

Exploring soil microbiome self-assembling in bioreactors as a strategy to decrease chemical fertilizers in tomato plants

Carlos F. Gutiérrez¹, Nicolás Rodríguez-Romero¹, Siobhon Egan², Elaine Holmes², Janeth Sanabria^{1,2*}

Statics for chemical parameters measured in Bioreactors

Table S1. Summary of average physical and chemical parameters.

Average values +/- Standard Deviation		
	Start a	Steady-state b
OD600 (nm)	C-	0
	C+	0.28 +/- 0.15
	R2	0.39 +/- 0.24
	R3	0.41 +/- 0.23
	R5	0.28 +/- 0.15
Biomass (mg/L)	C-	0
	C+	0.09 +/- 0.04
	R2	0.1 +/- 0.04
	R3	0.1 +/- 0.05
	R5	0.8 +/- 0.04
COD removal (%)	C-	18.57 +/- 9.84
	C+	57.92 +/- 30.97
	R2	56.92 +/- 32.01
	R3	61.56 +/- 30.43
	R5	59 +/- 29
TKN (mg/L)	C-	0.95 +/- 1.94
	C+	0.14 +/- 0.13
	R2	0.16 +/- 0.16
	R3	0.07 +/- 0.10
	R5	0.88 +/- 1.67

^a Start refers to the process of reactors inoculation until they reach a steady-state after 80 days of operation. ^b Steady-state refers to the process in which there are no significant changes in the values and are relatively constant over time.

Table S1. Summary of average physical and chemical parameters.

		Average values +/- Standard Deviation	
		Start a	Steady-state b
N-NH4 (mg/L)	C-	1.14 +/- 1.87	0.2
	C+	0.53 +/- 2.16	0.08
	R2	1.55 +/- 2.35	0.1
	R3	1.67 +/- 2.48	0.07
	R5	1.97 +/- 2.56	0.08
N-NO3 (mg/L)	C-	0.06 +/- 0.11	0.01
	C+	0.06 +/- 0.1	0.43
	R2	0.07 +/- 0.1	0.14
	R3	0.07 +/- 0.1	0.09
	R5	0.08 +/- 0.11	0.84
N-NO2 (mg/L)	C-	0.02 +/- 0.01	0.09
	C+	0.02 +/- 0.01	0.11
	R2	0.02 +/- 0.01	0.12
	R3	0.02 +/- 0.02	0.14
	R5	0.02 +/- 0.01	0.14
N Fixed = TKN + NH4 + NO2 + NO3	C-	0.02 +/- 0.01	1.13
	C+	0.02 +/- 0.01	14.37
	R2	0.02 +/- 0.01	15.34
	R3	0.02 +/- 0.02	7.16
	R5	0.02 +/- 0.01	25.02

^a Start refers to the process of reactors inoculation until they reach a steady-state after 80 days of operation. ^b Steady-state refers to the process in which there are no significant changes in the values and are relatively constant over time.

Table S2. Anova Coefficients for transformed OD₆₀₀ response

Term	Coef	SE Coef.	T	p	VIF
Constant	-2,332	117	-19,99	0	
Days	1,683	197	8,54	0	2,00
Treatment R	-99	165	-0,60	548	3,42
Days * Treatment					
SAM	0,00560	0,00279	2,01	0,047	4,42

Equation of regression for OD₆₀₀ response
Treatments

OD C+ $\ln(\text{Response}) = -2,332 + 0,01683 \text{ Days}$

OD SAM $\ln(\text{Response}) = -2,431 + 0,02243 \text{ Days}$

Table S3. ANOVA for biomass response coefficients for transformed biomass (mg/L)

Term	Coef	SE Coef.	T	p	VIF
Constant	-1,716	112	-15,30	0	
Days_1	767	159	4,81	0	2,00
Treatment					
SAM	8	159	0,05	960	2,14
Days_1*Treatment					
SAM	12	225	0,05	958	3,14

Equation of regression for biomass response equation of regression
Treatments

C+ $-\text{Response}^{-0,145643} = -1,716 + 0,00767 \text{ Days}_1$

SAM $-\text{Response}^{-0,145643} = -1,708 + 0,00779 \text{ Days}_1$

Table S4. ANOVA for COD % removal efficiency coefficients

Term	Coef	SE Coef.	T	p	VIF
Constant	10,87	3,95	2,75	10	
Days_1	1,665	863	1,93	63	3,00
Treatments					
C+	6,46	5,59	1,16	257	4,76
SAM	2,02	5,59	0,36	720	4,76
Days_1*Treatments					
C+	868	122	7,11	0	5,43
SAM	889	122	7,29	0	5,43

Equation of regression for COD % removal efficiency
Treatments

C+ $\text{Response} = 17,32 + 1,0343 \text{ Days}$

SAM $\text{Response} = 12,89 + 1,0556 \text{ Days}$

Table S5. Coefficients for transformed NT response

Term	Coef	SE Coef.	T	p	VIF
Constant	-1,53	1,01	-1,51	0,165	
Days_1	0,0014	0,0117	0,12	0,905	3,00
Treatment					
C+	-2,43	1,43	-1,70	0,123	2,67
SAM	-0,33	1,43	-0,23	0,823	2,67
Days_1*Treatment					
C+	0,0234	0,0166	1,41	0,193	3,33
SAM	0,0194	0,0166	1,17	0,272	3,33

Equation of regression for transformed NT**Treatment**

C+	$\ln(\text{Response}) = -3,96 + 0,0248 \text{ Days}_1$
SAM	$\ln(\text{Response}) = -1,85 + 0,0209 \text{ Days}_1$

