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

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Abstract

Aerobic sludge granulation is rather difficult or impossible for the treatment of low-strength wastewater. In this study, a novel technique involving granular activated carbon (GAC) was developed for rapid aerobic granulation under a low organic loading condition. Laboratory experiments were conducted with two sequencing batch reactors (SBRs) running side by side. One reactor had fine GAC added to the sludge mixture, and the other had no GAC added. A low-strength organic wastewater with a chemical oxygen demand (COD) 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.

Keywords: Aerobic granulation, granular activated carbon (GAC), low-strength wastewater, microbial community, sequencing batch reactor (SBR), wastewater treatment.

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].

Aerobic granulation relies on rapid biomass growth that requires a sufficient supply of substrates into the bioreactors. Granule formation has been reported with a high organic, or COD (chemical oxygen demand), loading rate ranging from 1 to 15 kg/m^3·d [3,11,12]. However, granulation may not be achieved with a relatively low organic loading of 1 kg COD/m^3·d or lower [10,13]. Apart from a low organic loading rate, a low influent organic concentration would greatly increase the difficulty of granule formation and growth [14]. Moreover, a low influent concentration often results in more filamentous growth, leading to deterioration and breakage of the granules [15]. There have been few reports of successful granulation for a low-strength wastewater influent with an organic concentration of less than 250 mg COD/L. However, considering the low organic concentration level in most municipal sewage, a simple and effective granulation startup strategy needs to be developed for low-strength wastewater influents.

Aerobic granules can be regarded as a special type of biofilm growth in a stable, contiguous, and multicellular association [16,17]. Granular activated carbon (GAC) has been used as the support medium for microbial immobilization and attached biofilm growth in biological wastewater treatment [18,19]. GAC has a large specific surface area and a fast settling velocity. Its coarse and irregular surface and characteristic adsorption property also provide a favorable microenvironment for bacterial growth. GAC has been successfully applied as the support media in biological aerated filters [20,21] and fluidized-beds [22,23] for water and wastewater treatment. Thus, GAC could be used as the carrier medium for aerobic granulation under unfavorable conditions, such as a low substrate concentration and a low organic loading rate. However, the technique of using GAC for rapid granule formation and long-term granule stability in biological wastewater treatment has yet to be developed.

In this study, laboratory experiments were conducted with two sequencing batch reactors (SBRs) running side by side. GAC was added to the sludge mixture in only one of the reactors. A low organic influent with a COD concentration of only 200 mg/L was tested in the SBRs. With the low-strength influent, granules could not be formed in the SBR without adding GAC. In contrast, complete granulation was achieved in the reactor with GAC added. The morphology, structure, physical properties, and bacterial community of the sludge in the two reactors were characterized and compared throughout the experiments. The aims of the experimental study were to develop an effective technique using GAC for rapid aerobic granulation in bioreactors with a low-strength influent and to investigate the underlying mechanisms of granule formation on GAC.

2. Materials and Methods

2.1. Experimental set-up and SBR operation

Two identical columns (6 cm in diameter and 80 cm in height) with a working volume of 2.4L each were used as SBRs for the experimental study (Fig. S1, Supplementary Data). The two reactors, R1 and R2, were operated in a fixed sequential mode for a 3 hr cycle with 4 min of feeding, 142 min of aeration, 30-min of sludge settling and 4 min of effluent withdrawal from the middle ports of the columns. The reactors were fed with a glucose-based synthetic wastewater prepared according to the chemical composition given by Tay et al. [24]. A low organic concentration with a COD of 200 mg/L was used for the SBR influent. Activated sludge from a full-scale sewage treatment plant (Stanley Sewage Treatment Works, Hong Kong) was used as the seed sludge. The sludge was acclimated in the two SBRs for one month with the glucose-based synthetic wastewater, and the initial sludge MLVSS (mixed liquor volatile suspended solids) concentration was 3000 mg/L.

Fine GAC particles were used to enhance aerobic sludge granulation for the low-strength influent in one of the reactors. The GAC had a mean size of 224 μm with a specific surface area of 1002 m^2/g and an apparent density of 1.183 g/cm^3 (Merck, NJ, USA). No GAC was added to R1, while 7.2 g of GAC was added to R2 to result in a GAC concentration of 3 g/L or a volume fraction of less than 0.3%. The experiments were performed at room temperature, and the water temperature was 20-22˚C. $NaHCO_3$ was dosed into the feed wastewater to maintain the reactor pH in the neutral range between 7.0 and 7.5. Air was supplied at a flow rate of 2.0 L/min into the reactors during the aeration phase to keep the dissolved oxygen (DO) concentration in the sludge suspension in the range of 2-5 mg/L.

