

INVESTIGATION INTO METHANOL AS A CARBON SOURCE FOR DENITRIFICATION IN WASTEWATER TREATMENT

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ABSTRACT

The Rotorua Wastewater Treatment Plant was upgraded in 2005 to reduce the mass of total nitrogen discharged to the Rotorua Lakes catchment. The upgrade included the installation of methanol dosing to the secondary anoxic zone of the Bardenpho activated sludge reactor for the reduction of nitrate and total nitrogen in the treated effluent.

Methanol is a popular choice as a carbon source for nitrogen removal as it contains no solids, nutrients (such as nitrogen and phosphorus), has a neutral pH, and can be regarded as containing 100% readily degradable substrate. Specialist organisms (methylophils) are likely to be responsible for denitrification with methanol as not all organisms are able to use it as a carbon source. This is likely to be due to the C1 or single carbon bond in the methanol molecule.

Methanol dosing at the Rotorua WWTP has been successful, with a reduction in the total nitrogen concentration in the final effluent. Investigations into the amount of methanol used to achieve this reduction indicated significant "bleeding" of methanol from the anoxic zone to the re aeration zone was occurring. Measurements of nitrate reduction across the anoxic zone suggest that the growth rate of the methanol utilizing organisms may be too low to make full use of the methanol.

A Titrimetric and Off Gas Analysis (TOGA ©) sensor was used to quantify the rate of denitrification on methanol, acetate and a spent fermentation liquor from the food industry. It was found that when methanol was used as the sole exogenous carbon source the rate of denitrification was approximately one third greater than the rate of endogenous nitrogen removal. This was the slowest relative rate of the three substrates trialed. Based on this result it could be expected that methanol addition to the Bardenpho, while increasing denitrification capacity, would do so at a slower rate than the other sources of carbon.

KEYWORDS

Methanol, Activated Sludge, Denitrification, Maximum Specific Growth Rate, TOGA

1 INTRODUCTION

The Rotorua Wastewater Treatment Plant (WWTP) is a high rate activated sludge process based on the BARDENPHO configuration.

The treatment process (at Rotorua) consists of inlet screening, grit removal and primary sedimentation followed by the BARDENPHO activated sludge process. Treated wastewater is discharged to the Whakarewarewa Forest and enters Lake Rotorua via groundwater and surface water systems.

The Whakarewarewa Forest is effective at removing phosphorus from the treated wastewater but only moderately effective at removing nitrogen. Therefore to minimize the mass of nitrogen entering the Lake, nitrogen removal at the treatment plant is required.

To achieve low levels of oxidized nitrogen (nitrite and nitrate) via biological nitrification/denitrification, sufficient degradable carbon (electron acceptor) must be available for the growth of new cells and as an energy

source. If insufficient carbon is available then an additional source such as methanol can be added to enhance the denitrifying potential of the process.

Methanol is often selected for municipal wastewater treatment plants as it has a neutral pH, contains no nutrients such as nitrogen and phosphorus, and can be considered to contain 100% readily degradable carbon. Unlike alternatives such as molasses, methanol is also a clear liquid and no discoloration of the treated effluent occurs when it is added to a biological process. This is particularly important when ultraviolet disinfection is included as a reduction in transmissivity will reduce the efficacy of the disinfection process. Methanol is also relatively cheap at a cost of approximately \$1.80-2.00 per kg of COD.

An upgrade to the wastewater treatment plant was undertaken in 2005 and included increasing the aeration capacity of the Bardenpho reactor by 25% and the addition of a methanol dosing.

Prior to methanol being added at Rotorua, the average total nitrogen concentration in the final effluent of the Bardenpho was approximately 10mgN/L. After methanol addition (and extension of the reactor) this dropped to 6.5mgN/L. This result appears to confirm that methanol is an effective carbon source for denitrification, although it is unclear what effect the extension of the reactor had on this total nitrogen reduction.

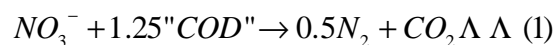
Recent investigations by Takacs et al (2006) have shown that the rate of methanol utilization under anoxic conditions may be very much slower than assumed in earlier versions of process simulation models such as BioWin. The current model version (v3) includes a methanol growth rate of 1.3 days⁻¹ compared to 6.4 days⁻¹ in version 2.

