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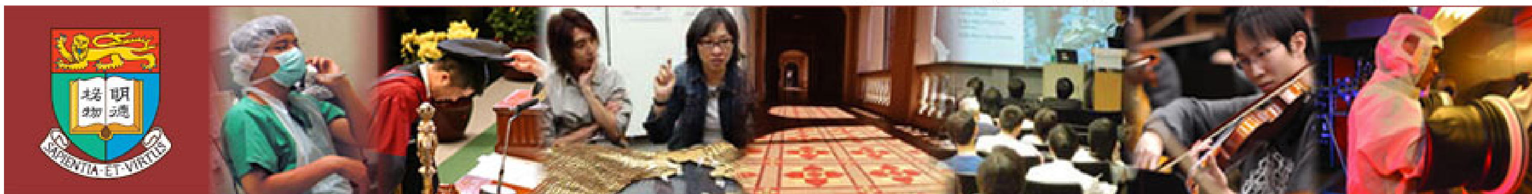

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Aerobic sludge granulation facilitated by activated carbon for partial nitrification treatment of ammonia-rich wastewater

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Abstract

Although the use of partial nitrification, or nitritation, for nitrogen removal via nitrite is an energy-saving method for treating high-strength ammonia wastewater, its stable operation with sufficient enrichment of ammonia-oxidizing bacteria (AOB) is difficult to maintain in activated sludge systems. In this study, an aerobic granulation technique was developed for the effective and stable nitritation treatment of ammonia-rich inorganic influent. Granular activated carbon (GAC) or powdered activated carbon (PAC) was added to the bioreactor to

enhance the granulation of slow-growing AOB. The results show that aerobic granules could be formed for partial nitrification through the selective discharge of small and slow-settling sludge flocs, with or without activated carbon addition. However, dosing GAC into the sludge greatly accelerated the granulation process and shortened the granulation period from about 6 weeks to less than 3 weeks with the formation of large and fast-settling granules. In contrast, dosing PAC led to the slower formation of smaller granules. Compared to activated sludge flocs, sludge granulation with selective sludge discharge was found to help halt ammonia oxidation to the level of partial nitrification rather than complete nitrification. Based on the molecular analysis, aerobic granulation resulted in AOB enrichment and the reduction of nitrite-oxidizing bacteria (NOB) in granules, which is highly favorable to a stable partial nitrification operation.

Keywords: Aerobic granulation; Partial nitrification; Activated carbon; Activated sludge; Biological nitrogen removal; Wastewater treatment.

1. Introduction

Nitrogen removal is one of main objectives in wastewater treatment. Partial nitrification for biological nitrogen removal (BNR) via nitrite has recently gained interest because of its considerable energy and cost savings compared to complete nitrification [1,2]. It has become a particularly attractive option for treating high-strength ammonia wastewater with low organic content. The nitrite produced by ammonia-oxidizing bacteria (AOB) in partial nitrification can be readily removed via denitrification by anaerobic ammonium oxidation (anammox) or other similar processes [3,4]. Partial nitrification or nitritation (ammonia oxidation to nitrite) can be achieved in activated sludge systems [5,6] or biofilm reactors [7,8]. However, problems have been reported with the stability of the partial nitrification system

because of the accumulation of nitrite-oxidizing bacteria (NOB) in the biomass under the nitrite-rich condition [9,10]. A strict operating condition such as a low DO level (<1.5 mg/L) is commonly requested. In addition, a sufficient washout of NOB is essential to stable nitritation [6,11], which typically requires a short sludge retention time (SRT). However, a longer SRT (>10 d) is commonly required for activated sludge to ensure a high biomass concentration and efficient and reliable treatment performance.

Aerobic granulation is a process in which loose sludge flocs are transformed into dense granules. Due to attributes such as a compact structure and fast settling velocity, granule formation allows a higher sludge concentration that increases the loading capacity of biological wastewater treatment systems [12]. The selective discharge of loose and slow-settling flocs has been found to be crucial to the transformation from activated sludge to granular sludge [13,14]. Granulation helps maintain and enrich the slow-growing, nitrifying bacteria in a reactor to enhance ammonia oxidation [15]. However, due to the slow growth rates of nitrifying bacteria, complete granulation is rather difficult to achieve in nitrification or partial nitrification, particularly for concentrated ammonia feeds with little organic substrates [16]. In some of the successful cases that have been reported, a long start-up period of 2 months or more was needed to achieve sludge granulation [17-19]. Other factors such as dissolved oxygen, sludge discharge and ammonia concentration can affect the granule formation and performance of ammonia oxidation [20,21]. Nonetheless, effective start-up strategies need to be developed to accelerate granule formation for reliable granulation and stable partial nitrification.

Activated carbon (AC) has a large specific surface area and a fast settling velocity. Dosing with AC enhances aerobic granulation under unfavorable conditions, such as low substrate concentrations and low loading rates [22]. Because AOB have a slow growth rate, it is difficult to increase their concentration in a bioreactor. With the use of AC, AOB would

attach to the AC and avoid washout from the reactor during the start-up of granulation. As a result, rapid granule formation could be achieved to ensure the stable operation of nitritation. In this study, laboratory experiments were conducted with three batch bioreactors to cultivate granular sludge for partial nitrification. Granular activated carbon (GAC) or powdered activated carbon (PAC) was added to the sludge mixture in one of the reactors. The aims of this study were to develop an effective technique for the rapid granulation of AOB sludge for nitritation, to investigate the microbial population change during the granulation process and to examine the capability and stability of partial nitrification by the granular sludge.

2. Materials and Methods

2.1. Experiment set-up

Three laboratory bioreactors, B1, B2 and B3, were used to grow granular sludge for partial nitrification. Each reactor was a small column (H 30 cm × i.d. 3.6 cm) with a working volume of 200 mL. The reactors were inoculated with nitrifying activated sludge as the seed sludge that had been cultivated in a lab-scale fermentor (Sartorius Biostat®, A Plus, Germany). The feed into the fermentor, named B0, was an NH_4Cl solution with a NH_4^+-N concentration of 200 mg/L and no organic content [23]. The initial sludge concentration added to the column reactors was around 2000 mg /L in terms of mixed-liquor volatile suspended solids (MLVSS).

