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Effect of the Food-to-Microorganism (F/M) Ratio on the Formation and Size of Aerobic Sludge Granules

An-jie Li^{1,2}, Xiao-yan Li^{2*}, and Han-qing Yu³

¹ Key Laboratory of Water and Sediment Sciences of Ministry of Education / State Key Joint
Laboratory of Environment Simulation and Pollution Control, School of Environment,
Beijing Normal University, Beijing, 100875, China

²Environmental Engineering Research Centre, Department of Civil Engineering,
The University of Hong Kong, Pokfulam Road, Hong Kong, China

³School of Chemistry, University of Science and Technology of China, Hefei, 230026, China

(*Corresponding Author: Tel: (852)28592659; Email: xlia@hkucc.hku.hk; Homepage: <http://web.hku.hk/~xlia/>)

1

Abstract

2

Laboratory experiments were carried out to investigate the effect of the sludge loading, or
3 the food-to-microorganism (F/M) ratio, on the rate of aerobic granulation and the size of the
4 granules in biological wastewater treatment. Four column batch reactors were used with a
5 similar sludge suspended solids (SS) concentration of around 2000 mg/L. The reactors were
6 fed with a glucose-based wastewater at different chemical oxygen demand (COD)
7 concentrations, resulting in F/M ratios from 0.3 to 1.1 g COD/g SS-d. A higher F/M ratio
8 appeared to promote faster formation of larger granules and a lower F/M ratio led to slower

9 formation of smaller granules. Upon complete granulation, the granules became rather stable in
10 size, and the mean diameter of the granules in different reactors increased from 1.2 to 4.5 mm
11 linearly with the F/M ratio applied. Molecular analysis of the sludge did not show the
12 domination of any particular bacterial species during the granulation process. In is apparent
13 that applying different F/M ratios in different granulation stages, e.g., a higher F/M in the
14 early stage and a reduced F/M in the later stage, can be an effective start-up strategy to facilitate
15 rapid granule formation and sustain small and healthy granules in bioreactors.

16
17 **Keywords:** Aerobic granulation; F/M ratio; activated sludge; microbial community;
18 wastewater treatment.

19 20 **1. Introduction**

21 Aerobic sludge granulation is a new microbial immobilization technique that has the
22 potential to fundamentally advance the biological wastewater treatment technology [1-3].
23 Compared to conventional activated sludge, the dense granule structure confers on granular
24 sludge an excellent settling ability that allows for rapid sludge-effluent separation, a high
25 level of biomass concentration, and a greater organic treatment loading capability [3-5].
26 Aerobic granules are considered as a special form of bacterial biofilm growth in suspension
27 [5,6]. It has been demonstrated that granulation can be achieved by means of selective
28 discharge of small and slow-settling sludge flocs [7]. However, the influences of process
29 conditions on the quality and property of granules are still issues of investigation. For
30 instance, the size of aerobic granules has a profound impact on the stability and treatment
31 performance of granular sludge, and larger granules may become less stable in wastewater
32 treatment. However, effective measures for controlling the size and improving the stability of
33 aerobic granules in a bioreactor remain to be developed.

34 During the start-up of aerobic granulation, a short settling period is commonly adopted to
35 force the discharge of small and loose sludge flocs from the reactors and hence to retain
36 denser sludge [3,7]. Such an early washout of small and slow-settling sludge from the
37 suspension leads to a loss of biomass, resulting in an increase in sludge loading rate [1,3]. A
38 high food-to-microorganism (F/M) ratio would enhance microbial growth [8] and hence
39 facilitate the aerobic granulation process [9,10]. In connection to biomass growth, the sludge
40 loading rate, or the F/M ratio, in a bioreactor could be an essential parameter that regulates
41 the size of granules. However, the correlations between the F/M ratio and the rate of aerobic
42 granulation and the size of granules have not been well established.

43 F/M ratio is a process variable that can be easily adjusted in operating bioreactors. A
44 suitable F/M can be favorable for both the progress of granulation and the size control of
45 granules. In this study, laboratory experiments were conducted with batch reactors to
46 investigate the effect of the F/M ratio on the formation, size, and stability of granules. The rates
47 of sludge granulation and the stable size of granules formed under different F/M conditions
48 were determined. The findings are essential to the development of an effective start-up strategy
49 for the formation and maintenance of small and healthy granules in long-term biological
50 wastewater treatment operation.

51

52 **2. Materials and Methods**

53 *2.1. Experimental set-up and operation*

54 Four 0.4-L graduate cylinders (H 22 cm × D 5.2 cm) were used as column batch reactors
55 for the experimental study on aerobic granulation. Activated sludge from a full-scale sewage
56 treatment plant (Stanley Sewage Treatment Works, Hong Kong) was used as the seed sludge
57 after one month of laboratory acclimation with a glucose-based synthetic wastewater. The seed
58 sludge was well mixed before loading into the four reactors to have the same initial mixed

59 liquor suspended solids (MLSS) concentration of 2000 mg/L. The reactors were fed once a day
60 with a synthetic wastewater consisting of glucose, NH_4Cl , KH_2PO_4 and $\text{NaHPO}_4 \cdot 6\text{H}_2\text{O}$, and
61 other nutrients that was prepared according to the formula given by [Tay et al. \[10\]](#).

62 The operating condition was the same for the four reactors, R1, R2, R3, and R4, except the
63 feed substrate concentration. Different influent organic concentrations in terms of the chemical
64 oxygen demand (COD) – 600, 1400, 2200, and 2200 mg/L – were used for the four reactors to
65 have F/M ratios of 0.3, 0.7, 1.1, and 1.1 g COD/g SS-d for R1, R2, R3, and R4, respectively.
66 For R4, however, the influent COD concentration changed from 2200 to 600 mg/L in the third
67 week after its start-up, which reduced the F/M ratio from 1.1 to 0.3 g COD/g SS-d. The
68 experiments were carried out at room temperature, and the water temperature was 20-22°C.
69 NaHCO_3 was dosed into the feed solution to maintain the pH of the reactors in the neutral range
70 between 7.0 and 7.5. Aeration was conducted from the bottom of the reactors at an air flow rate
71 of around 1.0 L/min during the aeration phase, and the dissolved oxygen (DO) concentration in
72 the sludge suspension was in a range of 2-5 mg/L.

73 At the end of each 24-hr cycle, the sludge was allowed to settle in the column without
74 aeration. During the early settling phase, a certain amount of the sludge suspension was
75 withdrawn by siphon below the water surface. The slow-settling sludge flocs in the suspension
76 were therefore removed from the reactors. The sludge concentration in each reactor and the
77 amount of daily sludge discharge from the reactor were measured. Accordingly, the rate of the
78 daily sludge discharge was then adjusted to maintain the MLSS concentration at about 2000
79 mg/L in the reactors. The purpose of this operation was to selectively remove the small and
80 slow-settling sludge from the sludge mixture while keeping the sludge concentration and F/M
81 ratio at the pre-determined levels in each reactor [\[7\]](#). After another 30 min of sludge
82 sedimentation, the supernatant was withdrawn from the reactors, and the wastewater influent
83 was added into each reactor to restore its original water volume of 0.4 L.