Based on these and other observations, experiments were undertaken to measure the maximum specific growth rates of methanol utilizers under anoxic conditions and to compare these measurements with those found by Takacs et al (2006).

2 BACKGROUND

2.1 DENITRIFICATION KINETICS

Biological denitrification involves the conversion of nitrate or nitrite to nitrogen gas as shown in equation 1. This is the most commonly used biological process for removing nitrogen from wastewater and is widely used in activated sludge, membrane and fixed film type processes.



On a mass basis, the above equation suggests that 2.86kg of COD are consumed per kg of nitrate that is converted to nitrogen gas.

The rate of biomass growth on nitrate can be described by Monod type kinetics as shown in equation 2. This mathematical model of substrate limited growth is the basis of the equations used in activated sludge models such as BioWin.

$$r_{v,xb} = m_{\max} \frac{S}{(K_S + S)} \cdot X_{bh} \quad (2)$$

Where:

$r_{v,xb}$ = volumetric rate of biomass growth (g/m³/day)

m_{\max} = maximum specific growth rate of denitrifying biomass (days⁻¹)

S = substrate (nitrate concentration) (mgN/L)

K_S = Substrate saturation constant (mgN/L)

The Monod equation presented in equation 2 describes the rate of biomass (X_b) growth, however in most instances in wastewater treatment it is the rate of substrate use (in this case COD) and the rate of oxygen or nitrate utilization that is useful for the prediction of effluent quality. In order to relate the rate of biomass growth to the rate of substrate use, the concept of biological yield (Y_h) is included in the basic Monod expression as shown in equation 3.

$$r_{v,COD} = \frac{m_{\max}}{Y_h} \frac{S}{(K_S + S)} \cdot X_{bh} \quad \Lambda \quad \Lambda \quad (3)$$

The nitrate (or oxygen) used in heterotrophic growth is formed into CO_2 with organic carbon as an energy source. In heterotrophic growth the carbon used to form CO_2 is the same as that used by the organism to grow new cells. The relationship between the carbon used as an energy source and that used to grow new cells is the difference between the total substrate available and the amount that is used for growth. Hence to describe the rate of nitrate utilization equation (3) can be written as follows;

$$r_{v,N} = \frac{(1 - Y_h)}{2.86} \frac{m_{\max}}{Y_h} \frac{S}{(K_S + S)} X_{bh} \quad \Lambda \quad \Lambda \quad (4)$$

If the concentration of nitrate (S) in equation (4) is very much larger than K_S then the rate of nitrate removal can be described as a zero order reaction with respect to the initial concentration of biomass as shown in equation (5).

$$r_{v,N} = \frac{(1 - Y_h)}{2.86} \frac{m_{\max}}{Y_h} X_{bh} \quad \Lambda \quad \Lambda \quad (5)$$

The change in biomass concentration (X_{bh}) is the result of growth and decay of methanol utilizers is given by:

$$\frac{dX_{bh}}{dt} = m_{\max} X_{bh} - bX_{bh} \quad \Lambda \quad \Lambda \quad (6) \quad \text{Re arranging and integrating from time zero to time t:}$$

$$X_{bh,t} = X_{bh,0} e^{(m-b)t} \quad \Lambda \quad \Lambda \quad (7)$$

Substituting (7) into (5) gives;

$$\frac{dS_N}{dt} = \frac{(1 - Y_h)}{2.86} \frac{m_{\max}}{Y_h} X_{bh,0} e^{(m-b)t} \quad \Lambda \quad \Lambda \quad (8)$$

The TOGA © continuously measures both the liquid phase and the gas phase nitrogen and this allows for the fitting of the nitrogen production rate (NPR) to equation (8) without the need for offline nitrate data. This simplifies the analysis of the kinetic rates and also eliminates the errors involved in sample measurement and storage that can often be a factor in the analysis of offline data.

2.2 INITIAL INVESTIGATIONS OF METHANOL USE AT ROTORUA WWTP

Based on the findings of Dold et al (2007), investigations into the nitrate/nitrite removal performance of the secondary anoxic zone were undertaken at the Rotorua WWTP. These investigations included:

- Online and offline measurements of nitrate across the secondary anoxic zone
- Offline analysis of methanol “bleeding” from the secondary anoxic zone
- Batch laboratory tests to determine the relative denitrification rate of methanol versus acetate and an industrial wastewater.

