Desalination and Water Treatment
www.deswater.com
doi: 10.1080/19443994.2014.927118

53 (2015) 2412–2420
March

Taylor & Francis
Taylor & Francis Group

Optimization of the mechanical strength of PVA/alginate gel beads and their effects on the ammonia-oxidizing activity

Hyokwan Bae[a,b], Heejeong Yang[c], Minkyu Choi[a], Yun-Chul Chung[a], Seockheon Lee[a,*], Young Je Yoo[b,*]

[a]*Center for Water Resource Cycle Research, Korea Institute of Science and Technology, 39-1 Hawolgok-Dong, Seongbuk-Gu, Seoul 136-791, Republic of Korea, Tel. +82 2 958 5829; Fax: +82 2 958 5839; email: seocklee@kist.re.kr (S. Lee)*
[b]*Interdisciplinary Program of Bioengineering, Seoul National University, San 56-1, Shilim-Dong, Kwanak-Gu, Seoul 151-742, Republic of Korea, Tel. +82 2 880 7411; Fax: +82 2 887 1659; email: yjyoo@snu.ac.kr (Y.J. Yoo)*
[c]*Water Environmental Engineering Research Division, National Institute of Environmental Research, Environmental Research Complex, Kyungseo-Dong, Seo-Gu, Incheon 404-708, Republic of Korea*

Received 8 September 2013; Accepted 7 October 2013

ABSTRACT

Poly (vinyl alcohol) (PVA)/alginate gel beads were fabricated to entrap ammonia-oxidizing bacteria. The PVA/alginate gel beads were prepared in different conditions to investigate the effects of the fabrication procedures on the mechanical strength and the initial ammonia-oxidizing activity. For the mechanical strength, the optimal conditions were analyzed using response surface analysis (RSA) considering the inter-correlated effects of the reaction times of cross-linking and phosphorylation. For RSA, nine trials resulted in a partial cubic polynomial equation, which best predicted the amount of residual debris after homogenization. In the model, the optimum conditions of 3.5 h of cross-linking and 5.6 h of phosphorylation were estimated to ensure the maximum mechanical strength. The initial ammonia-oxidizing activity was significantly affected by the cross-linking due to the highly acidic environment of pH 3.3, but it was not affected by the phosphorylation in pH 4.2. Batch experiments to measure the bioactivity showed that only 34.0% of the initial activity survived the fabrication procedure of the 4 h reaction in $B(OH)_3$ in comparison to the 1 h reaction. The lower initial ammonia-oxidizing activity contributed to the delayed acclimation period to achieve ammonia removal of more than 1 kg N/m^3 d, but the inhibitory effects were fully recovered in 5 d.

Keywords: Entrapment; PVA/alginate gel; Cross-linking; Phosphorylation; Response surface analysis; Ammonia-oxidation; Acclimation period

1. Introduction

Immobilization has been widely used in biological wastewater treatment processes to achieve a long solid retention time. Especially for slowly growing autotrophic nitrifying bacteria, a high concentration of biomass can be maintained through immobilization even at a high dilution rate. For biological processes, two types of immobilization techniques have been

*Corresponding authors.

utilized: the attached growth system (passive immobilization) and physical entrapment within porous matrices (active immobilization) [1].

For passive immobilization, supporting materials such as polyurethane are applied generally as foam carriers in the nitrification process [2]. The retention of the nitrifying biofilm on a solid substratum, which is supported by the binding forces between nitrifying bacteria and a support material and by interaction among cells, is affected by many factors. For example, the formation of biofilm is regulated by the pH, the ionic strength of the wastewater, the amount of extracellular polymeric substances (EPS), and the physicochemical characteristics of the surface material [3,4].

In comparison, active immobilization offers a rather simple methodology by physically locating nitrifying bacteria in a polymeric gel matrix. The conventional natural polymer used in the nitrification process is alginate, which forms Ca-alginate gel when sodium alginate is mixed with a $CaCl_2$ solution [5]. However, alginate gel is susceptible to mechanical destruction and biodegradation [6,7]. Therefore, nitrification processes have adopted synthetic polymeric gels such as poly (vinyl alcohol) (PVA) [8,9]. PVA is a promising type of synthetic polymer for the entrapment of nitrifying bacteria because it is cheap and nontoxic to micro-organisms. In this study, the active immobilization using PVA-$B(OH)_3$ cross-linking was applied to entrap ammonia-oxidizing bacteria (AOB).

PVA/alginate gel beads are formed by cross-linking PVA with $B(OH)_3$. Because PVA beads are sticky in the $B(OH)_3$ gelling solution, the agglomeration of PVA beads during the gelatin period can be prevented by the relatively rapid gelation of the Ca-alginate matrix [10]. For a further enhancement of the mechanical strength, phosphorylation in an orthophosphate solution was conducted with the PVA gel beads by the addition of a phosphate functional group through esterification of PVA [11]. Generally, an enhancement of the mechanical strength is achieved by the extended reaction time in the gelling solution with saturated $B(OH)_3$ and a phosphorylating solution. However, long-term incubation may have adverse effects on the bioactivity due to the destruction of the cell membrane and unbalanced metabolism which arise possibly due to the acidic environments of the solutions typically used [11]. AOB and nitrite-oxidizing bacteria (NOB) are responsible for the nitrification. The performance of the nitrification largely depends on the AOB activity because ammonia-oxidation is the rate-limiting step. AOB are sensitive to environmental conditions of pH, temperature, and toxic chemicals [12–14] and susceptible to washout due to their low growth rate of $2\ d^{-1}$, which is equivalent to a doubling time of 8 h [15,16]. Therefore, the initial activity of AOB is critical for the successful start-up of the nitrification process and it is meaningful to monitor the initial activity of AOB according to the fabrication conditions of PVA/alginate gel beads.

