Appl Biol Chem (2016) 59(5):703–709
DOI 10.1007/s13765-016-0216-1

Online ISSN 2468-0842
Print ISSN 2468-0834

ARTICLE

Biodegradation of 3-chloroaniline by suspended cells and biofilm of *Acinetobacter baumannii* GFJ1

Ha Danh Duc[1,2]

Received: 15 April 2016 / Accepted: 21 June 2016 / Published online: 6 July 2016

Abstract *Acinetobacter baumannii* strain GFJ1 was isolated from soil using 3-chloroaniline (3CA) as sole of carbon, nitrogen, and energy source under both aerobic and anaerobic conditions. The investigation of aerobic utilization profile showed that the utilization kinetics of 3CA followed the Edward model with a maximum specific degradation as 3.45 ± 0.33 μM.h^{-1}.mg cell protein^{-1}, and apparent half-saturation coefficient value was 0.062 ± 0.01 mM. The aerobic utilization toward 3CA was stimulated with the addition of sodium nitrate and citrate. Under anaerobic conditions, *A. baumannii* GFJ1 was able to utilize 3CA linked with nitrate reduction. The investigation of biofilm formation showed that biofilm formation was affected by cosubstrates and 3CA concentrations. Biofilm formation enhanced with the presence of cosubstrates, especially nitrogen sources. The biofilm formation and chemical degradation by biofilm increased in the following intervals of incubation with the supply of fresh medium. The results indicate that *A. baumannii* GFJ1 has a potential for the application to clean up 3CA.

Keywords *Acinetobacter baumannii* GFJ1 · Biodegradation · Biofilm · Cosubstrates · Edward model · 3-chloroaniline

✉ Ha Danh Duc
hadanhduc@gmail.com

[1] International Program in Hazardous Substance and Environmental Management, Graduate School, Chulalongkorn University, Bangkok 10330, Thailand

[2] Center of Excellence on Hazardous Substance Management (HSM), Chulalongkorn University, Bangkok 10330, Thailand

Introduction

Chloroanilines (CAs) are aromatic amines, which have been widely used as ingredients for chemical production including pesticides, dyes, and several pharmaceuticals (Boon et al. 2001; Zhang et al. 2010; Yao et al. 2011). They are also generated from natural transformation of several herbicides applied in agricultural areas. Thus, they are widely detected in the environment, including in surface water (Wegman and Corte 1981), sediment (European Chemicals Bureau 2006), especially in waste water (Livingston and Willacy 1991). Because of their toxicity to human and other living organisms and difficulty to be degraded, they are included in the EU legislation and recommended to be one of priority pollutants in the US EPA (Boon et al. 2001).

Because of their toxicity, the removal of CAs contaminated in environments is necessary. Biodegradation is considered as an effective and environmentally friendly method, in which the removal process depends on the biodegradation ability of microorganisms and environmental conditions. Biofilm provides a range of advantages in unfavorable conditions such as exposure to toxic chemicals. The extracellular polymeric substance (EPS) in biofilm protected cells against toxic chemicals, and high tolerance of biofilms is attributed to the intrinsic heterogeneity (Ding et al. 2014). Biofilm-mediated biodegradation is considered to be an efficient, safe, environmentally friendly, and cost effective (Pandey and Jain 2002; Paul et al. 2005).

There are a number of studies describing the CAs degradation. The degradation toward 3CA by biofilm has been mentioned (Bathe et al. 2009; Wu et al. 2014). However, there are no reports analyzing the effects of both nutrients and chemical concentrations on biofilm formation.

Moreover, no study of the degradation toward a toxic chemical by *A. baumannii* under anaerobic conditions has been reported. In this study, *A. baumannii* strain GFJ1 was investigated for its biodegradation toward 3CA by freely suspended cells and biofilm.

Materials and methods

Microorganism and culture media

A. baumannii strain GFJ1 was isolated from soil (by researchers in Chulalongkorn University, Bangkok, Thailand) using some CAs, including 3CA which was widely detected in environment as the sole carbon, nitrogen, and energy source.

For culture media, mineral medium (MM) containing 1.4196 g L^{-1} Na_2HPO_4, 1.3609 g L^{-1} KH_2PO_4, 98.5 mg L^{-1} $MgSO_4$, 5.88 mg L^{-1} $CaCl_2$. $2H_2O$, 1.16 mg L^{-1} H_3BO_4, 2.78 mg L^{-1} $FeSO_4.7H_2O$, 1.15 mg L^{-1} $ZnSO_4.7H_2O$, 1.69 mg L^{-1} $MnSO_4.H_2O$, 0.38 mg L^{-1} $CuSO_4.5H_2O$, 0.24 mg L^{-1} $CoCl_2.6H_2O$, and 0.10 mg L^{-1} MoO_3; (Dejonghe et al. 2003) and Luria–Bertani (LB) medium were used. Media were solidified with 15 g L^{-1} of agar for cell cultivation.