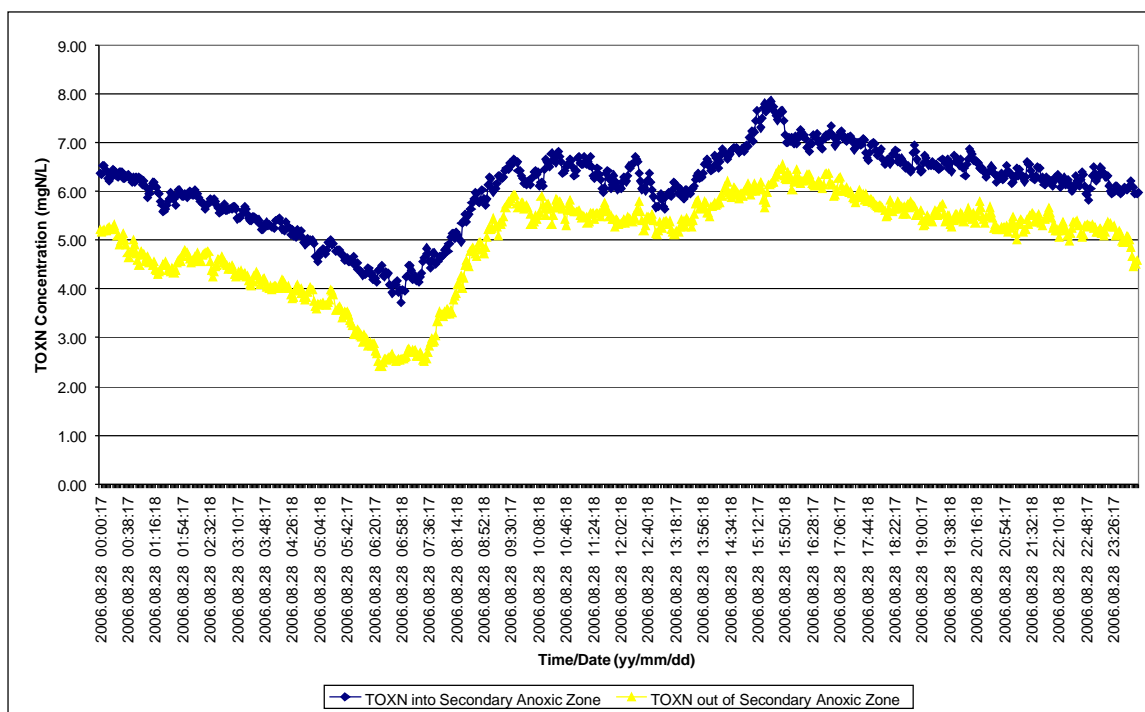
2.2.1 ONLINE NITRATE MEASUREMENTS

In order to test if methanol utilization is occurring at these slower rates at the Rotorua WWTP, online nitrate analyzers (S::CAN Spectrolysers) were placed at the inlet and outlet of the final anoxic zone in the Bardenpho (where methanol was added). The inlet versus outlet nitrate concentrations were compared to investigate if the mass of nitrate removed was comparable to a slow or a rapid utilization rate.

Using the stoichiometric relationship of 0.4kgCOD/kgCOD and the oxygen equivalence of nitrate (2.86 kgO₂/kgNitrate) and 1.188kgCOD/L of MeOH, the initial dose of methanol into the secondary anoxic zone of approximately 700L/day should be capable of removing approximately 156kgN/day or an equivalent of 3.5mgN/L (based on an influent flow of 18.5MLD and RAS of 25.9MLD). The requirement of the process is to remove approximately 5mgN/L in order to reduce the total nitrogen in the discharge to less than 4.5mgN/L.

The S::CAN spectrolysers were placed immediately upstream and downstream of the final anoxic reactors. The instruments were calibrated for nitrite and nitrate (TOXN) using a HACH 2400 Spectrophotometer. To minimize the error involved with the samples they were collected from the reactor and filtered through a membrane filter before analysis. Both spectrolysers were zeroed on the same sample. The concentration of nitrite/nitrate was measured every two minutes for a period of three weeks. A plot of a typical diurnal profile from the S::CAN's across the secondary anoxic zone is shown in Figure 1:

Figure 1: Typical 24 Hour Nitrate Profile – Secondary Anoxic Zone Rotorua WWTP

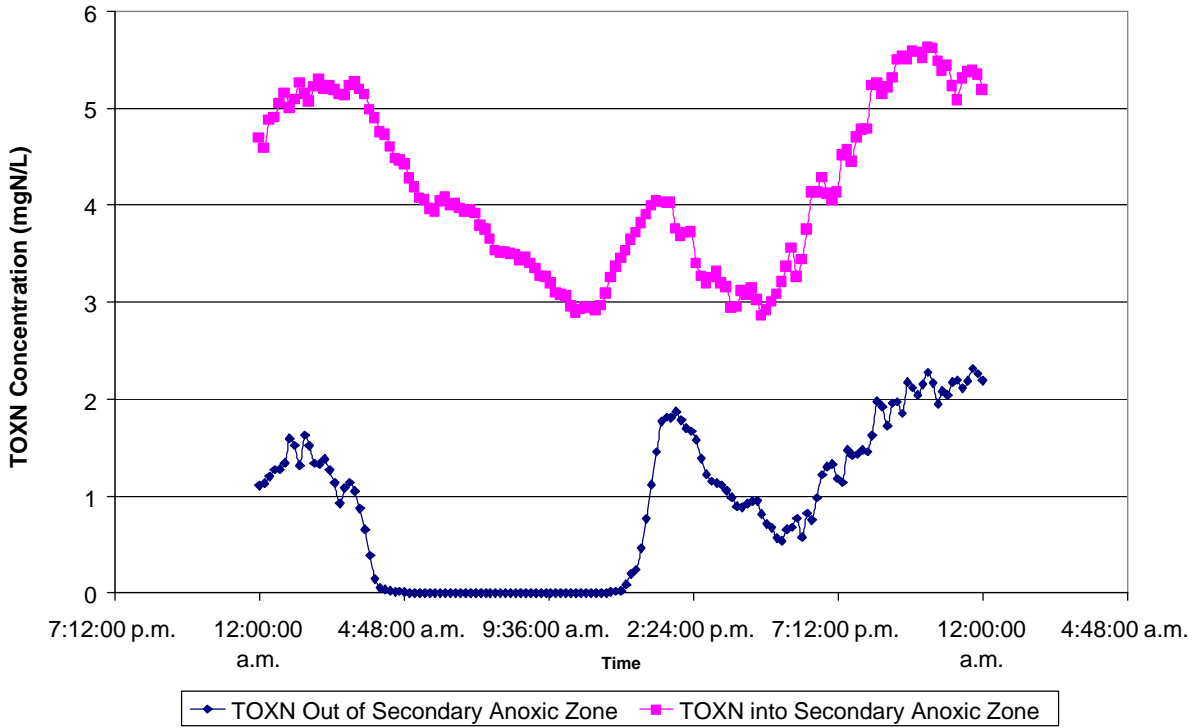


Based on the S::CAN measurements, the average TOXN entering the secondary anoxic zone from figure 1 was 6.0mgN/L, with an exit concentration of 4.5mgN/L. This equates to a removal of 1.5mgN/L.

From the data obtained from the S::CANs the dosing of methanol, while potentially providing some benefit in terms of nitrogen removal, was not nearly as effective as that predicted from stoichiometry (156kgN/day versus 66kgN/day).

The nitrate removal performance predicted by BioWin (version 2) using the model defaults for anoxic methanol utilizers is shown in Figure 2 as a comparison. The simulation was first run at “steady state” such that a population of methanol users could establish and then the model was run under dynamic conditions (with actual plant data taken every 2 minutes via the S::CAN). Note that the data used for the dynamic simulation is not for the same time period as the S::CAN data however the variation in both flow and load at the plant follow a repeatable pattern.

Figure 2: Modeled TOXN Across Secondary Anoxic Zone



The average TOXN into the secondary anoxic zone from Figure 2 is 4.2 mgN/L, with an exit concentration of 0.89 mgN/L. This equates to a removal of 3.31 mgN/L which compares very well with stoichiometry.

Given the results obtained above it was suspected that loss of methanol from the secondary anoxic zones to the final aeration reactors was occurring potentially resulting in aerobic use of the methanol as opposed to anoxic use with nitrate.

To test this hypothesis, samples were taken from the exit of the secondary anoxic reactors (during a period of flow proportional methanol dosing) and analyzed for methanol. Samples were filtered onsite and frozen before analysis. Table 1 below shows a summary of the results of this sampling and analysis.

