Relationships among filamentous microorganisms in rotating biological contactors

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Abstract The existing relationships were studied among the different types of filamentous microorganisms that appear in the biofilm of a biological contactor system. Using the hierarchical cluster analysis it was observed that, in all the stages, *Beggiatoa* sp. and the Eikelboom's types 0803 and 1863 always appeared associated, while *Sphaerotilus natans* was always associated with the morphological type 021N. The remaining microorganisms were associated in variable forms in the plants. In addition, different association models were obtained according to the season of the year and the stage-season interaction. It has also been observed that a significant correlation exists among the filamentous microorganisms we have studied and the different physical-chemical parameters.

Keywords: *Beggiatoa* · Biofilm · Filamentous bacteria · Rotating biological contactor · *Sphaerotilus* · Wastewater treatment

Introduction

The presence of filamentous microorganisms in biofilms from rotating biological contactor (RBC) plants is well established and some of these microorganisms are indicators of deteriorating conditions^{2,3,4,5,6,9}. Some filamentous microorganisms, for example *Beggiatoa* sp. and *Thiothrix* sp., have been considered high load indicators^{3,4,5,6}.

However, in RBC, there are few studies about the correlation between the presence of different microorganisms and the values of physical-chemical parameters. This paper describes the structure of the filamentous microorganisms community and their associations along the different stages of the RBC plants and their relationships with water-line physical-chemical parameters.

Materials and Methods

Biofilm were obtained, twice a month, during a year from three municipal wastewater treatment plants with an RBC system located in the province of Madrid (Spain). Samples were taken in several points along the different stages (Fig. 1)

The biofilms were scraped from a constant area of the contactor surface, and diluted with sterile distilled water. The area was defined with the sample bottle mouth (23.75 cm²), as described in Galván *et al.*⁷ (2000), and the filaments were identified using Jenkin's technique.

In addition, in the wastewater for the different stages, several physical-chemical parameters were determined by the methods as described in APHA¹. The measured parameters were: biochemical oxygen demand (BOD₅), dissolved oxygen (DO), temperature, pH, ammonia (NH₃), nitrous

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Fig. 1 Sampling points. (1) Initial stages, (2) middle stages, (3) Final stages.

nitrogen (NO_2^-), nitric nitrogen (NO_3^-), and orthophosphate (PO_4^{3-}).

Results and discussion

1. Relationship by stage

The existing relationships among the different types of filamentous microorganisms have been studied by means of hierarchical cluster analysis (complete linkage), with the purpose of obtaining more information about microorganisms relationships in these wastewater treatment processes.

For the initial stages (Fig. 2a), the results obtained show three groups composed of different microorganisms. One of the groups is formed by *Beggiatoa* sp. and the 0803 and 1863 morphological types. The second group is formed by

Haliscomenobacter hydrossis and *Thiothrix* sp., as well as the 0914, 0961, 1701, and 1851 types. *Sphaerotilus natans* and the 021N type constitute the third group. The Fig. 2 b,c show the results obtained for the middle and final stages. It is observed, in both cases, that only two groups appear, one of them formed by *Beggiatoa* sp., *Sphaerotilus natans* and the 021N, 0803, and 1863 types, while the other group consists of the remaining microorganisms. It is observed that, in all stages, *Beggiatoa* sp. & the Eikelboom's types 0803, and 1863 always appear associated. In addition, *Sphaerotilus natans* is always associated with the morphological type 021N.

2. Relationships by season

Additionally, the microorganisms relationships were studied in the different seasons of the year (Figs. 3a, b, c, & d). In spring (Fig. 3a), two groups appear. One of the groups is formed by *Beggiatoa* sp., *Sphaerotilus natans*, and 021N, 0803, and 1863 morphological types. The other group is constituted by the remaining microorganisms. During the summer (Fig. 3b), two groups are formed. One group is formed by *Beggiatoa* sp. and 0961 type while the other is formed by the remaining microorganisms. The same thing happens in autumn (Fig. 3c), but the difference is that 1851.

Eikelboom's type associates, in this case, with 0961 type and with *Beggiatoa* sp. Lastly, in winter (Fig. 3d), two groups are formed and one of them is constituted by *Beggiatoa* sp., and 021N and 0961 morphological types, and the other one by the remaining microorganisms.

3. Relationships by stage-season interaction

When considering the interactions between the season of the year and the stage (Figs. 4, 5, 6, & 7), it is observed that, in spring, two groups in each stage are defined, and are formed by the same microorganisms (Figs. 4a,b,c). In summer, clear differences appear among the formed groups, according to the stage. In the initial stage (Fig. 5a) four groups appear, one formed by *Beggiatoa* and *Sphaerotilus*, another by *Haliscomenobacter hydrossis* and 021N, 0914, 1701, and 1851 types, a third group by *Thiothrix* sp., and 0961 type, and the last group by 0803, and 1863 morphological types. In the middle stage (Fig. 5b), two groups appear that are similar to those that are observed in spring. On the other hand, three groups appear in final stages (Fig. 5c); one of them is constituted by *Beggiatoa* sp., *Sphaerotilus natans* and 021N type; 0803 and 1863 types form another group, and the remaining microorganisms constitute the last group.