Sludge was discharged once a day from the two SBRs at a predetermined rate to maintain a stable biomass concentration. Sludge loss in the effluent during effluent withdrawal was minimized by allowing a settling time of 30 min in each SBR cycle. The SBR experiment was conducted for a total of 120 days in two operating phases with different sludge discharge methods. In the first 30 days, Phase 1, the mixed sludge discharge method was used, and in the next 90 days, Phase 2, selective discharge of slow-settling sludge was applied. For the mixed sludge discharge in Phase 1, the sludge mixture was discharged from the middle ports of the SBR columns while the aeration was still being conducted. The GAC in the sludge mixture discharged from R2 was recovered and returned to R2. For the selective sludge discharge in Phase 2, the sludge was discharged from the middle ports during the settling phase without aeration after a few minutes of sludge settling. The settling period varied from 1 to 5 min depending on the sludge settling property and the targeted amount of sludge to be discharged. In comparison to the mixed sludge discharge method, the selective discharge had a higher fraction of small and slow-settling sludge flocs in the discharged sludge than in the bulk sludge mixture. The amount of sludge loss in the

effluent was measured every day. The amount of daily sludge discharge was adjusted accordingly to maintain a biomass MLVSS concentration of 3000 mg/L in each reactor.

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

The biomass sludge was collected from R1 and R2 periodically to test the organic substrate uptake capability of the sludge. The organic uptake tests were performed in 250-mL glass beakers as batch reactors, with sufficient aeration provided. In each reactor, sludge was added to an MLVSS concentration of 3000 mg/L before adding wastewater. Two different initial glucose concentrations - 200 and 500 mg/L - were used for the substrate uptake tests. After adding the wastewater, the sludge mixtures were sampled at various time intervals. The samples were filtered, and the glucose and COD concentrations in the filtrates were measured. A first-order kinetics may be assumed for the early phase of glucose uptake in the batch reactor, i.e. $\frac{dS}{dt} = -kXS$, where S is the glucose concentration, t is time, k is a rate constant and X is the sludge concentration. From a linear regression of $\ln(S_0/S)$ versus Xt, where S_0 is the initial glucose concentration, the substrate uptake rate constant of the sludge can be determined.

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

The microbial population of the sludge samples was analyzed for the two reactors on experimental days 10, 40 and 70 of the second phase. The genomic DNA of the sludge was extracted using a beadbeater (Mini-beadbeater™, Biospec, Bartlesville, OK, USA) and micro-centrifuge (MiniSpin plus®, Eppendorf, Hamburg, Germany) [25]. The bacterial 16S rDNA gene sequence (V3 region, corresponding to positions 341-534 of *E. coli* sequence) was amplified by polymerase chain reaction (PCR) (PTC-200, MJ Research, Waltham, MA, USA) following the procedure detailed previously [10]. The PCR amplified DNA products

were then separated by denaturing gradient gel electrophoresis (DGGE) through 8% polyacrylamide gels with a linear gradient of 30-50% denaturant, using the DCode™ Universal Mutation Detection System (Bio-Rad, Hercules, CA, USA). The gels were run for 6 h at 130V in 1× TAE buffer at 60°C, and then stained with ethidium bromide for 10 min and visualized by a UV illuminator. The DGGE images were acquired using the ChemiDoc (Bio-Rad) gel documentation system.

A 16S rRNA gene sequence clone library was constructed to identify the phylogeny of the DGGE bands of the sludge samples [26]. Representative clones of the operational taxonomic units (OTUs) underwent the same DGGE analysis under the conditions used for the biomass PCR products. The migration positions of the library clones were compared with the DGGE profiles of the sludge samples. Based on the comparison, an OTU in the clone library was assigned to a particular DGGE band for species identification.

2.4. Analytical methods

The COD concentration, sludge MLSS (mixed liquor suspended solids) and MLVSS concentrations, effluent suspended solids (ESS) concentration, and the sludge volume indexes after 5 min (SVI_5) and 30 min (SVI_{30}) of sedimentation were measured according to the Standard Methods [27]. The interfacial settling velocity of the sludge layer, which is defined as the falling velocity of the water-sludge interface during sludge sedimentation, was measured regularly during the early phase of sludge settling in the two SBR columns. The glucose content was determined using the phenol-sulphuric acid method [28]. The morphology of the sludge flocs and granules was examined under a stereomicroscope (S8 APO, Leica, Cambridge, UK) equipped with a digital camera (EC3, Leica, Cambridge, UK). A laser diffraction particle counter (LS13 320, Beckman Coulter, Miami, FL, USA) was

used to measure the size distribution of the sludge flocs and granules. Accordingly, the volume-based mean size of the sludge in a sample was calculated from its size distribution.

3. Results and Discussion

3.1. Formation of aerobic granules in the SBR

During the first 30 days of SBR startup (Phase 1), the mixed sludge discharge method was used in the two SBRs. Sludge remained in the form of flocs in both R1 without GAC and R2 with added GAC (Fig. 1). A few sludge flocs were found to attach to the GAC, and the amount of biomass that attached to or grew on the GAC was about 9% of the total biomass in R2 by the end of Phase 1. Both reactors were then changed to the elective sludge discharge mode in Phase 2 to facilitate aerobic sludge granulation. As expected, complete granulation was difficult with the low-strength (200 mg COD/L) influent. There was little sign of granule formation in R1 throughout Phase 2. In contrast, however, GAC-based granules began to form rapidly in R2, despite the same low-strength influent. Biofilm appeared to grow on the GAC surface after only 5 days of Phase 2 (Fig. 2), while the amount of biomass growing on GAC increased to 16% of the total biomass in R2. After 10 days of Phase 2, the GAC was fully covered by biofilm, and small granules became visible. Sludge granulation was almost fully achieved in R2 after 20 days (Fig. 1). The granules in R2 were round with a clear boundary, and were completely different from the loose and irregular sludge flocs in R1. The amount of biomass in the GAC-based granules accounted for more than 80% of the total sludge in R2.

