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IS THE USE OF COSUBSTRATES BENEFICIAL IN THE REMOVAL OF PHARMACEUTICALS FROM SYNTHETIC SANITARY SEWAGE?

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ABSTRACT

The current study attempted to evaluate if the addition of cosubstrates could be beneficial in anaerobic digestion, using an Anaerobic Sequencing Batch Biofilm Reactor (AnSBBR), to remove ibuprofen (IBU), diclofenac (DCF), and caffeine (CAF) from synthetic sanitary sewage. The AnSBBR reactor showed higher IBU removal without the presence of cosubstrates (21 vs. 17% with sucrose); glucose and lactose boosted DCF removal (57 and 53%, respectively vs. 22% without cosubstrate); and ethanol and cheese whey maintained the same CAF removal (94 and 93%, respectively vs. 95% without cosubstrate). Furthermore, ecotoxicological tests with *Chironomus sancticaroli* revealed a reduction in the toxic effect of the treated effluent in all assays, resulting in the lowest mortality rates of 6 and 11% when glucose and glycerin were used, respectively, vs. 56% without cosubstrate.

Keywords: Anaerobic digestion. Caffeine. Diclofenac. Ibuprofen. Sequencing batch reactor.

1 INTRODUCTION

Micropollutants or emerging contaminants, such as ibuprofen (IBU), diclofenac (DCF), and caffeine (CAF), are introduced into the environment via a range of sources, most notably wastewater discharge[1]. Due to proven adverse effects on human health and aquatic biota, wastewater containing micropollutants must be thoroughly cleaned before release. Several traditional physicochemical techniques have been widely investigated for micropollutant removal. However, due to one or more drawbacks, biological treatment using appropriate microorganisms has sparked recent attention[2]. The anaerobic bioreactor is a flexible treatment technology that is widely utilized in the treatment of many types of wastewaters as it requires less energy and produces less sludge than aerobic reactors[3]. Therefore, this project aimed at using a sequencing batch reactor to remove IBU, DCF and CAF from synthetic sanitary sewage by anaerobic treatment, as well as testing the environmental adequacy (ecotoxicity tests) of the resulting effluent. The influence of cosubstrates (sucrose; ethanol; lactose; cheese whey; glucose; and glycerin) on system stability and performance was investigated.

2 MATERIAL & METHODS

The studies were carried out using a 6-L Anaerobic Sequencing Batch Biofilm Reactor (AnSBBR) with mechanical agitation (200 rpm). Diaphragm pumps were utilized for both feeding and discharge. An automated system based on timers was used to initiate and stop the pumps and agitation (feed, reaction, and discharge phases). A thermostatic bath-controlled water jacket maintained a temperature of 25 ˚C (± 1 ˚C). Figure 1 depicts the schematic representation of the system.

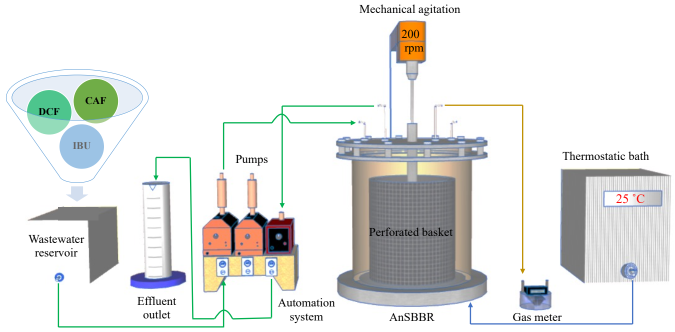

Figure 1 Schematic representation of the system.

Mesophilic granular sludge from an Upflow Anaerobic Sludge Blanket (UASB) reactor handling poultry slaughterhouse effluent was employed as inoculum, and it was immobilized in 1 cm^3 polyurethane foam cubes as inert support. The wastewater was formulated using synthetic sanitary sewage (500 mg-COD/L). Chebel et al. [4] provide details on its composition. DCF, IBU, and CAF concentrations at influent were 400, 1200, and 92000 ng/L, respectively[5]. The equivalent of 200 mg-COD/L of each cosubstrate was added to the reactor according to the desired condition[6], that is, the mixture fed to the reactor was composed of synthetic sewage and one cosubstrate at a time (700 mg-COD/L).

