

1 **Evaluating two concepts for the modelling of intermediates**
2 **accumulation during biological denitrification in wastewater**
3 **treatment**

4

5 Yuting Pan¹, Bing-Jie Ni¹, Huijie Lu², Kartik Chandran³, David Richardson^{1,4}, Zhiguo
6 Yuan^{1*}

7

8 ¹Advanced Water Management Centre (AWMC), The University of Queensland, St Lucia,
9 Brisbane, QLD 4072, Australia

10 ²Department of Civil and Environmental Engineering, University of Vermont, Burlington,
11 VT 05405, USA

12 ³Department of Earth and Environmental Engineering, Columbia University, New York, NY
13 10027, USA

14 ⁴Centre for Molecular Structure and Biochemistry (CMSB), School of Biological Sciences,
15 University of East Anglia, Norwich, NR4 7TJ, UK

16

17 * Corresponding author: phone + 61 7 3365 4374; fax +61 7 3365 4726; email:
18 zhiguo@awmc.uq.edu.au

19

20

21 **ABSTRACT**

22 The accumulation of the denitrification intermediates in wastewater treatment systems is
23 highly undesirable, since both nitrite and nitric oxide (NO) are known to be toxic to bacteria,
24 and nitrous oxide (N₂O) is a potent greenhouse gas and an ozone depleting substance. To date,

25 two distinct concepts for the modelling of denitrification have been proposed, which are
26 represented by the Activated Sludge Model for Nitrogen (ASMN) and the Activated Sludge
27 Model with Indirect Coupling of Electrons (ASM-ICE), respectively. The two models are
28 fundamentally different in describing the electron allocation among different steps of
29 denitrification. In this study, the two models were examined and compared in their ability to
30 predict the accumulation of denitrification intermediates reported in four different
31 experimental datasets in literature. The N-oxide accumulation predicted by the ASM-ICE
32 model was in good agreement with values measured in all four cases, while the ASMN model
33 was only able to reproduce one of the four cases. The better performance of the ASM-ICE
34 model is due to that it adopts an “indirect coupling” modelling concept through electron
35 carriers to link the carbon oxidation and the nitrogen reduction processes, which describes the
36 electron competition well. The ASMN model, on the other hand, is inherently limited by its
37 structural deficiency in assuming that carbon oxidation is always able to meet the electron
38 demand by all denitrification steps, therefore discounting electron competition among these
39 steps. ASM-ICE therefore offers a better tool for predicting and understanding intermediates
40 accumulation in biological denitrification.

41

42 **Key words:** denitrification modelling, electron competition, carbon source, nitrous oxide,
43 ASMN, ASM-ICE

44

45 **1. INTRODUCTION**

46 Denitrification is an important process of the global nitrogen cycle. Nitrate reduction consists
47 of four consecutive reduction steps, with nitrite (NO_2^-), nitric oxide (NO) and nitrous oxide
48 (N_2O) as three obligatory intermediates (Zumft 1997). Each reduction step is catalysed by
49 one or more specific enzymes, including nitrate reductase (Nar), nitrite reductase (Nir), NO

50 reductase (Nor) and N₂O reductase (Nos). In wastewater treatment systems, denitrification,
51 together with nitrification, are the key processes to remove nitrogen pollutants from
52 wastewater (Tchobanoglous et al. 2003).

53

54 A long-existing operational issue of wastewater denitrification is the accumulation of N-
55 oxide intermediates. Nitrite and NO are known to be toxic, which could suppress the activity
56 of denitrifiers (Zumft 1997, Ni and Yu 2008). In recent years, the emission of nitrous oxide
57 from wastewater treatment plants (WWTPs) has become an emerging problem, because N₂O
58 is a potent greenhouse gas with a 300-fold stronger radiative force than carbon dioxide, and is
59 also a primary ozone depleting substance in the 21 century (IPCC 2007, Ravishankara et al.
60 2009).

61

62 It has been demonstrated that the accumulation of denitrification intermediates is often a
63 result of electron competition among N-reductases involved in the four denitrification steps
64 (Pan et al. 2013a, Schalk-Otte et al. 2000). Pure culture-based studies of electron transport
65 network in typical denitrifying bacteria, such as *Paracoccus denitrificans*, have proven that
66 all denitrification enzymes derive their electrons from a common electron supply source, i.e.,
67 the ubiquinol pool of the respiratory electron transport chain (Richardson et al. 2009). The
68 structure of this electron transport network sets the stage for the electron competition between
69 the four denitrification steps. The electron competition occurs when the electron supply rate
70 is rate-limiting during denitrification.

71

72 Mathematical modelling has been widely applied to predict nitrogen removal in wastewater
73 treatment. Previous modelling efforts have primarily focussed on the prediction of nitrate
74 removal (Henze et al. 2000), and in some cases, nitrite as well (Ni and Yu 2008). However, it

75 is increasingly recognised that N₂O accumulation should also be modelled, especially due to
76 its detrimental influence on the atmosphere (Ni et al. 2011). It has been proposed to achieve
77 this goal through modelling denitrification as a four-step process, using NO₃⁻, NO₂⁻, NO, and
78 N₂O as the terminal electron acceptor, respectively (Vonschulthess et al. 1994, Schulthess
79 and Gujer 1996, Hiatt and Grady 2008, Pan et al. 2013b). With each step being modelled with
80 individual, reaction-specific kinetics, the accumulation of nitrite, NO and N₂O can be
81 predicted.

82

83 To date, two distinct concepts have been proposed for modelling the four-step denitrification,
84 with their structures shown in Figure 1.

85

86 *Model I: The “direct coupling approach”*, represented by Activated Sludge Model for
87 Nitrogen (ASMN) (Hiatt and Grady 2008), in which the carbon oxidation and nitrogen
88 reduction processes are directly coupled. This type of model describes each of the four steps
89 as a separate and independent oxidation-reduction reaction (Figure 1-a), and reaction-specific
90 kinetics are applied. Many of the multiple step denitrification models have adopted such
91 structure (e.g., Ni et al. (2011), Schulthess and Gujer (1996)).

92

93 *Model II: the “indirect coupling approach”*, proposed by Pan et al. (2013a) and named
94 Activated Sludge Model for Indirect Coupling of Electrons (ASM-ICE), in which the carbon
95 oxidation and nitrogen reduction processes are indirectly coupled. Electron carriers are
96 introduced as a new component in this model to link carbon oxidation to nitrogen oxides
97 reduction (Figure 1-b). As a result, each step of denitrification can be regulated by both the
98 nitrogen reduction and the carbon oxidation processes.

99

100 It is of importance to evaluate the abilities of these two models in predicting denitrification
101 activities and particularly the accumulation of denitrification intermediates. This can be done
102 by conducting parallel comparisons with existing data reported for different denitrifying
103 cultures and/or under different conditions. Therefore, the aim of this work is to reveal how
104 the two model structures presented in Figure 1 would affect their ability to reproduce
105 experimental data reported in literature. Four distinctive denitrifying cultures were used in
106 this examination, including one pure culture (*P. denitrijkans* (N.C.1.B. 8944)) and three
107 mixed denitrifying cultures/sludge fed with different substrates (e.g., acetate or methanol). In
108 particular, the ability of the two models in predicting electron competition during
109 denitrification was assessed. The findings are expected to improve the fundamental
110 understanding of electron competition involved in specific denitrification steps, which could
111 ultimately lead to better modelling and control of intermediate accumulation during
112 wastewater denitrification.

113

114 **2. MATERIALS AND METHODS**

115 **2.1. Mathematical models for denitrification**

116 The kinetic and stoichiometric matrices describing the nitrogen reduction and the carbon
117 oxidation processes for the two mathematical models are presented in Table 1. Nomenclature
118 for all state variables used in this article slightly differs from the original publications (Hiatt
119 and Grady 2008, Pan et al. 2013b). We employ the following symbols for concentrations of
120 various components: heterotrophic biomass (X), nitrate (S_{NO_3}), nitrite (S_{NO_2}), nitric oxide
121 (S_{NO}), nitrous oxide (S_{N_2O}), readily biodegradable carbon source (S_s), reduced form of
122 electron carriers ($S_{M_{red}}$), oxidized form of electron carriers ($S_{M_{ox}}$). Other processes involved
123 in denitrification, such as death and lysis of heterotrophs, hydrolysis of particulate organic
124 nitrogen, are included in both models with standard ASM kinetic expressions and parameter

125 values taken from published literature (Hiatt and Grady 2008). Table 2 lists the definitions,
126 values and units of the parameters used in the two models. Both models are based on mass
127 balance, but with different units. The ASMN model adopted weight unit (gram chemical
128 oxygen demand (COD) and g N) while the ASM-ICE model adopted mole units (mole COD
129 and mole N). The two sets of units can be easily converted.

