Biochemical Engineering Journal 159 (2020) 107566

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Regular article

Biodegradation of high acrylamide concentrations in integrated fixed film activated sludge (IFAS) wastewater treatment system

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HIGHLIGHTS

- IFAS was superior to conventional activated sludge system to biodegrade acrylamide
- Acrylamide biodegradation rates increased with the decrease of substrate complexity
- Acrylamide toxicity decreased sludge production in the wastewater treatment system
- Clogging of media in IFAS with calcium carbonate was firstly reported

ARTICLE INFO

Keywords:
Acrylamide biodegradation
IFAS
Ammonia stripping
BioPortz
Ammonia inhibition

ABSTRACT

The study was to evaluate the capacity of IFAS technology to enhance the acrylamide (AM) biodegradation in the biological wastewater treatment systems at high concentrations of 200, 300, 400, and 800 mg AM/L. Two sequencing batch reactor (SBR) systems operating as the IFAS and conventional activated sludge (AS) were operated at the solids retention time (SRT) of 9.0 days and hydraulic retention time (HRT) of 24 h at the operating temperature of about 28 °C. The experimental results revealed that the mixed culture bacteria in the IFAS system biodegraded acrylamide at the removal efficiencies of 64.9, 82.4, 99.5, and 86.3% while the AS system removed 36.4, 75.1, 71.8 and 55.9% at the acrylamide concentrations of 200, 300, 400, and 800 mg AM/L, respectively. As acrylamide concentrations increased in the wastewater, the sludge productions decreased due to the acrylamide toxicity, but the biodegradation rates increased resulting from less complexity of mixed substrates. Ammonia was stripped out due to relative high temperature and pH; therefore, the ammonia inhibition effects on the acrylamide biodegradation were not found. Due to the media clogging, the diffusion of substrates was limited; therefore, biodegradation rates in the IFAS system were remarkably less than the AS system. It can be concluded that the IFAS was superior to the AS system because of additional fixed film biomass.

1. Introduction

It is generally known that acrylamide monomer (AM) is not only a carcinogenic and mutagenic compound but also reported as a hazardous substance due to its irritant and neurotoxic properties [1]. Acrylamide is directly used for photopolymerization systems, grouts and adhesives, and polymer productions such as polyacrylamides (PAMs). Furthermore, acrylamide is primarily used for the PAMs production [2]; thus, acrylamide could possibly be found in the wastewater from the PAMs production. Without a proper treatment of this wastewater, the discharge of the wastewater can adversely affect environment and human health. In addition, PAMs are primarily used as a coagulant in the water and wastewater treatment facilities [3]. For the application of PAMs in drinking water treatment facilities, PAMs are allowed to contain acrylamide at the maximum concentration of 0.05% (w/w) and to apply at the maximum concentration of less than 1.0 mg/L [2]. In European Union (EU), PAMs must contain acrylamide less than 0.1% (w/w) depending on regulations in each country; however, some countries in EU such as UK or Netherlands require PAMs to contain acrylamide at the concentration of less than 0.025% (w/w) [4].

Acrylamide can be removed in aquatic environment via abiotic degradation and biodegradation. Brown et al. [5] reported that acrylamide at the concentrations of 0.5 and 5.0 mg AM/L in river water could not be degraded during the pH range of 4–10 with abiotic mechanisms, but were biologically degraded. Furthermore, it has been reported that 2 mg AM/L of acrylamide was completely biodegraded in

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https://doi.org/10.1016/j.bej.2020.107566
Received 21 November 2019; Received in revised form 18 February 2020; Accepted 21 March 2020
Available online 29 April 2020
1369-703X/

the closed bottles inoculated with activated sludge bacteria after 28 days during the OECD 301D test, indicating that acrylamide is readily biodegradable. However, acrylamide at a higher acrylamide concentration of 5 mg AM/L was considered toxic to microorganisms [6]. Most of the previous acrylamide biodegradation studies have focused on the microbial isolation and acrylamide biodegradation of several bacteria including *Arthrobacter* [7], *Rhodococcus* [8], *Nocardia, Bacillus, Xanthomonas, Rhodopseudomonas, Rastonia, Geobacillus, Pseudomonas*, *Enterobacter aerogenes* [3,9]. When acrylamide is biodegraded with amidase as a enzymatic catalyst by those microbes, both ammonia nitrogen and acrylic acid (AA) are generated as primary products [3,9–11]. Yan et al. [12] degraded the hydrolyzed polyacrylamide (HPAM) in a sequencing batch biofilm reactor (SBBR) feeding with the HPAM dosages increasing from 0 to 500 mg HPAM/L. The results revealed that the degradation of HPAMs occurred with the hydrolysis of amide groups; thereby, acrylamide was not produced from the biodegradation. Therefore, the HPAM biodegradation studies could not be used to represent the acrylamide biodegradation in the biological wastewater treatment plants. Brown et al. [13] reported that both activated sludge and biological filter systems could remove acrylamide at low acrylamide concentrations with the removal efficiencies of about 50%. Acrylamide in the biological wastewater treatment sequencing batch reactor (SBR) systems containing *E. aerogenes*, mixed culture bacteria, and a mixture of both bacteria at the operating solids retention time (SRT) of 10 days and hydraulic retention time (HRT) of 24 h were removed at the maximum concentration of 200 mg AM/L [14]. Ammonia nitrogen was dramatically accumulated in the SBR systems, inhibiting the acrylamide biodegradation and nitrification. It appears that mixed culture bacteria in the wastewater treatment system could biologically degrade acrylamide at low concentrations due to the acrylamide toxicity and the inhibition effects of ammonia. Another study indicated by respirometric evaluations that the unacclimatized mixed culture bacteria from SBR systems required the acclimation period of 2 h for acrylamide at the concentration of 400 mg AM/L [15].

Integrated Fixed Film Activated Sludge (IFAS) technology combines both suspended-growth and attached-growth microorganisms in the system to sustain a year-round nitrification [16,17] and to enhance system capacity and stability [18]. It is hypothesized that the IFAS system could increase nitrification, reducing the accumulation of ammonia, and to enhance the system capacity for acrylamide biodegradation; thereby, acrylamide would be removed in the biological wastewater treatment system at higher concentrations. In this study, the performance comparisons between conventional activated sludge (AS) and Integrated Fixed Film Activated Sludge (IFAS) systems for acrylamide biodegradation were evaluated at different acrylamide concentrations of 200, 300, 400, and 800 mg AM/L. Respirometric activities of mixed culture bacteria acclimatized with acrylamide were evaluated at different acrylamide concentrations.

