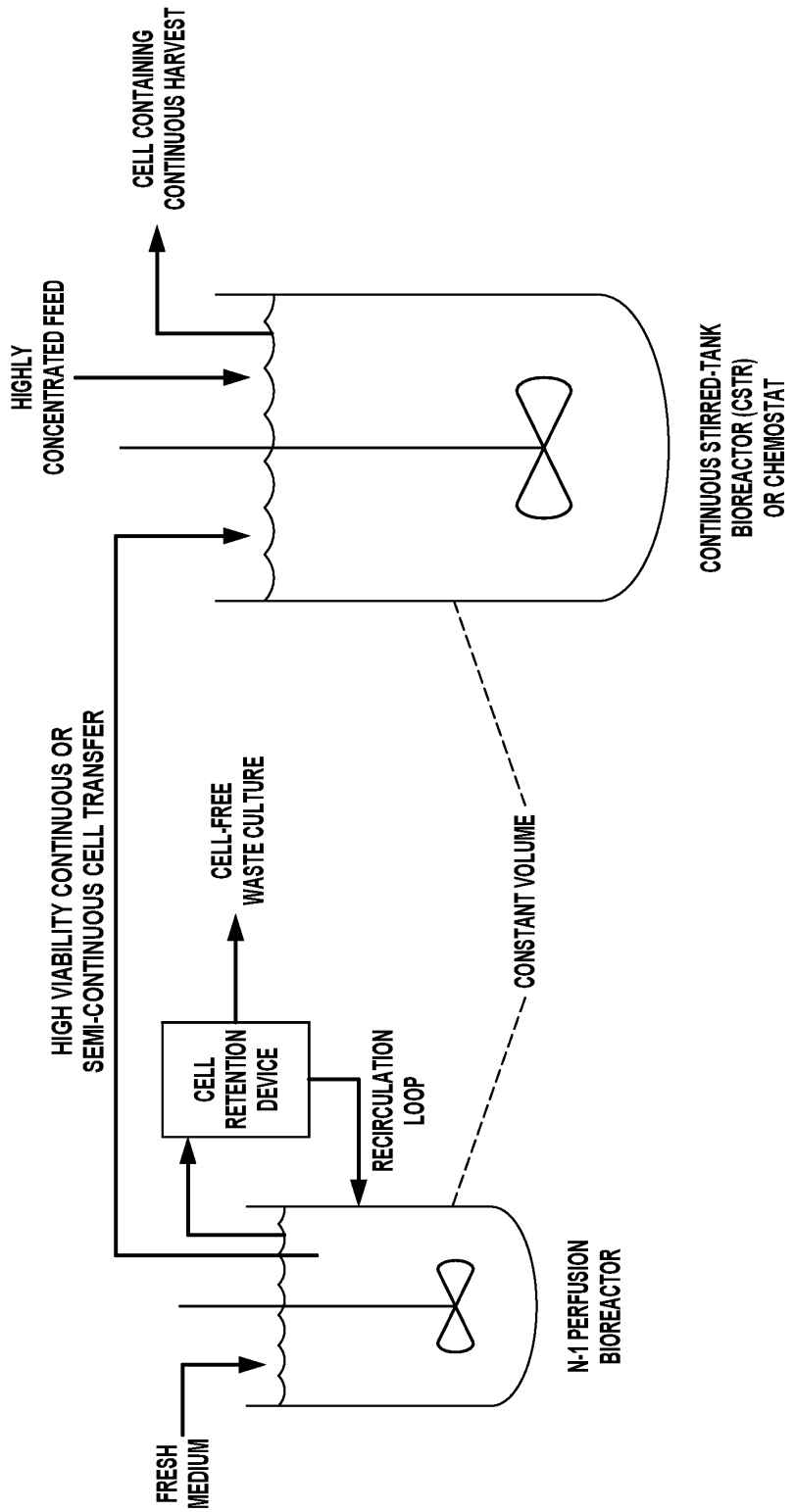

CELL CONTAINING CONTINUOUS HARVEST
HIGHLY CONCENTRATED FEED
CONTINUOUS STIRRED-TANK BIOREACTOR (CSTR) OR CHEMOSTAT
HIGH VIABILITY CONTINUOUS OR SEMI-CONTINUOUS CELL TRANSFER
CELL-FREE WASTE CULTURE
CELL RETENTION DEVICE
RECIRCULATION LOOP
CONSTANT VOLUME
N-1 PERFUSION BIOREACTOR
FRESH MEDIUM

Figure 1

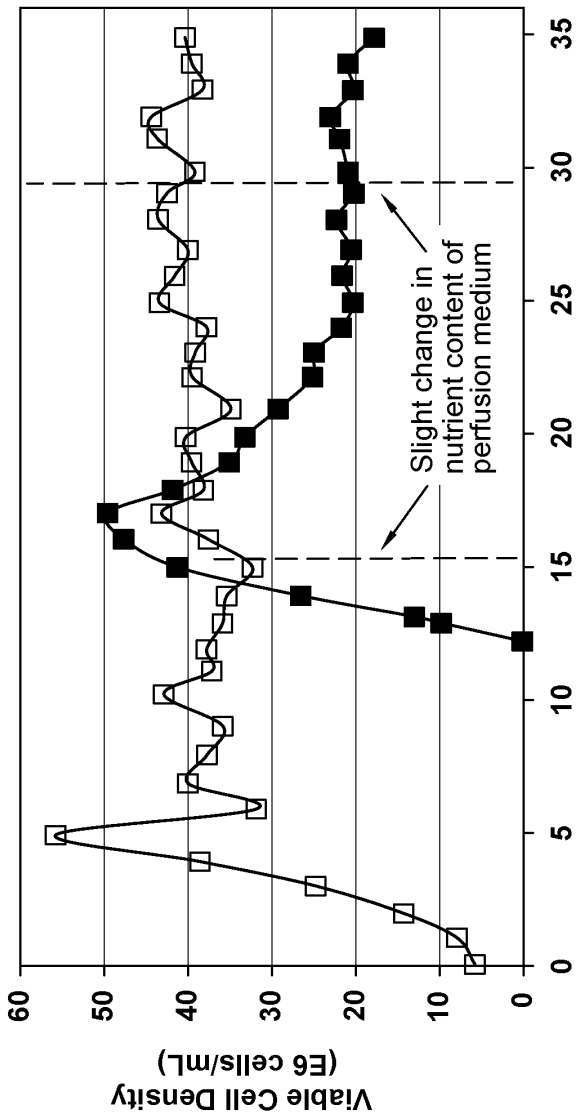

Viable Cell Density
(E6 cells/mL)
0
10
20
30
40
50
60
0
5
10
15
20
25
30
35
Time (days)
Slight change in
nutrient content of
perfusion medium

Figure 2A

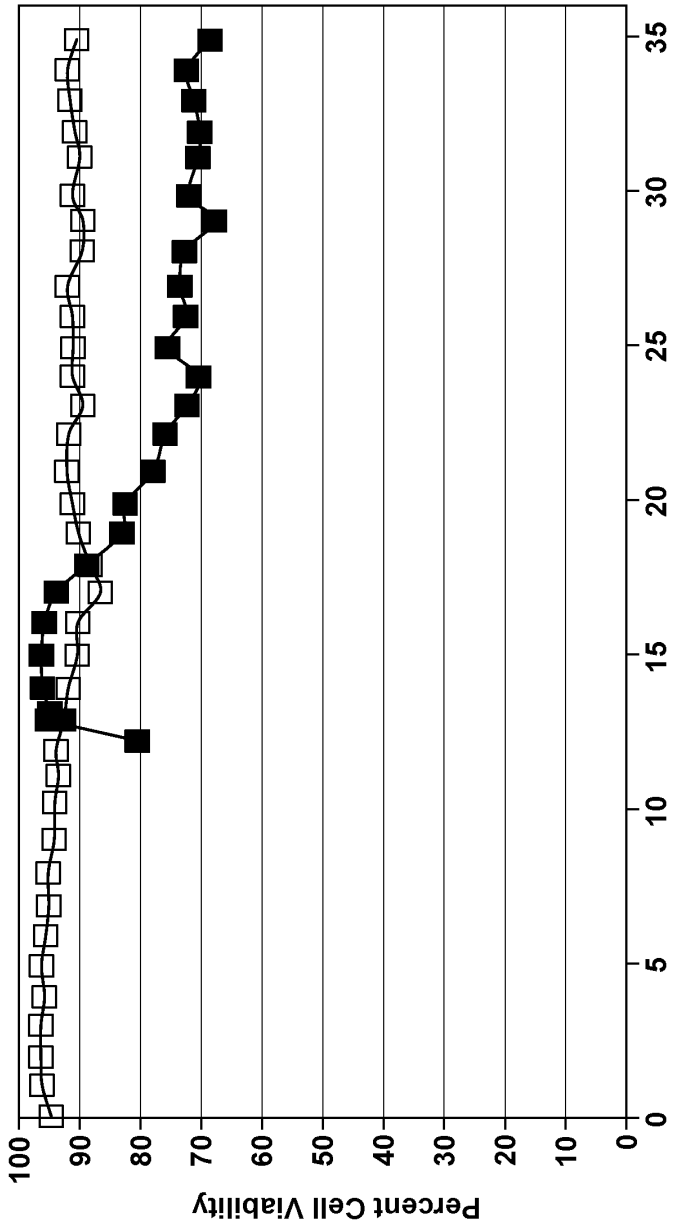

Percent Cell Viability
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
35
Time (days)

