Formaldehyde biodegradation in the presence of methanol under denitrifying conditions

Marta Eiroa, Almudena Vilar, Christian Kennes, María C Veiga

Journal of Chemical Technology & Biotechnology, Volume 81, Issue 3, March 2006, Pages 312–317
DOI: 10.1002/jctb.1395

Abstract

Simultaneous formaldehyde and methanol biodegradation and also denitrification were studied in batch assays and in a continuous laboratory-scale reactor. In batch assays, high formaldehyde concentrations (up to 1360 mg dm^{-3}) were removed under anoxic conditions in the presence of methanol. It was found that formaldehyde biodegradation produced methanol and formic acid as products. The denitrification process was affected by the initial formaldehyde concentration. In the continuous reactor, the biodegradation of different concentrations of formaldehyde (1500–275 mg dm^{-3}) and methanol (153–871 mg dm^{-3}) took place, maintaining the organic loading rate at 0.84 g COD dm^{-3} d^{-1} (COD/N 4). However, each increase in the methanol concentration in the influent caused a decrease in the denitrification level. An adaptation period to methanol was necessary before the denitrification percentage could be recovered. In contrast with batch assays, in the continuous reactor methanol and formic acid were not detected in the effluent. Moreover, in the continuous reactor the denitrification percentages were higher and the nitrite accumulation was lower.

Keywords:

formaldehyde; methanol; biodegradation; denitrification

INTRODUCTION

Wastewaters from resin-producing industries are characterized by the presence of high concentrations of nitrogen compounds (56–1462 mg Total Kjeldahl Nitrogen (TKN) dm^{-3}) and organic matter, mainly formaldehyde (7–2711 mg dm^{-3}).1 Therefore, the treatment of these wastewaters requires the simultaneous removal of nitrogen compounds and organic matter, which can be undertaken with biological processes. It is necessary to achieve both formaldehyde biodegradation and nitrogen removal by nitrification and denitrification. In previous experiments, simultaneous nitrification and formaldehyde biodegradation was investigated using aerobic batch assays2 and an activated sludge unit,3 whereas in this research, the simultaneous denitrification and formaldehyde biodegradation was studied.

In many cases, the organic matter present in wastewaters has been shown to be limiting to the denitrification process. An effective solution requires the addition of an external carbon source. Comparing possible carbon substrates, methanol turns out to be relatively cost effective and causes low sludge production.4 Therefore, methanol was chosen as the external carbon source in the present research.

Formaldehyde is known to be biodegradable in both aerobic5–7 and anaerobic systems.8–10 Only a few publications have dealt with the biological removal of formaldehyde under anoxic conditions. Garrido *et al.*11 studied the treatment of wastewaters from a formaldehyde–urea adhesives factory, using a nitrification–denitrification process in two reactors with suspended biomass. Most of the formaldehyde was used by microorganisms as carbon source for denitrification, obtaining formaldehyde removal rates between 0.2 and 0.6 g dm^{-3} d^{-1}. The denitrification process was also very efficient, reaching rates up to 1.0 g N-NO_x^- dm^{-3} d^{-1}. Formaldehyde and urea removal was also studied in an anoxic upflow sludge blanket reactor.12 Formaldehyde concentrations of 250–300 mg dm^{-3} in this reactor caused a decrease in the denitrification rate. Nevertheless, the denitrification process was totally restored after decreasing the concentration of formaldehyde accumulated. At the end of the experimental period formaldehyde removal rates of 2.4 g dm^{-3} d^{-1} were reached. In previous experiments, simultaneous formaldehyde biodegradation, urea hydrolysis and denitrification in an upflow granular sludge blanked reactor were investigated.13 Formaldehyde removal efficiencies >99.5% were obtained at organic loading rates between 0.34 and 2.78 g CH_2O dm^{-3} d^{-1} (625–5000 mg CH_2O dm^{-3}).

The purpose of this work was to study simultaneous formaldehyde biodegradation, methanol biodegradation and denitrification at the laboratory scale, first in anoxic batch assays and then in a continuous anoxic reactor. The biodegradability of formaldehyde and methanol and their effect on the denitrification process were investigated.

