

Comparison of sequentially combined carbon with sole carbon in denitrification and biological phosphorus removal

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Abstract The sequentially combined carbon (SCC) of methanol and acetic acid was used for the biological nutrient removal (BNR). Its BNR performance was compared with methanol or acetic acid as a sole carbon substrate. Compared to the sole carbon substrate, the use of SCC demonstrated the highest overall TIN removal of 98.3% at a COD ratio of 30 mg COD/l of methanol/50 mg CDO/l of acetic acid. Furthermore, denitrification was more enhanced when methanol was used as one of the SCC, rather than as a sole carbon source. Complete phosphorus removal was accomplished with a non-detectable o-P concentration when SCC was added. This research also showed that aerobic denitrifiers appear to prefer acetic acid to methanol, and the amount of poly- β -hydroxybutyrate (PHB) stored by P accumulating organisms (PAOs) using acetic acid in the anoxic zone could be another important factor in improving the aerobic denitrification. The SCC was a very favorable carbon source for the aerobic denitrification since acetic acid was utilized more efficiently for P-release in accordance with increase of PHB stored in the cell of PAOs by removing nitrogen first using methanol.

Keywords Acetic acid; aerobic denitrification; denitrifying phosphorus removing bacteria; methanol; poly- β -hydroxybutyrate; sequentially combined carbon

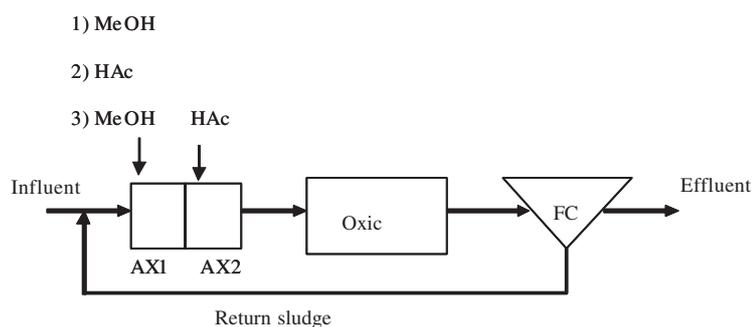
Introduction

Methanol or acetic acid is frequently chosen as an external carbon source to enhance biological nutrient removal (BNR). It has been reported that methanol is a suitable carbon source for improvement of denitrification but not in phosphorus removal. Acetic acid is suggested as the most efficient external carbon source for phosphorus removal as well as nitrogen removal. However, acetic acid that is a favourable substrate for both denitrification and phosphorus removal causes increase of substrate competition between denitrifying bacteria and P accumulating organisms (PAOs). As a result, a high amount of acetic acid is required to overcome substrate competition for successful BNR. In order to decrease the adverse effect of nitrate on P-release due to substrate competition, the use of sequentially combined carbon (SCC), methanol for N removal and acetic acid for P removal, was investigated and demonstrated excellent BNR performance (Cho, 2001). The best BNR removal was accomplished at the level of 30 mg COD/l of methanol in the first anoxic zone and 50 mg COD/l of acetic acid in the second anoxic zone. In the present research, the primary objective was to compare the effects of SCC with respective sole carbon sources, acetic acid and methanol all at quantity equivalent to 80 mg COD/l.

Materials and methods

Operation of bench-scale pilot plant

For the use of SCC, the experimental system employed in this research consisted of two anoxic zones to feed methanol in the first anoxic zone and acetic acid in the second anoxic



1) Sole methanol was added to the first anoxic zone, 2) Sole acetic acid was added to the first anoxic zone, 3) For the SCC addition, methanol and acetic acid was added to the first and second anoxic zones, respectively. AX1: first anoxic zone, AX 2: second anoxic zone, FC: final clarifier

Figure 1 Flow scheme of bench scale process

Table 1 Characteristics of MLE effluent as influent to bench scale reactor

Component	Concentration, mg/l
TIN	24–25 for Phase I 36–37 for Phase II
Ortho-P	4–6
*SCOD	105–110
*TCOD	160–170
pH	7–7.4

*The concentration of COD is comprised of external carbon substrate of 80 mg COD/l and influent carbon substrate that was mostly non-biodegradable.

