APPENDIX C:

DATA, FIGURES, AND TABLES FROM MANUSCRIPT #2:

MEASURING NITRIFYING POPULATIONS IN A BIOLOGICAL AERATED FILTER SYSTEM USING QUANTITATIVE DOT BLOTTING

PROFILE # 2

2nd Column Profile

Date: **8/6/98**
Flows: **C column:** **13.0 gpm** **N column:** **11.5 gpm**
10.2 m/hr **9.0 m/hr**

Quantification of Mass Standards

Dilutions of *E.coli* and *N. europaea* probed with Universal Probe EUB338

Mass blotted	E. coli signal	N. europaea signal
667.7	237894.2	475636.1
267.1	163722.8	291172.4
133.5	114276.2	215239.2
26.7	34736.54	111083.4
2.7	1616.91	22897.38

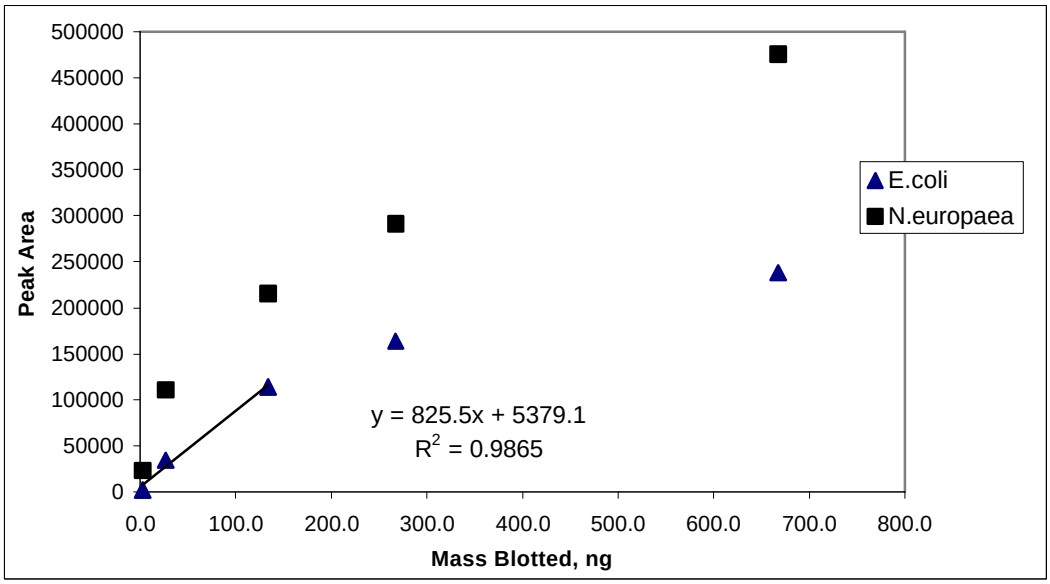


Figure C-1. EUB338 Mass Standard Fit, Profile 2, 8/6/98, 10.2 & 9.0 m/hr.

2nd Column Profile

Date: **8/6/98**
Flows: **C column:** **13.0 gpm** **N column:** **11.5 gpm**
10.2 m/hr **9.0 m/hr**

Quantification of Mass Standards

Dilutions of *E.coli* and *N. europaea* probed with Ammonia Oxidizer Probe Nso190.

Mass blotted	E. coli signal	N. europaea signal
667.7	53831.55	746961.5
267.1	23837.69	564764.8
133.5	13938.3	475629.5
26.7		209663.3
2.7		68826.6

