

Appendix

Appendix A: Preparation of phosphate buffer for dilution water.

<u>Phosphate buffer</u>	
Potassium dihydrogen phosphate, KH ₂ PO ₄	8.50 g
Dipotassium phosphate, K ₂ HPO ₄	21.75 g
Sodium phosphate, Na ₂ HPO ₄ .7H ₂ O	33.40 g
Ammonium chloride, NH ₄ Cl	1.70 g
Distilled water	1 liter
pH	7.2

Appendix B: Preparation of the dilution water.

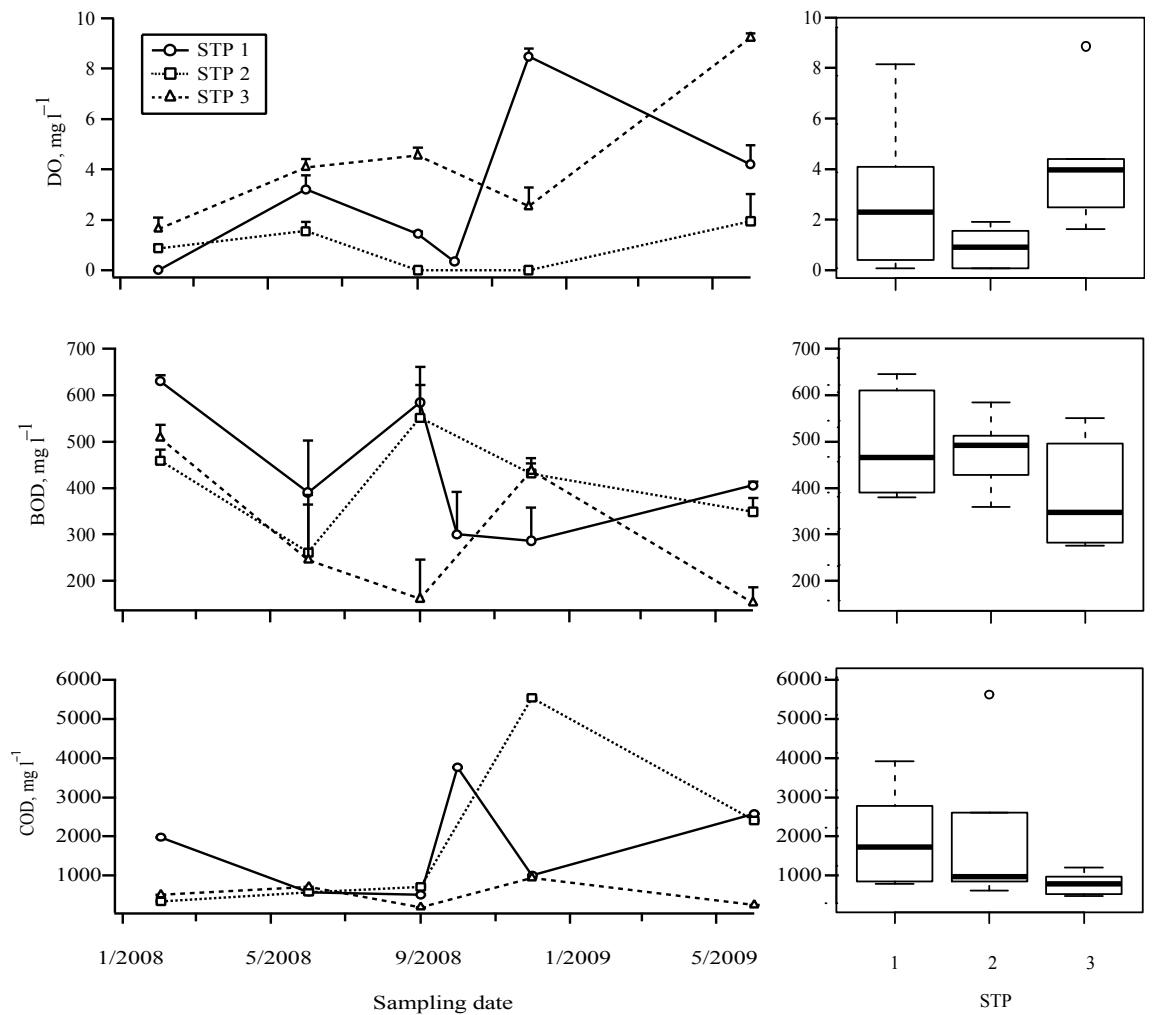
<u>Dilution water</u>	
Phosphate buffer, pH7.2	1 ml
Magnesium sulfate, MgSO ₄ .7H ₂ O (22.5 g l ⁻¹)	1 ml
Calcium chloride, CaCl ₂ (27.5 g l ⁻¹)	1 ml
Iron (III) chloride, FeCl ₃ .6H ₂ O (0.25 g l ⁻¹)	1 ml
Distilled water	to 1000 ml

