INHIBITION OF MICROBIAL ACTIVITY OF ACTIVATED SLUDGE BY AMMONIA IN LEACHATE

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ABSTRACT

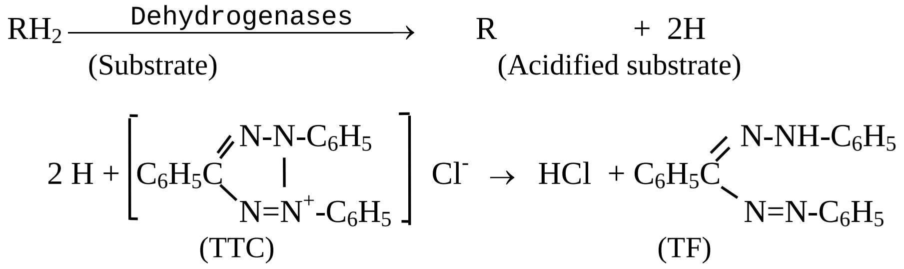
Leachate samples were collected from the West New Territory Landfill (WENT), Hong Kong and characterized in our laboratory. The analytical results confirmed that it has a typical nature of aged leachate with low BOD_5/COD ratio of 0.22 and high strength of ammonia-nitrogen around 5 g/L. A lab-scale study was conducted to investigate the inhibition of microbial activity of the activated sludge. In the first test, the glucose-based synthetic wastewater was used in two parallel reactors. The experimental results demonstrated that COD removal declined from 95.1 to 79.1% and the dehydrogenase activity of the sludge decreased from 11.04 to 4.22 μg TF/mg MLSS (mixed liquor suspended solids), when the ammonia-nitrogen concentration increased from 50 to 800 mg/L progressively. In the meantime, the remaining NH_3^+-N residue in the treated wastewater increased from 0.58 to 649 mg/L extensively. In the second test, mixed wastewater samples containing glucose and raw leachate was fed into six parallel biological reactors and operated also on batch mode. The experimental results showed COD removal decreased from 97.7 to 78.1% and the dehydrogenase activity decreased from 9.29 to 4.93 μg TF (triphenyl formazon)/mg MLSS (mixed liquor suspended solids) respectively, when the ammonia-nitrogen concentration increased within the same range. Microbial inhibition could also be substantiated by the decrease of specific oxygen uptake rate (SOUR) from 68 to 45 mg O_2/g MLSS. All of these results suggested leachate containing high-strength ammonia-nitrogen should be pretreated to an acceptable NH_4^+-N level before it is fed into biological reactors.

INTRODUCTION

Since landfilling is one of the main waste disposal methods used in Hong Kong, the treatment of the leachate from landfill sites becomes a concerned issue. Currently, there exist 13 landfills and three strategic landfills among those are in operation in the New Territory area of Hong Kong (EPD 1996). Based on the past trends and future projections, it is anticipated that daily waste arising in Hong Kong will continue to increase such that about 30 Gg/d will require disposal by the year 2001 (Robinson and Luo 1991). Landfills are dynamic chemical/biological reactors, leachate quality is dependent on refuse composition, age, site conditions such as precipitation, infiltration and runoff (Philips *et al.* 1995). The changing encounter from landfill to landfill are such that waste treatment technique applicable at one site may not be directly transferable to other locations (Qasim and Chiang 1994). An illustration of developments in leachate indicates that landfill experiences five biological processes from short-time aerobic to anaerobic acid fermentation, intermediate anaerobiosis, methanogic fermentation and second aerobic processes (Christensen and Kjedsen 1989). Because of the 'varying' reactor conditions and ‘varying’ compositions in the produced leachate, there exists the need to characterize the specific leachate to be dealt with. For instance, the West New Territory landfill (WENT) in Hong Kong has been receiving mainly domestic wastes and operated for about 4.5 years since it’s commission on 19 November 1993 and daily production of leachate is roughly 40 m^3/d at present time. This leachate contains a high level of ammonia-nitrogen around 5 g/L and soluble chemical oxygen demand (COD) of 6-7g/L. Obviously, it has a low ratio of COD to ammonia and conventional activated sludge processes have a difficulty to effectively eliminate the organic and ammonia strength to an acceptable level because high ammonia-nitrogen cause a significant toxicity to the microbe in biological treatment processes.