2. Materials and methods

2.1. Reactor setup and operating conditions

Two pilot-scale 10-L SBR reactors named herein as AS and IFAS systems were operated in the Environmental Engineering Laboratory at Burapha University of Thailand. Both systems were operated at the liquid temperature of about 28 °C. The AS SBR system was a conventional activated sludge process containing the suspended-growth mixed culture bacteria. The IFAS SBR system containing both suspended-growth and attached-growth mixed culture bacteria was added with BioPortz moving media (ENTEX Technologies, Inc., USA) at the filling media fraction of 30%, which was equivalent to 3 L or 510 media. The BioPortz media manufactured with high-density polyethylene (HDPE) has the specific surface area of 576 m^2/m^3 [19] and the specific gravity of 0.96; thus, the total specific surface area installed in the IFAS system was 1.73 m^2. The mixed culture bacteria taken from a pilot-scale biological nitrogen removal (BNR) wastewater treatment system located in the same laboratory were seeded to both AS and IFAS systems.

Table 1
List of chemicals in 40-L synthetic wastewater.

Chemicals	Chemical grades and sources	Amount (g)
Sucrose	Commercial Grade, Wangkanai, Thailand	12.0
CH_3COONa	Industrial Grade of 58.8%, Sinoway International, China	24.0
K_2HPO_4	Food Grade of 99.2%, Young Jin Chemical, South Korea	2.0
KH_2PO_4	ACS Grade, VWR Chemicals, EC	4.0
$NaHCO_3$	Food Grade of 99.5%, Tianjin Soda Plant, China	10.0
NH_4Cl	Industrial Grade of 99.5%, Tianjin Soda Plant, China	9.0
$MgCl_2$	Industrial Grade of 47%, Dead Sea Works, Ltd., Israel	2.8
$CaCl_2$	Food Grade of 74.0%, Young Jin Chemical, South Korea	1.6

Both AS and IFAS SBR systems were operated with two cycles per day consisting of five operating periods for each cycle (12 h), i.e., 15 min filling, 10 h aerobic reacting, 1 h settling, 15 min decanting, and 30 min idling. Three small air fine stone diffusers were installed into each SBR system supplied by an air pump at a capacity of 60 L/min to provide the dissolved oxygen (DO) concentrations of about 6.0–7.0 mg O_2/L and to suspend the biomass and media in the systems. Effluent from each SBR system was decanted at the exchange volume ratio of 50% (5.0 L), resulting in a nominal hydraulic retention time (HRT) of 24 h. Due to liquid displacement of BioPortz media (about 5%), the HRT of IFAS system was about 23 h. To control both systems at the operating SRT of about 9.0 days, the sludges were wasted from both reactors at the end of reacting period. The reactor volume of IFAS system was adjusted accordingly to take the bulk volume displacement of BioPortz media for calculating the SRT.

In this study, a synthetic wastewater was prepared daily from various chemicals listed in Table 1 dissolving in a 40-L tap water. The wastewater was allowed to acclimatize for a day with the room temperature before feeding to both AS and IFAS systems at the flowrate of 5 L/day. The analyzes of wastewater revealed that the total chemical oxygen demand (TCOD) of about 438 mg COD/L, soluble chemical oxygen demand (SCOD) of about 398 mg COD/L, total kjeldahl nitrogen (TKN) of about 58 mg N/L, ammonium nitrogen (NH_4^+-N) of about 35.9, pH of about 8.1, and total suspended solids (TSS) of about 27.3 mg SS/L were obtained. Initially, both AS and IFAS systems were fed with only synthetic wastewater until quasi-steady state conditions were achieved. Subsequently, three different acrylamide concentrations of 200, 300, and 400 mg AM/L were added incrementally into the synthetic wastewater while other carbon sources including sucrose and acetate were decreased proportionally to obtain the total COD concentration of about 400 mg COD/L. No other carbon sources were added at the acrylamide feeding concentration of 400 mg AM/L. To evaluate the capacity of IFAS system at a higher acrylamide concentration, the acrylamide concentration of 800 mg AM/L was finally added to the wastewater without other carbon sources in the wastewater. For each acrylamide concentration, both AS and IFAS systems were operated for a period of 7 days. Samples were collected periodically for parameter analyzes at days 0, 3, 5, and 7; however, the day 7 data after the acclimatized period of 6 days were used to evaluate the system performances for COD and acrylamide removals and nitrification.

2.2. Respirometric activities evaluation

The respirometric activities of mixed culture bacteria acclimatized with acrylamide were evaluated by using the OxiTop Control apparatus (OC 110, WTW, Germany). It was reported as a reliable respirometric test method for evaluating the biodegradability of chemical compounds [20]. A pressure drop of gases in the closed OxiTop vessels at a constant

temperature is used to indicate the respirometric activities. During the first a few hours, the pressure in the vessel generally increases as a result of the difference in temperature between sample and incubator [21,22]. After that, it is usually found that pressure in the vessel decreases nonlinearly because microorganisms require an acclimation period to adapt themselves to new conditions. When the pressure decreases linearly due to the steady microbial oxygen consumption, the oxygen uptake rate (OUR) could be determined from Eq. (1) using the slope of linear pressure drop ($\Delta P/t$) [15]. For comparison between the systems and between different acrylamide concentrations, the OUR was divided by the MLVSS concentration to determine the specific oxygen uptake rate (SOUR).

$$\mathrm{OUR}\left(\frac{\mathrm{mg\ O_2}}{\mathrm{L{\cdot}h}}\right) = \frac{\left(\frac{\Delta P}{t}\frac{\mathrm{hPa}}{\mathrm{h}}\right)\cdot\left(\frac{100\mathrm{Pa}}{\mathrm{hPa}}\right)\cdot\left(\frac{1\mathrm{N}}{\mathrm{m^2}}\frac{\mathrm{m^2}}{\mathrm{Pa}}\right)\cdot\left(\frac{32\mathrm{g}}{\mathrm{mol}}\right)\cdot\left(\frac{1000\mathrm{mg}}{\mathrm{g}}\right)}{\left(\frac{8.314\mathrm{J}}{\mathrm{mol{\cdot}^\circ K}}\right)\cdot\left(\frac{1\ \mathrm{N{\cdot}m}}{\mathrm{J}}\right)\cdot T(^\circ\mathrm{K})\cdot\left(\frac{1000\mathrm{L}}{\mathrm{m^3}}\right)} \quad (1)$$

Where T is the temperature in Kelvin (K). t is the incubation time in hour, and ΔP is the pressure drop in hPa.

To evaluate the respirometric activities, mixed culture bacteria were collected from the AS and IFAS SBR systems at the end of reacting periods to obtain a 0.5-L sludge after achieving the steady state conditions for each acrylamide concentration. Each sludge was washed three times with distilled water to remove remaining substrates. Additional 0.5 L of distilled water was added to the sludge to increase the sludge volume to 1 L. The sludge was then transferred equally into two 1-L OxiTop bottles (0.5 L each). Subsequently, all OxiTop bottles were injected with acrylamide concentration of 200 mg AM/L along with the synthetic wastewater that was fed to the SBR systems. For the IFAS system, 20 BioPortz media were randomly taken from the IFAS system and added into the OxiTop bottle. The nitrification inhibitor of N-Allylthiourea ($C_4H_8N_2S$) (98%, Alfa Aesar, UK) at a concentration of 5 g/L was added at the dosage of 2 mL to inhibit nitrification so the effects of ammonia on acrylamide biodegradation could be evaluated. Finally, the OxiTop system was incubated in the BOD incubator operated at the temperature of 28 °C for a period of 5 days. The pressure data were recorded at the 20-min time interval for a period of 5 days to obtain 360 datasets. A handheld remote controller was used to transfer all data from the OxiTop heads by using an infrared interface and software-controlled functions. Finally, the data in the remote controller were transferred to the personal computer (PC) via a cable and a communication program called Achat OC (version 2.03).