Measurement of the particle size showed that the mean size of the sludge in R2 with added GAC was larger than that in R1 (Fig. 3). During Phase 1, with mixed sludge discharge, the mean sludge sizes in both R1 and R2 were quite stable at no more than 130 μm after 30 days. Selective sludge discharge in Phase 2 led to an increase in sludge size in

both reactors, particularly in R2. The mean size of the R2 sludge increased from 134 to 153 μm after only 10 days in Phase 2. The size increased continuously with the formation and growth of granules, and the mature granules had a mean size of around 600 μm. The sludge in R1 also increased in size to about 250 μm after 20 days in Phase 2, and a few small granules were found in the sludge mixture (Fig. 1). However, complete aerobic granulation could not be achieved in R1 with no GAC added. The small granules apparently broke up and the mean sludge size eventually decreased to about 200 μm (Fig. 3).

The two reactors were operated under the same condition except for the GAC addition (Fig. 4). The two SBRs had the same organic loading of 0.8 kg COD/m^3·d, the same HRT of 6 h and a similar SRT of around 15 d. Sludge was discharged from the two SBRs once a day at an overall biomass sludge removal ratio of about 6%, and the MLVSS was kept at around 3000 mg/L in both reactors (Fig. 4a). The F/M (food-to-microorganism) ratio was maintained between 0.25 to 0.30 g COD/g SS·d (Fig. 4b). Both reactors performed well on organic removal with an effluent COD of below 30 mg/L. The amount of SS in the effluent was 60 mg/L or lower for R1, without GAC addition and granulation. In comparison, R2 had a lower effluent SS level of less than 40 mg/L after GAC-enhanced granule formation, which showed the benefit of sludge granulation (Fig. 4c).

3.2. Comparison of the sludge between the two SBRs

GAC addition during the SBR startup significantly improved the sludge settleability and compression (Fig. 5). The sludge in R2 with the initial GAC addition always had a lower SVI value than the sludge in R1 (Fig. 5a). With the mixed sludge discharge in Phase 1, the SVI_{30} was about 110 ml/g for the R1 sludge and 90 ml/g for the R2 sludge after 30 days. The SVI_5 values were more than twice the respective SVI_{30} values, implying typical activated sludge flocs without granulation in both SBRs [4]. Selective sludge discharge in

Phase 2 led to a considerable improvement in sludge compressibility and settleability. The SVI_{30} decreased to 40 ml/g for the sludge in R1 and to 30 ml/g for R2 after 25 days of Phase 2 operation (Fig. 5a). Meanwhile, the SVI_5 decreased from 250 to 100 ml/g in R1 and from 200 to 40 ml/g in R2. However, the SVI_5 remained about twice as large as SVI_{30} for the R1 sludge throughout the rest of the SBR test. This agreed with the microscopic observation that the sludge in R1 remained in the form of suspended flocs. Although selective discharge of small and loose flocs improved the sludge settleability, it was not enough to lead to complete granulation for the low-strength influent. In contrast, the sludge SVI_5 was similar to SVI_{30} for the R2 sludge after 25 days of Phase 2. This indicated complete granulation according to the typical defining feature of aerobic granules [4]. The comparative results demonstrate that the initial GAC addition was crucial to the granule formation in R2. In other words, aerobic granulation would not be achieved for a low-strength influent of 200 mg COD/L or less without the addition of GAC during the SBR startup.

Granular sludge showed its great advantage in sludge water separation. For the suspended sludge in R1, the sludge-water interfacial settling velocity was rather stable at a rate of no more than 1.5 m/h (Fig. 5b). In contrast, the bulk sludge settling velocity continuously increased in R2 with the formation and growth of granules. The mature granules had an interfacial settling velocity of about 6 m/h, which was at least 4 times as fast as that of the sludge flocs in R1. In comparison to conventional activated sludge, sludge after granulation could be separated much more rapidly from the wastewater after treatment. In other words, granular sludge would request a very short phase of sludge-water separation, which is particularly beneficial to low-strength wastewater treatment. In addition, aerobic granulation would allow a much higher level of biomass concentration at 5-8 g/L and in granular SBRs, which has been well demonstrated by previous studies [3-5,10].

Although the granular sludge in R2 performed better in sludge-water separation, the sludge flocs in R1 were found to have a greater substrate uptake capability than the granules in R2. For the same biomass SS content of 3 g/L, the feeding glucose concentration dropped more rapidly with the R1 sludge than with the R2 sludge (Fig. 6). For the initial glucose concentrations of 200 mg/L, the activated sludge flocs in R1 had a glucose uptake rate constant *k* at 6.7 L/g SS·h, which is considerably higher than that of the mature granules in R2, at 4.9 L/g SS·h. The different glucose uptake rates suggest that loose sludge flocs have a clear advantage over dense granules for the uptake of substrates and nutrients. Small and loose flocs can obtain substrates from the suspension more easily than tightly-packed granules [29]. With mixed sludge discharge, there is less substrate available for uptake by dense flocs and granules due to competition from loose sludge flocs [26]. Thus, as demonstrated in Phase 1, it is apparently impossible for granules to grow and become dominant in a reactor without selective discharge. Discharge of suspended small and loose sludge flocs removes these competitors from the system and makes the substrates more available for the biomass in attached-growth form, which leads to granulation [30].

