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AN EXPERIMENTAL STUDY ON AEROBIC DENITRIFICATION WITH POLYVINYL ALCOHOL AS A CARBON SOURCE IN BIOFILMS

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ABSTRACT

Aerobic denitrification occuring in the biofilms attached to a partially submerged RBC, was investigated. Denitrification using polyvinyl alcohol (PVA) as a organic carbon source, was well proceeded by aerobic RBC systems at 25 ℃. At an influent C/N ratio of around 1.2, the maximum net-denitrification efficiency was about 78% at a TOC loading of 2g/m^2/d. In a chemostat experiment, aerobic denitrification was well proceeded under the dissolved oxygen concentration of 3 to 6 mg/L. The PVA-decomposing bacteria, nitrifiers, and denitrifiers co-existed in the biofilm, but the population of PVA-decomposing bacteria and denitrifiers in the surface layer was 1 to 2 orders of magnitude higher than those in the middle and bottom layers. It may indicate that the surface layer had a higher denitrifying activity. The nitrogen mass balance obtained using the experimental data clearly indicates a reasoning for aerobic denitrification.

KEYWORDS

Biofilm, aerobic denitrification, nitrification, PVA(Polyvinyl alcohol), phenol, nitrogen mass balance, bacterial population

INTRODUCTION

Biological nitrification-denitrification is the most reliable and cost-effective process for removing nitrogen from wastewater. Usually biological denitrification processes have been performed in separate reactors following carbon oxidation and nitrification. When biodegradable organic carbon is not present in sufficient amounts, carbon from an external source must be added. Methanol has commonly been used as a typical carbon source for this purpose. In recent years, some studies of external carbon sources for denitrification were conducted. Acetate produced in the acidification process of primary sludge may be available as a external carbon source(Ødegaard, H., 1993). Werner (1991) reported that denitrification with biogas as external carbon source is possible. Pesari *et. al.*, (1993) investigated about denitrification with nongrowth

substrate of nitroglycerin.

PVA(Polyvinyl alcohol), a water-soluble synthetic polymer, used widely as a textile sizing agent, synthetic-fiber and for pulp and paper processing has been known to be non-biodegradable substance. Microbiological studies on the biodegradation of PVA have also been carried out. Suzuki *et al.*, (1973) isolated the *Pseudomonas* species from PVA-containing soil and subsequently used the species for the treatment of PVA containing wastewater. Watanabe *et al.*, (1975) reported that the PVA-decomposing enzyme is red in color and has molecular weight of 30,000 and that aerobic condition was necessary for the decomposition of PVA.

Bang *et al.*, (1994) studied the phenomenon of simultaneous nitrification and denitrification occurring in the fully aerobic biofilm system treating the wastewater containing PVA and demonstrated that nitrogen losses were observed even under high dissolved oxygen concentration of above 5 mg/L. If denitrification can proceed even under aerobic conditions, nitrification and denitrification can be achieved simultaneously in a single reactor and thus the supply of external organic source will not be required as in the common two-stage process. In this paper we deal with the investigation of aerobic-denitrification in an aerobic biofilm and a chemostat reactor, where PVA is used as an organic source.

MATERIALS AND METHODS

Acclimation techniques of organism. PVA-decomposing bacteria was acclimated in the biofilm attached to the RBC. To induce the denitrifier under aerobic conditions, the organism was cultivated as follows; a part of biofilm attached to the RBC treating the municiple wastewater was removed then added into a bench scale fully aerobic RBC. Synthetic wastewater with PVA, phenol and ammonia nitrogen was fed into the RBC. This acclimation operation has been conducted for 2 years at a temperature of 25℃ or 30℃. The bacteria inhabited the biofilm was used in the study.

Experimental procedures. Fig.1 illustrates the schematic description of a partially submerged RBC used in this study. The RBC consisted of the disks made of poly-acryl plates. A part of each disk was removable for the measurement of the biofilm properties. The composition of the synthetic wastewater is shown in Table 1.

