การสลายอะคริลาไมด์ด้วยแบคทีเรียผสมในระบบบำบัดน้ำเสียเอสบีอาร์

Acrylamide Degradation with Mixed Culture Bacteria in Sequencing Batch Reactor (SBR) Wastewater Treatment Process

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บทคัดย่อ

อะคริลาไมด์ถูกนำมาใช้ในกระบวนการผลิตของอุตสาหกรรมต่างๆ ทำให้มีการปนเปื้อนอะคริลาไมด์ในน้ำเสียและต้องถูกบำบัดก่อนปล่อยออกสู่แหล่งรองรับน้ำทิ้งเพราะอะคริลาไมด์และสารอนุพันธ์ของอะคริลาไมด์เป็นพิษและเป็นสารก่อมะเร็ง ดังนั้น งานวิจัยนี้จึงประเมินการสลายอะคริลาไมด์ด้วยแบคทีเรียผสมในระบบบำบัดน้ำเสีย Sequencing Batch Reactor (SBR) จำนวน 2 ระบบ ระบบหนึ่งเป็นระบบควบคุมที่มีการป้อนน้ำเสียสังเคราะห์ที่ไม่มีอะคริลาไมด์ อีกระบบหนึ่งป้อนอะคริลาไมด์ร้อยละ 25 ของความเข้มข้นสารอินทรีย์ทั้งหมดบ่งชี้ด้วยความต้องการใช้ออกซิเจนทางเคมี (Chemical Oxygen Demand, COD) (~100 mg acrylamide/L) ทั้งนี้ น้ำเสียมีค่า COD ทั้งหมดเท่ากับ 400 mg COD/L ผลการทดลองพบว่า แบคทีเรียผสมสามารถกำจัดอะคริลาไมด์ได้ทั้งหมดภายในระยะเวลา 10 ชั่วโมง มีประสิทธิภาพการกำจัด COD เพียงร้อยละ 76.4 จากการสะสมของกรดอะคริลิค โดยมีแอมโมเนียมและกรดอะคริลิคจากการสลายอะคริลาไมด์สะสมเท่ากับ 68 และ 108 mg/L ตามลำดับ นอกจากนั้น ยังพบการสะสมของไนไตรท์และไนเตรทจากปฏิกิริยาไนตริฟิเคชั่น เท่ากับ 6.8 และ 19.1 mg N/L ตามลำดับ สรุปได้ว่า ระบบบำบัดน้ำเสียแอคติเวเต็ทสลัดจ์สามารถกำจัดอะคริลาไมด์ที่มีความเข้มข้นประมาณ 100 mg/L ได้อย่างมีประสิทธิภาพเพราะจุลินทรีย์สามารถใช้อะคริลาไมด์เป็นแหล่งคาร์บอนได้

คำสำคัญ : อะคริลาไมด์ แบคทีเรียผสม แอคติเวเต็ทสลัดจ์ ระบบเอสบีอาร์

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Abstract

Acrylamide is widely used in various industrial processes resulting in the discharge of acrylamide in the wastewater. Due to its carcinogenicity and toxicity, the acrylamide including its derivatives must be removed from the wastewater prior to discharge to the receiving water. Therefore, this research aims to evaluate the acrylamide degradation from the synthetic wastewater by mixed culture bacteria in two Sequencing Batch Reactor (SBR) systems. One SBR system was operated as a control system feeding the synthetic municipal wastewater without any acrylamide. The synthetic wastewater contained total Chemical Oxygen Demand (COD) about 400 mg COD/L. Another SBR system was exposed to high concentration of acrylamide at 25% of total COD equivalent in synthetic municipal wastewater (~100 mg acrylamide/L). It is found that the mixed culture bacteria in the SBR system removed the acrylamide completely at the concentration of 25% total COD equivalent in 10 hours of reacting period, but the COD removal efficiency was only 76.4% because of the accumulation of acrylic acid. Both ammonium nitrogen (68 mg N/L) and acrylic acid (108 mg/L) were accumulated in the SBR system as a result of the degradation process by mixed culture bacteria. Nitrification was accomplished in the SBR systems resulting in the accumulations of nitrite and nitrate nitrogens at the concentrations of 6.8 mg N/L and 19.1 mg N/L, respectively. It is concluded that the activated sludge system can remove acrylamide efficiently at the acrylamide concentration of about 100 mg/L because microorganisms use acrylamide as a carbon source for growth.

