Evaluating the Potential of Multi-Anodes in Constructed Wetlands Coupled with Microbial Fuel Cells for Treating Wastewater and Bioelectricity Generation under High Organic Loads

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1. Materials and Methods

1.1. Wastewater Composition

Synthetic wastewater, which represents untreated household wastewater, was prepared using glucose as a carbon source. The composition of synthetic wastewater was adopted from literature includes glucose (1 g/l or 2g/l), $CaCl_2$ (0.0301 g/l), $MgCl_2.6H_2O$ (0.0371 g/l), KH_2PO_4 (0.0445 g/l), $(NH_4)_2SO_4$ (0.01119 g/l), $(NH_4)_2Fe(SO_4)_2.6H_2O$(0.0842 g/l) and $NaHCO_3$(.0111 g/l). The trace element solution contains H_3BO_3 (0.15 g/l), $CaCl_2.6H_2O$ (0.15g/l), $CuSO_4.5H_2O$ (0.03 g/l), $FeCl_3.6H_2O$ (1.5 g/l), $ZnSO_4.7H_2O$ (0.12 g/l) and KI (0.03gm/l).

1.2. Configuration, Inoculation & Operation

Constructed wetland (CW) as control was also fabricated along with CW-MFC(GG) and CW-MFC(GAC) using polyvinyl chloride pipes of 57 cm height and 11 cm diameter as shown in Figure S1. In CW microcosm, 5-7 mm size regular stone gravel was filled from bottom to top. No electrode was used in Control-CW microcosm. An inlet pipe is given in the center to provide influent wastewater. Three sampling points were given at the height of 7cm (final effluent), 34cm (middle) and 51 cm (bottom) from top. The microcosm was inoculated with a pre-acclimated microbial community of another CW, which was already running for over a year in the lab. After inoculation, each system was allowed to acclimate for one and a half months before the start of the experiment. During this acclimatization phase, synthetic wastewater was regularly added to both CW and CW-MFC microcosms in batch mode. After acclimatization, all three microcosms were switched to continuous flow mode using a peristaltic pump at a flow rate of 1.16 ml/min. The wastewater was passing in an up-flow manner, from bottom to upwards, eventually discharging from the top. The HRT for all microcosms was maintained for 24 hours. The systems were run under two organic loading rates. Initially, it was run, and all the experiments were conducted at an organic loading rate of 890.11 g COD/m^3-d. After that, the organic loading rate changed to 1781.32 g COD/m^3-d. All the experiments were conducted at room temperature.

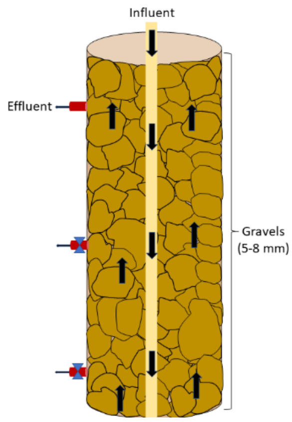

Figure S1. Schematic representation of control CW microcosm

2. Results and Discussion

2.1. Wastewater Treatment Performance

The COD removal efficiency of CW-microcosm for both organic loading rates is shown in Figure S2. For COD analysis, the samples were taken out from upper, middle and lower portions of CW-microcosm. The overall average COD removal efficiency was observed 55.13% ± 3.1% and 44.22 ± 2.3% at an organic loading rate of 890.11 g COD/m^3-d and 1781.32 g COD/m^3-d, respectively. The lower and middle portion of CW-microcosm showed 20.53 ± 3.1% and 40.66±2.68% COD treatment efficiency for an organic loading rate of 890.11 g COD/m^3-d whereas the COD removal efficiency for an organic loading rate of 1781.32 g COD/m^3-d was observed as 17.38 ± 1.8% and 29.30 ± 1.9% for lower and middle portion, respectively. Figure S3 and Table 1 shows comparison in treatment efficiency of CW microcosm, CW-MFC(GG) and CW-MFC(GAC). It is clear from the Figure S3 and Table 1 that for organic loading rate of 890.11 g COD/m^3-d CW-microcosm showed 15.31% lesser COD removal than CW-MFC(GG) and 40.1% less removal than CW-MFC(GAC). As the organic loading rate was changed to 1781.32 g COD/m^3-d, the COD removal efficiency in CW was observed as 21.21% and 43.88% less in CW-MFC(GG) and CW-MFC(GAC), respectively.