The bioreactors were dosed with two typical types of AC, GAC and PAC, with the goal of enhancing the sludge granulation process. The GAC and PAC had mean sizes of 224.4 μm and 50.5 μm, respectively, with an apparent density of 1.183 g/cm^3 according to the supplier (Merck, NJ, USA). No AC was added to reactor B1, 0.1 g of GAC was added to B2 and 0.1 g of PAC was added to B3 for an initial GAC or PAC concentration of 0.5 g/L. Aeration was supplied from the bottom of each column by an air pump at a flow rate of 8 L/min to keep the

DO concentration in the sludge suspension within a range of 2-4 mg/L. The reactors were fed once every 12 h. The influent to the reactors was a synthetic wastewater prepared with NH_4Cl and KH_2PO_4 without any organic substrates added. Clean seawater collected from Cape d'Aguilar Marine Reserve, Hong Kong, was filtered with a 0.22-μm membrane and added to the synthetic wastewater at a ratio of 1:2 to increase the wastewater salinity. The synthetic wastewater had a salinity of about 1% that is similar to the saline wastewater in Hong Kong. The wastewater influent contained an NH_4^+-N concentration of 400 mg N/L and a PO_4^{3-}-P concentration of 40 mg P/L, which resulted in a volumetric N loading of 0.8 g N/L·d in the reactors. The pH of the mixed liquor in the reactors was controlled at around 7.5 during the experimental period by adding a diluted $NaHCO_3$ (0.1 M) solution automatically.

The discharge of small and slow-settling sludge flocs was conducted at the end of each 12-h cycle from each of the column reactors. Before sludge discharge, the sludge was allowed to settle in the column without aeration for a period from 1 to 5 min, depending on the sludge's settling properties and the targeted sludge discharge rate. The slow-settling sludge in the top 40 mL suspension was then removed from the reactors. The sludge concentration in each reactor was measured; accordingly, the amount of daily sludge discharge was adjusted to maintain a biomass MLVSS concentration of around 2000 mg/L in each reactor. After the selective sludge discharge, the remaining sludge suspension was allowed to settle in the column for another 30 min, and the supernatant (~180 mL) was then withdrawn as the effluent from the reactor. The feed solution was added into each reactor to restore the original volume of 200 mL.

2.2. Determination of the nitritation capability of the granules

After the completion of aerobic granulation, the granular sludge was characterized for its partial nitrifying kinetics and settling velocity, in comparison to the seed nitrifying activated

sludge. For each sludge sample from B0, B1, B2 and B3, the nitrification and nitritation capability test was performed in a 100-mL glass beaker and the sludge and NH_4^+-N concentrations were 2 g MLVSS/L and 400 mg/L, respectively. The sludge mixture was sampled at various time intervals. The NH_4^+-N, NO_2^--N and NO_3^--N concentrations in the liquid phase of the sludge were measured, and subsequently the specific rate of ammonia degradation by the granules was determined.

2.3. Analysis of microbial population and identification of dominant species in the bioreactors

The microbial population of the seed sludge and the mature granules from the three reactors was analyzed. The genomic DNA of the sludge was extracted from the cells using a beadbeater (Mini-beadbeater™, Biospec, Bartlesville, OK, USA) and micro-centrifuge (MiniSpin plus, Eppendorf, Hamburg, Germany) [24]. The bacterial 16S rDNA gene sequence (V3 region, corresponding to positions 341-534 of the *Escherichia coli* sequence) was amplified by polymerase chain reaction (PCR) (PTC-200, MJ Research, Waltham, MA, USA) following the previously detailed procedure [25]. 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 130 V in a 1×TAE buffer at 60°C. Afterward, the gels were stained with ethidium bromide for 10 min and then visualized by a UV illuminator. The DGGE images were acquired using the ChemiDoc (Bio-Rad) gel documentation system and the DGGE profile was analyzed by “QuantityOne” (Version 4.6.3, Bio-Rad, Hercules, CA, USA).

A 16S rRNA gene sequence clone library constructed for the seed sludge was used to identify the phylogeny of the DGGE bands of the sludge samples [23]. The sequences of the

clones used as markers have been deposited in GenBank under accession numbers HM117161 to HM117169. Some of the clones were selected as markers for the DGGE analysis and the migration positions of these clones were compared to the DGGE profiles for the sludge samples. Based on this comparison, an OTU in the clone was assigned to a particular DGGE band for species identification. The percentages of the species in a sample were estimated according to the intensity of the individual bands over the total intensity of all detectable bands.

2.4. Analytical methods

The sludge MLVSS concentration and the sludge volume index after 5 min of sedimentation (SVI_5) were measured according to the Standard Methods [26]. The MLVSS was used to indicate the amount of biomass in the sludge or sludge-activated carbon mixture. The ammonium, nitrite and nitrate concentrations were measured following the Nesslerizaion, colorimetric and ultraviolet spectrophotometric screening methods, respectively [26]. The morphologies of the sludge flocs and granules were examined under a stereomicroscope (S8APO, Leica, Cambridge, UK) equipped with a digital camera (EC3, Leica, Cambridge, UK). A laser diffraction particle counter (LS13320, Beckman Coulter, Miami, FL, USA) was used to determine the size distribution of the sludge flocs and granules. Accordingly, the volume-based mean size of the sludge flocs and granules in a sample was calculated. In addition, the settling velocity was measured for individual granules by recording the time it had taken for the granules to settle through a distance of 60 cm in a water column.

3. Results and Discussion

3.1. Formation of aerobic granules for partial nitrification

The daily discharge of slow-settling sludge flocs was applied to the column bioreactors in

operation. As a result of the selective sludge discharge, aerobic sludge granulation was achieved in the three bioreactors fed on ammonia as a sole energy source (Fig. 1). The three reactors were operated under the same conditions, with the exception of the AC addition, e.g., the same nitrogen loading of 0.8 kg N/m^3·d and the same HRT of 12 h. The biomass content measured by MLVSS increased gradually from around 2000 to 2300 mg/L in the three reactors (Fig. 2a) and the F/M (food-to-microorganism) ratio decreased accordingly from more than 0.40 to about 0.35 g N/g VSS·d (Fig. 2b). With an increase in biomass content and a decrease in sludge discharge rate, the SRT increased similarly in the three bioreactors from about 13 to 23 d (Fig. 2c). Generally speaking, the addition of GAC or PAC did not appear to be the crucial factor for the final result of granule formation. As previously indicated [14], the selective discharge of small and slow-settling sludge flocs from the sludge suspension appeared to be the determining factor for granulation.

