Electronic Supplementary Material (ESI) for Environmental Science: Nano.


**Supporting information**

# Acute toxicity of Graphene Nanoplatelets on Biological Wastewater Treatment Process

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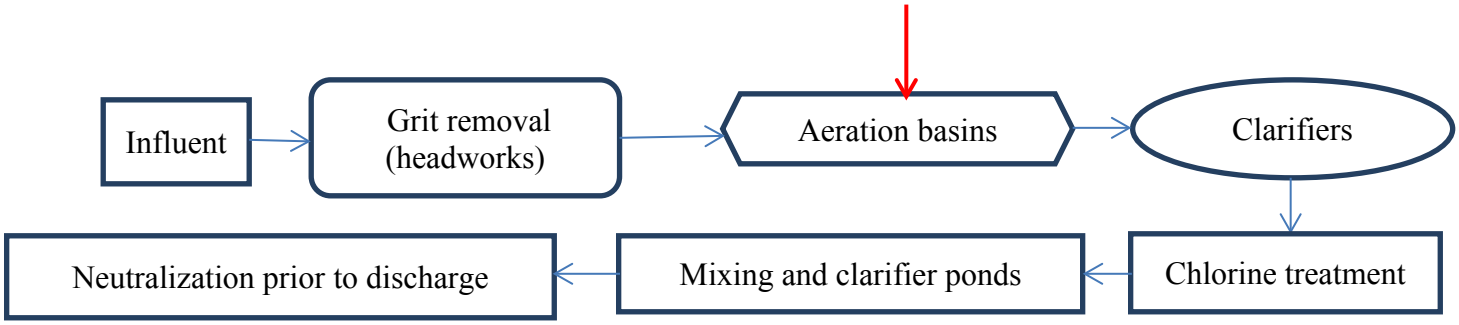


**Figure S1** – Diagram of the flow of the wastewater at the Sims South Bayou Wastewater Treatment Plant. Red arrow indicates where the activated sludge was collected for this study.