2.3. Sampling and sample analyzes

After both AS and IFAS systems achieved the quasi-steady state conditions, a set of samples was collected for parameter analyzes. Mixed liquor suspended solids (MLSS), MLVSS, TCOD and SCOD (Closed Reflux, Titrimetric Method), TKN (Semi-Micro-Kjeldahl Method), ammonium nitrogen (NH_4^+—N) (Phenate Method), nitrite nitrogen (NO_2^-—N) (Colorimetric Method), and nitrate nitrogen (NO_3^-—N) (Brucine Method) were measured in accordance with Standard Methods for the Examination of Water and Wastewater [23]. Both acrylamide and acrylic acid were quantified by a high performance liquid chromatography (HPLC) (Varian 9050). The HPLC was installed with a UV spectrophotometric detector (JENWAY 6305) operating at 254 nm installed with a Nova-Pack C18 (4 µm 60 Å) Guard-Pak Insert column (Waters, Ireland) in a reversed system. The mobile phase consisted of 50% deionized water and 50% acetonitrile. A 20-µL volume was delivered from a 60 µL injection loop of sample, which was filtered and injected into the HPLC system, to obtain the peak areas. A run time of 5 min under a constant flowrate of 1 mL/min was operated at room temperature. To determine both acrylamide and acrylic acid concentrations, the standard solutions of both compounds at different concentrations were prepared for the calibration curve. To measure acrylamide, acrylic acid, SCOD, NH_4^+—N, NO_2^-—N and NO_3^-—N, all samples were filtered with glass membrane filter paper with a pore size of 0.45-µm to remove all particulates after centrifuging samples at 10,000 rpm for 10 min. The pH and DO values were determined by a pH meter (Cyberscan pH510, Eutech Instruments) and a DO meter (Cyberscan DO110, Eutech Instruments), respectively.

The fixed film biomass on the BioPortz media was quantified by randomly sampling two BioPortz media from the IFAS system. A large syringe connecting with a small pipette tip was used to provide a strong water jet for cleaning the biomass from the BioPortz media. The washed out biomass was collected in a glass beaker. Subsequently, the beaker was filled up with distilled water to obtain a liquid volume of 100 mL for biomass dilution. A 5-mL sample volume was randomly collected for MLSS and MLVSS analyzes of biofilm. The MLVSS concentrations were used to calculate the biofilm density on the 510-BioPortz media and then to determine the equivalent MLVSS concentrations calculated by dividing the total amount of biomass with the volume of reactor.

3. Results and discussion

3.1. Acrylamide biodegradation in the AS and IFAS systems

Both AS and IFAS systems, which were operated in parallel under the controlled experimental conditions and fed only with synthetic wastewater without any acrylamide addition, achieved the quasi-steady state conditions after the operating period of about six months. Both systems were subsequently fed with synthetic wastewater supplemented incrementally with acrylamide at the concentrations of 200, 300, and 400 mg AM/L, while the total COD concentration was kept constant of about 400 mg COD/L, to compare the capacities of AS and IFAS systems for the acrylamide biodegradation. Acrylamide was a sole carbon source in the synthetic wastewater at the acrylamide concentration of 400 mg AM/L. It appears from Fig. 1 that the IFAS system removed acrylamide linearly at the biodegradation rate of 6.3 mg AM/L-h (R^2 = 0.96) when the system was fed with acrylamide at the concentration of 200 mg AM/L for 7 days (64.9% removal efficiency, 0.9 mM). Number with a unit of mM inside parenthesis indicates the amount of acrylamide removed in the system. At the acrylamide concentrations of 300 and 400 mg AM/L, the acrylamide concentrations also decreased linearly at the biodegradation rates of 8.9 and 14.2 mg AM/L-h, resulting in the acrylamide removal efficiencies of 82.4% (R^2 = 0.97) (1.5 mM) and 99.5% (R^2 = 0.97) (2.7 mM), respectively. It appears that the capacity of the IFAS system to degrade acrylamide increased with the acrylamide concentration in the wastewater even acrylamide was used as a sole carbon source at the concentration of 400 mg AM/L. In contrast, the AS system degraded acrylamide at the biodegradation rates of 3.1 (R^2 = 0.99), 10.2 (R^2 = 0.98), and 12.2 (R^2 = 0.98) mg AM/L-h with the removal efficiencies of 36.4% (0.5 mM), 75.1% (1.4 mM) and 71.8% (1.7 mM) at the acrylamide concentrations of 200, 300 and 400 mg AM/L, respectively. The results indicate that the acrylamide biodegradation rates increased with the increases of acrylamide fractions in the wastewater in both AS and IFAS systems. It is possible to explain that the complexity of mixed substrates in wastewater decreased at higher acrylamide fractions. It is noted that acrylamide concentrations at the beginning of reacting period were resulted from the dilution effects of mixing between influent acrylamide concentrations and acrylamide concentrations remaining in the reactor from previous operating cycle at the exchange volume ratio of 50%. Therefore, these findings suggest clearly that the IFAS system had a greater capacity for the acrylamide biodegradation than the AS system.

When acrylamide is degraded biologically with amidase, acrylic acid and ammonia nitrogen are generated [3,9–11]. The experimental results showed that acrylic acid was produced minimally at the concentrations of 36.5 ± 2.6, 37.1 ± 2.2 and 33.9 ± 2.3 mg AA/L in the AS system and at the concentrations of 37.1 ± 2.2, 34.9 ± 1.8 and 35.6 ± 3.5 mg AA/L in the IFAS system at the acrylamide concentrations of 200, 300 and 400 mg AM/L, respectively. It is generally known