MATERIALS AND METHODS

Analytical methods

Formaldehyde was determined spectrophotometrically according to the Hantzch reaction, using a Perkin-Elmer Lambda 11 UV–visible spectrophotometer.14 Methanol was measured using a Hewlett-Packard 5890-II gas chromatograph equipped with a Nukol column (30 m × 0.25 mm i.d.) and a flame ionization detector. Nitrogen (1.5 cm^3 min^{-1}) was utilized as carrier gas. The injector and detector temperatures were 250 and 270 °C, respectively. Formic acid was determined using a Hewlett-Packard 1100 liquid chromatograph equipped with a C-18 ODS column (25 cm × 4 mm i.d.) and a diode-array UV detector. The mobile phase was acetonitrile–phosphoric acid (80:20) at a flow rate of 1 cm^3 min^{-1}. Detection was performed at 210 nm.

Nitrite and nitrate were determined by capillary electrophoresis using a Hewlett-Packard ^{3D}CE system with a microcapillary tube of fused silica (40 cm × 50 µm i.d.). UV detection was undertaken at a wavelength of 214 nm with 450 nm as reference. The biogas composition (N_2, CH_4, CO_2 and N_2O) was determined on a Hewlett-Packard 5890-II gas chromatograph equipped with a Porapak Q W80/100 column (2 m × 1/8 in i.d.) and a thermal conductivity detector. Helium (15 cm^3 min^{-1}) was utilized as carrier gas. Injector, oven and detector temperatures were 90, 25 and 100 °C, respectively. pH, chemical oxygen demand (COD) and volatile suspended solids (VSS) were evaluated according to *Standard Methods*.15

Batch assays

After some preliminary experiments that allowed the most appropriate operating conditions to be defined, anoxic batch assays were performed in 300 cm^3 vials filled with 250 cm^3 of medium. Each flask was inoculated with 2 g VSS dm^{-3}, using sludge obtained from the anoxic chamber of the full-scale wastewater treatment plant of a resin-producing factory. The initial pH was adjusted to 7.5; sodium bicarbonate was used as pH buffer. The medium was supplemented with 2.5 cm^3 of nutrient solution composed of $CaCl_2 \cdot 2H_2O$ 1.00, $FeSO_4 \cdot 7H_2O$ 0.50, $MnCl_2 \cdot 4H_2O$ 0.25, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ 0.05, $CuSO_4 \cdot 5H_2O$ 0.05, $MgSO_4 \cdot 7H_2O$ 2.40 and $CoCl_2 \cdot 6H_2O$0.001 g dm^{-3}. Ammonium chloride and potassium phosphate monobasic were added in order to obtain a COD:N:P ratio of 200:5:1. Then potassium nitrate (400 mg N dm^{-3}) and different formaldehyde (1360–160 mg dm^{-3}) and methanol (270–950 mg dm^{-3}) concentrations were added, maintaining the COD:N ratio at 3.6 (COD 1440 mg dm^{-3}). Finally, the flasks were sealed and nitrogen gas was passed through the headspace for 5 min in order to remove oxygen. Assays were performed in a thermostated chamber at 20 °C and with constant shaking at 200 rpm.

Continuous reactor

An anoxic upflow sludge blanket reactor, made of glass with a length of 45 cm, an inner diameter of 5.5 cm and an effective volume of 0.92 dm^3, was used for continuous assays (Fig. 1). The system was provided with a liquid displacement biogas measurement device.16

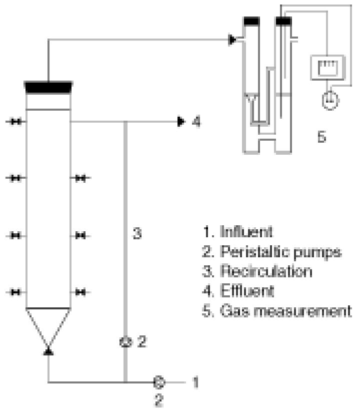

Figure 1.

Laboratory-scale anoxic upflow sludge blanket reactor.

The reactor was inoculated with 7.2 g VSS dm^{-3} of anoxic sludge from the full-scale wastewater treatment plant of a resin-producing factory. The hydraulic retention time was 1.9 days. The influent consisted of a synthetic solution containing formaldehyde, potassium nitrate, ammonium chloride, potassium phosphate buffer and 10 cm dm^{-3} of the nutrient solution mentioned above. The nitrate concentration was 400 mg N dm^{-3}, while the formaldehyde (1500–275 mg dm^{-3}) and methanol (153–871 mg dm^{-3})

concentrations in the influent were varied, maintaining the organic loading rate at 0.84 g COD dm^{-3} d^{-1} (COD/N 4).