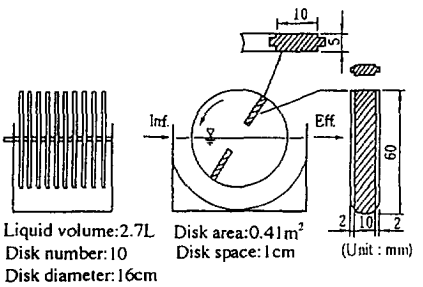

Fig.1 Process flow diagram and RBC design

Table 1 Composition of the synthetic wastewater

Constituents	mg/L
Polyvinyl alcohol	variable
Phenol	variable
$(NH_4)_2SO_4$	variable
KH_2PO_4	400
Na_2HPO_4	50
$CaCl_2$	10
$MgSO_4 \cdot 7H_2O$	180
$FeSO_4 \cdot 7H_2O$	10
NaCl	10
K_2HPO_4	500
$NaHCO_3$	variable
$MnSO_4 \cdot 7H_2O$	7

PVA used as an organic source was completely saponificated, which had 1,500 of the degree of polymerization with molecular weight of 66,000.

RBC experiments. Five series of experiments were performed with organic source of PVA and ammonia nitrogen under various TOC loadings which gave different biofilm thickness ranging between 85 μm and 3000 μm. Three of these series were conducted with phenol as the co-organic substrate. The temperature was maintained at 25℃ and the influent pH was kept at 7.6 to 7.8.

Chemostat experiments. In the chemostat experiments, the biofilm was homogenized then added into a chemostat reactor with an effective volume of 4.8L. Homogenized biomass was composed of PVA-decomposing bacteria, nitrifiers and denitrifiers. The aerobic condition of the reactor was kept by sparging with air and stirring. The reactor was operated for about 4 months under the following conditions; DO concentration =3 to 6 mg/L, HRT=2 days and influent wastewater pH=7.6 to 7.8.

Analytical methods. The concentration of PVA was measured by the absorbance of 650 nm with

iodine-color method proposed by Finley(1961). Nitrate-, nitrite- and ammonia-nitrogen were determined by the brucine sulfanilic acid method, Griess-Romijn method and Nessler method, respectively. TOC and phenol were determined by the TOC-Analyzer and 4-aminoantipyrine method, respectively. Determination of MLVSS was accomplished by filtration through 0.45 μm pore - size filters.

Table 2 Summary of experimental conditions

Exp. No.	Reactors	Oxygen condition	Oxygen supply	Temp. (℃)	Operation period(days)	HRT (hr)	TOC loading ($g/m^2/d$)	Organics and nitrgen source[d]	Film thickness (μm)
1	RBC	Aerobic	10rpm[a]	25	70	30	0.47	PVA(1)+ammonia(2)	85
2	RBC	Aerobic	10rpm	25	30	40	1.0	PVA(1)+phenol(3)+ ammonia(4)	380
3	RBC	Aerobic	10rpm	25	200	20	2.0	PVA(1)+phenol(3)+ ammonia(4)	2200
4	RBC	Aerobic	10rpm	25	120	10	4.2	PVA(1)+phenol(3)+ ammonia(4)	3000
5	RBC	Aerobic	10rpm	25	132	Variable	Variable	PVA(1)+ammonia(4)	NT[e]
6	Chemostat	Aerobic	Aeration[b]	30	120	48	Variable[c]	PVA(1)(5)+ammonia(2)	—

a); Rotating speed
b); The dissolved oxygen concentration was maintained at 3 and 6 mg/L
c); g TOC/gMLVSS/d of 0.51 and 0.69
d); Concentration(mg/L); (1)=200, (2)=50, (3)=200, (4)=80, (5)=400
e); Reused biofilm after exp. No.3, NT; not tested

Microscopic examination. For scanning electron microscopy(SEM), samples of biofilm were fixed with 2% glutaraldehyde solution for 1 hour. Dehydration was made in a graded ethanol series of 50%, 60%, 70%, 80%, 90%, 95% and 100% for 30 min for each step. After dehydration the samples were treated by critical-point drying. After all the dehydration steps, the samples were glued onto stubs and coated with gold for SEM examination.