An on-site aerobic treatment using activated sludge lagoon in Hong Kong has demonstrated a poor treatability with COD removal of 40-70% and ammonia removal of 30-40% only (Fung et al. 1997). Especially Hong Kong government tightened up the ammonia-nitrogen discharge standard to public sewers from 2 g/L to 200 mg/L recently. To comply with this new standard, those aerobic lagoons recirculate a large amount of treated effluent to the aeration tanks in order to dilute the ammonia strength and achieve higher removals of COD and ammonia. Since this recirculation operation can solve this problem technically, but it raises the operating cost up to about \$100/m^3. To eliminate the operating cost, the recirculation ratio in the process should be reduced as much as possible. Therefore, the maximum level of ammonia in the aerobic tanks to generate enough inhibition to activated sludge would be a critical parameter for the improvement of these biological processes.

A study was carried out in our laboratory to investigate the inhibition of microbial activity caused by high strength of ammonia in leachate. In the experiment, COD reduction and triphenyl tetrozolium chloride (TTC)-dehydrogenase activity were mainly assessed to evaluate the inhibition of microbial activity. Early works indicated that the measurement of dehydrogenases provided a good indication of sludge oxidation capacity, in which TTC is used as an artificial hydrogen acceptor (Eckenfelder et al. 1986, Ford et al. 1966; Weddle and Jenkins 1971). When TTC is introduced into the electron transport chain, the hydrogen transfer mechanism from flavin linked dehydrogenases to the cytochromes is replaced by TTC. The reduced TTC forms a reddish color substance named triphenyl formazon (TF) which is proportional to its concentration and can be extracted from the cell and measured colorimetrically. The chemical reactions are illustrated below:



Obviously, more of the TF formed during above reactions, more active the biomass present in the mixed liquor of biological reactor, when the environmental conditions as temperature and pH are relatively constant.

MATERIALS AND METHODS

Leachate samples were collected from the WENT landfill and prepared for the batch tests of biological treatment based on its characterization. The experiment consisted of two tests. In the first test, synthetic wastewater was used only, which had a glucose-based composition of $C_6H_{12}O_6 \cdot H_2O$ 1026 mg, NH_4Cl 141 mg, K_2HPO_4 68 mg, KH_2PO_4 34 mg and $NaHCO_3$ 790 mg in 1 L of tap water. This synthetic wastewater sample had a constant COD concentration of 1 g/L, but its ammonia strength varied from 50 mg/L to 800 mg/L according to the experimental requirement. Two reactors with an effective volume of 2 L were run in parallel to treat the synthetic wastewater on the basis of batch operation. While Reactor #0 used for control was fed with constant ammonia-nitrogen strength of 50 mg/L, Reactor #1 was run with the progressively increasing of ammonia-nitrogen concentration from 50 mg/L to 800 mg/L. The both reactors were aerated to keep DO around 2 mg/L and pH between 7.5 - 8.5. The experiment was continuously operated in 5 phases with the different ratios of COD:N:P as shown in Table 1. In each phase, the both reactors were initially filled with the synthetic wastewater containing mixed liquor suspended solids (MLSS) of 2 g/L and COD of 1 g/L, and continuously operated for 24 hours. In the mean time, the treated wastewater samples were consequently collected after every 6 hours for the analyses of COD, NH_4^+-N, MLSS and TTC-dehydrogenase activity. After 24 hours, the COD concentration in the reactors was made up to 1 g/L again and the second cycle started. Each phase of the experiment lasted for longer than a complete SRT period of 3-4 days.

[Table 1.]

