VP	8	40	70	28	99	100	30	25
Indole test	95	100	98	100	0	0	0	0
Acid from								
Glucose	95	100	100	76	81	0	100	100
Maltose	77	55	35	99	85	100	82	59
Lactose	49	57	2	78	70	55	2	23
Sucrose	95	75	75	99	84	100	81	87
Xylose	95	29	70	92	44	68	54	61
Arabinose	93	35	29	95	45	100	17	65
Gas production in glucose	0	1	0	0	0	1	0	1
Fermention of								
glucose and lactose	96	57	71	3	62	23	11	82
H_2S production	0	0	0	0	0	0	0	0

1985). The mean bacterial volume from samples in water was found to be $3.19 \pm 0.59 \times 10^{-2} - 6.19 \pm 0.76 \times 10^{-2}$ μm^3 for cocci, whereas $4.57 \pm 0.17 \times 10^{-2} - 12.94 \pm 0.21 \times 10^{-2}$ μm^3 for rods (Table 1). Such average biovolume values for esturine and coastal waters in Yellow Sea were almost corresponded with those of other investigators (Zimmermann, 1975, 1977; Ferguson and Rublee, 1976). It seemed that average biovolume showed also some seasonal fluctuation. However, we cannot explain why it showed seasonal fluctuation. It may reflect complex nutritional and physico-chemical variations within the surveying area. In addition to estimating the bacterial volumes, we have equated the data to biomass. Total biomass of investigating area amounted to $5.1 \times 10^4 \mu m^3/ml$ - $17.8 \times 10^4 \mu m^3/ml$ for cocci and $38 \times 10^4 \mu m^3/ml$ - $406.6 \times 10^4 \mu m^3/ml$ for rods (Table 1). Maximum values of total biomass appeared during spring (April) and summer (July), whereas minimum values occurred in fall (October) and winter (December). Beacterial total biomass fluctuated monthly to almost a small extent (except April and July). Some of the characteristics of the isolated becteria are summerized in Table 2. Fifty eight to 83.3% of isolates obtained from waters and 30.9 -89.4% from sediments were gram negative. A high percentage of isolates collected from water sample were rods during August and October, however, isolates from sediment were almost cocci during surveying periods except May. A greater percentage of isolates both waters and sediments were mesophiles and could grow at 20 -42°C. Most isolates also showed tolerance at broad range of salinity and showed various utilization of carbon sources such as glucose, maltose, lactose, xylse and arabinose. The analysis of 1,240 bacteria in waters and 1,328 bacteria in sediments which were isolated during surveying periods yielded in 33 groups in waters and 35 groups in sediments (Figs. 3,4). The diversity of these groups isolated both waters and sediments indicate high similarities between the populations of different season.

적 요

서해 군산 인근해역에서 조간대와 퇴적토를 대상으로 해양 종속영양세균의 년중 분포와 계절별 특성에 대하여 조사하였다. 해양 종속영양세균의 년간 분포는 해수에서는 $7.5 \times 10^2 - 1.1 \times 10^5$ c.f.u./ml 범주에서 변화하였으며 퇴적토에서는 $1.6 \times 10^4 - 4.8 \times 10^6$ c.f.u./g dry sediment 범주에서 변화하였다. 형광현미경에 의한 형태학적 분포를 측정한 결과 전 조사기간 중 간균이 74% 이상을 차지하였다. 또한 조사된 세균의 평균 생체량은 구균의 경우 $3.19 \pm 0.59 \times 10^{-2} - 6.19 \pm 0.76 \times 10^{-2}$ μm^3였으며, 간균은 $4.57 \pm 0.17 \times 10^{-2} - 12.94 \pm 0.21 \times 10^{-2}$ μm^3이었다. 동정된 균들은 glucose, maltose, lactose, xylose, arabinose를 탄소원으로 이용하였고 다양한 농도의 염분에 내성이 있었다. 해수에서는 82속이, 퇴적토에서는 114속이 동정되었고 우점속은 해수에서는 *Pseudomonas, vibrio, Flavobacterium*과 *Acinetobacter*이였고, 퇴적토에서는 *Pseudomonas, Acinetodacter, Vibrio, Mycobacterium*이었다.

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Filamentous Bacteria Found in Rotating Biological Contactors Treating Domestic Wastewater

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생활하수를 처리하는 회전원판체에서 발견된 사상세균

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ABSTRACT: Slime sampless from 10 rotating biological contactor (RBC) plants in New Jersey were microscopically examined. Filamentous bacteria such as Type 1701, Type 0041, Type 021N, *Nocardia*, *Beggiatoa*, and *Sphaerotilus*, which are commonly present as suspended forms also were found in RBC slimes growing as attached forms. However, the abundance was much different from that of activated sludge. In RBC slimes, *Beggiatoa* was most frequently observed filamentous bacteria and *Sphaerotilus*, Type 0041, Type 1701, Type 021N and *Nocardia* were present in decreasing order of abundance. There were morphologically two different types of *Beggiatoa* in RBC slimes. The statistical anaysis shows that filamentous bacterial poulations between the 100 cm inside and the outside the RBC were different in most cases with significant interactions between the location and stage.

KEY WORDS □ Filamentous Bacteria, Rotating Biological Contactor (RBC), *Beggiatoa*

Investigation of filamentous bacteria in activated sludge has been fairly extensive due to their association with bulking. Meanwhile, the study of filamentous bacteria in the Rotating Biological Contactor (RBC) is scanty and most of microbiological data is included in engineering papers as supplementary information.

According to the current research, *Begiatoa* and *Sphaerotilus* are the most frequently noted filamentous bacteria (Pretorius, 1971; Pescod and Nair, 1972; Antonie and Welch, 1969; Ouyang, 1980). They are found in the early stages of RBC's or throughout the entire proces (Pescod and Nair, 1972). *Nocardia* (Hoag et al., 1980), filamentous bacillae (Antonie and Welch, 1969) and *Streptomyces* (Phaup and Gannon, 1967) were also found with less frequency.

The purpose of this study was to identify filamentous bacteria in the RBC slimes using the methods developed for the identification of filamentous bacteria in activated sludge and to do a statistical analysis on the microorganism present in the RBC.

