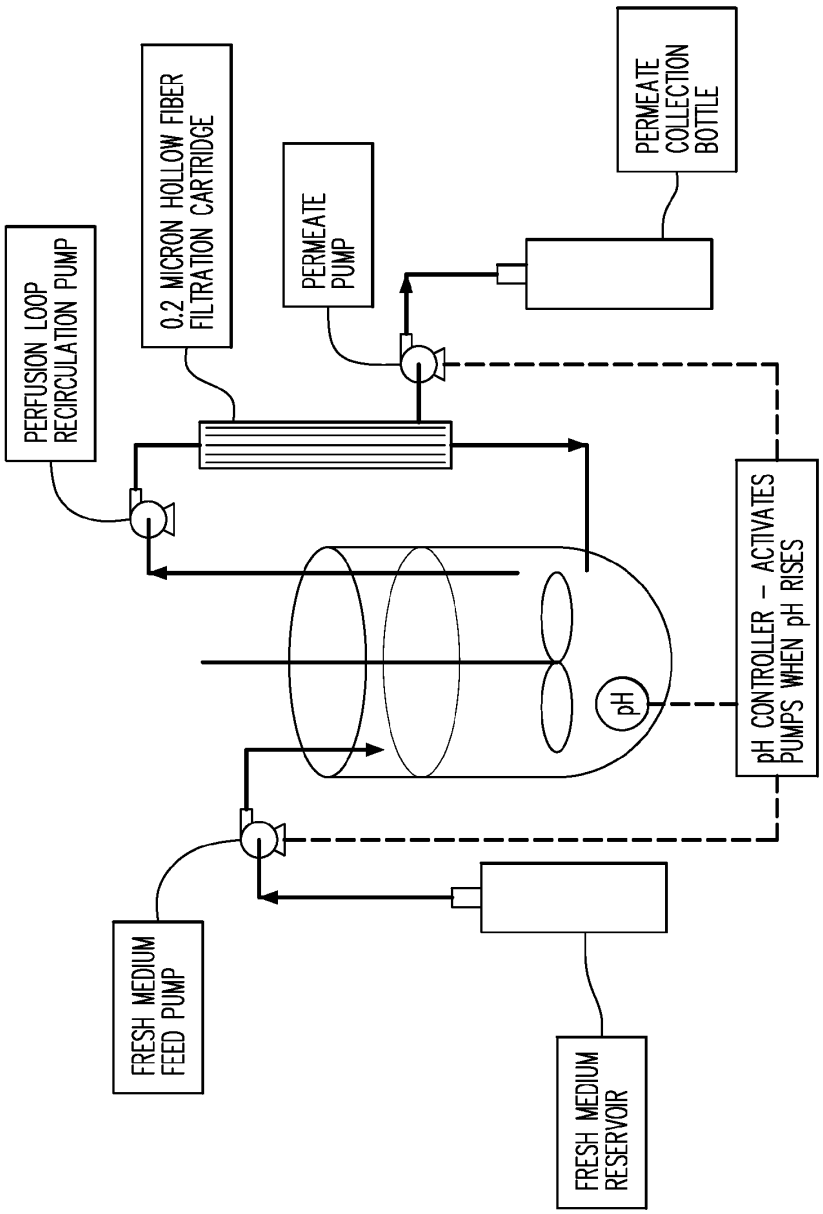

PERFUSION LOOP RECIRCULATION PUMP
0.2 MICRON HOLLOW FIBER FILTRATION CARTRIDGE
PERMEATE PUMP
PERMEATE COLLECTION BOTTLE
pH
pH CONTROLLER – ACTIVATES PUMPS WHEN pH RISES
FRESH MEDIUM FEED PUMP
FRESH MEDIUM RESERVOIR

