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Studies on influence and fate of carbamazepine in anaerobic digestion of sludge

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Abstract

Increased consumption of pharmaceutical compounds compounded by their persistence in biological treatment processes and potential toxicity is becoming a serious concern. The aim of the present study was to investigate the fate of an antiepileptic drug, carbamazepine (CBZ), in anaerobic digestion process and its impact on methanogenic metabolism. Biochemical methane potential of CBZ with or without glucose was studied for 40 days in designed batch experiments. About 67.98% and 66.37% of spiked CBZ (about 100 µg l^{-1}) were removed during this period from glucose amended or unamended sets, respectively. Loss of CBZ through adsorption onto suspended particles, as in sterilized seed sludge control (16.98%), was significantly lower in comparison to its biotic counterparts (P=0.002 and P=0.003). Analysis of methane or biogas production revealed no inhibitory effect of CBZ toward methanogenic process at its tested concentration. Differences in cumulative methane yields between glucose containing sets with or without CBZ were insignificant (P=0.885). This study suggests that any residual CBZ concentration lower than the present study, if detected in waste-water, might not significantly affect the methanogenic process.

Key words

Anaerobic digestion, BMP test, Carbamazepine, Pharmaceutical compound, Toxicity

Introduction

Diverse array of pharmaceutical compounds (that includes painkillers, tranquilizers, anti-depressants, anticonvulsant, antibiotics, contrast media, birth control pills and chemotherapy agents) are regularly discharged into water system through various routes such as clinical waste, hospital wastewater, households and manufacturing companies (Jjemba, 2006; Ellis, 2006). Some of these pharmaceutical compounds may not be removed during sewage treatment process and enters into rivers or drinking water supplies, increasing their ubiquity in biosphere (Kümmerer, 2009). The widespread occurrence and persistence of different pharmaceutical compounds in aquatic environment and their potential ecological toxicity has become a major environmental issue (Jones *et al.,* 2004; Gothwal, and Shashidhar, 2015). Occurrence of pharmaceutical products, their removal efficiency and mechanisms in sewage treatment processes has been investigated in recent past (Miège *et al.,* 2009; Guerra *et al.,* 2014).

Among them, antiepileptic drug carbamazepine (CBZ) is highly persistent and frequently found in sewage, surface waters and managed aquifer recharge systems (Leclercq *et al.,* 2009), and once it is discharged into environment it causes toxicity (Joss *et al.,* 2006; Verlicchi *et al.,* 2012). Removal of CBZ and its metabolites from municipal sewage treatment plant is very low (~8%), and effluent concentration could be as high as 5 µg l^{-1} (Heberer, 2002). Membrane bioreactor process remains ineffective in CBZ removal even at higher sludge retention time (Reif *et al.,* 2008). In recent time, though some advanced photocatalysis treatment systems (Laera *et al.,* 2011), like reverse osmosis and nano filtration membrane, ozonation, ultraviolet irradiation, activated carbon have shown to be partially effective in removal of CBZ (Radjenovic *et al.,* 2009; Sim *et al.,* 2010; Gur-Reznik *et al.,* 2011; Chong *et al.,* 2011),but

extent of removal for CBZ metabolites remains very poor.

It is suggested that biological treatment is essential for satisfactory removal of the transformation products (Miao and Metcalfe, 2003; Kosjek *et al.,* 2009). Biological process incurs lower cost for treatment, and maintenance in comparison to physical/chemical based treatment approaches (Oller *et al.,* 2011). However, CBZ removal in aerobic biological processes has remained unachievable (Narumiya, 2013; Kruglova *et al.,* 2014). Recent studies has indicated that CBZ persists during anaerobic digestion of sludge even after chemical or thermal pre-treatments (Carballa *et al.,* 2007, 2008). Uncertainty exists on whether persistence of CBZ in anaerobic digestion process can be linked to its toxicity toward the microbial biomass. Studies have suggested that toxicity can be significant where level of inhibition and affinity to adsorb on anaerobic sludge are linearly correlated (Fountoulakis *et al.,* 2004). Hydrophobic CBZ may likely be sequestrated within suspended solids biomass generated in the biological processes and may cause adverse effects (Drillia *et al.,* 2005). In contrast, some reports advocate that presence of CBZ does not elicit any impact on anaerobic sludge process (Stamatelatou *et al.,* 2003) or on methanogens even at high concentrations (Fountoulakis *et al.,* 2004).