In the second test, the leachate sample was used in the experiment. This raw leachate sample with an initial ammonia-nitrogen of 4925 mg/L was diluted by tap water accordingly to achieve their variable ammonia-nitrogen strengths in the range of 50 – 800 mg/L. However, to compare with the operating condition in the first test, a fixed amount of glucose, equivalent to 1 g/L of COD, was also added into all the diluted leachate samples. Six reactors with same volume in the first test were run in parallel. While one reactor was used for treating the synthetic wastewater with 50 mg/L NH_4^+-N as a control, the other five reactors were used for treating the diluted leachate samples with addition of glucose. In addition, phosphorus from K_2HPO_4 and KH_2PO_4 were also added into the diluted leachate at a ratio of glucose COD: P = 100:1 by weight, and $NaHCO_3$ or HCl was used for adjusting pH between 7.5 - 8.5 in the biological reactors. All other experimental conditions, sampling procedures and analyses were same as used in the first test.

The parameters such as COD, BOD_5, MLSS, TSS (total suspended solids), VSS (volatile suspended solids), TDS (total dissolved solids) and FDS (fixed dissolved solids) etc. were examined with the Standard Methods (APHA 1995). pH and NH_4^+-N were measured with an expandable ion analyzer (EA940, Orion Research Incorporation), while DO was monitored with a YSI DO meter. Dehydrogenase activity was employed in this study to indicate microbial activity because of its accurateness, rapidity and a minimum of instrumentation (Awong *et al*. 1985). Dehydrogenases are the oxidation-reduction enzymes, which participate in the mainstream of electron transport from organic substrate to molecular oxygen. Dehydrogenase activity assays were determined with a standard examination method (Japan Association 1984) with the following procedure: add 1-mL of triphenyl tetrozolium chloride (TTC, 5 g/L) and 3 drops of $NaSO_3$ solution (5 g/L) in a 15-ml tube containing 10 ml of mixed liquor from the reactor and then put the tube in dark place at 20^{o}C for 1 hour; centrifuge the tube 5 minutes at 3000 rpm, discard the supernatant and add 10 ml of ethanol into the tube for extraction of color product of triphenyl formazon (TF) from cells, then centrifuge again and finally measure the concentration of the produced TF at 480 nm by spectrophotometer.

In the second test, to substantiate the results of microbial activity expressed in dehydrogenase activity, specific oxygen uptake rate (SOUR) was also determined for each reactor according to the following procedure: take 300 ml of mixed liquor out from the respective reactor and put it into 6 BOD bottles respectively to allow for the sludge settling for 30 minutes; siphon the supernatant out, put magnetic stirrer in it and refill the bottles with pre-aerated synthetic wastewater of initial COD 500 mg/L; start mixing and record the depletion of dissolved oxygen in each bottle with DO meter; and finally calculate SOUR expressed in mgO_2/gMLSS-hr.

RESULTS AND DISCUSSION

Inhibition of microbial activity by ammonia-nitrogen in synthetic wastewater

In this test, the synthetic wastewater with different NH_4^+-N concentrations was tested to determine the inhibition of microbial activity. Since mixed liquor volatile suspended solids (MLVSS) differentiate neither between living and dead cells nor between biomass and inert volatile solids present in the mixed liquor of aeration tank, microbial activities can commonly be reflected by the magnification of specific oxygen uptake rate (SOUR), dehydrogenase activity and adenosine triphosphate (ATP) etc. The experimental results in the test showed that the activated sludge collected from a sewage treatment works needed about 3 days to be acclimated to receiving the synthetic wastewater as feed. While microbiological activity was greatly inhibited by progressively increasing NH_4^+-N from 50 mg/L in Phase I to 800 mg/L in Phase V, the significant declinations of COD reduction and dehydrogenase activity were found as shown in Fig. 1 and Fig. 2. The observed growth of activated sludge was also illustrated in Fig. 3. For comparison, average values of remaining COD, NH_4^+-N and dehydrogenase activity are summarized in Table 2. It could be noted from these figures and table that the COD removal was reduced from 95.1% to 79.1% and the NH_4^+-N removal was reduced from 98.8% to 18.8% while NH_4^+-N concentration increased from initial 50 mg/L to final 800 mg/L, accordingly the dehydrogenase activity decreased from 11.02 μg TF/mg MLSS to 4.22 μg TF/mg MLSS. These results revealed that increasing NH_4^+-N concentration resulted in great inhibition to the microbial activity. From a view of NH_4^+-N removal, initial NH_4^+-N concentration should be limited below 100 mg/L in order to avoid any significant inhibition of microbial activity of activated sludge by high ammonia-nitrogen strength and high residual NH_4^+-N appeared in the treated effluent.