84

85 2.2. Determination of the organic uptake capability and settling behavior of the granules

86 After the completion of aerobic granulation, the granular sludge was characterized for its
87 organic uptake rate and settling velocity. For each sludge sample from a reactor, the organic
88 uptake test was performed in a 250-mL glass beaker, and the sludge and glucose concentrations
89 were 1000 mg MLSS/L and 300 mg/L, respectively. The sludge mixture was sampled at
90 various time intervals and the glucose concentration in the liquid phase of the sludge was
91 measured. The mass-balance equation for organic in the reactor may be written as

92 $\frac{dS}{dt}V = QS_0 - QS + rV$, where S is the substrate concentration and S_0 is its initial concentration,

93 t is time and r the rate of the substrate removal. Since $Q = 0$ for a batch reactor and a

94 first-order correlation $r = -(kX)S$ may be assumed for the early phase of substrate uptake,

95 where k is a specific rate coefficient and X the biomass concentration in the batch reactor, the

96 rate of organic removal may be approximated by $\frac{dS}{dt} = -kXS$. By linear regression of $\ln(S_0/S)$

97 versus Xt , the apparent specific organic uptake rate coefficient of the sludge can be estimated.

98 The settling experiments were conducted for individual mature granules following the

99 procedure described by [Xiao et al. \[11\]](#) in water column. The acrylic settling column was 90

100 cm in height and 8.1 cm in diameter with a conical bottom and a valve. For each setting test, a

101 granule was placed at the top of the water column, and the settling velocity of the granule

102 through the lower 60 cm was measured. The granule reaching the bottom was then released

103 and retrieved. The granule was placed on a stereomicroscope (S8 APO, Leica, Germany)

104 equipped with a digital camera (EC3, Leica, Germany), and the granule was sized according

105 to its projected area, A , and expressed by the equivalent diameter of $d = \sqrt{4A/\pi}$ [12].

106

107 2.3. Water and sludge analysis

108 The COD and SS concentrations and the sludge volume index after 5 min (SVI₅) were
109 measured following the Standard Methods [13]. The total organic carbon (TOC)
110 concentration was measured using a TOC analyzer (IL550, HACH-Lachat, USA). The
111 glucose concentration was determined by a UV/VIS spectrophotometer (Lambda 25, Perkin
112 Elmer, USA) according to the phenol-sulphuric acid method [14]. The morphology of the
113 aerobic granules was observed under a stereomicroscope (S8 APO, Leica, Germany). The
114 particle size distributions (PSD) of the sludge samples (< 2000 µm) were measured using a
115 laser diffraction particle counter (LS13 320, Beckman Coulter, USA). When granules grew
116 larger, photographs of the granules in a sludge sample were taken by a digital camera with the
117 stereomicroscope. The photo images of the granules were analyzed by an image analysis
118 system (analySIS 3.1) for PSD of the granular sludge.

119 A heat extraction method was modified to extract extracellular polymeric substances (EPS)
120 from activated sludge and granules [15]. The sludge was first washed three times and
121 dewatered by centrifugation (5810R, Eppendorf, Germany) in a 25-mL tube at 4000 g for 5 min.
122 The sludge pellet in the tube was then homogenized into 2.5 mL of 0.05% NaCl solution by a
123 beadbeater (Mini-beadbeaterTM, Biospec, USA) without beads. The sludge mixture was then
124 diluted with the NaCl solution to its original volume of 25 mL. The sludge suspension was
125 heated to 60°C in a water bath for 30 min, and the sludge mixture was then centrifuged at 4000
126 g for 15 min. The supernatant collected was regarded as the EPS extract of the sludge, which
127 was analyzed for TOC, polysaccharides (PS), proteins, and humic-like substances (HS). The
128 PS content was determined using the phenol-sulphuric acid method [14] with glucose as the
129 standard. Proteins and HS were analyzed by a UV/VIS spectrophotometer (Lambda 25,
130 Perkin Elmer, USA) following the modified Lowry method [16] using bovine serum albumin
131 (Sigma) and humic acid (Fluka) as the standards, respectively.

132

133 2.4. DNA extraction and denaturing gradient gel electrophoresis (DGGE) analysis of the
134 sludge

135 DGGE band profiles were used to reveal the most abundant DNA types among the
136 microbial species in a sludge sample [17]. The genomic DNA of the biomass was extracted
137 following the protocol described by Zhuang et al. [18] using a beadbeater
138 (Mini-beadbeater™, Biospec, USA) and a microcentrifuge (MiniSpin plus®, Eppendorf,
139 Germany). Subsequently, the variable V3 region of the bacterial 16S rDNA gene sequence
140 was amplified by polymerase chain reaction (PCR) [19] with a DNA Engine® Peltier
141 Thermal Cycler (PTC-200, MJ Research, USA). A touchdown thermal profile technique was
142 used for the PCR procedure [20]. As described by Li et al. [21], the PCR-amplified DNA
143 products were then separated by DGGE, and the DGGE gel images were acquired using the
144 ChemiDoc (Bio-Rad, USA) gel documentation system. The DGGE band patterns were then
145 used to calculate the Shannon–Weaver index for the species diversity of different sludge
146 samples [21].

147

148 **3. Results and Discussion**

149 *3.1. Formation and physical properties of the aerobic granules*

150 Aerobic sludge granulation was well achieved in all four batch reactors operated at
151 different F/M ratios (Figs. 1 and 2). The sludge MLSS concentrations were kept largely
152 comparable between the four reactors during the experimental study. Within the range tested,
153 the F/M ratio, or the biomass loading rate, did not appear to be the crucial factor for granule
154 formation. As indicated previously [7], selective discharge of small and slow-settling sludge
155 flocs from the sludge suspension was the determining factor for aerobic granulation. However,
156 the F/M ratio displayed a profound effect on the rate of granulation and the morphological
157 property of the granules. In general, a higher F/M ratio brought about faster formation of larger

158 granules, and a lower F/M ratio led to slower formation of smaller granules (Figs. 2 and 3). In
159 R1 at a low F/M of 0.3 g COD/g SS-d, small granules were observed after 25 d and granulation
160 was fully achieved throughout the column reactor after 40 d. In R2 at a medium F/M of 0.7 g
161 COD/g SS-d, granules appeared after 16 d and granulation was completed after 20 d. In R3 and
162 R4 at a high F/M of 1.1 g COD/g SS-d, granules were observed after only 7 d and granulation
163 was completed in about 14 d. A comparison between the different column reactors suggests
164 that faster biomass growth under a higher F/M condition would facilitate a rapid granule
165 formation and growth. Nonetheless, a high biomass loading, e.g. $F/M > 0.5$ g COD/g SS-d [22],
166 may not be a necessity for complete sludge granulation.