Well-resolved DGGE bands were obtained from the biomass from R1 and R2 (Fig. 7). Changes in the DGGE banding profile are presumed to indicate the evolution of bacterial species in a reactor [10,31]. To determine the identity of the bands in the DGGE profiles, OTUs from 98 clones in the library were compared with the DGGE patterns. Of the 25 bands that appeared in the DGGE profiles, 16 dominant bands were identified (Table 1), which accounted for 70% of the microbial abundance represented by the DGGE banding profiles. The majority of the bacterial 16S rDNA sequences grouped with members of *Proteobacteria*, with two in the α subdivision and eight in the β subdivision. The next three groups clustered with *Sphingobacteria*, one clustered with *Flavobacteria* and two clustered 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-

strength wastewater. GAC facilitates biofilm growth and granule formation and helps to sustain the stability of mature granules for long-term wastewater treatment operation.

4. Conclusions

- Adding GAC is shown to be a necessary and effective technique to initiate granule formation for complete sludge granulation in SBRs with an influent COD of only 200 mg/L. In contrast, without GAC, aerobic granulation cannot be achieved in an SBR for treating low-strength influent.
- Selective discharge of slow-settling sludge is also essential for granulation. Adding GAC to the seed sludge mixture, together with the selective discharge of small and loose sludge flocs, facilitates the retention and growth of bacterial cells on GAC in attached-growth mode, leading to complete granulation.
- The use of GAC produces aerobic granules with a fast settling velocity and a much improved sludge-water separation property. The granules have strong cores that will help to maintain the long-term stability of mature granules for treatment of low-strength wastewater.

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Figure captions

Fig. 1. Photographs of the sludge after the following days in an SBR: (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.

Fig. 2. Photographs of GAC with biofilm growth in R2: (a) raw GAC, (b) after 30 days in Phase 1, (c) after 5 days in Phase 2, and (d) after 10 days in Phase 2; bar = 200 μm.

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

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

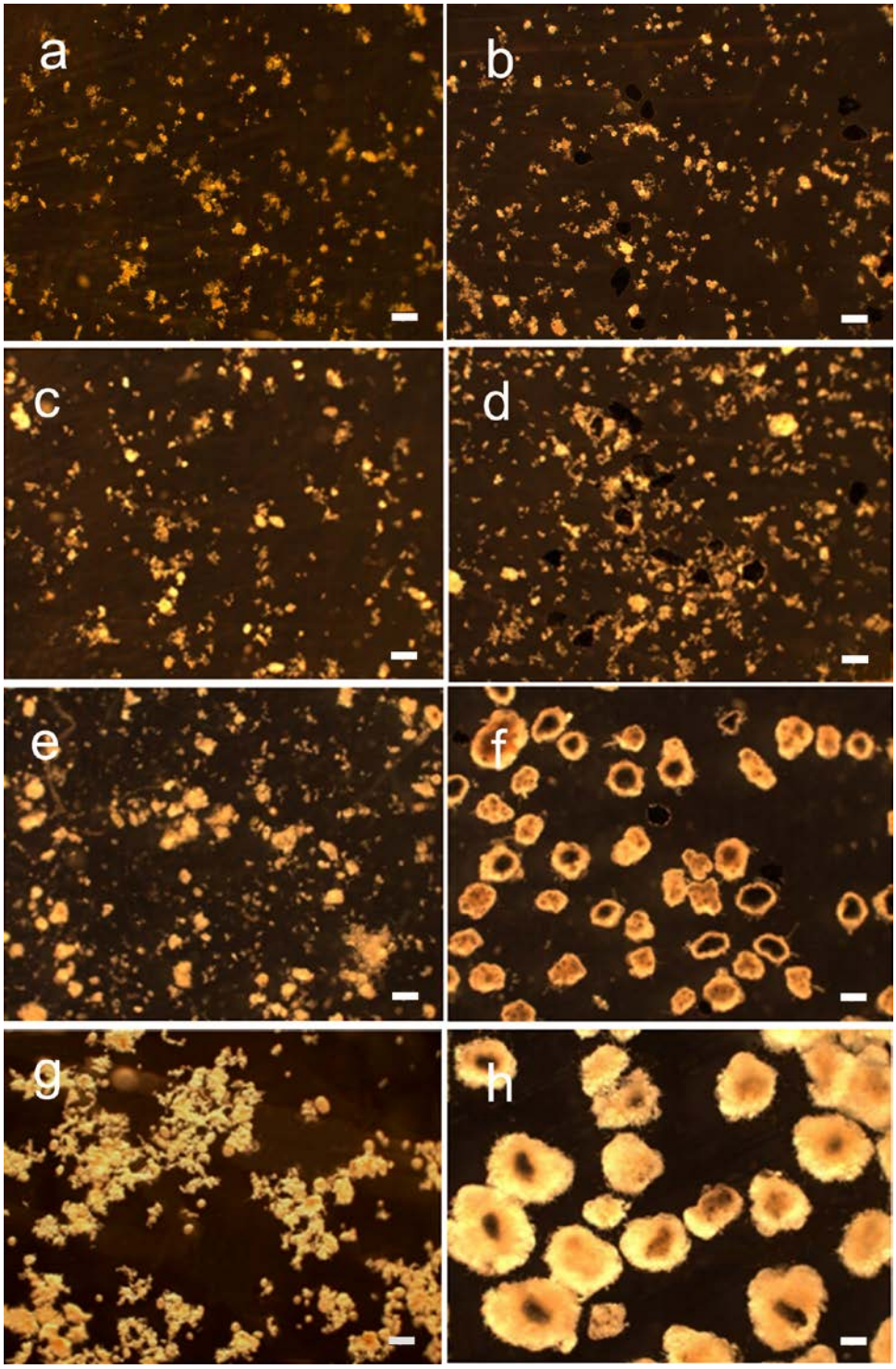
Fig. 5. The solid-liquid separation property of the sludge in the two SBRs: (a) the sludge volume indexes after 5 min and 30 min of sedimentation and (b) the sludge interfacial settling velocity.