In the present study, fate of CBZ in anaerobic digestion process was assessed using designed batch experiments. Two key aspect was addressed, i.e., to what extent CBZ can be metabolized and how CBZ can influence the activity of methanotrophic culture.

Materials and Methods

Chemicals and microbial culture : Carbamazepine powder at 99.9% purity was purchased from Sigma Aldrich Chemical. Stock solution of CBZ (1,000 μg l^{-1}) was prepared in HPLC grade methanol (Sigma Aldrich) and stored at 4°C until use. The same stock solution was utilized for preparation of working solution and spiking into different experiments.

Anaerobic sludge was collected from leachate treatment plant from Sudokwon Landfill Site, Incheon, Korea. Sludge was collected in 20 l plastic containers and immediately transported to the laboratory, where it was processed through centrifugation, and filtration in glass fiber membrane (Whatman®, grade: GF/C). Absence of CBZ in sludge was verified through solid phase extraction and HPLC.

Removal of CBZ in anaerobic digestion: Anaerobic biodegradation of CBZ was evaluated in a modified biochemical methane potential (BMP) test (Fountoulakis *et al.,* 2004). Briefly, 80 ml mineral media and 20 ml anaerobic seed sludge were dispensed in sets of 250 ml serum bottles. Subset of bottles were dosed with either glucose (0.1 g l^{-1}), or CBZ (100 μg l^{-1}), or both glucose and CBZ. The fourth set which received neither glucose nor CBZ was included as control to measure methane/biogas yield from seed sludge associated organic matter. All the bottles were sealed with rubber stopper and aluminum crimp and incubated at 36°C under dark condition. Samples (of 1 ml) from the mixed liquor were taken at regular intervals to determine the CBZ concentrations, while, head space gas samples were taken to determine the volume of biogas and its methane content.

Analysis of carbamazepine (CBZ) : Concentration of CBZ from BMP test samples was analyzed through solid phase extraction and HPLC system (Agilent technology 1200 series) following Miao and Metcalfe (2003). Samples were first centrifuged at 3,500 rpm for 15 min and the supernatants were filtered through glass fiber membrane. Samples (6 ml) were extracted with 500mg Oasis™ HLB (hydrophilic-lipophilic balance) SPE cartridge from Waters®. SPE cartridge was installed on a vacuum manifold and conditioned successively with 3 ml methanol and 3 ml deionized water. Thereafter, the samples which were adjusted pH 7.5 by 3.5 M H_2SO_4, were allowed to pass through the cartridge at a rate of 10 ml min^{-1}. Subsequently, cartridges were washed two times with 3 ml water, dried under vacuum for 1hr. Finally, CBZ was eluted with 3×3 ml of methanol dripping without vacuum. The eluted CBZ fractions were pooled and concentrated (~1 ml) in a nitrogen gas aided evaporation system (Turbo Vap system). HPLC analysis of the concentrated samples were carried out in a Zorbax Eclipse XDB-C18 column (4.8×150 mm, 5μm, Agilent technology). A binary gradient consisting of 0.1% v/v formic acid solution and methanol at a flow rate of 1 ml min^{-1} and UV-detection at 258 nm were utilized. The mobile phase gradient was as follows: 5% methanol held for 3 min, increased linearly to 50% by 5 min and held for 3 min, stepped to 100% for 7 min. Column was washed with 100% methanol to remove any residue in between sample runs.

Analysis of biogas : About 0.5 ml biogas accumulated in headspace were collected from serum bottle using gas tight syringe. The methane and carbon dioxide content in head space bio-gas were analyzed using a gas chromatograph (HP Agilent 6890) equipped with a packed column (Alltech 403412-1417) and a thermal conductivity detector (TCD). Injector and detector temperatures were maintained at 110 and 210°C, respectively. Helium was used as a carrier gas at a flow rate of 20.1 ml min^{-1} along with H_2 at a makeup flow of 5 ml min^{-1}.