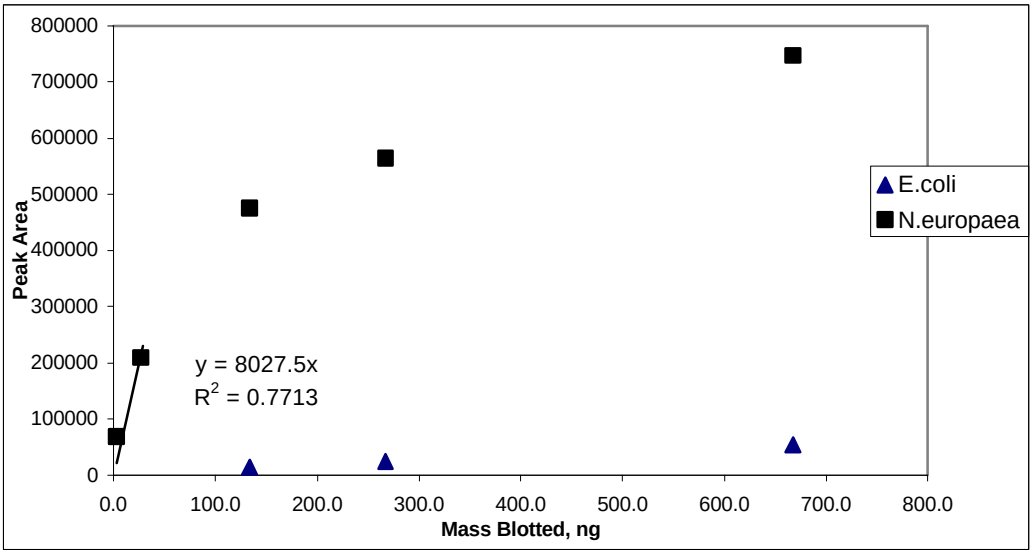


Figure C-2. Nso190 Mass Standard Fit, Profile 2, 8/6/98, 10.2 & 9.0 m/hr.

Table C-1. Profile 2 C Column EUB338 Densitometry Analysis Data 8/6/98

Flows: C column: 13.0 gpm N column: 11.5 gpm
10.2 m/hr 9.0 m/hr

Sample	Peak Area	Mass, per standard fit	Volume blotted, ul	Actual conc ng/ul	Total Average concentration
C1a	80888.63	91.47	15		10.91
	92546.06	105.59	15		
	47900.48	**51.51**	**5**	**10.30**	
	52945.72	**57.62**	**5**	**11.52**	
	13571.18	9.92	1		
	10534.96	6.25	1		
C1b	215055.7	254.00	15		20.99
	202347.6	238.61	15		
	77101.81	**86.88**	**5**	**17.38**	
	106919.7	**123.00**	**5**	**24.60**	
	19378.56	16.96	1		
	22002.54	20.14	1		
C2a	176356.4	207.12	42.8		10.13
	196639.2	231.69	42.8		
	123698.9	**143.33**	**14.3**	**10.02**	
	126217.2	**146.38**	**14.3**	**10.24**	
	36319.06	37.48	2.86		
C2b	140048.1	163.14	30.6		7.34
	86332.38	98.07	30.6		
	70779.7	**79.23**	**10.2**	**7.77**	
	63507.2	**70.42**	**10.2**	**6.90**	
	9348.644	4.81	2.04		
	1155.234	-5.12	2.04		
C3a	110381.9	127.20	15		10.59
	106051.7	121.95	15		
	47515.69	**51.04**	**5**	**10.21**	
	50689.75	**54.89**	**5**	**10.98**	
			1		
	14140.59	10.61	1		
C3b			60		16.12
			60		
	150246.6	175.49	20		
	157073.8	183.76	20		
	56736.52	**62.21**	**4**	**15.55**	
	60473.49	**66.74**	**4**	**16.69**	
C4a	117950.9	136.37	14.4		9.17
	99943.94	114.55	14.4		
	48151.19	**51.81**	**4.8**	**10.79**	
	35291.03	**36.23**	**4.8**	**7.55**	
	16060.86	12.94	0.96		
	12245.08	8.32	0.96		
C4b	98762.19	113.12	15		8.03
	88188.63	100.31	15		
	40022.34	**41.97**	**5**	**8.39**	
	36996.74	**38.30**	**5**	**7.66**	
	11270.91	7.14	1		
	13443.84	9.77	1		

Dilutions in bold print were selected for analysis based on quality of signal obtained on detection film.