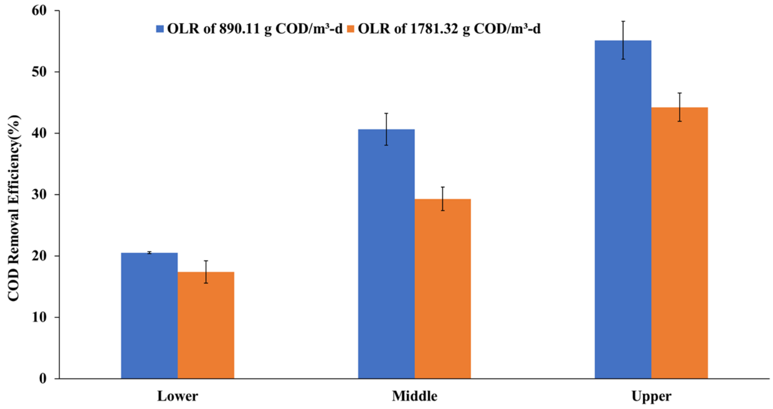

Figure S2. COD Removal Efficiency (%) in CW microcosm under OLR of 890.11 g COD/m^3-d and 1781.32 g COD/m^3-d.

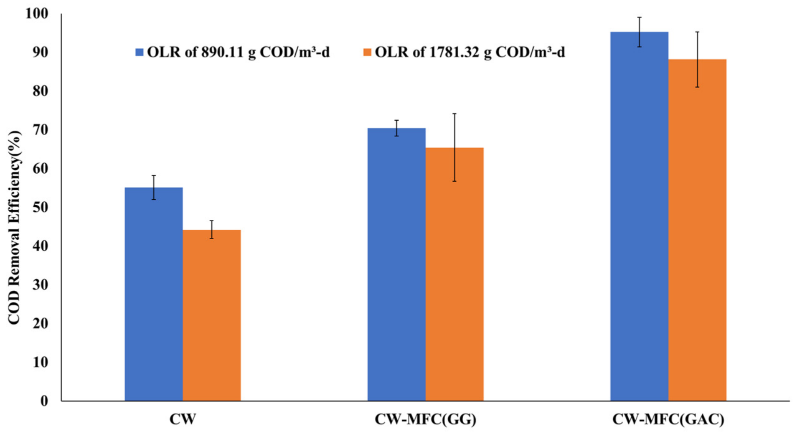

Figure S3. COD Removal Efficiency (%) of CW microcosm, CW-MFC(GG) and CW-MFC(GAC) under OLR of 890.11 g COD/m^3-d and 1781.32 g COD/m^3-d.

2.2.Nitrogen Removal

The NH_4^+-N removal of each microcosm is represented in Figure S4 and Table 1 for both of the organic loading rates. The ammonium removal efficiency of control CW microcosm was observed as 41.20% ± 3.6% and 29.92% ± 0.7% for the organic loading rate of 890.11 g COD/m^3-d and 1781.32 g COD/m^3-d, respectively. The NH_4^+-N removal was decreased by 11.28% as the organic loading rate was increased from 890.11 g COD/m^3-d to 1781.32 g COD/m^3-d in CW control microcosm.

All the three microcosms were also tested for NO_3^--N removal for both organic loading rates. The treatment performance for all three microcosm is represented in Figure S5 and Table 1. The CW-MFC(GG) showed highest NO_3^--N removal among all three microcosms. A total of 80.39 ± 1.7% removal efficiency was achieved at organic loading rate of 890.11 g COD/m^3-d. It was slightly increased to 83.17±1.4% as the organic loading rate changed from 890.11 g COD/m^3-d to 1781.32 g COD/m^3-d. In CW microcosm, 55.7 ± 2.6% NO_3^--N removal was observed at organic loading rate of 890.11 g COD/m^3-d whereas it was increased to 62.8 ± 2.5% when the organic loading rate was changed to 1781.32 g COD/m^3-d. With comparative analysis, it was observed that CW control microcosm has shown 24.69% and 20.37% less NO_3^--N removal than CW-MFC(GG) at an organic loading rate of 890.11 g COD/m^3-d and 1781.32 g COD/m^3-d, respectively. The lowest NO_3^--N removal was observed in case of CW-MFC(GAC).

