavings	275 kg	62	DD	1.09-1.38 kWh/kg DM	30 d	(Ahn et al., 2007)
Food waste, maize straw	17.2 kg	71	DD	1.78 kWh/kg DM	56 h	(Xie et al., 2017)
Wood	6 g	250$^\#$	OC	0.24-0.52 kWh/kg DM	36 d	(Caizán Juanarena et al., 2016)
Wood	6.7 g	250$^\#$	OC	1.49 kWh/kg DM	42 d	(Fan et al., 2020a)
Wood	4.7 g	250$^\#$	OC	1.44 kWh/kg DM	95 d	(Fan et al., 2020b)

Food waste, wood chips	76 kg	63	OC	About 1.19 kWh/kg DM	37 d	(de Guardia et al., 2012)
Separated pig solid	145 kg	68	OC	About 1.50 kWh/kg DM	27 d	(de Guardia et al., 2012)
Pig manure, wheat straw	8 kg	50-67	OC	0.008 kWh/kg VS/h*	NF	(Ge et al., 2016)
Chicken manure, rice bran, sawdust	0.24 m^3	60	OC	0.56-2.22 kWh/m^3/h	About 340 h	(Seki & Komori, 1995)
Sludge, compost product	5 t/2d	52	OC	0.70 kWh/kg DM/d	NF	(Bach et al., 1987)
Sludge, compost product	5 t/2d	52	HB	0.70 kWh/kg DM/d	NF	(Bach et al., 1987)
Sludge, fat, poplar sawdust	32 kg	65	HB	1.19 kWh/kg DM	180 h	(Viel et al., 1987)
Tomato plant waste, wood shavings, municipal waste, urea	NF	60	HB	4.01 kWh/kg DM	114 h	(Ghaly et al., 2006)
Kitchen waste, garden waste	70 kg	NF	HB	0.39 kWh/kg WW	41 d	(Neugebauer, 2018)
Wheat straw, poultry manure, gypsum	5254-8583 kg	67-71	HB	0.34 kWh/kg WW	180 h	(Harper et al., 1992)

Manure, straw	45 kg	NF	HB	0.12 kWh/h*	NF	(Boniecki et al., 2013)
Tomato residues	50 kg	60-65	HB	0.53 kWh/kg WW	108 h	(Alkoaik et al., 2018)
Solid poultry manure, wheat straw, gypsum, water	1000 t	74	HB	0.37 kWh/kg WW	80 h	(Radojičić et al., 2017)
Chicken manure, hay, wood chips	120 L	60	HB	0.11 kWh/kg	44 h	(Nwanze & Clark, 2019)
Green waste, industrial sludge, liquid waste	NF	60	TEM	1.94-2.78 kWh/kg	15 d	(Irvine et al., 2010)
Solid fraction of pig slurry, lignocellulosic residues, plant, water	1725 g	65-70	TEM	1.75-5.08 kWh/kg TS	18 d	(Hunce et al., 2020)
Food waste, saw dust, mature compost	400 kg	65	TEM	0.83 kWh/kg WW	30 d	(Yeh et al., 2020)
NF	100 kg	43	HV	0.50 kWh/kg WW	17 d	(Klejment & Rosiński, 2008)
Sawdust, grass, horse manure, potato paste, vegetables	15 m^3	65	HV	0.26 kWh/kg	84 d	(Raclavska et al., 2011)

Note: NF=not found; DD=degradation method; OC=oxygen consumption method; HB=heat balance method; TEM=temperature method; HV= heating value method;

MC=moisture content; BOM=biodegradable organic matter; VS=volatile solid; TS=total solid; WW=wet weight; DM=dry mater; * the data is the peak value of heat production rate; # the data is the moisture content of the system

2.3.1 Degradation method

Heat generated from composting is released with organic matter degradation. Therefore, the degradation models describing the composting process can be used for estimating the actual heat production. Different degradation models have been proposed (Ekinci et al., 2006; Haug, 1993; Khater et al., 2014; Sobel & Muck, 1983; Talib et al., 2014); and the general form can be expressed as shown in equation (2):

$$Q_{bio} = HV_r \times r = HV_r \times \frac{dm}{dt} \tag{2}$$

Where Q_{bio} is the heat production rate of composting (W); $\mathrm{HV_r}$ is the heating value of the substrates (MJ/kg), defined as amount of heat released per unit of substrate degradation; r is the coefficient of degradation rate (kg/s); t is the composting time (s); m is the weight of substrates (kg), which can be expressed as one of the follows: organic matter, dry matter, volatile solids, total solids, total organic carbon, carbon, wet weight, biodegradable volatile solids, or chemical oxygen demand (Higgins & Walker, 2001; Raclavska et al., 2011; Rada et al., 2014; Seki & Komori, 1984; Wang et al., 2014).

Among all those parameters, $\mathrm{HV_r}$ is usually regarded as a constant during composting process for simple and quick calculation (Raclavska et al., 2011), while $\mathrm{HV_r}$ actually decreases during composting. Ahn et al. (2007) proved that $\mathrm{HV_r}$ decreased with the increasing of composting time by using a calorimeter. Wang et al. (2014) proposed an empirical model to describe the change of $\mathrm{HV_r}$ during composting process to obtain a more accurate estimation. It is difficult to accurately measure the degradation rate during composting in large scale reactors. Thus, many degradation models have been developed to describe the degradation process, such as first-order models and Monod-type models (Ajmal et al., 2020; Mason, 2006).

In the first-order models, the degradation rate is related to the concentration of composting substrates (Haug, 1993). The first-order models are simple and easy because it only requires a single rate coefficient, which makes the first-order models the most prominent one (Walling et al., 2020). However, the first-order models cannot illustrate the effect of other factors on degradation rate, such as spatial distribution of substrates, decreasing availability of substrates, and concentration of microbial biomass (Haug, 1993; Mason, 2006; Wu et al., 2011). Hence, the first-order models need to be adjusted to give a more accurate description of the degradation process during composting.

Monod-type models describe the relationship between the concentration of microbial growth and the degradation process in composting (Ajmal et al., 2020; Kaiser, 1996; Seki, 2000). Monod-type models require four or more coefficients, including maximum specific growth rate, decay coefficient, half-saturation coefficient and maintenance coefficient (Mason, 2006). It is a challenge to obtain all those coefficients, because the microbial population growing on the composting substrates is variable. The degradation process may deviate from Monod-type when composting conditions (temperature, moisture, type of substrate and O_2 concentration) are limiting the microbial growth (Mohee et al., 1998; Wang et al., 2014). These challenges limit the broader use of Monod-type models (Mason, 2006).

Degradation models have been developed both in big scale reactors and small scales reactors. It can also be used for studying how the operational parameters, such as moisture and aeration rate, affect the heat production. However, it is difficult to propose deterministic models that can describe different composting processes with different parameters (substrates, aeration, temperature and moisture content), and different geometric configurations (size and shape of the reactors) (Seki, 2000). Thus, the degradation models are usually complex for describing single composting processes accurately.

2.3.2 O_2 consumption method

O_2 is particularly important since O_2 is needed in all aerobic degradations during composting. The biological reactions that take place in composting are often limited by the O_2 transfer rate (Paletski & Young, 1995). Besides, O_2 consumption has been proved as an useful indicator for the composting stability, microbial activity (Lasaridi & Stentiford, 1998; Lasaridi et al., 2000; Paletski & Young, 1995), degradation rate, microbial growth, and temperature change (Higgins & Walker, 2001; Tremier et al., 2005; VanderGheynst et al., 1997).

O_2 profiles are widely used for predicting the heat production during composting, which has been validated extensively. Cooney et al. (1969) found that the heat production rate during metabolism was linearly correlated with the O_2 consumption rate by using a dynamic calorimetric technique. Harper et al. (1992) and Weppen (2001) also reported similar results. Fan et al. (2020a) demonstrated that O_2 was linearly related to the wood weight loss during wood composting, which further linked to degradation rate. The heat generation during composting can be estimated and expressed as in the equation (3) (Bach et al., 1987; Haug, 1993; Nakasaki et al., 1987):

$$Q_{bio} = OCR \times HV_o = HV_o \times \frac{dO_2}{dt} \qquad (3)$$

where $\mathrm{HV_o}$ is the heating value defined as the amount of heat generated metabolically per mole of O_2 consumption (kJ/mol O_2), OCR is the O_2 consumption rate (mol/kg), defined as the O_2 consumption per unit of time (mol O_2/s). $\mathrm{HV_o}$ is generally identified as a constant during one composting process (Caizán Juanarena et al., 2016; de Guardia et al., 2012; Haug, 1993). $\mathrm{H_o}$ is usually between 304 and 448 kJ/mol O_2 consumed (Mason, 2006), and it can be determined on basis of the chemical composition of the organic matter. O_2 consumption rate can be estimated by first-order models and Monod-type models (Mason, 2006; Yamada & Kawase, 2006). In most cases, the O_2 consumption rate is determined by measuring the O_2 concentration difference in the inlet and outlet gas (Cooney et al., 1969).

Many researchers have improved this method for a better heat estimation. The O_2 model could be improved by considering the effect of temperature and moisture content in the composting air (Seki & Komori, 1995). de Guardia et al. (2012) calculated the heat generation via O_2 consumption and found that the heat coefficient could significantly affect the temperature in the composting pile. In addition, the O_2 model was more accurate when considering the peak temperature of the composting pile (Ge et al., 2016). The O_2 consumption method is a promising approach to calculate the heat production during composting. Firstly, the expression of O_2 consumption method is simple and easy to reproduce. The heat can be calculated without understanding the degradation process. Secondly, the O_2 consumption rate can be continuously measured, not only in larger reactors (de Guardia et al., 2012; Gómez et al., 2006), but also in small reactors at lab-scale studies (Fan et al., 2020b). The disadvantage of O_2 is that this method needs more specific instrumentation and more skilled labor (Gómez et al., 2006).

2.3.3 Heat balance method

The heat balance method, from the physical engineering point of view, has also been widely used. This method is under the condition that measuring or estimating the heat transfer in the composting environment is possible and valid. During the composting process, energy released by decomposition mainly results in the temperature increase of the organic matter and water, convection and conduction heat loss, and phase transformation of water to water vapor (Di Maria et al., 2008; Ghaly et al., 2006; Mason & Milke, 2005b). The heat essentially occurs via two forms: sensible heat (energy associated with an increase in temperature) and latent heat (energy associated with phase transformation). Therefore, the heat production can be calculated by measuring these components, which can be expressed in equation (4) (Bach et al., 1987; Ghaly et al., 2006; Haug, 1993; Wang et al., 2014):

$$Q_{bio} = Q_{sensible} + Q_{latent} = Q_{gas} + Q_{sub} + Q_{loss} + Q_{vap} + Q_{rad} \qquad (4)$$

Where $\mathrm{Q_{sensible}}$ is the amount of sensible heat (W), $\mathrm{Q_{latent}}$ is the amount of latent heat (W). To be more specific, heat balance components in composting model include heat production, convective heat loss of inlet and outlet streams (air, vapor, and water) $\mathrm{Q_{gas}}$ (W), sensible heat of composting materials $\mathrm{Q_{sub}}$ (W), conductive/convective losses through surface of reactor $\mathrm{Q_{loss}}$ (W), latent heat loss of water evaporation $\mathrm{Q_{vap}}$ (W), the radiant loss $\mathrm{Q_{rad}}$ (W) (Mason, 2006). Table 2-4 summarizes the models calculating the different components of heat balance.

Table 2-4 The models estimating different components of heat balance.

Component	Nomenclature	Reference
$Q_{gas} = q_a(e_{out} - e_{in})$	q_a is the airflow (kg/s); e_{out} is the enthalpy of the exit air (kJ/kg); e_{out} is the enthalpy of the in air (kJ/kg)	(Wang et al., 2014)
$Q_{sub} = m[(1 - MC)C_s + C_w MC]\frac{dT}{dt}$	m is the mass of composting pile (kg); MC is the moisture content (%); C_s is the specific heat capacity of solid (kJ/K/kg); C_w is the specific heat capacity of water (kJ/K/kg); T is the temperature of composting pile (K); t is the time (s)	(Zhou et al., 2014)
$Q_{loss} = UA(T_r - T_a)$	U is the surface aera of reactor wall (m^2); U is overall coefficient of heat transmittance (kJ/m^2/K/s); T_r is the reactor temperature (K); T_a is the ambient temperature (K).	(Mason, 2006)
$Q_{vap} = q_a Q_v(h_{out} - h_{in})$	q_a is the flow rate of air (kg/s); Q_v is the enthalpy change of water vaporization (kJ/kg); h_{out} is the absolute humidity of exit air (kg/kg), h_{in} is the absolute humidity of in air (kg/kg)	(Wang et al., 2014)
$Q_{rad} = \sigma A(T_c^4 - T_h^4)F_a F_e$	σ is the Stefan–Boltzmann constant, 5.67 * 10^{-11} kJ/(s m^2 K^4); T_c is the temperature of the compost top surface (K); T_h is the temperature of the headspace between compost top surface and reactor lid (K); F_a is a configuration factor accounting for the relative position and geometry of the objects (dimensionless); F_e is the emissivity factor accounting for non-black body radiation.	(Ahn et al., 2007)

Different composting system have different expressions of heat balance models. Generally, Q_{vap} takes up the largest part of the generated heat, especially in large scale reactors (Bach et al., 1987; Di Maria et al., 2008; Robinzon et al., 2000; Themelis & Kim, 2002; Wang et al., 2014). Q_{loss} from surface of reactor usually accounts more in small scale reactors than in big reactors (Ahn et al., 2007; Bach et al., 1987; Mason & Milke, 2005a). Q_{rad} is low and can be ignored compared to the total heat production in composting reactors (Ahn et al., 2007; Robinzon et al., 2000; van Lier et al., 1994). For example, Shaw and Stentiford (1996) reported that of the total heat loss from a pile, 88% was due to vaporization, 10% to dry air convection and 2% to conduction, with radiant heat transfer being assumed negligible. Q_{rad} is relatively large in open air composting studies (Kimman, 2019; Robinzon et al., 2000). The heat balance method displays the distribution of generated heat from composting, helping define which heat recovery method is suitable for different reactors.

Many studies have validated the heat balance method. Lemus and Lau (2002) compared the heat production calculated by heat balance method and by degradation method, they pointed out that both methods were suitable for heat calculation. Bath et al. (1987) compared the heat generated calculated by oxygen method and by heat balance method and reported that the difference between the two methods was less than 0.5%. Xie et al. (2017) also found the similar results, he reported the difference between oxygen method and heat balance method was less than 2%.

Heat balance is important in heat analysis of composting. This method is widely used for the mixed substrates in composting, especially at pilot and commercial reactors where the data of latent heat and heat loss is important and large. This method works well in continuously running reactors. By using the heat balance method, the heat production (rate) can be calculated without understanding the composting process. The disadvantage of the heat balance method is that it is usually difficult to measure all the required values.

2.3.4 Other methods

In addition, studies have been done to corelate heat production with other variables, such as CO_2 evolution (Vlyssides et al., 2009), temperature of the substrates (Hunce et al., 2020; Lemus & Lau, 2002; Seki & Shijuku, 2012; Yeh et al., 2020), and heating value of the substrates (Ahn et al., 2007; Klejment & Rosiński, 2008). These methods are summarized in Table 2-5.

Table 2-5 Other methods to calculate the heat production from composting.

Methods	Models	Nomenclature	Properties	Reference
CO_2 evolution	$Q_{bio} = HV_c \times CER$	HV_c is the heat released per unit of CO_2 evolution (kJ/mol); CER is the CO_2 evolution rate (mol/s)	CO_2 dissolves in water; the solubility is pH-dependent	(Kaiser, 1996; Vlyssides et al., 2009)
Temperature	$Q_{bio} = m_{mix} \times C_{mix} \times \Delta T$	ΔT is the temperature change of the substrates (K); C_{mix} is the specific heat capacity of substrates (kJ/kg/K); m_{mix} is the mass (kg)	Only valid if the heat loss is zero or known	(Hunce et al., 2020; Yeh et al., 2020)
HV	$Q_{bio} = HV_{ini} - HV_{fin}$	HV_{ini} is the HV of substrates at composting beginning (kJ/kg); HV_{fin} is the HV of substrate at the composting end (kJ/kg)	Low sample representativity; cannot describe the composting process	(Klejment & Rosiński, 2008)

The first method is CO_2 evolution method. During composting process, the microorganisms degrade OSW and generate CO_2 (Wang et al., 2019). The CO_2 concentration can be measured by CO_2 meters or estimated by respiratory quotient (Kaiser, 1996; Sundberg & Jönsson, 2008). Sundberg and Jönsson (2008) validated that CO_2 emission could be used as an indicator to heat production during composting; and together with heat production, CO_2 emission could be used for monitoring the microbial activity during the composting at the condition of continuous aeration. However, CO_2 dissolves in water (40 mg/L water at 25 °C and 1 bar), and the solubility of CO_2 depends on pH and temperature (Wiebe & Gaddy, 1940). Measuring CO_2 production is less accurate as measuring O_2 consumption. As a result, only few researchers linked CO_2 evolution to heat production.

Temperature change is the result of heat production and heat loss. It is important to know the temperature of composting because temperature is an indicator of microbial activity, composting stability, and composting maturation (Kumar et al., 2010). However, the temperature, by itself, cannot indicate the amount of heat generation (Finstein et al., 1986). The temperature method is only valid when the heat loss data is zero or known. In the temperature method, generated heat is considered to be conserved both in the solids (composting substrates) and liquids (water) (Sundberg, 2005). Moreover, the specific heat capacity of the mixed solids and liquids should be known. The specific heat capacity is assumed as constant, and the composting process is assumed at constant pressure (Haug, 1993; Irvine et al., 2010). Under these conditions, the heat production rate can be obtained by observing the rate of temperature change during composting process (Hunce et al., 2020; Roland Mote & Griffis, 1982; Yeh et al., 2020). Another principle of temperature method is to use a water bath to recover the generated heat and measure the temperature difference between the composting and water bath (Carlyle & Norman, 1941; Walker & Harrison, 1960). This method is usually used in laboratory bench-scale composting units.

Another method is heating value method. The principle of HV method is that the HV of composting substrates decrease due to the microbial degradation during composting process (Ahn et al., 2007; Wang et al., 2014). The difference between the HV of composting substrates at the beginning and at the end of the composting process indicates the amount of energy released during degradation (Klejment & Rosiński, 2008). Usually, a calorimeter is needed to accurately determine the HV of the mixed composting substrates (Raclavska et al., 2011). This method is handy and easy. However, it is highly dependent on the representativity of samples (although multi-sampling can reduce the deviation error), which limits its application remarkably, especially in large reactors with low homogeneity.

2.4 Heat Recovery from Composting by Using Different Methods

Heat accumulation in composting could hinder the composting process. If all the heat generated during the decomposition remains in the compost, the temperature of composting will rapidly rise to thermophilic conditions and reach a temperature where most microbial activity stops (> 70 °C) (Cooney et al., 1969). Another problem is that the generated heat might result in the drying out of the composting pile (Finstein et al., 1992). The energy generation rate declines quickly until the temperature reaches a proper range (generally between 40 to 60 °C). Thus, the excess generated heat must be removed to keep the composting at a proper temperature for high biological activity and further maximal heat generation (Mudhoo & Mohee, 2007; Shaw & Stentiford, 1996). The removed heat may be collected via several methods. These heat recovery methods varies depending to the composting substrate, composting method, temperature, reactor scale of composting, heat exchanger and even to geographical information (Smith et al., 2017; Yeh et al., 2020). Among these factors, several methods have been successfully applied to remove and/or recover the generated heat from composting. These methods include water-jacket, tube buried in the pile (TBP), heat exchanger in the headspace of composting (HEH), percolation water (PW), and low-temperature heat recovery technologies such as heat pump, organic Rankine Cycle (ORC), thermo electric generators (TEGs). Fig. 2-3 summarizes and describes these methods.

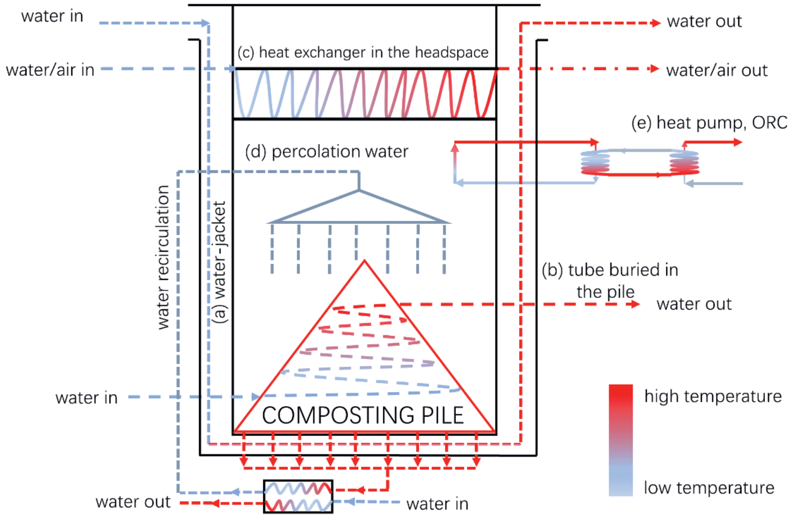

Fig. 2-3 The graphic illustration of different heat recovery methods.

In Fig. 2-3, the composting pile in the reactor produces heat. The first recovery method is water-jacket method. This method usually consists of water tube coated on the surface of the reactor/pile. The heat generated in the reactor heats the water via conduction, which is shown in the part (a) of Fig. 2-3 (Viel et al., 1987). The second method is TBP method. It involves recirculating water through a plastic/metal tube buried in the composting pile. Usually, the inlet cold water goes through the tube and carries the heat mainly via conduction (Pain, 1972). This method is shown in the part (b) of Fig. 2-3. The third method is HEH method. This is usually accomplished by placing a heat exchanger in the reactor headspace or in the exhaust air. By mechanical aeration, fresh cool dry air is pumped in, meanwhile the excess heat in hot humid air is removed (Zhao et al., 2017). The heat in the air can be efficiently recovered by heat exchangers (Radojičić et al., 2017). This method is described in the part (c) of Fig. 2-3. The next method is PW method. In this method, water is usually sprayed, percolated, collected and recirculated. The percolated water is heated via conduction during the percolated process, and the heat is removed in the collection process by using a heat exchanger. This method is described in the part (d) of Fig. 2-3. The last type of methods is the low-temperature heat recovery technologies, which can recover heat from a low-temperature heat source (< 100 °C). Some of these technologies, such as heat pump, organic Rankine cycle (ORC), and thermo electric generators (TEGs), have been successfully applied to composting (Varga & Palotai, 2017). The ORC and heat pump are shown in the part (e) of Fig. 2-3. Table 2-6 summarizes the characteristics of different heat recovery methods reviewed in this paper. Table 2-7 summarizes the different heat recovery methods from different composting studies.

Table 2-6 The characteristics of different heat recovery methods from composting.

Method	Property	Suitable for
Water jacket	Low recovery efficiency, not suitable for big scale reactors	Small scale reactors
TBP	Easy to build; low cost; labor intensive; tubes can be easily damaged during construction; cannot mix the composting pile after construction; low heat recovery efficiency	Pilot-scale reactors, backyard reactors
HEH	High heat recovery efficiency; the composting pile can be mixed; has little effect on the temperature of the composting pile; will dry out the composting process	Commercial reactors, pilot reactors
PW	High water recirculation; conservation of the nutrients; the composting pile can be mixed; only valid in composting substrates with big particle size and high porosity	Lignocellulosic biomass composting
HP	Can increase the heat temperature; the composting pile can be mixed; has little effect on the temperature of the composting pile	Commercial reactors; pilot reactors
ORC	The output energy is electricity; low maintenance and personnel cost; the composting pile can be mixed; has little effect on the temperature of the composting pile; low efficiency	Commercial reactors
TEGs	Low maintenance cost, the output energy is electricity, no greenhouse gases emission; low efficiency; does not contact pile temperature	Commercial reactors

Note: TBP= tube buried in the pile; HEH= heat exchanger in the headspace; PW=percolation water; HP=heat pump; ORC= organic Rankine Cycle; TEGs= thermoelectric generators

Table 2-7 Different heat recovery methods reviewed in this study.

Composting substrates	Mass	Moisture (%)	Method	Heat recovery (in rate or quantity)	Duration	Reference
Sludge, fat, poplar sawdust	32 kg	65	Water-jacket	0.22 kWh/kg DM	56 h	(Viel et al., 1987)
Wood	50 t	60	TBP	1.18 kWh/kg BOM	1 y	(Kimman, 2019)
Woodchips, horse manure, vegetable waste and leaves	5803 kg	47.5	TBP	0.45-0.62 W/kg DM	3 d	(Bajko et al., 2018)
Chicken manure, hay, wood chips, water	120 L	60	TBP	About 200 W*	NF	(Nwanze & Clark, 2019)
Chicken manure, rice bran, saw dust	0.24 m^3	60	TBP and HEH	0.01-0.03 kWh/m^3 WW	7-14 d	(Seki & Komori, 1995)
Green waste, industrial sludge, liquid waste	NF	60	HEH	1.94-2.78 kWh/kg WW	15 d	(Irvine et al., 2010)

Food waste, green waste	189 t	62	HEH	0.30 kWh/kg DM	21 d	(Rada et al., 2014)
NF	100000 t	NF	HEH	2.12 W/kg WW	NF	(Anderson et al., 2016)
Woodchips, horse manure, fresh grass, leaves, matured compost	6984 kg	58	HEH	0.14 kWh/kg DM	36 d	(Bajko et al., 2019)
Cow manure, horse manure/bedding mix, waste hay	136 t	60	HEH	0.13-0.24 kWh/kg DM	60 d	(Smith & Aber, 2018)
Manure, bedding and refusal feed	40 t	NF	HEH	0.22-0.29 W/kg WW	NF	(Brown, 2015)
Yard waste	About 0.5 m^3	15①	HP	About 1 kW/m^3	5 d	(Jaccard et al., 1993)
Food waste, yard waste, mixed paper	96 t	65	HP	1.11-1.38 kWh/kg OM	20 d	(Di Maria et al., 2008)
Organic fraction of MSW	32000 t	NF	ORC	0.61 W/t WW	1 y	(Micalea, 2014)
Fruit waste, meat waste, paper and yard waste	20000 t	63	ORC	0.05-1.25 W/t WW	1 y	(Di Maria et al., 2014a)

Municipal waste	20000 t	63	ORC	0.45-0.6 W/t WW	1 y	(Di Maria et al., 2014b)
Garden waste, food waste	450-650 m^3	NF	TEGs	175 $mW/m^{2*②}$	NF	(Rodrigues et al., 2018)
Chicken manure, rice hulls, sewage sludge	0.24 m^3	67	TEGs	7 W^*	NF	(Shangguan et al., 2020)

Note: NF=not found; TBP=tube buried in the pile; HEH= heat exchanger in the headspace; WIP= with-in tube; HP=heat pump; ORC= organic Rankine Cycle; TEGs= thermoelectric generators; BOM=biodegradable organic matter; WW=wet weight; DM=dry mater; * the data is the peak value of heat production rate;

① the initial moisture was low (15%), but water atomizers were used to humidify the composting material

② the data was the maximum power density, m^2 was the dimension of the TEGs system

2.4.1 Water-jacket method

Water-jacket is usually used for controlling temperature in a reactor. It can also be used for recovering heat from exothermic reaction reactor via conduction. This method is usually applied to pilot reactors and lab scale reactors because only in those reactors, the conducted heat loss through reactor wall is considerable (Ghaly et al., 2006). Moreover, the water-jacket method is not suitable for commercial reactors because of their huge surface area. The water-jacket method does not influence the composting process, however, it has low accuracy because its heat loss might be huge. To reduce the heat loss, Viel et al. (1987) used a double layer water-jacket to recover the generated heat from composting and reported the average heat recovery rate was 4 W/kg DM for 56 h.

2.4.2 Tube buried in the pile method

Another recovery method is tube buried in the pile (TBP) method. The cost of TBP is low, and the tubes are easy to be built. However, this recovery method is not suitable for commercial reactors, as it is labor/time intensive (Smith et al., 2017). It is a throwaway method which is optimal to long retention time composting. TBP method is usually used for lignocellulosic waste composting which aims to provide heat for household use (Smith, 2016). TBP method has low cost and maintaining cost because it is often ventilated naturally (Pain, 1972), which also means a huge heat loss. It has been reported that about half of the generated heat could be lost to the environment by using TBP method (Kimman, 2019). In order to reduce the heat loss, the composting heap should be in the shape of the truncated cone, covering with a layer of straw or leaves (Roman, 2015). Moreover, the recovery efficiency of TBP, defined as recovered heat over total generated heat, is low as it collects little latent heat in the air. The conductivity of the substrates also affects the recovery efficiency of this method.

TBP method was pioneered by Pain in the 1970’s (Pain, 1972) and still has been used today. Seki and Komori (1993) reported that only 0.3% of the whole amount of energy generated in the composting was recovered by TBP method; most of the energy was lost to the surroundings due to the convection loss and conduction loss. In Lekic’s study, about 73% of the theoretical value of heat energy was recovered by using polyethylene pipes buried in the composting piles, the high efficiency was due to the low heat loss (Lekic, 2005). Besides, a low-density polyethylene tube was proved to be well designed for a composting of wood chips, horse manure, leaves and vegetable waste, the whole system worked good without any significant problems in heat transferring (Bajko et al., 2018). Seki et al. (2014) used a flexible stainless tube to extract heat from a bamboo composting; the outlet temperature of water could reach 50-65 °C after 90 h, and it was possible to extract and utilize the generated heat for up to 1000 h. Nwanze and Clark (2019) found that the temperature was sensitive to the water flow rate of a copper tube during thermophilic stage, and more heat could be extracted with higher water flow rates (1147 ml/min).

2.4.3 Heat exchanger in the headspace

Another heat recovery method is heat exchanger in the headspace (HEH) method. HEH has high efficiency because it can collect the latent heat in water vapor and the sensible heat in air, which account for a large proportion of the total generated heat. The recovery efficiency can be even higher with multiple circulation times of the heat exchanger. HEH has little influence on the composting pile. Thus, this method has low maintaining cost and long life span with multiple composting processes (Smith et al., 2017). This approach is mostly used in commercial reactors as it combines heat recovery with aeration, which can save a lot of energy and cost. The main disadvantage of HEH method is that HEH would lead to condensation of water vapor, and the air recycling could dry out the composting heap.

It was reported that HEH together with a flexible pipe set along the side wall could recover 16-22% of the total generated heat (Seki & Komori, 1995). Irvine et al. (2010) used HEH method to collect heat from composting and reported that temperature of the water out from the heat exchanger was 47 °C, which could even increase to 60 °C when the heated water passing through the multiple tunnels in series of the heat exchangers; they also compared the cost of operating a composting, solar thermal and ground source heat pump system, and found that composting with HEH was the most reliable one. The heat from composting could be combined with solar updraft tower, it was reported that composting could supply 34% of the total energy gain with HEH method (Anderson et al., 2016). Smith and Aber (2018) found that heat recovered by HEH method could be combined with a heat sink for a food waste composting plant. The vapor temperature higher than 50 °C was sufficient for heat recovery. Bajko et al. (2019) built and tested the HEH method as a part of pilot-scale composting heat recovery system, and they suggested that this method could also be extended to larger scales composting operations.

2.4.4 Percolation water method

Another possible heat recovery method is percolation water (PW) method. In this method, water is sprayed to the composting pile, and the water percolates through the composting substrate via gravity. During percolation process, the percolated water, or leachate, is heated by the composting pile via conduction depending on the retention time. The water is usually collected on the bottom of the composting pile and flowed to a buffer tank for heat recovery. After heat recovery (usually by using a heat exchanger), the water is then pumped from the buffer tank to the top of the composting pile and sprayed again for next circulation. The PW method is only possible if the composting substrate has big particle size and high porosity, like wood chips. This principle was tested in Leeuwarden, the Netherlands in an insulated 80 m^3 tank (Scholtens, 2018). In this case, the percolated water could reach temperatures between 40 and 50 °C (unpublished paper).

The percolated water is usually considered as a source of nutrients or inoculum (Joanna et al., 2005; Ming et al., 2008; Roy et al., 2018). The advantage of this method is that it can conserve the microorganisms and nutrition, which is important in nutrients-poor composting systems (Ming et al., 2008). Furthermore, PW method enhances the circulation of nutrition and microbial activity (Zheng et al., 2020), which is optimal for a homogenized composting. The disadvantage of this method is that it is hard to control the retention time of the percolation process, and the humidity of the composting process and final compost product.

2.4.5 Low-temperature heat recovery technologies

Heat pump (HP) is one of the low-temperature heat recovery technologies. HP is commonly used for space cooling and heating application. HP can convert low-temperature heat to high-temperature heat by using an electrical pump, which makes it a promising heat recovery technology for composting. It can efficiently transfer heat from the exhaust air of the composting to the water that is needed to be heated (Willem et al., 2017). The coefficient of performance (COP) of HP is strongly dependent on the temperature of the composting. Once the COP has been determined, it is possible to estimate the heat recovery rate. It was reported that a HP could recover 1 kW/m^3 energy from a continuous yard waste composting in the thermophilic stage (Jaccard et al., 1993). Keil et al. (2008) reported that HP could provide a hot water with a constant temperature at 82 °C by recovering heat from a municipal waste composting plant in Germany with a COP of 1.6. The possibility of using HP to recover heat from composting for civil use was evaluated by Di Maria (Di Maria et al., 2008); result shows that the HP increased both the temperature and amount of heat released by composting (at 55-65 °C), achieving about 4000-5000 kJ/kg OM heat at 80-90 °C with a COP ranging between 3.5 and 6 (Di Maria et al., 2008).

Organic Rankine cycle (ORC) is a promising technology to recover heat from composting due to its simplicity, high reliability and economic advantages (Yari et al., 2015). ORC uses organic working fluids with low boiling points to recover low-temperature heat, and generates electricity by using an evaporator and a turbine (Chen et al., 2010). ORC has low maintaining and personnel costs, however, the energy conversion efficiency is usually low (Schuster et al., 2009). ORC is new and has been seldomly studied in composting systems. It was reported that the ORC could be a suitable approach to recover heat in the exhaust air of a MSW composting; in this case, the temperature of exhaust air ranged between 316-340 K while the energetic efficiency ranged between 1.5-11 % (Micalea, 2014). Di Maria et al. (2014a) analyzed the possibility of recovering heat from integrated composting and anaerobic digestion of OSW to generate electricity by using ORC. The result shows that combustion of biogas could increase the temperature of the exhaust air arising from a composting from 340 to 510 K, and increase the electricity production generated by ORC from 1 to 25 kW (Di Maria et al., 2014a). Moreover, combustion of solid fuel could also increase the temperature of exhaust air from 330 to 510 K, and could improve the electricity generation by OCR from 9 to 12 kW (Di Maria et al., 2014b).

