Tests for Nitrifying and Denitrifying Ability of Activated Sludge

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Introduction

The bacterial mass of a single-stage activated sludge system designed for carbonaceous contaminant removal, nitrification and denitrification may be thought of as consisting of three types of organisms: a) facultative anaerobes, capable of engaging in nitrate respiration; b) aerobic heterotrophs; c) aerobic autotrophs of the Nitrosomonas and Nitrobacter species, the nitrifyers.

The share of each of these three types of organisms in the total microbial mass may be manipulated by certain process design measures.

For plant control purposes, it is sometimes desirable to obtain an estimate of the mass of the nitrifying organisms and of the mass of the facultative anaerobes present as well as a measure of their performance under "ideal" conditions.

A direct determination of the mass of either facultative anaerobes or nitrifyers is impractical. In indirect mass determination tests, the basic technique used is an old one that has been described numerous times in the literature (WUHRMANN 1956): sludge is separated from its original supernatant in a centrifuge, washed with the help of the centrifuge in saline solution (0.9% NaCl) and resuspended in a specific reaction medium; after a specified reaction time under well defined test conditions, characteristic changes in the composition of the medium brought about by the reaction are determined.

Some of the test conditions that must be fulfilled can easily be recognized when considering the equation

$$\Delta C_1 = \Delta t \; k_1 \; M$$

where

ΔC_1 is the change in mass of the characteristic medium component that C_1 is monitored, in mg
k_1 is the specific reaction rate with respect to C_1 in mg/mg of biomass M, per day
M is the mass of the special biomass to be estimated, in mg
Δt is the duration of the test in days

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To make ΔC_1 proportional to M_1, Δt must be kept the same for all tests, k_1 and M must remain reasonably constant during the test. Test duration must be kept comparatively short so that changes of M and of the physiological state of the biomass during the test will have no distorting effect. k_1 must stay near constant from test to test.

These test conditions can be attained by maintaining the physical medium conditions constant from test to test and by keeping all medium constituents at such concentration levels that changes in their concentrations will not affect k_1.

The problem of type-of-substrate acclimation can be minimized by using endogenous respiration as test reaction, where possible. Endogenous reactions, however, are subject to the distorting effect of substrate materials that have been adsorbed but not yet metabolized.

Nitrifying Ability

Many of the details of the test for nitrifying ability described below have been developed by SRINATH et al. (1974).

The "nitrifying" ability test proposed herein is concerned only with one of the four reactions that make up nitrification; this reaction is the energy (respiration) part of the Nitrosomonas activity which may be described as follows:

$$NH_4HCO_3 + 1.5\ O_2 \rightarrow HNO_2 + H_2CO_3 + H_2O \qquad (3)$$

The other three reactions in "Nitrification" are of course the synthesis activity of Nitrosomonas, the respiration and the synthesis activity of Nitrobacter. The test is for the controlling reaction.
The test is conducted as a substrate mediated test, at a substrate NH_4-N level thought as providing for a zero order reaction. There seems to be a consensus that nitrification is a near zero order reaction above a concentration of 2.5 mg/l of NH_4-N. The NH_4-N concentration provided by the proposed medium is approximately 260 mg/l. U.S.Environmental Protection Agency(1975)

The one hour test length was selected to prevent change of the biomass due to test conditions, and to obtain a stabilized reaction.

The test should be made on Mixed Liquor, not on Return Sludge. The plant operator is primarily interested in the performance of the Mixed Liquor itself. The following test medium was used, which was originally formulated by STEPHENSON (1948) as enrichment medium for Nitrosomonas cultures. However, in the original medium $(NH_4)_2\ SO_4$ was listed instead of $NH_4\ HCO_3$. Other media were also tried, with lesser results.

The testing medium should be tested frequently for NO_2/NO_3-N content. The test is performed at room temperature; optimum pH is provided by composition of the medium.

TEST MEDIUM, NITRIFYING ABILITY TEST

$NH_4 HCO_3$	2.92 gr
$K_2H PO_4$	1.5
$K H_2PO_4$	0.5
$FeSO_4 \cdot 7H_2O$	0.02
$MnSO_4 \cdot 7H_2O$	0.02
$MgSO_4 \cdot 7H_2O$	0.06
$CaCl_2$	0.04
Water	2 liter
pH	7.7

Summary of Test Procedure (Duplicate Tests are Advisable)

1. Determine SS and VSS on sample of Mixed Liquor to be tested: SS mg/l VSS mg/l.

2. Place 10 ml of the well-mixed Mixed Liquor sample into a 15 ml centrifuge tube with conical bottom. Centrifuge - using a horizontal head - at about 1500 relative centrifugal force as long as required to obtain a clear supernatant. Decant supernatant.

