

Supplementary materials:

**Tannery wastewater recalcitrant compounds foster the selection
of fungi in non-sterile conditions: a pilot scale long-term test**

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Materials and Methods

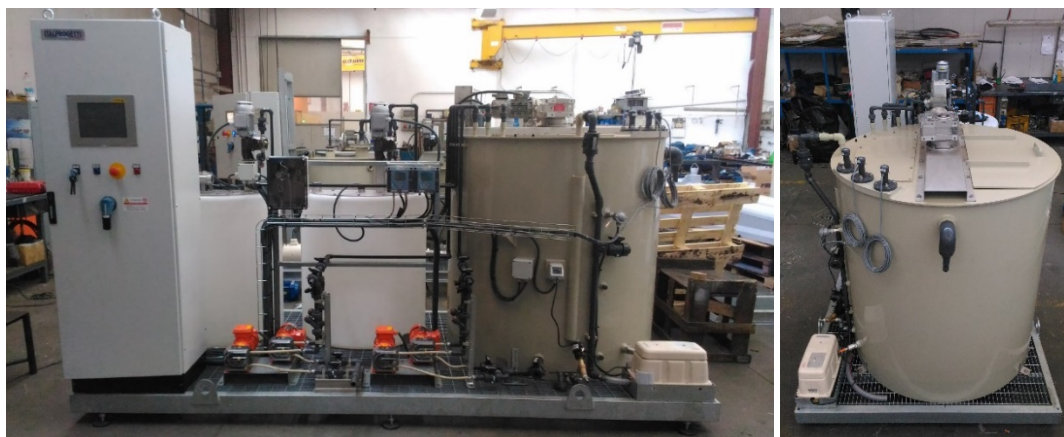


Figure S1. Lateral view of the pilot reactor (**left**) and front view of the pilot reactor (**right**).



Figure S2. Internal view of the rotating cage (**left**) and one sector full of PUF cubes (**right**).

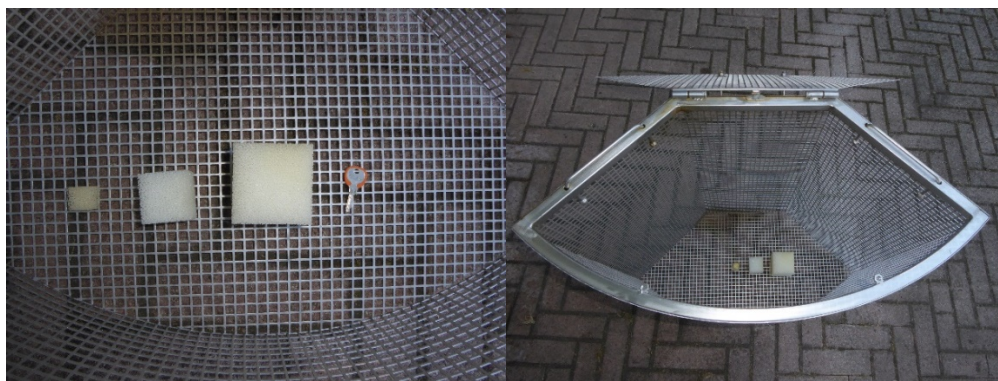


Figure S3. Representative pictures of the three sizes of PUF cubes (**left**) and one sector of the cage (**right**).

Table S1. Pilot-scale reactor: main sensors and actuators installed.

Actuators				
P&I	Description	Supplier	Model	n
P02	Vertical mixer	Italprogetti	Engine 63	1
G11A/B	Peristaltic pump	Watson marlow	521F/R2C	2
G21A/B	Peristaltic pump	Watson marlow	521F/R2C	2
P31	Air compressor	Techma gp	LAM200	1
G43	Dosing pump	Obl	RBB 30 P 95	1
P52	Vertical mixer	Italprogetti	Engine 71	1
G53	Dosing pump	Obl	RBB 30 AC 95	1
EV04	Valve	Omal	GF3/4"	1
Sensors				
DOIC03	DO probe	Hach	LXV416.99.20001	1
PHIC04	pH probe	Hach	DPD1R1.99	1
RXIC05	Redox probe	Hach	DRD1R5.99	1
TIC06	Temperature probe	Seico	PT 100	1

Table S2. Characteristic of the sectors of the pilot-scale reactor.

