

Volatile fatty acids carbon source for biological denitrification

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Abstract— In the suspended sludge system, when pH and temperature were suitable and carbon source was not limited, the denitrification and C-uptake rate followed a zero-order reaction. Mixed VFA C-source had higher denitrification rate than single VFA C-source consisting of them. When VFA were used as carbon source, consumed carbon quantity for denitrification was closed to 1.07 mgVFA-C/mg NO_3^- -N. About 20% of the applied C/N was used for assimilative purposes. As rising up influent C/N by increasing the C load, the sludge yield Y_N increased. The part of carbon consumption increased and the effluent oxidized nitrogen decreased. At pH 7.5, 25°C and sludge yield 0.72 mgVSS/mg NO_3^- -N, the calculated influent VFA-C / NO_3^- critical value was 1.43 for complete denitrification.

Keywords: biological denitrification; suspended sludge; volatile fatty acids(VFA); nitrate; nitrite; C/N ratio.

1 Introduction

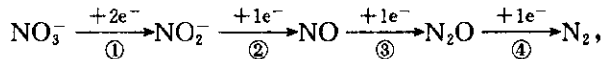
Increased nitrate concentration in public water supplies is becoming an important problem in some parts of Europe, nitrate concentration in ground water reached serious levels 200 years ago and have continued to increase. Nitrate is a cause of an inorganic nutrient for algae growth and can represent a danger to public health if present in excess in drinking water. It was found that the nitrate can cause infant methemoglobinemia (blue baby syndrome). Methemoglobinemia occurs when nitrite which is found in the stomach from ingested nitrate, reacts with hemoglobin in blood, converting hemoglobin into methemoglobin, which cannot carry oxygen to cell tissue. Nitrates may also lead to the possible formation of nitrosoamines, which are known carcinogens. In addition, it has been reported that nitrate have caused heart and behavioral problems in laboratory animals. The United States, Canada, the European Economic Community (EEC) and the World Health Organization (WHO) have set maximum acceptable limits for nitrate in drinking water to protect their constituent

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cies. The drinking water standard set by the U. S. Environmental Protection Agency is 50 mg/L as nitrate (11.3 mg/L as nitrate nitrogen).

Available treatment alternative for nitrate pollution control and treatment include anion exchange, reverse osmosis, biological denitrification, electro dialysis, distillation and possibly chemical reduction. A comparison of these alternatives concluded that only the first three processes are economically viable. Compared with anion exchange, biological denitrification offers some advantages. The polluting nitrate is actually removed, not only concentrated. It is converted into nitrogen gas which does not danger to health.

Biological dissimulatory denitrification occurs when nitrate is used instead of oxygen as a terminal electron acceptor. The circumstances affecting the onset of denitrification and the extent to which the process proceeds are complex and variable. There are few general rules for which exceptions have not been found. Generally, denitrification (dissimilatory reduction of oxidation state N) is considered to be an anoxic process, occurring in the presence of nitrate and the absence of molecular oxygen. The process proceeds through a series of four steps, from nitrate to molecular nitrogen gas:



where, 1 is nitrate reductase; 2 is the nitrite reductase; 3 is the nitric oxide reductase; 4 is the nitrous oxide reductase.

Surprisingly, most of the organisms known to denitrify are not strict, but facultative anaerobes organisms, which under anoxic conditions use nitrate as a final electron acceptor. Bacterial genera that are known to contain denitrifying species include (Yull-Rhee, 1978; Payne, 1976; *Achromobacter*, *Alcaligenes*, *Bacillus*, *Chromobacter*, *Corynebacterium*, *Halobacterium*, *Methanomonas*, *Moraxella*, *Paracoccus*, *Propionibacterium*, *Pseudomonas*, *Spirillum*, *Thiobacillus* and *Xanthomonas*).

Denitrification requires a carbon source for energy and cell synthesis. A wide variety of organic compounds has been used, such as alcohols, organic acids, carbonhydrates, amino acids. The most common carbon sources for the removal of nitrogen are methanol and ethanol (Timmermans, 1983; MacDonald, 1990; Hamon, 1991; Hanaki, 1989; Polprasert, 1986; Nurse, 1980) and acetic acid has been shown to give high reaction rates (Christensen, 1977; Lewandoswki, 1982).

