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Degradation of Cocaine and Benzoylecgonine in Sewage Sludge Batch Tests

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Abstract
The degradation studies of cocaine and its principal metabolites, benzoylecgonine, as emerging pollutants were carried out using various sludge types collected from the RAF Molesworth Sewage Treatment Work (STW) to determine their distribution and persistence in aquatic environment. The methodology, initially characterized the various sludge types before the laboratory batch tests were carried out at different temperatures and times. The results, after 3 hours equilibration of batch samples, showed the removal rates for cocaine and benzoylecgonine to be 91.0% and 90.6%, respectively. The partition coefficients (K_d) for the two substances ranged from 2.7 – 31.9 Kg L^{-1}. Degradation of compounds was found to be both biotic and abiotic and the two processes produced many short chain compounds and several metabolites that include cocaethylene and ecgonine methylester. Also at 4 ± 0.5° C, the degradation of substances occurred slowly, at 5 and 10% but was relatively greater at 19 ± 0.5° C. Compounds degradation was also influenced by both extracellular and intracellular enzyme activities. The batch study is an innovative method in calculating degradation rates of compounds from sewage treatment works. The capabilities of generating removal rates of drugs have also been clearly demonstrated in this work and can be applied to any organic compounds in real life (STWs) situations.

Keywords: Wastewater, Biodegradation, Intracellular, Extracellular, Sewage treatment.

Introduction

The persistence of emerging contaminants, their degradation products and disposal into receiving waters via sewage treatment works are of great concerns to the environment due to their non-biodegradability. Recently, the presence of pharmaceuticals, polychlorinated compounds, polyaromatic-hydrocarbons and increasing global usage of illicit drugs are compounding the situation. The hydrophilicity/lipophilicity properties of chemicals as being responsible for their enrichment in sewage sludge partitioning onto suspended solution and sludge solids were in agreement with

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reports obtained in previous studies [Zhong et al, 2017; Baena-Nogueras et al, 2017; Borusko et l, 2017; Madikizela et al, 2017]. The possibility of complete or partial degradation in solid and aqueous phases, mineralised or sorption to solids depends on the fate and behaviours of contaminants. Understanding the location of organic contaminants within the organic waste containing the fraction of dead and living micro-organisms is important in degradation processes.

The previous investigations on the contamination and response levels to the toxicity of wastewater effluents on aquatic organisms have shown correlation to the degree of removal of organic residues from sewage treatment works (STWs) [Sanchez-Reyes et al, 2017; Tang et al, 2017; Gomez-Acata et al, 2017]. Also, the stability experiment of cocaine and ecgonine methylester after 5 and 24h test period, showed biodegradation of the two substances changed by 40-95% in surface water [Xu et al, 2016; Azaria et al, 2017; Ashfaq et al, 2017; Gomez-Acata et al, 2017].

The batch studies enabled the physico-chemical properties and biological sludge characteristics to determine biodegradation using different sewage sludge samples. Currently, there is insufficient information in the literature on a laboratory-measured property that provides a thermodynamic measurement of distribution of some illicit substances that are now widely detected in the aquatic environment as pollutants. The present study is to furthering our quest of fate and behaviour of drugs and overall issues of complex-interplay of interactions, the batch experiments were undertaken to show an improvement in calculating degradation rates of cocaine and benzoylecgonine from sewage treatment works over previous studies where assumptions were made.

Materials and Methods

Standard compounds and reagents were purchased under license from both Sigma Aldrich (Gillingham Dorset, UK) and LGC standards (Teddington Middlesex, UK). Mustapha (2013) has shown experimental batch studies when drugs were spiked with 1 mL of 12.5 mg in 250 mL of the aliquots of each raw sewage type [PS (primary sludge); SAF-1 (submerged aerated filter-1); MSAF (mixed SAF); HS (humus sludge)] in the conical flasks and degradation measured for every 15min for 3h duration using gas chromatography - mass spectrometry. Gas chromatography mass spectrometry analyses were performed with an Agilent 6890 GC coupled to Agilent 5975 inert XL mass selector detector (MSD: 2564.7 eV), using a capillary column (HP5-MS) (30.0 x 0.25mm x 0.25 um film thickness) with helium as carrier gas (1 mL min^{-1}). With sample injection in splitless mode, the analyte separation was achieved with the temperature programming: 50°C (hold 2min), rise to 300°C at 10°C min^{-1} and then held at 300°C for 3 min. The physico-chemical characterization of the wastewaters and the recovery experiments were analysed exactly the same way like the full batch tests.