Keywords : acrylamide, mixed culture bacteria, activated sludge, sequencing batch reactor

Introduction

Acrylamide (C_3H_5NO) is widely used in various industrial processes including dyes manufacturing, plastic, and water treatment. Acrylamide is generally generated by the hydrolysis process of acrylonitrile with a nitrile hydratase enzyme. It is a neurotoxicant toxic and carcinogen substance for human (International Agency for Research on Cancer, 2008). Unfortunately, the widespread usages and indiscriminate discharges of acrylamide and polyacrylamide have resulted in the contamination of this compound in wastewater. Due to its carcinogenicity and toxicity, discharge of acrylamide to the natural water and soil systems may lead to an adverse environmental impact on water quality and thus endanger public health and welfare (Cherry, Gabaccia, & Senn, 1956); therefore, the acrylamide must be removed from the wastewater. Concerning the toxicity of acrylamide monomer, some microorganisms use acrylamide as their sole carbon source for outgrowth (Nawaz *et al.,* 1994; Nawaz, Billedeau, & Cerniglia, 1998; Shanker, Ramakrishna, & Seth, 1990)

Various types of microorganisms including *Arthrobacter*, *Xanthomonas*, *Rhodopseudomonas*, *Rasonia*, *Geobacillus* and *Enterobacteriaceae* have been reported that they are capable to degrade acrylamide effectively. (Charoenpanich, 2013). *Pseudomonas* sp. was reported by Shanker *et al.* (1990) that could degrade the

acrylamide at the concentration of 4000 mg/L to acrylamide and ammonia nitrogen. *Enterobacter aerogenes* and *Kluyvera georgiana* isolated from the domestic wastewater were reported as novel acrylamide degrading bacteria to remove the acrylamide at the concentration as high as 5000 mg/L (Buranasilp & Charoenpanich, 2011; Thanyacharoen, Tani, & Charoenpanich, 2012). *Ralstonia eutropha* was also capable to remove the acrylamide up to the concentration of 1446 mg/L. The bacterium was originally in the wastewater treatment system treating the polyacrylonitrile (PAN) fiber wastewater (Wang & Lee, 2007). In addition, most acrylamide degradation studies have been conducted in the laboratory with the pure culture of bacteria. Acrylamide degradation in the activated sludge wastewater treatment system has rarely been reported, especially in the SBR process. The Sequencing Batch Reactor (SBR) configuration is a true batch mode activated sludge process and is capable for organic matters removal and nitrification. It also offers various advantages including minimal space requirements, ease of management and possibility of modifications because the aeration and sludge settlement occur in the same tank. The objective of this study was to investigate the acrylamide degradation with mixed culture bacteria in the Sequencing Batch Reactor (SBR) wastewater treatment system.

Materials and Methods

SBR Configurations and Operation

Two batch reactors, each reactor had a working volume of 3.0 L as shown in Figure 1, were used as SBR systems in the Environmental Engineering Laboratory, Faculty of Engineering, Burapha University running in parallel at the room temperature of about 28 ^{o}C. It is referred as Control and AS systems in this study. Both SBR systems were operated with two cycles per day, each cycle consisting of 15 min fill, 10 h aerobic react, 1 h settle, 15 min decant, and 30 min idle resulting in a nominal hydraulic retention time (HRT) of 24 hours. The systems were operated at the solid retention time (SRT) of 10 days. The excess sludge was wasted daily from the systems twice a day at the end of each reacting period. Air pumps and air stone diffusers are used to provide the dissolved oxygen (DO) concentrations of 3.0-4.0 mg O_2/L. The SBR systems were initially seeded with the mixed culture of bacteria taken from another pilot scale continuous flow activated sludge system configured as the Modified Ludzack-Ettinger (MLE) configuration. Synthetic wastewater was fed into both SBR systems. Both SBR systems were operated until the steady state conditions were achieved. Samples were collected periodically for parameter analyses to monitor the steady state conditions. After the addition of acrylamide, the samples were taken every 2 days (0, 3, 5 and 7 days) from the influent and from the SBR reactors at the time intervals of 2.5 h.