167 As described previously, upon complete granulation, the F/M ratio for R4 was reduced
168 from 1.1 to 0.3 g COD/g SS-d after 20 d of the start-up. With the decrease in F/M, breakage
169 of large granules occurred, resulting in losses of the biomass. However, the aerobic granules
170 in R4 became stabilized eventually at smaller sizes after about 10 d, and the granules
171 appeared to be comparable to those formed in R1 at the low F/M of 0.3 g COD/g SS-d (Figs.
172 2 and 3). Based on the rate of sludge discharge from the four reactors, the sludge retention time
173 (SRT) was kept at 15, 8, and 5 d for R1, R2, and R3, respectively. The SRT of R4 changed from
174 5 to 15 d after running 20 d. The SVI_5 of the mature granules were 27.0 ± 2.4 , 36.8 ± 3.7 ,
175 42.5 ± 5.1 , and 29.2 ± 2.2 mL/g for R1, R2, R3, and R4, respectively. A lower F/M ratio produced
176 smaller granules with a better sludge compressibility.

177 Different from the loose and irregular activated sludge flocs, all of the aerobic granules
178 produced in the four reactors were round with a clear boundary and smooth surface (Fig. 2).
179 However, the morphology and structural features were different for the granules produced at
180 different F/M ratios. The slowly-forming granules in R1 were smaller and more tightly-packed
181 than the fast-forming granules in R2 and R3. The mature granules in R1 had an average
182 diameter of around 1.5 mm, whilst the granules grew to 2.8 mm in R2 and 4.5 mm in R3 (Fig.

183 3). Interestingly, the granules in R4 grew rapidly to 3 mm at the high F/M of 1.1 g COD/g
184 SS-d and decreased gradually from 3 mm to 1.2 mm after the F/M was reduced to 0.3 g COD/g
185 SS-d. Upon complete granulation, the granules appeared to be rather stable in size, and the
186 mean diameter of the mature granules could be correlated nearly linearly with the F/M ratio (R^2
187 = 0.99) applied to the different reactors (Fig. 3b). The result indicates that the sludge loading
188 rate can be an effective operating parameter to regulate the size of granules in a bioreactor.

189 190 3.2. Organic substrate uptake capability and settling behavior of mature granules

191 All of the four reactors after sludge granulation performed well in organic removal with
192 an effluent COD of lower than 50 mg/L. The specific laboratory test however showed
193 different organic substrate uptake capabilities of the mature granules formed in different
194 reactors. For the same initial glucose concentration and the same biomass concentration, the
195 glucose concentration decreased rapidly from 300 to below 10 mg/L after 30 min for R1 and
196 R4 granules, whilst the glucose concentration decreased from 300 to 20 mg/L after 60 min for
197 R2 granules and after 200 min for R3 granules (Fig. 4a). The small granules from R1 and R4
198 showed a considerably faster glucose uptake rate than did the larger granules from R2 and R3.

199 The comparison between different granules in the four reactors proved that small granules
200 had a clear advantage over larger granules in the uptake of organic substrates and nutrients. The
201 apparent specific glucose uptake rate coefficient of the granular sludge decreased nearly
202 linearly ($R^2 = 0.93$) with the mean size of the granules from 8.4/g SS-h for R4 granules to
203 0.78/g SS-h for R3 granules (Fig. 4b). The specific surface area of granules increased as the
204 granules became smaller in size. Hence, in comparison to larger granules at the same
205 concentration, smaller granules had more surface areas that would allow faster substrate uptake
206 and utilization. Assuming $\rho_c = 1.06 \text{ g/cm}^3$ for the density of the (wet) bacterial cells and a factor
207 $f = 3.45$ for the ratio between the wet mass and the dry mass of the cells in the granules [11],

208 using the mean size to calculate the surface area of mature granules from each reactor and
209 assuming a constant porosity of 0.8 for aerobic granules, the surface area-based specific
210 glucose uptake rate coefficient can be estimated as 0.135, 0.087, 0.046, and 0.128 /cm²-h for
211 the granules from R1, R2, R3, and R4, respectively. Apart from the difference in surface area
212 between granules of the same weight but different sizes, smaller granules apparently had a
213 faster substrate uptake capability per unit surface area. Compared to larger granules, smaller
214 granules would impose a less degree of mass transport limitation for the substrates and
215 dissolved oxygen into the granule interior [11]. Thus, from the point of view of biological
216 wastewater treatment, small granules are more preferred to large granules for more effective
217 organic degradation.

218 Granular sludge settled much faster in water than activated sludge flocs, and the settling
219 velocity generally increased with the size of granules. The settling velocities ranged from 0.42
220 to 0.89 cm/s for individual mature granules from R1 and R4, from 0.64 to 1.84 cm/s for the
221 granules from R2 and from 1.37 to 4.12 cm/s for the granules from R3 (Fig. 5). These values
222 are similar to the settling velocities of aerobic granules reported in previous studies [9,11]. In
223 comparison, activated sludge flocs were found to have a much slower settling velocity ranging
224 from 0.16 to 0.49 cm/s [23]. The slope of the linear regression between the granule size and
225 settling velocity after log-log transformation is 0.64, 0.80, and 0.76 for the granules cultivated
226 in R1, R2, and R3, respectively. It is apparent that the granules became denser and the settling
227 velocity increased more with the size for the large granules from R2 and R3 in comparison to
228 that for the smaller granules from R1.

229

230 *3.3. Sludge EPS during the granulation*

231 The EPS of the sludge in the four reactors had a similar trend of change during the
232 granulation process (Fig. 6). The EPS content decreased initially for the sludge in all reactors

233 and then increased to different levels with the granule formation. The total EPS in R1 and R2
234 sludge after 40 d were about 85 and 90 mg TOC/g SS, respectively. The EPS in R3 and R4 at
235 high F/M ratios reached 120 mg TOC/g SS after 20 d. The granules cultivated in R3 and R4
236 contained more EPS than the granules formed in R1 and R2 at lower F/M ratios. After the
237 completion of granulation in the four reactors, the EPS contents of the granular sludge all
238 decreased and fluctuated at a lower level of around 80 mg TOC/g SS. The sludge EPS
239 consisted of more proteins than polysaccharides and humic-like substances. Large granules
240 formed at a high F/M ratio had a higher protein proportion in EPS than that of the smaller
241 granules formed at a low F/M. This result is consistent with the previous findings for the EPS
242 composition in aerobic granules [24]. Proteins have been reported as the core EPS constituents
243 of the aerobic granules, which are believed to be the important building materials for the
244 internal structure of granules [24].