Figure 2B

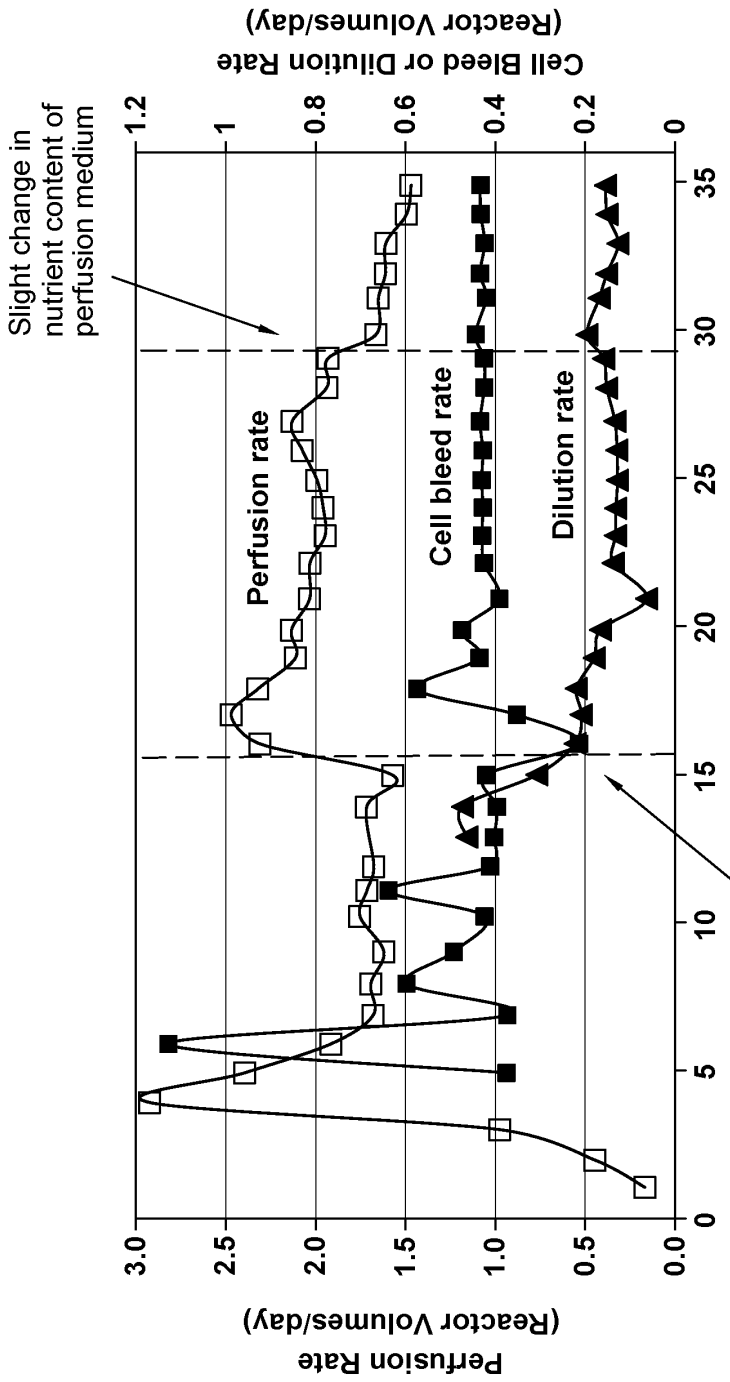

Perfusion Rate
(Reactor Volumes/day)
Cell Bleed or Dilution Rate
(Reactor Volumes/day)
Time (days)
Perfusion rate
Cell bleed rate
Dilution rate
Slight change in nutrient content of perfusion medium
Slight change in nutrient content of perfusion medium
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0
0.2
0.4
0.6
0.8
1
1.2
0
5
10
15
20
25
30
35

Figure 3

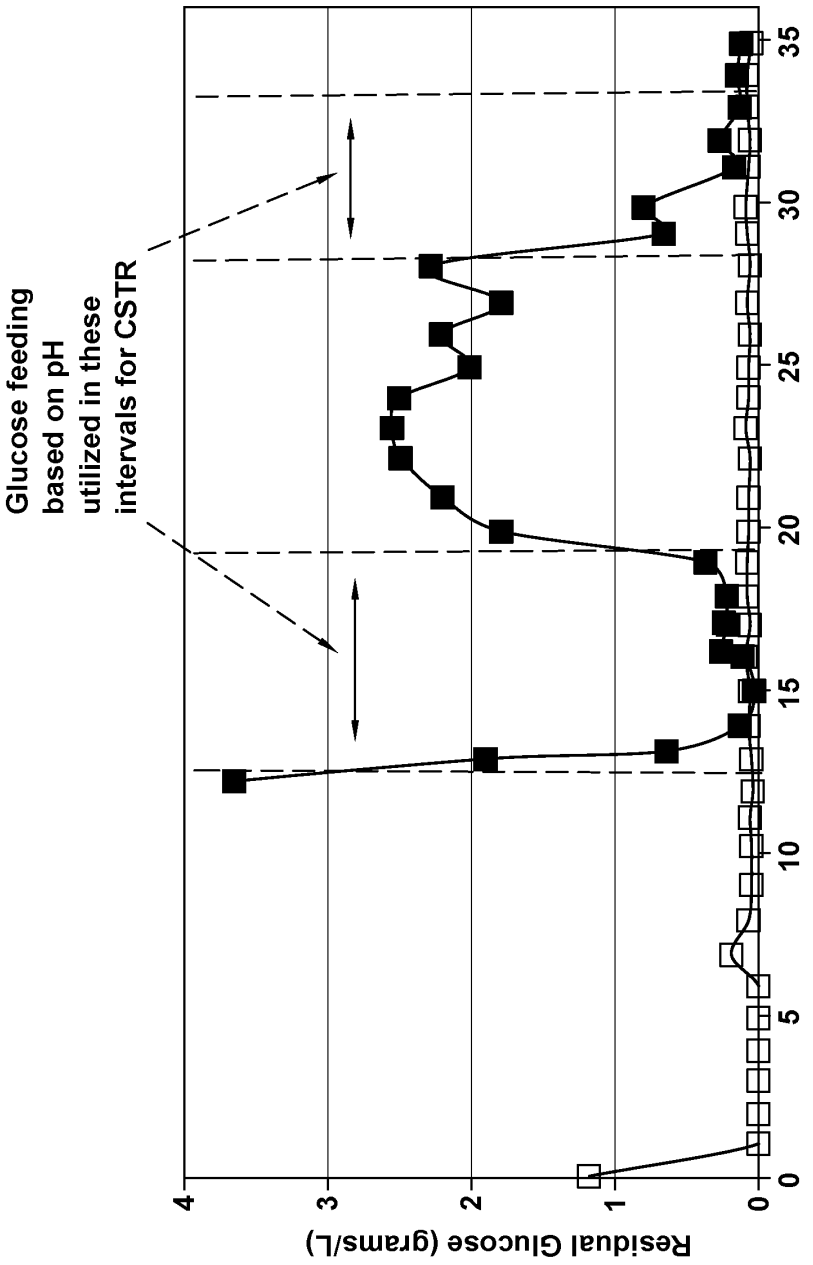

Glucose feeding based on pH utilized in these intervals for CSTR
Residual Glucose (grams/L)
Time (days)
0
1
2
3
4
0
5
10
15
20
25
30
35