zone, an oxic zone and a final settling tank (Figure 1). The settled biological solids were recycled back to the first anoxic zone at 100% of inflow flow rate. The influent wastewater was the effluent of the MLE (Modified Luzack-Ettinger) activated sludge pilot plant at the Rockland County, NY, Sewer District No. 1. The characteristics of the influent was presented in Table 1. Since readily biodegradable carbon was not available significantly in the MLE activated sludge effluent, nitrogen and phosphorus removal in this research was affected not by the influent carbon substrate but by the external carbon substrate. The HRT was 30 minutes for each anoxic zone and 80 minutes for the oxic zone. The MLSS was maintained in the range of 4000–4500 mg/l, corresponding to SRT of 7 to 15 days.

Analytical methods

The analyses were performed three times per week. All filtered samples from influent, all bioreactors and final settling tank were analyzed for ammonia, nitrite, nitrate, orthophosphate (ortho-P), soluble COD (SCOD). Unfiltered sample taken from influent and effluent were analyzed for total COD. Ammonia was measured by the Nessler method using a Hach Nessler Reagent and a Hach spectrophotometer (DR-2000). Nitrite and nitrate were determined by a colorimetric method using the Hach ready-made reagent (NitraVer 3 nitrite reagent powder and NitraVer 6 nitrate reagent powder) and a Hach spectrophotometer (DR-2000). Ortho-P was measured by Ascobic acid method using the Hach ready-made reagent (Posver 3 reagent) and a Hach spectrophotometer (DR-2000). COD was measured by the closed reflux colorimetric method using Hach digestion vials-low range and a Hach spectrophotometer (DR-2000). Dissolved oxygen (DO) concentration was measured using DO meter (YSI, Model 58, USA). pH was determined using a glass electrode pH meter (Orion, Model 525A, USA). Measurement of suspended solids (SS), volatile suspended solids (VSS) and sludge volume index (SVI) follow *Standard Methods* (APHA, 1995).

Results and discussion

The experiments were conducted in two phases to compare respective sludges derived from addition of SCC, sole acetic acid and sole methanol with respect to: (1) BNR efficiency and (2) the capacity of aerobic denitrification. All the results represent the mean of at least 6 experiment data obtained under steady state conditions.

Comparison of BNR efficiency (phase 1)

Nitrogen removal. Figure 2 showed that influent TIN concentrations were consistent in the range 24.21–25.1 mg N/l over three runs. In the final effluent, the lowest TIN concentration was obtained in the SCC addition run at 0.41 mg N/l (98.3%) and the second lowest one in the sole acetic acid addition run at 1.11 mg N/l (95.5%) followed by the sole methanol addition run at 3.95 mg N/l (84.3%). There was no significant difference in TIN effluent concentrations obtained from the anoxic, oxic, return and final settling tank in each run, indicating that nitrogen removal was due mainly to denitrification in the anoxic zone.

As shown in Figure 3, total amount of NO_x removal in the anoxic zones was 8.879 g N/d, 8.838 g N/d, 8.325 g N/d, corresponding to removing 98%, 93.3%, 88.5% of NO_x content in the SCC addition run, sole acetic acid addition run and sole methanol addition run, respectively. It should be noted that the amount of NO_x removed in the anoxic zone with methanol was higher in the first anoxic zone (HRT of 30 minutes) of the SCC addition run compared to that removed throughout both first and second anoxic zone (60 minutes of total anoxic HRTs) of the sole methanol addition run. This result could imply that the use of methanol as one of the SCC, rather than as a sole carbon, is more effective in enhancing the denitrification rate due to the biomass acclimated with the SCC substrate.