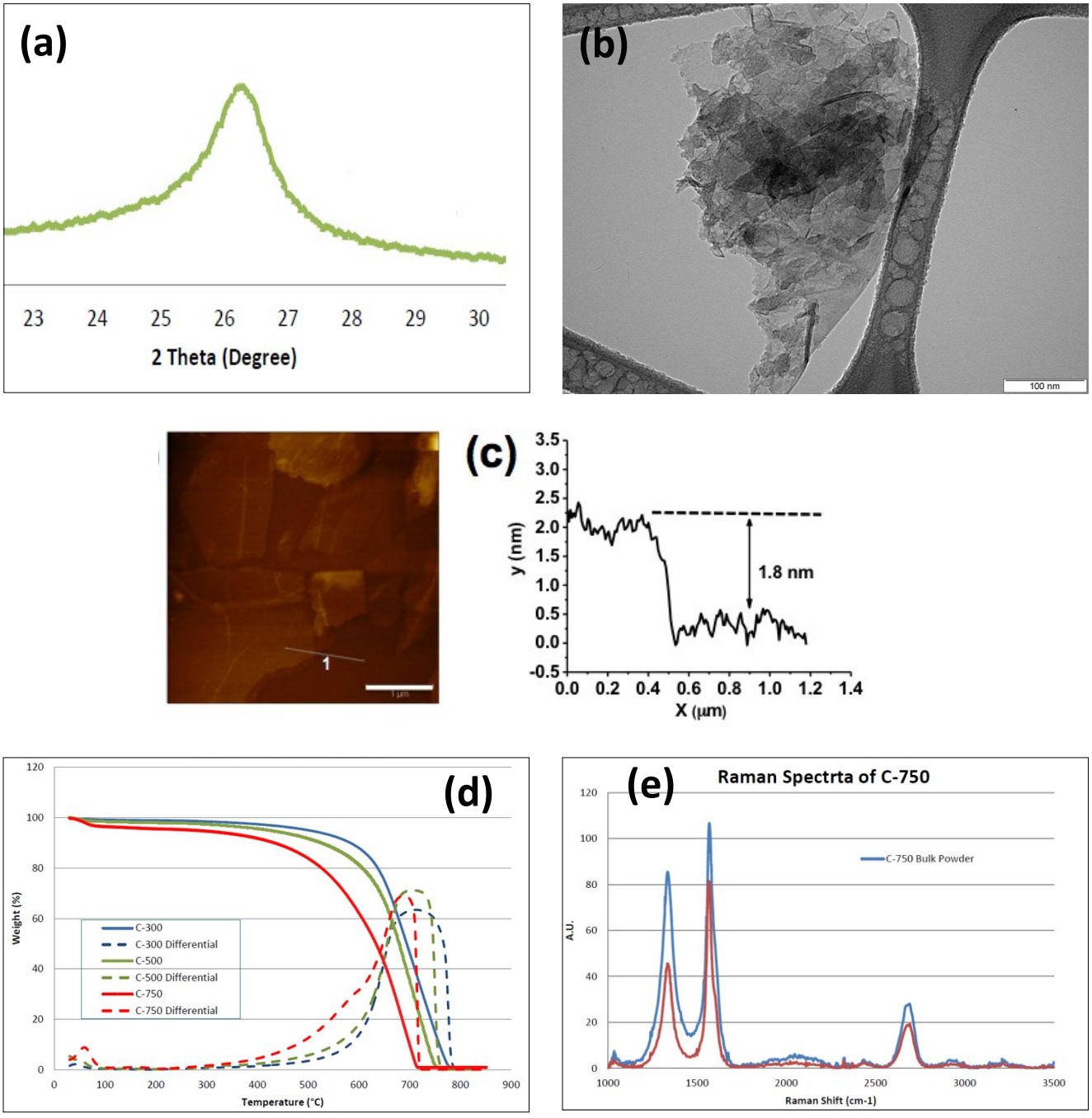


**Figure S2** – a) XRD spectrum showing the crystalline structure of Grade C Graphene nanopletelets (2 theta at 26.5); b) TEM image of graphene nanoplatelet (scale bar 100 nm) (provided by XG Science, Inc. U.S.A); c) AFM image and height profile of graphene nanoplatelet after 1 h sonication; d) TGA analysis of graphene nanoplatelets and e) Raman spectroscopy of the Grade C Graphene nanopletelets.

