# Characterization of Pile Fabric Yeast Carriers for Treatment of Wastewater from a Plum Production Facility

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#### Abstract

BF-T9P, a nonwoven fiber material, can be used as an effective biomass carrier for yeast (UY7 strain) in the treatment of wastewater generated during the production of seasoned salty plums. However, the cost is a major problem in small and medium scale companies. We investigated the feasibility of using a pile fabric biomass carrier that can be produced at low cost with existing equipment. The TOC removal rate of E-pile, a polyester pile fabric, was as high as that of the nonwoven material under a volumetric loading rate of 4 to 15 kg-TOC/m<sup>3</sup>/d. A packing ratio of 0.17 m<sup>3</sup>-carrier/m<sup>3</sup>-reactor was sufficient for TOC removal if the volumetric loading rate was less than 8 kg-TOC/m<sup>3</sup>/d. In a system using a biomass carrier, the decrease in TOC removal upon raising the temperature to  $10^{\circ}$ C was faster, compared with a system without a biomass carrier. These results may be attributed to the stable retention of yeast in the reactor by entrapment inside the biomass carrier.

Key Words: Yeast attached carrier, Pile fabric, Plum wastewater

# INTRODUCTION

Wastewater generated during the production of seasoned salty plums contains very high levels of sugar alcohol, with a total organic carbon (TOC) of about 50 g/l. We reported that a TOC removal rate of about 40% was successfully obtained under a volumetric loading rate of 5.1 to 12.5 kg-TOC/d/m<sup>3</sup> using a yeast strain (UY7) attached to Biofringe, which is a biomass carrier of a swim-bed type made of acrylic fiber.<sup>1)</sup> Treatment of wastewater from a plum production facility was carried out using a pilot plant combining yeast and an activated sludge process, illustrating that a system using biomass carriers could remove TOC from wastewater more efficiently than without a biomass carrier.<sup>2)</sup> A wastewater treatment system using a biomass carrier, BF-T9P, which is a nonwoven polyester material, was carried out in a stable operation over two years without the addition of yeast during the course of the process. Biofringe and BF-T9P have been used in several wastewater treatment processes. Biofringe is particularly noted as a biomass carrier that contributes to the reduction of excess sludge.<sup>3,4)</sup> However, these carriers are expensive and cost is a major problem in small and medium size companies. Therefore, the development of low-price biomass carriers is required.

We investigated the applicability of pile fabric, which is formed from a yarn-like permanent hair and is widely used in towels and carpeting. Pile fabric can be produced as a biomass carrier using existing apparatus, without equipment modification so it is expected that its price will be lower than that of Biofringe or BF-T9P. In initial work. the TOC removal performance using a pile fabric made of polyester fiber, E-Pile, was compared with that using BF-T9P. Subsequently, the TOC removal rate using a combined acrylic/polyester fiber, AE-Pile, was compared with E-Pile. The effectiveness of using pile fabrics as biomass carriers for wastewater treatment was shown bv illustrating and explaining the effect of temperature on TOC removal.

# MATERIALS AND METHODS

Yeast The UY7 strain, isolated from wastewater in a plum production company, was used in this study. The 26S rDNA sequence of UY7 was related to *Pichia jadinii* (99.2–99.7% of identity).<sup>1)</sup>

Wastewater The quality of wastewater discharged from the plum production company is shown in Table 1. Because the total nitrogen (TN)/TOC and total phosphorous (TP)/TOC ratios in Wastewater-1 (raw wastewater) were low, Wastewater-2 was prepared by adding 30.7 g/l of  $(NH_4)_2SO_4$  and 6.07 g/l of K<sub>2</sub>HPO<sub>4</sub> to Wastewater-1. The

Table 1 Water quality of seasoning solution used for experiment

Item		Wastewater-1	Wastewater-2
TOC	(g/l)	62~75	61~72
TN	(g/l)	$0.5 \sim 0.53$	6.0~6.4
TP	(mg/l)	$2.2 \sim 3.5$	720~1040
pН		2.4	2.8
NaCl	(g/l)	5.2	5.2

Wastewater-2 was prepared by adding 30.7 g/l of  $(NH_4)_2SO_4$  and 6.07 g/l of  $K_2HPO_4$  to Wastewater-1.

influent wastewater was prepared by diluting Wastewater-2 with distilled water.

