



# Effect of nitrogen, phosphorus and pH on biological wood oxidation at 42 °C

Shiyang Fan<sup>a</sup>, Yue Sun<sup>a</sup>, Annemiek ter Heijne<sup>a</sup>, Wei-Shan Chen<sup>a,\*</sup>, Cees J.N. Buisman<sup>a,b</sup>

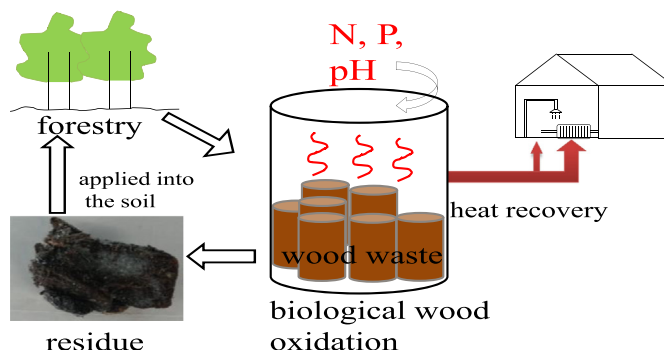
<sup>a</sup> Environmental Technology, Wageningen University & Research, Bornse Weilanden 9, 6708 WG Wageningen, the Netherlands

<sup>b</sup> Wetsus, European Centre of Excellence for Sustainable Water Technology, Oostergoweg 9, Leeuwarden, the Netherlands

## HIGHLIGHTS

- Biological wood oxidation for heat production can reduce air pollution and improve soil quality.
- Oxygen consumption was used as an indicator of heat production.
- Nitrogen was essential for higher heat production from biological wood oxidation.
- Phosphorus and pH were not important factors in this study.

## GRAPHICAL ABSTRACT



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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 is not 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 requirements 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 theoretical heat production rate (0.63 W/kg dry wood biomass) was achieved using 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 future studies.

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## 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.,

\* Corresponding author.

E-mail address: [wei-shan.chen@wur.nl](mailto:wei-shan.chen@wur.nl) (W.-S. Chen).

2019). Wood waste is frequently burned for energy, especially for heating (Sarsaiya et al., 2019). It is reported that about 60% of the bio-mass 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<sub>2</sub>, CO, NO<sub>x</sub> and fine particles, to the environment (Permchart and Kouprianov, 2004).

To overcome these environmental drawbacks, composting is proposed as an environment-friendly wood waste management method. In traditional composting, wood material 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 much slower in the low temperature phases (mesophilic phase, cooling phase and maturation phase) (Wei et al., 2019; Zhao et al., 2016).

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. CTC maintains the composting at thermophilic phase; the temperature of CTC is usually between 40 and 70 °C (Chang et al., 2019; Hosseini and Aziz, 2013; Jiang et al., 2015; Schulze, 1962; Suler and 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 and 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 energy.

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):



The temperature of BWO is usually between 40 and 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; Cooney and 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 and Zapponi, 2018; van der Wal et al., 2007). However, there is little information about BWO at temperature between 40 and 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 and Merrill, 1966). Phosphorus is also important

for fungal growth, as phosphorus helps fungi to take up nitrogen (Nicholas and Militz, 2008; Rayner and Boddy, 1988). In addition to nitrogen and phosphorus, 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 theoretical heat production, and the theoretical heat production was analyzed and correlated to the mass loss.

## 2. Materials and methods

### 2.1. Experimental setup

The experimental setup can be found in Fig. 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).

### 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 and 2.0 cm and a length of  $1.0 \pm 0.2$  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  $4.689 \pm 0.005$  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).