The effects of cosubstrates on 3CA degradation and biofilm formation of *A. baumannii* GFJ1 under aerobic media were carried out with the addition of carbon and nitrogen sources and 0.1 mM 3CA. The media included MM medium and MM medium supplemented with 0.01 % yeast extract (YE) (MY001), 0.1 % YE (MY01), 0.01 % YE and 0.1 % succinate (MYS), 0.01 % YE and 0.1 % citrate (MYC), 0.01 %YE and 0.1 % sodium nitrate (MYNa), 0.01 %YE and 0.1 % ammonium sulfate (MYA), and 0.01 %YE and 0.1 % ammonium chloride (MYAC). The biodegradation process by freely suspended cells was carried out at room temperature (30 °C) with a shaking speed of 150 rpm.

Determination of 3CA utilization rates by *A. baumannii* GFJ1 under aerobic conditions

Cells were cultivated in LB broth for 12 h to a turbidity of ~1.4 at 600 nm and used as the inoculum at 1.0 mL L^{-1} into the respective fresh media. The substrate utilization as sole carbon and nitrogen source was carried out in MM medium supplemented with various concentrations of 3CA (from 0.005 to 1.0 mM). The modified Michaelis–Menten equation was used to fit the degradation data. The kinetic parameters, including the maximum specific degradation (V_{max}) and apparent half-saturation coefficient value (K_s) of substrate concentrations (S) were derived by linear regression fitting of the Lineweaver–Burk plot or double reciprocal plot (Lineweaver and Burk 1934). The Dixon plot was used to determine the inhibition constant (K_i), in which the reciprocal of the velocity was plotted against the inhibitor concentrations (Dixon 1953). GraphPad Prism 6 software (CA, USA) was used to solve the model equation.

Determination of 3CA utilization by *A. baumannii* GFJ1 under anaerobic conditions

The anaerobic experiments were performed using 60 mL serum vials containing 15 mL of sterile MM medium with the addition of nitrate ($NaNO_3$) as an electron acceptor. The anaerobic media were prepared by boiling and then bubbling with nitrogen gas. These vials were immediately sealed with rubber septa and aluminum crimps. 3CA was then supplemented at 0.1 mM. The vials without electron acceptor or without bacteria served as controls and were run in parallel. Syringes and needles were used for substrate addition and sample collection. The anaerobic media were confirmed using the indicator rezasurin (0.4 mM) and a DO probe analyzer (Oxi 3210, WTW Co., Germany). The incubation was conducted in the same ways of aerobic degradation described above.

Effects of nutrients and 3CA concentrations on biofilm formation

The determination of biofilm formation on a 96-well polystyrene microplates was described by O'Toole and Kolter (O'toole and Kolter 1998) under aerobic conditions. 150 μL of a medium containing specific cosubstrates and 0.1 mM 3CA was added to each well. After inoculation, the plates were covered with plastic to prevent evaporation. The microplates were aerobically incubated at room temperature with a shaking speed of 100 rpm. After 24 h, the media were removed. The plates were rinsed three times with tap water in order to remove nonadhesion bacteria, and dried by converting. The biofilm formation was examined by staining with 150 μL of crystal violet (0.1 %) per well. 300 μl of mixture ethanol and acetone (>99 %) (80:20, v/v) was added to dilute the stain. The solution was transferred to a 1.5 mL Eppendorf tube, adjusted to 1.0 mL with distill water, and absorbance was determined using a spectrophotometer (at 600 nm). Another method to quantify biofilm formation was determined by scrapping and counting detached cells using the CFU count method. Wells containing only the media were used as background controls.

For the effects of chemical concentrations on biofilm formation, 3CA was supplemented with a series of concentrations.

Determination of degradation toward 3CA by biofilm

The experiments of chemical degradation by biofilm were carried out using MYNa medium under aerobic conditions. The medium was removed after 24 h of incubation and microplates were rinsed triplicate with sterilized saline solution (0.85 % NaCl). The wells were then filled with the same medium and incubated. Samples were taken after 3 h for the determination of 3CA remaining, and the microplates were continued to incubate for 21 h. The same operation was repeated in the following intervals. The experiment was carried out at the 3CA concentration of 0.1 mM.

