

APPENDIX E:

DATA, FIGURES, AND TABLES
FROM MANUSCRIPT #2:

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

PROFILE # 4

4th Column Profile

Date: 9/30/98
 Flows: C column: 12.0 gpm 9.4 m/hr N column: 10.5 gpm 8.2 m/hr

Quantification of Mass Standards

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

EUB338

Mass blotted	E. coli signal	N. europaea signal
667.7	217301.5	363512.5
267.1	102998.31	256134
133.5	116076.5	178695.1
26.7	29349.1	65959.6
2.7	3628.5	5457.3

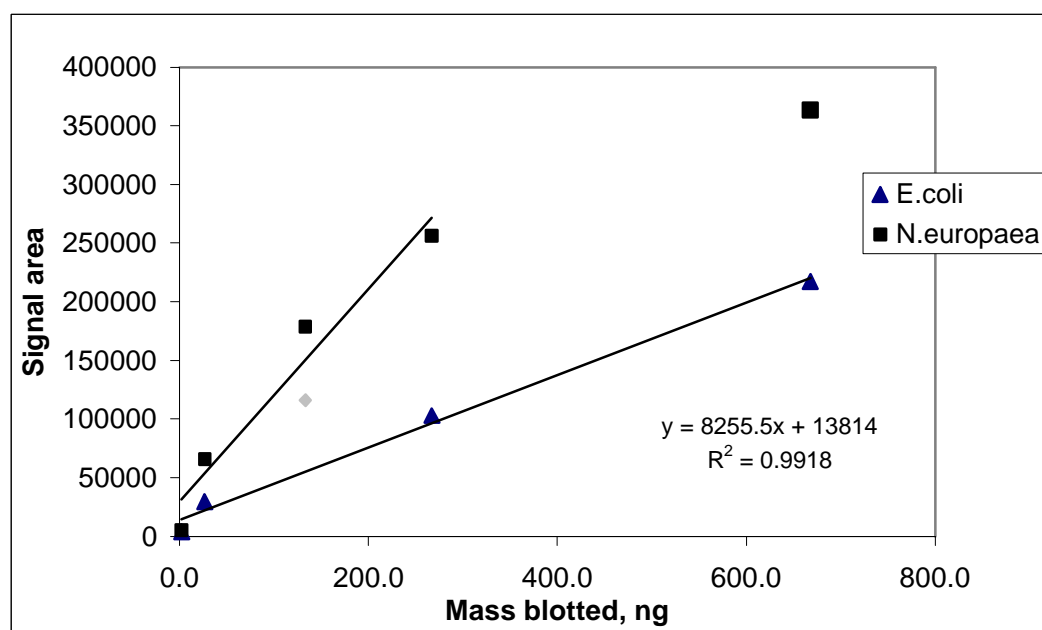


Figure E-1. EUB338 Mass Standard Fit, Profile 4, 9/30/98, 9.4 & 8.2 m/hr.

4th Column Profile

Date: 9/30/98

Flows: C column: 12.0 gpm N column: 10.5 gpm
 9.4 m/hr 8.2 m/hr

Quantification of Mass Standards

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

Mass blotted	E. coli signal	N. europaea signal
667.7	79209.9	792095.8
267.1	98759.4	782903.6
133.5	66119.3	459684.4
26.7		214431.9
2.7		80695.13