130

131 As shown in Table 1, with the ASMN model (Model I), the reduction of nitrogen oxide
132 compounds (nitrate, nitrite, nitric oxide and nitrous oxide) and the oxidation of organic
133 carbon are “directly coupled” in a single oxidation-reduction reaction with a process
134 stoichiometry based on electron balance (i.e. I-R₁, I-R₂, I-R₃, I-R₄). In particular, the role of
135 carbon oxidation in denitrification is reflected through the following two aspects: 1) the
136 affinity constants for carbon source of each denitrification step ($K_{S1}, K_{S2}, K_{S3}, K_{S4}$) can be
137 different; 2) the overall carbon oxidation rate is modelled as the sum of the four
138 denitrification steps. The underlying assumption of this modelling approach is that carbon
139 oxidation is always able to meet the demand for electrons by all the four denitrification steps.
140 However, in reality, carbon oxidation could be the rate-limiting step, affecting the
141 denitrification steps through electron competition. The conceptual reaction schemes of the
142 ASMN model are detailed in the supplementary materials.

143

144 In contrast, in the ASM-ICE model (Model II), the carbon oxidation process (II-R₁) is
145 decoupled from the nitrogen reduction processes (II-R₂ to II-R₅). Electron carriers, with *Mox*
146 representing oxidized form of electron carriers and *Mred* ($Mred \rightleftharpoons Mox + 2e^- + 2H^+$)
147 representing reduced form of electron carriers, are introduced as new components in this
148 model to link the carbon oxidation process and the nitrogen oxides reduction processes. *Mox*
149 gains electrons from carbon oxidation and meanwhile being reduced to *Mred* (II-R₁), while

150 *Mred* donates electrons to nitrogen reduction and meanwhile being oxidized back to *Mox* (II-
151 R₂ to II-R₅). The recirculation loop between *Mox* and *Mred* were realized in the ASM-ICE
152 model by implementing $S_{Mred} + S_{Mox} = C_{tot}$, where C_{tot} is a constant value related to the total
153 concentration of electron carriers. The relative ability of each denitrification step to compete
154 for electrons determines the electron distribution, and consequentially the denitrification
155 intermediate accumulation. The different values for the four affinity constants of each
156 denitrification step with respect to electrons ($K_{Mred,1}$, $K_{Mred,2}$, $K_{Mred,3}$ and $K_{Mred,4}$) largely affect the
157 competitiveness of different reduction steps for electrons when the overall carbon oxidation
158 rate become rate limiting. S_{Mox} and S_{Mred} are the concentrations of electron carriers related
159 to active biomass in the system. Therefore, they could be set to zero in influent, given the
160 small amount of active biomass in influent wastewater. The conceptual reaction schemes of
161 the ASM-ICE model are detailed in the supplementary materials.

162

163 **2.2. Testing the predictive abilities of the models**

164 Experimental data from four cases (Kucera et al. 1983, Pan et al. 2012, McMurray 2008, Oh
165 and Silverstein 1999) studying denitrification intermediates dynamics were used to test the
166 predictive abilities of the two mathematical models (Table 3).

167

168 Case 1 (Kucera et al. 1983): The branching of the electron flow to individual terminal
169 acceptors NO_3^- , NO_2^- and N_2O was investigated in a pure denitrifying culture *Paracoccus*
170 *denitrijkans* (N.C.1.B. 8944). The culture was cultivated anaerobically to early stationary
171 phase. A closed reactor with magnetic stirrer was used to carry out batch tests, during which
172 N_2 was provided into the reactor to ensure oxygen free environment. The reaction medium
173 contained 0.25 M sucrose, 20mM Tris/sulphate at pH 7.3. At the beginning of the reaction, 50
174 mM glucose and 1 mM KNO_3 were added. Nitrate concentration was determined with a

175 nitrate-specific electrode. Nitrite concentration was determined colorimetrically. Nitric oxide
176 and N₂O were not measured in the experiment. Two sets of batch tests (*Set A* and *Set B*) were
177 conducted to assess the nitrate and nitrite reduction dynamics.

178

179 Two sets of batch tests (*Set A* and *Set B*) were conducted to assess the nitrate and nitrite
180 reduction dynamics.

181

182 • **In batch test *Set A***, nitrate was firstly added to reach a concentration of 14 mg N/L at the
183 beginning of the test, and then nitrite was added at 0.5 hour to reach around 5 mg N/L.

184 The reduction profiles of both nitrate and nitrite were monitored.

185 • **In batch test *Set B***, nitrate was firstly added to achieve a concentration ranging from 10
186 to 14 mg N/L at the beginning of each test, with the nitrate reduction profile being
187 monitored. Then, either 1) nitrite, or 2) N₂O, or 3) a mixture of nitrite, N₂O and antimycin
188 (an inhibitor for nitrite and N₂O reduction) were added. Therefore, the nitrate reduction
189 rate was measured under the following four different conditions: 1) with only NO₃⁻ as the
190 substrate; 2) with NO₃⁻ and NO₂⁻; 3) with NO₃⁻ and N₂O; 4) with NO₃⁻, antimycin, NO₂⁻
191 and N₂O.

192

193 Case 2 (McMurray 2008): To investigate the denitrification intermediates dynamics, the
194 reduction of nitrate and nitrite, and the corresponding nitrogen gas production by a full-scale
195 activated sludge fed with acetate were studied. The activated sludge was collected from the
196 anoxic zone in a full-scale WWTP. All batch experiments were performed in a 2 litre, sealed
197 Perspex reactor fitted with pH (Ionode IJ44, TPS, Brisbane, Australia) and DO (YSI model
198 5739, Yellow Springs, USA) probes. The pH was maintained at 7.0 ± 0.01 throughout each
199 test, and temperature controlled at 22 °C. The nitrate and nitrite concentrations were analyzed

200 using a Lachat QuikChem8000 Flow Injection Analyzer (Lachat Instrument, Milwaukee,
201 Wisconsin). The N_2 gas was monitored using a mass spectrometer. ” Nitric oxide and N_2O
202 were not measured in the experiment.

203

204 At the beginning of the batch test, nitrate and nitrite were added to achieve initial
205 concentrations around 5.2 mg N/L and 8.9 mg N/L, respectively. Acetate was also added at
206 the same time, and was present in excess throughout the test. The conversions of nitrate,
207 nitrite and acetate were monitored, along with the production rate of nitrogen gas (N_2).

208

209 Case 3 (Pan et al. 2013a): This study aimed to understand the electron competition process
210 during denitrification, using an enriched denitrifying culture fed with methanol. Batch tests
211 were performed in a 300 mL sealed reactor under anaerobic conditions. The pH was
212 maintained at 8.0 ± 0.01 throughout each test. The batch tests were performed in a
213 temperature-controlled room at 22.0 - 23.0°C. Methanol and various nitrogen oxides were
214 supplied to the mixed liquor in each test. The nitrate and nitrite concentrations were analyzed
215 using a Lachat QuikChem8000 Flow Injection Analyzer (Lachat Instrument, Milwaukee,
216 Wisconsin). Methanol was analysed by gas chromatography (Perkin Elmer Autosystem). N_2O
217 in the liquid phase was measured online using a N_2O microsensor (N_2O -100, Unisense A/S,
218 Aarhus, Denmark).