Results and Discussion

Spike recovery studies and characterizations of compounds.

To reflect the actual rates, adjustments were made to the drug to make final results. In Table 1, the recoveries for the compounds show the data range between 75.8 – 96.2 % from different sewage matrices and discovery results compares well with similar experiments in the literature with most discovery between 50-80% [Lepik and Tenn, 2016; Ledjeri et al, 2016; Xiang et al, 2017]

Table 1: Recovery data for the compounds in aqueous phase **(%) (n=3,** mean ± STD**)**

Drug	**Aqueous matrix**			
	PS	SAF-1	MSAF	HS
Cocaine	91.5±1.8	90.1±1.3	86.6±6.5	95.4±1.2
Benzoylecgonine	96.2±0.8	88.1±2.7	85.3±2.4	92.5±3.1

Table 2 give the different samples of STW with the various contents. The sludge characterizations for total suspended solids (TSS), organic carbon (organic C), ash and chemical oxygen demand (COD) are also presented. The highest effluent of COD (162.6 $mgdm^{-3}O_2$) was far higher than the consent limit of BOD (15 mg L^{-1}), while the lowest concentration of COD (52.4 $mgdm^{-3}O_2$) for RAF Molesworth STW still higher than 48 $mgdm^{-3}O_2$ for

Nottingham STW, indicating how the kinetics of degradation can be influenced by different treatment processes and sewage nature.

Table 2. RAF Molesworth sludge characterization

Matrix	pH(Temp; °C)	TSS (mg/L)	Organic C (g)	Ash content (g)	COD ($mgdm^{-3}O_2$)
Molesworth STW					
PS	5.7 (14.4)	77386.5	0.95	0.07	162.6
MSAF	5.7 (14.4)	8465.0	0.93	0.05	138.0
SAF-1	7.1 (14.6)	70793.5	0.95	0.07	82.0
HS	7.1 (14.2)	3563.5	0.94	0.06	52.4

Though the obtained COD values compares well with the values reported in the literature for food processing sewage (7249 $mgdm^{-3}O_2$ COD), primary effluent (309 $mgdm^{-3}O_2$ COD), secondary effluent (35 $mgdm^{-3}O_2$ COD), swine waste (67,444 $mgdm^{-3}O_2$ COD), lagoon (27089 $mgdm^{-3}O_2$ COD) and another effluent (71 $mgdm^{-3}O_2$ COD) in previous studies [Martin et al, 2012; Mendoza et al, 2015; Patrolecco et al, 2015]. In a similar study, the amount of TSS (22.7 – 253 mg L^{-1}) produced was a function of organic matter in an activated sludge plant. But the remaining suspended particulates in the final effluent of TSS was 5.5 mg L^{-1} for Molesworth STW. The consent limit of 30 mg L^{-1} for TSS found in the effluents of the STW set by UK Environment Agency Compliance - Discharge Consent in comparison to our work was still far from being exceeded with results obtained in previous studies [Matin et al, 2012;Duan et al, 2016; O'Connor et al, 2016; Navarro et al, 2017]. However, Amarakoon et al (2016) has shown that due to myriads of materials, organic carbon was expectedly higher in the sludge as they facilitate compound degradation and removal. In our current work, the average concentration of organic carbon was 0.94 g per 1.0 gram of sewage sample. Also, the values indicate typical sewage characteristics and can as well be assumed to be representative of most municipal sewage types.

Batch experimental results

Evaluation of degradation in primary and secondary sludge samples.

Figure 1 shows the removal of drugs from the primary sludge and the removal mechanism for all drugs at different rates as drugs were distributed between aqueous and solid phases with simultaneous decline in various compounds concentrations.

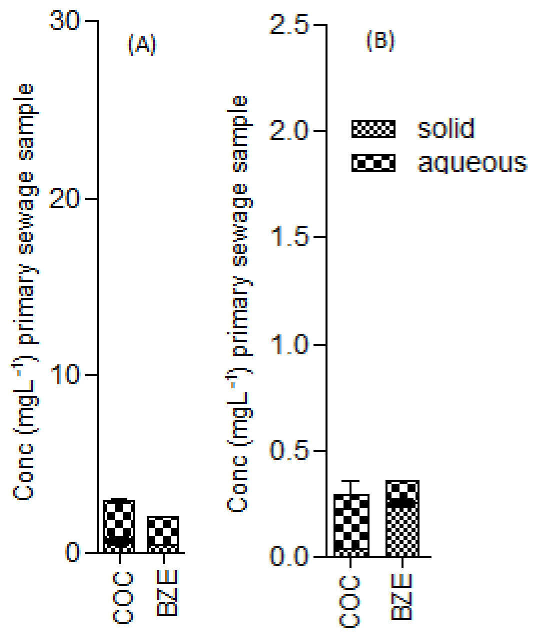


Figure 1: Degradation of compounds in primary sludge at 19± 0.5°C in (A) 15 min and (B) 3h as drugs degrade