Table 1: Methanol Analysis of Exit from Secondary Anoxic Zone

Time	Methanol Concentration (mgCOD/L)	Methanol Dose (L/min)
09:30	7.9	1.2
10:30	6.3	1.2
11:30	7.7	1.2
12:30	6.4	1.2
13:30	10.4	1.2
14:30	14.6	1.2
15:30	13.9	0.64

Based upon Table 1 there is clearly some methanol exiting the secondary anoxic reactors that is either being utilized in the final aeration reactors or is being utilized anoxically in the final settling tanks.

Based on the revised maximum specific growth rate of the methanol users in BioWin 3 (1.3 days^{-1}) the SRT of the final anoxic reactors is not enough to maintain a stable methylotroph population if only anoxic growth is considered. The BioWin model currently assumes that ordinary heterotrophic organisms (OHO's) utilize methanol under aerobic conditions, but only methylotrophs grow on methanol with nitrate. Based on these assumptions the BioWin model is unable to solve and achieve a steady methylotroph population for the Rotorua WWTP.

The first question of this research was therefore to undertake experiments to verify if any methanol could be utilized anoxically and if this proved to be the case how were these organisms able to establish in the Rotorua WWTP given the assumptions of the BioWin model.

3 MEASUREMENT OF DENITRIFICATION RATE

Based on the initial investigations detailed above a set of batch experiments were undertaken on the mixed liquor from Rotorua to determine the denitrification rate of methanol, acetate and the spent fermentation liquor.

The fermentation liquor was investigated as it appeared to have properties that would make it suitable for a carbon source at the WWTP, with a high COD (70,000-80,000mg/L) and relatively low nutrient content.

Acetate was chosen for these tests as it is widely reported in the literature as giving a very high rate of denitrification and therefore provided a bench mark to compare the other substrates against. The fermentation liquor was tested to ascertain if it could be used as a potential carbon source for denitrification at the WWTP and if it could what would its rate be relative to both methanol (current carbon source) and acetate (best case).

The experiments had the secondary aim of determining if methanol utilizing organisms were present in the mixed liquor and their rate of nitrate utilization compared to the other two substrates.

Batch experiments were undertaken at the SCION laboratory at Rotorua with a Titrimetric and Off Gas Analysis (TOGA ©) reactor. For these initial batch tests the rate of denitrification as a function of mixed liquor volatile suspended solids was determined rather than the maximum specific growth rate (μ_{\max}) for each substrate.

3.1 DESCRIPTION OF TOGA

A full description of the TOGA[®] sensor, and its measurement principles is provided by Gapes *et al.*, (2003 and Pratt *et al.*, (2003). Briefly, the sensor consists of a fermentation vessel, a pH control system, a dissolved species probe and an off-gas measurement arrangement. A Bioflow 110 (New Brunswick Scientific, NJ USA) with 1L working volume is utilised as the fermentation vessel. Controlled parameters include pH, temperature, reactor hydrodynamics (mechanical and pneumatic), and gas phase composition.

Nitrogen production rate (NPR) was determined from mass balance measurements of gas phase N_2 flows into and exiting the fermenter, using a quadrupole mass spectrometer (HPR20, Hiden Analytical, UK) in conjunction with a number of in-line mass flow controllers (Bronkhorst, Hi-tech, El-Flow, Netherlands).

The dissolved species probe consists of a membrane-covered capillary inlet attached directly to a mass spectrometer (HPR40, Hiden Analytical, UK), a technique defined as membrane inlet mass spectrometry (MIMS). This allows simultaneous measurement of multiple dissolved gas species, including O_2 , N_2 , CO_2 , CH_4 , H_2 as well as volatile organic compounds such as methanol and ethanol. The liquid phase analysis of NPR relied on the determination of mass transfer coefficient for N_2 (kLa_{N_2} -measured via MIMS probe response to controlled step change in gas phase composition, equivalent to that of standard oxygen kLa measurements), measurement of the dissolved N_2 concentration, and subsequent application of the equation:

$$NPR = kLa_{\text{N}_2} ([N_{2, \text{liq}}] - [N_{2, \text{liq}}^*]) ; \text{ where } [N_{2, \text{liq}}^*] = \text{liquid saturation nitrogen concentration at gas inlet conditions.}$$

Thus, two complementary methodologies (gas and liquid phase) were utilised to give confidence in the online measurement of denitrification rates.