3. Resuspend the deposit in 10 ml of saline solution (0.9% NaCl) from a wash bottle. Centrifuge and decant washwater supernatant. Continue centrifuge washing procedure until sure that washwater does not contain any NO_3/NO_2-N. Usually three washings suffice.

4. Fill reaction vessel (250 ml Erlenmeyer flask) with 100 ml of the test medium; set aside portion of test medium for determination of initial NO_3/NO_2-N determination.

5. Transfer washed deposit from centrifuge tube to reaction vessel, using portions of the testing medium already in the reaction vessel for this purpose. Place reaction vessel on magnetic stirring plate and let react for 60 minutes, using same stirring speed in all tests. At end of testing period, filter reaction product through glass fiber filter.

6. Determine NO_3/NO_2-N in testing medium set aside in (4) and in filtrate of reaction product (5); compute difference: NO_3/NO_2-N.

7. Compute nitrifying abilities, as desired:

$$NA_{ML} = \Delta NO_3/NO_2\text{-N} \times 10 \quad \text{mg/l/h}$$

$$NA_{SS} = \frac{\Delta NO_3/NO_2\text{-N} \times 10^4}{SS} \quad mg/gSS/h$$

$$NA_{VSS} = \frac{\Delta NO_3/NO_2\text{-N} \times 10^4}{VSS} \quad mg/gVSS/h$$

The following "idealizing" steps are taken in the nitrifying ability test:

a. NH_4-N concentration is increased to the point where it is certainly above the threshold value at which practical zero order reaction commences.

b. pH is optimized.

c. Mass transport difficulties that could affect the rate of nitrification in the reactor are largely eliminated in the nitrifying ability test, by placing the heterotrophic bacteria in a state of endogenous respiration; by increased turbulence and by dilution.

For 5 batch experiments performed in 6-liter reactors, the highest nitrification rates, found by determining the NO_3/NO_2-N concentration at 30 minute intervals, are indicated in the following tabulation together with the nitrifying abilities determined on the Mixed Liquor. All these Mixed Liquors had been produced by admixing primary effluent to Return Sludge, taken from the Coxsackie Experimental STP. The residence time of the heterotrophic matrix in the STP at that time was approximately 8 days. The MLSS concentration in the batch reactors was approximately the same as the MLSS in the experimental 570 m^3/d sewage treatment plant. On 6-12-75 a plant profile was taken, spaced at approximately 30 minute intervals. The highest nitrification rate in the plant observed was 17.4 mg/l hr.

NITRIFYING ABILITY vs NITRIFICATION RATE

Batch Experiments

Exp. No.	Date	VSS mg/l	Temp °C	Nitrifying Ability mg NH_4-N / l.h	Highest Nitrification Rate observed mg NH_4-N / l.h
361	5-11-75	2600	20	16	12.4
367	5-20-75	2500	22	19	13.0
371	6-5-75	1900	20	12	10.6
372	6-11-75	2600	22	19	12.8
373	6-11-75	2600	22	19	12.0

Denitrifying Ability (Percentage of Denitrifying Bacteria in Mixed Liquor Solids)

In order to avoid the difficulties that might be encountered in trying to overcome type-of-substrate acclimation problems, the simple test described is built around endogenous nitrate respiration.

An equation describing endogenous nitrate respiration has been formulated by MCKINNEY (1975) as follows:

$$C_5H_7O_2N + 4NaNO_3 + H_2O \rightarrow 4NaHCO_3 + NH_4HCO_3 + 2N_2$$

wherein $NaNO_3$ stands for all nitrates present.

In addition to "stored" carbon present in the biota, the period of aeration time that has elapsed since synthesis took place may also affect the rate of endogenous nitrate respiration(WUHRMANN 1963). These two factors will combine, to make the rate of NO_3-N reduction on the test not fully representative of the mass of denitrifiers present. Nevertheless, the simple test described should be useful for plant control purposes, at least. By testing Return Sludge, the stored carbon effect can largely be eliminated.