			Dry	
	Volume	Surface	Dry Mass	Mass/Volume
	(m ³)	(m ²)	(kg)	(kg m ⁻³)
Sector 1	0.094	56	0.468	4.99
Sector 2	0.078	47	0.377	4.86
Sector 3	0.072	43	0.243	3.39
Sector 4	0.094	56	0.468	4.99
Pilot				
bioreactor	0.337	202	1.556	4.56

Table S3. Characterisation of tannin-rich effluent collected from a tannery factory.

Parameter	Unit	Value
pH	pH	3.6
sCOD	mg O L ⁻¹	64,331
DOC	mg C _{org} L ⁻¹	14,546
Total N.	mg N L ⁻¹	1,071
TSS	mg TSS L ⁻¹	12,540
VSS	mg VSS L ⁻¹	9,050
Chlorides	mg Cl L ⁻¹	13,983
Sulphates	mg S L ⁻¹	5,369
Sulphides	mg S L ⁻¹	120

Nitrites	mg N L ⁻¹	4.7
Nitrates	mg N L ⁻¹	0.5
Ammonium	mg N L ⁻¹	834.7
Conductivity	mS cm ⁻¹	45.3

Metals

Chromium	mg L ⁻¹	0.4
Iron	mg L ⁻¹	15.8
Cadmium	mg L ⁻¹	< 0.2
Lead	mg L ⁻¹	< 0.2
Zinc	mg L ⁻¹	3.2
Aluminium	mg L ⁻¹	32.2
Copper	mg L ⁻¹	0.6
Boron	mg L ⁻¹	0.4

Results and Discussion

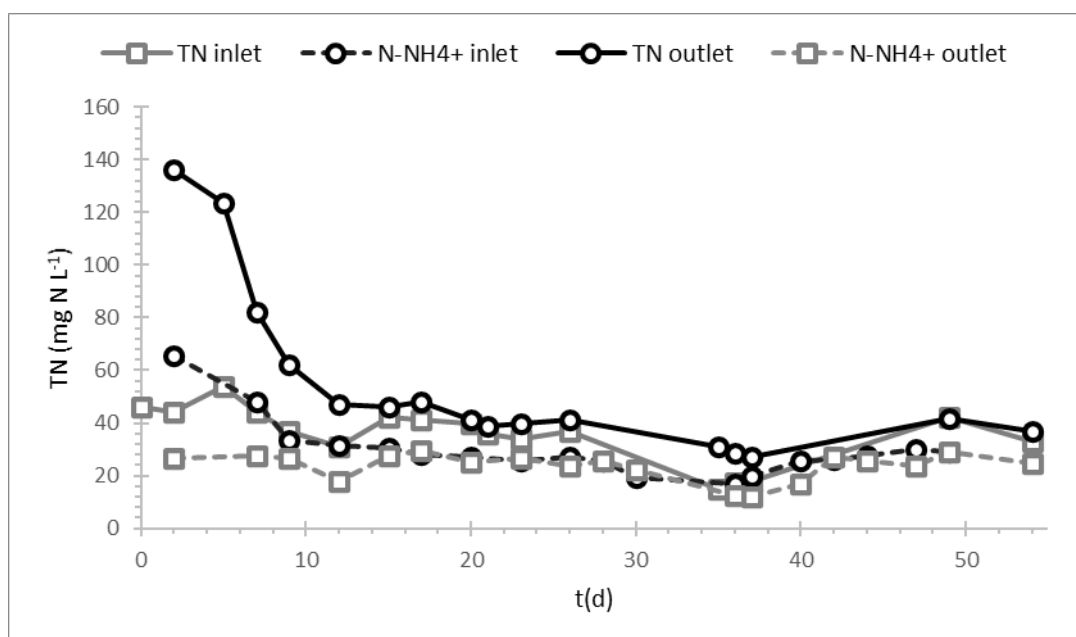


Figure S4. Total nitrogen and ammonium concentration inlet and outlet in the pilot reactor during the start-up phase. Noteworthy, it was observed a released of nitrogen (part detected as ammonium) from the reactor in the first two weeks. The release of nitrogen during the first two weeks could be related to the residuals of PUF production process possibly present on its surface, in fact, in the industrial PUF production process, isocyanate reacts with an amine leading to the synthesis of urea (double amide of the acid carbonate) as byproduct.