Another low-temperature heat recovery technology used for composting is thermoelectric generators (TEGs). By using TEGs, the heat from composting process can be directly converted into electrical energy. TEGs follows the principle of Seebeck effect (Martín-González et al., 2013). TEGs has long life, high safety, high reliability, and no harmful effect on the environment by producing any pollution and noise (He et al., 2015; Zarifi & Mirhosseini Moghaddam, 2020). However, the application of TEGs is limited in power generation due to the low energy conversion efficiency (Liu et al., 2020). It was reported that TEGs with composting process could generate a maximum voltage about 11.3 V, a maximum current of 18.5 mA, and a maximum power density of 175 mW/m^2 at a temperature gradient of 20 °C (Rodrigues et al., 2018). Besides, Shangguan et al. (2020) verified that the temperature difference between composting and environment could be used to generated electricity by using TEGS; they reported that TEGs could generate over 7 W energy with 8.8-18.6 V voltage from an aerobic composting under a temperature difference of 40 °C.

2.5 Heat Utilization from Composting

After recovery, the heat is ready for utilization as a renewable energy source. Without utilization of temperature increasing technologies like heat pump, the outlet temperature of heat generated from composting ranges from 20 to 70 °C depending on different composting processes and different recovery methods. The temperature fits well for building applications such as air heating, floor heating, and domestic hot water service (Kimman, 2019; Tucker, 2006; Walther et al., 2016). Besides, the heat from composting could be used for bath/swimming pool (Bajko et al., 2018; Loggia et al., 2019; Pain, 1972), and fishing pond (Seki et al., 2014). Often, a storage tank is needed to buffer the temperature and energy demand (Smith et al., 2017).

Heat from compost can also be used in agricultural field. The easiest utilization of the generated heat is the hotbed, which is commonly used in cold regions. It was reported that farmers in northern China used composting as hotbed to increase the yield of crops over 2000 years ago (Brown, 2014). It was also reported that using a composting of kitchen waste and garden waste as a hotbed in north-eastern Poland could accelerate radish yield by 5 days; the residue compost could be effectively used for garden purpose (Neugebauer, 2018). In addition, composting can also be used for greenhouses, because the generated CO_2 and heat from composting is amendable to greenhouses. It was reported that the heat generated from composting could meet the heat demand for a greenhouse to grow food during 12 months (Gilson, 2009). Moreover, the greenhouse supported by a composting could improve the yields of celery, leaf lettuce, stem lettuce oily sowthistle, and Chinese cabbage by 87%-270% compared to the control greenhouse (Jin et al., 2009). In addition to greenhouse, the heat generated from composting could be used for brooding chicken (Roland Mote & Griffis, 1982).

Heat from composting can also be used to heat other facilities that require moderate temperatures. It was reported that composting could be used to heat an anaerobic reactor. (Cheng et al., 2016). Smith (2016) also reported that heat from a composting could warm three 26500 L anaerobic digester tanks and maintain temperatures at 38 °C. Besides anaerobic digestions, the generated heat from composting could be used for melting snow and ice in winter (Allain, 2007) and dehydrating sludge (Rada et al., 2014).

The heat generated from composting, especially in commercial scale reactors, has great potential for wide utilization range. Heat reusing can be attractive for energy-saving, economic effects and environmental protection if the heat source and the heat demanding object are close to each other (Rada et al., 2014).

2.6 Outlook

Heat from composting has been studied extensively in last decades. However, there are challenges behind the heat generation, recovery and utilization, which requires future research. First of all, the heat production rate is usually dynamic during the composting due to the low heterogeneity of the OSW. For example, lignin degraders usually grow best between 35-50 °C (Tuomela et al., 2000), while temperature higher than 55 °C is suitable for manure composting (Li et al., 2019; Miyatake & Iwabuchi, 2005). The dynamic heat production rate and the volume change of the compost pile make the controlling of heat recovery difficult: insufficient heat recovery from composting will over-heat the composting pile; while too much heat recovery will cool the composting pile, which both will inhibit the degradation process. Therefore, studies relating to heat recovery and heat production rate are definitely important and necessary for future research. Although the heat production rate is high in the mesophilic phase and thermophilic phase, there is still a lot of heat generated in the cooling phase and maturation phase. Future work could also focus on extending heat recovery for longer than the first two phases (Nwanze & Clark, 2019).

Secondly, a thermophilic stage for at least three days is necessary for pathogen elimination (Burge et al., 1978; Qian et al., 2016). Therefore, the heat recovery may be limited during the thermophilic stage that is required to eliminate pathogens. Although we summarize the different heat recovery methods in this review, a model of the balance between the heat recovery and the risk elimination remains unknown.

Finally, although heat has been reused to various applications, there is still a knowledge gap between scientific results and actual implementation. Special attention should be paid to the Biomeiler. Biomeiler is a large composting pile of wood chips whose aim is to generate the high quality wood compost, and reuse the generated heat for hot water service (Pain, 1972). Biomeiler has now been successfully built in different countries, such as Canada, Chile, Netherlands, Germany and Italy (Biomeiler, 2020). But Biomeiler tends to dry out during the composting process which needs more research. Besides, new studies are needed for the economic, technological and environmental analysis of reusing heat from composting.

2.7 Conclusion

Heat from composting has gained more and more interests as it helps meet the energy demand and reduce the global warming. This review paper summarizes heat generated from composting, regarding the heat potential, heat production, heat recovery methods, and heat utilization.

Composting substrates generally consists of four OSWs namely food waste, lignocellulose waste, manure waste and sludge waste. These four OSWs have high heating values, which makes composting a process with high heat generation. The heating values can be estimated by ultimate analysis, proximate analysis, and composition analysis or determined by using calorimetric technologies.

Different methods estimating actual heat production from composting are comprehensively summarized and discussed. The degradation method is widely used to describe the composting process adequately. However, this method must be adjusted to different composting conditions to get a better prediction of heat production. O_2 method is easy and simple. It can be measured continuously to illustrate the composting process. This method has been extensively applied both on big scale reactors and small-scale reactors. Another method to calculate the heat production during composting is by monitoring the heat balance. This method measures the heat balance components other than heat production. This method is suitable for pilot scale and commercial scale because the heat monitoring data is only valid at large scale reactors. Moreover, the CO_2 evolution method, temperature method, and initial and final heating value method are also discussed in-depth.

We also summarized and discussed different heat recovery methods from composting. Water jacket method has low heat transfer efficiency and is only suitable for the small-scale reactors. Tube buried-in pile method collects the heat from the composting pile. This is commonly used in reactors with long retention time. Heat exchanger in the exhaust air is most common in big scale reactors. It has high heat recovery efficiency because it can collect the latent heat. For the composting of wood chips, the water percolation method is demonstrated. New technologies have also been developed to recover low-temperature heat from composting. Heat pump can increase the heat from composting both in quality and quantity. ORC and TEGs have low energy transfer efficiency but the output energy is electricity, which has wide utilization.

Recovering heat from composting is usually used for building applications, such as hot water service, floor heating and wall heating. Moreover, the heat can be used in agricultural field to increase the crop yield as hotbeds and greenhouses. Exploring other application of the heat from composting, given its characteristic, is also of use and interest.

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Chapter 3

Effect of Nitrogen, Phosphorus and pH on Biological Wood Oxidation at 42 °C

Shiyang Fan, Yue Sun, Annemiek ter Heijne, Wei-Shan Chen, Cees J.N. Buisman

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Abstract

Biological wood oxidation (BWO) is proposed as a cleaner alternative to wood combustion for heat production and wood waste management. Currently, BWO has not been extensively studied and little is known about it. Nevertheless, given the composition of wood residues, which is dominated by carbon, nutrient availability may become a limiting factor during BWO. Our objective was to study the nutrition requirement for sustaining the BWO. For this purpose, three different factors including nitrogen addition, phosphorus addition and pH, were studied. Oxygen consumption and mass loss were monitored and used to evaluate the impact of nutrition on BWO and to calculate the theoretical heat production. The result showed that nitrogen addition at a relatively low level (2.5-10 mg/g) enhanced the cumulative oxygen consumption by 60-124 % and mass loss by 28-95 %, when compared with the BWO without nitrogen addition. The highest nitrogen addition examined in this research (20 mg/g), on the other hand, did not enhance BWO. Different phosphorus addition (0.5-5 mg/g) and pH (4-6) had little impacts on BWO. The highest heat production rate (0.63 W/kg dry wood biomass) was obtained by 2.5 mg/g nitrogen addition with a 95-day incubation. This suggests that nitrogen addition is required and able to sustain BWO. Besides, the cumulative oxygen consumption showed a good linear relationship with mass loss. This study provides the first indication on the effective quantify of nitrogen addition for enhancing BWO, which contributes to the selection of nutrient source for BWO in the future studies.

Keywords: *biological wood oxidation, wood waste, nitrogen, phosphorus, pH, fungi*

3.1 Introduction

Wood waste is the abundantly available and highly renewable natural resource in the world (Fan et al., 2016). It is reported that the EU generated around 60 million tons of wood waste in 2014 (Nicolae et al., 2019). Wood waste is frequently burned for energy, especially for heating (Sarsaiya et al., 2019). It is reported that about 60% of the biomass energy is generated from combustion of wood and wood residues (Saratale et al., 2019). However, combustion of wood results in emissions of harmful components, such as SO_2, CO, NO_x and fine particles, to the environment (Permchart & Kouprianov, 2004).

To overcome these environmental drawbacks, composting is proposed as an environment-friendly wood waste management method. In traditional composting, wood waste is usually used as an additional material to achieve a suitable carbon-to-nitrogen ratio (C:N ratio), due to its high carbon content. However, the degradation of wood waste in composting is slow, mainly because lignin inhibits the enzymatic access to cellulose (Vermaas et al., 2015). Wood waste usually degrades faster in high-temperature phase (thermophilic phase) of composting and degrades much slower in the low temperature phases (mesophilic phase, cooling phase and maturation phase) (Zhao et al., 2016; Zhu et al., 2019).

Given this context, continuously thermophilic composting (CTC) is proposed as a process to achieve a rapid degradation of materials that are difficult to degrade at the mesophilic conditions, e.g. lignin-rich material like wood waste. CTC maintains the composting at thermophilic phase; the temperature of CTC is usually between 40-70°C (Chang et al., 2019; Hosseini & Aziz, 2013; Jiang et al., 2015; Schulze, 1962; Suler & Finstein, 1977; Waqas et al., 2018; Xiao et al., 2011; Xiao et al., 2009). Although CTC has been applied to substrates like municipal solid waste (Xiao et al., 2009), dairy manure (Li et al., 2019a; Qian et al., 2016), food waste (Jiang et al., 2015; Waqas et al., 2018) and mixture of these streams (Li et al., 2019b; Suler & Finstein, 1977; Xiao et al., 2011), it has not been applied to wood waste yet. Moreover, the heat produced in the degradation process of CTC is often overlooked, which is a potential source for renewable heat.

Therefore, we proposed biological wood oxidation (BWO) process for wood waste management. BWO is the CTC applied to wood waste. Like composting, BWO is an exothermic reaction in which only carbon dioxide and water vapor are released to the atmosphere with the presence of oxygen (Caizán Juanarena et al., 2016). The BWO can be described in the following reaction (1):

$$CH_{1.5}O_{0.7} + 1.03O_2 \rightarrow CO_2 + 0.734H_2O + \text{Heat} \qquad (1)$$

The temperature of BWO is usually between 40-55 °C, because a temperature higher than 55 °C may inhibit the growth of thermophilic fungi that play a crucial role in BWO (Caizán Juanarena et al., 2016; Donald G. Cooney & Emerson, 1965; Tuomela et al., 2000). The main aim of BWO is not only to degrade wood waste rapidly but also to generate sustainable heat from it.

Wood degradation at room temperatures (20-37 °C) has been widely studied (Beck et al., 2018; Graham et al., 2017; Hardersen & Zapponi, 2018; van der Wal et al., 2007). However, there is little information about BWO at temperature between 40-55°C. A recent study demonstrated the heat production of BWO and investigated the effect of addition of two fungi (one thermophilic and one thermotolerant) at 41 °C (Caizán Juanarena et al., 2016). The result showed that the natural biota in wood were as effective in degrading the wood as the artificially added fungi (Caizán Juanarena et al., 2016). However, the performance of BWO still requires improvement; for example, the effect of other factors, such as nutrient requirement, temperatures, wood sizes and moisture content have not been studied yet.

The nutrient requirement, such as nitrogen and phosphorus, is necessary for the microorganisms in BWO process. We hypothesized that nutrient availability will be a limiting factor in BWO, as very limited nitrogen and phosphorus is present in wood biomass. Nitrogen is an essential element for the growth and development of microorganisms, as nitrogen is a major constituent of the amino acids, peptides and proteins, purine and pyrimidine bases of the nucleic acid, chitin and other compounds (Cowling & Merrill, 1966). Phosphorus is also important for fungal growth, as phosphorus helps fungi to take up nitrogen (Nicholas & Militz, 2008; Rayner & Boddy, 1988). In addition to nitrogen and phosphorous, acidity also influences the decomposing activity of fungi. The pH may change the degradation speed of wood by alerting the activity of lignocellulase (Allison et al., 2009; Liers et al., 2011; van der Wal et al., 2007).

This study aims at investigating the effect of nitrogen addition (N addition), phosphorus addition (P addition) and pH on BWO at the temperature 42 °C. We analyzed the BWO based on wood mass loss and oxygen consumption. The oxygen consumption was correlated to the amount of heat production, and the heat production was analyzed and correlated to the mass loss.

3.2 Materials and Methods

3.2.1 Experimental setup

The experimental setup can be found in Fig. 3-1. Bottles with a volume of 650 mL were filled with 6 g of dried vermiculite to completely cover the bottom of the bottles. Each bottle had one group of 5 woodblocks. Then, 11.7 mL of the different nutrition solutions was slowly added on the top of the woodblock to ensure that the nutrition solution was well distributed. The amount of the nutrition addition was considered enough to maintain the moisture content of a bottle experiment during the whole experimental period. After that, all bottles were closed with lids and septa. Temperature was maintained at 42 °C by placing the bottles in an oven (Caizán Juanarena et al., 2016).

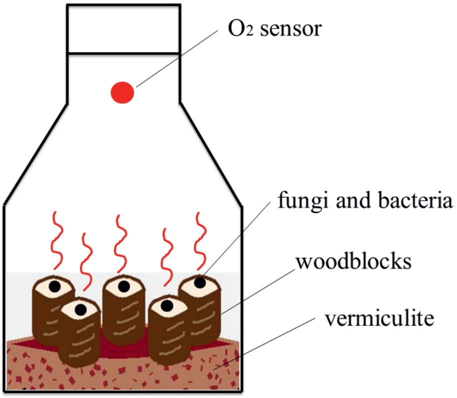


Fig. 3-1 Experiment set-up of bottles.

3.2.2 Woodblocks

Branches of a birch tree were collected in Wageningen, Netherlands. The branches were cut into cylinders by a handsaw. Every woodblock had a diameter between 1.5-2.0 cm and a length around 1.0 ± 0.2 cm. All woodblocks used in the experiments were individually numbered and weighed. After that, woodblocks were selected and distributed into different groups for further research. Each group had six woodblocks with a total dry weight of 4.689 ± 0.005 g. The chemical composition of birch was not determined; instead, the following general composition of wood was assumed: 50% carbon (C); 43% oxygen (O), 6% hydrogen (H), and around 1% of other elements such as nitrogen (N), sulfur (S) and phosphorus (P) (Caizán Juanarena et al., 2016).

3.2.3 Nutrient solution

Nutrient solution consisted of basic nutrition solution, N addition (in NH_4Cl) and P addition (in KH_2PO_4). Besides, the pH of the nutrition solution was adjusted by adding HCl and NaOH. The summary of each study is shown in Table 3-1.

Basic nutrition solution- The basic nutrient solution was modified from the nutrition recipe of Jin's work (Jin et al., 2006). The basic nutrient solution, supplying macronutrients, micronutrients and trace element, contained 1.0 mg/L $MgSO_4 \cdot 7H_2O$, 120 mg/L $FeCl_3$, 50 mg/L H_3BO_3, 10 mg/L KI, 45 mg/L $MnSO_4 \cdot H_2O$, 20 mg/L $Na_2MoO_4 \cdot 2H_2O$, 75 mg/L $ZnSO_4 \cdot 7H_2O$, 50 mg/L $CoCl_2 \cdot 6H_2O$, 20 mg/L $KAl(SO_4)_2$ $12H_2O$, 13.2 mg/L $CaCl_2 \cdot 2H_2O$, 10 mg/L NaCl.

N addition - Different amounts of N were added to the basic nutrient solution. The N addition amount was 0 mg/g, 2.5 mg/g, 3.3 mg/g, 5 mg/g, 10 mg/g and 20 mg/g basis of dry woodblock, respectively. 1 mg/g P was also added in the nutrition. Five replicates were used for each nitrogen level study.

P addition - Different amount of P was added to the basic nutrient solution. The P addition amount was 0 mg/g, 0.5 mg/g, 1.0 mg/g, 2.0 mg/g and 5.0 mg/g basis of dry woodblocks, respectively. 10 mg/g N basis of dry woodblock was also added in the nutrition. Five replicates were used for each phosphorus level study.

pH - The pH of the nutrition solutions was changed by adding 1 mmol/L HCl or 1mmol/L NaOH to obtain a nutrient solution at pH 4, 5 and 6, separately. The nutrient solution without pH adjustment was tested as control. 10 mg/g N in NH_4Cl and 1 mg/g P in KH_2PO_4 were added in the basis nutrition. Five replicates were used for each pH study.

The characteristics of the different nutrition solution can be found in Table 3-1.

Table 3-1 Characteristics of experiments.

Experiment	N amount (mg/g)	P amount (mg/g)	pH value
N addition	0, 2.5, 3.3, 5, 10, 20	1	Natural pH (5.73)
P addition	10	0, 0.5, 1, 2, 5	Natural pH (5.37-5.82)
pH	10	1	Natural pH (5.73), 4, 5, 6

3.2.4 Microbial Inoculum

The inoculum was initially from a garden waste composting pile. The composting materials were taken from this pile and further incubated at 42 °C for more than 4 months to yield the inoculum. Nutrient solution and fresh wood were supplied regularly during the incubation. For this study, around 0.1 ± 0.01 g inoculum (i.e. decayed materials from the incubation) were added in each bottle.

3.2.5 Oxygen measurement

The oxygen concentration in gas phase was measured by Fibox 4 trace and Sensor Spot SP-PSt 3 (PreSens, Germany) every 1-4 days. Once the oxygen level in the experimental bottle decreased to around 10 %, bottle was cooled to room temperature and opened in the flow cabinet to refresh the inside air and to ensure that the concentration of oxygen returned to atmosphere levels. After the refreshment, the bottle was returned to the oven to continue the experiment.

3.2.6 Mass loss

First, the woodblocks were washed to remove the vermiculite and fungal mycelium. Then the woodblocks were placed in the over at 105 °C for 24 hours to remove the moisture. After that, the mass loss was determined on the basis of the initial (in the beginning of the experiment) and final dry weight (at the end of the experiment) as indicated in the following equation.

$$\text{mass loss (\%)} = \frac{final\ dry\ weight - initial\ dry\ weight}{initial\ dry\ weight} \times 100\%$$

3.2.7 Heat production

It is not possible to measure the actual heat production or extract the heat production due to the small scale of the experiment. The theoretical heat production in BWO was calculated based on oxygen consumption because oxygen is independent of molecular substrate composition (Hamelers, 2001). The theoretical heat production (H, in kJ) of the reaction was calculated via the following equation:

$$\mathrm{H} = \Delta O_2 \times h$$

Where ΔO_2 is the oxygen consumption (mol) and h is the heat released by microorganisms expressed as kilo joule per mol of oxygen consumed (kJ/mol). This is commonly done in biomass combustion and composting modelling (Caizán Juanarena et al., 2016; de Guardia et al., 2012; Jenkins et al., 1998; Kaiser, 1996). Considering there was not any rigorous method in literature about the heat release via BWO, h was fixed at 467.5 kJ/mol O_2 and was maintained constant throughout the process. 467.5 kJ/mol is the heat released via glucose combustion per unit and glucose is the most abundant carbohydrate present in wood (Caizán Juanarena et al., 2016). Since the duration time of the experiments were different, the heat production rate (W), in W/kg DM (dry matter), was calculated by modifying with the duration time and dry weight of birch tree via the following equation:

$$\mathrm{W} = \frac{H}{t \times m} \times 1000$$

Here t is the duration time (s) and m is the initial dry weight (g).

3.2.8 Theoretical oxygen consumption

The theoretical oxygen consumption (TO, in mmol) was calculated via the wood biological oxidation reaction (1) by assuming that the wood was degraded homogeneously.

$$TO = \frac{mass\ loss \times initial\ dry\ weight \times 1.03}{M(C) * 1 + M(H) * 1.5 + M(O) * 0.7}$$

Where $M(C)$is the atomic weight of C (12g/mol), $M(H)$ is the atomic weight of H (1 g/mol), $M(O)$ is the atomic weight of O (16 g/mol).

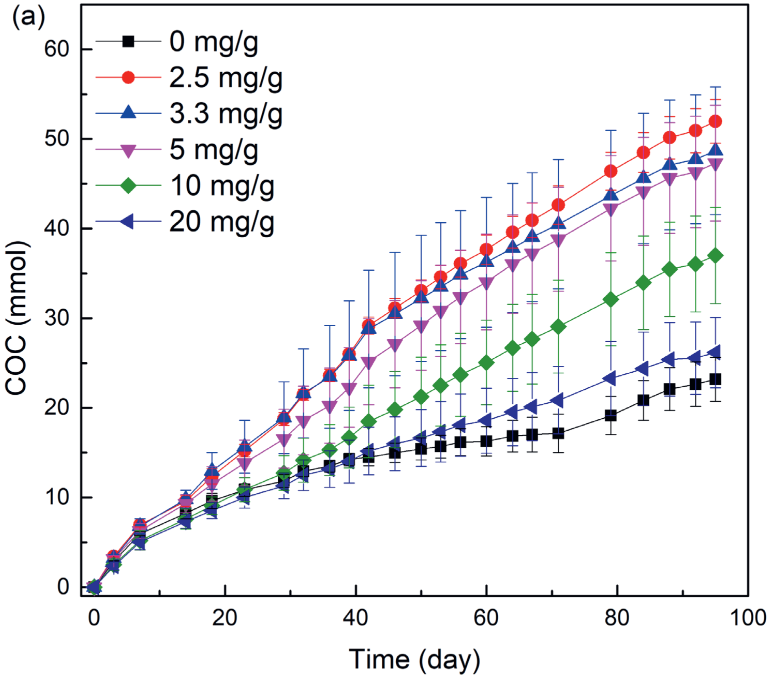
3.2.9 Statistical analysis

The results are the mean of five replicate analyses. All data were analyzed by using IBM SPSS Statistic 23. For some parameters the correlations were computed.

3.3 Results

3.3.1 Effect of N addition on oxygen consumption

Oxygen consumption was used as a key performance indicator for the wood biomass degradation in this research (Caizán Juanarena et al., 2016). The cumulative oxygen consumption (COC) of different N additions is shown in Fig. 3-2 (a), and the oxygen consumption rate (OCR) is shown in Fig. 3-2 (b).



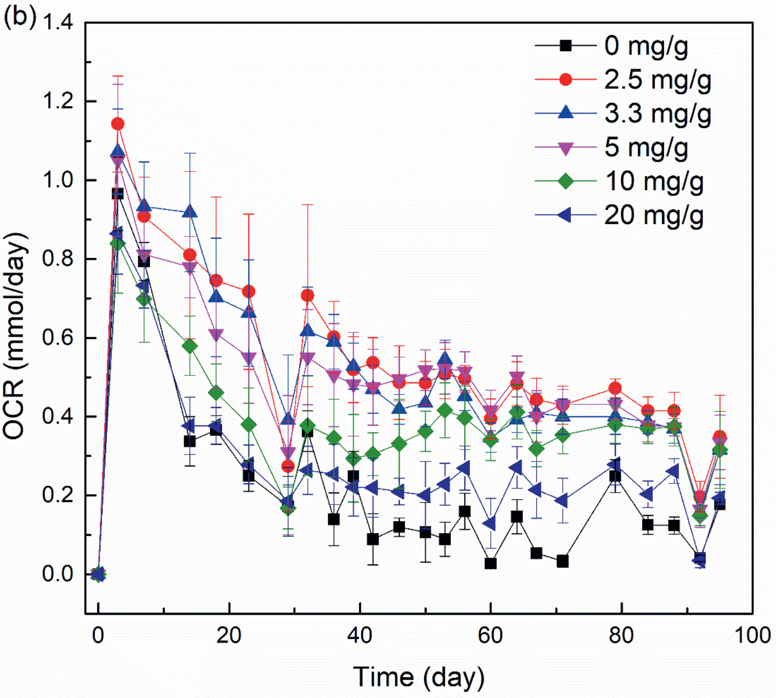


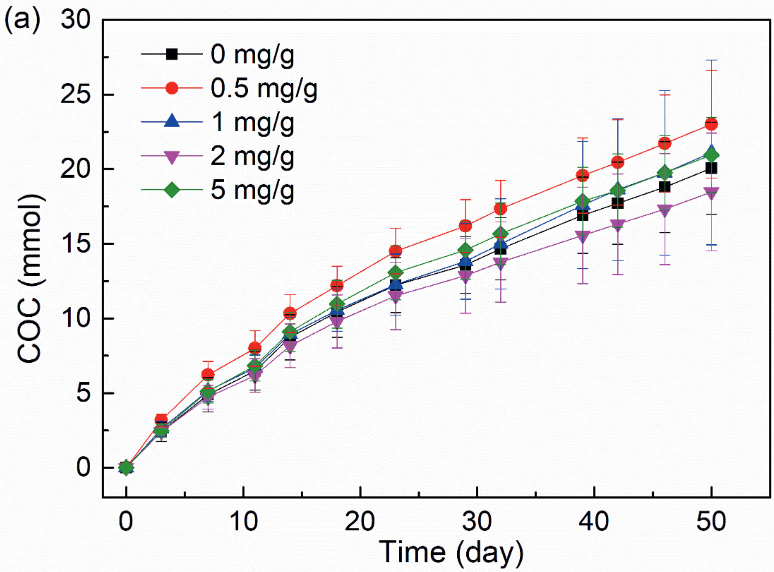
Fig. 3-2 (a) the effect of different N addition on cumulative oxygen consumption; (b) the effect of different N addition on oxygen consumption rate. Average and standard deviation was calculated for 5 replicates. Addition of N significantly improved the oxygen consumption at N addition from 2.5-10 mg/g at 42 °C.

As shown in Fig. 3-2 (a) and Fig. 3-2 (b), N addition changed the COC and OCR obviously. BWO without N addition (0 mg/g) consumed the lowest COC while the addition of 2.5 mg/g N addition obtained the highest COC. The COC of 2.5 mg/g N addition was 2.6 times as much as 0 mg/g N addition. For different N addition levels examined in this research, the lower N addition amount, the higher COC. There was a statistically significant difference in COC between 2.3, 3.3 and 5 mg/g N addition and without N addition after 95-day incubation (ANOVA, $P < 0.05$), which means that the N addition between 2.5-5 mg/g could significantly increase the oxygen consumption.

However, no statistically significant difference was found in the COC between 0 mg/g N addition group and 20 mg/g N addition group (LSD, P = 0.820 > 0.05), which shows that 20 mg/g N addition did not enhance the COC. As for the Fig. 3-2 (b), all OCR generally reached the highest in the first three days, then slowly decreased until day 30, and became relatively stable in the next 60 days, with some fluctuations. N addition significant changed the OCR in the whole duration time.

3.3.2 Effect of P addition on oxygen consumption

The effect of P addition on COC and OCR is shown in Fig. 3-3 (a) and Fig. 3-3 (b), respectively.



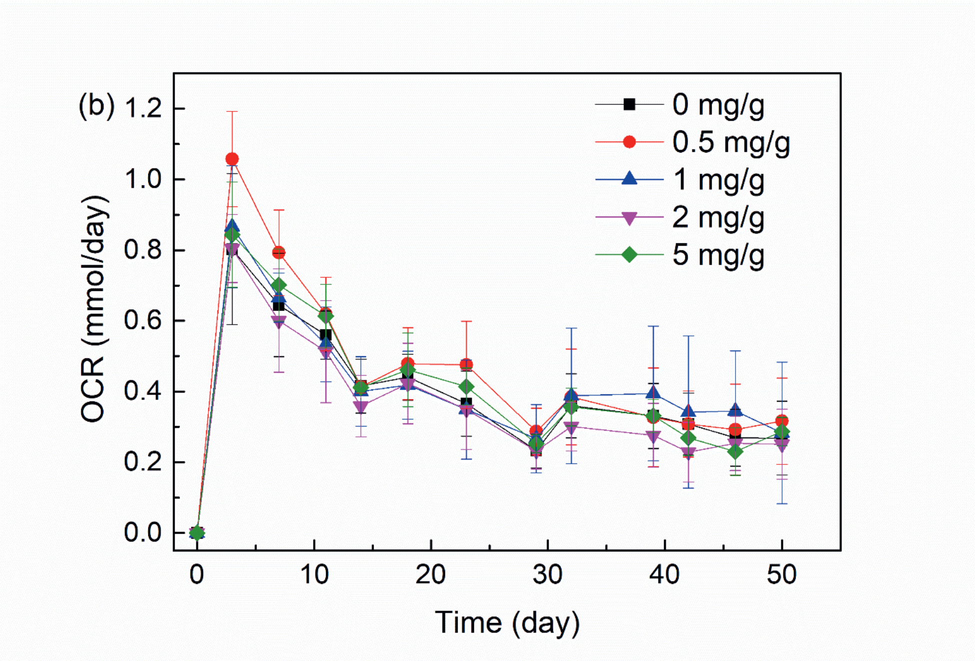


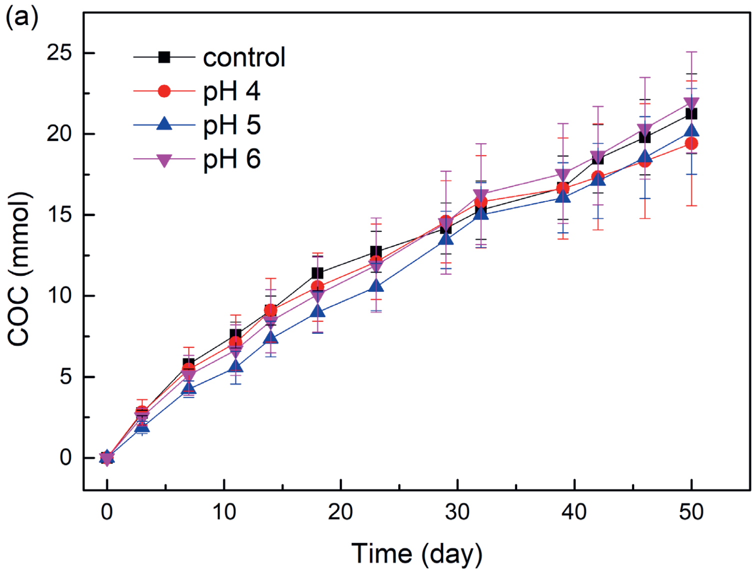
Fig. 3-3 (a) the effect of different P addition on cumulative oxygen consumption; (b) the effect of P addition on oxygen consumption rate. Average and standard deviation was calculated for 5 replicates. P addition between (0-5 mg/g) had little effect on oxygen consumption at 42°C.

As shown in Fig. 3-3 (a) and Fig. 3-3 (b), the P addition did not significantly change the COC and OCR as no statistically significant difference was found in COC between different P addition levels after 50-day incubation (ANOVA, $P > 0.05$). The different P addition had little effect on the COC in this study. The 0.5 mg/g P addition obtained the highest COC (23 mol). This number was only 1.25 times COC as much as 2 mg/g P addition did, which consumed the lowest COC (18 mmol). The OCR (Fig. 3-3 (b)) of different P additions followed a similar trend as for N. The OCR of P addition was highest in the start, then dropped continuously after three days and then became stable in the next stage. As no significant difference was found, the tests of P addition concentration at 0 mg/g, 1mg/g, 2mg/g and 5mg/g were stopped for mass loss determination on day 50. The test of 0.5 mg/g was continued for another 34 days

(Appendix Figure S1 (a) and Appendix Fig. 3-S1 (b)). We conclude that the P addition had no significant effect on the oxygen consumption.

3.3.3 Effect of pH on COC and OCR

The effect of different pH on COC and OCR is shown in Fig. 3-4 (a) and Fig. 3-4 (b), respectively.



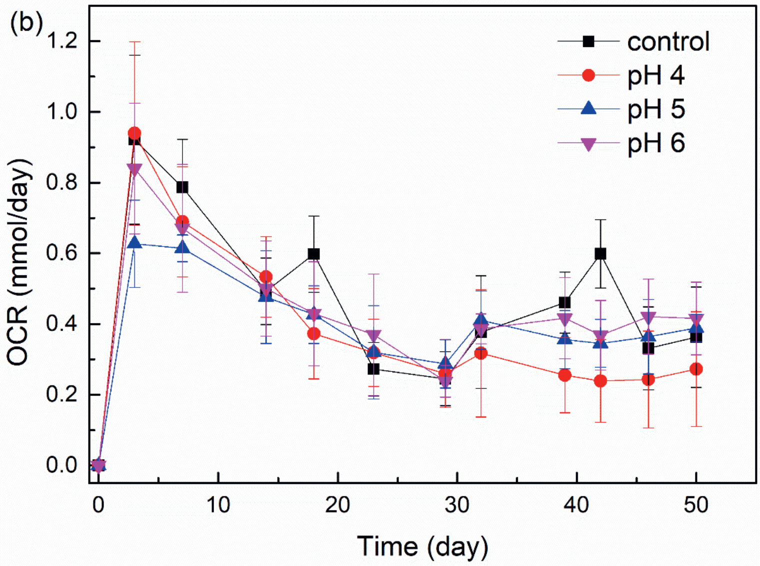


Fig. 3-4 (a) The effect of different pH on cumulative oxygen consumption; (b) the effect of different pH on oxygen consumption rate. Average and standard deviation was calculated for 5 replicates. pH range between 4-6 had little effect on oxygen consumption at 42 °C.

As shown in the Fig. 3-4 (a), adjusting pH did not obviously change the COC as no statistically difference was found in COC between different pH after 50-day incubation (ANOVA, $P > 0.05$). That means the pH between 4-6 did not have significant effect on the COC. The final COC of control, pH 4, pH 5 and pH 6 was 21, 19, 20 and 22 mol, respectively. As shown in the Fig. 3-4 (b), the OCR of different pH was highest in the beginning, then dropped continuously after three days and then became stable. As no significant difference was found, the experiment of pH control, pH 4 and pH 5 were stopped for mass loss determination on day 50. The test of pH 6 was continued for another 34 days (Appendix Figure 3-S1 (a) and Appendix Fig. 3-S1 (b)). We conclude that the pH range between 4-6 had little effect on the oxygen consumption.

3.3.4 Effect of nutrient addition on mass loss

The mass loss of N addition, P addition and pH is shown in Table 3-2 (a), Table 3-2 (b) and Table 3-2 (c), respectively. As shown in Table 3-2 (a), N addition between 2.5-10 mg/g significantly increased the mass loss compared with the 0 N addition group (ANOVA, $P < 0.05$), which means that N addition could enhance the wood degradation. But the difference between 0 mg/g N addition and 20 mg/g N addition was not significant (ANOVA, $P > 0.05$). It shows that 20 mg/g N addition had little effect on the mass loss. The highest mass loss was found under 2.5 mg/g N addition, which was 1.95 time as much as the mass loss without N addition. The mass loss difference between 2.5, 3.3 and 5 mg/g N addition was not significant (LSD, $P > 0.05$), which means the effect of 2.5, 3.3 and 5 mg/g N had significant effect on mass loss.