In Figure 2, biodegradation initially advanced rapidly with significant removals of drugs but slows down over 3 hour periods as degradation progressed with the accumulation of degradation products of cocaethylene, benzoyecgonine, ecgonine-methylester or cocaine metabolites. The significance of degradation on retention time and its effects on removal rates was carried out in the second experiment. Figure 3 shows removal rates from primary sewage solids (suspended solids concentration of 77387 mg L^{-1}) due to different association of compounds with more than 80% removal achieved.

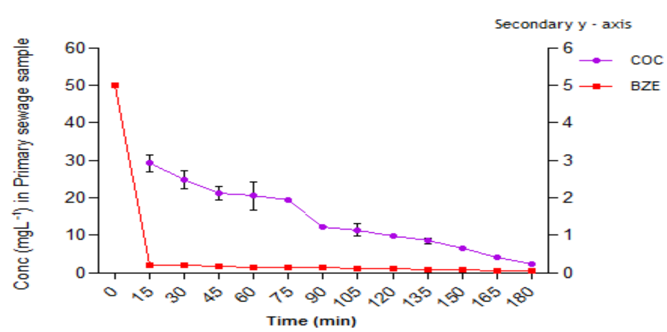


Figure 2: Plot of combined drugs in aqueous and solid phases at 19± 0.5°C in primary sewage sludge.

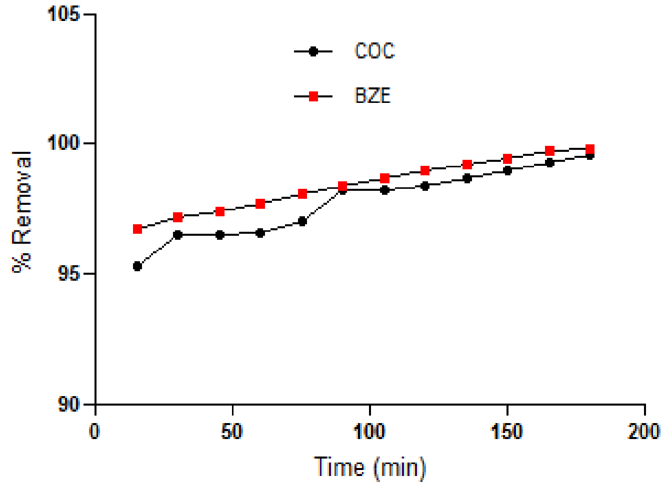
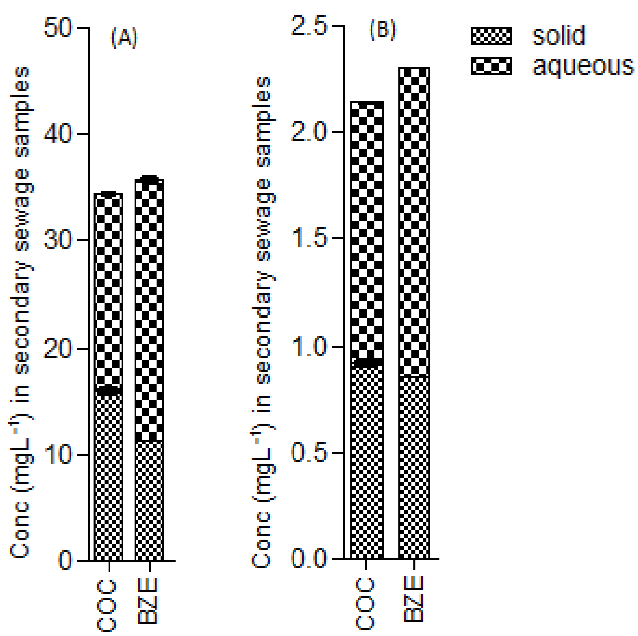


Figure 3: The effect of retention time during primary treatment processes.

For removal of drugs from secondary sludge, all the two compounds showed different removal rates over 3h retention time (Figure 4).