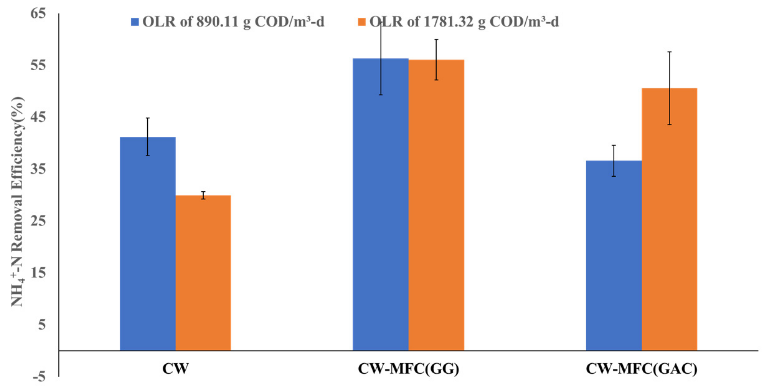

Figure S4. NH_4^+-N removal efficiency at an organic loading rate of 890.11 g COD/m^3-d and 1781.32 g COD/m^3-d.

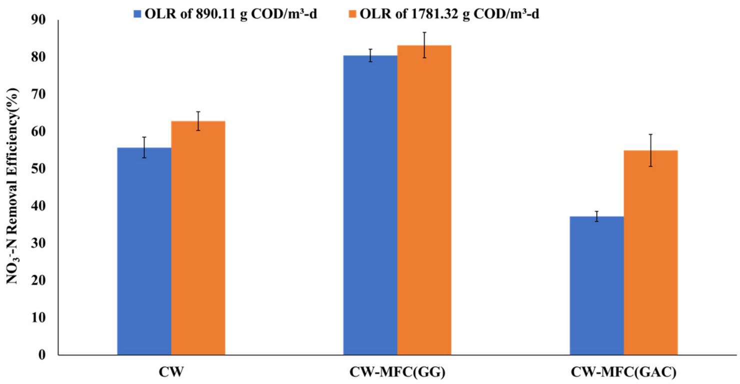

Figure S5. NO_3^--N removal efficiency at an organic loading rate of 890.11 g COD/m^3-d and 1781.32 g COD/m^3-d.

Table S1. Comparison of treatment performance of CW, CW-MFC(GG) and CW-MFC(GAC).

Parameters	Organic loading rate of 890.11 g COD/m^3-d			Organic loading rate of 1781.32 g COD/m^3-d		
	CW	CW-MFC(GG)	CW-MFC(GAC)	CW	CW-MFC(GG)	CW-MFC(GAC)
COD Removal Efficiency (%)	Lower- 20.53± 0.18% Middle- 40.66±2.6% Upper- 55.13± 3.1%	A1- 24.48 ± 2.3% A2- 28.10 ± 2.08% A3- 38.14 ± 4.45% A4- 47.69 ± 1.2% Cathode- 70.44±2%	A1- 53.68 ± 1.5% A2- 77.46 ± 1.8% A3- 81.23± 2.5% A4- 86.02 ± 5% Cathode- 95.24±3%	Lower- 17.38± 1.8% Middle- 29.30 ±1.9% Upper- 44.22 ± 2.3%	A1- 22.14 ± 1.05% A2- 26.61 ± 1.8% A3- 33.17 ± 2.2% A4- 46.95 ± 1.7% Cathode- 65.43±2.4%	A1- 46.81 ± 1.5% A2- 65.28 ± 5.8% A3- 69.36 ± 3.6% A4- 75.68 ± 4.9% Cathode- 88.1±1.7%
NH_4^+-N Removal (%)	41.20 ± 3.6%	56.29 ± 7%	36.59 ± 3%	29.92 ± 0.7%	56.09 ± 3.9%	50.59 ± 7%
NO3--N Removal (%)	55.7± 2.8%	80.39± 1.7%	37.21± 1.4%	62.8±2.5%	83.17± 3.4%	54.87± 4.3%