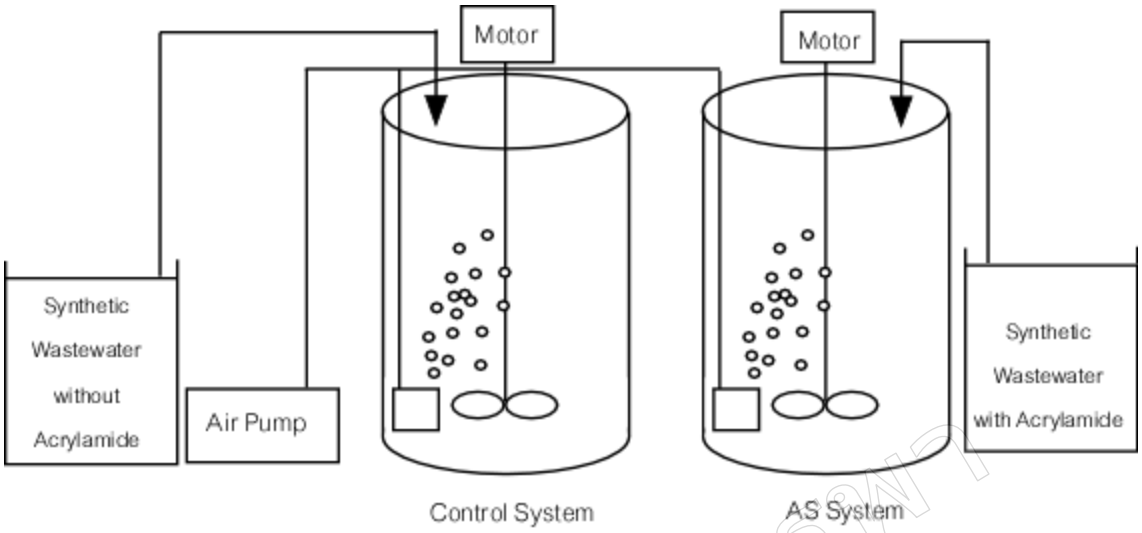

Figure 1 Two SBR systems seeding with the mixed culture bacteria and operating as activated sludge process

Synthetic Wastewater Characteristics

The synthetic wastewater used in the study had similar characteristics as that of municipal wastewater consisting of water together with relatively small concentrations of suspended, dissolved organic, and inorganic solids. The synthetic municipal wastewater were prepared daily by dissolving 4.1 g of sucrose, 1.7 g of CH_3COONa, 1.0 g of K_2HPO_4, 0.5 g of KH_2PO_4, 5.0 g of $NaHCO_3$, 2.1 g of NH_4Cl, 0.7 g of $MgCl_2$, and 0.4 g of $CaCl_2$ in 10 L of tap water resulting in the wastewater characteristics as listed in Table 1. After the steady state conditions were achieved in both SBR systems, organic matters in the synthetic wastewater for the experimental SBR system were partially replaced by the acrylamide in the amount of 25% total COD equivalent. The control system was still fed with the synthetic wastewater without any acrylamide supplementation.