Fig. 2 Groups of microorganisms formed according to the stages. (a) Initial stages. (b) Middle stages. (c) Final stages

In autumn, four groups appear in the initial stages (Fig. 6a). *Beggiatoa* and *Sphaerotilus* form the first group. The 0803 and 1863 morphological types constitute the second group, while the two remaining groups are constituted by (i) *Haliscomenobacter hydrossis* together with 021N, 0914, 1701 and 1851 types, and (ii) *Thiothrix* and 0961 type, respectively. On the contrary, in the middle and final stages (Figs. 6b,c), only two groups appear. One of them is formed by *Beggiatoa*, *Sphaerotilus*, and 1863 and 0803 types, and the other comprises of the remaining microorganisms, as seen during spring and summer.

In winter (Fig. 7), four groups appear in the initial stages (Fig. 7a). The first is constituted by *Beggiatoa* together with 0803 and 1863 Eikelboom's types. The following group is

formed by four microorganisms (*Haliscomenobacter*, 0914, 1701 and 1851 morphological types). The other two groups are constituted by two microorganisms each. The middle and final stages results are similar to those in autumn and spring (Fig. 7b,c).

4. Correlation between filamentous microorganisms and physical-chemical parameters

In order to determine if the presence of various microorganisms can be used as an index of the different studied physical-chemical parameters (Table 1), the fitting to a logistic regression model was carried out. The data for the different parameters were used as dependent variables,

Fig. 3 Groups of microorganisms formed according to the season of the year. (a) Spring. (b) Summer. (c) Autumn. (d) Winter.

while the presence of the different microorganisms was used as dependent variables. Table 2 shows the results of fitting the data to a logistic regression model. We can observe that BOD_5 is correlated with the *Beggiatoa* sp., *Haliscomenobacter hydrossis*, and the 0803, 0914, 0961, and 1863 Eikelboom's types presence. The correlation of *Beggiatoa* sp. With organic load had already been established by other authors^{3,4,5,6} however, the correlation between *Thiothrix* sp. and the organic load was not

found significant in our study. This was contrary to what was reported earlier^{3,4,5,6}. The content of ammonia is correlated with the presence of *Sphaerotilus natans*. It is observed that the nitrous nitrogen content is correlated with *Thiothrix* sp. and the type 0961. In addition, the *Beggiatoa*, *Haliscomenobacter*, and *Thiothrix* genera show a correlation with the nitric nitrogen content. The phosphate values and type 0914, 1701, and 1863 show correlation.

Fig. 4 Groups of microorganisms formed in soring according to the stages. (a) Initial stages. (b) Middles stages. (c) Final stages.

Fig. 5 Groups of microorganisms formed in summer according to the stages. (a) Initial stages. (b) Middles stages. (c) Final stages.

Fig. 6 Groups of microorganisms formed in autumn according to the stages. (a) Initial stages. (b) Middle stages. (c) Final stages.

Fig. 7 Groups of microorganisms formed according to the stages. (a) Initial stages. (b) Middles stages. (c) Final stages.

Table 1 Physical-chemical parameters.