Figure 4

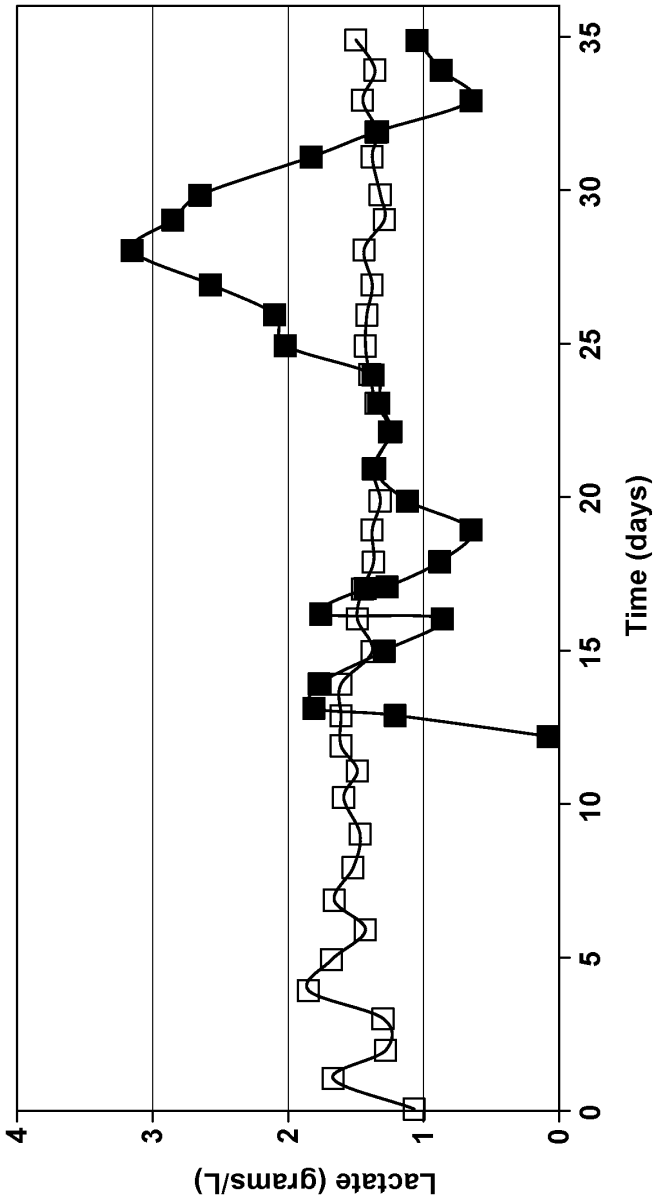


Figure 5

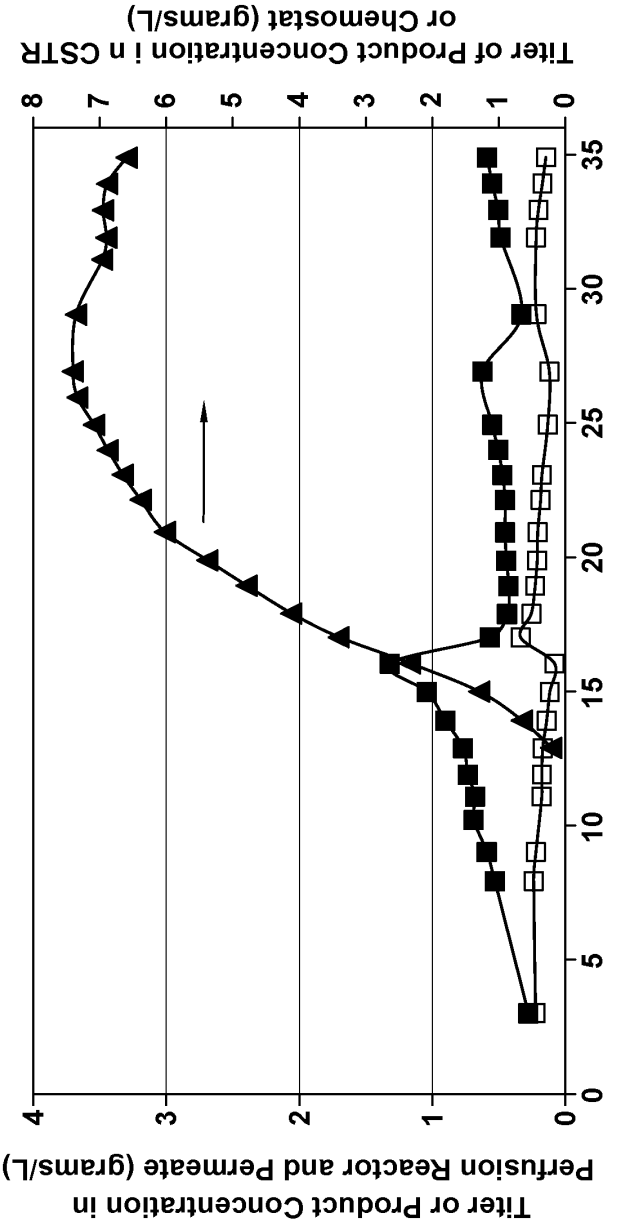

Titer or Product Concentration in
Perfusion Reactor and Permeate (grams/L)
Titer of Product Concentration i n CSTR
or Chemostat (grams/L)
Time (days)
0
1
2
3
4
5
6
7
8
0
5
10
15
20
25
30
35

Figure 6

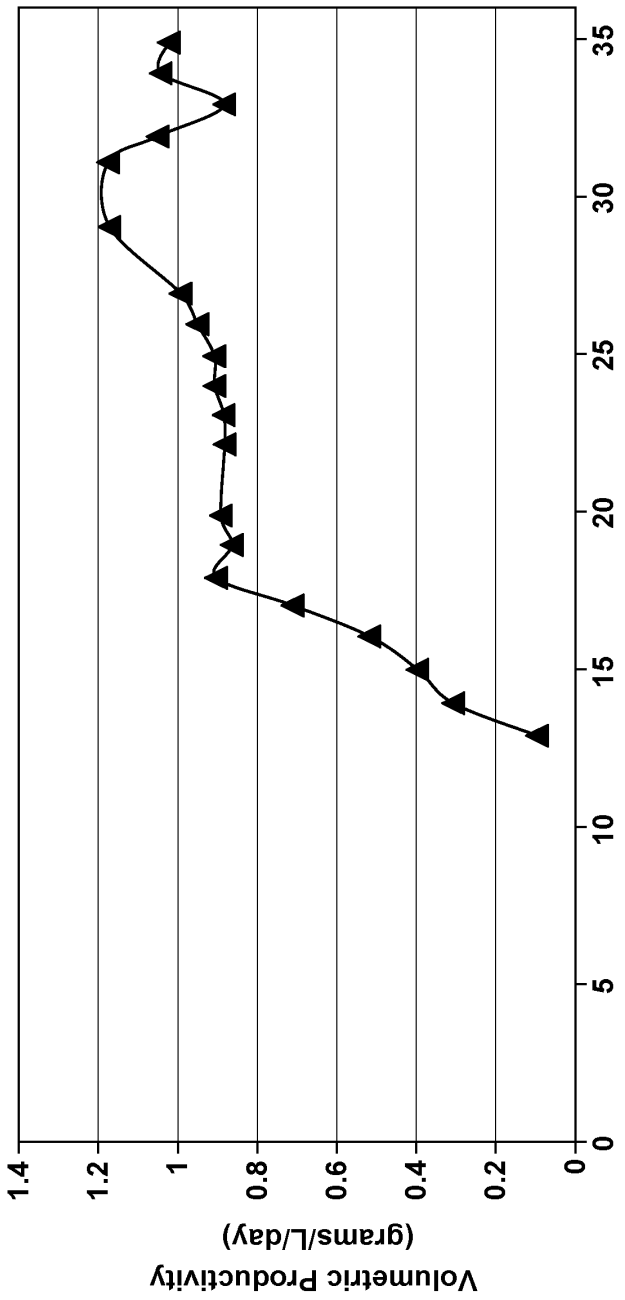

Volumetric Productivity
(grams/L/day)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
5
10
15
20
25
30
35
Time (days)

Figure 7

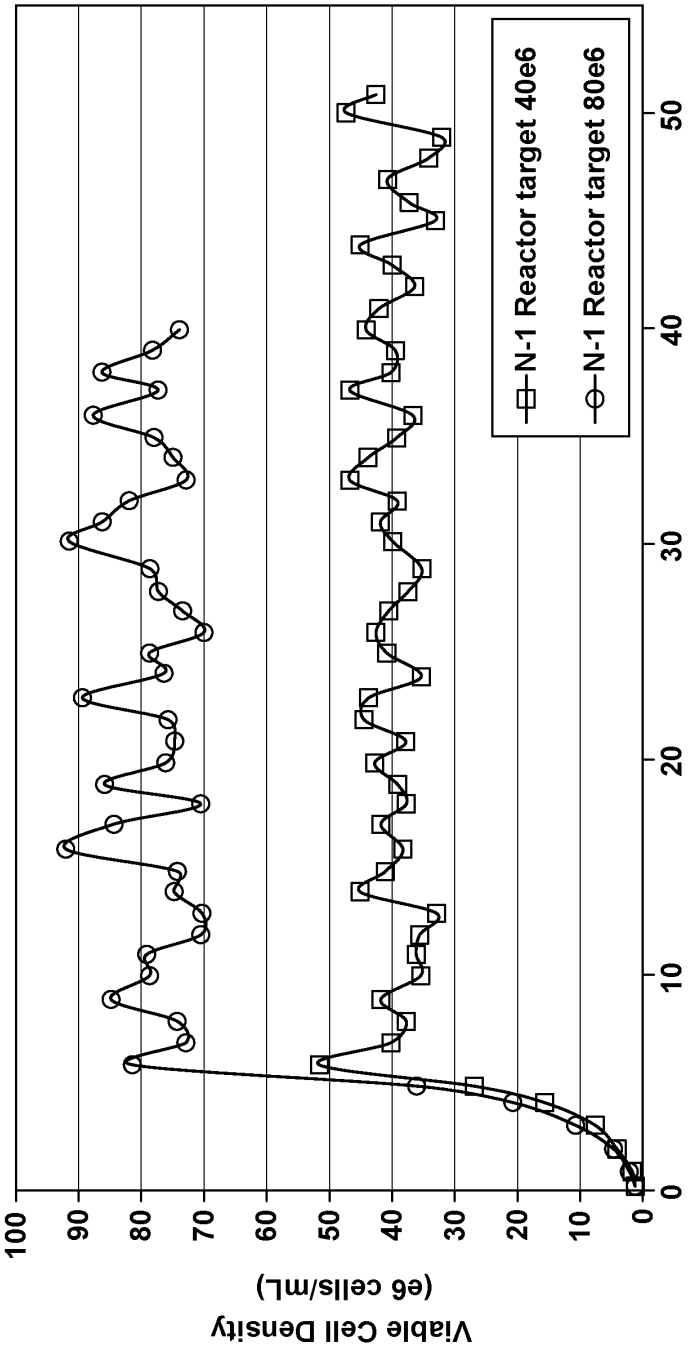

Viable Cell Density
(e6 cells/mL)
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
Time (days)
N-1 Reactor target 40e6
N-1 Reactor target 80e6