Analytical Methods

The wastewater in the storage tank was mixed to resuspend the settled solids before the wastewater samples were taken for the parameter analyses. The samples were filtered with the Whatman GF/C glass membrane to remove the particulates, which were larger than 1.2 μm in diameter. The filtrates were analyzed for acrylamide, acrylic acid, TCOD, SCOD, NO_2^--N, NO_3^--N, NH_4^+-N, MLSS, and MLVSS. The MLSS, MLVSS, TCOD, and SCOD were analyzed according to the Standard Methods for Water and Wastewater Examination (Eaton, Clesceri, & Greenberg, 2012). The Closed Reflux, titrimetric method (5220C) was used to measure the TCOD and SCOD. The MLSS and MLVSS parameters were determined according to the methods of Total Suspended Solids Dried at 103-105 ^{o}C (2540D) and Fixed and Volatile Solids Ignited at 550 ^{o}C (2540E), respectively. The supernatant characteristics, collected after the settling periods of batch reactor experiments, were also collected and analyzed for the same parameters.

Table 1 Synthetic municipal wastewater Characteristics

Parameter	Value
pH	6.5-7.5
Total Chemical Oxygen Demand (TCOD)	403.1 (mg COD/L)
Soluble Chemical Oxygen Demand (SCOD)	338.2 (mg COD/L)
Total Suspended Solids (TSS)	3.5 (mg TSS/L)
Total Volatile Suspended Solids (TVSS)	2.2 (mg VSS/L)
Ammonium Nitrogen (NH_4^+-N)	43.6 (mg N/L)
Nitrate Nitrogen (NO_3^--N)	-
Nitrite Nitrogen (NO_2^--N)	-
Phosphate (PO_4^{3-}-P)	7.5 (mg P/L)

Ammonium nitrogen, nitrite nitrogen and nitrates nitrogen were analyzed with an Ionic Chromatograph (Dionex 2010I and Electrochemical Conductivity Detector, Dionex Corp., Sunnyvale, CA) under the following conditions: 7 mL volume sample, cationic mobile phase (22 mN sulfuric acid, H_2SO_4), anionic mobile phase (9.0 mM carbonate, Na_2CO_3), and columns; IONPAC AS4A-SC (Dionex Corp., Sunnyvale, CA) and IONPAC CS12A-4X (Dionex Corp., Sunnyvale, CA). Samples were centrifuged at 10,000 rpm for 10 min and filtrated though 0.45 µm to remove suspended solids prior to inject to the chromatography columns. Acrylamide and acrylic acid concentrations were quantitatively determined using high-pressure liquid chromatography with UV-detection (HPLC-UV) in a reversed system under the following conditions: 40 µL volume sample and mobile phase concentrations (50% deionized water and 50% acetonitrile).

Results and Discussion

The SBR systems (Control and AS systems) were firstly operated for over four months in an aerated mode using sludge taken from the activated sludge system running in the Department of Chemical Engineering, Burapha University. At the steady state conditions, the Control and AS systems contained the suspended solids indicated as MLSS at the concentrations of 3169 mg/L and 3141 mg TSS/L at the end of reacting periods, respectively. The MLVSS concentrations in the Control and AS systems were 2808 and 2755 mg VS/L providing the MLVSS/MLSS ratios of 0.89 and 0.88, respectively. High solid concentrations were found in both systems because the systems were operated at high SRT of 10 days with the COD concentrations of about 400 mg/L. It appears that the acrylamide had no impact on the concentration of microorganisms; otherwise, the MLSS and MLVSS concentrations must be different between two systems. The mixed liquor pH was maintained about 7.2,

which was suitable for the biological wastewater treatment process. The experimental results for the removals of organic matters in both Control and AS SBR systems and acrylamide degradation in the AS system are listed in Table 2

Table 2 Acrylamide and COD removal efficiencies in SBR systems

Time (h)	SBR Period	Acrylamide (mg /L)		SCOD Concentrations (mg /L)	
		Control	AS	Control	AS
0.0		0.0	107.2	408.3*	412.6*
2.5		0.0	65.9	309.7	346.6
5.0	React	0.0	29.7	168.4	247.7
7.5		0.0	3.9	89.1	137.6
10.0		0.0	0.0	75.2	98.4
11.0	Settle	0.0	0.0	74.6	97.4
Removal Efficiency (%)		0.0	100.0	81.7	76.4

*The COD value at the beginning of reacting period was the Total Chemical Oxygen Demand (TCOD) in the influent.