As shown in Table 3-2 (b), the highest mass loss was found under 1 mg/g P addition after 50-day incubation, however, the mass loss was only 1.1 times higher compared to the lowest mass loss under 2 mg/g P addition. The addition of P had little significant influence on mass loss in this study (ANOVA, $P > 0.05$). The mass loss of 0.5 mg/g P addition was 24.3% after 84 days.

As shown in Table 3-2 (c), the mass loss of natural pH, pH 4 and pH 5 after 50 days was 18.9, 15.8 and 16.0%, respectively. Adjusting pH had little impacts on the mass loss (ANOVA, $P > 0.05$). For the pH 6 experiment, the mass loss after 84 days was 22.91%.

Table 3-2 (a) The effect of N addition on mass loss and heat production rate after 95-day incubation with 1mg/g P addition and pH 5.73.

N addition	0 mg/g	2.5 mg/g	3.3 mg/g	5 mg/g	10 mg/g	20 mg/g
Mass loss (%)	16.9 ± 1.4	33.0 ±7.5	28.7±3.4	29.4±3.4	21.6±1.4	16.4±2.2
W (W/kg DM)	0.28	0.63	0.59	0.58	0.45	0.33

Table 3-2 (b) The effect of P addition on mass loss and heat production rate after 50-day incubation with 10 mg/g N addition and pH range 5.37-5.82.

P addition	0 mg/g	0.5 mg/g	1 mg/g	2 mg/g	5 mg/g
Mass loss (%)	16.5 ± 2.1	24.3 ± 3.1*	17.3 ± 4.1	15.7 ± 2.6	15.9 ± 1.4
W (W/kg DM)	0.46	0.49*	0.48	0.43	0.48

Table 3-2 (c) The effect of pH on mass loss and heat production rate after 50-day incubation with 10 mg/g N addition and 1 mg/g P addition.

pH	Natural pH	pH 4	pH 5	pH 6
Mass loss (%)	18.9 ± 1.6	15.8 ± 2.1	16.0 ± 1.9	22.9 ± 2.6*
W (W/kg DM)	0.49	0.45	0.47	0.49*

* means the data was after day 84

3.3.5 Effect of nutrient addition on heat production rate

The heat production rate (W) for N addition, P addition and pH adjustment is shown in Table 3-2 (a), Table 3-2 (b) and Table 3-2 (c), respectively. As the heat production rate is linearly correlated to COC, the effect of N addition, P addition and pH on heat production rate was not analyzed. Generally, BWO with N addition obtained a heat production rate more than 0.45 W/kg DM. The highest heat production rate (0.63 W/kg DM) was obtained by 2.5 mg/g N addition after 95 days. Whereas the lowest heat production rate (0.28 W/kg DM) was found by BWO without N addition.

3.4 Discussion

3.4.1 Effect of nutrition addition on BWO

All OCR in this study was highest in the first three days. After that, all OCR decreased and then became stable. A possible reason for this peak in the first three days could be the degradation of the readily degradable organic matters, such as sugar, organic acids, and easily accessible cellulose and hemicellulose (Eiland et al., 2001; Gunnarsson et al., 2008; van der Wal et al., 2007). After the depletion of the readily degradable organic matters, the complex matrix of cellulose, hemicellulose and lignin gradually started to degrade and mainly contributed the oxygen consumption after three days.

Although the effect of N addition on wood degradation has been studied extensively at room temperature, little information is known at temperatures between 40-55 °C. In this study, the N addition between 2.5-10 mg/g apparently improved COC and mass loss, but a further increase in N addition (20 mg/g) did not result in a further increase in COC and mass loss. This is in accordance with some studies at room temperatures (Egli & Quayle, 1986; Labosky Jr et al., 1991; Leatham & Kent Kirk, 1983; Reid, 1983; Yang et al., 1980). We proposed that the mechanisms of N addition on wood degradation at higher temperature (42 °C) was the same as room temperature. Without N addition, wood decay microorganisms can only obtain N from wood (mainly from lignin) for their growth. The microorganisms, mainly fungi, can produce lignocellulase enzymes such as lignin peroxidase and manganese peroxidases to degrade wood for N capture (Egli & Quayle, 1986; Labosky Jr et al., 1991; Leatham & Kent Kirk, 1983; Reid, 1983; Yang et al., 1980). Although the nitrogen limitation could trigger the ligninolytic activity (Jeffries et al., 1981), the low available N amount resulted in slow growth of biota (van der Wal et al., 2007) and low degradation of BWO. With N addition, easily consumed nitrogen such as NH_4^+-N and amino-N addition could help the biota save energy to obtain N (van der Wal et al., 2007). However, the excessive nitrogen level generally suppresses the lignocellulase production by suppressing the gene expression (Edwards et al., 2011; FOG, 1988; Leung & Pointing, 2002; Li et al., 1994;

Sinsabaugh et al., 1993). For example, the RNA of manganese peroxidases produced from *Phanerochaete chrysosporium* was detected only under conditions of nitrogen depletion (Li et al., 1994). As a result, the BWO rate was slow due to the low amount of lignocellulase enzymes. In this study, the best N addition range was 2.5-5 mg/g at 42 °C.

For the effect of P addition, Reid et al. (1979) found that P addition (1.0 mM) improved the lignin degradation and fungal growth of *Phanerochaete chrysosporium* at 39°C, compared to 0.2mM P addition, but he did not give any reasons for this (Reid, 1979). However, Jeffries (1981) found that P limitation did not inhibit the ligninolytic activity (Jeffries et al., 1981). In our study, P addition and P limitation did not have significant effect on BWO. We postulated that the N source used in P addition study was easily consumed (NH_4^+-N). The wood decay fungi did not need extra P to take up N (Nicholas & Militz, 2008; Rayner & Boddy, 1988).

Wood decay fungi usually grow best in the pH range between 3-6 whereas many wood decay bacteria and actinomycetes prefer a more neutral pH (Nicholas & Militz, 2008). In the effect of pH study, adjusting pH at a range between 4-6 had no significant effect on the oxygen consumption and mass loss at 42 °C. We inferred that it was the fungi that played the major role in the BWO at 42 °C and pH ranges between 3-6 did not change the fungal community apparently. Otherwise, the COC and mass loss between pH 3 and pH 6 would be significantly different. Pictures displaying the great white mycelia fungal growth on woodblocks surface are shown in Fig. 3-S2 (a), Fig. 3-S2 (b) and Fig. 3-S2 (c). This was similar to the result of Donnelly (1990), who found that the amount of microbial biomass, the cellulose degradation, and lignin degradation were not statistically different when the pH values were 4.5, 5.5 and 6.5 at temperatures of 4°C, 12°C and 24°C (Donnelly et al., 1990). Pometto and Crawford (1986) found little difference in mass losses in cultures with initial pH between 6.0 and 8.0 at 37 °C, however, he did not test the result below pH 6.0 (Pometto & Crawford, 1986).

3.4.2 Relationship between mass loss and cumulative oxygen consumption

The relationship between mass loss and COC was further analyzed. All experimental data, including N addition, P addition and pH, was analyzed together. The theoretical oxygen consumption (TO) was also calculated and plotted in Fig. 3-5. First, as shown in the Fig. 3-5, the COC generally increased with the mass loss of the woodblocks. A strong linear relationship was found between the mass loss and COC (ANOVA, $P < 0.05$, $y = 0.9629x-5.4578$, $R^2 = 0.9307$), suggesting that the increase in mass loss was generally associated with increase of COC. Second, the slop of theoretical data pints trend line ($y = 0.9134x$, $R^2 = 1$) is closed to the experimental data points trend line, which means the wood was degraded aerobically.

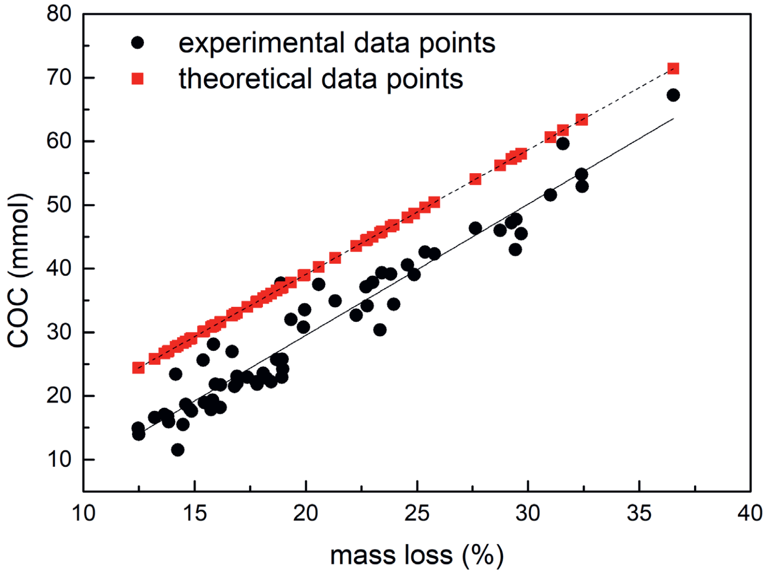


Fig. 3-5 The correlation between the mass loss and cumulative oxygen consumption. The mass loss and cumulative oxygen consumption had a strong linear relationship.

Third, there was a difference (average 11.7 mmol) between the theoretical data points and experiment data points, which means that at the same weight loss, the experimental COC was generally lower than the TO. We proposed one reason for this gap: part of the carbon was converted into biomass of microorganisms, which could be found in Fig. 3-S2 (a), Fig. 3-S2 (b) and Fig. 3-S2 (c).

3.4.3 Heat production via BWO

Wood biological oxidation by bacteria and fungi has been extensively studied at room temperatures (Ahn et al., 2007; Johnston et al., 2016; Kielak et al., 2016; Kirker & Winandy, 2014; Rinta-Kanto et al., 2016). There are very few studies that address heat production of BWO at temperatures between 40-55 °C. The only work about heat production was from Caizán Juanarena (Caizán Juanarena et al., 2016). Caizán studied the wood degradation and heat production caused by two (a thermotolerant and a thermophilic) fungi and natural biota at 41 °C. She calculated the heat production in the same way as we did. The heat production rate of 0.6 W/kg DM was obtained by *Phanerochaete chrysosporium* and 0.47 W/kg DM by unsterilized treatment after 36 days. Although there were some differences between her study and our study, such as the inoculum and the duration time, the heat production rate was at the same level (0.47 W/kg DM after day 36 in her study and 0.63 W/kg DM after 95 days in our study).

There are studies about composting addressing heat production. Among these studies, the numbers of heat production rate were generally highest in the first 1-3 weeks. With the increasing of composting time, the heat production rate decreased and the cumulative amount of the heat production increased. Irvine (2010) estimated around 7000-10000 kJ/kg heat was generated in 14 days during the composting of green waste, industrial sludge and solid waste; the heat production rate was 5.8-8.3W/kg DM after 14 days (Irvine et al., 2010). Maria (2008) obtained 4000-5000 kJ/kg OM (organic matter) from food and paper waste composting in 20 days, the heat production rate was around 2.3-2.9 W/kg DM after 20 days (Di Maria et al., 2008). Briski (2003) estimated around 7454 kJ/kg TS (total solid) heat was obtained from tobacco waste and flavoring agents composting lasting for 30 days and the heat production rate was 2.9 W/kg TS (Briški et al., 2003).

In our study, the maximum amount of heat production was 5184 kJ/kg DM after 95 days. This number is generally lower than the heat production in above studies because the wood waste is relatively difficult to degrade. Carbon in wood waste is the energy source for microorganisms. A small fraction of the carbon is used for microbial metabolism, and the majority is released as heat (Tuomela et al., 2000). The ultimate energy from BWO of wood waste is the same as from combustion, which is around 18.9 MJ/kg DM (Sobel & Muck, 1983). We expected the major energy would gradually release with the increasing of time. To extract the heat released in the BWO process, four heat recovery categories can be used, including direct heat utilization of vapor, hydronic heating through conduction of within-pile heat exchangers, BWO vapor exchange through latent heat, and a combination of several technologies (Smith et al., 2017).

3.5 Outlook

For sustainable heat production from wood waste, the process needs to occur at elevated temperatures (40-55° C) and N addition is necessary. In our research, only one source of N was studied (NH_4Cl). Among all possible nitrogen sources, human urine is cheap and rich in N, and may be an interesting N source for BWO.

Besides nutrient, further research on BWO should focus on finding the optimum conditions such as inoculum, temperature, surface-area-to-volume and moisture content. Only when the BWO is fast and efficient will it be possible to scale-up the process and make it applicable for good utilization. For the scale-up continuous reactor, it would be necessary to study how to control the process, such as controlling the oxygen concentration and temperature.

After the heat production, special attention should be paid to the efficient heat recovery methods and heat reuse applications. Because heat produced in the BWO process is a medium temperature heat source (usually between 40-55 °C). The medium temperature heat recovery methods have been studied extensively recently, these methods have been successfully utilized for waste heat recovery from air-conditioner (Asim et al., 2017) and composting (Radojičić et al., 2017; Rodrigues et al., 2018; Smith et al., 2017). The 40-55 °C temperatures are high enough for some heating applications like room heating. Among these methods, a heat pump can be utilized to increase both the quality and quantity of the heat generated in the wood biological degradation (Di Maria et al., 2008). It would be necessary to combine these methods with wood biological degradation for sustainable heat production.

3.6 Conclusion

In this study, the effect of N addition (NH_4Cl), P addition (in KH_2PO_4), and pH on the BWO were studied at 42 °C by analyzing the oxygen consumption and mass loss. 2.5 mg/g, 3.3 mg/g and 5 mg/g N addition significant changed the COC and mass loss of BWO ($P < 0.05$). 2.5 mg/g N addition obtained the highest COC (52 mmol O_2) and mass loss (33.0%) after 95 days, which was 2.6 times and 2.0 times more than the COC and mass loss obtained by no nitrogen addition (23 mmol O_2 and 16.9% mass loss), respectively. Too high N addition (20 mg/g) had little effect on the oxygen consumption and mass loss of BWO ($P > 0.05$). The best N addition range in this study was 2.5-5 mg/g. No significant difference was found between these N addition groups ($P > 0.05$). P addition (0-5 mg/g) and pH range between (4-6) had no significant effect on the BWO at 42 °C ($P > 0.05$). The mass loss and COC had a strong linear relationship ($R^2 = 0.9307$) among all tests. The highest heat production rate was 0.63 W/kg DM, obtained by 2.5 mg/g N addition. The heat production rate was lower than the heat production rate generated from composting because the wood is difficult to degrade. It was expected that the major energy would gradually release with the increasing of time.

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Supporting Information

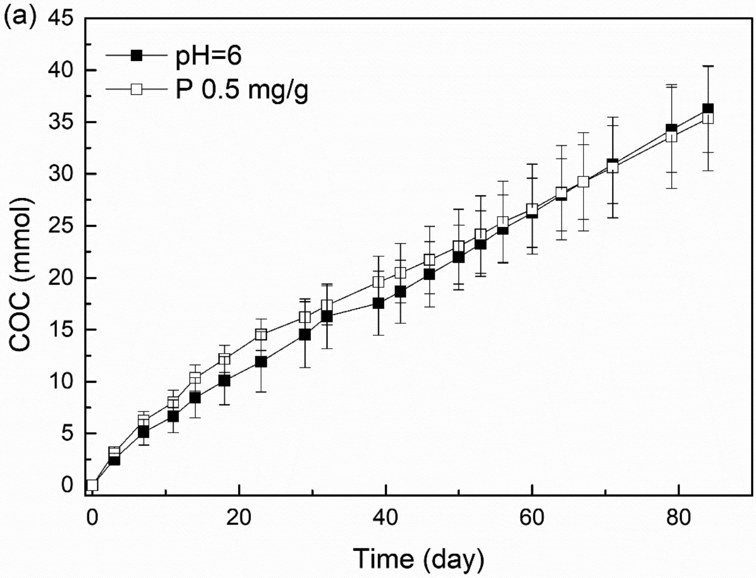

Fig. 3-S1 (a) the cumulative oxygen consumption of pH 6 and 0.5 mg/g P addition for 84 days.

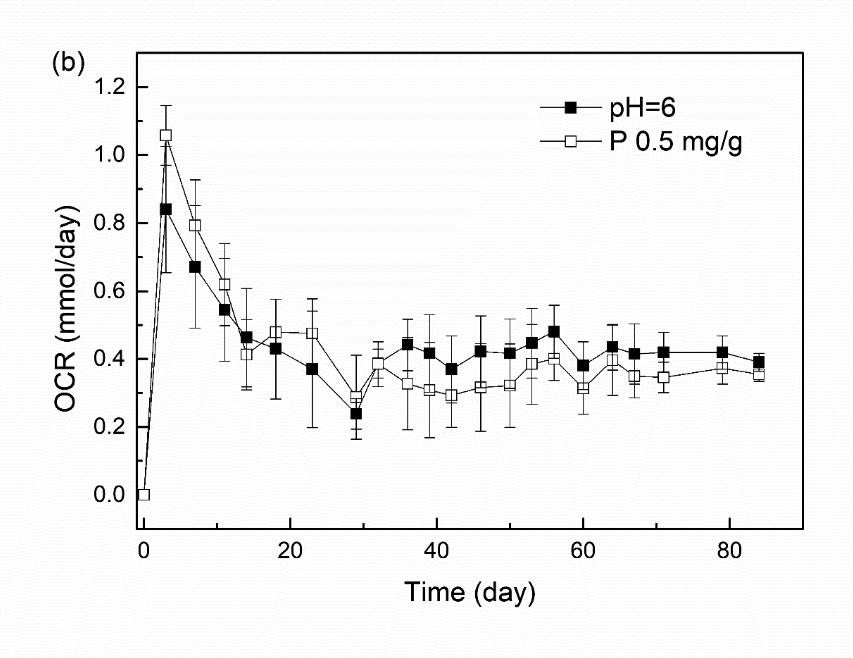

Fig. 3-S1 (b) the oxygen consumption rate of pH 6 and 0.5 mg/g P addition for 84 days.

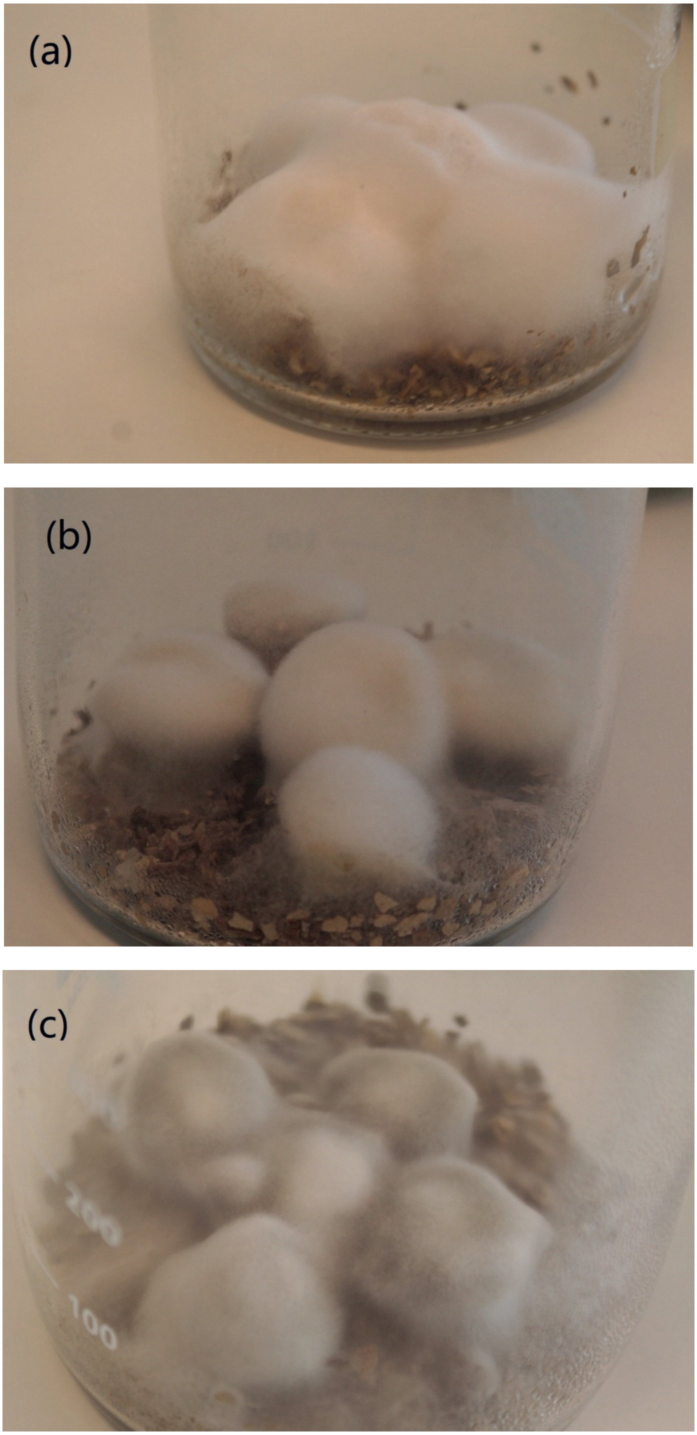
(a)
(b)
(c)
200
100

Fig. 3-S2 The fungal growth at (a) 2.5 mg/g N addition; (b) 0.5 mg/g P addition; and (c) pH 6 at 42°C at day 20.

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Chapter 4

Urine Addition as a Nutrient Source for Biological Wood Oxidation at 40 °C

Shiyang Fan, Anran Li, Annemiek ter Heijne, Cees J.N. Buisman, Wei-Shan Chen

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Abstract

Biological wood oxidation (BWO) offers great potential for sustainable heat generation, and our previous work has shown that nutrients addition (especially nitrogen; N) is necessary for fast BWO. To reduce the cost and environmental impacts of chemical nutrients, human urine was chosen as a source of nutrients in this study. We investigated the factors including urine dilution ratio, the types of urine (fresh urine and synthetic urine), and urine re-addition, by studying their effects on oxygen consumption and dry weight loss of the wood. After 42-day incubation, synthetic urine with 5 times dilution (corresponded to 1.2 ‰ N dry basis of wood) showed the best performance; it improved the oxygen consumption by 3.8 times and wood weight loss by 3.3 times than that without urine addition (ANOVA, $P < 0.05$). At the same N level, fresh urine addition was able to enhance the BWO more efficiently than synthetic urine addition, further improving the oxygen consumption by 64% and weight loss by 47% (ANOVA, $P < 0.05$). During the BWO process, the decrease in wood degradation rate was possibly due to the decrease in nutrients availability. With the re-addition of synthetic urine, the total oxygen consumption and weight loss after 100-day incubation increased by more than 40% compared with the group without re-addition. However, re-addition of only N-containing components did not increase the BWO, showing that elements (other than N) were important. To this end, we demonstrated the feasibility of human urine as a waste-based nutrients source for fast BWO and the possibility of long-term BWO operation via urine re-addition.

Keywords: *biological wood oxidation, synthetic urine, fresh urine, oxygen consumption, weight loss, heat production*

4.1 Introduction

Wood waste is one of the main renewable resources that is abundantly available in the world (Berger et al., 2020). Incineration is the most common management method of wood waste (Sarsaiya et al., 2019). However, wood incineration causes emission of harmful components, such as SO_2, CO, and NO_X (Permchart & Kouprianov, 2004). To avoid these harmful emissions, composting is proposed as an environment-friendly method of wood waste management. Woody materials are usually used as bulk agents to achieve a proper carbon-to-nitrogen ratio in composting (McMahon et al., 2008). However, in conventional composting, wood degrades slowly in low-temperature phases and relatively faster in high-temperature phases (Huang et al., 2010; Wei et al., 2019; Zhao et al., 2016). As a result, continuously thermophilic composting (CTC), which maintains composting at high temperatures, has been proposed to achieve a rapid decomposition of materials that are difficult to degrade at low temperatures (Chang et al., 2019; Xiao et al., 2009a; Xiao et al., 2011). CTC has been applied for dairy manure (Li et al., 2019), municipal solid waste (Xiao et al., 2009a), and food waste (Waqas et al., 2018), however, it has never been applied to wood waste. In addition, the heat generated during CTC is usually ignored.

Biological wood oxidation (BWO) is a CTC method applied to wood waste. BWO maintains wood degradation at an elevated temperature (40-55 °C) for fast decomposition and heat recovery. BWO is an environment-friendly disposal of wood waste, and has been proposed as a clean alternative to wood combustion for heat production (Caizán Juanarena et al., 2016; Fan et al., 2020). The complete BWO can be described as equation (1):

$$CH_{1.5}O_{0.7} + 1.03\ O_2 \rightarrow CO_2 + 0.734\ H_2O + \text{heat} \qquad (1)$$

BWO has been studied sparsely and there are still many unknown factors. Caizán-Juanarena et al. investigated the effect of thermophilic and thermotolerant fungi addition on BWO for heat production. The results showed that the wood degradation by natural biota in the wood was as effective as wood degradation by the added fungi

(Caizán Juanarena et al., 2016). Fan et al. showed that with ammonium chloride addition, the cumulative oxygen consumption (COC) was enhanced by up to 2.6 times and dry wood weight loss (DW) was enhanced 2.0 times (Fan et al., 2020). Nevertheless, considering the high price and environmental impacts of chemically synthesized nutrients, a more cost-effective source of nutrients is required in practice.

Urine is a potential nutrition source for BWO as it is cheap and rich in nutrients, such as K and P (Karak & Bhattacharyya, 2011; Nazari et al., 2020; Xu et al., 2018). Although urine only makes up about 1% of the total domestic wastewater, it contributes 79% of the nitrogen, 47% of the phosphorus, and 71% of the potassium (Barbosa et al., 2019; Ledezma et al., 2017; Nazari et al., 2020). Human urine is considered as a favourable source of nutrients because it is produced in every human being in society, even in the poorest areas (Jagtap & Boyer, 2018). So far, human urine has been extensively used in conventional composting and agriculture (AdeOluwa & Cofie, 2012; Daniels et al., 2009; Fatunbi & Mnkeni, 2011; Ranasinghe et al., 2016; Sangaré et al., 2002); however, it has never been studied in BWO to the authors' best knowledge.

Given the chemical complexity of urine, large uncertainties remain about its effect on BWO. First of all, real human urine is not stable because the urea can be hydrolysed to ammonia, ammonium, carbonate and bicarbonate (Barbosa et al., 2019; Udert et al., 2006). This makes the forms of urine-N dynamic and heavily dependent on the collection and storage process, e.g.: urea dominates in fresh human urine (FU) and ammonia in stored human urine (SU) (De Paepe et al., 2020; Randall et al., 2016). Another change caused by urea hydrolysis is the shift in pH (Johansson et al., 2002; Lehmann, 1976; Wagner & Mohebbi, 2010). Fresh urine often has an initial pH between 5.5 to 6.5 and the pH shifts to alkaline during collection and storage process, which could potentially affect the microbial activity (Tuomela et al., 2000). Secondly, the concentration of N addition should be in a proper range. Too little N addition shows insignificant enhancement on wood degradation while too much N inhibits some microbes, especially wood decaying fungi (Ejechi, 1998; Elisashvili et al., 2008; Fog, 1988; Hauptman et al., 2015; Johansson et al., 2002; Leatham & Kent Kirk, 1983;

Leung & Pointing, 2002; Rinkes et al., 2016; Veverka et al., 2007). Finally, there is little information about the relationship between nutrients availability and wood degradation rate. Our previous work has shown that the biological degradation rates gradually dropped during the BWO process and never returned to the previous peak thereafter (Caizán Juanarena et al., 2016; Fan et al., 2020). It is unknown whether the decrease is related to the nutrients availability.

This study aims at investigating the feasibility of urine as a nutrition source for BWO process. Hereby we studied three variables: (1) the dilution ratio of synthetic urine (SU), as it determines the nutrients concentration in BWO; (2) fresh human urine addition at the optimal dilution ratio of synthetic urine, as they differ in N forms and pH; (3) the effect of urine re-addition during the BWO for designing a long-term BWO process. We also investigated the relationship between wood degradation rate and nutrients availability. Since BWO is an aerobic process, we assessed the performance of BWO by measuring the oxygen consumption and dry weight loss of the wood.(Fan et al., 2020)

4.2 Materials and Methodology

4.2.1 Experimental setup

The setup of the experiment is shown in Fig. 4-1. BWO occurred in a glass bottle (Schott, Germany). The bottom of the bottle was completely covered by 5-gram dry vermiculite to maintain the moisture content (Caizán Juanarena et al., 2016). Each bottle contained 10 pre-treated woodblocks as one group. Bottles with closed lids and septa were placed in an incubator (Elbanton, Netherlands), at a constant temperature of 40 °C.

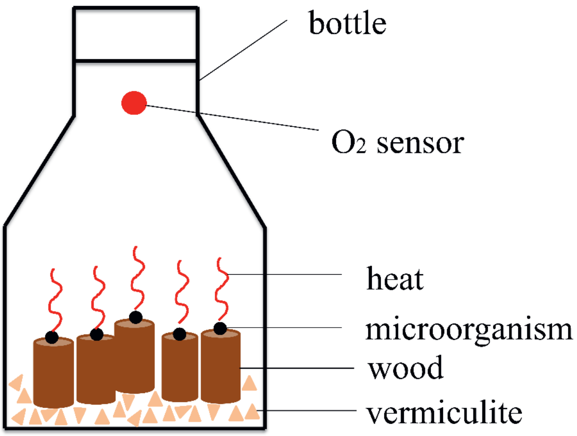


Fig. 4-1 Schematic representation of the experimental set-up.

4.2.2 Urine samples

4.2.2.1 Synthetic urine

To simulate urine in which urea is completely hydrolysed to NH_4^+-N, we prepared a synthetic urine solution (SU), containing the following components (in g/L or otherwise specified): CH_3COONH_4, 5.4; NH_4OH, 1.4; NH_4HCO_3, 22.1; Na_2SO_4, 1.2; NaOH, 3.4; KCl, 0.3; K_2HPO_4, 4.2; $MgCl_2 \cdot 6H_2O$, 0.8; $CaCl_2 \cdot 2H_2O$, 0.4; and trace elements solution, 1mL(Rodríguez Arredondo et al., 2019).

At first, experiments were conducted to study the different dilution ratios of SU. We prepared three dilution ratios (5 times, 10 times, and 20 times) of SU with demineralized water, labelled as SU-5x, SU-10x, SU-20x, respectively. Undiluted SU, labelled as SU-1x, was also tested.

To further study the effect of urine re-addition, especially the effect of N, we made an additional simplified solution from SU. In this solution, only N-containing components were included (in g/L): NH_4CH_3COOH, 5.4; NH_4OH, 1.4; NH_4HCO_3, 22.1. This simplified synthetic urine was labelled as SUN. After that, SUN was diluted 5 times and labelled as SUN-5x for nutrients re-addition study.

4.2.2.2 Fresh urine

To make a comparison between synthetic urine and fresh urine, we collected fresh human urine (FU) from a healthy male adult (28 years old, with no medicine taken over 2 years). After collection, FU was diluted 8.5 times to reach the same total nitrogen (TN) concentration of SU-5x. This dilution sample was labelled as FU-8.5x.

The main characteristics of SU, SUN and FU are investigated and available in Table 4-S1.

4.2.3 Wood and urine addition

4.2.3.1 Preparation of wood

The wood used in this study was collected from an ash tree (*Fraxinus excelsior*) located in Mirns, Friesland, Netherlands. Tree branches were cut into woodblocks using a cutting machine (BAS318, Metabo, Germany). Each woodblock had a square cross-section (side length around 10 ± 2 mm), with a thickness of 5 ± 0.5 mm. Fresh woodblocks were individually numbered and dried at 105 °C in an oven (E28, Binder, Germany) to constant weight. After that, dry woodblocks were weighed and distributed into different experimental groups. Each experimental group had 10 woodblocks with a total dry weight (DW) around 6.735 ± 0.005 g.

4.2.3.2 Addition of synthetic urine and fresh urine

For the SU addition study, woodblocks of the experimental groups were immersed in different SU solutions (with a volume of 500 mL) while the blank group was immersed in demineralized water (with a volume of 500 mL). For the FU addition study, woodblocks were immersed in 500 mL FU-8.5x solution. After 2 days, woodblocks were removed from the immersing liquids then weighted for recording moisture content and added into the bottles. Approximately 8.4 ± 0.3 g of synthetic/fresh urine solution or water was absorbed by each group of woodblocks (1.25 g/g DW). At beginning of the experiment, nine replicates were used for each dilution ratio or fresh urine study. Every 7-14 days, one replicate was stopped and used for TN, pH and weight loss measurement. At the end of the experiment (day 42), all the replicates were stopped and used for the analysis.

4.2.3.3 Re-addition of synthetic urine

For the re-addition study, woodblocks with SU-5x addition were firstly incubated in the experimental set-up. After 24 days, woodblocks with microorganism biomass were transferred to a new bottle covered with 5 g dry vermiculite. Before that, 8.4 g SU-5x solution or 8.4 g SUN-5x solution were re-added to the vermiculite, labelled as SU-R, and SUN-R, respectively. At beginning of the experiment, seven replicates were used for re-addition of synthetic urine study. At day 24 and 48, one replicate was stopped and used for TN, pH and weight loss measurement. At the end of the experiment (day 100), all the replicates were stopped and used for the analysis.

4.2.4 Microbial inoculum

Microorganism sample was originally obtained from a garden waste composting pile. The sampled composting materials were incubated in a bottle at 40 °C for more than 4 months. Oxygen, nutrients and water were supplied regularly during the incubation. To prepare the inoculum, well-decayed woodblocks (fully covered with microbial biomass) were taken out from the bottle and immersed in demineralized water (150 mL water per woodblock). The mixture was then stirred for 5 minutes to get the fungal spores and bacteria well-suspended. After that, the solution was filtered by glass-fibre membranes to remove the undissolved solid impurities. The filtrate was used as inoculum and added to each Schott bottle, with a weight around 8.4 g. The total moisture content in the system was thereby 250 % of DW.

4.2.5 Oxygen measurement

An oxygen sensor (Sensor Spot SP-PSt 3, PreSens, Germany) and an oxygen meter (Fibox 4 trace, PreSens, Germany) were used to measure the oxygen concentration in the Schott bottle. Once the oxygen level decreased to below 10%, the bottles were cooled to room temperature and opened in the flow cabinet to refresh the inside air. We did this to ensure the O_2 level was not a limiting factor in the BWO. Demineralized water was added to compensate for the water lost during refreshing. Once the oxygen returned to atmosphere levels, we moved the bottles to the incubator to continue the experiment.

4.2.6 Weight loss

Dry weight of woodblocks was determined by drying at 105 °C for 24 h in an oven (E28, Binder, Germany). The weight loss was determined on the basis of initial dry weight and final dry weigh, as shown in the equation (2):

$$weight\ loss\ (\%) = \frac{initial\ dry\ weight - final\ dry\ weight}{initial\ dry\ weight} \times 100 \tag{2}$$

4.2.7 Theoretical heat production and theoretical heat production rate

The theoretical heat production (H), in kJ/kg DW, was calculated via the measured oxygen consumption, as shown in the equation (3):

$$H = \frac{\Delta O_2 \times h}{m} \times 1000 \tag{3}$$

Where ΔO_2 is the accumulative oxygen consumption (mol), m is the initial dry weight of woodblocks (g) and h is the heat released by microorganisms expressed as kilo joule per mol of oxygen consumed (kJ/mol). h was fixed at 467.5 kJ/mol O_2 and was maintained constant throughout the process (Caizán Juanarena et al., 2016).