3.2 RESULTS OF BATCH TESTS

Figure 3 shows a plot of the liquid and gas phase response from the TOGA batch experiment. From this time-series data, the Nitrate Removal Rate for each condition was obtained. The denitrification rate for each substrate was then determined from the mixed liquor volatile suspended solids concentration (MLVSS) to provide rates on a VSS-specific basis, the major results of which are presented in Table 2.

Table 2: Denitrification Rates Obtained from Batch Experiments

CONDITION	DENITRIFICATION RATE (mgN/gVSS/h)
Acetate addition	5
Addition of industrial wastewater	3.5
Methanol addition	2.8
Endogenous decay	1.6

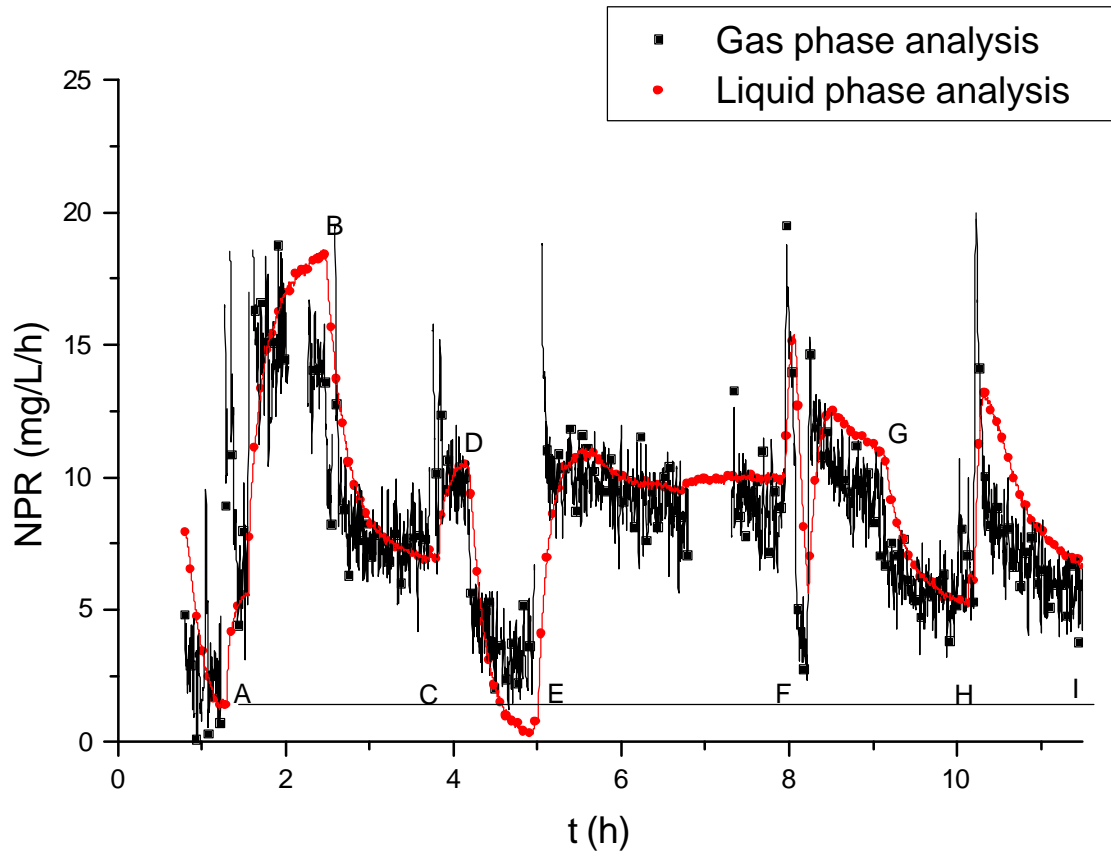
It can clearly be seen that all three carbon sources supported denitrification, with the highest rate being observed after acetic acid addition, followed by that of the industrial waste. The rate of methanol use is lower relative to both of the other substrates tested. This may indicate that either there is a very small population of organisms that use methanol at a high rate or that the population that is present grows at a lower rate thus supporting the hypothesis presented above.

With reference to figure 3 it is worth noting that denitrification continued to proceed even after acetic acid had been consumed [Point B in Figure 2] (endogenous respiration) but ceased once nitrate had been consumed [Point D, Figure 2].

