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Biodegradation of *p*-Nitrophenol by Aerobic Granules in a Sequencing Batch Reactor

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In this study, aerobic granules to treat wastewater containing *p*-nitrophenol (PNP) were successfully developed in a sequencing batch reactor (SBR) using activated sludge as inoculum. A key step was the conditioning of the activated sludge seed to enrich for biomass with improved settleability and higher PNP degradation activity by implementing progressive decreases in settling time and stepwise increases in PNP concentration. The aerobic granules were cultivated at a PNP loading rate of 0.6 kg/m^3·day, with glucose to boost the growth of PNP-degrading biomass. The granules had a clearly defined shape and appearance, settled significantly faster than activated sludge, and were capable of nearly complete PNP removal. The granules had specific PNP degradation rates that increased with PNP concentration from 0 to 40.1 mg of PNP/L, peaked at 19.3 mg of PNP/(g of VSS)·h (VSS = volatile suspended solids), and declined with further increases in PNP concentration as substrate inhibition effects became significant. Batch incubation experiments show that the PNP-degrading granules could also degrade other phenolic compounds, such as hydroquinone, *p*-nitrocatechol, phenol, 2,4-dichlorophenol, and 2,6-dichlorophenol. The PNP-degrading granules contained diverse microbial morphotypes, and PNP-degrading bacteria accounted for 49% of the total culturable heterotrophic bacteria. Denaturing gradient gel electrophoresis analysis of 16S rRNA gene fragments showed a gradual temporal shift in microbial community succession as the granules developed from the activated sludge seed. Specific oxygen utilization rates at 100 mg/L PNP were found to increase with the evolution of smaller granules to large granules, suggesting that the granulation process can enhance metabolic efficiency toward biodegradation of PNP. The results in this study demonstrate that it is possible to use aerobic granules for PNP biodegradation and broadens the benefits of using the SBR to target treatment of toxic and recalcitrant organic compounds.

Introduction

Nitroaromatic compounds, such as nitrophenol, nitrobenzene, nitrotoluene, and nitrobenzoates, are of considerable industrial importance as the main raw materials in the manufacture of dyes, pharmaceuticals, pesticides, and explosives (*1*). *p*-Nitrophenol (PNP) is one of the most important nitroaromatic compounds, both in terms of quantities used and potential environmental impacts (*2*). The annual demand for PNP in the U.S. from 1989 to 1994 ranged from 22 to 25.5 million pounds (*3*). PNP is used mainly for the manufacture of drugs (e.g. acetaminophen) and pesticides (e.g. methyl and ethyl parathion) and is also used in leather treatment, in dyestuff production, and for military purposes (*4*). Because of its frequent and widespread use, PNP can be found as a pollutant in industrial wastewater streams associated with its formulation, distribution, and application.

PNP can also be released into the subsurface and contaminate groundwater resources as a result of hydrolysis of pesticides and herbicides (*5*). If released into the environment, PNP contamination can pose a significant environmental and public health risk, due to its acute toxicity and its mutagenic potential. Animal studies have demonstrated that PNP can cause blood disorders. Acute exposure of PNP may lead to methemoglobin formation, liver and kidney damage, anemia, skin and eye irritation, and systemic poisoning (*1*, *3*). In addition, PNP contamination of rivers and groundwater resources may cause deleterious effects to ecological systems (*6*). For these reasons, the U.S. EPA has listed PNP as a priority pollutant and recommended restricting its concentration in natural waters to below 10 ng/L (*7*). Regulations have also been established to restrict PNP levels in industrial effluents. For example, average monthly concentrations of PNP should not exceed 162 μg/L (*8*).