Despite the wide application of the PVA-$B(OH)_3$ method followed by phosphorylation, little information is available about the systematic optimization of the cross-linking reaction time in $B(OH)_3$ solution and the phosphorylation reaction time in orthophosphate solution and about the consequent reduction in the initial ammonia-oxidizing activity. For this reason, the aim of the present study was to evaluate the optimum conditions for the best mechanical strength of PVA/alginate gel beads using the statistical optimization approach (multi-fitting) of response surface analysis (RSA). The initial ammonia-oxidizing activity was subsequently investigated in batch and continuous nitrifying reactors.

2. Experimental

2.1. Entrapment of AOB

Fig. 1 shows a schematic flow of the fabrication process for the preparation of the PVA/alginate gel beads used here. 250 ml/L of solution containing 15.0% PVA (w/v) (with a polymerization degree of 2000, Wako, Japan) and 2.0% of sodium alginate (w/v) (Showa, Japan) was autoclaved at 121℃ for 30 min. After cooling to 40℃, one portion of the concentrated inoculum was slowly added into the PVA/alginate solution. The resulting mixture was added to a solution of saturated $B(OH)_3$ and 1% of $CaCl_2$ (w/v) (pH 3.3 in 25℃) to form spherical beads. The beads were kept in the $B(OH)_3$-$CaCl_2$ solution for at least 1 h. Then, the beads were transferred to a 0.5 M orthophosphate solution (KH_2PO_4) (pH 4.2 in 25℃) and immersed for at least 1 h. The reactions in saturated $B(OH)_3$ and 1% $CaCl_2$ (w/v) (Step 5 in Fig. 1) and in 0.5 M KH_2PO_4 (Step 6 in Fig. 1) were extended for the statistical optimization of the mechanical strength. Finally, the fabricated PVA/alginate gel beads were washed with a large amount of distilled water and stored at 4℃ before use.

2.2. Statistical optimization of the mechanical strength of PVA/alginate beads

The dependent variable representing the mechanical strength of the PVA/alginate gel beads was the total chemical oxygen demand (TCOD). The TCOD level is caused by residual debris in the solution after the homogenization. The high intensity rotor–stator dispersing head (model no. S10N-19G, steel) of the homogenizer (IKA, T10 Basic, Germany) was

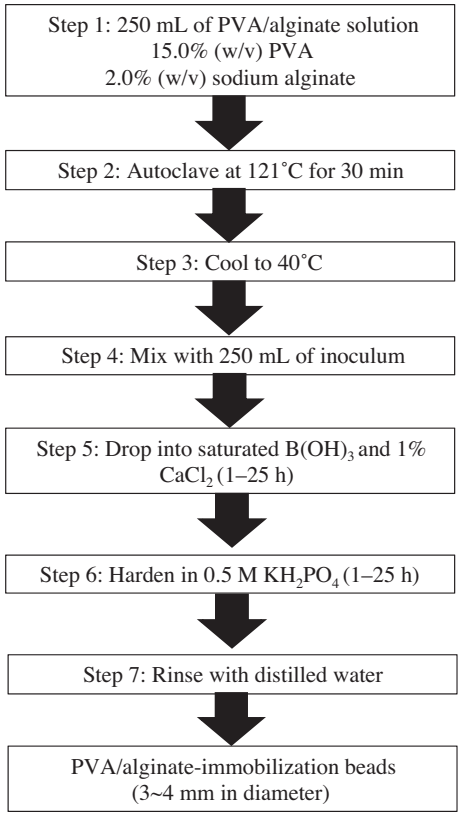

Fig. 1. Entrapment of nitrifying bacteria by means of PVA/alginate entrapment.

submerged in a 100 ml beaker containing 100 beads and 40 ml of distilled water. The dispersing head destroyed parts of the PVA/alginate gel matrix at a rotating intensity of 10,000 rpm. Due to the high mechanical strength and the elasticity of the gel matrix, the homogenizer produced only small fragments and the whole structure of the gel beads was maintained. Then, 2 ml of the solution was immediately collected for the TCOD measurement. The TCOD was determined with a digestion solution kit for COD (Lot A2245, HACH, Germany) and a HACH 5000 spectrophotometer. Various reaction periods of 1, 2, 4, 13, and 25 h were applied in Steps 5 and 6 in Fig. 1 to test the feasibility of the enhancement of the mechanical strength. The measurement was triplicated for the feasibility test.