Table C-2. Profile 2 C Column Nso190 Densitometry Analysis Data 8/6/98

Date: 8/6/98
Flows: C column: 13.0 gpm N column: 11.5 gpm
10.2 m/hr 9.0 m/hr

Sample	Peak Area	Mass, per standard fit	Volume blotted, ul	Actual conc. ng/ul	Total Average concentration	Fraction of nitrifier	Overall average
C1a	**48236.04**	**6.01**	**15**	**0.4006**	0.43	3.9%	3.5%
	54953.76	**6.85**	**15**	**0.4564**			
	28941.63	**3.61**	5				
	14031.49	**1.75**	5				
	7606.494	**0.95**	1				
	2092.914	**0.26**	1				
C1b	**75372**	**9.39**	**15**	**0.6259**	0.63	3.0%	
	75356.56	**9.39**	**15**	**0.6258**			
	41824.43	**5.21**	5				
	37637.06	**4.69**	5				
			1				
	14755.24	**1.84**	1				
C2a	**43417.6**	**5.41**	**42.8**	**0.1264**	0.14	1.4%	1.7%
	53528.0	**6.67**	**42.8**	**0.1558**			
	16654.1	**2.07**	14.3				
	30194.64	**3.76**	14.3				
			2.86				
			2.86				
C2b	**33649.6**	**4.19**	**30.6**	**0.1370**	0.15	2.0%	
	39223.7	**4.89**	**30.6**	**0.1597**			
	6797.0	**0.85**	10.2				
	18622.33	**2.32**	10.2				
			2.04				
			2.04				
C3a	**23631.45**	**2.94**	**15**	**0.1963**	0.15	1.4%	1.2%
	12891.64	**1.61**	**15**	**0.1071**			
	13997.78	**1.74**	5				
	4491.028	**0.56**	5				
			1				
			1				
C3b	**77435.1**	**9.65**	**60**	**0.1608**	0.16	1.0%	
	72043.9	**8.97**	**60**	**0.1496**			
	53522.2	**6.67**	20				
	34982.4	**4.36**	20				
			4				
	8625.4	**1.07**	4				
C4a	**50100.78**	**6.24**	**14.4**	**0.4334**	0.47	5.1%	4.5%
	58597.44	**7.30**	**14.4**	**0.5069**			
	20730.34	**2.58**	4.8				
	17760.01	**2.21**	4.8				
	5734.852	**0.71**	0.96				
	8861.006	**1.10**	0.96				
C4b	20702.01	**2.58**	15		0.32	3.9%	
			15				
	10880.93	**1.36**	**5**	**0.2711**			
	14558.82	**1.81**	**5**	**0.3627**			
	11093.61	**1.38**	1				
	2620.144	**0.33**	1				

Dilutions in bold print were selected for analysis based on quality of signal obtained on detection film.

Table C-3. **Profile 2** **N Column EUB338 Densitometry Analysis Data** **8/6/98**
Mass Standard Method

Flows: **C column:** **13.0 gpm** **N column:** **11.5 gpm**
10.2 m/hr **9.0 m/hr**

Sample	Peak Area	Mass, per standard fit	Volume blotted, ul	Actual conc. ng/ul	Total Average concentration
N1a	**106804.6**	**122.87**	**15**	**8.191**	9.35
	135466.9	**157.59**	**15**	**10.506**	
	67781.81	75.59	5		
	74511.69	83.75	5		
	11329.1	7.21	1		
	18106.33	15.42	1		
N1b	**76012.25**	**85.56**	**15**	**5.704**	8.17
	95071.94	**108.65**	**15**	**7.244**	
	56551.9	**61.99**	**5**	**12.398**	
	69529.13	77.71	5		
	11444.51	**7.35**	**1**	**7.348**	
	15744.19	12.56	1		
N2a	**84721**	**96.11**	**30**	**3.204**	3.50
	55589.33	**60.82**	**30**	**2.027**	
	48046.5	**51.69**	10	**5.169**	
	35173.4	**36.09**	**10**	**3.609**	
	10786.3	6.55	2		
	5649.156	0.33	2		
N2b	**57079.3**	**62.63**	**15**	**4.175**	3.18
	32314.24	**32.63**	**15**	**2.175**	
	21928.38	20.05	5		
	30227.33	30.10	5		
			1		
	12388.2	8.49	1		
N3a	**45898.1**	**49.08**	**44.1**	**1.113**	1.05
	41177.8	**43.37**	**44.1**	**0.983**	
	50695.6	54.90	14.7		
	25794.4	24.73	14.7		
			2.94		
			2.94		
N3b	**16780.27**	**13.81**	**16.7**	**0.827**	0.83
			16.7		
			5.6		
			5.6		
			1.11		
			1.11		
N4a	**30085**	**29.93**	**29.4**	**1.018**	0.93
	25859.4	**24.81**	**29.4**	**0.844**	
	20367.2	18.16	9.8		
	14195.2	10.68	9.8		
	7960.4	3.13	1.96		
	4000.6	-1.67	1.96		
N4b	**31414.65**	**31.54**	**22.1**	**1.427**	1.74
	39788.6	**41.68**	**22.1**	**1.886**	
	16452.8	**13.41**	**7.35**	**1.825**	
	16429.7	**13.39**	**7.35**	**1.821**	
	2878.465	-3.03	1.47		
	2842.641	-3.07	1.47		