MATERIALS AND METHODS

RBC slime samples were taken from 10 RBC plants in New Jersey. The samples were scraped off to the bottommost layer from the surfaces and from c.a. 100 cm inside the biodisc with a long, thin scoop. The slimes then were placed in plastic bottles. After the slime settled to the bottom, the supernatant was decanted and the slimes were mildly mixed with a spatula to homogenize the sample. Vigorous mixing was avoided as not to break filamentous forms inside the slimes. Two m/ of the homogenized slime was pipetted into a plastic bottle with a wide-bore pipette and 2 ml of tap water was added and again mildly mixed with a spatula. Then, the mixed slurry was mounted and uniformly spread to make a thin layer over the slides for microscopic observation. To deter-

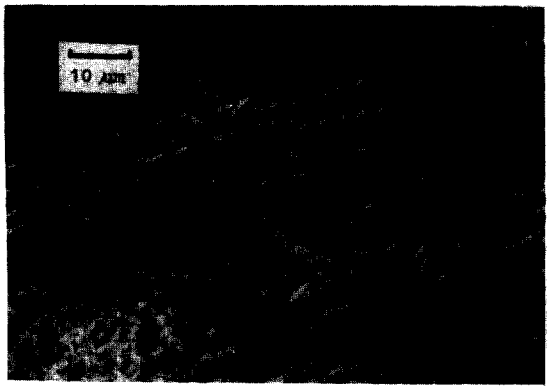

Fig. 1. *Sphaerotilus in the RBC Slime, 1000X phase contrast*

Fig. 2. *Type 0041 in the RBC Slime, 1000X phase contrast*

Fig. 3. *Type 1701 in the RBC Slime, 1000X phase contrast*

mine the relative abundance, 10 microscopic fields were randomly chosen and microorganisms counted. A magnification of 1000X was used for filamentous bacteria and algae, while, 100 magnification was used for protozoa and metazoa. Field diameter was 1950 μm at 100X and 195 μm at 1000X.

For the activated sludge samples, mixed liquor samples were taken form the aeration basin. One drop of the mixed liquor sample was mounted on the slide after vigorous shaking.

For the trickling filter sample, settled sludge from the secondary settling basin was taken in a bottle. One drop of the settled sludge was mounted after decanting the supernatant.

Filamentous organisms were identified using the microscopic method of Eikelboom (1975) and Eikelboom and van Buijsen (1981) as modified by Strom and Jenkins (1984). the presence of sulfur granules was tested by the method shown by Farquhar and Boyle (1971). *Sphaerotilus* enrichment and isolation was performed by the methods of Dondero *et al* (1961).

Protozoa were identified to the Genus by the key presented by Jahn and Jahn (1949) and Lee *et al* (1985).

Factorial design was used for the statistical analysis by the methods given by Hicks (1982). Statistical calculation was done by using the computor package SAS/9000.

RESULTS

Types found

Table 1 summarizes the filamentous bacteria found in the RBC slimes. Seven types of filamentous bacteria were found. Among these, *Beggiatoa* was the most abundant, with 9 of 10 plants showing its growth. *Sphaerotilus* was the second most abundant with 7 plants having it (Fig. 1). Type 0041 was found in 4 plants (Fig. 2). Type 1701 (Fig. 3), *Nocardia* (Fig. 4) and Type 021N (Fig. 5) were also found (2 plants each). One unidentified type (Fig.6) was observed (1 plant).

Beggiatoa, a gliding filamentous bacteria, was divided into 2 types by morphological differences (Fig. 2). *Beggiatoa* I is larger than *Beggiatoa* II in diameter and extremely packed with bright granules. Its diameter is 1.8 μm-2 μm and trichome length is 50-1000 μm. It contains sulfur granules and numerous large PHB granules. PHB granules are not melted by ethanol and the shape is irregular polygon. It is stained to bright green color by Neisser staining. Cross wall is normally not visible except incapacitated or degrading forms. Individual cell size is 1.8 μm-2.0 μm. Normally this is gram negative, however, it may be gram positive if it contains extremelv

Table 1. *Filamentous Bacteria in RBC Slimes*

Dec. 1984

Organism+	Location	Plant																								
		E.W.				H.T.							L.T.				N.B.**						S.H.			
		1*	2	3	4	1	2	3	4	5	6	7	1	2	3	4	1a	1b	1c	2a	2b	2c	1	2	3	4
Beggiatoa I	Inside	0	0	0	0	0	0	0.6	0	1.8	3.0	1.6	4.4	3.9	3.7	4.5	7.1	7.0	6.7	1.5	1.3	0.7	1.7	3.1	4.5	1.2
	Outside	0	0	0	0	0	0	3.1	0	2.1	1.4	3.9	7.9	3.9	3.7	6.9	8.1	8.2	6.9	1.5	0.8	1.1	1.6	3.0	4.7	3.1
Beggiatoa II	Inside	0	0.1	0	0	1.6	3.0	3.2	9.3	7.3	3.4	2.5	7.3	6.6	7.3	4.5	0.7	0.6	0	0	0	0	0.5	0	0	0.5
	Outside	0.2	0.1	0	0	1.0	2.0	4.6	9.4	6.8	5.6	7.4	7.3	7.2	7.3	6.9	0.6	0.6	0.7	0	0	0	0.5	0	0	0
Sphaerotilus	Inside	5.2	1.1	0.8	5.8	0	0	0	0	0	0	0	0	0	0	2.2	7.3	0.8	0.7	9.5	6.9	6.9	0.6	0	0	0
	Outside	2.9	1.0	0.9	2.7	0	0	0	0	0	0	0	0	0	1.8	0	0	0.6	0.7	6.6	9.7	7.0	0.6	0	0	0
Type 1701	Inside	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.4	0.7	9.3	6.6	6.8	1.4	0	0	0.5
	Outside	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5	0	0	1.7	3.1	4.5	1.2
	Outside	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.6	3.0	4.7	3.1
Type 021N	Inside	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.2	0	0.3	0	0	0	0	0	0	0	0
	Outside	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nocardia	Inside	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.4	0	0	0	0	0	0	0	0	0	0
	Outside	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.3	0	0	0	0	0	0	0	0	0	0
Unidentified	Inside	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	10	0
(bead-shaped)	Outside	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	10	0