Analytical methods

3CA concentration in media was analyzed using reverse phase high performance liquid chromatography (HPLC) (LC-10AD, Shimadzu, Japan) with a C18 column (5 µm, 250 mm × 4.6 mm; Hyperclone, Phenomenex, USA). Absorbance was measured at 240 nm. A mixture of acetonitrile and ultrapure water (7:3, v/v) served as mobile phase at a flow rate of 1 mL min^{-1}. The Lowry method (Lowry et al. 1951) was used to determine protein concentrations extracted from cultured bacteria, and bovine serum was used as the protein standard. Cell turbidity was determined at 600 nm (DU800, Beckman Coulter, Inc, USA) and the exponential growth rate was determined according to the method previously reported (Zeyer et al. 1985).

The determination of nitrate and nitrite concentrations was performed during the anaerobic degradation. Nitrate was analyzed using the APHA method (APHA 1992), and nitrite was measured according to the ISO 6777 (ISO 6777 1984).

Results

Utilization kinetics of 3CA by *A. baumannii* GFJ1 under aerobic conditions

Strain GFJ1 showed the ability of growth and use of 3CA as sole carbon, nitrogen, and energy source. In order to obtain the kinetic model parameters of chemical utilization, 3CA was supplemented at different initial concentrations. The specific degradation rates for each concentration were determined during the exponential growth phase. Figure 1 shows that the 3CA degradation kinetic profile fitted with the Edward model well, which was given by the equation: $V = V_{max}[\exp(-S/K_i) - \exp(-S/K_s)]$ (Edwards 1970). The utilization rates of *A. baumannii* GFJ1 first increased with the increase in initial 3CA concentrations up to a certain range. After this range, the rates tended to decrease gradually (Fig. 1). The calculated maximum degradation rate V_{max} of 3CA was 3.45 ± 0.33 µM.h^{-1}.mg cell $protein^{-1}$, K_s value was 0.062 ± 0.01 mM, and K_i was 0.96 ± 0.02 mM. It is interesting to note that the bacterial strain still showed apparent utilization rates at high concentrations of 3CA. Further experiments showed that bacteria could grow and degrade the chemical up to 2.8 mM.

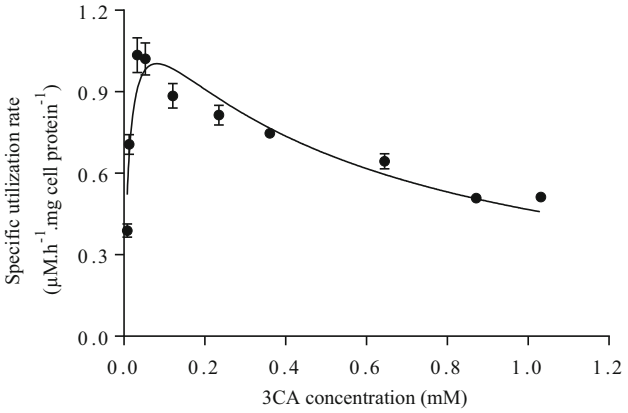

Fig. 1 Specific utilization rates of 3CA at different concentrations by *A. baumannii* GFJ1

Effects of cosubstrates on degradation toward 3CA under aerobic conditions

The effect of secondary carbon and nitrogen sources on bacterial growth and 3CA biodegradation by *A. baumannii* GFJ1 was investigated. Table 1 presents that the addition of any cosubstrate stimulated cell growth. Succinate and citrate were carbon sources which might present in the soil or water environments and might exert positive or negative effects on the microbial degradation (Dinkla and Janssen 2003). The supplementation of 0.01 % YE in the culture medium was required to maintain cell growth. The degradation rates increased with the addition of citrate and sodium nitrate. In these media, 3CA was nearly completely degraded within 9 h. However, the degradation was inhibited by the presence of ammonium sulfate and ammonium chloride even though these cosubstrates stimulated cell growth (Table 1).

Utilization of CAs under anaerobic conditions

In this study, *A. baumannii* GFJ1 was found to utilize 3CA under anaerobic conditions with nitrate as an electron acceptor. Under this condition, the bacterial strain took 20 days to degrade 42.3 ± 0.5 % of 0.1 mM 3CA (Fig. 2), which was significantly lower than the degradation under aerobic conditions. During the degradation process, nitrate was concomitantly reduced, and nitrite was produced. The

Table 1 Exponential growth and degradation rates toward 3CA by *A. baumannii* GFJ1 in various growth conditions

Media*	Exponential growth rate (h^{-1})	Degradation rate (%)	Specific degradation rate [$\mu M.(h.mg\ cell\ protein)^{-1}$]
MM	0.004 ± 0.00	98.0 ± 0.3***	1.07 ± 0.09
MY001	0.039 ± 0.00	96.8 ± 0.9***	0.35 ± 0.03
MY01	0.115 ± 0.02	93.7 ± 3.9***	0.13 ± 0.00
MYS	0.069 ± 0.01	98.5 ± 0.1***	0.31 ± 0.06
MYC	0.064 ± 0.01	84.6 ± 1.1**	1.22 ± 0.06
MYNa	0.071 ± 0.01	89.2 ± 3.5**	1.01 ± 0.22
MYA	0.109 ± 0.01	13.1 ± 3.2***	0.02 ± 0.00
MYAC	0.084 ± 0.00	42.1 ± 9.9***	0.04 ± 0.01

