Appendix A. Supplementary data

Impact of seeding on development of biological activated carbon filter for simultaneous removal of organics, nitrogen, & emerging contaminants from secondary effluent

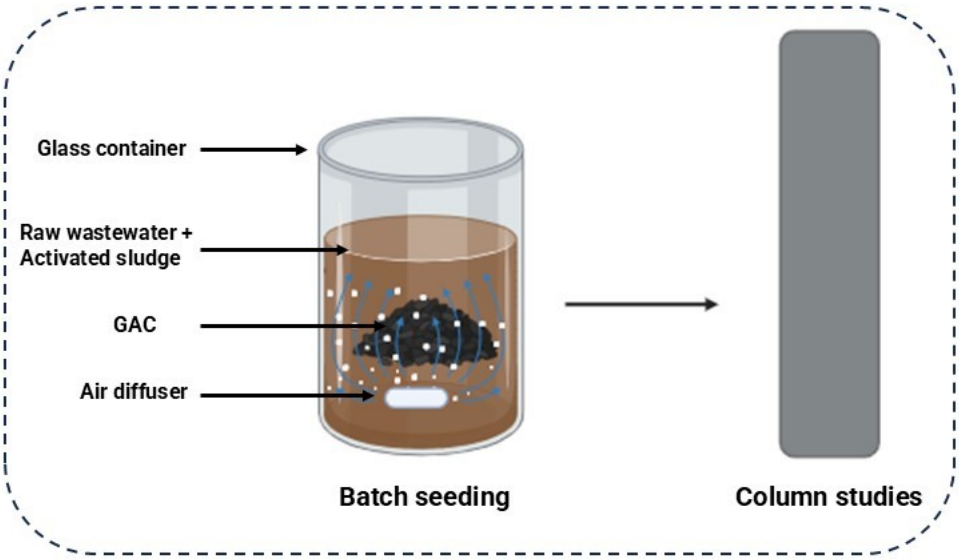
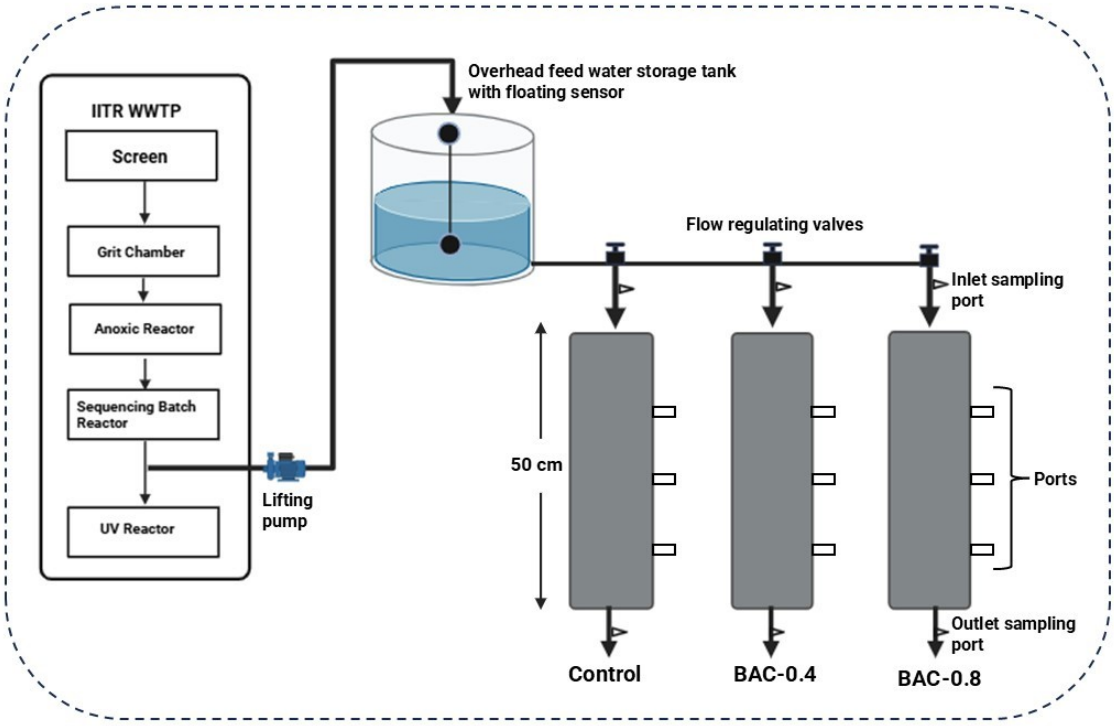


Figure S1: Schematic diagram of the batch seeding process



F

igure S2: Schematic diagram of bench-scale BAC filter setup

Text S1: BET surface area

The BET surface of virgin GAC was 455.2 m^2/g, and after 7 days of the batch seeding experiment at F/M, 0.4 and 0.8, decreased to 394.7 m2/g and 365.4 m^2/g, respectively. At the end of 14000 BV, the BET surface area of BAC media in control, BAC-0.4, and BAC-0.8 decreased to 251.4, 245.6, and 238.9 m^2/g, respectively.