It appears that the Control SBR system containing mixed culture of bacteria could remove the organic matters about 82% removal efficiency, leaving the COD concentration of about 75 mg/L in the effluent. While the COD was not exhausted from the system, the COD removal rate can be determined with the linear squares method during the reacting period of 10 h. It is found that the COD removal rate was 35.5 mg COD/L-h with the R^2 value of 0.94. From the experimental results in Table 2, it would appear that the mixed culture bacteria in the SBR system could complete the acrylamide degradation within 10 hours of reacting period. While the acrylamide was not exhausted from the AS system, the acrylamide removal rates during first 7.5 hours of reacting period was 13.8 mg/L-h with the R^2 value of 0.99. The experimental results confirm that mixed culture bacteria containing several microorganisms can use acrylamide as their carbon source for growth (Nawaz et al., 1994; Nawaz, Billedeau, & Cerniglia, 1998; Shanker, Ramakrishna, & Seth, 1990). The findings are in agreement with the report of Brown et al. (1982) that the AS process containing the mixed culture bacteria could remove the acrylamide at the removal efficiencies of 50-70% with the acrylamide concentration in the influent of 120 μg/L. This study reports that the AS system could remove the acrylamide at a much higher amount in the level of 100 mg/L. It is well known that the microorganisms, which are capable of acrylamide degradation, use the deamination via amidase enzyme to transform the acrylamide to acrylic acid and ammonia nitrogen (Cha & Chambliss, 2011; Hirrlinger *et al.,* 1996; Nawaz *et al*., 1994; Nawaz *et al.,* 1996; Shanker, et al., 1990). It is also suggested that the acrylamide has no impact on the unacclamitized sludge in the activated sludge system; However, the acrylamide

degradation in the AS system reduced the SCOD removal efficiency to 76.4% as a result of acrylic acid production as shown in Figure 2. The acrylic acid was one of primary products resulting from the acrylamide degradation (Asano, Tachibana, Tani, & Yamada, 1982a; Asano, Yasuda, Tani, & Yamada, 1982b; Nagasawa & Yamadam, 1989; Shanker *et al.,* 1990; Hirrlinger, Stolz, & Knackmuss, 1996). The production rate during first 7.5 hours was 8.4 mg/L-h with the R^2 value of 0.99. It is possibly calculated from the acrylamide removal rate that the acrylamide was completely removed approximately within 7.8 h [107.2/13.8 = 7.8 h]. In addition, the remaining COD should be also totally oxidized within 8.6 h as calculated from the COD removal rate of 35.5 mg COD/L-h [(412.6-107.2)/35.5 = 8.6 h], if it is assumed that the acrylamide to COD ratio (Acrylamide:COD) is 1:1. At reacting time greater than 8.6 h, without available COD and acrylamide, the remaining COD must belong to the acrylic acid.