However, dosing with GAC had a favorable effect on the rate of sludge granulation (Fig. 3). Compared to B1, dosing with GAC in B2 brought about the faster formation of larger granules. The mean size of the B2 sludge first increased from 181 to 272 μm after only 12 days and granule formation became apparent after about 2 weeks. The size increased continuously with the formation and growth of granules and the mature granules had a mean size of around 360 μm. In comparison, the sludge in B1 and B3 increased in size to about 330 and 280 μm, respectively, after 60 days (Fig. 3). Thus, the use of GAC helped the retention and growth of bacteria in attached-growth mode, which shortened the granulation period from about 6 weeks to less than 3 weeks. Unlike the addition of GAC, dosing with PAC did not appear to accelerate the granulation process.

The concentrations of nitrogen in various forms (e.g. NH_4^+-N, NO_2^--N and NO_3^--N) in the effluent from the bioreactors were measured during the experiment. The bioreactors performed well in ammonia removal, as was evident from the decrease in NH_4^+-N

concentration from around 400 to less than 4 mg N/L (Fig. 4). Initially, complete nitrification was observed in the bioreactors with a low NO_2^--N content in the effluent. Along with the formation of granules, partial nitrification became more predominant and the NO_3^--N in the effluent dropped to a level below 30 mg N/L. In the early start-up phase, little nitrite accumulated in the reactors and most of the NH_4^+-N was oxidized to NO_3^--N. Upon the completion of sludge granulation, more nitrite had accumulated in the reactors and the nitrate concentration had decreased continuously to a low level. After about 30 days, more than 90% of the N content was in the form of NO_2^--N and less than 10% of the nitrogen was NO_3^--N. It is apparent that sludge in a granular form favors partial nitrification over complete nitrification. In other words, compared to activated sludge flocs, granular sludge in the bioreactors would help halt ammonia oxidation at the level of nitritation or partial nitrification and hence keep it from reaching complete nitrification.

Sludge granulation is a method of microbial immobilization used to preserve a high population of nitrifying bacteria within a reactor [15]. The biomass yields of AOB and NOB are rather low with yield coefficients of 0.14 and 0.072 g/g N, respectively [27]. The selective sludge discharge not only contributed to the formation of granules, but also led to the accumulation of more AOB than NOB in the granular sludge. Meanwhile, the high-strength ammonia influent would promote more AOB growth. In addition, larger granules helped maintain a low DO condition within the granules, which is unfavorable to NOB function and growth. Biological nitrogen removal by mature granules was reported to be performed through denitrification via mainly NO_2^--N instead of NO_3^--N, probably due to the large size of granules formed [28]. Therefore, a strict DO control (< 1.5 mg/L) which is commonly needed for partial nitrification would not be required [8]. Therefore, granular sludge provided an environment highly favorable to an effective nitritation operation. Subsequently, nitrite produced by partial nitrification can be readily treated for denitrification by anammox or

similar processes [3,4]. In fact, the nitritation-anammox process for nitrogen removal has been recently reported for treating a low C/N wastewater by dosing hydroxylamine into aerobic granules at a high DO and ambient temperature [29].

3.2. Characteristics of the AOB granular sludge

The settleability and compressibility of the sludge improved significantly with the aerobic granulation in the bioreactors. The average settling velocities of mature granules from B1, B2 and B3 were 2.15±0.90, 5.15±0.41 and 3.80±0.26 mm/s, respectively (Table 1) – much faster than the activated sludge flocs with a settling velocity of slower than 1.0±0.12 mm/s. The addition of AC made the B2 and B3 granules settle faster than the B1 granules. Dosing with GAC in B2 formed larger granules with the best sludge settleability (Fig. 3, Table 1). Compared to the seed sludge SVI_5 of 80.5±8.0 ml/g, the granular sludge from B1, B2 and B3 had SVI_5 values of 30.0±2.1, 34.5±1.8 and 26.2±1.5 ml/g, respectively. Dosing with PAC produced smaller granules with better sludge compressibility. GAC has a large, specific surface area and a fast settling velocity. Its coarse pores and rough surface provided a favorable microenvironment for AOB attachment and growth. In other words, GAC functioned as biomass carriers to provide a core for microbial growth and granule formation. In contrast, the smaller PAC mixed together with microbial cells in the granules and appeared to weaken the structure of the granules to a certain extent, resulting in smaller granules than those with a GAC base.

Batch tests were conducted on the seed sludge and mature granules to investigate the nitrogen transformation dynamics during ammonia oxidation in the bioreactors. The results show that, while typical nitrifying activate sludge delivered complete nitrification, granular sludge performed reliably in partial nitrification (Fig. 5). NH_4^+-N with a high initial concentration of 400 N/L was oxidized within 10 h by all of the sludge samples. With the

activated sludge flocs from B0, more than 95% NH_4^+-N was converted to NO_3^--N. However, for mature granules from B1, B2 and B3, more than 95% of NH_4^+-N was converted to NO_2^--N, and NO_3^--N accounted for only about 5% or less of the N content in the treated effluent. Among the 3 granular sludge reactors with the same biomass concentration of 2 g VSS/L, B2 displayed the fastest NH_4^+-N oxidation rate while B3 showed the slowest NO_2^--N production rate. For the feed NH_4^+-N concentrations of 400 mg N/L, the mature granules from B2 had a specific NH_4^+-N degradation rate of 0.77 g NH_4^+-N/g VSS·d, which is considerably higher than that of the mature granules from B1 and B3 with values of 0.70 and 0.58 g NH_4^+-N/g VSS·d, respectively. Aerobic nitrifying granules were reported to have a specific NH_4^+-N degradation rate of 0.70 g NH_4^+-N/g VSS·d [30]. However, a lower specific NH_4^+-N degradation rate of only 0.14 g NH_4^+-N/g VSS·d was reported for granular sludge with a nitritation function treating the sludge reject water [19].