[Fig. 1.]

[Fig. 2.]

[Fig. 3.]

[Table 2.]

In the phase II of test, hourly changes of COD, MLSS, pH and dehydrogenase activity in both reactors were also monitored as shown in Fig. 4. It was further shown that COD removal was almost maintained at same rate and no substantial inhibition of microbial activity was found with initial NH_4^+-N of 100 mg/L as indicated by dehydrogenase activity. However, it should be kept in mind that remaining NH_4^+-N in Reactor #0 was only 0.46 mg/L while that in Reactor #1 was 41 mg/L as shown in Table 2. If the initial NH_4^+-N concentration exceeded 100 mg/L, the treated effluent would contain more residual NH_4^+-N, which may not comply with the required discharge standard.

[Fig. 4.]

Inhibition of microbial activity by ammonia-nitrogen in raw leachate

The leachate used in this study was collected from the WENT landfill in Hong Kong. The basic characteristics of the leachate are summarized in Table 3. It was obvious that this leachate can not be efficiently treated by a direct application to biological processes, because the high ammonia levels in leachate are inhibitors to biological decarbonation as well as to biological nitrification. It has been revealed that with total ammonia-nitrogen concentration more than 200 mg/L at temperature of 15 ^{o}C and pH of 8, the percentage distribution of ammonia is more than 6% with corresponding values of 120 mg/L of NH_3, impairing biological oxidation (Metcalf and Eddy Inc. 1979).

[Table 3.]

In this study, the diluted leachate samples with addition of glucose had COD concentration in the range of 1 – 2 g/L and variable NH_4^+-N concentrations from 50 to 800 mg/L and was studied to determine the inhibition of microbial activity. The activated sludge from a sewage treatment works was acclimated by feeding the synthetic wastewater in the control reactor during the first test. The 6 reactors were operated with same initial MLSS of 2 g/L. One reactor was continuously fed with the synthetic wastewater containing 50 mg/L NH_4^+-N as a control and the other five reactors were used for treating the diluted leachate samples with increasing NH_4^+-N concentrations from 50 to 800 mg/L. After every 6 hours operation, samples from each reactor were collected for the analyses of remaining COD and dehydrogenase activity. The test was conducted under room temperature (20^{o}C) and lasted for 36 hours. The experimental results of COD, dehydrogenase activity as well as MLSS under stable operating conditions are shown in Fig. 5, Fig. 6 and Fig. 7 respectively. For comparison, average values of COD, NH_4^+-N and dehydrogenase activity are summarized in Table 4.

The initial concentrations of COD comprised of glucose and leachate were different in the reactors. To evaluate the removal of biodegradable COD in each biological reactor and compare the results with those in the first test, it had been assumed that the initial glucose COD of 1 g/L was completely biodegradable while the COD of the diluted leachate was partially biodegradable. The portion of biodegradable COD from the diluted leachate was estimated to be the respective BOD_u, which was calculated from the equation of $\frac{0.22 \cdot COD_{measured}}{1 - e^{-0.2x5}}$, because the ratio of BOD_5/COD in the leachate was 0.22. Therefore, the values of final remaining COD concentrations plotted in Fig. 5 had been converted into the biodegradable COD by the subtraction of non-biodegradable COD in the leachate from the measured COD.