The theoretical heat production rate (W), in W/kg DM, was calculated via the equation (4):

$$W = \frac{H}{t} \times 1000 \qquad (4)$$

Here H is the theoretical heat production (kJ/kg DW) and t is the incubation time (s).

4.2.8 Determination of nutrients availability and pH values

In our study, it was hard to measure the nutrients availability during BWO process. Thus, we chose the most important and the richest urine component - N, as an indicator to nutrients availability. The amount of total nitrogen (TN) was positively related to nutrients availability. Once the TN in the BWO was depleted, the nutrients availability was considered as insufficient. It is not possible to directly measure the TN amount of BWO, we therefore used the water extraction method (Campbell & Bryant, 1941). Woodblocks were first immersed in demineralized water (100 ml water per one group of woodblocks) for 3 days. To avoid the TN consumption caused by microorganisms during the immersion process, we added 1ml of 1% (w/w) NaN_3. Then, the solution was filtered with a glass-fibre membrane (0.22 μm). After that, we used spectrophotometer (DR 3900, Hach, USA) and TN kits (LCK138, Hach, USA) to determine the TN concentration of the liquids. To know the N fractions better, we also used kits to measure the different N fractions: NH_4^+-N (LCK304, Hach, USA), NO_3^--N (LCK339, Hach, USA) and NO_2^--N (LCK341, Hach, USA). N excluding NH_4^+-N, NO_2^--N, and NO_3^--N was considered as organic N. The average N recovery effectivity by this extraction method was 87.6 ± 3%. Moreover, we also measured the pH value of extracted liquids using a standard pH meter (PHM210, Radiometer, France) (Johansson et al., 2002; Pedieu et al., 2008).

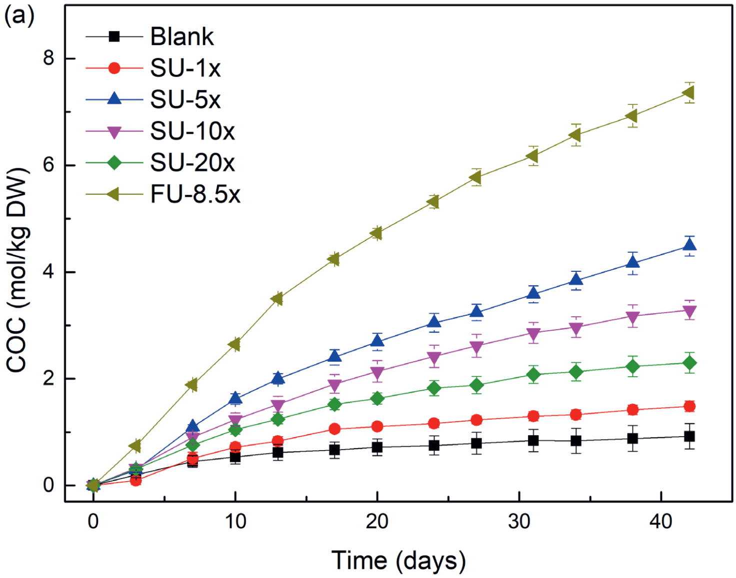
4.2.9 Statistical analysis

All data were analysed using IBM SPSS Statistic 23 (IBM, USA) for Microsoft Windows. Differences were compared statistically using ANOVA testing at the 5% level of significance. Differences between values at P greater than 0.05 were considered as not significantly different.

4.3 Results and Discussion

4.3.1 Five times was the best dilution ratio of synthetic urine

Oxygen consumption and weight loss are two important factors for assessing BWO performance. The cumulative oxygen consumption (COC) of different synthetic urine (SU) additions is shown in Fig. 4-2a, and the weight loss is shown in Fig. 4-2b. The oxygen consumption rate (OCR), displaying similar information as that of COC, is shown in Fig. 4-S1.



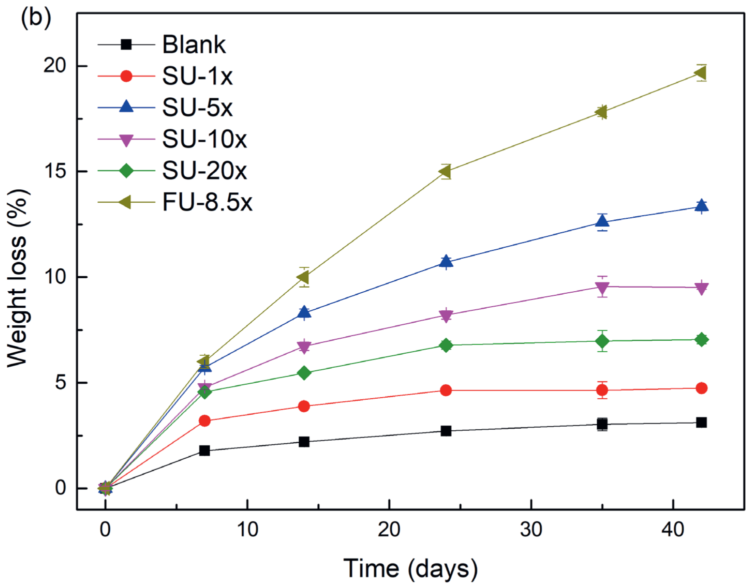


Fig. 4-2 The effect of different synthetic urine additions and fresh urine-8.5x addition on a) cumulative oxygen consumption; b) weight loss. Average and standard deviation were calculated for different replicates.

As shown in Fig. 4-2, all different SU additions and FU-8.5x addition significantly increased the oxygen consumption and weight loss at 40 °C (ANOVA, $P < 0.05$). SU-5x exhibited the highest COC and weight loss among all SU additions. FU-8.5x displayed higher oxygen consumption and weight loss than SU-5x (ANOVA, $P < 0.05$). All SU additions significantly improved the COC and weight loss of BWO after 42 days (ANOVA, $P < 0.05$). For different SU additions, SU with 5 times dilution (SU-5x) exhibited the highest COC (4.5 mol/kg DM) and weight loss (13.4%) while undiluted SU (SU-1x) had the lowest COC (1.5 mol/kg DM) and weight loss (4.8%). Besides, increased dilution ratios resulted in lower BWO performances. All oxygen consumption and weight loss increased rapidly in the first 7 days, gradually slowed down thereafter, except for SU-5x. The theoretical maximum oxygen consumption caused by oxidation of undiluted SU was only 0.15 mol/kg DW, and this number was even lower in diluted

SUs, which could be ignored compared with the COC in the experimental groups (e.g. 4.49 mol/kg DW for SU-5x).

Changes in TN amount with SU additions are shown in Fig. 4-3a, the changes in pH with different urine additions are shown in Fig. 4-3b; and the distribution of N fractions treated by SU-1x and SU-5x are shown in Fig. 4-S2. All TN remarkably decreased during the BWO process with most of TN (58%-98%) being consumed in the first week. The residual TN of SU-10x and SU-20x was almost depleted after 7 days. While for SU-1x, the consumption rate of TN gradually decreased, and the TN did not further decrease after 24 days. There was 25% (10.7 mg) of initially added TN left unutilized in SU-1x at the end.

The initial pH values of woodblocks treated by different SU solutions were higher than 7; the lower the dilution ratio, the higher was the initial pH. This was because of different NH_4^+-N and OH^- content in different SU solutions. The pH of SU-5x, SU-10x, and SU-20x decreased rapidly in the first 7 days and became stable in the next 35 days. This was possibly due to the quick consumption of NH_4^+-N and fatty acid production (malonate, lactate and oxalate) during the BWO process (Adejoye & Fasidi, 2009; Cao et al., 2020; Johansson et al., 2002; Kües, 2007; Schmidt, 2006; Xiao et al., 2009b). While the pH in SU-1x stayed stable around of 8.7-8.9 for the entire 42 days.

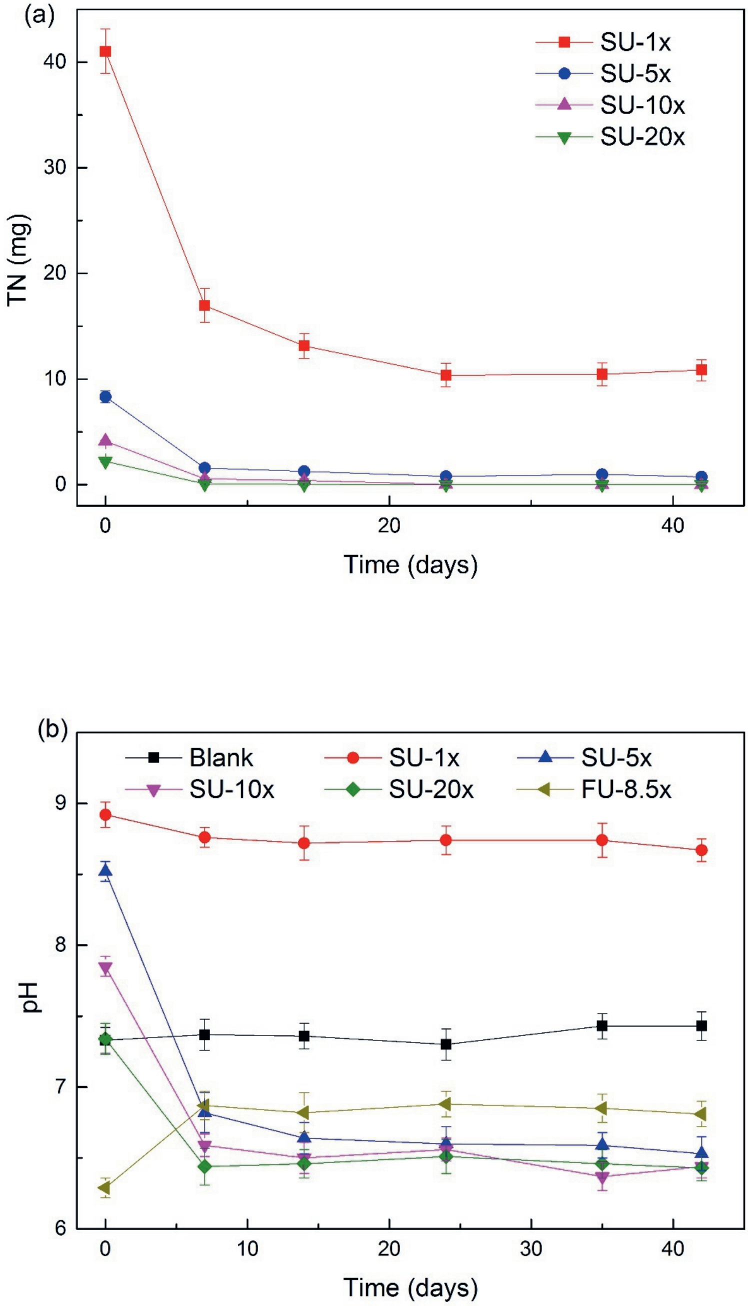

(a)
TN (mg)
0
10
20
30
40
Time (days)
0
20
40
SU-1x
SU-5x
SU-10x
SU-20x
(b)
pH
6
7
8
9
Time (days)
0
10
20
30
40
Blank
SU-1x
SU-5x
SU-10x
SU-20x
FU-8.5x

Fig. 4-3 a) Changes in TN amount treated by synthetic urine additions; b) changes in pH with different urine additions. Average and standard deviation were calculated for 5 measurements.

Wood is inherently poor in many macro- and micro-elements that are essential for microbial activity (Egli & Quayle, 1986; Fan et al., 2020; Labosky Jr et al., 2007). Urine, on the other hand, supplies some nutrients that are poor in wood. Among these nutrients, the most important one is N, as N is the major nutrient required by fungi and bacteria to assimilate carbon source (Sangaré et al., 2002; Tiquia et al., 1998). Wood contains only 0.3-1‰ N by dry weight (Cowling & Merrill, 1966; Sangaré et al., 2002). Without SUs addition, wood decaying microorganisms, mainly fungi, can only obtain N from wood for their growth, resulting in slow growth of microorganisms and slow BWO. With urine addition, wood decaying microorganisms can obtain easily available N sources like NH_4^+-N from urine,(van der Wal et al., 2007) causing the quick decrease of TN. Addition of SU solutions, from SU-1x (corresponded to 6‰ N) to SU-20x (corresponded to 0.3‰ N), could stimulate the oxygen consumption and weight loss during the BWO process (Reid, 1983a). As a result, all the experimental setups have higher COC and weight loss than the blank (Fig. 4-2a and Fig. 4-2b). However, the enhancement was different, with the largest wood degradation occurring at the SU-5x (corresponded to 1.2 ‰ N). Other researchers also reported similar N levels for optimal wood oxidation (Allison et al., 2009; Bebber et al., 2011; Kirk et al., 1978; Labosky Jr et al., 2007; Thomas et al., 2020; Wu et al., 2005), e.g. 1.2 ‰ reported by Yang et al.,(Yang et al., 1980) and 1.0 ‰ reported by Allison et al. (Allison et al., 2009). SU-1x did not improve the BWO as much as SU-5x. The reason could be the alkaline environment in SU-1x group during the entire BWO process (Fig. 4-3b). This alkaline environment might inhibit the growth of wood decaying fungi (Fig. 4-S3), because many fungi species are not able to grow at high pH (Ejechi, 1998; Schmidt, 2006; Verbist et al., 2019). Although the initial pH of SU-5x was 8.52, it dropped quickly below 7 in 7 days, which was good for wood decaying fungi (Schmidt, 2006). Moreover, it is reported that excessive N could suppress the production of lignocellulase (Edwards et al., 2011; Leung & Pointing, 2002). For example, *Phanerochaete chrysosporium* only produce the RNA of manganese peroxidases (MnP) under depleted N conditions.(Li et al., 1994) N in SU-1x addition experiment was never depleted, and the residual N inhibited the wood degradation during BWO process (Reid, 1983b; van der Wal et al., 2007).

4.3.2 Fresh urine was more effective than synthetic urine at 1.2 ‰ N

The fresh urine was diluted 8.5 times (FU-8.5x) to reach the same TN concentration of SU-5x (1.2 ‰ N dry basis of wood), which was the best-performing dilution ratio. The COC of FU-8.5x is shown in Fig. 4-2a, the weight loss is shown in Fig. 4-2b and the OCR is shown in Fig. 4-S1. Compared with SU-5x, FU-8.5x significantly enhanced the COC by 64% and weight loss by 47% after 42 days (ANOVA, $P < 0.05$). The theoretical maximum oxygen consumed by oxidation of FU-8.5x was 0.12 mol/kg DW, which could be ignored compared with the COC in the FU-8.5x group (7.36 mol/kg DW). Changes in N fractions of FU-8.5x addition is shown in Fig. 4-4. The TN in FU-8.5x was consumed quickly in the first 7 days and the consumption rate gradually decreased in the next 35 days, with only 3% of initially added TN left unutilized after 42 days. Not only N consumption but also N transformation occurred in the BWO process. In the first 7 days, the organic N (mainly urea) decreased rapidly from 7.8 to 2.7 mg, accompanied with a NH_4^+-N increase from 0.5 to 0.9 mg and a NO_3^--N increase from 0 to 1.0 mg. In the next 35 days, both N sources were consumed gradually and nearly depleted on day 42. The pH change of FU-8.5x is shown in Fig. 4-3b. The initial pH of woodblocks treated by FU-8.5x was acidic and the pH increased slightly from 6.29 to 6.80 and remained stable.

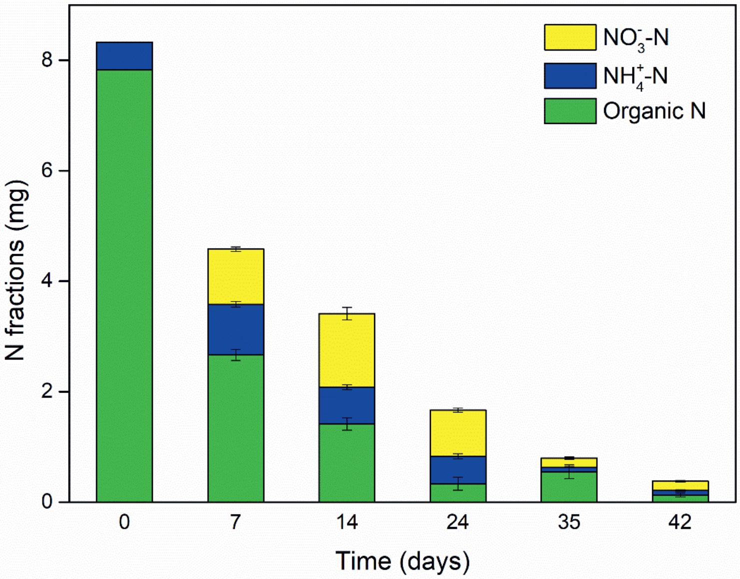


Fig. 4-4 Changes in N fractions treated by fresh urine-8.5x addition. Average and standard deviation were calculated for 5 measurements.

Urea is a toxic agent to microbe because the volatile NH_3 produced by bacterial ureolytic activity in an alkaline environment can significantly reduce the microbial activity (Ejechi, 1998; Johansson et al., 2002; Veverka et al., 2007). However, in an acidic environment, there was no volatile NH_3 generated from urea hydrolysis (Chang & Chang, 1999). In our study, the pH of FU-8.5x was acidic in 42 days due to the relatively low urea addition amount. The NH_4^+-N generated from urea hydrolysis was consumed quickly and never got over-accumulated. As a result, the FU-8.5x addition did not inhibit the BWO.

The FU-8.5x addition exhibited higher oxygen consumption and weight loss than SU-5x addition during the BWO process. We hypothesised two explanations: 1) the initially acidic pH helped FU-8.5x obtained a higher level of fungal growth than SU additions (Fig. 4-S3) (Schmidt, 2006); 2) the easily degradable organic matters in FU, such as amino acid, sugars and organic acid (Bouatra et al., 2013), could enhance the BWO to some extent. For example, the presence of amino acids and vitamin in FU-8.5x could stimulate the ligninolytic enzyme production generated by white-rot fungi (Levin et al., 2010).

4.3.3 Nutrients other than N were necessary for sustaining BWO

As shown in Fig. 4-2, Fig. 4-3 and Fig. 4-4, OCR was higher at relatively high nutrients levels, and decreased when the nutrients were gradually consumed. We suspected that the decline of OCR was due to the decrease in nutrients availability. To test this, we re-added SU-5x (SU-R) and SUN-5x (SUN-R) after 24 days and continued the incubation to 100 days. The COC of re-addition study is shown in Fig. 4-5, the OCR is shown in Fig. 4-S4 while the changes in TN availability is shown in Fig. 4-S5.

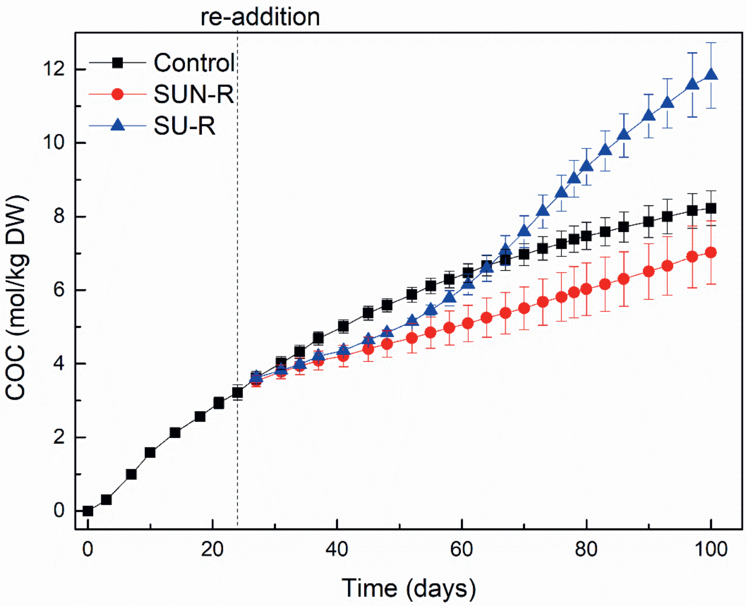


Fig. 4-5 Effect of nutrients re-addition on cumulative oxygen consumption. Average and standard deviation was calculated for different replicates.

Compared with the control group, the overall effect of SU-R on oxygen consumption was shown to be positive, while the SUN-R did not result in higher COC. The final weight loss of the control group, SU-R and SUN-R was 21.4%, 31.3% and 17.5%, respectively, showing the same trend as COC.

There was a lag phase of COC after re-addition. This could be due to the sudden increase of TN (mainly NH_4^+-N). The inhibition lasted for 24 days (day 24-48) for SU-R until the TN concentration was consumed to a proper level. Totally, the SU-R exhibited a COC increase of 8.7 mol/kg DW, 71% higher than the control group without re-addition. It indicates that the depletion of nutrients availability might slow down the BWO, and nutrients re-addition could stimulate the BWO in longer duration, with an OCR peak lasting from day 65 to day 80 (Fig. 4-S4). Once again, the OCR of SU-R decreased (from day 80 to 100) when the nutrients became limited (Fig. 4-S5). It was expected that more nutrients were needed further for fast BWO, as the highest weight loss of woodblocks was 31.3%. However, SUN-R exhibited a COC increase of 3.9 mol/kg (76% of the control group) only, showing that elements other than N also mattered in BWO. These elements could be important in two aspects. First of all, they are necessary for microbial N uptake. Despite the quick TN decrease from 9.0 to 2.7 mg between day 25 to 48, TN of SUN-R did not show a significant drop further, with only 0.4 mg consumed between day 48 to 100 (Fig. 4-S5). This might because of the depletion of these other elements. The TN consumption (mainly NH_4^+-N) of AUN-R after 100 days was 13.8 mg, which was much lower than the TN consumption of SU-1x in the first week (30.87 mg). This indicates that the deficiency of other elements led to a low TN consumption. It is reported that phosphorus can help fungi to uptake N (Nicholas & Crawford, 2003; Rayner & Boddy, 1988). Besides, the ionic strength and dissolved amino acids could also affect the N uptake by bacteria (Kirchmann & Pettersson, 1995; Müller et al., 2006). Secondly, the elements other than N could affect the function of lignocellulosic enzymes. For example, manganese (Mn^{2+}) is crucial for the production and expression of manganese peroxidase (Tekere et al., 2001), which is the most common lignin-modifying enzyme produced by wood-colonizing fungi

(Hofrichter, 2002). Ions including Cu^{2+} and Cd^{2+} can enhance the synthesis of laccase and MnP generated by some fungal species (Kenkebashvili et al., 2012; Montoya et al., 2015). Moreover, interactions between ions (e.g. Mg^{2+}, PO_4^{3-}, Na^+, Ca^{2+}, SO_4^{2-}, Cl^-, K^+) and organic acids can affect the wood degradation, though the mechanisms remain unknown (Tanaka & Yano, 2005).

4.3.4 Heat production potential in BWO

There was a strong linear relationship between weight loss and COC (Fig. 4-S6), showing that the oxygen consumption can be linearly linked to molar loss of the wood. The theoretical heat production, proportional to oxygen consumption, was therefore mainly due to the wood degradation. In our studies, the highest theoretical heat production (obtained by FU-8.5x) after 42-day incubation was 3441 kJ/kg DW (0.95 W/kg), which was almost doubled than heat production reported by Caizán-Juanarena et al. (0.47 W/kg after 36 days) (Caizán Juanarena et al., 2016). The theoretical heat production of SU-R was 5535 kJ/kg DW (0.64 W/kg DW after 100 days), which was comparable to our previous study with NH_4Cl and nutrients addition (0.63 W/kg DW after 95 days) (Fan et al., 2020). This indicates that the urine could be a better alternative to chemical nutrients source due to the higher performance and lower cost.

A limited number of studies investigated the heat production during composting of waste materials (Ekinci et al., 2006; Harper et al., 1992; Irvine et al., 2010; Klejment & Rosiński, 2008; Zhao et al., 2017), but the results are as diverse as the compost materials, the contexts, the experimental scales and the heat determination methods, making the comparison hard. Alkoaik and Ghaly reported that 1400 kJ/kg DM heat production was generated from tomato plant residues composting mixed with wood shavings (Alkoaik & Ghaly, 2011). Hogan et al. reported that around 2400 kJ/kg DM heat generated from rice hulls and flour composting (Hogan et al., 1989). Smith el al. summarized 45 cases of composting and reported that the average heat recovery is 1159 kJ/kg DM for lab-scale systems, 4302 kJ/kg DM for pilot-scale systems, and 7084 kJ/kg DM for commercial systems (Smith et al., 2017). Taking these as a reference, BWO with urine addition has great potential for scaled-up applications.

4.4 Outlook

In reality, human urine is naturally diluted by flushing, with a dilution ratio of 5-10 times, dependent on the water volume used per flushing time (Kuntke et al., 2016; Tice & Kim, 2014). This is similar to the best dilution ratio in our study (the best SU dilution ratio was 5 times, and the dilution ratio for fresh urine was 8.5 times), implying that the source-diverted urine can be ready-to-use for BWO without extensive further dilution. For sustainable BWO, urine re-addition is necessary to get a high weight loss of wood waste. Our results show that each addition of SU-5x and FU-8.5x could support the nutrients demand of BWO for at least 24 days. Taking this as a reference, one adult can produce 1.5 kg/d urine averagely (Shi et al., 2018), which could continuously support the nutrients demand of a 0.24 tons BWO system. Considering the population of human beings (>7 billion) (Pham et al., 2020), human urine could be a promising and sustainable nutrient sources for BWO. Combining human urine with BWO can also reduce the burden on water treatment plants, since the amount of urine waste will be reduced.

Our work has proven that human urine, fresh or synthetic, is a good alternative to chemical nutrients source for BWO, considering their cost and environmental impacts. The heat produced during BWO can be recovered and used for building applications, such as room heating and hot water service (Walther et al., 2016). Some challenges, however, still need further research. First of all, studies regarding the effect of single urine composition on BWO are rare at this moment. Secondly, the non-sterile condition during urine storage and transportation enables bacterial growth and urea hydrolysis, which may further lead to the nutrients loss (such as NH_3 loss) and the possibility of bacterial contamination (Lind et al., 2001). Therefore, the system design needs to be optimized to control the quality and quantity of urine addition. Finally, more attention should be paid on increasing the weight loss and heat production of BWO to reach industrial interest.

4.5 Conclusion

The present study confirmed the positive effect of urine addition on the oxygen consumption and weight loss of BWO. We showed that the addition of both fresh urine and synthetic urine could enhance BWO (ANOVA, $P < 0.05$). Synthetic urine with 5 times dilution (corresponded to 1.2 ‰ N) showed the highest oxygen consumption (4.5 mol/kg DW) and weight loss (13%) after 42-day incubation. At this TN level, the fresh urine significantly enhanced the oxygen consumption by 64% and weight loss by 47% after 42-day incubation compared with the synthetic urine with 5 times dilution (ANOVA, $P < 0.05$). The initially added nutrients could help enhance the BWO, however, this enhancement decreased with the nutrients consumption. Urine re-addition significantly improved the performance of BWO in the long run (ANOVA, $P < 0.05$), achieving 71 % higher oxygen consumption and 46% higher weight loss compared without urine re-addition. However, re-addition of only N inhibited the BWO significantly (ANOVA, $P < 0.05$), showing that other elements excluding N were important.

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Supporting Information

Table 4-S1 Characteristics of synthetic urine, fresh urine and synthetic urine with only N components.

	SU	**FU**	**SUN**
COD (mg/L)	4011 ± 21	3084 ± 16	3977 ± 23
TN (mg/L)	4900 ± 25	8430 ± 67	4900 ± 25
NH_4^+-N (mg/L)	4870 ± 50	540 ± 32	4870 ± 50
NO_3^--N (mg/L)	0	150 ± 9	0
NO_2^--N, mg/L	0	1.38 ± 0.25	0
Phosphate (PO_4^{3-}-P, mg/L)	760 ± 13	845 ± 11	0
pH	9.05 ± 0.1	6.29 ± 0.1	8.35± 0.3

SU: synthetic urine; FU: fresh urine; SUN: synthetic urine with only N components

After preparation, SU was diluted to different ratios for different stored urine addition experiment (5x, 10x, and 20x) and nutrition re-addition experiment (5x); FU was diluted 8.5x for the fresh human urine experiment; SUN was diluted 5 times for re-addition experiment.

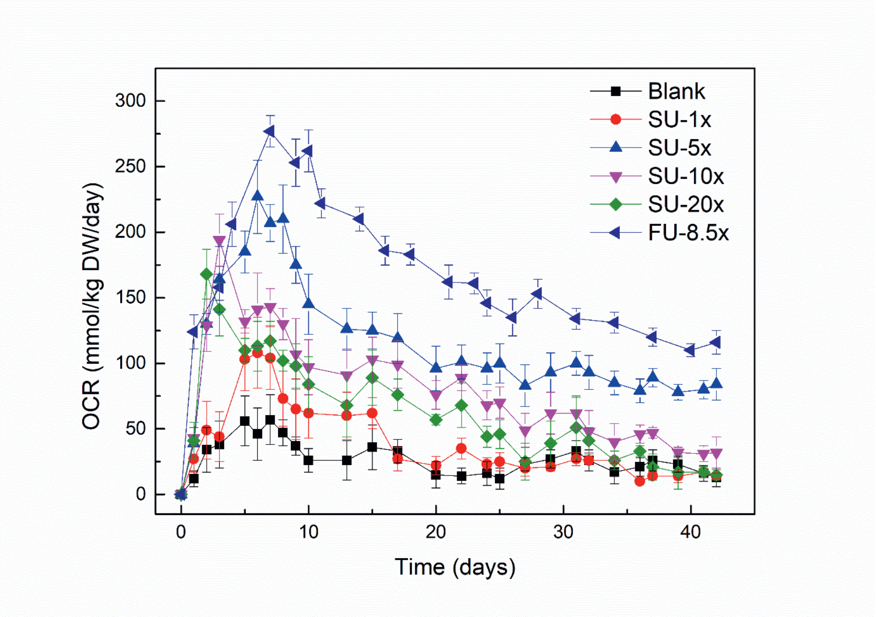

Fig. 4-S1 Effect of different urine additions on the oxygen consumption rate during 42-day incubation at 40 °C. Average and standard deviation were calculated for different replicates.

As shown in Fig. S1, all SU additions enhanced the OCR compared to blank group. SU-5x generally showed the highest OCR among all SU additions. At the same N level, FU-8.5x generally showed a higher OCR than SU-5x.

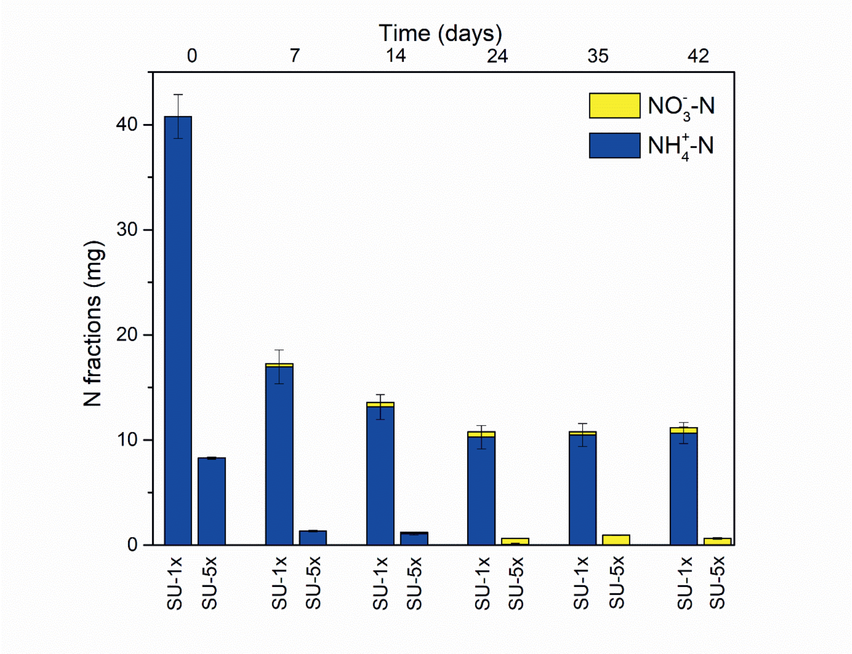

Fig. 4-S2 Changes in N fractions with synthetic urine-1x and synthetic urine-5x addition. Average and standard deviation were calculated for 5 measurements.

As shown in Fig. 4-S2, NH_4^+-N was the major N fraction in SU-1x and SU-5x. NO_3^--N was low and stayed low in the whole process. N fractions in SU-10 and SU-20x were not shown as they were depleted quickly. NO_2^--N was not shown either, as it was below 1% of TN.


(a)

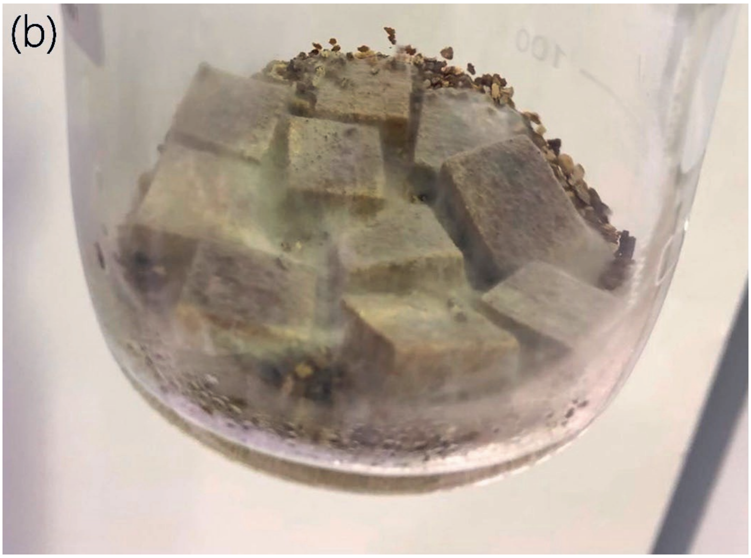
(b)

Fig. 4-S3 Pictures of microbial growth on woodblocks with different synthetic urine additions and fresh urine-8.5x addition at different times. (a) woodblocks treated by synthetic urine-5x after 7 days, little visible microorganisms growth could be found on the wood surface; (b) woodblocks treated by synthetic urine-5x after 14 days, visible microorganisms growth could be found on the wood surface; (c) woodblocks treated by synthetic urine-1x after 14 days, no visible microorganisms growth on

the wood surface; (d) woodblocks treated by fresh urine-8.5x after 7 days, much visible microbial biomass growth could be found on the surface of wood. In biological wood oxidation system, visible microbial growth only could be found when pH dropped below 7.