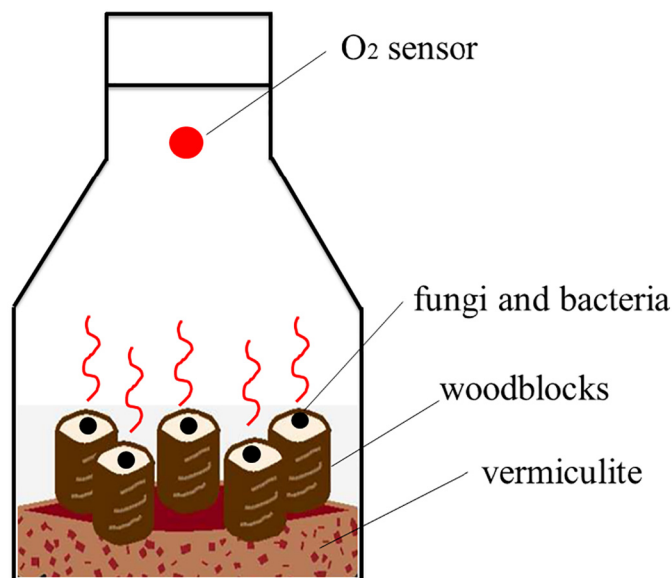


Fig. 1. Experiment set-up of bottles.

### 2.3. Nutrient solution

Nutrient solution consisted of basic nutrition solution, N addition (in  $\text{NH}_4\text{Cl}$ ) and P addition (in  $\text{KH}_2\text{PO}_4$ ). Besides, the pH of the nutrition solution was adjusted by adding HCl and NaOH. The summary of each study is shown in Table 1.

#### 2.3.1. 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  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 120 mg/L  $\text{FeCl}_3$ , 50 mg/L  $\text{H}_3\text{BO}_3$ , 10 mg/L KI, 45 mg/L  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ , 20 mg/L  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ , 75 mg/L  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 50 mg/L  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ , 20 mg/L  $\text{KAl}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$ , 13.2 mg/L  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 10 mg/L NaCl.

#### 2.3.2. N addition

Different amount of N was 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.

#### 2.3.3. 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.

#### 2.3.4. pH

The pH of the nutrition solutions was changed by adding 1 mmol/L HCl or 1 mmol/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  $\text{NH}_4\text{Cl}$  and 1 mg/g P in  $\text{KH}_2\text{PO}_4$  were added in the basis nutrition. Five replicates were used for each pH study.

### 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 >4 months to yield the inoculum. Nutrient solution and fresh wood were supplied regularly during the incubation. For this study, around  $0.1 \pm 0.01$  g inoculum (i.e. decayed materials from the incubation) were added in each bottle.

### 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 bottles decreased to around 10%, bottles were 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 bottles were returned to the oven to continue the experiment.

**Table 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

### 2.6. Mass loss

First, the woodblocks were washed to remove the vermiculite and fungal mycelium. Then the woodblocks were placed in the oven at 105 °C for 24 h 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.

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

### 2.7. Theoretical heat production and heat production rate

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 (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:

$$H = \Delta\text{O}_2 \times h$$

where  $\Delta\text{O}_2$  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  $\text{O}_2$  and was maintained constant throughout the process. 467.5 kJ is the amount of heat released per mol of  $\text{O}_2$  consumed in glucose combustion reaction and glucose is the most abundant carbohydrate present in wood (Caizán Juanarena et al., 2016). Since the duration time of the experiments was different, the theoretical 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:

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

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

### 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.

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

where  $M(\text{C})$  is the atomic weight of C (12 g/mol),  $M(\text{H})$  is the atomic weight of H (1 g/mol),  $M(\text{O})$  is the atomic weight of O (16 g/mol).

### 2.9. Statistical analysis

All indicators were determined on five replicate samples. All data were analyzed using IBM SPSS Statistic 23 (IBM, Armonk, New York, USA) for Microsoft Windows. Differences were compared statistically using ANOVA testing at the 5% level of significance, and using the Least Significant Difference (LSD) test. Origin 9.0 (OriginLab, Northampton, Massachusetts, USA) was used to plot the analytical results. Differences between values at  $P > 0.05$  were considered not significantly different.