[Fig. 5.]

[Fig. 6.]

[Fig. 7.]

[Table 4.]

It could be noted from the results in Fig. 5, 6 and 7 and Table 3 that average COD removal decreased from 97.7 to 78.1% (95.7% for the control) and the dehydrogenase activity decreased from 9.29 to 4.93 μg TF/mg MLSS (10.46 μg TF/mg MLSS for the control) respectively, when the ammonia-nitrogen concentration increased from 50 to 800 mg/L progressively. Microbial inhibition could also be substantiated by the decrease of specific oxygen uptake rate (SOUR) from 68 to 45 mgO_2/g MLSS (70 mgO_2/g MLSS for the control) accordingly. Compared to the results in the first test when synthetic wastewater was applied, the decreases of COD reduction and dehydrogenase activity had similar trends against the increase of ammonia-nitrogen strength in the reactor. However, two sets of data showed a slight difference, which may be resulted from the effects of leachate composition on the microbial activities.

CONCLUSION

The characterization of the leachate from WENT landfill indicated that it belonged to the methanogenic site with a low BOD_5/COD ratio of 0.22 and high strength of ammonia-nitrogen around 5 g/L. The operation of biological reactors receiving both synthetic wastewater and mixed wastewater of glucose and leachate demonstrated that the high levels of ammonia-nitrogen could significantly inhibit the microbial activity of activated sludge, which were indicated by the declination of COD removal efficiency, dehydrogenase activity and SOUR. All results implies that the high strength of ammonia-nitrogen in leachate needs to be eliminated to a level of less than 100 mg/L, either by a pretreatment such as air-stripping or chemical precipitation prior to biological treatment or recirculation of the treated effluent in the biological treatment process.

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Table 1. Initial NH_4^+-N concentrations during different phases in Reactor #1

Study Phase	COD:N:P (mg/L)	Initial NH_4^+-N (mg/L)
I	100: 5: 1	50
II	100:10:1	100
III	100:20:1	200
IV	100:40:1	400
V	100:80:1	800

Table 2. Summary of the average values in the five study phases

	COD (mg/L)			NH_4^+-N (mg/L)			Dehyd. activity (μg TF/mg MLSS)		MLSS (mg/L)	
Phase	Initial	#0	#1	Initial	#0	#1	#0	#1	#0	#1
I	1000	53	52	50	0.43	0.58	11.97	11.04	2562	2547
II	1000	53	60	100	0.46	41	12.89	13.96	2529	2501
III	1000	64	114	200	0.62	126	10.19	9.51	2641	2654
IV	1000	53	165	400	0.54	322	9.28	7.32	2601	2649
V	1000	50	209	800	0.59	649	9.64	4.22	2695	2566

Table 3. Characteristics of leachate from the WENT landfill site

Parameter	Value	Parameter	Value
Physical		Ammonia-N (as N)	4925 mg/L
Color	7800 Hazen (Peaty Brown)	Total PO_4^{3-}-P	16.3 mg/L
Odour	Slightly ammoniacal	Alk.(as $CaCO_3$)	13195 mg/L
pH	8.22	Chloride	3032 mg/L
Conductivity	37000 µS/cm	VFA	420 mg/L
Turbidity	4100 NTU	Potassium	3920 mg/L
Chemical		Sodium	2505 mg/L
Total COD	7439 mg/L	Calcium	13.7 mg/L
Soluble COD	6446 mg/L	Magnesium	93 mg/L
BOD_5	1436 mg/L	Iron	3.811 mg/L
TOC	2535 mg/L	Manganese	0.182 mg/L
TSS	784 mg/L	Nickel	0.365 mg/L
VSS	654 mg/L	Copper	0.120 mg/L
TDS	12352 mg/L	Zinc	1.155 mg/L
FDS	9420 mg/L	Chromium	0.553 mg/L
		Cadmium	0.103 mg/L
		Lead	<0.01 mg/L