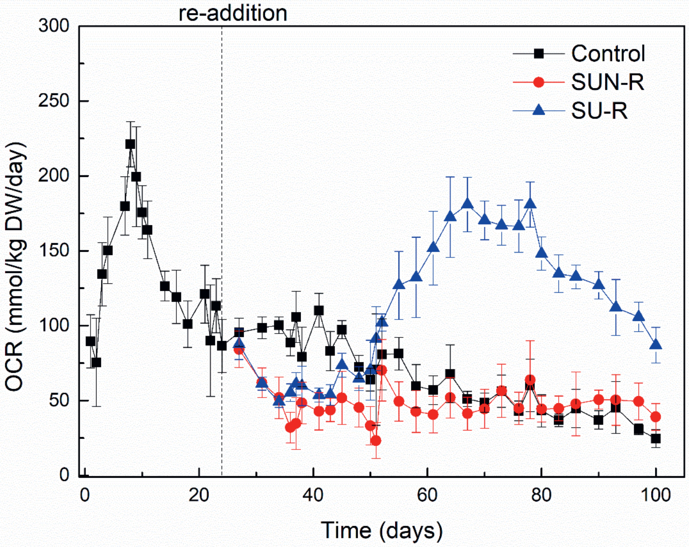

Fig. 4-S4. Effect of nutrient re-addition on the oxygen consumption rate. Average and standard deviation were calculated for different replicates.

As shown in Fig. 4-S4, all OCR first increased and decreased thereafter in the first 24 days. At day 24, when the nutrient availability was low, the SU-R and SUN-R were tested. OCRs of SU-R and SUN-R decreased immediately after the re-addition. After day 50, OCR of SU-R increased and a second peak occurred between day 65 and 80. SUN-R inhibited the OCR in the entire process compared with the control group.

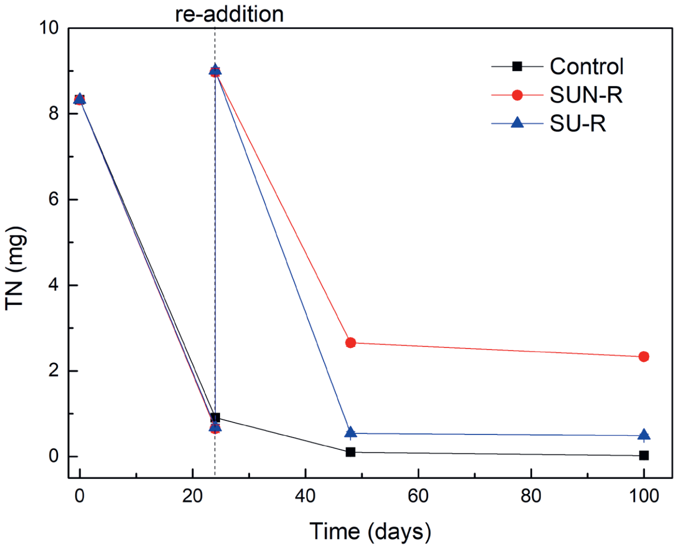

Fig. 4-S5 Changes of TN during the nutrient re-addition study. Average and standard deviation were calculated for 5 measurements.

As shown in Fig. 4-S5, most TN decreased quickly in the first 24 days. After re-addition, TN of SU-R decreased quickly again and reached to the almost zero in the next 24 days. While SUN-R test did not show a completely TN consumption in the next 76 days. There was still about 2.3 mg (28%) TN left after 100 days.

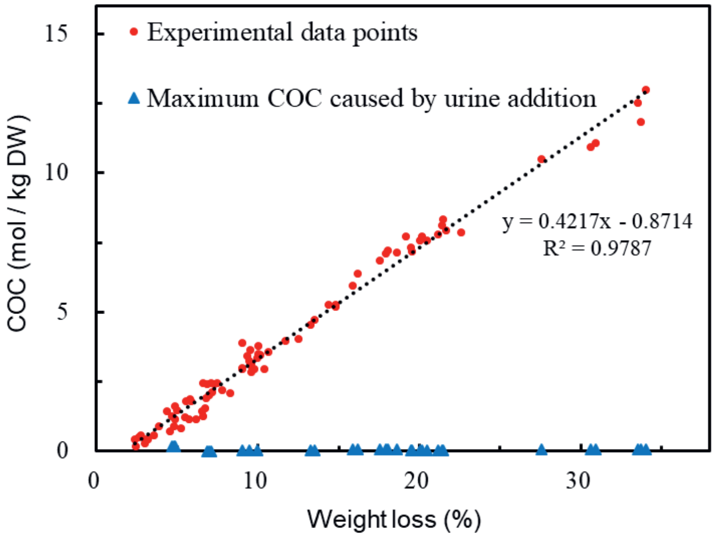

Fig. 4-S6 Relationship between cumulative oxygen consumption caused by urine addition and weight loss and total cumulative oxygen consumption.

As shown in Fig. S6, the COC caused by urine oxidation was small compared to the total COC. A strong relationship was found between the weight loss and COC (ANOVA, $P < 0.05$, $y=0.4217x - 0.8714$, $R^2 = 0.9787$). This figure proves that the oxygen was mainly caused by BWO, and BWO is an aerobic process.

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Chapter 5

Effect of Temperature on Biological Wood Oxidation

Shiyang Fan, Yujia Luo, Annemiek ter Heijne, Cees J.N. Buisman, Wei-Shan Chen,

A modified version of this chapter is ready to submit:
Shiyang Fan, Yujia Luo, Annemiek ter Heijne, Cees J.N. Buisman, Wei-Shan Chen.
“Effect of temperature on biological wood oxidation.”

Abstract

Biological wood oxidation (BWO) is an alternative to wood combustion for heat production. So far, wood degradation has mostly been studied at ambient temperatures. There is little information about BWO at elevated temperatures. Our objective was to study the BWO at three temperatures (30, 40 and 50 °C) and their response to the change of temperatures. Moreover, the microbial gene density was also studied. In the experiments without changing temperatures, the highest oxygen consumption and wood weight loss were observed at 40 °C compared to 30 °C and 50 °C. The highest microbial gene density was observed at 30 °C. Changing temperature from 30 °C to 40 °C significantly increased the oxygen consumption and wood weight loss while the fungal and bacterial gene density decreased obviously. Changing temperature from 50 °C to 30 °C increased the bacterial gene density remarkably, however, it did not affect the oxygen consumption and wood weight loss.

Keywords: *biological wood oxidation, heat production, temperature, qPCR, wood weight loss*

5.1 Introduction

As the one of the most abundant renewable energy sources on earth, lignocellulosic biomass has gained more and more attention due to the continuous increase in energy demand and environmental pressure. Woody biomass is the largest part of lignocellulosic biomass on earth (Bilgen et al., 2015). It is reported that about 4 billion m^3 roundwood is generated over the world annually (IEA, 2002). Most of wood is burned for heat generation. However, wood burning results in the emissions of harmful components, such as SO_2, NOx and fine particles, which is not good for the environment and human health (Permchart & Kouprianov, 2004). To avoid these harmful emissions, biological wood oxidation (BWO) has been proposed as an environment-friendly alternative to wood combustion (Caizán Juanarena et al., 2016). BWO is a type of composting for wood-rich materials at elevated temperatures. During BWO process, microorganisms (mainly fungi and bacteria) decompose wood materials in the presence of oxygen. The product of BWO is CO_2, H_2O and heat, which can be described by the equation (1) (Caizán Juanarena et al., 2016):

$$CH_{1.5}O_{0.7} + 1.03\ O_2 \rightarrow CO_2 + 0.734\ H_2O + \text{heat} \qquad (1)$$

BWO is a complex process affected by many factors, such as microbial community (Caizán Juanarena et al., 2016), nutrients (Fan et al., 2020a; Fan et al., 2020b), temperature, O_2 availability (Reid & Seifert, 1982), and moisture content (Viitanen & Ritschkoff, 1991). Among all those factors, temperature is important since it affects both the population of microorganisms and their activity (Blanchette et al., 1989).

The temperature of traditional composting is usually between 25-70 °C (Beffa et al., 1998; Derikx et al., 1990; Hassen et al., 2001; Nakasaki et al., 1985; Palmisano et al., 1993). The composting is divided into four stages according to the different temperature: mesophilic phase, thermophilic phase, cooling phase, and mature phase. It is widely reported that the high temperature can enhance the wood degradation in composting (Albrecht et al., 2010; Tiquia et al., 2002; Tuomela et al., 2000; Xiao et al., 2009). However, the high temperature of composting usually occurs in thermophilic stage where the dominant microorganisms are bacteria. In the thermophilic stage, the dominant microorganisms are more likely to degrade easily degradable organic matter other than difficult degradable substrate like wood. When the easily degradable organic matter becomes depleted, the temperature of composting decrease quickly and the fungi with lignocellulosic biomass decaying ability start to become dominant, usually in the cooling phase and mature phase (Albrecht et al., 2010; Huang et al., 2010; Villar et al., 2016). The temperature of cooling and mature phase of composting is below 40 °C. As a result, most part of lignocellulosic biomass might stay undegradable after thermophilic phase (Huang et al., 2010), and the remained lignocellulosic biomass in the last two phases degrade slowly (Zhang et al., 2017; Zhang et al., 2018).

The elevated temperature in BWO process means that thermophilic microbes are the important biodegradation agent. Generally, fungi tolerate a narrower temperature range (15-55 °C) compared to bacteria (0-70 °C) (Ryckeboer et al., 2003b; Tuomela et al., 2000). The effect of temperature on composting has been extensively studied. However, there is very little information relating temperature and BWO. Previous studies indicate that increasing temperature may stimulate the microbial activity in composting, however there is a threshold where the temperature prohibits the microbial activity (Liu et al., 2011; Miller et al., 1989; Nakasaki et al., 1985; Rao et al., 1996; Strom, 1985). Since BWO is an undefined mixed culture, it is important to explore the optimal temperature of BWO and its response towards temperature change. Therefore, we firstly studied the BWO performance at three different temperatures (30 °C, 40 °C, and 50 °C) by assessing the oxygen consumption and wood weight loss. When the oxygen consumption rate became stable, we changed the working temperature to further understand the effect of temperature on BWO. Lastly, we determined the fungal and bacterial gene density by using quantitative real-time PCR (qPCR) to elucidate their roles in the BWO process.

5.2 Materials & Methods

5.2.1 Setup of the batch experiment

The setup of the experiment is shown in Fig. 5-1. BWO was studied in glass bottles with a volume of 650 mL (Schott, Germany). Each bottle contained one group of 5 pre-treated woodblocks. All bottles were closed with lids and septa, and were placed in incubators (Elbanton, Netherlands) working at different temperatures.

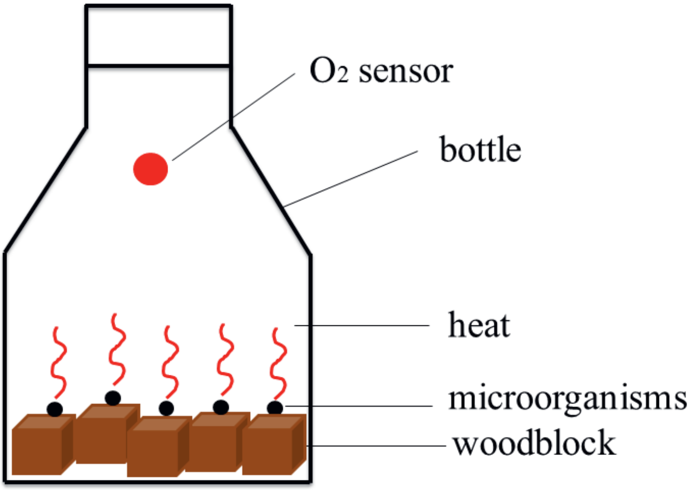


Fig. 5-1 Schematic representation of the experimental set-up.

5.2.2 Characteristics of the wood and nutrition

The wood used in this study was collected from an ash tree (*Fraxinus excelsior*) located in Mirns, Friesland, Netherlands. Tree branches were cut into cubic woodblocks by a cutting machine (BAS318, Metabo, Germany). Every cubic woodblock had a size around 1.5 cm * 1.5 cm * 1.5 cm (L * W * H). Fresh woodblocks were individually numbered and dried at 105 °C in an oven (E28, Binder, Germany) to constant weight. After that, dry woodblocks were weighed and distributed into different experimental groups. Each experimental group had 5 woodblocks with a total dry matter (DM) around 9.000 ± 0.005 g.

Before experiment, each group of woodblocks were immersed into the nutrient solution for 72 hours until reaching a constant weight. The nutrient solution, supplying macronutrients and micronutrients, contained 2 g/L NH_4Cl, 4.5 g/L KH_2PO_4, 0.5 g/L K_2HPO_4, 1.0 mg/L $MgSO_4 \cdot 7H_2O$, 120 mg/L $FeCl_3$, 50 mg/L H_3BO_3, 10 mg/L KI, 45 mg/L $MnSO_4 \cdot H_2O$, 20 mg/L $Na_2MoO_4 \cdot 2H_2O$, 75 mg/L $ZnSO_4 \cdot 7H_2O$, 50 mg/L $CoCl_2 \cdot 6H_2O$, 20 mg/L $KAl(SO_4)_2 \cdot 12H_2O$, 13.2 mg/L $CaCl_2 \cdot 2H_2O$, 10 mg/L NaCl (Jin et al., 2006). After immersion, approximately 9.0 ± 0.2 g of nutrition solution was absorbed by each group of woodblocks (1.0 g/g dry wood), thus the initial moisture content of woodblocks was 100 ± 5% (dry basis).

5.2.3 Microbial inoculum

Microbial inoculum was originally obtained from a garden waste composting pile (Fan et al., 2020a). The sampled composting materials were incubated in a bottle at 40 °C for more than 4 months. Oxygen, nutrition solution was supplied regularly. To prepare the inoculum, well-decayed woodblocks (fully covered with microbial biomass) were taken out from the bottle and immersed into demineralized water (150 mL water per woodblock). The mixture was then stirred for 5 minutes, to get the fungal spores and bacteria well-suspended. After that, the solution was filtered by glass-fibre membranes to remove the undissolved solid impurities. About 0.1 mL filtrate used as inoculum was then added to the bottle.

5.2.4 Experimental strategy

To study the effect of temperature on BWO, we placed the BWO bottles in three incubators with working temperatures of 30 °C, 40 °C and 50 °C (10 bottles per temperature). After day 52, when the oxygen consumption rate became stable, 1 bottle was selected for qPCR test. We changed the BWO temperature by moving 6 bottles from the initial incubator to the other incubators (3 bottles per incubator). The last 3 bottles without temperature change were kept in the initial incubator. After that, we continued the BWO process. The whole incubation process lasted for about 220 days. The bottles incubated at 30 °C, 40 °C and 50 °C without temperature change were labelled as B30, B40, and B50, respectively. The bottles with temperature change were labelled by two temperatures. For example, B50-40 was the group moving from 50 °C to 40 °C. All the labels and groups of bottles are summarized in Table 5-1.

Table 5-1 The labels of bottles used in this study.

Temperature	Label	Temperature	Label	Temperature	Label
30 °C	B30	40 °C to 30 °C	B40-30	50 °C to 30 °C	B50-30
30 °C to 40 °C	B30-40	40 °C	B40	50 °C to 40 °C	B50-40
30 °C to 50 °C	B30-50	40 °C to 50 °C	B40-50	50 °C	B50

5.2.5 Oxygen consumption

An oxygen sensor (Sensor Spot SP-PSt 3, PreSens, Germany) and an oxygen meter (Fibox 4 trace, PreSens, Germany) were used to measure the oxygen concentration (in the unit of %) in the Schott bottle. Once the oxygen level in the bottle decreased to below 10%, bottles were cooled to room temperature and opened in the flow cabinet to refresh the inside air. We did this to ensure the O_2 level was not a limiting factor in the BWO (Caizán Juanarena et al., 2016). When the oxygen returned to atmosphere levels, we moved the bottles to the incubator to continue the experiment (Fan et al., 2020b). Demineralized water was added to compensate for the water lost during refreshing process. The amount of added water was determined according to the weight change of bottles before and after refreshing procedure.

5.2.6 Theoretical heat production

It is not possible to measure the actual heat production directly due to the small scale of the experiment. The theoretical heat production (H) in BWO was calculated based on oxygen consumption because oxygen is independent of molecular substrate composition (Hamelers, 2001). The theoretical heat production (in kJ) of the reaction was calculated via the following equation:

$$H = \Delta O_2 \times h \qquad (2)$$

Where ΔO_2 is the oxygen consumption (mol) and h is the heat released by microorganisms expressed as kilo joule per mol of oxygen consumed (kJ/mol). This is commonly done in composting modelling (de Guardia et al., 2012; Kaiser, 1996). Considering there was not any rigorous method in literature about the heat released via

BWO, h was fixed at 467.5 kJ/mol O_2 and was maintained constant throughout the process. 467.5 kJ is the amount of heat released per mol of O_2 consumed in glucose combustion reaction and glucose is the most abundant carbohydrate present in wood (Caizán Juanarena et al., 2016).

5.2.7 Wood weight loss

First, the woodblocks were washed to remove the microbial biomass. Then the woodblocks were placed in the over at 105 °C for 24 hours to remove the moisture. After that, the weight loss was determined on the basis of the initial (in the beginning of the experiment) and final dry weight (at the end of the experiment) as indicated in the following equation.

$$\text{weight loss (\%)} = \frac{final\ dry\ weight - initial\ dry\ weight}{initial\ dry\ weight} \times 100\% \qquad (3)$$

5.2.8 DNA extraction and qPCR test

Before DNA extraction, woodblocks were ground by using a miller with stainless steel blade (Grinder 75, Krups, Solingen, Germany). About 1 g of ice was added to prevent the blade from too hot. Every 30 seconds of operation time, the grinder was stopped until the blade cooled to room temperature. The total grinding time of each sample was 2 mins. After milling, the wood particles (< 1mm) were collected and stored at -20 °C for further analysis. The grinder was disinfected with 70% alcohol between each use to avoid cross contamination.

DNA was extracted from about 0.2 g of each sample using DNeasy Power Soil Kit (Qiagen, the Netherlands) following the manufacturer's protocol. DNA concentration and purity were quantified by Quantus (Promega, the Netherlands) and a Nanodrop spectrophotometer (NanoDrop 1000 spectrophotometer, Thermo Scientific) with OD260/OD280, respectively. DNA samples were stored at -20°C before downstream analysis. Quantitative real-time PCR (qPCR) analysis was used to quantify total bacterial 16S rRNA gene copies and total fungal ITS copies, respectively. qPCR assays were performed in triplicates with a total volume of 20 μL reactions using iQ SYBR

Green master mix (Bio-rad) on a CFX96 Real-Time System (C1000 Touch, Bio-rad). Gene copy numbers were calculated as copies/g dry wood. The bacterial qPCR efficiency was 95.05% (± 4.25%), with $R^2 > 0.997$. The fungal qPCR efficiency was 97.7% (± 3.50%), with $R^2 > 0.998$. qPCR primers and amplification protocols are detailed in Table 5-S1.

5.2.9 Statistics analysis

All data were analysed using IBM SPSS Statistic 23 (IBM, USA) for Microsoft Windows. Differences were compared statistically using analysis of variance (ANOVA) testing at the 5% level of significance. The assumption of normality and equality of variances were checked for each ANOVA model. Differences between values at P greater than 0.05 were considered as not significantly different.

5.3 Results

5.3.1 Oxygen consumption at different temperatures

The cumulative oxygen consumption (COC) at different temperatures is shown in Fig. 5-2 (a), and the oxygen consumption rate (OCR) is shown in Fig. 5-2 (b). As shown in Fig. 5-2 (a), temperature had a significant effect on COC (ANOVA, $P < 0.05$). The highest oxygen consumption was found at 40 °C, while the lowest oxygen consumption was observed at 50 °C. The COC of B30, B40 and B50 at the end of incubation was 3421 mmol/kg DM, 4900 mmol/kg DM and 1169 mmol/kg DM, respectively. As shown in Fig. 5-2 (b), the oxygen consumption rate was highest in the first 10 days, then slowly decreased and became stable, with some fluctuations for the remaining period. It was expected that the OCR would not increase anymore without any parameter changes. The highest OCRs obtained by B30, B40 and B50 were between 50-60, 100-110 and 35-70 mmol/kg DM/day, respectively. As observed for COC, the OCR for B40 was highest, while the OCR for B50 was lowest. After 120 days, all OCRs were low and there was no significant difference between the three temperatures on the OCR (ANOVA, $P > 0.05$).

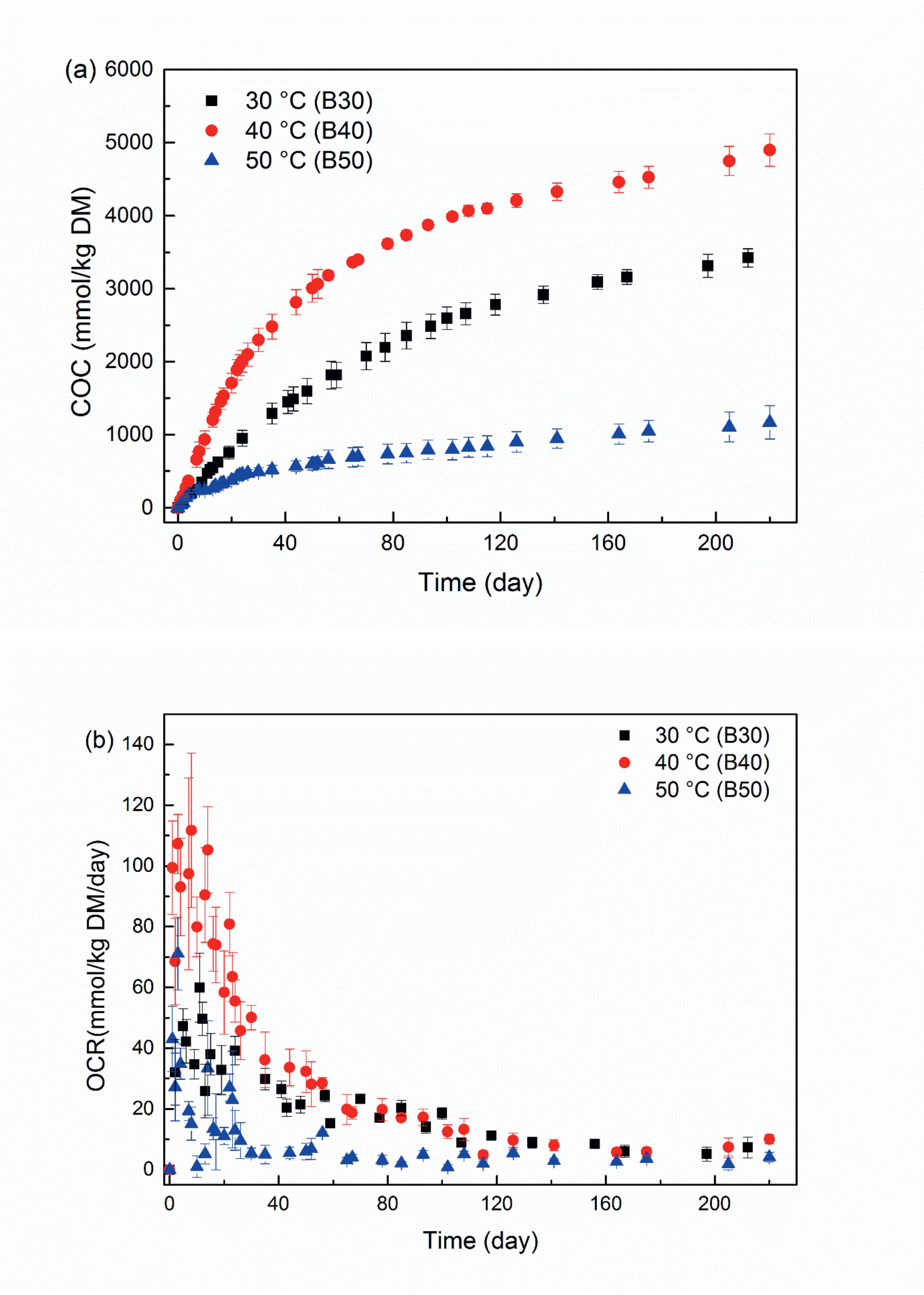


Fig. 5-2 (a) The cumulative oxygen consumption at 30, 40 and 50 °C; (b) the oxygen consumption rate at 30 °C, 40 °C and 50 °C. Average and standard deviation were calculated for 10 replicates in the first 52 days and for 3 replicates after day 52. 40 °C had the highest oxygen consumption while 50 °C had the lowest oxygen consumption.

5.3.2 Effect of temperature change on oxygen consumption

5.3.2.1 From 30 °C to 40 °C and 50 °C

The COC of B30, B30-40, and B30-50 is shown in Fig. 5-3 (a), and the OCR is shown in Fig. 5-3 (b). As shown in Fig. 5-3 (a), Changing temperature from 30 °C to 40 °C (B30-40) significantly increased the COC compared to the group without changing temperature (B30) (ANOVA, $P < 0.05$); however, changing temperature from 30 °C to 50 °C (B30-50) did not influence the COC of BWO process significantly (ANOVA, $P > 0.05$). Totally, the COC of B30-40 was 5367 mmol/kg DM and this number was 3206 mmol/kg DM for B30-50. As shown in Fig. 5-3 (b), the OCR of B30-40 remarkably increased after day 60, and there was a second peak (around 40 mmol/kg DM/day) which lasted for about 60 days until day 120. After day 120, the OCR of B34 gradually decreased. Changing temperature from 30 to 50 °C resulted in an increase in OCR immediately after day 60. However, after day 78, the OCR of B30-50 dropped and became stable with OCR around 10 mmol/kg DM/day.

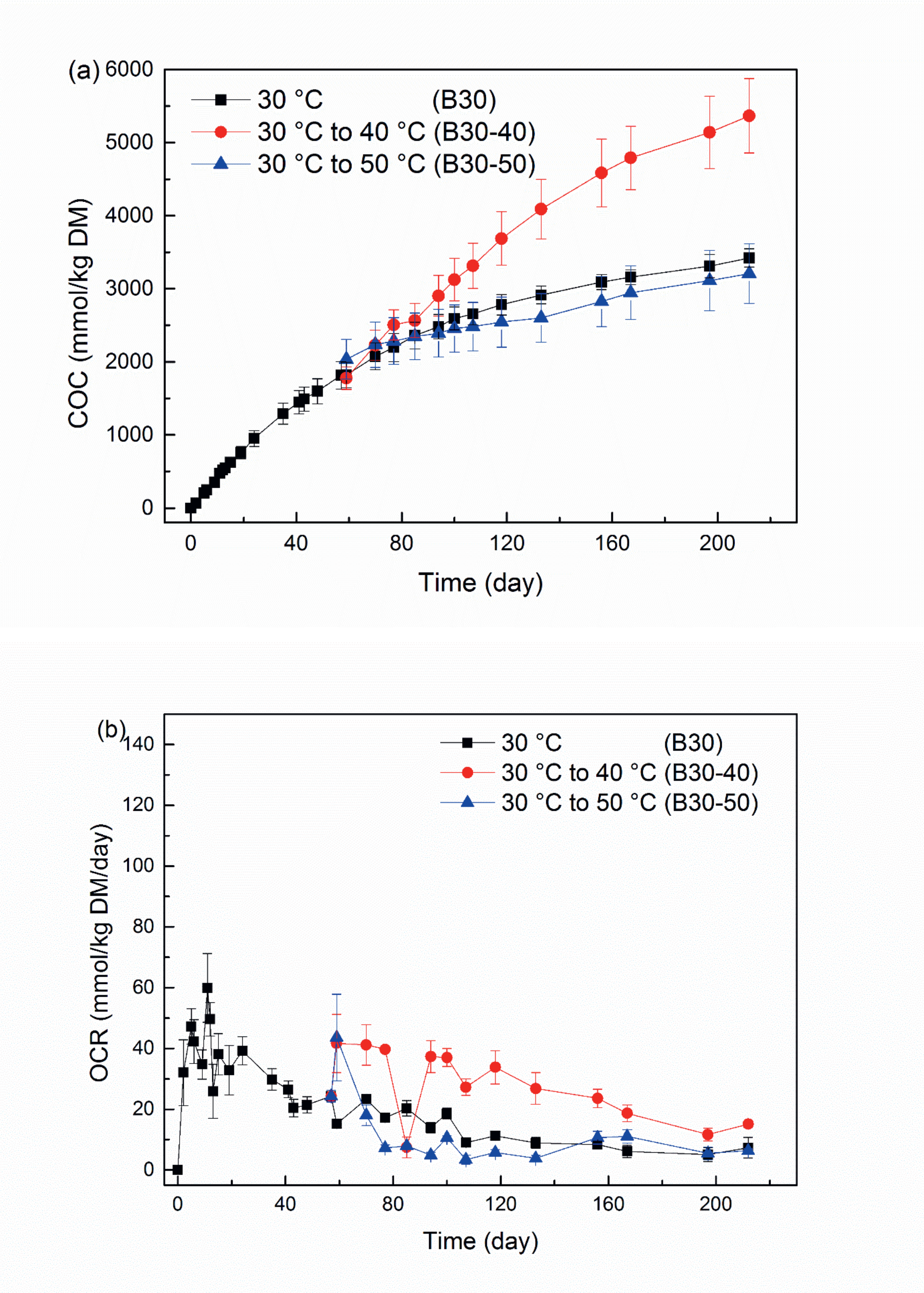
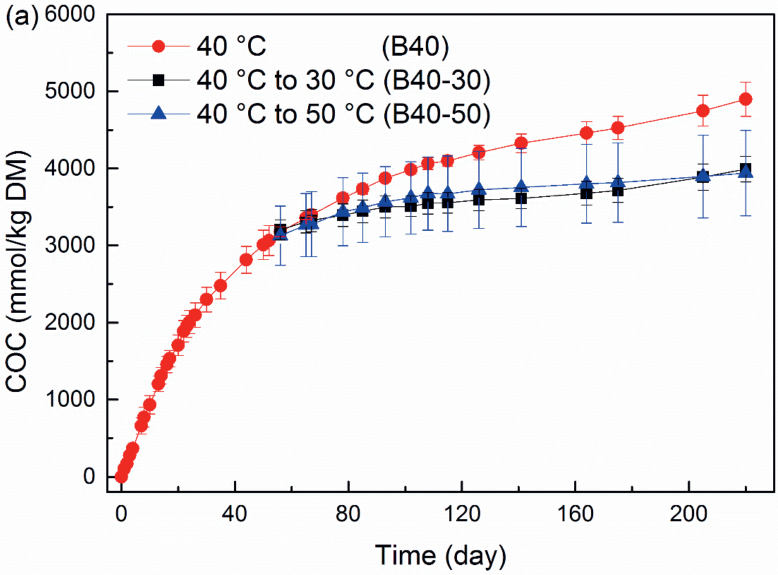


Fig. 5-3 (a) Effect of temperature change on cumulative oxygen consumption; (b) effect of temperature change on oxygen consumption rate. Average and standard deviation were calculated for 10 replicates in the first 52 days and for 3 replicates after day 52. Changing temperature from 30 °C to 40 °C significantly increased the oxygen consumption; while changing temperature from 30 °C to 50 °C did not have a significant effect on the oxygen consumption.

5.3.2.2 From 40 °C to 30 °C and 50 °C

The COC of B40-30, B40-40 and B40-50 is shown in Fig. 5-4 (a), and the OCR is shown in Fig. 5-4 (b). As shown in Fig. 5-4 (a), changing temperature from 40 °C to 30 °C (B40-30) and from 40 °C to 50 °C (B40-50) significantly inhibited the oxygen consumption (ANOVA, $P < 0.05$). However, difference between B40-50 and B40-30 was not statistically significant (ANOVA, $P > 0.05$). At the end of the experiment, the COC of B40-30 and B40-50 was 3993 mmol/kg DM COC and 3940 mmol/kg DM, respectively. As shown in Fig. 5-4 (b), changing temperature from 40 °C to 50 °C slightly increased the OCR at day 56, however, the OCR dropped immediately after day 60. After day 120, all OCRs were around 10 mmol/kg DM/day.



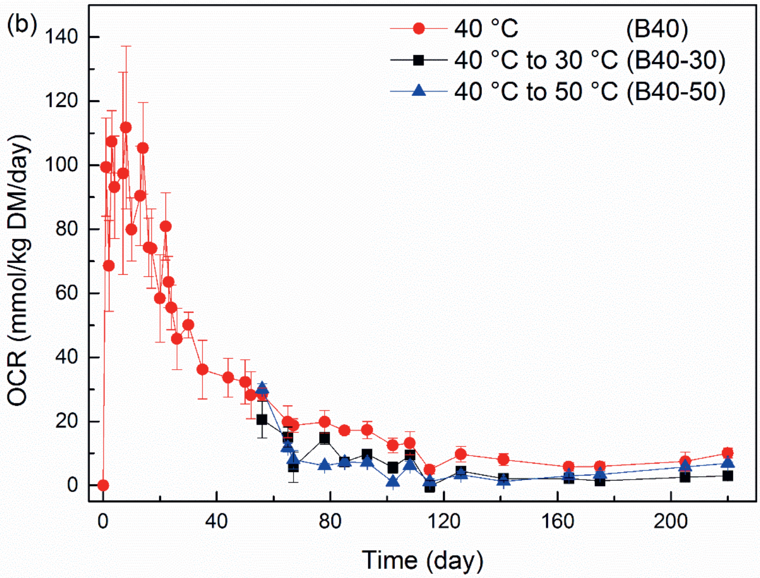
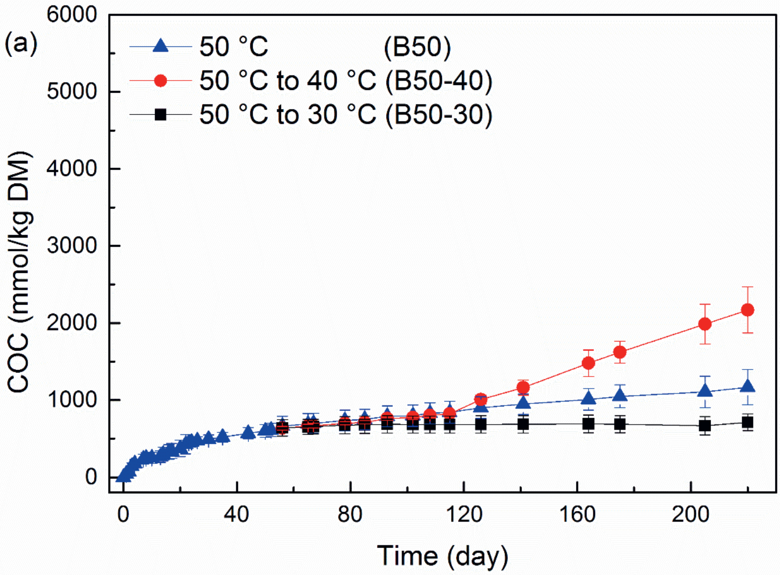


Fig. 5-4 (a) Effect of temperature change on cumulative oxygen consumption; (b) effect of temperature change on the oxygen consumption. Average and standard deviation were calculated for 10 replicates in the first 52 days and for 3 replicates after day 52. Changing temperature from 40 °C to 30 °C and from 40 °C to 50 °C significantly inhibited the oxygen consumption.