RSA was applied to analyze and to optimize the factors that affect the mechanical strength of the PVA/alginate gel beads associated with simultaneous changes in the incubation time in $B(OH)_3$-$CaCl_2$ and KH_2PO_4. A sequential procedure of collecting the data, estimating the polynomials (Eq. (1)), and checking the adequacy of the model was used [17].

$$\eta_\alpha = C_0 + \sum_{i=1}^{n} \alpha_i x_i + \sum_{i=1}^{n} \alpha_{ii} x_i^2 + \sum_i \sum_j \alpha_{ij} x_i x_j, \quad i<j \qquad (1)$$

Here, η_α is the measured TCOD value of the residual debris after homogenization (mg/L), x_k is the independent variable k (1 = incubation time in $B(OH)_3$ and $CaCl_2$; 2 = incubation time in KH_2PO_4), C_0 is the regression constant, and α_k denotes the regression coefficient of the independent variable k. The least squares method was used to estimate the parameters in the appropriate polynomials (Eq. (1)). The experimental conditions for RSA were designed based on the central composite cube (CCC) design [18]. The central conditions of the incubation times were set based on the feasibility test. This type of design was used to minimize the number of trials needed to obtain statistically significant results. The experiment and the measurement were performed once under each experimental condition for RSA except for the central condition (triplicate measurements).

Nine trials were run to approximate the response of the mechanical strength. Because significant effects of cross-linking and phosphorylation were identified with reaction times of 2 and 4 h (see Section 3.1), a broader range from 1.4 to 5.6 h was applied to the RSA (Table 1). The basic condition for the center point was 3.5 h (Trial 5 in Table 1).

2.3. Inoculum

Nitrifying bacteria were enriched in a moving bed biofilm reactor which was inoculated with conventional activated sludge from a wastewater treatment plant receiving sewage from a metropolitan city. The enrichment process showed a total nitrogen removal of approximately 95% at a total nitrogen loading rate (NLR) of 2.0 kg N/m^3 d (data not shown). The nitrifying biofilm was manually detached from the carriers into the bulk liquid and centrifuged for 30 min at 13,000 rpm. The nitrifying bacteria were agglomerated to form a biofilm on the carriers. Thus, the agglomerated nitrifying biofilm was homogenized before the entrapment process.

2.4. Evaluation of the ammonia-oxidizing activity

Batch experiments were conducted to assay the initial bioactivity of AOB immobilized in the PVA/alginate gel beads. For these experiments, possible toxic effects of individual reaction times of the cross-linking and phosphorylation steps were examined by varying one factor at a time while keeping the other factor at a constant

Table 1
Experimental design and observed TCOD concentration derived by the residual debris after homogenization

	Trial	Reaction time (h)		Responses TCOD (mg/L)
		Cross-linking	Phosphorylation	
Linear design	1	2.0	2.0	171
	2	5.0	2.0	152
	3	2.0	5.0	148
	4	5.0	5.0	115
	5*	3.5	3.5	166.3 ± 3.06
Quadratic and partial-cubic design	6	5.6	3.5	116
	7	1.4	3.5	201
	8	3.5	5.6	101
	9	3.5	1.4	187

*The center point was repeated three times.

level of 1 h. The concentrations of volatile suspended solids (VSS) of the inoculum were from 2173.3 to 3228.4 mg/L. To compare the initial bioactivity in different conditions, the NH_4^+-N removal rates in a series of batch experiments were normalized by the VSS concentration in the PVA/alginate gel beads, i.e. specific ammonia-oxidizing activity [mg-N/mg-VSS d]. The total reaction volume was 75 ml with 100 beads in a 100 ml flask. For the effective diffusion of oxygen into the growth medium, vigorous mixing at 250 rpm was applied in a shaking incubator at 35˚C. The NH_4^+-N removal rate was calculated based on the reduction of the NH_4^+-N concentration after 2 d. For the analysis of NH_4^+-N, samples were prepared by filtering through a 0.45 μm syringe filter. The NH_4^+-N concentration was measured by a Kjeldahl nitrogen analysis (Kjeltec 1035, Sweden).

To evaluate the effects of different bead preparation conditions on the acclimation period of continuous nitrifying reactors, the NH_4^+-N removal performance was monitored. Fig. 2 shows a schematic of the continuous nitrifying bioreactor used in this study. Nitrifying sludge in the amount of 2876.3 mg-VSS/L was immobilized in the PVA/alginate gel beads. The airlift reactor used here had a total working volume of 0.5 L. The gel beads of 0.15 L were added to continuous nitrifying bioreactors (a packing ratio of 30% v/v). A water jacket was utilized to maintain the reactor temperature at 35˚C. The influent was fed by a peristaltic pump. The pH was not controlled or monitored because the drop of pH was prevented by adding sufficient alkalinity at a molar ratio of NH_4^+-N/HCO_3^--C = 1:2 in this study [19]. The reactor was wrapped with aluminum foil to maintain a dark condition. Because active immobilization systems for the nitrification are applicable within a wide range of hydraulic retention time (HRT) between 2.5 and 35 h [20], HRT was fixed at 4.17 h in this study. The NH_4^+-N concentration in the influent was increased according to the AOB activity. Air was provided at an intensity of 2 L/min.

The composition of the medium was the modification of the medium described by van de Graaf et al. [21]. For both the batch and continuous experiments, the basal medium consisted of 6 mg-P/L of KH_2PO_4, 12 mg-Mg/L of $MgSO_4{\cdot}7H_2O$, 48 mg-Ca/L of $CaCl_2{\cdot}2H_2O$, 1 ml/L of trace element solution I, and 1 ml/L of trace element solution II. Trace element solution I was composed of 5 g/L EDTA and 5 g/L $FeSO_4{\cdot}7H_2O$. Trace element solution II was composed of 5 g/L EDTA, 0.43 g/L $ZnSO_4{\cdot}7H_2O$, 0.24 g/L $CoCl_2{\cdot}6H_2O$, 0.99 g/L $MnCl_2{\cdot}4H_2O$, 0.25 g/L $CuSO_4{\cdot}5H_2O$, 0.22 g/L $Na_2MoO_4{\cdot}2H_2O$, 0.19 g/L $NiCl_2{\cdot}6H_2O$, 0.21 g/L $Na_2SeO_4{\cdot}10H_2O$, and 0.014 g/L H_3BO_3. NH_4^+-N and HCO_3^--C were added to the basal medium in the required amounts in the forms of $(NH_4)_2SO_4$ and $NaHCO_3$, respectively.