Dilutions in bold print were selected for analysis based on quality of signal obtained on detection film.

Table C-4. Profile 2 N Column Nso190 Densitometry Analysis Data 8/6/98
Mass Standard Method

Flows: C column: 13.0 gpm N column: 11.5 gpm
10.2 m/hr 9.0 m/hr

Sample	Peak Area	Mass, per standard fit	Volume blotted, ul	Actual conc. ng/ul	Total Average concentration	Fraction of nitrifier	Overall average
N1a	28952.4	3.61	15		0.76	8.2%	8.9%
	28916.1	3.60	15				
	29074.1	3.62	**5**	**0.724**			
	32267	4.02	**5**	**0.804**			
			1				
			1				
N1b	**79185.7**	9.86	**15**	**0.658**	0.79	9.7%	
	69742.63	8.69	**15**	**0.579**			
	39617	4.94	**5**	**0.987**			
	38059.5	4.74	**5**	**0.948**			
			1				
			1				
N2a	56907.4	7.09	30		0.26	7.4%	6.3%
	24299.2	3.03	**30**	**0.101**			
			10				
			10				
			2				
	6747	0.84	**2**	**0.420**			
N2b	**19414.7**	2.42	**15**	**0.161**	0.16	5.1%	
			15				
			5				
			5				
			1				
			1				
N3a	**31702.2**	3.95	**44.1**	**0.090**	0.08	7.2%	7.2%
	22016.5	2.74	**44.1**	**0.062**			
			14.7				
			14.7				
	16140.5	2.01	2.94				
			2.94				
N3b			16.7				
			16.7				
			5.6		Poor signal quality		
			5.6				
			1.11				
			1.11				
N4a	17238	2.15	29.4	**0.073**	0.08	8.3%	10.0%
	13117.5	1.63	29.4	**0.056**			
			9.8				
	8153.8	1.02	9.8	**0.104**			
			1.96				
			1.96				
N4b	**52166.6**	6.50	22.1	**0.294**	0.20	11.6%	
			22.1				
	6524.8	0.81	7.35	**0.111**			
			7.35				
			1.47				
			1.47				

Dilutions in bold print were selected for analysis based on quality of signal obtained on detection film.

Table C-5. **Profile 2** **C Column Sample Concentration Determination** **8/6/98**
Spectrophotometry Method

Flows: **C column:** **13.0 gpm** **N column:** **11.5 gpm**
10.2 m/hr **9.0 m/hr**

Sample	Absorbance A_{260}	Average A260	Absorbance A_{280}	Average A280	Conc., ng/ul
C1a	0.0402	0.0389	0.0261	0.025	309.9
	0.0399		0.0259		
	0.0379		0.0239		
	0.0376		0.0238		
C1b	0.0236	0.0232	0.013	0.012	202.4
	0.0237		0.0132		
	0.0228		0.0118		
	0.0227		0.0117		
C2a	0.0048	0.004625	0.0023	0.002	41.3
	0.0047		0.0024		
	0.0044		0.0023		
	0.0046		0.0024		
C2b	0.0078	0.006275	0.0048	0.004	53.4
	0.0077		0.0044		
	0.0048		0.0025		
	0.0048		0.0025		
C3a	0.0283	0.0241	0.0151	0.013	213.2
	0.0283		0.0151		
	0.0198		0.0097		
	0.02		0.0101		
C3b	0.0035	0.003575	0.0011	0.002	34.2
	0.0034		0.0013		
	0.0037		0.0018		
	0.0037		0.0018		
C4a	0.0136	0.013775	0.0068	0.007	122.0
	0.0136		0.0068		
	0.0141		0.0077		
	0.0138		0.0072		
C4b	0.0233	0.025025	0.0116	0.013	224.6
	0.0234		0.0118		
	0.0265		0.0131		
	0.0269		0.0136		