4: Degradation in SAF-1 sludge at 19± 0.5°C in (A) 15 min and (B) 3h as drugs degrade

Figure 5 shows the illustration of combined concentrations of drugs in aqueous and solid phases in the same secondary sewage sample at 19± 0.5°C. Most compounds expectedly showed greater degradation in biological secondary sewage compared to the pattern observed in primary sludge in Figure 5.

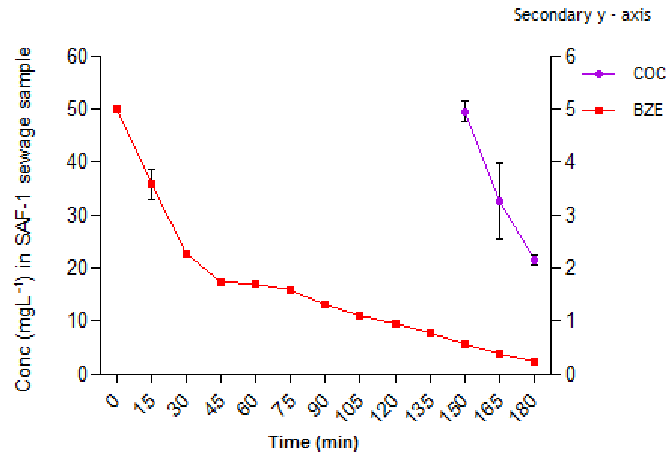


Figure 5. Plot showing combined concentrations of drugs in aqueous and solid phases in secondary sewage sludge (SAF-1) at 19± 0.5°C.

In Figure 6, the effect of retention time on the removal of compounds during the secondary sewage processes can be seen with benzoylecgonine and cocaine showing average removal rate in the first hour of contact. Since others exhibited maximum removals at the same period of exposure, it was found that the removal of compounds at 19± 0.5°C was favourable being the normal operational temperature of most STWs.

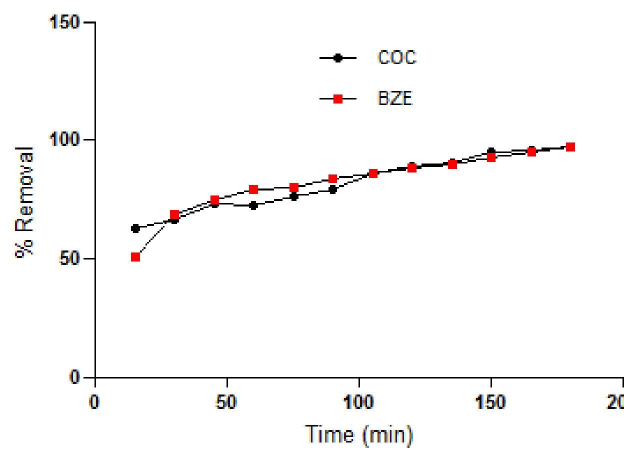


Figure 6: The effect of retention time on the removal of compounds during secondary treatment.

Comparison of Kd values to LogKow data.

The tendency for substance to prefer non-aqueous phase to aqueous phase (i.e. its hydrophilic/lipophilic balance) is known as the octanol-water partition coefficient (Log Kow). It is a laboratory-measured property that provides a thermodynamic measurement of distribution of substances, and widely used in environmental. But the water-solid partition coefficient (Kd) measures distribution between water and solid partition for the selected drugs. In the current work, comparison with literature, the Log Kow data range from 0.9 – 2.9 in Table 3 as against 1.2 – 69.9 in Table 4 for the drugs. However, Table 4 below generally show the relatively higher Kd values in secondary sludge compare to primary sludge with diazepam showing greater values in both sludge types.

Evaluation of biotic versus abiotic degradation.

Evaluation of biotic and abiotic degradation with unfiltered primary sludge samples with the suspended solids concentration of 77387 mgL^{-1} at 4 ± 0.5°C for 30 min to inhibit biological activity before the sample were taken at 15 min for 3h

Table 3: Compounds used in batch studies

Class of drugs	Compounds	Log Kow
Cocaine	Cocaine	2.3
	Benzoylecgonine	1.3

For cocaine, faster cocaine degradation at biotic temperature of 19 ± 0.5°C but relatively slowly at abiotic process (4± 0.5°C) were observed. The low temperature inhibited the biological process but gradual biodegradation process and chemical hydrolysis were the two major removal mechanism since no loss due to volatilisation occurred as reported in the literature [Tiwari et al, 2016; Han and Currell, 2017]. Figure 7 further demonstrates how microbial inactivity has slowed down metabolic processes. Indication that both biological and chemical processes increased degradation products and greater partitioning in solid phases were shown.