It was reported (Monteith, 1980) that there were specific different C/N ratio for each kind of carbon source. If there is a limitation of the carbon source, denitrification would not be completed and nitrogen removal would not be succeeded. On the contrary, if the C concentration is too high, the excess would run off in the effluent. It would decrease effluent quality and increase the treatment costs. For this reason the influence of the C/N ratio on denitrification was examined in order to determine its optimal value when pure VFA of digested sludge supernatant area used as carbon source for denitrification.

2 Materials and methods

2.1 Reactors

This study was carried out in completely mixed laboratory scale reactors. The pH was automatically controlled by adding HCl or NaOH. The temperature was controlled with thermostatic bath. The sludge retention time was always equal to 5 days. The sludge was stirred with mechanical mixers at a rate sufficient to keep the micro-organisms in suspension. The reactors were sealed and bubbled with nitrogen gas for five minutes every day. The schematic of the reactor is shown in Fig. 1.

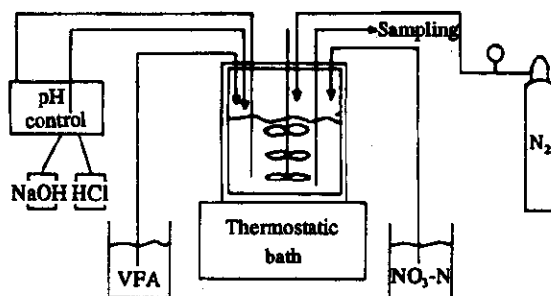


Fig. 1 The schematic of the reactor

2.2 Substrate

The sludge was supplied continuously with mixed VFA which consisted of formic, acetic, propionic, butyric and valeric acid in a ratio of 1:6:5:4:2 according to the TOC theoretical value. Phosphate was added to the reactor with an applied P/C ratio of 0.01. VFA and nitrate, phosphate stock solutions were diluted with tap water in order to provide trace elements essential for microorganisms. The carbon sources and NO_3^- -N were added separately using volumetric pump.

2.3 Analytical methods

2.3.1 Total suspended solids and volatile suspended solids determination

The sludge sample was dried in a silica crucible at 105°C for 24 hours and when weighed (suspended solids, SS). The sample was then burned at 550°C for two hours. The concentration of volatile suspended solids or volatile matter (VSS) was contained by calculating the difference between the total suspended solids and the weight after incineration.

2.3.2 Measurement of total organic carbon (TOC)

After samples were centrifuged (18000r/min) and filtered ($0.22\mu\text{m}$) the TOC was measured using a TOC meter (Beckman 915 B) in the liquid phase.

2.3.3 Determination of volatile fatty acids (VFA)

After centrifugation (18000r/min) and filtration ($0.2\mu\text{m}$), formic acid was added ($10\mu\text{m}$ formic acid per ml sample). The VFA concentration was determined by gaseous-phase chromatography (Carlo Erba VEGA 6300) in the soluble phase of the sludge. The two-meter glass column was filled with chromosorb. The vector gas was helium. The flame ionization sensor and the injector were at a temperature of 250°C . The temperature of the oven increased during the analysis from 120 to 140°C at rate of 2°C per min.

The VFA measured were acetic, propionic, butyric and valeric acids.

2.3.4 Measurement of nitrogen content

Nitrogen concentration was determined with the cadmium reduction method; Nitrite was determined with the ferrous sulphate methods; Ammonia was determined by the Nessler method.