There is therefore an obvious interest to effectively remediate industrial wastewaters and groundwaters that are contaminated with PNP. Biological treatment can be a viable alternative for PNP removal, since PNP is known to be mineralized by microorganisms under aerobic conditions (*9*). Many studies on PNP degradation have been reported, particularly on aspects relating to degradation pathways in pure bacterial cultures (*4*, *10*−*13*). But reports on aerobic PNP degradation in bioreactor systems are relatively limited (*14*−*16*), probably because microbial activity can be hindered by PNP's acute toxicity. The few bioreactor studies reported to date employed biomass immobilization strategies using attached biofilms to overcome the effects of PNP's acute toxicity. Compared with suspended systems, immobilized systems were able to retain higher amounts of PNP degrading bacteria, hence achieving higher PNP degradation activity and higher tolerance to PNP toxicity (*14*, *15*).

Aerobic granulation represents an innovative biomass immobilization strategy in biological wastewater treatment technology. Aerobic granules are self-immobilized microbial aggregates that are cultivated in sequencing batch reactors (SBRs) without reliance on artificial surfaces for biofilm attachment, hence rendering carrier material and bulky settling devices unnecessary (*17*−*19*). The basis for the formation of aerobic granules in the SBR is a repetitive selection for sludge particles such that denser components are retained in the system while lighter and dispersed particles are washed out. The aggregation of microorganisms into compact aerobic granules also confers additional benefits such as protection against predation and resistance to chemical toxicity. Aerobic granules had been previously shown to tolerate and degrade phenol at levels that were known to cause the breakdown of conventional activated sludge processes (*18*). In the case of PNP, the presence of the nitro group makes it more challenging to microbial degrada-

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tion than its unsubstituted analogue, phenol. The main objective of this study was to investigate the feasibility of developing aerobic granules for PNP biodegradation. Because activated sludge might not be suitable for direct inoculation into a reactor with a high input of PNP, a key step in the development of aerobic PNP-degrading granules was the conditioning of activated sludge seed to enrich for biomass that possessed both improved settleability and higher PNP degradation activity. The results from this study showed that the aerobic PNP-degrading granules could be cultivated from conditioned sludge at a PNP loading rate of 0.6 kg/m^3·day with glucose to promote growth of active biomass. This work will be useful in understanding how aerobic granules can be deployed to effectively treat PNP and other challenging organic compounds. The process reported in this study can potentially be applied to eliminate PNP in industrial effluents and contaminated groundwaters.

Experimental Section

Experimental Setup. A 2 L column-type SBR (120 cm height; 5 cm diameter) used for the study was housed in a temperature-controlled room at 25 °C and operated sequentially in 4 h cycles, with 2 min of influent filling, 213−229 min of aeration, 4−20 min of settling, and 5 min of effluent withdrawal. Fine air bubbles for aeration were supplied through a dispenser at the reactor bottom at a superficial gas velocity of 2.5 cm/s. Effluent was discharged at a volumetric exchange ratio of 50%, giving a hydraulic retention time (HRT) of 8 h. The abiotic loss of PNP in SBR was negligible under identical operation conditions.

The SBR was first inoculated with activated sludge from a full-scale wastewater reclamation plant in Singapore. The seed sludge was conditioned over a 23-day acclimation period to allow the biomass to adapt to PNP and to the short settling times required for successful granulation. During this acclimation period, the reactor was fed with a synthetic wastewater containing 500 mg/L of glucose and PNP. An initial PNP concentration of 50 mg/L was applied, and this was stepwise increased to a final PNP concentration of 200 mg/L toward the end of the acclimation period. In addition, the settling time in each 4-h cycle was reduced progressively from 20 min initially to 5 min toward the end of the acclimation period.

During the granule cultivation phase, the SBR was fed with a synthetic wastewater containing 500 mg/L glucose and 200 mg/L PNP. The settling time in each 4-h cycle was set at 5 min for the first 17 days, and then shortened to 4 min from day 18 until the end of the study.