Text S2: Scanning Electron Microscope

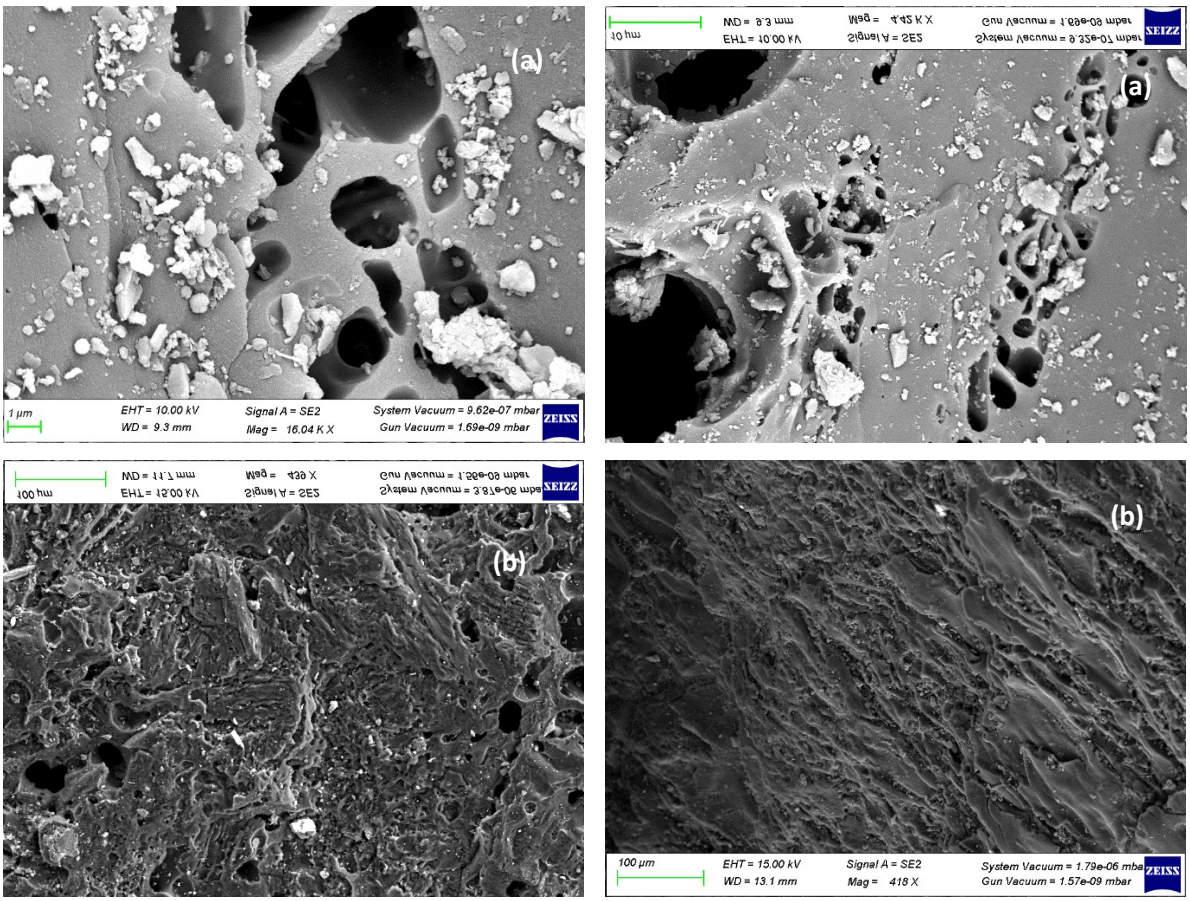
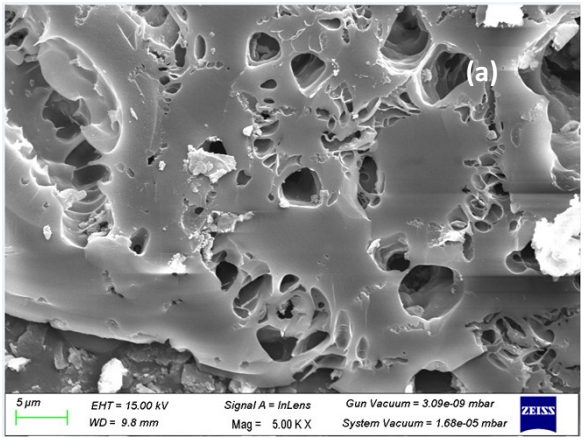
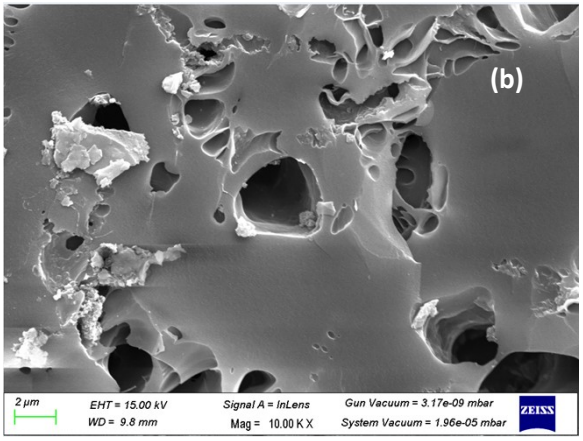
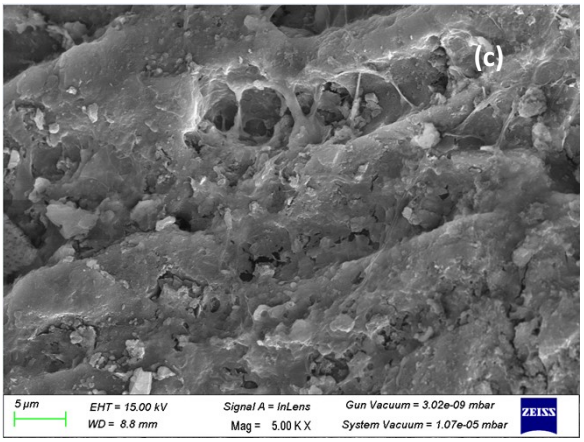


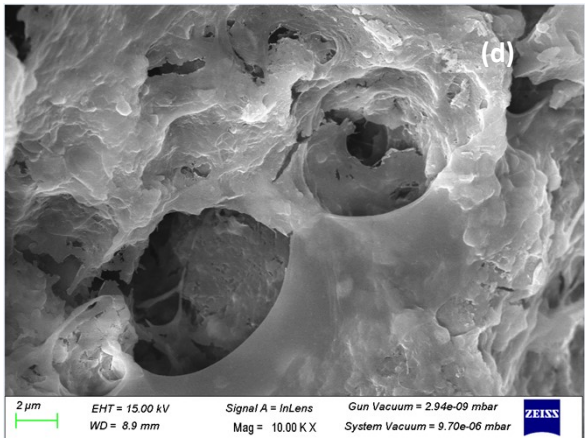
Figure S3: SEM images of the GAC before batch seeding (a) and after 7-day batch seeding (b)

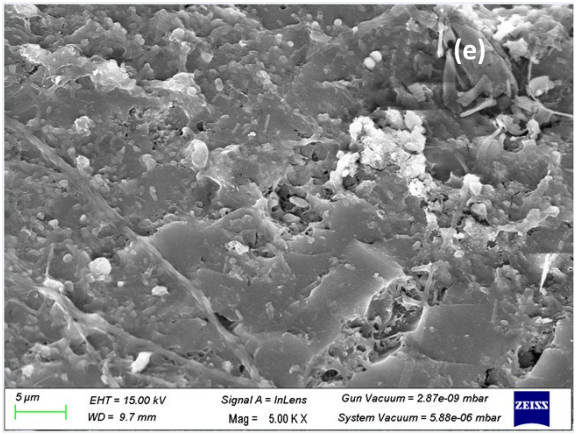
SEM images of virgin GAC and BAC before and after 14000 BV are presented in Figure S4. The fresh GAC exhibited irregular shapes with visible pores and crevices, as shown in Figure S4 (a & b). A biofilm is observed covering the entire media surface, signifying the transformation of GAC into BAC. The BAC media in the control sample displayed a biomass layer along with adsorbed materials from secondary treated wastewater coating the activated carbon surface, Figure S4(c-d). The accumulation of extracellular polymeric substances (EPS) is evident in Figure S4 (f & g), appearing as a bridge-like structure connecting the biofilm on the BAC granules and thin film on the BAC surface Figure S4 (h).

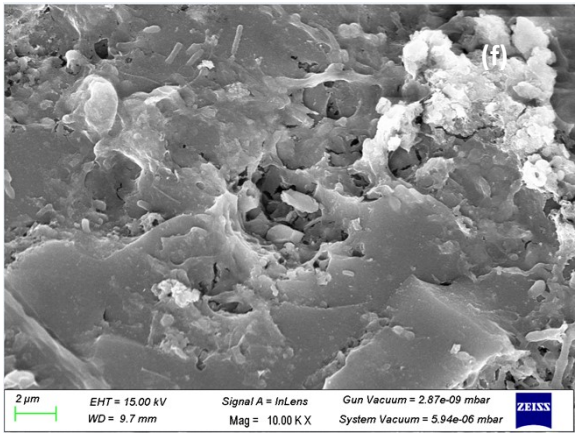

(a)
5 µm
EHT = 15.00 kV
WD = 9.8 mm
Signal A = InLens
Mag = 5.00 K X
Gun Vacuum = 3.09e-09 mbar
System Vacuum = 1.68e-05 mbar
ZEISS