It is recommended that this test be performed at room temperature in a 300 ml BOD bottle, closed by a BOD bottle DO probe, equipped with an agitator. The probe allows for monitoring of the DO and provides a uniform method of agitation.

The medium used for this test is based on tapwater, taken from the sewage treatment plant deoxygenated with sodium thiosulfate, or nitrogen gas. The medium contains 20 mg/l NO_3-N. This medium should be free of chlorine. STP tapwater is thought to represent carriage water.

The test must be performed under anoxic conditions. Special care must be used so as to not introduce O_2 into the medium when washing the sludge and transferring it to the reaction vessel. The washed solids of 100 ml of return sludge should be used in this test. In a 300 ml BOD bottle, the resulting solids concentration is of the same magnitude as in the Mixed Liquor of a plant.

Summary of Test Procedure (Denitrifying Ability)

1. Determine SS and VSS on sample to be tested; mg/l

2. Place 100 ml of the well mixed sample into two 50 ml centrifuge tubes with conical bottom. Centrifuge tubes - using a horizontal head - at about 1500 relative centrifugal force as long as required to obtain a clear supernatant. Decant supernatant.

3. Resuspend deposits in saline solution (0.9%) from a wash

bottle. Centrifuge and decant washwater supernatant. Repeat centrifuge washing procedure twice.

4. Transfer the washed sludge to a 300 ml BOD bottle, using the testing medium and pouring down the side of the bottle to incorporate as little O_2 as possible. Wash centrifuge tube with testing medium, adding wash to BOD bottle, being careful to avoid DO enrichment. Fill BOD bottle completely with testing medium and insert BOD bottle probe. Set aside portion of testing medium for determination of initial NO_3/NO_2-N

5. Start test reaction by putting BOD bottle stirrer in operation for 60 minutes. Monitor DO and temperature at 0, 1, 3, 6, 10, 30, 60 minutes. (If DO has not reached the zero value after 3 minutes, discontinue test.) At end of test, immediately filter reaction product through glass fiber filter.

6. Determine NO_3/NO_2-N concentration in testing medium set aside under step 4 and on filtrate of reaction product obtained under step 6; compute difference: NO_3/NO_2-N (mg/l)

7. Compute Denitrifying Ability

$$DA_{SS} = \frac{NO_3/NO_2\text{-N} \times 3 \times 1000}{SS} \qquad (mg/g^{SS}hr)$$

$$DA_{VSS} = \frac{NO_3/NO_2\text{-N} \times 3 \times 1000}{VSS} \qquad (mg/g^{VSS}\ hr)$$

Testing Medium (Denitrifying Ability)

0.1214 gr of $NaNO_3$ in 1 liter of testing medium, using warm tapwater for dilution (20 mg/l of NO_3-N); let cool to room temperature. Just prior to use, add 2 ml/l of a saturated sodium thiosulfate solution.

The following tabulation shows a number of test results comparing the sludge of the experimental sewage treatment plant at the Coxsackie Correctional Facility (CCF) with sludges of small activated sludge treatment plants in surrounding villages (V); they show the effect of the anoxic Return Sludge treatment at the CCF plant which was put into operation November 1, 1975.

Date of Test	Plant	Type of Sludge	Denitrifying Ability mg/h gvss	mg/h gss
1-6-76	Coxsackie V.	R.S. 65% vol.	1.51	0.98
1-6-76	Athens V.	R.S. 71% vol.	1.14	0.81
1-15-76	Athens V.	R.S. 75% vol.	.97	0.73
1-6-76	Ravena V.	R.S. 77% vol.	3.03	2.33
1-20-76	Ravena V.	R.S. 80% vol.	2.39	1.91
10-15-75	CCF	R.S. 60% vol.	1.77	1.06
10-17-75	CCF	R.S. 62% vol.	2.18	1.35
1-6-76	CCF	ML 63% vol.	4.08	2.57
1-15-76	CCF	RS 63% vol.	3.86	2.43
1-15-76	CCF	RS 64% vol.	3.38	2.16
1-15-76	CCF	ML 65% vol.	2.89	1.88

The plant at Athens is an Extended Aeration plant; the other village plants are conventional Activated Sludge plants.

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