2.4 Semi-continuous test

Semi-continuous test means that the substrates were added continuously, but effluent was drawn out once a day. Seeding sludge came from a pilot scale denitrification system. With a reactor valid volume equal to 5L, a sludge age of 5 days, a volumetric loading of 0.60 $\text{gNO}_3^- \text{-N}/(\text{L} \cdot \text{d})$ and 0.90 $\text{g VFA-C}/(\text{L} \cdot \text{d})$. Every day, 300 ml VFA stock solution (15 gVFA-C/L) and 200 ml tap water were mixed then 300 ml nitrate stock solution (10 $\text{g NO}_3^- \text{-N/L}$), 15 ml P stock solution (3 $\text{g PO}_4^{3-} \text{-P/L}$) and 185 ml tap water were mixed and were added separately by using volumetric pump. In order to keep a 5 days sludge age, 1 L sludge were removed per day. When different C/N were studied, the semi-continuous culture was run more than 10 days (2 times the sludge age). Then $\text{NO}_3^- \text{-N}$, $\text{NO}_2^- \text{-N}$, TOC, VFA, and SS, VSS of sludge were measured every day. Sludge yield and C, N distribution were calculated.

2.5 Batch test

In batch test, substrates were added at the beginning of the test. The tested sludge came from semi-continuous culture. If batch test reactor valid volume equal to 1 L, at the beginning of the test 15–25 ml VFA stock solution (15 gVFA-C/L), 15 ml nitrate stock solution (10 $\text{gNO}_3^- \text{-N/L}$) and 3 ml P salt stock solution (3 $\text{g PO}_4^{3-} \text{-P/L}$) are added. Temperature and pH are kept constant and sampling is realisted at constant interval for 2–7 hours until the nitrate concentration is constant. As soon as samples were collected, sulphuric acid (98%) was added to stop denitrification (for 10 ml sample, 0.05 ml acid was added). They are centrifuged (18000r/min) and filtered (0.22 μm) for measuring $\text{NO}_3^- \text{-N}$, $\text{NO}_2^- \text{-N}$, TOC and VFA. At the beginning and end of the batch test, the same amount of suspended solid was sampled and mixed for the determination of SS and VSS. Denitrification rates are calculated as the mass of nitrate or total oxidated nitrogen (nitrate + nitrite) removed per mass of VSS per day. In cases where the nitrate reduction curve shows a distinct decrease in rate, the first part of the line is assumed as the correct denitrification rate. The second part of the curve (with slower rate) is assumed to be a carbon-limiting situation and is disregarded for rate calculation. A uptake rate is calculated with the same method. When nitrite production is significant, the denitrification rate is calculated by subtracting the nitrite production rate from the measured nitrate removal rate.

2.6 Research procedure

2.6.1 Optimum C/N

Optimal C/N study is examined with a semi-continuous system. Temperature and pH are kept constant at 25°C and 7.5. Influence C/N is 1.0, 1.2, 1.3, 1.4 and 1.5 by increasing C load. It is to say, at C/N 1.0, 1.2, 1.3, 1.4 and 1.5, C volumetric loads are 0.60, 0.72, 0.78, 0.84 and 0.90 $\text{g VFA-C/L} \cdot \text{d}$ separately, but N load is always kept at 0.60 g

$\text{NO}_3^- \text{-N} / (\text{L} \cdot \text{d})$. After 15 days (3 times the sludge age), effluent $\text{NO}_3^- \text{-N}$, $\text{NO}_2^- \text{-N}$, TOC and SS, VSS of sludge are measured every day. Sludge yield and C, N distribution are calculated.

2. 6. 2 The influence of different carbon source on denitrification rate and carbon uptake rate

The tested sludge came from semi-continuous culture acclimated at pH 7.5, 25°C, influent C/N 1.5, using VFA as carbon source more than 15 days and washed twice with 0.03 mol/L phosphate buffer (pH 7.5). Briefly, 1 L sludge first was centrifuged (5000 r/min). Supernatant is discarded. Sediment sludge is mixed with 0.03mol/L phosphate using magnetic mixer. Then the sludge was translated into a batch reactor to examine denitrification rate and C uptake rate. For 1 L reactor, tested C substrate; mixed VFA (10gVFA-C/L stock solution 20-25ml), acetic acid (0.8 ml, pure), propionic acid (0.7ml, pure), butyric acid(0.6ml, pure), valeric acid(0.5ml, pure), methanol (0.8ml, pure), ethanol (0.8 ml, pure) were added separately. Nitrate (10g $\text{NO}_3^- \text{-N/L}$ stock solution, 15ml) and P salt (3g $\text{PO}_4^{3-} \text{-P/L}$ stock solution 0.75ml) and suitable amount of tap water were added at the same time. To prevent C limitation, C/N of substrate was between 2.0-3.0. Denitrification rate and C uptake rate were measured. Observed C/N is calculated by dividing C uptake rate/denitrification rate. For examining endogenous denitrification, no C source is added except N and P.

