Granular Activated Carbon for Aerobic Sludge Granulation in A Bioreactor with A Low-strength Wastewater Influent

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1   **Abstract**

2   Aerobic sludge granulation is rather difficult or impossible for the treatment of low-strength

3   wastewater. In this study, a novel technique involving granular activated carbon (GAC) was

4   developed for rapid aerobic granulation under a low organic loading condition. Laboratory

5   experiments were conducted with two sequencing batch reactors (SBRs) running side by

6   side. One reactor had fine GAC added to the sludge mixture, and the other had no GAC

7   added. A low-strength organic wastewater with a chemical oxygen demand (COD)

8   concentration of only 200 mg/L was used as the influent to the SBRs. The morphology,

physical properties, and bacterial community structure of the sludge in the two reactors were characterized and compared throughout the experiments. The results showed that granules could not be formed in the SBR without added GAC. However, complete granulation was achieved in the SBR with GAC addition. Selective discharge of slow settling sludge was also essential to the granulation process. Adding GAC to the seed sludge mixture, together with the selective discharge of small and loose sludge flocs, facilitated the retention and growth of bacterial cells on GAC in attached-growth mode, leading to complete granulation. In addition, the use of GAC produced aerobic granules with strong cores to help maintain the long-term stability of mature granules. With granulation, the solid-liquid separation property of the sludge was greatly improved. Once granules were formed, the granules were quite stable and GAC addition was no longer needed. Therefore, adding GAC is a simple and effective strategy to initiate granule formation for complete sludge granulation in bioreactors treating low-strength organic wastewater.

1. Introduction

Aerobic granulation is an appealing new technology that transforms loose sludge flocs into dense granules for biological wastewater treatment. Due to attributes such as a compact structure and fast settling velocity [1-5], granular sludge allows a high level of biomass concentration, a very short phase of sludge-water separation, and a much higher organic loading rate in bioreactors [3,6-8]. Given its potential in the development of novel, compact, and high-rate biological treatment systems, aerobic granulation may lead to fundamental advances in wastewater treatment [5,9,10].

34   Aerobic granulation relies on rapid biomass growth that requires a sufficient supply of

35   substrates into the bioreactors. Granule formation has been reported with a high organic, or

36   COD (chemical oxygen demand), loading rate ranging from 1 to 15 kg/m^3·d [3,11,12].

37   However, granulation may not be achieved with a relatively low organic loading of 1 kg

38   COD/m^3·d or lower [10,13]. Apart from a low organic loading rate, a low influent organic

39   concentration would greatly increase the difficulty of granule formation and growth [14].

40   Moreover, a low influent concentration often results in more filamentous growth, leading to

41   deterioration and breakage of the granules [15]. There have been few reports of successful

42   granulation for a low-strength wastewater influent with an organic concentration of less

43   than 250 mg COD/L. However, considering the low organic concentration level in most

44   municipal sewage, a simple and effective granulation startup strategy needs to be developed

45   for low-strength wastewater influents.

46   Aerobic granules can be regarded as a special type of biofilm growth in a stable,

47   contiguous, and multicellular association [16,17]. Granular activated carbon (GAC) has

48   been used as the support medium for microbial immobilization and attached biofilm growth

49   in biological wastewater treatment [18,19]. GAC has a large specific surface area and a fast

50   settling velocity. Its coarse and irregular surface and characteristic adsorption property also

51   provide a favorable microenvironment for bacterial growth. GAC has been successfully

52   applied as the support media in biological aerated filters [20,21] and fluidized-beds [22,23]

53   for water and wastewater treatment. Thus, GAC could be used as the carrier medium for

54   aerobic granulation under unfavorable conditions, such as a low substrate concentration and

55   a low organic loading rate. However, the technique of using GAC for rapid granule

56   formation and long-term granule stability in biological wastewater treatment has yet to be

57   developed.

58   In this study, laboratory experiments were conducted with two sequencing batch

59   reactors (SBRs) running side by side. GAC was added to the sludge mixture in only one of

60   the reactors. A low organic influent with a COD concentration of only 200 mg/L was tested

61   in the SBRs. With the low-strength influent, granules could not be formed in the SBR

62   without adding GAC. In contrast, complete granulation was achieved in the reactor with

63   GAC added. The morphology, structure, physical properties, and bacterial community of

64   the sludge in the two reactors were characterized and compared throughout the experiments.

65   The aims of the experimental study were to develop an effective technique using GAC for

66   rapid aerobic granulation in bioreactors with a low-strength influent and to investigate the

67   underlying mechanisms of granule formation on GAC.

68

69   **2. Materials and Methods**

70   *2.1. Experimental set-up and SBR operation*

71   Two identical columns (6 cm in diameter and 80 cm in height) with a working volume

72   of 2.4L each were used as SBRs for the experimental study (Fig. S1, Supplementary Data).

73   The two reactors, R1 and R2, were operated in a fixed sequential mode for a 3 hr cycle with

74   4 min of feeding, 142 min of aeration, 30-min of sludge settling and 4 min of effluent

75   withdrawal from the middle ports of the columns. The reactors were fed with a glucose-

76   based synthetic wastewater prepared according to the chemical composition given by Tay et

77   al. [24]. A low organic concentration with a COD of 200 mg/L was used for the SBR

78   influent. Activated sludge from a full-scale sewage treatment plant (Stanley Sewage

79   Treatment Works, Hong Kong) was used as the seed sludge. The sludge was acclimated in

80   the two SBRs for one month with the glucose-based synthetic wastewater, and the initial

81   sludge MLVSS (mixed liquor volatile suspended solids) concentration was 3000 mg/L.

82   Fine GAC particles were used to enhance aerobic sludge granulation for the low-

83   strength influent in one of the reactors. The GAC had a mean size of 224 µm with a specific

84   surface area of 1002 m^2/g and an apparent density of 1.183 g/cm^3 (Merck, NJ, USA). No

85   GAC was added to R1, while 7.2 g of GAC was added to R2 to result in a GAC

86   concentration of 3 g/L or a volume fraction of less than 0.3%. The experiments were

87   performed at room temperature, and the water temperature was 20-22˚C. $NaHCO_3$ was

88   dosed into the feed wastewater to maintain the reactor pH in the neutral range between 7.0

89   and 7.5. Air was supplied at a flow rate of 2.0 L/min into the reactors during the aeration

90   phase to keep the dissolved oxygen (DO) concentration in the sludge suspension in the

91   range of 2-5 mg/L.

92   Sludge was discharged once a day from the two SBRs at a predetermined rate to

93   maintain a stable biomass concentration. Sludge loss in the effluent during effluent

94   withdrawal was minimized by allowing a settling time of 30 min in each SBR cycle. The

95   SBR experiment was conducted for a total of 120 days in two operating phases with

96   different sludge discharge methods. In the first 30 days, Phase 1, the mixed sludge

97   discharge method was used, and in the next 90 days, Phase 2, selective discharge of slow-

98   settling sludge was applied. For the mixed sludge discharge in Phase 1, the sludge mixture

99   was discharged from the middle ports of the SBR columns while the aeration was still being

100   conducted. The GAC in the sludge mixture discharged from R2 was recovered and returned

101   to R2. For the selective sludge discharge in Phase 2, the sludge was discharged from the

102   middle ports during the settling phase without aeration after a few minutes of sludge settling.