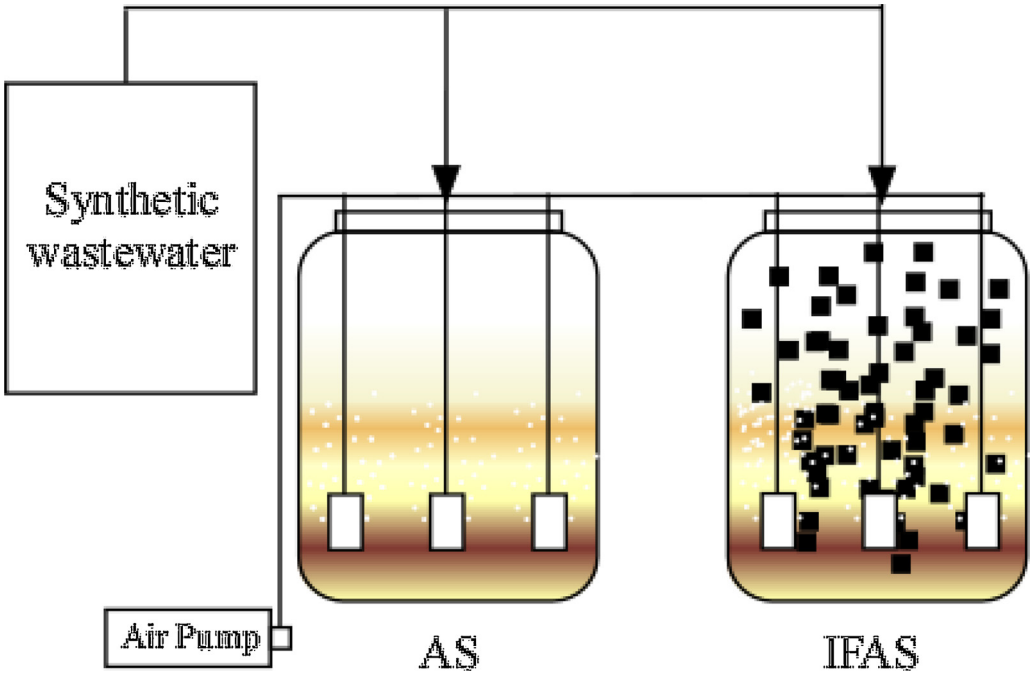

Fig. 1. Acrylamide concentrations in the AS and IFAS systems during the reacting periods with the acrylamide concentrations of 200, 300, and 400 mg AM/L; number in a parenthesis indicates the acrylamide concentration.

that one mole of acrylamide is biodegraded to one mole of acrylic acid and one mole of ammonia [24]; therefore, acrylic acid must be produced at the same amount of acrylamide biodegraded. According to the stoichiometric relationship listed above, the acrylic acid concentrations in the AS and IFAS systems from the calculations would be 35.1 and 62.8 mg AA/L, 101.1 and 110.3 mg AA/L, and 120.7 and 193.8 mg AA/L at the acrylamide concentrations of 200, 300 and 400 mg AM/L, respectively. It would appear that acrylic acids produced from the biodegradation of acrylamide were removed partially in both AS and IFAS systems. It is believed that microbes degraded the acrylic acid in the solutions to produce carbon dioxide.

Table 2 lists the average ammonium concentrations in both AS and IFAS systems at the acrylamide concentrations of 0, 200, 300, and 400 mg AM/L. It appears that the ammonium concentrations were also minimally changed with time during 7 days of operations in both AS and IFAS systems at different acrylamide concentrations. From the stoichiometric calculations, ammonia concentrations would be generated at the concentrations of 6.8 and 12.2 mg N/L, 19.7 and 21.4 mg N/L, and 23.5 and 37.7 mg N/L in both AS and IFAS systems at the acrylamide concentrations of 200, 300 and 400 mg AM/L, respectively. The ammonium concentrations in effluents of both AS and IFAS systems after acrylamide additions were approximately the same as the effluents from both systems feeding with only synthetic wastewater, suggesting that ammonia produced from the acrylamide biodegradation contributing to the wastewater was removed from both systems.

3.2. COD removal and nitrification in the AS and IFAS systems

Fig. 2 presents the COD profiles in both AS and IFAS systems after feeding with acrylamide at the concentrations of 0, 200, 300, and 400 mg AM/L. When both AS and IFAS systems were fed with only synthetic wastewater, it was found that both AS and IFAS systems removed COD equally at the removal efficiency of 87.5%, leaving COD of about 50 mg COD/L in the effluents. No further COD biodegradation was observed after the reacting period of 2 h, indicating that only non-biodegradable organics were remained in the solution because all carbon substrates including sucrose and acetate are biodegradable compounds. Furthermore, both systems were operated at high SRT of 9 days with respect to the washout SRT of heterotrophic bacteria [25] and at the temperature of about 28 °C.

After the additions of acrylamide at the concentrations of 200, 300 and 400 mg AM/L, it appears from Fig. 2 that both AS and IFAS systems degraded COD equally at the concentration of 200 mg AM/L. The organic matters in the AS and IFAS systems were degraded slowly resulting in the COD removal efficiencies of 54.5% with the effluent COD concentrations of about 200 mg COD/L, respectively. It confirms that the addition of acrylamide lowered the COD removals of mixed culture bacteria in both AS and IFAS systems. The COD analyzes of acrylamide

Table 2
Average ammonium concentrations with standard deviations in the AS and IFAS systems at the acrylamide concentrations of 0, 200, 300, and 400 mg AM/L.

Time (h)	Ammonium concentrations (mg N/L)							
	0 mg AM/L		200 mg AM/L		300 mg AM/L		400 mg AM/L	
	AS	IFAS	AS	IFAS	AS	IFAS	AS	IFAS
0	35.4 ± 3.4	35.0 ± 3.7	36.9 ± 3.8	36.4 ± 2.6	31.9 ± 2.6	31.9 ± 2.8	32.9 ± 3.2	32.9 ± 3.2
2	35.4 ± 3.4	34.9 ± 3.8	35.7 ± 3.1	35.7 ± 3.3	32.3 ± 3.7	32.3 ± 3.7	32.6 ± 3.8	31.9 ± 3.6
4	35.0 ± 3.7	34.2 ± 4.0	33.8 ± 1.8	35.7 ± 3.3	31.8 ± 3.9	32.3 ± 3.7	32.6 ± 3.8	32.6 ± 3.8
6	34.5 ± 2.7	32.1 ± 2.4	34.5 ± 1.3	34.5 ± 1.3	31.8 ± 3.9	32.3 ± 3.7	31.9 ± 3.6	32.6 ± 3.8
8	33.6 ± 3.0	30.2 ± 2.8	34.5 ± 1.3	34.5 ± 1.3	31.5 ± 4.4	31.5 ± 4.4	31.9 ± 3.6	32.6 ± 3.8
10	33.6 ± 3.0	28.5 ± 2.0	34.5 ± 1.3	34.5 ± 1.3	31.5 ± 4.4	31.5 ± 4.4	31.7 ± 4.1	32.6 ± 3.8

