Production of biosurfactants in batch reactor for food waste composting

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Abstract

Biosurfactants produced by microbial activities may potentially enhance solid waste composting. A study was performed to characterize the production of biosurfactants and the enhancement of food waste composting process in a batch reactor. Production of biosurfactants was examined on the basis of surface tension values. Experimental results indicated that biosurfactants could be produced by microorganisms during composting and enhance microbial activities of food waste. After separated from food waste, the produced biosurfactants were degraded by microorganisms naturally within 24 hours. Based on experimental results, certain relationship between surfactants and composting progress was also disclosed.

1 Introduction

Microbial activities were performed in a layer of liquid film at the surface of solid waste particles during composting processes [1], which indicates the physicochemical conditions around the liquid film have great effects on composting efficiency. Many traditional techniques including the control of C/N, humidity, oxygen, temperature and porosity in the composting system were used to improve conditions of the microenvironment for enhancing the degrading efficiency [2]. However, details about the related mechanism are not well studied and more effective methods that can ameliorate the physicochemical conditions
of composting are desired.

Biosurfactants, materials produced by many genera of microorganisms in response to growth on hydrophobic organic compounds [3, 4], were expected to have important role to enhance solid waste composting. They are amphiphilic molecules that partition preferentially at interfaces between different phases, such as solid-water and air-water interfaces, to reduce surface and interfacial tension [5]. Biosurfactants could also increase the surface area between HOC and water phase, thereby increasing the aqueous concentration of HOC and its availability to microorganisms [6]. However, little information is available on the linkage between biosurfactants and solid waste composting. Also, no records about biosurfactant production during a composting process and its role in enhancing the composting were reported.

This study attempts to verify the production of biosurfactants by microorganisms during composting processes, and to disclose the effects of biosurfactants. A batch scale reactor was designed for the composting of food waste under controlled conditions. Through the investigation of the effect of biosurfactants on composting, the insight about the role of biosurfactants can be gained.

2 Methodology

2.1 The batch reactor

A mixture of food wastes was used, with their characteristics being shown in Table 1. This waste mixture was then introduced into a reactor for the aerobic composting. The structure of the reactor is illustrated in Figure 1.

<table>
<thead>
<tr>
<th>Compositions</th>
<th>Rice</th>
<th>Vegetables</th>
<th>Fruits</th>
<th>Potato</th>
<th>Meats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contents (%)</td>
<td>33.7</td>
<td>25.3</td>
<td>16.9</td>
<td>16.9</td>
<td>7.2</td>
</tr>
<tr>
<td>C/N</td>
<td>36.14</td>
<td>34.79</td>
<td>36.92</td>
<td>15.28</td>
<td>5.69</td>
</tr>
</tbody>
</table>

It consists of a covered 8-L glass cylinder with a working volume of 6 L. There are five ports on the reactor’s lid, with each being fitted with a septum-filled screw cap to minimize airflow. A mechanical mixture set at 2 r/min occupies the central port. The other two ports are occupied by fine-bubble diffuser stones, through which air would be forced into the system for providing sufficient oxygen and preventing from anaerobic condition. The reactor offgas is issued through the fourth port, which delivers the gas into a sealed conical flask with 2% H₂SO₄ solution to absorb NH₃ produced during the composting process. Finally, the offgas would transfer through a GAC (Granular Active Carbon) trap before flowing out. The fifth port of the reactor was used to fill/draw food wastes.
2.2 Experimental methods

Five kilogram of food wastes were smashed into particles with a diameter of less than 1.0 cm and mixed evenly before filled into the batch reactor. During the composting process, the reactor was placed in a bath to maintain a system temperature of 50 °C. The investigation involved the analysis of surface tension, total C and N, ash, and water content in waste samples at different time stages.

2.2.1 Surface tension

Five gram of food wastes were drawn from the reactor each day and put into a 50 mL centrifuge tube with 25 ml distilled water. The tube was then placed first on a shaker for 5 min to mix the wastes and distilled water intensively, and next left in a centrifuge for 2 min. After that, the upper layer supernatant solution was filtered (0.45 μm). Surface tension is then measured on duplicate 5-mL filtrate samples through an Automatic Surface Tensiometer (accuracy ± 0.2 dyn/cm).

2.2.2 Concentration of biosurfactants

Biosurfactant concentrations were quantified on duplicate 5 mL filtrate samples using the critical micelle dilution method [4]. Surface tension remains constant at some minimum value when surfactant concentrations are above the critical micelle concentration (CMC). The surface tension increases only when the surfactant concentration drops below the CMC dilution. The initial surface tension was measured at the beginning of the composting process. Then surface
tension of each sample was measured in increasing dilution rates (1:2, 1:4, and 1:6, and so on) until the surface tension rose above the initial value. The dilution factor required to produce an increase in surface tension is the surfactant concentration, expressed as times the CMC ($\times$ CMC).