219

220 Four batch tests reported therein are chosen in this paper to evaluate the two types of models,
221 including 1) the reduction of NO_3^- with itself being added as the sole externally-supplied
222 electron acceptor; 2) the reduction of NO_2^- with itself being added as the sole externally-
223 supplied electron acceptor; 3) the reduction of N_2O with itself being added as the sole
224 externally-supplied electron acceptor; 4) the reduction of NO_3^- , NO_2^- and N_2O with all of

225 them being added simultaneously. The initial concentrations of the nitrogen compounds were
226 between 30 and 50 mg N/L. Methanol was used as the carbon source and was in excess in all
227 these four tests. The reduction of the nitrogen compounds were monitored throughout the
228 tests.

229

230 Case 4 (Oh and Silverstein 1999): The effect of COD to N ratio on nitrite accumulation
231 during nitrate reduction by an enriched denitrifying culture fed with acetate was investigated.
232 Experiments were carried out in a 10-L sequencing batch reactor (SBR) operated for
233 activated sludge denitrification. At the beginning of the test, 50 mg N/L nitrate and 130 mg
234 COD/L acetated were provided to the reactor. The reduction of nitrate, the accumulation of
235 nitrite and the oxidation of carbon source were measured throughout the experiment. The
236 SBR system was maintained in a temperature-controlled laboratory at $21\pm 2^{\circ}\text{C}$. SBR
237 operations were controlled with a programmable timer (ChronTrol, XTseries, San Diego,
238 Calif.). Nitrate, nitrite, and acetate were measured using an ion chromatograph (IC)
239 (DionexModel DX-300, AS-10 column, Dionex Corp., Sunnyvale, Ca-lif.). Nitric oxide and
240 N_2O were not measured in the experiment.

241

242 Parameter estimation were performed with AQUASIM for aquatic systems (Reichert et al.
243 1995). Not all the parameters were identifiable from the experimental data, however, most of
244 them have been well established in previous studies, and therefore they were adopted from
245 literature (Hiatt and Grady 2008, Pan et al. 2013b) (Table 2). For example, since relative high
246 COD concentration were used in all the cases, the affinity constants for carbon source of each
247 step (K_{S1} , K_{S2} , K_{S3} and K_{S4}) in the ASMN model were not identifiable based on the
248 experimental data. Therefore, these parameters were adopted from literature. Similar rules
249 applied for some other literature derived parameter values in both models, as listed in Table 2.

250 In this work, only parameters that are unique for each model and sensitive to the experimental
251 data (η_{g^1} , η_{g^2} , η_{g^4} for the ASMN model and $r_{COD,max}$, $K_{Mred,1}$, $K_{Mred,2}$ and $K_{Mred,4}$ for the ASM-
252 ICE model) were calibrated. The calibrated parameter values are presented in Table 2 as well.
253 It should be highlighted that the aim of the modelling work is to verify if various model
254 structures (rather than parameter calibrations) could explain the trend of the experimentally
255 observed denitrification dynamics, because having a solid model structure is a key step
256 towards reliable prediction of denitrification intermediates accumulation.

257

258 **3. RESULTS**

259 **3.1. Evaluation of the Mathematical Models: Case 1**

260 In the first case, the ASMN model and the ASM-ICE model were evaluated based on their
261 abilities in predicting the nitrogen conversion by *P. denitrificans* (N.C.1.B. 8944) (Kucera et
262 al. 1983). The experimental data along with the model predictions are presented in Figure 2,
263 demonstrating the influences of nitrite and N₂O on nitrate reduction.

264

265 The experimental observations from batch test Set A are shown in Figure 2-a1 & a2. The
266 nitrate reduction rate significantly decreased from 10.8 mg N/hour in phase 1 when only
267 nitrate was present, to 2.6 mg N/hour in phase 2 with nitrite addition. After the depletion of
268 nitrite, the nitrate reduction rate recovered immediately and almost to its original level in
269 phase 3. Results given by the ASM-ICE model agree well with the experimental nitrate and
270 nitrite profiles (Figure 2-a2). In contrast, the ASMN model failed to predict the dynamic
271 change of nitrate profile although the nitrite profile was reasonably reproduced (Figure 2-a1).

272

273 The measured nitrate reduction rates under the four different conditions in batch test Set B
274 (Table 3) are shown in Figure 2-b1 & b2. The experimental results showed that the addition

275 of other chemicals (nitrite, N_2O and antimycin) significantly influenced the nitrate reduction
276 rate. Specifically, considering the value of the nitrate reduction rate as 100% when only
277 nitrate itself was added, the nitrate reduction rate decreased to 32% after nitrite addition, and
278 to 6% after N_2O addition. However, when N_2O , nitrite and antimycin (a chemical which
279 inhibits nitrite and N_2O reduction) were added together, the nitrate reduction rate increased
280 up to 233%.

281

282 The ASMN model completely failed to describe these experimentally observed variations in
283 the nitrate reduction rates, but predicted a constant nitrate reduction rate under all conditions
284 (Figure 2-b1). This clearly indicates that the ASMN model is not able to capture the influence
285 of nitrite and N_2O on nitrate reduction. On the contrary, as shown in Figure 2-b2, the ASM-
286 ICE model successfully predicted the influence of nitrite, N_2O and antimycin on nitrate
287 reduction, with 38% of the nitrate reduction rate left after nitrite addition (in comparison to
288 the 32% experimentally observed), 7% left after N_2O addition (in comparison to the
289 experimental data of 6%). The model also correctly predicted the substantial increase (240%
290 in comparisons to the experimentally observed 233%) in the nitrate reduction rate, when
291 antimycin was used to inhibit nitrite and N_2O reduction.

292

293 **3.2. Evaluation of the Mathematical Models: Case 2**

294 In the second case, the denitrification dynamics by a full-scale activated sludge fed with
295 acetate was studied by McMurray (2008). The experimental data along with the model
296 predictions are presented in Figure 3. No N_2O accumulation was observed throughout the
297 experiment, and the N_2O concentration predicted by both models was also negligible.

298

299 The experimental results showed that nitrate was reduced but nitrite accumulated in the first
300 0.3 hour. After the depletion of nitrate, nitrite was then reduced (Figure 3-a1 & a2). COD was
301 consumed during nitrate and nitrite reduction (Figure 3-b1 & b2). The N₂ production rate was
302 around 22 mg N/hour when both nitrate and nitrite were present, and increased to around 28
303 mg N/hour when only nitrite was present (Figure 3-c1 & c2).

304

305 The ASMN model captured the trends of nitrate and nitrite reduction (Figure 3-a1), and the
306 trend of acetate consumption (Figure 3-b1). However, the fitting errors between the model
307 predictions and experimental data were relatively large. These errors can be clearly seen in
308 the mismatch between the model-predicted and experimentally observed N₂ production rates
309 (Figure 3-c1). In comparison, the ASM-ICE model successfully reproduced all the nitrogen
310 profiles observed, including the changes in N₂ production rate (Figure 3-a2, b2 & c2).

311

312 **3.3. Evaluation of the Mathematical Models: Case 3**

313 In the third case, the ASMN model and the ASM-ICE model were evaluated based on their
314 ability to capture the nitrogen conversion dynamics by an enriched denitrifying culture fed
315 with methanol as the carbon source (Pan et al. 2013a). The experimental data along with the
316 model predictions are presented in Figure 4.

317

318 In the tests when only one nitrogen oxide species was added, the reduction rate of nitrate
319 (Figure 4-a1 & a2), nitrite (Figure 4-b1 & b2) and N₂O (Figure 4-c1 & c2) was 45, 74 and
320 370 mg N/(gVSS×h), respectively. However, when nitrate, nitrite and N₂O were added
321 simultaneously (Figure 4-d1 & d2), the reduction rate of all the nitrogen oxides decreased to
322 19, 39 and 256 mg N/(gVSS×h), respectively (Pan et al. 2013a).

323

324 Generally, both the ASMN model and the ASM-ICE model were able to reproduce the nitrate
325 (Figure 4-a1 & a2), nitrite (Figure 4-b1 & b2) and N₂O (Figure 4-c1 & c2) profiles when only
326 one nitrogen oxide species was added. However, the ASMN model failed to reproduce the
327 experimental results when the three nitrogen oxide species were added together (Figure 4-d1).
328 The predicted NO₃⁻ reduction rate was significantly higher than the predicted NO₂⁻ reduction
329 rate, being inconsistent with the experimental observation. In addition, the predicted N₂O
330 reduction rate was significantly lower than the experimentally observed N₂O reduction rate.
331 In comparison, the ASM-ICE model reproduced all experimental data reasonably well, with
332 slightly poor fitting for nitrite only (Figure 4-d2).