**Preculture** The UY7 strain was inoculated in 50 ml of medium, which consisted of sterilized Koji solution with a sugar content of 10 wt%. The solution was incubated by rotary shaking (150 min<sup>-1</sup>) for 2 days at 30°C.

**Biomass carrier** The characteristics and packing ratios of the biomass carriers are shown in Table 2. BF-T9P (Japan Vilene Co., Ltd., Tokyo, Japan) is a nonwoven material made of polyester coated with a copolymer, 4-vinypyridine-styrene, which is used in the wastewater treatment system of a plum production facility. The other carriers (Ohya Pile Co., Ltd., Wakayama, Japan) were novel pile fabric materials. E-pile1 and E-pile2 were made of polyester fiber. 7E3A-pile and 4E6A-pile were composed of 30 wt% and 60 wt% acrylic fiber, respectively, and the balance polyester fiber. The surface area of E-pile1 and the volume of E-pile2 were approximately the same as those of BF-T9P. The volumes of 7E3A-pile and 4E6A-pile were the same as that of E-pile2. As shown in Fig. 1, BF-T9P has a structure with 8 fins but the pile fabrics have planar structures. When pile fabrics were attached in the reactor, they were arranged in a structure with three fins.

**Reactor startup and operation** The reactor used is shown in Fig. 2. The working volume was  $1.55 \ l$ . The biomass carrier was attached on a steel pipe for aeration and  $1 \ l$  of wastewater diluted Wastewater-2 to 5 times with distilled water was added to the reactor. The reactor was sterilized by autoclave ( $121^{\circ}$ C, 30 min). After being cooled to room temperature, 30 ml of preculture

Table 2 Characteristics and packing ratio of test biomass carriers

		packing ratio			
carrier	ester noer : acrylic noer	m <sup>2</sup> -carrier/m <sup>3</sup> -reactor	m <sup>2</sup> -carrier/m <sup>3</sup> -reactor		
BF-T9P	100 : 0	47.4	0.17		
E-pile 1	100 : 0	46.7	0.53		
E-pile 2	100 : 0	18.8	0.17		
7E3A-pile	70:30	19.5	0.16		
4E6A-pile	40:60	19.5	0.16		

Surface area and volume of biomass carrier were calculated using the size shown in Fig.1 without taking each pile size into consideration.



Fig. 1 Shapes of biomass carriers



Fig. 2 Schematic diagram of fixed bed reactor

solution was added to the reactor in a clean room and aeration was carried out for 1 day at 25°C. The air was passed through a 0.5 µm filter and the aeration flow was 2.0 *l*/min. Thereafter, the sterilized influent wastewater was continuously fed to reactor by a peristaltic pump.

The weight of yeast attached on the biomass carrier To measure the weight of yeast attached on the E-pile, a 4cm x 4cm sample of the carrier was placed in the reactor. After mixed liquor suspended solids (MLSS) in the reactor reached a steady state, the E-pile sample was taken out and drained. After no water drops were observed falling from the carrier for 5 seconds, it was transferred to a beaker and washed 5 times with distilled water to remove the yeast. The total washing solution was made up to 50ml and the MLSS of the 50 ml washing solution was measured. This procedure was performed 5 times and the weight of attached yeast was determined as the average value. To measure the suspended yeast concentration in the reactor, 5.1 ml of mixed liquid (the water volume held in a 4 cm x 4 cm E-pile sample) was diluted to 50 ml with distilled water and the MLSS was measured.

Analytical methods The sample was prepared without filtration and by diluting with distilled water. TOC and TN were analyzed by a TOC/TN analyzer (TOC-V<sub>CPH</sub>/ <sub>CPN</sub>, Shimadzu Co., Kyoto, Japan). TP was determined in accordance with Japanese Industrial Standards.<sup>6</sup>) NaCl and pH were determined using a Sension Cl sensor (HACH Co., Loveland, USA) and F-53 sensor (Horiba Co., Kyoto, Japan), respectively. MLSS was calculated using eq.1, which previously related MLSS to the absorbance or optical density (OD) at 660 nm (OD<sub>660</sub>).