Results and Discussion

Degradation of pharmaceutical compounds in anaerobic digestion process is closely linked with activity of

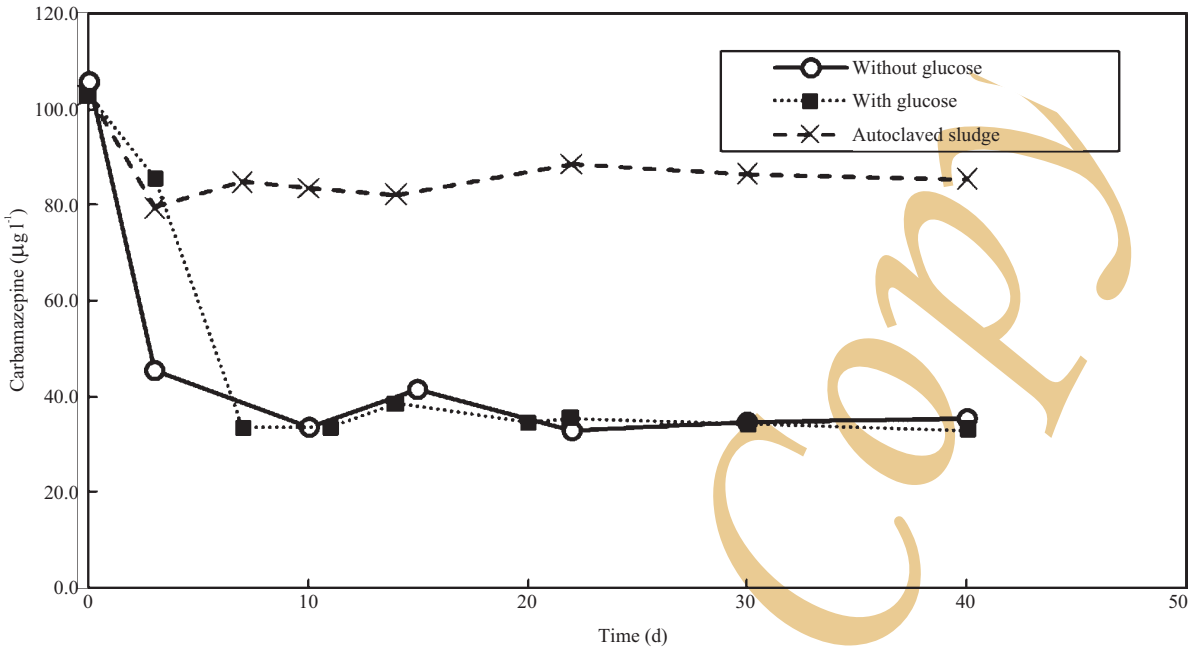

Fig. 1 : Removal of carbamazepine in the BMP test with or without glucose. Autoclaved seed sludge served as abiotic control

microorganism, which in turn depends on availability of biodegradable substrates as well as toxicity of different compounds (including the pharmaceutical compound under study) present in the system. Inhibitory effect of pharmaceutical substance on anaerobic microorganisms is manifested through reduction in the amount of biogas production for BMP test. In the present study, concentration of CBZ in experimental sets (with or without glucose) reduced from 105.8 to 33.4 µg l^{-1} within 6 to 10 days of incubation (Fig. 1). However, no further change in CBZ level was observed during the extended test period.

Loss of appreciable degradation rate in extended time may reflect kinetic limitations imposed at lower bio available CBZ level. Being sequestered into biosolid, fraction of bio available carbamazepine as well as its biodegradation rate decreases with time (Plósz *et al.,* 2012). Co-existence of a readily biodegradable carbon source (*i.e.,* glucose) can potentially improve anaerobic degradation of recalcitrant compounds due to stimulation of overall metabolism (Chang *et al.,* 2005; Barret *et al.,* 2010). Abiotic control experiment with autoclaved seed sludge (treated for 30 min at 130°C) showed decrease in CBZ concentration to 79.5 µg l^{-1} after 3 days and final concentration of 88.6 µg l^{-1}. This clearly suggests that drop of CBZ concentration in experimental sets could majorly (76.24%) be attributed to biotic removal, as well as, in part (23.76%) to abiotic process. Removal in of CBZ in the present study was much higher than reported earlier studies (Carballa *et al.,* 2008; Ternes *et al.,* 2002).

