การสำรวจแบคทีเรียสายใยในระบบแอกติเวเต็ดสลัดจ์ในโรงงานอุตสาหกรรมน้ำยางข้นและอุตสาหกรรมอาหารทะเล
Survey on Filamentous Bacteria in Activated Sludge Processes in Concentrated Latex Rubber and Seafood Industries

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บทคัดย่อ

การศึกษาครั้งนี้ได้สำรวจโรงบำบัดน้ำเสียในโรงงานอุตสาหกรรมน้ำยางข้นและอุตสาหกรรมอาหารทะเลทั้งสิ้น 17 โรงงานในภาคใต้ของประเทศไทย ผู้ศึกษาได้สนใจเกี่ยวกับปัญหาตะกอนเบาไม่จมตัวซึ่งเกิดขึ้นเนื่องจากแบคทีเรียสายใย น้ำเสียจากโรงงานทั้งสองประเภทนี้มีความเข้มข้นของสารอินทรีย์สูงมาก ทั้งในรูปของค่าบีโอดี และค่าทีเคเอ็น มีถึง 16 โรงงานที่ออกแบบระบบแอกติเวเต็ดสลัดจ์เพื่อบำบัดคาร์บอนและสารแขวนลอยเท่านั้น และต้องมีหน่วยบำบัดก่อน-หลังอื่นๆ ร่วมด้วย โดยระบบได้ถูกออกแบบให้มีค่าระยะเวลาการเก็บกักน้ำสูงและมีค่าอัตราส่วนอาหารต่อจุลินทรีย์ต่ำซึ่งเป็นสาเหตุหลักของการเกิดปัญหาตะกอนเบาไม่จมตัวที่ผู้ควบคุมระบบไม่สามารถคาดเดาสาเหตุและหาทางแก้ไขได้ จากการตรวจสอบด้วยกล้องจุลทรรศน์ พบว่าชนิด 0041 และ *Nostocoida limicola II* พบเฉพาะในโรงงานอาหารทะเล และ ชนิด 1863 พบเฉพาะในโรงงานน้ำยางข้น

Abstract

This study surveyed seventeen wastewater treatment plants, in concentrated latex rubber and seafood industries, in southern Thailand. The researchers concerned mainly in the bulking sludge problems caused by filamentous bacteria. Wastewaters from those factories contained high organic contents, high BOD_5 and TKN concentrations. All of the existing activated sludge process were equipped with other pre- and post-treatments. Sixteen of them were concerned with only carbon and suspended removal. They were operated with high hydraulic retention time (HRT) and low food per microorganism ratio (F/M ratio), which are the main causes of bulking sludge problem. Most operators faced up with this problem, but could not realize the reasons and solutions. This study observed their settle ability and presented filamentous bacteria in each plant. It can be evidently seen that some filamentous bacteria such as type 0041 and *Nostocoida limicola II* appeared at seafood treatment plants only, and type 1863 found in rubber treatment plants only. This can be affirmed to the nature of influent composition of wastewater.

คำสำคัญ : ระบบแอกติเวเต็ดจ์สลัดจ์ น้ำยางข้น แบคทีเรียสายใย อุตสาหกรรมอาหารทะเล

Key words : Activated Sludge Process, Concentrated Latex Rubber, Filamentous Bacteria, Seafood Industry

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1. Introduction

The Activated Sludge Process (ASP) is one of the most highly efficient biological processes currently used. The ASP was developed by ARDERN and LOCKETT in 1914. Activated sludge was so named because it involved the use of a mass of activated microorganisms, which is capable of removing waste in an aerobic condition (Phanapavudhikul, 1978). The ASP mainly consists of an aeration tank, a settling tank and a return sludge line. It is very flexible and can be adapted to almost any types of biological wastes. There are several modifications of the ASP, aimed at serving different treatment objectives. However, the very well-known problem in the ASP, the bulking sludge problem, brought to the poor settle- ability.

ATV Working Group 2.6.1 (1989) reported that the bulking sludge problem occurs when the sludge volume index (SVI), analysed by the dilution method, is more than 150 mL/g. Such an explanation is based solely on sludge physical characteristics. About 30 different filamentous species of bacteria are known to cause bulking sludge, in Germany. Not all 30 species are equally common.

