Improved biological phosphorus removal persisted at a low SRT regime in a full-scale Sequencing Batch Reactor-based IIT Roorkee STP complying with low influent rbCOD/TP

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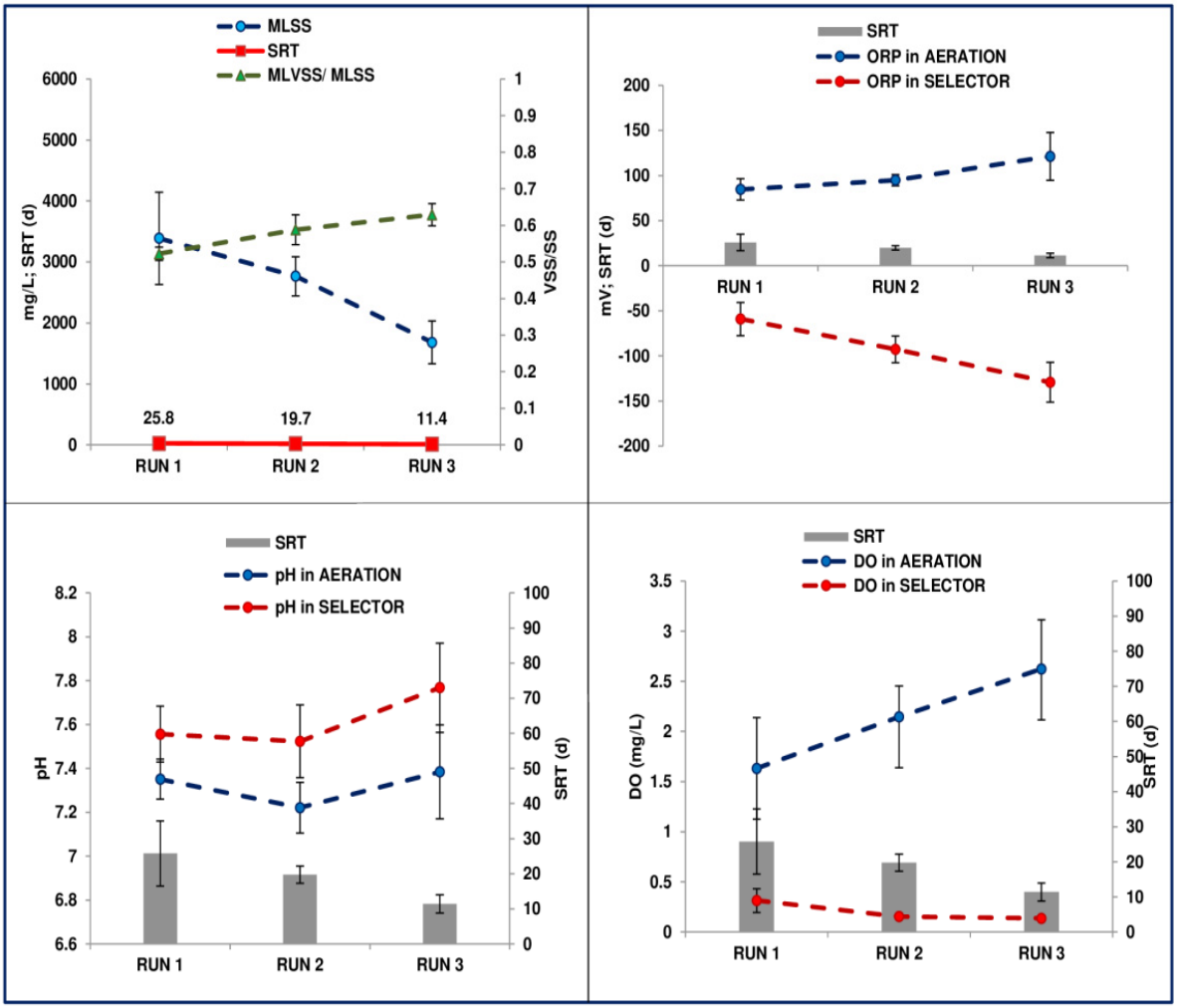
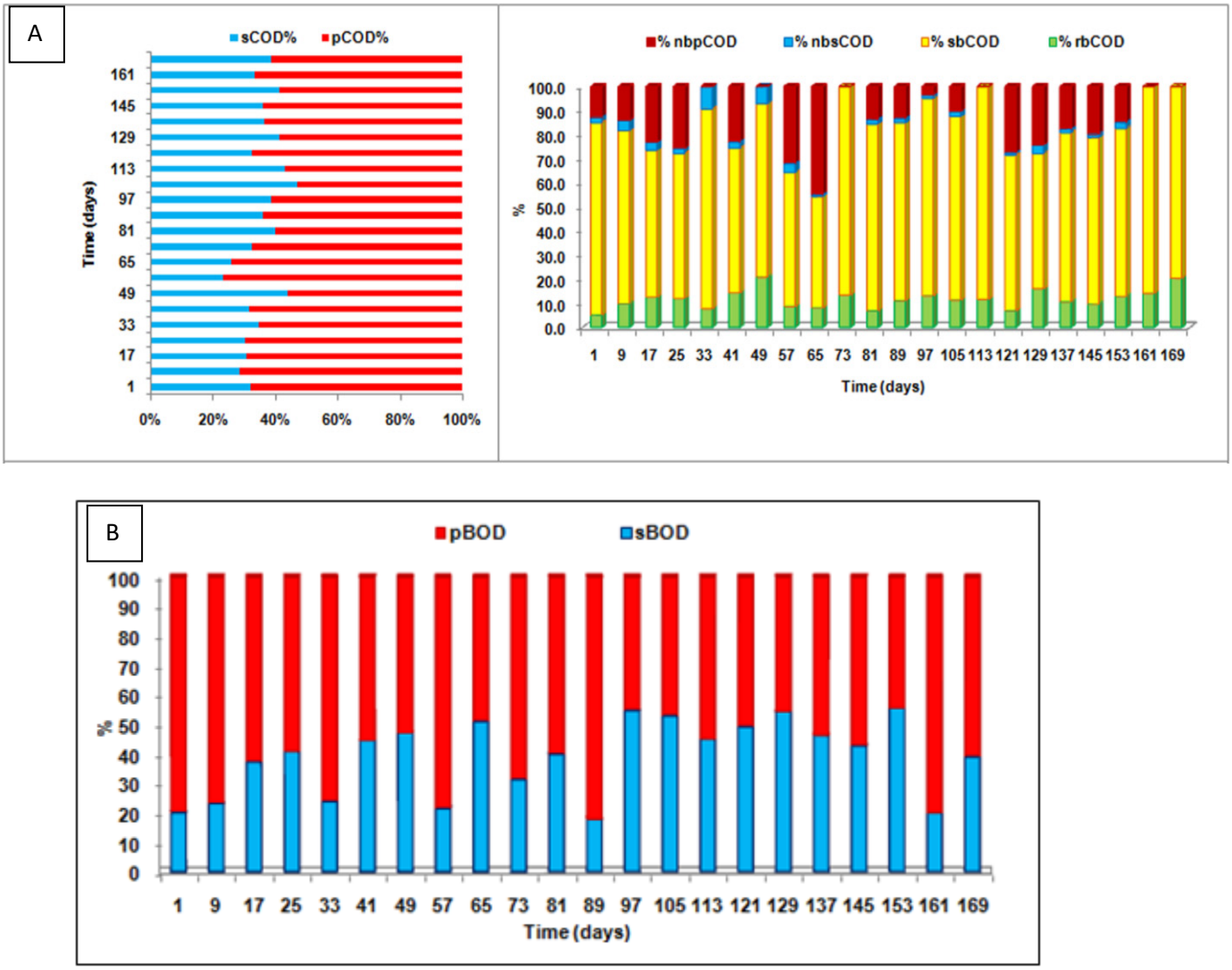
Supplementary Material