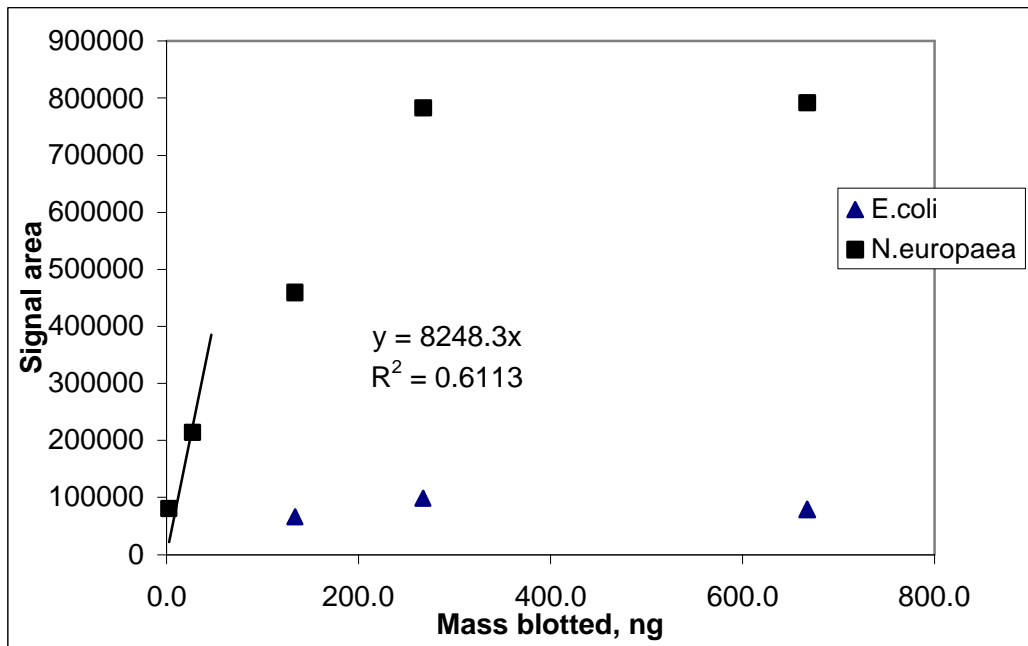


Figure E-2. Nso190 Mass Standard Fit, Profile 4, 9/30/98, 9.4 & 8.2 m/hr.

Table E-1. Profile 4 C Column EUB338 Densitometry Analysis Data

9/30/98

Flows: C column: 12.0 gpm 9.4 m/hr N column: 10.5 gpm 8.2 m/hr

Sample	Approx. Mass NA blotted, ng	Peak Area	Mass, per standard fit	Volume Blotted, ul	Actual conc. ng/ul	Total Average concentration
C1a	15	83085.25	8.39	1.65	5.09	4.94
	15	79147.56	7.91	1.65	4.80	
	5	28262.93	1.75	0.551		
	5	40440.46	3.23	0.551		
	1	10981.84	-0.34	0.11		
	1	8380.175	-0.66	0.11		
C1b	15	132740.4	14.41	2.28		7.79
	15	134666.5	14.64	2.28		
	5	65705.32	6.29	0.76	8.27	
	5	59611.9	5.55	0.76	7.30	
	1	53467.53	4.80	0.152		
	1	19175.37	0.65	0.152		
C2a	15	72103.38	7.06	22.4	0.32	0.34
	15	81329.38	8.18	22.4	0.37	
	5	35682.33	2.65	7.46		
	5	34057.03	2.45	7.46		
	1	14189.67	0.05	1.49		
	1	4327.805	-1.15	1.49		
C2b	15	158293	17.50	27.3		1.21
	15	202377.8	22.84	27.3		
	5	116411.2	12.43	9.09	1.37	
	5	92203.7	9.50	9.09	1.04	
	1	83721.44	8.47	1.82		
	1	41407.99	3.34	1.82		
C3a	15	133541.3	14.50	8.62		2.29
	15	142437.4	15.58	8.62		
	5	59007.9	5.47	2.87	1.91	
	5	77194.63	7.68	2.87	2.68	
	1	66282.25	6.36	0.575		
	1	87013.56	8.87	0.575		
C3b	15	105090.2	11.06	4.64		3.48
	15	124031	13.35	4.64		
	5	61183.59	5.74	1.55	3.70	
	5	55420.93	5.04	1.55	3.25	
	1	17815.63	0.48	0.31		
	1	18077.01	0.52	0.31		
C4a	15	103030.88	10.81	7.46	1.45	1.64
	15	126286.3	13.62	7.46	1.83	
	5	39521.57	3.11	2.49		
	5	127930.9	13.82	2.49		
	1	17620.64	0.46	0.498		
	1	9903.9	-0.47	0.498		
C4b	15	159917.4	17.70	7.98	2.22	2.21
	15	158760.6	17.56	7.98	2.20	
	5	59667.51	5.55	2.66		
	5	41892.2	3.40	2.66		
	1	12755.54	-0.13	0.532		
	1	16381.89	0.31	0.532		

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

Table E-2.