333

334 **3.4. Evaluation of the Mathematical Models: Case 4**

335 In Case 4, the ASMN model and the ASM-ICE model were evaluated based on their ability
336 to capture the nitrogen conversions by an enriched denitrifying culture fed with acetate as the
337 carbon source. The experimental data along with the model predictions are presented in
338 Figure 5. Nitrite accumulated from 7 mg N/L to around 34 mg N/L in the batch test, while
339 nitrate reduced from 52 mg N/L to 10 mg N/L and COD concentration reduced from 130 mg
340 COD/L to 5 mg COD/L. As shown in Figure 5-a1 & a2, both models were able to reproduce
341 these experimental trends.

342

343 **4. DISSCUSSION**

344 **4.1. Modelling of intermediates dynamics in denitrification**

345 In this work, the two distinct concepts of four-step denitrification models (ASMN and ASM-
346 ICE) were evaluated for their ability to predict denitrification dynamics in four cases from
347 literature. The results obtained using the ASM-ICE model are in better agreement with the
348 experimental data for all four cases. In contrast, the ASMN model failed to reproduce the

349 experimental data in Cases 1, 2 and 3, and only succeeded in predicting the experimental
350 observation in Case 4.

351

352 The question arising herein is why the two models performed differently. The answer to this
353 question lies in their consideration of the electron competition process, which is reflected by
354 the differences in the structure of the two models. In the ASMN model, there is no specific
355 kinetic equation to describe the carbon oxidation process. Instead, the carbon oxidation
356 kinetics and nitrogen oxides reduction kinetics are directly lumped into each denitrification
357 step. Such a structure disables the model to describe the electron competition process,
358 particularly when the carbon oxidation rate limits the overall denitrification rate through a
359 limiting electron supplying flux. In contrast, the carbon oxidation process (II-R₁) and the
360 nitrogen reduction processes (II-R₂ to II-R₅) are modelled separately in the ASM-ICE model
361 (Table 1). The model is able to predict both the electron supply (determined by carbon
362 oxidation process) and consumption rate (determined by nitrogen reduction process). The
363 relative ability of each denitrification step to compete for electrons was modelled with
364 different affinity constants for reduced carriers ($k_{Mred,1}$, $k_{Mred,2}$, $k_{Mred,3}$, $k_{Mred,4}$).

365

366 The advantage of the ASM-ICE model over the ASMN model in describing the electron
367 competition process is strongly supported by Case 1, Case 2 and Case 3 studied. In Case 1,
368 the electron supply rate was the rate limiting process in all the tests. This is evident in the
369 experimental data, which showed that the nitrate reduction rate increased by around 233%
370 when antimycin (which inhibits nitrite reduction and the downstream denitrification steps)
371 was added (Figure 2-b2). The ASM-ICE model revealed that the nitrate reduction step (II-R₂)
372 received more electrons with antimycin blocking the electron flows to the other
373 denitrification steps. Thus a higher nitrate reduction rate (240%) was predicted by the ASM-

374 ICE model. However, the ASMN model failed to reproduce the changes of the nitrate
375 reduction rate (Figure 2-b1), because the structure of the ASMN model itself failed to
376 describe the electron competition process between the four denitrification steps. In addition,
377 the failure of the ASMN model could not be changed by adding any inhibition terms to the
378 kinetic equations as long as the “direct coupling approach” is applied. For example, it is not
379 possible for the ASMN model to predict the 233% increase in the nitrate reduction rate after
380 the addition of antimycin by adding an inhibition term in the model.

381

382 Similar to the pure culture study in Case 1, the study of a full activated sludge in Case 2 and
383 an enriched mixed culture in Case 3 also suggested that the electron competition process
384 significantly affects the denitrification intermediates dynamics. For Case 2, the increase of
385 NO_2^- reduction rate (reflected by the N_2 production rate with no nitric oxide and N_2O
386 accumulation) from 22 mg N/hour to 28 mg N/hour indicates that there was electron
387 competition when NO_2^- and NO_3^- were both present leading to a lower nitrite reduction rate
388 (and N_2 production rate) in this case. The competition between nitrate reduction and nitrite
389 reduction disappeared after the depletion of NO_3^- and therefore a higher nitrite reduction rate
390 was achieved. For Case 3, the decline of the reduction rates of nitrate, nitrite and N_2O when
391 all of them were added was also due to electron competition (Figure 4-d1 & d2). Therefore,
392 the ASM-ICE model gives a better prediction of the case compared to the ASMN model.

393

394 Different from the above cases, both models performed equally well in Case 4. A feature in
395 this case is that the availability of electron acceptors did not change throughout the
396 experiment, with the concentrations of both nitrate and nitrite were substantially above the
397 respective affinity constants. Also, the electron donor was also in excess during most of the
398 experimental period. Under such conditions, the electron allocation to different denitrification

399 steps is expected to be constant, which could be adequately captured by both models.
400 However, the predictive ability of ASMN under changed electron acceptor conditions may be
401 questionable, based on the results obtained in Cases 1-3.

402

403 **4.2. Application of the ASM-ICE denitrification model**

404 This work revealed that the ASMN-type model is structurally deficient in describing the
405 electron competition process in denitrification. This is normally not a problem if the model is
406 aimed to predict the overall nitrogen and COD removal performance in a wastewater
407 treatment plant, as in most cases the low level accumulation of denitrification intermediates
408 do not significantly affect the overall nitrogen removal rate. However, in the context of
409 predicting the accumulation of denitrification intermediates, the structure of ASMN is
410 inadequate. For example, the accumulation of N₂O to 0.1 mg N/L in the anoxic zone in a pre-
411 denitrification system, while not having a significant effect on the nitrogen removal
412 performance, could potentially lead to an N₂O emission factor of 1% of the nitrogen load
413 (Pan et al. 2013c). With the increasing use of nitrogen removal by the nitrification pathway (Ni
414 and Yu 2008), the prediction of nitrite accumulation is also becoming more important. In
415 such situations, the concept of the ASM-ICE model should be adopted.

416

417 The application of the ASM-ICE type model requires information on both the carbon
418 oxidation reaction kinetics and the nitrogen reduction kinetics. Due to the lack of
419 understanding of the electron competition process in most of the previous studies, the
420 respective reaction kinetics of the carbon oxidation and nitrogen reduction processes were not
421 well established. For instance, the maximum carbon source oxidation rate ($r_{COD,max}$), which is
422 the key parameter to restrict the overall model predicted carbon oxidation (electron supply)
423 rate, does not exist in the previous ASMN type of models and therefore is not available in

424 literature. The electron affinity constant ($K_{Mred,1}$, $K_{Mred,2}$, $K_{Mred,3}$ and $K_{Mred,4}$), which are newly
425 proposed in the ASM-ICE model to replace the affinity constant to carbon source of each
426 denitrification step (K_{S1} , K_{S2} , K_{S3} and K_{S4}) in the ASMN model, are also not available in
427 literature. Therefore, more efforts are needed to provide more information on the key
428 parameters of the ASM-ICE model for its further implementation. In addition, efforts are
429 needed to obtain more information on the reaction kinetics of the nitric oxide reduction. NO
430 is a compulsory intermediate of denitrification but is usually difficult to measure. While the
431 current ASM-ICE model may not yet serve as a precise and quantitative predictor of
432 intermediates accumulation in various wastewater treatment systems (due to parameter value
433 uncertainties), it can nevertheless serve as tool to explore the effect of operational conditions
434 on intermediates dynamics, and its continued testing against more experimental data will
435 serve to confirm the consensus mechanism of electron competition across denitrification
436 systems, and delineate a range/pattern in parameter values. Nitric oxide, which is an
437 inevitable intermediate of denitrification but usually very hard to be measured,

438

439 In future work, experiment designs should be optimized to provide more information on the
440 kinetics of both the carbon oxidation and the nitrogen reduction processes from different
441 cultures/sludges and under different conditions. The parameters obtained with different
442 experiments and cultures should then be compared and synthesised, aiming at form a
443 consistent pattern which could then be implemented as default values of the parameters of the
444 ASM-ICE model for practical applications. Further improvement/simplification of the ASM-
445 ICE model structure might be achieved depending on the new parameter pattern and the
446 model performance. A fully calibrated and verified ASM-ICE model is expected to provide
447 strong support to both future experimental studies and modelling practice aiming at get better
448 understanding of biological denitrification in wastewater treatment.