* MM: mineral medium, MY001: MM + 0.01 % YE, MY01: MM + 0.1 % YE, MYS: MM + 0.01 % YE and 0.1 % succinate, MYC: MM + 0.01 % YE and 0.1 % citrate, MYNa: MM + 0.01 %YE and 0.1 % sodium nitrate, MYA: MM + 0.01 %YE and 0.1 % ammonium sulfate, and MYAC: 0.01 %YE and 0.1 % ammonium chloride

** 3CA was degraded after 6 h

*** 3CA was degraded after 48 h

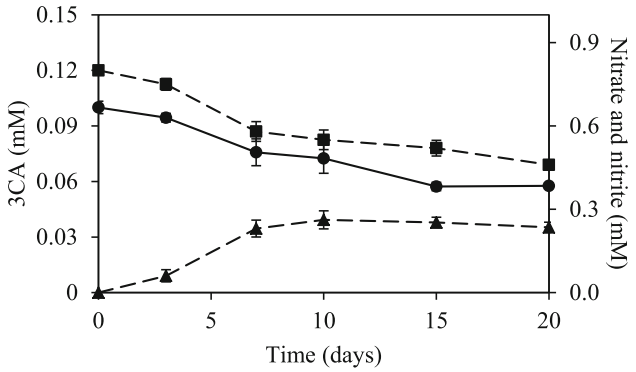

Fig. 2 3CA (*filled circle*) degradation under anaerobic, denitrifying conditions by strain GFJ1. Nitrate (*filled square*) was transformed to nitrite (*filled triangle*) during the processes

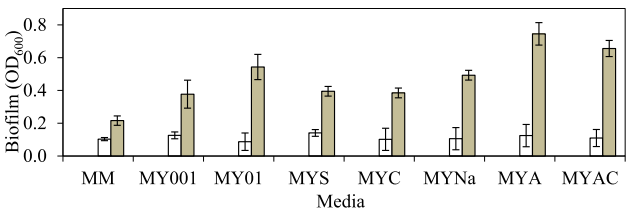

Fig. 3 Biofilm formation of *A. baumannii* GFJ1 (*gray square*) on microplates in various media with different cosubstrates. The negative controls without bacteria were carried out in parallel (*white square*). The media included MM: mineral medium, MY001: MM + 0.01 % yeast extract, MY01: MM + 0.1 % YE, MYS: MM + 0.01 % YE and 0.1 % succinate, MYC: MM + 0.01 % YE and 0.1 % citrate, MYNa: MM + 0.01 % YE and 0.1 % sodium nitrate, MYA: MM + 0.01 % YE and 0.1 % ammonium sulfate, and MYAC: 0.01 % YE and 0.1 % ammonium chloride. Data are means of the results from at least three individual experiments, and mean values and standard deviations are shown

amount of nitrite formed from nitrate utilization increased to a maximum concentration at day 10. In all the trials here, the controls without bacteria, and anaerobic controls without the electron acceptor did not reduce a respective 3CA concentration. Moreover, no nitrate reduction was found in the medium without 3CA or without bacteria. These results indicate that 3CA was utilized under anaerobic conditions linked to nitrate reduction.

Effects of nutrients and chemical concentrations on biofilm development

The effect of cosubstrates on biofilm formation of *A. baumannii* GFJ1 was quantified (Fig. 3). The biofilm formation was promoted with the presence of cosubstrates, especially nitrogen sources. The biofilm development was highest in the medium with the addition of ammonium sulfate. The presence of $NaNO_3$ in medium showed the intermediated biofilm formation and it stimulated 3CA degradation. Therefore, MYNa medium was used in next experiments.