103   The settling period varied from 1 to 5 min depending on the sludge settling property and the

104   targeted amount of sludge to be discharged. In comparison to the mixed sludge discharge

105   method, the selective discharge had a higher fraction of small and slow-settling sludge flocs

106   in the discharged sludge than in the bulk sludge mixture. The amount of sludge loss in the

107   effluent was measured every day. The amount of daily sludge discharge was adjusted

108   accordingly to maintain a biomass MLVSS concentration of 3000 mg/L in each reactor.

109

110   *2.2. Determination of the organic uptake capability of the sludge in batch test*

111   The biomass sludge was collected from R1 and R2 periodically to test the organic

112   substrate uptake capability of the sludge. The organic uptake tests were performed in 250-

113   mL glass beakers as batch reactors, with sufficient aeration provided. In each reactor, sludge

114   was added to an MLVSS concentration of 3000 mg/L before adding wastewater. Two

115   different initial glucose concentrations - 200 and 500 mg/L - were used for the substrate

116   uptake tests. After adding the wastewater, the sludge mixtures were sampled at various time

117   intervals. The samples were filtered, and the glucose and COD concentrations in the filtrates

118   were measured. A first-order kinetics may be assumed for the early phase of glucose uptake

119   in the batch reactor, i.e. $\dfrac{dS}{dt} = -kXS$, where S is the glucose concentration, t is time, k is a

120   rate constant and X is the sludge concentration. From a linear regression of $\ln(S_0/S)$ versus

121   Xt, where S_0 is the initial glucose concentration, the substrate uptake rate constant of the

122   sludge can be determined.

123

124   *2.3. Analysis of microbial population and identification of dominant species in reactors*

125   The microbial population of the sludge samples was analyzed for the two reactors on

126   experimental days 10, 40 and 70 of the second phase. The genomic DNA of the sludge was

127   extracted using a beadbeater (Mini-beadbeater[TM], Biospec, Bartlesville, OK, USA) and

128   micro-centrifuge (MiniSpin plus®, Eppendorf, Hamburg, Germany) [25]. The bacterial 16S

129   rDNA gene sequence (V3 region, corresponding to positions 341-534 of *E. coli* sequence)

130   was amplified by polymerase chain reaction (PCR) (PTC-200, MJ Research, Waltham, MA,

131   USA) following the procedure detailed previously [10]. The PCR amplified DNA products

132 were then separated by denaturing gradient gel electrophoresis (DGGE) through 8%

133 polyacrylamide gels with a linear gradient of 30-50% denaturant, using the DCode^{TM}

134 Universal Mutation Detection System (Bio-Rad, Hercules, CA, USA). The gels were run for

135 6 h at 130V in $1\times$ TAE buffer at 60°C, and then stained with ethidium bromide for 10 min

136 and visualized by a UV illuminator. The DGGE images were acquired using the ChemiDoc

137 (Bio-Rad) gel documentation system.

138   A 16S rRNA gene sequence clone library was constructed to identify the phylogeny of

139 the DGGE bands of the sludge samples [26]. Representative clones of the operational

140 taxonomic units (OTUs) underwent the same DGGE analysis under the conditions used for

141 the biomass PCR products. The migration positions of the library clones were compared

142 with the DGGE profiles of the sludge samples. Based on the comparison, an OTU in the

143 clone library was assigned to a particular DGGE band for species identification.

144

145 *2.4. Analytical methods*

146   The COD concentration, sludge MLSS (mixed liquor suspended solids) and MLVSS

147 concentrations, effluent suspended solids (ESS) concentration, and the sludge volume

148 indexes after 5 min (SVI_5) and 30 min (SVI_{30}) of sedimentation were measured according to

149 the Standard Methods [27]. The interfacial settling velocity of the sludge layer, which is

150 defined as the falling velocity of the water-sludge interface during sludge sedimentation,

151 was measured regularly during the early phase of sludge settling in the two SBR columns.

152 The glucose content was determined using the phenol-sulphuric acid method [28]. The

153 morphology of the sludge flocs and granules was examined under a stereomicroscope (S8

154 APO, Leica, Cambridge, UK) equipped with a digital camera (EC3, Leica, Cambridge, UK).

155 A laser diffraction particle counter (LS13 320, Beckman Coulter, Miami, FL, USA) was

156 used to measure the size distribution of the sludge flocs and granules. Accordingly, the

157 volume-based mean size of the sludge in a sample was calculated from its size distribution.

158

159 **3. Results and Discussion**

160 *3.1. Formation of aerobic granules in the SBR*

161 During the first 30 days of SBR startup (Phase 1), the mixed sludge discharge method

162 was used in the two SBRs. Sludge remained in the form of flocs in both R1 without GAC

163 and R2 with added GAC (Fig. 1). A few sludge flocs were found to attach to the GAC, and

164 the amount of biomass that attached to or grew on the GAC was about 9% of the total

165 biomass in R2 by the end of Phase 1. Both reactors were then changed to the elective sludge

166 discharge mode in Phase 2 to facilitate aerobic sludge granulation. As expected, complete

167 granulation was difficult with the low-strength (200 mg COD/L) influent. There was little

168 sign of granule formation in R1 throughout Phase 2. In contrast, however, GAC-based

169 granules began to form rapidly in R2, despite the same low-strength influent. Biofilm

170 appeared to grow on the GAC surface after only 5 days of Phase 2 (Fig. 2), while the

171 amount of biomass growing on GAC increased to 16% of the total biomass in R2. After 10

172 days of Phase 2, the GAC was fully covered by biofilm, and small granules became visible.

173 Sludge granulation was almost fully achieved in R2 after 20 days (Fig. 1). The granules in

174 R2 were round with a clear boundary, and were completely different from the loose and

175 irregular sludge flocs in R1. The amount of biomass in the GAC-based granules accounted

176 for more than 80% of the total sludge in R2.

177 Measurement of the particle size showed that the mean size of the sludge in R2 with

178 added GAC was larger than that in R1 (Fig. 3). During Phase 1, with mixed sludge

179 discharge, the mean sludge sizes in both R1 and R2 were quite stable at no more than 130

180 µm after 30 days. Selective sludge discharge in Phase 2 led to an increase in sludge size in

181　both reactors, particularly in R2. The mean size of the R2 sludge increased from 134 to 153

182　µm after only 10 days in Phase 2. The size increased continuously with the formation and

183　growth of granules, and the mature granules had a mean size of around 600 µm. The sludge

184　in R1 also increased in size to about 250 µm after 20 days in Phase 2, and a few small

185　granules were found in the sludge mixture (Fig. 1). However, complete aerobic granulation

186　could not be achieved in R1 with no GAC added. The small granules apparently broke up

187　and the mean sludge size eventually decreased to about 200 µm (Fig. 3).

188　　The two reactors were operated under the same condition except for the GAC addition

189　(Fig. 4). The two SBRs had the same organic loading of 0.8 kg $COD/m^3 \cdot d$, the same HRT

190　of 6 h and a similar SRT of around 15 d. Sludge was discharged from the two SBRs once a

191　day at an overall biomass sludge removal ratio of about 6%, and the MLVSS was kept at

192　around 3000 mg/L in both reactors (Fig. 4a). The F/M (food-to-microorganism) ratio was

193　maintained between 0.25 to 0.30 g COD/g SS·d (Fig. 4b). Both reactors performed well on

194　organic removal with an effluent COD of below 30 mg/L. The amount of SS in the effluent

195　was 60 mg/L or lower for R1, without GAC addition and granulation. In comparison, R2

196　had a lower effluent SS level of less than 40 mg/L after GAC-enhanced granule formation,

197　which showed the benefit of sludge granulation (Fig. 4c).