4: Data of water-solid distribution coefficients of drugs in both PS and SAF at 19± 0.5°C (K_d, Kg/L)

Time (min)	Primary sludge (PS)		Submerged aerated filter (SAF)	
	COC	BZE	COC	BZE
15	3.2	2.9	12.2	6.5
30	5.6	4.0	12.2	5.3
45	2.8	3.6	13.2	9.0
60	2.7	4.0	9.9	8.2
75	3.8	7.6	9.9	8.1
90	4.6	7.9	7.7	-
105	3.7	10.6	7.0	-
120	3.3	12.9	10.2	-
135	4.4	15.6	8.1	-
150	4.0	18.9	12.4	8.7
165	3.6	25.7	8.8	8.2
180	2.7	31.9	10.6	6.3

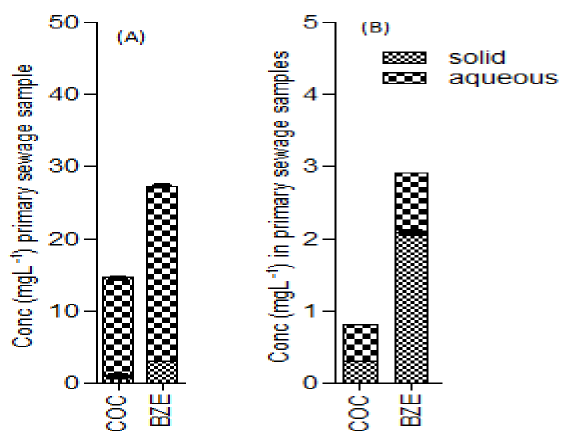


Figure 7: Degradation of compounds in primary sludge at abiotic conditions (4± 0.5°C) at time (A) 15 min and (B) 3h.

Figure 8 below further confirmed temperature dependency of degradation but chemical hydrolysis also occurred as well at low temperature.

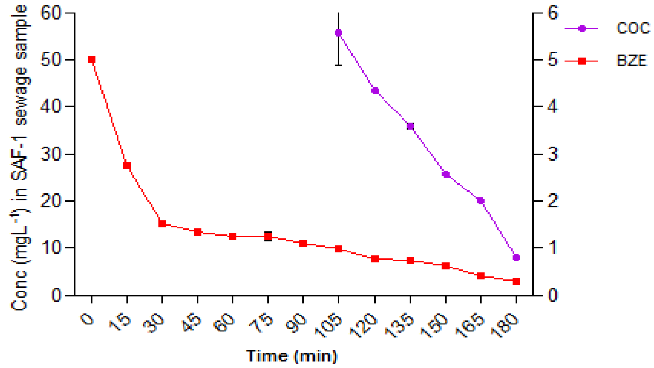


Figure 8: Combined concentrations of compounds in aqueous and solid phases (4± 0.5°C).

Inhibition of the biological activities in the samples was expected at 4 ± 0.5° C to slow the metabolic processes. Chemical with possible biological processes may be involved. If degradation were only biological, no changes would have been observed but the metabolic processes though were slowed down compared to degradation at 19± 0.5°C. The rate of removal of compounds was affected though, but the K_d values of benzoylecgonine showing greater sorption at 19± 0.5°C while cocaine was better sorped at 4± 0.5°C.

Evaluation of suspended solids on the removal of compounds

Song et al (2016) has shown that high removals of organic depended on water-solid partition coefficient (K_d) values. In the current work, the effect of suspended solids on the removal of compounds by evaluation of adsorbed compounds onto the solids in primary sludge at 19±0.5°C were carried out.

Table 5 indicate Primary Sludge (0.08%) of cocaine showed significant removal compare to benzoylecgonine in secondary sludge.

Table 5: Adsorption of compounds onto sludge with different suspended solids (%) in 3h.

	Suspended solids (mg L^{-1})			
Compounds	77386.5 (PS)	70793.5 (SAF-1)	8465.0 (MSAF)	3563.5 (HS)
Cocaine	0.08	1.83	0.17	0.09
Benzoylecgonine	0.51	1.69	0.59	0.73

The sorption by organic compounds has therefore be reported with log K_{ow} < 2.5 (low sorption potential), log K_{ow} > 2.5 and < 4.0 (medium sorption potential) and log K_{ow} > 4.0 (high sorption potential) for chemicals that partition to organic phases to estimate a clear relationship with the degree of partitioning of contaminants during treatment. Also, The solid-water partition coefficients (K_d) in the current study were in the range for cocaine (0.2 – 0.9) and benzoylecgonine (0.2 – 2.5) with over 75% removal efficiency observed.