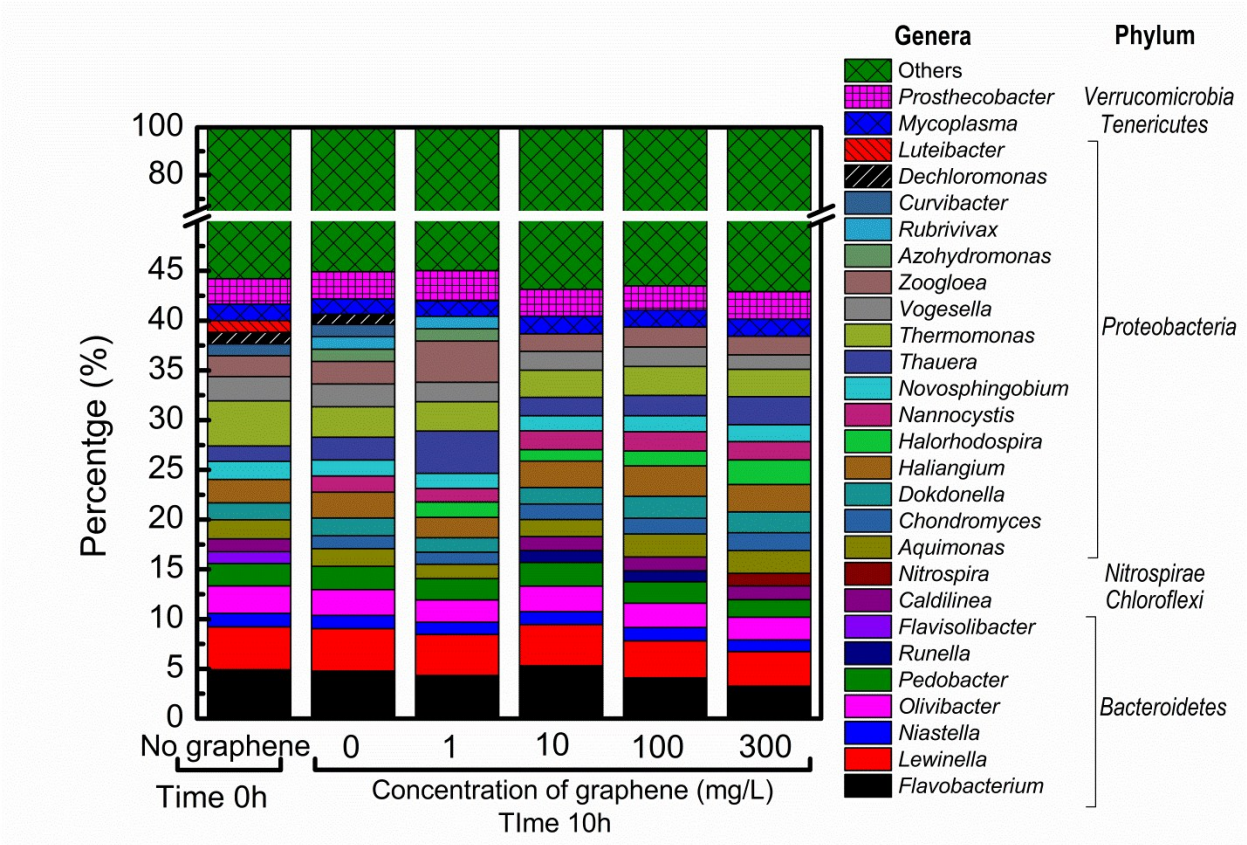


**Figure S3 ─** The top 20 genera with highest abundance in the reactors containing different concentrations of graphene. Results are presented at 0 h and 10 h. The abundance was expressed in term of percentage of total effective reads for each sample (excluding unclassified genera). Less abundant genera were represented as others (≤ 1%).