198

199　*3.2. Comparison of the sludge between the two SBRs*

200　　GAC addition during the SBR startup significantly improved the sludge settleability and

201　compression (Fig. 5). The sludge in R2 with the initial GAC addition always had a lower

202　SVI value than the sludge in R1 (Fig. 5a). With the mixed sludge discharge in Phase 1, the

203　SVI_{30} was about 110 ml/g for the R1 sludge and 90 ml/g for the R2 sludge after 30 days.

204　The SVI_5 values were more than twice the respective SVI_{30} values, implying typical

205　activated sludge flocs without granulation in both SBRs [4]. Selective sludge discharge in

206 Phase 2 led to a considerable improvement in sludge compressibility and settleability. The

207 SVI_{30} decreased to 40 ml/g for the sludge in R1 and to 30 ml/g for R2 after 25 days of Phase

208 2 operation (Fig. 5a). Meanwhile, the SVI_5 decreased from 250 to 100 ml/g in R1 and from

209 200 to 40 ml/g in R2. However, the SVI_5 remained about twice as large as SVI_{30} for the R1

210 sludge throughout the rest of the SBR test. This agreed with the microscopic observation

211 that the sludge in R1 remained in the form of suspended flocs. Although selective discharge

212 of small and loose flocs improved the sludge settleability, it was not enough to lead to

213 complete granulation for the low-strength influent. In contrast, the sludge SVI_5 was similar

214 to SVI_{30} for the R2 sludge after 25 days of Phase 2. This indicated complete granulation

215 according to the typical defining feature of aerobic granules [4]. The comparative results

216 demonstrate that the initial GAC addition was crucial to the granule formation in R2. In

217 other words, aerobic granulation would not be achieved for a low-strength influent of 200

218 mg COD/L or less without the addition of GAC during the SBR startup.

219   Granular sludge showed its great advantage in sludge water separation. For the

220 suspended sludge in R1, the sludge-water interfacial settling velocity was rather stable at a

221 rate of no more than 1.5 m/h (Fig. 5b). In contrast, the bulk sludge settling velocity

222 continuously increased in R2 with the formation and growth of granules. The mature

223 granules had an interfacial settling velocity of about 6 m/h, which was at least 4 times as

224 fast as that of the sludge flocs in R1. In comparison to conventional activated sludge, sludge

225 after granulation could be separated much more rapidly from the wastewater after treatment.

226 In other words, granular sludge would request a very short phase of sludge-water separation,

227 which is particularly beneficial to low-strength wastewater treatment. In addition, aerobic

228 granulation would allow a much higher level of biomass concentration at 5-8 g/L and in

229 granular SBRs, which has been well demonstrated by previous studies [3-5,10].

230   Although the granular sludge in R2 performed better in sludge-water separation, the

231   sludge flocs in R1 were found to have a greater substrate uptake capability than the granules

232   in R2. For the same biomass SS content of 3 g/L, the feeding glucose concentration dropped

233   more rapidly with the R1 sludge than with the R2 sludge (Fig. 6). For the initial glucose

234   concentrations of 200 mg/L, the activated sludge flocs in R1 had a glucose uptake rate

235   constant k at 6.7 L/g SS·h, which is considerably higher than that of the mature granules in

236   R2, at 4.9 L/g SS·h. The different glucose uptake rates suggest that loose sludge flocs have

237   a clear advantage over dense granules for the uptake of substrates and nutrients. Small and

238   loose flocs can obtain substrates from the suspension more easily than tightly-packed

239   granules [29]. With mixed sludge discharge, there is less substrate available for uptake by

240   dense flocs and granules due to competition from loose sludge flocs [26]. Thus, as

241   demonstrated in Phase 1, it is apparently impossible for granules to grow and become

242   dominant in a reactor without selective discharge. Discharge of suspended small and loose

243   sludge flocs removes these competitors from the system and makes the substrates more

244   available for the biomass in attached-growth form, which leads to granulation [30].

245   Well-resolved DGGE bands were obtained from the biomass from R1 and R2 (Fig. 7).

246   Changes in the DGGE banding profile are presumed to indicate the evolution of bacterial

247   species in a reactor [10,31]. To determine the identity of the bands in the DGGE profiles,

248   OTUs from 98 clones in the library were compared with the DGGE patterns. Of the 25

249   bands that appeared in the DGGE profiles, 16 dominant bands were identified (Table 1),

250   which accounted for 70% of the microbial abundance represented by the DGGE banding

251   profiles. The majority of the bacterial 16S rDNA sequences grouped with members of

252   *Proteobacteria*, with two in the α subdivision and eight in the β subdivision. The next three

253   groups clustered with *Sphingobacteria*, one clustered with *Flavobacteria* and two clustered

254   with *Actinobacteria*.

The DGGE banding patterns show some difference between the microbial community structure in R1 and R2 in the early stage of Phase 2. After running 10 days of phase 2, the R2 sludge with GAC addition appeared to have fewer band numbers and a lower species diversity than R1 without GAC (Fig. 7). Some species ((B4, B5, B12, B22) became more dominant in R2 compared to R1 after 10 days of Phase 2. GAC addition had an apparent effect on species selection and accumulation in the initial phase of sludge granulation. These four dominant microbial species indicted by the DGGE analysis in R2 were the organisms related to the genera *Variovorax*, *Rhodobacter*, *Pedobacter* and *Thauera* (Table 1). A previous study also found that *Pedobacter* (B12) clustered with *Sphingobacteria* increased rapidly in the early phase of aerobic granulation [26]. The class *Sphingobacteria* is composed of environmental bacteria capable of producing sphingolipids [32]. Certain complex glycosphingolipids have been found to be involved in specific microbial functions, such as cell recognition and signaling for attached-growth and biofilm formation [33]. Thus, the use of GAC helped to facilitate the retention and growth of some species in attached-growth mode to enhance biofilm growth and granulation.

There were minor changes in the DGGE banding pattern for R2 after 40 days of Phase 2, which indicates the stability of the microbial population of the mature granules formed on GAC in R2. Despite the apparent difference in physical characteristics between the R1 and R2 sludge, comparison of the DGGE showed little difference between the microbial diversity of R1 activated sludge and R2 granules after 40 days of Phase 2. The comparison implies that aerobic granulation may not require the dominance of particular bacterial species. Rather, granules can be formed from the bacteria ordinarily present in biological wastewater treatment systems, such as activated sludge. Nonetheless, without the addition of GAC in R1, sludge still remained in the form of suspended-growth (flocs) rather than attached-growth (granules) for the low-strength influent.