The synthetic wastewater consisted of a buffered mineral salt medium (pH 7.1±0.3) with the following composition: Na_2HPO_4, 650 mg/L; KH_2PO_4, 200 mg/L; $CaCl_2$·H_2O, 30 mg/L; $MgSO_4$·$7H_2O$, 25 mg/L; and $FeSO_4$·$7H_2O$, 20 mg/L. Micronutrients were supplemented to the medium at 1 mL/L (*19*). Ammonium chloride (NH_4Cl) was used as nitrogen source, and the COD (chemical oxygen demand):NH_4^+−N ratio was maintained at 20:1. Wastewater containing 200 mg/L of PNP had a corresponding COD concentration of 0.83 kg COD/m^3 and an organic loading rate (OLR) of 2.5 kg COD/m^3·day. The OLR attributed to PNP alone was 0.9 kg COD/m^3·day.

Analytical Methods. During the granule cultivation phase, pH, biomass concentration (mixed liquor suspended solids [MLSS] and volatile suspended solids [VSS]), sludge volume index (SVI), specific gravity, and specific PNP-associated oxygen utilization rate (SPOUR) were periodically analyzed in accordance with the standard methods (*20*). The mean biomass size was measured with a laser particle size analysis system (Malvern Mastersizer 2600).

The TOC (total organic carbon) concentration was measured using a TOC analyzer (TOC-500, Shimadzu, Singapore). Nitrite and nitrate concentration were measured using an IC analyzer (LC-2010, Shimadzu, Singapore). To measure PNP concentration, samples were passed through a 0.22 μm syringe filter (Millipore) and adjusted to pH 9 or higher with 0.1 N NaOH as described by Ray et al. (*15*). The concentrations of PNP were spectrophotometrically determined using the absorbance values at 400 nm with a UV/vis spectrophotometer (Lambda Bio 20, Perkin-Elmer, USA). The concentrations of TOC, nitrite, and nitrate, as well as PNP, were quantified using standards of known concentrations.

For morphological observations, granules were fixed, dried, and then viewed with a scanning electron microscope (Stereoscan 420, Leica, Cambridge Instruments, U.K.) as described previously (*18*). Bacterial counts for total bacteria and PNP-degrading bacteria were determined with Bacto plate count agar and PNP mineral salt agar (this is the mineral salt medium described in Experiment Setup supplemented with 100 mg/L PNP and 1.5% Bacto agar), respectively. The ability of granules to degrade phenolic or nitroaromatic compounds was evaluated in 100-mL serum bottles which contained 50 mg/L of one of the following chemicals: phenol, hydroquinone, *p*-nitrocatechol, *p*-aminophenol, 2,4-dichlorophenol, or 2,6-dichlorophenol. The bottles were incubated overnight on a rotary shaker and substrate removal was confirmed through TOC analysis at the beginning and end of incubation.

Specific PNP degradation rates for granules were calculated from PNP degradation curves obtained from experiments carried out in a small reaction volume of 100 mL at a range of PNP concentrations up to 300 mg/L. A kinetic analysis of the degradation data was performed on the basis of Haldane's equation for an inhibitory substrate, $V = V_{max}S/[K_s + S + (S^2/K_i)]$, where V and V_{max} are the specific and the maximum specific substrate degradation rates (mg of PNP/(g of VSS)·h), respectively, and S, K_s and K_i are the substrate concentration, half-saturation constant, and inhibition constant (mg of PNP/L), respectively (*21*).

DNA Extraction, PCR and Denaturing Gradient Gel Electrophoresis (DGGE). Genomic DNA of PNP-degrading biomass was extracted on the basis of a protocol described previously (*22*). Approximately 200−300 mg (wet weight) of biomass was harvested in duplicate during the aeration stage of the SBR cycle and used immediately for DNA extraction. This involved bead beating followed by extraction with saturated phenol (pH 8.0), phenol:chloroform (1:1), and chloroform:isoamyl alcohol (24:1). The extracted DNA was precipitated overnight with a sodium acetate−ethanol mix at −20 °C and dissolved in sterile deionized water. Extracted DNA samples were stored in a −20 °C freeze before use.