*25.8d, 19.7d, and 11.4d are the average SRTs in different runs

Figure S1. The ORP, DO and pH profiles with SRT in subsequent runs of the SBR plant (aeration tank and selector's last compartment).



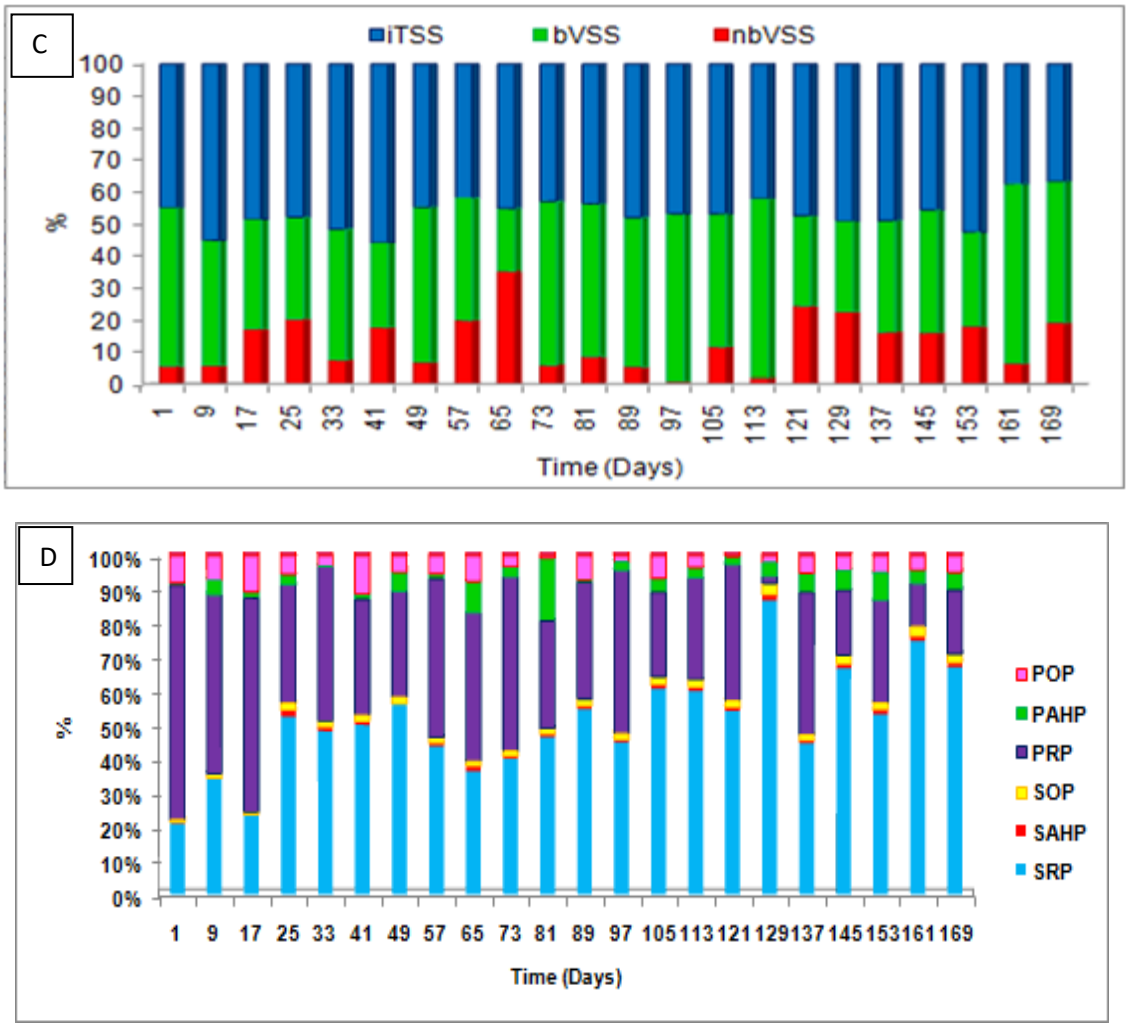


Figure S2. Wastewater fractions in terms of COD, BOD, TSS, and TP during the SRT>50d as described in Srivastava et al., 2022.

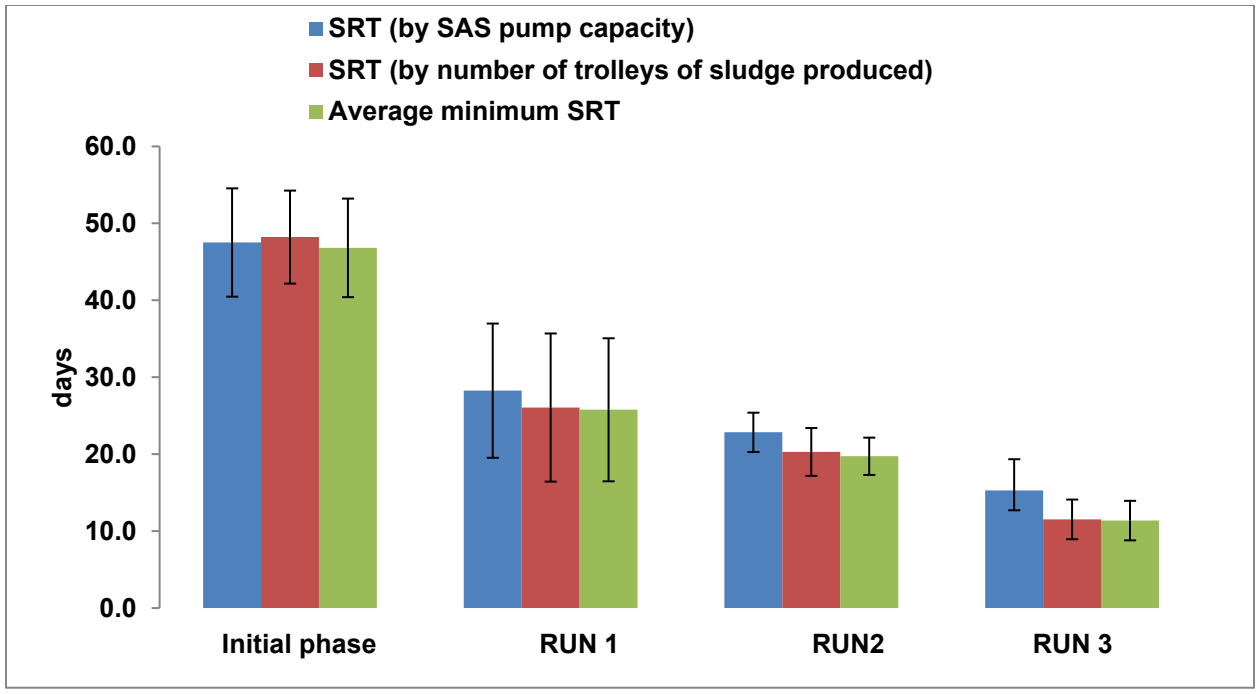


Figure S3. SRT calculated by two ways and average minimum SRT observed.