3.3. Importance of GAC to aerobic granulation for low-strength wastewater influent

It is generally believed that SBRs are the most suitable type of bioreactors for aerobic granule formation [5]. The initial washout of slow-settling sludge is important in starting up the SBR for aerobic granulation [26]. However, granule formation is still difficult or impossible for low-strength wastewater influent even with the selective discharge of loose and small flocs, as demonstrated by R1. In contrast, the initial addition of GAC to the sludge mixture, together with the selective sludge discharge, facilitated the attached biomass growth that led to complete sludge granulation in R2. Thus, the addition of GAC is shown as a necessary and effective technique to initiate granule formation for complete granulation in SBRs with a low-strength influent. Previous studies have found that GAC is an effective carrier for the growth of biofilm in wastewater treatment bioreactors [20-23]. In the present study, only the initial GAC addition was needed for aerobic granulation. Once granules had formed, they were rather stable and GAC addition was no longer needed. Under the low influent condition, GAC provided the core for granule formation and growth.

Moreover, the use of GAC would greatly improve the stability of granules under unfavorable conditions. Due to the large size and dense structure of aerobic granules, mass transport limitation is often a problem for granular sludge [34,35]. The centers of individual granules have a limited or no supply of organic substrates, DO, and nutrients. Hence, large granules often suffer from cell death and decay, resulting in hollow centers and even breakage of the granules [13]. The mass transfer limitation and instability of aerobic granules can only be worse for sludge treating low-strength wastewater. GAC, however, can provide the support medium and strong cores for aerobic granules. The GAC cores do not require substrates or DO, which helps to stabilize the biofilm growing on GAC. Thus, the use of GAC offers an effective solution for aerobic granulation in SBRs for treating low-

305 strength wastewater. GAC facilitates biofilm growth and granule formation and helps to

306 sustain the stability of mature granules for long-term wastewater treatment operation.

307

308 **4. Conclusions**

309 • Adding GAC is shown to be a necessary and effective technique to initiate granule

310 formation for complete sludge granulation in SBRs with an influent COD of only 200

311 mg/L. In contrast, without GAC, aerobic granulation cannot be achieved in an SBR for

312 treating low-strength influent.

313 • Selective discharge of slow-settling sludge is also essential for granulation. Adding

314 GAC to the seed sludge mixture, together with the selective discharge of small and

315 loose sludge flocs, facilitates the retention and growth of bacterial cells on GAC in

316 attached-growth mode, leading to complete granulation.

317 • The use of GAC produces aerobic granules with a fast settling velocity and a much

318 improved sludge-water separation property. The granules have strong cores that will

319 help to maintain the long-term stability of mature granules for treatment of low-strength

320 wastewater.

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328 **References**

329   [1] E. Morgenroth, T. Sherden, M.C.M. van Loosdrecht, J.J. Heijnen, P.A. Wilderer,

330   Aerobic granular sludge in a sequencing batch reactor, Water Res. 31 (1997) 3191-

331   3194.

332   [2] B. Arrojo, A. Mosquera-Corral, J.M. Garrido, R. Mendez, Aerobic granulation with

333   industrial wastewater in sequencing batch reactors, Water Res. 38 (2004) 3389-3399.

334   [3] Y. Liu, J.H. Tay, State of the art of biogranulation technology for wastewater treatment,

335   Biotechnol. Adv. 22 (2004) 533-563.

336   [4] M.K. de Kreuk, N. Kishida, M.C.M. van Loosdrecht, Aerobic granular sludge - state of

337   the art, Water Sci. Technol. 55(8-9) (2007) 75-81.

338   [5] S.S. Adav, D.J. Lee, K.Y. Show, J.H. Tay, Aerobic granular sludge: recent advances,

339   Biotechnol. Adv. 26 (2008) 411-423.

340   [6] J.J. Beun, A. Hendriks, M.C.M. van Loosdrecht, E. Morgenroth, P.A. Wilderer, J.J.

341   Heijnen, Aerobic granulation in a sequencing batch reactor, Water Res. 33 (1999)

342   2283-2290.

343   [7] K.Z. Su, H.Q. Yu, Formation and characterization of aerobic granules in a sequencing

344   batch reactor treating soybean-processing wastewater, Environ. Sci. Technol. 39

345   (2005) 2818-2827.

346   [8] M.K. de Kreuk, M.C.M. van Loosdrecht, Formation of aerobic granules with domestic

347   sewage, J. Environ. Eng.-ASCE, 132 (2006) 694-697.

348   [9] B.Y.P. Moy, J.H. Tay, S.K. Toh, Y. Liu, S.T.L. Tay, High organic loading influences

349   the physical characteristics of aerobic sludge granules, Lett. Appl. Microbiol. 34

350   (2002) 407-412.

351   [10] A.J. Li, S.F. Yang, X.Y. Li, J.D. Gu, Microbial population dynamics during aerobic

352   sludge granulation at different organic loading rates, Water Res. 42 (2008) 3552-3560.

353   [11] M.K. de Kreuk, M. Pronk, M.C.M. van Loosdrecht, Formation of aerobic granules and

354   conversion processes in an aerobic granular sludge reactor at moderate and low

355   temperatures, Water Res. 39 (2005) 4476-4484.

356   [12] M. Torregrossa, G. Di Bella, G. Viviani, A. Gnoffo, Performances of a granular

357   sequencing batch reactor (GSBR), Water Sci. Technol. 55 (8-9) (2007) 125-133.

358   [13] J.H. Tay, S. Pan, Y.X. He, S.T.L. Tay, Effect of organic loading rate on aerobic

359   granulation. II: Characteristics of aerobic granules, J. Environ. Eng.-ASCE 130 (2004)

360   1102-1109.

361   [14] S.G. Wang, L.H. Gai, L.J. Zhao, M.H. Fan, W.X. Gong, B.Y. Gao, Y. Ma, Aerobic

362   granules for low-strength wastewater treatment: formation, structure, and microbial

363   community, J. Chem. Technol. Biot. 84 (2009) 1015-1020.

364   [15] Y. Liu, Q.S. Liu, Causes and control of filamentous growth in aerobic granular sludge

365   sequencing batch reactors, Biotechnol. Adv. 24 (2006) 115-127.

366   [16] S.T.L. Tay, W.Q. Zhuang, J.H. Tay, Start-up, microbial community analysis and

367   formation of aerobic granules in a tert-butyl alcohol degrading sequencing batch

368   reactor, Environ. Sci. Technol. 39 (2005) 5774-5780.

369   [17] S.F. Yang, X.Y. Li, H.Q. Yu, Formation and characterisation of fungal and bacterial

370   granules under different feeding alkalinity and pH conditions, Process Biochem. 43

371   (2008) 8-14.

372   [18] S.W. Maloney, N.R. Adrian, R.F. Hickey, R.L. Heine, Anaerobic treatment of

373   pinkwater in a fluidized bed reactor containing GAC, J. Hazard. Mater. 92 (2002) 77-

374   88.

375   [19] M.A. Moteleb, M.T. Suidan, J. Kim, S.W. Maloney, Pertubated loading of a

376   formaldehyde waste in an anaerobic granular activated carbon fluidized bed reactor,

377   Water Res. 36 (2002) 3775-3785.

378   [20] A. Andersson, P. Laurent, A. Kihn, M. Prevost, P. Servais, Impact of temperature on

379   nitrification in biological activated carbon (BAC) filters used for drinking water

380   treatment, Water Res. 35 (2001) 2923-2934.

381   [21] K. Yapsakli, B. Mertoglu, F. Cecen, Identification of nitrifiers and nitrification

382   performance in drinking water biological activated carbon (BAC) filtration, Process

383   Biochem. 45 (2010) 1543-1549.

384   [22] W.P. Flanagan, Biodegradation of dichloromethane in a granular activated carbon

385   fluidized-bed reactor, Water Environ. Res. 70 (1998) 60-66.