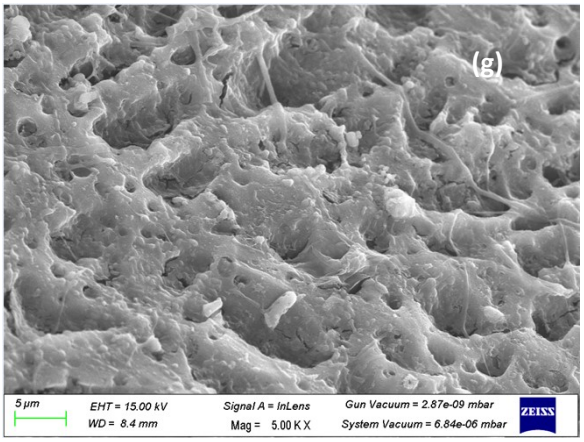

(b)
2 µm
EHT = 15.00 kV
WD = 9.8 mm
Signal A = InLens
Mag = 10.00 K X
Gun Vacuum = 3.17e-09 mbar
System Vacuum = 1.96e-05 mbar
ZEISS


(c)
5 µm
EHT = 15.00 kV
WD = 8.8 mm
Signal A = InLens
Mag = 5.00 K X
Gun Vacuum = 3.02e-09 mbar
System Vacuum = 1.07e-05 mbar
ZEISS


(d)
2 µm
EHT = 15.00 kV
WD = 8.9 mm
Signal A = InLens
Mag = 10.00 K X
Gun Vacuum = 2.94e-09 mbar
System Vacuum = 9.70e-06 mbar
ZEISS


(e)
5 µm
EHT = 15.00 kV
WD = 9.7 mm
Signal A = InLens
Mag = 5.00 K X
Gun Vacuum = 2.87e-09 mbar
System Vacuum = 5.88e-06 mbar
ZEISS


(f)
2 µm
EHT = 15.00 kV
WD = 9.7 mm
Signal A = InLens
Mag = 10.00 K X
Gun Vacuum = 2.87e-09 mbar
System Vacuum = 5.94e-06 mbar
ZEISS


(g)
5 µm
EHT = 15.00 kV
WD = 8.4 mm
Signal A = InLens
Mag = 5.00 K X
Gun Vacuum = 2.87e-09 mbar
System Vacuum = 6.84e-06 mbar
ZEISS

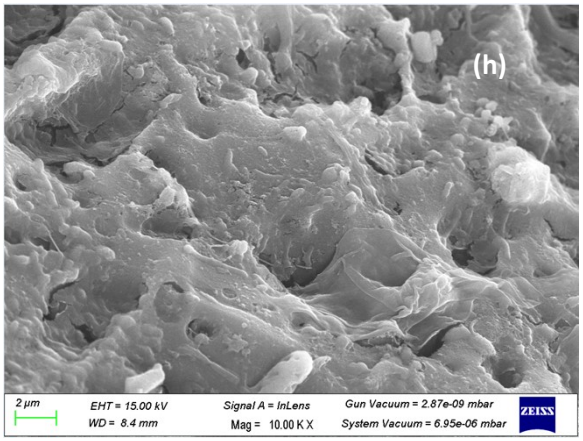

(h)
2 µm
EHT = 15.00 kV
WD = 8.4 mm
Signal A = InLens
Mag = 10.00 K X
Gun Vacuum = 2.87e-09 mbar
System Vacuum = 6.95e-06 mbar
ZEISS

Figure S4: SEM images of the fresh GAC (a) and (b), control (c) and (d), BAC-0.4 (e) and (f), and BAC-0.8 (g) and (h) media.

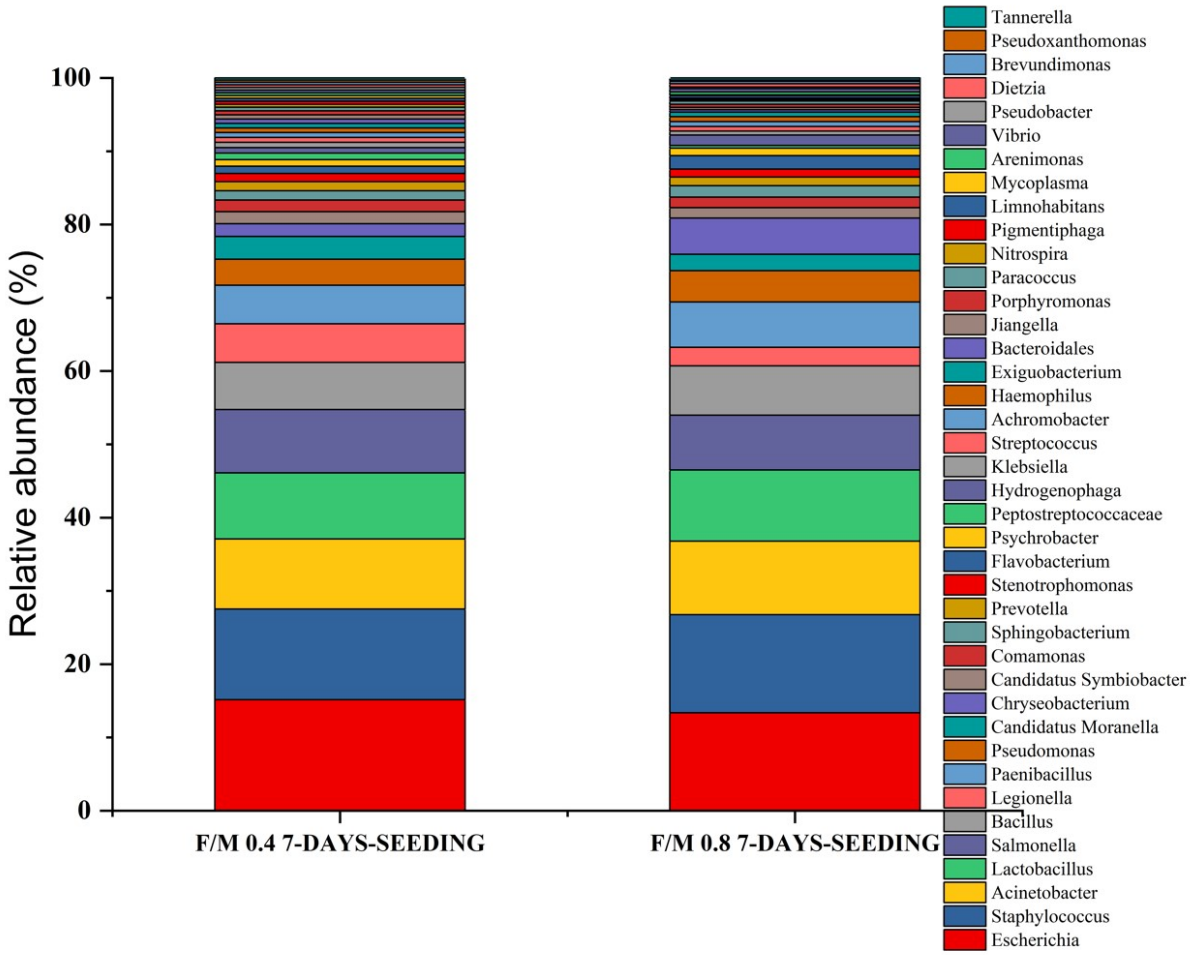


Fig. S5. Relative abundance of bacterial community (genus level) on BAC biofilms after 7 days of batch seeding experiments.