5.3.2.3 From 50 °C to 30 °C and 40 °C

The COC of B50-30, B50-40 and B50 is shown in Fig. 5-5 (a), and the OCR is shown in Fig. 5-5 (b). As shown in Fig. 5-5 (a), changing temperature from 50 °C to 40 °C (B50-40) significantly increased the COC (ANOVA, $P < 0.05$), while changing temperature from 50 °C to 30 °C (B50-30) significantly decreased the COC (ANOVA, $P < 0.05$). At the end of the incubation, the COC of B50-30 and B50-40 was 712 mmol/kg DM and 1169 mmol/kg DM, respectively. As for Fig. 5-5 (b), the OCR of B50-30 quickly dropped to around 0 mmol/kg DM after changing temperature. There was no significant difference between the OCR of B50-40 and B50 in the period of day 65 to 115 (ANOVA, $P < 0.05$). After day 115, the OCR of B50-40 increased rapidly and became stable until day 220. We concluded that changing the temperature from 50 °C to 40 °C resulted in an increase in oxygen consumption, while changing the temperature from 50 °C to 30 °C almost stopped the BWO process.



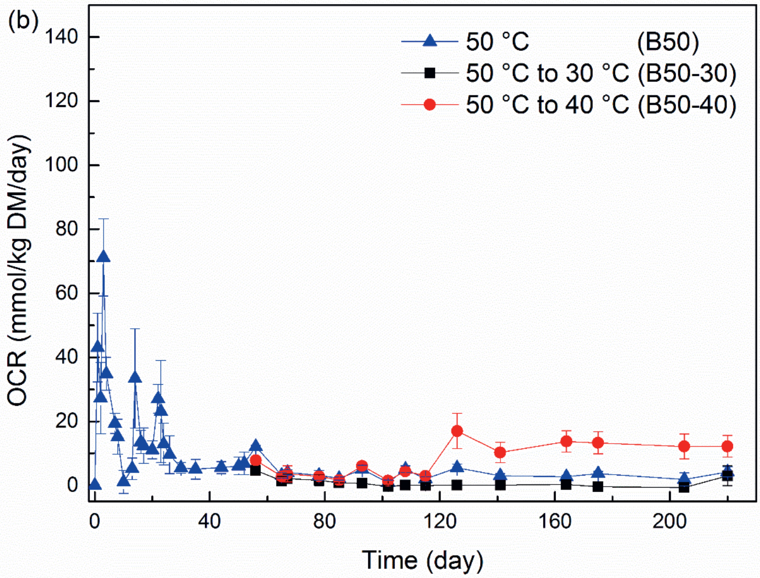


Fig. 5-5 (a) Effect of temperature change on cumulative oxygen consumption; (b) effect of temperature change on the oxygen consumption rate. Average and standard deviation were calculated for 10 replicates in the first 52 days and for 3 replicates after day 52. Changing temperature from 50 °C to 40 °C significantly enhanced the oxygen consumption while changing temperature from 50 °C to 30 °C did not.

5.3.3 Wood weight loss

The results of weight loss are shown in Fig. 5-6. The weight loss of B30-40 group was 15.5 ± 1.3%, which was 55% higher than that of B30. The weight loss of B40-30 and B40-50 were only 9.8 ± 2.6% and 9.4 ± 0.1%, respectively, which was 18% and 22% lower than that without temperature change (B40). B50-40 increased the weight loss from 3.3 ± 1.1% to 6.5 ± 1.1% compared to B50, however, the overall weight loss was still low. B50-30 only had a weight loss of 1.8 ± 0.5%.

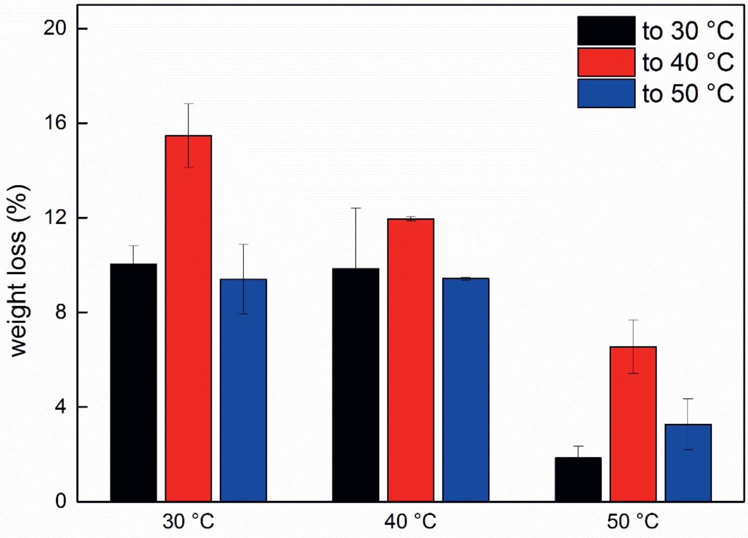
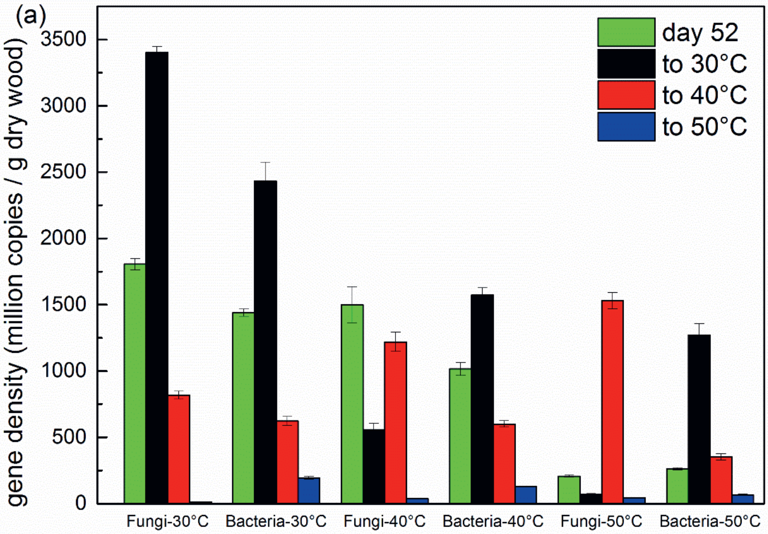


Fig. 5-6 The weight loss at the end of incubation. Average and standard deviation were calculated for 3 replicates.

5.3.4 qPCR test

Temperature is an important factor affecting the microbial population and activity (Xiao et al., 2011). In this study, qPCR was used to determine the microbial gene density (copies / g dry wood) during the BWO process. qPCR has been used to estimate the fungal biomass and bacterial biomass (Black et al., 2013; Cotto et al., 2015; Horevaj et al., 2011). However, it is difficult to estimate the fungal biomass in mixed culture by using qPCR since the number of copies per fungal cell are largely unknown for most species, making the estimation unreliable (Manerkar et al., 2008). Despite this, qPCR results still quantitatively demonstrate the relative development of bacteria and fungi within the same operational condition. The fungal ITS gene and bacterial 16S rRNA gene is shown in Fig. 5-7 (a); the gene ratio between fungi and bacteria is shown in Fig. 5-7 (b). Despite this, qPCR results still quantitatively demonstrated the relative change of bacteria and fungi.



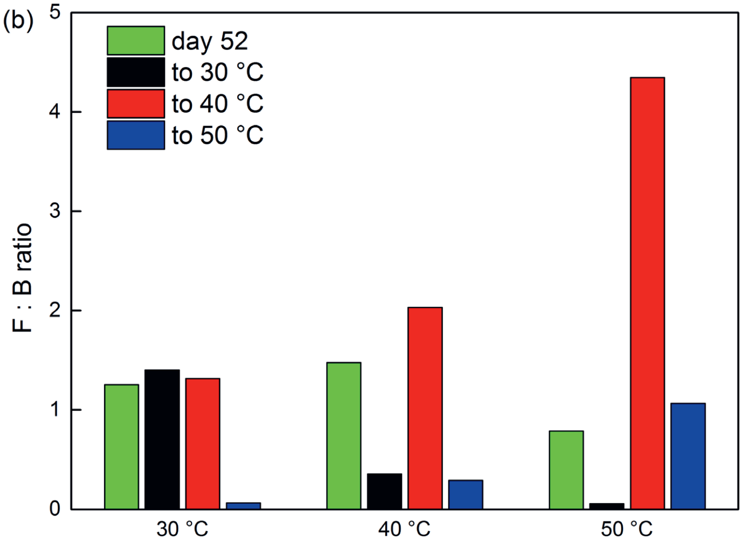


Fig. 5-7 (a) The fungal ITS gene and bacterial 16S rRNA gene; (b) the ratio between fungal gene density and bacterial gene density. Average and standard deviation was calculated for 3 measurements.

As shown in Fig. 5-7 (a), at day 52, the highest fungal ITS gene and bacterial 16S rRNA gene were observed at 30 °C, then followed by 40 °C. The lowest microbial gene density was observed at 50 °C. As for the response of BWO to temperature change, changing temperature from 30 °C to 40 °C (B30-40) and from 30 °C to 50 °C (B30-50) significantly decreased the fungal ITS gene and bacteria 16S rRNA gene (ANOVA, $P < 0.05$). Changing temperature from 40 °C to 30 °C (B40-30) significantly decreased the fungal ITS gene and increased the bacterial 16S rRNA gene (ANOVA, $P < 0.05$). Changing temperature from 40 °C to 50 °C (B40-50), however, decreased both the fungal and bacterial gene density. The microbial gene density was low at B50. Changing temperature from 50 °C to 30 °C (B50-30) showed an obvious increase in bacterial 16S rRNA gene and a relatively small increase in fungal ITS gene. Changing temperature from 50 °C to 40 °C increased the fungal ITS gene remarkably while this increase was not as high for bacterial gene density.

Fig. 5-7 (b) shows the ratio between fungal gene density and bacterial gene density (F:B ratio). The F:B ratio of B30-50 and B50-30 was low (< 0.1), while the F:B ratio of B50-40 was over 4. The F:B ratios of B30-40, B40, and B50-40 were generally over 1, resulting in large increase in the fungal proportion compared to bacteria proportion.

5.4 Discussion

5.4.1 BWO at different temperatures

There was a strong linear relationship between COC and weight loss, which implies that the oxygen consumption can be used as an indicator for wood degradation (Fig. 5-S1). This was in accordance with our previous studies (Fan et al., 2020a; Fan et al., 2020b). The highest OCR was observed in the first ten days for all temperatures, which was due to the degradation of readily organic matter, such as starches, pectins, and some easily available hemicellulose (McCarthy, 1987; Nada et al., 2012; Tanaka et al., 2020; Tondi et al., 2012). Moreover, the depletion of nutrients could also result in the decrease of OCR (Fan et al., 2020a). The COC of the first 52 days accounted for about 58-68%

of the total COC in the whole duration (220 days), showing that the BWO process under these conditions was fastest in the first 52 days. This was also found in our previous studies (Fan et al., 2020a; Fan et al., 2020b) and other composting studies (VanderGheynst et al., 1997; Yang et al., 2015).

In the experiment without temperature change, the highest oxygen consumption and the highest weight loss were observed at 40 °C while the highest fungal and bacterial development were observed at 30 °C. The highest microbial gene density did not relate to the highest microbial activity, which was reported by others (Albrecht et al., 2010; Godden et al., 1983; Palmisano et al., 1993). Usually, the fungal and bacterial population in composting drop as the temperature begins to peak and then increase when the temperature decreases, however, the microbial activity can be enhanced by higher temperature (Albrecht et al., 2010; Tiquia et al., 2002; Tuomela et al., 2000). It has been extensively reported that higher temperatures, usually between 40 to 50 °C, could stimulate the wood degradation by enhancing the cellulose degradation and lignin degradation (Ryckeboer et al., 2003a; Ryckeboer et al., 2003b; Tomati et al., 1995; Tuomela et al., 2000; Yu et al., 2007). That could explain our observation that 40 °C had higher BWO rate with lower microbial gene density compared to 30 °C. However, 50 °C displayed the lowest COC and weight loss, which might be because 50 °C was too high for microbial growth in this experiment. Tuomela et al. (2000) reported that some wood-decaying microorganisms, especially fungi, have an optimal growth temperature between 40-45 °C and a maximum growth temperature of about 50 °C (Tuomela et al., 2000). Ryckeboer et al. (2003) also reported that some bacteria and fungi could only be found below 50 °C (Ryckeboer et al., 2003b). Another possible reason for the lowest BWO performance at 50 °C might be that woodblocks were tending to be too dry at 50 °C in our setup. Although we did not measure the moisture content of woodblocks during BWO process, we took pictures of B50 to support our point (Fig. 5-S2). Wood is a hygroscopic material, with the ability to interact with water (adsorb or desorb) from air, which is affected by the temperature (Zupanc et al., 2019). At higher temperature (50 °C), the water absorbed by woodblocks evaporated, resulting in low moisture content of the woodblocks. The evaporation rate at 30 °C and 40 °C was relatively lower compared to 50 °C. The moisture content did not prohibit the BWO process at 30 °C and 40 °C. Moisture content is important in wood decaying process (Schmidt, 2006). Microorganisms are able to utilize organic molecules which dissolve in water. If the moisture content falls below a critical level (usually 30%), microbial activity will decrease and the microbes become dormant (Viitanen, 1994a; Woodard & Milner, 2016). Wood-decaying fungi are especially sensible to moisture, and thus control of humidity could be a route towards increasing fungal activity at 50 °C. 40 °C was high enough for fast wood degradation and not too high for inhibition to microbial growth. As a result, the highest BWO was found at 40 °C compared to 30 °C and 50 °C.

5.4.2 BWO under temperature change conditions

Changing temperature from 40 °C or 30 °C to 50 °C decreased the microbial gene density. At the end of incubation, B30-50 resulted in only about 0.35 % fungal ITS gene and 7.9 % bacterial 16S rRNA gene compared to B30 group. The fungal gene density were nearly depleted after changing temperature from 30 to 50 °C, showing that the mesophilic fungi, with a growing temperature between 5-35 °C and an optimum temperature of 25-30 °C (Dix & Webster, 1995; Viitanen, 1994), could not adapt the temperature of 50 °C, at which the thermophilic fungi were growing (Crisan, 1973). However, the bacterial gene density stayed similar in this group, which suggests that bacteria could possess a stronger temperature tolerance and recover their prosperity better than fungi (Dix & Webster, 1995; Ryckeboer et al., 2003b; Sun et al., 2017). As a result, fungi could not compete over bacteria in the group of B30-50. This was also proved by the fact that the bacterial gene density increased obviously while the fungal gene density almost remained the same level when changing temperature from 50 to 30 °C (B50-30).

Changing temperature from 50 °C or 30 °C to 40 °C could stimulate the BWO process. Although the temperature was changed at day 52, the OCR did not increase significantly until day 58 for B30-40 experiment and day 126 for B50-40 experiment, which means that microbes growing at 30 °C could adapt the 40 °C quickly (6 days) compared to the microbes growing at 50 °C (74 days). The long adapting phase shows that mesophilic microbes, mainly fungi, with a growing temperature at below 40 °C (Dix & Webster, 1995), did not survive in B50, it therefore took a long time for them to flourish again. B30-40 increased the BWO process while the microbial gene density decreased remarkably. Likely, 40 °C could stimulate the microbial activity and the degradation of cellulose and lignin compared to 30 °C (Albrecht et al., 2010; Ishii et al., 2000; Ryckeboer et al., 2003a; Ryckeboer et al., 2003b; Tomati et al., 1995; Tuomela et al., 2000; Yu et al., 2007). B50-40 significantly enhanced the BWO process which might be due to that B50-40 increased the population of wood-decaying microorganisms (microbial gene density) since visible microbial biomass could be only found in the B50-40 group (Fig. 5-S3).

5.4.3 The role of fungi and bacteria during BWO process

Both fungi and bacteria were found in BWO process, however, their contribution to BWO were different. Fungi are generally more efficient wood decomposer agents than bacteria (Mester et al., 2004; Mitsuhashi & Morrell, 2012; Pérez et al., 2002; Voběrková et al., 2017). In the BWO, we found that fungi, other than bacteria, were more related to fast BWO process. This is clear in the results of F:B ratio. The low F:B ratio (< 0.1) means that the microbial growth was largely attributed to the bacteria while the fungi largely contributed to the microbial growth in the groups with high F:B ratio. In the present study, groups with low F:B ratio (B30-50 and B50-30) inhibited the BWO process while the groups with high F:B ratio (B40 and B50-40) had higher BWO rate. Moreover, 40 °C always had high fungal gene density (over 750 million copies number / g dry wood), which could be the reason why 40 °C was the preferred temperature for the BWO process.

5.4.4 Theoretical heat production and theoretical heat production rate

The objective of BWO is to recover the generated heat. The theoretical heat production was calculated proportionally to oxygen consumption. In the present study, the highest theoretical heat was 2510 kJ/kg DM obtained by B30-40, followed by 2290 kJ/kg DM obtained by B40. From the view of energy, B30-40 generated higher amount of heat compared to B40, showing that it is not optimal to start at 40 °C initially. However, the reason needs further research.

When comparing the theoretical heat production rate to our previous studies, we calculated the rate in the first 44 days. This was due to the different experiment duration in the present study and our previous studies. The theoretical heat production rate decreased in the course of time. Thus, the longer experiment duration had the lower heat production rate. At day 44, the theoretical heat production rate of B40 was 0.4 W/kg DM, which was a lower than that in our previous studies: 0.6 W/kg DM in (Fan et al., 2020a) and 0.7 W/kg DM in (Fan et al., 2020b). The reason could be the different amount of nutrition addition. In the present study, the initially added nitrogen was 0.52 ‰ (basis of dry wood) while this number was 1.2 ‰ in (Fan et al., 2020a) and 2.5 ‰ in (Fan et al., 2020b). Nutrition is important for fast BWO (Fan et al., 2020b). During BWO process, the nutrition was depleted quickly in the first week of BWO due to the fast growth of microbes and wood degradation (Fan et al., 2020a). In our study, the initially added nutrition might not be able to supply a fast BWO.

5.5 Conclusion

In this study, we investigated the effect of three different temperatures (30 °C, 40 °C, 50 °C) on BWO process and the response of BWO to temperature change. Temperature affect the BWO performance and microbial gene density obviously. In the experiments without changing temperature, the highest oxygen consumption and weight loss were observed at 40 °C, which means that 40 °C was the preferred temperature to BWO compared to 30 °C and 50 °C. Although the highest microbial gene density was observed at 30 °C, the BWO performance at 30 °C was not the highest. 50 °C had the lowest BWO performance and the microbial amount.

In the experiment with temperature changes, changing temperature had a significant effect on the BWO performance and microbial gene density. Changing temperature from 30 °C or 50 °C to 40 °C could enhance the BWO process obviously, while from 40°C to 30 °C or 50 °C inhibited the BWO process. Changing temperature from 50 °C to 30 °C increased the bacterial gene density obviously, however, the fungal gene density did not increase that much. Although the microbial gene density was decreased remarkably when changed the temperature from 30 °C to 50 °C, the BWO performance did not show a significantly change.

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Support information

Table 5-S1 qPCR primers and amplification protocols.

Target gene	Primer	Thermal profile	Cycles	Reference
Bacteria 16S rRNA	338F (5’-ACTCCTACGGGAGGCAGCAG-3’) 518R (5’-ATTACCGCGGCTGCTGG-3’)	95°C 5 min; 95°C 15 s, 64°C 30 s	1 40	(Muyzer et al., 1993)
Fungi ITS region	ITS86F (5’-GTGAATCATCGAATCTTTGAA-3’) ITS4R (5′-TCCTCCGCTTATTGATATGC-3′)	95°C 5 min; 95°C 15 s, 55°C 30 s, 72°C 60 s	1 40	(Turenne et al., 1999)

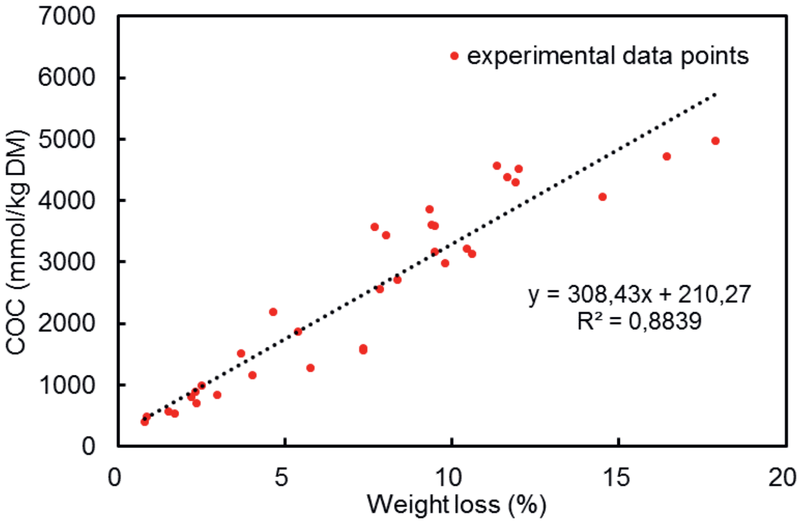

Fig. 5-S1 The relationship between weight loss and cumulative oxygen consumption.

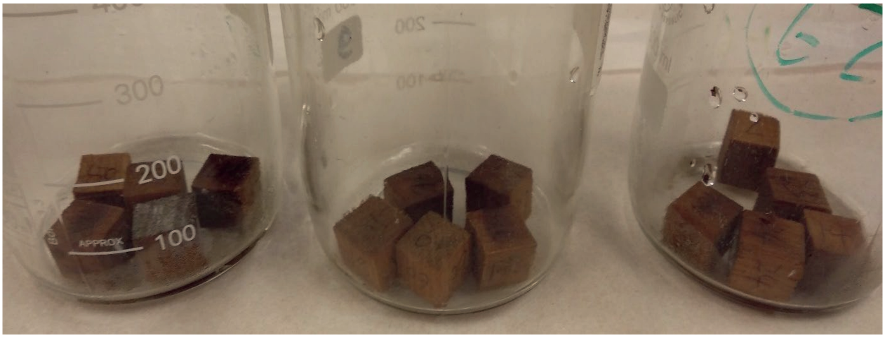

Fig. 5-S2 Pictures of bottles at 50 °C. Woodblocks were dry and visible microbial biomass could hardly be detected.

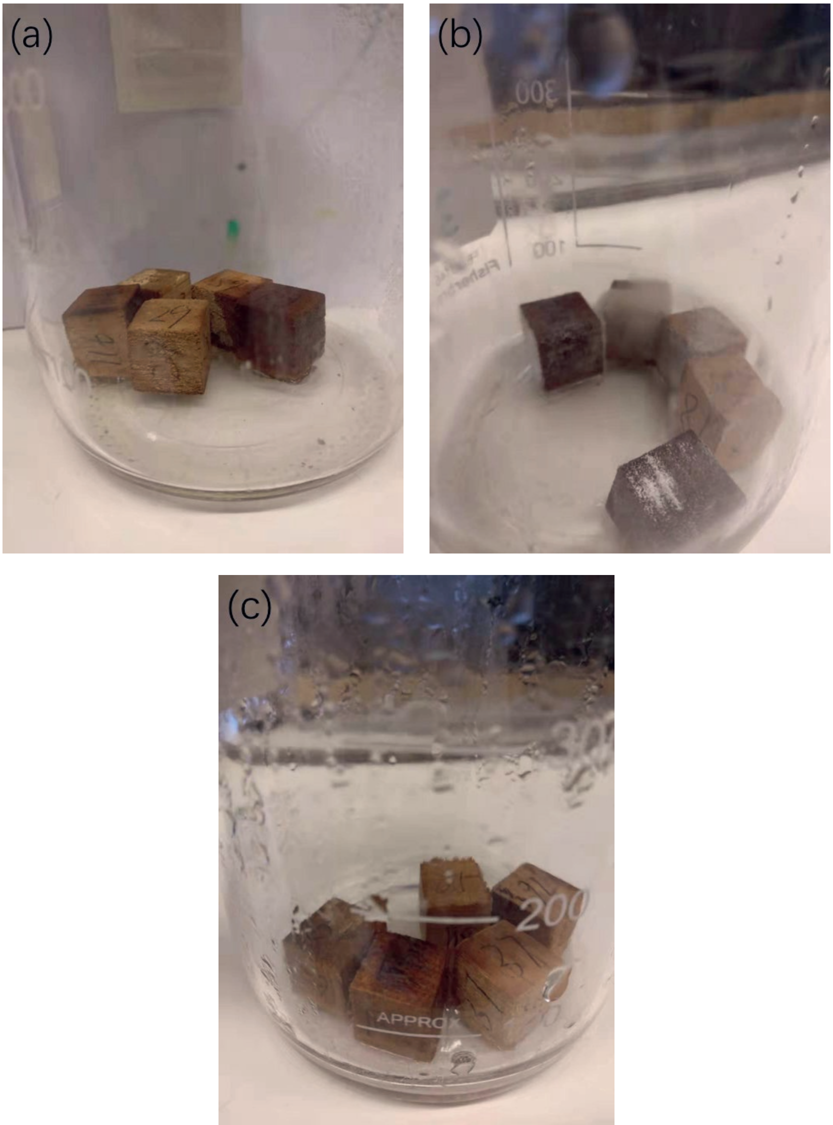

Fig. 5-S3 Pictures of bottles at the end of experiment. (a) at 50 °C (B50); (b) changing temperature from 50 °C to 40 °C (B50-40); (c) changing temperature from 50 °C to 30 °C (B50-30). Visible microbial growth was only observed after changing temperature from 50 °C to 40 °C. No visible microbial biomass was found at 50 °C and temperature change from 50 °C to 30 °C.

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Chapter 6

Biological Wood Oxidation at 40°C and 50 °C: A Reactor-Scale Study

Shiyang Fan, Yujia Luo, Annemiek ter Heijne, Cees J.N. Buisman, Wei-Shan Chen,

A modified version of this chapter is ready to submit:
Shiyang Fan, Yujia Luo, Annemiek ter Heijne, Cees J.N. Buisman, Wei-Shan Chen.
“Biological wood oxidation at 40 °C and 50 °C: A reactor-scale study.”

Abstract

Biological wood oxidation (BWO) is an alternative for sustainable heat generation from woody biomass. Our previous work has shown that temperature is a critical factor for the performance of BWO at batch scale (< 10 g woodblocks in 650 mL batch volume). Considering its potentials for application, we focused on BWO at a larger reactor scale (880 ± 5 g woodblocks in 6 L reactor volume). In this study, we ran four reactors at two temperatures (40 °C and 50 °C) and monitored the CO_2 evolution continuously. Moreover, we determined the weight loss and microbial development during BWO process. The fungal ITS gene and bacterial 16S rRNA gene on wood was determined by qPCR. After 150-day incubation, 40 °C had higher CO_2 evolution (rate) and weight loss than 50 °C. In addition, the fungal gene density and bacterial gene density at 40 °C was higher than them at 50 °C, which means that 40 °C was more suitable for microbial growth. At the same nutrients level, BWO at reactor scale had a slightly higher theoretical heat production than BWO at batch scale.

Keywords: *biological wood oxidation, temperature, wood weight loss, oxygen consumption, heat production, reactor study*

6.1 Introduction

Biological wood oxidation (BWO) is an environmental-friendly wood waste management approach at a temperature around 40-55 °C, and it has been proposed as a clean alternative to wood combustion for heat production (Caizán Juanarena et al., 2016; Fan et al., 2020b). BWO is a type of composting for woody biomass at elevated temperatures. During BWO process, microorganisms (mainly bacteria and fungi) decompose wood materials in the presence of oxygen. The product of BWO is CO_2, H_2O and heat, which can be described by the equation (1) (Caizán Juanarena et al., 2016):

$$CH_{1.5}O_{0.7} + 1.03\ O_2 \rightarrow CO_2 + 0.734\ H_2O + \text{heat} \qquad (1)$$

BWO is new and has been limitedly studied. In the previous studies, we confirmed the significance of nutrient addition. With nutrient addition, the oxygen consumption and weight loss of dry wood was enhanced (Fan et al., 2020a; Fan et al., 2020b). We also investigated the effect of temperature on BWO. We reported that at the batch scale (< 10 g woodblocks in 650 mL batch volume), the preferred temperature for BWO was 40 °C while 50 °C inhibited the BWO remarkably. We found that the weight loss at 50 °C was only 27.3% of the weight loss at 40 °C, which was due to the microbial development because the fungal ITS gene copies at 50 °C were only 3.5% of the fungal gene copies at 40 °C; and this number was only 10.8% for bacteria (Chapter 5). So far, all the studies have been done at small batch scale (the highest initial weight of the woodblocks was only 9 gram). Little information about BWO on a larger scale is known, which is important considering its potential future application. Moreover, to the author's best knowledge, there are little scientific literature about the microbial development during BWO in a larger scale.

Thus, the objective of this article was to study the effect of temperature, i.e.: 40 and 50 °C, on BWO. In order to ensure that the humidity was not influencing the microbial activity, we continuously supplied the humidified air to minimize drying of wood. This was widely done in composting reactor studies (Klejment & Rosiński, 2008; Petric et al., 2009; Villaseñor et al., 2012). To assess the BWO performance, we monitored the

CO_2 evolution and wood weight loss. We also determined the fungal and bacterial gene density of woodblocks during BWO process. Also, the theoretical heat production was estimated and discussed. This article is the first article focusing on the BWO performance and microbial development at a larger scale reactor.

6.2 Materials and Methods

6.2.1. Woodblocks

The wood used in this study was collected from an ash tree (*Fraxinus excelsior*) located in Mirns, Friesland, Netherlands. The trunk was cut into cubes using a cutting machine (BAS318, Matabo, Germany). Each woodblock had a size around 1.5 cm * 1.5 cm * 1.5 cm (L * W * H). All woodblocks used in the study were individually numbered and weighed.

6.2.2 Nutrients and inoculum

Before experiment, the woodblocks were immersed in the nutrients solution for 3 days until reaching a constant weight. The nutrient solution, supplying macronutrients and micronutrients, contained 2 g/L NH_4Cl, 4.5 g/L KH_2PO_4, 0.5 g/L K_2HPO_4, 1.0 mg/L $MgSO_4 \cdot 7H_2O$, 120 mg/L $FeCl_3$, 50 mg/L H_3BO_3, 10 mg/L KI, 45 mg/L $MnSO_4 \cdot H_2O$, 20 mg/L $Na_2MoO_4 \cdot 2H_2O$, 75 mg/L $ZnSO_4 \cdot 7H_2O$, 50 mg/L $CoCl_2 \cdot 6H_2O$, 20 mg/L $KAl(SO_4)_2 \cdot 12H_2O$, 13.2 mg/L $CaCl_2 \cdot 2H_2O$, 10 mg/L NaCl (Jin et al., 2006). After immersion, the initial moisture content of woodblocks was 100 ± 5% (dry basis).

The inoculum was the same as our previous work (Fan et al., 2020b). Microbial inoculum was originally obtained from a garden waste composting pile (Fan et al., 2020a). The sampled composting materials were incubated in a bottle at 40 °C for more than 4 months. Oxygen and nutrient solution were supplied regularly. To prepare the inoculum, well-decayed woodblocks (fully covered with microbial biomass) were taken out from the bottle and immersed into demineralized water (150 mL water per woodblock). The mixture was then stirred for 5 minutes, to get the fungi and bacteria suspended. After that, the solution was filtered by glass-fiber membranes (0.45 μM) to remove the undissolved solid impurities. About 20 mL filtrate used as inoculum was then added to the woodblocks averagely.

6.2.3 Reactor setup and BWO system

Four laboratory reactors made of steel sheet carried out the BWO experiment. Each reactor has a total volume of about 6.2 L, with 0.35 m in height and 0.15 m in diameter. An expanded metal floor was installed 0.10 m from the bottom to create an aeration plenum. About 880 ± 5g of dry woodblocks were filled in each reactor. Two reactors were put in an oven at a constant temperature of 50 °C, marked as 50 °C-R1 and 50 °C-R2, respectively. The other two reactors were kept at 40 °C, marked as 40 °C-R3 and 40 °C-R4, respectively. During the BWO process, the reactors were opened at day 20, 30, 50, 90, and 150. 20 woodblocks were taken out from the top, middle and bottom layers of each reactor each time for samples analysis. The woodblocks were used for weight loss test and qPCR test.

The schematic of the BWO system is shown in Fig. 6-1. O_2 and N_2 were served as artificial air at a volume of 0.3 L/h and 1.2 L/h, respectively, which was controlled by two mass flow controllers (EL-Flow select F-201CV, Bronkhorst, Veenendaal, the Netherlands). The artificial air was humidified by passing through a water-washer, which was also located in the oven. Then the humidified air was fed into the reactor from the bottom. After reaction, the exit gas passed over acid in an acid-washer before analysis. The acid was used to remove the ammonia that might generate during the degradation process. Moreover, the low pH of the acid could inhibit the CO_2 dissolve in water. Then the air stream was passed through a gas cooler (ElektrogasKühler TYP EGK-4, Bühler, Ratingen, Germany) before gas analysis (CO_2 measurement). The CO_2 concentration in the outlet gas was measured continuously by using a CO_2 meter (Dräger Polytron IR CO_2, Dräger, Lübeck, Germany) with a measuring range of 0-30% (v/v) and an upper temperature limit of 65 °C. The CO_2 data were recorded by using Labview 2013 software (National Instruments, Texas, USA) running on a desktop computer. Temperature in the woodblocks pile was monitored continuously by using a temperature sensor at the height of 150 mm above the base plate.

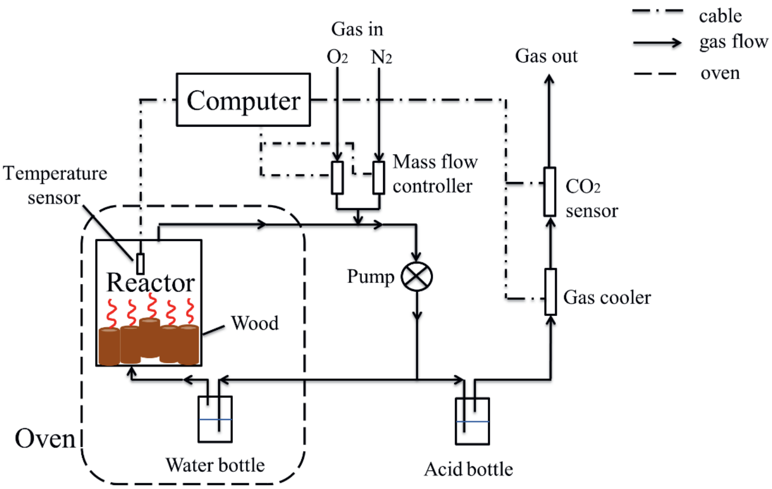


Fig. 6-1 Schematic illustration of wood oxidation reactor used in this experiment.

6.2.4 Weight loss

Dry weight of woodblocks was determined by drying at 105 °C for 24 h in an oven (E28, Binder, Tuttlingen, Germany). The weight loss was determined on the basis of initial dry weight and final dry weight, as shown in the equation (1):

$$weight\ loss\ (\%) = \frac{initial\ dry\ weight - final\ dry\ weight}{initial\ dry\ weight} \times 100 \qquad (1)$$

6.2.5 Theoretical heat production and theoretical heat production rate

Due to the small scale, the insulation and the ventilation of the reactor, the generated heat was hardly to be collected and measured. Heat is calculated by using the heat released in aerobic respiration of glucose ($C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$), as glucose is the most abundant carbohydrate present in lignocellulosic materials (Willför et al., 2005). Thus, the theoretical heat production (rate) was estimated according to the CO_2 evolution. This method has been already validated (Kaiser, 1996; Vlyssides et al., 2009). The theoretical heat production (H), in kJ/kg dry matter (DM), was shown in the equation (2):

$$H = \frac{\Delta CO_2 \times h}{m} \times 1000 \tag{2}$$

Where ΔCO_2 is the accumulative carbon dioxide consumption (mol), m is the initial dry weight of woodblocks (g) and h is the heat released by oxidation process expressed as kilo joule per mol of CO_2 generated (kJ/mol). h is assumed to be 467.5 kJ/mol CO_2, since this is the amount of heat generated per mol of carbon dioxide generation during the reaction of glucose oxidation (Luong & Volesky, 1983).