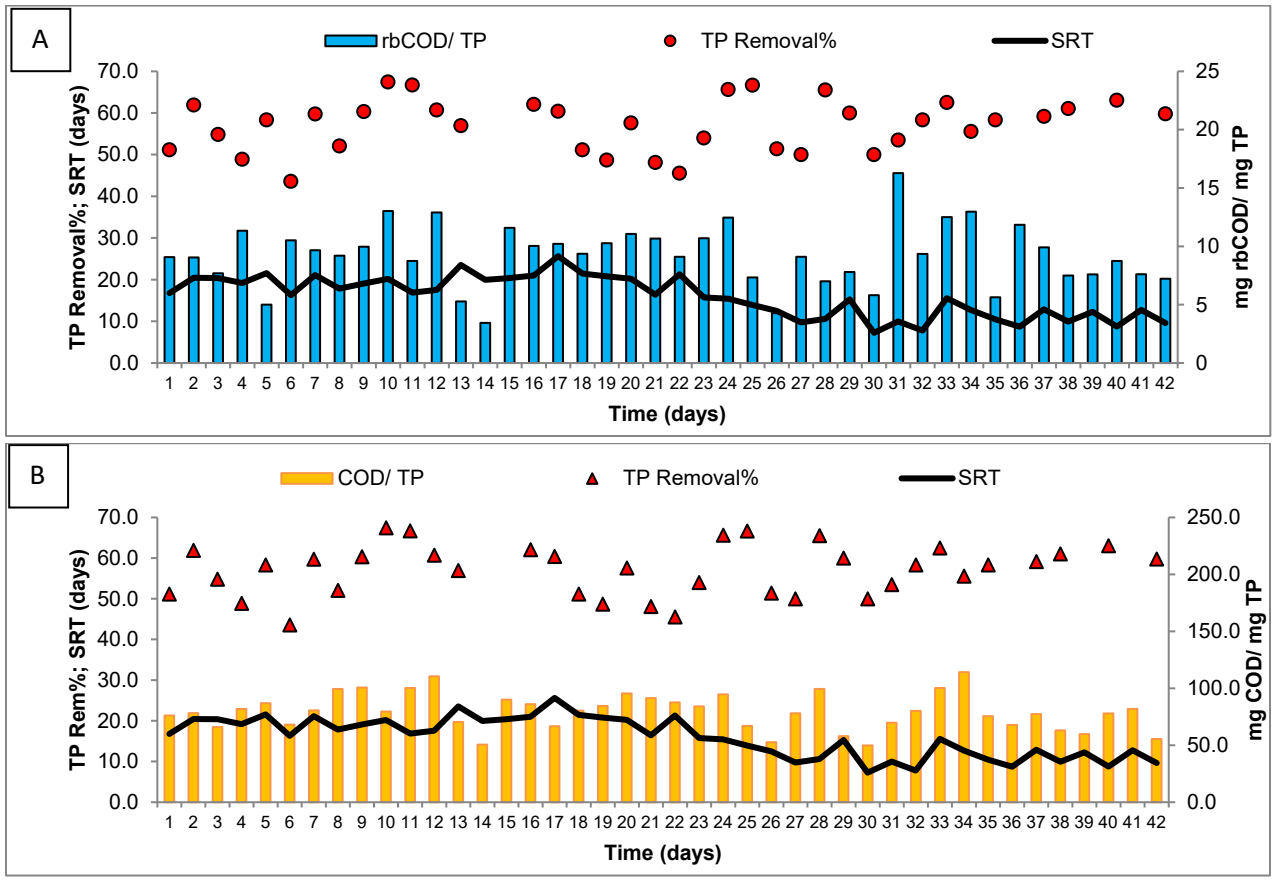


Figure S4. Profiles of PO_4-P and TP removal with SRT and (a) rbCOD/ TP (b) COD/ TP.