Concentration determined by: C = A260*62.9 - A280*36

Table C-6. **Profile 2** **C Column Nso190 Densitometry Analysis Data** **8/6/98**
Spectrophotometry Method

Flows: **C column:** **13.0 gpm** **N column:** **11.5 gpm**
10.2 m/hr **9.0 m/hr**

Sample	Dilution Made	Diluted Conc., ng/ul	Denatured Conc., ng/ul	vol. Blotted ul	Blot Mass ng	Peak Area	Area/mass	Overall port averages	Standard deviation
C1a	0.313	96.999	24.250	**15**	**363.75**	**48236.04**	132.61	159.3	21.5
		96.999	24.250	**15**	**363.75**	**54953.76**	151.08		
		96.999	24.250	5	121.25	28941.63			
		96.999	24.250	5	121.25	14031.49			
		96.999	24.250	1	24.25	7606.494			
		96.999	24.250	1	24.25	2092.914			
C1b	0.562	113.747	28.437	**15**	**426.55**	**75372**	176.70		
		113.747	28.437	**15**	**426.55**	**75356.56**	176.67		
		113.747	28.437	5	142.18	41824.43			
		113.747	28.437	5	142.18	37637.06			
		113.747	28.437	1	28.44				
		113.747	28.437	1	28.44	14755.24			
C2a	1	41.263	10.316	**42.8**	**441.51**	**43417.6**	98.34	99.5	16.1
		41.263	10.316	**42.8**	**441.51**	**53528.0**	121.24		
		41.263	10.316	14.3	147.51	16654.1			
		41.263	10.316	14.3	147.51	30194.64			
		41.263	10.316						
		41.263	10.316	2.86	29.50				
C2b	1	53.380	13.345	**30.6**	**408.35**	**33649.6**	**82.40**		
		53.380	13.345	**30.6**	**408.35**	**39223.7**	**96.05**		
		53.380	13.345	10.2	136.12	6797.0			
		53.380	13.345	10.2	136.12	18622.33			
		53.380	13.345	2.04	27.22				
		53.380	13.345	2.04	27.22				
C3a	0.546	116.395	29.099	15	436.48	23631.45		145.8	7.4
		116.395	29.099	15	436.48	12891.64			
		116.395	29.099	**5**	145.49	13997.78	signals not reliably		
		116.395	29.099	**5**	145.49	4491.028	quantifiable: too light		
		116.395	29.099	1	29.10		sample C3b used		
		116.395	29.099	1	29.10				
C3b	1	34.174	8.543	**60**	**512.60**	**77435.1**	**151.06**		
		34.174	8.543	**60**	**512.60**	**72043.9**	**140.55**		
		34.174	8.543	20	170.87	53522.2			
		34.174	8.543	20	170.87	34982.4			
		34.174	8.543	4	34.17				
		34.174	8.543	4	34.17	8625.4			
C4a	1	121.990	30.497	**14.4**	**439.16**	**50100.78**	**114.08**	113.5	25.1
		121.990	30.497	**14.4**	**439.16**	**58597.44**	**133.43**		
		121.990	30.497	**4.8**	**146.39**	**20730.34**	**141.61**		
		121.990	30.497	**4.8**	**146.39**	**17760.01**	**121.32**		
		121.990	30.497	0.96	29.28	5734.852			
		121.990	30.497	0.96	29.28	8861.006			
C4b	0.532	119.506	29.876	15	448.15	20702.01			
		119.506	29.876	15	448.15				
		119.506	29.876	**5**	**149.38**	**10880.93**	**72.84**		
		119.506	29.876	**5**	**149.38**	**14558.82**	**97.46**		
		119.506	29.876	1	29.88	11093.61			
		119.506	29.876	1	29.88	2620.144			