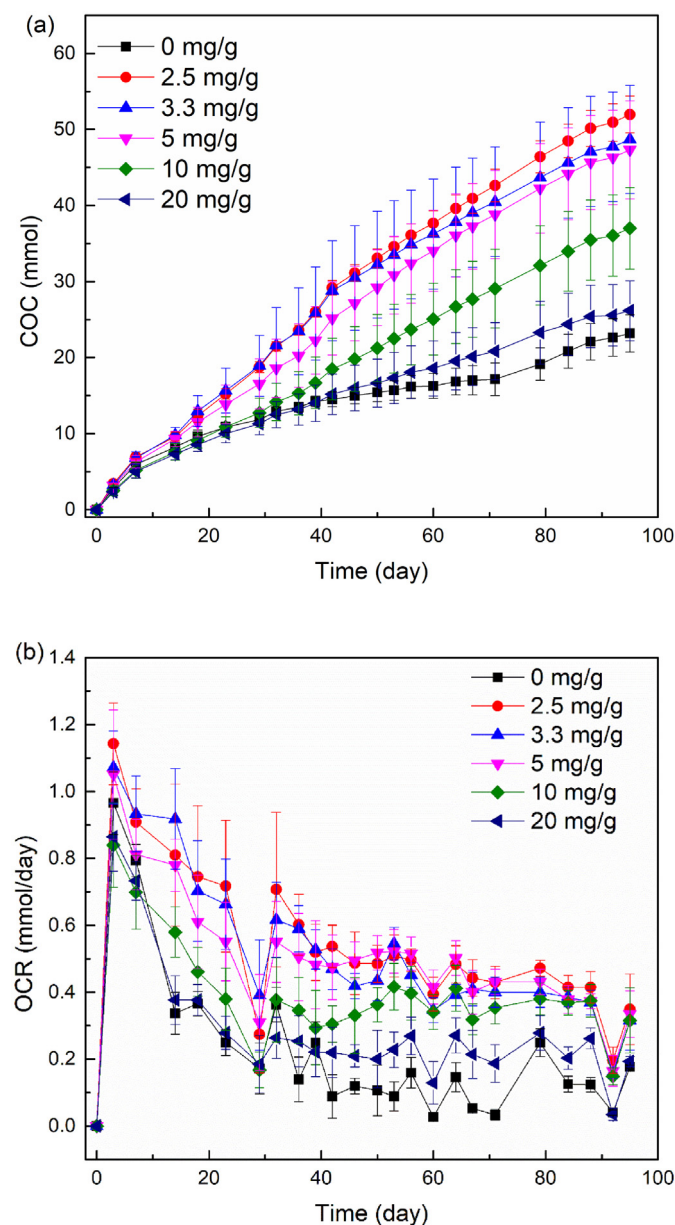


### 3. Results

#### 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. 2a, and the oxygen consumption rate (OCR) is shown in Fig. 2b.

As shown in Fig. 2a and b, N addition changed the COC and OCR obviously. BWO without N addition (0 mg/g) consumed the lowest COC while 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 additions resulted in higher COCs. 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,  $P < 0.05$ ). However, no statistically significant difference was found in the COC between 0 mg/g N addition



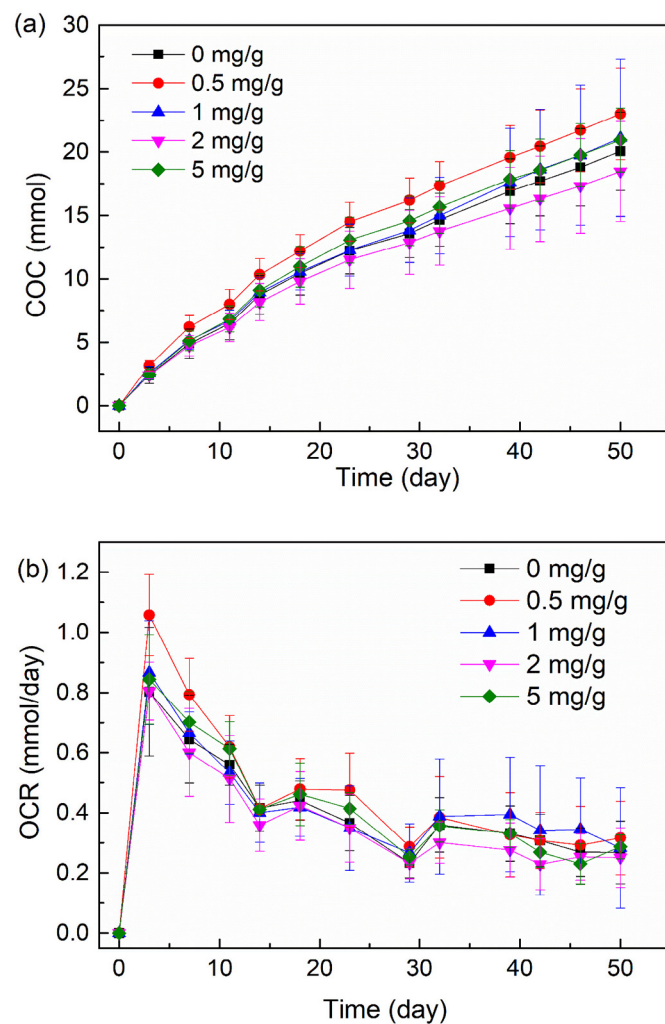
**Fig. 2.** (a) The effect of different N addition on COC. (b) The effect of different N addition on OCR. Average and standard deviation was calculated for 5 replicates. Addition of N significantly improved the oxygen consumption at N addition from 2.5 to 10 mg/g at 42 °C.