3.3. Microbial population dynamics during sludge granulation

Well-resolved DGGE bands were obtained for the biomass sludge from B0 (seed sludge), B1, B2 and B3 (Fig. 6a). In a mixture of extracted DNA, less abundant sequences may not be amplified sufficiently to form visible DGGE bands [25,31]. The abundance of the dominant microbial species in the sludge samples were determined based on a quantitative DGGE analysis (Fig. 6b). Sludge granulation had an apparent effect on both species selection and accumulation. The DGGE profile and quantitative analysis show that Band-1 (*Nitrosomonas sp.*, one of the AOB species) occurred in all of the DGGE profiles for both the seed and the granular sludge. The abundance of *Nitrosomonas sp.* remained stable and reached about 20% after sludge granulation in all three reactors. This suggests that aerobic granulation through selective sludge discharge might help keep AOB in the bioreactors. The species indicated by Band-3 was dominant in seed nitrifying sludge with a 25% proportion; however, it decreased

greatly and almost disappeared in mature granules in all three reactors. This species is closely related to the genera *Nitrospira sp.* (Table 2), which is one of the NOB species that oxidizes nitrite to nitrate. Thus, sludge granulation did not result in NOB enrichment in the reactors. The results of bacterial DGGE analysis are consistent with the performance of nitrification in the bioreactors. During the sludge granulation process, the reactors were converted from complete nitrification to nitritation with little nitrate in the effluent (Fig. 4). Partial nitrification was achieved and stable for mature granules because AOB became much more dominant than NOB in the granular sludge within the bioreactors.

The microbial communities were similar between the mature granules in B1, B2 and B3, despite the apparent differences in the physical characteristics of the granules. The addition of AC might have affected the rate of granulation and the morphology of the granules, but did not significantly change the bacterial communities in the sludge. The proportions of some of the species (Bands-4, -5 and -7) remained at high levels or even increased in the bioreactors after sludge granulation (Fig. 6b). They were the microbial species related to the genera *γ-proteobacteria*, *β-proteobacteria* and *Sphingobacteria* (Table 2). The class of *Sphingobacteria* is composed of bacteria capable of producing sphingolipids [32]. Certain complex glycosphingolipids have been found to be involved in specific microbial functions such as cell recognition, signaling for attached-growth and biofilm formation [33]. The species related to Band-5 match *Denitromonas indolicum*, which is a marine microbial species that has been observed in granular sludge for the likely simultaneous removal of sulfur, nitrogen and carbon [34,35]. These species might have contributed to the formation and structure of the aerobic granules for the partial nitrification treatment of high-strength ammonia wastewater.

4. Conclusions

- Aerobic sludge granules were produced for partial nitrification through the selective discharge of small and slow-settling sludge flocs in the treatment of high-strength, ammonia-based (400 mg NH_4^+-N/L) inorganic wastewater.
- Dosing the sludge with GAC effectively accelerated the granulation process, which shortened the granulation period from about 6 weeks to less than 3 weeks with the formation of large and fast-settling granules. In contrast, dosing with PAC led to the slower formation of smaller granules.
- Sludge granulation with selective sludge discharge helped halt ammonia oxidation at the level of partial nitrification instead of complete nitrification, which is highly favorable to a stable and effective nitritation operation.
- According to DNA-based molecular analysis, aerobic granulation resulted in an enrichment of AOB and a reduction of NOB in the granular sludge.

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References

[1] O. Turk, D.S. Mavinic, Benefits of using selective inhibition to remove nitrogen from highly nitrogenous wastes, Environ. Technol. Lett. 8 (1987) 419-426.

[2] S.W.H. Van Hulle, H.J.P. Vandeweyer, B.D. Meesschaert, P.A. Vanrolleghem, P. Dejans, A. Dumoulin, Engineering aspects and practical application of autotrophic nitrogen removal from nitrogen rich streams, Chem. Eng. J. 162 (2010) 1-20.

[3] Z.Y. Ji, Y.G. Chen, Using sludge fermentation liquid to improve wastewater short-cut nitrification-denitrification and denitrifying phosphorus removal via nitrite, Environ. Sci. Technol. 44 (2010) 8957-8963.

[4] L. Zhang, J.C. Yang, K. Furukawa, Stable and high-rate nitrogen removal from reject water by partial nitrification and subsequent anammox, J. Biosci. Bioeng. 110 (2010) 441-448.

[5] C. Muñoza, D. Rojasa, O. Candiab, L. Azocarb, C. Bornhardtb, C. Antileob, Supervisory control system to enhance partial nitrification in an activated sludge reactor, Chem. Eng. J. 145 (2009) 453-460.

[6] I. Jubany, J. Lafuente, J.A. Baeza, J. Carrera, Total and stable washout of nitrite oxidizing bacteria from a nitrifying continuous activated sludge system using automatic control based on oxygen uptake rate measurements, Water Res. 43 (2009) 2761-2772.

[7] K. Furukawa, Y. Inatomi, S. Qiao, L. Quan, T. Yamamoto, K. Isaka, T. Sumino, Innovative treatment system for digester liquor using anammox process, Bioresource Technol. 100 (2009) 5437-5443.

[8] A. Bartroli, J. Perez, J. Carrera, Applying ratio control in a continuous granular reactor to achieve full nitritation under stable operating conditions, Environ. Sci. Technol. 44 (2010) 8930-8935.

[9] H.J. Yun, D.J. Kim, Nitrite accumulation characteristics of high strength ammonia wastewater in an autotrophic nitrifying biofilm reactor, J. Chem. Technol. Biotechnol. 78 (2003) 377-383.

[10] C. Fux, D. Huang, A. Monti, H. Siegrist, Difficulties in maintaining long-term partial

nitritation of ammonium-rich sludge digester liquids in a moving-bed biofilm reactor (MBBR), Water Sci. Technol. 49 (11-12) (2004) 53-60.

[11] M.S. de Graaff, G. Zeeman, H. Temmink, M.C.M. van Loosdrecht, C.J.N. Buisman, Long term partial nitritation of anaerobically treated black water and the emission of nitrous oxide, Water Res. 44 (2010) 2171-2178.

[12] Y. Liu, J.H. Tay, State of the art of biogranulation technology for wastewater treatment, Biotechnol. Adv. 22 (2004) 533-563.

[13] L. Qin, J.H. Tay, Y. Liu, Selection pressure is a driving force of aerobic granulation in sequencing batch reactors, Process Biochem. 39 (2004) 579-584.

[14] A.J. Li, X.Y. Li, Selective sludge discharge as the determining factor in SBR aerobic granulation: Numerical modeling and experimental verification, Water Res. 43 (2009) 3387-3396.

[15] S. Tsuneda, T. Nagano, T. Hoshino, Y. Ejiri, N. Noda, A. Hirata, Characterization of nitrifying granules produced in an aerobic upflow fluidized bed reactor, Water Res. 37 (2003) 4965-4973.

[16] M. Belmonte, J.R. Vazquez-Padin, M. Figueroa, A. Franco, A. Mosquera-Corral, J.L. Campos, R. Mendez, Characteristics of nitrifying granules developed in an air pulsing SBR, Process Biochem. 44 (2009) 602-606.