3. Results and discussion

3.1. Mechanical strength enhancement of PVA/alginate gel beads

Because measurements of the mechanical strength using homogenization tools have not been conducted with PVA/alginate gel, basic experiments to assess the effects of the two parameters of the incubation times in the cross-linking solution and in the phosphorylation solution were conducted. The effects of the reaction time in saturated $B(OH)_3$ and 1% $CaCl_2$ (w/v) (Step 5 in Fig. 1) on the mechanical strength of the PVA/alginate gel beads were investigated. The PVA/alginate gel beads were fabricated without the inoculum to avoid the interference of TCOD measurement by the AOB cell debris and the EPS excreted by AOB.

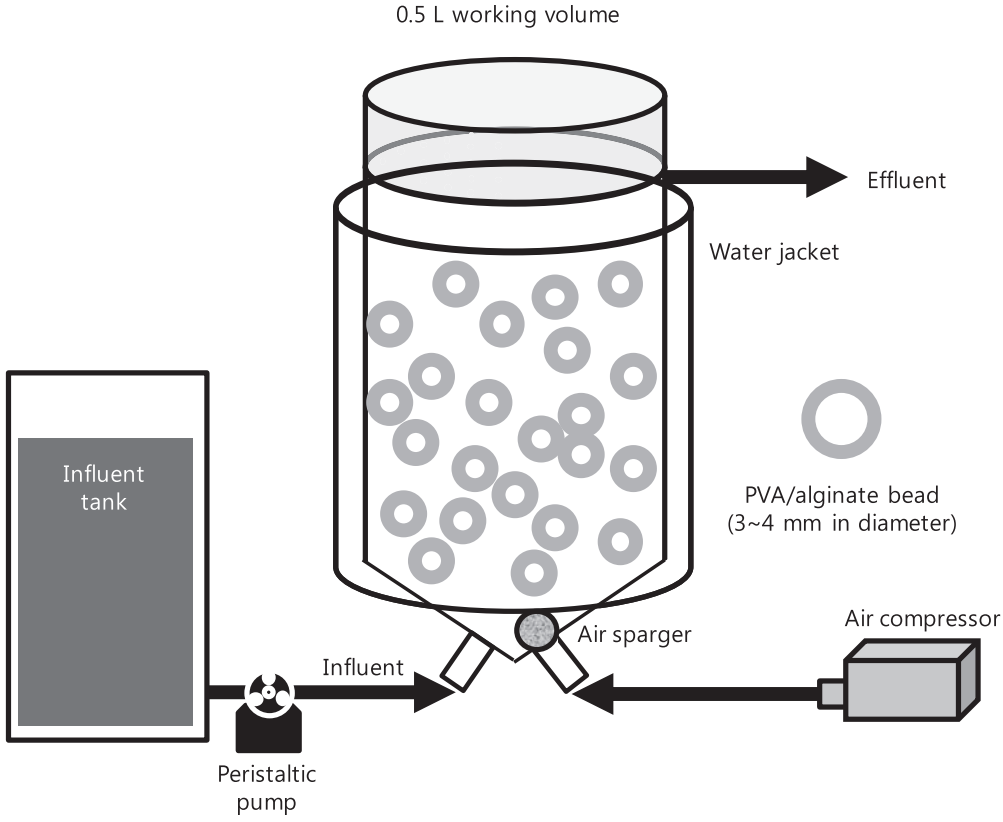

Fig. 2. Schematic of the nitrification bioreactor used in this study.

PVA/alginate gel beads were prepared at various cross-linking reaction times of 1, 2, 4, 13, and 25 h followed by phosphorylation in 0.5 M KH_2PO_4 for 1 h. The additional cross-linking reaction in $B(OH)_3$ and $CaCl_2$ significantly enhances the mechanical strength of PVA/alginate beads. A sharp reduction of the residual debris of PVA/alginate gel beads, as produced by homogenization, was observed at reaction times of 2 and 4 h (Fig. 3(a)). The 13 and 25 h reactions exhibited little difference in the production of residual debris in terms of the TCOD compared to the 4 h condition.

PVA/alginate gel beads without inoculum fabricated in the $B(OH)_3$ and $CaCl_2$ solution for 1 h were phosphorylated with different reaction times in 0.5 M KH_2PO_4 (Step 6 in Fig. 1) (Fig. 3(b)). The extended phosphorylation exhibited the drastic increase in the mechanical strength with reaction times of 2 and 4 h. Phosphorylation reactions of 12 and 24 h resulted in an insignificant improvement in the mechanical strength compared to the 4 h condition.

It was concluded that cross-linking for 4 h in saturated $B(OH)_3$ and 1% $CaCl_2$ (w/v) followed by phosphorylation for 4 h in 0.5 M KH_2PO_4 would result in the best mechanical strength assuming that the two reactions are independent. However, the total reaction time for cross-linking by $B(OH)_3$ and $CaCl_2$ and phosphorylation by KH_2PO_4 may be shorter or longer than 8 h for the best mechanical strength if the cross-linking and phosphorylation affect each other. The intercorrelated effects of the cross-linking and the phosphorylation were verified and the optimal conditions for the mechanical strength were estimated using a CCC design and RSA.

