Influence of deterministic and stochastic processes on microbial community assembly during aerobic granulation

Raquel Liébana [a]*, Enikö Szabó [a], Frank Persson [a], Oskar Modin [a], Malte Hermansson [b] and Britt-Marie Wilén [a]

[a] Division of Water Environment Technology, Department of Architecture and Civil Engineering, Chalmers University of Technology, SE-41296 Gothenburg, Sweden

[b] Department of Chemistry and Molecular Biology, University of Gothenburg, SE-40530 Gothenburg, Sweden

*Corresponding author. Postal address: Division of Water Environment Technology, Department of Civil and Environmental Engineering, Chalmers University of Technology, Sven Hultins gata 8, SE- 412 96 Gothenburg, Sweden; Telephone number: +46 031-772-1912, Email address: raquel.liebana@chalmers.se

Running title: Microbial community assembly during granulation

The authors declare no competing financial interests.

This work was supported by FORMAS (Contract no. 245-2013-627), the Swedish Research Council for Environment, Agriculture Sciences and Spatial Planning.

ABSTRACT

Aerobic granular sludge is an energy efficient and compact biofilm process for wastewater treatment which has received much attention during the last decades and is now being implemented in full-scale. However, the factors involved in microbial community assembly during formation of granules are poorly understood and little is known about the reproducibility in treatment performance and community structure. Here we show that both deterministic and stochastic factors exert a dynamic influence during microbial community assembly into granular sludge. During granulation, the microbial communities in three replicate sequencing batch reactors followed similar successional trajectories of the most abundant taxa and showed similar dynamics in diversity. Deterministic factors dominated the assembly of the most abundant community members as the microbial community transitioned from floccular to granular form. Stochastic factors mostly affected rare members of the communities and caused the microbial community structure to diverge in one of the reactors; however, this did not have an impact on the treatment performance. This demonstrates that the reactor function and the dynamics of the most abundant community members are in fact reproducible during the formation of aerobic granules.

37 # Introduction

38 The structure of microbial communities depends on complex and dynamic ecological
39 mechanisms. Studying the compositional differences between two different communities (β-
40 diversity) is useful in order to understand the underlying ecological mechanisms that have
41 shaped the communities, since it provides a link between biodiversity at the local scale (α-
42 diversity) and at the regional scale (γ-diversity) [1, 2]. Vellend (2010) developed a conceptual
43 framework, modified by Nemergut *et al* (2013), where the ecological mechanisms responsible
44 for community assembly are framed into four fundamental processes: selection, dispersal,
45 diversification and drift. Selection results from deterministic factors that modify the
46 community structure according to the environmental conditions, differences in fitness
47 between individuals and microbial interactions. Dispersal results both from deterministic and
48 stochastic factors which enable the movement and establishment of organisms among
49 communities. Diversification results mainly from stochastic factors which generate genetic
50 variation. Drift refers to stochastic changes due to birth, death and reproduction. These four
51 processes act simultaneously in natural ecosystems and their influences vary in time and space
52 [4, 5].

53 Generally, the design and development of wastewater treatment systems is hindered by the
54 lack of understanding of the ecology of the microbial communities on which they rely [6, 7].
55 The importance ascribed to the different ecological mechanisms for shaping the microbial
56 community composition in wastewater treatment bioreactors vary in the literature. Some
57 studies report similar microbial communities in replicate reactors, such as membrane
58 bioreactors [8], anaerobic digesters [9-11], microbial fuel cells [12] and sequencing batch
59 reactors [13, 14], due to selection caused by the reactor environmental conditions. On the

60 contrary, diverging trajectories have been reported in replicate microbial electrolysis cell
61 reactors [15], sequencing batch reactors [16] and anaerobic digesters [17, 18], due to the roles
62 of stochastic factors. However, deterministic and stochastic factors are not mutually exclusive
63 but exert a combined effect on the microbial community assembly and need to be assessed in
64 conjunction [4, 5].

65 Wastewater treatment bioreactors are engineered to select for the functional groups needed
66 for water purification. Also, the reactor operational conditions are adjusted to cultivate the
67 microorganisms in the desired aggregate modes. The microbial communities in wastewater
68 treatment are either assembled as flocs in activated sludge or as biofilms on different
69 substrata. Free-floating spherical biofilms, so called granules, combine many of the properties
70 of these two growth modes [19, 20], which has gained increasing interest and implementation
71 in recent years [21]. The possibility to remove carbon, nitrogen and phosphorous
72 simultaneously is one of the most important attributes of granular sludge, which renders highly
73 compact and less energy demanding wastewater treatment [22]. The granules are compact
74 and spherical suspended biofilms with a diameter of approximately 1-3 mm obtained in
75 defined reactor conditions of high up-flow air velocity, large shear forces, large temporal
76 variation of electron donors and -acceptors causing feast-famine operation and short settling
77 time to select for well settling biomass [23, 24]. Despite well-established methods for granule
78 cultivation, detailed studies on the microbial community ecology in aerobic granules are scarce
79 and the ecological processes underpinning the microbial community assembly during
80 granulation are poorly understood [25].

81 Laboratory experiments in wastewater treatment are often performed in one reactor due to
82 practical reasons. Hence, conclusions regarding reactor performance and microbial community

83 structure are generally drawn from single reactors operated at different conditions [26-29]. It
84 is unclear how reliable these conclusions are, especially regarding complex processes such as
85 microbial community assembly. Furthermore, laboratory scale wastewater studies that enable
86 controlled environmental conditions and tests of reproducibility are valuable test-benches for
87 studies of microbial ecology [30]. To better understand he microbial community assembly and
88 reproducibility of granule formation, we have used amplicon sequencing to investigate the
89 succession during the transition from floccular to granular sludge in three replicate reactors
90 seeded with the same inoculum and operated at identical conditions for 35 days. At the same
91 time, the function of the microbial communities was monitored. We hypothesized that similar
92 communities would develop in all three reactors if deterministic factors were dominating,
93 whereas the opposite would support the importance of stochastic factors. We also
94 investigated possible shifts in the importance of deterministic/stochastic factors for
95 community assembly during the operation period.

96 Material and methods

97 Experimental set-up

98 Three identical sequencing batch reactors (6 cm diameter and 132 cm height) with a working
99 volume of 3 L were operated for 35 days at room temperature (20-22˚C) in 4-hour cycles (0:05
100 h filling, 0:55 h anaerobic, 2:53 h aerobic, 0:02 h settling, 0:05 h withdrawal) with a volumetric
101 exchange ratio of 43%. The settling time was gradually decreased to promote granulation and
102 at the same time allow retention of slow growing organisms (Figure S1), and the aerobic phase
103 was adjusted accordingly to permit even 4-hour cycle lengths. Air was provided from the
104 bottom of the reactors with porous diffusers (pore size 1 µm) at a flow rate of 2.5 L min^{-1} and

105 superficial up-flow velocity of 1.5 cm s^{-1}. The reactors were inoculated with aerobic/anoxic
106 activated sludge from a full-scale wastewater treatment plant (Gryaab AB, Gothenburg,
107 Sweden). Synthetic wastewater was pumped in at the bottom of the reactor with a chemical
108 oxygen demand (COD) to nitrogen ratio of 100:25 (3 kg COD $m^{-3}d^{-1}$ and 0.75 kg NH_4-N $m^{-3}d^{-1}$)
109 and pH of approximately 7 and consisted of 1.6 g l^{-1} NH_4CH_3COO, 12.5 mg l^{-1} $MgSO_4 \cdot 7H_2O$, 15.0
110 mg l^{-1} $CaCl_2$, 10.0 mg l^{-1} $FeSO_4 \cdot 7H_2O$, 22.5 mg l^{-1} K_2HPO_4, and 1 ml l^{-1} micronutrient solution
111 (0.05 g l^{-1} H_3BO_3, 0.05 g $l^{-1}ZnCl_2$, 0.03 g l^{-1} $CuCl_2$, 0.05 g l^{-1} $MnSO_4.H_2O$, 0.05 g l^{-1}
112 $(NH_4)_6Mo_7O_{24}.4H_2O$, 0.05 g l^{-1} $AlCl_3$, 0.05 g l^{-1} $CoCl_2.6H_2O$, and 0.05 g l^{-1} $NiCl_2$). Biomass samples
113 (100 ml) were withdrawn from the reactors daily for analysis. The replicate reactors were
114 operated identically.