Figure 8

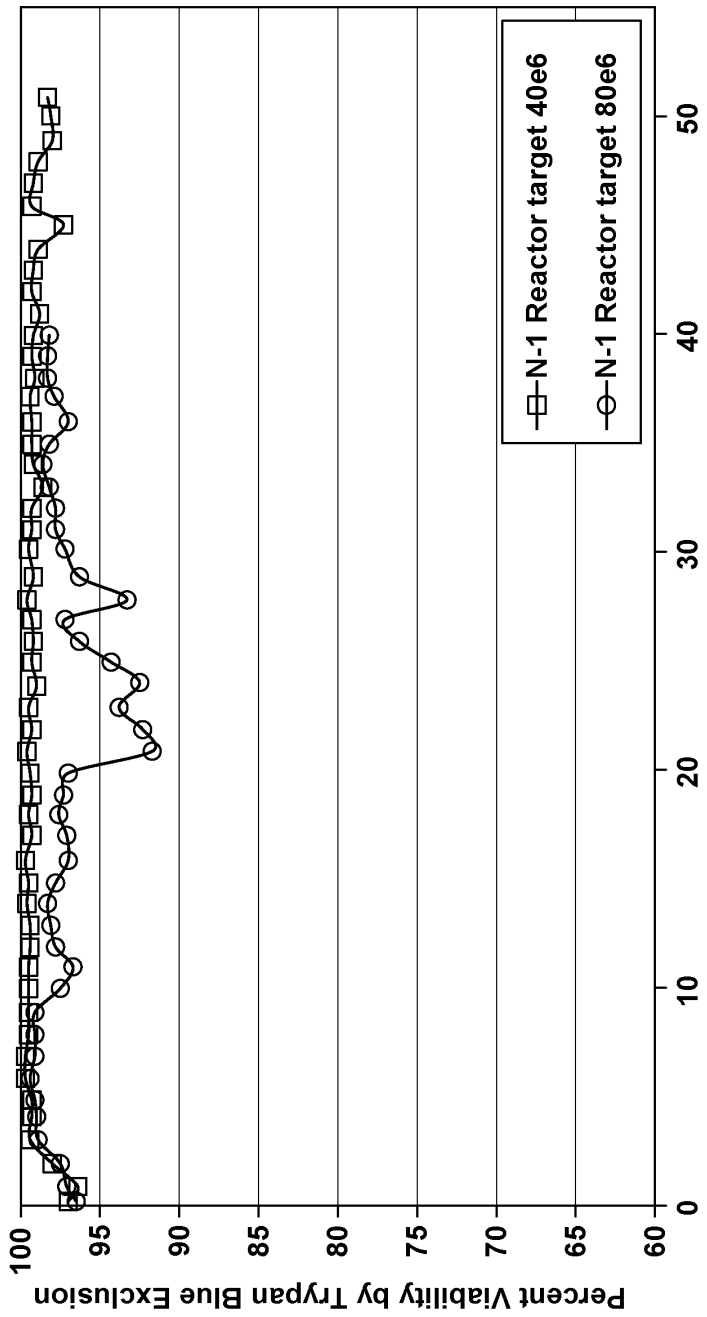

Percent Viability by Trypan Blue Exclusion
100
95
90
85
80
75
70
65
60
0
10
20
30
40
50
Time (days)
N-1 Reactor target 40e6
N-1 Reactor target 80e6

Figure 9

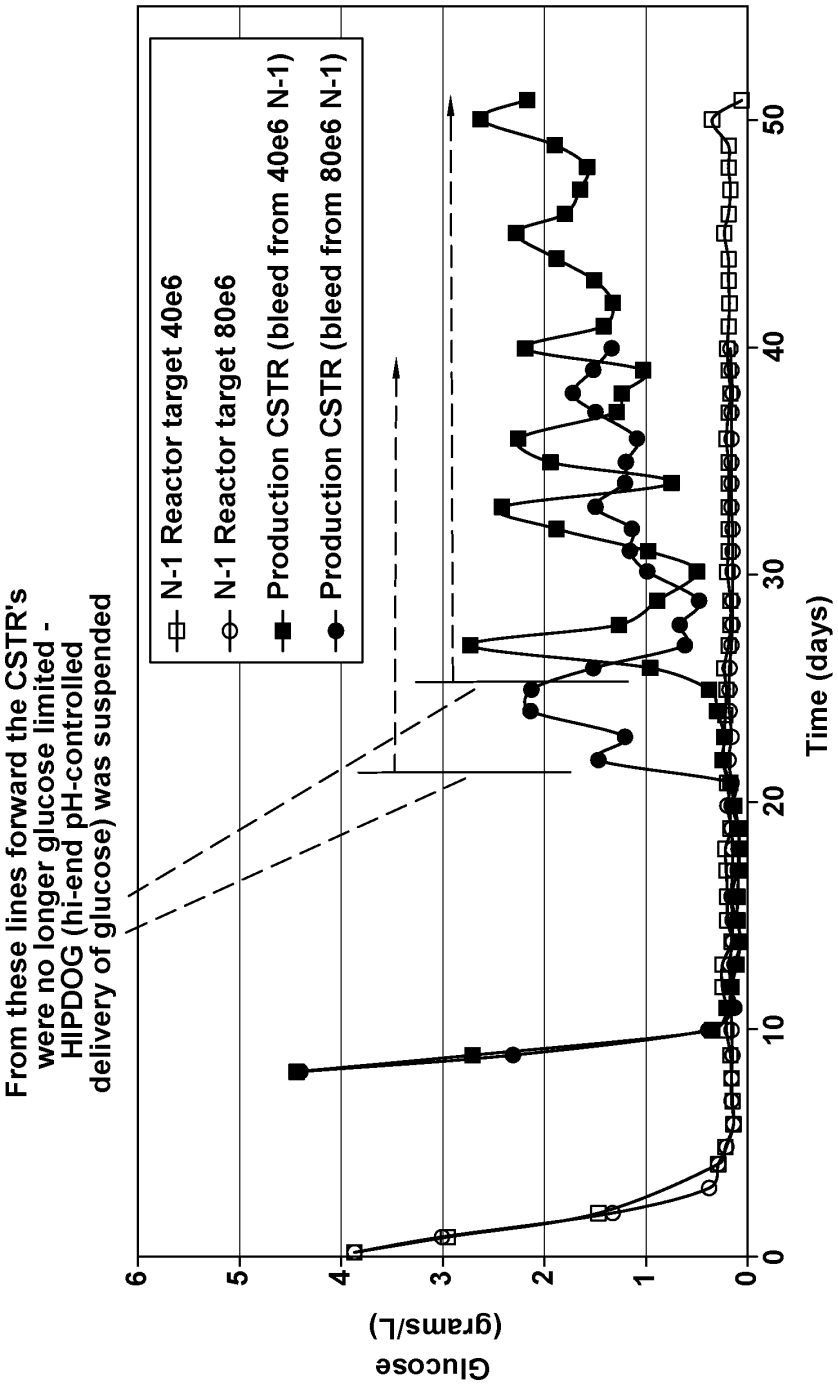

From these lines forward the CSTR's were no longer glucose limited - HIPDOG (hi-end pH-controlled delivery of glucose) was suspended
N-1 Reactor target 40e6
N-1 Reactor target 80e6
Production CSTR (bleed from 40e6 N-1)
Production CSTR (bleed from 80e6 N-1)
Glucose (grams/L)
Time (days)
0
1
2
3
4
5
6
0
10
20
30
40
50