PCR primers P2 and P3 (containing 40 bp of GC clamp) were used to amplify the variable V3 region of the bacterial 16S rRNA gene (corresponding to positions 341−534 in the *Escherichia coli* sequence) (*23*). Touchdown PCR was performed (*24*) with a Mastercycler gradient thermal cycler (Eppendorf AG, Germany) using a 100 μL (total volume) mixture containing Taq DNA polymerase (Promega Co., USA), 1 × thermophilic DNA Taq polymerase buffer B, $MgCl_2$, deoxynucleotide triphosphates, primers, and DNA extract as described previously (*22*). Successful PCR was confirmed by 2.0% agarose gel electrophoresis.

The PCR-amplified fragments were separated by DGGE using a DCode universal mutation detection system (Bio-Rad Laboratories) as described previously (*23*). A 25 mL 30−70% urea−formamide denaturant gradient gel [10% (w/v) acrylamide solution (40% acrylamide and bisacrylamide, 37.5:1 stock solution) in TAE buffer (40 mM Tris base, 20 mM sodium acetate, 1 mM Na_2−EDTA, pH 8.0)] was covered by a 4 mL of acrylamide stacking gel (10%) without denaturant. A 40 μL aliquot of PCR amplicons from DNA of biomass samples were loaded with 8 μL loading dye in each well. The

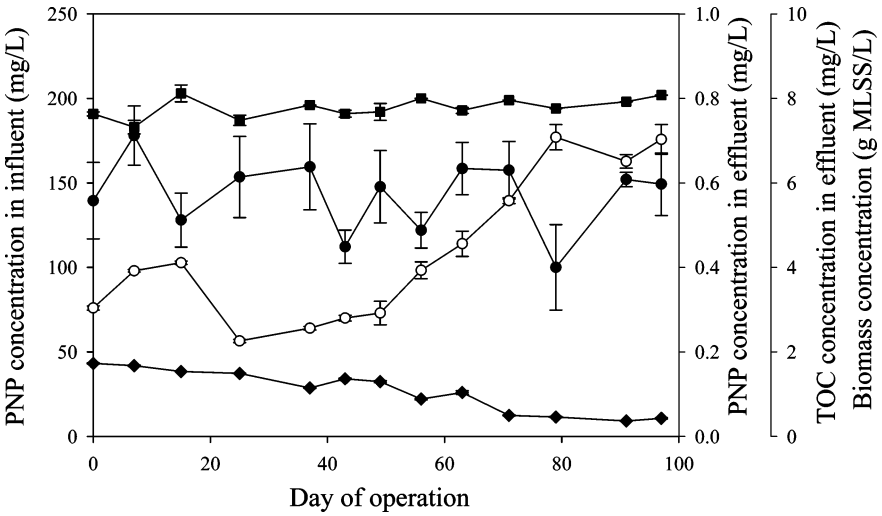

FIGURE 1. Profiles of PNP concentration in influent (■) and effluent (◆), TOC concentration in effluent (●), and in-reactor biomass concentration (○).

gel was placed in TAE buffer and run at 40 V for 30 min and then at 85 V for 15 h at 60 °C. After electrophoresis, the gel was stained with ethidium bromide for 30 min and photographed with an EDAS 290 gel imaging system (Eastman Kodak, USA). GelCompar II (version 1.5) software (Applied Maths, Belgium) was used for DGGE band pattern analysis. Binary coefficient Dice was applied to calculate the similarity of band patterns. The unweighted pair group method using arithmetic averages (UPGMA) was used to construct the DGGE dendrograms.

Results

Cultivation of Aerobic PNP-Degrading Granules. Activated sludge from a wastewater reclamation plant was first conditioned over a 23-day period to allow the biomass to adapt to PNP and to the short settling times required for successful granulation. The initial seed sludge had a mean biomass size of 0.140 mm, an SVI value of 134 mL/g, and a specific gravity of 1.0002 (Table 1 of the Supporting Information). SPOUR activity was not detected during incubation with 100 mg of PNP/L, indicating that the unacclimated sludge was initially unable to oxidize PNP. The acclimated sludge showed marked improvements in settleability and PNP degradation activity. The mean biomass size fell to 0.070 mm, the SVI value dropped to 32 mL/g, the specific gravity increased to 1.0011, and SPOUR activity was detected at 12.7 mg of O_2/(g of VSS)·h.