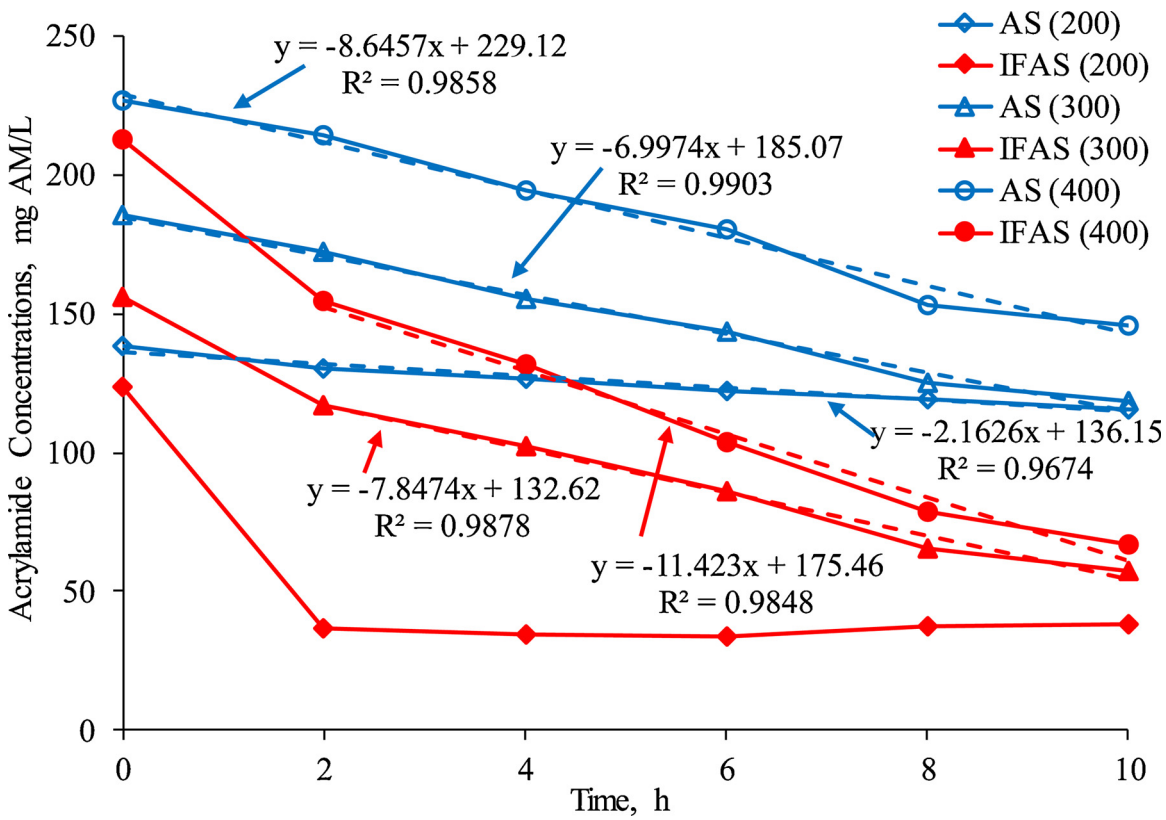

Fig. 2. COD concentrations in the AS and IFAS systems during the reacting periods with the acrylamide concentrations of 200, 300, and 400 mg AM/L; number in a parenthesis indicates the acrylamide concentration.

and acrylic acid in this study revealed that the COD values of both compounds were about 1.00 g COD/g AM and 1.45 g COD/g AA, respectively. According to the COD mass balance calculations, the organic matters remaining in effluents of the AS and IFAS systems were contributed by remaining acrylamide and acrylic acid at the percentages of 85.9 and 60.2%, respectively. At the acrylamide concentration of 300 mg AM/L, much less COD was found to remain in the IFAS system than the AS system. The COD removal efficiency of the IFAS system was only 63.6%, leaving the COD concentration of 160 mg COD/L in the effluent. Total fraction of both acrylamide and acrylic acid in the effluent was 63.1%. On the other hands, the COD removal efficiency of AS was 36.4%, resulting in the COD in effluent of 280 mg COD/L with the fraction of acrylamide and acrylic acid of 43.5%. Finally, it appears that COD concentrations decreased dramatically in the IFAS system when acrylamide was a sole carbon source in the wastewater at the concentration of 400 mg AM/L, providing the greatest removal as compared with the COD removal efficiency of AS system (50.0%). The COD removal efficiency of IFAS system was 83.3%, leaving the COD concentration in the effluent of 80 mg COD/L. The remaining fractions of acrylamide and acrylic acid in both AS and IFAS systems at 69.1 and 62.7%, respectively. The experimental results support the conclusion that the IFAS system was superior to the AS system for COD removals at the acrylamide concentrations greater than 200 mg AM/L. Also, the remaining acrylamide and acrylic acid produced from the acrylamide biodegradation contributed COD dramatically in the effluents, resulting in lower COD removal efficiencies.

It was found from Table 2 that when both systems were fed with synthetic wastewater without acrylamide, ammonium removal efficiencies were only 36.2 and 46.4% in the AS and IFAS systems, respectively. The experimental results revealed that nitrite concentrations were negligible in both AS and IFAS systems. As illustrated in Fig. 3, nitrate was accumulated minimally in the AS system, but were greatly produced in the IFAS system. The observations indicated that nitrification was completely lost from the AS system, but ammonium was partially nitrified in the IFAS system. It clearly indicates that IFAS system performed nitrification better than the conventional AS system due to additional biomass in the BioPortz media [16–18]. From the nitrogen mass balance calculations [26], the nitrogen mass balances of AS and IFAS systems in Fig. 4 were about 92.7 and 101.6%, respectively. The total nitrogen loadings to both AS and IFAS systems were 0.26 g N/cycle. In addition, the total nitrifications in the AS and IFAS systems were 0.03 and 0.05 g N/cycle, respectively. However, the total nitrogens including ammonium, nitrite and nitrate in the effluents of AS and IFAS systems were only 0.17 and 0.20 g N/cycle, respectively. The results indicate that ammonium nitrogens were lost from the AS system as indicated by the unidentified nitrogen loss fraction of Fig. 4. It is hypothesized that ammonium nitrogen in the AS system was partially stripped out from the solution. Only IFAS system nitrified ammonium nitrogen without any ammonia stripping. Throughout the studies, the average pH values of synthetic wastewater fed to the AS and IFAS systems were 8.2 ± 0.1 and 8.0 ± 0.1, respectively. The pH of solution in the AS system increased to 8.4 during the reacting period due to the failure of nitrification (no acid produced from nitrification) and the stripping of carbon dioxide (CO_2). According to the Henry constants of CO_2 and free ammonia nitrogen (FAN), CO_2 is stripped faster than the FAN [27]; thereby, the raise of pH is expected. According to US.EPA [26], it is listed in the table at the temperature of 28 °C and pH of 8.2 that the fraction of un-ionized ammonia is about 10.0% of total ammonium and ammonia species. When the pH increased from 8.2 to 8.4 [28], the fraction of FAN increases to 15%. On the other hands, the pH of IFAS system decreased from 8.0 to 7.95 due to the partial nitrification as a result of BioPortz addition. The fraction of FAN at the pH of 8.0 is 6.56% [28]. Therefore, it can be concluded reasonably that ammonium nitrogen was stripped out from the solution of AS system due to the moderate temperature of about 28 °C, pH of about 8.4, and turbulence of mixing in the systems [27,29].