At 3CA concentrations of 0.0, 0.05, and 0.1 mM, the biofilm development was not significantly different. The maximum biofilm formation was found at 24 h (Fig. 4), and did not significantly change in the following days. The investigation of cell growth in bulk solution showed that *A. baumannii* GFJ1 grew without a lag phase and reached at maximum within 12 h at these 3CA concentrations. At higher chemical concentrations (≥0.3 mM), bacteria had a lag phase and required more time to reach the maximum biomass in bulk solution and biofilm. However, the maximum biofilm formation was not significantly different among treatments with and without supplementing with 3CA (Fig. 4). The plates were continued to incubate up to 7 days, and no significant change in biofilm quantification

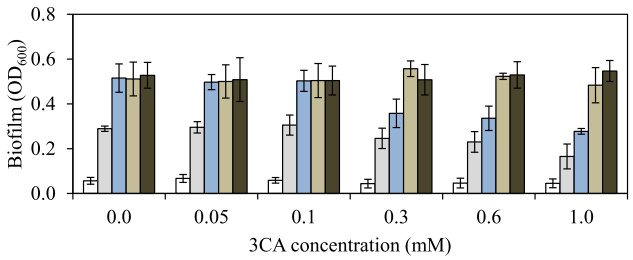

Fig. 4 Biofilm formation in MYNa medium supplemented with 3CA on the 96-well microplates. The incubation time was 0 h (*white square*), 12 h (*gray square*), 24 h (), 48 h (), and 72 h (). Data are means of the results from at least three individual experiments, and mean values and standard deviations are shown

was detected in both staining with crystal violet and counting cell numbers in biofilm. In the range of 3CA concentrations (from 0 to 1.0 mM), 3CA affected the time to form biofilm but did not significantly affect the final biofilm levels of *A. baumannii* GFJ1.

Degradation toward 3CA by biofilm

Figure 5 presents that the biofilm levels of *A. baumannii* GFJ1 increased in the following intervals, which resulted in the enhancement of biodegradation toward 3CA. In the fourth interval, bacteria in biofilm were almost three times higher than the first interval (Fig. 5A). The 3CA degradation by biofilm was nearly completely in the fourth interval, and was nearly three times higher than the first interval (Fig. 5B). Because bacteria in biofilm might die after several days and the microplates were used for next intervals, the biofilm development was determined by counting CFUs instead of using crystal violet.

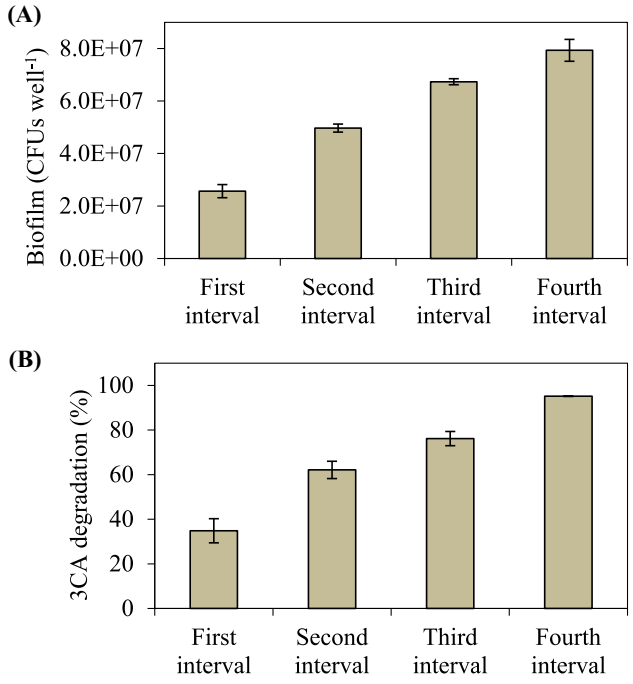

Fig. 5 Biofilm formation (**A**) and 3CA degradation by biofilm (**B**). The experiments were carried out in MYNa medium supplemented with 0.1 mM 3CA, using microplates. Each interval lasted 24 h and samples were taken after 3 h for the determination of 3CA remaining. Data are means of the results from at least three individual experiments, and mean values and standard deviations are shown

Discussion

The degradation toward 3CA by *A. baumannii* GFJ1 was consistent with Edward inhibition kinetics. The specific utilization rates were decreased at high concentrations of the chemical due to the toxicity of the substrate. Similarly, the degradation of 34DCA by *A. baylyi* GFJ2 (Hongsawat and Vangnai 2011), and 4CA and 34DCA by aerobic granules (Zhu et al. 2011, 2012) was inhibited at high substrate concentrations. In batch cultivation with a low initial cell number, cells grew exponentially and the kinetic models of the degradation of organic compounds depended upon the concentrations of chemicals (Schmidt et al. 1985).

The effects of cosubstrates on 3CA degradation were determined by supplementation with other nutrients. With the presence of cosubstrates, bacteria had more nutrients to stimulate the growth. The addition of YE and succinate did not reduce the total degradation rates, but resulted in lower specific utilization rates because of higher biomass in media. With the presence of ammonium sulfate and ammonium chloride, bacteria preferred to use these compounds instead of 3CA for growth.