Profile 4 C Column Nso190 Densitometry Analysis Data

9/30/98

Flows: C column: 12.0 gpm N column: 10.5 gpm
 9.4 m/hr 8.2 m/hr

Sample	Approx. Mass NA blotted, ng	Peak Area	Mass, per standard fit	Volume Blotted, ul	Actual conc. ng/ul	Total Average concentration	Fraction of nitrifier	Overall average
C1a	50	324003.8	1.47	5.5		0.57	11.6%	9.5%
	50	268576.1	1.22	5.5				
	10	135789.2	0.62	1.1				
	10	86904.9	0.39	1.1				
	5	96559.8	0.44	0.6	0.80			
	5	42513.1	0.19	0.6	0.35			
C1b	50	416615.9	1.89	7.6		0.57	7.3%	
	50	387814.2	1.76	7.6				
	10	154812.76	0.70	1.5				
	10	123088.7	0.56	1.5				
	5	96320.3	0.44	0.8	0.58			
	5	94591.32	0.43	0.8	0.56			
C2a	50	366288.8	1.66	74.6		0.04	13.2%	10.9%
	50	263980.1	1.20	74.6				
	10	144867.8	0.66	14.9				
	10	146177.7	0.66	14.9				
	5	53746.3	0.24	7.5	0.03			
	5	93790.38	0.43	7.5	0.06			
C2b	50	418330.2	1.90	90.1		0.10	8.6%	
	50	386978.1	1.76	90.1				
	10	265132.6	1.20	18.2				
	10	271132.7	1.23	18.2				
	5	209372.4	0.95	9.1	0.10			
	5	206702.4	0.94	9.1	0.10			
C3a	50	289710.6	1.32	28.0		0.09	3.9%	4.1%
	50	279573.6	1.27	28.0				
	10	121827	0.55	5.8	0.10			
	10	107848.2	0.49	5.8	0.09			
	5	55706.54	0.25	2.9	0.09			
	5	55532.25	0.25	2.9	0.09			
C3b	50	215646.8	0.98	15.5		0.15	4.3%	
	50	226853.9	1.03	15.5				
	10	91811.78	0.42	3.1	0.13			
	10	96104.56	0.44	3.1	0.14			
	5	59864.78	0.27	1.6	0.18			
	5	42030.81	0.19	1.6	0.12			
C4a	50	283219.5	1.29	24.9		0.13	7.8%	6.3%
	50	272287.5	1.24	24.9				
	10	108437.7	0.49	5.0	0.10			
	10	113481.5	0.52	5.0	0.10			
	5	90639.81	0.41	2.5	0.17			
	5	49253.99	0.22	2.5	0.09			
C4b	50	263048.4	1.19	26.6		0.11	4.9%	
	50	254285.3	1.15	26.6				
	10	115782.3	0.53	5.3	0.10			
	10	89940.68	0.41	5.3	0.08			
	5	61017.39	0.28	2.7	0.10			
	5	64905.72	0.29	2.7	0.11			

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

Italics indicate poor sample signal quality.