Table 1. *(Continued)*

Organism+	Plants														
	W.T.@				V.H.@				S.O.@				C.H.@		S.R.@
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	1
Beggiaton I	0	0	0.6	1.1	6.9	6.4	9.7	7.3	0	0	0	0	0	0	0
Beggiatoa II	3.1	4.4	0.6	1.2	9.8	9.0	7.2	4.9	1.8	1.6	1.5	1.6	0	0	0.6
Sphaerotilus	1.2	3.9	9.8	11.0	0	0	0.8	1.1	0	0	0	0	0.6	0	7.7
Type 0041	0	0	0	0	0	0	0	0	1.8	1.6	1.9	1.8	3.5	3.7	0
Nocardia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.3

*Stage

**This plant has 2 stages with wastewater flow parallel to the shaft.

The slime samples were taken from 3 points for each stage. Point a is on the influent side, point b is at the midpoint, and point c is on the effluent side.

@ No inside samples collected.

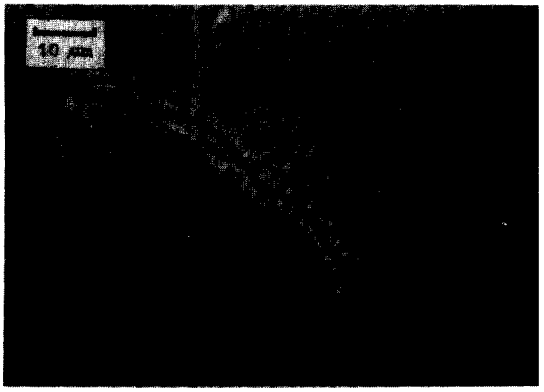

Fig. 4. *Nocardia in the RBC Slime, 1000X phase contrast*

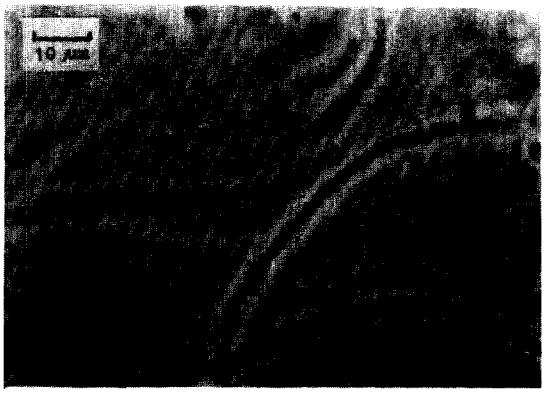

Fig. 6. *Unidentified Filamentous Bacteria in the RBC Slime, 1000X phase contrast*

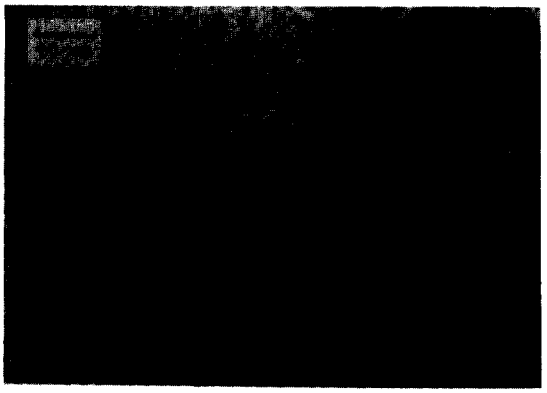

Fig. 5. *Type 021N in the RBC Slime, 1000X phase contrast*

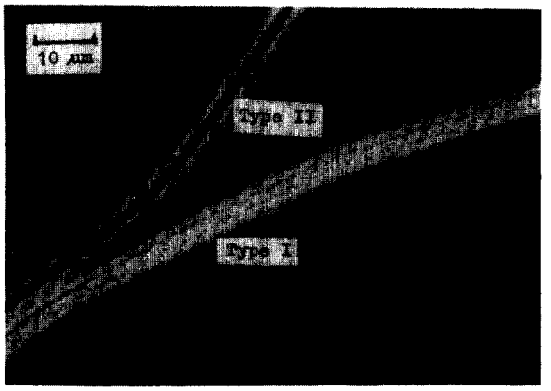

Fig. 7. *Beggiatoa Type I and II in the RBC Slime, 1000X phase contrast*

high amounts of PHB granules. This type is also Neisser negative.

Sphaerotilus has some outstanding morphological characteristics; long chains of rod shaped cells, the presence of sheath and false branching. *Sphaerotilus* found in the RBC slimes in this study had long chains of rod shaped cells and sheath. However, false branching was not observed. To confirm the Genus, isolation was made from the RBC slimes.

The slime samples from L.T. plant and N.B. plant which have the suspected *Sphaerotilus* in their slimes (determined by microscopic observation) were planted on the Dondero's isolation media. The slime sample which has no observed *Sphaerotilus* was also plated for control. The L.T. and N.B. plant slime samples produced 10-20 suspected filamentous colonies on the first inoculation while the H.T. plant sample produced only one suspected colony. The pure cultures obtained from those 3 plant samples by the second inoculation showed basically typical morphology of *Sphaerotilus* including the characteristics of false branching. There were also some slight morphlogical differences betwen the type isolated and the type normally present in activated sludge. It had shorter trichome length than in the activated sludge, ranging 20 μm-150 μm, the false branching was not as prolific as the type in the activated sludge, and trichomes were circuitous rather than slightly bent.

Type 0041, Type 1701, *Nocardia* and Type 021N showed the identical morphological characteristics as the types found in the activated sludge.

The unidentified type (bead-shaped) (Fig. 6) is a long chain of cocci. The individual cell is a sphere which diameter is 1.5 μm-2.0 μm. The trichome color is opaque and 50-300 μm long. It is not motile. The diameter of cocci become slightly larger in the basal part. At the base, the cocci become a larger, elongated ellipse or peanut-shape cells with the cell size of 2.5 μm-3.5 μm $\times$ 3.5 μm-7.0 μm. It contains some granules. It is gram positive and Neisser negative.