FIG. 1

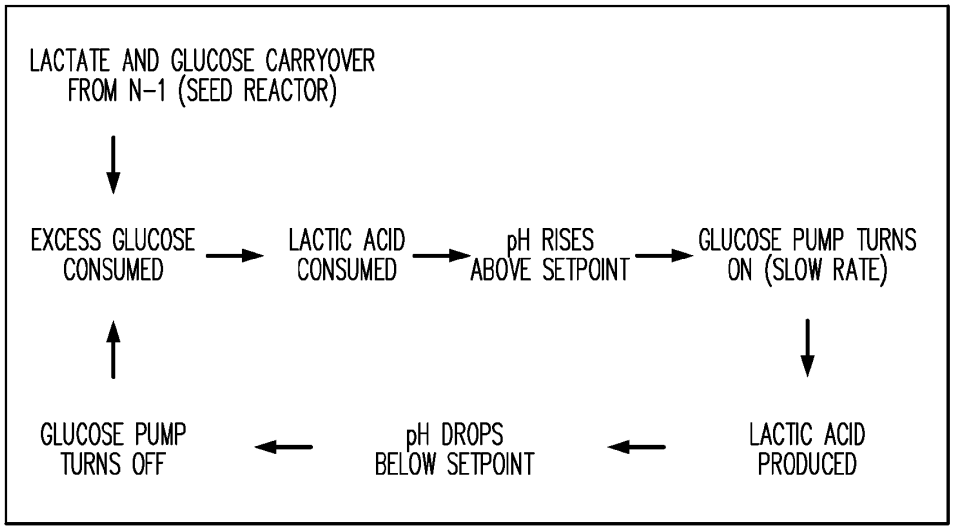

LACTATE AND GLUCOSE CARRYOVER FROM N-1 (SEED REACTOR)
EXCESS GLUCOSE CONSUMED
LACTIC ACID CONSUMED
pH RISES ABOVE SETPOINT
GLUCOSE PUMP TURNS ON (SLOW RATE)
LACTIC ACID PRODUCED
pH DROPS BELOW SETPOINT
GLUCOSE PUMP TURNS OFF

FIG. 2A

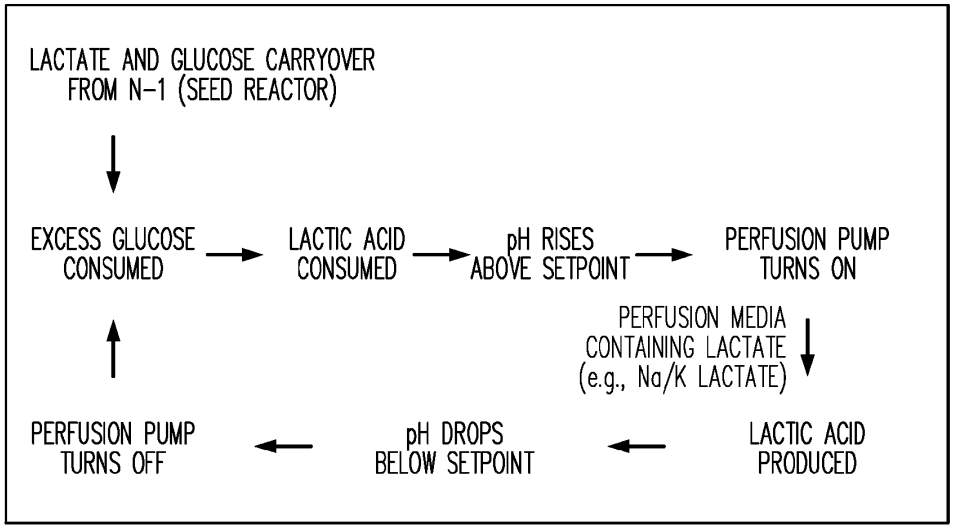

LACTATE AND GLUCOSE CARRYOVER FROM N-1 (SEED REACTOR)
EXCESS GLUCOSE CONSUMED
LACTIC ACID CONSUMED
pH RISES ABOVE SETPOINT
PERFUSION PUMP TURNS ON
PERFUSION MEDIA CONTAINING LACTATE (e.g., Na/K LACTATE)
LACTIC ACID PRODUCED
pH DROPS BELOW SETPOINT
PERFUSION PUMP TURNS OFF