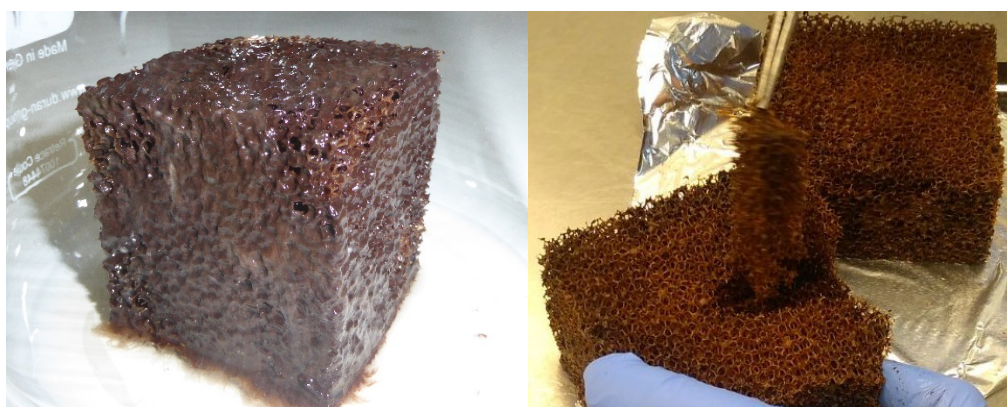


Figure S5. PUF cubes with 7.5 cm size: external view (left) and internal view (right).

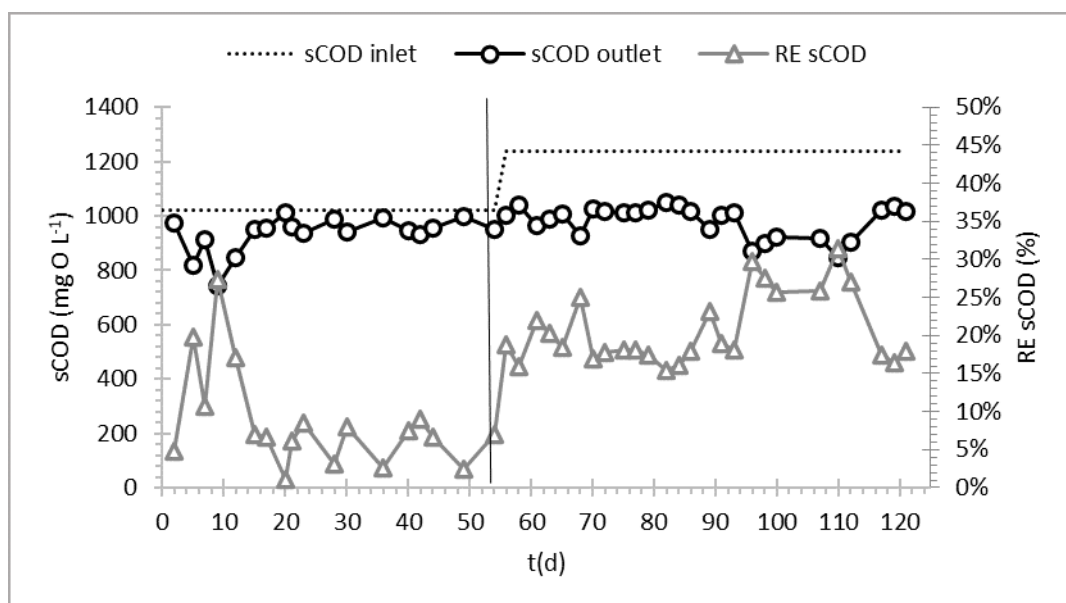


Figure S6. Inlet and outlet sCOD and sCOD removal percentage during continuous treatment in the pilot reactor. Vertical line divided the start-up phase from the treatment phase.