386   [23] A. Patel, G. Zuo, S.G. Lehman, M. Badruzzaman, D.A. Clifford, D.J. Roberts,

387   Fluidized bed reactor for the biological treatment of ion-exchange brine containing

388   perchlorate and nitrate, Water Res. 42 (2008) 4291-4298.

389   [24] J.H. Tay, Q.S. Liu, Y. Liu, Characteristics of aerobic granules grown on glucose and

390   acetate in sequential aerobic sludge blanket reactors, Environ. Technol. 23 (2002)

391   931-936.

392   [25] W.Q. Zhuang, J.H. Tay, S. Yi, S.T.L. Tay, Microbial adaptation to biodegradation of

393   tert-butyl alcohol in a sequencing batch reactor, J. Biotechnol. 118 (2005) 45-53.

394   [26] A.J. Li, X.Y. Li, Selective sludge discharge as the determining factor in SBR aerobic

395   granulation: numerical modelling and experimental verification, Water Res. 43 (2009)

396   3387-3396.

397   [27] APHA-AWWA-WEF, Standard methods for the examination of water and wastewater,

398   20th ed.; American Public Health Association/American Water Works

399   Association/Water Environment Federation: Washington D.C., USA, 1998.

400   [28] P. Gerhardt, R.G.E. Murray, W.A. Wood, N.R. Krieg, Methods for General and

401   Molecular Bacteriology, Washington D.C., USA, 1994.

402 [29] S.F. Yang, Q.S. Liu, J.H. Tay, Y. Liu, Growth kinetics of aerobic granules developed

403   in sequencing batch reactors, Lett. Appl. Microbiol. 38 (2004) 106-112.

404 [30] A.J. Li, T. Zhang, X.Y. Li, Fate of aerobic bacterial granules with fungal

405   contamination under different organic loading conditions, Chemosphere 78 (2010)

406   500-509.

407 [31] C.A. Eichner, R.W. Erb, K.N. Timmis, I. Wagner-Dobler, Thermal gradient gel

408   electrophoresis analysis of bioprotection from pollutant shocks in the activated sludge

409   microbial community, Appl. Environ. Microbiol. 65 (1999) 102-109.

410 [32] D.R. Boone, R.W. Castenholz, G.M. Garrity, Bergey's Manual of Systematic

411   Bacteriology, 2nd ed., Volume 1: Springer, New York, USA, 2001.

412 [33] Y.A. Hannun, L.M. Obeid, The ceramide-centric universe of lipid-mediated cell

413   regulation: stress encounters of the lipid kind, J. Biol. Chem. 277 (2002) 25847-25850.

414 [34] Z.C. Chiu, M.Y. Chen, D.J. Lee, C.H. Wang, J.Y. Lai, Oxygen diffusion and

415   consumption in active aerobic granules of heterogeneous structure, Appl. Microbiol.

416   Biotechnol. 88 (2007) 789-797.

417 [35] Y.Q. Liu, J.H. Tay, Influence of starvation time on formation and stability of aerobic

418   granules in sequencing batch reactors, Bioresource Technol. 99 (2008) 980-985.
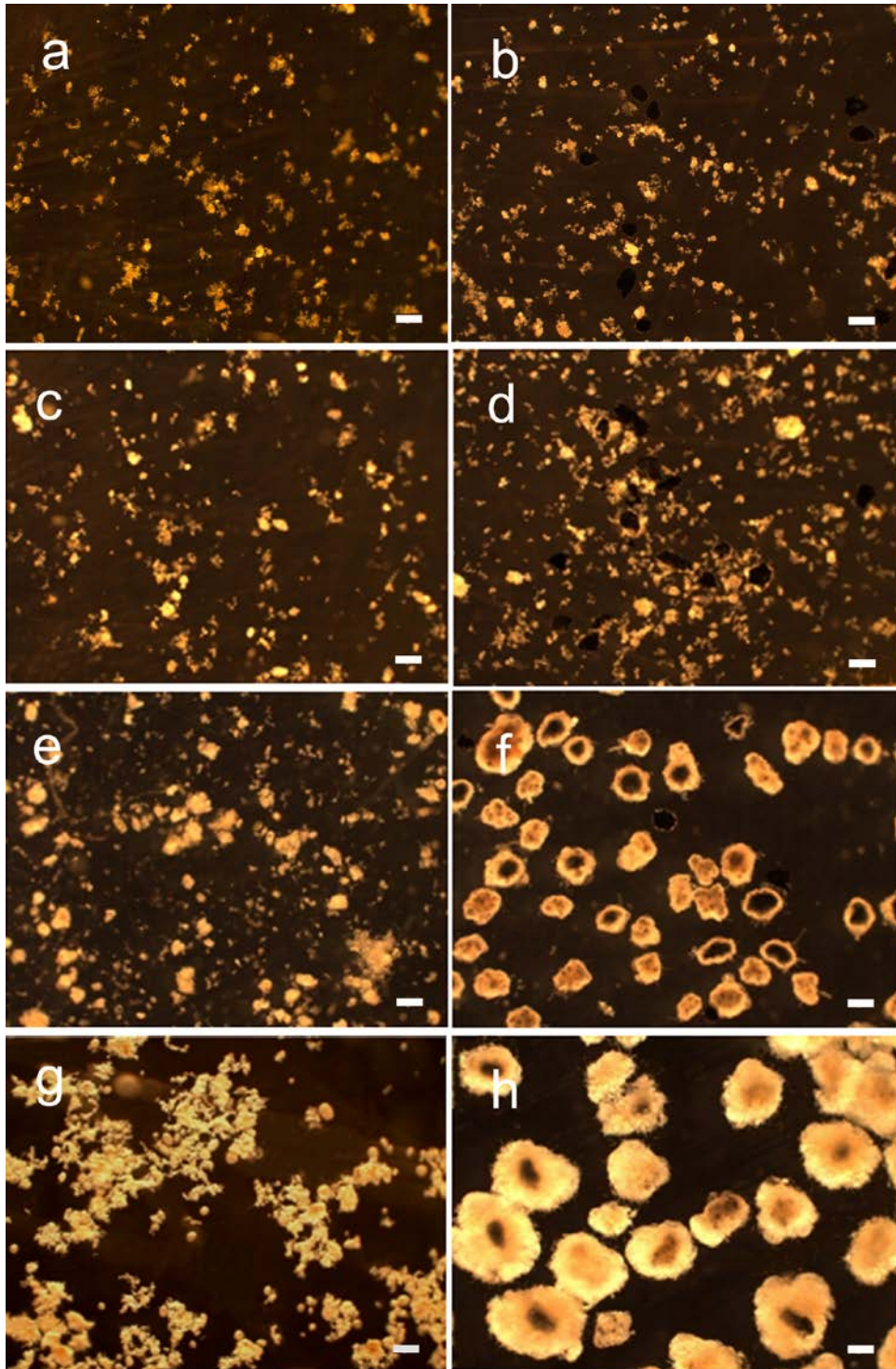
419

Figure captions

421   Fig. 1. Photographs of the sludge after the following days in an SBR: (a) seed, (c) 30 days,

422   (e) 50 days, and (g) 120 days in R1 (without GAC addition), and (b) seed, (d) 30

423   days, (f) 50 days, and (h) 120 days in R2 (with GAC addition); bar = 200 µm.