Fig. 6. Comparison of the organic substrate uptake rate between activated sludge flocs from R1 and aerobic granules from R2: (a) for a low initial glucose concentration of 200 mg/L and (b) for a high initial glucose concentration of 500 mg/L.

Fig. 7. DGGE images of the microbial sludge from the two SBRs during Phase 2 with selective sludge discharge; m-n: sludge from Rm (R1 or R2) after n days in Phase 2, 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		



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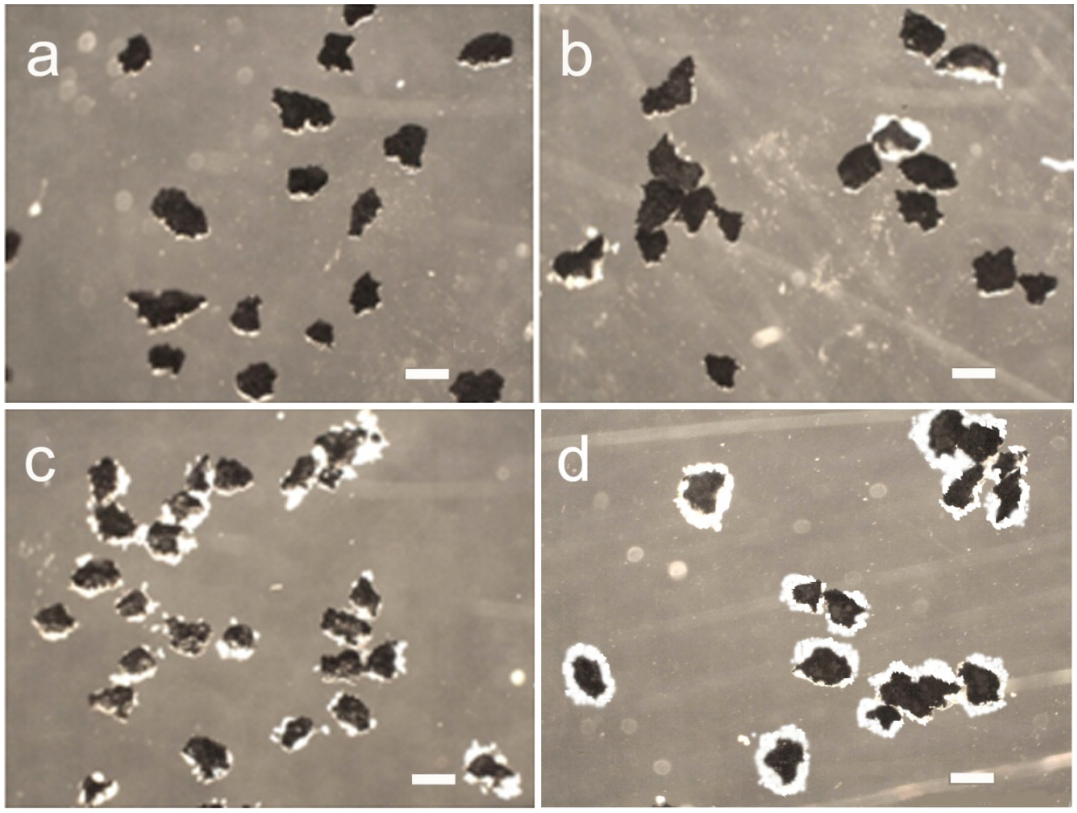