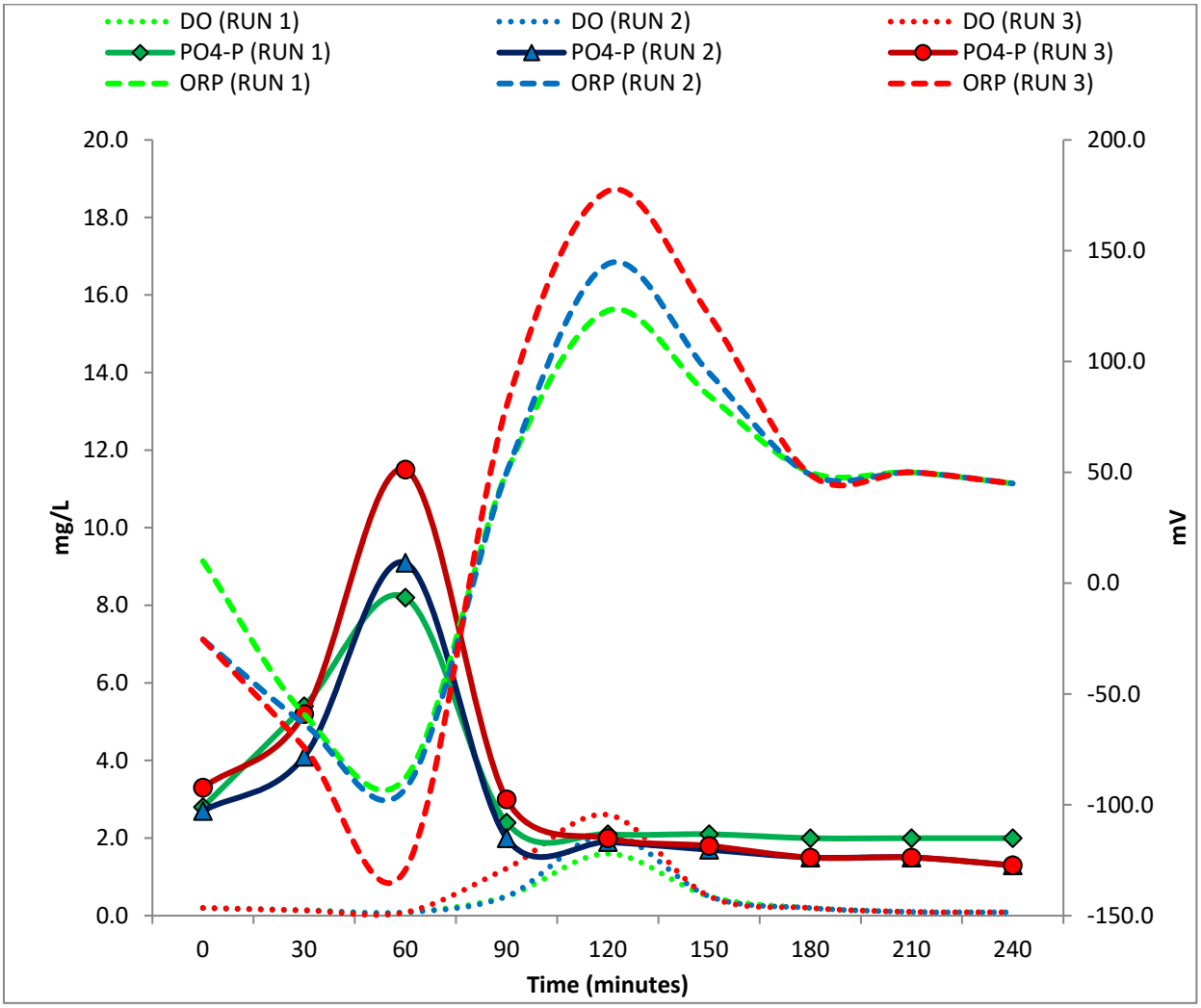
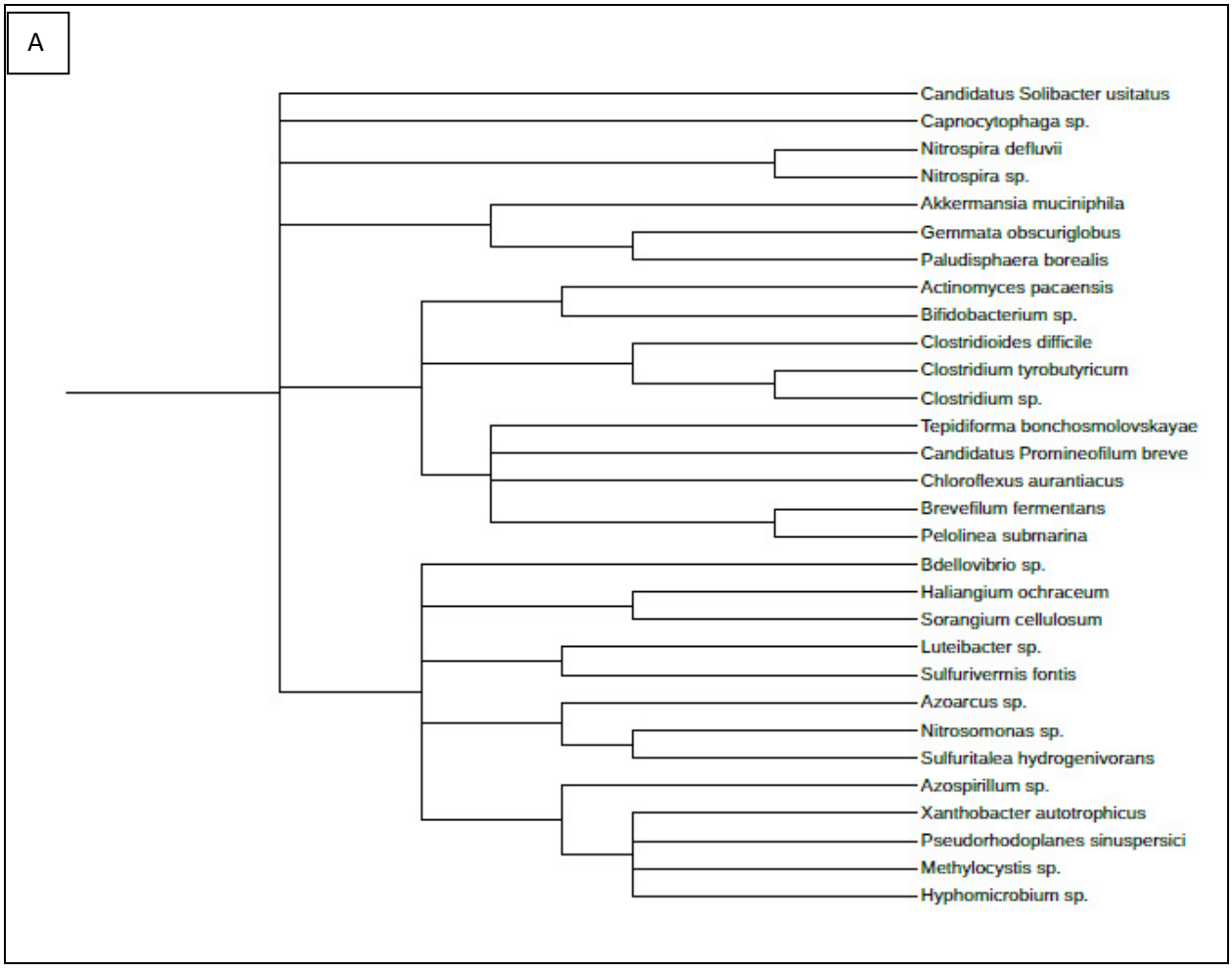


Figure S5. Cycle-wise profile of DO, ORP and PO_4-P in 3 MLD SBR in different runs.