449

450 **5. CONCLUSIONS**

451 In this work, two distinct mathematical model structures for denitrification were compared
452 for their ability to predict nitrogen conversion dynamics in one pure culture and three mixed
453 culture studies. It was demonstrated that the ASM-ICE model was able to describe the
454 experimental data in all four cases studied; however, the ASMN model failed to describe the
455 experimental data from three cases. The results suggest that the ASM-ICE model is
456 advantageous over the ASMN model in describing the electron competition between the four
457 steps of denitrification and in predicting the accumulation of denitrification intermediates.
458 The ASM-ICE model is expected to provide strong support to both future experimental
459 studies and modelling practice aiming at get better understanding of biological denitrification
460 in wastewater treatment.

461

462 **ACKNOWLEDGEMENT**

463 This study was funded by the Australian Research Council, Western Australia Water
464 Corporation and Melbourne Water Corporation through projects LP0991765 and DP0987204.
465 Bing-Jie Ni acknowledges the support of Australian Research Council Discovery Early
466 Career Researcher Award (DE130100451).

467

468

469 **REFERENCES**

470 Zumft, W.G. (1997) Cell biology and molecular basis of denitrification. *Microbiology and Molecular*
471 *Biology Reviews* 61(4), 533-616.
472 Tchobanoglous, G., Burton, F. and Stensel, H.D. (2003) *Wastewater engineering: treatment and*
473 *reuse*, Metcalf & Eddy, Inc., McGraw Hill Education, New York.
474 Ni, B.-J. and Yu, H.-Q. (2008) An approach for modeling two-step denitrification in activated sludge
475 systems. *Chemical Engineering Science* 63(6), 1449-1459.
476 IPCC (2007) *Climate Change 2007: The Physical Science Basis*. Contribution of Working Group I to the
477 *Fourth Assessment Report of the Intergovernmental Panel on Climate Change* [Solomon, S., D. Qin,

478 M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor and H.L. Miller (eds.)). Cambridge
479 University Press, Cambridge, United Kingdom and New York, NY, USA.
480 Ravishankara, A.R., Daniel, J.S. and Portmann, R.W. (2009) Nitrous oxide (N₂O): The dominant ozone-
481 depleting substance emitted in the 21st century. *Science* 326(5949), 123-125.
482 Pan, Y., Ni, B.-J., Bond, P.L., Ye, L. and Yuan, Z. (2013a) Electron competition among nitrogen oxides
483 reduction during methanol-utilizing denitrification in wastewater treatment. *Water Research* 47(10),
484 3273-3281.
485 Schalk-Otte, S., Seviour, R.J., Kuenen, J.G. and Jetten, M.S.M. (2000) Nitrous oxide (N₂O) production
486 by *Alcaligenes faecalis* during feast and famine regimes. *Water Research* 34(7), 2080-2088.
487 Richardson, D., Felgate, H., Watmough, N., Thomson, A. and Baggs, E. (2009) Mitigating release of
488 the potent greenhouse gas N₂O from the nitrogen cycle - could enzymic regulation hold the key?
489 *Trends in Biotechnology* 27(7), 388-397.
490 Henze, M., Gujer, W., Mino, T. and van Loosdrecht, M.C.M. (2000) Activated sludge models ASM1,
491 ASM2, ASM2d, and ASM3, IWA Scientific and Technical Report No. 9. IWA Publishing, London, UK.
492 Ni, B.-J., Rusalleda, M., Pellicer-Nàcher, C. and Smets, B.F. (2011) Modeling nitrous oxide production
493 during biological nitrogen removal via nitrification and denitrification: extensions to the general ASM
494 models. *Environmental Science & Technology* 45(18), 7768-7776.
495 Vonschulthess, R., Wild, D. and Gujer, W. (1994) Nitric and nitrous oxides from denitrifying
496 activated-sludge at low-oxygen concentration. *Water Science and Technology* 30(6), 123-132.
497 Schulthess, R.V. and Gujer, W. (1996) Release of nitrous oxide (N₂O) from denitrifying activated
498 sludge: Verification and application of a mathematical model. *Water Research* 30(3), 521-530.
499 Hiatt, W.C. and Grady, C.P.L. (2008) An updated process model for carbon oxidation, nitrification,
500 and denitrification. *Water Environment Research* 80(11), 2145-2156.
501 Pan, Y., Ni, B.-J. and Yuan, Z. (2013b) Modeling electron competition among nitrogen oxides
502 reduction and N₂O accumulation in denitrification. *Environmental Science & Technology* 47(19),
503 11083-11091.
504 Kucera, I., Dadak, V. and Dobry, R. (1983) The distribution of redox equivalents in the anaerobic
505 respiratory chain of *paracoccus denitrificans*. *European Journal of Biochemistry* 130(2), 359-364.
506 Pan, Y., Ye, L., Ni, B.-J. and Yuan, Z. (2012) Effect of pH on N₂O reduction and accumulation during
507 denitrification by methanol utilizing denitrifiers. *Water Research* 46(15), 4832-4840.
508 McMurray, S.H. (2008) Formation of denitrification intermediates and their impact on process
509 performance. PhD Thesis, The University of Queensland, Brisbane, Australia.
510 Oh, J. and Silverstein, J. (1999) Acetate limitation and nitrite accumulation during denitrification.
511 *Journal of Environmental Engineering-Asce* 125(3), 234-242.
512 Reichert, P., von Schulthess, R. and Wild, D. (1995) The use of AQUASIM for estimating parameters
513 of activated sludge models. *Water Science and Technology* 31(2), 135-147.
514 Pan, Y., Ye, L. and Yuan, Z. (2013c) Effect of H₂S on N₂O reduction and accumulation during
515 denitrification by methanol utilizing denitrifiers. *Environmental Science & Technology* 47(15), 8408-
516 8415.

517

518 **Table 1: Process matrices for the two types of denitrification models evaluated in this study**

Processes	Model components								Kinetic rate expressions	
	S _{NO3}	S _{NO2}	S _{NO}	S _{N2O}	S _{N2}	S _S	S _{Mox}	S _{Mred}		X
Model I (ASMN)- the “direct coupling approach” adapted from Hiatt and Grady (2008)										
I-R ₁	-A	+A				-1/(Y _H · η _Y)				1 R ₁ = μ _H · η _{g1} · X ($\frac{S_S}{K_{S1} + S_S}$) ($\frac{S_{NO3}}{K_{NO3}^{HB} + S_{NO3}}$)
I-R ₂		-B	+B			-1/(Y _H · η _Y)				1 R ₂ = μ _H · η _{g2} · X ($\frac{S_S}{K_{S2} + S_S}$) ($\frac{S_{NO2}}{K_{NO2}^{HB} + S_{NO2}}$) ($\frac{K_{NO,2}}{K_{NO,2} + S_{NO}}$)
I-R ₃			-B	+B		-1/(Y _H · η _Y)				1 R ₃ = μ _H · η _{g3} · X ($\frac{S_S}{K_{S3} + S_S}$) ($\frac{S_{NO}}{K_{NO}^{HB} + S_{NO} + S_{NO}^2 / K_{NO,3}}$)
I-R ₄				-B	+B	-1/(Y _H · η _Y)				1 R ₂ = μ _H · η _{g4} · X ($\frac{S_S}{K_{S4} + S_S}$) ($\frac{S_{N2O}}{K_{N2O}^{HB} + S_{N2O}}$) ($\frac{K_{NO,4}}{K_{NO,4} + S_{NO}}$)
Model II (ASM-ICE)- the “indirect coupling approach” adapted from Pan et al.(2013b)										
II-R ₁						-1		-(1-Y _H)	1-Y _H	Y _H R ₁ = r _{COD,max} X ($\frac{S_S}{K_S + S_S}$) ($\frac{S_{Mox}}{K_{Mox} + S_{Mox}}$)
II-R ₂	-1	1						1	-1	R ₂ = r _{NO3,max} X ($\frac{S_{NO3}}{K_{NO3}^{HB} + S_{NO3}}$) ($\frac{S_{Mred}}{K_{Mred,1} + S_{Mred}}$)
II-R ₃		-1	1					$\frac{1}{2}$	$-\frac{1}{2}$	R ₃ = r _{NO2,max} X ($\frac{S_{NO2}}{K_{NO2}^{HB} + S_{NO2}}$) ($\frac{S_{Mred}}{K_{Mred,2} + S_{Mred}}$)
II-R ₄			-1	$\frac{1}{2}$				$\frac{1}{2}$	$-\frac{1}{2}$	R ₄ = r _{NO,max} X ($\frac{S_{NO}}{K_{NO}^{HB} + S_{NO}}$) ($\frac{S_{Mred}}{K_{Mred,3} + S_{Mred}}$)
II-R ₅				-1	1			1	-1	R ₅ = r _{N2O,max} X ($\frac{S_{N2O}}{K_{N2O}^{HB} + S_{N2O}}$) ($\frac{S_{Mred}}{K_{Mred,4} + S_{Mred}}$)