245 The contents of polysaccharides and humic substance in EPS were similar for the granules
246 in the four reactors, ranging from 20 to 40 mg/g SS. Between polysaccharides and humic
247 matter, the fraction of humic materials was somewhat higher than that of polysaccharides. The
248 humic substances in EPS are expected to play an important role in immobilizing exoenzymes
249 through their reversible complexation with the enzymes [25]. It is generally considered that
250 microbial EPS play an essential role in microbial granulation [26]. EPS are expected to bind
251 cells closely for the formation and the structure stability of granules [27]. However, the results
252 of this experimental study suggest that the roles of EPS in sludge granulation are complex.
253 During the phase of granule formation, the EPS abundance increased with time; while upon
254 granulation the EPS content decreased from the peak levels. Wang et al. [28] also observed
255 that the amount of EPS increased during the early phase of aerobic sludge granulation, but
256 changed little after the granules had matured.

257

258 *3.4. Microbial population dynamics during aerobic granulation at different F/M ratios*

259 Well-resolved DGGE bands were obtained that show the changes of bacterial communities
260 during sludge granulation in the four batch reactors (Fig. 7). The DGGE banding patterns
261 showed the positions of major bands shifting during the course of granulation in the four
262 reactors, which suggests changes in the dominant species with the granule formation and
263 growth. Nonetheless, for the reactors under different F/M conditions, the bacterial populations
264 changed differently, producing different trends of DGGE banding profiles. In the early phase
265 of granule growth, or for the precursor granules in R1 (25 d), R2 (16 d), R3 (7 d), and R4 (30 d),
266 there were not the same dominant species that could be found in all four reactors. The
267 comparison suggests that aerobic granulation did not request the domination of one or more
268 particular microbial species. The mature granules in all of the four reactors after 100 d also had
269 different population structures.

270 The difference in bacterial community among the four reactors probably was related to the
271 operating condition, such as the F/M ratio. According to the Shannon–Weaver index, the
272 population diversity changed in different ways for the biomass in the four reactors with
273 different F/M ratios (Fig. 8). During the start-up of sludge granulation, it is apparent that a
274 higher F/M ratio would facilitate the change of the bacterial community and lead to a lower
275 species diversity in the bioreactors (R3 and R4). Upon the completion of granulation, the F/M
276 ratio became less important to the microbial structure, and the population diversities of the
277 sludge approached a similar level in the four reactors. It also has been recognized in ecology
278 that competition for growth-limiting resources would bring about chaotic fluctuations in
279 species abundances [29].

280 The biomass loading rate, or F/M ratio, exhibited a profound effect on the aerobic
281 granulation process. An F/M ratio as high as 1.1 g COD/g SS-d facilitated rapid formation of
282 large granules in about 15 d. In comparison, a low F/M ratio of 0.3 g COD/g SS-d in R1 would

283 result in a slow granulation process of more than 40 d (Fig. 3a). However, although large
284 granules can be formed more quickly at a higher F/M, the large sizes of granules are not
285 desirable for the wastewater treatment purpose. It has been found that large granules usually
286 are less stable and have more problems in long-term operation, such as breakage, erosion,
287 floating, and fungal contamination [30,31]. In comparison, small and healthy granules are
288 more favorable for use in biological wastewater treatment. Under a low F/M condition, as
289 shown in R1 and R4, small granules could be formed and stabilized with a mean size of 1.5 mm
290 or smaller. Because of the less degree of mass transport limitation and a higher level of stability,
291 small granules are much more preferred to large granules during aerobic granulation in
292 bioreactors.

293 The present study demonstrated that the size of granules can be controlled by the sludge
294 F/M ratio, together with the selective sludge discharge technique, during the granulation
295 process. A low F/M of 0.3 g COD/g SS-d led to the formation and stabilization of small
296 granules after about 40 d. Moreover, adjusting the F/M ratio at different stages of granulation
297 can be a more effective start-up strategy, as shown by the operation of R4. A high F/M ratio
298 could be applied in the early stage, which would bring about fast granule formation and growth.
299 Afterwards, the F/M ratio could be reduced to a lower level to allow the formation and
300 stabilization of smaller granules. The effective operating strategy for the control of the size of
301 aerobic granules is of great importance to the development and actual application of the
302 granulation technology in biological wastewater treatment.

303

304 **Conclusions**

- 305 • Aerobic granules were cultivated with selective discharge of small and slow-settling
306 sludge flocs in four batch column reactors at different F/M ratios from 0.3 to 1.1 g COD/g

307 SS-d. A higher F/M ratio promoted faster formation of larger granules and a lower F/M
308 ratio led to slower formation of smaller granules.

309 • Upon complete granulation, the mature granules became rather stable in size and EPS
310 content. The mean size of the granules in different reactors increased from 1.2 to 4.5 mm
311 nearly linearly with the F/M ratio applied to the reactors, and the specific organic uptake
312 rate of the granular sludge decreased nearly linearly with the mean size of the granules.

313 • Adjusting the F/M ratio in different granulation stages, e.g., >1.1 g COD/g SS-d in the
314 early stage and < 0.3 g COD/g SS-d in the later stage, can be a simple and effective start-up
315 strategy to facilitate rapid granule formation and sustain small and healthy granules in
316 bioreactors.

317

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401 **Figure captions**

402

403 **Fig. 1.** (a) Sludge MLSS and (b) the F/M ratio in the four batch column reactors during the
404 experiment.

405 **Fig. 2.** Photographs of the mature granules produced in the four reactors after 120 d: (a) R1, (b)
406 R2, (c) R3, and (d) R4.

407 **Fig. 3.** (a) Change of the mean size of the sludge in the four reactors during the granulation
408 process; and (b) the correlation between the mean size of mature granules from
409 different reactors and the F/M ratio applied for granulation.

410 **Fig. 4.** (a) Reduction in glucose concentration during the glucose uptake tests on the granular
411 sludge from the four reactors; and (b) the correlation between the apparent specific
412 glucose uptake rate coefficient and the mean size of granules from different reactors.

413 **Fig. 5.** Settling velocities of individual granules formed different reactors.

414 **Fig. 6.** Analysis of the EPS contents, including TOC, proteins, polysaccharides, and
415 humic-like substances, for the sludge from different reactors during the granulation
416 process.

417 **Fig. 7.** DGGE profiles of the bacterial communities in the four reactors during the sludge
418 granulation process. AS: seed activated sludge, m-n: sludge from reactor m (R1, R2, R3
419 or R4) after n days of the start-up operation, e.g., 2-16: sludge from R2 after 16 d (top:
420 image; bottom: schematic).