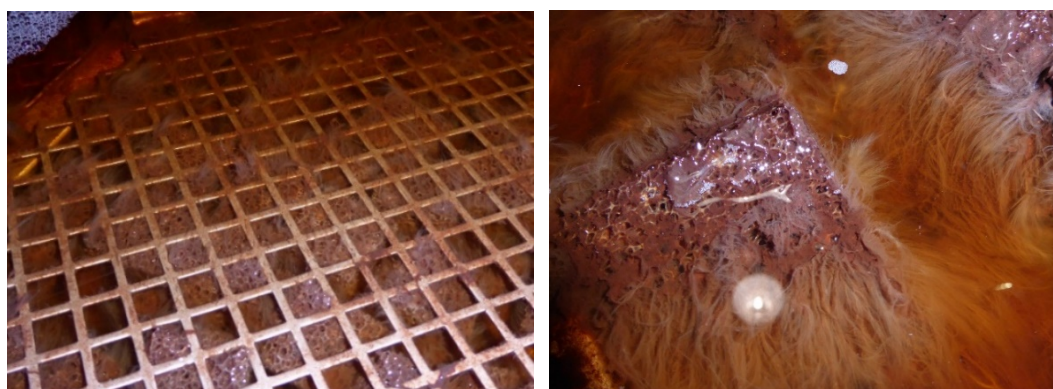


Figure S7. Pictures of immobilised PUF cubes in the pilot at the end of the start-up phase. On the left a picture of PUF cubes located in the middle of the sector (less exposed to the shear stress of rotation) on the right a detail of a PUF.

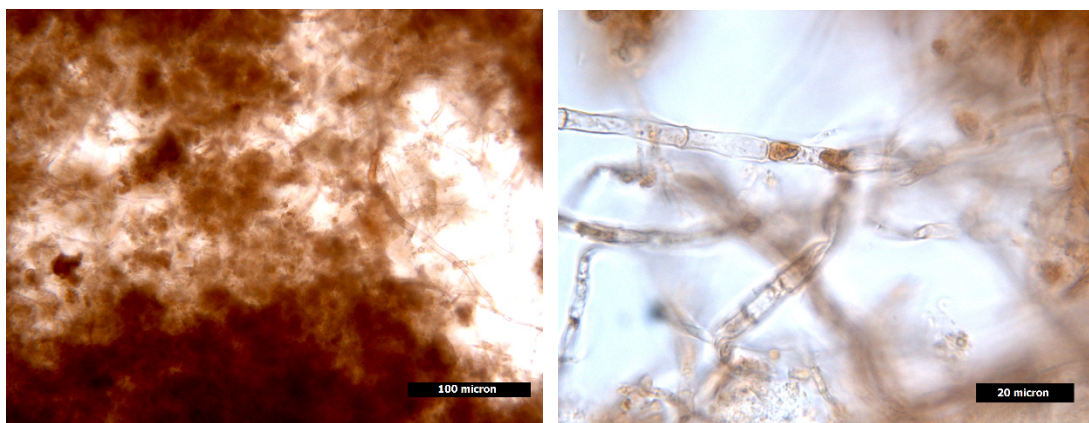


Figure S8. Microscope pictures of biofilm from immobilised PUF cubes in the pilot at the end of the start-up phase.