Table E-3. Profile 4 N Column EUB338 Densitometry Analysis Data

9/30/98

Flows: C column: 12.0 gpm 9.4 m/hr N column: 10.5 gpm 8.2 m/hr

Sample	Peak Area	Mass, per standard fit	Volume Blotted, ul	Actual conc. ng/ul	Total Average concentration
N1a	65809.5	6.30	5.49	1.15	1.33
	64430.69	6.13	5.49	1.12	
	63614.99	6.03	3.66	1.65	
	56380.98	5.16	3.66	1.41	
	21681.83	0.95	0.37		
	20568.91	0.82	0.37		
N1b	92607.19	9.54	5.81	1.64	1.94
	115091.8	12.27	5.81	2.11	
	79833.56	8.00	3.88	2.06	
	155554.4	17.17	3.88		
	58059.51	5.36	0.39		
	36972.59	2.81	0.39		
N2a	87267.31	8.90	6.25		2.15
	77037.69	7.66	6.25		
	77214.44	7.68	4.17	1.84	
	88878.38	9.09	4.17	2.18	
	21553.61	0.94	0.42	2.25	
	21770.23	0.96	0.42	2.31	
N2b	57660	5.31	5.54	0.96	1.23
	79802.5	7.99	5.54	1.44	
	53158.51	4.77	3.69	1.29	
	61461.64	5.77	3.69		
	25710.65	1.44	0.37		
	15124.41	0.16	0.37		
N3a	92255.94	9.50	5.26		2.27
	81238.75	8.17	5.26		
	51038.5	4.51	3.51	1.28	
	79418.44	7.95	3.51	2.26	
	17543.73	0.45	0.35	1.29	
	26081.21	1.49	0.35	4.23	
N3b	158266.9	17.50	1.26		12.37
	155480	17.16	1.26		
	99047.06	10.32	0.84	12.29	
	100109.38	10.45	0.84	12.44	
	13954.05	0.02	0.084		
	38809.98	3.03	0.084		
N4a	49447.86	4.32	36.6	0.12	0.11
	38309.23	2.97	36.6	0.08	
			24.4		
	42025.14	3.42	24.4	0.14	
	11857.38	-0.24	2.4		
	11454.02	-0.29	2.4		
N4b	43253.01	3.57	10.6	0.34	0.27
	36827.5	2.79	10.6	0.26	
	23778.19	1.21	7.1	0.17	
	31121.41	2.10	7.1	0.30	
	4627.586	-1.11	0.709		
	4887.824	-1.08	0.709		

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

Table E-4. Profile 4 N Column Nso190 Densitometry Analysis Data

9/30/98

Flows: C column: 12.0 gpm 9.4 m/hr N column: 10.5 gpm 8.2 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	177953.3	0.8	18.3		0.13	9.6%	8.3%
	231337.2	1.1	18.3				
	98437.94	0.4	3.66	0.12			
	71856.56	0.3	3.66	0.09			
	66201.69	0.3	1.83	0.16			
	54546.75	0.2	1.83	0.14			
N1b	279516.6	1.3	19.4		0.13	7.0%	
	244873	1.1	19.4				
	89105.44	0.4	3.88	0.10			
	122949.2	0.6	3.88	0.14			
	61848.12	0.3	1.94	0.14			
	62523.58	0.3	1.94	0.15			
N2a	223158.2	1.0	20.8		0.14	6.7%	5.8%
	280454.8	1.3	20.8				
	86410.7	0.4	4.17	0.09			
	113768.2	0.5	4.17	0.12			
	99824.81	0.5	2.08	0.22			
	62584.86	0.3	2.08	0.14			
N2b	145519.4	0.7	18.4	0.04	0.06	4.9%	
	141476.7	0.6	18.4	0.03			
	45522.4	0.2	3.69	0.06			
	40033.45	0.2	3.69	0.05			
	46975.43	0.2	1.85	0.12			
	29727.56	0.1	1.85	0.07			
N3a	143218.11	0.7	17.5		0.07	3.0%	1.9%
	210273.4	1.0	17.5				
	45928.67	0.2	3.51	0.06			
	45897.01	0.2	3.51	0.06			
	29149.65	0.1	1.75	0.08			
	29398.59	0.1	1.75	0.08			
N3b	41937.71	0.2	4.17		0.11	0.9%	
	51079.74	0.2	4.17				
	19419.76	0.1	0.834	0.11			
	17350.91	0.1	0.834	0.09			
	10022.273	0.0	0.417	0.11			
	11639.375	0.1	0.417	0.13			
N4a	147869.2	0.7	122		0.01	9.9%	8.7%
	150324.3	0.7	122				
	53107.39	0.2	24.4	0.01			
	48166.18	0.2	24.4	0.01			
	43199.9	0.2	12.2	0.02			
	26233.19	0.1	12.2	0.01			
N4b	90313.38	0.4	35.5		0.02	7.5%	
	125413.5	0.6	35.5				
	35174.93	0.2	7.09	0.02			
	20307.638	0.1	7.09	0.01			
	17933.35	0.1	3.55	0.02			
	17059.069	0.1	3.55	0.02			

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

Table E-5.