Table C-7. **Profile 2** **N Column Sample Concentration Determination** **8/6/98**
Spectrophotometry Method

Flows: **C column:** **13.0 gpm** **N column:** **11.5 gpm**
10.2 m/hr **9.0 m/hr**

Sample	Absorbance A_{260}	Average A260	Absorbance A_{280}	Average A280	Conc., ng/ul
N1a	0.0471	0.0451	0.0254	0.024125	393.7
	0.047		0.0256		
	0.0431		0.0227		
	0.0432		0.0228		
N1b	0.0584	0.055175	0.0304	0.02865	487.8
	0.0583		0.0304		
	0.0521		0.0268		
	0.0519		0.027		
N2a	0.0065	0.006675	0.0037	0.003375	59.7
	0.0067		0.0037		
	0.0067		0.003		
	0.0068		0.0031		
N2b	0.0283	0.0147	0.0236	0.009417	117.1
	0.0165		0.0097		
	0.0085		0.0038		
	0.0088		0.0039		
	0.0131		0.0078		
	0.013		0.0077		
N3a	0.0042	0.004275	0.0027	0.0026	35.1
	0.0043		0.0026		
	0.004		0.0023		
	0.0046		0.0028		
N3b	0.0119	0.00723333	0.0086	0.00485	56.1
	0.0122		0.0088		
	0.0039		0.0024		
	0.0039		0.0023		
	0.0056		0.0036		
	0.0059		0.0034		
N4a	0.0064	0.00605	0.0044	0.0042	45.9
	0.0077		0.0058		
	0.0049		0.0032		
	0.0052		0.0034		
N4b	0.0126	0.00853333	0.0083	0.005183	70.0
	0.0147		0.0096		
	0.0058		0.0031		
	0.006		0.0032		
	0.006		0.0034		
	0.0061		0.0035		

Concentration determined by: C = A260*62.9 - A280*36

Table C-8. **Profile 2** **N Column Nso190 Densitometry Analysis Data** **8/6/98**
Spectrophotometry Method

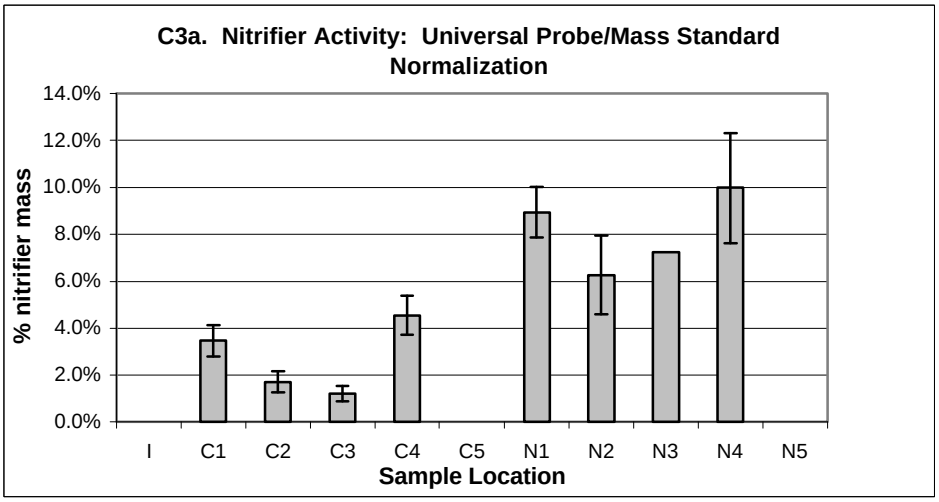
Flows: **C column:** **13.0 gpm** **N column:** **11.5 gpm**
10.2 m/hr **9.0 m/hr**