### 2.2.3 Ash and water contents in food wastes

During the composting period in each day, duplicate 10 g food wastes were drawn and put into crucibles; they were then burnt in a muffle furnace at the temperature of 800 ± 10 °C and stopped till the content weight (less than 0.2 mg difference between two weight values) was reached. The ash content was expressed as the ratio of the inorganic matter left in the crucible to the total weight of the food wastes. To quantify water content, another set of duplicate 10 g food waste samples was put into beakers, and then settled in the oven at a temperature of 105 ± 5 °C till the beakers weighted constantly.

### 2.2.4 Carbon content in dry food waste

Food waste was mulled with liquid nitrogen to 10 meshes after drying under 105 ± 5 °C. Duplicate 2 ± 0.2 mg mulled food waste samples were sealed into tin foil bags. Air was expelled from the foil bags before being introduced into a mass spectrometer with a High Temperature Conversion Elemental Analyzer (TC/EA) to measure the carbon content in each dry waste sample.

### 2.2.5 Total carbon in composting system

The equation below was used to calculate the total carbon content in the reactor each day:

$$\text{Total C} = (W_0 + W_1) \times (1 - C_w) \times C_c$$

where $W_0$ is the total weight of food wastes (g); $W_1$ is the weight of food wastes drawn from the reactor for experimental measurements (g); $C_w$ is the water content of food waste (%); and $C_c$ is the value of C contents in dry food wastes (%).

### 3 Results and Discussions

#### 3.1 Existence of Biosurfactant

Track study about the change of surface tension was performed during the composting process. Time profile of surface tension during the first week of composting was shown in Figure 2. The original surface tension of filtrated solution of food waste was 56.92 dyn/cm (average value). At the first two days, surface tension of filtrated samples decreased rapidly to 44.93 dyn/cm, and then became stable at around 44 dyn/cm. Until the end of first week, surface tension had not risen up.
Surfactants were defined as the materials that would lead to reduction of surface and interfacial tension levels. Therefore, surface tension was considered as one of the most important indices for proving the existence of surfactants. The original food wastes showed no sign of containing the surfactants. However, during the period of food waste composting, surface tension decreased remarkably, providing evidence of biosurfactant production. When concentration of the produced biosurfactant was above the critical micelle concentration (CMC), the surface tension would then remain constant [4]. This explains the relative stability of surface tension during the second to seventh days. Values of surface tension in the last five days of the study week were in the range of 44.72 ± 0.48 dyn/cm (average value ± standard deviation).

3.2 Production rate of biosuractants

Based on the minimized value of surface tension around 45 dyn/cm, each filtrated sample was diluted till surface tension of the diluted solution raised up to 45 dyn/cm. Thus, the change of biosurfactant concentrations during the week was obtained (Figure 3).

In the first day, the concentration of biosurfactant was less than the CMC. Since the second day, biosurfactants had been accumulating in the system and their concentrations were raised to the highest at the fifth day, with the average being over 16 times the CMC in the filtrated samples. Then, the concentration began to decrease again.

Compared to the two curves in Figure 2 and 3, although the surface tension was stable during days 2 and 7, concentrations of the biosurfactants showed...
regular variations, revealing some valuable information of the related composting mechanism. Before the 5th day, sufficient bio-available organic materials and proper environment existed in the system so that a rapid rate of aerobic composting was maintained, and more and more biosurfactants accumulated in the system. From the 6th day, microorganisms could not longer get sufficient organic matter from food wastes. Biosurfactants hence became the alternative food source for the microorganisms such that the biosurfactant concentration began to decrease.

![Figure 3: Biosurfactant concentration vs. time during the first week of the composting process](image)

### 3.3 Degradation of biosurfactant

During the first week of composting, biosurfactants were drawn from the reactor together with food wastes each day. They were then dissolved in distilled water and next separated from particle food wastes through a filtration process. After measuring the surface tension, each filtrated solution was placed in the laboratory for 24 hr, open to the air, before the second measurement of surface tension. Table 2 showed surface tension levels of the filtrated samples before and after the 24 hr period. It is indicated that surface tension levels in the filtrated samples during days 1 to 7 were raised rapidly after the 24 hr period. The statistic analysis showed that the average value of surface tension in the seven days was 55.73 dyn/cm with a relative standard deviation of only 0.39%. In the filtrated solutions, microorganisms were separated from the reactor and thus the organic substrate. Therefore, microorganisms began to degrade the biosurfactants in the filtrated solutions. After 24 hours, almost all biosurfactants had been degraded so that the surface tension became constant in all the seven solutions with different concentrations of biosurfactants. These results indicate that the biosurfactants could be degraded by microorganisms naturally.

To obtain more details about the natural degradation process, surface tension and biosurfactant concentration levels of the filtrated solution in the 5th day were
measured hourly (Figure 4). Within the first hour, the surface tension changed slowly and biosurfactant concentrations remained constant, indicating that some organic matter could pass through the 0.45 μm filter paper and enter the filtrated solution then be biodegraded by microorganisms during this period. It provided the evidence that organic matter in aqueous films of composting system was emulsified by the biosurfactants and present in micelles because a well-mixed organic-matter-in-water emulsion consisted of droplets smaller than 0.1 μm in diameter, which was proved in the previous studies [7, 8]. This showed that the biosurfactants produced in the composting system were also emulsifying agents. Emulsion vastly increased the surface area of organic matter available for direct microbial growth or dissolution [3, 6]. Therefore, it was likely that biosurfactant production in the composting system could result in enhanced microbial activities of food waste although the effect of biosurfactant production on rates of microbial growth was not quantified in this study.