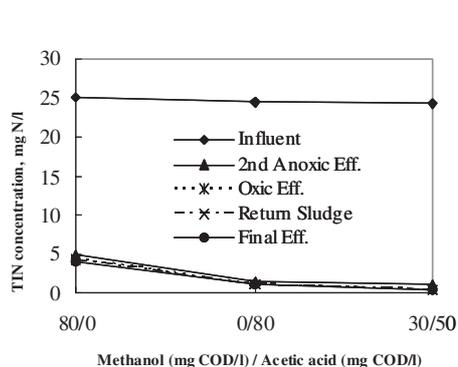


Figure 2 Concentration of TIN effluent in phase 1

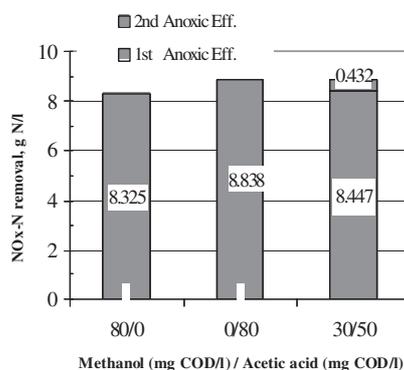


Figure 3 NO_x-N removal in the anoxic zone

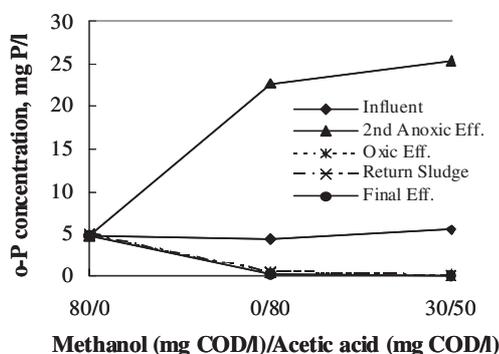


Figure 4 Concentration of o-P effluent in phase 1

Phosphorus removal. Figure 4 represented the o-P concentrations observed in the influent, the first anoxic zone, the second anoxic zone, the oxic zone, the return sludge and final settling tank, over 3 runs.

The maximum net P-release and uptake was observed in the SCC addition run with 24.54 mg P/l and 25.25 mg P/l, respectively. Despite the addition of sole acetic acid at a high dose of 80 mg COD/l, the amount of P released during total anoxic HRT of 60 minutes was lower than that released during the anoxic HRT of 30 minutes in the second anoxic zone of the SCC addition run where an acetic acid concentration of 50 mg COD/l was added. This might be ascribed to the increase of competition between denitrifiers and PAOs for acetic acid uptake, which resulted from the introduction of high nitrate concentrations into the anoxic zone. That is, a larger portion of acetic acid in the sole acetic acid addition run might be used for denitrification than for P-release since denitrifiers utilize acetic acid faster than PAOs. On the other hand, most of the nitrate in the SCC addition run was removed in the first anoxic zone with methanol, thus almost all acetic acid added in the second anoxic zone would be available for P-release. This resulted in the higher P-release even though acetic acid dosage was 30 mg COD/l less compared to the sole acetic acid addition run. The non-detectable o-P concentration in the final effluent was achieved in the SCC addition run, second low effluent phosphorus concentration in the sole acetic acid addition run at 0.21 mg P/l, corresponding to overall removal efficiency of 95.1%, and lastly in the sole methanol addition run at 4.71 mg P/l yielding an overall efficiency of 0.8%, indicating methanol had a negligible effect on P removal.

Comparison of aerobic denitrification capacity (phase 2)

Nitrogen removal. In Figure 5, since internal recirculation from oxic to anoxic zone was not performed and a very low concentration of influent nitrate was almost denitrified in the anoxic zone regardless of three different types of external carbon substrate, TIN concentration remaining in the anoxic effluent in each run was not significantly different. Therefore, TIN removal efficiency would be dependent mainly on aerobic nitrogen removal.