Table 2. *Factorial Design Analysis of Variance for Sphaerotilus at the E.W. Plant*

Source	df	Sum of Squares	Mean Square	F Value	Significance Level
Total	79	741.80	—	—	—
Location (inside vs outside)	1	36.45	36.45	5.69	0.0197*
Stage	3	205.60	68.53	10.69	0.0001*
Interaction (Lo. × S)	3	38.15	12.72	1.98	0.1225
Error	72	461.60	6.41	—	—

*Statistically significant at 5% level

The difference between outside and inside population

To test whether the population in the outside slimes and the population in the inside (100 cm deep) slimes are different, the microscopic count was performed both for the outside and the inside slimes for 5 plants. A factorial design methods was employed to test the statistical difference. One sample calculation for the *Sphaerotilus* population in the E.W. plant was shown in Table 2. The inside and outside populations of *Beggiatoa* and *Sphaerotilus* were statistically different at 5% level except the N.B. plant. There were also significant interactions between the location (outside vs. inside) and the stages. In the case of Types 0041 and 1701, the outside and inside population were not statistically different between the stages and there were some interactions between them

Table 3. *Factorial Design Analysis of Variance for Effect of Samplling Location and Stage on Filamentious Bacteria Abundance*
(Probability value of significance level)

Orgainsm	Source	Plant (No. of Stages) E.W.* (4)	H.T. (7)	L.T. (4)	L.B. (6)**	S.H. (4)
Beggiatoa I	Location (inside/ outside)		0.0002*	0.0001*	0.1903	0.0842
	Stage		0.0001*	0.0001*	0.0001*	0.0001*
	Interaction (L. × S)		0.0001*	0.0005*	0.5601	0.4813
Beggiatoa II	Location		0.0004*	0.5230	0.0001*	0.0004*
	Stage		0.0001*	0.0001*	0.0001*	0.0001*
	Interaction		0.0001*	0.3801	0.0001*	0.0001*
Sphaerotilus	Location	0.0197*		0.4280	0.0027*	
	Stage	0.0001*		0.0001*	0.0001*	
	Interaction	0.1225		0.3462	0.0001*	
Type 1701	Location				0.1029	0.6512
	Stage				0.0001*	0.0001*
	Interaction				0.0001*	0.4914
Type 0041	Location					0.0907
	Stage					0.0001*
	Interaction					
Nocardia	Location			0.0001*		
	Stage			0.0001*		
	Interaction			0.0001*		

* Values less than 0.05 indicate statistical significance at 5% level.

** N.B. plant has 2 stages with wastewater flow parallel to the shaft. By dividing each stage into 3 equal lengths for sampling purposes, the 2 stages were considered to be 6 stages.

Table 4. *Microorganisms Found in the H.T. Plant RBC and Trickling Filter Receiving the Same Influent*

Organism*	Process: RBC (average for all stages)	Process: Trickling Filter (rock scrapings and settled sludge)
Beggiatoa	4.9	2.6
Bodo	0.6	0
Paramecium	2.0	0.3
Aspidisca	0.6	0
Vorticella	0.2	0
Epistylis	3.0	0
Opercularia	8.2	0
Fungi	0	some
Nematodes	1.0	0.2
Rotifers	0.3	0
Remarks		Filamentous green algae, filamentous fungi, and fly larvae were amply present on the rocks (down to 15 cm depth)

* Average number per field for 10 fields at 1000X for filamentous bacteria and 100X for protozoa and metazoa

(Table 3).

To test the sampling variability in the sampling, 2 subsamples were taken from the sampling bottles containing the slimes of 2 plants (N.B., H.T.). The variability of the numbers of the microorganisms present were statistically tested (ANOVA). The results showed that the 2 subsamples from all the stages in the 2 plants tested were not statistically different.

Comparison of the RBC fauna with the activated sludge and trickling filter fauna

Comparison of the microfauna was performed between the RBC system and the activated sludge system for 2 plants which have both systems in their plants receiving the same influent. The results are shown in Table 4. For the filamentous bacteria, Types 1701, 0041 and *Nocardia* were common in the activated sludge, while *Beggiatoa* and *Sphaerotilus* were common in the RBC. Due to the different growth pattern between the 2 processes (attached vs suspended growth) and diffrent dilution factor, the further quantitative comparison was not performed.

The RBC and the trickling filter biota were compared in the H.T. plant which has both processes receiving the same influent. The results are shown in Table 5. In both processes, *Beggiatoa was the most* dominant bacteria. Fungi were present in the trickling filter only. Generally, the biota of the 2 processes appeared to be more similar than that of between the activated sludge and RBC process.

Table 5. *Microorganisms Found in V.H. and S.H. RBC and Activated Sludge Plants*

Organism @	Plant: V.H. Activated Sludge Summer (July, 83)	V.H. Activated Sludge Winter (Dec. 84)	V.H. RBC* Summer (July, '83)	V.H. RBC* Winter (Dec, '84)	S.R. Activated Sludge Summer (July, '84)	S.R. RBC
Beggiatoa (Type I + II)	0	0.5	abundant	15.3	0	0.6
Sphaerotilus	0	0	some	0	0	7.7
Type 1701	0	0	some	0	moderate	0
Type 0041	few	3.0	0	0	0	0
Nocardia	some	0.6	0	0	some	0.3
Bodo	N.I.**	0.2	N.I.**	12.83	abundant	1.1
Stylonychia	"	1.3	"	0	0	0
Vorticella	"	0	"	0	0	0.8
Epistylis	"	3.2	"	17.8	moderate	0
Opercularia	abundant	0	"	36.7	0	0
Nematodes	N.I.**	0.2	"	3.3	0	0
Rotifers	"	0.6	"	2.28	0	0.3
Annelid Worms	"	0	"	0.25	0	0

*4 Stage Average. **Not Investigated.

°Average number per field for 10 fields at 1000X for filamentous bacteria and 100X for protozoa metazoa.

DISCUSSION

The results show that quite a few types of filamentous bacteria which exist in the activated sludge are also found in the RBC. This implies that the filamentous bacteria which grow in suspended forms in the activated sludge can grow also in attached forms in the RBC.