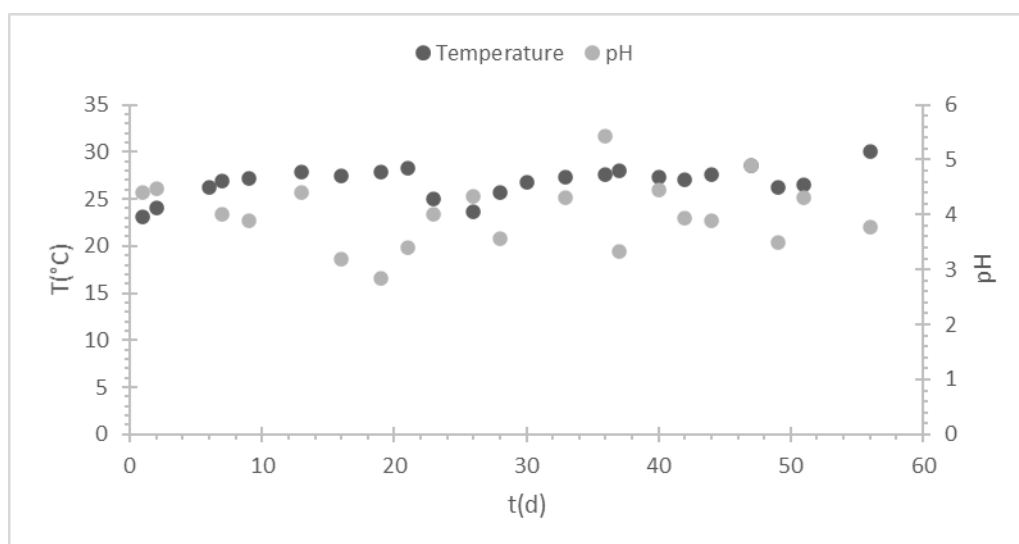


Figure S9. The average temperature and pH during the start-up in the pilot-scale reactor.

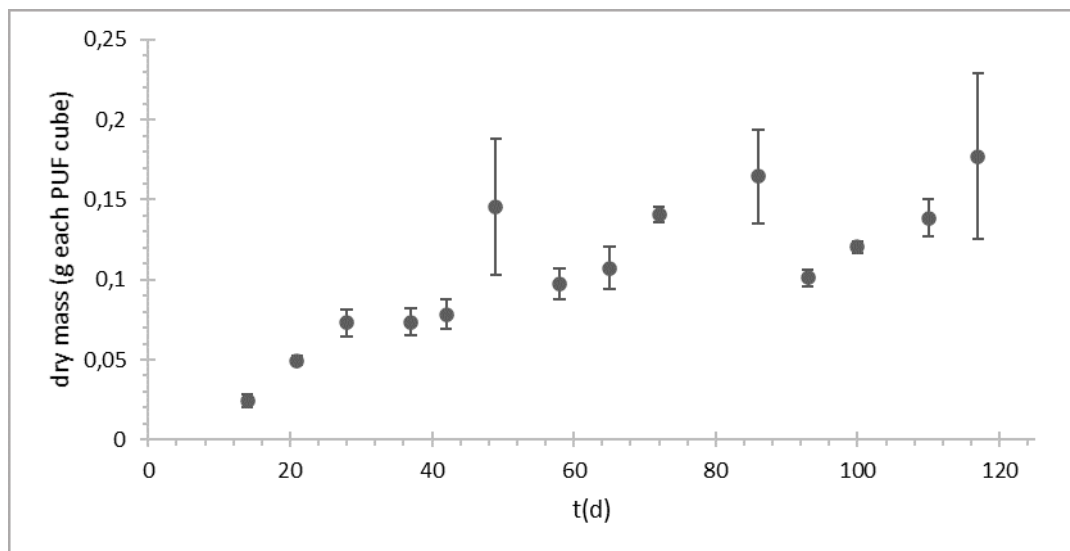


Figure S10. Dry mass in PUF cubes collected from the pilot reactor. Values are given as average among three replicates +/- Standard Deviation (SD).