115 ## DNA extraction, amplification and sequencing

116 Samples for microbial community analysis were collected from the reactor three times per
117 week. Total genomic DNA was extracted using the FastDNA Spin Kit for Soil (MP Biomedicals)
118 following the manufacturer's protocol. The V4 region of the 16S rRNA gene was amplified in
119 duplicates, using the forward primer 515'F (5´-GTGBCAGCMGCCGCGGTAA-3´) and the reverse
120 primer 806R (5´-GGACTACHVGGGTWTCTAAT-3´), indexed according to Kozich *et al* (2013). The
121 PCR reactions were conducted in a 20 µl reaction volume using 17 µl of AccuPrime Pfx
122 SuperMix (Life Technologies), 1 µl of genomic DNA (20 ng template), and 1 µl each of the
123 forward and reverse primers (10 µM). The PCR reactions were carried out in a Biometra T3000
124 Thermocycler with the following thermal cycling parameters: initial denaturation at 95°C for 5
125 min, followed by 30 cycles of denaturation (95°C, 20 s), annealing (50°C, 15 s) and elongation
126 (68°C, 60 s), and finished by a 10 min final elongation at 68°C. PCR products were purified with
127 the MagJET NGS Cleanup and Size Selection Kit (Thermo Scientific) and the DNA concentration

128 was measured using a Qubit 3.0 fluorometer (Invitrogen), using the dsDNA HS assay kit
129 (Invitrogen). The PCR products were pooled in equimolar amounts, the concentration and size
130 was confirmed by TapeStation 2200 (Agilent Technologies) and sequencing was performed
131 with a MiSeq using reagent kit v3 (Illumina). To increase the confidence in the obtained results,
132 a second MiSeq sequencing run was performed on the same pool. Raw sequencing data were
133 deposited in the NCBI Sequence Read Archive (SRA), BioProjectID PRJNA472243, accession
134 number SRP148672.

135 ## Sequence processing and data analysis

136 Raw sequence reads were processed following the UNOISE pipeline [32, 33] with USEARCH
137 v.10 [34]. Briefly, paired-end reads were merged with a maximum number of mismatches of
138 15 (5% of the read length) and a minimum of 80% of identity in the alignment. Merged reads
139 were filtered (maximum expected error per read of 0.5), dereplicated (default settings) and
140 denoised (minimum abundance of 4), resulting in zero-radius operational taxonomic units
141 (OTUs). OTUs were taxonomically classified with the SINTAX algorithm [35] based on the MiDAS
142 database v.2.1 [36] and analysed in R version 3.4.1 (http://www.r-project.org). The dataset was
143 rarefied, subsampling each sample to 17395 sequences. Basic R functions were used to
144 perform Wilcoxon signed-rank tests and to calculate Pearson correlation coefficients. Non-
145 metric multi-dimensional scaling (NMDS) ordination and heatmaps were created using the R
146 package ampvis [37]. Constrained analysis of proximities (CAP) was performed using the R
147 package vegan [38]. The sequences were aligned with the R package DECIPHER [39] and a
148 maximum likelihood tree was generated with FastTree 2 software [40] using the GTR+CAT
149 (General Time Reversible with per-site rate CATegories) model of approximation for site rate
150 variation and computation of Gamma20-based likelihood.

151 α-diversity for each sample was calculated using numbers equivalents (Hill numbers) [41]. We
152 refer to these indices as naïve diversity (^{q}ND) because the phylogenetic distance between OTUs
153 are not considered. The parameter q is the diversity order. At a q of 0, all OTUs are considered
154 equally important and, hence, ^{0}ND is the sample richness (i.e. the number of OTUs in a sample).
155 For higher values of q, more weight is put on abundant OTUs. We also calculated diversity
156 numbers which take the sequence dissimilarity into account using the framework described by
157 Chiu and Chao (2014). The metric, here referred to as ^{q}PD (phylogenetic diversity), represents
158 the total effective phylogenetic distance between all OTUs in a sample. For all pairwise
159 comparisons of samples, β-diversity was obtained using the same calculation framework. The
160 β-diversities were converted into dissimilarity indices (^{q}βdis) constrained between 0 (two
161 identical samples) and 1 (two samples with no shared OTUs) [42, 43]. The parameters $^{q}\beta dis_{ND}$
162 and $^{q}\beta dis_{PD}$ are based on the naïve and the phylogenetic β-diversity values, respectively.

163 ## Null model analysis

164 Null models were used to analyse the taxonomic- and phylogenetic successional turnover
165 between consecutive samples in the reactors. Taxonomic turnover was estimated with Raup-
166 Crick-based measures based on Bray-Curtis dissimilarities (RC_{bray}), calculated with the R
167 package vegan as in Stegen *et al* (2013). Shortly, a null distribution (999 randomizations) of
168 expected Bray-Curtis dissimilarities was created for each pair of communities, which was
169 compared to the empirically observed Bray-Curtis dissimilarities. The RC_{bray} index is calculated
170 by adding the number of times the null distribution is greater than the empirical Bray-Curtis
171 distribution to half the number of times the null and the empirical distribution are equal. Then
172 this count is normalized to a value between -1 and 1. A negative value means that the two
173 communities share more taxa than expected by chance whereas a positive value means that

174 they share less taxa. Values > |0.95| were considered statistically significant. Values < |0.95|
175 indicates that the taxonomic turnover between the community pair was not different from the
176 null expectation and therefore, influenced by stochastic factors [5].

177 The R package PICANTE [45] was used to calculate the β nearest-taxon index (βNTI), which
178 measures if the phylogenetic turnover is greater or less than the null expectation. For this, the
179 β mean nearest-taxon distance (βMNTD), which measures the mean phylogenetic distance
180 between the most closely related OTUs in two communities, was first calculated based on
181 relative abundance data, as in Stegen *et al* (2013). In each iteration (999 randomizations) the
182 OTUs were moved randomly across the tips of the regional pool phylogeny and the resulting
183 phylogenetic relationships were used to calculate the $\beta MNTD_{null}$. The βNTI was calculated as
184 the difference in standard deviation units between the observed βMNTD and the mean of
185 $\beta MNTD_{null}$ in the pairwise sample comparisons. A negative βNTI value means that the samples
186 are more phylogenetically close to each than expected by chance and a positive value means
187 that the two samples are more phylogenetically distant from each other. Previous studies have
188 found that short phylogenetic distances between OTUs, quantified using βMNTD and βNTI,
189 reflect ecological distances [44, 46, 47]. Pairwise comparisons with βNTI > |2| were considered
190 statistically significant. Values < |2| were not significantly different from the null expectation,
191 which indicate that stochastic factors influenced the phylogenetic turnover [5].

192 Analytical methods

193 Concentrations in the effluent of total organic carbon (TOC) and total nitrogen (TN) were
194 measured with TOC-TN analyser 87 (TOC-V, Shimadzu, Japan). Total suspended solids and
195 volatile suspended solids in the in the reactor (MLSS, MLVSS) and in the effluent (effTSS, effVSS)
196 were measured according to standard methods. Microscopy was performed using an Olympus

197 BX60 light microscope (Olympus Sverige AB, Solna, Sweden) with particle size assessment by
198 measuring the diameter of 10 random flocs/granules using the CellSens (Olympus) software.

199 Results

200 Granulation of the sludge and reactor performance

201 The sludge started to granulate around day 7, when small granules emerged (Figure S2) and
202 the mean particle sizes of the reactor biomass increased similarly in the three reactors (Figure
203 S3). At day 25, the granulated biomass dominated in the reactors and at the end of the
204 experiment (day 35), the granules had an average diameter of 1.5 mm. The three reactors also
205 followed similar patterns over time in suspended solids concentrations in the reactor and in
206 the effluent (Figure 1A-B), although R1 had significantly lower reactor concentrations (MLSS
207 and MLVSS) than R2 and R3 ($p<0.05$) (Table S1). Removal of TOC and TN was similar among the
208 reactors (Figure 1, C-D). TOC removal was above 90% since day 4, with an average of 95% for
209 R1 and R3 and 96% for R2. The average TN removal was above 50% for the three reactors.