A
Candidatus Solibacter usitatus
Capnocytophaga sp.
Nitrospira defluvii
Nitrospira sp.
Akkermansia muciniphila
Gemmata obscuriglobus
Paludisphaera borealis
Actinomyces pacaensis
Bifidobacterium sp.
Clostridioides difficile
Clostridium tyrobutyricum
Clostridium sp.
Tepidiforma bonchosmolovskayae
Candidatus Promineofilum breve
Chloroflexus aurantiacus
Brevefilum fermentans
Pelolinea submarina
Bdellovibrio sp.
Haliangium ochraceum
Sorangium cellulosum
Luteibacter sp.
Sulfurivermis fontis
Azoarcus sp.
Nitrosomonas sp.
Sulfuritalea hydrogenivorans
Azospirillum sp.
Xanthobacter autotrophicus
Pseudorhodoplanes sinuspersici
Methylocystis sp.
Hyphomicrobium sp.

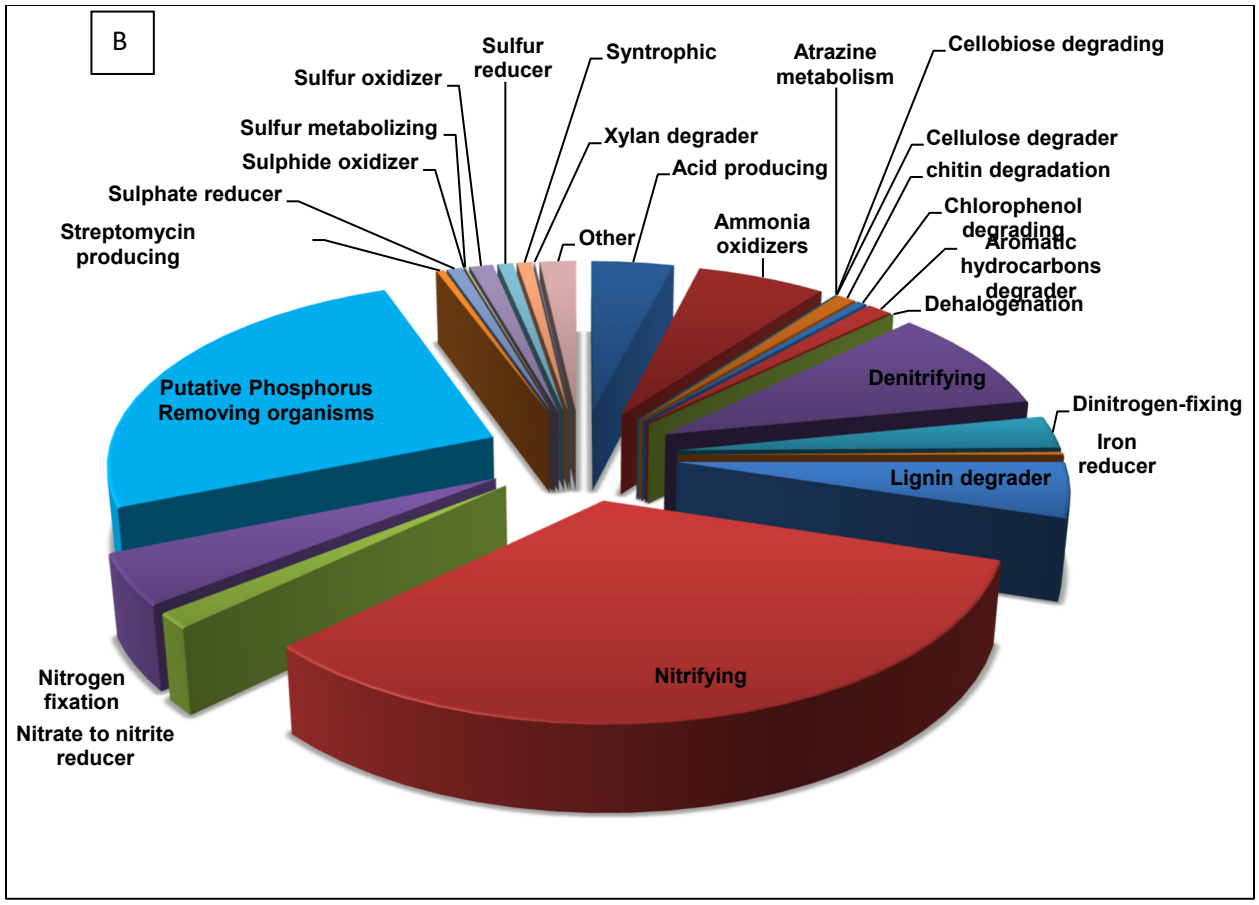


Figure S6. (A) Phylogenetic tree analyses of top 30 species in Aeration Sludge of 3 MLD Full-scale SBR at 20 days SRT, and (B) Functional micro-organisms prevalence in aeration SBR sludge at 20 days SRT.

