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Quantitative analysis of microbial biomass yield in aerobic bioreactor

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Abstract

We have studied the integrated model of reaction rate equations with thermal energy balance in aerobic bioreactor for food waste decomposition and showed that the integrated model has the capability both of monitoring microbial activity in real time and of analyzing biodegradation kinetics and thermal-hydrodynamic properties. On the other hand, concerning microbial metabolism, it was known that balancing catabolic reactions with anabolic reactions in terms of energy and electron flow provides stoichiometric metabolic reactions and enables the estimation of microbial biomass yield (stoichiometric reaction model). We have studied a method for estimating real-time microbial biomass yield in the bioreactor during food waste decomposition by combining the integrated model with the stoichiometric reaction model. As a result, it was found that the time course of microbial biomass yield in the bioreactor during decomposition can be evaluated using the operational data of the bioreactor (weight of input food waste and bed temperature) by the combined model. The combined model can be applied to manage a food waste decomposition not only for controlling system operation to keep microbial activity stable, but also for producing value–added products such as compost on optimum condition.

Key words: food waste; compost; biomass yield; bioenergetics; reaction rate; aerobic biodegradation; modeling; bioreactor

Introduction

It is of great importance from the standpoint of environmentally friendliness to reuse disposal waste obtained from biodegradation of food waste as raw materials for value-added products such as compost for agriculture. Nitrogen element contained in the disposal waste is most important as resources for value-added products and elementary concentration of nitrogen is closely related to microbial biomass yield in the bioreactor.

It was reported that balancing catabolic reactions with anabolic reactions in terms of energy and electron flow provides stoichiometric metabolic reactions and enables the estimation of microbial biomass yield (McCarty, 1969, 1972; Woo and Rittmann, 2000).

We have studied the integrated model of reaction rate equations and thermal energy balance in aerobic bioreactor for food waste decomposition and showed that the integrated model had the capability both of monitoring microbial activity in real time and of analyzing biodegradation kinetics and thermal-hydrodynamic properties (Watanabe and Isoda, 2011). Accordingly, it was strongly suggested that combining the two models of stoichiometric metabolic reactions with the integrated model (combined model) provides a theoretical framework to estimate the microbial biomass yield in the bioreactor during biodegradation.

This article deals with quantitative analysis based on the combined model for estimating the real-time microbial biomass yield and the real-time concentration of nitrogen element during food waste decomposition.

1 Materials and methods

1.1 Theoretical background

1.1.1 Metabolic reactions by biodegradation

In heterotrophic organisms, stoichiometric relationships for metabolic reactions by biodegradation can be obtained based on balancing respiration reactions with synthesis reactions in terms of energy and electron flow (McCarty, 1969; Woo and Rittmann, 2000). Assuming microbial cells are produced from carbonate and ammonium, a half reaction for cell synthesis (R_c) can be expressed as:

$$(R_c):\ \frac{5}{20}H_2CO_3+\frac{1}{20}NH_4^+ +\frac{19}{20}H^+ + e^- \rightarrow \frac{1}{20}C_5H_7O_2N+\frac{13}{20}H_2O \qquad (1)$$

where, $C_5H_7O_2N$ is a molecular formula of a microbial cell.

The energy required to drive this reaction is supplied from the respiration reaction. The respiration reaction can be expressed as a pair of half reactions for electron donor and electron acceptor:

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$$(R_\mathrm{d}) : (\mathrm{e.d.})_{\mathrm{oxidized}} + \mathrm{e}^- \rightarrow (\mathrm{e.d.})_{\mathrm{reduced}} \tag{2}$$

and

$$(R_\mathrm{a}) : \frac{1}{4}\mathrm{O_2}\,(\mathrm{g}) + \mathrm{H}^+ + \mathrm{e}^- \rightarrow \frac{1}{2}\mathrm{H_2O} \tag{3}$$

where, $(\mathrm{e.d.})_{\mathrm{reduced}}$ is a reduced form of substrate (electron donor).