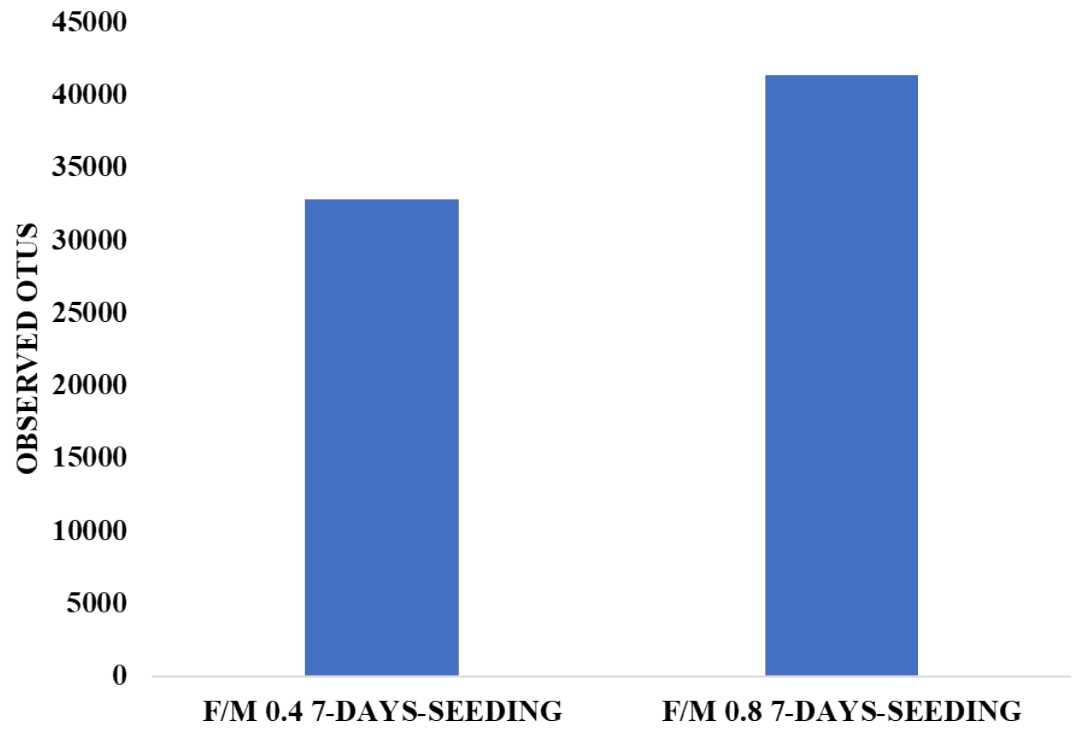


Figure S6: Observed OTUs on the GAC surface after 7 days of the batch seeding at F/M 0.4 and 0.8.

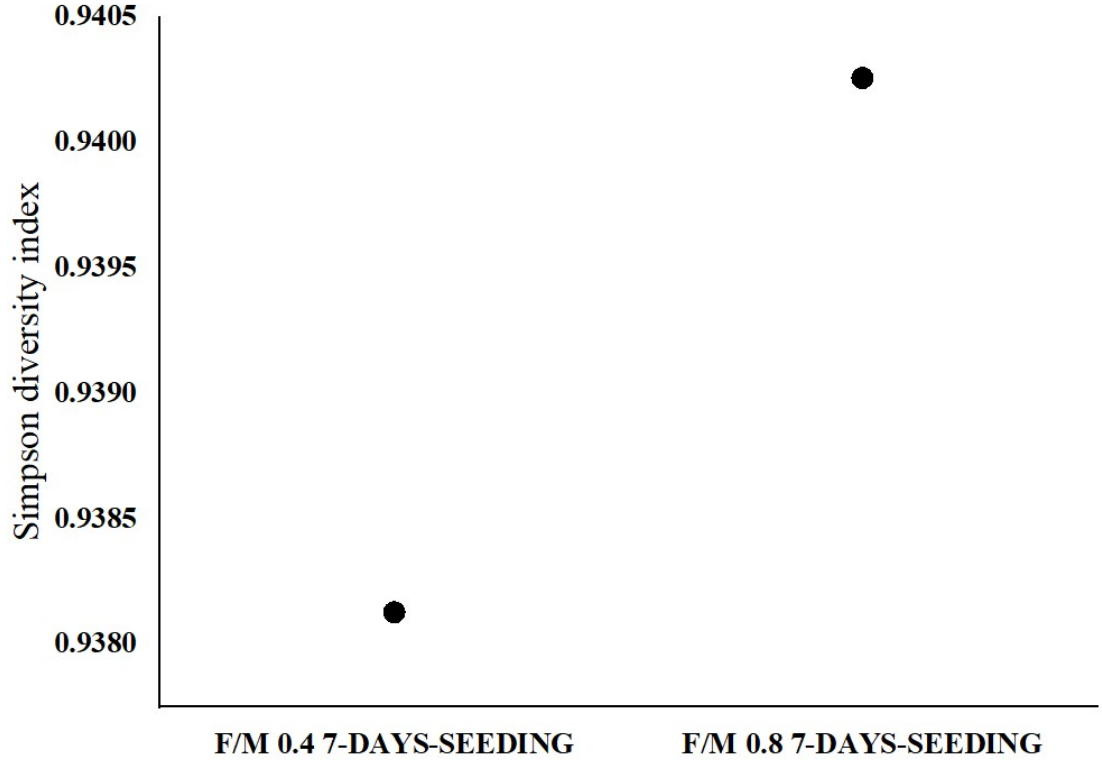


Figure S7: Simpson diversity index of BAC biofilm after 7 days of batch seeding at F/M 0.4 and 0.8

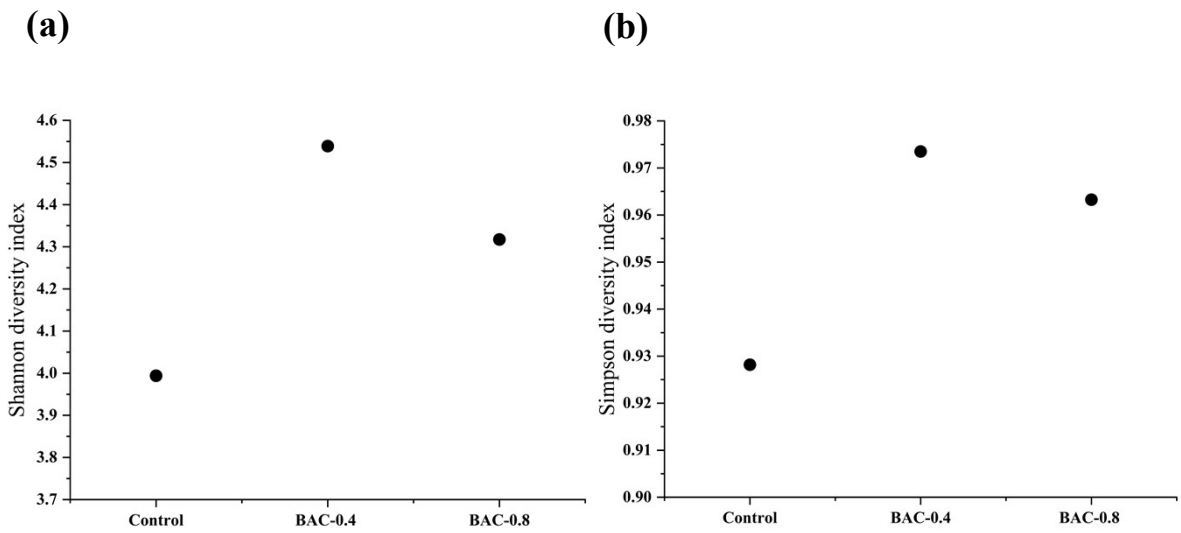


Figure S8: Shannon diversity index (a), Simpson diversity index (b) of control, BAC-0.4, and BAC-0.8 (> 19,206 BV).