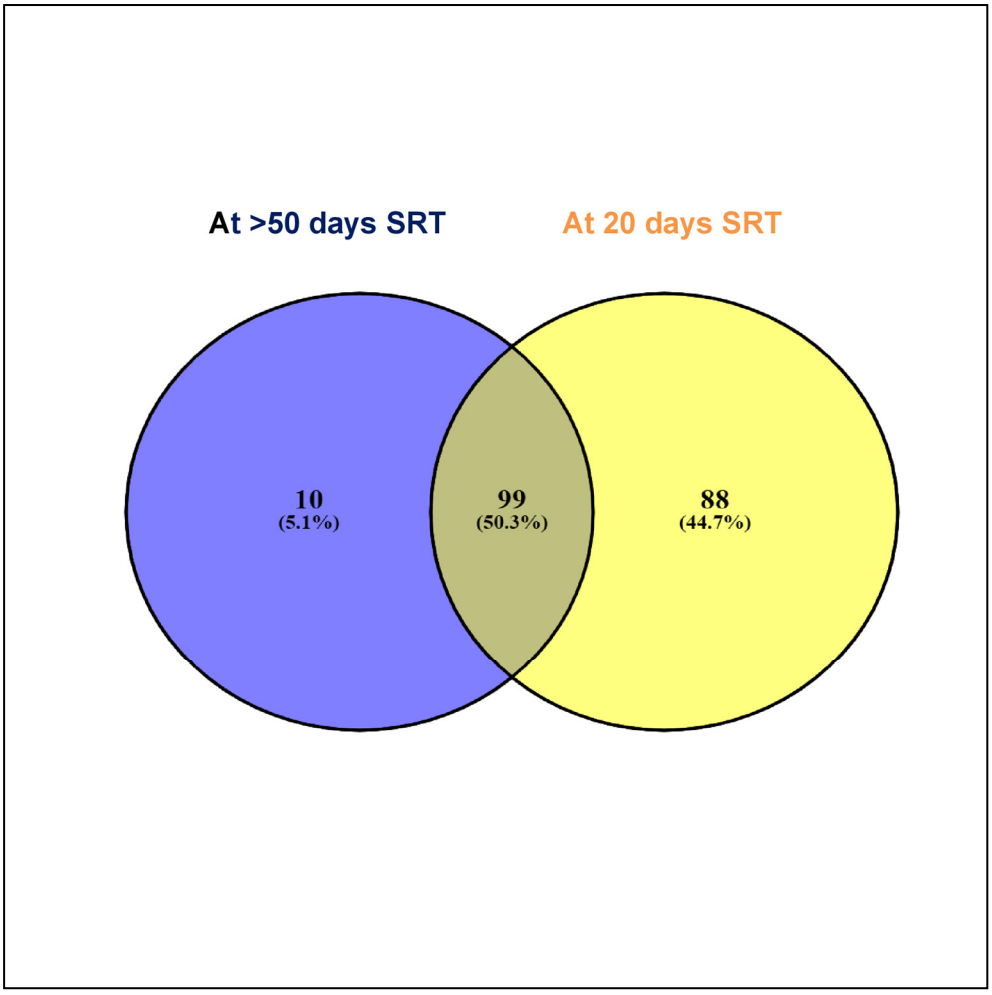


Figure S7. Venn diagram showing relationship between species observed in Aeration Sludge of 3 MLD Full-scale SBR at >50 days SRT and 20 days SRT.

***PCR Amplification of V3-V4 section of 16s Gene (16SrRNA) is performed by the following process:

Composition of TAQ Master MIX:

1) High-Fidelity DNA Polymerase

2) 0.5mM dNTPs

3) 3.2mM $MgCl_2$

4) PCR Enzyme Buffer

Primer Details:

16sF:- 5’ AGAGTTTGATGMTGGCTCAG3’

16sR:- 5’ TTACCGCGGCMGCSGGCAC3’

40ng of extracted DNA is employed for amplification consisting of 10pM of each primer. The following conditions were provided for each 25 cycles of PCR: Denaturation was set at 95 ºC for 15 seconds, annealing was performed at 60 ºC set for 15 seconds, Elongation was provided at 72 ºC for 2 minutes duration, final extension was performed at 72 ºC for 10 minutes and then Hold was conducted at 4 ºC.

DNA QC:

Extracted DNA from the samples was issued to Nanodrop and gel electrophoresis checking before being taken for PCR amplification: The Nanodrop readings of 260/280 coming in the range of 1.8 to 2.0, was conducted to examine the DNA’s performance.

PCR Ampliqon QC:

The amplified 16s PCR product is cleaned, purified and gel electrophoresis checking was exhibited.

The overview of 16S Sequencing Protocol:

The amplicons from each sample were cleaned with Ampure beads to reduce extra primers and a supplementary batch of 8 cycles of PCR was executed using Illumina barcoded adapters to design the sequencing libraries. Libraries were also treated and purified using Ampure beads and the values were quantified using Qubit dsDNA High Sensitivity assay kit. Sequencing was characterized using Illumina Miseq with 2x300PE V3 sequential kits.