 $\begin{array}{ll} \text{MLSS (mg/l)} = 44.8 \ \exp(1.74 \ \text{OD}_{660}) & (1) \\ 0.18 < \text{OD}_{660} < 2.73 & r^2 = 0.9816 \end{array}$ 

### **RESULTS AND DISCUSSION**

TOC removal performance using pile fabric (E-pile) and nonwoven (BF-T9P) Influent and effluent TOC concentrations and TOC removal rates under different HRT ( $\theta$ ) and temperature using E-pile1 as a biomass carrier are shown in Fig. 3. The average values of TOC, TN and TP concentrations under each HRT were shown in Table 3. Though the effluent TOC, TN and TP concentrations were influenced by HRT, the difference of TN/TOC or TP/TOC between influence and effluent was small. Fig. 4 shows the daily changes in TOC removal under different HRT at 25°C using E-pile2 and BF-T9P as biomass carriers. In Fig. 4, for the case of  $\theta = 1.7$  d, using BF-T9P, when the influent TOC concentration was 13 g/l, the TOC removal rate was 46% but at 14.5 g/l, it was 38%. It is believed that TOC removal was influenced not only by HRT but also by influent concentration, because the TOC removal could not respond to the change to such a high concentration. In Fig. 5, the relationship between the volumetric loading rate (VLR) and TOC removal is shown. TOC removal was calculated using the average value of the effluent TOC concentrations in 5 days with an error of 1 %. The data were correlated by a single curve. This indicates that the TOC removal capability of UY7 using E-pile was about the same as using the commercial BF-T9P. The TOC removal capabilities of UY7 using E-pile1 and E-pile2 were equal, except that the VLR was 17 kg-TOC/m<sup>3</sup>/d. It was confirmed that the packing ratio of E-pile under stable operation was



Fig. 3 Daily changes in TOC removal using E-pile 1 as biomass carrier ○, influent TOC; △, effluent TOC;

TOC removal



Fig. 4 Daily changes in TOC removal using E-pile 2 and BF-T9P as biomass carrier at 25°C ○, influent TOC; △, effluent TOC; ●, TOC removal



Fig. 5 Relationship between volumetric loading rate and TOC removal at 25°C ○, E-pile1 (pile fabric); ◇, E-pile2 (pile fabric); ●, BF-T9P(nonwonven)

нвт	d	0.8	1.5	23	3.0
	u	0.0	1.0	2.0	0.0
Influent					
TOC	g/l	14.8 (100)	14.9 (100)	14.6 (100)	13.6 (100)
TN	g/l	1.42 (9.6)	1.47 (9.9)	1.41 (9.7)	1.28 (9.4)
TP	g/l	0.226 (1.5)	0.228 (1.5)	0.238 (1.6)	0.236 (1.7)
Effluent					
TOC	g/l	10.7 (100)	9.60 (100)	8.12 (100)	6.74 (100)
TN	g/l	1.16 (11)	1.09 (11)	1.01 (12)	0.870 (13)
ŤP	g/l	0.187 (1.7)	0.133 (1.4)	0.105 (1.3)	0.0943 (1.4)

Table 3 TOC, TN and TP under different HRT

The values in parentheses are the ratios of TN and TP to TOC.

about 0.17 m<sup>3</sup>-carrier/m<sup>3</sup>-reactor for VLRs less than 8 kg-TOC/m<sup>3</sup>/d.

TOC removal capability using pile fabrics with or without acrylic fiber Fig. 6 shows the relationship between VLR and TOC removal using 7E3A-pile, 4E6A-pile and Epile2. The difference in TOC removal amongst these pile fabric biomass carriers was hardly discernible. Although the TOC removal rate at VLR = 23 kg-TOC/m<sup>3</sup>/d using 4E6A-pile was larger than for the other pile fabrics, this might be related to the effect of influent TOC concentration rather than the acrylic fiber content, because the influent concentration was lower than for the others (12-13 g/l).

Stability of treatment using biomass Fig.7 shows the effect of the carriers biomass carrier 7E3A-pile on TOC removal. When HRT was varied from 0.7 d to 1.6 d at  $25^{\circ}$  (0-64 d), the TOC removal did not depend on the presence of the biomass carrier, because TOC removal rates with and without the biomass carrier were 35% for  $\theta$  = 1.5 d or 1.6 d and they were 23% for  $\theta = 0.7$ d or 0.8 d. Subsequently, without the biomass carrier, the temperature was varied from 5 $^\circ$ C to  $25^{\circ}$  at  $\theta = 1.7$  d (after 64 d). When the temperature was reduced to 5 $^{\circ}$ C from 25 $^{\circ}$ C, the TOC removal rate was decreased from 36% to 5% after four days. When the temperature was increased to  $10^{\circ}$ , the TOC removal rate increased to 30% after 14 days. In the case using E-pile1 as a biomass carrier (after 87 d, Fig. 3), when the temperature was reduced to 5 $^{\circ}$ C, the TOC removal rate decreased from 36% to 4% after 7 days. When the temperature was increased to  $10^{\circ}$ , the TOC removal rate increased to 30% after 4 days. This indicates that the rate of decrease in TOC removal after a drop in temperature can be reduced and more stable treatment is possible by using a biomass carrier.