Enumeration of bacterial population. The experimental apparatus consisted of the RBC with the disks made of poly-acryl plates. A part of each disk was removable for the measurement of the biofilm properties. The wastewater containing PVA and ammonia nitrogen was fed into it to develop the biofilm. The biofilm segments attached to the removable part of the disks were cut into slices with a thickness of 20-50 μm using a Micro-slicer (D.S.K Micro-slicer model DTK 1000), which was also used for measuring the biofilm thickness. The biofilm thickness was defined as the maximum height of a biofilm segment. The sliced biofilms were divided into three groups, depending on their appearance. The first group was the scraped biomass consisting of slices of the surface layer. The second group had less dense biofilm slices of the middle layer. The third group comprised dense biofilm slices of the bottom layer. Each group of sliced biofilms was homogenized. Then the bacterial population of aerobic heterotrophs, PVA-decomposing bacteria, nitrifiers and denitrifiers was measured by the most probable number(MPN) method. The count of PVA-decomposing bacteria was measured at an optical density of 600 nm with the MPN method. The medium of PVA-decomposing bacteria contained PVA 1g, $(NH_4)_2SO_4$ 0.15g, KH_2PO_4 0.2g, $CaCl_2$ 0.05g, $MgSO_4 \cdot 7H_2O$ 0.05g, $FeSO_4 \cdot 7H_2O$ 0.02g, NaCl 0.02g, K_2HPO_4 1.6g, yeast extract 0.5g, distilled water 1000mL.

RESULTS AND DISCUSSION

(1) Aerobic denitrification

RBC experiments. Fig.2 illustrates the efficiencies of the nitrification, overall nitrogen removal, PVA and TOC removal, in experiment No.1. Experiment No.1 was conducted using PVA as the sole organic carbon source at the influent C/N ratio of around 2.0. In this condition, biofilm did not grow very well and the film thickness was about 85 μm. Dissolved oxygen concentration was kept at above 5.5 mg/L. The

PVA and TOC removal efficiencies at steady-state were 99% and 89%, respectively. The overall nitrogen removal efficiency was 34%, although DO concentration was as high as 5.5 mg/L. These results demonstrate that oxidation of organics, nitrification and denitrification can be achieved simultaneously under aerobic conditions.

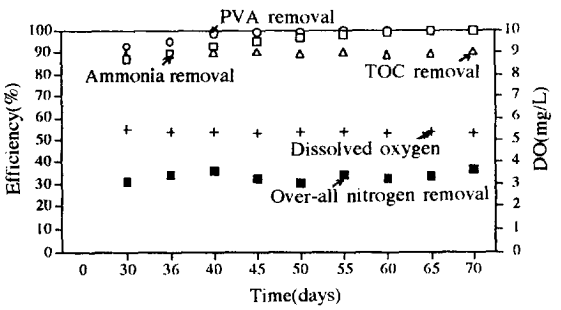

Fig.2 Performance with PVA under aerobic condition in experiment No.1

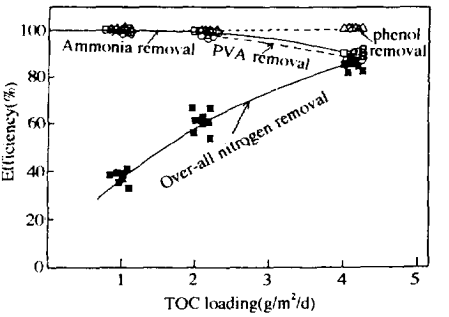

Fig.3. Effect of TOC loading on the efficiencies of PVA , phenol and nitrogen removal under aerobic condition in experiment Nos. 2,3 and 4