519 $A = \frac{1 - Y_H \cdot \eta_Y}{1.143 \cdot Y_H \cdot \eta_Y}$, $B = \frac{1 - Y_H \cdot \eta_Y}{0.571 \cdot Y_H \cdot \eta_Y}$, $S_{Mred} + S_{Mox} = C_{tot}$

521 **Table 2:** Best-fit parameters of the two models describing denitrification dynamics

Parameter	Definition	Case 1	Case 2	Case 3	Case 4	Source
Model I (ASMN)- the “direct coupling approach” adapted from Hiatt and Grady (2008)						
μ_H	Maximum specific growth rate (hour ⁻¹)	0.26	0.26	0.26	0.26	Hiatt and Grady (2008)
Y_H	Heterotrophic yield (g COD/g COD)	0.6 ^a	0.6 ^a	0.5 ^b	0.6 ^a	a: Hiatt and Grady (2008) b: Pan et al. (2013b)
η_Y	Anoxic yield factor (dimensionless)	0.9	0.9	0.9	0.9	Hiatt and Grady (2008)
η_{g1}	Anoxic growth factor, R1 (dimensionless)	0.029	0.14	0.18	0.14	Estimated
η_{g2}	Anoxic growth factor, R2 (dimensionless)	0.024	0.058	0.15	0.016	Estimate
η_{g3}	Anoxic growth factor, R3 (dimensionless)	0.35	0.35	0.35	0.35	Hiatt and Grady (2008)
η_{g4}	Anoxic growth factor, R4 (dimensionless)	0.35 ^a	0.35 ^a	0.81 ^b	0.35 ^a	a: Hiatt and Grady (2008) b: Estimated
K_{S1}	Affinity constant for Ss, R1 (mgCOD/L)	20	20	20	20	Hiatt and Grady (2008)
K_{S2}	Affinity constant for Ss, R2 (mgCOD/L)	20	20	20	20	Hiatt and Grady (2008)
K_{S3}	Affinity constant for Ss, R3 (mgCOD/L)	20	20	20	20	Hiatt and Grady (2008)
K_{S4}	Affinity constant for Ss, R4 (mgCOD/L)	40	40	40	40	Hiatt and Grady (2008)
$K_{NO_3}^{HB}$	Affinity constant for nitrate-nitrogen (mg N/L)	0.2	0.2	0.2	0.2	Hiatt and Grady (2008)
$K_{NO_2}^{HB}$	Affinity constant for nitrite-nitrogen (mg N/L)	0.2	0.2	0.2	0.2	Hiatt and Grady (2008)
K_{NO}^{HB}	Affinity constant for nitric oxide-nitrogen (mg N/L)	0.05	0.05	0.05	0.05	Hiatt and Grady (2008)
$K_{N_2O}^{HB}$	Affinity constant for nitrous oxide-nitrogen (mg N/L)	0.05	0.05	0.05	0.05	Hiatt and Grady (2008)
$K_{NO,2}$	NO inhibition coefficient, R2 (mg N/L)	0.5	0.5	0.5	0.5	Hiatt and Grady (2008)
$K_{NO,3}$	NO inhibition coefficient, R3 (mg N/L)	0.3	0.3	0.3	0.3	Hiatt and Grady (2008)
$K_{NO,4}$	NO inhibition coefficient, R4 (mg N/L)	0.075	0.075	0.075	0.075	Hiatt and Grady (2008)
Model II (ASM-ICE)- the “indirect coupling approach” adapted from Pan et al.(2013b)						
$r_{COD,max}$	Maximum carbon source oxidation rate (mmol COD/(L*hour)	0.064	0.090	0.34	0.129	Estimated

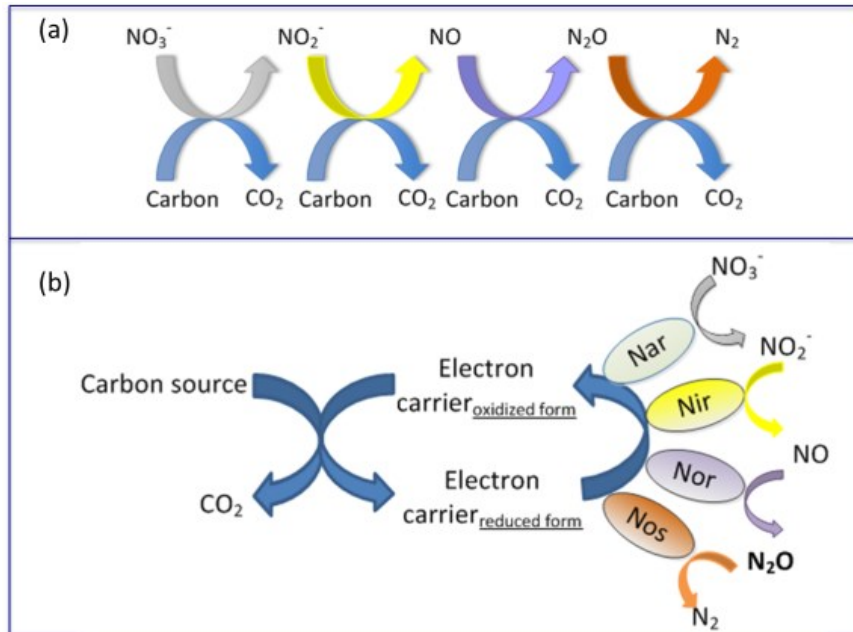
$r_{NO_3,max}$	Maximum nitrate reduction rate (mmol NO ₃ ⁻ /mmol biomass*hour)	0.045	0.045	0.045	0.045	Pan et al. (2013b)
$r_{NO_2,max}$	Maximum nitrite reduction rate (mmol NO ₂ ⁻ /mmol biomass*hour)	0.059	0.059	0.059	0.059	Pan et al. (2013b)
$r_{NO,max}$	Maximum nitric oxide reaction rate (mmol NO /mmol biomass*hour)	0.56	0.56	0.56	0.56	Pan et al. (2013b)
$r_{N_2O,max}$	Maximum nitrous oxide reaction rate (mmol N ₂ O /mmol biomass*hour)	0.23	0.23	0.23	0.23	Pan et al. (2013b)
K_S	Affinity constant for S _s (mmol COD/L)	0.1	0.1	0.1	0.1	Pan et al. (2013b)
$K_{NO_3}^{HB}$	Affinity constant for nitrate-nitrogen (mmol NO ₃ ⁻ /L)	0.018	0.018	0.018	0.018	Pan et al. (2013b)
$K_{NO_2}^{HB}$	Affinity constant for nitrite-nitrogen (mmol NO ₂ ⁻ /L)	0.0041	0.0041	0.0041	0.0041	Pan et al. (2013b)
K_{NO}^{HB}	Affinity constant for nitric oxide-nitrogen (mmol NO/L)	0.000011	0.000011	0.000011	0.000011	Pan et al. (2013b)
$K_{N_2O}^{HB}$	Affinity constant for nitrous oxide-nitrogen (mmol N ₂ O/L)	0.0025	0.0025	0.0025	0.0025	Pan et al. (2013b)
K_{Mox}	Affinity constant for S _{Mox} , R1 mmol/(mmol biomass)	0.0001	0.0001	0.0001	0.0001	Pan et al. (2013b)
$K_{Mred,1}$	Affinity constant for S _{Mred} , R2	0.0015 ^a	0.0068 ^a	0.0046 ^b	0.0018 ^a	a:Estimated b: Pan et al. (2013b)
$K_{Mred,2}$	Affinity constant for S _{Mred} , R3	0.00058 ^a	0.016 ^a	0.00040 ^b	0.0033 ^a	a:Estimated b: Pan et al. (2013b)
$K_{Mred,3}$	Affinity constant for S _{Mred} , R4	0.000010	0.000010	0.000010	0.000010	Pan et al. (2013b)
$K_{Mred,4}$	Affinity constant for S _{Mred} , R5	0.00024 ^a	0.0032 ^b	0.0032 ^b	0.0032 ^b	a: Estimated b: Pan et al. (2013b)
Y_H	Heterotrophic yield	0.6 ^a	0.6 ^a	0.5 ^b	0.6 ^a	a: Hiatt and Grady (2008) b: Pan et al. (2013b)
C_{tot}	Total electron carrier concentration mmol/mmol biomass	0.01	0.01	0.01	0.01	Pan et al. (2013b)