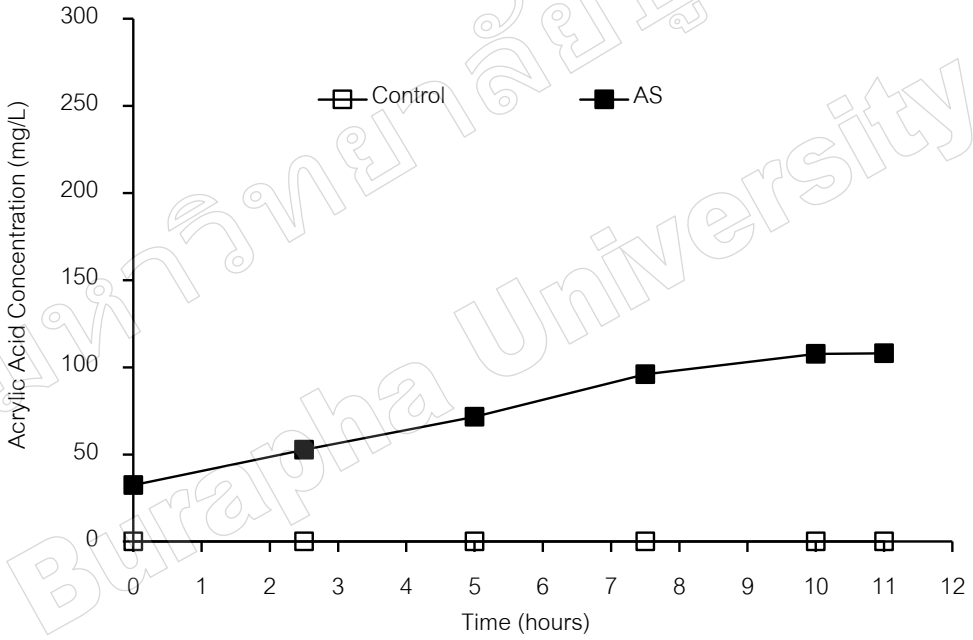

Figure 2 Acrylic acid production in Control and AS SBR systems

The acrylamide degradation by microorganisms has a potential to result in the occurrence of ammonium nitrogen and acrylic acids (Asano, *et al*., 1982a; Asano, *et al.,* 1982b; Nagasawa & Yamadam, 1989; Shanker *et al*., 1990; Hirrlinger, *et al*., 1996). Figure 3 shows that the Control SBR system could complete nitrification within 10 hours of reacting period. The ammonium concentration was less than 1.0 mg N/L (0.43 mg N/L) at the end of reacting periods because the system was operated at high SRT of 10 days and high temperature of 28 $^{\circ}$C. The nitrification rate during first 10.0 hours of reacting period was 4.6 mg N/L-h with the R^2 value of 0.93. It is well known that ammonium nitrogen was nitrified to nitrite nitrogen (nitritation) and then to nitrate nitrogen (nitratation)

consecutively. However, there was nitrite accumulation in the Control system in this experiment. The nitrite and nitrate accumulation rates were 0.20 mg N/L-h and 1.35 mg N/L-h with the R^2 values of 0.95 and 0.99, respectively. The DO concentration was maintained above 2.0 mg/L; therefore, the amount of oxygen was not limited. It is postulated that the temperature above 25 ^{o}C would result in the faster growth rate of ammonium oxidizer bacteria (AOB) than nitrite oxidizer bacteria (NOB) (Sriwiriyarat, Ungkurarate, Fongsatitkul, & Chinwetkitvanich, 2011).

