

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37

Electronic Supporting Information for

**Anaerobic Membrane Bioreactor Treatment of
Domestic Wastewater at Psychrophilic Temperatures
Ranging from 15 to 3°C**

Adam L. Smith, Steven J. Skerlos, and Lutgarde Raskin

Table of Contents

Supplementary Figures.....	S3
Supplementary Calculations.....	S8

List of Figures

38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

66

67

68

69

70

71

72

73

74

75

76

Figure S1. P1, P2, and P3 permeate VFA concentrations (concentrations are expressed as the actual compound, not as COD), calculated COD contribution from measured VFAs (indicated as Total as COD in legend), and measured COD during days 162-313. Error bars represent standard deviations of triplicate IC injections. The method detection limit for each VFA was <0.15 mg/L.....S3

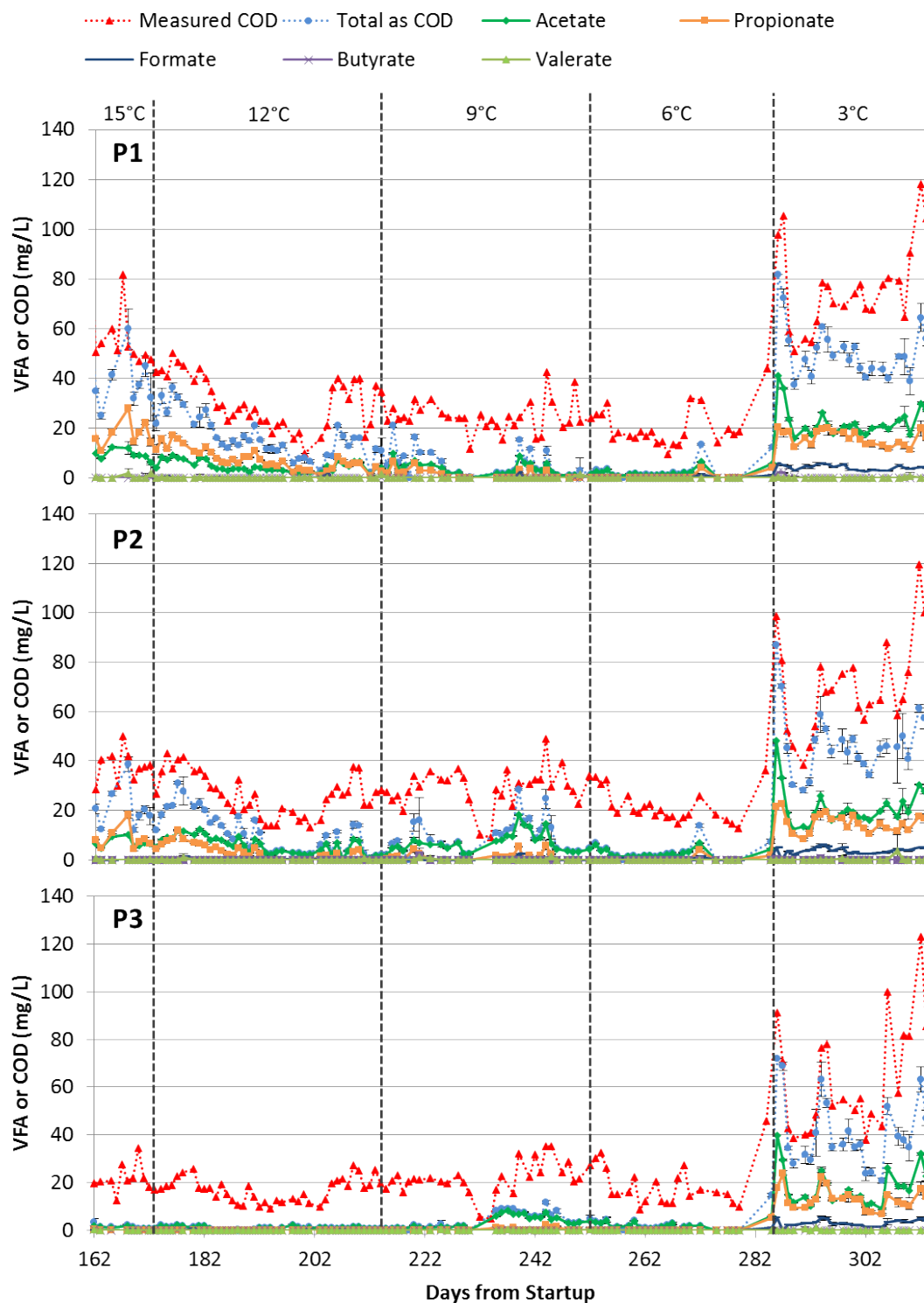
Figure S2. COD mass balance for days 162-313. Total COD_{out} is the summation of measured permeate COD, measured dissolved methane, measured gaseous methane, theoretical COD removal from measured sulfate reduction, and theoretical COD from measured biomass wasting. An issue with the biogas collection system during days 162-205 prevented accurate measurement of biogas production during that time.....S4