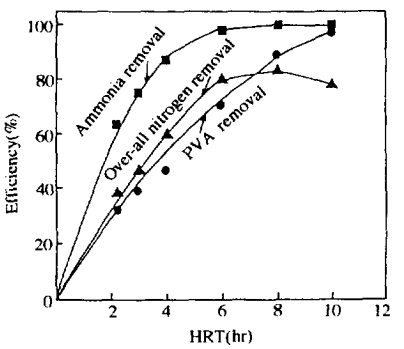

Fig.4. Effect of HRT on the efficiencies of PVA and nitrogen removal under aerobic condition in experiment No. 5 (Using PVA as a sole organic carbon source)

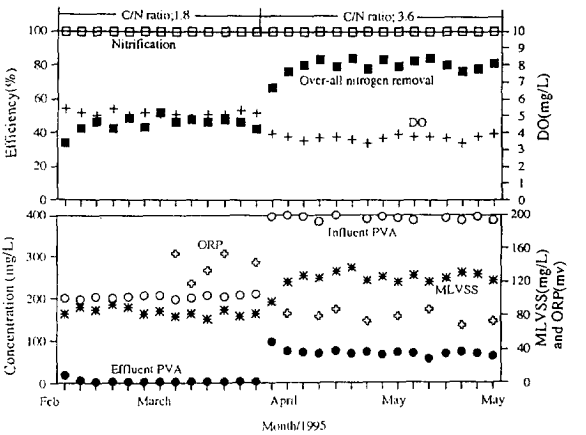

Fig.5 Reactor performance with PVA in the chemostat under aerobic conditions

Fig.3 illustrates the relationship between the TOC loading and the removal efficiencies of the nitrification, over-all nitrogen removal, PVA and phenol in experiment Nos. 2, 3 and 4. Since phenol was added as a co-substrate for the degradation of PVA, the biofilm growth was very well and biofilm thickness reached to 380 μm - 3000 μm, which was much thicker than that of experiment No.1. The efficiency of over-all nitrogen removal increased from 38% to 84% as the TOC loading rate was increased from 1g/m^2.d to 4.2g/m^2.d, while PVA-removal and nitrification efficiencies were above 90% throughout all the experiments. After experiment No.3, the organic carbon source was changed to PVA only. The biofilm thickness decreased notably, but later on became stable. Fig.4 illustrates the relationship between the HRT and the efficiencies of the nitrification, overall nitrogen and PVA in experiment No.5. The removal efficiencies of PVA removal and nitrification increased with increasing HRT, and reached to the levels of 95%, and 99%, respectively at the HRT of 10 hours, where over-all nitrogen removal efficiency reached to its maximum value of 78% at a HRT of 8 hours, corresponding to TOC loading of 2g/m^2/d. These results indicate that PVA could be used as a sole electron donor for denitrification and that the optimum HRT for the simultaneous removal of PVA and nitrogen was between 8 and10 hours.

Chemostat experiments. The chemostat experiment was conducted where PVA was the sole organic

carbon source(experiment No. 6). In this experiment, the influent C/N ratio was first kept at 1.8 for 2 months, and then changed to 3.6. The MLVSS and DO concentration were changed from 80 to136 mg/L, and from 6 to 3 mg/L with the increase in the C/N ratio from 1.8 to 3.6, respectively. The suspension of organism was strongly agitated by continuous stirring (stirrer speed> 600rpm). Because of the above mentioned experimental conditions, the floc formation did not occur in the suspension during the chemostat experiment. Fig.5 illustrates the efficiencies of the nitrification, over-all nitrogen removal, organics removal, and water quality of the influent and effluent. Although the DO concentration was higher than 3.0, over-all nitrogen removal efficiency was 45% at the C/N ratio 1.8, and 84% at the C/N ratio 3.6, respectively.