Figure 10

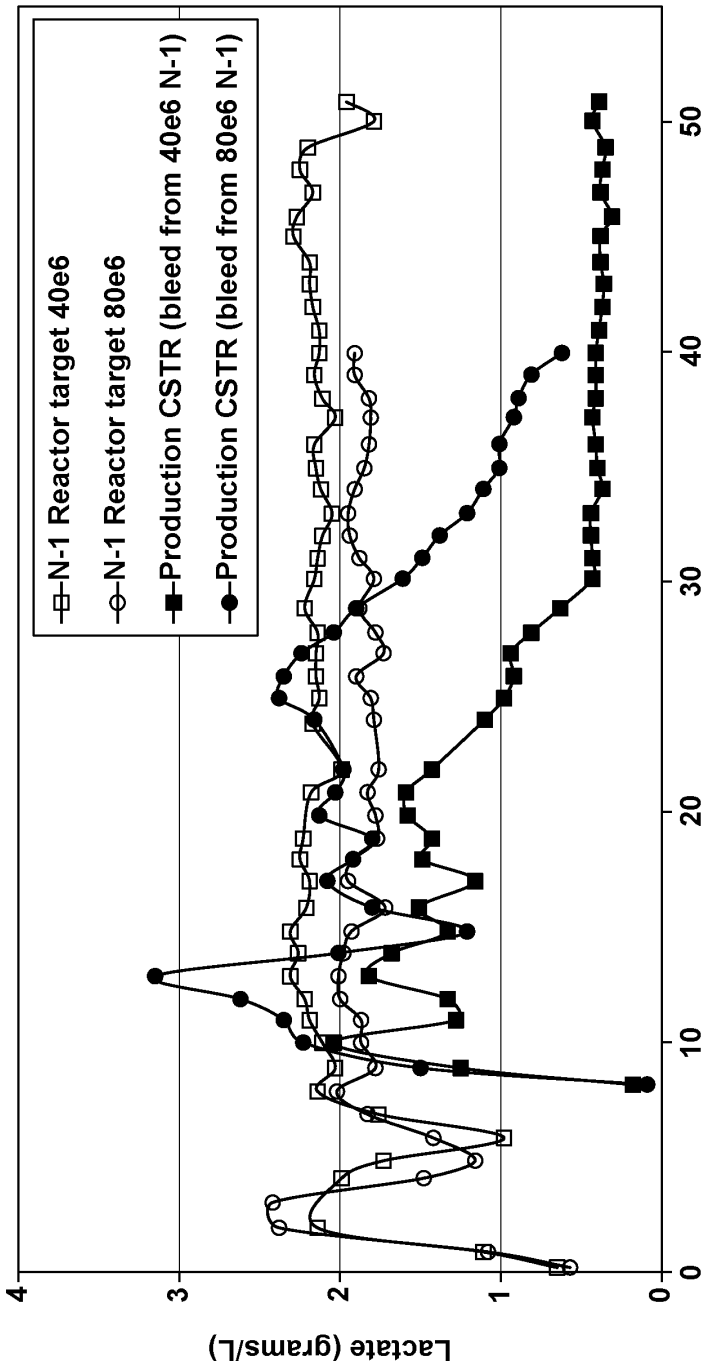

N-1 Reactor target 40e6
N-1 Reactor target 80e6
Production CSTR (bleed from 40e6 N-1)
Production CSTR (bleed from 80e6 N-1)
Lactate (grams/L)
0
1
2
3
4
Time (days)
0
10
20
30
40
50

Figure 11

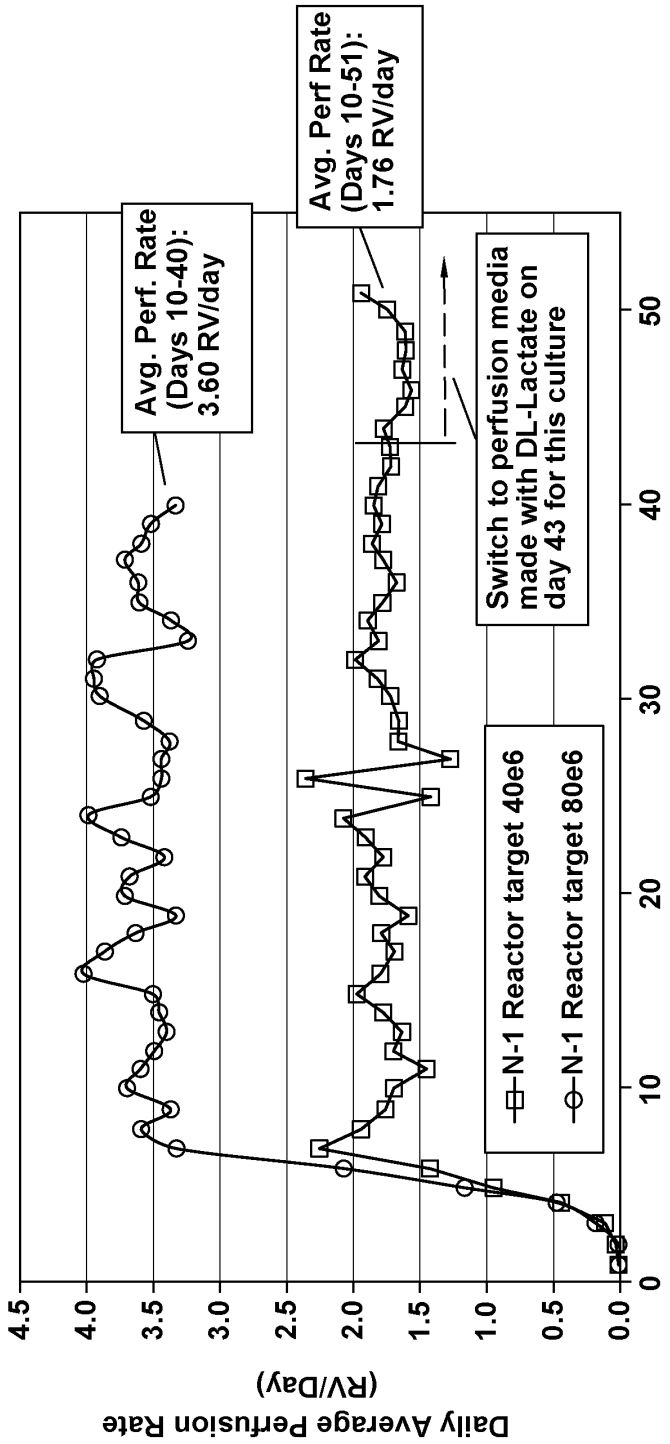

Daily Average Perfusion Rate
(RV/Day)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
10
20
30
40
50
Time (days)
Avg. Perf. Rate
(Days 10-40):
3.60 RV/day
Avg. Perf Rate
(Days 10-51):
1.76 RV/day
Switch to perfusion media
made with DL-Lactate on
day 43 for this culture
N-1 Reactor target 40e6
N-1 Reactor target 80e6

Figure 12

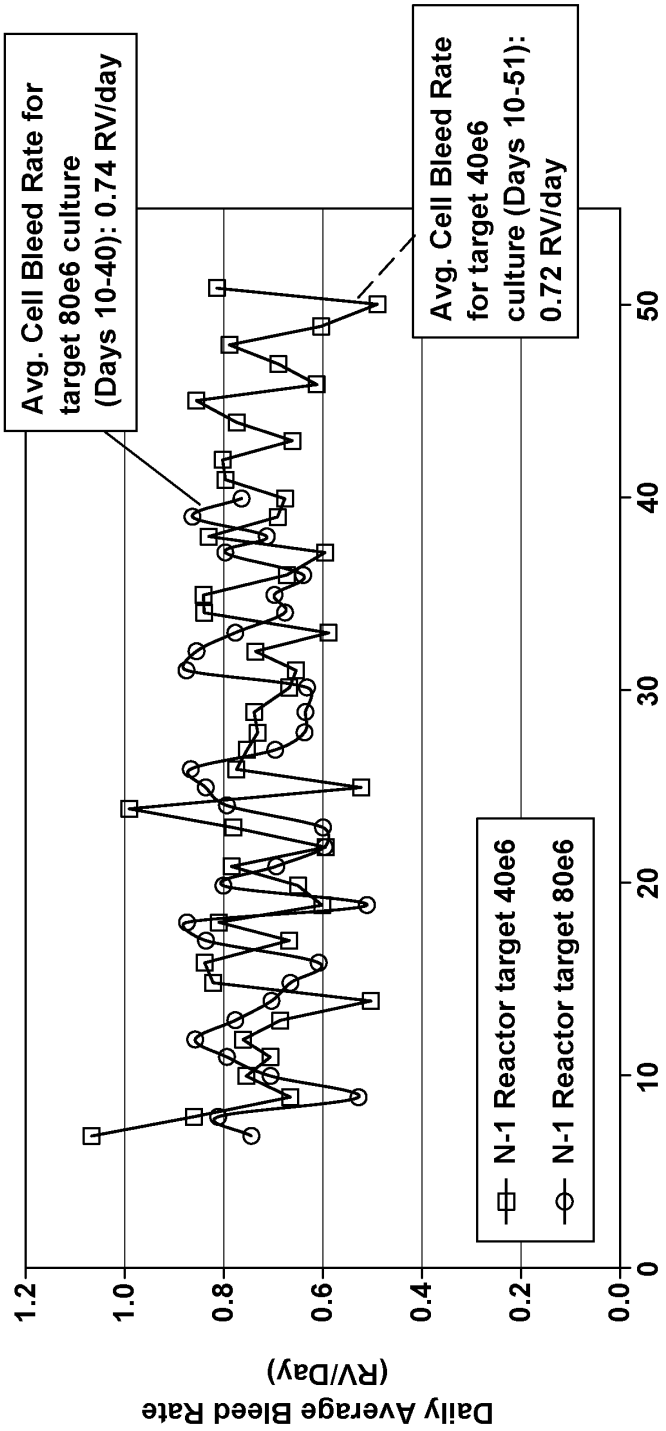

Avg. Cell Bleed Rate for target 80e6 culture (Days 10-40): 0.74 RV/day
Avg. Cell Bleed Rate for target 40e6 culture (Days 10-51): 0.72 RV/day
N-1 Reactor target 40e6
N-1 Reactor target 80e6
Daily Average Bleed Rate (RV/Day)
Time (days)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0
10
20
30
40
50