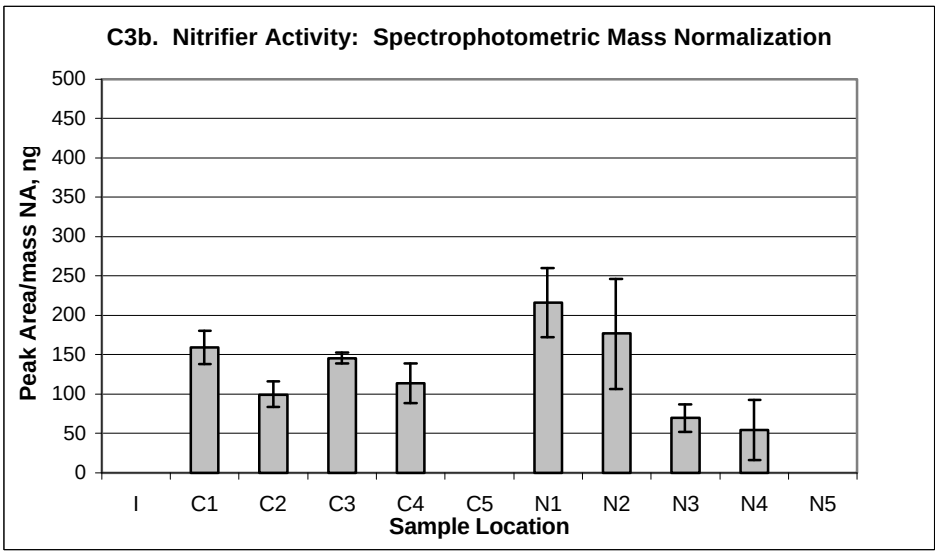
Sample	Dilution Made	Diluted Conc., ng/ul	Denatured Conc., ng/ul	vol. Blotted ul	Blot Mass ng	Peak Area	Area/mass	Overall port averages	Standard deviation
N1a	0.289	113.77	28.442	15	426.63	28952.4		217.5	44.3
		113.77	28.442	15	426.63	28916.1			
		113.77	28.442	**5**	**142.21**	**29074.1**	**204.45**		
		113.77	28.442	**5**	**142.21**	**32267**	**226.90**		
		113.77	28.442	1	28.44				
		113.77	28.442	1	28.44				
N1b	0.239	116.59	29.147	**15**	**437.21**	**79185.7**	**181.12**		
		116.59	29.147	**15**	**437.21**	**69742.63**	**159.52**		
		116.59	29.147	**5**	**145.74**	**39617**	**271.84**		
		116.59	29.147	**5**	**145.74**	**38059.5**	**261.15**		
		116.59	29.147	1	29.15				
		116.59	29.147	1	29.15				
N2a	1	59.67	14.918	**30**	**447.54**	**56907.4**	**127.16**	176.6	70.0
		59.67	14.918	30	447.54	24299.2			
		59.67	14.918	10	149.18				
		59.67	14.918	10	149.18				
		59.67	14.918	2	29.84				
		59.67	14.918	**2**	**29.84**	**6747**	**226.14**		
N2b	0.826	96.75	24.187	15	362.80	19414.7			
		96.75	24.187	15	362.80				
		96.75	24.187	5	120.93				
		96.75	24.187	5	120.93				
		96.75	24.187	1	24.19				
		96.75	24.187	1	24.19				
N3a	1	35.06	8.765	**44.1**	386.53	**31702.2**	**82.02**	69.5	17.7
		35.06	8.765	**44.1**	386.53	**22016.5**	**56.96**		
		35.06	8.765	14.7	128.84				
		35.06	8.765	14.7	128.84				
		35.06	8.765	2.94	25.77	16140.5			
		35.06	8.765	2.94	25.77				
N3b	1	56.08	14.019	16.7	234.11				
		56.08	14.019	16.7	234.11				
		56.08	14.019	5.6	78.51				
		56.08	14.019	5.6	78.51				
		56.08	14.019	1.11	15.56				
		56.08	14.019	1.11	15.56				
N4a	1	45.87	11.467	**29.4**	**337.14**	**17238**	**51.13**	53.3	14.0
		45.87	11.467	**29.4**	**337.14**	**13117.5**	**38.91**		
		45.87	11.467	9.8	112.38				
		45.87	11.467	**9.8**	**112.38**	**8153.8**	**72.56**		
		45.87	11.467	1.96	22.48				
		45.87	11.467	1.96	22.48				
N4b	1	70.03	17.507	22.1	386.91	52166.6			
		70.03	17.507	22.1	386.91				
		70.03	17.507	**7.35**	**128.68**	**6524.8**	**50.71**		
		70.03	17.507	7.35	128.68				
		70.03	17.507	1.47	25.74				
		70.03	17.507	1.47	25.74				