3 Results and discussion

3. 1 Denitrification rate

Using batch studies, we examined denitrification rate in various conditions. For a typical experiment in non-carbon limiting condition, the linear part of the curve of nitrate concentration versus time was used to calculate the nitrate removal rate and that value was divided by VSS concentration for calculation to the denitrification rate. Results are shown in Fig. 2 and Fig. 3.

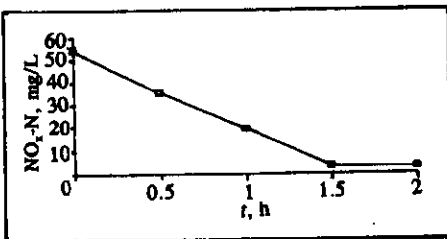


Fig. 2 Batch denitrification under non-carbon limiting
 $T = 20.6^\circ\text{C}$, $\text{pH} = 8.0$, $\text{VSS} = 0.639\text{g/L}$

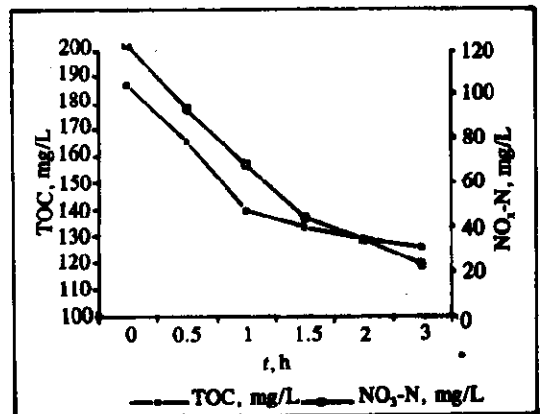


Fig. 3 Batch denitrification under carbon-limiting
 $T = 25^\circ\text{C}$, $\text{pH} = 7.5$, $\text{VSS} = 0.376\text{g/L}$

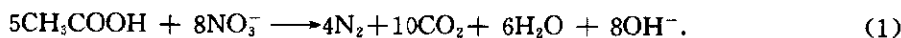
In suitable environmental condition (pH, temperature and non-carbon limiting, initial $C/N > 1.5$), it was found that the denitrification rate did not relate to substrate concentration except when the NO_3^- -N concentration was too low. In these conditions, the denitrification rate of a suspended culture could be considered as a zero-order reaction with respect to the nitrate concentration, as concluded by authors (Beccari, 1983; Abufayed, 1986; Christensen, 1977).

In carbon-limiting conditions, at the beginning, denitrification rate was as high as that of non-carbon-limiting condition. With denitrification reaction going on, carbon was exhausted, the denitrification rate reduced rapidly. So its kinetics did not seem to be a zero-order reaction.

3.2 Optimum C/N

In biological denitrification system, carbon source can be used as hydrogen donor for dissimilatory nitrate reduction, for bacterial accimulatory synthesis, for deoxygenation.

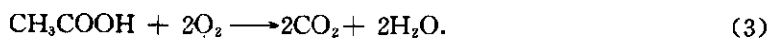
When acetate was used as carbon source, the dissimilative part of the process can be expressed by the following equation (Frick, 1985);



According to this equation, the acetate consumption is 1.07 mg CH_3COO^- -C per mg NO_3^- -N reduced to nitrogen gas. If oxygen is taken into consideration, the total acetate consumption for dissimilative purpose (A_d) is;

$$A_d = 1.07C_{\text{NO}_3} + 0.375 C_{\text{O}_2}, \quad (2)$$

where C_{NO_3} is the nitrate concentration in influent; C_{O_2} is the dissolved oxygen in influent; 0.375 is the carbon consumption coefficients when acetate is oxidized by oxygen (mg CH_3COO^- -C/mg O_2).