An electron donor substrate is oxidized to release available electrons (Eq. (2)). Coupling this reaction, a part of electrons are transferred to electron acceptor substrate for energy generation, and a part of electrons are used for reducing oxygen molecules during the processes such as the electron transport and the oxidative phosphorylation (Eq. (3)). The remainder of electrons is used for cell formation (Eq. (1)). Woo and Rittmann (2000) reported oxygenation reactions and/or hydroxylation reactions involved in the biodegradation pathway significantly affect the energy and electron flows for the metabolic reactions.

McCarty (1969) defined an electron equivalent (eeq) as the amount of substrate which releases one mole of electrons during a specific oxidation reaction. Then, the ratio (A) of eeq of electron donor converted to energy versus eeq of cells synthesized is expressed as:

$$A = \frac{-\Delta G_\mathrm{s}}{\varepsilon \Delta G_\mathrm{r}} \tag{4}$$

where, ΔG_s (kcal/eeq) is the standard free energy for the cell synthesis; ΔG_r (kcal/eeq) is the standard free energy for the respiration reaction; ε is the efficiency of energy capture in the respiration reaction. As a result, metabolic reaction (R_t) can be obtained by coupling electron donor half reaction (R_d) with the half reaction for electron acceptor (R_a) and the half reaction for the cell synthesis (R_c) via the ratio A, and expressed as:

$$\mathrm{R_t} = -\mathrm{R_d} + \frac{A}{1+A}\mathrm{R_a} + \frac{1}{1+A}\mathrm{R_c} \tag{5}$$

In order to obtain the electron donor half reactions and the corresponding free energies, biodegradation pathway of food waste is determined based on microbial metabolism as follows:

Carbohydrate: Glucose → Pyruvate → $\mathrm{CO_2, H_2O}$.

Protein: Protein → Amino acid (glycine etc) → Pyruvate → $\mathrm{CO_2, H_2O}$

Lipid: Lipid → Aliphatic acid + Glycerol;

Aliphatic acid → Acetyl − CoA → $\mathrm{CO_2, H_2O}$

Glycerol → Pyruvate → $\mathrm{CO_2, H_2O}$

The standard free energy for the electron-donor reduction half reactions ΔG_d can be calculated using the standard free energy of formation ΔG_f for reactants in the reduction half reactions and the equation $\Delta G_\mathrm{d}(\mathrm{reaction}) = \sum \Delta G_\mathrm{f}(\mathrm{products}) - \sum \Delta G_\mathrm{f}(\mathrm{reactants})$. The standard free energy of formation for unknown organic compound is estimated using group contribution method (Mavrovouniotis, 1990).

1.1.2 Reaction rates for food waste biodegradation

Food waste is mainly composed of protein, lipid, carbohydrate and water. According to the paper by Watanabe and Isoda (2011), the degradation of carbohydrate can be treated as a one-step reaction of oxidation and degradations of protein and lipid can be treated as two-step reactions of hydrolysis and oxidation. These reactions can be expressed using rate constant k as:

$$\text{Carbohydrate (HC)}\quad \mathrm{HC} \xrightarrow{k_0} \mathrm{D_1} \tag{6}$$

$$\text{Protein (P)}\quad \mathrm{P} \xrightarrow{k_1} \mathrm{AA} \xrightarrow{k_2} \mathrm{D_2} \tag{7}$$

$$\text{Lipid (L)}\quad \mathrm{L} \xrightarrow{k_3} \mathrm{LA} \xrightarrow{k_4} \mathrm{D_3} \tag{8}$$

where, AA is amino acid, LA is aliphatic acid; $\mathrm{D_1}$, $\mathrm{D_2}$, and $\mathrm{D_3}$ are inorganic compounds respectively. Since rate constants are closely related to microbial activities in the bioreactor, rate constant k is time-dependent parameter expressed as:

$$k = k_\mathrm{i} \times \exp(k_\mathrm{a} \times t) \tag{9}$$

where, k_i (day^{-1}) is an initial rate constant and k_a (day^{-1}) is a damping factor.