522

523

524 **Table 3: Four experimental cases used for evaluation of the two denitrification models**

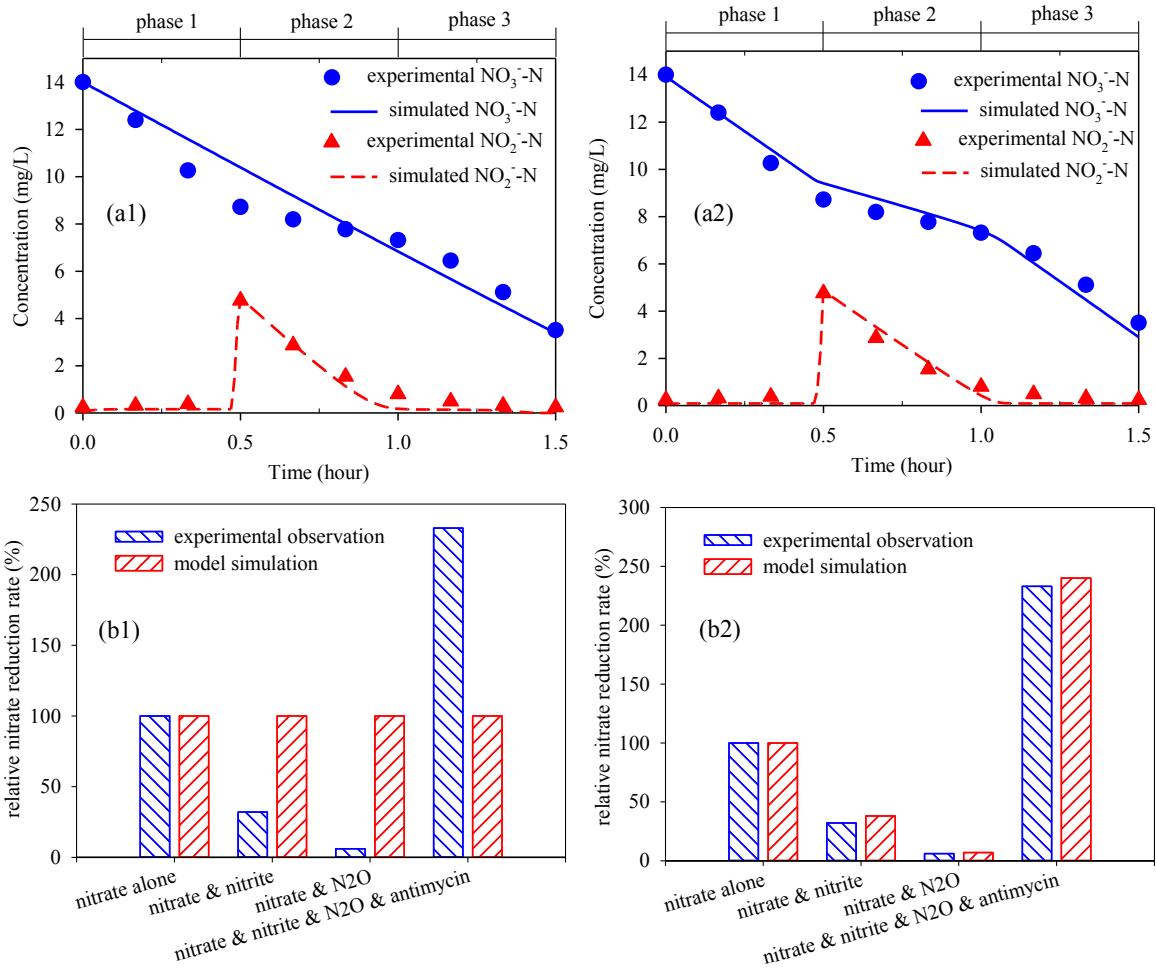
	Culture	Carbon source	Nitrogen oxides added	Batch tests
Case 1	pure denitrifying culture	glucose	NO_3^- , NO_2^- , N_2O and antimycin	a) the effect of nitrite addition on nitrate reduction, b) the impact of nitrite, N_2O or antimycin on nitrate reduction rate
Case 2	full-scale activated sludge	acetate	NO_3^- , NO_2^-	the relationship between nitrate and nitrite reduction, acetate oxidation and nitrogen gas production
Case 3	enriched denitrifying culture	methanol	NO_3^- , NO_2^- , N_2O	1) NO_3^- reduction with only NO_3^- being added 2) NO_2^- reduction with only NO_2^- being added 3) N_2O reduction with only N_2O being added 4) NO_3^- , NO_2^- and N_2O reduction with NO_3^- , NO_2^- and N_2O being added simultaneously
Case 4	enriched denitrifying culture	acetate	NO_3^-	Investigating the nitrite accumulation during nitrate reduction



526

527 **Figure 1.** Conceptual reaction schemes used in the two 4-step denitrification models
 528 evaluated in this study: (a) The ASMN model - Using the "direct coupling approach" to
 529 model the carbon oxidation and nitrogen reduction processes during denitrification; (b) The
 530 ASM-ICE model - Using the "indirect coupling approach" to model the carbon oxidation and
 531 nitrogen reduction processes during denitrification.

