Supplementary Information (SI) for

The Effect of Carrier Filling Ratios on Dissolved Organic Nitrogen Removal in Integrated Fixed-film Activated Sludge Systems Treating Municipal Wastewater

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Notes

The authors declare no competing financial interests.

Method S1. Algal bioassay measurements

Briefly, 1.5 ml of *Selenastrum capricornutum* algal seeds collected from Freshwater Algae Culture Collection of the Institute of Hydrobiology, Chinese Academy of Sciences (FACHB-Collection, Wuhan, China) and 1 ml of mixed culture bacteria collected from a municipal wastewater treatment plant were mixed with 100 ml of DON samples discharged from IFAS in a 250 ml conical flask to start bioassay according to Liu et al.[1] Then, each bioassay was cultivated in a temperature-controlled shaker (approximately 24 °C) with a 12-h light/dark cycle. *Selenastrum capricornutum* was chosen as the algal species for it has been used to study eutrophication as a standard test ordanism and is widely used in wastewater-derived DON algal bioassays experiments.[1,2] The ABDON procedures depend on the change of DON in the specimen before (DON_i) and after (DON_f) the algal bioassays incubation period. The concentration of background DON brought by inoculation was assessed with the Milli-Q water control. A seed control with Milli-Q water was prepared for each bioassay and was prepared as the same as the sample (DON_{ci} and DON_{cf}). ABDON was calculated as shown in Equation (1).[2] Each sample was tested in quintuplicate, along with the control consisting of Milli-Q water.

$$ABDON = (DON_i - DON_f) - (DON_{ci} - DON_{cf}) \quad (1)$$

Method S2. Solid-phase extraction

DON extraction and concentration was conducted by a solid-phase extraction (SPE) cartridge

(functionalized styrenedivinyl-benzene polymer resin (PPL), Supelco Analytical, Bellefonte, PA, USA). It should be mentioned that, PPL was used to enrich and desalt DON samples in this study since salt-free samples are imperative for ESI-FTICR-MS analysis. Therefore, only PPL extractable DON molecules are included in the MS spectra. In a previous study, comparison of different SPE methods showed that PPL was the most efficient and reproducible cartridge for extracting nitrogenous organic compound among all the methods tested.[3] Therefore, PPL SPE has been widely chosen to extract DON in both wastewater[4] and stormwater[5] in previous studies on DON molecular characteristics using FTICR-MS.

Method S3. Untargeted metabolites analysis

Mass spectrum scanning range from 50 to 1000 m/z with positive and negative ion scanning mode. The source parameters were as follows: spray voltage: 1000 v; ion source temperature: 120°C; carrier gas flow rate: 900 L/h. To obtain information regarding system repeatability, quality control (QC) samples were injected at regular intervals throughout the analytical run. Using Progenesis QI software to analyse LC-MS raw data (Waters Corporation, Milford, MA, United States). Screened differential metabolites were characterized using a self-built database of the Majorbio I-Sanger Cloud Platform (www.i-sanger.com). Utilizing a combination of (O)PLS-DA and Student's t-tests analyzed the discrepancy of metabolites between groups.

Method S4. Wastewater analysis. The dissolved organic carbon (DOC) was analyzed by a Shimadzu 5000−A total organic carbon analyzer (Shimadzu Corporation, Kyoto, Japan). Mixed liquor suspended solids were determined according to standard methods.[6] The dissolved oxygen (DO) was measured by a dissolved oxygen analyzer (Mettler-Toledo, Columbus, OH, USA). The pH and temperature were monitored by an advanced electrochemical apparatus (Orion VERSA STAR, Thermo, USA). The total phosphorus was tested by the ascorbic acid method.[6]

Method S5. Gray relational analysis (GRA)