Methanol addition [Point C, Figure 2] resulted in an increase in denitrification, but only about 70% greater than that of the endogenous rate.

The industrial waste, added twice [Points F and H Figure 2] showed elevated but variable denitrification capability, a reflection of the complex nature of the carbon source in this sample, as compared with pure acetic acid or methanol.

Figure 3: Raw TOGA Data



POINT	COMMENT	POINT	COMMENT
A	Acetic acid addition	F	Fermentation waste addition
B	Acetic acid depletion	G	Fermentation waste depletion
C	Methanol addition	H	Fermentation waste addition (2)
D	Nitrate depletion	I	Experimental completion
E	Nitrate addition		

3.3 PROCESS MODELING

To further investigate the above a spreadsheet model was used to simulate the batch test and to estimate the starting concentration of methanol utilisers under a high growth rate (6.4 days^{-1}) and low growth rate (1.3 days^{-1}) scenario respectively. The results of this analysis are shown in table 3 below.

Table 3: Calculated Initial Concentration of Methyloprophs from Batch Experiments

MAXIMUM SPECIFIC GROWTH RATE (day^{-1})	INITIAL METHLYTROPH CONCENTRATION (mgCOD/L)
6.4 (BioWn version 2 default)	30
1.3 (BioWin version 3 default)	120

From the results of the above analysis it appears very unlikely that the population of methyotrophs could be as low as 20mgVSS/L (0.7% of MLVSS, based on 3000mgVSS/L in the reactor), thus supporting the hypothesis of the slower growth rate in the latest version of BioWin.

3.4 MEASUREMENT OF MAXIMUM SPECIFIC GROWTH RATE

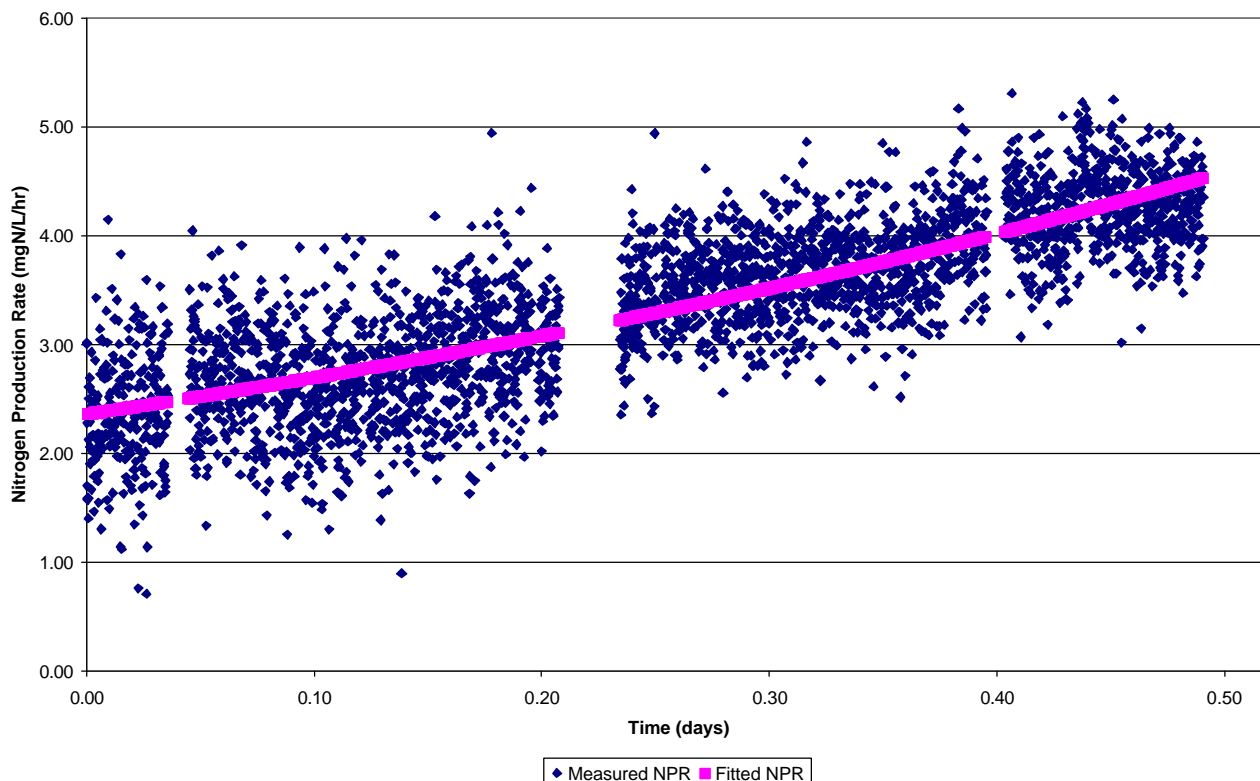
The initial denitrification rate tests described above confirmed that organisms were present in the Rotorua WWTP that could utilise methanol as a carbon source for denitrification. These tests based on the rate of reaction per mass of VSS do not however give any information as to the maximum specific growth rate of these organisms or their initial concentration in the activated sludge.