**Profile 4 C Column Sample Concentration Determination
Spectrophotometry Method**

9/30/98

Flows: C column: 12.0 gpm 9.4 m/hr N column: 10.5 gpm 8.2 m/hr

Sample	Absorbance A_{260}	Average A260	Absorbance A_{280}	Average A280	Conc., ng/ul
C1a	0.1224	0.1225	0.0592	0.059	980.0
	0.1226		0.0591		980.0
					980.0
					980.0
					980.0
C1b	0.088	0.08815	0.0434	0.043	705.2
	0.0883		0.0434		705.2
					705.2
					705.2
					705.2
C2a	0.0089	0.00905	0.0042	0.004	72.4
	0.0092		0.0045		72.4
					72.4
					72.4
					72.4
C2b	0.0069	0.00695	0.004	0.004	55.6
	0.007		0.004		55.6
					55.6
					55.6
					55.6
C3a	0.0222	0.0222	0.0126	0.013	177.6
					177.6
					177.6
					177.6
					177.6
C3b	0.0439	0.037	0.0396	0.028	296.0
	0.0301		0.0156		296.0
					296.0
					296.0
					296.0
C4a	0.0248	0.02575	0.014	0.014	206.0
	0.0267		0.0149		206.0
					206.0
					206.0
					206.0
C4b	0.025	0.02505	0.0125	0.013	200.4
	0.0251		0.0125		200.4
					200.4
					200.4
					200.4

Concentration determined by: $C = A_{260} * 62.9 - A_{280} * 36$

Table E-6.

**Profile 4 C Column Nso190 Densitometry Analysis Data
Spectrophotometry Method**

9/30/98

**Flows: C column: 12.0 gpm N column: 10.5 gpm
9.4 m/hr 8.2 m/hr**

Sample	Dilution Made	Diluted Conc., ng/u	Denatured Conc., ng/ul	vol. Blotted ul	Blot Mass ng	Peak Area	Area/mass	Overall por averages	Standard deviation
C1a	0.1	98.0	24.5	5.5	134.75	324003.8		614.2	199.2
	0.1	98.0	24.5	5.5	134.75	268576.1			
	0.1	98.0	24.5	1.1	26.95	135789.2			
	0.1	98.0	24.5	1.1	26.95	86904.9			
	0.1	98.0	24.5	5.5	134.75	96559.8	716.58		
	0.1	98.0	24.5	5.5	134.75	42513.1	315.50		
C1b	0.1	70.5	17.6	7.6	133.988	416615.9		543.0	121.2
	0.1	70.5	17.6	7.6	133.988	387814.2			
	0.1	70.5	17.6	1.5	26.7976	154812.8			
	0.1	70.5	17.6	1.5	26.7976	123088.7			
	0.1	70.5	17.6	7.6	133.988	96320.3	718.87		
	0.1	70.5	17.6	7.6	133.988	94591.32	705.97		
C2a	1	72.4	18.1	74.6	1350.26	366288.8		435.0	47.3
	1	72.4	18.1	74.6	1350.26	263980.1			
	1	72.4	18.1	14.9	269.69	144867.8	537.16		
	1	72.4	18.1	14.9	269.69	146177.7	542.02		
	1	72.4	18.1	7.5	135.026	53746.3	398.04		
	1	72.4	18.1	7.5	135.026	93790.38	694.61		
C2b	1	55.6	13.9	90.1	1252.39	418330.2		423.7	49.4
	1	55.6	13.9	90.1	1252.39	386978.1			
	1	55.6	13.9	18.2	252.98	265132.6	all at saturation		
	1	55.6	13.9	18.2	252.98	271132.7			
	1	55.6	13.9	9.1	126.351	209372.4			
	1	55.6	13.9	9.1	126.351	206702.4			
C3a	1	177.6	44.4	28.0	1243.2	289710.6		423.7	49.4
	1	177.6	44.4	28.0	1243.2	279573.6			
	1	177.6	44.4	5.8	255.3	121827	477.19		
	1	177.6	44.4	5.8	255.3	107848.2	422.44		
	1	177.6	44.4	2.9	127.428	55706.54	437.16		
	1	177.6	44.4	2.9	127.428	55532.25	435.79		
C3b	1	296.0	74.0	15.5	1147	215646.8		423.7	49.4
	1	296.0	74.0	15.5	1147	226853.9			
	1	296.0	74.0	3.1	229.4	91811.78	400.23		
	1	296.0	74.0	3.1	229.4	96104.56	418.94		
	1	296.0	74.0	1.6	114.7	59864.78	521.92		
	1	296.0	74.0	1.6	114.7	42030.81	366.44		
C4a	1	206.0	51.5	24.9	1282.35	283219.5		423.7	49.4
	1	206.0	51.5	24.9	1282.35	272287.5			
	1	206.0	51.5	5.0	256.47	108437.7	422.81		
	1	206.0	51.5	5.0	256.47	113481.5	442.47		
	1	206.0	51.5	2.5	128.235	90639.81			
	1	206.0	51.5	2.5	128.235	49253.99	384.09		
C4b	1	200.4	50.1	26.6	1332.66	263048.4		423.7	49.4
	1	200.4	50.1	26.6	1332.66	254285.3			
	1	200.4	50.1	5.3	266.532	115782.3	434.40		
	1	200.4	50.1	5.3	266.532	89940.68	337.45		
	1	200.4	50.1	2.7	133.266	61017.39	457.86		
	1	200.4	50.1	2.7	133.266	64905.72	487.04		