424   Fig. 2. Photographs of GAC with biofilm growth in R2: (a) raw GAC, (b) after 30 days in

425   Phase 1, (c) after 5 days in Phase 2, and (d) after 10 days in Phase 2; bar = 200 µm.

426   Fig. 3. Changes in the mean particle size of the sludge in R1 and R2 during the SBR startup.

427   Fig. 4. (a) Biomass concentration, (b) sludge F/M ratio, and (c) effluent SS (ESS) of the two

428   SBRs.

429   Fig. 5. The solid-liquid separation property of the sludge in the two SBRs: (a) the sludge

430   volume indexes after 5 min and 30 min of sedimentation and (b) the sludge

431   interfacial settling velocity.

432   Fig. 6. Comparison of the organic substrate uptake rate between activated sludge flocs from

433   R1 and aerobic granules from R2: (a) for a low initial glucose concentration of 200

434   mg/L and (b) for a high initial glucose concentration of 500 mg/L.

435   Fig. 7. DGGE images of the microbial sludge from the two SBRs during Phase 2 with

436   selective sludge discharge; m-n: sludge from Rm (R1 or R2) after n days in Phase 2,

437   e.g., 1-10: sludge from R1 after 10 days in Phase 2 (Left: image; Right: schematic).

Table 1. Phylogenetic analysis of the dominant DGGE bands of the biomass in R2 (Figure 7)

based on the comparison with the clone library.

Band No.	Closest relatives (accession no.)	Identity (%)	Phylogenetic division
2	Diaphorobacter sp. R-25011 (AM084025.1)	96	*β-Proteobacteria*
3	Uncultured Rhodocyclaceae bacterium (AM268350.1)	93	*β-Proteobacteria*
4	Uncultured Variovorax sp. clone HKT603 (DQ098969.1)	99	*β-Proteobacteria*
5	Rhodobacter sp. TUT3732 (AB251408.1)	96	*α-Proteobacteria*
6	Runella sp. EMB111 (DQ372985.1)	98	*Sphingobacteria*
7	Acidovorax sp. BSB421 (Y18617.1)	99	*β-Proteobacteria*
8	Ideonella sp. 0-0013 (AB211233.1)	97	*β-Proteobacteria*
10	Burkholderiales bacterium YT0099 (AB362826.1)	98	*β-Proteobacteria*
11	Riemerella anatipestifer strain RAf68 (EU016551.1)	98	*Flavobacteria*
12	Pedobacter sp. DS-57 （DQ889723.1）	89	*Sphingobacteria*
13	Kaistomonas ginsengisoli （AB245370.1）	98	*Sphingobacteria*
17	Zoogloea ramigera （D14257.1）	99	*β-Proteobacteria*
19	Paracoccus sp. BBTR62 （DQ337586.1）	98	*α-Proteobacteria*
20	Microsphaera sp. G-96 (EF600014.1)	100	*Actinobacteria*
22	Thauera sp. R-28312 （AM084110.1）	98	*β-Proteobacteria*
25	Actinomadura macra (AB364594.1)	99	*Actinobacteria*
1, 9, 14, 15, 16, 18, 21, 23, 24	Unknown		