Acetate consumption for assimilation (A_a) is :

$$A_a = 0.5 Y_{\text{NO}_3} \cdot C_{\text{NO}_3}. \quad (4)$$

Where, Y_{NO_3} is the sludge yield mg VSS/mg NO_3^- -N; 0.5 is the fraction of carbon in VSS.

Nitrate consumption for assimilation (N_a) is expressed as follows;

$$N_a = 0.1 Y_{\text{NO}_3} \cdot C_{\text{NO}_3}. \quad (5)$$

where, 0.1 is the fraction of nitrogen in VSS.

Total stoichiometric acetate consumption in denitrification is;

$$A = 1.07C_{\text{NO}_3} + 0.375C_{\text{O}_2} + 0.5Y_{\text{NO}_3} \cdot C_{\text{NO}_3}, \quad (6)$$

In our test, dissolved oxygen = 0, so $C_{\text{O}_2} = 0$.

$$A = 1.07C_{\text{NO}_3} + 0.5Y_{\text{NO}_3} \cdot C_{\text{NO}_3} = C_{\text{NO}_3}(1.07 + 0.5 Y_{\text{NO}_3}). \quad (7)$$

When sludge yield equals zero, the consumption of acetate can be calculated; $A = 1.07$ mg CH_3COO^- -C/mg NO_3^- -N. When sludge yield equals 0.4 mg VSS /mg NO_3^- -N, then 0.20 mg C and 0.04 mg N are assimilated for every 1 mg NO_3^- -N removal respectively. Total consumption of acetate is ; $A = C_{\text{NO}_3}(1.07 + 0.2) = 1.27$ mg CH_3COO^- -C/mg NO_3^- -N.

When sludge yield equals 0.8 mg VSS/mg NO_3^- -N then 0.40 mg C and 0.08 mg N are

assimilated for every 1 mg NO_3^- -N removed respectively. Total stoichiometric amount of acetate $A=1.47$ mg CH_3COO^- -C/mg NO_3^- -N. From these calculations, we can see, in denitrification unit, with sludge yield increases, total carbon consumption also increases.

Through semi-continuous studies, we respectively examined the influence of C/N 1.0, 1.2, 1.3, 1.4, 1.5 on denitrification. Results are summarized in Table 1 and 2.

Table 1 Effluent NO_3^- -N, NO_2^- -N, TOC and sludge yield (Y_N) in different influence C/N condition. pH = 7.5, $T=25^\circ\text{C}$, N volumetric load = 0.60gNO_3^- -N/(L · d)

Influent C/N	NO_3^- -N, mg/L	NO_2^- -N, mg/L	TOC, mg/L	VSS, g/L	Y_N , mgVSS/mg NO_3^- -N
1.0	96.0	0.3	48	1.458	0.485
1.2	19.0	5.0	119	1.802	0.600
1.3	10.6	1.2	104	1.940	0.657
1.4	0.6	0.1	162	2.145	0.715
1.5	0.0	0.0	395	2.185	0.728

Table 2 C and N distribution in different influent C/N condition pH=7.5, $T=25^\circ\text{C}$, sludge volume=5L, N volumetric load= 0.60gNO_3^- -N/L · d

C/N	C distribution, gC/d				N distribution, gN/d				DN C/N
	Influent	Wasted sludge	Effluent	DN	Influent	Wasted sludge	Effluent	DN	
1.0	3.0	0.730	0.048	2.22	3.0	0.150	0.096	2.75	0.81
1.2	3.6	0.909	0.119	2.57	3.0	0.180	0.024	2.79	0.92
1.3	3.9	0.970	0.104	2.83	3.0	0.190	0.012	2.79	1.01
1.4	4.2	1.076	0.162	2.98	3.0	0.220	0.001	2.78	1.07
1.5	4.5	1.093	0.395	3.01	3.0	0.220	0.000	2.78	1.08