Reactor monitoring was carried out by examining influent and effluent samples for organic matter concentration (COD and total carbohydrates), bicarbonate alkalinity (BA), and total volatile acids (TVA). Biogas concentration was analyzed using gas chromatography and gas volume was measured by a Ritter MilligasCounter[7]. The pharmaceuticals were investigated using Ultra Efficiency Chromatography Coupled with Mass Spectrometry (UPLC-MS/MS)[6]. Acute ecotoxicity tests (96 h) were conducted on *Chironomus sancticaroli*, a neotropical insect occurring in the state of São Paulo, Brazil[8]. Seven assays were carried out (Table 1) totaling 225 days of study.

Table 1 Operational conditions.

Assay	Compound	Cosubstrate	Feeding strategy	Cycle length (h)	Temperature (˚C)	Duration (days)
1	IBU+DCF+CAF	-	Fed-batch	8	25	31
2	IBU+DCF+CAF	Sucrose	Fed-batch	8	25	33
3	IBU+DCF+CAF	Ethanol	Fed-batch	8	25	33
4	IBU+DCF+CAF	Lactose	Fed-batch	8	25	35
5	IBU+DCF+CAF	Cheese whey	Fed-batch	8	25	35
6	IBU+DCF+CAF	Glucose	Fed-batch	8	25	29
7	IBU+DCF+CAF	Glycerin	Fed-batch	8	25	29

3 RESULTS & DISCUSSION

All assays (Table 2) showed COD removal efficiency above 84% for filtered samples and nearly complete carbohydrates removal (> 98%). Low TVA concentrations in the effluent and a rise in BA in the effluent were detected, therefore maintaining the pH in the range considered ideal for methanogenic reactors (7.0-7.5)[9]. As the synthetic sewage load is low, it was not possible to quantify the concentration and percentage of CO_2 present in the biogas. Therefore, based on previous work[10], which studied the treatment of the same synthetic sewage and the same operating conditions, a percentage of CH_4 in the reactor was assumed to be 50%. From the results presented in Table 2, it can be seen that the productivity and methane yield values were very close, regardless of the cosubstrate added, showing reactor stability.

Table 2 Performance indicators.

Parameters	1	2 (Sucrose)	3 (Ethanol)	4 (Lactose)	5 (Cheese whey)	6 (Glucose)	7 (Glycerin)
OLR_A	1.4	1.7	1.7	2.0	1.9	1.8	1.7
C_{SINF}	$580\pm50^{(19)}$	$728\pm62^{(22)}$	$714\pm71^{(22)}$	$828\pm127^{(23)}$	$781\pm88^{(23)}$	$759\pm86^{(20)}$	$722\pm61^{(20)}$
ε_{SF}	$91\pm1^{(19)}$	$88\pm3^{(22)}$	$91\pm2^{(22)}$	$85\pm5^{(23)}$	$92\pm2^{(23)}$	$84\pm4^{(20)}$	$92\pm2^{(20)}$
C_{CINF}	$176\pm28^{(19)}$	$361\pm44^{(22)}$	$173\pm43^{(22)}$	$406\pm27^{(23)}$	$375\pm46^{(23)}$	$446\pm41^{(20)}$	$208\pm59^{(20)}$
ε_{CF}	$98\pm1^{(19)}$	$99\pm0.3^{(22)}$	$98\pm0.6^{(22)}$	$98\pm1^{(23)}$	$99\pm0.3^{(23)}$	$99\pm0.6^{(20)}$	$98\pm0.6^{(20)}$
TVA_{INF}	$25\pm 2^{(19)}$	$30\pm2^{(22)}$	$28\pm3^{(22)}$	$32\pm3^{(23)}$	$36\pm4^{(23)}$	$32\pm2^{(20)}$	$31\pm4^{(20)}$
TVA_{EFF}	$16\pm4^{(19)}$	$34\pm13^{(22)}$	$28\pm9^{(22)}$	$44\pm15^{(23)}$	$27\pm7^{(23)}$	$44\pm12^{(20)}$	$30\pm11^{(20)}$
BA_{INF}	$218\pm10^{(19)}$	$228\pm11^{(22)}$	$228\pm9^{(22)}$	$223\pm4^{(23)}$	$219\pm6^{(23)}$	$227\pm8^{(20)}$	$223\pm11^{(20)}$
BA_{EFF}	$308\pm19^{(19)}$	$306\pm14^{(22)}$	$309\pm13^{(22)}$	$285\pm14^{(23)}$	$305\pm8^{(23)}$	$288\pm14^{(20)}$	$304\pm13^{(20)}$
V_F	$1.9\pm0.2^{(19)}$	$2.0\pm0.1^{(22)}$	$2.0\pm0.1^{(22)}$	$2.0\pm0.1^{(23)}$	$2.0\pm0.1^{(23)}$	$2.0\pm0.1^{(20)}$	$2.0\pm0.1^{(20)}$
V_G	$612\pm73^{(19)}$	$614\pm99^{(18)}$	$629\pm104^{(19)}$	$664\pm163^{(19)}$	$680\pm160^{(18)}$	$666\pm58^{(16)}$	$684\pm108^{(16)}$
MPr	15.7	16.5	16.6	17.8	18.4	17.6	18.4
MY	12.4	10.8	10.6	10.5	10.7	11.4	11.6