421 **Fig. 8.** Shannon-Weaver index (H) for the bacterial species diversity calculated from the
422 DGGE band profiles for the four bioreactors.

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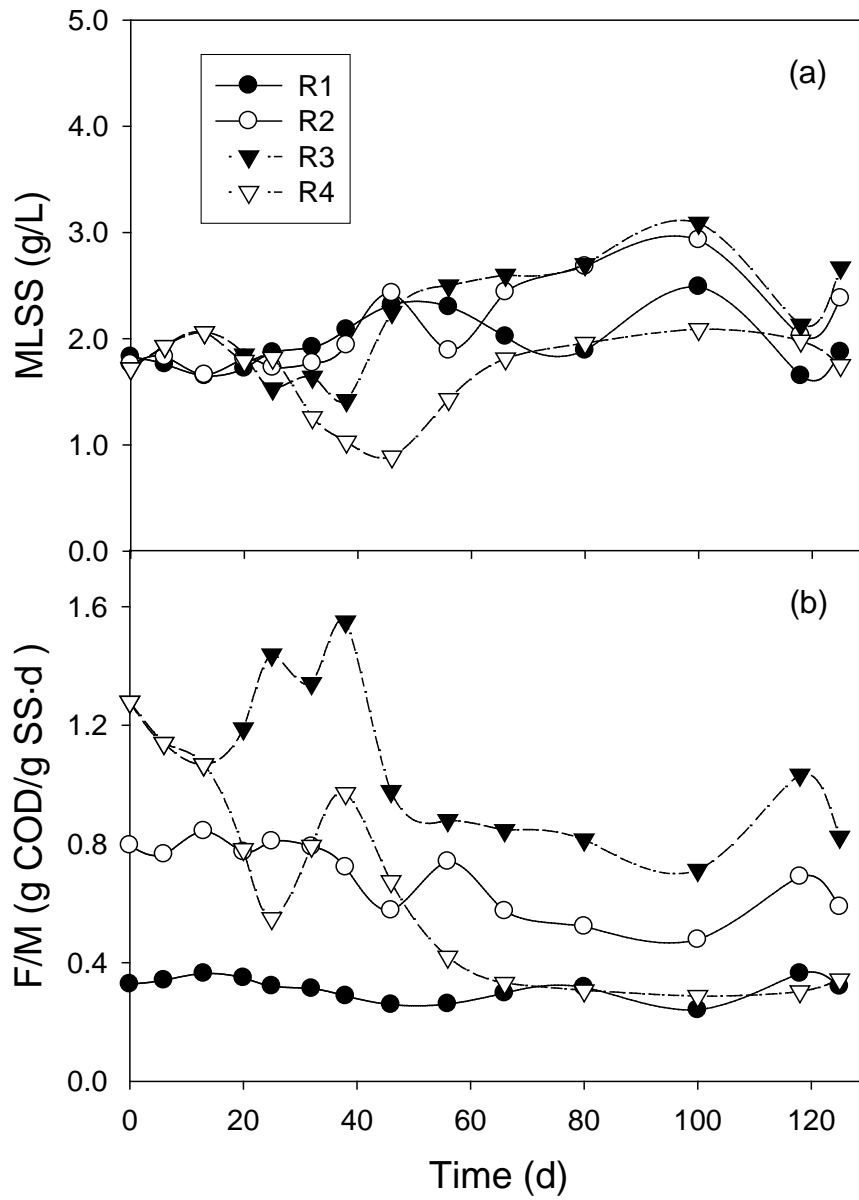


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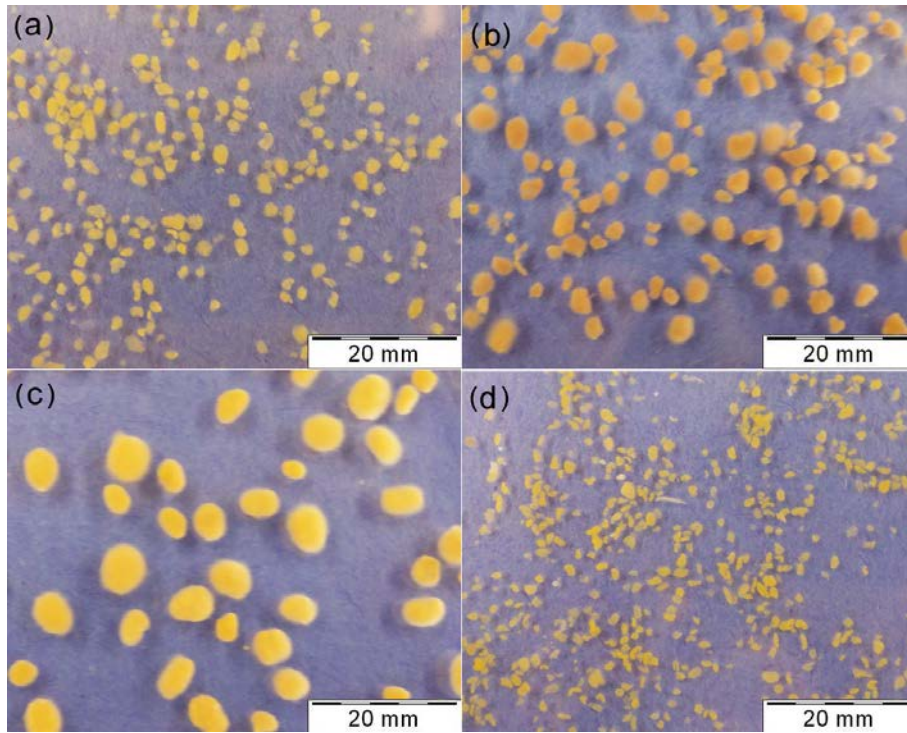


Fig. 2. Photographs of the mature granules produced in the four reactors after 120 d: (a) R1, (b) R2, (c) R3, and (d) R4.