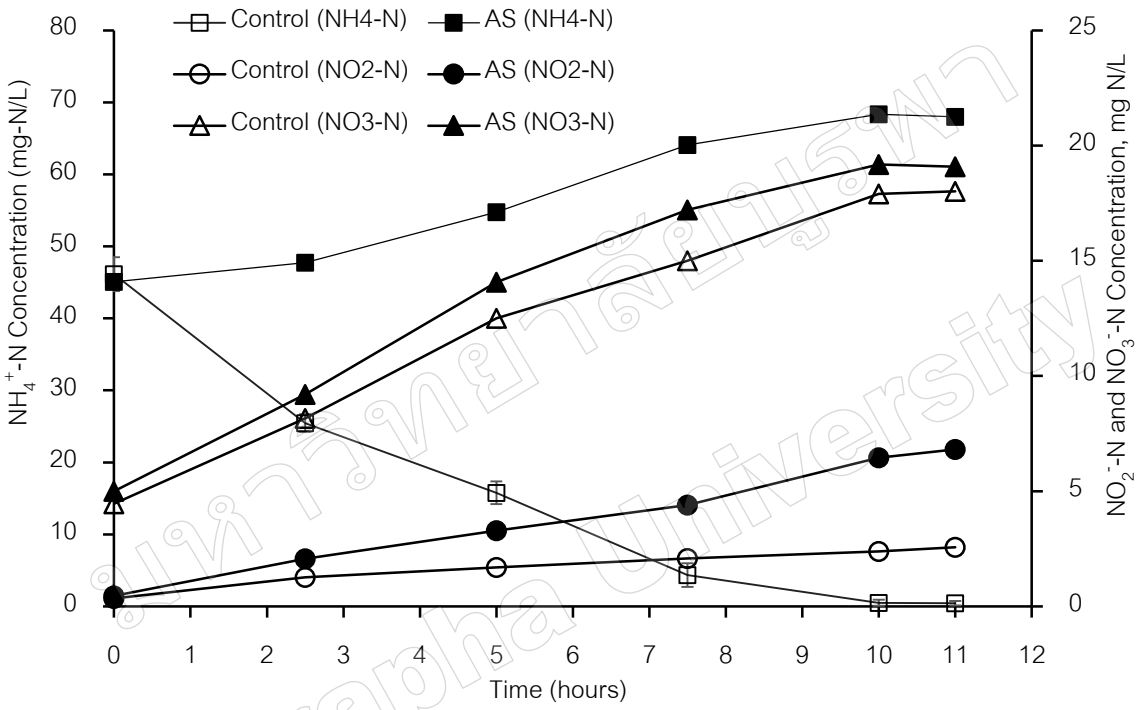

Figure 3 Ammonium, nitrite and nitrate nitrogen profiles in Control and AS SBR systems

As compared with the experimental SBR system (AS), it is found that the ammonium nitrogen concentrations increased over a period of reacting period and accumulated in the AS system resulting from the acrylamide degradation. The accumulation rate of ammonium nitrogen was 2.51 mg N/L-h with the R^2 value of 0.97. In addition, the nitritation and nitratation rates were 0.57 mg N/L-h and 1.46 mg N/L-h with the R^2 values of 0.99 and 0.98, respectively. It is apparent that both rates were higher than the Control systems because the ammonium nitrogen was not limited in the AS system as a result of acrylamide degradation. Greater nitrite and nitrate nitrogen accumulations in the AS system than the control system were also obtained. In summary, additional ammonium nitrogen from the acrylamide degradation could be used as a nitrogen source for microbial growth and nitrification.

These experimental results support the conclusion that the mixed culture of bacteria in the SBR system could degrade efficiently the acrylamide in the SBR system as shown by the accumulations of acrylic acids and ammonium nitrogen. Moreover, if the aeration period is extended, the acrylic acid and ammonium-nitrogen intermediates would potentially be used as carbon and nitrogen sources, respectively (Chun & Chi, 2001).

Conclusion

The experiments aim to evaluate the performances of SBR system containing mixed culture bacteria for acrylamide degradation. The experiments support the conclusion that the acrylamide can be degraded efficiently by the mixed culture bacteria at the acrylamide concentration of about 100 mg/L and would result in the ammonium nitrogen and acrylic acid accumulation because microorganisms employ the deamination process with amidase enzyme to transform the acrylamide to acrylic acid and ammonia nitrogen. The SBR system could remove the acrylamide completely within 10 hours of reacting period, but the COD removal efficiency was reduced because of the accumulation of acrylic acid. The accumulations of ammonium, nitrite, and nitrate nitrogens were found in the AS system as a result of acrylamide degradation.

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References

Asano, Y., Tachibana, M., Tani, Y., & Yamada, H. (1982a). Purification and characterization of amidase which participates in nitrile degradation. *Agricultural and Biological Chemistry*. *46*(5), 1175-1181.

Asano, Y., Yasuda, T., Tani, Y., & Yamada, H. (1982b). A new enzymatic method of acrylamide production. *Agricultural and Biological Chemistry*. *46*(5), 1183-1189.