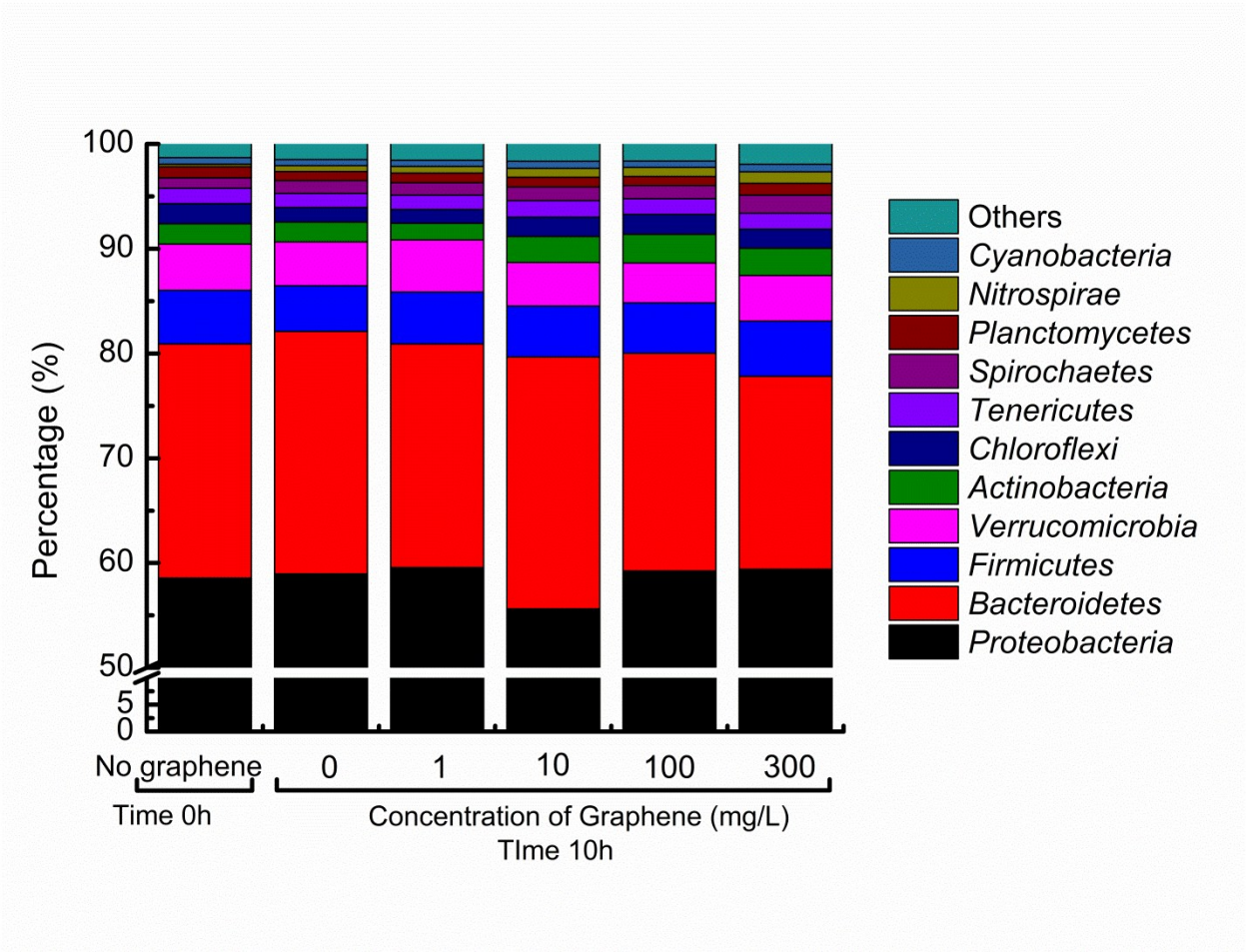


**Figure S4** — The top 10 phylum with the highest abundance in the reactors with different concentrations of graphene. Results are presented at 0 h and 10 h. The abundance was expressed in terms of percentage of total effective sequences in each sample (excluding unclassified phyla). Less abundant phyla were presented as others.