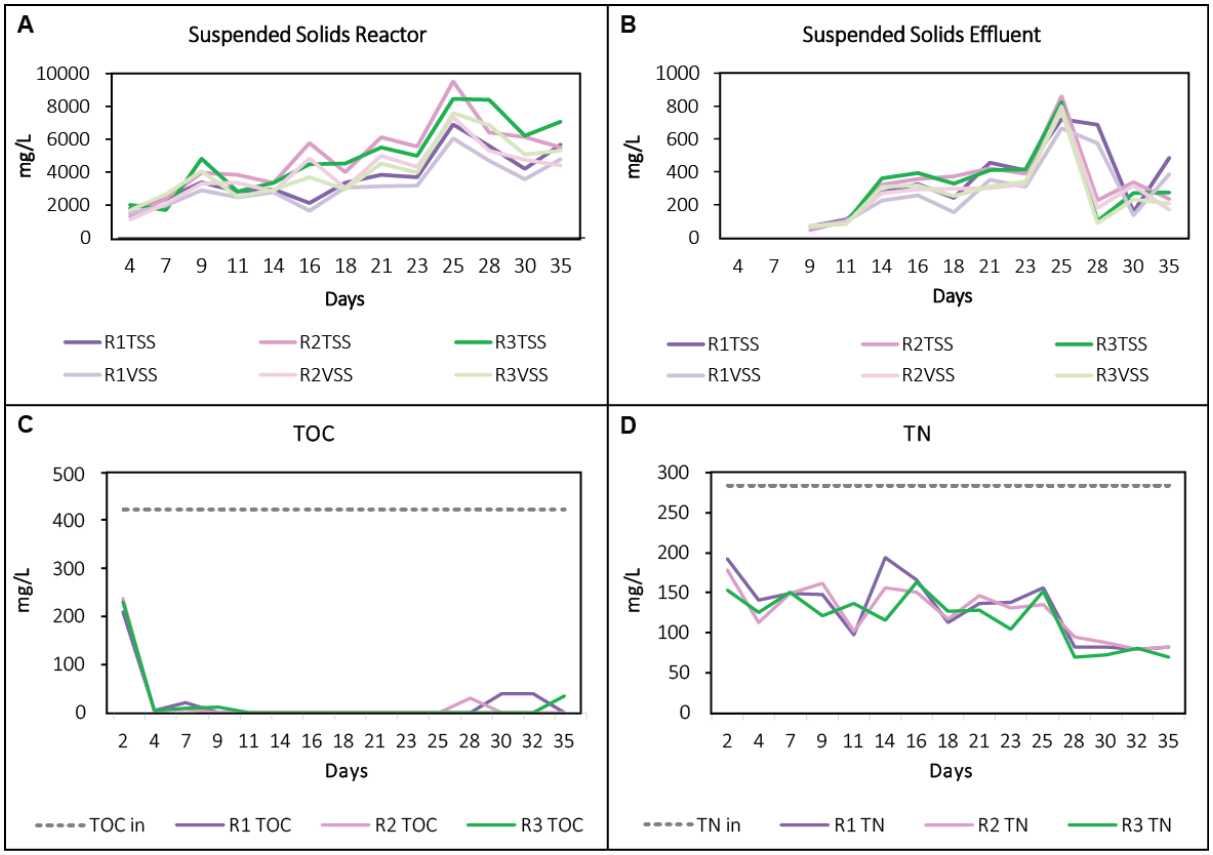


Figure 1. Sludge properties and reactor performance over time for R1, R2 and R3. A: reactor suspended solids concentrations; B: effluent suspended solids concentrations; C: TOC concentrations in the influent and effluent; D: TN concentrations in the influent and effluent.

Temporal variation of abundant taxa

The microbial community composition was highly dynamic during the transition from floccular to granular sludge. The seed sludge, mainly composed of *Betaproteobacteria* (38% of total reads), *Alphaproteobacteria* (14%), *Sphingobacteria* (11%) and *Clostridia* (7%) at class level, changed rapidly after inoculation to a community dominated by *Gammaproteobacteria* (70%) with *Acinetobacter* as the dominant genus during the first 7 days (Figure 2, S4). Thereafter, *Betaproteobacteria* dominated in the three reactors, with high abundances of the genera *Thauera*, *Acidovorax*, *Simplicispira*, *Comamonas*, *Curvibacter* and *Alicycliphilus* (Figure 2). From

222 days 21-25, *Actinobacteria* dominated until the end of the experiment, with *Corynebacterium*

223 sp. as only representative of this class, having more than 30% of the total reads in the three

224 reactors. Many taxa that were rare or undetected in the seed sludge became abundant during

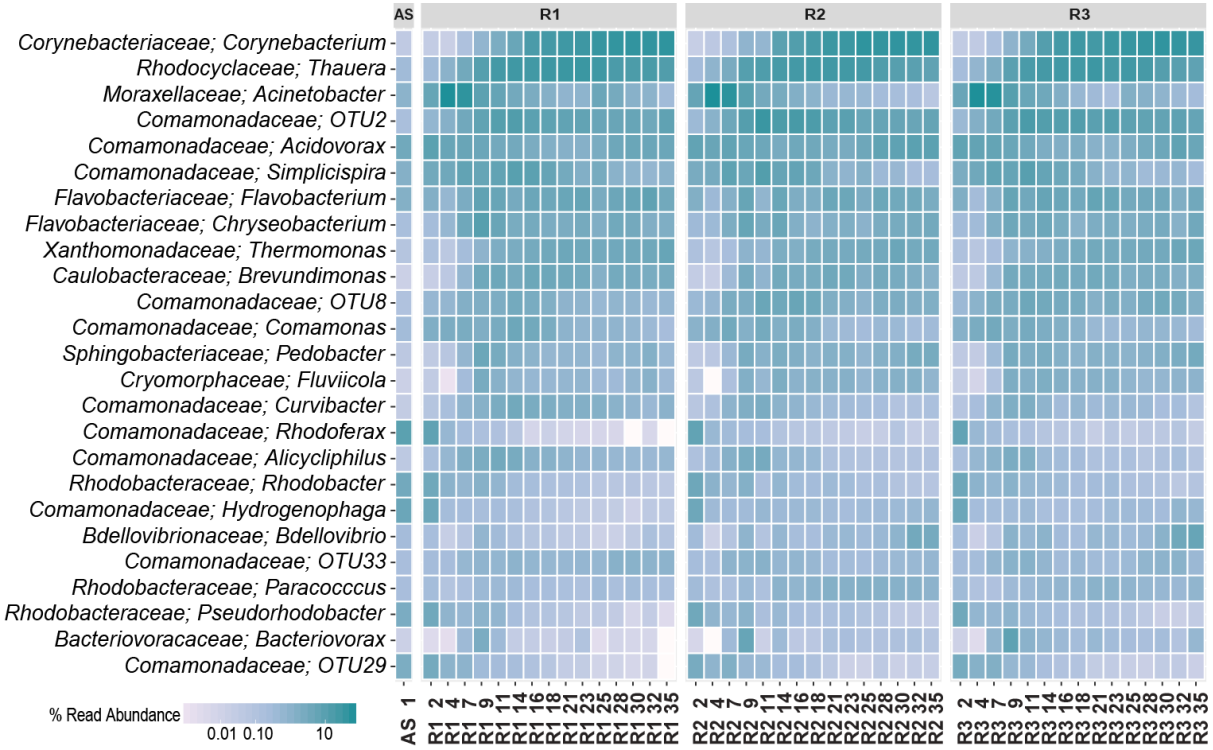
225 the granulation, e.g. *Corynebacterium* sp., *Brevundimonas* sp., *Pedobacter* sp., *Fluviicola* sp.

226 and *Bacteriovorax* sp., while other taxa that were common in the seed sludge decreased

227 drastically in relative abundance, e.g. *Rhodoferax* sp., *Rhodobacter* sp., *Pseudorhodobacter* sp.

228 and *Hydrogenophaga* sp.

229



230

231 **Figure 2.** Temporal variation of the 25 most abundant genera as percentages of reads in the

232 replicate reactors (R1, R2 and R3) and the seed sludge (AS), days 1 to 35.