The gray relational coefficients $\gamma(z_0(k), z_j(k))$, which were used to demonstrate the relationship between the metabolites and mDON bioavailability, were caculated as follows:

$$\gamma(z_0(k), z_j(k)) = \frac{\min_j \min_k |z_0(k) - z_j(k)| + \xi \max_j \max_k |z_0(k) - z_j(k)|}{|z_0(k) - z_j(k)| + \xi \max_j \max_k |z_0(k) - z_j(k)|}$$

$$= \frac{\Delta_{min} + \xi \Delta_{max}}{\Delta_{0j}(k) + \xi \Delta_{max}} \quad (2)$$

In this equation, Δ_{min} is the element of minimum value in the matrix Δ, Δ_{max} is defined as the element of maximum value in the matrix Δ, and ξ ($0 < \xi \leq 1$) is a discerning coefficient to adjust the range of the comparison environment. A more detailed analytical protocol for GRA can be found in a previous study.[7]

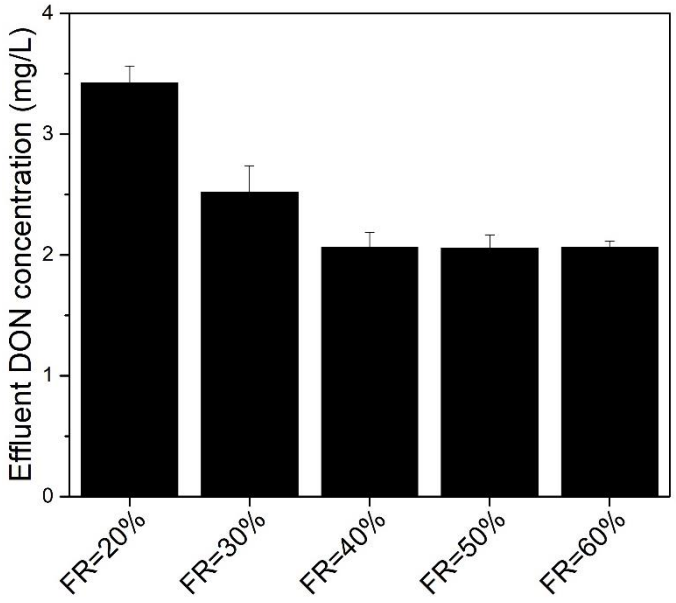
Table S1. Mobile phase elution gradient used in the untargeted metabolites analysis.

Time (min)	Flow rate (mL/min)	A (%)	B (%)
0	0.4	95	5
3	0.4	80	20
9	0.4	5	95
13.0	0.4	5	95
13.1	0.4	95	5
16	0.4	95	5

Table S2. The biomass concentrations of the suspended activated sludge and biofilm in IFAS with different carrier filling ratios (FRs).

	FR=20%	FR=30%	FR=40%	FR=50%	FR=60%
Activated sludge (mg MLVSS/L)	1392±25	1440±85	1400±110	1340±50	1360±120
Biofilm (mg MLVSS/L)	1700±80	1900±110	2510±120	2200±100	2510±50

(a)



(b)

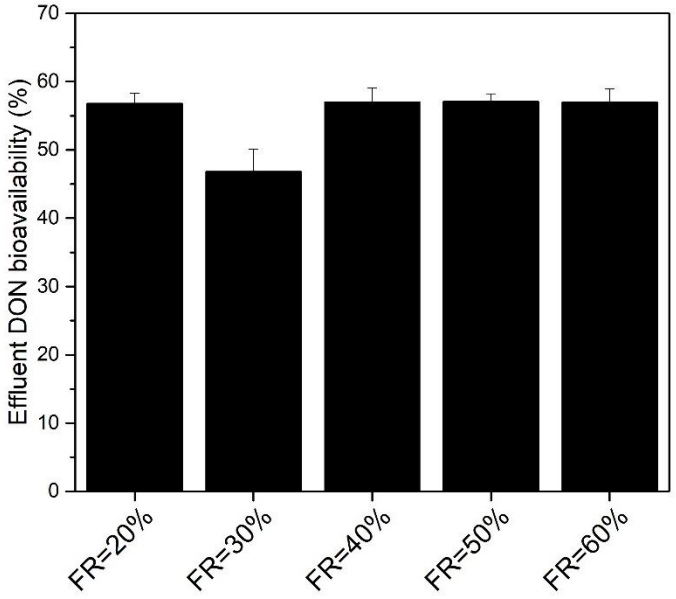


Figure S1. Variation of (a) DON concentration and (b) DON bioavailability in effluent with carrier filling ratios (FRs) from 20% to 60% under steady-state conditions (n = 6).