Appendix C: Preparation of stock solution for 0% and 100% denaturant solutions.

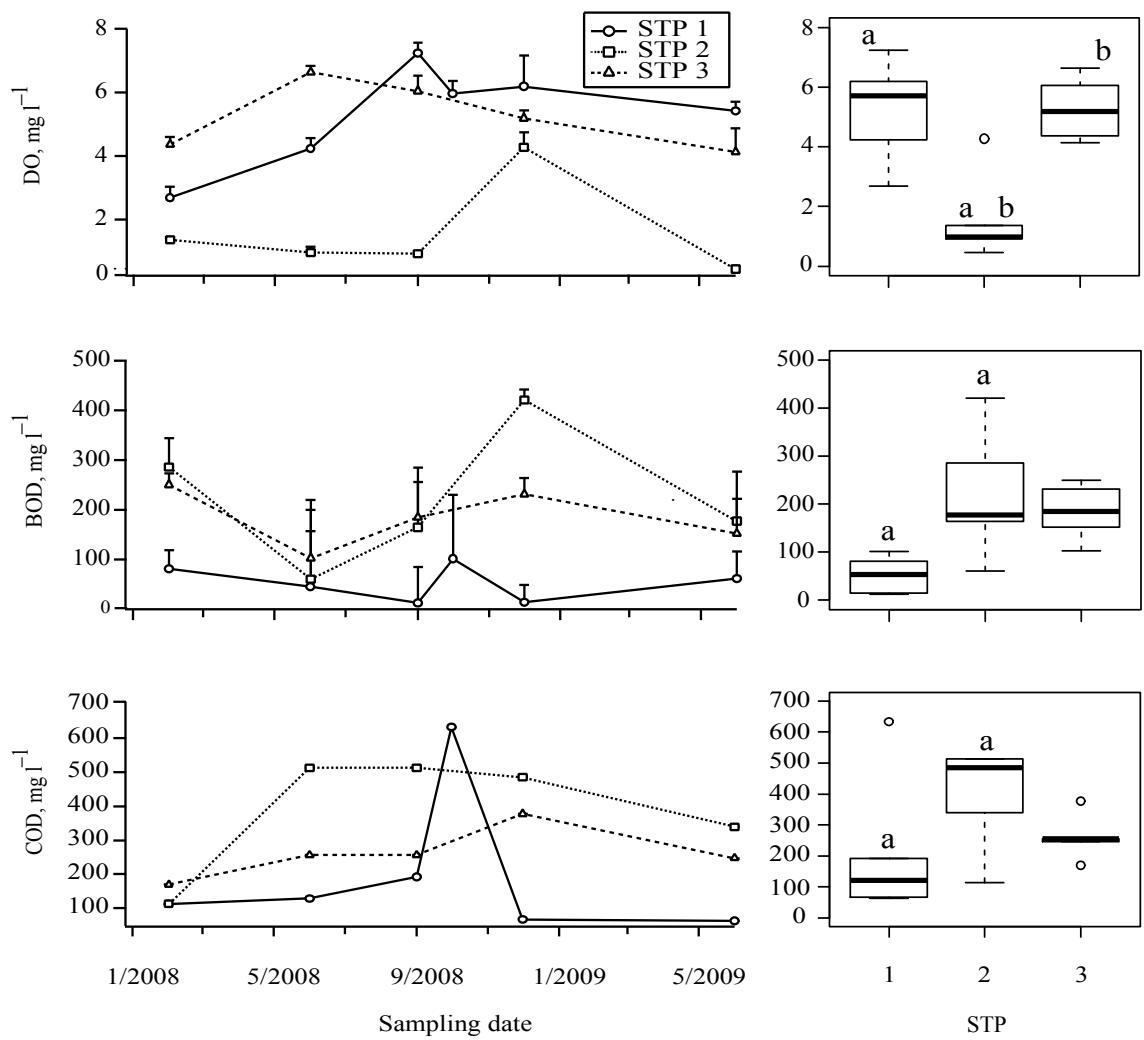
Percentage of denaturant solution	0%	100%
40% Acrylamide / bis solution, 37:5:1	4.0 ml	4.0 ml
50X Tris Acetate EDTA (TAE) buffer	0.4 ml	0.4 ml
Formamide	-	8.0 ml
Urea	-	8.4 ml
Sterile Milli-Q water top up to	20.0 ml	20.0 ml