233 ## Microbial diversity and dynamics during granulation

234 The three reactors followed the same trends in α-diversity (Figure 3A-C). After inoculation,
235 there was a drop in diversity, followed by a peak around day 9 and then a continuous decrease.
236 R1 differed somewhat from R2 and R3 during the dynamic period after the inoculation and
237 during days 21-35 when ^{0}ND was consistently lower in R1, while ^{1}ND and ^{2}ND were similar in
238 the three reactors, showing that the rare OTUs were fewer in R1. The observed trends for naïve
239 diversity were very similar to the trends for the phylogenetic diversity (Figure S5A-C).

240 Succession, measured as β-diversity between successive sample points, showed that after
241 inoculation there were large temporal dynamics in each reactor with dissimilarities of around
242 0.5 for ^{0}βdisND and even higher peak dissimilarities (0.8) for ^{2}βdisND, which gives large weight
243 to abundant OTUs (Figure 3D-F). Thereafter, the dissimilarities between successive samples
244 decreased considerably to values of about 0.3 for ^{0}βdisND and 0.05 for ^{1}βdisND and ^{2}βdisND.

245 For β-diversity between the reactors (Figure 3G-I), ^{0}βdisND, was around 0.3-0.4, showing that
246 60-70% of the OTUs were shared among the reactors at any given time. ^{1}βdisND was around
247 0.1 and ^{2}βdisND around 0.05, i.e. the reactors were more similar to each other when
248 considering the more abundant OTUs. R2-R3 were more similar than R1-R2 or R1-R3 for
249 diversity order 0 and 1, but not for diversity order 2. Also for β-diversity between successive
250 sampling points or between reactors, the naïve diversity patterns were highly similar to the
251 ones for phylogenetic diversity (Figure S5).

252 NMDS shows initial, rapid changes after inoculation and that R1 and R2 deviated from the other
253 reactors at day 7 and day 11 (Figure 4). From day 14, R2 and R3 converged and evolved similarly
254 after that, whereas R1 diverged. CAP analysis based on Bray-Curtis dissimilarities confirmed R1

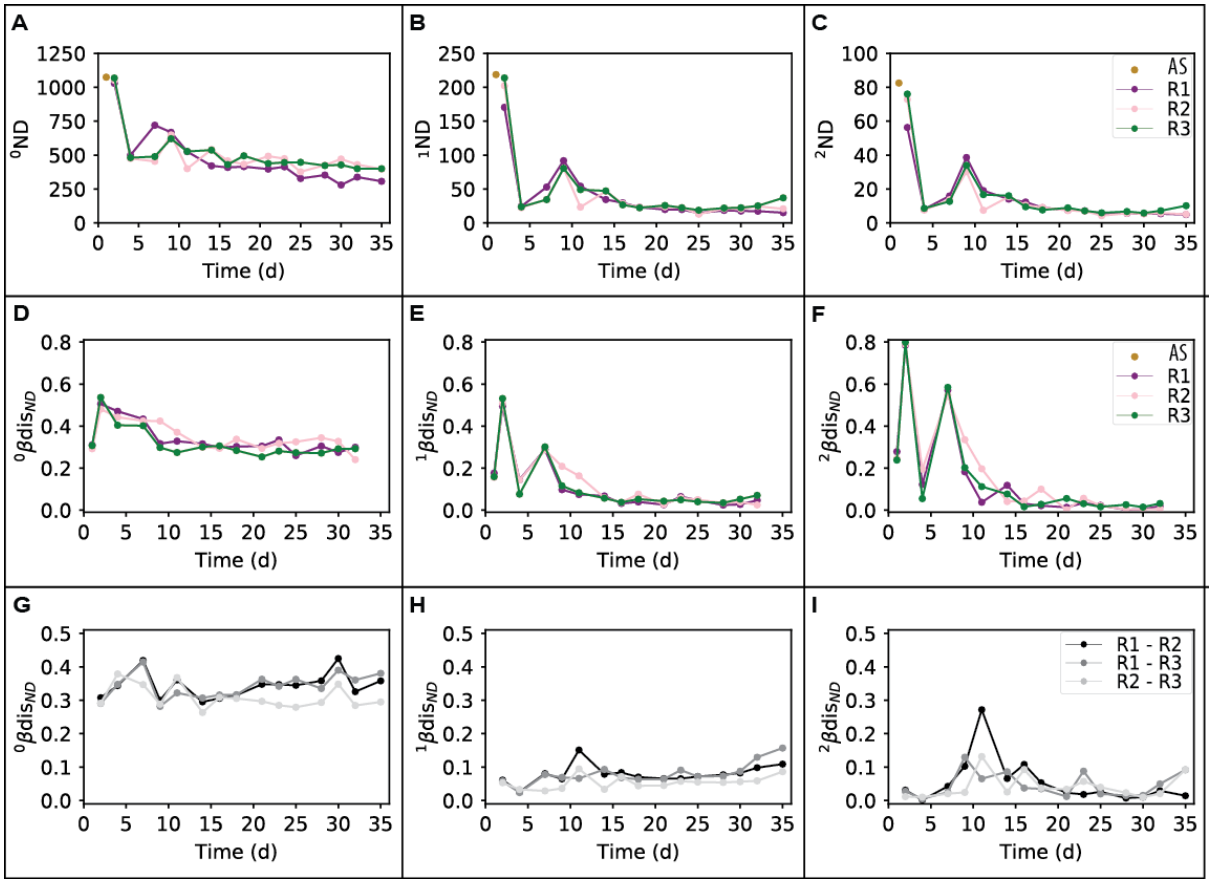
255 to be different to R2 and R3 (Figure S6). Pearson correlation based on CAP showed that 144

256 out of the 2540 OTUs were significantly different ($p<0.05$ in the correlation to CAP2 axis, see

257 Table S2) in relative abundance between reactors. 58 OTUs were more abundant in R1 than

258 R2-R3 and 82 OTUs more abundant in R2-R3 than R1. These OTUs, mainly of low relative

259 abundance added up to 8% of the total reads.



260

261 **Figure 3.** Dynamics of the naïve diversity (taxonomic distance between OTUs) for the replicate

262 reactors. A, B and C: α-diversity; D, E and F: β-diversity over time between two successive

263 sample points; G, H and I: β-diversity over time between the reactors.