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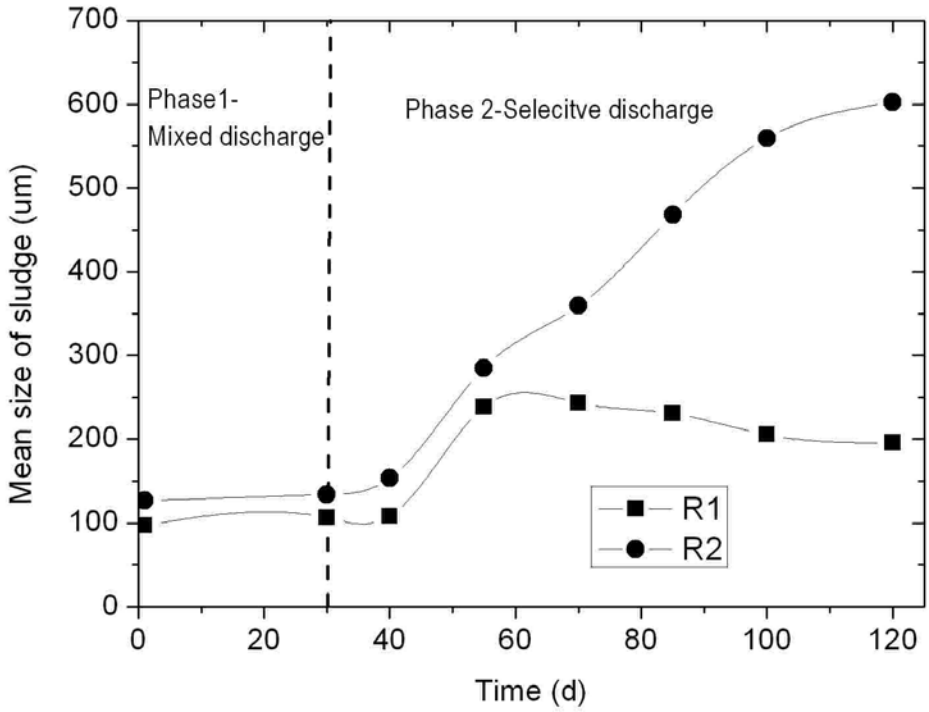

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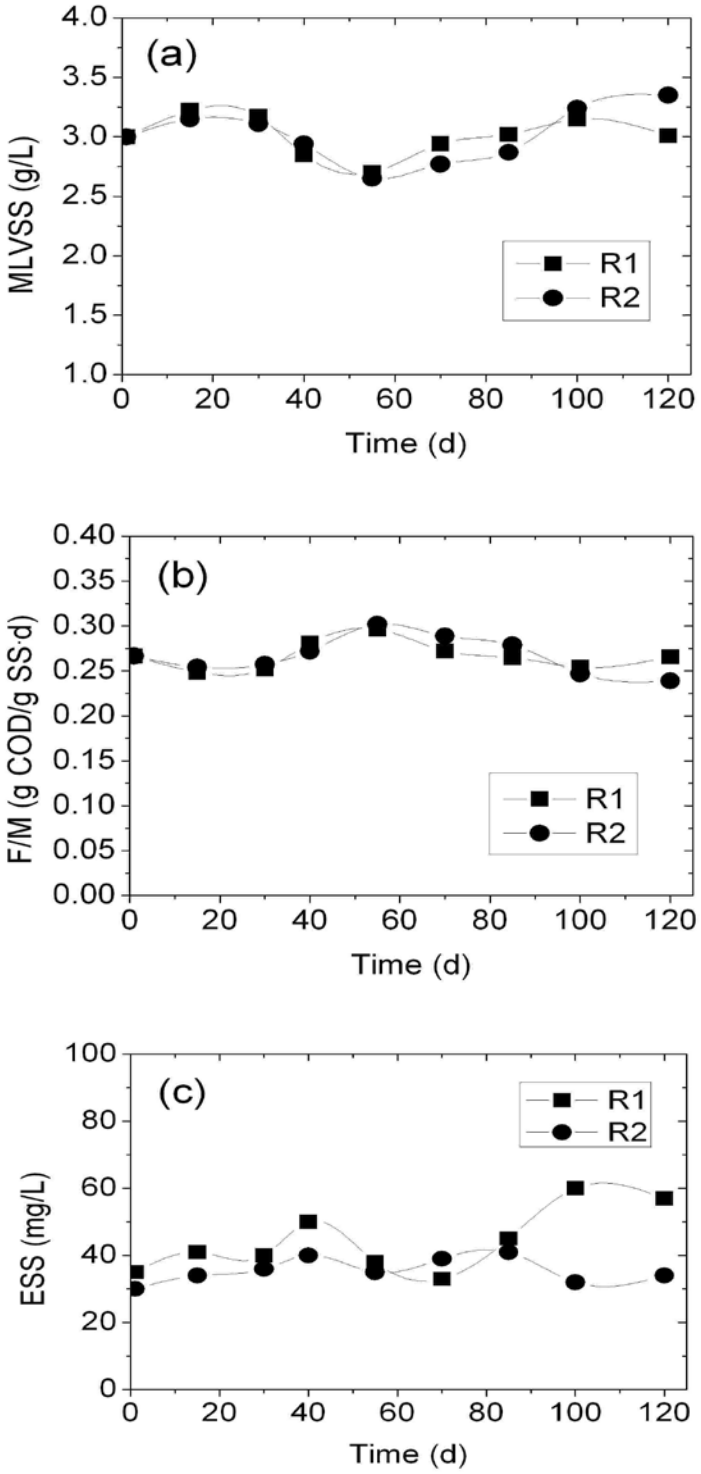