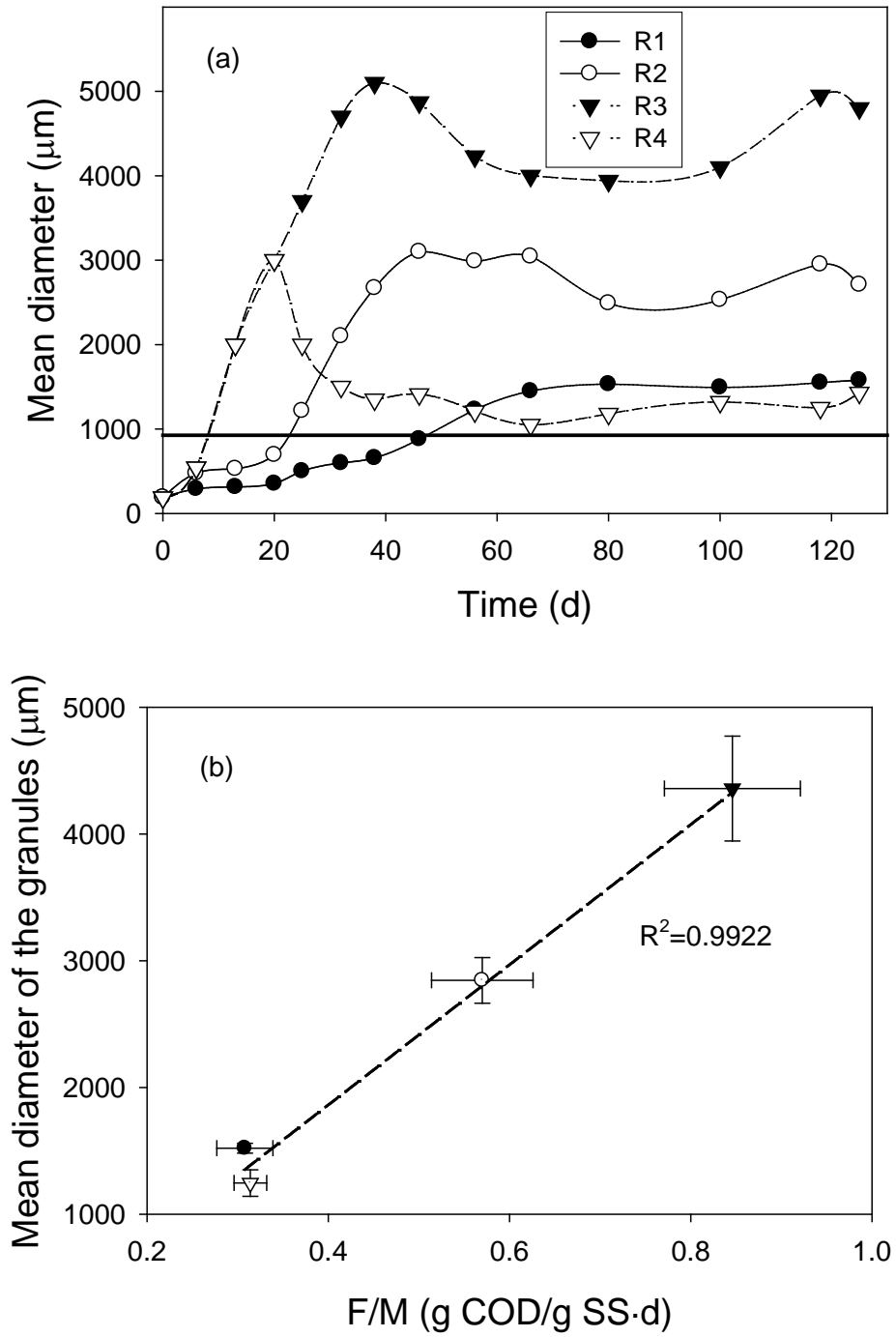


Fig. 3. (a) Change of the mean size of the sludge in the four reactors during the granulation process; and (b) the correlation between the mean size of mature granules from different reactors and the F/M ratio applied for granulation.

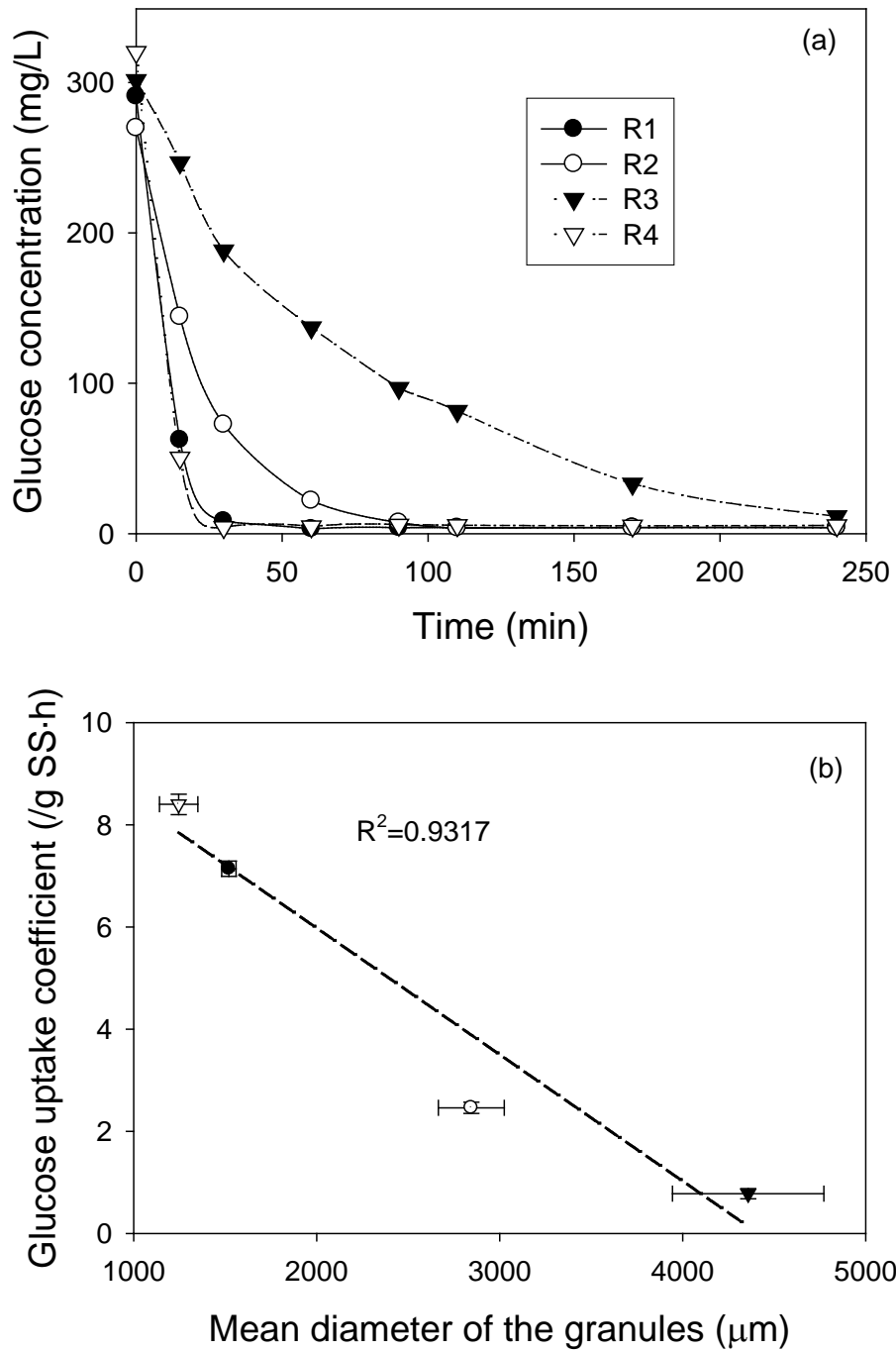


Fig. 4. (a) Reduction in glucose concentration during the glucose uptake tests on the granular sludge from the four reactors; and (b) the correlation between the apparent specific glucose uptake rate coefficient and the mean size of granules from different reactors.