[17] D.J. Kim, D. Seo, Selective enrichment and granulation of ammonia oxidizers in a sequencing batch airlift reactor, Process Biochem. 41 (2006) 1055-1062.

[18] X.Y. Shi, H.Q. Yu, Y.J. Sun, X. Huang, Characteristics of aerobic granules rich in autotrophic ammonium-oxidizing bacteria in a sequencing batch reactor, Chem. Eng. J. 147 (2009) 102-109.

[19] S. Lopez-Palau, A. Pericas, J. Dosta, J. Mata-Alvarez, Partial nitrification of sludge reject water by means of aerobic granulation, Water Sci. Technol. 64 (9) (2011)

1906-1912.

[20] J.R. Vazquez-Padin, M. Figueroa, J.L. Campos, A. Mosquera-Corral, R. Mendez, Nitrifying granular systems: A suitable technology to obtain stable partial nitrification at room temperature, Sep. Purif. Technol. 74 (2010) 178-186.

[21] A. Bartroli, J. Carrera, J. Perez, Bioaugmentation as a tool for improving the start-up and stability of a pilot-scale partial nitrification biofilm airlift reactor, Bioresource Technol. 102 (2011) 4370-4375.

[22] A.J. Li, X.Y. Li, H.Q. Yu, Granular activated carbon for aerobic sludge granulation in a bioreactor with a low-strength wastewater influent, Sep. Purif. Technol. 80 (2011) 276-283.

[23] L. Ye, T. Zhang, Estimation of nitrifier abundances in a partial nitrification reactor treating ammonium-rich saline wastewater using DGGE, T-RFLP and mathematical modeling, Appl. Microbiol. Biotechnol. 88 (2010) 1403-1412.

[24] W.Q. Zhuang, J.H. Tay, S. Yi, S.T.L. Tay, Microbial adaptation to biodegradation of tert-butyl alcohol in a sequencing batch reactor, J. Biotechnol. 118 (2005) 45-53.

[25] A.J. Li, S.F. Yang, X.Y. Li, J.D. Gu, Microbial population dynamics during aerobic sludge granulation at different organic loading rates, Water Res. 42 (2008) 3552-3560.

[26] APHA, Standard methods for the examination of water and wastewater, 21st ed. Washington, DC, USA: American Public Health Administration, 2005.

[27] R. Blackburne, V.M. Vadivelu, Z. Yuan, J. Keller, Determination of growth rate and yield of nitrifying bacteria by measuring carbon dioxide uptake rate, Water Environ. Res. 79 (2007) 2437-2445.

[28] E. Isanta, M.E. Suarez-Ojeda, A.V. del Rio, N. Morales, J. Perez, J. Carrera, Long term operation of a granular sequencing batch reactor at pilot scale treating a low-strength wastewater, Chem. Eng. J. 198 (2012) 163-170.

[29] G.J. Xu, X.C. Xu, F.L. Yang, S.T. Liu, Y. Gao, Partial nitrification adjusted by hydroxylamine in aerobic granules under high DO and ambient temperature and subsequent Anammox for low C/N wastewater treatment, Chem. Eng. J. 213 (2012) 338-345.

[30] F. Fang, B.J. Ni, X.Y. Li, G.P. Sheng, H.Q. Yu, Kinetic analysis on the two-step processes of AOB and NOB in aerobic nitrifying granules, Appl. Microbiol. Biotechnol. 83 (2009) 1159-1169.

[31] C.A. Eichner, R.W. Erb, K.N. Timmis, I. Wagner-Döbler, Thermal gradient gel electrophoresis analysis of bioprotection from pollutant shocks in the activated sludge microbial community, Appl. Environ. Microbiol. 65 (1999) 102-109.

[32] D.R. Boone, R.W. Castenholz, G.M. Garrity, Bergey's Manual of Systematic Bacteriology, 2nd ed., vol. 1, Springer, New York, USA, 2001.

[33] Y.A. Hannun, L.M. Obeid, The ceramide-centric universe of lipid-mediated cell regulation: Stress encounters of the lipid kind, J. Biol. Chem. 277 (2002) 25847-25850.

[34] C. Chen, N.Q. Ren, A.J. Wang, Z.G. Yu, D.J. Lee, Simultaneous biological removal of sulfur, nitrogen and carbon using EGSB reactor, Appl. Microbiol. Biotechnol. 78 (2008) 1057-1063.

[35] G. Zuo, D.J. Roberts, S.G. Lehman, G.W. Jackson, G.E. Fox, R.C. Willson, Molecular assessment of salt-tolerant, perchlorate- and nitrate-reducing microbial cultures, Water Sci. Tech. 60 (7) (2009) 1745-1756.

Table 1 Characteristics of the seed activated sludge from B0 and mature granules cultivated in B1, B2 and B3.

Bioreactor	**B0 (Seed sludge)**	**B1**	**B2**	**B3**
Mean size (μm)	181.0	332.4	358.6	281.4
Settling velocity (mm/s)	1.0±0.12	2.15±0.90	5.15±0.41	3.80±0.26
SVI_5 (ml/g VSS)	80.5±8.0	30.0±3.1	34.5±1.8	26.2±2.5

Table 2 Phylogenetic analysis of the dominant DGGE bands compared to the clone library.

Band No.	**Closest relatives (accession no.)**	**Identity (%)**	**Phylogenetic division**
B-1	*Nitrosomonas sp.* NM 107 16S ribosomal RNA gene (AF272416.1)	99	*β-proteobacteria*
B-3	Uncultured *Nitrospira sp.* partial 16S ribosomal RNA, clone 28 T9d-oil (FM242344.1)	98	*Nitrospira*
B-4	Uncultured gamma proteobacterium clone NdSurf190 16S ribosomal RNA gene (FJ753238.1)	96	*γ-proteobacteria*
B-5	*Denitromonas indolicum* strain MPKc 16S ribosomal RNA gene (AY972852.1)	97	*β-proteobacteria*
B-7	*Flexibacter aggregans* gene for 16S rRNA, strain: IFO 15974 (AB078038.1)	97	*Sphingobacteria*

Figure Captions

Fig. 1. Seed sludge and mature granules produced in the bioreactors: (a) seed activated sludge from B0, (b) granules from B1, (c) granules from B2 and (d) granules from B3 (Bar = 0.5 mm).