1.1.3 Thermal energy balance in the bioreactor

According to the paper by Watanabe and Isoda (2010), four thermal heat flows in the bed at time t changes into two terms, i.e., latent heat of vaporization HVP and tangible heat HTG under a thermal equilibrium condition. Then thermal energy balance is expressed as:

$$\mathrm{HBC} + \mathrm{HCV} + \beta \times \mathrm{HCD} - \mathrm{HLoss} = \mathrm{HVP} + \mathrm{HTG} \tag{10}$$

where, HBC (kcal/min) is the rate for bacteria heat evolution, HCV (kcal/min) is convective heat transferred between bed and the air above it, $\beta \times$ HCD (kcal/min) is a part (ratio = β) of vapor condensation heat HCV (kcal/min), which is transferred back to the bed, HVP (kcal/min) is latent heat for vaporization which removes heat from the bed, HLoss (kcal/min) is conductive heat loss through bioreactor's wall, and HTG (kcal/min) is tangible heat which changes bed temperature.

1.2 Biochemical constituents of food waste and dead microbial biomass

In order to analyze microbial biomass yield and other contents in the bioreactor, it is necessary to obtain amount of constituents of food waste and dead microorganisms in the bioreactor. Dead microorganisms in the bioreactor are considered to be digested as nutrient by living microorganisms. We define a standard table for biochemical constituent ratios of food waste and dead microorganisms in order to convert amount of food waste and dead microorganism to amount of the corresponding biochemical constituents. **Table 1** shows the standard table for biochemical constituent ratios of food waste and dead microorganisms.

2 Results and discussion

2.1 Development of combined model

2.1.1 Analyzing microbial biomass yield during food waste decomposition

In order to complete the framework for the analysis of microbial biomass yield in the bioreactor for food waste decomposition based on the combined model, we must know microbial metabolic reactions by food waste biodegradation and molar concentration of living microorganisms in the bioreactor. For the metabolic reactions by food waste biodegradation, it is necessary to know electron donor half reactions and the corresponding free energies for the pathway of food waste biodegradation.

Concerning the standard free energy for the biodegradation half reactions, the calculated values shown in **Table 2** are larger than those reported by McCarty (1969). Here, we take into account the effects of oxygenation reaction. According to the paper by Woo and Rittmann (2000), an oxygenation reaction, which involves direct incorporation of atomic oxygen to product molecule from O_2 is more energy yielding than oxidation without oxygenation. As shown in **Table 2**, the electron donor half reactions, in which electron donor substrate are carbohydrate, pyruvate, aliphatic acid and glycerol, can be treated as oxygenation reactions. And also the electron donor half reaction where electron donor substrate is amino acid is treated as a set of oxygenation reactions which incorporate totally 2.5 moles of oxygen molecules per mole of amino acid and hydroxylation reactions which incorporate totally 1.3 moles of water per mole of amino acid.

2.1.2 Calculated values of ratio A for food waste degradation

Calculated values of the ratio A for metabolic reactions by food waste biodegradation are shown in **Table 3**. The standard free energy for respiration reaction ΔG_r((kcal/eeq) can be obtained using the equation $\Delta G_r = \Delta G_a - \Delta G_d$,

Table 1 Standard table for biochemical constituent ratios of food waste and dead microorganisms

Food group	Constituent ratios			Minerals			
	Proteins	Lipids	Carbohydrates	Cytoplasmic water	Total	Kalium ($\times 10^{-5}$)	Phosphorus ($\times 10^{-5}$)
Vegetable group (vg)	0.012	0.002	0.063	0.915	0.008	313	29
Meat group (mt)	0.207	0.145	0.002	0.635	0.011	390	220
Grains group (grn)	0.026	0.004	0.294	0.675	0.001	19	26
Microorganisms	0.103	0.009	0.076	0.800	0.012	–	–