Figure 13

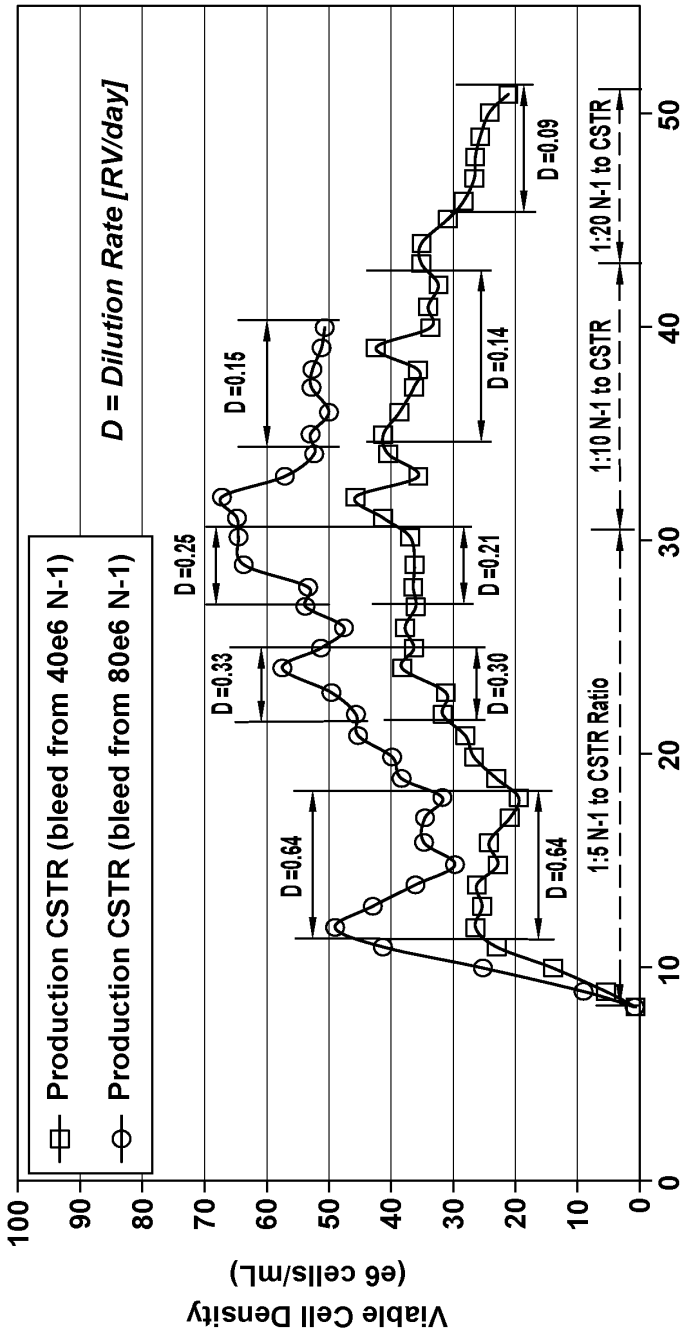

Viable Cell Density
(e6 cells/mL)
Time (days)
Production CSTR (bleed from 40e6 N-1)
Production CSTR (bleed from 80e6 N-1)
D = Dilution Rate [RV/day]
1:5 N-1 to CSTR Ratio
1:10 N-1 to CSTR
1:20 N-1 to CSTR
D=0.64
D=0.33
D=0.25
D=0.15
D=0.64
D=0.30
D=0.21
D=0.14
D=0.09

Figure 14

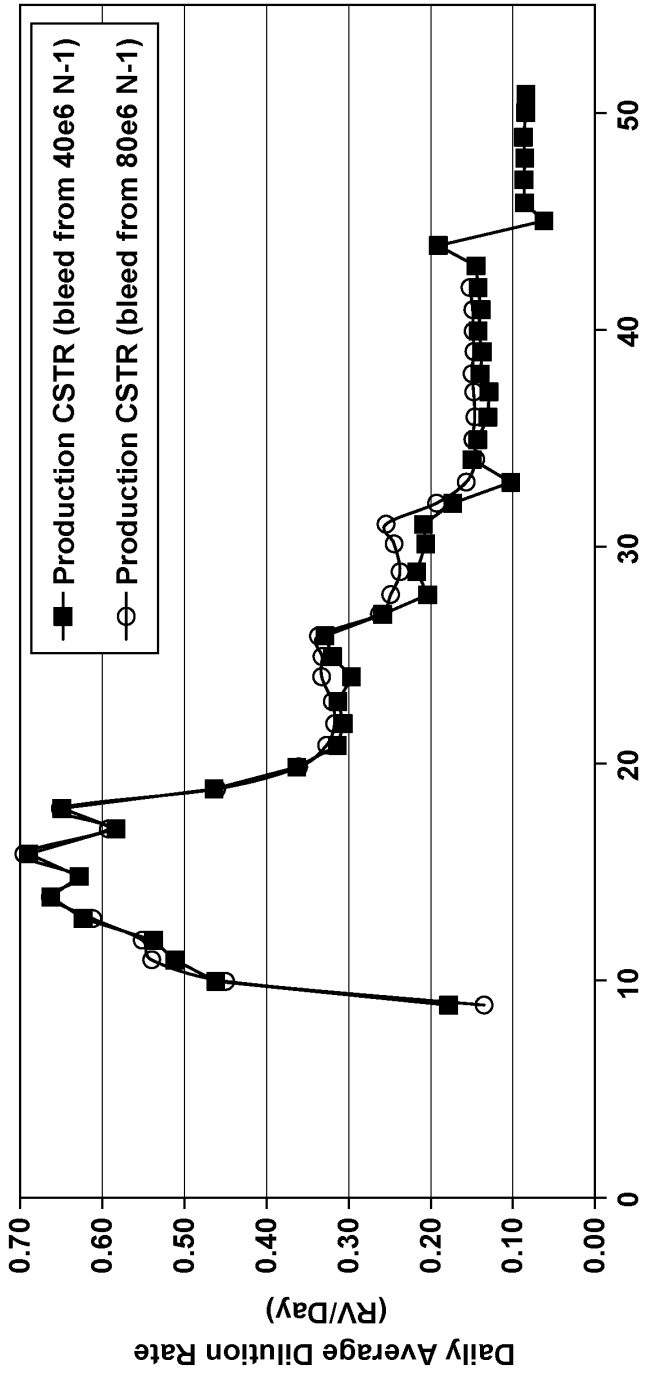

Production CSTR (bleed from 40e6 N-1)
Production CSTR (bleed from 80e6 N-1)
Daily Average Dilution Rate
(RV/Day)
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0
10
20
30
40
50
Time (days)

Figure 15

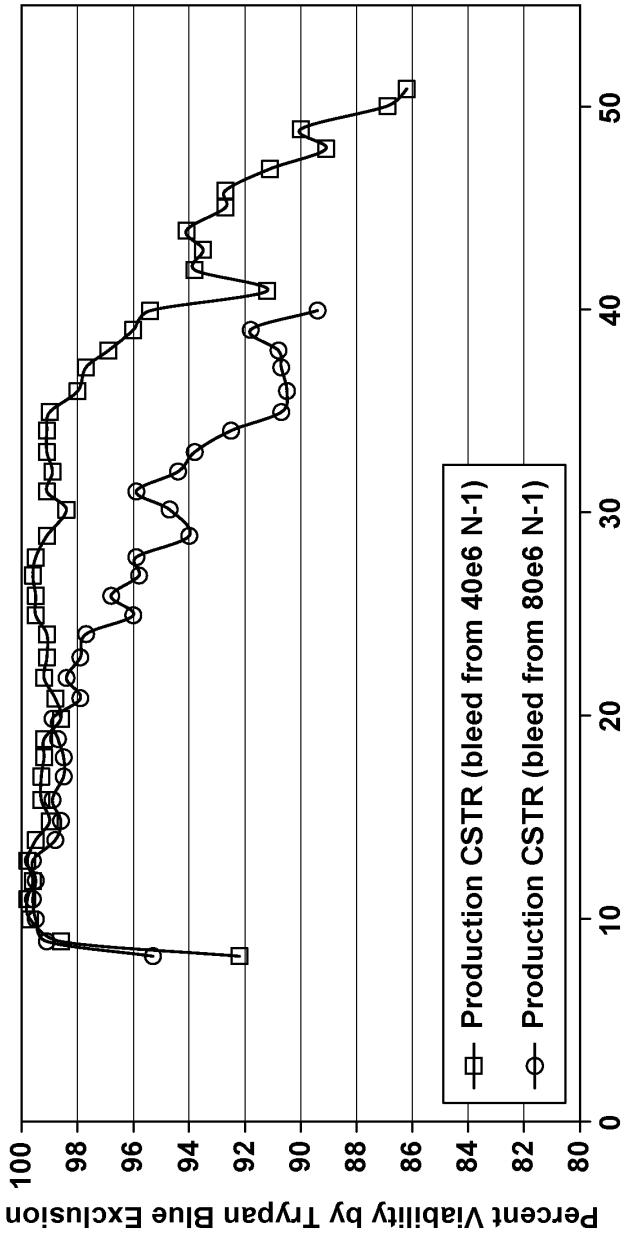

Percent Viability by Trypan Blue Exclusion
100
98
96
94
92
90
88
86
84
82
80
0
10
20
30
40
50
Time (days)
Production CSTR (bleed from 40e6 N-1)
Production CSTR (bleed from 80e6 N-1)