RESULTS AND DISCUSSION

Batch assays

Batch assays with different initial formaldehyde and methanol concentrations were undertaken in order to study formaldehyde biodegradation in the presence of a co-substrate. Formaldehyde and methanol concentrations between 1360 and 160 and between 270 and 950 mg dm^{-3}, respectively, were added, maintaining the COD at 1440 mg dm^{-3} [Fig. 2(A)]. In all assays formaldehyde was completely biodegraded in a few days. The data show that high formaldehyde concentrations could be removed under anoxic conditions in the presence of methanol.

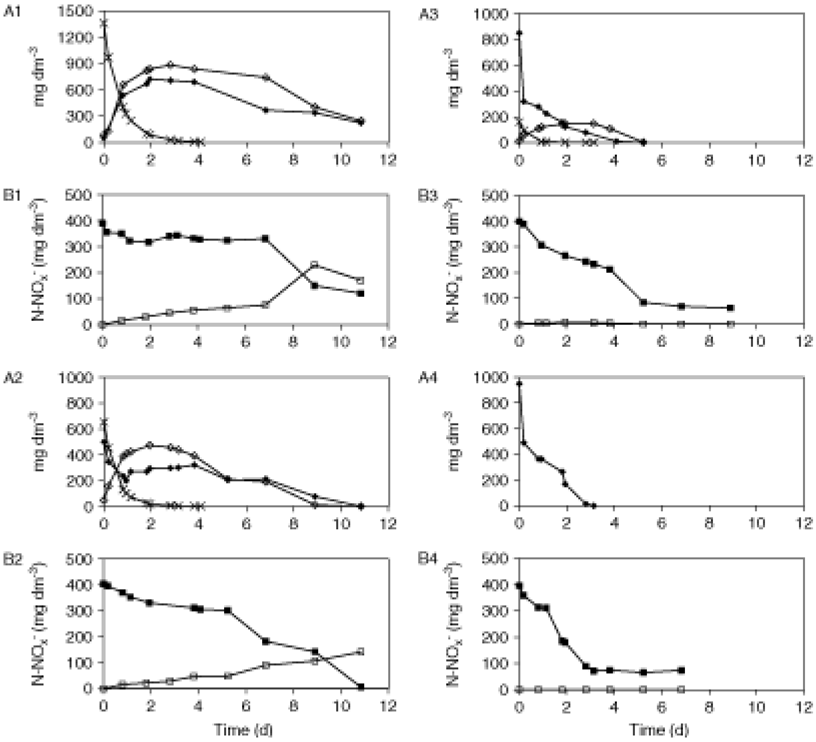

Figure 2.

(A) Concentrations of formaldehyde (×), methanol (♦) and formic acid (◊). (B) Concentrations of nitrite (□) and nitrate (▪). Initial concentrations: (1) 1360 mg CH_2O dm^{-3}; (2) 650 mg CH_2O dm^{-3} and 500 mg CH_3OH dm^{-3}; (3) 160 mg CH_2O dm^{-3} and 850 mg CH_3OH dm^{-3}; (4) 950 mg CH_3OH dm^{-3}.

According to the data for formaldehyde, methanol and COD concentrations, it was concluded that another organic compound was forming in the medium. Kato *et al.*17 studied aerobic formaldehyde biodegradation by *Pseudomonas putida* F61. They found an enzyme which catalysed the dismutation of formaldehyde to form methanol and formic acid. Similarly, Adroer *et al.*18 studied the mechanism of aerobic formaldehyde biodegradation by a strain of *Pseudomonas putida*. Their results indicated that this biodegradation led to the simultaneous appearance of methanol and formic acid.

In our research, it was confirmed that formaldehyde removal led to the formation of methanol and formic acid [Fig. 2(A)]. The degradation of methanol and formic acid began after the exhaustion of formaldehyde in the medium. Whereas methanol as the single carbon source was removed in few days, in the presence of formaldehyde its removal took longer. This could be due to the inhibition of microorganisms caused by formaldehyde. However, this inhibition could also be caused by the presence of formic acid, since the inhibition remained after formaldehyde had been completely removed. Moreover, this inhibition was also observed in batch assays undertaken with formic acid as the single carbon source. Therefore, at higher initial formaldehyde concentration, the formic acid accumulation was also higher and the removal of methanol was slower.