In Table 1 and Table 2, C, N distribution means C (or N) amount which enter in and get out the reactor. For example, when influent C/N was 1.5, from the added 4.5g VFA-C (TOC theoretical value), 1.093 g C was used to produce biomass (wasted sludge), 0.395 g C was discharged in the effluent and 3.01 gC were consumed by denitrification. According to C (or N) balance, DN-C (or DN-N) represents VFA-C (or NO_3^- -N) used for denitrification were determined by calculation. They are equal to influent C (or N)-effluent C (or N)-wasted sludge C (or N). DN C/N represents the VFA-C/ NO_3^- -N ratio which is used for denitrification and is equal to DN-C/DN-N. Microbial growth was described by the sludge yield Y_N (mgVSS per mg nitrogen removed) which represents sludge production per unit nitrogen removed.

From Table 1, 2 and Fig. 4, we can see that when the influent C/N was increased, the effluent oxidized N cut down. When influent C/N was higher than 1.3, the residual oxidized N was closed to zero. The sludge yield Y_N in these conditions was 0.72 mgVSS/mg NO_3^- -N

and from Equation (7) the total VFA consumption was equal to 1.43 (0.86 gC/(L · d) divided by 0.60gN/(L · d)). This calculated C/N ratio agrees with the results of Dahab *et al.* (Dahab, 1988) and Narkis *et al.* (Narkis, 1979), who found respectively C/N ratio equal to 1.45 and 1.35 with acetic acid as a carbon source.

However, when the influent C/N was increased, the amount of residual organic C increased too. At the same time, sludge yield (Y_N) and the carbon load used for assimilation both increased (Fig. 5). This result is in good agreement with Grabinska Loniewska (Grabinska, 1991).

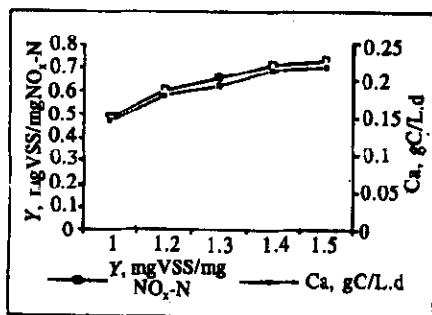


Fig. 5 The relationship between influent C/N and sludge yield (Y), assimilatory carbon consumption (Ca)

From C and N distribution, the calculated C/N ration used for denitrification represents in average $77 \pm 3\%$ of the influent C/N, which means that an increase of the C loading at a constant N loading does not change the C/N requirement for denitrification. From N distribution it can be seen that most of the influent nitrogen was removed by denitrification.

3.3 The influence of different carbon source on denitrification rate and carbon uptake rate

As a wide variety of organic compounds can be used for denitrification, the influence of different carbon source on denitrification rate and carbon uptake

rate were examined. The sludge used for these comparisons was acclimated at pH 7.5, 25°C and C/N 1.5. Denitrification rate and C uptake rate were determined by using batch test. The results are shown in Table 3.

Table 3 The influence of different carbon source on denitrification rate and C uptake rate
(Unless specified, the sludge is washed before testing)

C-source	DN rate			C uptake rate			Observed C/N
	mgNO ₃ ⁻ -N /mgVSS. d	n	Standard deviation	mg/C mgVSS. d	n	Standard deviation	
Mixed VFA ¹	0.754	32	0.109	1.792	14	0.387	2.37
Acetate	0.603	4	0.029	1.236	4	0.252	2.05
Propionate	0.362	3	0.061	0.505	3	0.126	1.40
Butyrate	0.519	4	0.036	0.928	4	0.405	1.79
Valerate	0.487	3	0.091	0.929	3	0.273	1.91
Methanol ¹ , 20°C	0.289	3	0.006				
Ethanol	0.349	2	0.001	0.601	2	0.066	1.72
Ethanol ²	0.415	2	0.019	0.635	2	0.317	1.53

Table 3 (continued)