Abiotic loss could possibly be non-extractable fraction, which gets adsorbed onto suspended solid of sludge and/or microorganisms through extra cellular polymeric substances produced in microbial metabolism. Extent of sorption to anaerobic sludge is known to be significantly different from that of primary and excess activated sludge, which depends on the physico-chemical characteristics of the suspended solids and nature of the pharmaceutical involved (Stasinakis, 2012).

Distribution coefficient (K_d) values for moderately hydrophilic CBZ (35.4 and 20.2 l kg^{-1}) suggest that sorption on digested sludge can be of minor importance (Carballa *et al.,* 2008). However, the non-extractable (*i.e.*, bound) CBZ fraction in present study appeared to be higher when compared to other studies, where the difference could be attributed to inherent variation in physico-chemical properties *i.e.,* solid content (Matamoros *et al.,* 2012; Li *et al.,* 2013).

One-way repeated measure analysis of variance among different experimental sets for CBZ removal suggests that differences among the treatment groups were greater than would be expected by chance; there was a statistically significant difference (P = <0.001). All pairwise multiple comparison procedures (α=0.05) based on Holm-Sidak method revealed that CBZ removal in experimental sets (with or without glucose) were significantly different from the abiotic one (P=0.003 and 0.002, respectively). However, the

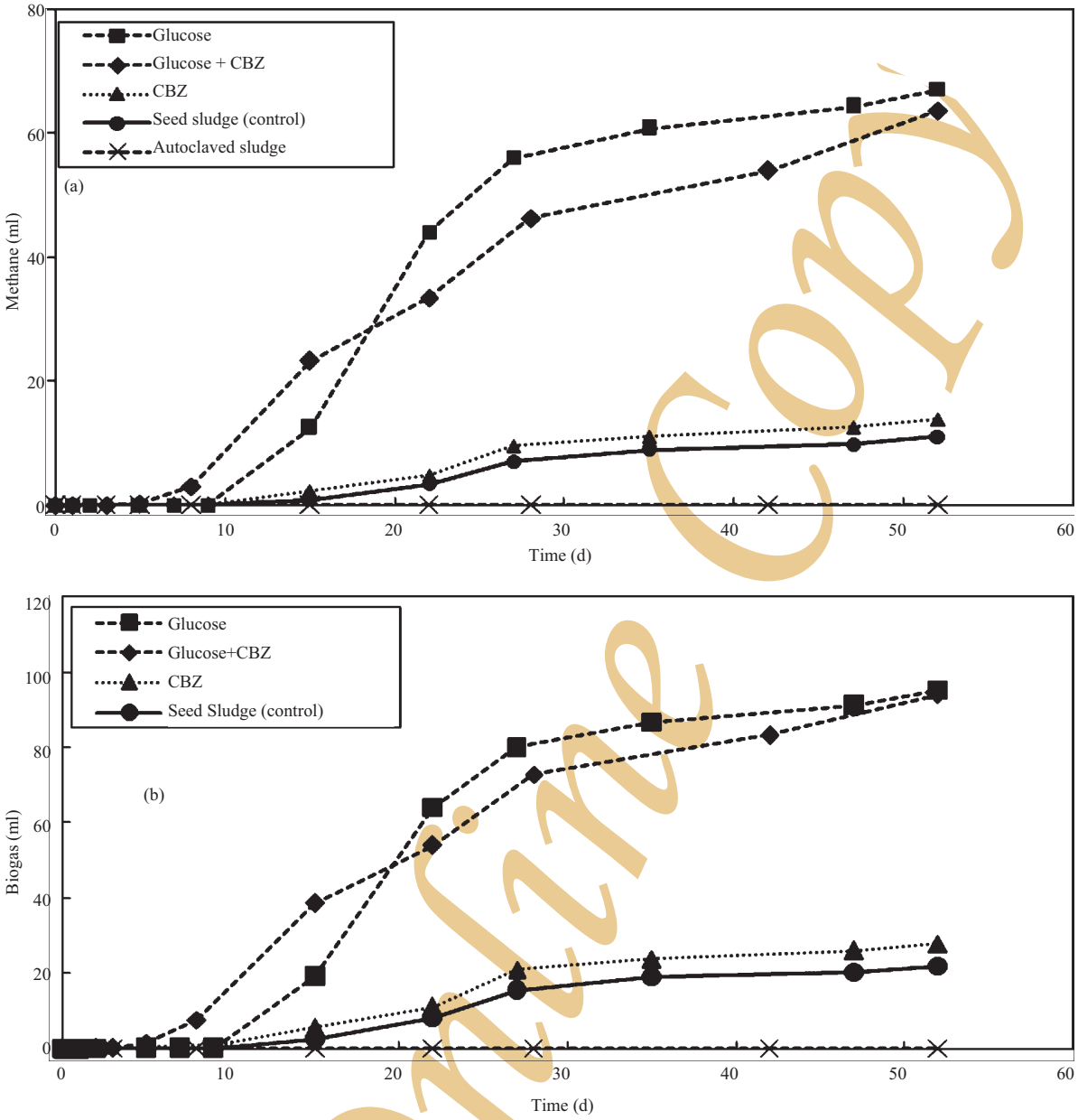

Fig. 2 : Effects of carbamazepine (CBZ) addition on cumulative (a) methane (b) biogas production by anaerobic microbial sludge in the BMP test. Three different experimental sets received either glucose (0.1 g l^{-1}), CBZ or both. Autoclaved seed sludge (as negative control) and original seed sludge without any additive served as control

difference in CBZ removal between sets with or without glucose had no significant difference (P=0.35). Fig. 2 suggests that when CBZ was added to seed sludge biogas/ methane production somewhat increased in comparison to seed sludge in control set. In fact the difference in amount of biogas generated was even higher than the theoretical amount of methane (0.0355 ml) that would have been produced from added CBZ (based on its chemical oxygen demand). In other words, no toxicity of CBZ was noted onto the anaerobic sludge microorganism. This result is in accordance with other studies, where no effect of CBZ was noted at 1 and 10 mg l^{-1} in short-or long- term experiments, respectively (Stamatelatou