The acclimated sludge was used as the starting seed for the cultivation of aerobic PNP-degrading granules. This starting seed had a biomass concentration of 3040 mg of MLSS/L and demonstrated good PNP degradation activity (Figure 1). Compared to a PNP concentration of 200 mg/L in the SBR influent, PNP concentrations in the effluent were less than 0.2 mg/L during the first several weeks of the granule cultivation phase and further declined to below 0.05 mg/L beyond day 71. TOC concentrations in the effluent ranged from 4.0 to 7.1 mg/L and were probably attributed to soluble organic matter from biomass lyses.

Aerobic granules were first observed in the SBR as small spherical particles dispersed within amorphous sludge flocs on day 7 of the granule cultivation phase (Figure 1 of the Supporting Information). A distinct separation of granules from the sludge flocs was observed on day 25. The granules eventually grew to become the dominant form of biomass in the reactor, as evidenced by the gradual increase in mean biomass size and decrease in SVI beyond day 25 (Figure 2 of the Supporting Information). Except for a brief duration of several days when the system adjusted to the reduction in settling time from 5 to 4 min implemented on day 18, the biomass concentration in the reactor generally showed an upward trend to plateau at 7080 mg of MLSS/L from day 79. Stable granules were obtained in the SBR when conditions stabilized from day 79 to the end of the reactor operation. The mean biomass size, SVI, and specific gravity eventually stabilized at 0.386 mm, 12 mL/g, and 1.0035, respectively (Table 1 of the Supporting Information).

SPOUR activity was measured for reactor biomass in batch incubations with a PNP concentration of 100 mg/L to monitor changes in metabolic activity. The acclimated sludge on day 0 exhibited an initial metabolic activity toward PNP (Figure 2 of the Supporting Information). From days 7 to 25, the SPOUR values fluctuated from 10.6 to 14.1 mg of O_2/(g of VSS)·h. These fluctuations were associated with events such as the onset of granulation on day 7, the reduction in settling time on day 18, and the brief decline in biomass concentration from days 18 to 25. After day 25, the SPOUR values showed an upward trend and increased to finally stabilize at 22 mg of O_2/(g of VSS)·h from day 79 to the end of the reactor operation. The steady-state SPOUR values achieved at the end of the experiment were approximately 2-fold higher than the initial values. A plot of SPOUR against mean biomass size showed that larger aerobic granules had higher SPOUR activity (Figure 3 of the Supporting Information).

PNP Degradation and TOC Removal in One SBR Cycle. The removal of PNP and TOC and the release of nitrite and nitrate during one SBR cycle were examined on day 85 after reactor conditions have stabilized (Figure 2). PNP and TOC removal commenced immediately after influent feeding. Since the influent contained both glucose and PNP, the immediate removal of PNP and TOC indicated that glucose was not preferentially degraded at the expense of PNP when the benign substrate was supplied together with PNP. The PNP that was fed into the reactor was rapidly degraded within 1 h, while TOC removal took much longer.

Although the stoichiometric release of nitrite has previously been observed during the aerobic biodegradation of PNP in biofilm reactors (*15*, *16*), no nitrite release was detected during the SBR cycle investigated in the current study. However, increases in nitrate concentration were measured that were stoichiometrically related to the amounts of PNP degraded (inset in Figure 2).