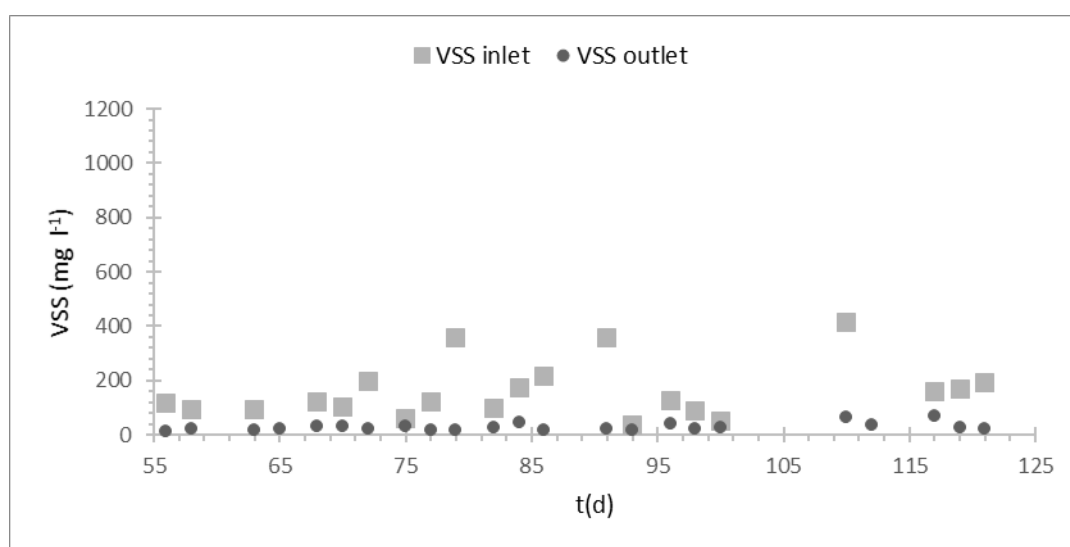


Figure S11. VSS inlet and outlet in the pilot reactor during the treatment

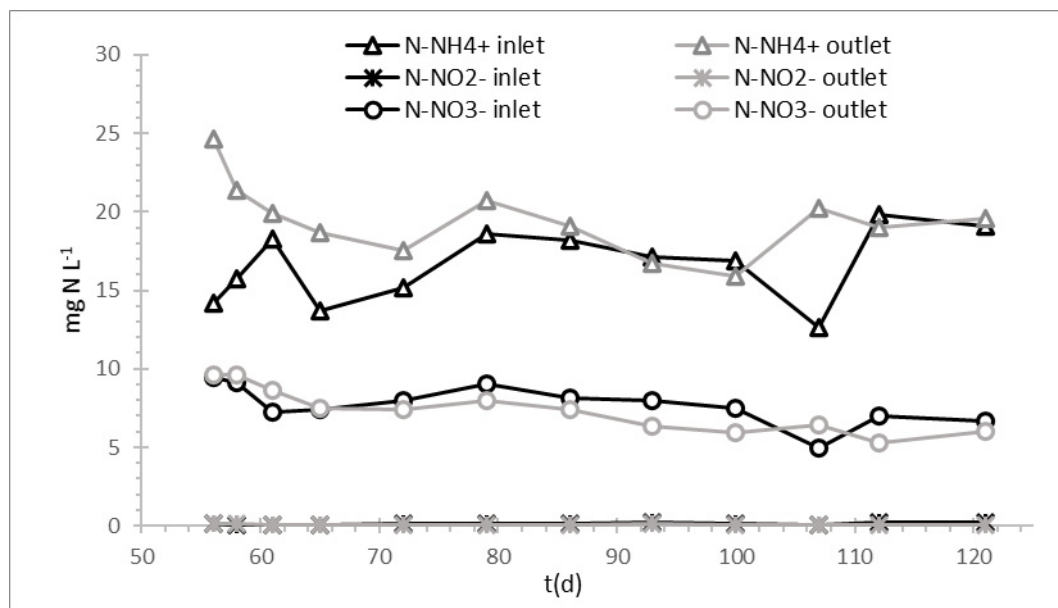


Figure S12. Ammonium, Nitrate and Nitrite concentration inlet and outlet in the pilot reactor during the treatment phase.