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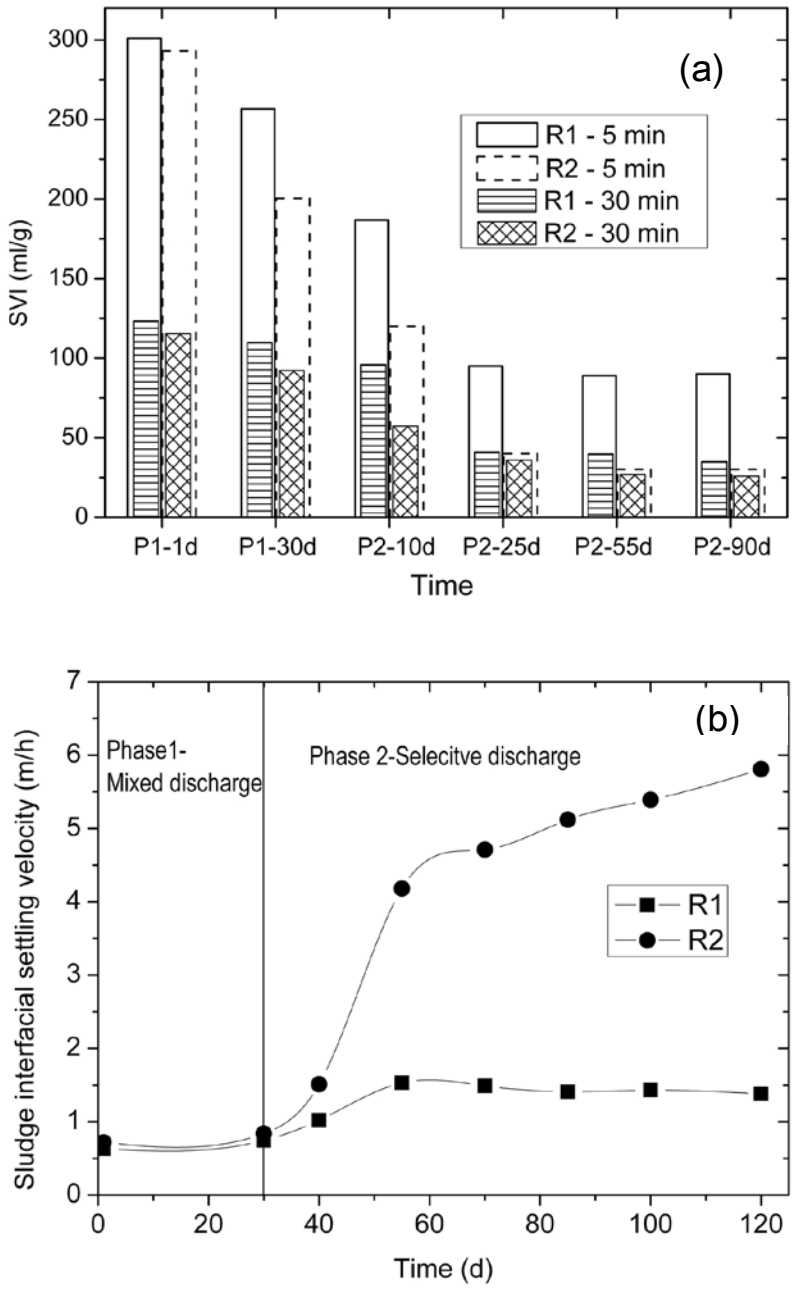