Morphological and Physiological Characteristics of Aerobic Granules. SEM images showed that the PNP-degrading granules had a compact structure with many

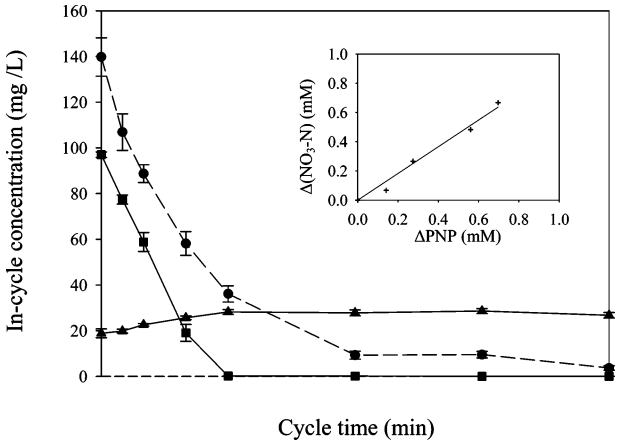

FIGURE 2. Profiles of PNP (■), TOC (●), and NO_3−N (▲) concentrations within a representative 4-h SBR cycle. Inset: plot of N released against PNP degraded (+) at various times in the first hour of the SBR cycle; values were calculated from the data in the main figure. The stoichiometric coefficient (*E*) of N released from PNP biodegradation with granules was obtained through linear regression. $E = 0.91 \pm 0.04$.

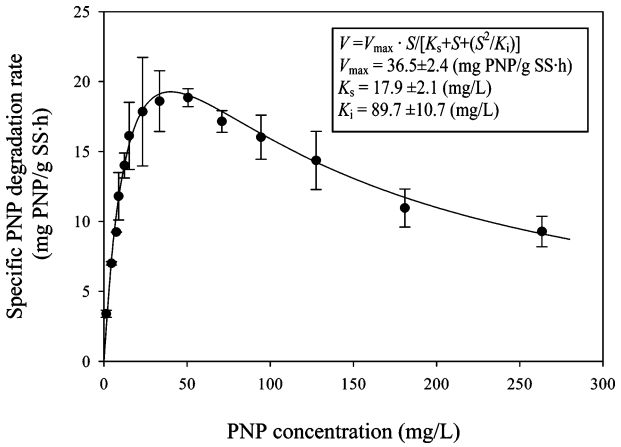

FIGURE 3. Specific PNP degradation rates of aerobic granules at different PNP concentrations (experimental results, ●; Haldane fit, —).

cauliflower-like clusters (Figure 4a of the Supporting Information). The outer surface of these clusters typically consisted of short rod-shaped bacteria embedded in an extracellular polymeric matrix (Figure 4b of the Supporting Information). Many slender rod-shaped bacteria could be observed below this matrix. In contrast, the zones between adjacent clusters contained a large diversity of microbial morphotypes, including bacterial rods and cocci and protozoa (Figure 4c of the Supporting Information).

The ability of granules to degrade PNP was evaluated by monitoring PNP disappearance at different PNP concentrations in batch culture for granules sampled on day 95. The specific PNP degradation rate increased with PNP concentration from 0 to 40.1 mg of PNP/L, peaked at 19.3 mg of PNP/(g of VSS)·h, and declined with further increases in PNP concentration as substrate inhibition effects became significant (Figure 3). The Haldane equation was used to fit the degradation data, with calculated kinetic parameters of V_{max} = 36.5 mg of PNP/(g of VSS)·h, K_s = 17.9 mg/L, and K_i = 89.7 mg/L, and a correlation coefficient (R^2) of 0.97.

The possible PNP degradation pathways employed by the granules were assessed indirectly by examining the ability of granules to utilize potential intermediates. Under aerobic conditions, the major intermediates would include hydroquinone (HQ) and *p*-nitrocatechol (PNC) (*9*), while *p*-aminophenol (PAP) would be an intermediate encountered under anaerobic conditions (*25*). The PNP-degrading granules could degrade both HQ and PNC, but not PAP. The PNP-degrading granules also possessed broad substrate utilization, as they could degrade phenolic compounds such as phenol, 2,4-dichlorophenol, and 2,6-dichlorophenol.