Appendix D: Preparation of 20% and 70% denaturant solutions.

Stock of denaturant solution / %	20%	70%
0%	14.4 ml	5.4 ml
100%	3.6 ml	12.6 ml



Appendix E: Left panel shows the DO, BOD₅ and COD concentrations in the aeration tank at each STP throughout the sampling period. Right panel shows the respective box plot indicating median, and range of the profiles. Outliers are also shown as open circles. The same letters of the alphabet are used to indicate values whose means were significantly different.



Appendix F: Left panel shows the DO, BOD₅ and COD concentrations in the effluent at each STP throughout the sampling period. Right panel shows the respective box plot indicating median, and range of the profiles. Outliers are also shown as open circles. The same letters of the alphabet are used to indicate values whose means were significantly different.

Appendix G: Complete nutrients concentration measured in influent of the three STPs.

Sampling date (date/month/year)	STP 1	STP 2	STP 3
NH_3^+			
22/2/2008	44.21	49.43	46.26
9/6/2008	41.82	48.46	45.87
11/9/2008	41.80	47.63	43.31
17/10/2008	43.06	-	-
3/12/2008	43.47	52.57	35.57
24/6/2009	54.00	52.53	52.37
NO_2^-			
22/2/2008	0.26	0.34	0.17
9/6/2008	0.26	0.34	0.17
11/9/2008	0.24	0.36	0.33
17/10/2008	0.18	-	-
3/12/2008	0.10	0.44	0.19
24/6/2009	0.14	0.29	0.75
NO_3^-			
22/2/2008	0.22	0.95	0.12
9/6/2008	1.42	9.25	0.35
11/9/2008	0.86	1.45	1.58
17/10/2008	0.88	-	-
3/12/2008	0.35	0.21	0.01
24/6/2009	1.25	0.72	8.13
PO_4^{3-}			
22/2/2008	56.08	47.97	128.03
9/6/2008	59.64	81.47	34.25
11/9/2008	47.11	64.40	31.87
17/10/2008	30.69	-	-
3/12/2008	45.38	56.40	9.51
24/6/2009	62.88	63.75	59.75

Appendix H: Complete nutrients concentration measured in aeration tank of the three STPs.

Sampling date (date/month/year)	STP 1	STP 2	STP 3
NH ₃ ⁺			
22/2/2008	1.92	19.20	0.58
9/6/2008	1.53	7.47	0.19
11/9/2008	0.93	27.64	0.45
17/10/2008	0.35	-	-
3/12/2008	0.14	34.34	32.52
24/6/2009	0.60	20.07	7.66
NO ₂ ⁻			
22/2/2008	0.31	0.20	0.36
9/6/2008	0.31	0.20	0.36
11/9/2008	0.36	0.04	0.08
17/10/2008	0.24	-	-
3/12/2008	0.13	0.13	0.89
24/6/2009	0.34	0.46	0.31
NO ₃ ⁻			
22/2/2008	51.34	0.60	64.26
9/6/2008	5.69	58.97	46.87
11/9/2008	6.07	53.80	61.41
17/10/2008	4.05	-	-
3/12/2008	8.66	9.07	3.34
24/6/2009	8.32	27.66	53.09
PO ₄ ³⁻			
22/2/2008	71.53	85.46	18.26
9/6/2008	69.47	47.11	11.45
11/9/2008	51.75	63.10	7.67
17/10/2008	38.79	-	-
3/12/2008	50.78	17.94	2.05
24/6/2009	17.94	1.04	13.29

Appendix I: Complete nutrients concentration measured in effluent of the three STPs.