group and 20 mg/g N addition group (LSD,  $P = 0.820 > 0.05$ ). As for Fig. 2b, 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. We concluded that the N addition between 2.5 and 5 mg/g could increase the oxygen consumption while 20 mg/g N addition had no significant effect within our BWO system.

#### 3.2. Effect of P addition on oxygen consumption

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

As shown in Fig. 3a and 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 0.5 mg/g P addition obtained the highest COC (23 mol). This number was only 1.3 times COC as much as 2 mg/g P addition did, which consumed the lowest COC (18 mmol). The OCR (Fig. 3b) 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, 1 mg/g, 2 mg/g and 5 mg/g were stopped for mass loss determination on day 50. The test of 0.5 mg/g was continued for another 34 days



**Fig. 3.** (a) The effect of different P addition on COC. (b) The effect of P addition on OCR. Average and standard deviation was calculated for 5 replicates. P addition between 0 and 5 mg/g had little effect on oxygen consumption at 42 °C.

(Appendix Fig. S1a and b). We concluded that the P addition had no significant effect on the oxygen consumption within our BWO system.

### 3.3. Effect of pH on COC and OCR

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

As shown in Fig. 4a, adjusting pH did not obviously change the COC as no statistically difference was found in COC between different pH after 50-day incubation (ANOVA,  $P > 0.05$ ). The final COC of control, pH 4, pH 5 and pH 6 was 21, 19, 20 and 22 mol, respectively.

As shown in Fig. 4b, 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 Fig. S1a and b). We concluded that the pH range between 4 and 6 had no significant effect on the oxygen consumption.

### 3.4. Effect of nutrient addition on mass loss

The mass loss of N addition, P addition and pH is shown in Tables 2a, 2b and 2c, respectively. As shown in Table 2a, N addition between 2.5 and 10 mg/g significantly increased the mass loss compared with the 0 N addition group (ANOVA,  $P < 0.05$ ). But the difference between

**Table 2a**

The effect of N addition on mass loss and theoretical heat production rate after 95-day incubation with 1 mg/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	33.0	28.7	29.4	21.6	16.4
	$\pm 1.4$	$\pm 7.5$	$\pm 3.4$	$\pm 3.4$	$\pm 1.4$	$\pm 2.2$
W (W/kg DM)	0.28	0.63	0.59	0.58	0.45	0.33

0 mg/g N addition and 20 mg/g N addition was not significant (ANOVA,  $P > 0.05$ ). The highest mass loss was found under 2.5 mg/g N addition, which was 2.0 times 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,  $P > 0.05$ ). We concluded that 2.5–10 mg/g N addition increased the mass loss while 20 mg/g N addition had no significant effect in our BWO system.

As shown in Table 2b, 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 no 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 2c, 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 no significant impacts on the mass loss (ANOVA,  $P > 0.05$ ). For the pH 6 experiment, the mass loss after 84 days was 22.91%.