Characterization of Microbial Community. Counts of colony-forming units (CFUs) revealed that the granules contained $(8.2 \pm 2.2) \times 10^{10}$ CFU/(g of MLSS) of heterotrophic bacteria and $(4.0 \pm 1.4) \times 10^{10}$ CFU/(g of MLSS) of PNP-degrading bacteria. PNP-degrading bacteria accounted for 49% of the total heterotrophic CFUs.

DGGE was employed to generate genetic fingerprints to provide information on the composition and diversity of microbial communities in the reactor biomass. Figure 5 of the Supporting Information shows DGGE fingerprints of amplified 16S rRNA gene fragments from the reactor biomass sampled over a 97-day period. Fingerprint similarity was analyzed by applying the UPGMA algorithms. A gradual succession in the microbial community was observed, and DGGE fingerprints from similar sampling periods were highly similar to one another. The UPGMA analysis clustered the 12 DGGE fingerprints into three groups, with intragroup similarity of 71−97% and intergroup similarity of 61% (Figure 6 of the Supporting Information). Group I contained DGGE fingerprints from seed sludge (AS) and acclimated sludge (day 0; similarity ≈ 83%) and was associated with reactor start-up. Group II contained DGGE fingerprints from days 7 to 63 and was associated with the development of granules (similarity ≥ 71%). Group III contained DGGE fingerprints from days 79 to 97 and was associated with stable granules (similarity ≥ 97%).

Discussion

Cultivating Aerobic PNP-Degrading Granules. This study demonstrates that it was possible to cultivate aerobic granules in a SBR for biodegradation of PNP. A key strategy in the development of stable aerobic PNP-degrading granules was the addition of glucose as a cosubstrate to promote granule formation. This was different from past studies in which target contaminant was used as a sole carbon and energy source to cultivate the aerobic granules for biodegradation (*18*, *26*).

The strategy adopted herein was aimed to avoid the problems related to the retention of sufficient active biomass in SBR since combined effects from the inhibitory effect of PNP as well as hydraulic selection pressures in SBR can post a significant challenge to retain active biomass in SBR for granulation. PNP is an uncoupling agent for oxidative phosphorylation, which can cause serious inhibition to the microbial growth and activity (*27*). In addition, hydraulic selection pressures in SBR will boost the washout of biomass if it is not able to well-adapt to the presence of PNP.

Therefore, to improve the retention of active biomass in the SBR, glucose was added as a benign substrate to boost the growth of PNP-degrading biomass. Similar strategy has been adopted in PNP-degrading biofilm rectors to facilitate the biofilm adhesion and formation processes. For example, Bhatti et al. (*16*) added glucose along with PNP to cultivate the PNP-degrading sludge for immobilization on carriers and also added glucose to ensure an adequate acclimation of attached biofilms to PNP.

It needs to be pointed out that within-cycle measurements of PNP and TOC in this study showed that PNP was degraded simultaneously with TOC (Figure 2). The presence of glucose did not hinder PNP degradation, and there was no preferential degradation of glucose. These findings validate the strategy of adding glucose to develop stable aerobic granules efficient at PNP degradation.

PNP Degradation Kinetics in Aerobic Granules. The granules had a maximum specific PNP removal rate (V_{max}) of 36.5 mg of PNP/(g of VSS)·h. V_{max} in other studies involving SBRs for PNP removal ranged from 85.4 to 194 mg of PNP/(g of VSS)·h (Table 2 of the Supporting Information). These rates were associated with sludge flocs and were 2.3−5.3 times larger than the V_{max} reported for the aerobic granules in this study. The smaller rate in this study could be attributed to two main reasons. First, the compact structure of aerobic granules meant that a smaller specific surface area was presented to the substrate than in the case of the sludge flocs. Moreover, the compact granular structure created a diffusion barrier such that microorganisms in the granule interior would encounter a lower concentration of PNP than existed in the bulk milieu. Second, the ratio of PNP to the total COD used in this study was 0.36, which was lower than the ratios used elsewhere (*28*). Tomei and co-workers (*21*) found that V_{max} correlated positively with the PNP:COD_{total} ratio. The colony counts showed that the culturable PNP-degrading bacteria accounted for 49% of the total heterotrophic bacterial population. This pointed to the existence of a large group of bacteria within the granules that did not contribute directly to PNP degradation. Therefore, normalizing the rate of PNP removal to the total biomass concentration resulted in a lower specific PNP degradation rate. However, the ability to retain high amounts of biomass allowed aerobic granules to paradoxically achieve higher volumetric PNP removal rates than reported for other nongranular systems. The volumetric PNP removal rate for the SBR in this study was 256 mg of PNP/L·h. This was significantly higher than the range of 94−188 mg of PNP/L·h reported in other studies (*21*, *28*).