Text S3: FTIR analysis

The type of functional groups on the surface of GAC and BAC after conducting the 7 days of batch seeding process at F/M 0.4 and 0.8 was determined by FTIR, as shown in Figure S8. The type of functional group on the activated carbon helps in the adsorption of microbes and organic contaminants from secondary treated wastewater. Differences were observed in the characteristic peaks between GAC and BAC. The peak at around 3440 corresponds to the stretching vibration of the hydrogen-bonded hydroxyl (-OH) group, indicating the presence of carboxyl, phenol, or alcohol on the surface (1). This peak was more pronounced in GAC than in BAC. The -OH group intensity decrease indicates that the surface is getting hydrophobic. The peaks in the region from 1500 to 1800 cm^{-1} suggest the presence of carbonyl (C=O) and aromatic (C=C) stretching, which are typically associated with proteins, polysaccharides, and humic substances; the bac samples show stronger peaks than GAC, indicating biofilm formation and organic matter accumulation. The BAC samples exhibit strong peaks in the region from 1000 – 1300 cm^{-1}, suggesting the presence of EPS and polysaccharides. Overall, the results indicate an increase in the media's lipophilicity due to biomass's growth on GAC media after 7 days of the batch seeding process.

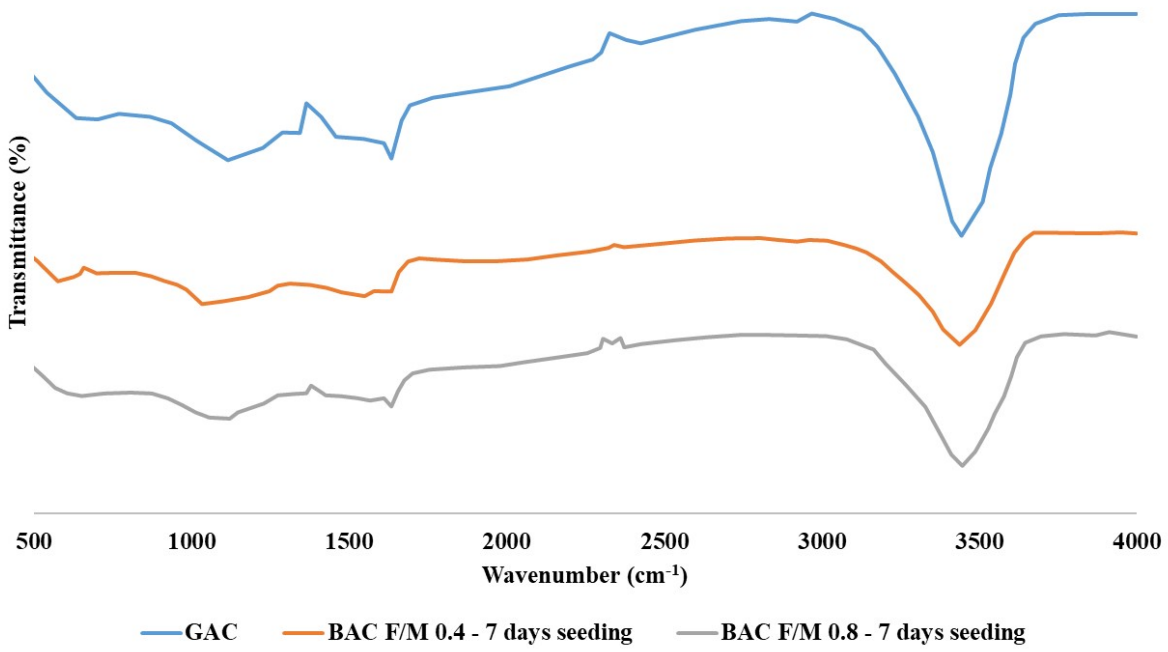


Figure S9: FTIR spectra of Virgin GAC, BAC media after seeding for 7 days at F/M 0.4 and F/M 0.8.

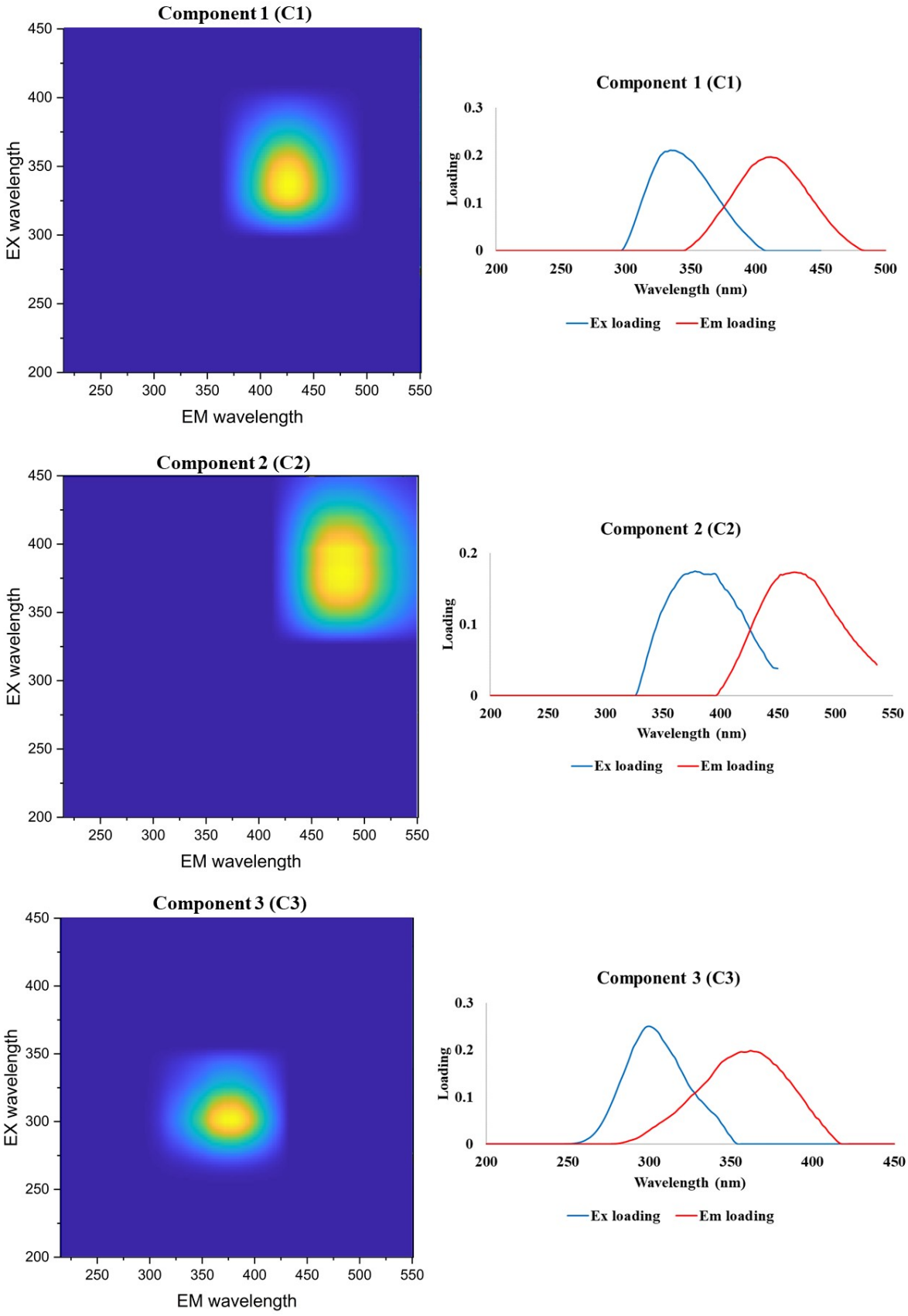
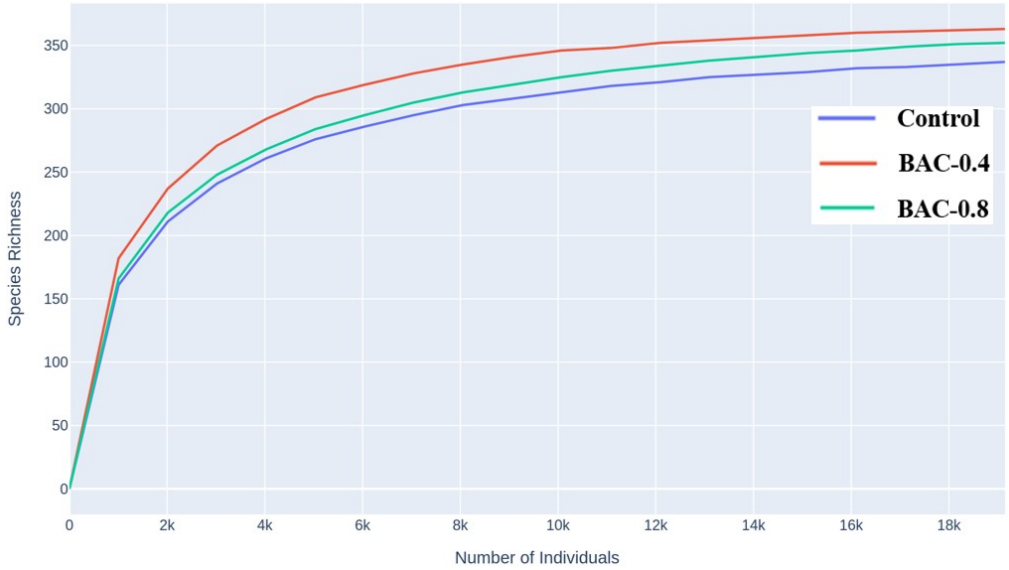