FIG. 2B

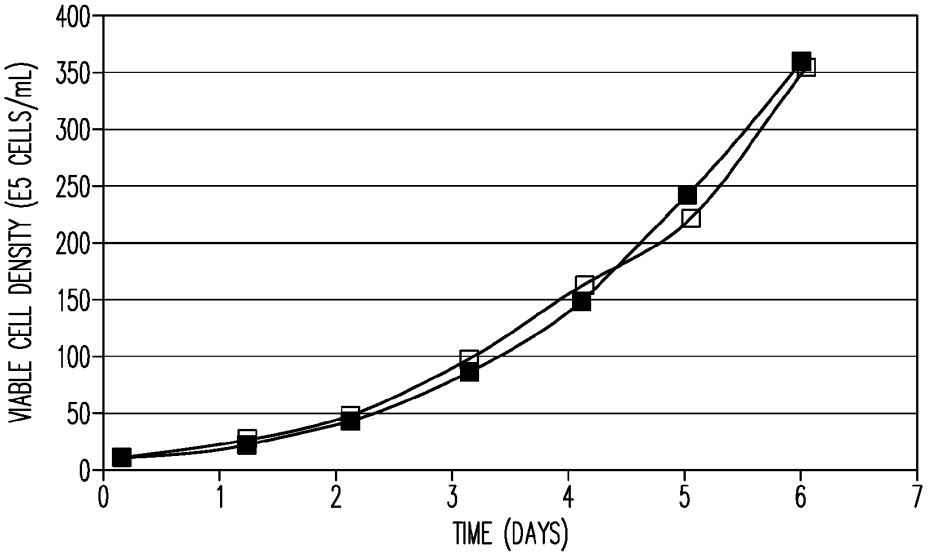

VIABLE CELL DENSITY (E5 CELLS/mL)
400
350
300
250
200
150
100
50
0
0
1
2
3
4
5
6
7
TIME (DAYS)

FIG. 3A

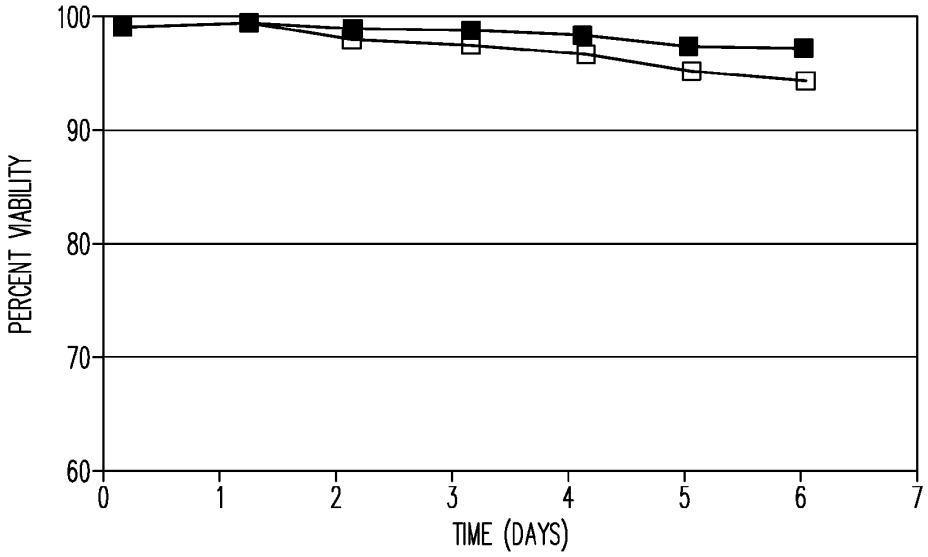

PERCENT VIABILITY
100
90
80
70
60
0
1
2
3
4
5
6
7
TIME (DAYS)

FIG. 3B

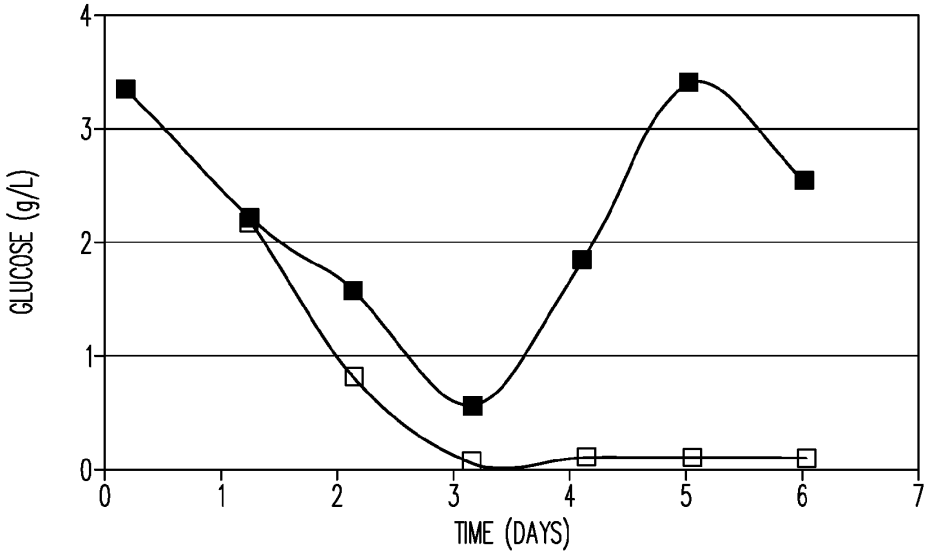

GLUCOSE (g/L)
0
1
2
3
4
0
1
2
3
4
5
6
7
TIME (DAYS)