(2) Bacterial populations

Table 3 summarizes the population of the PVA-decomposing bacteria, nitrifiers, and denitrifiers in the biofilms in experiment No. 2. Bacteria counts are expressed by the number per unit mass of total solids. The biofilm was grown with PVA, phenol and ammonia nitrogen at a TOC loading of 1.0 g/m^2.d. In the steady state condition, the biofilm thickness reached to about 380 μm. Then biofilm was gently scraped from the plates of RBC. The surface area of the plates, used for the experiment described here, was 1.2 cm^2. In this experiment bacterial population for the entire thickness of the biofilm was determined without slicing the biofilm layers because the biofilm thickness was only 380 μm. The biofilm was homogenized by the sonic of 20W for 1 min before enumeration. Fig 6 shows the population of the PVA-decomposing bacteria, nitrifiers, and denitrifiers in the biofilms of experiment No. 3. In this experiment, the thickness of the biofilms was about 2200 μm. PVA-decomposing bacteria, nitrifiers, and denitrifiers co-existed in the biofilm, but the populations of PVA-decomposing bacteria and denitrifiers in the surface layer was 1 to 2 orders of magnitude higher than those in the middle and bottom layers, indicating that the surface layer had a higher denitrifying activity.

Table 3 Bacterial composition of biofilm in experiment No. 2

Constituents	Bacteria numbers(MPN/mg/SS)
PVA-decomposing bacteria	5.98 X 10^7
Nitrate reducing bacteria	3.99 X 10^6
Denitrifiers	2.73 X 10^7
Ammonium oxidizing bacteria	1.97X 10^7
Nitrite oxidizing bacteria	8.72X 10^7

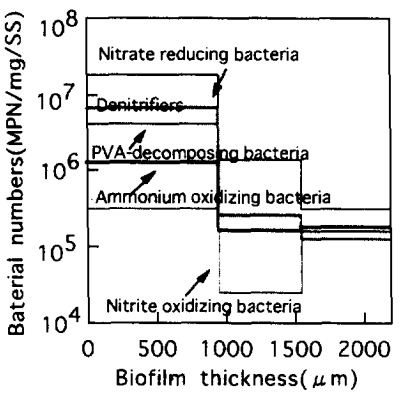

Fig. 6 profiles of intrinsic various population in the RBC in exp. No.3

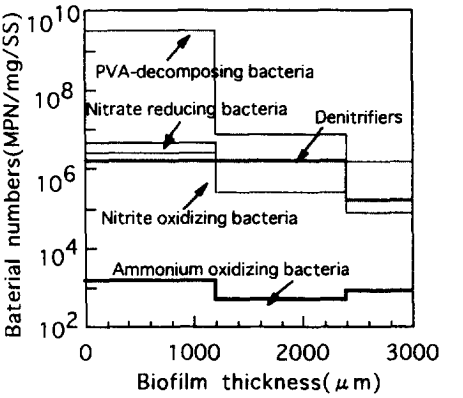

Fig. 7 profiles of intrinsic various population in the RBC in exp. No.4

Fig.7 illustrates the bacterial populations in experiment No.4 under steady-state condition at the TOC

loading of 4.2 g/m^2.d. The biofilm thickness reached to approximately 3000 μm. The PVA-decomposing bacteria, nitrifiers, and denitrifiers co-existed in the biofilm, but the population of PVA-decomposing bacteria and denitrifiers in the surface layer was 1 to 2 orders of magnitude higher than that in the middle and bottom layers. The thickness and density of each layer of the sliced biofilms are summarized in Table 4. The biofilm density is expressed in TSS basis. The thickness and density of the surface layer were about 1600 μm and 35.5 mg/cm^3, respectively. The thickness and density of the middle layer were about 800 μm and 41.0 mg/cm^3, respectively. That of the bottom layer were about 450 μm and 53.5 mg/cm^3, respectively.

Table 4 Variation of biofilm density with biofilm depth

	Depth from the biofilm surface in μm	Density in mg/cm^3
Surface layer	0-1600	35.5
Middle layer	1600-2400	41.0
Bottom layer	2400-2850	53.5

(3) Morphological observation of organism retained in RBC Fig.8 showes the microcolonies in the surface layer of biofilms under SEM. SEM photos illustrate that microcolonies were composed of the PVA-decomposing bacteria, nitrifier and denitrifier in the same biofilm.