532



533

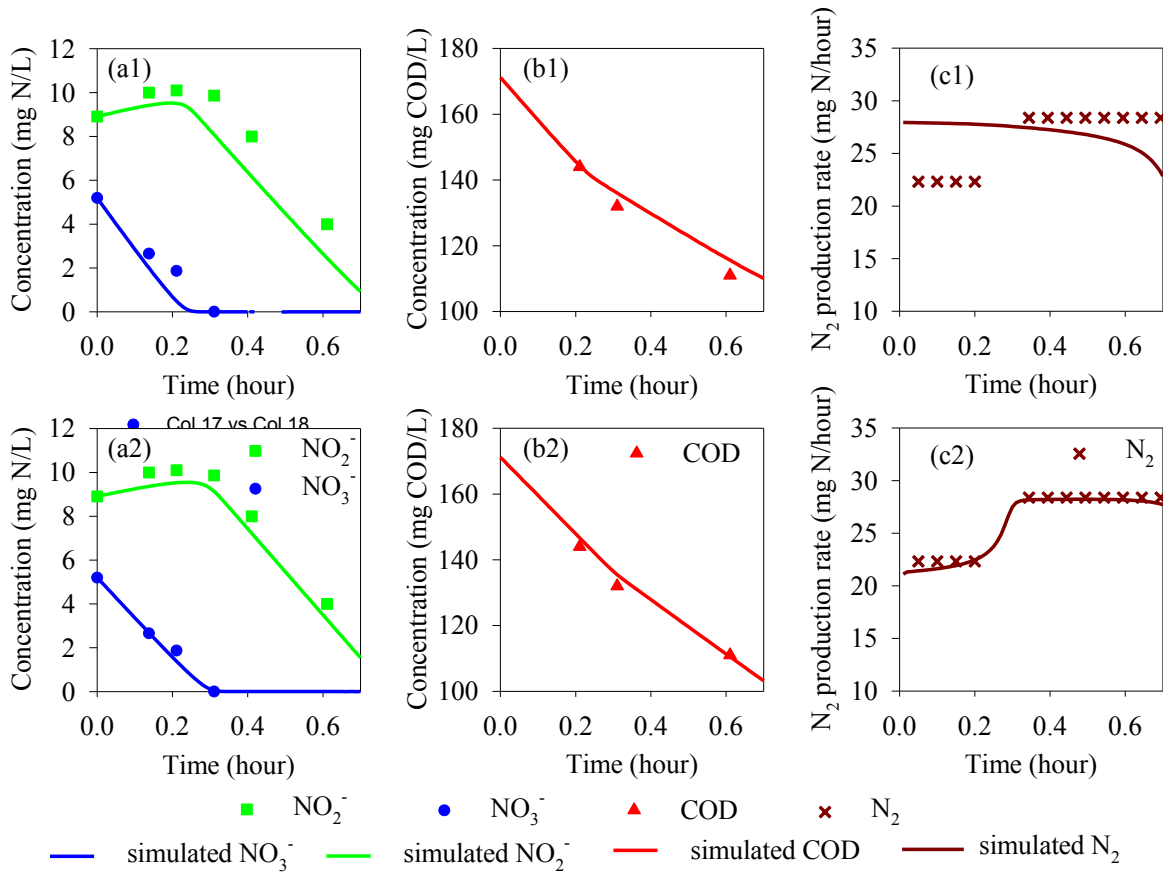
534 **Figure 2:** Experimental results and model predictions for Case 1 (Kucera et al. 1983). (a1) &

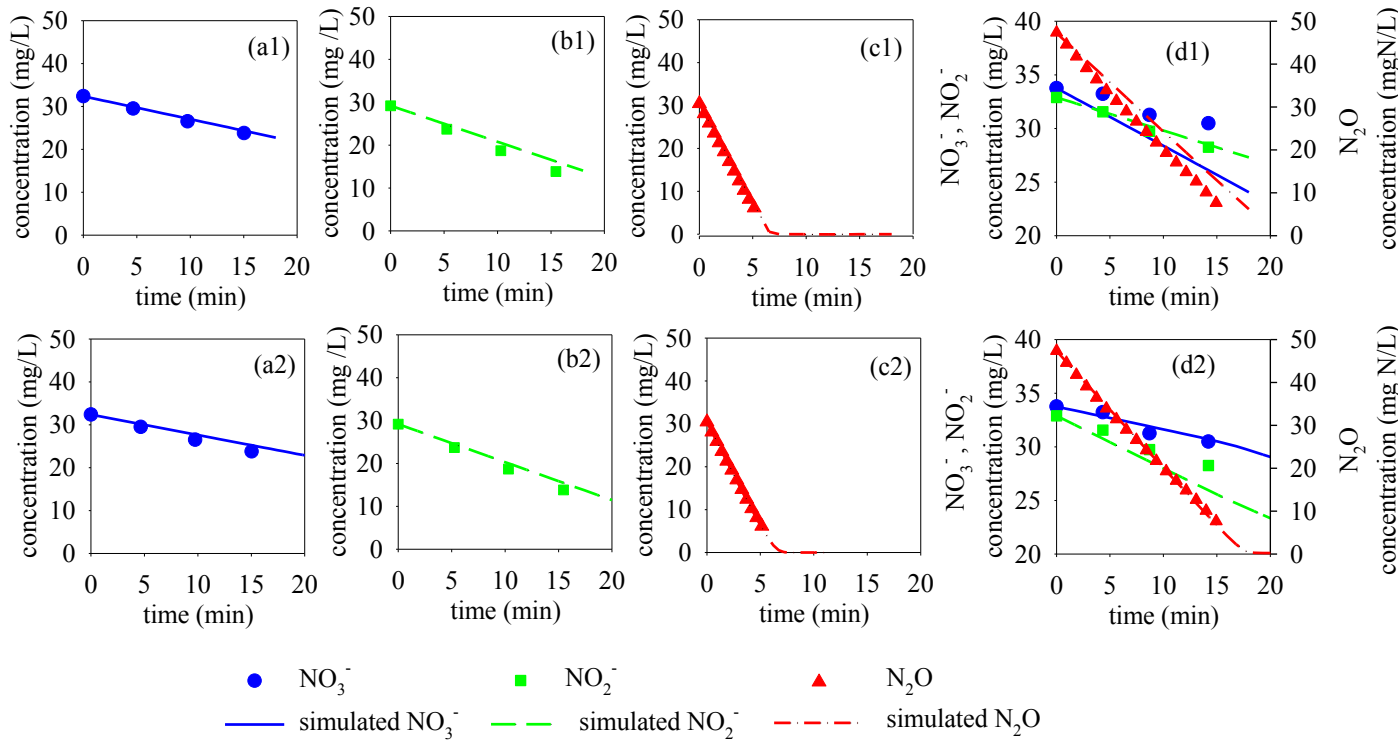
535 (b1) – Evaluation of ASM-N; (a2) & (b2) – Evaluation of ASM-ICE.

536

537

538





544

545 **Figure 4:** Experimental results and model predictions in Case 3 (Pan et al. 2013a): (a1) & (b1)

546 & (c1) & (d1) - Evaluation of ASM-N; (a2) & (b2) & (c2) & (d2) - Evaluation of ASM-ICE.

547

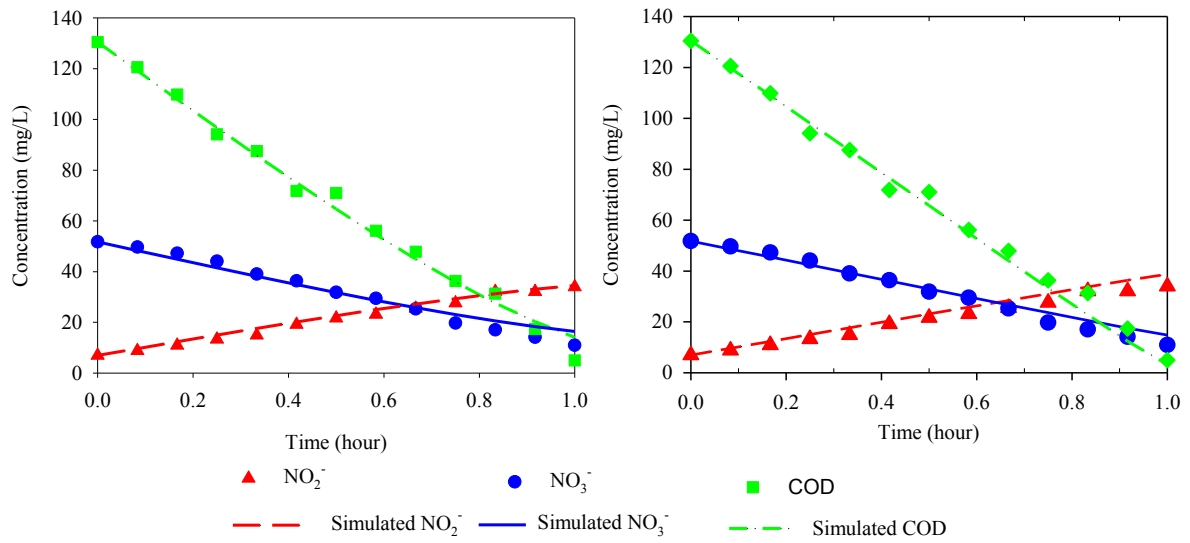
548

549

550

551

552



553

554 **Figure 5:** Experimental results and model predictions in Case 4 (Oh and Silverstein 1999):

555 (a1) & (b1) & (c1) & (d1) - Evaluation of ASMN; (a2) & (b2) & (c2) & (d2) - Evaluation of

556 ASM-ICE.

557

558

559