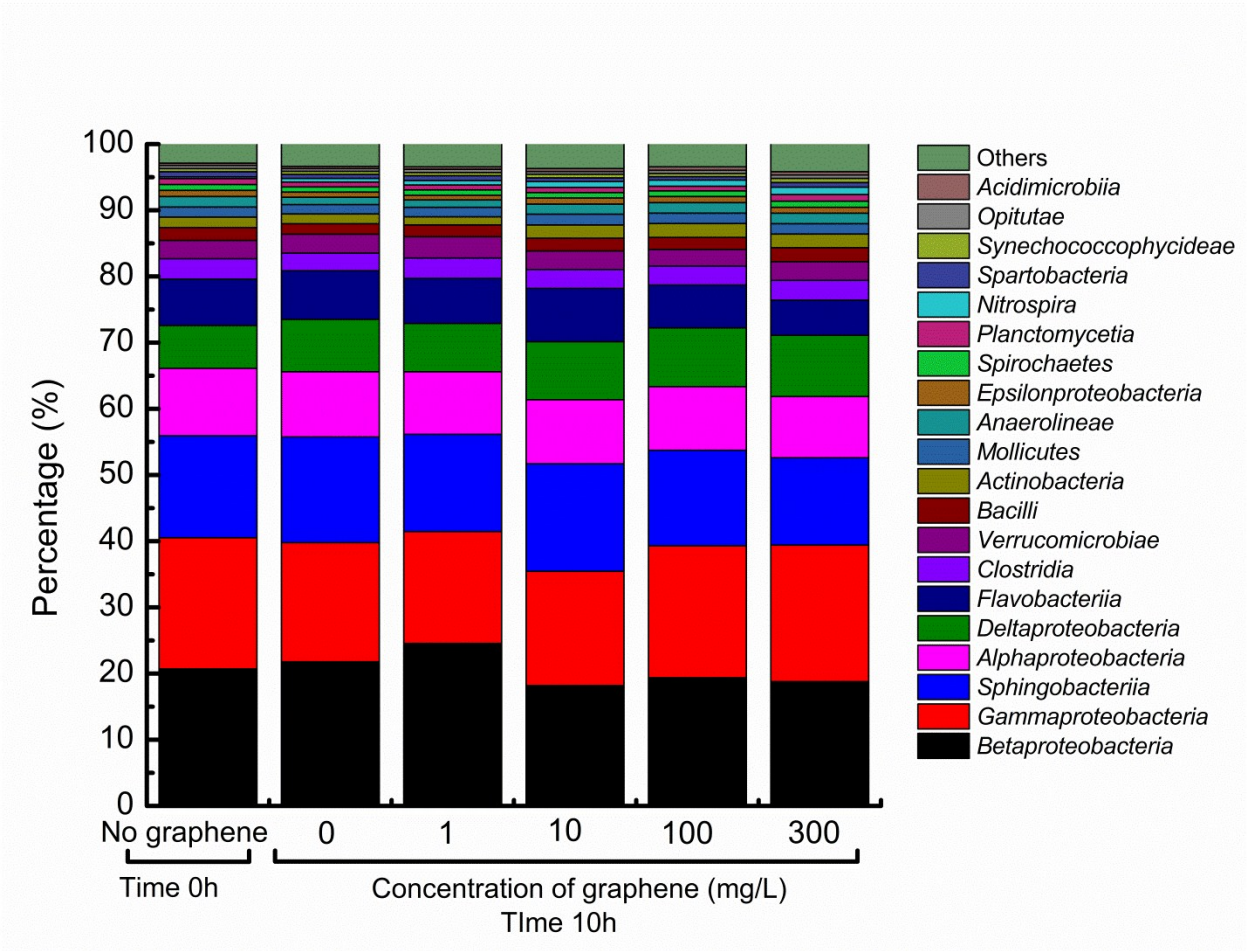


**Figure S5** — The top 20 classes with highest abundance in the reactors with different concentrations of graphene. Results are presented at 0 h and 10 h. The abundance was expressed in term of percentage of total effective sequences of each samples (excluding unclassified classes). Less abundant classes were represented as others.