Notation: OLR_A= Applied organic loading rate (g-COD/L/d); C_{SINF}= Influent concentration (mg-COD/L); ε_{SF}= Filtered COD removal efficiency (%); C_{CINF}= Carbohydrate concentration (mg-Carb/L); ε_{CF}= Filtered carbohydrate removal efficiency (%); TVA_{INF}= Total volatile acids at influent (mg- HAc/L); TVA_{EFF}= Total volatile acids at effluent (mg-HAc/L); BA_{INF}= Bicarbonate alkalinity at influent (mg-$CaCO_3$/L); BA_{EFF}= Bicarbonate alkalinity at effluent (mg-$CaCO_3$/L); V_F= Fed volume of the liquid medium (L/cycle); V_G= Gas volume produced (mL-NTP/cycle); MPr = Methane molar productivity (mol-CH_4/m^3/d); MY = Molar yield of methane per removed substrate (mol-CH_4/kg-COD). Values in parentheses refer to the number of samples analyzed.

The micropollutant removal analysis revealed that there was a reduction of IBU removal even with the addition of cosubstrates. This is likely due to the high branching in the structure, presence of substitutions in the *para* position of the aromatic ring, and spatial configuration, which suggest its high resistance to biodegradation[11]. Furthermore, lactose and glucose-based assays showed a significant increase in DCF removal. This finding might be attributed to the cosubstrates' synergistic effect with DCF, which may have aided in the breakdown of the molecule's two benzene rings[12]. In turn, it appears that CAF was identified as the micropollutant with the highest removal efficiency, as predicted given its higher biodegradability[13]. However, the addition of sucrose, lactose, and glucose reduced CAF removal, most likely due to microorganisms' preference for the major carbon source of cosubstrates over caffeine. As a result, there was no clear trend in micropollutant removal with the addition of cosubstrates, indicating that each cosubstrate interacted differently with the IBU, DCF, and CAF molecules.

From an ecotoxicological viewpoint, there was a decrease in the mortality rate of the assays in comparison to the influent (89%) (Figure 2), confirming that the treatment of removing IBU, DCF and CAF was indeed effective. Furthermore, it is worth noting that the control tests had minimal or no mortality rates, suggesting that the specimens perished as a result of the presence of the micropollutants under study rather from natural causes or being fragile. Also, a decrease in the mortality rate of *Chironomus sancticaroli* was observed when the cosubstrates were added. Glucose and glycerin were the cosubstrates that resulted in the lowest mortality rates (6 and 11%, respectively) of *Chironomus sancticaroli* in comparison to the assay without cosubstrate (56%), when considering 100% of raw sample, that is, a sample without any dilution. Table 4 shows non-significant differences from the control tests, with *p-values* higher than 0.05 (Dunn's post hoc test), with means that most of the assays were considered non-toxic for the specimens. Thus, the use of the AnSBBR reactor in the treatment of synthetic sanitary sewage with the goal of removing IBU, DCF, and CAF was effective as micropollutant removal was proven (maximum of 21, 57 and 95%, respectively), as well as a reduction in the negative effects on *Chironomus sancticaroli*.