The factors influencing each type of filamentous bacteria are different. The nature of the wastewater and the mode of the operating process are the main factors. Casey *et al.* (1994) concluded that the major factor influencing low F/M filament bulking was the alternating anoxic/oxic conditions forcing the heterotrophic organism to switch between oxygen and nitrate as terminal acceptors.

Martins *et al.* (2003) compiled worldwide surveys of filamentous microorganisms in activated sludge systems. Only few surveys were done in Asia or tropical areas. Especially, in Europe, the investigators often found *Microthrix parvicella.* That could be explained that *Microthrix parvicella* grows well at low BOD_5-sludge loading (less than 0.1 kg/(kg•d)) and low temperature (Knoops and Kunst, 1998 and Mamais *et al.*, 1998).

Mino (1995) investigated the filamentous situation in the tropical zone, Bangkok Thailand, the first time in Asia. Six types of wastewater treatment processes were collected, concerning both industry and domestic wastewaters. His result showed that each type of wastewater treatment plant provided different type of filamentous bacteria. Microthrix parvicella was not found in the domestic sewage treatment plant, in Thailand. However, only one treatment plant for each type of wastewater was investigated.

Chevakidagarn *et al.* (2004) surveyed four ASP treatment plants, 2 from concentrated latex rubber factories and 2 from seafood industry. Their microscopic investigations of mixed liquor showed the same dominant filamentous bacterium, *Nostocida Limicola II*, was found in both concentrated latex rubber factories. The dominant filamentous bacterium was Type 1851 and *Nostocoida limicola I* in seafood industries. However, only 2 treatment plants from each type of wastewater could not be finalized that type of wastewater could influence type of filamentous bacteria. They suggested for further survey on more treatment plants.

Therefore, the main idea of this study is to survey the actual situations of the existing ASP treatment plants in Para rubber and seafood industries, in southern Thailand. The secondary objective is to gain an understanding of the bulking sludge problems.

2. Methodology

2.1 Site descriptions

Wastewater samples were collected in two intervals, for dry and wet weathers in southern Thailand, in 2005, from seven ASPs in latex rubber industry and ten ASPs from seafood industry. In southern Thailand, two main industries are para rubber and seafood products. They are main sources of industrial wastewater fed to natural resources. In each of the seventeen plants, the ASPs are equipped with other pre- and post-treatment units, such as Upflow Anaerobic Sludge Blanket (UASB), anaerobic ponds, oxidation pond, or wetland. Due to the high concentrations (of organic matters) in influents, the ASPs alone could not be a single solution able to reduce contaminants. Sixteen from seventeen ASPs were conventional ASPs without nutrient removal unit. They were designed and operated with conditions of high hydraulic retention time (HRT) (1 to 8 days) and low F/M ratio (average about 0.3 kgBOD5 /(kgMLSS.•d)). Only one treatment plant from seafood industry was designed for nitrogen removal.

2.2 Sampling

Wastewater characteristics for each treatment plants were analyzed by both laboratory and on site measurements. In this study, the influents and effluents from the ASP were mainly determined. Parameters measured in this study were shown in Table 1 and 2.

3. Results and Discussion

The wastewater characteristics from each treatment plant are presented in this section. Seven ASPs plants named A, B, C, D, E, F and G were represented of ASPs from latex rubber industries and ten ASPs plants named H, I, J, K, L, M, N, O, P and Q were from seafood industries (Table 1).

3.1 Onsite Measurement

Dissolved oxygen (DO) concentration, pH and temperature were measured onsite by using a pH/Oxi 340i meter from WTW GmbH & Co.Ltd. The results showed that there was no significantly different among 7 ASPs plants in latex rubber industries and 10 from those in seafood industries. However, some conclusions could be drawn here:

1. Temperatures in ASPs from seafood industries were lower than those from latex wastewaters, due to ice used to preserve the qualities of raw material.

2. DO concentrations in aeration tanks in seafood industries were higher than those from latex industries (due to higher influent concentration, in term of COD and sulfate).

3.2 Treatability Evaluation

All chemical tests in the laboratory were performed according to Standard Methods for the Examination of Water and Wastewater (APHA, 1998). The BOD_5 and COD analysis was undertaken for both filtered and non-filtered samples (filtrated with 0.45 μm filter paper). In this study, the total removal efficiencies of BOD_5, COD, total nitrogen, total phosphorus and total suspended solids are defined by the following equation.

$$R = \frac{(\text{Influent concentration} - \text{Effluent concentration})}{\text{Influent concentration}} \times 100\%$$

Where R = Total removal efficiency of the activated sludge process from each treatment plant.