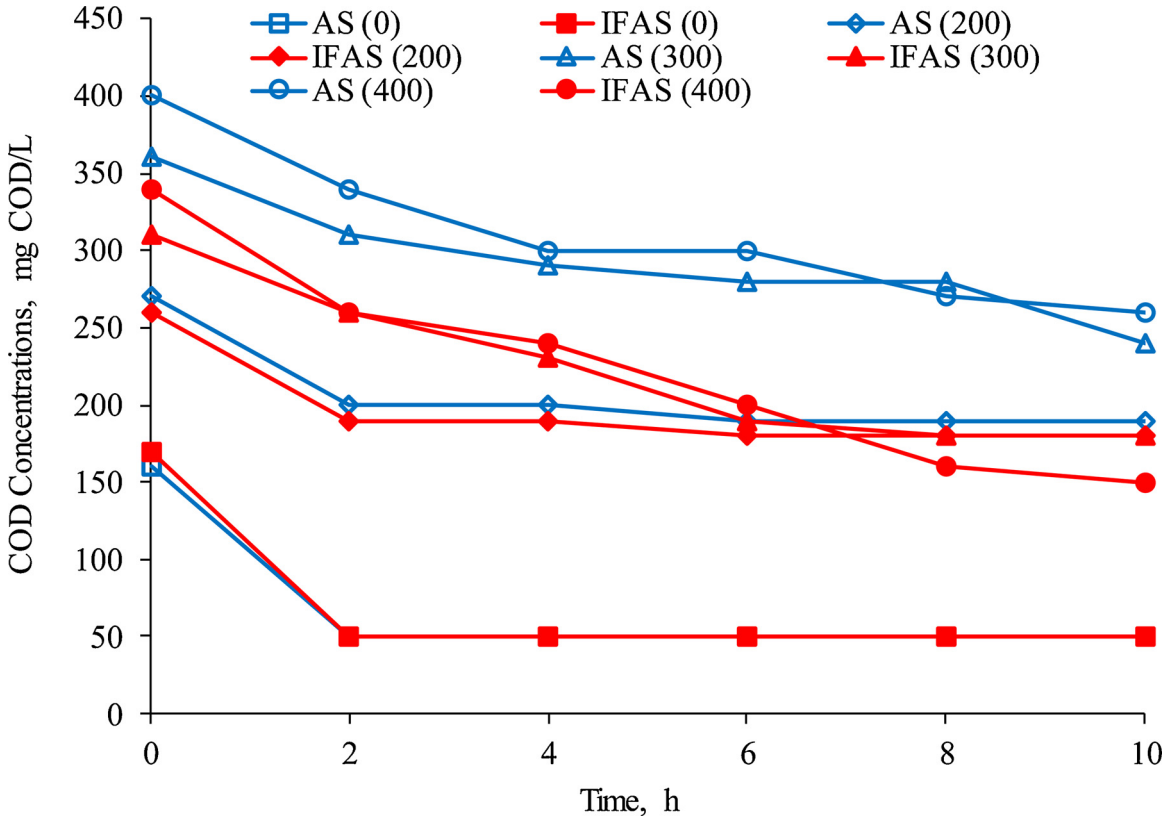

Fig. 3. Nitrate nitrogen concentrations in the AS and IFAS systems during the reacting periods with the acrylamide concentrations of 200, 300, and 400 mg AM/L; number in a parenthesis indicates the acrylamide concentration.

It was found from Fig. 4 that the ammonium removal efficiencies decreased dramatically when the acrylamide concentrations were added in the wastewater as compared with the results from the systems feeding with only synthetic wastewater. The ammonium removal efficiencies of the AS and IFAS systems were 23.7 and 26.3%, 25.7 and 27.3%, and 23.0 and 23.0% at the acrylamide feeding concentrations of 200, 300, and 400 mg AM/L, respectively. It is known that the ammonia from the acrylamide biodegradation contributed nitrogen loadings to

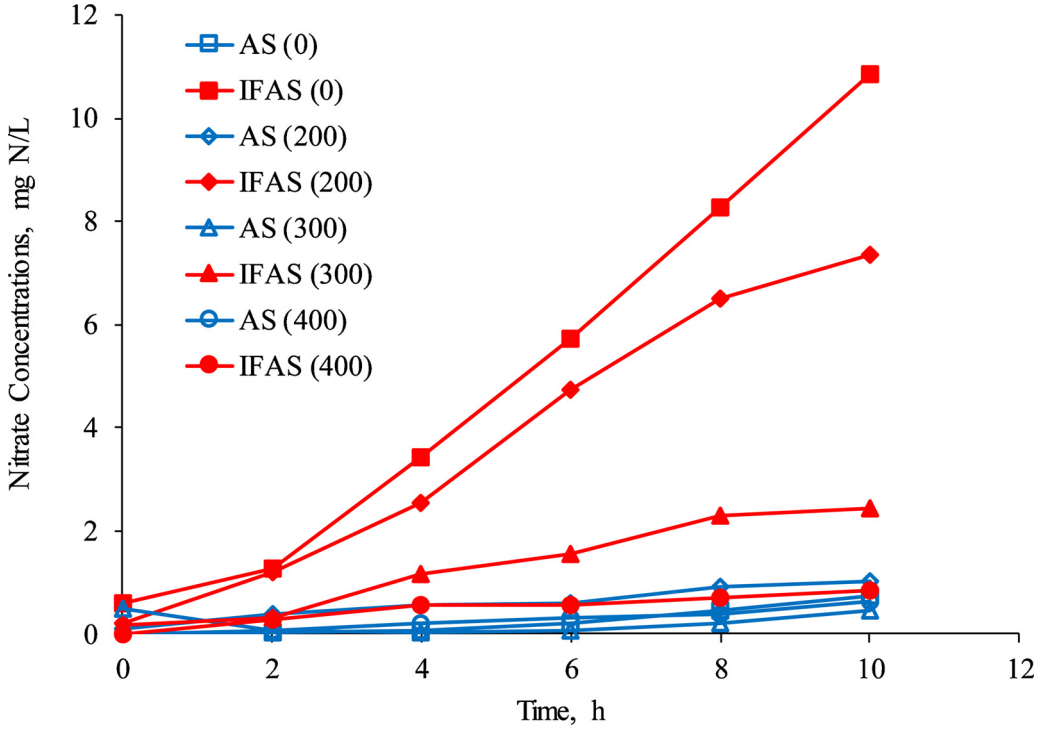

Fig. 4. Nitrogen mass balances and ammonium removal efficiencies with different acrylamide concentrations.

both AS and IFAS systems, depending on the acrylamide removal efficiencies. The total nitrogen loadings to the AS system were 0.26, 0.29 and 0.29 g N/cycle and to the IFAS system were 0.32, 0.31, and 0.36 g N/cycle at the acrylamide concentrations of 200, 300 and 400 mg AM/L, respectively. However, Fig. 3 indicates that nitrate nitrogen was accumulated minimally in the AS system, indicating that nitrifications were lost from the AS system at the acrylamide concentrations of 200, 300 and 400 mg AM/L. On the other hands, the nitrate concentrations in the IFAS system decreased as the acrylamide concentrations increased in the wastewater, suggesting that nitrifications in the IFAS system decreased with the increasing of acrylamide concentrations. Nitrification was completely lost from the IFAS system at the acrylamide concentration of 400 mg AM/L. Furthermore, the percentages of nitrogen mass balances in Fig. 4 at the acrylamide concentrations of 200, 300, and 400 mg AM/L around the AS system were 94.4, 71.2 and 71.4% and around the IFAS system were 87.7, 71.4 and 59.3%, respectively, corresponding to the unidentified nitrogen losses illustrated in Fig. 4. The fractions of nitrogen losses in both AS and IFAS systems increased with acrylamide concentrations. It is suggested that ammonia resulting from the acrylamide biodegradation were removed from both AS and IFAS systems due to ammonia stripping.