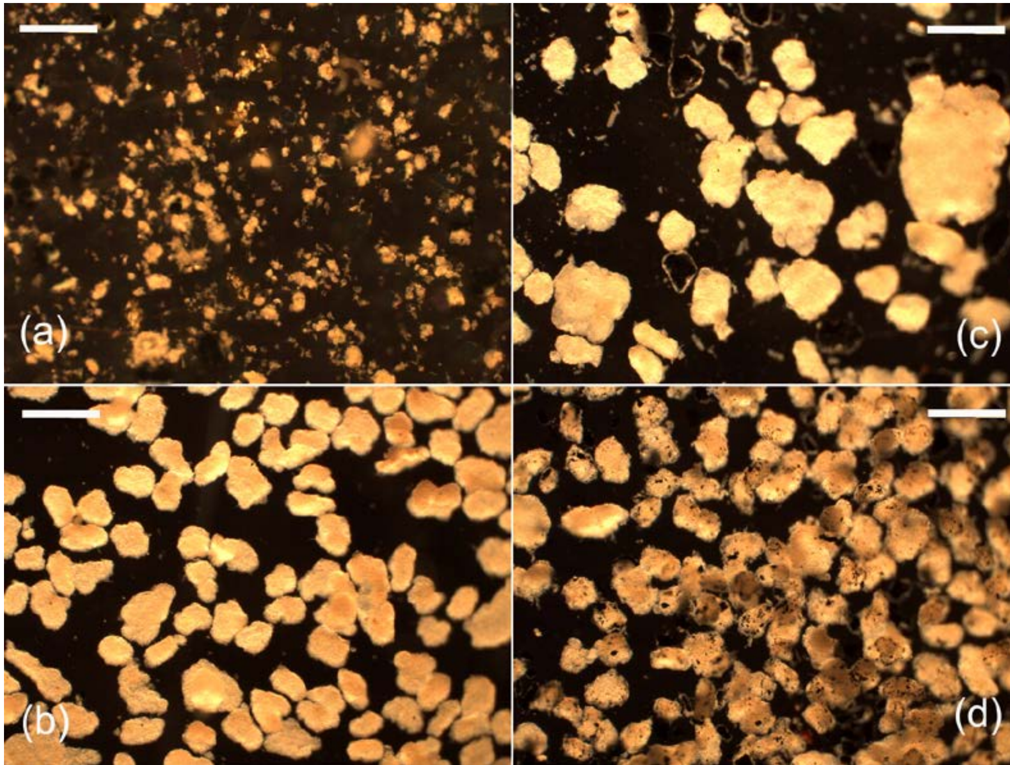
Fig. 2. Process parameters of the three bioreactors: (a) MLVSS, (b) F/M ratio and (c) SRT.

Fig. 3. Changes in the mean sizes of the sludge flocs and granules in the three bioreactors during the granulation process.

Fig. 4. The ammonia oxidation performance of the three bioreactors during sludge granulation: (a) effluent NH_4^+-N, (b) effluent NO_2^--N and (c) effluent NO_3^--N.

Fig. 5. Changes of NH_4^+-N, NO_2^--N and NO_3^--N concentrations during the batch tests of ammonia oxidation by the seed activated sludge (B0) and mature granules (B1, B2 and B3).

Fig. 6. DNA-base molecular analysis: (a) DGGE profiles of the bacterial communities for the seed sludge (SS) and granular sludge (GS) from B1, B2 and B3; (b) relative abundance of the dominant species determined from the DGGE bands.



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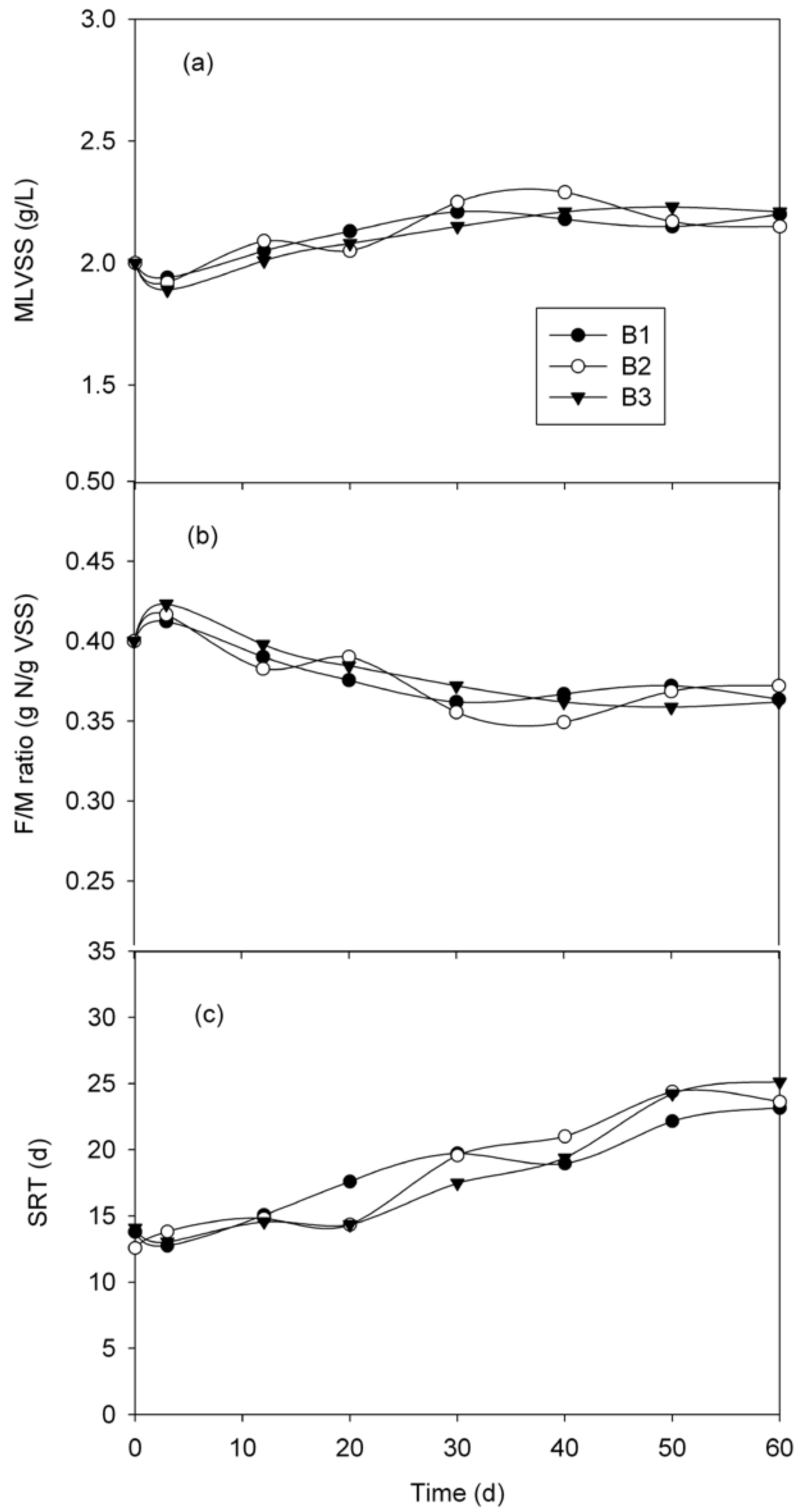