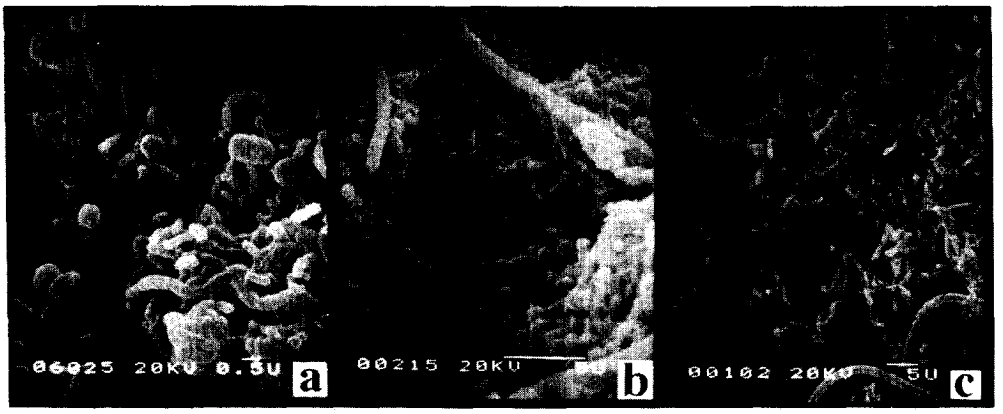

Fig.8 A microcolonies in the surface layer of biofilms under SEM
a; Structure of microcolonies at experiment No.1 (bar=0.5 μm)
b and c ; Structure of microcolonies at experiment No.2 (bar=5 μm)

(4) Nitrogen mass balance

In order to confirm the experimental results reported in the previous part, we made a nitrogen mass balance using the chemostat reactor. Table 5 summarizes the nitrogen mass balance in the experiment using PVA as a sole organic carbon source. For the estimation of net-denitrification amount, the biomass-yield was estimated using the chemostat experiment data under steady-state.
Because the biomass(VSS) was composed of two groups of bacteria, heterotropic bacteria(X_1) and autotropic bacteria(X_2), the following relations exist;

Y_h (heterotropic bacteria yield) = mg VSS_{X1} formed/ mg PVA removed ------(1)

Y_n (autotropic bacteria yield) = mg VSS_{X2} formed/ mg NH_4-N oxidized ----(2)

According to the data obtained in this study, the growth yield of PVA-decomposing heterotropic bacteria , Y_h, was determined as 0.37mg VSS / mg PVA removed. The growth yield of nitrifying autotropic bacteria, Y_n, was reported to be 0.20 mg VSS / mg NH_4-N oxidized(by Tchobanoglous *et. al*, 1991).
Using these parameters, the nitrogen amounts used for bacterial growth were calculated to be 18.1% at the C/N ratio of 1.8 and 30.51% at the C/N ratio of 3.6, respectively. The net-denitrification percentage was, therefore, estimated to be 37.8% at the C/N ratio of 1.8 and 52.1% at the C/N ratio of 3.6, respectively.

Table 5 Nitrogen mass balance in the experiment using PVA as a sole organic carbon source in the chemostat reactor

C/N ratio	HRT (d)	Input NH_4-N (%)	Output NH_4^+ (%)	NO_2^- (%)	NO_3^- (%)	N in biomass (%)	Net-denitrification (%)
1.8	2	100	0	32.0	12.1	18.1	37.8
3.6	2	100	0	13.8	3.6	30.5	52.1

Table 6 summarizes the nitrogen mass balance in the experiment where organic source is PVA and phenol, or PVA only in the RBC. The net-denitrification percentage was estimated using the biomass-yields for PVA and phenol decomposing heterotropic bacteria which were reported to be 0.298 mg VSS / mg PVA removed(by Hasimoto *et. al*, 1980) and 0.600mg VSS / mg phenol removed(by Pawlowsky *et. al*, 1973),respectively. Using these parameters, the nitrogen percentage used for bacterial growth and net-denitrification were calculated as shown in Table 7. These results of Tables 6 and 7 show that the denitrification can proceed even under aerobic conditions.