Table 4. Summary of the average values at different NH_4^+-N levels

Reactors	pH (Med.)	Init. Sol. COD$^+$ (mg/L)	Init. Biod. COD^{++} (mg/L)	Resid. Biod. COD^{+++} (mg/L)	Initial NH_4^+-N (mg/L)	Resid. NH_4^+-N (mg/L)	Deh. Activity (μg/mg)	Final MLSS (mg/L)	SOUR (mgO_2/g MLSS)
#0	7.92	1000	1000	43	50	0.19	10.46	2477	70
#1	8.13	1065	1023	28	50	0.30	9.29	2467	68
#2	7.79	1131	1046	33	100	46	8.80	2554	64
#3	8.51	1262	1091	59	200	143	8.71	2521	65
#4	8.27	1524	1182	177	400	353	7.12	2583	56
#5	8.52	2047	1364	299	800	718	4.93	2564	45

Note: COD$^+$ = Glucose COD + COD measured in leachate

COD^{++} = Glucose COD + Biodegradable COD in leachate

COD^{+++} = Biodegradable COD in effluent

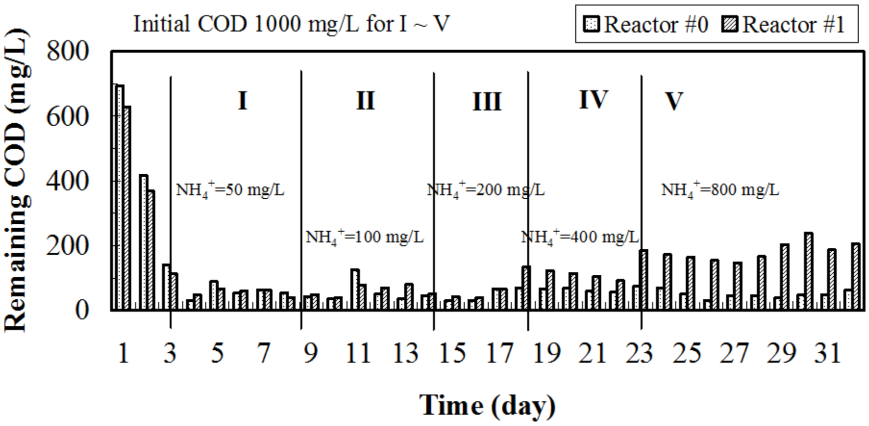


Fig. 1. COD reduction in the five phases

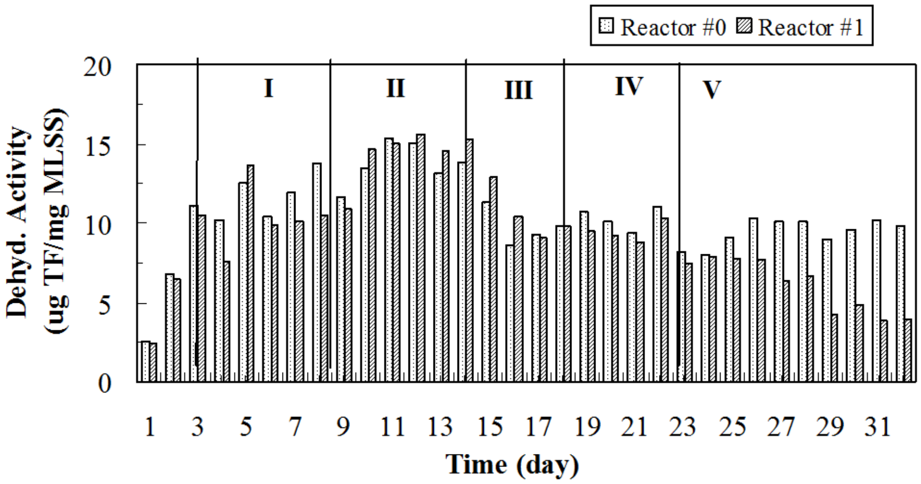


Fig. 2. Dehydrogenase activity of microbe in the five phases

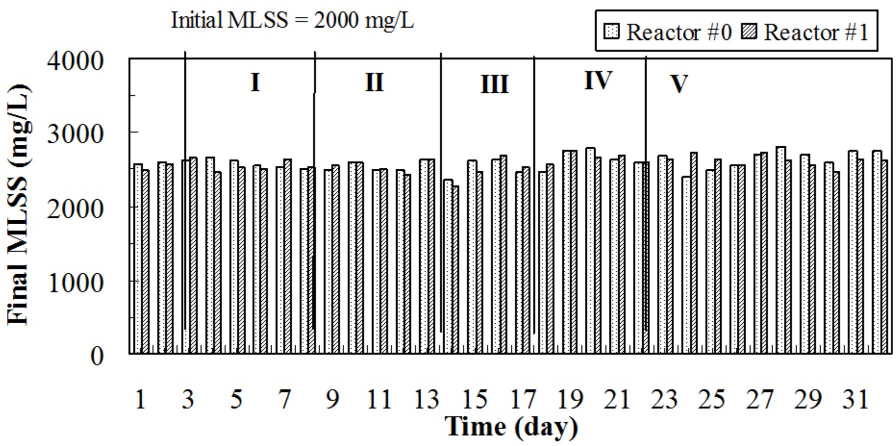
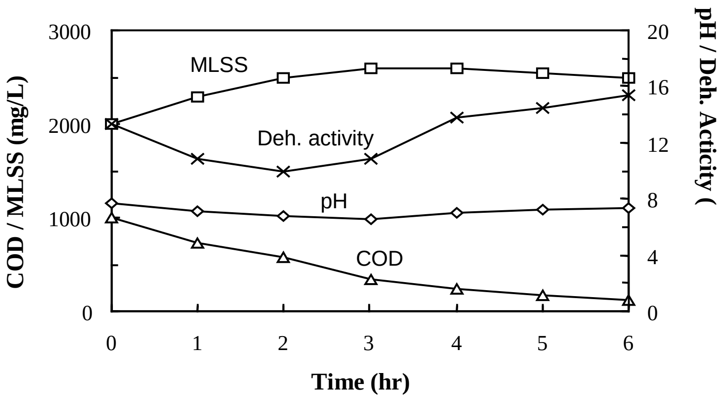
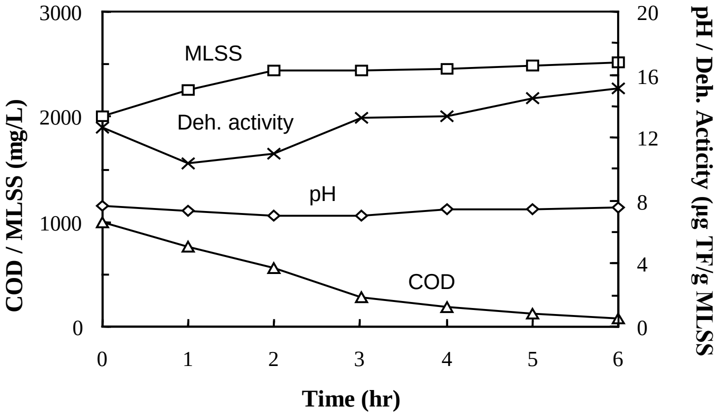