However, the abundance of filamentous bacteria in the 2 systems are different. In the activated sludge, Type 1701, Type 021N, *Sphaerotilus,* Type 0041 and *Nocardia* are common filaments and *Beggiatoa* belong to minor type (Strom and Jenkins, 1984), while it is a most common type in the RBC and Type 1701, Type 021N, Type 0041 and *Nocardia* are minor organisms. Presumably, this difference comes from the different microenvironment created by the 2 different wastewater systems.

Principally, the classification of *Beggiatoa* is solely based on the diameter of trichomes (Buchanan and Gibbons, 1974). According to this method, both the *Beggiatoa* I and *Beggiatoa* II in this study belong to *Beggiatoa leptomitiformis.* However, they could form different supspecies or strains since their morphological differences are outstanding and the physiological difference (deposition of PHB granules) is also salient, usually coexisting in the same slimes.

False branching of the *Sphaerotilus* in the slimes and the pure cultures from the isolation in our study was absent or scarce. It appears that the false branching can be amply formed when it grows in the suspended form and becomes scarce when grown in the attached form. This is because the space for the filamentous organism becomes smaller as the slime grows thicker or it may comprise a new supspecies or new strain.

The population between the inside and outside slimes of *Beggiatoa* and *Sphaerotilus* were statistically significantly different with important interactions between the location and the stage. This is rather natural for all organisms. Organisms choose their own niche, which is the sum total of the complex factors such as organic load, DO, temperature, nutrient composition etc. Assuming that organic load, temperature, and nutrient composition are same both for the inside and outside the RBC surface, dissolved oxygen(DO) might be the factor that make the difference. Even in the same stage, DO can be slightly lower inside than outside since the volume of diffusable air exposed per unit area inside is less than outside.

The following conclusions can be drawn based upon the study of the 10 RBC plants in New Jersey:

(1) Filamentous bacteria such as Type 1701, Type 0041, type 021N, *Nocardia, Beggiatoa* and *Sphaerotilus,* which are present as suspended forms in activated sludge also grew as attached forms in RBC slimes. However, the abundance was different from that of activated sludge. Here, *Beggiatoa, Sphaerotilus,* Type 0041, Type 1701, Type 021N, and *Nocardia* were present in decreasing order of abundance.

(2) For the *Beggiatoa* which was common in RBC slimes, two morphologically different types were found.

(3) The false branching of *Sphaerotilus* in the RBC slimes was scarce, and is speculated to be an ecological type when it grows as attached forms.

(4) The statistical analysis shows that the population between the inside and the outside the RBC were different with significant interactions between the location and stage.

적 요

미국 뉴저지주에 있는 10개의 회전원판 처리장에서 생물막 시료를 채취하여 사상세균에 대해 현미경적 연구를 실시하였다. 활성오니에서 부유형 성장을 하며 흔히 출현하는 Type 1701, Type 0041, Tpe 021N, *Nocardia, Beggiatoa* 및 *Sphaerotilus*가 회전원판체 생물막에서도 출현하였으나 수도(abundance)는 크게 달랐다. 여기서는 *Beggiatoa*의 출현빈도가 가장 높았으며 *Sphaerotilus,* Type 0041, Type 1701, Type 021N, *Nocardia*의 순이었다. 회전원판 생물막에서 발견된 *Beggiatoa*는 형태적으로 뚜렷히 구별되는 2가지 Type이 존재하였다. 회전원판체의 중심축으로 100 cm 깊이에 존재하는 생물막과 외부의 생물막에 존재하는 사상세균의 군집에 대해 인자분석법에 의한 통계처리를 수행한 결과 대부분의 경우, 내부의 군집과 외부의 군집이 통계적으로 유의하게 달랐으며 장소(내부, 외부)와 단(stage) 사이에도 많은 경우 통계적으로 유의한 상호작용이 있었다.

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D-xylose 발효효모의 분리 및 성질

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Isolation and Properties of D-xylose Fermenting Yeast

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ABSTRACT: In order to ferment D-xylose directly to ethanol, Yeasts capable of utilizing D-xylose as a sole carbon source and energy source were isolated from soil, sawdust and rotten woods. Among them, the yeast strain, which showed the best ability to produce ethanol, was identified as *Candida* sp. L-16 isolated from rotten woods. The optimal conditions for production of ethanol were 60rpm of agitation speed, 28°C of temperature, 4.5 of initial pH and 5% of D-xylose concentration. Ethanol production was reached to maximum state for 4 days culture. Under these optimal conditions, the maximum ethanol concentration and theoretical ethanol yield were 2.4%(v/v) and 74.4% of theoretical value, respectively.

KEY WORDS D-xylose, *Candida* sp. L-16, theoretical ethanol yield

5탄당인 D-xylose는 재생 biomass의 약 30%를 구성하는 hemicellulose의 주성분으로서 목재와 농산물 폐기물로 부터 얻어지는 재생 biomass에 존재하는 당질 가운데서 glucose 다음으로 풍부하며 5탄당 중에서는 가장 풍부한 당질이다. D-xylose는 묽은산이나 효소에 의해 가수분해되어 얻어질 수 있으며, 특히 묽은산에 의한 선택적 가수분해 (Lee 등, 1978)와 같은 방법으로 비교적 쉽게 hardwood로 부터 고수율로 회수될 수 있는 당질이다.

D-xylose는 재생 biomass로 부터 얻어질 수 있는 풍부한 탄소원이지만 glucose와 비교해서 *Saccharomyces*속과 같은 종래의 ethanol 발효효모로서는 aldopentose인 D-xylose로 부터는 직접 ethanol 생산이 어렵기 때문에 발효기질로서는 적당하지 못한 것으로 인식되어 왔었다. 그러나 근래에 와서 *Pachysolen tannophillus* (Schneider 등, 1981 ; Slininger 등, 1982 ; Dekker, 1982 ; Maleszka 등, 1982 ; Deverell, 1983 ; Jefferies, 1984 ; Mütze 등, 1985), *Candida* sp.(Jefferies, 1981 ; Cong 등, 1981 a, b ; Alexander 등, 1987), *Phichia* sp. (Dellweg 등, Linko 등, 1986 ; Tran과 Chambers, 1986), *Kluyveromyces* sp. (Magaritis와 Bajpai, 1982 ; Marikawa 등, 1985) 및 *Clavispora* sp. (Nigam 등, 1985)의 일부효모균주와 *Mucor* sp. (Ueng과 Cong, 1982)과 *Fusarium* sp. (Suihko와 Enari, 1981 ; 이 등, 1987)과 같은 곰팡이를 이용한 alcohol 발효에 대한 연구가 활발히 진행되고있는 실정이다.