3CA was considered as a recalcitrant compound in anaerobic and anoxic environments such as in sediment and aquifer (Kuhn and Suflita 1989; Struijs and Rogers 1989; Susarla et al. 1997). *A. baumannii* GFJ1 showed the degradation capacity of 3CA under both aerobic and anaerobic conditions, and oxygen was the more favorable electron acceptor compared to nitrate. Similarly, *Bacillus licheniformis* strain ycsd02 degraded 4CA under both aerobic and anaerobic conditions with much higher rates in aerobic media (Ding et al. 2011). Nitrate was used as an additional nitrogen source to stimulate aerobic degradation toward 3CA. It was an electron acceptor and might be a nitrogen source in anaerobic degradation.

Although a number of studies have described the degradation of 3CA under aerobic conditions by pure cultures (Latorre et al. 1984; Zeyer et al. 1985; Ferschl et al. 1991; Hinteregger et al. 1992; Boon et al. 2000; Shah 2014), only *Paracoccus* sp. showed the capacity to degrade several CAs under both aerobic and anaerobic conditions with the simultaneous reduction of nitrate to nitrite (Bollag and Russel 1976). *Acinetobacter* species are known as

aerobes, but a previous study showed that *A. johnsonii* could convert nitrate to nitrite under anoxic conditions (Boswell et al. 1999). *A. baumannii* has been reported on aerobic degradation of toxic chemicals such as polyethylene (Pramila et al. 2012), phenanthrene (Kim et al. 2009), or 4CA (Vangnai and Petchkroh 2007). However, the degradation of a toxic chemical by *A. baumannii* under anaerobic media has not been reported before.

A. baumannii GFJ1 formed biofilms with different levels belonging to nutrients and chemicals supplemented. Ammonium sulfate and ammonium chloride inhibited 3CA degradation; however, they stimulated the biofilm formation. Previous reports showed that the biofilm formation was influenced by nutrients presenting in the bulk fluid and environmental conditions, especially during initial attachment stages (Donlan 2002). The roles of nitrogen sources have been mentioned as an important nutrient for biofilm formation (Fujishige et al. 2006). In another report, the increase of nitrogen concentrations enhanced the rate and extent of biofilm accumulation of *P. putida* isolated from a paper machine (Rochex and Lebeault 2007). The positive influence of additional sodium nitrate on biodegradation and biofilm formation of GFJ1 revealed the potential application of its biofilm developing in MYNa medium.

Bacteria formed biofilm faster in the medium with low 3CA concentrations. At higher chemical concentrations, the medium was more toxic, so they required more time to grow and form biofilm. The increase of cell numbers in the exponential growth phase resulted in the higher contact with material surface to stimulate the adhesion. After 24 h, the biofilm formation was consistent in the same medium (MYNa medium) supplemented with 0.1 mM 3CA described above. However, biofilm increased with the supply of fresh medium because bacteria had more nutrients. Once anchored on the surface, cell division and recruitment of planktonic bacteria resulted in growth and development of the biofilm community (Andersson 2009). Cells required nutrients in the conditioning film and the aqueous bulk to grow and produce more EPS resulting in the formation of microcolonies to extend the biofilm thickness (Kumar and Anand 1998). In the following intervals, the degradation rates of 3CA increased due to the increase of cell numbers in biofilm. Biofilm with a high density of microbial biomass, localizing solute concentration and increasing the bioavailability of xenobiotic compounds could facilitate biodegradation (Nisha et al. 2015).

Previous reports presented that cells detached from biofilm in following time due to the factors like nutrient depletion, increased concentration of detrimental metabolites, and changes in culture conditions (Delaquis et al. 1989; Nisha et al. 2015; Wang et al. 2016). However, the biofilm of *A. baumannii* GFJ1 was stable after reaching the maximum biofilm formation. Wu et al. (2014) described that the detachment of CAs-degrading *Comamonas testosteroni* WDL7 from biofilm increased after the supplementation of 3CA. The degradation toward 3CA by GFJ1 in microplates was carried out by cells in biofilm and even cells detached from biofilm. The increase of biofilm formation and degradation rates of chemicals with the supply of fresh medium (containing 3CA) had a significant role for further application in wastewater treatment using a continuous biofilm reactor.

In conclusion, the novel CAs-degrading *A. baumannii* GFJ1 was able to utilize 3CA as sole carbon, nitrogen, and energy source under both aerobic and anaerobic conditions. To my knowledge, this report is the first to show that *A. baumannii* utilized a toxic chemical under both aerobic and anaerobic media. This is also the first report investigating the effects of substrate concentrations and cosubstrates on biofilm formation of a CAs-degrading bacterial strain. The investigation of chemical concentrations and nutrients on biofilm development and biodegradation toward 3CA provided preliminary information to facilitate the degradation processes by maintaining optimal conditions, which can make *A. baumannii* GFJ1 as a potent bacterial strain for bioremediation toward 3CA in polluted areas.