Fig. 3. Growth of activated sludge in the five phases



(a) COD:N:P=100:5:1 for Reactor #0



(b) COD: N: P=100:10:1 for Reactor #1

Fig. 4 Hourly change of COD, MLSS, pH and dehydrogenase activity in Phase II

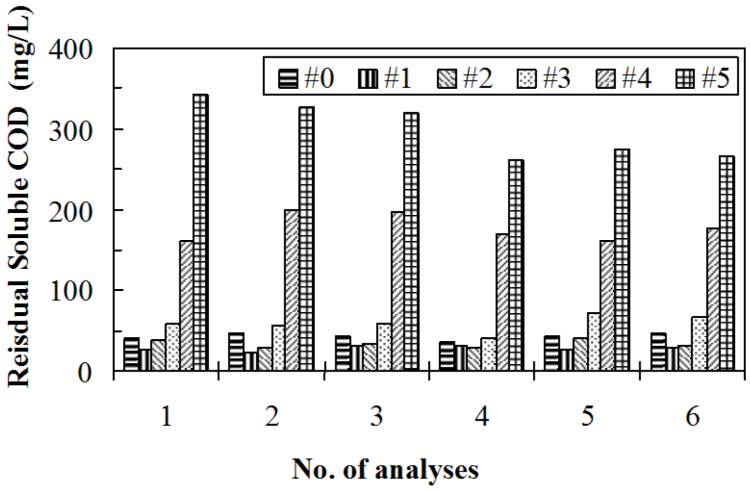


Fig. 5. COD reduction at different NH_4^+-N levels

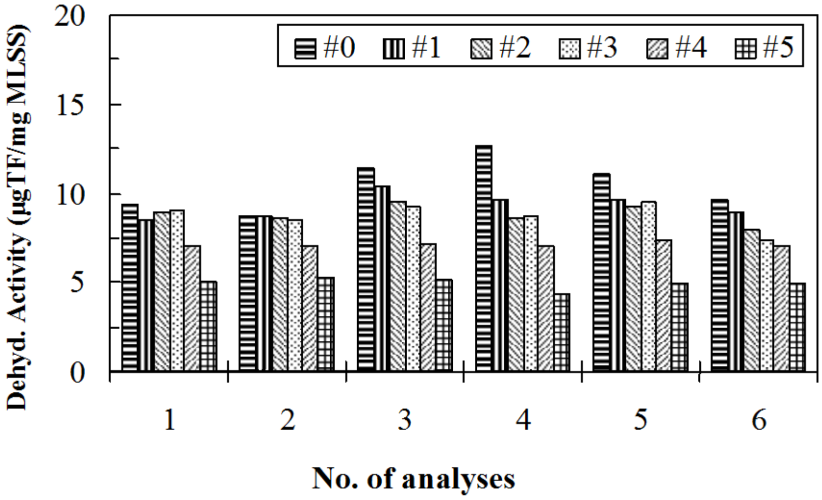


Fig. 6. Dehydrogenase activity of microbe at different NH_4^+-N levels

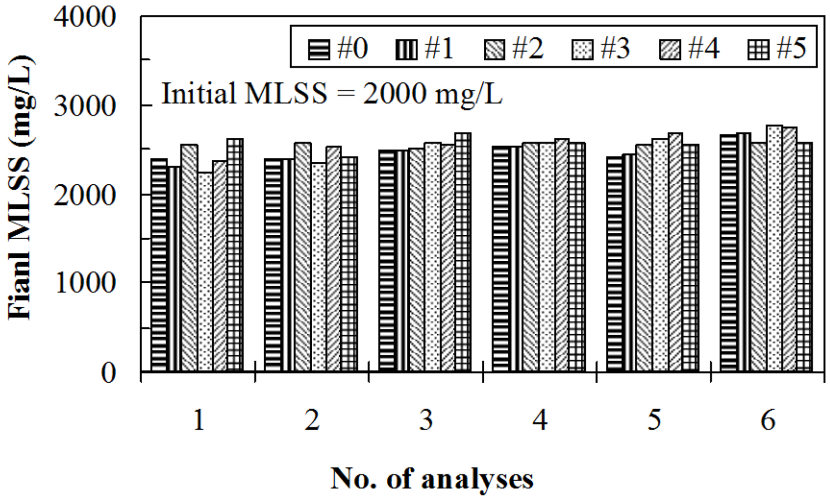


Fig. 7. Growth of activated sludge at different NH_4^+-N levels

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