Table 3 Ibuprofen, diclofenac, and caffeine concentration in influent and effluent.

Assay	Compounds	Concentration			Removal efficiency		
		Ibuprofen (ηg/L)	Diclofenac (ηg/L)	Caffeine (µg/L)	Ibuprofen (%)	Diclofenac (%)	Caffeine (%)
Influent	-	966	293	55	-	-	-
1	IBU+DCF+CAF	760	227	3	21	22	95
2	IBU+DCF+CAF+Sucrose	798	215	17	17	27	69
3	IBU+DCF+CAF+Ethanol	818	268	3	15	9	94
4	IBU+DCF+CAF+Lactose	819	138	23	15	53	58
5	IBU+DCF+CAF+Cheese whey	827	226	4	14	23	93
6	IBU+DCF+CAF+Glucose	889	125	25	8	57	54
7	IBU+DCF+CAF+Glycerin	884	277	10	9	5	82

Assays	Compounds	*p-values* for proportion of sewage in the samples				
		100%	50%	25%	12.5%	6%
Influent	-	0.0007291	0.007556	0.05935	0.2386	0.4321
1	IBU+DCF+CAF	0.002375	0.04207	0.9907	1.0000	0.9907
2	IBU+DCF+CAF+Sucrose	0.003663	0.4384	0.4978	1.0000	1.0000
3	IBU+DCF+CAF+Ethanol	0.1860	0.4275	1.0000	0.00817	0.4275
4	IBU+DCF+CAF+Lactose	0.006273	0.1583	1.0000	0.4806	0.4806
5	IBU+DCF+CAF+Cheese whey	0.02070	0.6553	0.04673	0.2913	0.1332
6	IBU+DCF+CAF+Glucose	0.4644	0.2453	0.02819	0.02819	0.1434
7	IBU+DCF+CAF+Glycerin	0.1160	0.4320	1.0000	0.4320	0.06446

Table 4 Statistical analysis using Dunn's post hoc test.

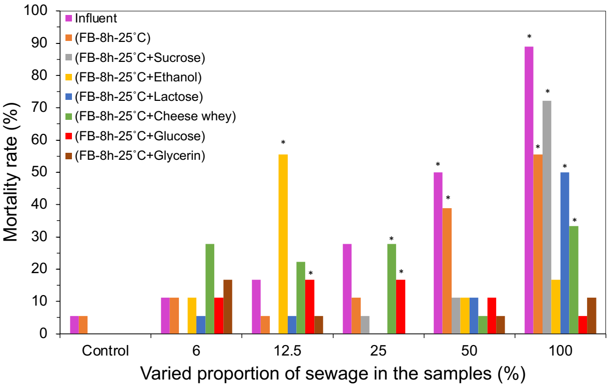

Figure 2 Mortality rate of *Chironomus sancticaroli* after 96 h of exposure.

Notation: The pink color in Table 4 means non-significant differences from the control; * in Figure 2 indicates significant differences to control; Control tests mean that only the cultivation medium was used. 6, 12.5, 25, 50, and 100% mean the proportion of sewage present in the samples.

4 CONCLUSION

In conclusion, the findings of this study shed light on the effectiveness of cosubstrates in enhancing the removal of IBU, DCF, and CAF in AnSBBR systems. While no clear pattern in the removal of these pharmaceutical compounds was observed with the addition of cosubstrates, the inclusion of glucose and glycerin significantly reduced the mortality rate of *Chironomus sancticaroli* compared to the assay without cosubstrate addition. Specifically, the mortality rate dropped to 6 and 11% with the addition of glucose and glycerin, respectively, in contrast to 56% mortality in samples lacking cosubstrates. These results underscore the beneficial impact of cosubstrates on the removal of pharmaceutical compounds in AnSBBR systems. Further research could delve into optimizing cosubstrate addition to maximize removal efficiency and minimize ecological impacts.

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