Figure S3. Total suspended solids (TSS) and volatile suspended solids (VSS) in the bioreactor during days 162-313. Error bars represent the standard deviations of triplicate measurements...S5

Figure S4. Relative activity of *Archaea* in the suspended biomass (S) and biofilm (P1, P2, and P3) based on 16S rRNA sequencing at operational temperatures of 12, 9, 6, and 3°C. Data are expressed as a percentage and were normalized using the total number of archaeal 16S rRNA sequences.....S6

Figure S5. (a) Relative abundance based on 16S rDNA sequencing and (b) relative activity based on 16S rRNA sequencing of the top 20 phylotypes to total community in the suspended biomass (S) and biofilm (P1, P2, and P3) at operational temperatures of 12, 9, 6, and 3°C. Data are expressed as a percentage and were normalized using the total number of 16S rDNA sequences (a) and 16S rRNA sequences (b) (including both Archaea and Bacteria).....S7

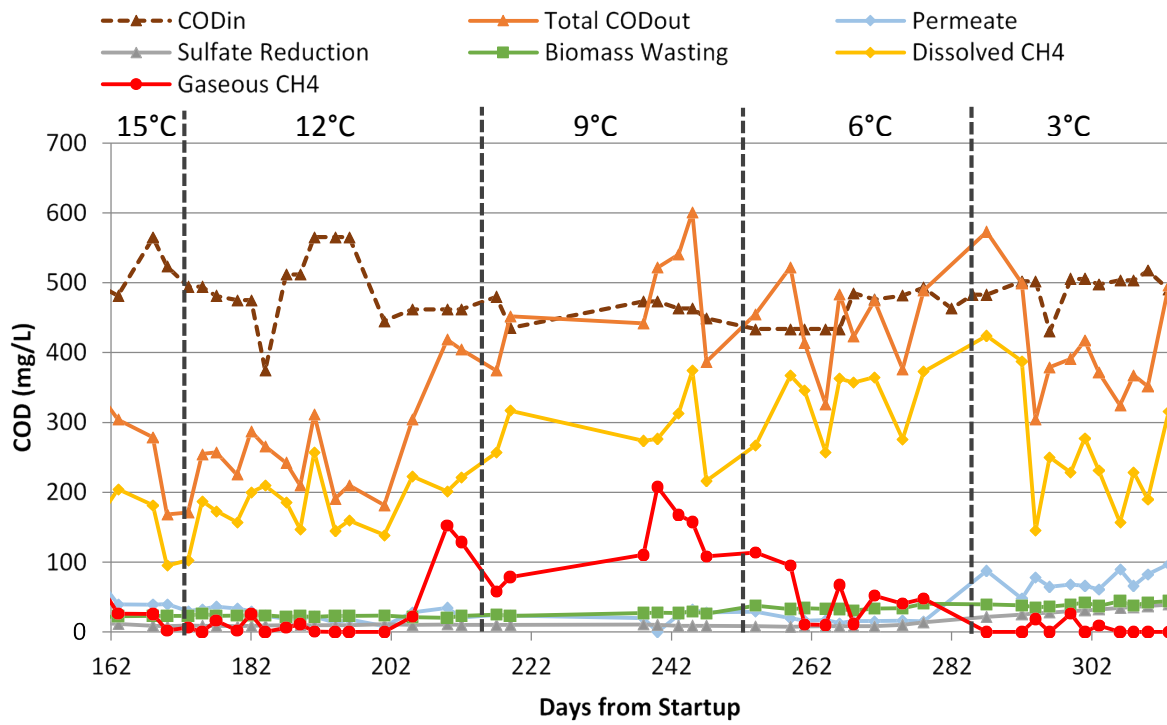
77 Supplementary Figures



78

79 Figure S1. P1, P2, and P3 permeate VFA concentrations (concentrations are expressed as the actual
 80 compound, not as COD), calculated COD contribution from measured VFAs (indicated as Total as COD in
 81 legend), and measured COD during days 162-313. Error bars represent standard deviations of triplicate IC
 82 injections. The method detection limit for each VFA was <0.15 mg/L.