et al., 2003). Studies have indicated that the inhibition mechanism are directly correlated with the affinity of pharmaceuticals to adsorb onto anaerobic sludge. However, CBZ being moderately hydrophilic, such limitations could be ruled out.

In experimental sets with added glucose (0.1 gl^{-1}), cumulative amount of biogas generated was higher than their respective theoretical values of 74.8 ml. Increased microbial activity in presence of glucose would have empowered the microorganism to better utilize some of the sludge associated chemical oxygen demand, which was otherwise non-bioavailable. Biogas production in presence of CBZ and glucose was slightly higher, though the trend reverted soon after about 20 days of incubation and final cumulative methane yields were very similar at the end. Thus, an anticipated concentration of CBZ in wastewater ($\mu g\ l^{-1}$) would have no significant effect on metabolism of anaerobic microorganisms.

Pairwise multiple comparison (α=0.05) among different experimental sets for CH_4 production suggested that methane production in presence of CBZ was not significantly different than in control without CBZ, irrespective of with or without glucose (P=0.885 and 0.87, respectively). Interestingly, when time scale of CBZ concentration (Fig. 1) and biogas/methane profiles (Fig. 2) were compared, it appear that CBZ gets metabolized even before appreciable amount of biogas formation. The increased biogas production with CBZ spiked sets suggested its contribution toward the available carbon source, or due to co-metabolism with other carbon source (Bergersen *et al.,* 2012). Additional carbon source could be an essential prerequisite for effective CBZ removal (Santos *et al.,* 2012).

The present study provides new findings on the fate of CBZ in anaerobic digestion of sludge. A significant proportion of CBZ can be removed without eliciting any toxicity toward methanotrophic culture. However, CBZ removal did not translate into biogas production through methanotrophic metabolism. Further studies targeting CBZ metabolic pathway assessment and identification of the rate limiting steps would certainly improve our understanding of eliminating of CBZ in anaerobic digestion.

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