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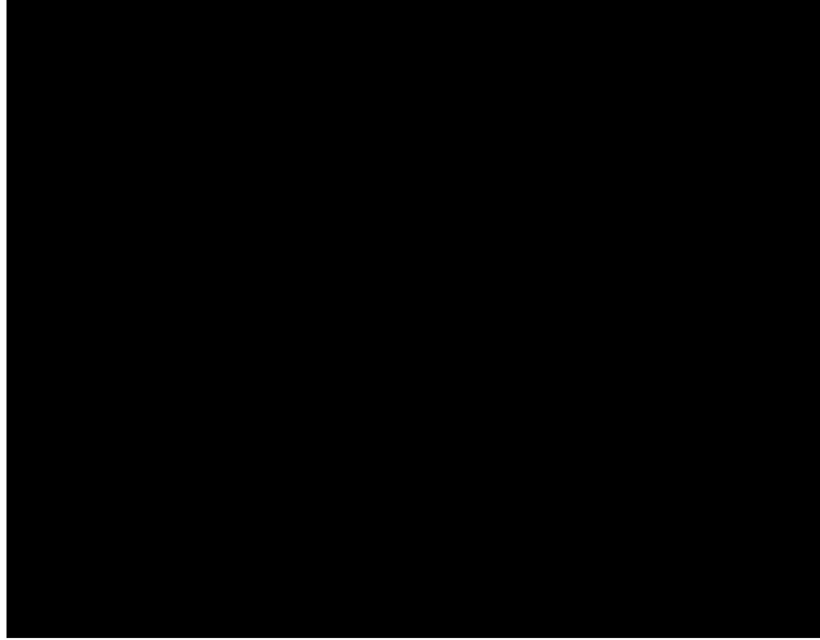

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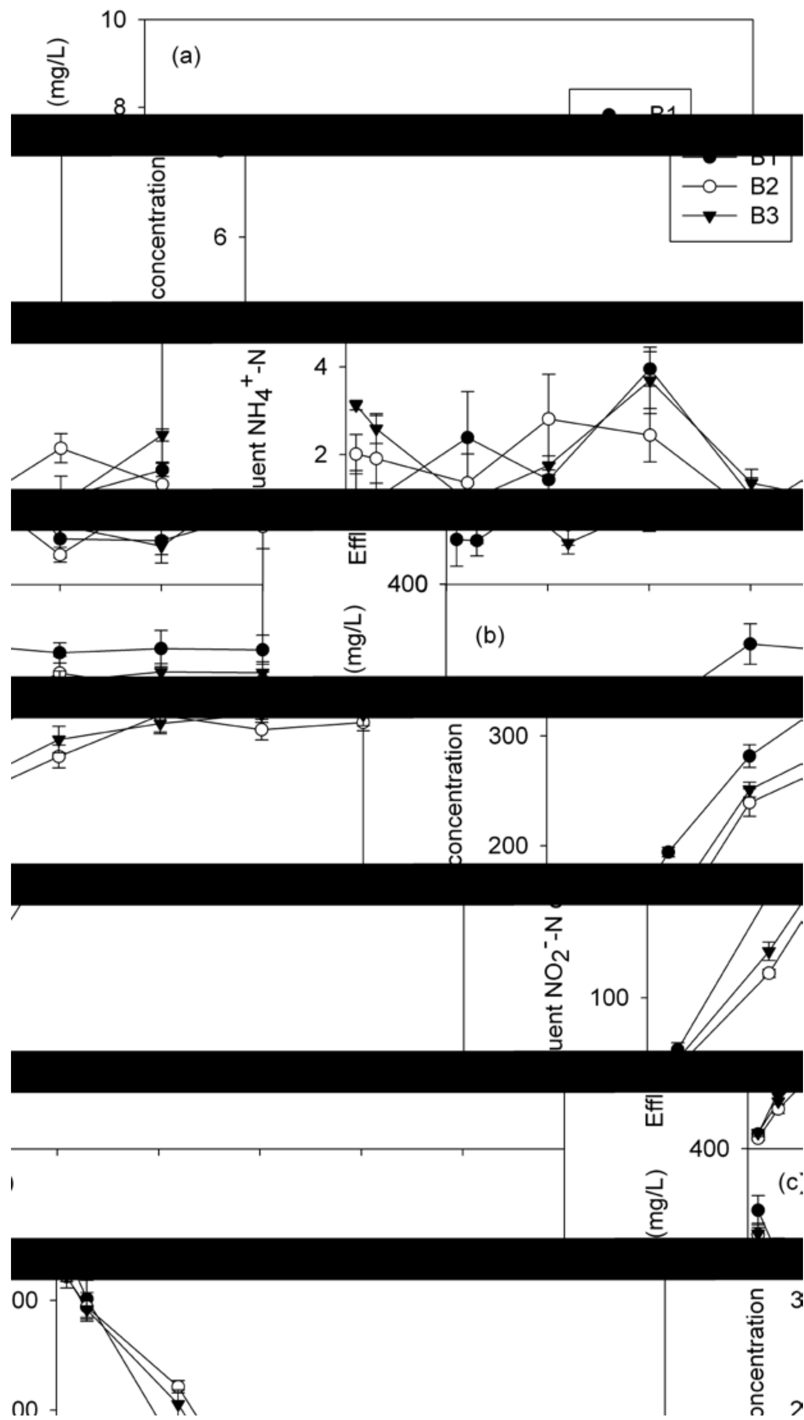