83



84

85 **Figure S2. COD mass balance for days 162-313. Total COD_{out} is the summation of measured permeate COD,**
 86 **measured dissolved methane, measured gaseous methane, theoretical COD removal from measured sulfate**
 87 **reduction, and theoretical COD from measured biomass wasting. An issue with the biogas collection system**
 88 **during days 162-205 prevented accurate measurement of biogas production during that time.**

89

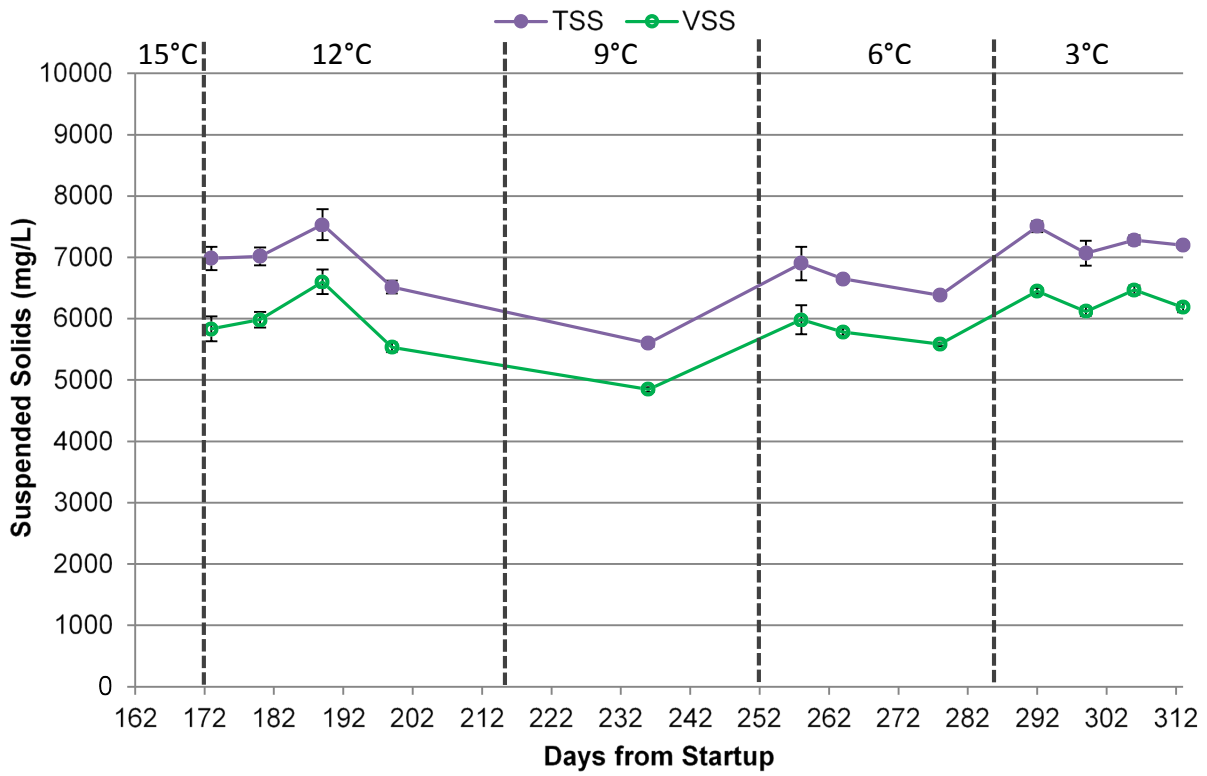
90

91

92

93

94



95

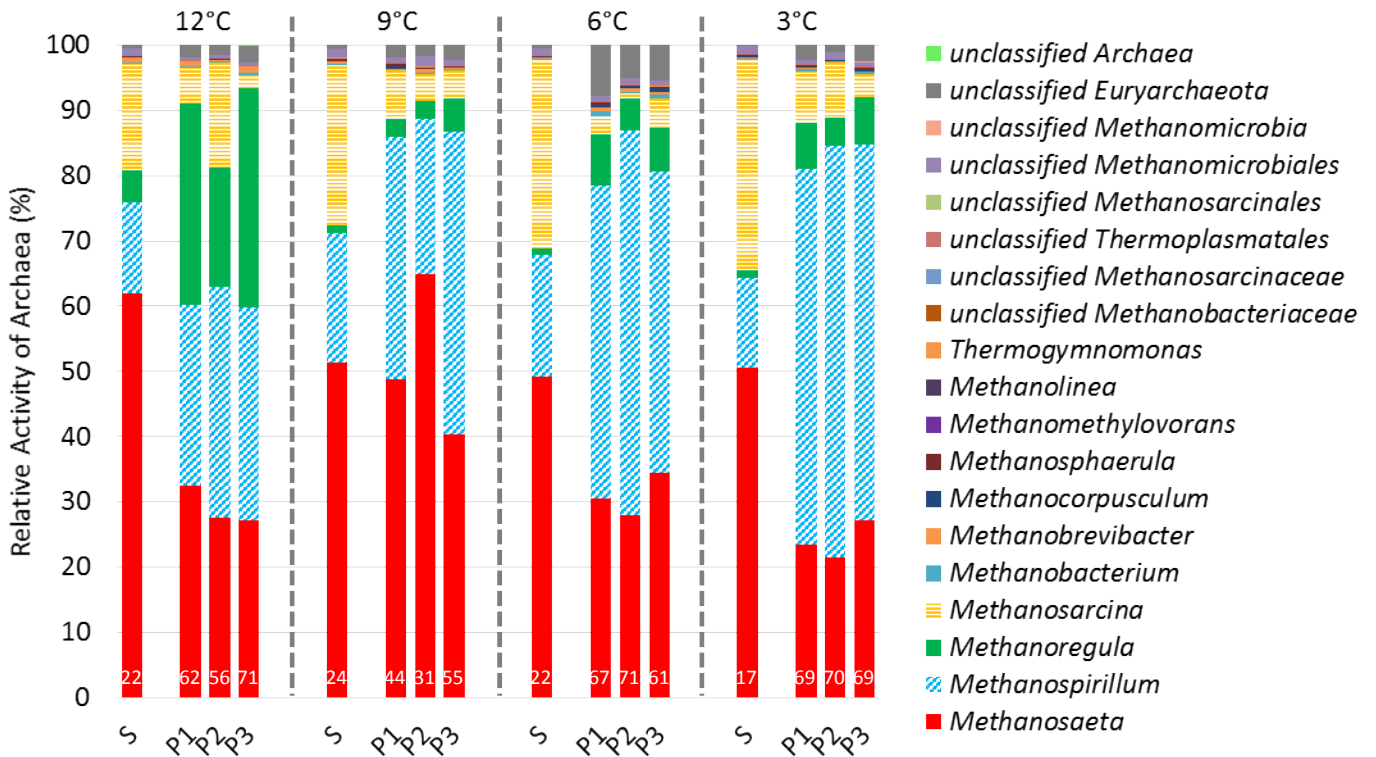
96 **Figure S3. Total suspended solids (TSS) and volatile suspended solids (VSS) in the bioreactor during days**
 97 **162-313. Error bars represent the standard deviations of triplicate measurements.**

98

99

100

101



102

103 **Figure S4. Relative activity of *Archaea* in the suspended biomass (S) and biofilm (P1, P2, and P3) based on**
 104 **16S rRNA sequencing at operational temperatures of 12, 9, 6, and 3°C. Data are expressed as a percentage**
 105 **and were normalized using the total number of archaeal 16S rRNA sequences. Numbers at the bottom of each**
 106 **bar represent the relative activity of hydrogenotrophic methanogens (all methanogens except *Methanosaeta***
 107 **spp. and *Methanosarcina* spp.).**