Brown, L., Rhead, M.M., Hill, D., & Bancroft, K.C.C. (1982). Qualitative and quantitative studies on the in situ adsorption, degradation and toxicity of acrylamide by the spiking of the waters of two sewage works and a river, *Water Research*, *16(*5), 579-591.

Buranasilp, K. & Charoenpanich, J. (2011). Biodegradation of acrylamide by *Enterobacter aerogenes* isolated from wastewater in Thailand, *Journal of Environmental Sciences*, *23*(3), 396–403.

Cha, M., & Chambliss, G.H. (2011). Characterization of acrylamide isolated from a newly isolated acrylamide-utilizing bacterium, *Rastonia eutropha* AUM-01, *Current Microbiology*, *62*(2), 671-678.

Charoenpanich, J. (2013). Removal of acrylamide by microorganisms. In Y.B. Patil & P. Rao (Eds), *Applied bioremediation – active and passive approaches* (104-108). Rijeka, Croatia: Intech.

Cherry, A.B., Gabaccia, A.F., & Senn, H.W. (1956). The assimilation behavior of certain toxic organic compounds in natural waters, *Sewage Industrial Wastes*. *28*, 1137.

Chun, C.W., & Chi, M.L. (2001). Denitrification with acrylamide by pure culture of bacteria isolated from acrylonitrile-butadiene-styrene resin manufactured wastewater treatment system. *Chemosphere*, *44*, 1047-1053.

Eaton, A.D., Clesceri, L.L., & Greenberg, A.E., (Eds.) (1995). *Standard methods for the examination of water and wastewater*. (19th ed.). Washington, DC: American Public Health Association.

Hirrlinger, B., Stolz, A., & Knackmuss, H.J. (1996). Purification and properties of an amidase from Rhodococcus erythropolis MP50 which enantioselectively hydrolyzes arylpropionamides. *Journal of Bacteriology*, *178*(2). 3501-3507.

International Agency for Research on Cancer (IARC). (2008). *World Cancer Report 2008*, France, World Health Organization. Retrieved January 8, 2015 from http//www.iarc.fr/en/publications/pdfs-online/wcr/2008/wcr_2008.pdf.

Nawaz, M.S., Billedeau, S.M., & Cerniglia, C.E. (1998). Influence of selected physical parameter on the biodegradation of acrylamide by immobilized cells of Rhodococcus sp. *Biodegradation*, *9*, 381-387.

Nawaz, M.S., Khan, A.A., Seng, J.E., Leakey,J.E., Siitonen, P.H., & Cerniglia, C.E. (1994). Purification and characterization of an amidase from an acrylamide-degrading Rhodococcus sp. *Applied and Environmental Microbiology*, *60*(9), 3343-3348.

Nagasawa, T., & Yamadam, H., (1989). Microbial transformation of nitriles. *Trends Biotechnology*. *7*(6). 153-158.

Shanker, R., Ramakrishna, C. & Seth, P.K. (1990). Microbial degradation of acrylamide monomer. *Archives of Microbiology*, *154*, 192-198.

Thanyacharoen, U., Tani, A., & Charoenpanich, J. (2012). Isolation and characterization of Kluyvera georgiana strain with the potential for acrylamide biodegradation. *Journal of Environmental Science and Health, Part A. Toxic/hazardous substances & environmental engineering*. *47*(11)1491-1499.

Sriwiriyarat, T., Ungkurarate, W., Fongsatitkul, P., & Chinwetkitvanich, S. (2011). Effects of dissolved oxygen on biological nitrogen removal in integrated fixed film activated sludge (IFAS) wastewater treatment process. *Journal of Environmental Science and Health, Part A. Toxic/hazardous substances & environmental engineering*. *43*(5). 518-527.

Wang, C.C, & Lee, C. M. (2007). Isolation of the acrylamide denitrifying bacteria from a wastewater treatment system manufactured with polyacrylonitrile fiber. *Current Microbiology*. *55*(4), 339-443.