본 연구는 재생 biomass에 풍부한 탄소원인 D-xylose를 자화 발효하여 직접 ethanol을 생산할 목적으로 삼림, 제재소 및 오래된 사찰을 중심으로 하여

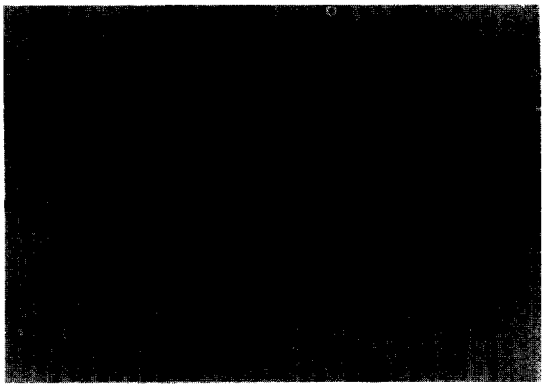

Fig. 1. *Photograph of Candida sp. L-16.*

토양, 썩은 톱밥 및 썩은 나무들로부터 D-xylose를 자화 발효하는 효모균주들 가운데서 오래된 사찰 주위의 썩은 밤나무 그루터기로 부터 ethanol 생산력이 가장 우수한 효모균주를 분리 선별하고 균주의 동정과 ethanol 생산시의 최적조건에 대한 연구를 행하였다.

재료 및 방법

사용균주

삼림, 제재소 및 오래된 사찰을 중심으로하는 토양, 톱밥 및 썩은 나무들로 부터 D-xylose 자화능을 가진 150여 효모균주들 가운데서 경북 달성군 옥포면 용연사 주위의 산에 있는 썩은 밤나무 그루터기에서 D-xylose로 부터 ethanol 생산성이 가장 우수한 균주인 L-16을 분리, 선별하여 본 실험의 공시균주로 사용하였다.

균주의 동정 방법

효모균주의 형태적, 생리적 및 배양상의 특성을 조사하여 Lodder씨(1970)와 Kregervan Rij씨(1984)의 분류 기준에 준하여 동정하였다.

배지 및 배양 방법

전배양은 2% D-xylose, 0.3% yeast extract 0.3% maltextract 및 0.5% bacto peptone(pH 5.0)의 조성을 지닌 배지 5 ml를 시험관(2×15 cm)에 분주하고 121℃에서 15분간 가압살균한 후 공시균주를 접종하여 30℃에서 24시간 진탕(120 strokes/min, amplitude 5 cm) 배양하여 종균으로 사용하였다.

Ethanol 생산력을 조사하기 위한 본 배양은 5% D-xylose, 0.3% yeast extract, 0.3% malt extract 및 0.5% bacto peptone(pH 5.0)의 조성을 지닌 배지를 100 ml용 삼각플라스크에 40 ml씩 분주하고 121℃에서 15분간 고압 살균한 후 상기 전 배양액 1.6 ml를 접종하고, semi aerobic상태로하여 28℃에서 회전진탕 배양기로 진탕배양 하였다.

Table 1. *Morphological and cultural characteristics of the isolated strain L-16*

Shape of vegetative cells	oval
Cell size (μm)	1.8-2.8
Vegetative reproduction	budding
Ascospore	absent
Pseudomycellium	present
True-mycellium	absent
Culture in YM media	
Pellicle	absent
Ring	present
Growth on YM agar	
Form	regular
Edge	entire
Elevation	raised
Surface	smooth
Color	white creamy

분석 방법

발효액내의 잔당(D-xylose)은 DNS법(Summer, 1925)으로 측정하였고 균수측정은 혈구계수측정기를 사용하여 현미경 하에서 계측(本村輝正, 1969)하였으며, 발효액내의 Ethanol 함량은 Rapid oxidation method(Amerine 등, 1980)로 정량하였다.

결과 및 고찰

분리균의 동정

썩은 밤나무 그루터기로 부터 분리된 D-xylose 발효효모 L-16의 형태적, 생리적 및 배양상의 특성을 기초로하여 Lodder씨(1970)와 Kregervan Rij씨(1984)의 분류기준에 준하여 동정한 결과 Fig. 1과 Table 1, 2, 3에서 나타난 바와 같이 L-16 효모균주는 *Candida* sp.으로 동정되었다.

Ethanol 생산을 위한 배양조건의 검토

Ethanol 생산에 미치는 aeration의 영향

본 공시균의 Ethanol 생산에 미치는 aeration의 영향을 검토하기 위하여 100 ml용 삼각 플라스크에 본 배양액을 40 ml씩 분주하고 멸균 후 30℃에서 24시간 진탕 배양한 전 배양액 1.6 ml를 접종하고 회전진탕속도를 40, 60, 80, 및 100 rpm으로 각각 달리하여 초기 pH 5.0, D-xylose농도 5% 및 배양온도 28℃에서 배양액 내의 ethanol 농도가 최대가 될 때까지 배양한 결과는 Table 4와 같다.