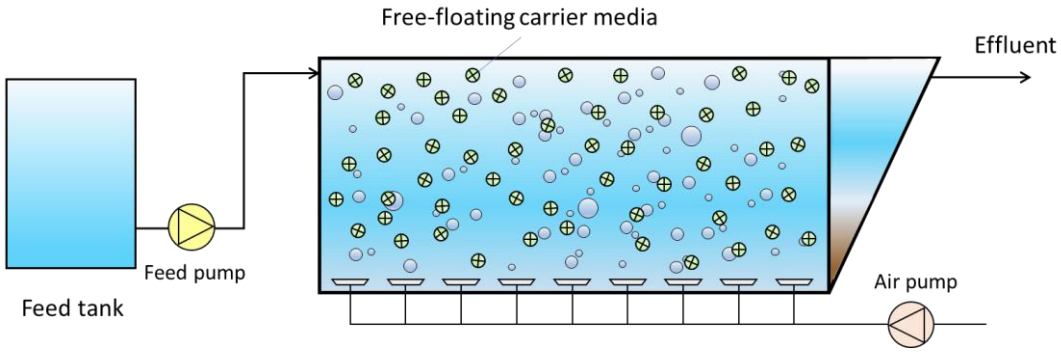


Figure S2. The schematic diagram of IFAS reactor.

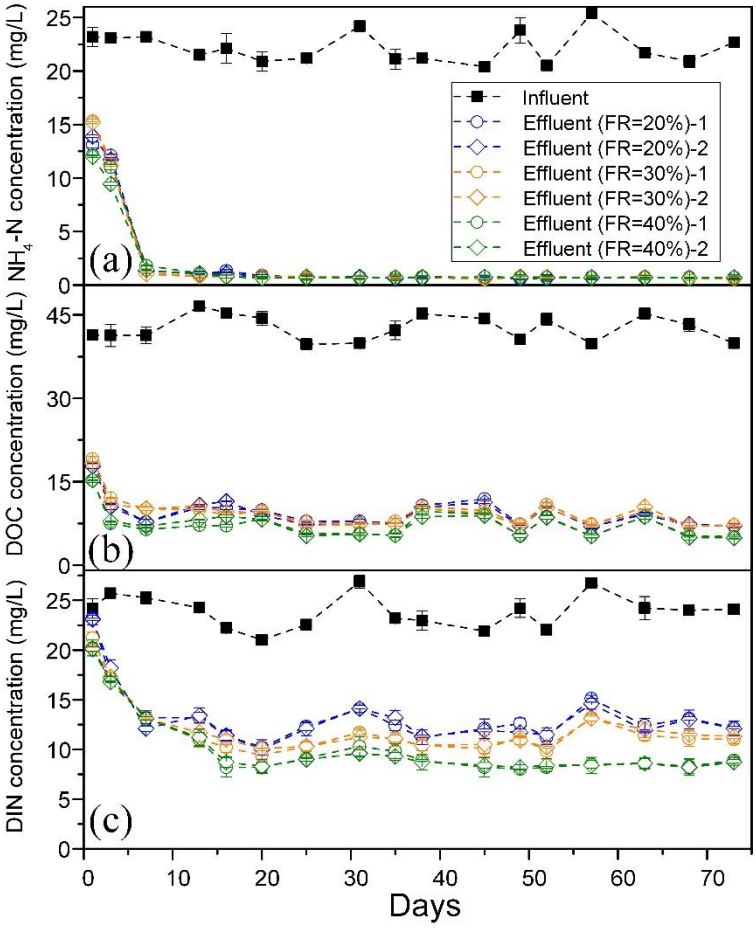


Figure S3. Removal of (a) NH4-N, (b) dissolved organic carbon (DOC) and (c) total dissolved inorganic nitrogen (DIN) in IFAS at different carrier filling ratios (FRs). Duplicate IFAS reactors were used for each FR.