Acknowledgments This work was financially supported by the Center of Excellence on Hazardous Substance Management (HSM), Chulalongkorn University, Bangkok, Thailand and the Vietnamese Government.

References

Andersson S (2009) Characterization of bacterial biofilms for wastewater treatment. School of Biotechnology, Royal Institute of Technology (KTH), Stockholm

APHA (1992) Standard methods for the examination of water and wastewater, 20th edn. American Public Health Association, Washington

Bathe S, Schwarzenbeck N, Hausner M (2009) Bioaugmentation of activated sludge towards 3-chloroaniline removal with a mixed bacterial population carrying a degradative plasmid. Bioresour Technol 100:2902–2909

Bollag JM, Russel S (1976) Aerobic versus anaerobic metabolism of halogenated anilines by a *Paracoccus* sp. Microb Ecol 3:65–73

Boon N, Goris J, De Vos P, Verstraete W, Top EM (2000) Bioaugmentation of activated sludge by an indigenous 3-chloroaniline-degrading *Comamonas testosteroni* strain, I2gfp. Appl Environ Microbiol 66:2906–2913

Boon N, Goris J, De Vos P, Verstraete W, Top EM (2001) Genetic diversity among 3-chloroaniline- and aniline-degrading strains of the *Comamonadaceae*. Appl Environ Microbiol 67:1107–1115

Boswell CD, Dick RE, Macaskie LE (1999) The effect of heavy metals and other environmental conditions on the anaerobic phosphate metabolism of *Acinetobacter johnsonii*. Microbiology 145:1711–1720

Dejonghe W, Berteloot E, Goris J, Boon N, Crul K, Maertens S, Hofte M, De Vos P, Verstraete W, Top EM (2003) Synergistic degradation of linuron by a bacterial consortium and isolation of

a single linuron-degrading *Variovorax* strain. Appl Environ Microbiol 69:1532–1541

Delaquis PJ, Caldwell DE, Lawrence JR, McCurdy AR (1989) Detachment of *Pseudomonas fluorescens* from biofilms on glass surfaces in response to nutrient stress. Microb Ecol 18:199–210

Ding C, Li ZX, Yan JL (2011) Isolation, identification and degradation characterization of a p-chloroaniline degrading strain. Bull Environ Contam Toxicol 86:454–459

Ding Y, Peng N, Du Y, Ji L, Cao B (2014) Disruption of putrescine biosynthesis in *Shewanella oneidensis* enhances biofilm cohesiveness and performance in Cr(VI) immobilization. Appl Environ Microbiol 80:1498–1506

Dinkla IJT, Janssen DB (2003) Simultaneous growth on citrate reduces the effects of iron limitation during toluene degradation in *Pseudomonas*. Microb Ecol 45:97–107

Dixon M (1953) The determination of enzyme inhibitor constants. Biochem J 55:170–171

Donlan RM (2002) Biofilms: microbial life on surfaces. Emerg Infect Dis 8:881–890

Edwards VH (1970) The influence of high substrate concentrations on microbial kinetics. Biotechnol Bioeng 12:679–712

European Chemicals Bureau (2006) 3,4-Dichloroanilines, summary risk assessment report. Institute for Health and Consumer Protection, Ispra

Ferschl A, Loidl M, Ditzelmüller G, Hinteregger C, Streichsbier F (1991) Continuous degradation of 3-chloroaniline by calcium-alginate-entrapped cells of *Pseudomonas acidovorans* CA28: influence of additional substrates. Appl Microbiol Biotechnol 35:544–550

Fujishige NA, Kapadia NN, De Hoff PL, Hirsch AM (2006) Investigations of *Rhizobium* biofilm formation. FEMS Microbiol Ecol 56:195–206

Hinteregger C, Loidl M, Streichsbier F (1992) Characterization of isofunctional ring-leaving enzymes in aniline and 3-chloroaniline degradation by *Pseudomonas acidovorans* CA28. FEMS Microbiol Lett 87:261–266

Hongsawat P, Vangnai AS (2011) Biodegradation pathways of chloroanilines by *Acinetobacter baylyi* strain GFJ2. J Hazard Mater 186:1300–1307

ISO 6777 (1984) Water quality – determination of nitrite – molecular absorption spectrometric method. ISO Standard Compendium, Environment, Water Quality, Chemical Methods, 1st edition, ISO 2:137–141

Kim YM, Ahn CK, Woo SH, Jung GY, Park JM (2009) Synergic degradation of phenanthrene by consortia of newly isolated bacterial strains. J Biotechnol 144:293–298

Kuhn EP, Suflita JM (1989) Sequential reductive dehalogenation of chloroanilines by microorganisms from a methanogenic aquifer. Environ Sci Technol 23:848–852