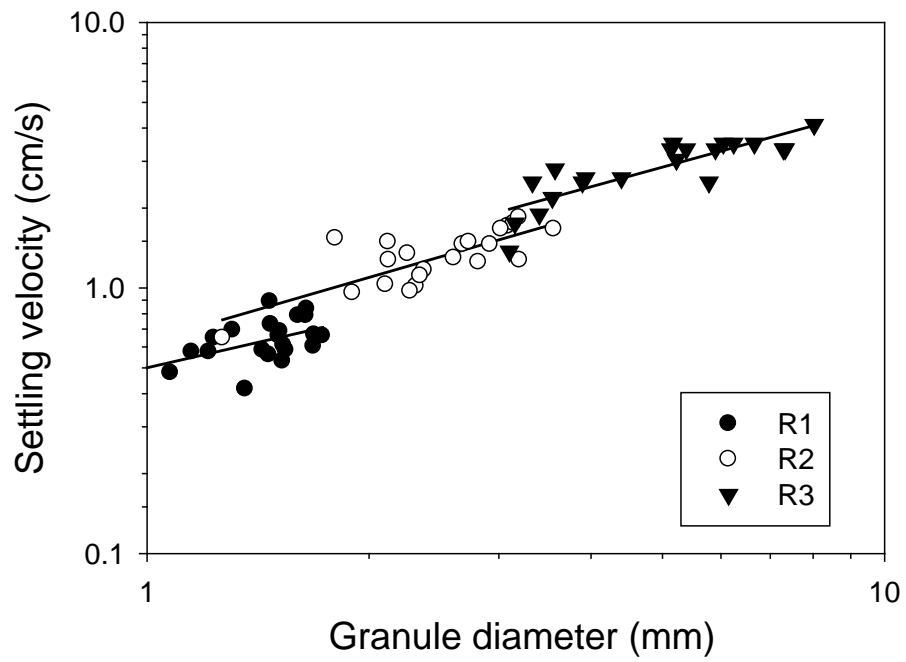


Fig. 5. Settling velocities of individual granules formed in different reactors.

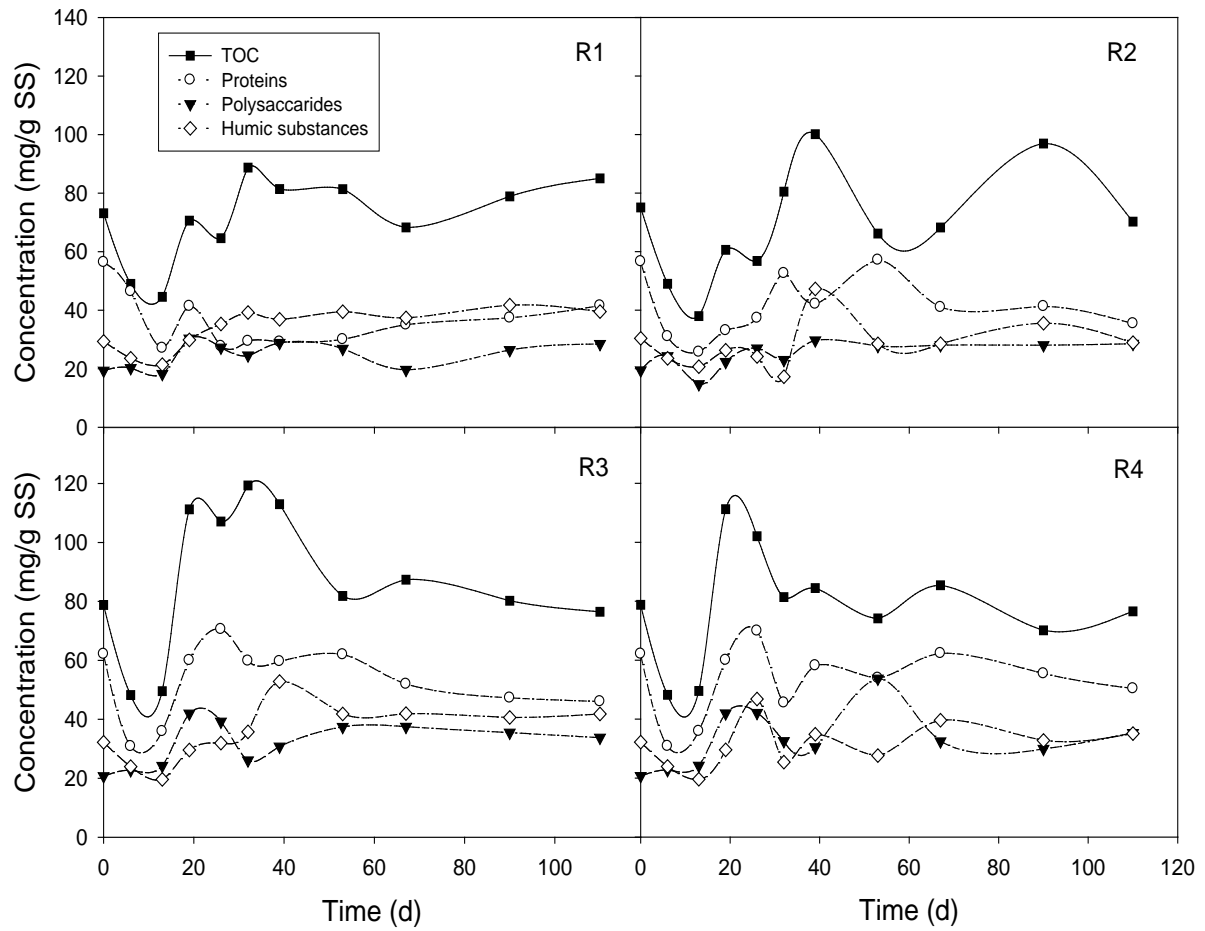


Fig. 6. Analysis of the EPS contents, including TOC, proteins, polysaccharides, and humic-like substances, for the sludge from different reactors during the granulation process.