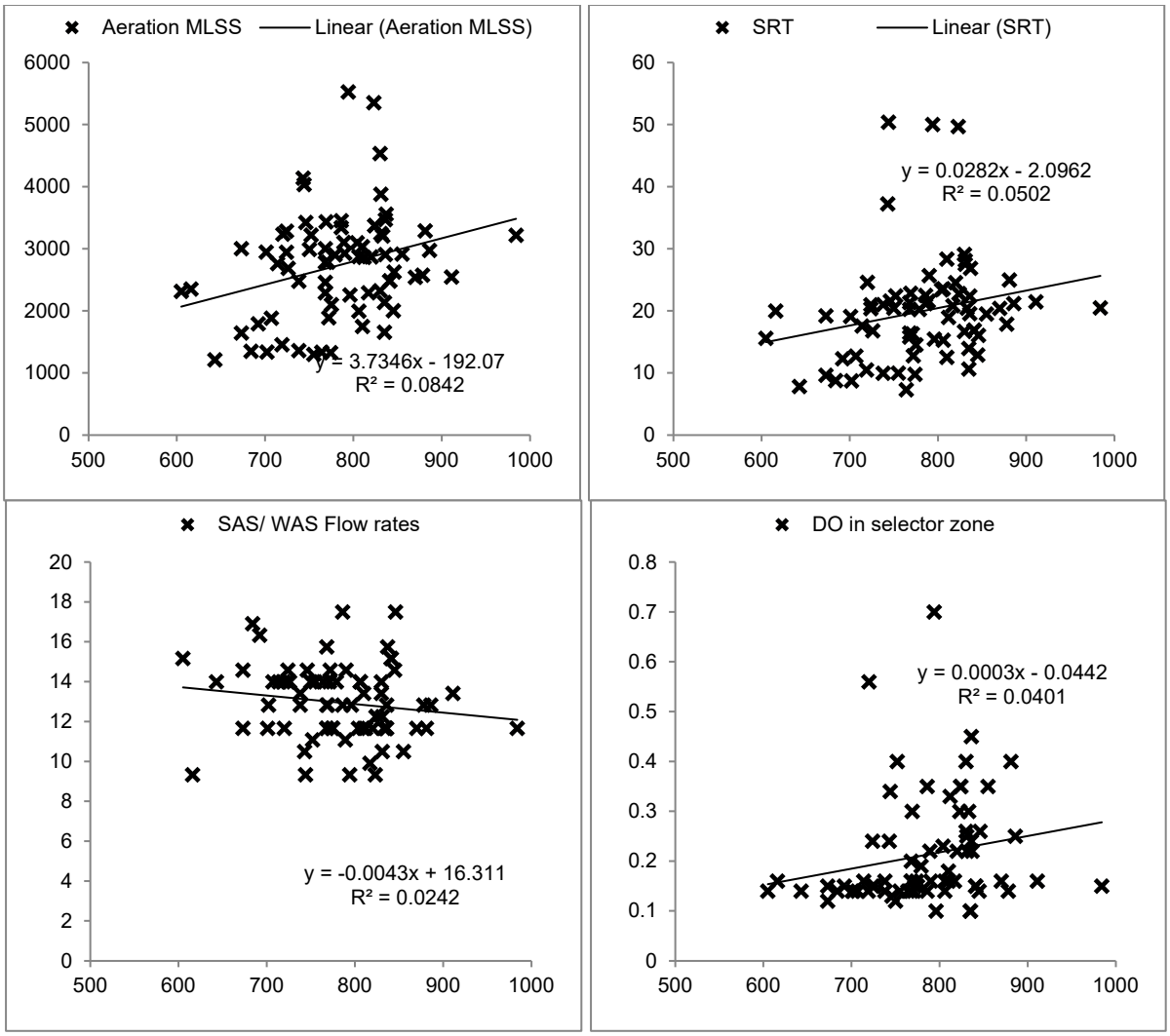


Figure S8. Relationships among energy consumption and SRT, aeration MLSS, WAS/ SAS flow rates and DO in selector zones.

Table S1. Protozoa identification in 3 MLD SBR.

S. No.	Microbiota	At 20 days SRT	Conditions when they appear
1	Arcella	++++	Low DO (1–2 mg/L), satisfactory water quality and occurrence of nitrification in the aeration tank
2	Vorticella	+++	Strong against contamination due to cilia; appears when floc and water condition is satisfactory

3	Peranema	+	Indicator of satisfactory effluent , at load low & high DO, sludge separates but population doesn't become large
4	Colpidium	Nil	Sludge condition is not satisfactory
5	Linototus	++	Appears from the time the load was high until condition becomes good
6	Aspidisca	++	DO is low, small population appears and then disappearing when the water quality is good and satisfactory
7	Rotaria	++	Found in the later stages of our treatment process. Appears at a low load and indicates that nitrification is occurring
8	Opercularia	+++	Sludge condition is satisfactory
9	Filamentous	++	Multiplies in the aerobic system leading to sludge bulking when higher in number