Table E-7. Profile 4 N Column Sample Concentration Determination Spectrophotometry Method

9/30/98

Flows: C column: 12.0 gpm 9.4 m/hr N column: 10.5 gpm 8.2 m/hr

Sample	Absorbance A ₂₆₀	Average A260	Absorbance A ₂₈₀	Average A280	Conc., ng/ul
N1a	0.0319	0.03545	0.0172	0.019	283.6
	0.039		0.0211		283.6
					283.6
					283.6
					283.6
					283.6
N1b	0.0335	0.03375	0.0178	0.018	270.0
	0.034		0.018		270.0
					270.0
					270.0
					270.0
					270.0
N2a	0.0311	0.03115	0.0166	0.017	249.2
	0.0312		0.0169		249.2
					249.2
					249.2
					249.2
					249.2
N2b	0.0367	0.03605	0.0182	0.018	288.4
	0.0354		0.018		288.4
					288.4
					288.4
					288.4
					288.4
N3a	0.0374	0.0374	0.0194	0.020	299.2
	0.0374		0.0196		299.2
					299.2
					299.2
					299.2
					299.2
N3b	0.068	0.07795	0.0362	0.036	623.6
	0.0879				623.6
					623.6
					623.6
					623.6
					623.6
N4a	0.0046	0.0051	0.0026	0.003	40.8
	0.0056		0.0033		40.8
					40.8
					40.8
					40.8
					40.8
N4b	0.0088	0.00895	0.0051	0.005	71.6
	0.0091		0.0052		71.6
					71.6
					71.6
					71.6
					71.6

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

Table E-8.