Figure S10: Fluorescent components identified by PARAFAC based on EEM spectra of water samples of influent and effluents of control, BAC-0.4, and BAC-0.8 after 19,206 BVs

(a)



(b)

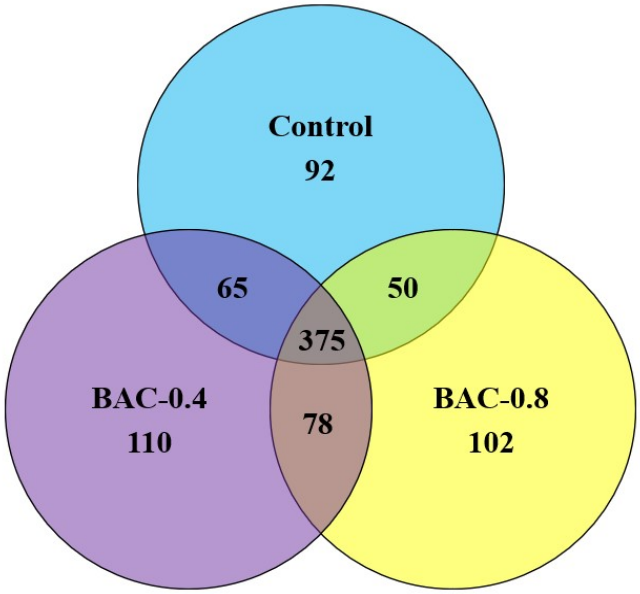


Figure S11: Microbial community dilution curves (a), OUT Venn diagram (b), of control, BAC-0.4, and BAC-0.8 (> 19,206 BV).

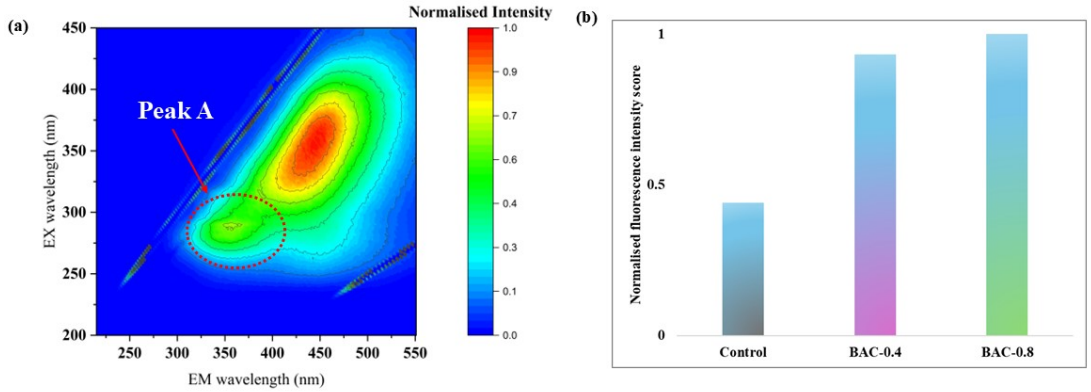


Figure S12: Fluorescence spectra of EPS matric (a), Fluorescence intensity scores of peak A (b) (> 19,206 BV).

Table S1

Recent progress in biological activated carbon for water and wastewater treatment