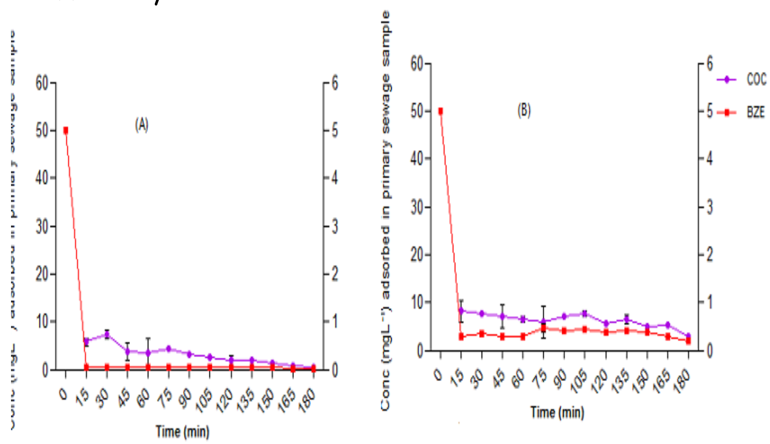


Figure 9: Effect of adsorption on the removal of compounds in primary sludge at (A) 4±0.5°C (B) 19±0.5°C.

Effect of nature of sludge on degradation.

The nature of sludge sample show results obtained from the primary sludge samples collected at Molesworth and Stoke Bardolph Nottingham STWs. The ability of sewage treatment works removal processes depends on design, this made physical-chemical biodegradation to be disimilar (Figure 10).

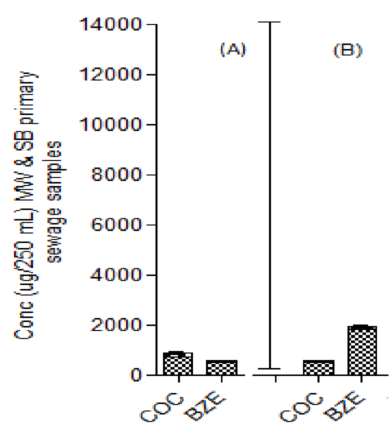


Figure 10: Cocaine degradation in (a) Molesworth (b) Nottingham STWs at 19± 0.5° for 3h.

Surprisingly, the similarity in removal profiles showed no significant effects of nature of primary sludge samples on degradation. Both had high values after 15 mins, and low values after 3hours. Variability in the values of TSS and COD as shown in the characterization (Table 3) suggested many different treatment processes exist with different requirements based on peculiar inflow variability and operational requirements.

Conclusions

The novel aspects of this work are data derived from batch studies, removal rates and their application to Molesworth STW. The main conclusions for the novel work are:

At biotic (19 ± 0.5°C) and abiotic (4 ± 0.5° C) temperatures, degradation of cocaine and benzoylecgonine were conducted on sludge samples. At 19 ± 0.5° C the degradation were relatively greater and faster than at 4 ± 0.5° C, by between 5 and 10% indicating both biotic and abiotic degradations occurred but slowly at 4 ± 0.5° C. This confirmed that the rate of removal of compounds is temperature dependent with both chemical and biological degradation/partitioning occurring together.

The removal rates of cocaine (91.0%) and benzoylecgonine (90.6%) were by, and sorption, biodegradation and chemical degradation as dominant removal processes (volatilization is unlikely) for 8 HRT, with corresponding partitioning coefficients (K_d) that ranged from 2.7 – 31.9 Kg L^{-1}. With identical K_d values, the compounds exhibited tendencies of accumulating in sludges could possibly provide empirical relationship between drug removal and HRT.

The RAF Molesworth STW sludge characterization showed changes in pH (7.8 - 8.4) between the influent and effluent wastewaters, TSS [36243.0 mg L^{-1} (influent) and 5.5 mg L^{-1} (effluent)], COD [216.8 mgdm$^{-3}O_2$ (influent) and 1.8 mgdm$^{-3}O_2$ (effluent)]. Average ash (0.94 g/g) and organic carbon (0.06 g/g) contents were observed.

Work encompassed directly measures illicit drug removal rates in laboratory studies for the first time. The capabilities of the current experimental batch data in generating removal rates of drugs have however been clearly demonstrated and can be applied to any organic compounds in real life (STWs) situations.

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