Figure 16

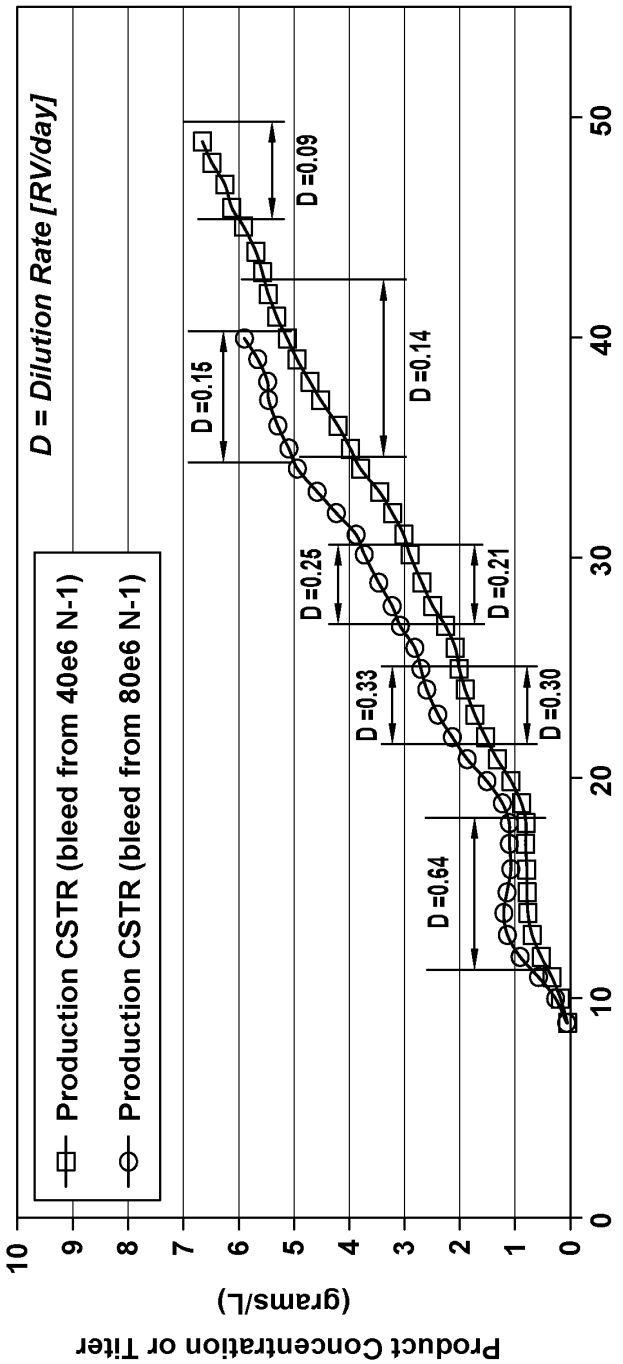

D = Dilution Rate [RV/day]
Production CSTR (bleed from 40e6 N-1)
Production CSTR (bleed from 80e6 N-1)
D =0.15
D =0.09
D =0.14
D =0.25
D =0.21
D =0.33
D =0.30
D =0.64
Product Concentration or Titer (grams/L)
Time (days)
0
1
2
3
4
5
6
7
8
9
10
0
10
20
30
40
50

Figure 17

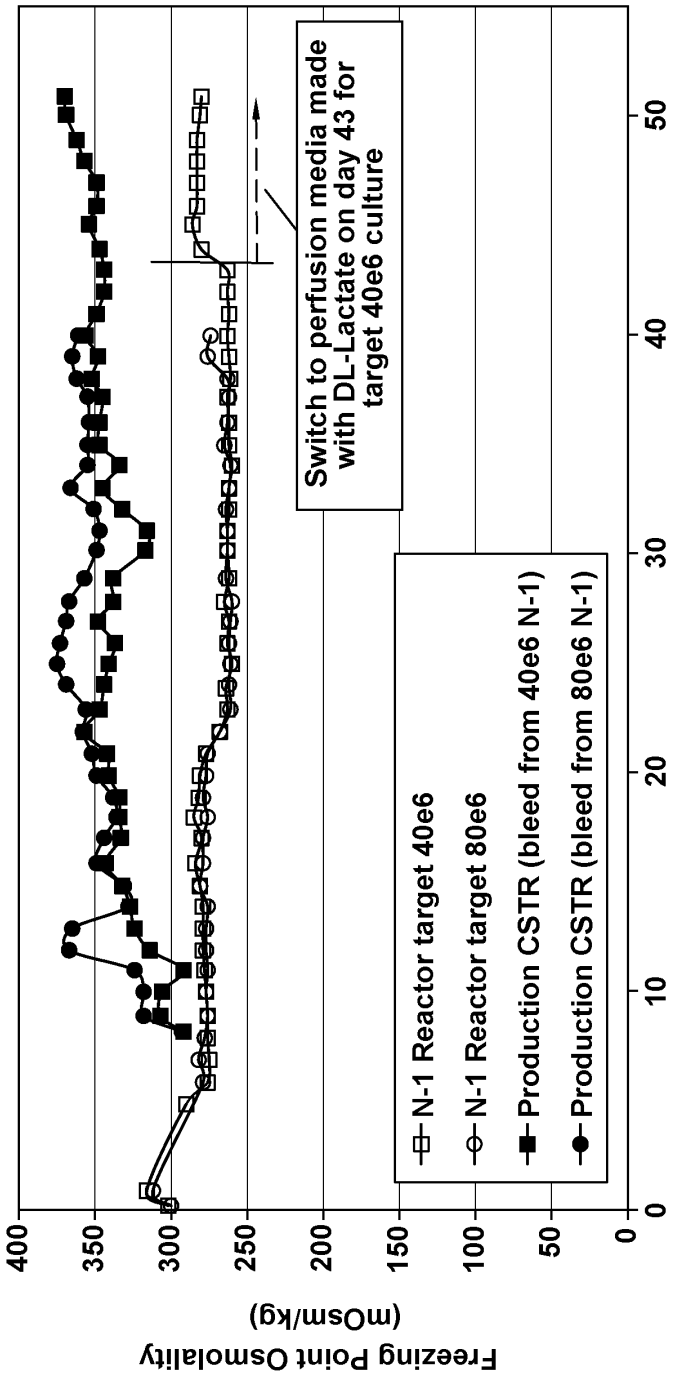

Freezing Point Osmolality (mOsm/kg)
400
350
300
250
200
150
100
50
0
0
10
20
30
40
50
Time (days)
N-1 Reactor target 40e6
N-1 Reactor target 80e6
Production CSTR (bleed from 40e6 N-1)
Production CSTR (bleed from 80e6 N-1)
Switch to perfusion media made with DL-Lactate on day 43 for target 40e6 culture