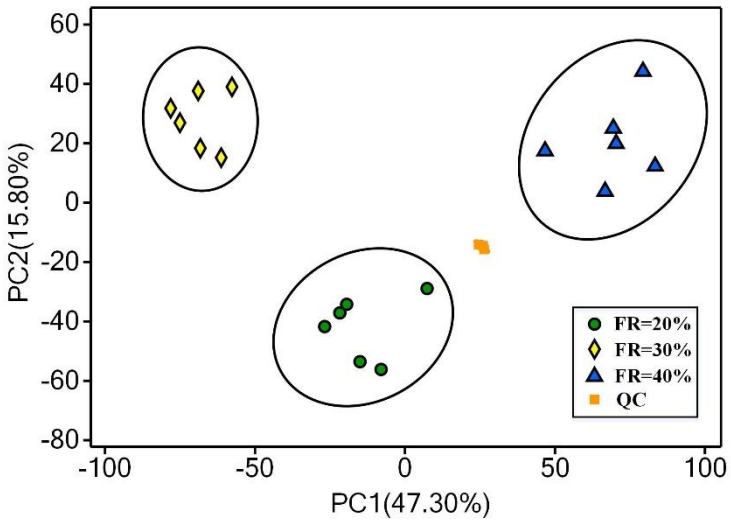


Figure S4. Principal component analysis (PCA) plot of the types of effluent metabolites from IFAS at different carrier filling ratios (FRs) under steady-state conditions. The two principal components together explain 63.10% of the variance in the types of metabolites.

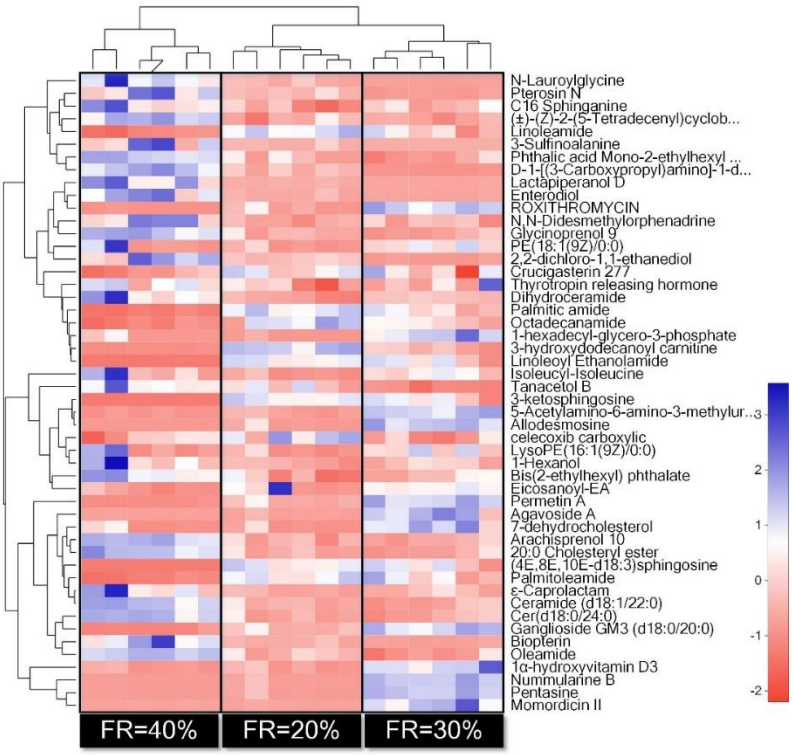


Figure S5. The relative abundances of metabolites in effluent from IFAS at different carrier filling ratios (FRs) under steady-state conditions (n = 6).

References

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