Biological denitrification in the presence of different formaldehyde and methanol concentrations was investigated in batch assays. The initial nitrate concentration was 400 mg N dm^{-3} and the COD:N ratio was maintained at 3.6. The evolution of nitrite and nitrate concentrations is presented in Fig. 2(B). Complete denitrification did not take place in any case. It can be observed that the decrease in the nitrate concentration occurred simultaneously with the decrease in the methanol and formic acid concentrations. Therefore, it was concluded that formaldehyde was transformed into methanol and formic acid, which were then used as carbon sources for denitrification.

In all assays, except with methanol as the single carbon source, accumulation of nitrite took place. This accumulation increased with increase in the initial formaldehyde concentration. At the highest formaldehyde concentration (1360 mg dm^{-3}), around 230 mg N dm^{-3} nitrite accumulated, whereas only 6 mg N dm^{-3} were reached at the lowest formaldehyde concentration (160 mg dm^{-3}). The nitrite accumulation could be due to the inhibition of microorganisms caused by formaldehyde. However, this accumulation could also be caused by the presence of formic acid produced during formaldehyde dismutation, since the formation of nitrite continued after formaldehyde had been completely removed. Moreover, in batch assays that were undertaken with formic acid, nitrite accumulation was also observed. Therefore, at higher initial formaldehyde concentration, the formic acid accumulation was also higher and a higher nitrite concentration was reached.

Continuous reactor

In order to study formaldehyde biodegradation in the presence of methanol, the continuous reactor was fed different concentrations of both compounds, maintaining the organic loading rate at 0.84 g COD dm^{-3} d^{-1} (COD/N 4). The evolution of formaldehyde concentration in the influent and effluent and methanol concentration in the influent of the reactor is shown in Fig. 3. The methanol concentration in the effluent is not represented because it was not detected throughout the operation period. Formaldehyde concentrations up to 1500 mg dm^{-3} (0.84 g COD dm^{-3} d^{-1}) were biodegraded with removal efficiencies >99.8%, its concentration in the effluent being <0.8 mg dm^{-3}. With regard to methanol, its biodegradation was complete at concentrations between 153 and 871 mg dm^{-3}. The results indicate that the presence of methanol at the concentrations studied did not affect the anoxic biodegradation of formaldehyde. Therefore, methanol could be used as external carbon source for denitrification in wastewaters with formaldehyde, without affecting its removal.

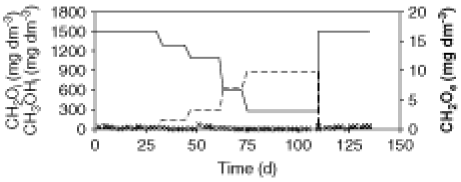

Figure 3.

Formaldehyde concentration in the influent (solid line) and effluent (×) and methanol concentration in the influent (dashed line) of the continuous reactor.

The mean COD removal was 96.7%, which is lower than the percentage of formaldehyde removal. The average COD value in the effluent was 54.1 mg dm^{-3}. As has been indicated before, formaldehyde removal led to the appearance of methanol and formic acid in the medium. Therefore, formic acid was measured but it was not detected in the effluent throughout the operation period. This remaining COD in the effluent could be due to extra enzymes or inert bioproducts released from the sludge in the reactor caused by turbulent shear stress.

Biological denitrification in the presence of formaldehyde and methanol was also studied in the continuous reactor. During all the operation period, the pH in the effluent was between 7.6 and 8.8, with a mean value of 8.2. The nitrate concentration in the influent was 400 mg N dm^{-3} (0.21 g N-NO_3^-dm^{-3} d^{-1}) and the formaldehyde and methanol concentrations were changed, maintaining the COD:N ratio at 4. The evolution of nitrate and nitrite concentrations is presented in Fig. 4, together with the methanol concentration in the influent.

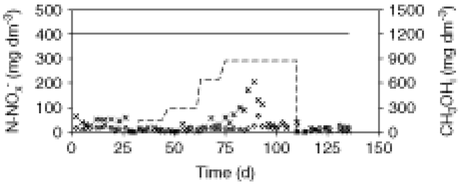

Figure 4.

Nitrate in the influent (solid line) and nitrate (×) and nitrite (◊) in the effluent and methanol in the influent (dashed line) of the continuous reactor.