3.3. Sludge production in the AS and IFAS systems

Table 3 lists the MLSS and MLVSS of suspended-growth biomass concentrations in both AS and IFAS systems. Table 3 also provides biofilm densities on the BioPortz media in the IFAS system. It appears that MLSS and MLVSS concentrations were approximately the same between the AS and IFAS systems because the suspended-growth biomass in both systems removed the same amounts of substrates and were operated approximately at the same SRT. However, the BioPotz media supplemented a great amount of biomass in the systems as indicated by total MLVSS. It is noted that the total MLVSS in the IFAS system was a combination of MLVSS and equivalent MLVSS. As acrylamide increased in the wastewater, the biomass concentrations decreased in both AS and IFAS systems. It could possibly be explained that acrylamide was toxic to microorganisms at high concentrations. As the acrylamide concentrations increased in the systems, acrylamide inhibited the microbial growth and resulted in lower biomass concentrations. However, the biofilm densities did not change with acrylamide concentrations; thereby, the total MLVSS concentrations in the IFAS systems remained approximately constant at different acrylamide concentrations. In addition, the MLVSS/MLSS ratios in both AS and IFAS systems were 0.97 and 0.99, respectively. However, the MLVSS/MLSS ratio of biofilm was only 0.37, indicating that substantial amount of inorganic matters was accumulated in the BioPortz media. It was observed from samples after cleaning BioPortz media with acid at the end of experiments that calcium carbonate precipitates were accumulated in the BioPortz media; thereby, specific surface area in the BioPortz media was reduced. It is explained that the total hardness of synthetic wastewater was about 120 mg $CaCO_3$/L. At the moderate temperature of 28 °C and pH of about 8.0, it was possible that the hardness was precipitated as calcium carbonate and was accumulated inside the BioPortz media because the solubility product (K_S) of calcium carbonate is decreased with increasing temperature. Without calcium carbonate clogging in the BioPortz media, it is possible that the IFAS system could provide higher capacity to biodegrade acrylamide than the AS system.

3.4. Respirometric activities of mixed culture bacteria

The respirometric activities were evaluated in the OxiTop apparatus for the effects of different acrylamide concentrations on the acclimatized mixed culture bacteria from the AS and IFAS systems. Fig. 5 presents the pressure drops in the OxiTop bottles containing mixed culture bacteria taken from the AS and IFAS systems during initial two days of incubation after 7-day of operating periods at the acrylamide concentrations of 0, 200, 300 and 400 mg AM/L. Datasets of initial two days were chosen for evaluating respirometric activities in this study to avoid the oxygen limitations in the OxiTop bottles. When feeding both AS and IFAS systems with only synthetic wastewater, Fig. 5 indicates that the pressures in the OxiTop bottles of both systems decreased linearly with time as a result of microbial metabolism consuming oxygen and producing carbon dioxide after achieving the temperature equilibrium, suggesting that the acclimated mixed culture bacteria from the AS and IFAS systems did not require the acclimation period for degrading the synthetic wastewater because both systems were fed with this synthetic wastewater for about 6 months. After both systems were fed with the wastewater containing acrylamide at the concentrations of 200, 300 and 400 mg AM/L, it appears from the pressure drops in Fig. 5 that acclimatized suspended-growth microbes from the AS system could degrade organic matters in the wastewater immediately without any acclimation period. Madmanang et al. [15] reported that unacclimatized suspended-growth mixed culture bacteria required 2 h for acrylamide adaptation when acrylamide was a sole carbon source at the concentration of 400 mg AM/L. In contrast, the microbes from the IFAS system required longer time of 2.5–3.0 h before degrading organic matters in the wastewater steadily. About 1 h of substrate acclimatization was required for the unacclimatized mixed culture bacteria from the IFAS system [15]. Therefore, it is believed that the mass transport resistance limited the substrate diffusion into the biofilm matrix.

The linear slopes of pressure drops in Fig. 5 were used to calculate the OURs and SOURs according to Eq. 1 of mixed culture bacteria from both AS and IFAS systems at the acrylamide concentrations of 0, 200, 300 and 400 mg AM/L, respectively. The OURs and SOURs listed in Table 4 indicate that the biodegradation rates of microbes from both systems increased with acrylamide concentrations in the synthetic wastewater. It is believed that the complex nature of mixed substrates decreased as acrylamide concentrations increased in the wastewater. It is noted that acrylamide was a sole carbon source at the acrylamide feeding concentration of 400 mg AM/L. It was reported that the substrate affinity increased when the complexity of mixed substrates decreased, resulting higher acrylamide biodegradation rate [30]. The higher the proportion of acrylamide in the wastewater, the more the

Table 3
Average MLSS and MLVSS concentrations of mixed culture bacteria in the AS and IFAS systems.

AMconcentrations (mg AM/L)	System	MLSS (mg SS/L)	MLVSS (mg VSS/L)	Biofilm (g/m^2)	Total MLVSS* (mg VSS/L)
0	AS	1443 ± 47	1395 ± 17	–	1395
	IFAS	1305 ± 90	1295 ± 90	21.1	4942
200	AS	1210 ± 141	1188 ± 151	–	1188
	IFAS	1220 ± 69	1203 ± 74	22.0	5002
300	AS	875 ± 26	865 ± 26	–	865
	IFAS	945 ± 31	935 ± 31	20.9	4556
400	AS	788 ± 70	773 ± 62	–	773
	IFAS	868 ± 41	838 ± 36	21.5	4561

* Total MLVSS = suspended MLVSS + equivalent MLVSS (biofilm).