108

109

110

111

112

113

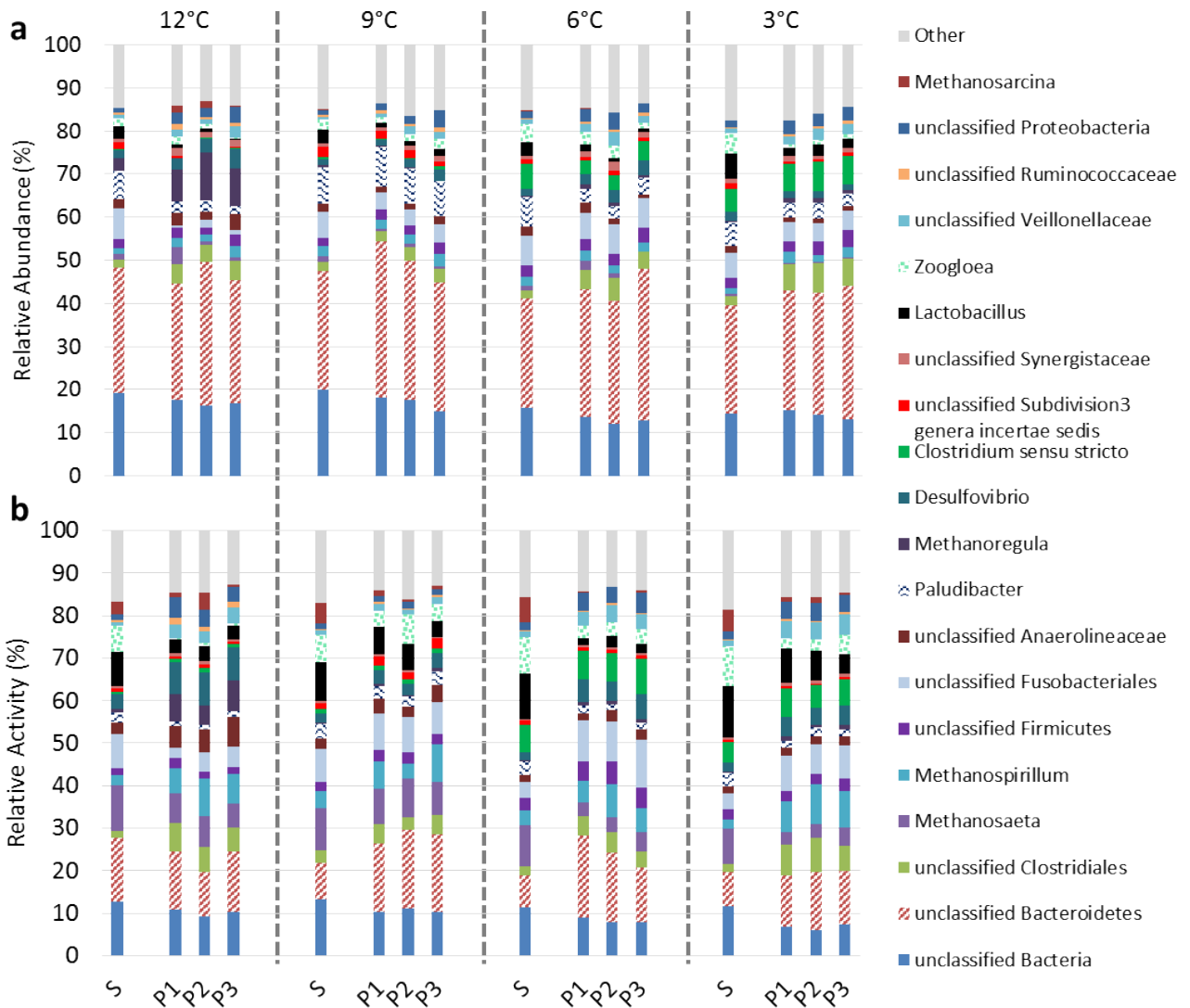
114

115

116

117

118



119

120 **Figure S5. (a) Relative abundance based on 16S rDNA sequencing and (b) relative activity based on 16S**
 121 **rRNA sequencing of the top 20 phylotypes to total community in the suspended biomass (S) and biofilm (P1,**
 122 **P2, and P3) at operational temperatures of 12, 9, 6, and 3°C. Data are expressed as a percentage and were**
 123 **normalized using the total number of 16S rDNA sequences (a) and 16S rRNA sequences (b) (including both**
 124 ***Archaea* and *Bacteria*).**

125

126

127

128

129

130

131

132

133

134

135

136

137

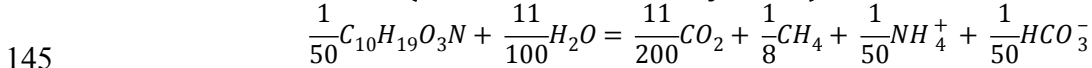
138 **Supplementary Calculations**

139

140 The following calculations demonstrate that for the operational conditions and observed
 141 performance data for the bench-scale AnMBR at 15°C, the biogas can contain >90% as
 142 experimentally observed.