### 3.5. Effect of nutrient addition on theoretical heat production rate

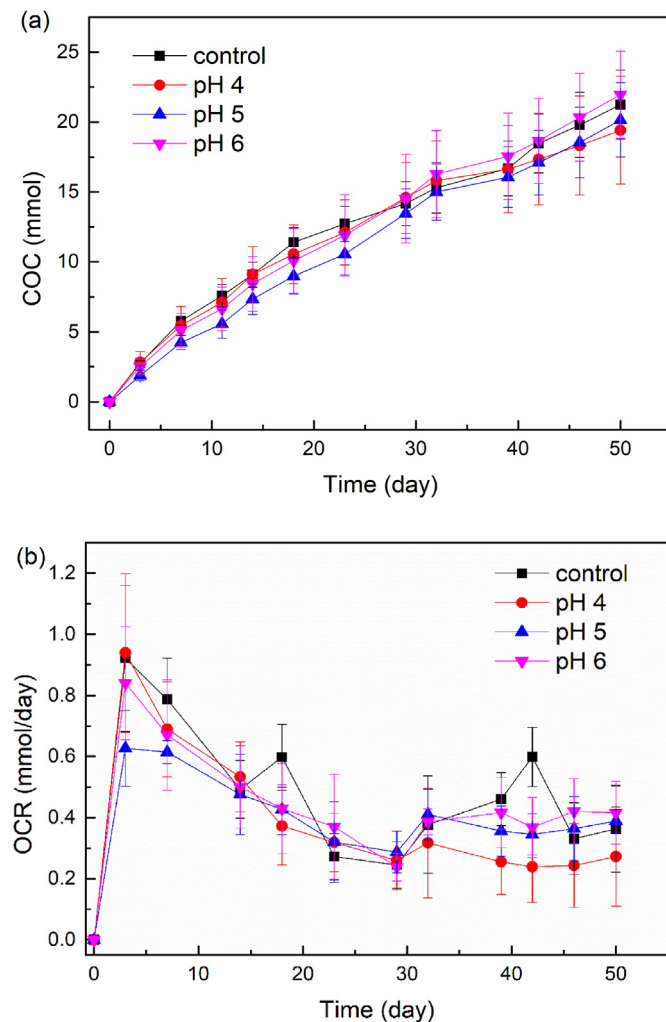
The theoretical heat production rate (W) for N addition, P addition and pH adjustment is shown in Tables 2a, 2b and 2c, respectively. As the theoretical heat production rate is linearly correlated to COC, the effect of N addition, P addition and pH on theoretical heat production rate was not analyzed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

## 4. Discussion

### 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 and 55 °C. In this study, the N addition between 2.5 and 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



**Fig. 4.** (a) The effect of different pH on COC. (b) The effect of different pH on OCR. Average and standard deviation was calculated for 5 replicates. pH range between 4 and 6 had little effect on oxygen consumption at 42 °C.

**Table 2b**

The effect of P addition on mass loss and theoretical 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 $\pm$ 2.1	24.3 $\pm$ 3.1*	17.3 $\pm$ 4.1	15.7 $\pm$ 2.6	15.9 $\pm$ 1.4
W (W/kg DM)	0.46	0.49*	0.48	0.43	0.48

**Table 2c**  
The effect of pH on mass loss and theoretical 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 <sup>a</sup>
W (W/kg DM)	0.49	0.45	0.47	0.49 <sup>a</sup>

<sup>a</sup> The data was after day 84.

increase in COC and mass loss. This is in accordance with some studies at room temperatures (Egli and Quayle, 1986; Labosky Jr et al., 1991; Leatham and 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) were 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 and Quayle, 1986; Labosky Jr et al., 1991; Leatham and 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<sub>4</sub><sup>+</sup>-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 and 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 (1979) found that P addition (1.0 mM) improved the lignin degradation and fungal growth of *Phanerochaete chrysosporium* at 39 °C, compared to 0.2 mM P addition, but he did not give any reasons for this (Reid, 1979). However, Jeffries et al. (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<sub>4</sub><sup>+</sup>-N). The wood decay fungi did not need extra P to take up N (Nicholas and Miltz, 2008; Rayner and Boddy, 1988).

Wood decay fungi usually grow best in the pH range between 3 and 6 whereas many wood decay bacteria and actinomycetes prefer a more neutral pH (Nicholas and Miltz, 2008). In the effect of pH study, adjusting pH at a range between 4 and 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 and 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. S2a, b and c. This was similar to the result of Donnelly et al. (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 and Crawford, 1986).