264

265

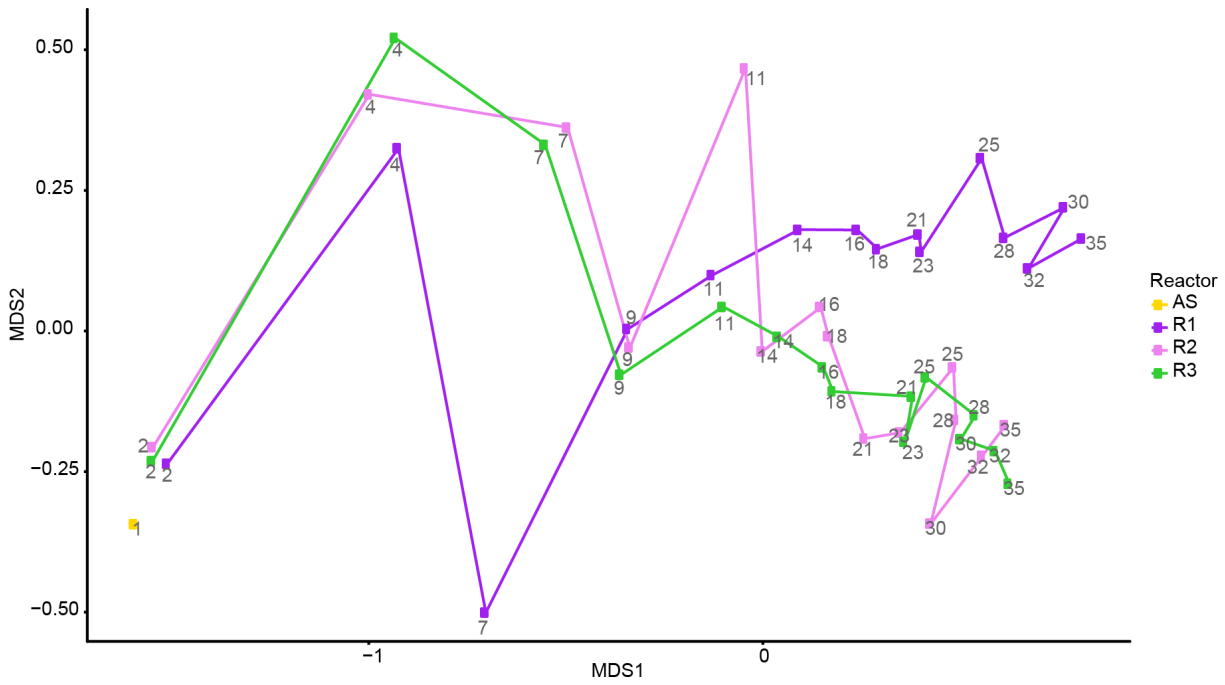
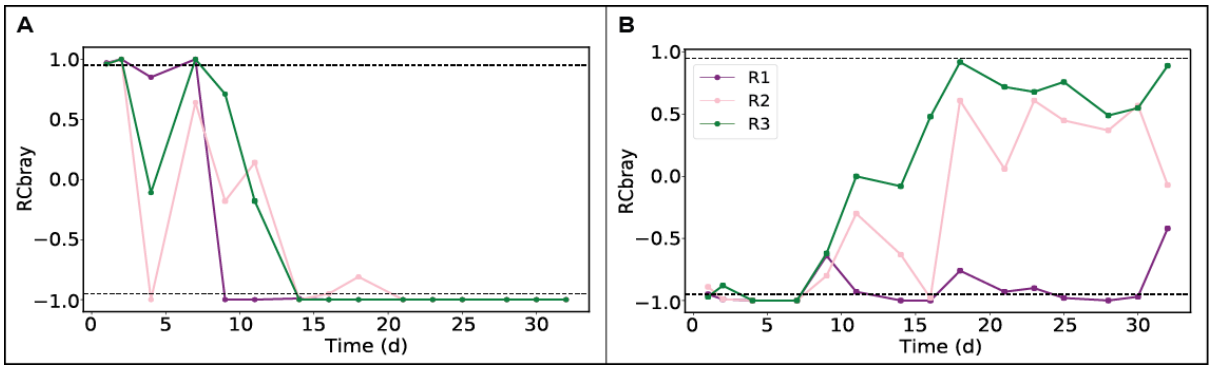


Figure 4. NMDS ordination based on Bray-Curtis dissimilarities after square root transformation. Each line represents the community succession of each reactor (R1, R2 and R3) starting from the seed sludge (AS, day 1). Numbers refer to days after startup. Stress = 0.047.

Null model analysis

We wished to determine the factors behind the shifts in β-diversity over time in each reactor. However, α-, β- and γ-diversity are interconnected so that e.g. a difference in richness between samples may in itself cause a difference in β-diversity between the samples [15, 48]. Null model analysis was therefore used to disentangle turnover due to succession from changes in α- and γ-diversity. We used two different types of null models for this, RC_{bray}, based on taxonomic turnover, and βNTI, based on phylogenetic turnover. Turnover between sequential samples from the same reactor would show successional phases. Thus, RC_{bray} and βNTI values lower than the null expectation, would indicate "steady-state" microbial community composition. In

280 R1, RC_{bray} values were close to 1 during the first 9 days (Figure 5A), indicating higher taxonomic
281 turnover than the null expectation, and thereafter turnover remained lower than the null
282 expectation (RC_{bray} < -0.95). R2 an R3 showed a somewhat different trend with turnover closer
283 to the null expectation (RC_{bray} between 0.95 and -0.95) during the first 11 days and after this
284 period, the taxonomic turnover between successive sample points remained lower than
285 expected by chance (RC_{bray} < -0.95). Similar patterns were observed using βNTI, with higher
286 phylogenetic turnover for R1 than for R2 and R3 until day 14, followed by lower turnover than
287 expected by chance in all reactors (Figure S7). RC_{bray} was also used for the fraction of OTUs
288 having significantly different relative abundance between the reactors, as determined by
289 correlations to the CAP axes (Figure S6 and Table S2). For this fraction of the community, the
290 taxonomic turnover between succesive sample points in R2 and R3 gradually increased from
291 day 9 to values not significantly different to the null expectation whereas in R1, the taxonomic
292 turnover remained lower than the null expectation throughout the reactor operation (Figure
293 5B).

294 

295 **Figure 5.** Taxonomic turnover (RC_{bray}) in community composition between two succesive
296 sample points of; A: the entire community and; B: the subset of OTUs having significant
297 correlation for the CAP2 axis of the replicate reactors.

298 ### Sequencing reproducibility

299 To ensure that the observed differences between the reactors, mainly caused by OTUs of low
300 abundance, were not caused by limited precision of sequencing, the samples were sequenced
301 twice. Highly similar results were obtained from the two sequencing runs with regard to
302 relative read abundance of major OTUs, NMDS ordination and CAP (Figures S8-S10).

303 ## Discussion

304 In this study, we investigated how communities of microorganisms assembled over time as
305 granules formed in three replicate reactors for wastewater treatment. Granule growth,
306 successional patterns of taxa, microbial diversity and reproducibility are discussed together
307 with an assessment of the mechanisms that influence the community assembly.

308 ### Succession of taxa during the granulation process

309 During the early transformation from flocs to granule-like particles, *Acinetobacter* sp. became
310 increasingly abundant in all reactors (Figure 2). *Acinetobacter* sp. has been reported as primary
311 colonizers and bridging bacteria in aggregate/biofilm development [49, 50] and have
312 previously been observed during initial stages of granulation [51]. *Acinetobacter* sp. is a
313 hydrophobic bacterium and produces EPS, which are decisive properties for early stage
314 aggregate formation [52] and likely explains their frequent occurrence during the initial phase.

315 As the granules started to increase in size, after one week of operation (Figure S3), the
316 microbial communities shifted and bacteria within *Comamonadaceae*, *Rhodocyclaceae*,
317 *Flavobacteriaceae*, *Xanthomonadaceae* and *Caulobacteraceae* became more abundant. Many
318 of these bacteria are associated with granulation [26, 51]. *Thauera* sp., which was particularly

319 abundant in this period (Figure 2), is an important EPS producer commonly found in acetate-
320 fed aerobic granules and is also a well-known denitrifier, as are many of the other common
321 microorganisms in this period [26, 53]. For instance, bacteria within the *Comamonadaceae*
322 family are considered important for acetate-fed denitrification in aerobic granules [54].
323 Despite this, the total nitrogen removal was incomplete, around 50% until day 28. Thereafter
324 nitrogen removal increased to 70% until the end of the study period, day 35. In this last period,
325 *Corynebacterium* sp. was highly abundant in the reactors (Figure 2). *Corynebacteria* can
326 remove nitrogen both by assimilatory reduction and denitrification [55].

327 None of the common members of the microbial community in the granules were highly
328 abundant, or even detected in the seed sludge. This is commonly observed in reactor studies
329 [9, 27, 56] and experimental evidence suggests that during succession, rare taxa can be
330 promoted to the dominant fraction of the microbial community following a perturbation or a
331 change of environment [57-59].

332 Microbial community assembly during granulation

333 The microbial communities in the replicate reactors followed similar succession patterns but
334 with a clear divergence of R1 from R2 and R3 (Figure 4). Since the inoculum, feed composition,
335 and reactor operation were the same among the reactors, this suggests that stochastic factors
336 had a role in the community assembly. Let us first consider the two extreme cases of either
337 stochastic or deterministic factors acting alone. With only stochastic factors, i.e. drift, neutral
338 theory predicts that the microbial communities in the three reactors would eventually diverge
339 and the dissimilarity index between reactors would increase and reach unity. On the other
340 hand, if deterministic factors, i.e. selection, were acting alone, the reactors would follow the
341 same trajectory and the dissimilarity index between reactors would be zero. The ecological

342 processes responsible for microbial community assembly can be classified as selection,
343 dispersal, drift, and diversification [3]. Our experimental design excluded dispersal as a major
344 factor because synthetic feed was used. Diversification can most likely also be neglected
345 because of the short time frame of the study (35 days). This leaves selection as the major
346 deterministic process and drift as the major stochastic process affecting community assembly.