FIG. 3C

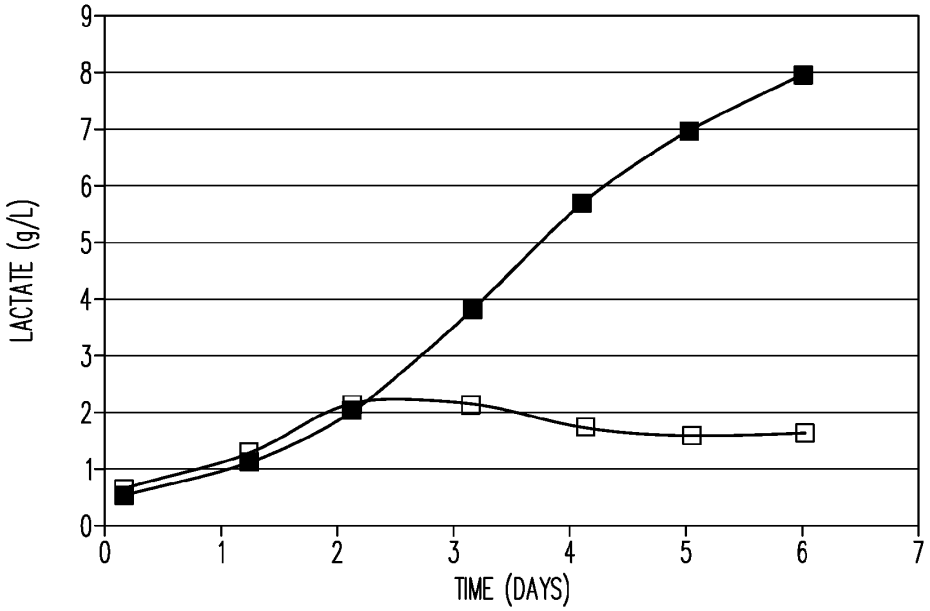

LACTATE (g/L)
0
1
2
3
4
5
6
7
8
9
0
1
2
3
4
5
6
7
TIME (DAYS)

FIG. 3D

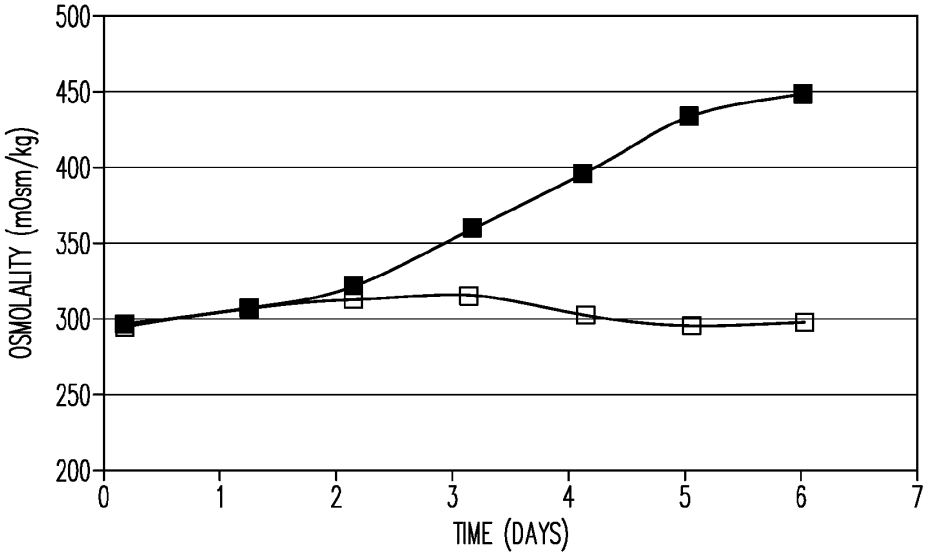

500
450
400
350
300
250
200
OSMOLALITY (mOsm/kg)
0
1
2
3
4
5
6
7
TIME (DAYS)