143

144 **Stoichiometric reaction (Rittmann and McCarty, 2001):**



145

146

147 Hydraulic retention time, HRT = 16 h

148 Reactor volume, V = 4 L

149 Influent flow rate, $Q_{liquid} = V/HRT = 6 \text{ L/d}$

150 Influent COD concentration = 450 mg/L as COD

151 Permeate COD concentration = 50 mg/L as COD

152

$$COD \text{ removal} = Influent - Permeate = \frac{450 \text{ mg COD}}{L} - \frac{50 \text{ mg COD}}{L} = \frac{400 \text{ mg COD}}{L}$$

153

154

$$C_{10}H_{19}O_3N \text{ removal} = \frac{400 \text{ mg COD}}{L} * \frac{1 \text{ g } C_{10}H_{19}O_3N}{1.99 \text{ g COD}} * \frac{1 \text{ mol } C_{10}H_{19}O_3N}{201 \text{ g } C_{10}H_{19}O_3N} = 1 \text{ mM } C_{10}H_{19}O_3N$$

155

156

$$1 \text{ mM } C_{10}H_{19}O_3N * \frac{\frac{1}{8}}{\frac{1}{50}} = 6.25 \text{ mM } CH_4$$

157

158

$$1 \text{ mM } C_{10}H_{19}O_3N * \frac{\frac{11}{200}}{\frac{1}{50}} = 2.75 \text{ mM } CO_2$$

159

160

161 **Total CH₄ and CO₂ production:**

162

$$Total \text{ CH}_4 \text{ production} = 6.25 \text{ mM } CH_4 * \frac{16 \text{ g}}{\text{mmol}} * \frac{6 \text{ L}}{\text{d}} = \frac{600 \text{ mg}}{\text{d}}$$

163

164

$$Total \text{ CO}_2 \text{ production} = 2.75 \text{ mM } CO_2 * \frac{44 \text{ g}}{\text{mmol}} * \frac{6 \text{ L}}{\text{d}} = \frac{726 \text{ mg}}{\text{d}}$$

165

166

167 **Dissolved CH₄ and CO₂ production:**

168

$$Dissolved \text{ CH}_4 \text{ production} = \frac{P_{CH_4} * \frac{55.6 \text{ mol}}{L} * \frac{16 \text{ g}}{\text{mol}} * \frac{1 \text{ 000 mg}}{\text{g}} * \frac{6 \text{ L}}{\text{d}} * CH_4 \text{ oversaturation}}{H_{CH_4}}$$

169

170

171 P_{CH_4} = Partial pressure of CH₄

172 H_{CH_4} = Henry's law constant, 34 269 atm at 15°C

173 $CH_4 \text{ oversaturation} = 2$ (assumption based on bench-scale data)

174

$$\text{Dissolved } CO_2 \text{ production} = \frac{P_{CO_2} * \frac{55.6 \text{ mol}}{L} * \frac{44 \text{ g}}{\text{mol}} * \frac{1\,000 \text{ mg}}{g} * \frac{6 \text{ L}}{d}}{H_{CO_2}}$$

175

176

177 P_{CO_2} = Partial pressure of CO_2

178 H_{CO_2} = Henry's law constant, 1 273 atm at 15°C

179

180 **Gaseous CH_4 and CO_2 production:**

181

$$\text{Gaseous } CH_4 \text{ production} = P_{CH_4} * Q_{gas} * \frac{1 \text{ mol} * K}{0.0821 \text{ L} * \text{atm}} * \frac{1}{288 \text{ K}} * \frac{16 \text{ g}}{\text{mol}} * \frac{1\,000 \text{ mg}}{g}$$

182

183

$$\text{Gaseous } CO_2 \text{ production} = P_{CO_2} * Q_{gas} * \frac{1 \text{ mol} * K}{0.0821 \text{ L} * \text{atm}} * \frac{1}{288 \text{ K}} * \frac{44 \text{ g}}{\text{mol}} * \frac{1\,000 \text{ mg}}{g}$$

184

185

186 Q_{gas} = 0.45 L/d (assumption based on bench-scale data)

187

188 **Total mass balance:**

189

190 $\text{Total } CH_4 \text{ production} = \text{Dissolved } CH_4 \text{ production} + \text{Gaseous } CH_4 \text{ production}$

191

192 $\text{Total } CO_2 \text{ production} = \text{Dissolved } CO_2 \text{ production} + \text{Gaseous } CO_2 \text{ production}$

193

194 Solving for P_{CH_4} and P_{CO_2} ,

195

196 $P_{CH_4} = 0.94$

197 $P_{CO_2} = 0.059$

198

199

(error of ± 20 mg/d in mass balance)

200

201

202