The theoretical heat production rate (W), in W/kg DM, was calculated via the equation (3):

$$W = \frac{H}{t} \times 1000 \tag{3}$$

Here H is the theoretical heat production (kJ/kg DM) and t is the incubation time (s).

6.2.6 DNA extraction and qPCR test

Before DNA extraction, woodblocks were ground by using a miller with stainless steel blade (Grinder 75, Krups, Solingen, Germany). About 1 g of ice was added to prevent the blade from significant temperature increase. Every 30 seconds, the grinder was stopped until the blade cooled to room temperature. The total grinding time of each sample was 2 mins. After milling, the wood particles (< 1mm) were collected and stored at -20 °C for further analysis. The grinder was disinfected with 70% alcohol between each use to avoid cross contamination.

DNA was extracted from about 0.2 g of each sample using DNeasy Power Soil Kit (Qiagen, the Netherlands) following the manufacturer’s protocol. DNA concentration and purity were quantified by Quantus (Promega, the Netherlands) and a Nanodrop spectrophotometer (NanoDrop 1000 spectrophotometer, Thermo Scientific) with OD260/OD280, respectively. DNA samples were stored at -20°C before downstream analysis. Quantitative real-time PCR (qPCR) analysis was used to quantify total bacterial 16S rRNA gene copies and total fungal ITS copies, respectively. qPCR assays were performed in triplicates with a total volume of 20 μL reactions using iQ SYBR Green master mix (Bio-rad) on a CFX96 Real-Time System (C1000 Touch, Bio-rad). Gene copy numbers were calculated as copies/g dry wood. The bacterial qPCR efficiency was 95.05% (± 4.25%), with $R^2 > 0.997$. The fungal qPCR efficiency was 97.7% (± 3.50%), with $R^2 > 0.998$. qPCR primers and amplification protocols are detailed in Table 6-S1.

6.2.7 Statistical analysis

All data were analysed using IBM SPSS Statistic 23 (IBM, USA) for Microsoft Windows. Differences were compared statistically using ANOVA (alpha = 0.05). Differences between values at P greater than 0.05 were considered as not significantly different.

6.3 Results and Discussion

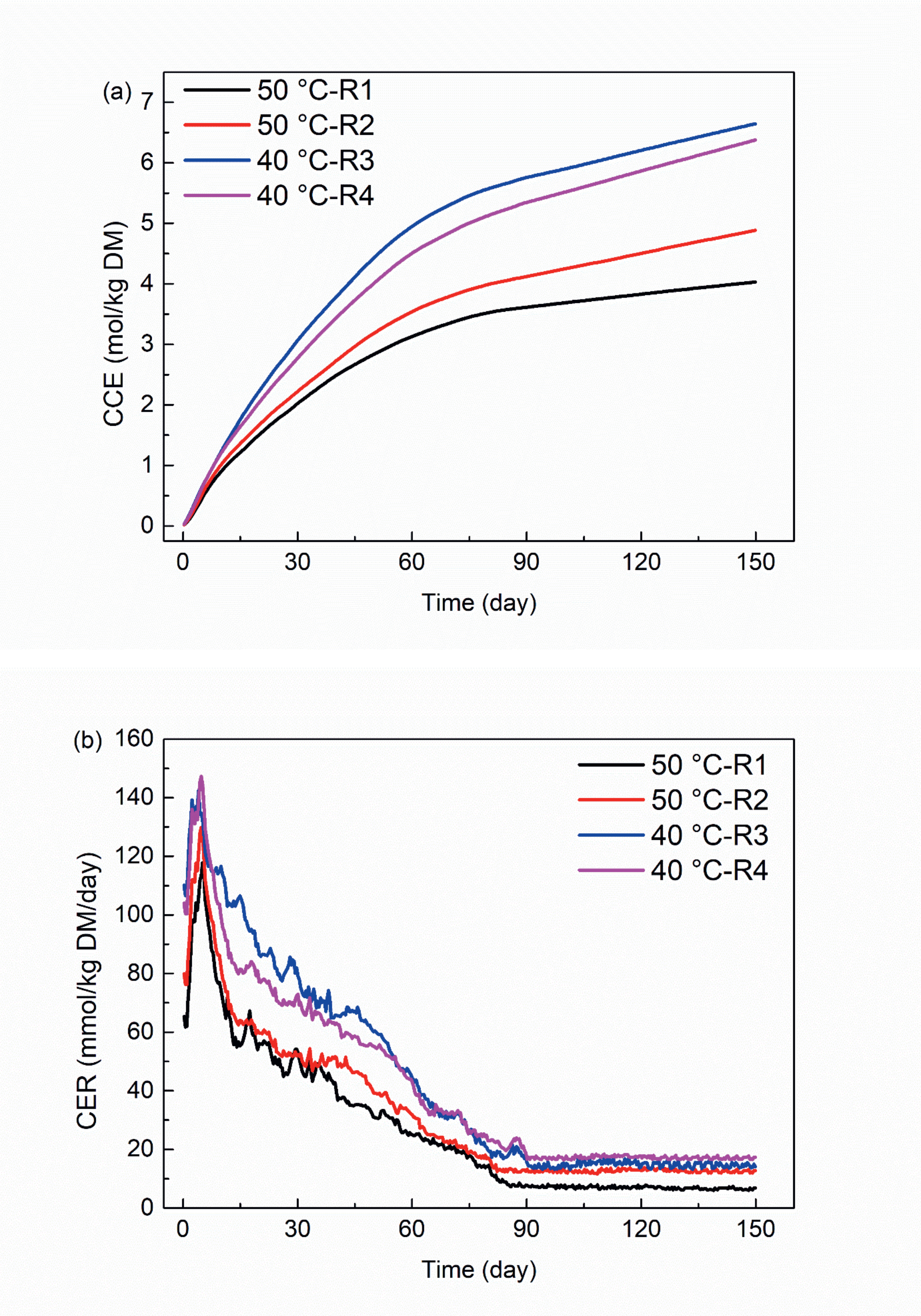
6.3.1 Carbon dioxide evolution and wood weight loss

The cumulative CO_2 evolution (CCE) of the reactors is shown in Fig. 6-2 (a), the CO_2 evolution rate (CER) is shown in Fig. 6-2 (b); and the weight loss is shown in Fig. 6-2 (c). As shown in Fig. 6-2 (a), the CCE of the four reactors increased with the incubation time. 40 °C-R3 and 40 °C-R4 generally displayed higher CCE compared to 50 °C-R1 and 50 °C-R2. After 150 days, the highest CCE was 6.65 mol/kg DM observed by 40 °C-R3, which was about 1.63 times as much as the 50 °C-R1. The CCE of each temperature revealed that the decomposition of wood blocks was vigorous in the first 90 days, because the CCE during this period accounted for about 85-88% of total CCE for all the reactors.

As shown in Fig. 6-2 (b), the time course of CER increased quickly in the first 5 days and then gradually decreased and became stable in the last 60 days. The CER of four reactors peaked at around day 5, the highest CER was 147 mmol/kg DM/day (40 °C-

R4) while the lowest CER was 113 mmol/kg DM/day (50 °C-R1). The higher CER in the first 5 days might attribute to the decomposition of easily degradable substrates, such as starches, pectins and some easily available hemicellulose (McCarthy, 1987; Nada et al., 2012; Tanaka et al., 2020; Tondi et al., 2012). With the depletion of these easily degradable substrates, the CER decreased. Whereas, other non-readily degradable carbon sources, such as lignin and cellulose, will be degraded more gradually. After 90 days, CER became lower and stable, despite of some fluctuations. The reason for the low CER could be the nutrients limitation. The initially added nutrients (corresponding to 0.05% N) may have been consumed in the first week quickly due to the rapid propagation of microorganisms (Fan et al., 2020a). The initially added nutrients (mainly N) decreased due to the growth of microorganisms that degrade readily degradable organic matter and repress the formation of lignolytic enzymes (Fioretto et al., 2005; Keyser et al., 1978). With the depletion of nutrients availability, the microorganisms could only obtain nitrogen from wood (mainly from lignin), which was difficult due to the low activity (amount) of lignolytic enzymes.

As shown in Fig. 6-2 (c), the total weight loss of 50 °C-R1 and 50 °C-R2 was 12.4% and 11.9%, respectively. While the number of 40 °C-R3 and 40 °C-R4 was 16.7% and 16.0%, respectively. 40 °C resulted in higher weight loss compared to 50 °C after 150 day's incubation (ANOVA, $P < 0.05$). Correlated to the CO_2 evolution and weight loss results, BWO at 40 °C preferred compared to 50 °C. In the present study, the moisture content of woodblocks varied between 50-80%, showing that the moisture content was not a limiting factor (Viitanen & Ritschkoff, 1991). However, in our previous study where the moisture content was not continuously supplied, thus the moisture was limiting at higher temperatures (50 °C) (Chapter 5). In this study, the wood weight loss was enhanced by 36% at 40 °C and 270% at 50 °C, which proves that the continuously moisture supply especially at 50 °C was important. If the moisture content falls below a critical level (usually 30%), microbial activity will decrease and the microbes become dormant (Viitanen, 1994; Woodard & Milner, 2016)


(a)
CCE (mol/kg DM)
0
1
2
3
4
5
6
7
Time (day)
0
30
60
90
120
150
50 °C-R1
50 °C-R2
40 °C-R3
40 °C-R4
(b)
CER (mmol/kg DM/day)
0
20
40
60
80
100
120
140
160
Time (day)
0
30
60
90
120
150
50 °C-R1
50 °C-R2
40 °C-R3
40 °C-R4

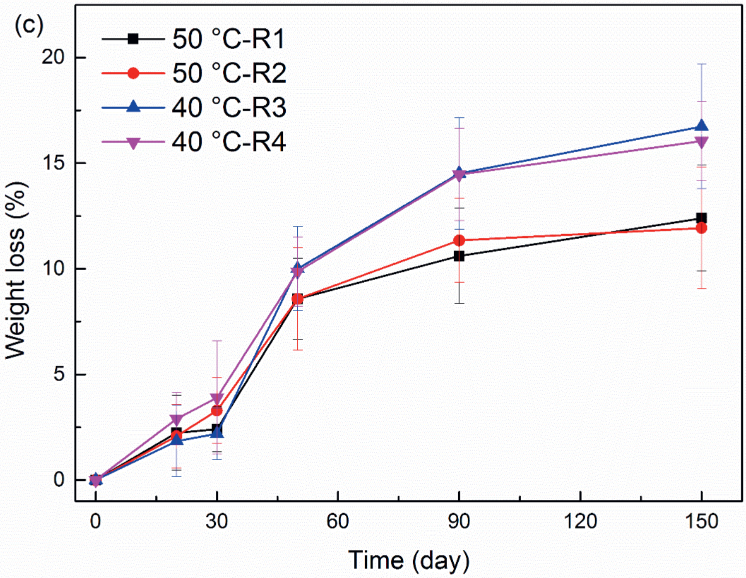
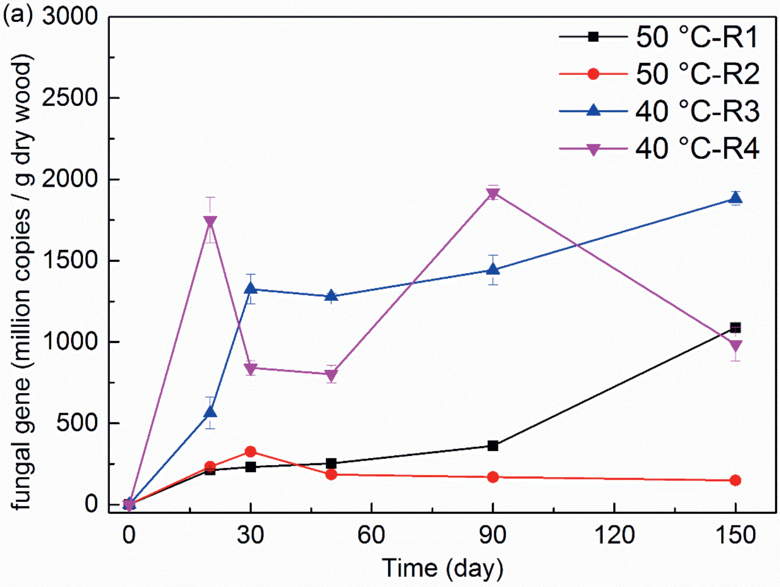
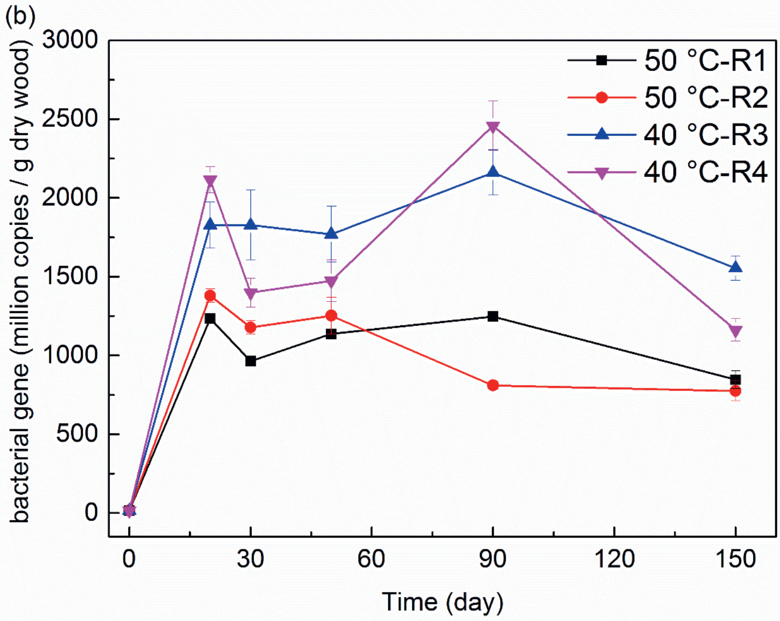


Fig. 6-2 (a) The change in cumulative CO_2 evolution of different reactors; (b) the change in CO_2 evolution rate of different reactors; (c) the change in weight loss of different reactors, average and standard deviation were calculated for 18 measurements. Biological wood oxidation at 40 °C significantly displayed higher CO_2 evolution and weight loss than at 50 °C after 150-day incubation (ANOVA, $P < 0.05$).

6.3.2 qPCR

Temperature is an important factor in determining the population and activity of fungi and bacteria (Xiao et al., 2011). In this study, we used qPCR to determine the gene density (copies / g dry wood) of fungi and bacteria during the BWO process. qPCR has been used to estimate the fungal biomass and bacterial biomass (Cotto et al., 2015; Horevaj et al., 2011). However, estimating fungal biomass in mixed culture via qPCR has its limitation since the number of copies per fungal cell are largely unknown for most species, making quantification of fungal biomass from a mixed community less reliable (Manerkar et al., 2008). Despite this, qPCR results still quantitatively demonstrate the relative development of bacteria and fungi within the same operational condition. The higher microbial biomass resulted in higher copies number / g dry wood. The fungal ITS gene and bacterial 16S rRNA gene of different reactors are shown in Fig. 6-3 (a) and Fig. 6-3 (b), respectively; the fungal to bacterial copies ratio is shown in Fig. 6-3 (c).





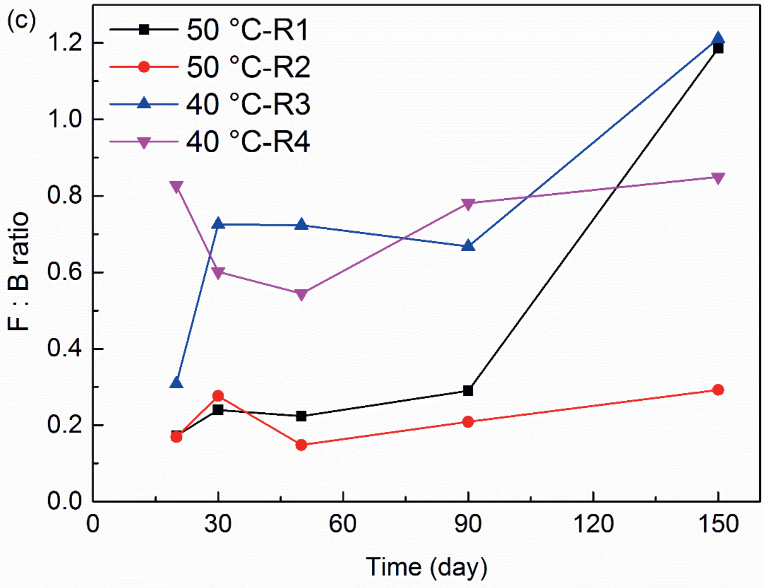


Fig. 6-3 (a) The changes in fungal ITS gene density of different reactors; (b) the change in bacterial 16S rRNA gene density of different reactors; (c) the fungal and bacterial copies ratio of different reactors. Average and standard deviation were calculated for 3 measurements. 40 °C generally had higher microbial gene density than at 50 °C. The F:B ratio at 40 °C was higher than 50 °C.

As shown in Fig. 6-3 (a) and Fig. 6-3 (b), the initial microbial gene density (copies / g dry wood) was low and it greatly thrived until day 20. This was due to the rapid development of both fungi and bacteria in the early stage (Liu et al., 2011; Sudharsan Varma & Kalamdhad, 2014; Vargas-García et al., 2010). After incubation, the fungal copies number at 50 °C ranged from 0.2×10^9 to 1.0×10^9 per g of dry wood, and the number of bacteria ranged from 0.8×10^9 to 1.4×10^9 per g of dry wood. As for 40 °C, the fungal copies number ranged from 0.6×10^9 to 1.9×10^9 per g of dry wood, and the bacterial copies number ranged from 1.2×10^9 to 2.5×10^9 per g of dry wood. Between day 20 to day 150 where the CER gradually decreased, however, the fungi and bacteria reached a plateau. This could indeed point to the nitrogen limitation and stabilized conditions (e.g. temperature and moisture) of the system. Palmisnao et al. (1993) kept a composting pile (1.8 kg) in an incubator at 37 °C and found the colony forming units of the compost remained unchanged for 20 days (out of 30 days) when the temperature of the composting ranged between 37-40 °C (Palmisano et al., 1993). Tang et al. (2004) kept a manure composting at 60 °C and found that the total microbial biomass (indicated by total quinone) was stable after the initial stage (Tang et al., 2004). Moreover, Tosin et al. (1996) reported that the amount of bacteria and fungi was stable during a composting process kept in a thermostat with a working temperature of 50 °C (Tosin et al., 1996). However, in traditional composting, where the temperature changes with time, the microbial population changed remarkably (Albrecht et al., 2010; Derikx et al., 1990; Hassen et al., 2001; Hellmann et al., 1997; Ryckeboer et al., 2003; Wong & Fang, 2000). Generally, the amount of microorganisms reduces in the thermophilic stage and new mesophilic microorganisms take time to grow again (Sudharsan Varma & Kalamdhad, 2014). Thus, it is suggested that inoculation after thermophilic stage might be a better way to avoid the inhibition to microbial growth at high temperature (Zhao et al., 2016).

In the present study, we continuously supplied humid air into the reactor which avoided moisture limitation. 40 °C had higher fungal and bacterial gene density compared to 50 °C (ANOVA, $P < 0.05$), which shows that 40 °C was more suitable for microbial growth on wood. This might be the reason that 40 °C had higher CCE and weight losses over 50°C.

As shown in Fig. 6-3 (c), the fungal gene to bacterial gene ratio (F:B ratio) at 50 °C was generally lower than the F:B ratio at 40 °C during the BWO process. Note that F:B ratio equals to one did not mean that the fungal amount and the bacterial amount were the same. In traditional composting (not wood only), ratio of fungal amount to bacterial amount was often reported at low level, especially at thermophilic stage where the temperature was too high (> 60 °C) for fungi to grow (Hassen et al., 2001; Klamer & Bååth, 1998; Rao et al., 1996; Ryckeboer et al., 2003; Wong & Fang, 2000). This implies that bacteria are the main decomposer in non-wood composting process. In our previous study, we found that the high BWO occurred at higher F:B ratio (Chapter 5), which implies that fungi play an important role in BWO process. Fungi are the most important agents of wood decomposition because of their capability to generate copious amount of cellulases, hemicellulases and lignin-modify enzymes (Mäkelä et al., 2020; Maki et al., 2009; Mitsuhashi & Morrell, 2012). However, fungi are known to be less adaptable to the relatively extreme environmental conditions (pH, temperature, osmotic pressures) than bacteria (Maki et al., 2009). In the present study, the temperature of BWO was constant, which was good for the growth of thermophilic wood-decaying fungi (Ryckeboer et al., 2003; Tuomela et al., 2000). However, some bacteria species could not be ignored. For example, Gram-positive bacteria such as *Bacillus* has been reported to be the most abundant group of bacteria in composting (Ryckeboer et al., 2003). It has also been observed that the most efficient composting process is achieved by mixed communities of bacteria and fungi (Partanen et al., 2010).

6.3.3 Heat production potential from BWO

In this study, the CO_2 evolution (rate) was used to estimate the theoretical heat production (rate). There was a strong linear relationship between the CCE and weight loss (Fig. 6-4), which means that CO_2 evolution is a good indicator to weight loss. Since the theoretical heat is proportional to the degradation of organic matter (wood), CO_2 evolution could be used as an indicator to theoretical heat production during BWO process.

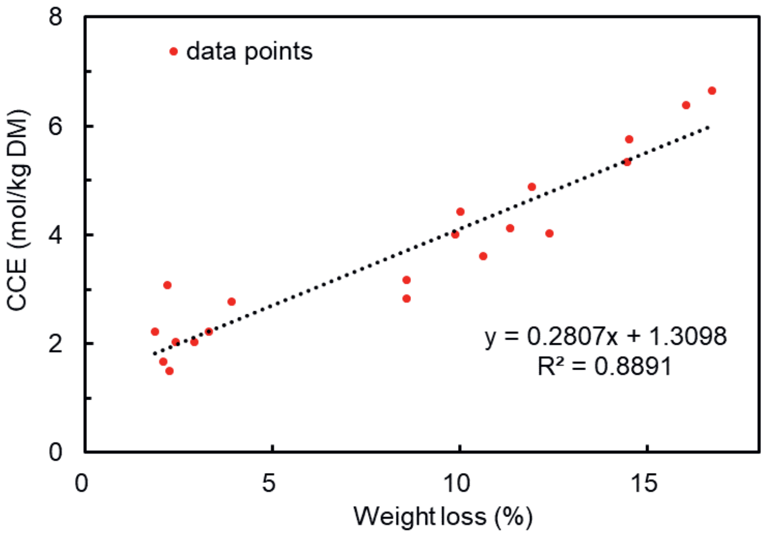


Fig. 6-4 The relationship between cumulative CO_2 evolution and weight loss.

The theoretical heat production after 150-day incubation of 50 °C-R1, 50 °C-R2, 40 °C-R3 and 40 °C-R4 was 1884, 2281, 3104, and 2982 kJ/kg DM, respectively. One objective of this study was to find out if the theoretical heat production rate at larger scale was comparable to the rate at batch scale. Since BWO rate decreased with time, the longer duration results in lower theoretical heat production rate. To make the comparison fair, we compared the theoretical heat production rate in different studies at the same day. The theoretical heat production rate at day 42 in the present study was 0.50 W/kg DM (40 °C-R3) and 0.45 W/kg DM (40 °C-R4), which was a little bit higher than the small-scale study (0.35 W/kg DM at 40 °C) (Chapter 5). This means that BWO in large-scale could get similar theoretical heat production rate compared to the BWO in batch-scale if the nutrients addition was the same.

6.4 Conclusion

Temperature tested in this study, 40 and 50 °C, does have an effect on the BWO performance and microbial development. Under the condition that the moisture content of wood was not limited (in this study), 40 °C resulted in the higher CCE and CER than 50 °C. This might be because 40 °C had higher microbial development than 50 °C, especially the higher fungal gene copies under 40°C. The microbial gene density did not show a remarkably change after the incubation time. If the nutrient addition was at the same level, BWO in large-scale at 40 °C could get slightly higher theoretical heat production rate compared to the BWO in batch-scale.

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Support information

Table 6-S1 qPCR primers and amplification protocols.

Target gene	Primer	Thermal profile	Cycles	Reference
Bacteria 16S rRNA	338F (5’-ACTCCTACGGGAGGCAGCAG-3’) 518R (5’-ATTACCGCGGCTGCTGG-3’)	95°C 5 min; 95°C 15 s, 64°C 30 s	1 40	(Muyzer et al., 1993)
Fungi ITS region	ITS86F (5’-GTGAATCATCGAATCTTTGAA-3’) ITS4R (5′-TCCTCCGCTTATTGATATGC-3′)	95°C 5 min; 95°C 15 s, 55°C 30 s, 72°C 60 s	1 40	(Turenne et al., 1999)

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Chapter 7

General Discussion & Outlook

7.1 Summary of the Research Outcomes

This thesis investigated the potential of biological wood oxidation as an alternative technology to wood combustion for heat production. We mainly investigated two factors: nutrients and temperature. The important outcomes (including the main finding) from each research chapter in this PhD dissertation are summarized as follows:

Heat potential, production, recovery and utilization from composting: A review (Chapter 2)

Composting substrates generally consists of four organic solid waste namely food waste, lignocellulose waste, manure waste and sludge waste. All organic solid waste has a great potential to generate heat during their composting process.

Degradation method, oxygen method, and heat balance method are three main methods that can be used to calculate the amount of heat production during composting.

For the heat recovery methods, water-jacked method has low heat transfer efficiency and is only suitable for small-scale reactors; tube buried-in pile method is commonly used in reactors with long retention time; heat exchanger in the exhaust air has high efficiency as it collects the latent heat; water percolation method is suitable to composting of wood chips.

For new heat recovery technologies, heat pump can increase the heat from composting both in quality and quantity; organic Rankine Cycle (ORC) and thermoelectric generators (TEGs) can generate electricity from recovered heat but with low energy efficiencies.

Heat recovered from composting can be used for building applications such as hot water service, flooring heating and wall heating. Besides, it can also be used in agricultural field, such as hotbeds and greenhouse.

Effect of nitrogen, phosphorus and pH on biological wood oxidation at 42 °C (Chapter 3)

Nitrogen is an important nutrient affecting BWO; N addition (2.5-10 mg/g N, expressed in mg N per d dry wood) could enhance the oxygen consumption by 60-124% and wood weight loss by 28-95%, compared with the BWO without N addition.

The highest oxygen consumption and weight loss was observed by adding 2.5 mg/g N (in NH_4Cl). No significant difference was found between 2.5 mg/g N, 3.3 mg/g N, and 5 mg/g N addition, while too much N addition (20 mg/g) did not enhance the BWO process.

Phosphorus (in the form of KH_2PO_4; between 0 mg/g-20 mg/g) and pH (between 4-6) did not have a significant effect on BWO.

Urine addition as a nutrient source for biological wood oxidation at 40 °C (Chapter 4)

Both synthesis urine and fresh urine could be used as an alternative to chemical nutrient used in chapter 2.

Addition of synthetic urine with five times dilution (corresponding to 1.2 ‰ N basis of dry wood) resulted in the highest oxygen consumption and weight loss. At this N level, the fresh urine addition had higher BWO performance than synthetic urine.

The degradation rate decreased after 24 days, possibly because of the depletion of nutrient availability. Re-addition of synthesis urine with five times dilution could stimulate the BWO process. However, re-addition of only N components (NH_4OH and NH_4HCO_3) did not further stimulate the BWO process.

Effect of temperature on biological wood oxidation (Chapter 5)

The oxygen consumption and wood weight loss at 40 °C was higher than that at 30 °C and 50°C; while 50 °C had the lowest oxygen consumption and wood weight loss.

Changing temperature from 40 or 50 °C to 30 °C decreased the wood weight loss by 17.6% and 43.4%, respectively; while changing temperature from 30 or 50 °C to 40 °C enhanced the wood weight loss by 54.1% and 100%, respectively.

Changing temperature from 40 or 50 °C to 30 °C increased the bacterial gene density significantly, while the fungal gene density did not increase remarkably and even decrease.

Changing temperature from 30 to 40 °C decreased the fungal and bacterial gene density, however, the oxygen consumption and weight loss was improved significantly.

Biological wood oxidation at 40 °C and 50 °C: A reactor-scale study (Chapter 6)

At the condition that the humid air is supplied continuously, 40 °C resulted in higher CO_2 evolution and weight loss than 50 °C.

The fungal ITS gene and bacterial 16S rRNA gene at 40 °C was higher than the microbial gene at 50 °C.

The microbial gene density was generally stable in the course of time during BWO process.

Under the condition that the nutrient addition was at the same level, BWO in large-scale at 40 °C could get higher theoretical heat production rate compared to the BWO in batch-scale.

Table 7-1 summarizes the theoretical heat production (in kJ/kg DM) and theoretical heat production rate (in W/kg DM) in different chapters. In this PhD thesis, all the wood weight loss had a strong linear relationship with the oxygen consumption/carbon dioxide evolution. This is because the wood was the only carbon source during BWO process and the oxidation of wood proportionally consumed oxygen and generated CO_2. The good linear relationship means that oxygen consumption or carbon dioxide evolution is a good indicator to BWO. Usually, the BWO rate (oxygen consumption rate or CO_2 evolution rate) decreased with time. Therefore, the longer duration results in lower theoretical heat production rate. To make the comparison of heat production rate in different chapters fair, we calculated the theoretical heat production rate at the same day (day 42). In Chapter 3, the highest heat production rate at day 42 was 0.80 W/kg DM observed by 2.5 mg/g N (in NH_4Cl, corresponding to 0.25% N addition); in Chapter 4, the highest heat production rate was 0.95 W/kg DM observed by fresh human urine addition (8.5 times dilution) and 0.58 W/kg DM observed by synthetic urine addition (five times dilution); while the number was 0.35 W/kg DM observed at 40 °C in Chapter 5; in Chapter 6, the reactors with a working temperature of 40 °C resulted in a heat production rate of 0.50 W/kg DM at day 42. From Table 7-1, the most efficient nutrients might be the fresh human urine with certain times dilution. Moreover, different types of wood and sizes of wood affect the BWO process. The trees used in this PhD thesis were birch tree and ash tree. Although birch tree and ash tree are hardwood, they have different chemical composition (Kizha, 2008; ODE, 2003; Oliveira et al., 2017; Wittmann et al., 2008; WoodDatabase, 2020). Studies regarding the effect of different wood types on degradation are rare, especially in mixed culture. We chose the birch and ash tree as the experiment substrates, but we could not really give specific information on it. In addition, the size of woodblocks decides the contact area between wood and microorganisms. Fungi and bacteria start degrading the outer layer of the wood before reaching the inside (Sachs et al., 2007; Singh et al., 2016). Therefore, it is logical that with a decreasing particle size, and thus a larger surface to volume ratio, the wood is more accessible. As a result, the smaller woodblock size can lead to a faster degradation of woodblocks (Reid, 1989). In this PhD thesis, two different sizes of ash tree were chosen (1.0 cm * 1.0 cm * 0.5 cm and 1.5 cm * 1.5 cm * 1.5 cm, L*W*H). It was expected that smaller size of woodblocks might contribute to the highest heat production rate (0.95 W/kg DM) to some extent.

Table 7-1 Theoretical heat production (kJ/kg DM) and theoretical heat production rate (W/kg DM) for various operational conditions.

Wood	Wood size (cm)	Nutrients addition (% w/w dry wood)	TEM (°C)	MC (%)	Duration (d)	Weight loss (%)	Theoretical heat production (kJ/kg DM)	Theoretical heat production rate (W/kg DM)	Chapter
Birch (*Betula spp.*)	Diameter between 1.5-2.0 * 1.0 ± 0.2 long	0% N②	42	250	95	16.9	2298	0.28	3
Birch (*Betula spp.*)	Diameter between 1.5-2.0 * 1.0 ± 0.2 long	0.25% N②	42	250	95	33	5171	0.63	3
Birch (*Betula spp.*)	Diameter between 1.5-2.0 * 1.0 ± 0.2 long	0.33% N②	42	250	95	28.7	4843	0.59	3

Birch (*Betula spp.*)	Diameter between 1.5-2.0 * 1.0 ± 0.2 long	0.5% N②	42	250	95	29.4	4761	0.58	3
Birch (*Betula spp.*)	Diameter between 1.5-2.0 * 1.0 ± 0.2 long	1% N②	42	250	95	21.6	3694	0.45	3
Birch (*Betula spp.*)	Diameter between 1.5-2.0 * 1.0 ± 0.2 long	2% N②	42	250	95	16.4	2709	0.33	3
Birch (*Betula spp.*)	Diameter between 1.5-2.0 * 1.0 ± 0.2 long	0 mg/g P (1% N)②	42	250	50	16.5	1987	0.46	3
Birch	Diameter	0.5 mg/g P	42	250	84	24.3	3556	0.49	3

(*Betula* spp.)	between 1.5-2.0 * 1.0 ± 0.2 long	(1% N)②						
Birch (*Betula* spp.)	Diameter between 1.5-2.0 * 1.0 ± 0.2 long	1.0-5 mg/g P (1% N)②	42	250	50	15.7-17.3	1858-2074	0.43-0.48	3
Birch (*Betula* spp.)	Diameter between 1.5-2.0 * 1.0 ± 0.2 long	natural pH (1% N)②	42	250	50	18.9	2117	0.49	3
Birch (*Betula* spp.)	Diameter between 1.5-2.0 * 1.0 ± 0.2 long	pH=4-5 (1% N)②	42	250	50	15.9-16.0	1944-2030	0.45-0.47	3
Birch (*Betula*	Diameter between 1.5-	pH=6 (1% N)②	42	250	84	22.9	3556	0.49	3

spp.)	2.0 * 1.0 ± 0.2 long								
Ash (*Fraxinus excelsior*)	1.0 * 1.0 * 0.5	SU*-undiluted (0.6% N)	40	250	42	4.8	701	0.19	4
Ash (*Fraxinus excelsior*)	1.0 * 1.0 * 0.5	SU*-5 times (0.12% N)	40	250	42	13.4	2104	0.58	4
Ash (*Fraxinus excelsior*)	1.0 * 1.0 * 0.5	SU*-10 times (0.06% N)	40	250	42	9.5	1542	0.43	4
Ash (*Fraxinus excelsior*)	1.0 * 1.0 * 0.5	SU*- 20 times (0.03% N)	40	250	42	7.1	1075	0.3	4
Ash (*Fraxinus excelsior*)	1.0 * 1.0 * 0.5	RU*- 8.5 times (0.12% N)	40	250	42	19.7	3441	0.95	4

Ash (*Fraxinus excelsior*)	1.0 * 1.0 * 0.5	SUN-R*- (0.12% + 0.12% N)	40	250	100	18.3	3273	0.38	4
Ash (*Fraxinus excelsior*)	1.0 * 1.0 * 0.5	SU-R*- (0.12% + 0.12% N)	40	250	100	31.2	5517	0.64	4
Ash (*Fraxinus excelsior*)	1.0 * 1.0 * 0.5	Control*- 10 times (0.12% N)	40	250	100	21.2	3834	0.44	4
Ash (*Fraxinus excelsior*)	1.5 * 1.5 * 1.5	0.05% N③	30	100	212	10.0	1589	0.09	5
Ash (*Fraxinus excelsior*)	1.5 * 1.5 * 1.5	0.05% N③	40	100	220	12.0	2291	0.12	5
Ash (*Fraxinus	1.5 * 1.5 * 1.5	0.05% N③	50	100	220	3.3	561	0.03	5

excelsior)								
Ash (*Fraxinus excelsior*)	1.5 * 1.5 * 1.5	0.05% N③	30 to 40	100	212	15.5	2525	0.14	5
Ash (*Fraxinus excelsior*)	1.5 * 1.5 * 1.5	0.05% N③	30 to 50	100	212	9.4	1496	0.08	5
Ash (*Fraxinus excelsior*)	1.5 * 1.5 * 1.5	0.05% N③	40 to 30	100	220	9.8	1870	0.10	5
Ash (*Fraxinus excelsior*)	1.5 * 1.5 * 1.5	0.05% N③	40 to 50	100	220	9.4	1823	0.10	5
Ash (*Fraxinus excelsior*)	1.5 * 1.5 * 1.5	0.05% N③	50 to 40	100	220	6.5	1029	0.05	5
Ash	1.5 * 1.5 * 1.5	0.05% N③	50 to	100	220	1.8	327	0.02	5

(*Fraxinus excelsior*)			30				
Ash (*Fraxinus excelsior*)	1.5 * 1.5 * 1.5	0.05% N③	50	100 + 150 continuously supply	13.0	1884	0.14	6
Ash (*Fraxinus excelsior*)	1.5 * 1.5 * 1.5	0.05% N③	50	100 + 150 continuously supply	12.3	2281	0.18	6
Ash (*Fraxinus excelsior*)	1.5 * 1.5 * 1.5	0.05% N③	40	100 + 150 continuously supply	16.7	3104	0.24	6
Ash (*Fraxinus excelsior*)	1.5 * 1.5 * 1.5	0.05% N③	40	100 + 150 continuously supply	16.0	2982	0.23	6

1* NH_4NO_3 solution to reach a nitrogen content of 3% (dry weight basis)

2* 1.0 mg/L $MgSO_4 \cdot 7H_2O$, 120 mg/L $FeCl_3$, 50 mg/L H_3BO_3, 10 mg/L KI, 45 mg/L $MnSO_4 \cdot H_2O$, 20 mg/L $Na_2MoO_4 \cdot 2H_2O$, 75 mg/L $ZnSO_4 \cdot 7H_2O$, 50 mg/L $CoCl_2 \cdot 6H_2O$, 20 mg/L $KAl(SO_4)_2 \cdot 12H_2O$, 13.2 mg/L $CaCl_2 \cdot 2H_2O$, 10 mg/L NaCl.