The percentage of aerobic denitrification was calculated by dividing total inorganic nitrogen loss in the oxic zone by total inorganic nitrogen entering the oxic zone. The high percentages were found at 53.5% and 50% in the SCC addition run and in the sole acetic acid addition run, respectively. These percentages were almost twice higher compared to 25.1% of aerobic denitrification obtained from the sole methanol addition run. As a result, overall TIN removal efficiency was about 10% higher in both the SCC and the sole acetic acid addition runs than in the sole methanol addition run. This would imply that the aerobic denitrifiers appeared to prefer acetic acid to methanol. It is, however, interesting that compared to the sole acetic acid, the use of SCC achieved similar amount of aerobic nitrogen removal even though acetic acid dosage was 30 mg COD/l less. As shown in Figure 6, the amount of P-release was higher in the SCC addition run than in the sole acetic acid addition run. Thus, the amount of poly- β -hydroxybutyrate (PHB) stored by PAOs is believed to be higher in the SCC addition run than in the sole acetic acid addition run. The PHB metabolism investigated by many researchers (Dawes and Senior, 1973; Tam *et al.*, 1992; Kern-Jespersen and Henze, 1993; Mino *et al.*, 1995; Barker and Dold, 1996; Chuang *et al.*, 1996) is that PHB is synthesized if a carbon substrate is present, while PHB is degraded in the presence of oxygen or nitrate when the carbon substrate is limited. Accordingly, it could be possibly stated that a fraction of PAOs is able to denitrify under carbon limited and aerobic conditions as well as under carbon limited and anoxic conditions since they can use nitrate as well as oxygen as an electron acceptor to oxidize PHB. Zhao (1998) also suggested that a fraction of PAOs could be aerobic denitrifiers. Therefore, the increase of PHB associated with acetic acid uptake for P-release could be

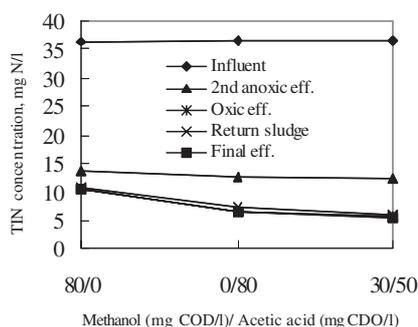


Figure 5 Concentration of TIN effluent in phase 2

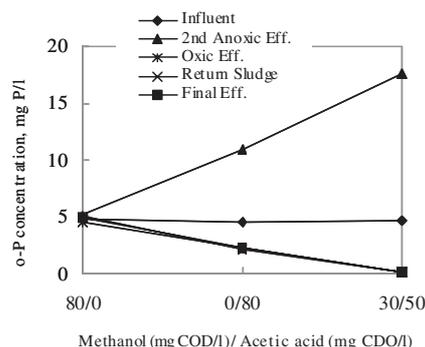


Figure 6 Concentration of o-P effluent in phase 2

another important factor to improve the simultaneous aerobic denitrification and phosphorus uptake. The overall efficiencies of TIN were 85% in the SCC addition run, 82.5% in the sole acetic acid addition run and 71.5% in the sole methanol addition run.

Phosphorus removal. The overall phosphorus removal efficiency was 96.6% in the SCC addition run, whereas 49.2% and negative removal efficiency were observed in the sole acetic acid addition run and in the sole methanol addition run, respectively (Figure 6). Compared to the experiment phase 1, a significant reduction of phosphorus removal was observed in the sole acetic acid addition in spite of the same amount of acetic acid 80 mg COD/l. This phenomenon might be due to the decrease of pH in experiment phase 2 than in experiment phase 1. From the results of batch tests (Cho, 2001), pH was decreased instantly by adding acetic acid and was increased gradually due to the recovery of alkalinity by denitrification. Accordingly, the lower pH in experiment phase 2 may result from a lower buffer capacity due to the less denitrification corresponding to a much lower concentration of influent nitrate compared to experiment phase 1.

The effect of pH on P-release and per cent overall removal efficiency is illustrated in Figure 7. The concentration of P-release in the second anoxic zone and % overall efficiency were increased as pH increased. In the SCC addition, there was only 3.4% decrease in the overall removal efficiency although o-P concentration in the second anoxic zone was decreased from 25.25 to 17.58 mg/l with decreasing pH from 7.17 to 6.82. However, the overall removal efficiency in the 80 mg COD/l of sole acetic acid was significantly decreased from 95% to 49.2% when the pH decreased from 7.05 to 6.45. Furthermore, the values of pH in the SCC addition run were higher than those in the sole acetic acid addition at 80 mg COD/l. Consequently, an excess acetic acid causing a decrease of pH to around 6.5 could be detrimental to excess biological phosphorus removal. These results could explain the Randall and Chopin study (1994) reporting that the high acetic acid addition enhanced