Table 4에서 보는 바와 같이 최대 알코올 농도는 진탕속도가 60 rpm이고, 배양 4일째 이루어졌으며

Table 2. *Fermentation and assimilation of carbon compounds by the strain L-16*

Fermention			
D-Glucose	+	Soluble starch	−
D-Galactose	+	Inositol	−
Maltose	+	Sorbitol	−
Sucrose	−	Glycerol	−
Cellobiose	−	Raffinose	−
Mannose	+	Melibiose	−
Lactose	−	D-Xylose	+
Inulin	−	L-Arabinose	−
Assimilation			
D-Glucose	+	Ducitol	−
D-Galactose	+	D-Sorbitol	+
Maltose	+	Salicine	−
Sucroce	+	Succinic acid	−
Lactose	−	Citric acid	−
Starch	+	D-Ribose	+
D-Xylose	+	Melibiose	−
L-Arabinose	−	Raffinose	−
Ethanol	+	Rhamnose	+
Adonitol	+	Manitol	+
Glycine	−		

(+; positive, −; negative)

Table 3. *Physiological characteristics of the strain L-16*

Potassium nitrate assimilation	−
Growth in vitamin free medium	−
Growth at 37 ℃	w
Gelatin liquefaction	−
Growth in NaCl free medium (NaCl 0-8%)	+
Acid production	−
Urea hydrolysis	−
Pigment formation	−
Production of extracellular amyloid compounds	−

ethanol 생산량은 18.2g/*l*, ethanol 수율은 0.36g/g이었다. 진탕속도가 빠를 수록 발효시간은 단축되나 ethanol 생산량과 ethanol 수율은 점차 떨어지고 진탕속도가 60 rpm보다 적을 때는 발효시간이 더 연장됨은 물론 ethanol 생산량과 수율도 떨어지는 경향을

Table 4. *Effect of aeration on the production of ethanol by Candida sp. L-16*

Rate of shaking	Fermentation time(day)	Final ethanol		Maximum ethanol yield	
		v/v%	g/L	g/g	%
40 rpm	6	2.18	17.2	0.34	67.5
60 rpm	4	2.30	18.2	0.36	71.3
80 rpm	4	2.25	17.8	0.35	69.7
100 rpm	3	2.11	16.7	0.33	65.4

1. Ethanol yield (g/g) $= \frac{\text{Final ethanol concentration (g/L)}}{\text{Initial D-xylose concentration (g/L)}}$

2. Ethanol yield (%) $= \frac{\text{Final ethanol concentration (g/L)} \times 100}{\text{Initial D-xylose concentration (g/L)} \times 0.51}$

The strain cultured at 28 ℃ and wit shaking.
The inintial pH was 5.0 and the initial D-xylose concentration was 50 g/L.

Table 5. *Effect of temperature on the production of ethanol by Candida sp. L-16.*

Temperature	Fermentation time(day)	Final ethanol		Maximum ethanol yield	
		v/v%	g/L	g/g	%
25 ℃	7	2.04	16.1	0.32	63.1
28 ℃	4	2.30	18.2	0.36	71.3
30 ℃	4	2.28	18.0	0.36	70.6
33 ℃	4	1.89	14.9	0.30	58.6

The strain cultured on a rotary shaker at 60 strokes/min.
The initial pH was 5.0

보였다. 이와 같은 경향에 미루어 보아 ethanol 생산은 산소 공급과 밀접한 관계가 있으며 산소 공급이 균체 증식과 D-xylose 소비를 촉진하나 ethanol 생산량을 감소 시키는 것으로 생각되었다. 그리고, 산소 공급이 지나치게 제한된 경우는 균체 증식과 D-xylose 소비가 억제되며, xylitol 생산이 증가하여 ethanol 생산 역시 감소되는 경향이 있으므로 본 공시 균주는 산소 공급에 의해 ethanol 생산이 크게 조절받는 것으로 사료되었다. Maleszka와 Schneider(1982)는 *Paschysolen tannophilus*에서 산소 공급이 증가 할 수록 ethanol 수율이 떨어지는 이유는 D-xylose 존재 하에서 ethanol을 소비하기 때문이라고 하였으며, Jefferis (1984)는 혐기적 조건 하에서 ethanol 수율이 떨어지는 것은 xylitol 생산이 증가하기 때문이라고 하였다.

배양 온도의 영향

Ethanol 생산에 미치는 배양온도의 영향을 조사하기 위하여 초기 pH 5.0, 진탕속도 60 rpm 및 D-xylose

Table 6. *Effect of pH on the production of ethanol by Candida sp. L-16*

pH	Fermentation time(day)	Final ethanol		Maximum ethanol yield	
		v/v%	g/L	g/g	%
3.0	6	1.02	8.0	0.16	31.5
3.5	5	1.83	14.5	0.29	56.7
4.0	4	2.11	16.7	0.33	65.4
4.5	4	2.40	19.0	0.38	74.4
5.0	4	2.30	18.2	0.36	71.3
5.5	4	1.89	14.9	0.30	58.6
6.0	4	1.82	14.4	0.29	56.4

농도 5%로 하여 배양 온도를 25, 28, 30 및 33℃로 각각 조정하고 진탕 배양하여 ethanol 생산량을 비교 검토한 결과는 Table 5와 같다.

Table 5에서 나타난 바와 같이 ethanol 생산은 28℃에서 ethanol 수율이 0.36 g/g으로 가장 양호 하였으며, 배양온도가 증가 함에 따라 발효시간은 별로 단축되지 않았으며 ethanol 생산량은 감소되어 33℃에서는 ethanol 수율이 0.299 g/g으로 급격히 감소되어졌다. 이결과는 du Preez 등 (1986)이 *Candida shehate*를 이용한 *ethanol* 생산에서 *28*℃에서 ethanol 생산은 최대가 되었으며 이온도 이상에서는 ethanol 생산성이 크게 떨어졌으며, *Phichia stipitis*에서는 30℃에서 최대가 되었으며 이 온도 이상에서는 ethanol 생산이 다소 떨어졌다는 것은 속이 다르다는 점을 감안하여 본 연구와 어느 정도 일치하는 경향을 보이는 것으로 생각되어진다. 그러나 25℃에서는 알코올 생산량이 다소 떨어지고 발효시간이 연장되는 것으로 보아 본 공시 균주는 25℃ 이하에서 균체증식과 D-xylose 소비가 억제되어 ethanol 생산도 감소되는 경향을 보였다. 이와 같은 결과로 미루어 보아 본 공시 균주는 ethanol 생산에 있어서 온도에 대단히 민감한 영향을 받는 것으로 생각되어진다.