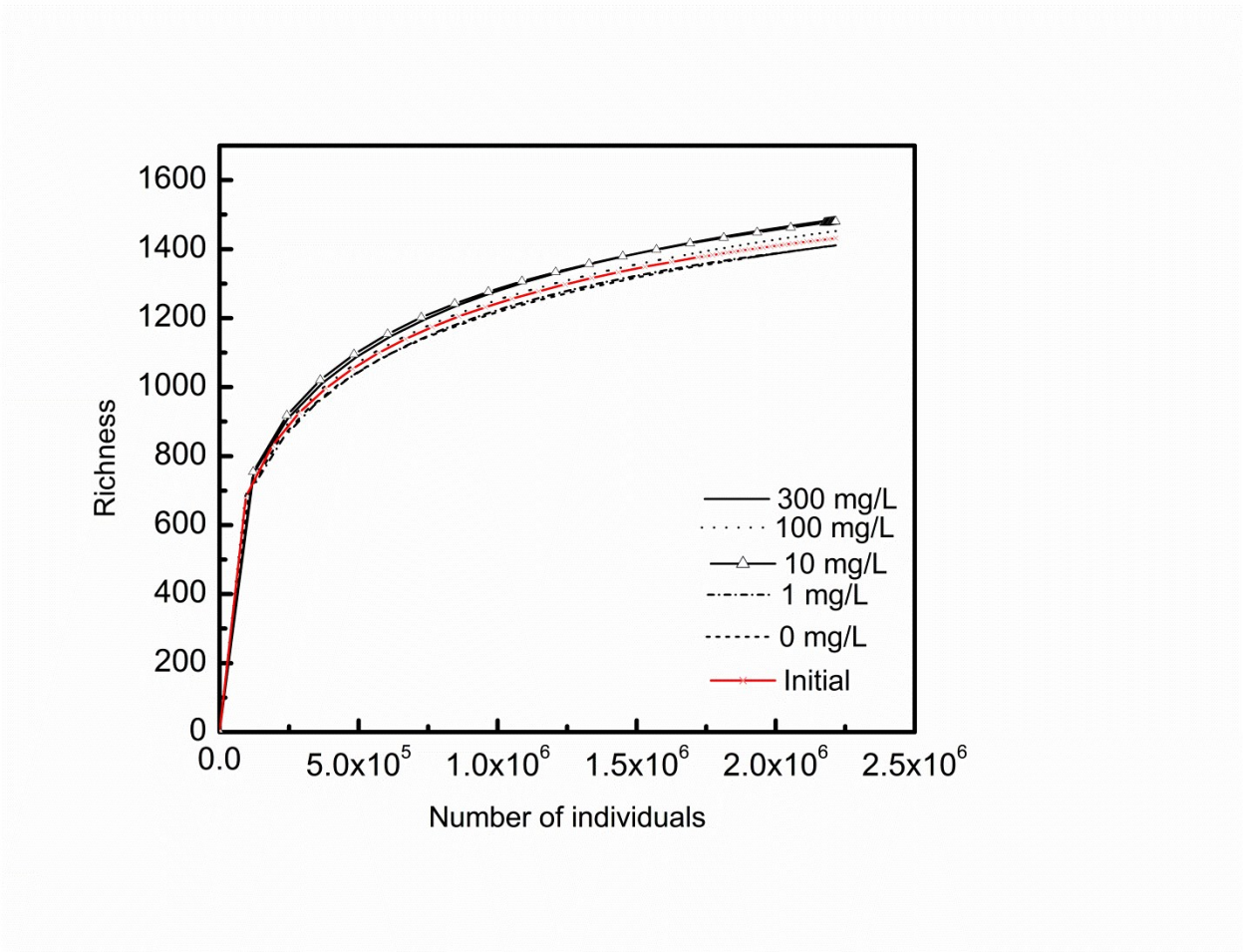


**Figure S6 ─** Sample-size-based rarefaction curves of activated sludge from reactors exposed to different concentrations of Graphene.

**Table S1 ─** Target gene, concentration of primers and thermal cycle

| Target | Name | Primer (5'-3') | Primer final concentration (nM) | RT-PCR | References | Regular PCR |
|---|---|---|---|---|---|---|
| Ammonia-oxidizing bacteria (AOB) 16S rRNA | CTO 189-F A/B | GGAGRAAAGCAGGGGATCG | 300 | 50°C 2mins, 95°C 10mins, 40 cycles of 95°C 1min, 50°C 1min and 60°C 1min | [1, 2, 3, 4] | 10 min of 95 °C; 35 cycles of 94 °C in 30 s, 55 °C in 40 s, and 72 °C in 1 min; 72 °C for 7 min, and 4 °C infinite |
| | CTO 654-R | CTAGCYTTGTAGTTTCAAACGC | 300 | | | |
| Ammonium monooxygenase (amoA) | amoA-1F | GGGGTTTCTACTGGTGGT | 300 | 95°C 15mins, 95°C 4mins, 50 cycles of 94°C 30s, 56°C 30s and 72°C 30s | [2, 5-7, 4, 8] | |
| | amoA-2R | CCCCTCKGSAAAGCCTTCTTC | 300 | | | |
| *Accumulibacter* 16S rRNA genes (PAO) | 518-F | CCAGCAGCCGCGGTAAT | 50 | 95°C 3min, 45 cycles of 95°C 30s, 60°C 40s | [9, 10] | |
| | PAO846-R | GTTAGCTACGGCACTAAAAGG | 50 | | | |
| Universal 16S rRNA gene (Universal) | E8-F | AGAGTTTGATCCTGGCTCAG | 300 | 95°C 3mins 44 cycles of 95°C 15s 55°C 1min | [11-13] | |
| | E533-R | TTACCGCGGCTGCTGGCAC | 300 | | | |

## References


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