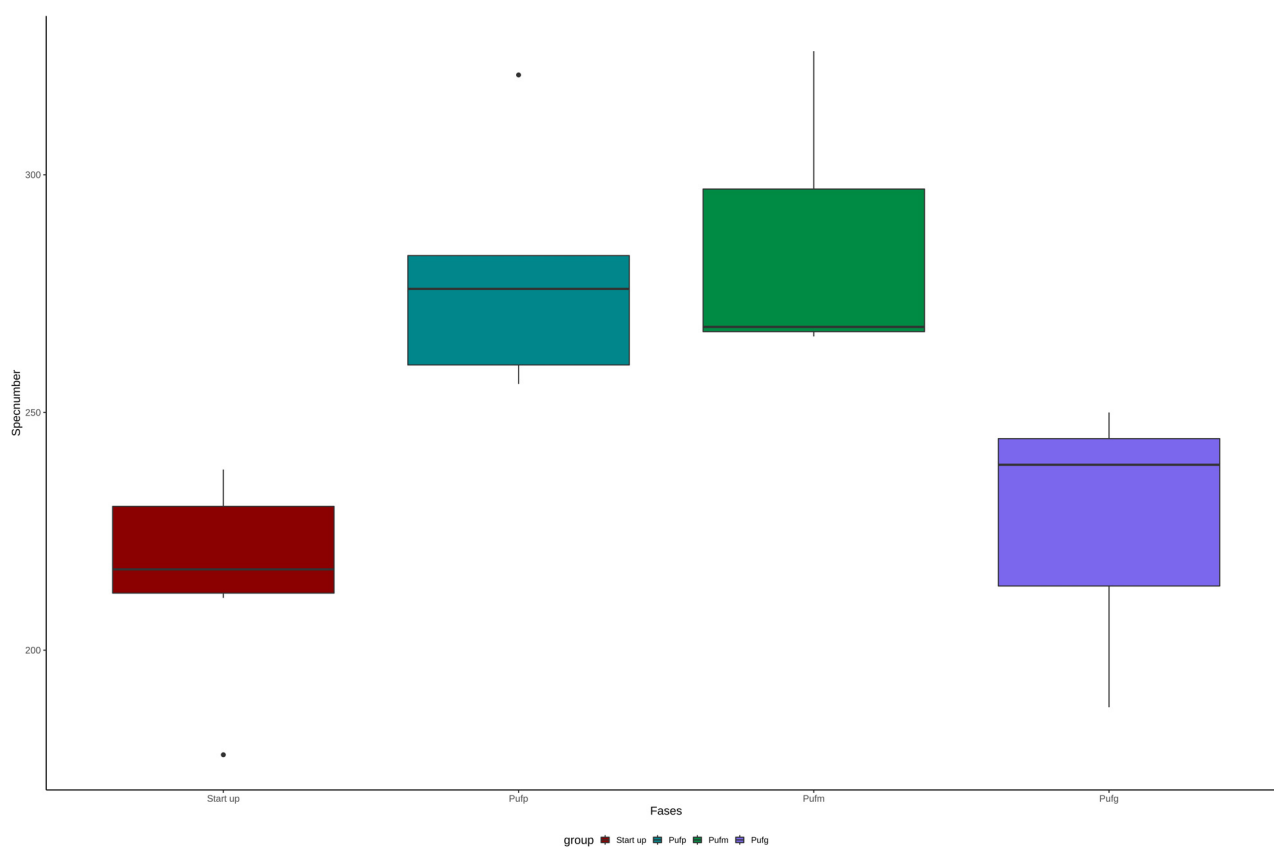


Figure S13. Number of species.

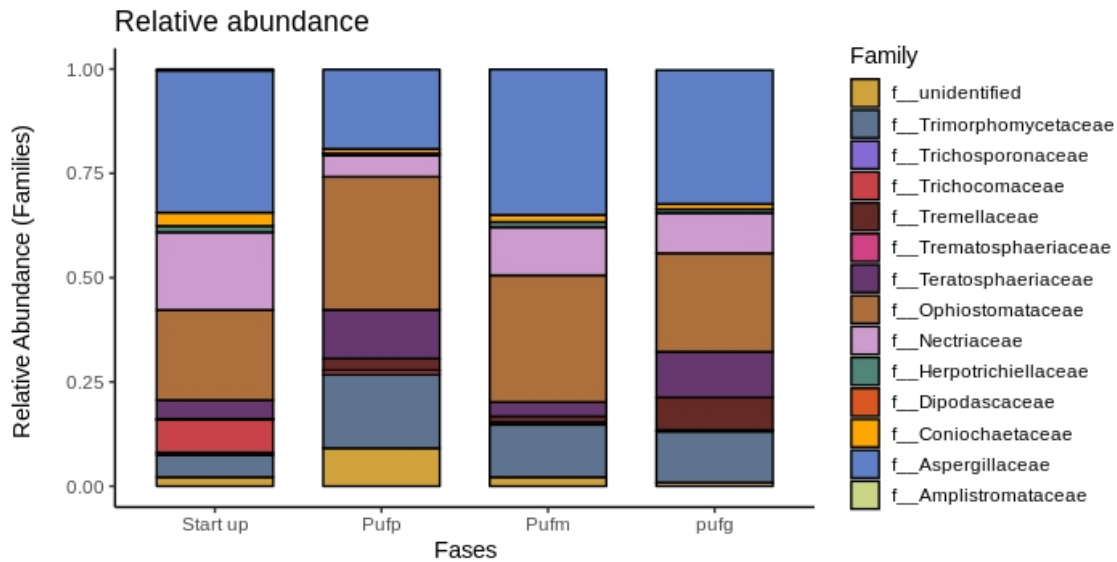
Table S4. Characteristic Alpha diversity indices among the phases. The groups are indicated by letters. Groups with the same letters are not statistically significant different ($p < 0.05$). Values are gives as average among replicates \pm Standard Deviation (S.D.).