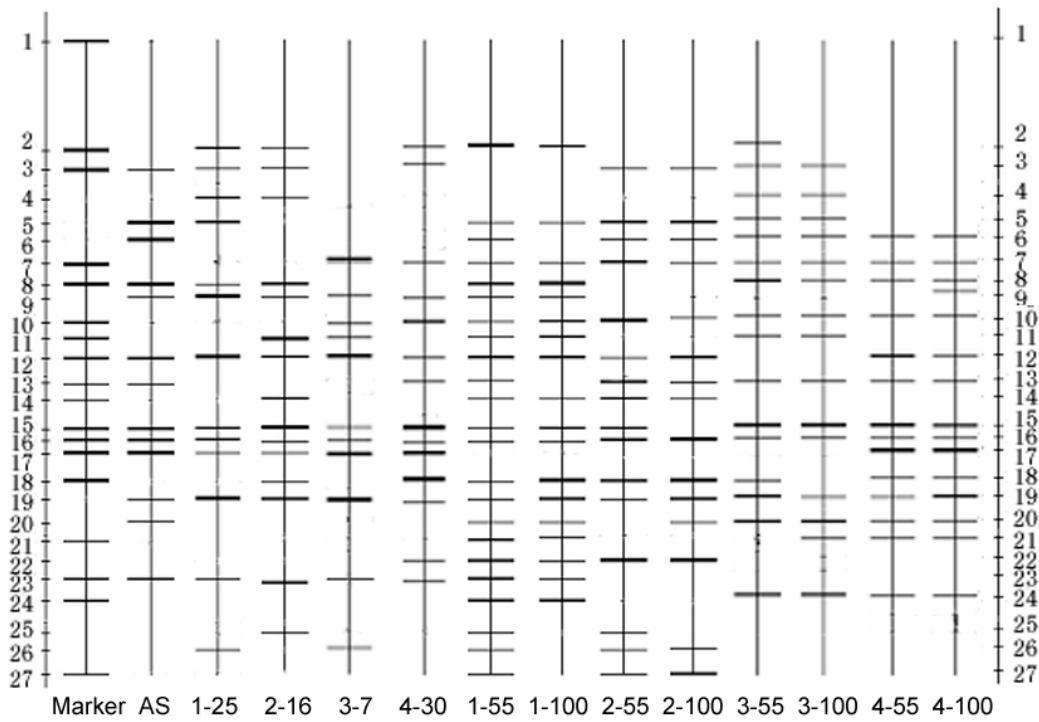
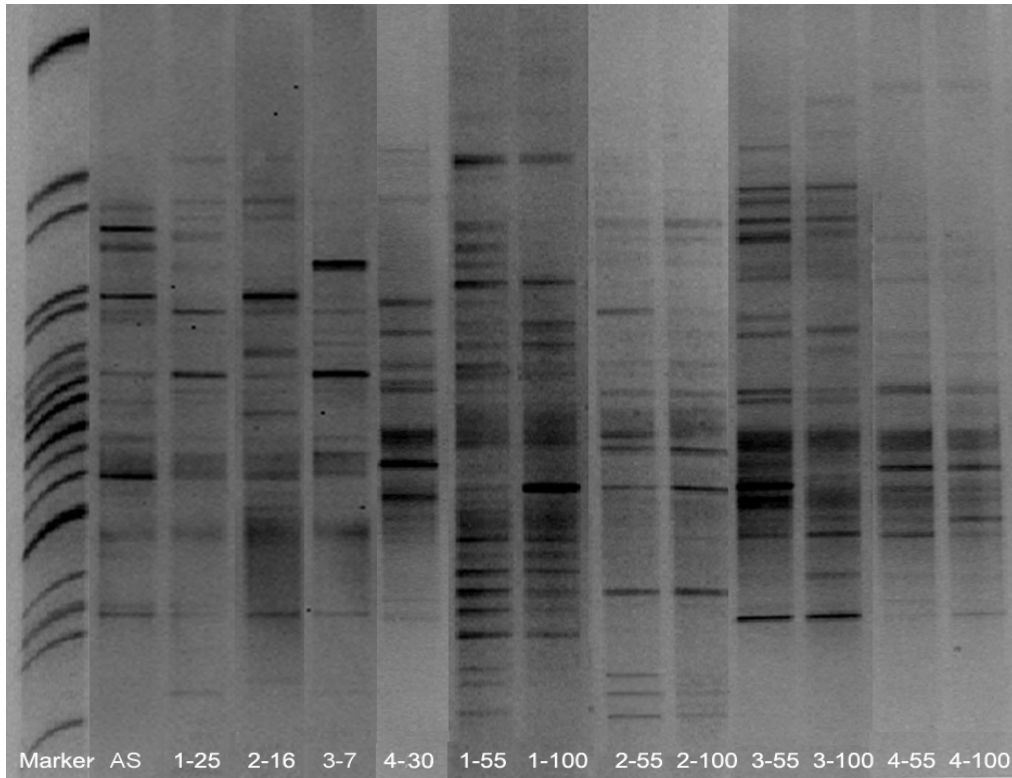


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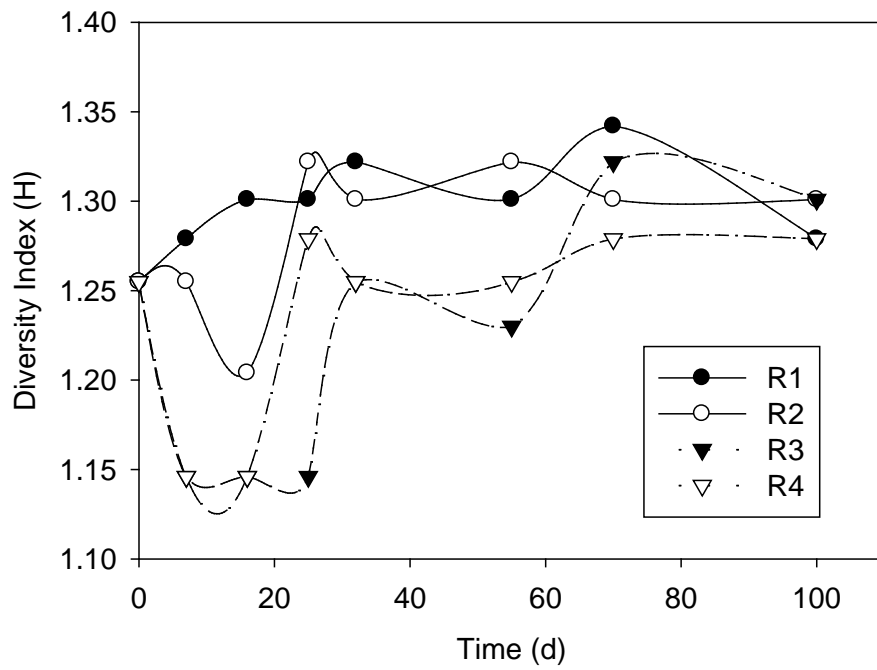


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