Kinetic studies of granules with PNP as the sole substrate yielded a half-saturation coefficient K_s = 17.9 mg/L and a substrate inhibition coefficient K_i = 89.7 mg/L. K_s was similar to values reported elsewhere (*21*, *28*), but K_i was significantly higher than the range of 12−30.7 mg of PNP/L reported previously (*21*, *28*). The higher K_i indicated that the aerobic granules were more resilient than other forms of biomass toward PNP toxicity. One explanation, which has been elucidated elsewhere, was that the compact granular structure served to shelter the microorganisms against the chemical toxicity that might exist in the surrounding milieu (*18*). Compared to other biofilm systems, aerobic granules are less susceptible to the effects of substrate inhibition and can potentially be deployed to degrade higher concentrations of PNP.

Granulation Enhances Metabolic Activity toward PNP. As shown in Figure 3 of the Supporting Information larger aerobic granules had higher SPOUR activity. This finding that metabolic activity increased with granule size contrasted with observations of biofilms growing at the expense of benign substrates, where an increase in biomass size was usually associated with a decrease in specific biomass activity because of mass-transfer limitation (*18*). In the case of single-species biofilms cultivated on toxic substrates, immobilization of PNP-degrading bacteria was also correlated with a decrease in specific activity because of mass-transfer limitation (*29*). However, the current study was consistent with observations of multispecies biofilms cultivated on toxic substrates. For example, sludge biomass immobilized on carriers possessed higher specific PNP degradation rates than suspended sludge flocs when acclimated to increased PNP concentrations (*14*). Similar observations were also reported for granulation systems where higher specific phenol degradation activities were attained after microbial cells aggregated into compact aerobic granules (*18*).

Many mechanisms could be involved in the enhancement of metabolic efficiency in the granules. First, PNP metabolic activity could be enhanced by the retention of specific PNP-degrading microorganisms in the granules. Second, this enhancement could be the result of syntrophic interactions between the community members in the granules. Finally, metabolic enhancement could result from increase of specific degradation activity through the exchange of genetic material among the bacteria in granules. The increased PNP-degrading activity associated with increasing granule size could be the result of the specific or synergistic effects of the mechanisms mentioned above.

In summary, this study demonstrates the successful cultivation of aerobic granules in an SBR for PNP biodegradation. Operating strategies, such as the addition of glucose as well as incremental adjustments in settling time and PNP concentrations were adopted to develop aerobic PNP-degrading granules with excellent settling characteristics and high volumetric PNP biodegradation activity. This study broadens the benefits of using the SBR to target the biodegradation of toxic and recalcitrant organic compounds. From a practical perspective, refinements that leverage on the operational flexibility of the SBR, such as introduction of anoxic−aerobic phases within an SBR cycle, can be employed to remove nitrate and improve the treatment process.

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Supporting Information Available

Figures showing microscope images of aerobic granules, time profiles of granule characteristics, a plot of SPOUR over mean biomass size, a DGGE fingerprint, and a DGGE dendrogram and tables listing characteristics of seed sludge, acclimated sludge and granules, and parameters for PNP degradation kinetics. This material is available free of charge via the Internet at http://pubs.acs.org.

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