Fig. 1. Photographs of the sludge after the following days in the SBRs: (a) seed, (c) 30 days, (e) 50 days, and (g) 120 days in R1 (without GAC addition), and (b) seed, (d) 30 days, (f) 50 days, and (h) 120 days in R2 (with GAC addition); bar = 200 µm.
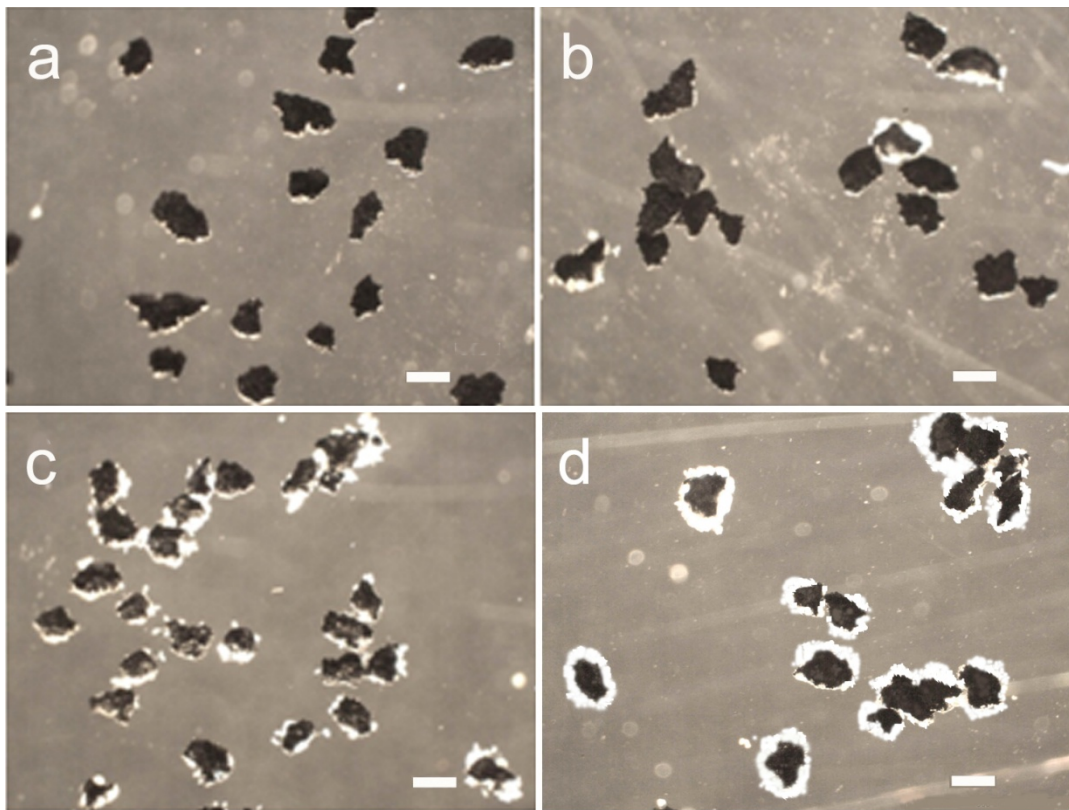
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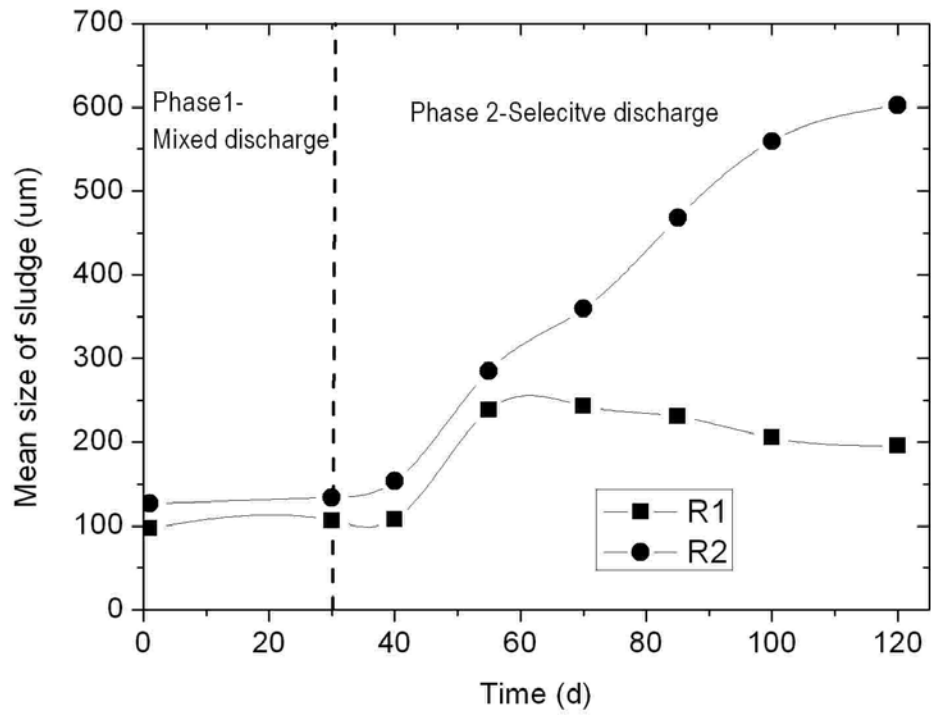
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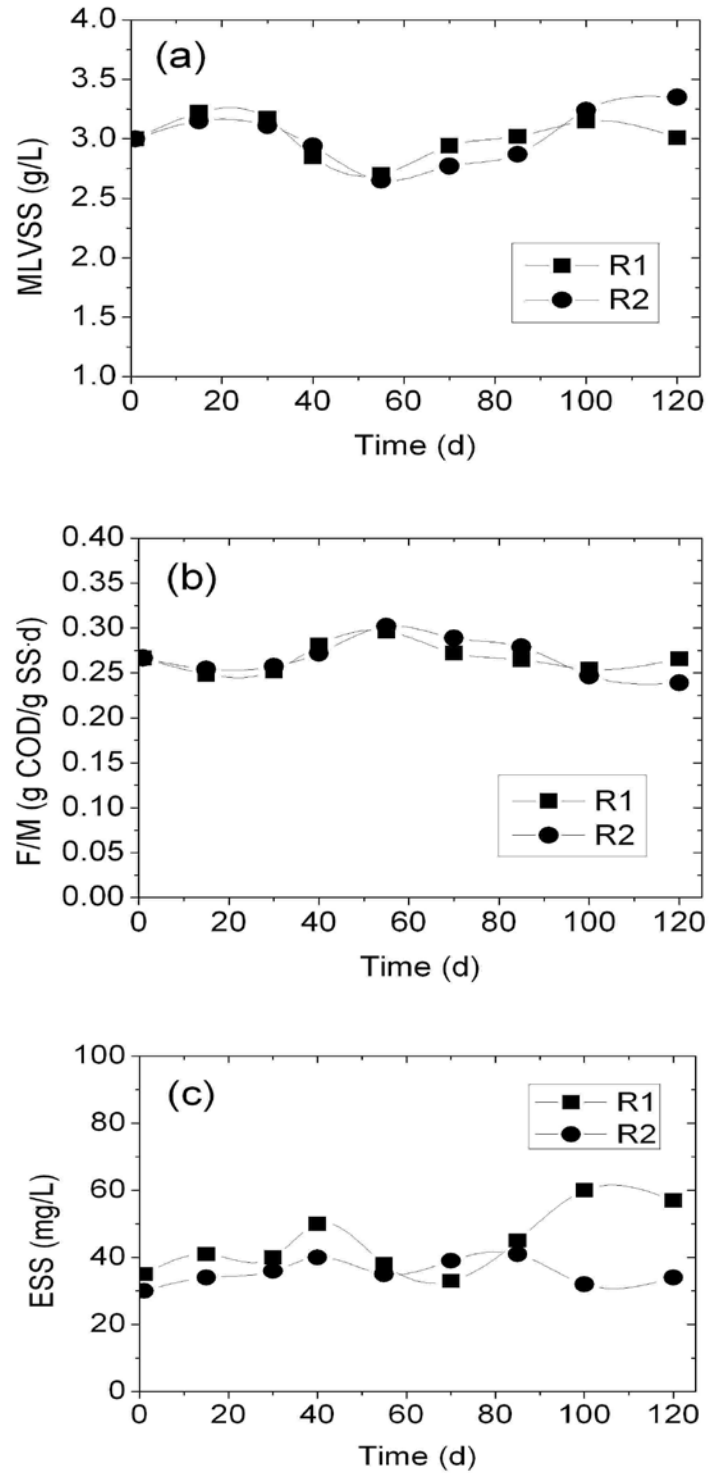