Table 6 Nitrogen mass balance in experiments using PVA and phenol (1) and PVA only (2) in the RBC

(1)

Exp.No.	TOC loading (g/m2.d)	Input NH4-N (%)	Output NH_4^+ (%)	NO_2^- (%)	NO_3^- (%)	N in biomass (%) a*	b*	total	Net-denitrification (%)
2	1.0	100	0.0	14.3	45.8	9.5	19.1	28.6	11.3
3	2.0	100	1. 2	28.2	9. 2	9.5	19.1	28.6	32. 8
4	4. 2	100	16.4	0.5	0. 1	8.6	19.1	27.7	55.3

(2)

Exp. No.	HRT (hr)	Input NH_4-N (%)	Output NH_4^+ (%)	NO_2^- (%)	NO_3^- (%)	N in biomass (%)	Net- denitrification (%)
1	30	100	0.0	40.2	25.8	14.8	19. 2
5	2	100	36.4	22.6	2.1	9.0	29.9
5	3	100	24.1	26.8	2.7	8.4	38.0
5	4	100	13.0	23.9	2.9	6.8	53.4
5	6	100	1.5	15.2	4.9	4.4	74.0
5	8	100	0.0	12.4	6.1	3.7	77.8
5	10	100	0.0	11.4	12.0	3.2	73.4

a*: PVA- decomposing bacteria, b*: phenol -decomposing bacteria

(5) Proposed mechanism of PVA degradation and denitrification.

Based on the results of this study, the possible metabolic pathway in the PVA degradation and aerobic denitrification may be illustrated as shown in Fig.9. From this pathway,The breakdown of PVA, is consider to take place stepwise via the intermediate materials with ketone, carboxyl and methyl radical produced by the PVA-decomposing bacteria. The intermediates produced through the PVA degradation may be used by the denitrification as the carbon source under aerobic and anoxic conditions. In this study, we have shown the persistence of aerobic denitrification at higher dissolved oxygen concentrations by both the biofilm and chemostat experiments. The hypothesis (**a**) assumes that bacteria used either oxygen or nitrate(or nitrite), and the presence of oxygen automatically excluded the possibility of denitrification . The hypothesis (**b**) and (**c**) assume that the existence of organisms with the ability of aerobic denitrification. These hypotheses can explain that some denitrifiers do not lock up " denitrifying ability" even under aerobic condition. Several researchers have reported that the existence of organisms with the ability of aerobic denitrification (Robertson and Kuenen, 1984 ; Krul , 1976).

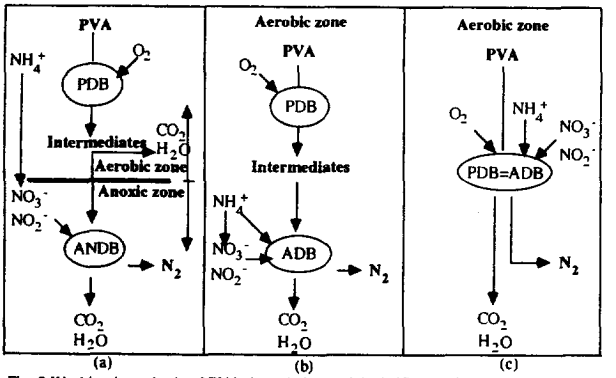

Fig. 9 Working hypothesis of PVA degradation and denitrification in aerobic biofilms
PDB; PVA-decomposing bacteria, ADB; Aerobic denitrifying bacteria,
ANDB; Anaerobic denitrifying bacteria

CONCLUSIONS

This study provided quantitative reasoning for the phenomenon of aerobic denitrification occurring in the fully aerobic biofilm treating the wastewater containing PVA and ammonia nitrogen. The DO concentrations higher than 3.0 mg/L did not prevent the denitrification from the treatment system. PVA can be used for denitrification as a sole carbon source. For many years the evidence of the aerobic denitrification has been reviewed. However, most of their studies focus on "aerobic denitrification" by the pure culture, but in this paper we dealt with the investigation of aerobic denitrification in the mixed culture.

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