Plants	Stage	Season	pH	Temp. (° C)	BOD ₅ (mg/L)	NH ₃ (mg/L)	NO ₃ ⁻ (mg/L)	NO ₂ ⁻ (mg/L)	D.O. (mg/L)	PO ₄ ³⁻ (mg/L)
Camarma	Initial	spring	7.82	18.72	127.17	25.77	0.31	0.26	0.73	6.07
		summer	7.69	23.30	132.75	40.80	0.50	0.02	0.31	6.24
		autumn	7.69	15.98	131.25	28.57	0.50	0.06	1.66	5.36
		winter	7.62	13.70	52.50	22.32	2.32	0.80	2.68	3.65
	Middle	spring	7.88	18.58	117.50	26.08	0.31	0.25	0.25	5.76
		summer	7.72	23.18	115.38	40.25	0.47	0.02	0.11	6.02
		autumn	7.74	15.98	118.60	29.83	0.38	0.07	1.16	5.15
		winter	7.66	13.66	53.78	21.28	1.98	0.63	2.45	3.49
	Final	spring	7.90	18.66	110.00	25.03	0.27	0.20	0.21	5.39
		summer	7.76	22.86	104.60	38.60	0.45	0.02	0.14	5.55
		autumn	7.76	15.72	105.80	27.57	0.28	0.07	0.97	4.75
		winter	7.63	13.54	37.25	19.64	1.71	0.56	2.88	3.10
Boadilla	Initial	spring	7.63	18.69	56.26	19.96	0.78	0.51	1.08	5.68
		summer	7.57	20.68	28.28	14.80	1.15	0.37	1.40	4.21
		autumn	7.62	15.95	38.75	14.82	1.16	0.34	2.25	3.99
		winter	7.70	12.36	43.30	14.21	1.46	0.23	3.05	3.91
	Middle	spring	7.65	19.07	30.31	21.85	0.64	0.38	2.18	5.56
		summer	7.56	21.02	19.19	15.00	1.45	0.33	2.07	4.26
		autumn	7.57	15.92	21.58	16.34	1.32	0.31	3.40	3.91
		winter	7.71	12.36	18.50	14.86	1.93	0.22	3.89	3.91
	Final	spring	7.55	18.22	15.50	18.60	3.40	0.51	2.70	5.51
		autumn	7.58	15.85	18.50	12.80	4.25	0.47	3.60	3.95
		winter	7.57	12.30	13.60	11.14	5.62	0.37	4.40	3.88
Las Matas	Initial	spring	7.34	17.31	44.83	19.10	0.30	0.10	0.66	4.10
		summer	7.29	21.26	57.67	17.40	0.63	0.03	0.28	5.57
		autumn	7.17	15.97	66.20	16.29	1.11	0.05	2.58	3.54
		winter	7.44	11.40	28.50	8.72	0.89	0.12	4.45	2.14
	Middle	spring	7.38	17.56	27.25	17.00	0.23	0.09	1.64	4.02
		summer	7.46	21.16	38.67	17.50	0.79	0.06	1.18	5.51
		autumn	7.39	15.85	43.50	14.15	0.89	0.06	3.21	3.49
		winter	7.40	11.46	15.67	7.68	0.94	0.13	5.04	2.07
	Final	spring	7.37	17.49	17.80	17.66	0.49	0.20	2.94	4.17
		summer	7.41	21.24	25.50	15.10	1.66	0.26	1.80	5.90
		autumn	7.41	15.92	31.60	12.98	1.07	0.17	3.99	3.77
		winter	7.32	11.58	12.67	5.54	2.23	0.16	5.34	2.22

Table 2 Estimated Regression Model (Maximum Likelihood)

Microorganism	Parameter	Estimate	Standard Error	Estimated Odds Ratio
<i>Beggiatoa</i> sp.	Constant	0.335154	0.0389282	
	BOD	0.0344294	0.0111868	1.03503
	NO ₃ ⁻	-0.320296	0.12653	0.725934
<i>Haliscomenobacter hydrossis</i>	Constant	1.18246	0.110609	
	BOD	-0.0203962	0.00514142	0.97981
	NO ₃ ⁻	-0.241409	0.104998	0.785521
<i>Sphaerotilus natans</i>	Constant	2.0633	0.0298772	
	NH ₃	-0.0550894	0.0201801	0.946401
<i>Thiothrix</i> sp.	Constant	-0.23071	-0.0791418	
	NO ₃ ⁻	-0.397011	0.146997	0.672326
	NO ₂ ⁻	1.38464	0.588765	3.99339
Eikelboom's type 0803	Constant	-0.00418566	0.0423187	
	BOD	-0.0122884	0.00468466	0.987787
Eikelboom's type 0914	Constant	-1.36551	0.426513	
	BOD	-0.0381353	0.00933342	0.962583
	PO ₄ ³⁻	0.368874	0.129458	1.44611
Eikelboom's type 0961	Constant	-0.573876	-0.111421	
	BOD	0.0144618	0.00459705	1.01457
	NO ₂ ⁻	-1.13665	0.591241	0.320891
Eikelboom's type 1701	Constant	-0.392645	-0.624422	
	PO ₄ ³⁻	-0.459675	0.170183	0.631489
Eikelboom's type 1863	Constant	-1.17648	0.325878	
	BOD	-0.0157911	0.00507772	0.984333
	PO ₄ ³⁻	0.306778	0.108837	1.35904

Conclusions

In all stages, *Beggiatoa* sp. and the Eikelboom's types 0803 and 1863 always appear associated. In addition, *Sphaerotilus natans* is always associated with the morphological type 021N. *Beggiatoa* sp., *Haliscomenobacter hydrossis* could be used as an indicator for the organic load. The presence of *Sphaerotilus natans* could be used as an indicator for ammonia content. *Thiothrix* sp. could be used as indicator of nitrous and nitric nitrogen. Eikelboom's types 0914, 1701, and 1863 could be phosphate indicators.

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