A second set of experiments was conducted to determine the maximum specific growth rate of the methanol utilizers by fitting measured TOGA data (NPR) to equation (7) using the SOLVER tool on EXCEL. This second set of tests was conducted over several days where a high initial concentration of methanol and nitrate were dosed into a batch reactor until an exponential response in the nitrate consumption with time was observed. The experimental data was then exported to a spreadsheet and the SOLVER used to find the unknown parameters in equation (7).

The nitrate consumption rate was determined using both liquid and gas phase analysis of the TOGA reactor obtained from the mass spectrophotometer outputs. The background endogenous rate of nitrate consumption was measured at the start and end of the test and this rate was taken off the total NPR measured by the TOGA.

Figure 4 shows the exponential growth phase of the test and the fitted (model) curve obtained from a least squares fit of equation (7).

Figure 4: Measured and Fitted NPR data



The fitted initial biomass concentration, maximum specific growth rate and assumed decay rate of the methanol utilising organisms based on figure 4 are shown in table 5 below.

Table 5: Summary of Fitted Parameters

PARAMETER	UNITS	VALUE
Stoichiometric Yield (Y)	kgCOD/kgCOD	0.4
Decay (anoxic) (b)	day ⁻¹	0.03
Initial Methyloph Concentration (in MLSS) (X ₀)	mgCOD/L	120
Maximum Specific Growth rate (μ_{max})	day ⁻¹	1.36

The above results confirm that the methanol utilizing organisms in the mixed liquor analyzed have a very similar growth rate to that assumed in the current version of BioWin and confirm the hypothesis of a small group of slower growing organisms being responsible for anoxic methanol utilization.

The implication of this work for the design and optimization of wastewater treatment plants is that a sufficiently long SRT and HRT are required to utilize methanol for denitrification. The ability of the Rotorua WWTP to remove nitrate from the final effluent via methanol dosing into the secondary anoxic zone of the Bardenpho reactor is limited by both the growth rate of the anoxic methanol utilizers and the hydraulic retention time in the secondary anoxic zone. As the number of methanol utilizing bacteria is finite within the mixed liquor (approximately 120mg/L), and the growth rate, yield and decay rate are constant, it follows that irrespective of how much methanol is added there is finite rate that it can be utilized. If the retention time in the anoxic zone is low then by simply adding more methanol no additional nitrate will be removed. This has significant cost implications for the Rotorua District Council in that flow pacing of the methanol dose (i.e. adding more methanol at peak flow and load) is unlikely to remove more nitrate and in fact given the decrease in HRT with flow will remove less nitrate hence wasting methanol.

Further work is currently being undertaken into the aerobic growth of methanol utilizers and their effect on the viability of methanol dosing into short SRT/HRT reactors designed for denitrification.

4 CONCLUSIONS

Methanol addition at the Rotorua WWTP has been successful at establishing a viable population of methanol utilizing organisms. The reduction in nitrate due to methanol dosing into the secondary anoxic zone of the Bardenpho has been measured at about 1.5mgN/L and this is significantly less than the amount predicted from stoichiometry.

Recent tests undertaken using a Titrimetric Off Gas Analyzer (TOGA) have shown that the maximum specific growth rate of methanol utilizing organisms is approximately 1.4 days⁻¹ and this compares well with the default value in the latest version of BioWin.

Based on the results of this study the efficacy of methanol in removing nitrate relative to other carbon sources means that alternatives to methanol (industrial wastewater and ethanol) may be better suited to the Rotorua Wastewater Treatment Plant.

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