Sampling date (date/month/year)	STP 1	STP 2	STP 3
NH ₃ ⁺			
22/2/2008	0.15	41.98	3.08
9/6/2008	0.35	20.44	2.69
11/9/2008	0.12	39.12	1.97
17/10/2008	1.41	-	-
3/12/2008	16.05	39.93	1.32
24/6/2009	0.19	45.97	3.83
NO ₂ ⁻			
22/2/2008	0.10	3.33	0.97
9/6/2008	0.10	5.27	0.97
11/9/2008	0.26	0.15	0.26
17/10/2008	0.18	-	-
3/12/2008	0.51	1.49	0.14
24/6/2009	0.32	0.42	1.05
NO ₃ ⁻			
22/2/2008	54.26	0.74	41.71
9/6/2008	38.51	48.13	27.51
11/9/2008	51.12	42.10	62.12
17/10/2008	4.60	-	-
3/12/2008	1.24	49.45	1.64
24/6/2009	5.15	1.62	46.63
PO ₄ ³⁻			
22/2/2008	40.30	105.78	15.67
9/6/2008	56.94	61.05	11.24
11/9/2008	41.06	91.41	11.02
17/10/2008	42.35	-	-
3/12/2008	49.81	82.12	3.89
24/6/2009	15.13	84.06	5.51

Appendix J: Present-absent matrix of the bands on the DGGE gel picture in Figure 3.6.

O T U month/year	STP 1						STP 2						STP 3					
	2/08	6/08	9/08	10/08	12/08	6/09	2/08	6/08	9/08	12/08	6/09	2/08	6/08	9/08	12/08	6/09	2/08	
1	0	0	1	0	1	0	0	0	1	1	0	0	0	0	1	0	1	
2	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	1	
3	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	
4	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	
5	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	
6	0	0	0	1	0	1	0	1	0	1	1	0	1	1	1	1	1	
7	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1	0	0	
8	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	
9	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	
10	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	
11	0	1	1	1	0	0	1	1	1	0	1	0	0	0	0	0	0	
12	0	0	0	0	0	1	1	1	1	1	0	0	1	1	0	1	1	
13	0	1	1	1	0	0	0	0	0	1	0	1	1	1	1	1	1	
14	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	
15	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	
16	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	
17	0	0	1	1	1	1	0	1	0	0	0	0	1	1	0	0	0	
18	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	
19	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	
20	1	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0	
21	0	0	1	0	0	1	1	0	0	0	0	1	0	0	0	0	0	
22	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	
23	1	0	0	0	0	0	0	0	0	1	1	1	0	1	0	0	0	

Appendix J, continued.

OTU month/year	STP 1						STP 2						STP 3					
	2/08	6/08	9/08	10/08	12/08	6/09	2/08	6/08	9/08	12/08	6/09	2/08	6/08	9/08	12/08	6/09		
24	1	0	0	0	0	1	1	1	1	1	0	0	1	0	0	0	1	
25	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
26	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	
27	0	1	1	0	0	1	1	1	1	1	1	1	1	0	1	0	0	
28	1	1	0	1	1	1	1	1	0	0	1	1	0	0	0	0	0	
29	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	
30	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
31	1	1	0	0	1	1	1	1	0	0	1	1	1	0	0	0	0	
32	1	1	1	1	1	1	1	1	1	1	0	1	1	0	0	0	0	
33	0	1	1	1	0	1	1	1	0	0	0	0	0	0	0	0	0	
34	0	0	1	0	0	1	0	0	0	0	1	1	1	0	0	0	0	
35	1	1	1	1	1	0	0	0	1	0	1	1	1	1	0	0	0	
36	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	
37	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	
38	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	
39	1	0	0	1	1	0	1	0	0	1	1	1	1	0	0	1	1	
40	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
41	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	
42	0	0	1	0	0	0	0	0	1	0	1	1	1	1	1	1	1	
43	1	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	