3.2. RSA of the mechanical strength

Stepwise changes of 1.5 h from the center point were initially applied for a first-order model, $\eta_i = \beta_0 + \beta_1 x_1 + \beta_2 x_2$ (Trials 1–5 in Table 1). The following model was generated for the concentration of the residual TCOD in the solution after the homogenization process.

$$\eta_{\text{residual TCOD}} = 155 - 8.67x_1 - 10.0x_2 \quad (2)$$

In this equation, η_i is the concentration of i produced (mg/L, where i = residual TCOD) and x_j is the

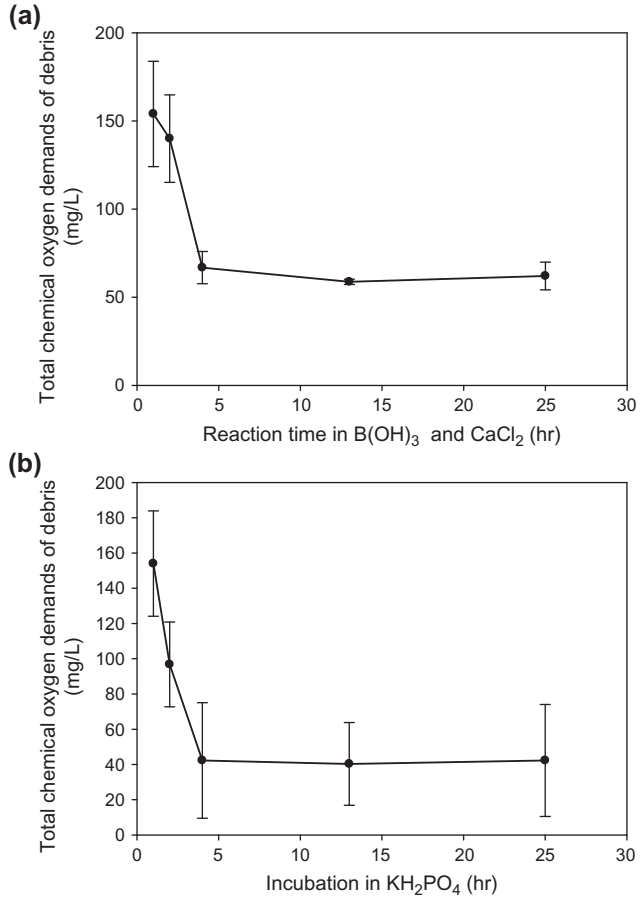

Fig. 3. The effects of (a) the gelling solution and (b) the phosphorylation on the mechanical strength of PVA/alginate beads.

corresponding variable term (j = reaction times for cross-linking (h) and phosphorylation (h) in order). The first-order model resulted in a significant p-value of the lack-of-fit for the residual TCOD at the 2.5% α level, whereas the regression coefficient was not significant at the 10% α level. Thus, four trials of the stepwise changes of 2.1 h ($1.5\,\text{h} \times 2^{1/2}$) from the center point were run to fit the second and partial cubic order regressions (Trials 6–9 in Table 1). To find the optimum condition for the lowest level of TCOD production, a quadratic model was tested with Eq. (3).

$$\eta_{\text{residual TCOD}} = 166 - 14.4x_1 - 15.2x_2 - 1.56x_1x_2 - 2.29x_1^2 - 5.58x_2^2 \quad (3)$$

Here, η_{ai} is the concentration of the residual TCOD produced with model ai (ai = quadratic and partial cubic models in order). The regression coefficient for the quadratic model (Eq. (3)) was improved to the 2.5% α level compared to the first-order regression, but it exhibited a significant p-value of the lack-of-fit for the residual TCOD at the 2.5% α level.

Therefore, the partial cubic model was tested by adding the terms of $x_1^2x_2$ and $x_1x_2^2$ (Eq. (4)). Only the partial cubic model adequately fit the response surface with an insignificant p-value of the lake of fit and the significant regression coefficient at the 0.5% α level (p-value, 0.0024). The R^2 and the adjusted R^2 values are 0.99 and 0.98, respectively. Therefore, the partial cubic model was selected to describe the response surface of the residual TCOD within this region and to find the optimum conditions. Three- and two-dimensional response surfaces of the partial cubic model with corresponding estimated optimums, the lowest and the greatest production of residual TCOD, are shown in Fig. 4(a) and (b), respectively.

$$\eta_{\text{residual TCOD}} = 166 - 29.2x_1 - 20.5x_2 - 1.56x_1x_2 - 2.29x_1^2 - 5.58x_2^2 + 4.66x_1^2x_2 + 5.14x_1x_2^2 \quad (4)$$

The conditions that minimize the production of residual TCOD were calculated for the partial cubic model (Eq. (4)). The optimum conditions were 3.5 h for cross-linking and 5.6 h for phosphorylation which are the identical conditions of Trial 8 in Table 1. The predicted model output under the optimum conditions was 98.6 mg/L in terms of the residual TCOD which was close to the observed value of 101 mg/L. A further inspection of the coefficients of the model (Eq. (4)) for possible two-way interactions among the independent variables showed that the interaction term of x_1x_2 was not significant. The inter-correlated effects on the response were significant only for the interaction terms of $x_1^2x_2$ and $x_1x_2^2$; they were found to be interdependent at the 5% α level (p-values of 0.0149 and 0.0113, respectively). This interaction can be seen in the form of an elongated ellipse on the response surface (Fig. 4(b)). The rounded ridge starting from the conditions of x_1 of 1.4 h and x_2 of 3.5 h is positioned diagonally on the plot to the upper right. The steepest ascent to the upper right (the solid line in Fig. 4(b)) results in a drastic decrease in the residual TCOD while the gentlest ascent (the dashed line in Fig. 4(b)) to the lower right makes an insignificant contribution to the decrease in the response.