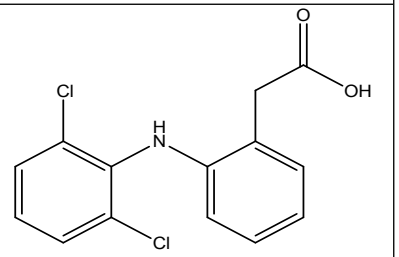
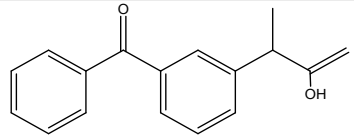
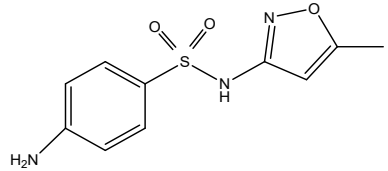
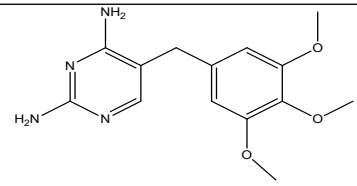
Microbial Inoculation Technique	% Removal of (DOC/COD/BOD)	% Removal of Nitrogen	% Removal of ECs	Reference
GAC was directly packed into the column, and over time, GAC was allowed to transform into BAC.	DOC= 25 – 42% EBCT 6 – 35 min.	NA	NA	(2)
No bacterial seeding was conducted. BAC reached steady state at 13,840 BV (320 days)	DOC = 38% EBCT =18 min.	NH_4-N = 57 - 65% NO_3-N = 25 %	NA	(3)
GAC media was soaked in activated sludge for 24 hr to start initial bacterial colonization. BAC reached steady state after operating 320 days (11,000 BV)	DOC = 40% EBCT = 25 min	NH_4-N = 60 % NO_3-N = 70 %	Antibiotics = 50-80% Other classes of drugs, such as analgesics and anti-inflammatories, account for around 80 %.	(4)
GAC was directly used. BAC reached steady state after operating for 200 days	DOC = 50% EBCT = 18 min.	NA	NA	(5)
GAC media was soaked in activated sludge for 24 hr to start initial bacterial colonization. Feed water was spiked with sludge and methanol to boost bacterial growth in GAC filter.	DOC ~ 50 % EBCT =15 min.	NA	ECs, including NPX, KTP, EFX, SMX, TMP, TCX, CPX, and IBN, were completely removed at 30 min EBCT.	(6)
The tap water was spiked with pyrazole and ammonia to promote pyrazole-degrading biomass growth.	Pyrazole > 90 % removal EBCT =30 min.	NA	NA	(5)
Washed GAC was soaked in 22.8 mg/L activated sludge for 3 h to form biofilm.	COD = 98 % HRT = 6.7 hr	NH_4-N = 99%	NA	(7)
Pre-loading process: glucose solution circulated through the filter as a carbon source for 24 hr. Artificial immobilization: activated carbon particles were soaked in bacterial solution for 2 hr cycles over 24 hr.	NA	NH_4-N = 61 % EBCT = 30 min.	NA	(8)
GAC was directly used BAC reached steady state after operating 200 days.	DOC = 42% in up flow BAC DOC = 33% in down flow BAC EBCT = 15 min	NH_4-N = 55 %	NA	(9)

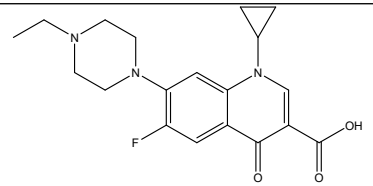
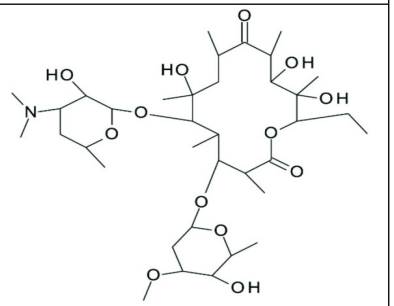
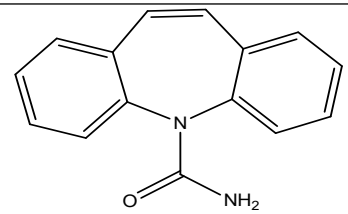
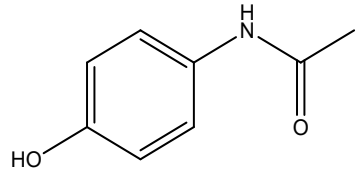
*NA – not analyzed

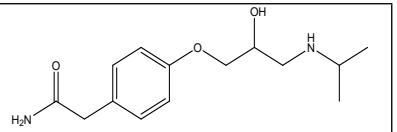
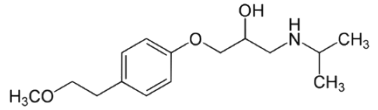
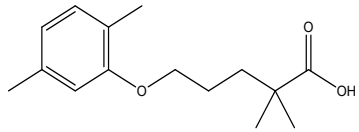
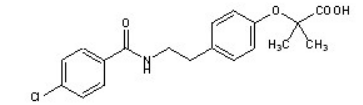
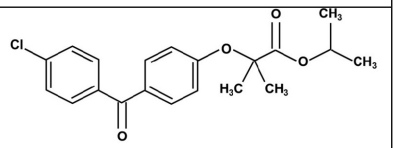
Table S2: Physico-chemical parameters of the raw wastewater and secondary effluent (feed water for column studies)

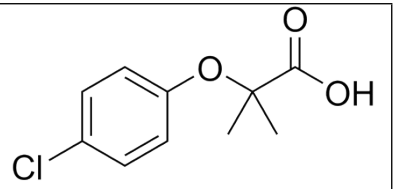
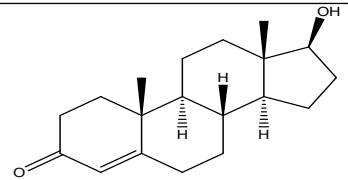
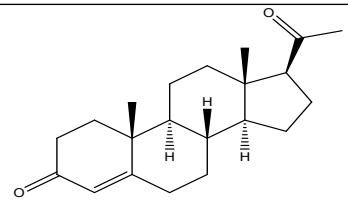
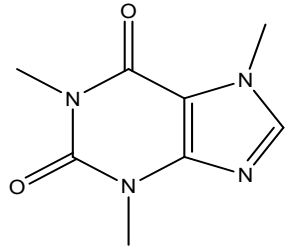
Parameters	Unit	Raw wastewater	Secondary effluent	BAC-0.8 Effluent	Backwash water
pH	-	7.3	7.5	6.7	-
TSS	mg/L	175	10	2	15
COD	mg/L	245	32.4	12	-
BOD	mg/L	140	8.9	2	-
NH_4-N	mg/L	25	1.6	0.55	-
NO_3-N	mg/L	0.8	5.2	2.6	-

Table S3: List of contaminants and chemical properties

Class	Name	Supplier	Mol. Wt.	PKa	Log Kow	Solubility (mg/L) at 25°C	Molecular structure
Anti-inflammatory	Diclofenac (DCF)	TCI Chemicals	296.14	4.15	4.51	2.37	
	Ketoprofen (KTP)	TCI Chemicals	254.28	4.45	3.12	51 (22°C)	
Antibiotic	Sulfamethoxazole (SMZ)	TCI Chemicals	253.28	1.6, 5.7	0.89	610 (37 °C)	
	Trimethoprim (TMP)	TCI Chemicals	290.32	7.12	0.91	400	

	Enrofloxacin (ENR)	TCI Chemicals	359.4	3.85, 6.19	0.83	146	
	Erythromycin (ERY)	TCI Chemicals	733.93	8.9	3.06	2000	
Psychiatric drug	Carbamazepine (CBZ)	TCI Chemicals	236.27	15.96, -3.8; 13.9	2.45	18	
Analgesic	Acetaminophen (ACP)	TCI Chemicals	151.16	9.38	0.46	14000	

β-blocker	Atenolol (ATL)	TCI Chemicals	266.34	9.6	0.16	13300	
	Metoprolol (MTP)	TCI Chemicals	267.36	9.7	1.88	> 10,00,000	
Lipid regulator	Gemfibrozil (GBF)	TCI Chemicals	250.33	4.5	4.77	11	
	Bezafibrate (BZF)	TCI Chemicals	361.82	3.6	4.3	1.55	
	Fenofibrate (FFB)	TCI Chemicals	360.83	-4.9	5.3	Insoluble in water	

	Clofibric acid (CFA)	TCI Chemicals	214.64	3.2	2.88	<500	
Natural hormone	Testosterone (TST)	TCI Chemicals	288.42	3.87	3.32	8.8	
	Progesterone (PGR)	TCI Chemicals	314.46	3.32	3.87	23	
Stimulant	Caffeine (CAFF)	TCI Chemicals	194.19	-0.92	-0.07	21.6	

Text S4: SPE protocol and method for HPLC-MS

To evaluate the % removal of ECs by BAC, the samples were subjected to clean-up and extraction process using solid phase extraction (SPE) followed by an analysis using HPLC-MS. The SPE protocol involved conditioning the cartridges using 6 mL methanol followed by equilibration with 6 mL of pH 2 water for acidic conditions using HCl or 10 using NH_4OH for basic conditions before the sample (250 mL influent and 500 mL effluent) was loaded onto the cartridges at a volumetric flow of 3-4 mL/min with a vacuum pump, the samples were spiked with Na_4EDTA at 500 mg/L to suppress interferences of metal complexation with pharmaceuticals (US EPA 2007). The cartridges were then washed with 4 mL water (pH 2 or 10) and sequentially eluted with 4 mL (2 mL x 2) of methanol and 2 mL (1 mL x 2) of tetrahydrofuran. The eluent was concentrated to almost dryness using a gentle nitrogen stream at 35°C with a nitrogen evaporator, and the sample was reconstituted to 1 mL with an 80:20 (v/v) mixture of water and methanol. The extraction analysis was completed within 72 hr of sample collection.