pH의 영향

ethanol 생산에 미치는 초기 pH의 영향을 검토하기 위하여 기본 배양액의 pH를 3.0에서 6.0까지 0.5간격으로 조절하여 ethanol 수율을 비교 연구한 결과는 Table 6과 같이 최대 ethanol 농도는 2.4 %(v/v), ethanol 수율은 0.38 g/g이었다. 그리고 이 보다 낮은 산성 영역에서는 ethanol 생산이 지연 됨과 동시에 ethanol 수율이 떨어지는 것은 pH3.5 이하의 낮은 pH영역에서는 균의 생육이 저조하여 D-xylose 소비가 낮기 때문일 것으로 생각 되어진다. pH 4.0-6.0에서는 ethanol 발효속도는 별 영향이 없으나 pH4.5 이상에서는 pH가 증가함에 따라 ethanol 생산량은 감소하는 경향을 보였다. 이와 같은 결과는 김 등(1986)의 *Candida* sp.에서 최적 pH가 4.0, du Preez 등(1986)의 *Candida shehate*를 이용한 D-xylose 발효에서 최적 pH가 4.0이였으며, pH 4.5-5.5 범위에서 ethanol 생산속도가 가장 빨랐다는 보고와 비교해 볼 때 본 공시 균주의 D-xylose 발효 최적 pH보다 조금 높지만은 pH와 발효속도와의 관계는 다소 일치하는 경향을 보였다.

Table 7. *Effect of D-xylose concentration on the production of ethanol by Candida sp. L-16*

D-xylose concentration (%)	Fermentation time(day)	Final ethanol		Maximum ethanol yield	
		v/v%	g/L	g/g	%
2	2	0.95	7.5	0.38	73.2
5	4	2.40	19.0	0.38	74.4
7	9	3.06	24.1	0.34	67.6
10	10	2.98	23.6	0.24	46.2

Table 8. *Ethanol production from different sugars by Candida sp. L-16*

Ferman table sugars	Fermentation time(day)	Final ethanol		Maximum ethanol yield	
		v/v%	g/L	g/g	%
Xylose	4	2.40	19.0	0.93	74.4
Glucose	3	1.75	13.8	0.28	54.1
Galactose	3	1.89	15.0	58.6	
Maltose	3	1.92	1.52	0.30	59.5

The initial pH was 4.5 and the sugars concentration was 50 g/L.
Incubation was carried out in flask culture at 28 ℃, shaken at 60 strokes/min.

D-xylose 농도의 영향

ethanol 생산에 미치는 초기 D-xylose 농도의 영향을 검토하기 위하여 초기 pH4.5, 온도 28℃ 및 진탕속도 60 rpm에서 D-xylose 농도 2, 5, 7 및 10%로 각각 조정하여 ethanol 생산을 비교 검토하였다.

Table 7에서 보는 바와 같은 D-xylose 농도 2%에서 5%까지는 D-xylose 농도가 증가 함에 따라 ethanol 수율은 증가 되나 5% 이상의 농도에서는 기질농도가 증가 함에 따라 최대 ethanol 수율과 ethanol 생산 속도는 급격히 감소하는 경향을 보였으며 최대 ethanol 수율은 기질농도 5%에서 이루어졌고 이때 ethanol 수율은 0.38g/g이었으며, 이론적인 ethanol 수율은 총당에 대하여 74.4%이었다.

발효성 당류들로 부터 ethanol 생산성 비교

초기 pH4.5, 온도 28℃, 진탕속도 60 rpm 및 농도를

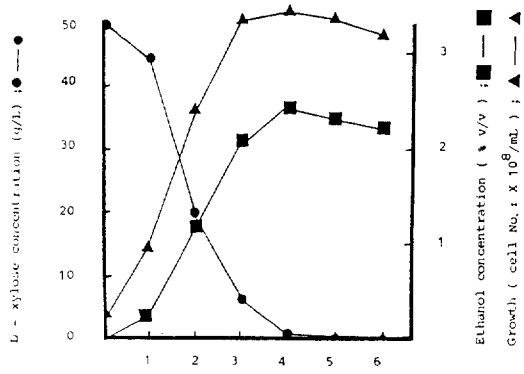

Fig. 2. *Effect of incubation time on the production of ethanol by Candida sp. L-16.*

5%로 본 공시 균주의 발효성 당류인 glucose, galactose, maltose 및 D-xylose로 부터 ethanol 수율을 비교 조사한 결과는 Table 8.에서와 같이 D-xylose로 부터 가장 높은 ethanol 수율을 얻었으며 hexose인 glucose, galactose 및 maltose는 D-xylose에 비해 발효 속도는 빠르나 ethanol 수율은 상당히 떨어지는 경향을 보였다. 이와 같은 결과는 본 공시 균주가 D-xylose 발효 균주이며 D-xylose의 최적 발효 조건하에서 이루어 졌기 때문인 걸로 생각되어진다.

배양 시간이 D-xylose 발효에 미치는 영향

앞에서의 실험 결과에서 얻어진 최적 조건 하에서 배양 시간에 따른 ethanol 발효, D-xylose 소비 및 균체 성장을 조사한 결과는 Fig.2에서 보는 바와 같이 균체 수율과 ethanol 생산량은 배양 4일째 최대값을 나타내었으며 이때 ethanol 농도는 2.4%(v/v)이었고 균체량은 3.408 × 10^8 cell/ml이었다. 배양 4일째 이후부터는 ethanol 농도와 균체량은 서서히 감소하는 경향을 보였다. D-xylose 소비는 배양 2일째 부터 급격히 증가하여 배양 4일째 부터는 D-xylose 농도가 급격히 감소하다가 배양 5일째에는 D-xylose를 완전히 소비하였다.

적 요

D-xylose로 부터 직접 ethanol을 생산할 목적으로 D-xylose를 유일한 탄소원과 에너지원으로 이용하는 효모들을 토양, 톱밥, 썩은 나무들로 부터 분리하였다. 이 균주들 가운데서 ethanol 생산력이 가장 우수한 균주는 썩은 나무로 부터 분리한 *Candida* sp. L-16으로 동정되었다. 이균주를 이용한 ethanol 발효의 최적 조건은 진탕속도 60 rpm, 배양온도 28℃, 초기 pH 4.5 및 D-xylose 농도 5%이었다. 알코올 생산은 배양 4일째에 최대치에 도달했고 이때의 ethanol 농도는 2.4%(v/v)로서 이론적인 ethanol 수율은 총당에 대해 74.4%이었다.

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