3.3. The effects of cross-linking and phosphorylation on the ammonia-oxidizing activity

For an effective wastewater treatment, immobilized micro-organisms have to survive their fabrication

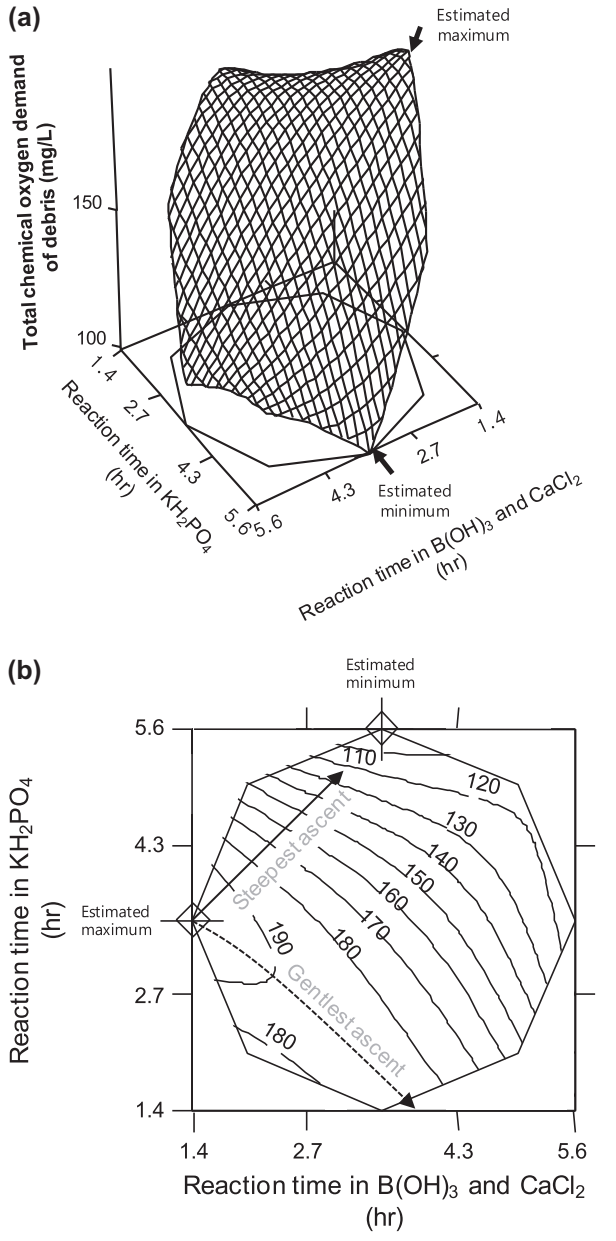

Fig. 4. The projection of the response surface for the residual TCOD produced by the homogenization of the phosphorylated PVA/alginate gel bead in (a) three- and (b) two-dimensional contour plots.

procedure. Thus, possible toxic effects of the cross-linking and phosphorylation steps on the bioactivity of ammonia-oxidation were examined (Fig. 5). The nitrifying sludge was immobilized in the PVA/alginate beads under different reaction times of cross-linking and phosphorylation.

A decrease in the initial ammonia-oxidizing activity according to the reaction time of the cross-linking step in $B(OH)_3$ and $CaCl_2$ was observed, whereas the phosphorylation step had insignificant effects on the initial level of bioactivity. It was expected that AOB are tolerable to pH 4.2 during a period of 1–25 h in an orthophosphate solution. However, the low pH of 3.3 caused by the high concentration of $B(OH)_3$ resulted in serious cell damage of AOB. In comparison to the reaction of 1 h in the cross-linking solution, 2 and 4 h incubation periods led to the reduction of the initial ammonia-oxidizing activity to 44.6 and 34.0%, respectively. However, the exact mechanism for the severe inhibition of ammonia-oxidizing activity by $B(OH)_3$ is unclear.

The inhibitory effects of $B(OH)_3$ could be prevented through applying shorter reaction times ranging from 10 min to 2 h in saturated $B(OH)_3$ compared to the conventional period of 24 h [11]. However, the shorter reaction time results in the expense of lowering the mechanical strength as described above. Thus, the initial ammonia-oxidizing activity may recover by repeated incubations in favorable conditions or with continuous cultivation with a high dilution rate which maintains the exponential growth rate of the AOB; Leenen et al. [6] demonstrated the recovery and/or the regrowth of AOB, that was completely inhibited by $B(OH)_3$, in appropriate growth media. To evaluate the regrowth of AOB, whose activity was partially inhibited by saturated $B(OH)_3$ in this study, operation in continuous nitrifying reactors was carried out.