Kumar CG, Anand SK (1998) Significance of microbial biofilms in food industry: a review. Int J Food Microbiol 42:9–27

Latorre J, Reineke W, Knackmuss HJ (1984) Microbial metabolism of chloroanilines: enhanced evolution by natural genetic exchange. Arch Microbiol 140:159–165

Lineweaver H, Burk D (1934) The determination of enzyme dissociation constants. J Am Chem Soc 56:658–666

Livingston AG, Willacy A (1991) Degradation of 3,4-dichloroaniline in synthetic and industrially produced wastewaters by mixed cultures freely suspended and immobilized in a packed-bed reactor. Appl Microbiol Biotechnol 35:551–557

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265–275

Nisha KN, Devi V, Varalakshmi P, Ashokkumar B (2015) Biodegradation and utilization of dimethylformamide by biofilm forming *Paracoccus* sp. strains MKU1 and MKU2. Bioresour Technol 188:9–13

O'toole GA, Kolter R (1998) Initiation of biofilm formation in *Peudomonas fluorescens* WCS365 proceeds via multiple, convergent signalling pathways: a genitic analysis. Mol Microbiol 28:449–461

Pandey G, Jain RK (2002) Bacterial chemotaxis toward environmental pollutants: role in bioremediation. Appl Environ Microbiol 68:5789–5795

Paul D, Pandey G, Pandey J, Jain RK (2005) Accessing microbial diversity for bioremediation and environmental restoration. Trends Biotechnol 23:135–142

Pramila R, Padmavathy K, Ramesh KV, Mahalakshm K (2012) *Brevibacillus parabrevis, Acinetobacter baumannii and Pseudomonas citronellolis*-Potential candidates for biodegradation of low density polyethylene (LDPE). J Bacteriol Res 4:9–14

Rochex A, Lebeault JM (2007) Effects of nutrients on biofilm formation and detachment of a *Pseudomonas putida* strain isolated from a paper machine. Water Res 41:2885–2892

Schmidt SK, Simkins S, Alexander M (1985) Models for the kinetics of biodegradation of organic compounds not supporting growth. Appl Environ Microbiol 50:323–331

Shah MP (2014) Microbial degradation of 3-chloroanilne by two bacterial strains isolated from common effluent treatment plant. J Appl Environ Microbiol 2:155–165

Struijs J, Rogers JE (1989) Reductive dehalogenation of dichloroanilines by anaerobic microorganisms in fresh and dichlorophenol-acclimated pond sediment. Appl Environ Microbiol 55:2527–2531

Susarla S, Yonezawa Y, Masunaga S (1997) Reductive dehalogenation of chloroanilines in anaerobic estuarine sediment. Environ Technol 18:75–83

Vangnai AS, Petchkroh W (2007) Biodegradation of 4-chloroaniline by bacteria enriched from soil. FEMS Microbiol Lett 268:209–216

Wang X, Teng Y, Luo Y, Dick RP (2016) Biodegradation of 3,3′,4,4′-tetrachlorobiphenyl by *Sinorhizobium meliloti* NM. Bioresour Technol 201:261–268

Wegman RCC, Corte DALD (1981) Aromatic amines in surface waters of the Netherlands. Water Res 15:391–394

Wu Y, Ding Y, Cohen Y, Cao B (2014) Elevated level of the second messenger c-di-GMP in *Comamonas testosteroni* enhances biofilm formation and biofilm-based biodegradation of 3-chloroaniline. Appl Microbiol Biotechnol 99:1967–1976

Yao XF, Khan F, Pandey R, Pandey J, Mourant RG, Guo RKJJH, Russell RJ, Oakeshott JG, Pandey G (2011) Degradation of dichloroaniline isomers by a newly isolated strain, *Bacillus megaterium* IMT21. Microbiology 157:721–726

Zeyer J, Wasserfallen A, Timmis KN (1985) Microbial mineralization of ring-substituted anilines through an *ortho*-cleavage pathway. Appl Environ Microbiol 50:447–453

Zhang LL, He D, Chen JM, Liu Y (2010) Biodegradation of 2-chloroaniline, 3-chloroaniline, and 4-chloroaniline by a novel strain *Delftia tsuruhatensis* H1. J Hazard Mater 179:875–882

Zhu L, Yu Y, Xu X, Tian Z, Luo W (2011) High-rate biodegradation and metabolic pathways of 4-chloroaniline by aerobic granules. Process Biochem 46:894–899

Zhu L, Lv M, Dai X, Xu X, Qi H, Yu Y (2012) Reaction kinetics of the degradation of chloroanilines and aniline by aerobic granule. Biochem Eng J 68:215–220