Fig. 8 shows photographs of the reactor using E-pile1 at 5°C and 10°C. When the temperature was reduced to 5°C, the reactor effluent was clear, because the MLSS decreased to 0.54 g/l by yeast washout, but it was observed that yeast was attached to the E-pile1 carrier. When the temperature was increased to 10°C, the MLSS rose to 5.16 g/l







, 4E6A-pile (60% acryl)



Fig. 7 Effect of biomass carrier 7E3A-pile on TOC removal ○, influent TOC; △, effluent TOC; ●, TOC removal



Fig. 8 Photographs of the reactor using E-pile1 at 5  $^\circ\!\!C$  and 10  $^\circ\!\!C$ 

and the reactor effluent became turbid because of yeast growth. This suggests that the yeast could be retained in the reactor by the biomass carrier.

Yeast retention on biomass carriers The weights of yeast attached on E-pile (4 cm x 4 cm) and suspended yeast in the reactor at  $25^{\circ}$ C and  $5^{\circ}$ C are shown in Table 4. At  $25^{\circ}$ C, yeast grew well and the weights of attached yeast and suspended yeast were 70 and 69 mg, respectively. But at  $5^{\circ}$ C, the weights of attached yeast and suspended yeast were 3.3

and 1.4 mg, respectively. The microscope images of E-pile, solution in the reactor and washings from E-pile after this operation are shown in Fig. 9. The yeast were present in the solution held within the E-pile but were rarely attached on the fibers themselves. At  $5^{\circ}$ , the growth of yeast stopped and most of it was washed out of the reactor but yeast were still present in the washings from the E-pile carrier. It should be noted that the yeast in the E-pile is retained within the intra-fabric hold-up solution rather than by

Table 4	Quantity of	yeast attached on	E-pile	(4cm×4cm	) and	suspended	yeast
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	attached yeast	suspended yeast	attached yeast	suspended yeast
Operational condition				
HRT (d)	2	2.1	1	.6
Temperature (°C)	25	i	5	i i
TOC removal (%)	40 0		1	
Yeast weight				
in E-pile or 5.1 cm <sup>3</sup> -solution (mg)	70±12(n=5)	69	3.3 ±0.17(n=5)	1.4

As  $4\text{cm}\times4\text{cm}$  of E-pile could hold 5.1 cm<sup>3</sup> of water, the weight of suspended yeast was obtained from 5.1 cm<sup>3</sup> of solution in the reactor.



Carrier washed with water after the operation at  $25^{\circ}$ C. (×100 times)

the operation at 5℃



Carrier washed with water after the operation at  $5^{\circ}$ C. (× 400 times)



Fig. 9 Microscopic photographs of yeast in washed E-pile, solution in reactor and washing of E-pile

( × 100 times)

attaching on to the fabric itself. Therefore it is thought that a carrier of a high waterholding capacity is effective for yeast retention.

#### CONCLUSIONS

A pile fabric biomass carrier with a lower cost than Biofringe or BF-T9P was investigated.

E-pile, a polyester pile fabric, could be used as a biomass carrier for yeast, with TOC removal during operation found to be equal to that using the commercial biomass carrier BF-T9P. It was determined that the packing ratio of E-pile,  $0.17 \text{ m}^3$ -carrier/m<sup>3</sup>reactor, was sufficient to treat wastewater at VLRs under 8 kg-TOC/m<sup>3</sup>/d.

Because the yeast was retained by entrapment in the hold-up solution within the E-pile fabric, it is concluded that highly efficient biomass carriers for yeast will have high water hold-up volumes.

The biomass carrier system was stable with respect to temperature variation because, although the MLSS fell to 0.54 g/l at 5°C, it rose readily to 5.16 g/l at 10°C. This temperature stability is expected to be very useful for practical operation during winter.

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