Statics for chemical parameters measured in Bioreactors

Table S6. Comparing fertilizer treatment effects of the tomato growth rates in the first gathering.Trend line equations and R square values $C_- = 1,1117x - 0,3918 \quad R^2 = 0,9982$ $C_+ = 1,4638x - 0,8408 \quad R^2 = 0,9802$ $P1 = 1,3189x + 0,3694 \quad R^2 = 0,9709$ $P2 = 1,5791x - 0,398 \quad R^2 = 0,9962$ $P3 = 1,2194x + 0,2286 \quad R^2 = 0,9841$

Table S7. Tukey multiple pairwise comparisons of Stem length in the first gathering.

		diff	lwr	upr	Padj
C+	-C	-2.7857143	-0.3451074	5.916536	0.0997065
P1	-C	-2.1	-1.0308216	5.230822	0.3163822
P2	-C	-3.5142857	0.3834641	6.645107	0.0217685
P3	-C	-1.7285714	-1.4022502	4.859393	0.5080016
P1	-C+	-0.6857143	-3.8165359	2.445107	0.9680413
P2	-C	+0.7285714	-2.4022502	3.859393	0.9603222
P3	-C+	-1.0571429	-4.1879645	2.073679	0.8623589
P2	-P1	1.4142857	-1.7165359	4.545107	0.6870557
P3	-P1	-0.3714286	-3.5022502	2.759393	0.9968156
P3	-P2	-1.7857143	-4.9165359	1.345107	0.4760769

Table S8. Tukey multiple pairwise comparisons of Stem length in the first gathering.

	diff	lwr	upr	padj
C+-C-	1.3714286	-0.4462773	3.18913445	0.2115377
P1-C-	2.0000000	0.1822941	3.81770588	0.0254190
P2-C-	2.5285714	0.7108655	4.34627731	0.0029723
P3-C-	0.7000000	-1.1177059	2.51770588	0.7963371
P1-C+	0.6285714	-1.1891345	2.44627731	0.8518911
P2-C+	1.1571429	-0.660563	2.97484874	0.367049
P3-C+	-0.6714286	-2.4891345	1.14627731	0.8195156
P2-P1	0.5285714	-1.2891345	2.34627731	0.9147368
P3-P1	-1.3000000	-3.1177059	0.51770588	0.2572031
P3-P2	-1.8285714	-3.6462773	-0.01086555	0.0480815

Table S9. Tukey multiple pairwise comparisons of Total length in the first gathering.

	diff	lwr	upr	padj
C+-C-	3.600000	-0.9800697	8.180070	0.1794070
P1-C-	4.000001	-0.4800697	8.680070	0.0965278
P2-C-	6.042857	1.4627875	10.622927	0.0051454
P3-C-	2.428571	-2.1514983	7.008641	0.5468984
P1-C+	0.500000	-4.0800697	5.080070	0.9976950
P2-C+	2.442857	-2.1372125	7.022927	0.5413182
P3-C+	-1.171429	-5.7514983	3.408641	0.9447722
P2-P1	1.942857	-2.6372125	6.522927	0.7340233
P3-P1	-1.671429	-6.2514983	2.908641	0.8258500
P3-P2	-3.614286	-8.1943554	0.965784	0.1764419

Table S10. Tukey multiple pairwise comparisons of Root width in the first gathering.

	diff	lwr	upr	P adj
C+-C-	0.32857143	-0.8764859	1.53362876	0.9312654
P1-C-	0.78571429	-0.41934304	1.99077161	0.3436463
P2-C-	1.62857143	0.4235141	2.83362876	0.0040306
P3-C-	0.38571429	-0.81934304	1.59077161	0.8835997
P1-C+	0.45714286	-0.74791447	1.66220019	0.8049507
P2-C+	1.30000000	0.09494267	2.50505733	0.0294874
P3-C+	0.05714286	-1.14791447	1.26220019	0.9999144
P2-P1	0.84285714	-0.36220019	2.04791447	0.2772783
P3-P1	-0.4000000	-1.60505733	0.80505733	0.8694778
P3-P2	-1.24285714	-2.44791447	-0.03779981	0.0406474

Table S11. Tukey multiple pairwise comparisons of Root volume in the first gathering.

	diff	lwr	upr	padj
C+-C-	2.4549360	-10.64491224	15.554784	0.9819044
P1-C-	6.6637450	-6.43610324	19.763593	0.5856284
P2-C-	15.6326016	2.53275336	28.732450	0.0131211
P3-C-	2.9239694	-10.17587884	16.023818	0.9657966
P1-C+	4.2088090	-8.89103924	17.308657	0.8822008
P2-C+	13.1776656	0.07781736	26.277514	0.0480932
P3-C+	0.4690334	-12.63081484	13.568882	0.9999720
P2-P1	8.9688566	-4.13099164	22.068705	0.2970215
P3-P1	-3.7397756	-16.83962384	9.360073	0.9197947
P3-P2	-12.7086322	-25.80848044	0.391216	0.0606439

Table S12. Comparing fertilizer treatment effects of the tomato growth rates in the second gathering.

$$\mathbf{C+} = -0,002x_4 + 0,0718x_3 - 0,3251x_2 + 0,2514x + 3,6092 \quad R^2 = 0,9978$$

$$\mathbf{P1} = -0,0021x_4 + 0,093x_3 - 0,8747x_2 + 3,8588x - 2,0546 \quad R^2 = 0,9992$$

$$\mathbf{P2} = -0,0049x_4 + 0,2217x_3 - 2,7156x_2 + 12,122x - 12,06 \quad R^2 = 0,9976$$

$$\mathbf{P3} = -0,0007x_4 + 0,0227x_3 + 0,2141x_2 - 1,9876x + 4,8939 \quad R^2 = 0,9955$$