347 During the early start-up, the microbial community shifted rapidly from the inoculum in a
348 similar manner in all reactors (Figure 4) and the phylogenetic successional turnover was
349 relatively high, especially in R1 (Figure S7). The settling time for the biomass was long (Figure
350 S1), thus the microorganisms were not washed out due to their settling properties, but
351 according to their ability to grow at these reactor conditions [60]. Therefore, the changes in
352 the microbial community structure, resulting in a harsh decrease in diversity (Figure 3), were
353 to a large extent caused by the switch from municipal wastewater to a synthetic feed with
354 acetate as carbon source. A switch from a complex substrate to a simple and easily
355 biodegradable substrate would favour specific taxa, and consequently, the biodiversity, both
356 taxonomic and phylogenetic, would decrease [26, 51]. Indeed, the drop in diversity was
357 particularly evident for highly abundant OTUs (^{2}ND and ^{2}PD, Figure 3, S5) and the dissimilarity
358 between the consecutive days 2 and 4 was also higher for the more abundant OTUs (^{2}βdisND
359 and ^{2}βdisPD, Figure 3). In addition, the lack of immigration of new taxa (dispersal) to the
360 reactors due the synthetic feed probably contributed to the decrease in α-diversity [61].

361 The settling time was thereafter drastically reduced, from 30 min to 5 min between days 5 and
362 11, to promote granulation (Figure S1) and the first granules were observed at day 7. This led
363 to increased α-diversity in the reactors as new, previously undetected, OTUs grew in relative
364 abundance (Figure 3), presumably due to the formation of new niches in the emerging

365 granules. The communities changed rapidly over time, in particular around day 7 (Figure 3),
366 and it was clear that the community assembly took different paths in the three reactors in this
367 phase (Figure 4). The null-models indicated that stochastic factors, here mainly drift, were
368 important for the succession during this dynamic phase (Figure 5, S7). This could be due to the
369 random distribution of microorganisms between aggregates of different sizes of which only
370 the larger and denser ones were retained by sedimentation [60].

371 From day 11, the decrease in settling time was less dramatic (Figure S1) and the granules
372 increased gradually in size (Figure S3). In this phase, the microbial communities changed more
373 slowly with time (Figure 3, S5) and turnover was lower than predicted by chance by the null-
374 models (Figure 5, S7), suggesting that deterministic forces, i.e. selection, were important. The
375 microbial succession in R2 and R3 followed the same trajectory until the end of the experiment,
376 but R1 diverged (Figure 4). The divergence of R1 was not due to the most abundant OTUs, the
377 community was dominated by the same genera in the three replicate reactors (Figure 2).
378 However, α-diversity (^{0}ND) was lower in R1 than R2 and R3 and β-diversity between reactors
379 (Figure 3) showed that the divergence of R1 was mainly caused by rare and intermediate OTUs.
380 A subset of the OTUs were significantly different among the reactors, as assessed by
381 correlation to the CAP2 axis (Figure S6), and these OTUs were of low or intermediate relative
382 abundance (Table S2). For these OTUs, successional turnover was lower than by chance in R1,
383 while in R2 and R3, turnover was higher and the null-model predicted stochasticity (drift) to
384 have larger influence (Figure 5B) Within the same microbial community, selection and drift can
385 affect different subpopulations differently [5]. The less abundant OTUs were likely more
386 susceptible to drift, since many events could result in extinction [4]. Interestingly, predatory
387 bacteria belonging to the genus *Bdellovibrio*, *Bacteriovorax* and *Peredibacter* were detected in

388 the three reactors at as high as 10.45% maximum relative abundance. *Bdellovibrio* and like
389 organisms (BALOs) prey on gram negative bacteria, with some BALOs being generalist and
390 some specialists in their prey preferences [62]. BALOs have been found in granules [28, 63, 64]
391 and have recently been shown to affect the granular microbial structure [65]. R2 and R3
392 harboured considerably more BALOs than R1, especially in the later stages of the study (Figure
393 2 and Figure S8). Also, out of a total of 60 OTUs belonging to BALOs, 11 were significantly lower
394 in abundance in R1 compared to R2 and R3 according to CAP (Table S2). It could hence be
395 hypothesized that BALOs caused a higher turnover in R2 and R3, contributing to the divergence
396 of these reactors from R1.

397 We tentatively interpret the difference between the reactors in terms of underlying
398 mechanisms: During the early phase the change of feed selected for certain taxa, which caused
399 the biodiversity to decrease (Figure 3). The successional turnover was high, but the microbial
400 communities in replicate reactors were similar (Figure 2 and 4), therefore the acclimation to a
401 new feed seems to be mostly deterministic. A second transitional phase occurred when the
402 settling time was decreased and granules began to emerge. This likely created new niches,
403 which allowed low-abundant taxa, previously below the detection threshold, to increase in
404 abundance resulting in temporarily increased α-diversity (Figure 3). During this phase,
405 stochasticity seemed to cause the microbial community in reactor 1 to take a different path
406 (Figure 4). However, selection eventually led to the same dominating taxa in the parallel
407 reactors (Figure 2). The successional turnover was low during this last period, presumably
408 because selection had led to the development of a microbial community adapted to the
409 specific reactor conditions, but drift caused reactors 2 and 3 to slowly diverge from reactor 1.

410 ## Ecological processes and wastewater treatment

411 Biological processes for wastewater treatment are often tested in laboratory- or pilot-scale
412 bioreactors. Bioreactors are designed as if they were solely affected by deterministic processes
413 and the treatment process relies on this assumption. But the literature is inconsistent
414 regarding the reproducibility of wastewater biological reactors. Vanwonterghem *et al* (2014)
415 observed that the microbial community structure and dynamics in three replicate anaerobic
416 digesters were highly reproducible, influenced by deterministic processes. Ayarza *et al* (2010)
417 reported the microbial community dynamics to follow similar patterns in four replicate
418 sequencing batch reactors treating synthetic wastewater due to deterministic processes,
419 although, drift caused the reactors to display a differences. Zhou *et al* (2013) demonstrated
420 divergent microbial communities in 14 replicate microbial electrolysis cell reactors treating
421 wastewater due to a dominant role of stochastic processes. Despite highly dynamic microbial
422 communities, stable performance of parallel bioreactors treating wastewater are often found
423 for such general functions as aerobic/anoxic oxidation of organic matter in activated sludge
424 [16, 66-68]. On the other hand, for complex processes that require multispecies cooperation,
425 such as anaerobic digestion or specialized processes such as microbial electrolysis, stochastic
426 variations in community composition has resulted in altered microbial functions [15, 68]. In the
427 present study the differences in the microbial community dynamics were due to differences in
428 the abundance of a small fraction of the total OTUs, which did not have major impact on the
429 reactor functions since the replicate reactors behaved similarly (Figure 1). The same dynamics
430 in dominant OTUs and a higher variability of the less abundant fraction of the community has
431 also been observed in full-scale activated sludge reactors [69]. From the engineering
432 perspective our results are important since they not only confirm the possibility to select for

433 certain dominating taxa by manipulating the reactors conditions, even for a process as
434 common as acetate oxidation, but also provide information about fundamental ecological
435 processes involved in sludge granulation. Moreover, the results presented here suggest that it
436 is possible to draw conclusions about the reactor performance and microbial community
437 dynamics (at least for abundant community members) in experiments with single aerobic
438 granular sludge reactors.

439 In summary, our study shows that three parallel granular sludge reactors operated identically
440 and inoculated with the same seed sludge behaved similarly, both in terms of treatment
441 performance and microbial community assembly. Deterministic and stochastic processes
442 exerted a dynamic influence during the different successional stages. Deterministic factors
443 shaped the community composition of abundant members whereas stochastic factors mostly
444 affected rare members of the communities. Drift caused the microbial community in one of
445 the reactors to diverge from the other two. Predation seems to have an influence in the
446 divergence of the reactors. Despite these differences in the microbial community, the reactors
447 functions were stable and similar between reactors. More studies dealing with fundamental
448 ecological processes involved in granulation are needed. For instance, it is important to know
449 how perturbations affect the microbial community and the resilience of the system. This
450 knowledge will help to better understand the technology which ultimately will improve its
451 application and management.

452 Acknowledgements

453 This work was supported by FORMAS (Contract no. 245-2013-627), the Swedish Research
454 Council for Environment, Agriculture Sciences and Spatial Planning. The authors acknowledge

the Genomics core facility at the University of Gothenburg for support and use of their equipment.