Initially, the reactor was fed only formaldehyde and the nitrate and nitrite concentrations in the effluent were between 13.0 and 63.7 and between 2.7 and 24.5 mg N dm^{-3}, respectively. However, these concentrations decreased after 26 days of operation. After increasing the methanol concentration in the influent, an increase in the nitrate concentration in the effluent was observed. After increasing the methanol concentration to 871 mg dm^{-3} (day of operation 75), the increase in the nitrate and nitrite concentrations in the effluent was higher, reaching 203.1 and 65.7 mg N dm^{-3}, respectively. After 18 days of operation with this methanol concentration, the denitrification was recovered and the nitrate and nitrite concentrations in the effluent decreased to <40.6 and <38.0 mg N dm^{-3}, respectively. Subsequently, the reactor was again fed only formaldehyde, the nitrate and nitrite concentrations in the effluent being

<16.7 and <26.4 mg N dm^{-3}, respectively. Therefore, a mean denitrification efficiency of 95.4% was reached during this last period.

Each increase in the methanol concentration in the influent caused a decrease in the denitrification percentage. However, after an adaptation period the denitrification percentage was recovered. This is in agreement with data found by other workers, who also observed this adaptation period to methanol, after which the denitrification in the system improved. Nyberg *et al.*4 operated a wastewater treatment plant with a post-denitrification system of activated sludge. Methanol was supplied as external carbon source and they observed that the denitrification with methanol required an adaptation period of the biomass to this compound. In their case, this adaptation period lasted around 1 month until reaching 90% denitrification. Purtschert *et al.*19 operated a wastewater treatment plant with a pre-denitrification system of activated sludge. The supply of methanol as external carbon source improved the denitrification percentage from 35 to 55%, after an adaptation period of a few days.

With regard to the biogas, it was mainly composed of nitrogen, with a mean value of 92.6%, the average carbon dioxide content being 4.3% (Fig. 5). Methane was never detected in the gas phase and nitrous oxide was only detected during days of operation 14–26 and 75–110, at levels <0.9%.

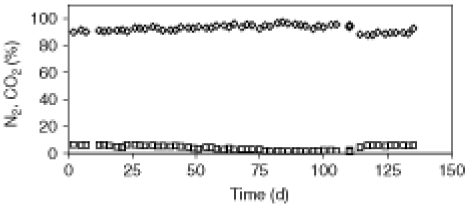

Figure 5.

Evolution of biogas composition: nitrogen (◊) and carbon dioxide (□).

The biomass concentration in the reactor remained almost constant at around 7.2 g VSS dm^{-3}. The biomass concentration in the effluent ranged between 0.054 and 0.180 g VSS dm^{-3} during all the operation period. During this study, the dark brown colour of the sludge changed to pale. Timmermans and van Haute20 observed a similar behaviour in an anoxic reactor fed with high concentrations of methanol and nitrate. After several months of operation, a pale sludge with high denitrifying efficiency was formed. Microscopic studies revealed that the sludge was composed of *Hyphomicrobium* sp., facultative methylotrophic bacteria which grow in media in which denitrification with methanol as carbon source takes place.21

In both batch and continuous studies, it has been proved that the anoxic biodegradation of formaldehyde in the presence of methanol is possible. However, unlike batch assays, in the continuous reactor methanol and formic acid were not detected in the effluent. Moreover, in the reactor the denitrification percentages were higher and the nitrite accumulation was lower, probably because formaldehyde and formic acid did not accumulate and, consequently, the denitrification process was not inhibited. Therefore, the denitrification process can be undertaken using formaldehyde and methanol as carbon sources, provided that the sludge is adapted to the presence of methanol.

CONCLUSIONS

Batch assays

High formaldehyde concentrations (up to 1360 mg dm^{-3}) were removed under anoxic conditions in the presence of methanol. It was confirmed that formaldehyde biodegradation led to the appearance of methanol and formic acid in the medium.

The denitrification process was affected by the initial formaldehyde concentration. At the highest formaldehyde concentration (1360 mg dm^{-3}), around 230 mg N dm^{-3} nitrite were reached.

Continuous reactor

The continuous reactor was fed different concentrations of formaldehyde (1500–275 mg dm^{-3}) and methanol (153–871 mg dm^{-3}), maintaining the organic loading rate at 0.84 g COD dm^{-3} d^{-1} (COD/N 4). The biodegradation of both compounds took place. The presence of methanol at the studied concentrations did not affect the anoxic biodegradation of formaldehyde.

Each increase in the methanol concentration in the influent caused a decrease in the denitrification percentage. An adaptation period to methanol was necessary; subsequently, the denitrification percentage was recovered.

It has been proved that the anoxic biodegradation of formaldehyde in the presence of methanol is possible. The denitrification process can be undertaken using formaldehyde and methanol as carbon sources.