C-source	DN rate			C uptake rate			Observed
	mgNO ₃ ⁻ -N /mgVSS. d	n	Standard deviation	mg/C mgVSS. d	n	Standard deviation	C/N
DSS	0.575	11	0.105	1.212	11	0.424	2.12
DSS ^{1,2}	0.646	4	0.071	1.178	4	0.338	1.82
DSS, 20°C	0.386	2	0.016	0.675	2	0.165	1.75
Mixed VFA, 20°C, ¹	0.530	9	0.079	1.591	9	0.567	3.00
Endogenous	0.084	3	0.010				

1. Sludge was not washed by P salt buffer; 2. sludge was acclimated by DSS; 3. sludge was acclimated by ethanol

From Table 3, the denitrification rate with mixed VFA was the highest. In the digested sludge supernatant (DSS), the VFA occupy about 80% of total organic compounds, so denitrification rate was also very high. As mentioned above, the sludge was acclimated by using mixed VFA as carbon source. In this condition, the population of bacteria has a large diversity. Some bacteria use acetic acid, while other prefer propionic acid, butyric acid or valeric acid. When a single kind of VFA is supplied, only a part of the biomass can use it directly and the observed denitrification rate is lower than that of mixed VFA.

For biodegradation, fatty acids are first linked to coenzyme A and formed acyl CoA, then they are degraded by oxidation at the β -carbon to form acetyl-CoA and a fatty acid two carbon shorter. Acetyl-CoA enters the TCA cycle. Energy which is generated by the coupled oxidation phosphorylation is used for denitrification.

Among tested VFA, acetic acid can be used directly after acetyl CoA was formed. So, acetic acid showed higher denitrification rate. The biodegradation pathway of propionic acid is more complex. First, propionyl CoA is formed. Through a series of enzymatic carboxylation and enzymatic epimerization reactions, it is converted into succinyl CoA, entering the citric acid cycle, then it is oxidized continuously. So, it had lower denitrification rate when it is used for denitrification. To butyric and valeric acids, first butyryl CoA and valeryl CoA are formed. Through β -oxidation, one acetyl CoA and one 2-C (or 3-C) fatty acids are formed. As mentioned above, they are oxidized and used for denitrification energy source. They showed middle denitrification rate.

Denitrification rate with methanol and ethanol was lower than that with VFA. For methanol and ethanol biodegradation, they have to be oxidized and converted into corresponding fatty acids, then degraded. The sludge which was applied to measure denitrification rate came from ethanol carbon source denitrification unit and then was acclimated by VFA, the population of bacteria was not adapted to methanol and ethanol, they showed lower denitrification rate. After acclimatization, we could assumed that their denitrification rate would be improved (Table 4, 0.349 mgNO₃⁻-N/mg/VSS. d with the sludge which is not acclimated and 0.415 mgNO₃⁻-N/mg/VSS. d with the sludge which is acclimated).

When no exogenous carbon is added, stored C can be used for denitrification. Because of

starvation, lysis could occur, then some substrates would be produced and used by denitrifying bacteria. But this process is very slow and the concentration of the biomass would decrease. This is endogenous denitrification. It presented the lowest denitrification rate (0.084 mgNO₃⁻-N/mgVSS.d). In the anoxic section of lagoon and inside layer of biofilm of rotating biological contractors and trickling filters, the endogenous denitrification could be found. When denitrification unit is large enough, in order to reduce cost and wasted sludge production, endogenous denitrification could be used.

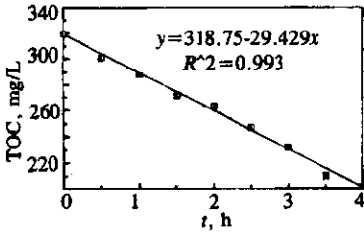


Fig. 6 C-uptake rate batch test using acetic acid as C-source
T=25°C, pH=7.5

When C,N substrates were not limited, single kind of exogenous carbon was supplied. The C uptake kinetics followed a zero-order reaction. Fig. 6 shows one of C uptake rate batch test.