Competing Interests

The authors declare no competing financial interests.

460 # REFERENCES

461 1. Anderson MJ, Crist TO, Chase JM, Vellend M, Inouye BD, Freestone AL *et al*. Navigating the
462 multiple meanings of β diversity: a roadmap for the practicing ecologist. *Ecology Letters* 2011;
463 **14**(1)**:** 19-28.

464 2. Shade A. Diversity is the question, not the answer. *The Isme Journal* 2016; **11:** 1.

465 3. Vellend M. Conceptual synthesis in community ecology. *The Quarterly review of biology*
466 2010; **85**(2)**:** 183-206.

467 4. Nemergut DR, Schmidt SK, Fukami T, O'Neill SP, Bilinski TM, Stanish LF *et al*. Patterns and
468 processes of microbial community assembly. *Microbiology and Molecular Biology Reviews*
469 2013; **77**(3)**:** 342-356.

470 5. Zhou J, Ning D. Stochastic Community Assembly: Does It Matter in Microbial Ecology?
471 *Microbiology and Molecular Biology Reviews* 2017; **81**(4).

472 6. McMahon KD, Martin HG, Hugenholtz P. Integrating ecology into biotechnology. *Current*
473 *Opinion in Biotechnology* 2007; **18**(3)**:** 287-292.

474 7. Xia Y, Wen X, Zhang B, Yang Y. Diversity and assembly patterns of activated sludge microbial
475 communities: A review. *Biotechnology Advances* 2018; **36**(4)**:** 1038-1047.

476 8. Falk MW, Song KG, Matiasek MG, Wuertz S. Microbial community dynamics in replicate
477 membrane bioreactors - Natural reproducible fluctuations. *Water Research* 2009; **43**(3)**:** 842-
478 852.

479 9. Vanwonterghem I, Jensen PD, Dennis PG, Hugenholtz P, Rabaey K, Tyson GW. Deterministic
480 processes guide long-term synchronised population dynamics in replicate anaerobic digesters.
481 *ISME J* 2014; **8**(10)**:** 2015-2028.

482 10. Lucas R, Kuchenbuch A, Fetzer I, Harms H, Kleinsteuber S. Long-term monitoring reveals
483 stable and remarkably similar microbial communities in parallel full-scale biogas reactors
484 digesting energy crops. *FEMS Microbiology Ecology* 2015; **91**(3)**:** fiv004-fiv004.

485 11. Luo G, De Francisci D, Kougias PG, Laura T, Zhu X, Angelidaki I. New steady-state microbial
486 community compositions and process performances in biogas reactors induced by
487 temperature disturbances. *Biotechnology for Biofuels* 2015; **8**(1)**:** 3.

488 12. El-Chakhtoura J, El-Fadel M, Rao HA, Li D, Ghanimeh S, Saikaly PE. Electricity generation
489 and microbial community structure of air-cathode microbial fuel cells powered with the
490 organic fraction of municipal solid waste and inoculated with different seeds. *Biomass and*
491 *Bioenergy* 2014; **67:** 24-31.

492 13. Wittebolle L, Van Vooren N, Verstraete W, Boon N. High reproducibility of ammonia-
493 oxidizing bacterial communities in parallel sequential batch reactors. *Journal of Applied*
494 *Microbiology* 2009; **107**(2)**:** 385-394.

495 14. Ayarza JM, Guerrero LD, Erijman L. Nonrandom Assembly of Bacterial Populations in
496 Activated Sludge Flocs. *Microbial Ecology* 2010; **59**(3)**:** 436-444.

497 15. Zhou J, Liu W, Deng Y, Jiang Y-H, Xue K, He Z *et al*. Stochastic Assembly Leads to Alternative
498 Communities with Distinct Functions in a Bioreactor Microbial Community. *mBio* 2013; **4**(2).

16. Kaewpipat K, Grady CPL. Microbial population dynamics in laboratory-scale activated sludge reactors. *Water Science and Technology* 2002; **46**(1-2)**:** 19-27.

17. Solli L, Håvelsrud OE, Horn SJ, Rike AG. A metagenomic study of the microbial communities in four parallel biogas reactors. *Biotechnology for Biofuels* 2014; **7**(1)**:** 146.

18. Han S, Liu Y, Zhang S, Luo G. Reactor performances and microbial communities of biogas reactors: effects of inoculum sources. *Applied Microbiology and Biotechnology* 2016; **100**(2)**:** 987-995.

19. Chen Y, Lan S, Wang L, Dong S, Zhou H, Tan Z *et al*. A review: Driving factors and regulation strategies of microbial community structure and dynamics in wastewater treatment systems. *Chemosphere* 2017; **174:** 173-182.

20. Ferrera I, Sánchez O. Insights into microbial diversity in wastewater treatment systems: How far have we come? *Biotechnology Advances* 2016; **34**(5)**:** 790-802.

21. Zhang Q, Hu J, Lee D-J. Aerobic granular processes: Current research trends. *Bioresource Technology* 2016; **210:** 74-80.

22. Bengtsson S, de Blois M, Wilén B-M, Gustavsson D. A comparison of aerobic granular sludge with conventional and compact biological treatment technologies. *Environmental Technology* 2018**:** 1-10.

23. Nancharaiah YV, Kiran Kumar Reddy G. Aerobic granular sludge technology: Mechanisms of granulation and biotechnological applications. *Bioresource Technology* 2018; **247:** 1128-1143.

24. Wilén B-M, Liébana R, Persson F, Modin O, Hermansson M. The mechanisms of granulation of activated sludge in wastewater treatment, its optimization, and impact on effluent quality. *Applied Microbiology and Biotechnology* 2018: 1-16.

25. Sarma SJ, Tay JH, Chu A. Finding Knowledge Gaps in Aerobic Granulation Technology. *Trends in Biotechnology* 2017; **35**(1): 66-78.

26. Szabó E, Liébana R, Hermansson M, Modin O, Persson F, Wilén B-M. Microbial Population Dynamics and Ecosystem Functions of Anoxic/Aerobic Granular Sludge in Sequencing Batch Reactors Operated at Different Organic Loading Rates. *Frontiers in Microbiology* 2017; **8:** 770.

27. Fan X-Y, Gao J-F, Pan K-L, Li D-C, Zhang L-F, Wang S-J. Shifts in bacterial community composition and abundance of nitrifiers during aerobic granulation in two nitrifying sequencing batch reactors. *Bioresource Technology* 2018; **251:** 99-107.

28. Weissbrodt DG, Shani N, Holliger C. Linking bacterial population dynamics and nutrient removal in the granular sludge biofilm ecosystem engineered for wastewater treatment. *FEMS Microbiology Ecology* 2014; **88**(3): 579-595.

29. Gonzalez-Martinez A, Muñoz-Palazon B, Maza-Márquez P, Rodriguez-Sanchez A, Gonzalez-Lopez J, Vahala R. Performance and microbial community structure of a polar Arctic Circle aerobic granular sludge system operating at low temperature. *Bioresource Technology* 2018; **256:** 22-29.

30. Widder S, Allen RJ, Pfeiffer T, Curtis TP, Wiuf C, Sloan WT *et al*. Challenges in microbial ecology: building predictive understanding of community function and dynamics. *The Isme Journal* 2016; **10:** 2557.

540 31. Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD. Development of a dual-index
541 sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq
542 Illumina sequencing platform. *Applied and environmental microbiology* 2013; **79**(17)**:** 5112-
543 5120.

544 32. Edgar RC. UNOISE2: improved error-correction for Illumina 16S and ITS amplicon
545 sequencing. *bioRxiv* 2016.

546 33. Edgar RC, Flyvbjerg H. Error filtering, pair assembly and error correction for next-generation
547 sequencing reads. *Bioinformatics (Oxford, England)* 2015; **31**(21)**:** 3476-3482.

548 34. Edgar RC. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*
549 *(Oxford, England)* 2010; **26**(19)**:** 2460-2461.

550 35. Edgar R. SINTAX: a simple non-Bayesian taxonomy classifier for 16S and ITS sequences.
551 *bioRxiv* 2016.