3.4. Acclimation period of the nitrification process

The acclimation period is a critical factor for the implementation of full-scale wastewater treatment

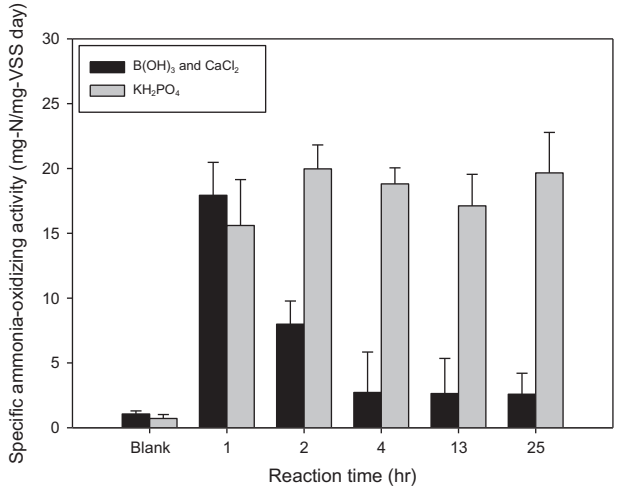

Fig. 5. The effects of cross-linking and phosphorylation on the activity of AOB entrapped in PVA/alginate beads. Blank experiments were conducted without PVA/alginate gel beads under the identical conditions.

processes. The growth rate is proportional to the amount of inoculum and its viability. Thus, it was hypothesized that a reactor containing the PVA/alginate gel beads that were fabricated with the optimized and extended incubation times (Trial 8 in Table 1), would exhibit a longer acclimation period due to the inhibitory effects on the cell viability of AOB (Reactor 2 in Fig. 6). For a comparison, PVA/alginate gel beads which underwent 1 h of cross-linking and 5.6 h of phosphorylation were applied in a the parallel continuous nitrifying reactor (Reactor 1 in Fig. 6) under the same conditions of a low HRT of 4.17 h and NH_4^+-N concentrations ranging 204.8–574.6 mg/L in the influent. The initial activity levels of the PVA/alginate gel beads were 15.9 and 4.95 mg-N/mg-VSS d for Reactors 1 and 2, respectively.

In Phase I, a constant NLR of 1.18 kg N/m^3 d was applied between days 1 and 15 (Fig. 6). After 8 d, gradual increases in the NH_4^+-N removal rates were observed for both Reactor 1 and Reactor 2. The increase in the NH_4^+-N removal rate of Reactor 2 was less steep than that of Reactor 1; thus, a longer period was required for Reactor 1 to achieve more than 1.0 kg N/m^3 d. Accordingly, it was concluded that the lower cell viability as affected by the reaction time in $B(OH)_3$ resulted in a longer acclimation period. The inhibition of the ammonia-oxidizing activity by the extended reaction in $B(OH)_3$ of Reactor 2 was overcome to the degree of Reactor 1 in 5 d (i.e. days 8–13 in Fig. 6). To test the maximum treatability of the two reactors, the NH_4^+-N concentration in the influent was gradually increased for 6 d in Phase II. The maximum NH_4^+-N removal rates were identical at 2.8 kg N/m^3 d for the two reactors.

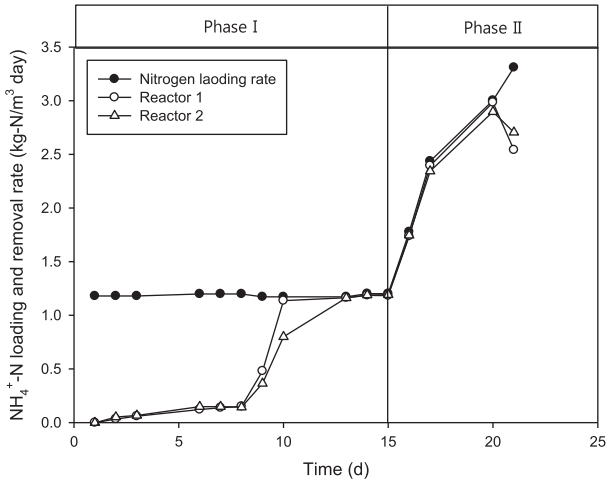

Fig. 6. The continuous nitrification process using PVA/alginate beads.

4. Conclusions

The optimal conditions to enhance the mechanical strength of PVA/alginate gel beads were investigated to entrap AOB. Significant effects of an extended incubation period in cross-linking agents of saturated $B(OH)_3$ and 1% $CaCl_2$ (w/v) and a phosphorylation agent of 0.5 M KH_2PO_4 on the mechanical strength of PVA/alginate gel beads were identified in one-factor-at-a-time experiments. The optimal reaction time for each step was identical, at 4 h. However, a combination of the CCC design and RSA for the two parameters of cross-linking and phosphorylation reactions revealed an interdependent relationship based on the mathematical modeling of a partial cubic polynomial equation, which demonstrated optimal conditions of 3.5 and 5.6 h, respectively. The mechanical strength was the first priority, but the effects of the optimal fabrication conditions on ammonia-oxidizing activity were carefully evaluated for the stable operation of nitrifying processes. According to the initial ammonia-oxidizing activity of nitrifying sludge immobilized in the beads, saturated $B(OH)_3$ contributed to the severe inhibition of bioactivity by exhibiting 34.0% of the initial activity for a 4 h reaction in comparison to a 1 h reaction. In contrast, the phosphorylation step had an insignificant effect on the initial bioactivity. Finally, due to the lower bioactivity caused by the extended reaction time in the cross-linking agents for the optimum mechanical strength, the acclimation period in a continuous nitrifying reactor was delayed to achieve process performance of more than 1 kg N/m^3 d. On the other hand, the findings that the inhibitory effects was fully recovered in 5 d and that identical maximum treatability levels were exhibited compared to the conditions for the minimum mechanical strength imply that the optimized fabrication method for the best mechanical strength is feasible for implementation with continuous nitrification processes.