The results showed that average removal efficiencies of treatment plants from para rubber industries were lower than those from seafood industries. It might be explained that the waste-water characteristic from para rubber industries was not suitable for activated sludge process due to the high concentrations of COD (varied from 3,240 to 14,400 mg/L) and of sulfate (varied from 827 to 7,293 mg/L). Those high concentrations brought to the lack of oxygen in the aeration tanks, as shown in the results of onsite measurements. However, they were forced to install ASPs as the first unit to reduce the odor problem from hydrogen sulfide.

Table 1 Wastewater characteristics in each aeration tank from 17 representative treatment plants

Plant	pH		Temperature °C		DO mg/L	
	Dry	Wet	Dry	Wet	Dry	Wet
A	7.6	7.8	35.50	45.80	1.50	0.41
B	7.5	7.5	37.30	36.00	0.40	0.54
C	7.4	7.2	35.35	35.10	5.75	5.75
D	7.4	7.4	34.50	34.70	0.37	0.30
E	7.9	7.9	30.60	31.60	0.94	0.95
F	8.0	7.9	36.60	30.60	0.20	1.88
G	7.3	7.2	37.53	37.10	0.63	0.61
H	7.6	5.6	25.30	26.30	0.65	4.20
I	4.3	7.3	25.00	24.90	0.86	1.39
J	7.1	7.1	30.70	29.20	2.50	1.17
K	7.4	7.2	28.20	25.90	1.19	1.39
L	7.4	7.4	26.20	25.50	2.45	4.21
M	6.6	6.5	30.80	33.00	2.03	1.08
N	7.6	7.6	26.30	28.30	2.70	2.02
O	8.4	7.5	25.70	26.20	2.56	1.26
P	7.6	7.5	24.80	26.20	2.56	0.97
Q	6.6	6.7	30.80	24.70	1.32	1.73

Dry = dry weather (February-July)

Wet = wet weather (August-January)

As mentioned, in this study, the influent and effluent from the ASP were mainly determined. The effluents concentrations calculated in table 2 were the effluents from the ASPs, not directly discharged to natural water resources. The removal efficiencies of BOD_5 were above 80 percent. In contrast, the COD removal efficiencies were lower than 80 percent, as shown in Table 2.

Table 2 Average and Standard deviation of removal efficiencies from 17 representative treatment plants

Values	Para rubber industries (A to G)		Seafood industries (H to Q)	
	Dry	Wet	Dry	Wet
BOD_f	88.43±5.09	88.14±5.27	88.10±5.34	90.50±5.76
BOD_{nf}	84.86±7.80	85.14±3.02	86.80±4.80	84.10±8.05
COD_f	56.57±15.65	79.71±16.49	75.20±14.61	57.00±15.82
COD_{nf}	55.86±14.16	69.14±18.62	77.80±7.36	46.60±7.31
SS	72.57±15.45	61.57±16.82	81.20±19.54	79.10±19.31
TKN	70.57±16.44	50.43±18.96	71.00±20.95	56.30±24.06
TP	67.14±14.72	62.43±23.97	42.60±20.44	60.40±19.92
TN	56.00±17.31	40.00±10.58	70.50±19.48	46.60±15.21
SO_4^{2-}	62.00±14.70	53.14±18.36	-	-

When BODf / CODf = BOD_5/COD when filtrated sample with 0.45 µm. filter paper
BOD_{nf}/COD_{nf} = total BOD_5/COD (non-filtrated)

The suspended solids removal efficiency from each treatment plant was not high enough to pass Thai effluent standard (the SS in effluents varied from 40 to 2, 260 mg/L). The poor settle abilities from those plants can be proved by measuring the sludge volume index (SVI).

$$\text{When} = \text{SVI (mL/g)} = \frac{SV_{30} \times 1000}{\text{MLSS (mg/L)}}$$

SV_{30} = Volume of sludge in cylinder 1 L after 30 minutes settled

MLSS = Mixed Liquor Suspended Solid

The observation showed that most treatment plants operated with low F/M ration and low HRT, the main factors cause bulking sludge problem (Jenkins *et al.*, 1993). The reason was most local operators do not withdraw their excess sludge continuously. They normally wait till the SV30 became 800 to 900 mL/L, and then, they pump out their excess sludge in one time. Meanwhile, the long HRTs provided extended aeration condition to the ASPs. The excess sludge was oxidized and consumed as carbon source.