Table 2. Variations of surface tension in filtrated solutions after 24 hr

<table>
<thead>
<tr>
<th>Days</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface tension (dyn/cm)</td>
<td>56.92</td>
<td>50.88</td>
<td>44.93</td>
<td>44.12</td>
<td>44.15</td>
<td>44.78</td>
<td>45.22</td>
<td>45.13</td>
</tr>
<tr>
<td>Surface tension after 24 hr (dyn/cm)</td>
<td>56.94</td>
<td>55.52</td>
<td>55.63</td>
<td>55.48</td>
<td>55.73</td>
<td>56.10</td>
<td>55.69</td>
<td>55.87</td>
</tr>
</tbody>
</table>

After 4 hr, the rate of biosurfactant degradation became fast and the surface tension increased from 45.98 to 54.88 dyn/cm within 9 hr. Then most of the biosurfactants were degraded by microorganisms so that the surface tension tended to a constant value eventually.
3.4 Relationship between biosurfactant production and composting indices

Parameters reflecting processes of the composting are listed in Table 3. Based on information of waste weight, water content, and carbon percentage (in dry waste), the total carbon and ash levels vs. time can be obtained as shown in Figure 5.

Table 3. Parameters of food wastes in the first week of composting operation

<table>
<thead>
<tr>
<th>Time (day)</th>
<th>Total weight of wet waste (g)*</th>
<th>Water content (%)</th>
<th>Carbon content in dry waste (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4150.0</td>
<td>0.57</td>
<td>46.61</td>
</tr>
<tr>
<td>1</td>
<td>3722.8</td>
<td>0.48</td>
<td>42.79</td>
</tr>
<tr>
<td>2</td>
<td>3512.4</td>
<td>0.44</td>
<td>41.61</td>
</tr>
<tr>
<td>3</td>
<td>3364.0</td>
<td>0.42</td>
<td>40.89</td>
</tr>
<tr>
<td>4</td>
<td>3176.4</td>
<td>0.38</td>
<td>40.31</td>
</tr>
<tr>
<td>5</td>
<td>3056.6</td>
<td>0.35</td>
<td>40.22</td>
</tr>
<tr>
<td>6</td>
<td>3034.9</td>
<td>0.35</td>
<td>40.11</td>
</tr>
<tr>
<td>7</td>
<td>3015.0</td>
<td>0.34</td>
<td>40.07</td>
</tr>
</tbody>
</table>

* Wastes drawn from the reactor for experimental analysis were included.

In Figure 5, the total carbon level decreased and the ash content increased with time remarkably. Since carbon can be lost as carbon dioxide following the composting process, variation of total carbon level would be considered as one of main indicators of composting rate. Meanwhile, the change of ash content is another indicator to quantify the composting process. After the rapid change of total carbon and ash content from the beginning to the 5th day, the trends of slow increase in ash content and decrease in total carbon were encountered (Figure 5), indicating that the rate of composting slowed down after the 5th day. Examining the results as shown in Figure 3, where biosurfactant concentrations also started to decrease at the 5th day, demonstrating that microbial activities also slowed down from the fifth day; thus, variation in biosurfactant concentrations could be a potential indicator for measuring progress of the composting process.

Several factors could count for the slowdown of biodegrading activities after the 5th day. One is the change of water content in the system. Table 3 shows that water contents decrease for 38% during the first five days. Since the microbial activities were performed mainly in the liquid film on the solid surface, the decrease in water content would change characteristics of the liquid film, making it unsuitable for further composting. Another factor is system temperature. In this study, temperature was fixed at 50°C, which might prevent from further composting progress, since the growth of some thermophilic bacteria that have high composting capability was restrained. Meanwhile, the decrease of total available carbon in the system during the first five days was also an important factor influencing the process.
4 Conclusions

A batch-scale composting system was designed for investigating the existence and the roles of biosurfactants for composting processes. The results indicated that biosurfactants could be produced during composting processes. Experimental results showed that biosurfactants were accumulated in the system and raised to the highest concentration at the 5th day with a value of over 16 times the Critical Micelle Concentration. It was also indicated that the variations of total carbon and ash content in the system could be used to measure the rate of composting progress. After the rapid change of total carbon and ash content during days 1 to 5, the rate of composting became slow and had a similar trend to the biosurfactant production rate, revealing a correlation between composting progress and biosurfactant production.

The results also demonstrate that biosurfactants could be degraded by microorganisms naturally. Through hourly measurements of surface tensions, it was revealed that most of the produced biosurfactants were degraded by microorganisms within 13 hours. Besides, some organics of food waste, which passed through the 0.45 μm filter paper and found in the filtrated solution, was emulsified by biosurfactants; this provided evidence that the produced biosurfactants could enhance microbial activities in the food wastes.

This study is the first attempt to develop a new type of surfactant — biosurfactant — for enhancing medium conditions for food waste composting. Many extensions to the composting of other organic wastes can be further examined.
References