	Start up	Pufp	Pufm	Pufg
Species nr.	215.83 \pm 21.39(b)	279.20 \pm 25.88(a)	286.67 \pm 34.08(a)	225.67 \pm 33.08(b)
Simpson	0.69 \pm 0.05(a)	0.72 \pm 0.06(a)	0.72 \pm 0.01(a)	0.73 \pm 0.02(a)

Table S5. SIMPER analysis. The upper part of the table shows the percentage of difference among pairwise comparisons among phases. The lower part of the table reports the families that contribute to these differences with the respective percentages. Only the statically significant differences are reported ($p < 0.05$).

	Inoculum	Start up	End Treatment
Inoculum		6.2%	7.5%
Start up	<i>Xanthomonadaceae</i> (6.2%)		39.4%
		<i>Xanthomonadaceae</i> (13.5%)	
End treatment	<i>Xanthomonadaceae</i> (7.53%)	<i>Rhodochloramydiaceae</i> (8.01%)	
		<i>Acidobacteriaceae</i> (4.83%)	

(A)



(B)

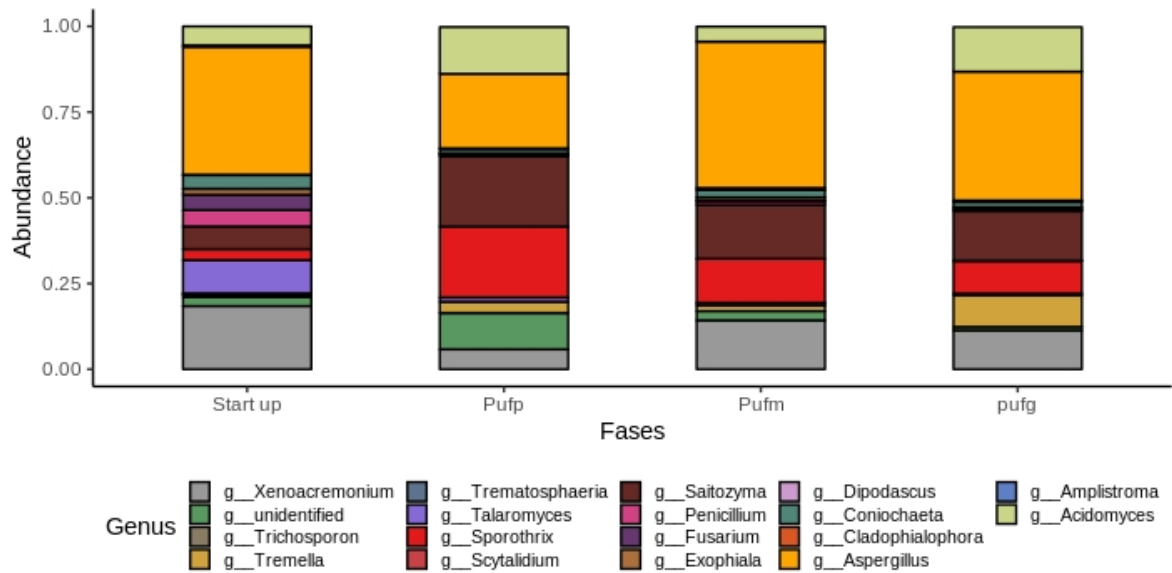


Figure S14. Fungal (A) families and (B) genera.