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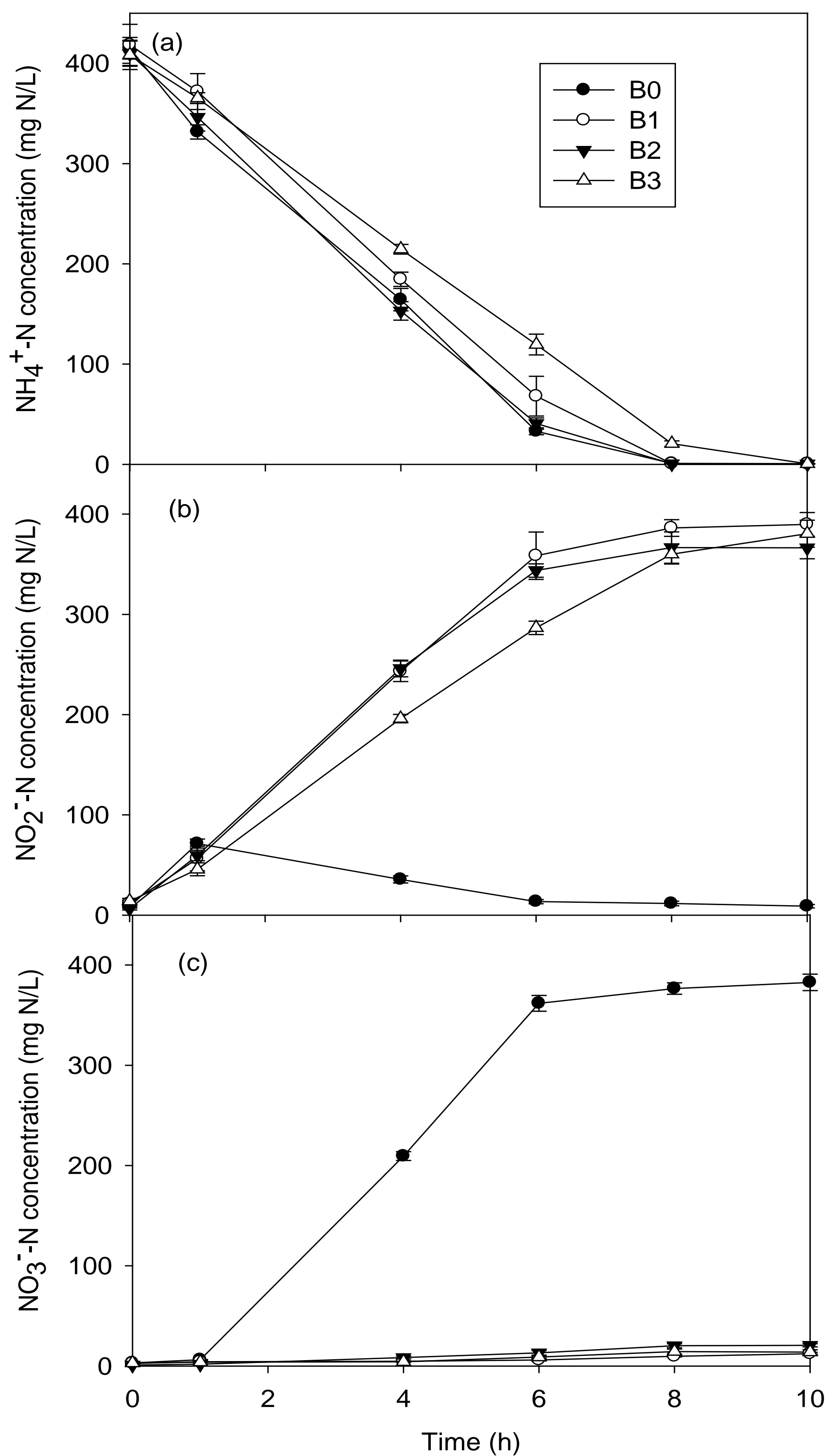

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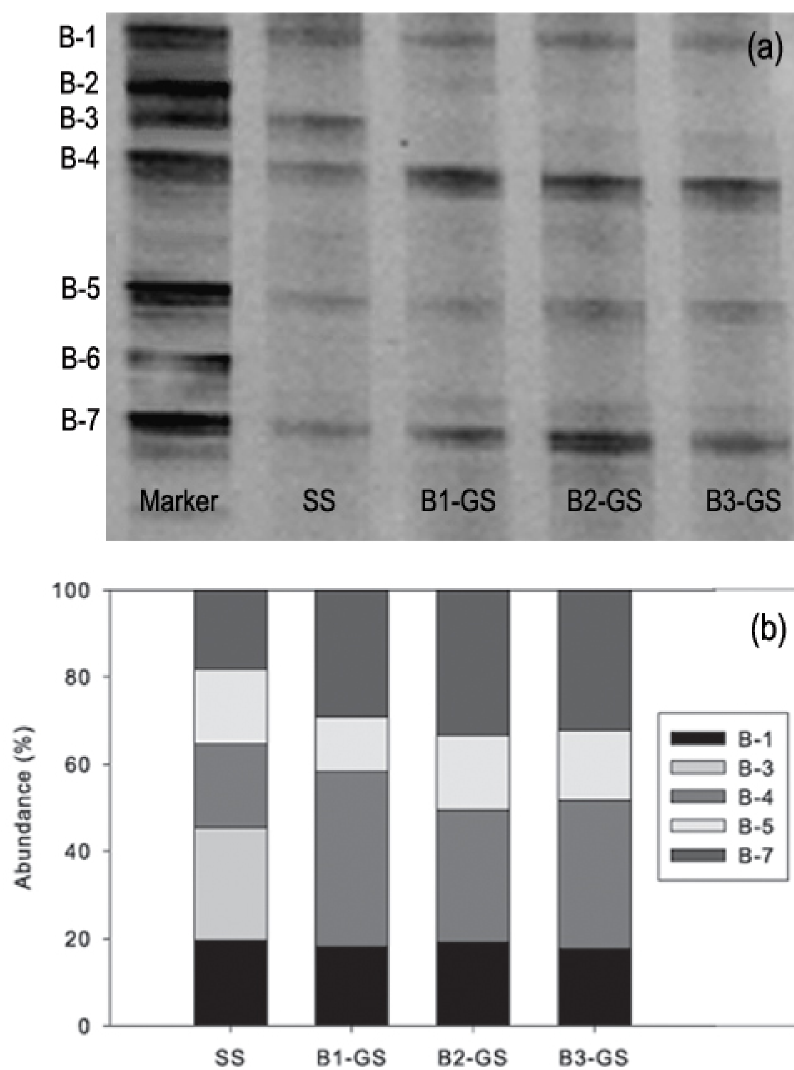

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