Chromatographic separation was performed using a Sunfire C18 column (4.6 mm X 250 mm) at a temperature of 40°C. The sample temperature and injection volume were kept at 15°C and 10 µL, respectively. The 40 min gradient was started with 80% of mobile phase A (water) and 20% of mobile phase B (methanol). At 7.5 minutes, the mobile phase B was linearly ramped to 85%, and at 9 minutes, it further increased to 98% gradually and held up to 26 min. At 33 min, the mobile phase B linearly declined to 20% and held up to 40 min for equilibrating the column. The other MS parameters, such as flow rate: 0.3 ml/min, capillary voltage: 3 KV, desolvation temperature: 400°C, cone gas flow: 50 L/h, and desolvation gas flow: 800 L/h, were kept fixed.

Eight-point calibration curves covering the 5 to 500 µg/L concentration range were obtained. The linearity (R^2) value is provided in **Table S4**. The limit of detection (LOD) and limit of quantification (LOQ) were calculated as 3 and 10 times the signal-to-noise ratio, and this value is found to be in the range from 1.5 to 14.5 ng/L and 5 to 30 ng/L, respectively. A list of LOD, LOQ values, SPE recovery, and R^2 values for different ECs was provided in **Table S4**.

SPE recovery:

$$R\ (\%) = \frac{A\ pre - SPE\ spiked\ -\ Anon - spiked}{Anon\ SPE\ std}$$

Where $A_{(pre\text{-}SPE\ spiked)}$ is the peak area of the analyte in the matrix spiked before SPE, $A_{(non\text{-}spiked)}$ is the peak area of the analyte in the non-spiked matrix, and $A_{(non\text{-}SPE\ std)}$ is the peak area of the analyte in the standard solution without SPE. (6).

Table S4: % Recovery of ECs in wastewater samples, ionization mode, cone voltage, and limit of quantification of the compounds.

Name	Molecular wt.	Product ion (m/z)	Cone voltage (V)	SPE recovery (%)	LOQ (ng/L)	R^2
ACP	151.16	152.07	42	73.74 ± 4.44	10	0.993
ATL	266.34	267.17	45	77.60 ± 3.25	10	0.999
BZF	361.82	362.11	39	72.78 ± 9.91	20	0.999
CAFF	194.19	195.08	50	101.63 ± 4.19	5	0.995
CBZ	236.27	237.10	43	98.43 ± 2.44	25	0.985
CFA	214.64	213.03	29	78.62 ± 4.03	20	0.998
DCF	296.15	294.01	33	80.37 ± 0.78	10	0.999
ENR	359.40	360.17	34	105.53 ± 2.21	10	0.980
ERY	733.93	716.40	29	86.18 ± 1.24	25	0.999
KTP	254.28	255.10	33	82.59 ± 0.86	10	0.998
MTP	267.36	268.19	45	95.05 ± 2.66	20	0.999
PGR	314.46	315.23	36	83.52 ± 9.47	5	0.999
SMZ	253.28	254.05	38	88.09 ± 5.87	5	0.983
TST	288.42	289.21	46	95.65 ± 8.95	5	0.995

TMP	290.32	291.14	47	77.16 ± 7.15	5	0.993
FFB	360.83	361.12	42	74.62 ± 2.91	15	0.997
GBF	250.33	251.16	26	101.27 ± 3.92	15	0.997

Text S5: DO mass balance analysis

The total theoretical oxygen requirement for complete nitrification is 4.57 mg of O_2/mg of NH4-N. This value does not include the portion of ammonia used for cell synthesis. Werzernak and Gannon (1967) found that the total oxygen consumption for nitrification, after considering the portion of ammonia used for cell synthesis, is 4.33 mg of O_2 per mg of NH_4-N.

Text S6: Detailed information of gravel and sand layer which were used in the BAC filter.

The gravel and sand layer with a total depth of 10 cm was filled at the bottom of all BAC filters to support the 35 cm depth GAC/BAC media. The detailed information about the gravel and sand bed is as follows

Supporting bottom sand and gravel bed

Layer	**Grain size (mm)**	**Thickness (cm)**
Bottom layer	8 - 10	5
Intermediate layer	4 - 5	2.5
Top layer	0.25 - 4	2.5
D60 (mm)	0.88	
D30 (mm)	0.65	
D10 (mm)	0.50	
Cu (D60/D30)	1.35	

Text S7:

In the first 7 months of column studies from March to September the average temperature during the treated was recoded as 26.28 °C. from October onward winter season had started, during this period (October to February) the average temperature was recorded as 18 °C.

Text S8: DOC characterisation by Fluorescence – PARAFAC analysis

Fluorescence excitation-emission (EEM) spectra were measured using a fluorescence spectrophotometer (Fluromax Horiba, Japan). The range of excitation (Ex) and emission wavelengths (Em) was 200-450 nm and 215-550 nm, respectively. Measurements were taken with a scanning interval of 2 nm and a slit width of 2 nm at a scanning speed of 1200 nm/min. All water samples were adjusted to a pH of 7.0 ± 0.2. To reduce the impact of elastic (Rayleigh) and non-elastic (Raman) scattering, EEM results of all samples were subtracted from the 3D fluorescence spectra of ultra-pure water. All findings were averaged from three repetitions. The PARAFAC analysis was executed using the PLS toolbox. The initial steps in PARAFAC analysis involved loading the data, scattering removal, performing explorative data analysis, and computing PARAFAC models with 3 components. The residual analysis, visual evaluation, and split-half analysis were conducted to conclude the correct number of components.

Text S9: Bacterial Community Analysis

Biofilm samples were obtained from the top, middle, and bottom sampling ports of the BAC column after reaching steady-state conditions at 200 days (19,206 BV). The microbial community analysis was then conducted on the combined samples of the three sources with equal mixing ratios. The methodology followed for this study includes: (1) deoxyribonucleic acid (DNA) extraction and assessment of its quality, (2) Purification of PCR products and amplification via PCR using V3-V4 primers (16sF: -5' AGAGTTTGATGMTGGCTCAG3') and (16sR: -5' TTACCGCGGCMGCSGGCAC3'), and (3) 16s sequencing accomplished with Illumina MiSeq Platform. The original data were subsequently pre-processed and quality checked. The taxonomic classification of operational taxonomic unit (OTU) was determined using quality-controlled sequences, and bacterial diversity and taxonomic composition were analysed based on the results of OTU clustering.

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