SU: CH_3COONH_4, 5.4; NH_4OH, 1.4; NH_4HCO_3, 22.1; Na_2SO_4, 1.2; NaOH, 3.4; KCl, 0.3; K_2HPO_4, 4.2; $MgCl_2 \cdot 6H_2O$, 0.8; $CaCl_2 \cdot 2H_2O$, 0.4; and trace element solution, 1mL

3* 2 g/L NH_4Cl, 4.5 g/L KH_2PO_4, 0.5 g/L K_2HPO_4, 1.0 mg/L $MgSO_4 \cdot 7H_2O$, 120 mg/L $FeCl_3$, 50 mg/L H_3BO_3, 10 mg/L KI, 45 mg/L $MnSO_4 \cdot H_2O$, 20 mg/L $Na_2MoO_4 \cdot 2H_2O$, 75 mg/L $ZnSO_4 \cdot 7H_2O$, 50 mg/L $CoCl_2 \cdot 6H_2O$, 20 mg/L $KAl(SO_4)_2$ $12H_2O$, 13.2 mg/L $CaCl_2 \cdot 2H_2O$, 10 mg/L NaCl

All the inoculation were from the wood compost.

TEM = temperature; MC = moisture content; SU = synthetic human urine; RU = fresh human urine; SUN-R = re-addition of N components of synthetic human urine; SU-R = re-addition of synthetic human urine; control = addition of synthetic urine without re-addition

7.2 Challenges, Opportunities and Outlook

7.2.1 Advantage of BWO for heat production

In the EU, the main use of energy by households is for space heating and water heating, which account for 64.1% and 14.8% of the total energy consumption, respectively (Eurostat, 2019). Usually, the final energy consumption of space heating and water heating in the household is covered by natural gas and electricity. A major advantage of BWO over electricity and natural gas is the low cost. For example, the price of electricity in Netherlands is 0.14 euro/kWh (Eurostat, 2021a), and the price of natural gas is 0.10 euro/kWh (Eurostat, 2021b). The cost of local wood in Netherlands is 35-45 euro/ton according to USDA (United States Department of Agriculture) (USDA, 2019), corresponding to 0.01-0.02 euro/kWh energy (depending on the moisture content of wood). Considering the installation cost and maintaining cost, hot water from BWO is still much cheaper than the hot water supplied by natural gas and electricity. It is estimated that the material cost (except for wood waste) of a 90 m^3 BWO is only 457 euro and the cost of tool / machine is 525 euro (LoBauerInnen, 2013). van Ziel (2018) reported that the total cost of building a BWO pile is 3000 euro and with reuse it can be possible to have a cost of 700 euro for next BWO process (Ziel, 2018). Jorritsma et al. (2018) also reported that the total cost (including woodchips, materials and labor) of a 135 m^3 BWO pile is 6225 euro and it can save 6900 euro compared to using gas (Fedde Jorritsma et al., 2018). Even if the BWO cannot heat the water to a high temperature when the heat generation rate decreases, it can save energy and money by preheating the water for further use.

Another advantage of BWO is that the final product of BWO is wood compost, which creates a new possibility for converting wood to soil improver (Fedde Jorritsma et al., 2018). Based on this, it was reported that BWO could retain about 70% of the CO_2 absorbed by wood (through photosynthesis process) in the form of compost (Scholtens, 2017). From this point of view, BWO is an environment-friendly technology which can reduce the CO_2 emission generated from combustion of fossil fuels.

7.2.2 Challenges and solutions

7.2.2.1 Low heat production rate

The main challenge of BWO is the low heat production rate. In this PhD dissertation, the theoretical heat production rate varied from 0.02 to 0.95 W/kg DM, depending on different experiment conditions (Table 7-1). The highest theoretical heat production rate (0.95 W/kg DM after 42 days' incubation) was generally 200-6000 times lower compared to the heat production by a domestic stove for wood burning (Alibaba, 2021). In order to get a reasonable hot water service, the pile mass of BWO is usually large. For example, tests performed in the 1970s showed that a heap of approximately 50 tons could be used to heat a 5-roomed house (100 m^2) and produce enough hot water for at least 6 months (Pain, 1972). Moreover, Kimman et al. (2019) estimated that 50 tons of BWO pile (20 tons of dry weight) could generate almost 45 MWh of energy after one year when combined with a heat pump (Kimman et al., 2019). The most interesting application for BWO is to provide hot water for domestic use. Assuming that the initial temperature of water is 8 °C (temperature of ground water) with a flowrate of 8 L/min (flowrate of a showerhead), and the heat transfer efficiency is 100%. To get hot water at different temperatures, the required mass of BWO at different heat production rate is shown in Fig. 7-1.

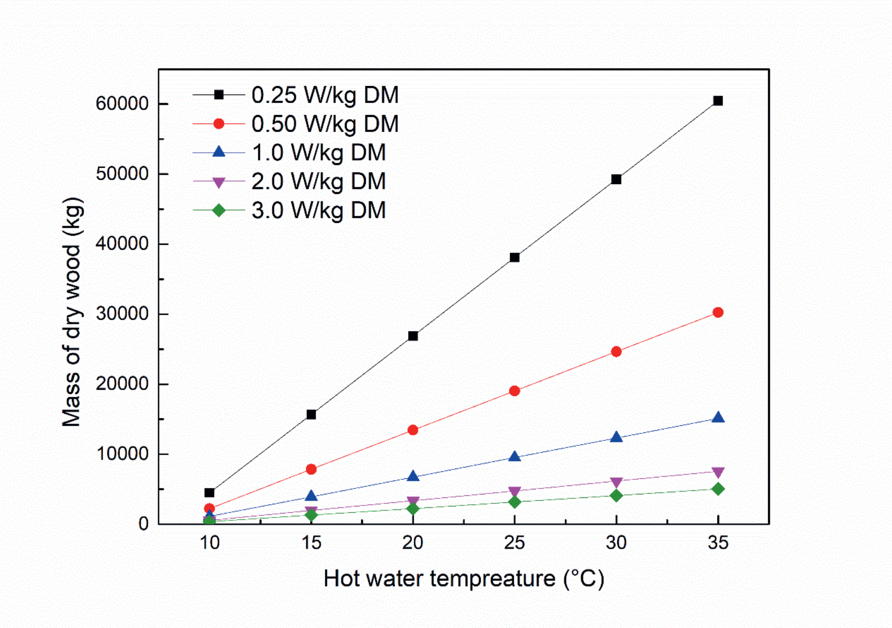

Fig. 7-1 The relationship between theoretical heat production rate and pile mass.

As shown in Fig. 7-1, the hot water temperature is higher for a higher mass of dry wood results at constant heat production rate. For example, 5 ton of dry wood is able to supply a 35 °C water with a flowrate of 8 L/min for 42 days continuously if the heat production rate of BWO is 3 W/kg DM, while this number is 60 ton when the heat production rate drops to 0.25 W/kg DM. Usually, the mass of BWO is usually high (20 ton-100 ton) in the case studies (Fedde Jorritsma et al., 2018; Kimman, 2019). 5 ton is relatively small and feasible for domestic use. The higher mass of dry wood also results in some issues such as higher cost, lower oxygen transfer efficiency, and lower heat recovery efficiency due to the higher heat loss. Thus, it is important and necessary to improve the BWO rate.

Table 7-2 summarizes the wood weight loss in related studies. It is clear that the wood weight loss varies with the different wood degradation conditions, such as temperature, wood types and microorganisms. Most available literature focuses on the wood degradation in pure culture, especially by white-rot fungi and brown-rot fungi. White-rot fungi, such as *Phanerochaete chrysosporium, Coniophora puteana, and Trametes versicolor*, have the ability to degrade both lignin and cellulose biopolymers in wood

(Manavalan et al., 2015). Brown-rot fungi, such as *Gloeophyllum trabeum*, *Coniophera puteana,* and *Postia placenta,* can also effectively depolymerize cellulose biopolymers into simple sugars, however, the lignin can only be partially degraded (Sánchez, 2009). Compared to fungi, the metabolism of wood by bacteria is less well characterized (Brown & Chang, 2014; Manavalan et al., 2015). From the available literature, bacteria generally degrade wood slower than fungi.

Most wood-decaying fungi with high ability to degrade wood is mesophilic fungi. There are few studies working on thermophilic fungi. Mesophilic fungi degrade wood fast, however, they may not work well in BWO process. In order to generate enough amount of heat, the pile of BWO is usually large and thus the heat is easily to be cumulated, which increase the temperature remarkably. Some thermophilic fungi were reported to have the ability to generate lignocellulases (Caizán Juanarena et al., 2016; Ofosu-Asiedu & Smith, 1973a; Tuomela et al., 2000; Wei et al., 2019). However, their mechanisms to degrade wood remain unknown.

There are generally two types of wood in nature: hardwood (angiosperms), and softwood (gymnosperms), which have different proportions of elemental composition (Roger et al., 2012; Zhu & Pan, 2010). For example, acetylated glucuronoxylan is the main hemicellulose polymer in hardwood tissues, while galactoglucomannan is the most abundant in coniferous wood (Timell, 1967). Generally, most hardwood has higher density than most softwood (WoodDatabase, 2020). This does not mean that the softwood is easier for microbial degradation than hardwood. In general, the wood fiber from hardwood is more readily biological degraded compared to coniferous softwood, which might be partially because that hardwood has lower lignin content (Mooney et al., 1998; Timell, 1967; Zhu & Pan, 2010). Since few studies are related to the difference between softwood and hardwood, we could not give too much information.

Among all these studies, the highest wood weight loss was 46.4% after 60 days observed by (SolÁR et al., 2007) and 61.3% after 84 days observed by (Howell et al., 2009). This is corresponding to a heat production rate around 1.56 W/kg DM and 1.47 W/kg DM, respectively, which proves that 2 W/kg DM seems feasible, but further increase in rate is required.

Table 7-2 Wood degradation in related literature.

Wood	Microorganisms	Weight loss (%)	Duration (d)	Temperature (°C)	Reference
Birch (hybrid of *Betula pendula* and *Betula pubescens*)	Indigenous microbe	4.7	36	41	(Caizán Juanarena et al., 2016)
Birch (hybrid of *Betula pendula* and *Betula pubescens*)	Indigenous microbe + *Chaetomium thermophilum*	11	36	41	(Caizán Juanarena et al., 2016)
Birch (hybrid of *Betula pendula* and *Betula pubescens*)	*Phanerochaete chrysosporium* (W)	12.2	36	41	(Caizán Juanarena et al., 2016)
Birch (hybrid of *Betula pendula* and *Betula pubescens*)	*Chaetomium thermophilum*	6.5	36	41	(Caizán Juanarena et al., 2016)
Birch (hybrid of *Betula pendula* and *Betula pubescens*)	Indigenous microbe + *Phanerochaete chrysosporium* (W)	8.3	36	41	(Caizán Juanarena et al., 2016)
Chinese Poplar (*Populus simonii*)	*Gloeophyllum trabeum* (B)	44	90	25	(Xu et al., 2019)
Pine (*Pinus spp.*)	*Postia placenta* (B)	30	70	25	(Curling et al., 2002)

Beech	*Coniophora puteana* (W)	26	60	26	(SolÁR et al., 2006)
Alder plywood (*Alnus glutinosa*)	*Trametes versicolor* (W)	38.5	56	25	(Kose Demirel et al., 2017)
Alder plywood (*Alnus glutinosa*)	*Coniophera puteana* (B)	31.7	56	25	(Kose Demirel et al., 2017)
Beech (*Fagus orientalis*)	*Trametes. versicolor* (W)	27.3	56	25	(Kose Demirel et al., 2017)
Beech (*Fagus orientalis*)	*Coniophera puteana* (B)	23.8	56	25	(Kose Demirel et al., 2017)
Beech	*Coniophera puteana* (B)	46.4	60	22	(SolÁR et al., 2007)
Pine (*Pinus roxburghii*)	*Phanerochaete chrysosporium* (W)	33.8	84	25	(Ahmed et al., 2011)
Pine (*Pinus roxburghii*)	*Scizophyllum commune* (W)	25.9	84	25	(Ahmed et al., 2011)
Pine (*Pinus roxburghii*)	*Ganoderma lucidum* (W)	26.4	84	25	(Ahmed et al., 2011)
Pine (*Pinus roxburghii*)	*Coriolus versicolor* (W)	28.2	84	25	(Ahmed et al., 2011)
Pine (*Pinus roxburghii*)	*Fomes fomentarius* (W)	26.5	84	25	(Ahmed et al., 2011)
Pine (*Pinus roxburghii*)	*Pleurotus ostreatus* (W)	31.5	84	25	(Ahmed et al., 2011)

Pine (*Pinus silvestris*)	*Phanerochaete chrysosporium* (W)	15.3	42	25	(Ander & Eriksson, 1977)
Pine (*Pinus sylvestris*)	Actinomycete Fb352	1.19	60	30	(Roussel et al., 2000)
Pine (*Pinus sylvestris*)	Actinomycete Fb352	1.82	60	30	(Roussel et al., 2000)
Pine (*Pinus spp.*)	*Gloeophyllum trabeum* (B)	30	72	25	(Curling et al., 2001)
Aspen (*Populus tremuloides*)	*Phanerochaete chrysosporium* (W)	35.3	30	39	(Reid, 1983)
Pine	*Serpula lacrymans* (B)	49.9	84	-	(Howell et al., 2009)
Pine	*Gloeophyllum trabeum* (B)	61.3	84	-	(Howell et al., 2009)
Pine	*Coniophera puteana* (B)	44.0	84	-	(Howell et al., 2009)
Spruce (*Picea glauca*)	*Aspergillus fumigatus* (W)	2.51	42	45	(Ofosu-Asiedu & Smith, 1973a)
Lodgepole pine (*Pinus contorta*)	*Aspergillus fumigatus* (W)	3.91	42	45	(Ofosu-Asiedu & Smith, 1973a)
Ponderosa pine (*Pinus ponderosa*)	*Aspergillus fumigatus* (W)	3.62	42	45	(Ofosu-Asiedu & Smith, 1973a)
Lodgepole pine (*Pinus contorta*)	*Chrysosporium pruinosum* (W)	3.74	42	45	(Ofosu-Asiedu &

					Smith, 1973a)
Pine (*Pinus ponderosa*)	Allescheria terrestris (W)	4.39	42	25-60	(Ofosu-Asiedu & Smith, 1973b)
Pine (*Pinus ponderosa*)	Thermoascus aurantiacus (W)	4.08	42	45	(Ofosu-Asiedu & Smith, 1973b)
Pine (*Pinus spp.*)	*Trametes. versicolor* (W)	9.97	252	35	(Mitsuhashi & Morrell, 2012)
Pine (*Pinus spp.*)	*Postia placenta* (B)	35.7	252	35	(Mitsuhashi & Morrell, 2012)
Pine (*Pinus spp.*)	*Gloeophyllum trabeum* (B)	53.6	252	35	(Mitsuhashi & Morrell, 2012)

Note: (W) means white rot fungus; (B) means brown rot fungus

Some species of fungi, bacteria, and actinomycetes are known to able to degrade wood (Ahmed et al., 2011; Antai & Crawford, 1981; Goodell, 2003; Maataoui et al., 2014; McCarthy, 1987; Ofosu-Asiedu & Smith, 1973a; Roussel et al., 2000; Shields, 1969; Zeng et al., 2010). Actinomycetes are important part of the microbial community responsible for nutrient recycling in natural substrates (McCarthy, 1987). Although actinomycetes have received only limited attention, their ability to solubilize lignin may have a role in BWO process of lignocellulose conversion (McCarthy, 1987). Both fungi and bacteria have been heavily exploited for their abilities to degrade wood (Humar et al., 2001; Maki et al., 2009; Singh et al., 2016; Worrall et al., 1997). Some bacteria are also known to be efficient cellulase producers (Maki et al., 2009). Bacteria generally degrade wood slower than fungi, but they have faster growth rate and are more adaptable to the relatively extreme environmental conditions (pH, temperature, osmotic pressures) (Maki et al., 2009; Oke et al., 2017). This was also observed in our experiment (Chapter 5). Fungi are the most important agents of wood structure deterioration (Mitsuhashi & Morrell, 2012) because of their capability to generate copious amount of cellulases, hemicellulases and lignin-modify enzymes (Mäkelä et al., 2020; Maki et al., 2009). Most fungi do not affect wood strength significantly, while rot fungi, especially brown rot fungi, can cause serious damage to wood (Viitanen, 1994). Moreover, white rot fungi are the most efficient wood decomposer in nature because they are the only organisms that able to degrade the whole wood components (cellulose, hemicellulose and lignin) (Korcan et al., 2013; Mäkelä et al., 2020).

Based on the microorganisms summarized in Table 7-2, it might be promising to use one single fungus or bacterium to get a high BWO rate. However, this requires strict sterilization which is unlikely to implement and has a very high cost. Considering these aspects, it is necessary and important to exploit a better understanding of BWO in mixed culture. Although the inter-species fungal interactions in mixed culture are most likely to be antagonism and competition, cooperative interactions are also possible that could benefit the degradation of lignocellulose (Boddy, 2000; Daly et al., 2017). Mixed cultures could be advantageous over monocultures because of enzymatic and gene regulatory reasons (Daly et al., 2017). For example, it was found that co-cultivation of

Aspergillus niger and *Aspergiuul oryzae* resulted in higher production of lignocellulases compared to single fungus (Hu et al., 2011). More importantly, the highest wood weight loss in our experiment was 19.7% after 42-day incubation (Chapter 4), which is comparable to some pure fungal strains that have high capacity to degrade wood (Ahmed et al., 2011; Curling et al., 2002; Kose Demirel et al., 2017; Mitsuhashi & Morrell, 2012; Xu et al., 2019). The work of Caizan Juanarena et al. (2016) also demonstrated that the natural biota in wood were as effective in degrading wood as the studied fungi (one thermophilic and one thermotolerant) (Caizán Juanarena et al., 2016).

7.2.2.2 Inoculation

Inoculating the (lignocellulolytic) microorganisms in the mixed culture of BWO is a possible strategy to enhance the wood degradation (Nakasaki et al., 2013; Xi et al., 2015; Zeng et al., 2007). For example, Caizán Juanarena et al. (2016) inoculate *Chaetomium thermophilum* on nonsterile wood and found that the weight loss was enhanced by 2.3 times compared to the group without inoculation (Caizán Juanarena et al., 2016). The study by Zeng et al. (2010) demonstrated that the inoculation of *Phanerochaete chrysosporium* could accelerate the degradation of lignocellulose in agriculture waste composting (Zeng et al., 2010). Hachicha et al. (2012) also showed that the inoculation of *Trametes versicolor* could improve the lignin degradation and enhance the humification process (Hachicha et al., 2012). The proper inoculation could enhance the activity of lignin peroxidase (LiP), cellulas and hemicellulolase (Zeng et al., 2010), and improve the proliferation of mesophilic and thermophilic bacteria (Nakasaki et al., 2013). It was also reported that multi inoculation in composting could enhance the community structure and functional diversity of bacteria and fungi, and avoid the competition within the inoculant microbes and the competition between inoculant microbes and the indigenous microbes (Xi et al., 2015). However, not all inocula enhanced the degradation. For example, Golueke et al. (1953) found that all of the inocula tested did not accelerate the composting process or improve the final product since they were already rich in bacteria (Golueke et al., 1954). The study of Faure and Deschamps (1991) reported that the addition of an inoculum rich in cellulolytic and ligninolytic bacteria showed no effect on the degradation of organic matter in grape pulps composting (Faure & Deschamps, 1991). The reason might be that lignocellulose degrading communities in environments like soil and compost are known to contain organisms that represent all domains of life (Alessi et al., 2018). As a result, other inoculation shows little effect. In this way, the effect of other inoculation on BWO needs further testing.

7.2.2.3 Nutrients

In this PhD dissertation, we emphasized the effect of nutrients on BWO. We found that the optimal N level was around 0.12-0.33% N basis of dry wood (Chapter 3, Chapter 4) and the synthesis human urine and fresh human urine could be used as the alternative to the chemical nutrients that discussed in Chapter 3 (Chapter 4). An important finding was that the nutrients availability might decrease the BWO rate; while nutrients re-addition could enhance and sustain the degradation of wood for longer time (Chapter 4). However, the effect of nutrients on BWO still need more research. Firstly, studies regarding to the effect of single element / human urine composition on BWO are rare at this moment. Little information has been known so far, especially in mixed culture. For example, ions (Mg^{2+}, PO_4^{3-}, Na^+, Ca^{2+}, SO_4^{2-}, Cl^- and K^+) and organic acids can affect the wood degradation, however, their mechanisms remain unknown (Tanaka & Yano, 2005). Secondly, the degradation of individual wood components (cellulose, hemicellulose and lignin) during BWO should be investigated. The nutrients addition (especially N) could stimulate the BWO compared to the experiment without nutrients addition (Chapter 3). However, the N amount could suppress the formation of lignolytic enzymes that can degrading the lignin, the inert component in wood (Fioretto et al., 2005; Keyser et al., 1978). If only lignin remains undegraded, nutrients addition will not work since the amount of lignoclytic enzymes is very low.

7.2.2.4 Temperature

In this PhD thesis, we also investigated the effect of temperature on BWO and microbial development, which is complex due to the mixed culture. Temperature is an important factor affecting the microbial activity and population (Blanchette et al., 1989). It has been pointed out that temperatures above 60 °C in a chip pile may completely sterilize the wood (Ofosu-Asiedu & Smith, 1973b). Thus, it is possible to control the BWO rate by changing its working temperatures. In our studies, we found that 40 °C was the preferred temperature compared to 30 °C and 50 °C. However, changing temperature from 30 to 40 °C had even higher oxygen consumption and wood weight loss compared to the experiment at 40 °C. The reasons behind this were unclear, which needs further study. In addition, the influence of temperature on the succession of microbial community should be investigated.

7.2.2.5 Other information

Apart from the suggestions indicated in this thesis, there are also other recommendations to improve the BWO performance, such as addition of readily organic matters like glucose or glycerol (Jang et al., 2002; Sadano et al., 2010). It was reported that the certain amount addition of these readily degradable organic matter could improve the number of microorganisms and resulted in a further increase the degradation of the indigenous substrates (Meng et al., 2021). However, too much addition did not show a further improvement because these carbon source may give rise to a limited availability of nutrients, such as N and P, which is necessary for the microorganisms (Jang et al., 2002). Another possible reason could be that addition of glucose increased the amount of bacteria other than fungi, which might not increase the degradation of wood significantly since fungi are the main wood decomposer (Jang et al., 2002).

Another challenge is that the quality of the generated heat is not high, which means the temperature of the heat is low (< 50 °C). The low temperature heat limits it application. This challenge can be solved by low-temperature heat recovery technologies, such as heat pump, organic Rankine Cycle (ORC), thermo electricity generators (TEGs).

Detailed information about the low-temperature heat recovery technologies can be found in Chapter 2. With the development of fundamental research and engineering research, implementation of BWO may become more attractive in the coming decades.

7.2.3 Last remarks

BWO was firstly developed by Jean Pain, a French inventor in 1970s (Pain, 1972). BWO has gained large attention and has been considered a promising process of heat generation which can last for hundreds of days (Arie van Ziel & Zampieri, 2020; Biomeiler, 2020; Cuhls, 2021; Kimman, 2019). Through this PhD thesis, more insight in the BWO process was obtained. With further fundamental and application-oriented research, the BWO will become an attractive alternative technology to wood combustion or even natural gas and electricity used for heat generation.

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Summary

Summary

Our society relies heavily on fossil fuels to fulfill the demand of energy consumption on earth. The combustion of fossil fuels has led to environmental issues such as global warming and air pollution. Wood materials, as one of the most abundant renewable energy sources on earth, is a promising alterative to fossil fuels. Wood is mostly used for combustion for heat production. However, the combustion of wood also results in harmful emissions. To further eliminate the environmental issues caused by wood combustion, biological wood oxidation (BWO) has been proposed as an alternative to wood combustion. However, there are little scientific literature focusing on BWO. To this end, this thesis investigated the performance and possibility of BWO for heat production. More importantly, the effect of two important factors, nutrients addition and temperature, were studied and recommendations to enhance the heat production rate of BWO were proposed.

In Chapter 2, the heat potential, heat production, heat recovery and heat utilization from composting are reviewed and discussed. Four types of organic solid waste (OSW), namely lignocellulose waste, manure waste, food waste and sludge waste are usually used in composting. These four OSWs have high heating values, which makes composting a promising process with huge heat potential. Some methods, such as ultimate analysis, proximate analysis, and composition analysis are used to calculate the heat potential of different OSWs. Moreover, different methods such as degradation method, oxygen consumption methods, and heat balance methods can be used to calculate the specific amount of heat production during composting. The degradation method is widely used to describe the composting process adequately. O_2 method is easy and simple. It can be measured continuously to illustrate the composting process. This method has been extensively applied both on big scale reactors and small-scale reactors. Heat balance method measures the heat balance components other than heat production. This method is suitable for pilot scale and commercial scale because the heat monitoring data is only valid at large scale reactors. Moreover, the CO_2 evolution method, temperature method, and initial and final heating value method are also

reviewed. The different heat recovery methods from composting are also summarized in Chapter 2. Water jacket method has low heat transfer efficiency and is only suitable for the small-scale reactors. Tube buried-in pile method collects the heat from the composting pile, which is commonly used in reactors with long retention time. Heat exchanger in the exhaust air is most common in big scale reactors, which has high heat recovery efficiency. The water percolation method is well used for composting of wood chips. Low-temperature heat recovery technologies, such as heat pump, organic Rankine Cycle (ORC), thermo electrical generators (TEGs), are also discussed. Heat generation from composting is usually used for building applications, such as hot water service, floor heating and wall heating. Moreover, the heat can be used in agricultural field to increase the crop yield as hotbeds and greenhouses. Exploring other application of the heat from composting, given its characteristic, is also of use and interest.

To understand the effect of nutrients on BWO, we investigated the effect of N addition (in NH_4Cl), P addition (in KH_2PO_4), and pH on biological wood oxidation in Chapter 3. BWO with 2.5 mg/g, 3.3 mg/g and 5 mg/g N addition significantly increased the cumulative oxygen consumption (COC) and wood weight loss. The highest COC (52 mmol O_2) and wood weight loss (33.0%) was observed by 2.5 mg/g N addition after 95 day-incubation. While the group without N addition had the lowest COC (23 mmol) and wood weight loss (16.9%). The highest N addition (20 mg/g) did not show a significant influence on the COC and wood weight loss. The best N addition range in this study was 2.5–5 mg/g. P addition (0–5 mg/g) and pH (4-6) had no significant effect on the BWO at 42 °C ($P < 0.05$). The mass loss and COC had a strong linear relationship (R^2 =0.9307) among all tests. P addition and pH had no significant effect on BWO. The highest theoretical heat production rate was 0.63W / kg DM, obtained by 2.5 mg/g N addition.

In Chapter 4, we investigated the factors including urine dilution ratio, the types of urine (fresh human urine and synthetic human urine), and urine re-addition at 40 °C, by studying their effects on oxygen consumption and dry weight loss of the wood. After 42-day incubation, synthetic urine with 5 times dilution (corresponding to 1.2 ‰ N dry basis of wood) showed the best performance; it improved the oxygen consumption by

3.8 times and wood weight loss by 3.3 times than that without urine addition (analysis of variance (ANOVA), $P < 0.05$). At the same N level, fresh human urine addition was able to enhance the BWO more efficiently than synthetic urine addition, further improving the oxygen consumption by 64% and weight loss by 47% (ANOVA, $P < 0.05$). During the BWO process, the decrease in wood degradation rate was possibly due to the decrease in nutrient availability. With the re-addition of synthetic urine, the total oxygen consumption and weight loss after 100-day incubation increased by more than 40% compared with the group without re-addition. However, re-addition of only N-containing components did not increase the BWO, showing that elements (other than N) were important.

To further understand the effect of temperature on BWO, we studied the BWO at three temperatures (30, 40 and 50 °C) at batch scale experiment and investigated their response to the change of temperatures in Chapter 5. Moreover, we detected the microbial gene density at different conditions to understand the microbial development better. In the experiments without changing temperatures, the highest oxygen consumption and wood weight loss were observed at 40 °C compared to 30 °C and 50 °C. The highest microbial gene density was observed at 30 °C. The lowest oxygen consumption and wood weight loss were observed at 50 °C. Changing temperature from 30 °C to 40 °C significantly increased the oxygen consumption and wood weight loss while the fungal and bacterial gene density decreased obviously. Changing temperature from 50 °C to 40 °C increased the BWO performance and microbial gene density obviously. Changing temperature from 50 °C to 30 °C increased the bacterial gene density remarkably, however, it did not increase the fungal gene density that much and increase the oxygen consumption and wood weight loss. The microbial gene density decreased obviously when changing temperature from 30 °C to 50 °C, however, the oxygen consumption and wood weight loss did not decrease too much.

To compare the BWO at reactor scale and batch scale, we ran a continuously BWO for 150 days. The CO_2 evolution was monitored continuously. Moreover, we determined the weight loss and microbial development during BWO process. The fungal ITS gene and bacterial 16S rRNA gene on wood was determined by qPCR. The results were shown in Chapter 6. After 150-day incubation, 40 °C had higher CO_2 evolution (rate) and wood weight loss than 50 °C. In addition, the fungal gene density and bacterial gene density at 40 °C was higher than at 50 °C, which means that 40 °C was more suitable for microbial growth. At the same nutrients level, BWO at reactor scale had a slight higher theoretical heat production than BWO at batch scale.

Finally in Chapter 7, the outcomes of previous chapters were summarized. We also explored the challenges and its solution. We concluded that BWO is a promising alternative to wood combustion for heat production due to its low cost and the valuable final product (wood compost). However, the biggest challenge of BWO is that the heat production rate is low. We proposed and discussed several strategies, such proper inoculation, proper nutrients addition and re-addition, proper temperature, to increase its heat production rate. The outcomes of this thesis may contribute to the future application of BWO and help release the environmental issues caused by combustion of fossil fuels.

Appendix

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List of publications

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Shiyang Fan, Anran Li, Annemiek ter Heijne, Cees J.N. Buisman, Wei-Shan Chen. Urine addition as a nutrient source for biological wood oxidation at 40 °C. ACS Sustainable Chemistry & Engineering 2020, 8, 17079-17087.

Shiyang Fan, Annemiek ter Heijne, Cees J.N. Buisman, Wei-Shan Chen. Heat potential, production, recovery and utilization from composting: A comprehensive review. To be submitted.

Shiyang Fan, Yujia Luo, Annemiek ter Heijne, Cees J.N. Buisman, Wei-Shan Chen. Effect of temperature on biological wood oxidation. To be submitted.

Shiyang Fan, Yujia Luo, Annemiek ter Heijne, Cees J.N. Buisman, Wei-Shan Chen. Biological wood oxidation at 40 °C and 50 °C: A reactor-scale study. To be submitted.

About the author

Shiyang Fan was born on 30th May 1991 in Hunan, China. He received his bachelor diploma in Environmental Science from Beijing Forestry University in 2013. His bachelor thesis was about the effect of metal ions on biological degradation of corn straw. After graduation, he continued his postgraduate study in Beijing Forestry University. His MSc thesis was aiming to improve the methane production from anerobic digestion of corn straw by hydrothermal pretreatment. Based on this research, a review paper was published. After that, he got the grant from Chinese Scholarship Council and started his PhD research at the Department of Environmental Technology, Wageningen University, the Netherlands. The project focused on the heat production of wood compost at elevated temperatures.

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- Scientific Writing, Wageningen Graduate Schools (2020)
- Project & Time Management, Wageningen Graduate Schools (2020)
- Workshop ‘LCA for biomeiler and turbomeiler’ (2019)
- Workshop ‘Quinone test using HPLC’ (2019)

Management and Didactic Skills Training

- Organising exchange visits ‘Wageningen meets Ghent’ (2018)
- Supervising three MSc students with thesis titled ‘Sustainable heat production by using biological wood oxidation’ (2017), ‘Human urine for sustainable heat production via biological wood oxidation’ (2019) and ‘Assessing heat production & recovery from biological wood oxidation’ (2019)
- Assisting at MSc course ‘Renewable Energy’ (2018-2020)Assisting at MSc course ‘Renewable Energy’ (2018-2020)

Oral and Poster Presentations

- *Effect of nitrogen, phosphorus and pH on biological wood oxidation at elevated temperature.* Environmental Technology for Impact, 3rd -4th June 2020, Wageningen, The Netherlands

SENSE coordinator PhD education

Dr. ir. Peter Vermeulen