The settleabilities of the activated sludge samples investigated given in Table 3. In general, the settleabilities of the investigated activated sludges were poor to very poor. The SVI values varied from 22 to 805 mL/g. Nine from seventeen plants faced up with the bulking sludge problems (when the SVI values were more than 150 mL/g).

Table 3 Sludge volume index (SVI) and main factors affected SVI from each treatment plant

Plant	SVI (mL/g)		F/M ratio ($kgBOD_5$/(kg MLSS.•d))		MLSS (mg/L)		HRT (day)
	Dry	Wet	Dry	Wet	Dry	Wet	
A	118	101	0.02	0.06	7500	6110	7
B	106	65	0.05	0.14	4500	4740	6
C	118	125	0.06	0.11	3300	3360	7
D	600	69	0.11	0.32	3080	2620	3
E	588	526	0.14	0.33	1738	1900	7
F	91	158	0.10	0.14	5400	5175	5
G	132	178	0.03	0.06	8600	1900	7
H	20	123	0.94	0.06	1807	4900	8
I	182	787	0.89	0.08	4810	940	3
J	47	69	0.09	0.03	9260	8142	3
K	22	29	0.21	0.16	5010	1740	4
L	124	457	0.43	0.24	6110	2140	1
M	68	209	0.37	0.30	5860	4740	1
N	110	80	1.62	2.39	725	580	1
O	805	66	0.34	0.30	1242	2740	1
P	151	132	0.07	0.09	4810	2420	4
Q	282	515	0.29	0.24	2060	1940	1

3.3 Microscopic examination

Microscopic examinations of filamentous bacterial were made under phase contrast at 100X, 400X and 1000X magnification. The Gram and Neisser staining techniques were used for identification. The filaments were identified according to Jenkins *et al.* (1993). Table 4 showed the results of filamentous bacteria found in each treatment plant.

Table 4 Morphological staining characteristics of filamentous bacteria observed in activated sludge

Plant	Type of filamentous bacteria	Morphological staining characteristics
A	Type 1701	Gram negative
B	Type 021N	Gram negative
C	Type 1863	Gram negative
D	Type 1701	Gram negative
	Microthrix parvicella	Gram positive
E	Unidentified	Gram positive
F	Type 0092	Gram negative
G	Type 1863	Gram negative
H	Type 0041	Gram negative
I	Type 0041	Gram negative
J	*Nostocoida limicola II*	Gram positive
K	Type 1851	Gram positive
L	*Nostocoida limicola II*	Gram positive
M	Type 021N	Gram negative
N	*S. natans*	Gram negative
O	Type 0803	Gram negative
P	Type 0041	Gram negative
Q	Type 1701	Gram negative

Results indicate that a diverse population of filamentous organisms was present in the activated sludge process. In 13 from 17 plants (or 76.5 percent) found gram-negative filamentous bacteria. Gram-negative filamentous bacteria are commonly observed in activated sludge (Wagner et.al., 1994). In this study, a total of 9 filamentous bacteria were identified, from 17 treatment plants. Two most frequently dominant filamentous bacteria identified are type 1701 and type 0041. Unexpectedly, *Microthrix parvicella* was found in plant D with scale 5 (abundant), when using the scoring technique outline by Jenkins *et al.* (1993). In contrast with Knoops and Kunst (1998) and Mamais *et al.* (1998), they reported that *Microthrix parvicella* was commonly found in low temperature condition. But wastewater temperatures at plant D were high (about 34 - 35°C).

It can be evidently seen that some filamentous bacteria such as type 0041 and *Nostocoida limicola II* appeared at seafood treatment plants only, and type 1863 found in rubber treatment plants only. This can be affirmed to the nature of influent composition of wastewater. However, type 1701 and type 021 N were found in both types of wastewater treatment plants.

4. Conclusion

The study shows that several types of filamentous bacteria found in ASPs operated in southern Thailand. Most of treatment plants operated with the conditions pleasing bulking sludge problems; such as low DO, low F/M ratio and high HRT. Changes in the filamentous bacteria population (SVI values changed) could be caused by changing operating conditions; pH, DO concentrations, F/M ratio and HRT. Bulking sludge problems caused reducing removing capacities of ASPs, especially for COD and SS removing.

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