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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-

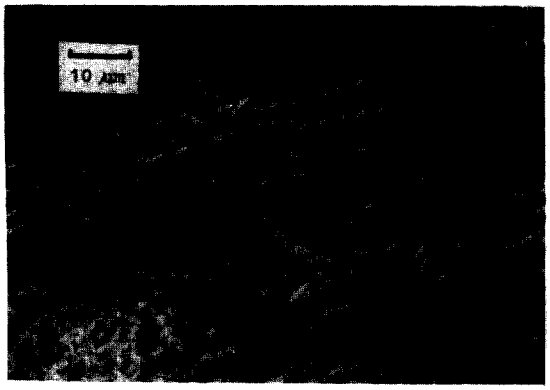

Fig. 1. *Sphaerotilus in the RBC Slime, 1000X phase contrast*

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.

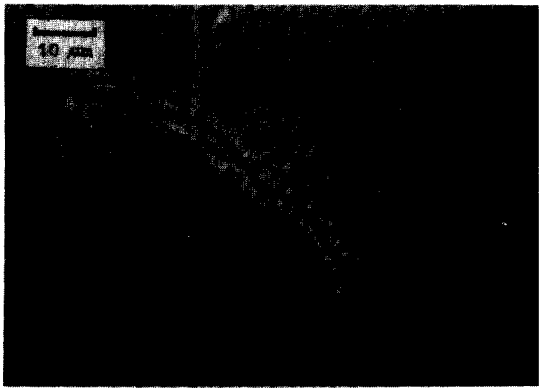

Fig. 4. *Nocardia in the RBC Slime, 1000X phase contrast*

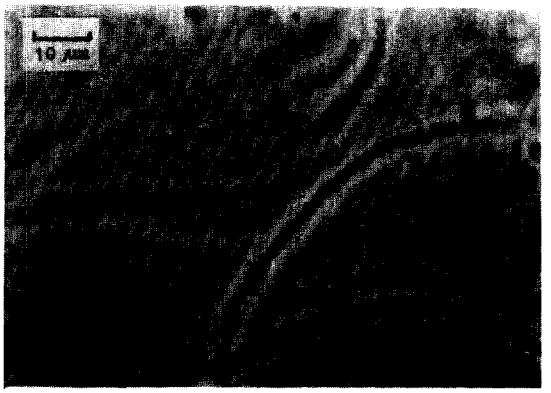

Fig. 6. *Unidentified Filamentous Bacteria in the RBC Slime, 1000X phase contrast*

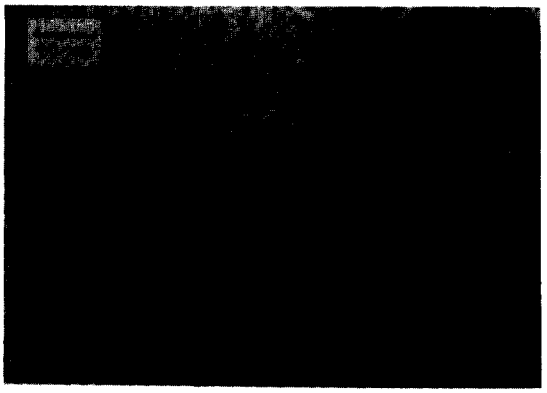

Fig. 5. *Type 021N in the RBC Slime, 1000X phase contrast*

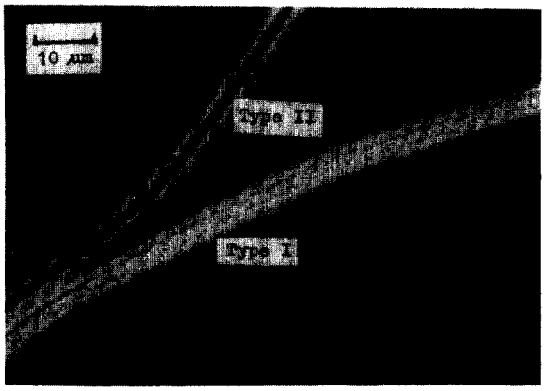

Fig. 7. *Beggiatoa Type I and II in the RBC Slime, 1000X phase contrast*

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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