Profile 4 N Column Nso190 Densitometry Analysis Data
Spectrophotometry Method

9/30/98

Flows: C column: 12.0 gpm N column: 10.5 gpm
9.4 m/hr 8.2 m/hr

Sample	Dilution Made	Diluted Conc., ng/u	Denatured vol. Conc., ng/ul	Blotted vol. ul	Blot Mass ng	Peak Area	Area/mass	Overall por averages	Standard deviation
N1a	1	283.6	70.9	18.3	1297.47	177953.3		470.1	37.1
	1	283.6	70.9	18.3	1297.47	231337.2			
	1	283.6	70.9	3.66	259.494	98437.94			
	1	283.6	70.9	3.66	259.494	71856.56			
	1	283.6	70.9	1.83	129.747	66201.69	510.24		
	1	283.6	70.9	1.83	129.747	54546.75	420.41		
N1b	1	270.0	67.5	19.4	1309.5	279516.6		457.1	234.3
	1	270.0	67.5	19.4	1309.5	244873			
	1	270.0	67.5	3.88	261.9	89105.44			
	1	270.0	67.5	3.88	261.9	122949.2			
	1	270.0	67.5	1.94	130.95	61848.12	472.30		
	1	270.0	67.5	1.94	130.95	62523.58	477.46		
N2a	1	249.2	62.3	20.8	1295.84	223158.2		457.1	234.3
	1	249.2	62.3	20.8	1295.84	280454.8			
	1	249.2	62.3	4.17	259.791	86410.7			
	1	249.2	62.3	4.17	259.791	113768.2			
	1	249.2	62.3	2.08	129.584	99824.81	770.35		
	1	249.2	62.3	2.08	129.584	62584.86	482.97		
N2b	1	288.4	72.1	18.4	1326.64	145519.4		176.6	32.8
	1	288.4	72.1	18.4	1326.64	141476.7			
	1	288.4	72.1	3.69	266.049	45522.4			
	1	288.4	72.1	3.69	266.049	40033.45			
	1	288.4	72.1	1.85	133.385	46975.43	352.18		
	1	288.4	72.1	1.85	133.385	29727.56	222.87		
N3a	1	299.2	74.8	17.5	1309	143218.1		176.6	32.8
	1	299.2	74.8	17.5	1309	210273.4			
	1	299.2	74.8	3.51	262.548	45928.67	174.93		
	1	299.2	74.8	3.51	262.548	45897.01	174.81		
	1	299.2	74.8	1.75	130.9	29149.65	222.69		
	1	299.2	74.8	1.75	130.9	29398.59	224.59		
N3b	0.1	62.4	15.6	41.7	650.103	41937.71		244.1	60.3
	0.1	62.4	15.6	41.7	650.103	51079.74			
	0.1	62.4	15.6	8.34	130.0206	19419.76	149.36		
	0.1	62.4	15.6	8.34	130.0206	17350.91	133.45		
	0.1	62.4	15.6	4.17	65.0103	10022.27	154.16		
	0.1	62.4	15.6	4.17	65.0103	11639.38	179.04		
N4a	1	40.8	10.2	122	1244.4	147869.2		244.1	60.3
	1	40.8	10.2	122	1244.4	150324.3			
	1	40.8	10.2	24.4	248.88	53107.39	213.39		
	1	40.8	10.2	24.4	248.88	48166.18	193.53		
	1	40.8	10.2	12.2	124.44	43199.9	347.15		
	1	40.8	10.2	12.2	124.44	26233.19	210.81		
N4b	1	71.6	17.9	35.5	635.45	90313.38		244.1	60.3
	1	71.6	17.9	35.5	635.45	125413.5			
	1	71.6	17.9	7.09	126.911	35174.93	277.16		
	1	71.6	17.9	7.09	126.911	20307.64	160.01		
	1	71.6	17.9	3.55	63.545	17933.35	282.21		
	1	71.6	17.9	3.55	63.545	17059.07	268.46		

Table E-9.

Profile 4. Ammonia Oxidizer Activity

9/30/98

Flows: C column: 12.0 gpm N column: 10.5 gpm
 9.4 m/hr 8.2 m/hr

Ammonia Oxidizer Activity				
Sample	Mass Standard Method		Spectrophotometry Method	
	Nitrifier Fraction	Standard Deviation	Peak Area/Mass	Standard Deviation
C1	9.5%	3.0%	614.2	199.2
C2	10.9%	3.2%	543.0	121.2
C3	4.1%	0.3%	435.0	47.3
C4	6.3%	2.1%	423.7	49.4
N1	8.3%	1.9%	470.1	37.1
N2	5.6%	1.4%	457.1	234.3
N3	1.9%	1.5%	176.6	32.8
N4	8.7%	1.7%	244.1	60.3