Figure 18

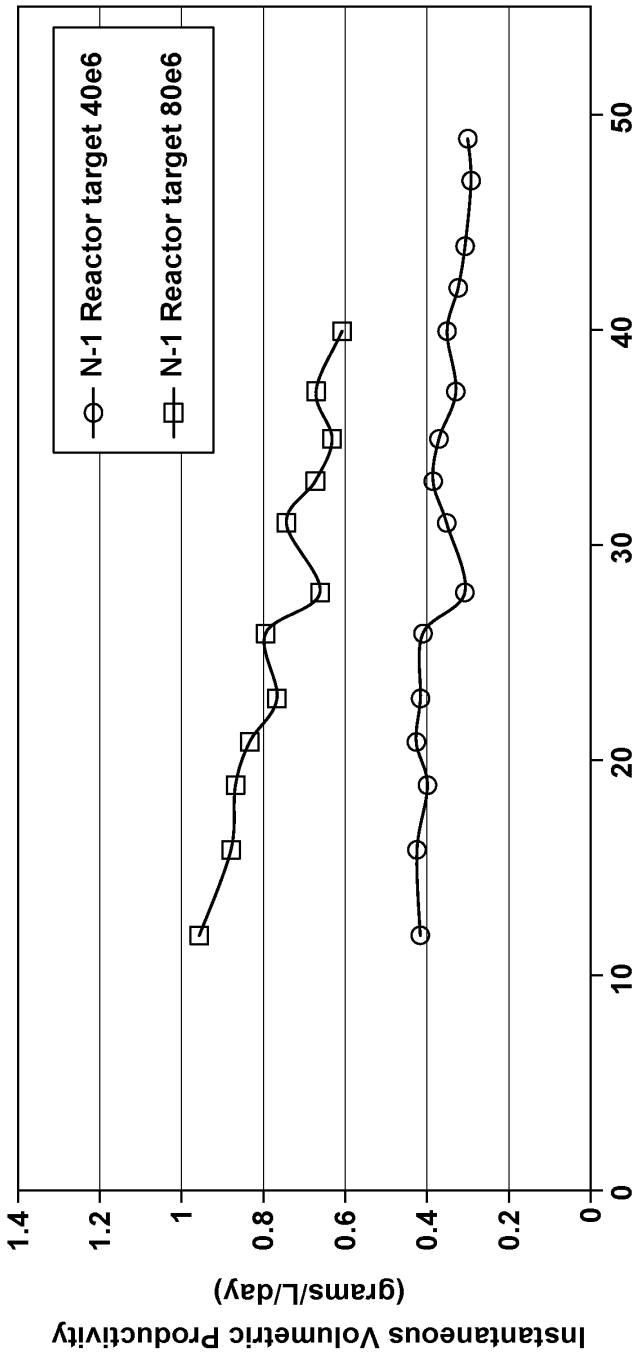

N-1 Reactor target 40e6
N-1 Reactor target 80e6
Instantaneous Volumetric Productivity
(grams/L/day)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
10
20
30
40
50
Time (days)

Figure 19

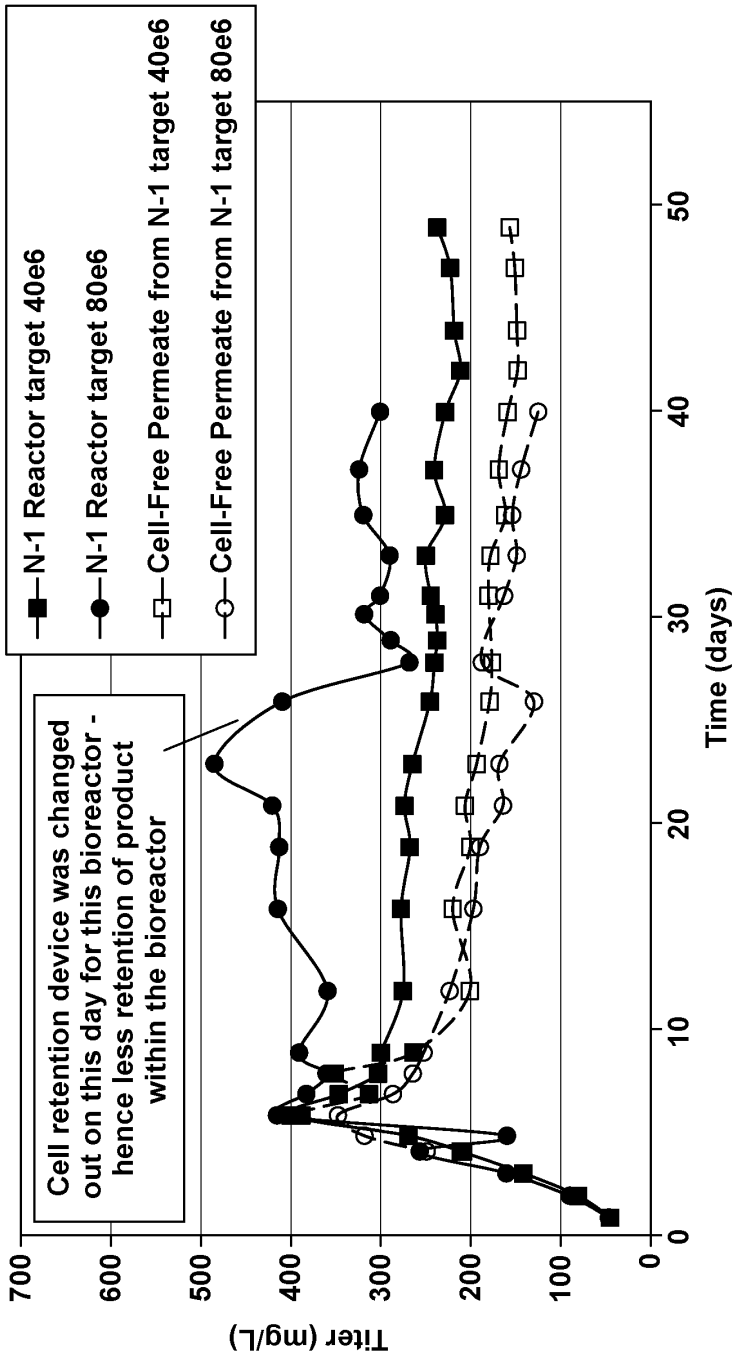

N-1 Reactor target 40e6
N-1 Reactor target 80e6
Cell-Free Permeate from N-1 target 40e6
Cell-Free Permeate from N-1 target 80e6
Cell retention device was changed out on this day for this bioreactor - hence less retention of product within the bioreactor
Titer (mg/L)
Time (days)
0
100
200
300
400
500
600
700
0
10
20
30
40
50

Figure 20

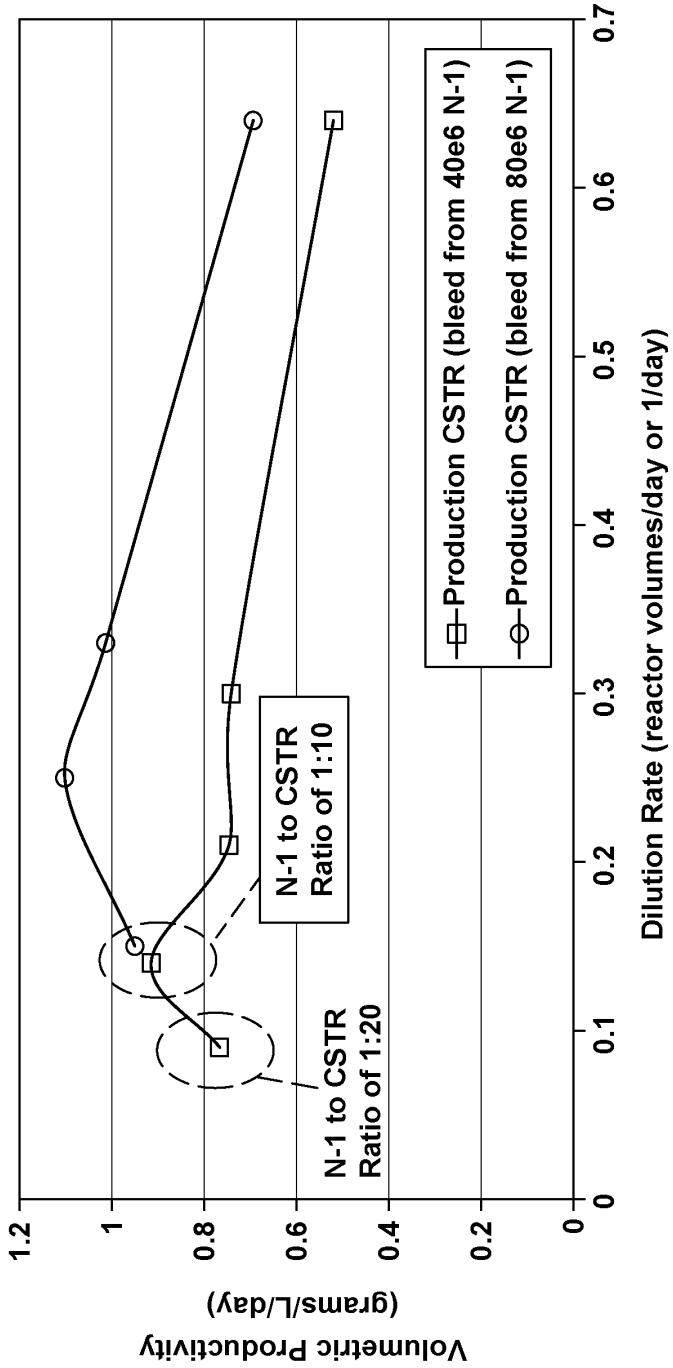

Volumetric Productivity
(grams/L/day)
1.2
1
0.8
0.6
0.4
0.2
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Dilution Rate (reactor volumes/day or 1/day)
Production CSTR (bleed from 40e6 N-1)
Production CSTR (bleed from 80e6 N-1)
N-1 to CSTR
Ratio of 1:10
N-1 to CSTR
Ratio of 1:20

Figure 21