Table 2 Standard free energy of formation for electron donor substrate (ΔG_f), electron donor half reaction, moles of reduced oxygen molecule and standard free energy for electron-donor reduction half reaction (ΔG_d)

Electron donor substrate	ΔG_f (kcal/eeq)	Step	Electron donor half reaction (reduction form)	O′	ΔG_d (kcal/eeq)
Carbohydrate	–219	HC → Pyr	$\frac{1}{2}C_3H_4O_3 + H^+ + e^- \rightarrow \frac{1}{4}C_6H_{12}O_6$	0	–13.1
Pyruvate	–113	Pyr → D1	$\frac{3}{4}CO_2 + H^+ + e^- \rightarrow \frac{1}{4}C_3H_4O_3 + \frac{3}{8}O_2$	3/2	–51.2
		HC → D1 1 step	$\frac{1}{2}CO_2 + H^+ + e^- \longrightarrow \frac{1}{12}C_6H_{12}O_6 + \frac{1}{4}O_2$		–38.5
Protein	–10630	P → AA	$P + 100H_2O \rightarrow 100AA$	–	–
Amino acid	–263	AA → D2	$\frac{460}{864}CO_2 + \frac{120}{864}NH_3 + \frac{3}{864}H_2S + H^+ + e^- \rightarrow$ $\frac{100}{864}AA + \frac{250}{864}O_2 + \frac{130}{864}H_2O$	2.5	–21.7
		P → D2 1 step	$\frac{460}{864}CO_2 + \frac{120}{864}NH_3 + \frac{3}{864}H_2S + H^+ + e^- \rightarrow$ $\frac{1}{864}P + \frac{250}{864}O_2 + \frac{230}{864}H_2O$	250	–33.3
Lipid	–115	L → LA + G	$(CH_2{-}O{-}CO{-}C_{14}H_{29})_3 + 3H_2O$ $\rightarrow (CH_2OH)_3 + 3C_{14}H_{29}COOH$	–	–
Aliphatic acid	–70.6	LA → D3	$\frac{1}{2}CO_2 + H^+ + e^- \longrightarrow \frac{1}{30}C_{14}H_{29}COOH + \frac{7}{15}O_2$	14	–54.4
Glycerol	–119	G → D3	$\frac{1}{3}CO_2 + H^+ + e^- \longrightarrow \frac{1}{9}(CH_2OH)_3 + \frac{1}{6}O_2$	3/2	–28.3
		L → D3 1 step	$\frac{16}{33}CO_2 + H^+ + e^- \longrightarrow$ $\frac{1}{99}(CH_2{-}O{-}CO{-}C_{14}H_{29})_3 + \frac{29}{66}O_2 + \frac{1}{33}H_2O$	87	–52.4

P: $(COOH(CONH{-}C_{3.6}H_{6.7}N_{0.2}O_{0.9}S_{0.03})_{100}{-}NH_2)$, AA: $C_{2.6}H_{5.7}N_{0.2}O_{0.9}S_{0.03}{-}(CHCOOH){-}NH_2$. O′: moles of oxygen molecule reduced per 1 mole electron donor substrate for each step of electron donor half reaction.

Table 3 Calculated values of the ratio A for metabolic reactions by food waste biodegradation

Primary electron donor substrate	Protein	Lipid	Carbohydrate
$\Delta G_{\mathrm{d(intermediate)}}$ (kcal/eeq)	+21.7	+54.4 (LA) +28.3 (G)	+51.2
$\Delta G_{\mathrm{d(primary\ substrate)}}$ (kcal/eeq)	+21.7	+52.0	+38.5
ΔG_{r} (kcal/eeq)	–40.4	–70.7	–57.2
$\Delta G_{\mathrm{S}}{}^{*}$ (kcal/eeq)	+8.91	+10.74	+7.5
Ratio A	0.368	0.253	0.219

* Values reported by McCarty (1969).

where, ΔG_{a}(kcal/eeq) is the standard free energy for electron acceptor half reaction (Eq. (3)) and $\Delta G_{\mathrm{a}} = -18.7$ kcal/eeq.