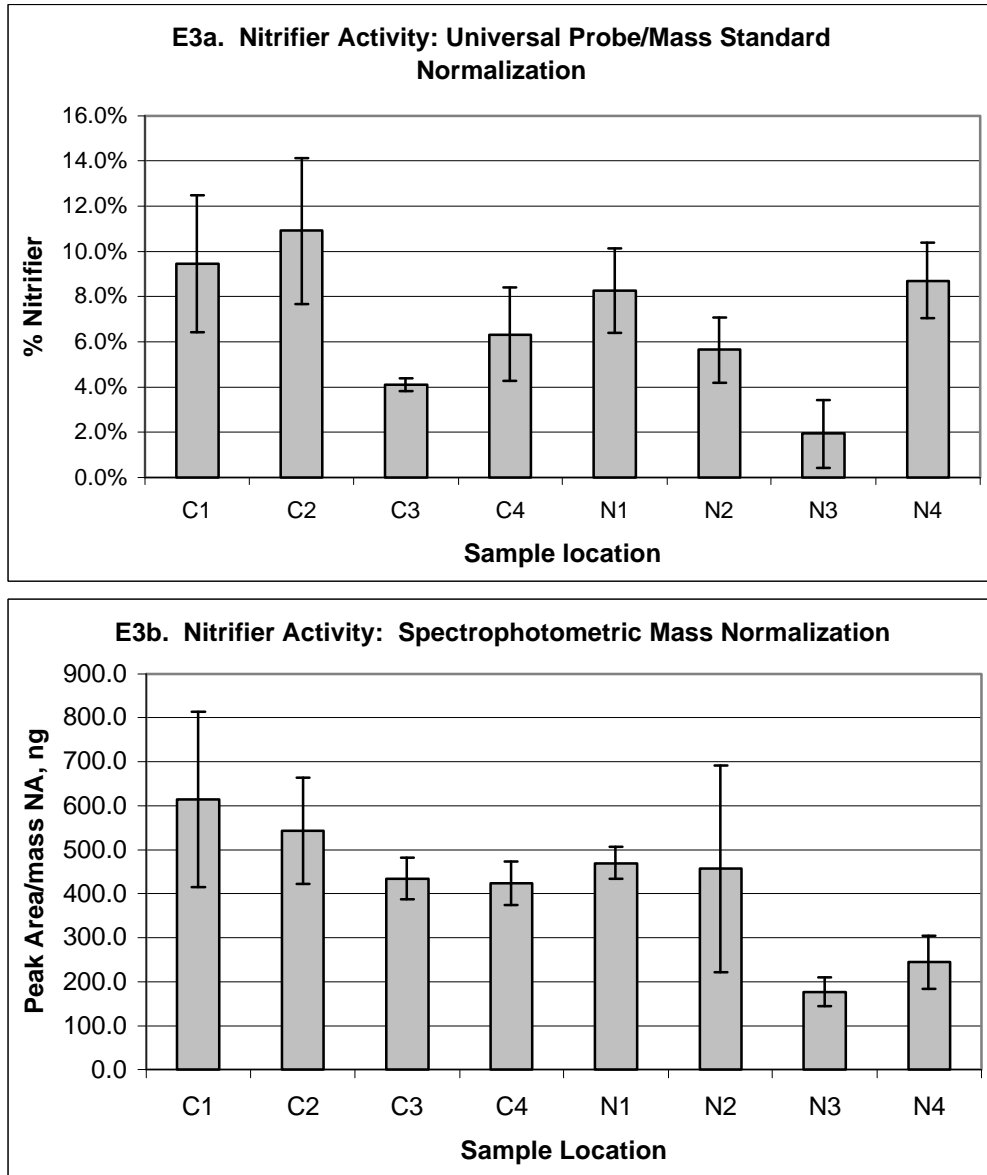


Figure E-3. Column Profiles at 9.4 & 8.2 m/hr, 9/30/98. a. Nitrifier activity normalized to mass standards; b. Nitrifier activity normalized to nucleic acid mass.