Acknowledgements

This research was funded by the Spanish Ministry of Science and Technology (projects REN2000-0218-P4-02 and PPQ2002-02882) and by the Xunta de Galicia (projects PGIDIT03PXIC10304PN and PGIDIT03AM13E).

REFERENCES

- 1*Cantó M, Gómez J, Kennes C and Veiga MC, Integrated anoxic–aerobic treatment of wastewaters from a synthetic resin producing factory, in European Conference on New Advances in Biological Nitrogen and Phosphorus Removal for Municipal or Industrial Wastewaters, Narbonne, pp. 289–292 (1998).*
- 2*Eiroa M, Kennes C and Veiga MC, Formaldehyde biodegradation and its inhibitory effect on nitrification. J Chem Technol Biotechnol 79: 499–504 (2004).*
- 3*Eiroa M, Kennes C and Veiga MC, Simultaneous nitrification and formaldehyde biodegradation in an activated sludge unit. Bioresource Technol 96: 1914–1918 (2005).*
- 4*Nyberg U, Aspegren H, Andersson B, Jansen JC and Villadsen IS, Full-scale application of nitrogen removal with methanol as carbon source. Water Sci Technol 26: 1077–1086 (1992).*
- 5*Azachi M, Henis Y, Oren A, Gurevich P and Sarig S, Transformation of formaldehyde by a Halomonas sp. Can J Microbiol 41: 548–553 (1995).*

- 6*Yamazaki T, Tsugawa W and Sode K, Biodegradation of formaldehyde by a formaldehyde-resistant bacterium isolated from seawater. Appl Biochem Biotechnol 91–93: 213–217 (2001).*
- 7*Hidalgo A, Lopategi A, Prieto M, Serra JL and Llama MJ, Formaldehyde removal in synthetic and industrial wastewater by Rhodococcus erythropolis UPV-1. Appl Microbiol Biotechnol 58: 260–263 (2002).*
- 8*Qu M and Bhattacharya SK, Toxicity and biodegradation of formaldehyde in anaerobic methanogenic culture. Biotechnol Bioeng 55: 727–736 (1997).*
- 9*Lu Z and Hegemann W, Anaerobic toxicity and biodegradation of formaldehyde in batch cultures. Water Res 32: 209–215 (1998).*
- 10*Omil F, Méndez D, Vidal G, Méndez R and Lema JM, Biodegradation of formaldehyde under anaerobic conditions. Enzyme Microbiol Technol 24: 255–262 (1999).*
- 11*Garrido JM, Méndez R and Lema JM, Treatment of wastewaters from a formaldehyde–urea adhesives factory. Water Sci Technol 42: 293–300 (2000).*
- 12*Campos JL, Sánchez M, Mosquera A, Méndez R and Lema JM, Coupled BAS and anoxic USB system to remove urea and formaldehyde from wastewater. Water Res 37: 3445–3451 (2003).*
- 13*Eiroa M, Kennes C and Veiga MC, Formaldehyde and urea removal in a denitrifying granular sludge blanket reactor. Water Res 38: 3495–3502 (2004).*
- 14*Nash T, The colorimetric estimation of formaldehyde by means of the Hantzch reaction. Biochem J 55: 416–421 (1953).*
- 15*APHA, Standard Methods for the Examination of Water and Wastewater, 20th edn. APHA, Washington DC (1998).*
- 16*Veiga MC, Soto M, Méndez R and Lema JM, A new device for measurement and control of gas production by bench-scale anaerobic digesters. Water Res 24: 1551–1554 (1990).*
- 17*Kato N, Shirakawa K, Kobayashi H and Sakazawa C, The dismutation of aldehydes by a bacterial enzyme. Agric Biol Chem 47: 39–46 (1983).*
- 18*Adroer N, Casas C, de Mas C and Solà C, Mechanism of formaldehyde biodegradation by Pseudomonas putida. Appl Microbiol Biotechnol 33: 217–220 (1990).*
- 19*Purtschert I, Siegrist H and Gujer W, Enhanced denitrification with methanol at WWTP Zurich–Werdholzli. Water Sci Technol 33: 117–126 (1996).*
- 20*Timmermans P and van Haute A, Denitrification with methanol. Water Res 17: 1249–1255 (1983).*
- 21*Purtschert I and Gujer W, Population dynamics by methanol addition in denitrifying wastewater treatment plants. Water Sci Technol 39: 43–50 (1999).*