552 36. McIlroy SJ, Saunders AM, Albertsen M, Nierychlo M, McIlroy B, Hansen AA *et al*. MiDAS:
553 the field guide to the microbes of activated sludge. *Database* 2015; **2015**.

554 37. Albertsen M, Karst SM, Ziegler AS, Kirkegaard RH, Nielsen PH. Back to Basics–The Influence
555 of DNA extraction and primer choice on phylogenetic analysis of activated sludge communities.
556 *PloS one* 2015; **10**(7)**:** e0132783.

557 38. Oksanen F, Blanchet G, Friendly M, Kindt R, Legendre P, McGlinn D *et al*. vegan: Community
558 Ecology Package. R package version 2.4-4. http s. *CRAN R-pro j ect org/pack age= vega n* 2017.

559 39. Wright ES. DECIPHER: harnessing local sequence context to improve protein multiple
560 sequence alignment. *BMC Bioinformatics* 2015; **16**(1)**:** 322.

561 40. Price MN, Dehal PS, Arkin AP. FastTree 2 – Approximately Maximum-Likelihood Trees for
562 Large Alignments. *PLOS ONE* 2010; **5**(3)**:** e9490.

563 41. Jost L. Entropy and diversity. *Oikos* 2006; **113**(2)**:** 363-375.

564 42. Chiu C-H, Chao A. Distance-Based Functional Diversity Measures and Their Decomposition:
565 A Framework Based on Hill Numbers. *PLOS ONE* 2014; **9**(7)**:** e100014.

566 43. Jost L. Partitioning diversity into independent alpha and beta components. *Ecology* 2007;
567 **88**(10)**:** 2427-2439.

568 44. Stegen JC, Lin X, Fredrickson JK, Chen X, Kennedy DW, Murray CJ *et al*. Quantifying
569 community assembly processes and identifying features that impose them. *The Isme Journal*
570 2013; **7:** 2069.

571 45. Kembel S, Ackerly D, Blomberg S, Cornwell W, Cowan P, Helmus M *et al* (2015). Picante: R
572 tools for integrating phylogenies and ecology. R package version 1.6-2.

573 46. Dini-Andreote F, Stegen JC, van Elsas JD, Salles JF. Disentangling mechanisms that mediate
574 the balance between stochastic and deterministic processes in microbial succession.
575 *Proceedings of the National Academy of Sciences* 2015; **112**(11)**:** E1326-E1332.

576 47. Wang J, Shen J, Wu Y, Tu C, Soininen J, Stegen JC *et al*. Phylogenetic beta diversity in
577 bacterial assemblages across ecosystems: deterministic versus stochastic processes. *ISME J*
578 2013; **7**(7)**:** 1310-1321.

579 48. Chase JM, Kraft NJB, Smith KG, Vellend M, Inouye BD. Using null models to disentangle
580 variation in community dissimilarity from variation in α-diversity. *Ecosphere* 2011; **2**(2)**:** 1-11.

581 49. Liébana R, Arregui L, Santos A, Murciano A, Marquina D, Serrano S. Unravelling the
582 interactions among microbial populations found in activated sludge during biofilm formation.
583 *FEMS Microbiology Ecology* 2016; **92**(9): fiw134-fiw134.

584 50. Katharios-Lanwermeyer S, Xi C, Jakubovics NS, Rickard AH. Mini-review: Microbial
585 coaggregation: ubiquity and implications for biofilm development. *Biofouling* 2014; **30**(10):
586 1235-1251.

587 51. Weissbrodt DG, Lochmatter S, Ebrahimi S, Rossi P, Maillard J, Holliger C. Bacterial selection
588 during the formation of early-stage aerobic granules in wastewater treatment systems
589 operated under wash-out dynamics. *Front Microbiol* 2012; **3**: 332.

590 52. Ding Z, Bourven I, Guibaud G, van Hullebusch ED, Panico A, Pirozzi F *et al*. Role of
591 extracellular polymeric substances (EPS) production in bioaggregation: application to
592 wastewater treatment. *Applied Microbiology and Biotechnology* 2015; **99**(23): 9883-9905.

593 53. Xia J, Ye L, Ren H, Zhang X-X. Microbial community structure and function in aerobic
594 granular sludge. *Applied Microbiology and Biotechnology* 2018; **102**(9): 3967-3979.

595 54. Lu H, Chandran K, Stensel D. Microbial ecology of denitrification in biological wastewater
596 treatment. *Water Res* 2014; **64**.

597 55. Seenivasagan R, Rajakumar S, Kasimani R, Ayyasamy PM. Screening of assimilatory and
598 dissimilatory denitrifying microbes isolated from nitrate-contaminated water and soil.
599 *Preparative Biochemistry and Biotechnology* 2014; **44**(6): 586-597.

600 56. Szabó E, Liébana R, Hermansson M, Modin O, Persson F, Wilén B-M. Microbial population
601 dynamics and ecosystem functions of anoxic/aerobic granular sludge in sequencing batch
602 reactors operated at different organic loading rates. *Front Microbiol* 2017; **8**.

603 57. Coveley S, Elshahed MS, Youssef NH. Response of the rare biosphere to environmental
604 stressors in a highly diverse ecosystem (Zodletone spring, OK, USA). *PeerJ* 2015; **3:** e1182.

605 58. Pagaling E, Strathdee F, Spears BM, Cates ME, Allen RJ, Free A. Community history affects
606 the predictability of microbial ecosystem development. *The Isme Journal* 2013; **8:** 19.

607 59. Shade A, Jones SE, Caporaso JG, Handelsman J, Knight R, Fierer N *et al*. Conditionally Rare
608 Taxa Disproportionately Contribute to Temporal Changes in Microbial Diversity. *mBio* 2014;
609 **5**(4).

610 60. Szabó E, Liébana R, Hermansson M, Modin O, Persson F, Wilén B-M. Comparison of the
611 bacterial community composition in the granular and the suspended phase of sequencing
612 batch reactors. *AMB Express* 2017; **7**(1)**:** 168.

613 61. Albright MBN, Martiny JBH. Dispersal alters bacterial diversity and composition in a natural
614 community. *The Isme Journal* 2017; **12:** 296.

615 62. Johnke J, Cohen Y, de Leeuw M, Kushmaro A, Jurkevitch E, Chatzinotas A. Multiple micro-
616 predators controlling bacterial communities in the environment. *Current Opinion in*
617 *Biotechnology* 2014; **27:** 185-190.

618 63. Li Y, Zou J, Zhang L, Sun J. Aerobic granular sludge for simultaneous accumulation of mineral
619 phosphorus and removal of nitrogen via nitrite in wastewater. *Bioresource Technology* 2014;
620 **154:** 178-184.

621 64. Wan C, Yang X, Lee D-J, Liu X, Sun S. Partial nitrification using aerobic granule continuous-
622 flow reactor: Operations and microbial community. *Journal of the Taiwan Institute of Chemical*
623 *Engineers* 2014; **45**(5)**:** 2681-2687.

624 65. Feng S, Tan CH, Constancias F, Kohli GS, Cohen Y, Rice SA. Predation by Bdellovibrio
625 bacteriovorus significantly reduces viability and alters the microbial community composition
626 of activated sludge flocs and granules. *FEMS Microbiology Ecology* 2017; **93**(4)**:** fix020-fix020.

627 66. Ayarza JM, Erijman L. Balance of neutral and deterministic components in the dynamics of
628 activated sludge floc assembly. *Microbial Ecology* 2011; **61**(3)**:** 486-495.

629 67. Bagchi S, Tellez B, Rao H, Lamendella R, Saikaly P. Diversity and dynamics of dominant and
630 rare bacterial taxa in replicate sequencing batch reactors operated under different solids
631 retention time. *Applied Microbiology and Biotechnology* 2015; **99**(5)**:** 2361-2370.

632 68. Goux X, Calusinska M, Lemaigre S, Marynowska M, Klocke M, Udelhoven T *et al*. Microbial
633 community dynamics in replicate anaerobic digesters exposed sequentially to increasing
634 organic loading rate, acidosis, and process recovery. *Biotechnology for Biofuels* 2015; **8**(1)**:** 122.

635 69. Griffin JS, Wells GF. Regional synchrony in full-scale activated sludge bioreactors due to
636 deterministic microbial community assembly. *The Isme Journal* 2016; **11:** 500.

637