Table C-9. Profile 2. Nitrogen Species and Ammonia Oxidizer Activity 8/6/98

Flows: C column: 13.0 gpm N column: 11.5 gpm
10.2 m/hr 9.0 m/hr

		Chemical Nitrogen Species					Ammonia Oxidizer Activity			
							Mass Standard Method		Spectrophotometry Method	
Sample	Media Distance, m	NH_3 mg/L-N	sol. TKN mg/L-N	N-org mg/L-N	Nitrate mg/L - N	Nitrite mg/L -N	Nitrifier Fraction	Standard Deviation	Peak Area/ Mass	Standard Deviation
I (grab)	0	15.1	21.7	6.6	0.1	0.1				
C1	0.58	14.6	20.9	6.3	0.1	0.1	3.5%	0.67%	159.3	21.5
C2	1.42	16.0	20.6	4.6	0.1	0.1	1.7%	0.45%	99.5	16.1
C3	2.26	16.8	20.0	3.2	0.08	0.1	1.2%	0.33%	145.8	7.4
C4	3.07	17.1	19.8	2.7	0.08	0.1	4.5%	0.83%	113.5	25.1
C5	3.91	16.0	18.9	2.9	0.08	0.1				
N1	4.39	15.5	18.6	3.1	0.35	0.64	8.9%	1.1%	215.67	44.28
N2	5.28	11.4	14.1	2.7	1.58	0.67	6.3%	1.7%	176.65	69.99
N3	6.10	6.7	9.2	2.5	5.37	0.98	7.2%		69.49	17.72
N4	6.91	5.2	7.0	1.8	5.96	0.8	10.0%	2.3%	54.20	38.42
N5	7.75	1.5	3.6	2.1	9.86	0.83				





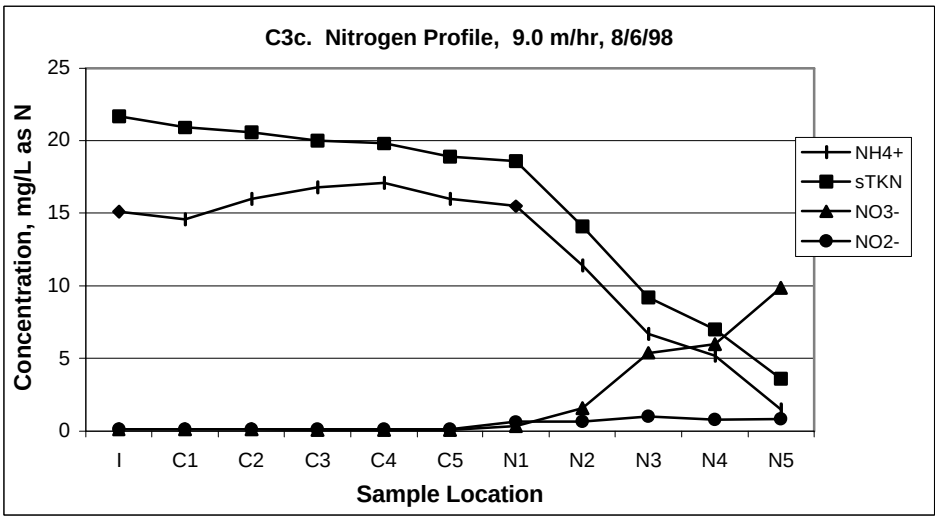


Figure C-3. Column Profiles at 10.2 & 9.0 m/hr, 8/6/98. a. Nitrifier activity normalized to mass standards; b. Nitrifier activity normalized to nucleic acid mass; c. Chemical nitrogen species.