In biodegradation pathway of protein or lipid, microbial cells gain energy and synthesize new cells while intermediate (amino acid, aliphatic acid) is digested, because cell synthesis is done inside the cells (Conn et al., 1987). When calculating the ratio A of the primary electron donor substrate (protein, lipid, carbohydrate), it is necessary to obtain the standard free energy for the electron donor half reaction in which the electron donor substrate is the primary one $\Delta G_{\mathrm{d(primary\ substrate)}}$. The first column of **Table 3** shows the standard free energy for the electron donor (intermediate) half reaction ($\Delta G_{\mathrm{d(intermediate)}}$. Then, $\Delta G_{\mathrm{d(primary\ substrate)}}$ can be obtained by converting $\Delta G_{\mathrm{d(intermediate)}}$ to $\Delta G_{\mathrm{d(primary\ substrate)}}$ on eeq basis using related electron donar half reactions shown in **Table 2**.

2.1.3 Metabolic reactions by food waste biodegradation

The metabolic reactions by food waste biodegradation can be obtained by substituting electron donor half reactions, electron acceptor half reaction (Eq. (3)), half reaction for cell synthesis (Eq. (1)) and the ratio A into Eq. (5). **Table 4** shows the metabolic reactions by food waste biodegradation.

2.1.4 Molar concentration of living microorganisms in the bioreactor

We reported the reaction rates for food waste biodegradation in the previous paper (Watanabe and Isoda, 2011). The ratio A gives the stoichiometric relationship between an amount of electron donor substrate consumed and an amount of microbial cell synthesized during metabolic reactions. Using the reaction rates and the ratio A, rate equations for cell synthesis can be expressed as:

$$\frac{\mathrm{dM_{s1}}}{\mathrm{d}t} = -\frac{1}{1+A}\frac{\mathrm{dHC}}{\mathrm{d}t} \tag{11}$$

$$\frac{\mathrm{dM_{s2}}}{\mathrm{d}t} = -\frac{1}{1+A}\frac{\mathrm{dAA}}{\mathrm{d}t} \tag{12}$$

$$\frac{\mathrm{dM_{s3}}}{\mathrm{d}t} = -\frac{1}{1+A}\frac{\mathrm{dLA}}{\mathrm{d}t} \tag{13}$$

where, $\mathrm{M_s}$ (mol) is the concentration of the molecule representing a microbial cell (Eq. (1)). Since the solutions of rate equations for biodegradation reactions of HC, AA, and LA were presented in the previous paper, temporal change of $\mathrm{M_s}$ can be obtained using the reaction rate model. Using the reaction rate of microbial cell synthesis ($\frac{\mathrm{dM_s}}{\mathrm{d}t}$), the reaction rate of living microorganisms in the bioreactor can be expressed as:

$$\frac{\mathrm{dM}}{\mathrm{d}t} = \frac{\mathrm{dM_s}}{\mathrm{d}t} - \frac{\mathrm{dM_d}}{\mathrm{d}t} \tag{14}$$

where, $\mathrm{M_d}$ (mol) is the concentration of dead microorganisms in the bioreactor. Assuming the lifetimes of various microorganisms in the bioreactor are identical, the reaction rate is expressed as:

$$\frac{\mathrm{dM_d}}{\mathrm{d}t} = \frac{\mathrm{dM_s}(t-\tau)}{\mathrm{d}t} \tag{15}$$

where, τ (day) is a lifetime of microorganisms in the bioreactor. Thus, molar concentration of living microorganisms M in the bioreactor is written as:

$$\mathrm{M}(t) = \int_0^t \frac{\mathrm{dM}}{\mathrm{d}t}\mathrm{d}t = \int_0^t \left(\frac{\mathrm{dM_s}(t)}{\mathrm{d}t} - \frac{\mathrm{dM_s}(t-\tau)}{\mathrm{d}t}\right)\mathrm{d}t = \int_{t-\tau}^t \frac{\mathrm{dM_s}(t)}{\mathrm{d}t}\mathrm{d}t = \mathrm{M_s}(t) - \mathrm{M_s}(t-\tau) \tag{16}$$

where, second term is concentration of dead microbial biomass in the bioreactor ($\mathrm{M_d}(t) = \mathrm{M_s}(t-\tau)$).

Table 4 Metabolic reactions by food waste biodegradation

Electron donor substrate	Reaction equations
Protein	$\mathrm{P + 355.7O_2}$ $\rightarrow \mathrm{22.1C_5H_7O_2N + 249.6CO_2}$ $\mathrm{+97.9NH_3 + 3H_2S + 157.8H_2O}$
Lipid	$\mathrm{(CH_2OCOC_{14}H_{29})_3 + 48.5O_2 + 4.0NH_3}$ $\rightarrow \mathrm{4.0C_5H_7O_2N + 28.2CO_2 + 38.6H_2O}$
Carbohydrate	$\mathrm{C_6H_{12}O_6 + 3.5O_2 + 0.5NH_3}$ $\rightarrow \mathrm{0.5C_5H_7O_2N + 3.5CO_2 + 5.0H_2O}$

P:$\mathrm{(COOH(CONH{-}C_{3.6}H_{6.7}N_{0.2}O_{0.9}S_{0.03})_{100}{-}NH_2)}$.

2.1.5 Nitrogen element contained in disposal waste

The contents in the bioreactor during food waste decomposition are compounds of disposal waste and culture medium. The contents of disposal waste in the bioreactor are compounds of undigested organic materials, intermediates and water (liquid) produced during decomposition processes, minerals contained in food waste and microbial biomass. Here, we denote weight of protein, lipid, carbohydrate, amino acid, aliphatic acid, mineral and solid portion of microbial biomass in the bioreactor at time t of operation period as P(t) (kg), L(t) (kg), HC(t) (kg), AA(t)

(kg), LA(t) (kg), Mineral(t) (kg) and M(t) (kg) respectively, then the solid weight of disposal waste $(\mathrm{W_s})(t)$ in the bioreactor at time t can be expressed as:

$$\mathrm{W_s}(t) = \mathrm{P}(t) + \mathrm{L}(t) + \mathrm{HC}(t) + \mathrm{AA}(t) + \mathrm{LA}(t) + \mathrm{Mineral}(t) + \mathrm{M}(t) \qquad (17)$$

Denoting $\mathrm{N_p}(t)$ (kg), $\mathrm{N_{AA}}(t)$ (kg) and $\mathrm{N_M}$ (t) (kg) as the weight of nitrogen element contained in P(t), AA(t) and M(t) respectively, the weight of nitrogen element contained in disposal waste excluding microbial biomass is represented as $\mathrm{N_p}(t)+ \mathrm{N_{AA}}(t)$.

2.2 Analysis of microbial biomass yield and concentration of nitrogen element

It is noted that the rate constants for food waste decomposition during operation period can be estimated through thermal energy balance equation (Eq. (10)) with time course of bed temperature in the bioreactor. Then, using the estimated rate constants, reaction rates for biodegradation of food waste can be obtained through the reaction rate equations for biodegradation represented in the previous paper (Watanabe and Isoda, 2011). And then, reaction rates for cell synthesis can be obtained via Eq. (11) to (13). Finally, microbial biomass yield in the bioreactor during food waste decomposition can be estimated using Eq. (16).

In order to verify the combined model proposed in Section 2.1, microbial biomass yield in the bioreactor for food waste decomposition has been simulated using experimental data of the bioreactor. **Figure 1** shows successive input data of food waste in the experiment for food waste decomposition. The operation period was 12 days and food waste was added 9 times during the operation period. **Figure 2** shows the time course of experimental and simulated bed temperature.