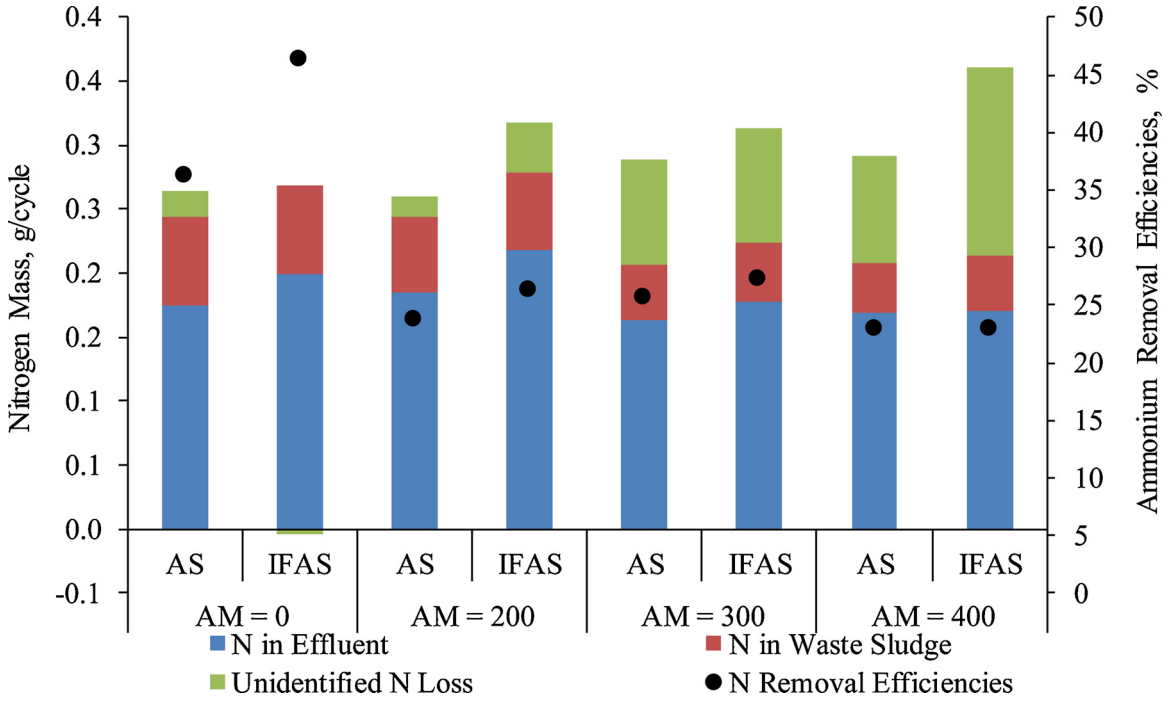

Fig. 5. Pressure drops during a 2-day incubation period of mixed culture bacteria from the AS and IFAS systems at the temperature of 28 °C and at the acrylamide concentrations of 0, 200, 300 and 400 mg AM/L.

Table 4
Oxygen uptake rate (OUR) and specific oxygen uptake rate (SOUR) of mixed culture bacteria from the AS and IFAS systems.

Acrylamide (mg AM/L)	AS system			IFAS system			
	MLVSS	OUR	SOUR	MLVSS	Biofilm	OUR	SOUR
0	880	2.09	0.86	740	2860	0.43	0.05
200	1050	2.55	1.63	1150	2980	0.24	0.19
300	840	4.84	2.23	940	2840	0.60	0.29
400	730	4.38	2.47	820	2920	0.53	0.22

MLVSS unit is mg VSS/L; OUR unit is mg O_2/L-h; SOUR unit is mg O_2/g VSS-h; Biofilm is equivalent MLVSS with a unit of mg VSS/L.

amount of amidase was induced [31]. The OURs of mixed culture bacteria in Table 4 reveal that the suspended-growth mixed culture bacteria from the AS system removed substrates at higher biodegradation rates than the IFAS system at all acrylamide concentrations due to the limitation of substrate diffusion. Higher biomass concentrations in the IFAS systems also resulted in dramatically lower the SOURs than the AS system.

3.5. Enhanced capacity of IFAS system for acrylamide biodegradation

Both AS and IFAS systems were continuously operated and were finally fed with acrylamide as a sole carbon source at the concentration of about 800 mg AM/L or 840 mg COD/L. It was found that the biomass concentrations decreased to 490 ± 14 and 1125 ± 49 mg VSS/L in the AS and IFAS systems, respectively. The total MLVSS concentration was 5562 mg VSS/L. It indicates that the mixed culture bacteria were almost washed out from the AS system due to the toxicity of acrylamide, but biomass concentrations increased in the IFAS system. The COD removal efficiencies in the AS and IFAS systems were 26.2 and 66.7%, resulting in COD in effluents of 620 ± 28 and 280 ± 57 mg COD/L due to the remaining acrylamide and acrylic acid in effluents, respectively. The failures in nitrification were still found in both systems; therefore, there were no nitrite and nitrate accumulation. Interestingly, the acrylamide removal efficiencies in the AS and IFAS systems were 53.8 and 85.0%, respectively. The OURs of AS and IFAS systems were 4.75 and 7.39 mg O_2/L-h, respectively. Both AS and IFAS removed acrylamide at the concentrations of 1.7 and 3.7 mM, respectively. Acrylic acid was remained in the effluents of the AS and IFAS systems at the concentrations of 47.7 ± 2.5 and 52.7 ± 1.8, respectively. Thus, it could be concluded that the IFAS system containing BioPortz media enhanced the acrylamide biodegradation at high concentrations due to additional biomass in the media. Furthermore, it is well known that microbial communities in the fixed film media are resistant intrinsically to changing environmental conditions, which are more resilient to variation in toxicity concentrations in the wastewater [32].

4. Conclusions

The conventional activated sludge and IFAS sequencing batch reactor systems were comparatively evaluated to degrade acrylamide at the relative high concentrations of 0, 200, 300, 400 and 800 mg AM/L, at the operating SRT of 9 days and at a nominal HRT of 24 h. Due to the acrylamide toxicity at high concentrations, the sludge productions of suspended-growth biomass decreased with acrylamide concentrations. Nitrification was completely lost from both systems. The experimental results supported the conclusions that the IFAS system was superior to the conventional AS system to degrade acrylamide at high concentrations due to the additional biomass in the media resisting the acrylamide toxicity. However, ammonia from the acrylamide biodegradation was stripped out due to the relative high temperature and pH; therefore, the ammonia inhibition effects on the acrylamide biodegradation were removed. Acrylic acid was partially biodegraded. The respirometric activities indicated that acrylamide reduced the complexity of mixed substrates; thus, biodegradation rates in both AS and IFAS systems increased as the acrylamide concentrations increased in the wastewater. However, the diffusion of substrates was limited in the IFAS system due to the clogging of BioPortz media; therefore, the oxygen uptake rates of mixed culture bacteria in the IFAS system were dramatically less than the AS system.

CRediT authorship contribution statement

Tongchai Sriwiriyarat: Conceptualization, Methodology, Formal analysis, Resources, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Romsan Madmanang:** Investigation, Validation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This research was supported by the Research Funds of Burapha University through National Research Council of Thailand [No. 121/2558]; the Office of the Higher Education Commission (OHEC), Ministry of Education, Thailand.

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