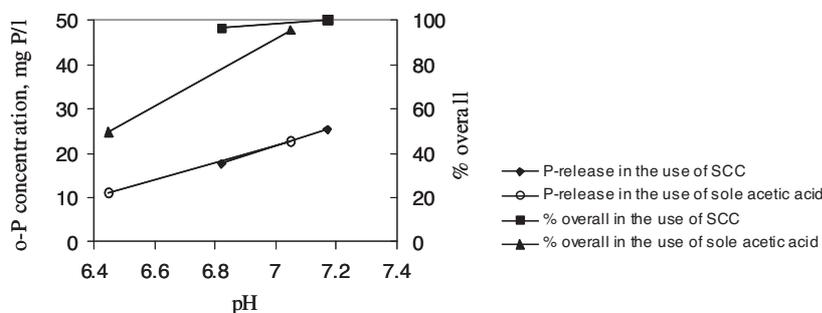


Figure 7 Effects of pH on P-release and P removal efficiency

the growth of non-phosphorus removal bacteria more than the growth of phosphorus removal bacteria, leading to gradually washout of the phosphorus removal bacteria from the activated sludge and finally a failure of BPR.

Conclusions

The SCC was compared with sole carbon substrate in terms of nutrient removal efficiency and aerobic denitrification. The use of SCC demonstrated superior removal efficiency of biological nitrogen and phosphorus removal, and it could reduce the requirement of acetic acid for excess phosphorus removal by removing NO_x first with methanol in order to reduce of the competition for acetic acid uptake between denitrification and P-release. In addition, denitrification with methanol was enhanced if methanol was used as one of the SCC substrate, rather than as the sole methanol substrate, indicating that the SCC addition provides more favorable environmental conditions for denitrifiers using methanol than the sole methanol addition. In the aerobic denitrification, the use of SCC was also more effective compared to the use of sole carbon substrate.

References

- Barker, P.S. and Dold, P.L. (1996). Denitrification behavior in biological excess phosphorus removal activated sludge systems. *Wat. Res.* **30**(4), 769–780.
- Cho, E.S. (2001). *Effects of combined carbon on biological nitrogen and phosphorus removal*. PhD thesis, Department of Civil and Environmental Engineering, Polytechnic University, Brooklyn, NY.
- Chuang, S., Ouyang, C. and Wang, Y. (1996). Kinetic competition between phosphorus release and denitrification on sludge under anoxic condition. *Wat. Res.* **30**(12), 2961–2968.
- Dawes, E.A. and Senior, P.J. (1973). The role and regulation of energy reserve polymers in microorganisms. *Adv. Microb. Physiol.*, **10**, 135–266.
- Gerber, A., Mostert, E.S. Winter C.T., deVilliers R.H. (1987) Interactions between phosphate, nitrate and organic substrate in biological nutrient removal processes. *Wat. Sci. Tech.*, **19**(1/2), 183–194.
- Kernn-Jespersen, J.P. and Henze, M. (1993). Biological phosphorus uptake under anoxic and aerobic conditions. *Wat. Res.* **27**(4), 617–624.
- Mino, T., Liu, W.T., Kurisu, F. and Matsuo, T. (1995) Modelling glycogen storage and denitrification capability of microorganisms in enhanced biological phosphate removal processes. *Wat. Sci. Tech.*, **31**(2), 25–34.
- Randall, C.W., and Chapin, R.W. (1994). Acetic acid inhibition of biological phosphorus removal. *Proc. Water Environ. Fed. 67th Annu. Conf. Exposition*, Chicago, III, 459.
- Standard Methods for The Examination of Water and Wastewater* (1995). 19th edn, American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC, USA.
- Tam, N.F.Y., Wong, Y.S. and Leung G. (1992). Effect of exogenous carbon sources on removal of inorganic nutrient by the nitrification-denitrification process. *Wat. Res.* **26**(9), 1229–1236.
- Zhao, H.W. (1998). *Oxidation-reduction potential and organic carbon source as two control parameters for simultaneous nitrification and denitrification in biological nutrient removal processes*. PhD thesis, Department of Civil Engineering, The University of British Columbia Vancouver, Canada.