Figure 3 shows simulated solid weight of contents in the bioreactor, i.e., solid weight of disposal waste, living microbial biomass yield, nitrogen element contained in disposal waste and nitrogen element contained in microbial biomass in the bioreactor during the operation period. In **Fig. 3**, the solid weight ratio of microbial biomass to disposal waste is about 30% on average during the operation period. Weight ratio of nitrogen element contained in both disposal waste and microbial biomass to solid disposal waste is about 10%. Thus, it was found that microbial biomass yield is an essential factor for food waste decomposition.

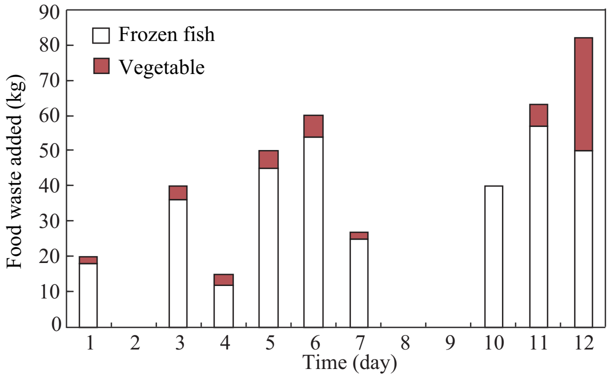

Fig. 1 Successive input data of food waste in the experiment for food waste decomposition.

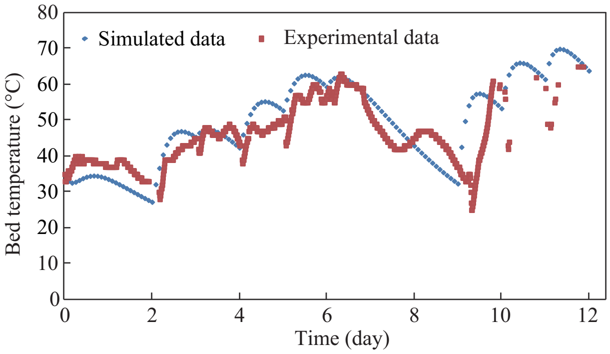

Fig. 2 Time course of experimental and simulated bed temperature. Simulation parameters: life time = 3 days, rate constants (day^{-1}) k_0:k_1:k_2:k_3:k_4 = 2:0.7:3:2:1, air temperature T_a = 25°C, air flow rate V_0 = 0.5 m^3/min.

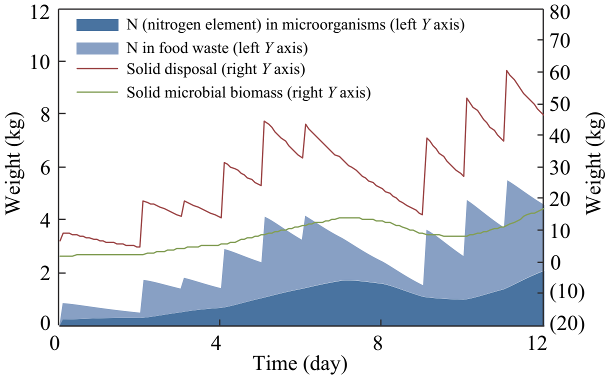

Fig. 3 Simulated solid weight of contents in the bioreactor. The simulation parameters are the same as **Fig. 2**.

3 Conclusions

The combined model based on stoichiometric metabolic reaction model and the integrated model provides the quantitative relationship between microbial biomass yield and physicochemical parameters such as bed temperature in the bioreactor during food waste decomposition. Accordingly, it is possible to monitor microbial biomass yield in real time. Thus, the combined model can be applied to manage a food waste decomposition not only for controlling the system operation to keep microbial activity stable, but also for producing value–added products such as compost.

Acknowledgments

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