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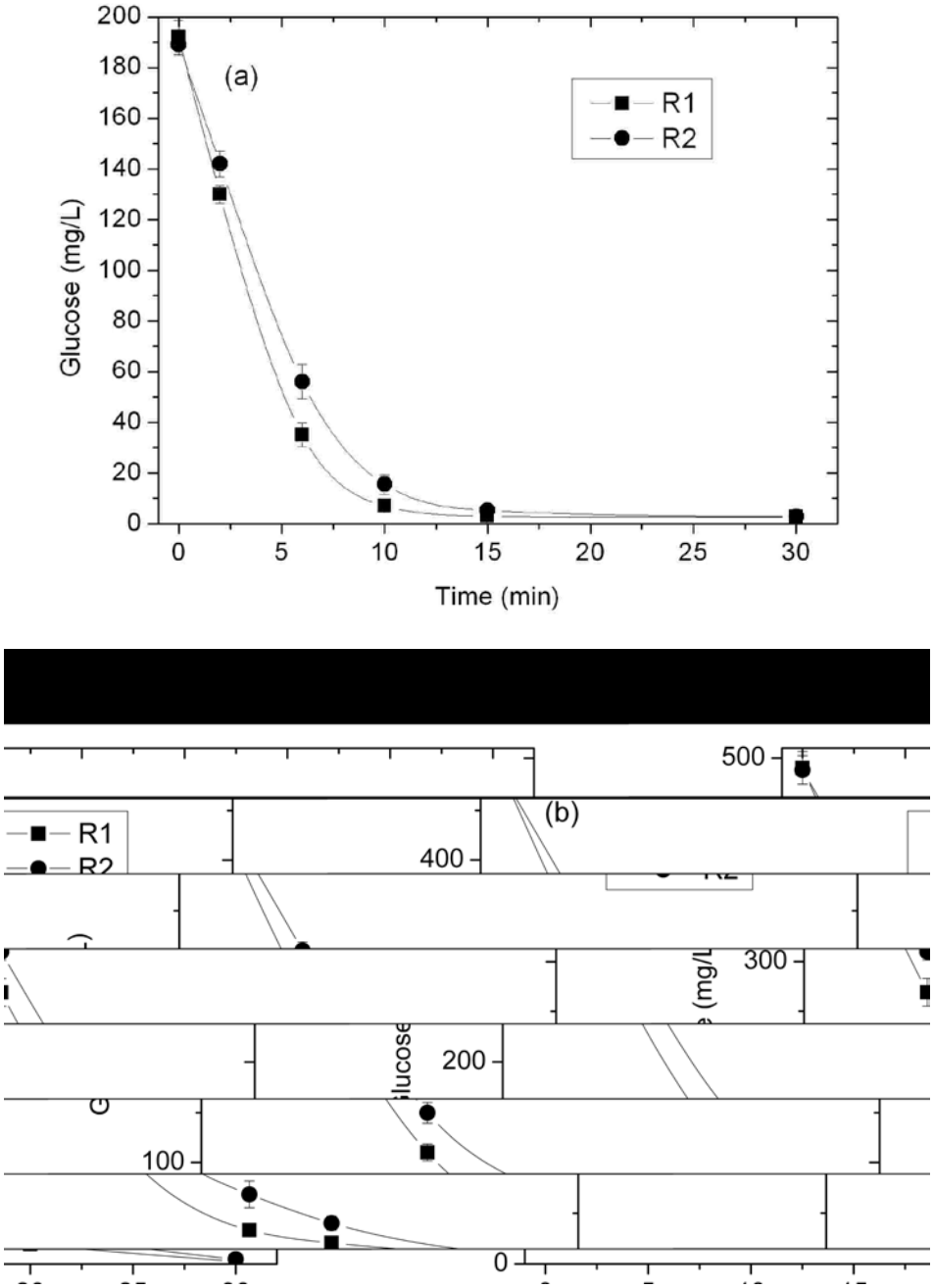

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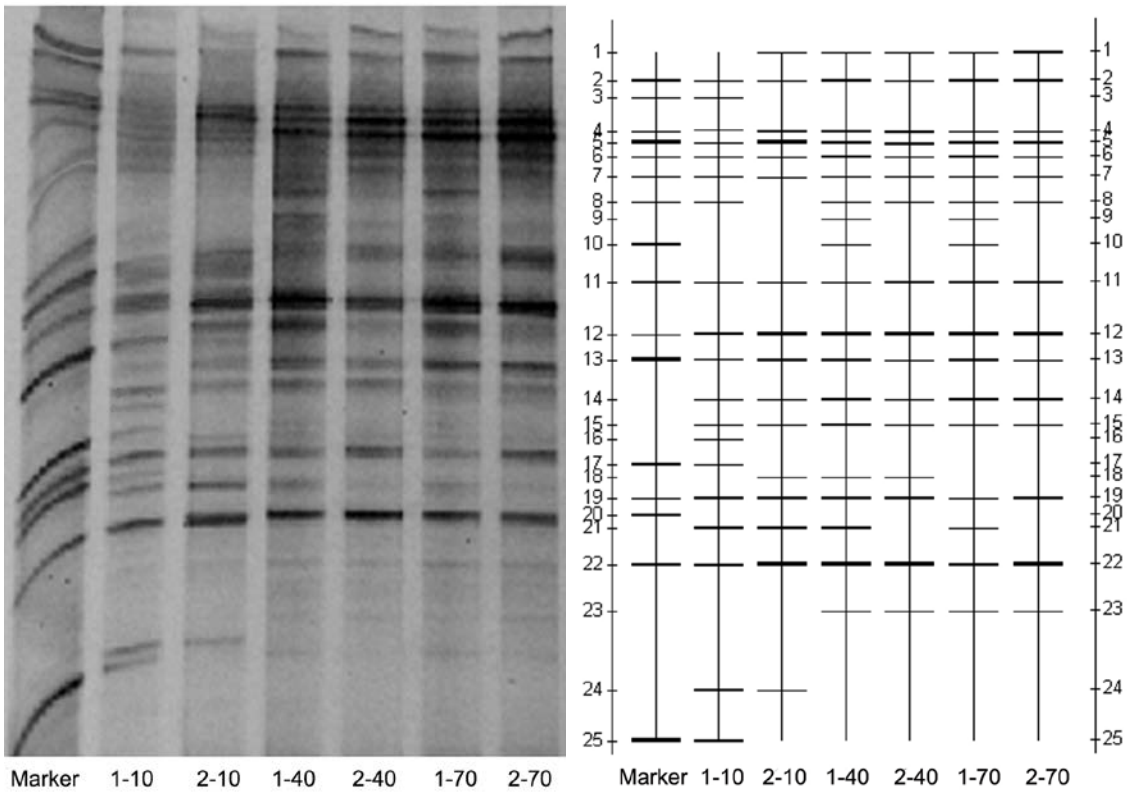

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