#### 4.2. Relationship between mass loss and COC

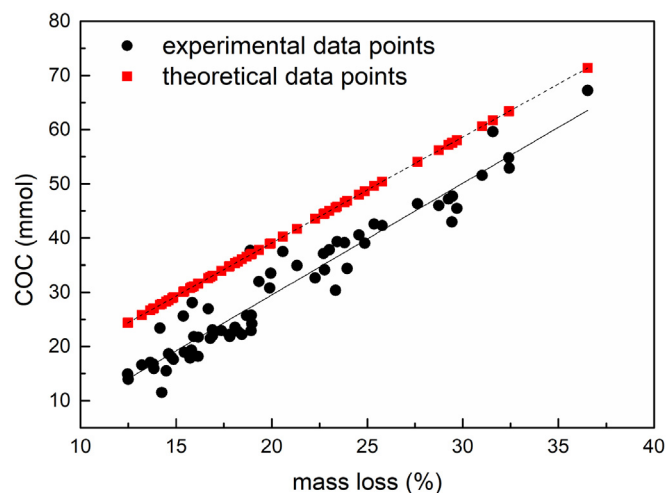
The relationship between mass loss and COC was further analyzed. All experimental data, including N addition, P addition and pH, was

analyzed together. The theoretical oxygen consumption (TO) was also calculated and plotted in Fig. 5. First, as shown in Fig. 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. In other words, the majority of oxygen consumed was indeed used for degrading wood materials. Second, the trend line slope of theoretical data points ( $y = 0.9134x$ ,  $R^2 = 1$ ) is almost the same as the trend line slope of experimental data points, which means the wood was degraded aerobically. Third, there was a difference (average 11.7 mmol) between the theoretical data points and experiment data points, which could be due to the difference between the actual, heterogeneous wood composition and the hypothetical chemical formula used to estimate the theoretical heat production from wood.

#### 4.3. Theoretical heat production and heat production rate via BWO

Wood biological degradation by bacteria and fungi has been extensively studied at room temperatures (Ahn et al., 2007; Johnston et al., 2016; Kielak et al., 2016; Kirker and Winandy, 2014; Rinta-Kanto et al., 2016). There are very few studies that address heat production of BWO at temperatures between 40 and 55 °C. The only work about heat production was from Caizán Juanarena et al. (2016). Caizán studied the wood degradation and estimated 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 theoretical heat production rate was 0.6 W/kg DM, 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 theoretical 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 et al. (2010) estimated around 7000–10,000 kJ/kg heat was generated in 14 days during the composting of green waste, industrial sludge and solid waste; the heat production rate was 5.8–8.3 W/kg DM after 14 days (Irvine et al., 2010). Di Maria et al. (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). Briški et al. (2003) estimated around 7454 kJ/kg TS (total solid)



**Fig. 5.** The correlation between the mass loss and COC. The mass loss and COC had a strong linear relationship.



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 theoretical 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 is relatively difficult to degrade (Tuomela et al., 2000).

## 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<sub>4</sub>Cl). 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.

Moreover, special attention should be paid to the efficient heat recovery methods because heat produced in the BWO process is a medium temperature heat source (usually between 40 and 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 (Radojčić 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 BWO (Di Maria et al., 2008). It would be necessary to combine these methods with BWO for sustainable heat production.

## 6. Conclusion

In this study, the effect of N addition (NH<sub>4</sub>Cl), P addition (in KH<sub>2</sub>PO<sub>4</sub>), 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<sub>2</sub>) 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<sub>2</sub> and 16.9% mass loss), respectively. Too high N addition (20 mg/g) had no significant 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 and 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 0.63 W/kg DM, obtained by 2.5 mg/g N addition.

## CRedit authorship contribution statement

**Shiyang Fan:** Conceptualization, Methodology, Software, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **Yue Sun:** Methodology, Formal analysis, Investigation, Writing - original draft. **Annemiek ter Heijne:** Conceptualization, Methodology, Writing - original draft, Writing - review & editing, Supervision, Project administration. **Wei-Shan Chen:** Resources, Writing - review & editing, Supervision. **Cees Buisman:** Conceptualization, Supervision, Funding acquisition.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2020.138569>.

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