Acknowledgment

The authors appreciate the financial support from the Korea Institute of Science and Technology (project nos. 2E23942 and 2Z03740).

References

[1] M.L. Shuler, F. Kargi, Bioprocess Engineering: Basic Concepts, Prentice Hall, NJ, pp. 263–268.
[2] Q. Feng, Y. Wang, T. Wang, H. Zheng, L. Chu, C. Zhang, H. Chen, X. Kong, X.H. Xing, Effects of packing rates of cubic-shaped polyurethane foam carriers on the microbial community and the removal of organics and nitrogen in moving bed biofilm reactors, Bioresour. Technol. 117 (2012) 201–207.

[3] D. Janjaroen, F. Ling, G. Monroy, N. Derlon, E. Mogenroth, S.A. Boppart, W.T. Liu, T.H. Nguyen, Roles of ionic strength and biofilm roughness on adhesion kinetics of *Escherichia coli* onto groundwater biofilm grown on PVC surfaces, Water Res. 47 (2013) 2531–2542.
[4] R. Oliveira, L. Melo, A. Oliveira, R. Salgueiro, Polysaccharide production and biofilm formation by *Pseudomonas fluorescence*: Effects of pH and surface material, Colloids Surf., B 2 (1994) 41–46.
[5] F. Benyahia, R. Polomarkaki, Mass transfer and kinetic studies under no cell growth conditions in nitrification using alginate gel immobilized *Nitrosomonas*, Process Biochem. 40 (2005) 1251–1262.
[6] E.J.T.M. Leenen, V.A.P. Dos Santos, K.C.F. Grolle, J. Tramper, R.H. Wijffels, Characteristics of and selection criteria for support materials for cell immobilization in wastewater treatment, Water Res. 30 (1996) 2985–2996.
[7] I. Cruz, Y. Bashan, G. Hernàndez-Carmona, L.E. de-Bashan, Biological deterioration of alginate beads containing immobilized microalgae and bacteria during tertiary wastewater treatment, App. Microbiol. Biotechnol. 97 (2013) 9847–9858.
[8] M. Levstek, I. Plazl, Influence of carrier type on nitrification in the moving-bed biofilm process, Water Sci. Technol. 59 (2009) 875–882.
[9] Y. Kimura, H. Itokawa, K. Noto, T. Murakami, K. Isaka, Stability of autotrophic nitrogen removal system under four non-steady operations, Bioresour. Technol. 137 (2013) 196–201.
[10] K.Y.A. Wu, K.D. Wisecarver, Cell immobilization using PVA crosslinked with boric acid, Biotechnol. Bioeng. 39 (1992) 447–449.
[11] K.C. Chen, Y.F. Lin, Immobilization of microorganisms with phosphorylated polyvinyl alcohol (PVA) gel, Enzyme Microb. Technol. 16 (1994) 79–83.
[12] K. Egli, C. Langer, H.-R. Siegrist, A.J.B. Zehnder, M. Wagner, J.R. van der Meer, Community analysis of ammonia and nitrite oxidizers during start-up of nitritation reactors, Appl. Environ. Microbiol. 69 (2003) 3213–3222.
[13] H.D. Park, D.R. Noguera, Evaluating the effect of dissolved oxygen on ammonia-oxidizing bacterial communities in activated sludge, Water Res. 38 (2004) 3275–3286.
[14] M. Wagner, G. Rath, R. Amann, H.P. Koops, K.H. Schleifer, In situ identification of ammonia-oxidizing bacteria, Syst. Appl. Microbiol. 18 (1995) 251–264.
[15] J.I. Prosser, Autotrophic nitrification in bacteria, Adv. Microb. Physiol. 30 (1989) 125–181.
[16] S. Siripong, B.E. Rittmann, Diversity study of nitrifying bacteria in full-scale municipal wastewater treatment plants, Water Res. 41 (2007) 1110–1120.
[17] K. Yang, C. Oh, S. Hwang, Optimizing volatile fatty acid production in partial acidogenesis of swine wastewater, Water Sci. Technol. 50 (2004) 169–176.
[18] S. Lee, H. Bae, M. Song, S. Hwang, Bioconversion of starch processing waste to *Phellinus linteus* mycelium in solid-state cultivation, J. Ind. Microbiol. Biotechnol. 35 (2008) 859–865.
[19] K. Chandran, B.F. Smets, Applicability of two-step models in estimating nitrification kinetics from batch respirograms under different relative dynamics of ammonia and nitrite oxidation, Biotechnol. Bioeng. 70 (2000) 54–64.
[20] J. Yan, Y.Y. Hu, Partial nitrification to nitrite for treating ammonium-rich organic wastewater by immobilized biomass system, Bioresour. Technol. 100 (2009) 2341–2347.
[21] A.A. van de Graaf, P. de Bruijn, L.A. Robertson, M.S.M. Jetten, J.G. Kuenen, Autotrophic growth of anaerobic ammonium-oxidizing micro-organisms in a fluidized bed reactor, Microbiology 142 (1996) 2187–2196.