C consumption in denitrification may be more complex. C source can be used for dissimulatory nitrate reduction, assimilatory cell synthesis, deoxygenation or converted into bacteria stored carbon. Organic compounds can be divided into 3 kinds; easily biodegradable, non-easily biodegradable and hardly one. For example, in the DSS, large amount of

sludge digested products are VFA, they belong to the easily biodegradable organic compounds, other constituents; cell wall lysis products, belong to the hardly biodegradable. Methanol ethanol and VFA belong to the easily biodegradable compounds. It was reported that when different kinds of organic compounds were used for denitrification they showed different denitrification rate and different C uptake rate. Suitable C/N for completely eliminating N was also different (Monteith, 1980). The same result was observed in this study (Table 3). So, in a denitrification system using mixed carbon source, the kind and amount of carbon source would influence not only denitrification rate but also carbon uptake rate.

By the way, environmental condition will also influence carbon uptake rates. When bacteria live in starvation condition, if a lot of nutrients are supplied, they would uptake it more than in non-starvation condition and then accumulate it. This situation is called "luxury uptake or overplus accumulation" (Xu, 1989).

When the observed C/N in Table 3 is compared to that of the semi-continuous test in Table 2, the value of the former is higher. Carbon consumption for denitrification was constant. So, carbon consumption for assimilatory synthesis (sludge yield, Y_N) was measured. The result is shown in Table 4.

Table 4 Comparison of sludge yield (Y_N) between batch test and semi-continuous test

Tested Process	Sludge yield mgVSS/mgNO ₃ ⁻ -N	n	Standard deviation
Batch	1.117	11	0.610
Semi-continuous	0.726	7	0.067

In batch test, substrate were added only once at the beginning, their concentrations were very high. So, the N, C loads also were very high. In semi-continuous test, substrate were added continuously. After they enter completely mixed reactors, they were diluted immediately. According to Table 1 and Fig. 5, higher the load was, larger the sludge yield was, more C was used for assimilatory synthesis. As we mentioned above, in semi-continuous system, influent C, N loads were lower than the maximum removal loads (from batch test), organisms live in starvation condition. When we use it to do batch test, excess of C was added, organisms could luxury uptake it and converted it to stored C. The amount of C consumption would be increased. It is to say, when we use continuous denitrification system it will not only decrease C consumption amount and treatment cost but also reduce wasted sludge production.

4 Conclusion

In the suspended sludge system when the pH, temperature were suitable and carbon source was not limited, single kind of carbon source was supplied, the denitrification and carbon uptake rate flowed a zero-order reaction.

Carbon source was mainly used for dissimulatory denitrification. When we applied VFA as a carbon source, consumed carbon quantity for denitrification was closed to 1.07mgVFA-C/mgNO₃⁻-N.

The carbon quantity for assimilatory synthesis occupied part of carbon source added. When influent C/N and carbon load increased, sludge yield also increased. The part of carbon consumption rose at the same time.

In tested condition, when influent C/N increased, the effluent oxidized nitrogen decreased. When influent C/N > 1.4, the effluent oxidized nitrogen was close to zero. Under pH 7.5, temperature 25°C, sludge yield 0.72 mgVSS/mgNO₃⁻-N, influent VFA-C/NO₃⁻-N 1.43 may be suitable for complete denitrification.

In denitrification, different organic carbon source had different denitrification rate. Mixed VFA C-source had higher denitrification rate than single VFA C-source consisting of them.

In order to reduce carbon consumption in denitrification system, reduce cost of performance, it is suggested to apply immobilized denitrification bacteria system or to use attached sludge denitrification system (denitrification filter or rotating discs); select continuous or semi-continuous influent denitrification units, in which sludge yield is lower than batch denitrification unit. In suspended culture system, sludge age can be prolonged. Use endogenous denitrification. Keep suitable influent C/N in order to prevent excess C converting to cell stored carbon. By the way, if the nitrogen draining standard permitted, carbon supply was appropriately reduced and the denitrification was not completed, it also would reduce carbon consumption. As the results of mentioned above, the cost of denitrification system performance can be cut down.

Acknowledgements—The authors want to thank " Center International Research for Water

and Environmental, Lyonnaise des Eaux Dumez", Joel Maallevialle (Director of International Research Centre), Jacques Manem (Department Head Biology-Biotechnology) accept author to do this research. The research was followed up by Vincent Urban and Jian Chang.

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(Received January 23, 1995)