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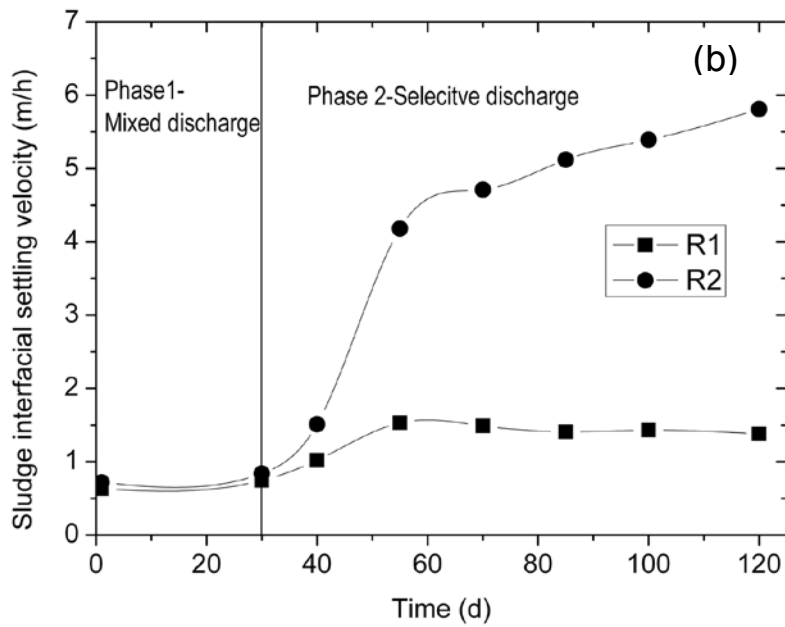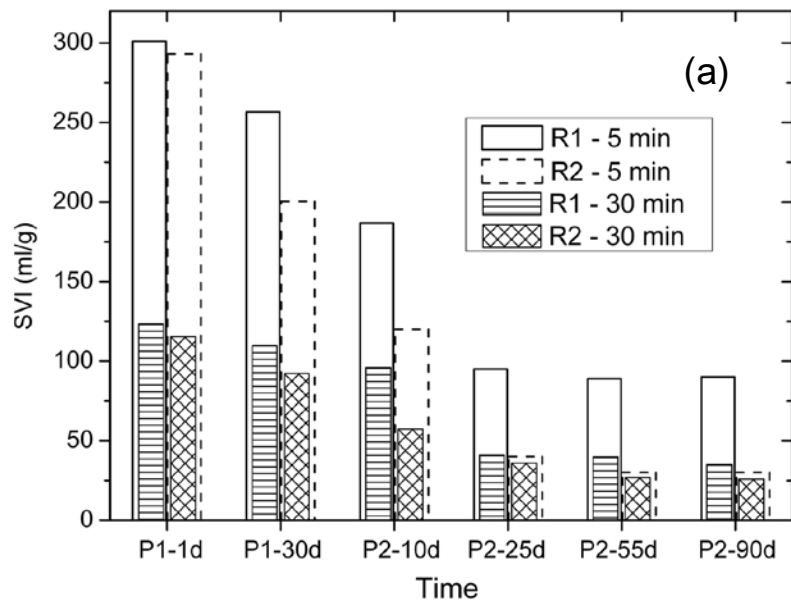
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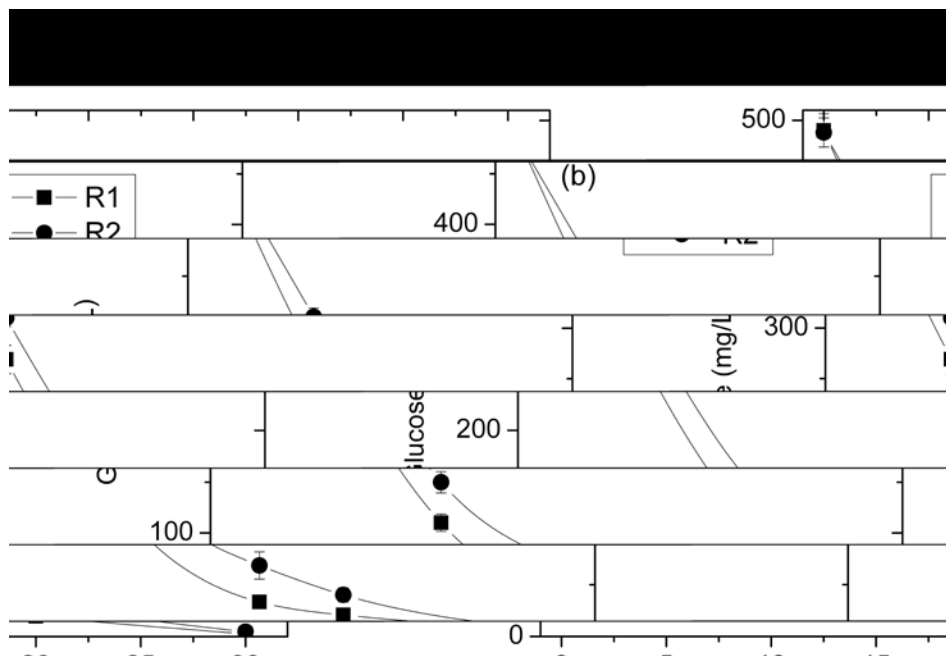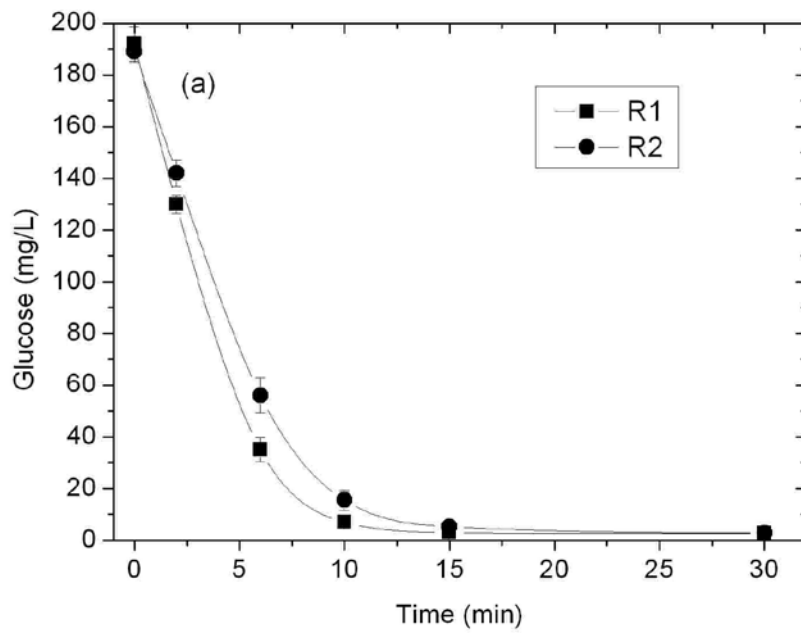
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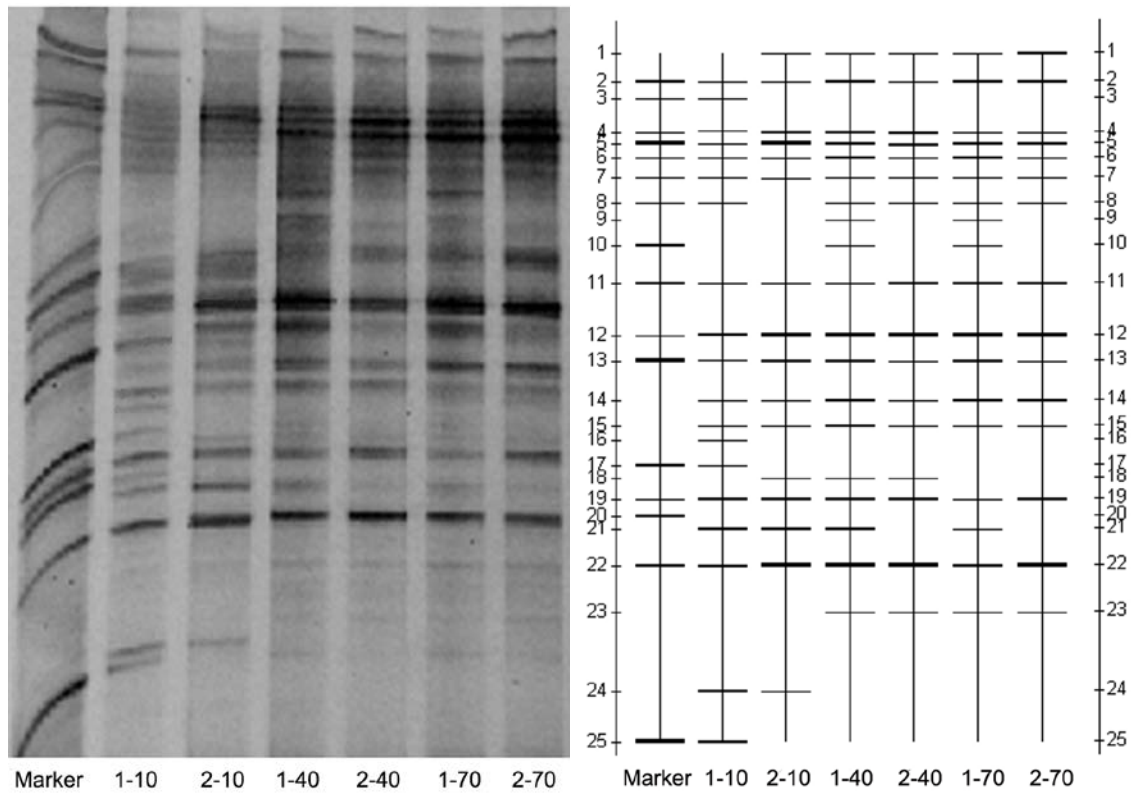
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