FIG. 3E

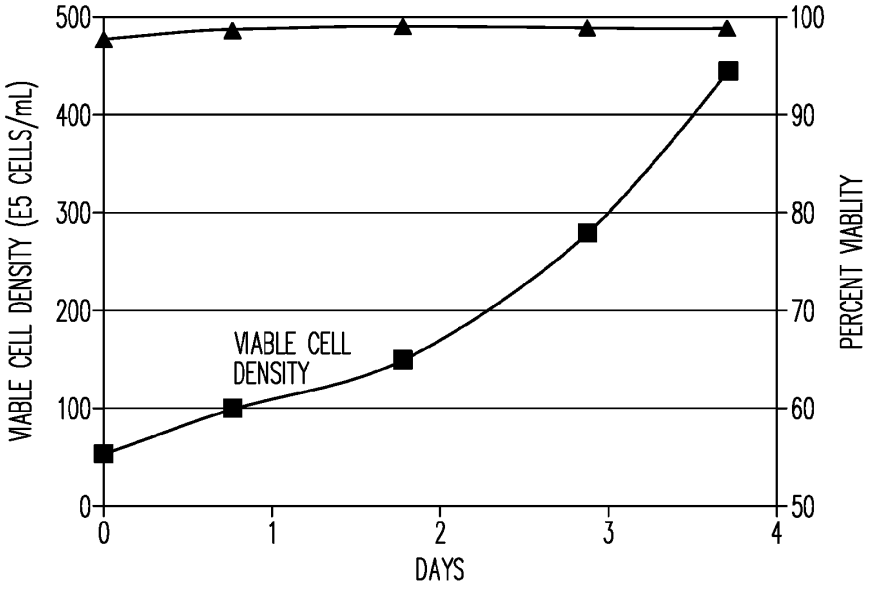

VIABLE CELL DENSITY (E5 CELLS/mL)
PERCENT VIABLITY
VIABLE CELL DENSITY
DAYS
500
400
300
200
100
0
100
90
80
70
60
50
0
1
2
3
4

FIG. 4A

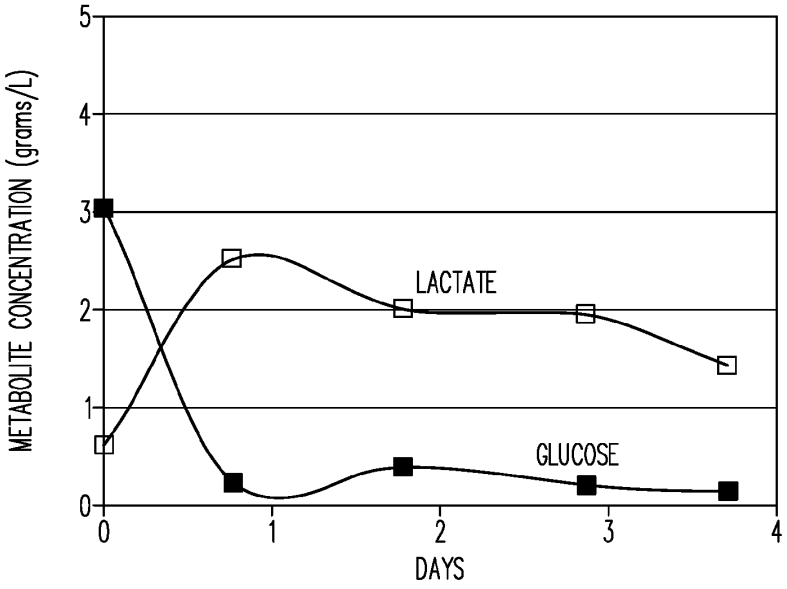

METABOLITE CONCENTRATION (grams/L)
LACTATE
GLUCOSE
DAYS
5
4
3
2
1
0
0
1
2
3
4

FIG. 4B

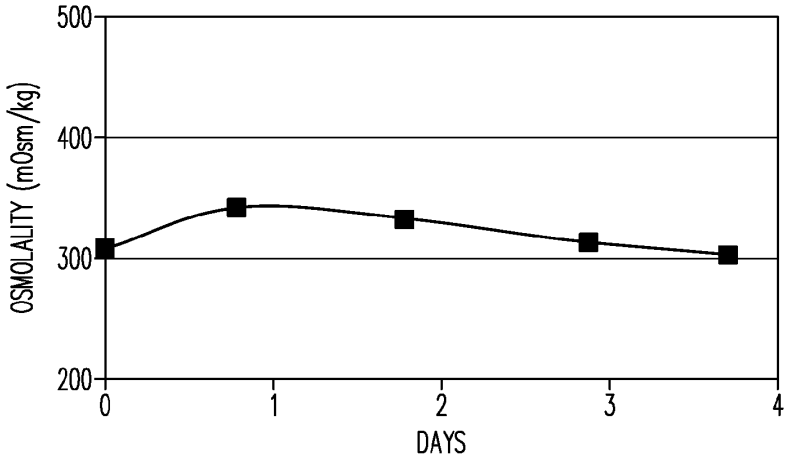


FIG. 4C

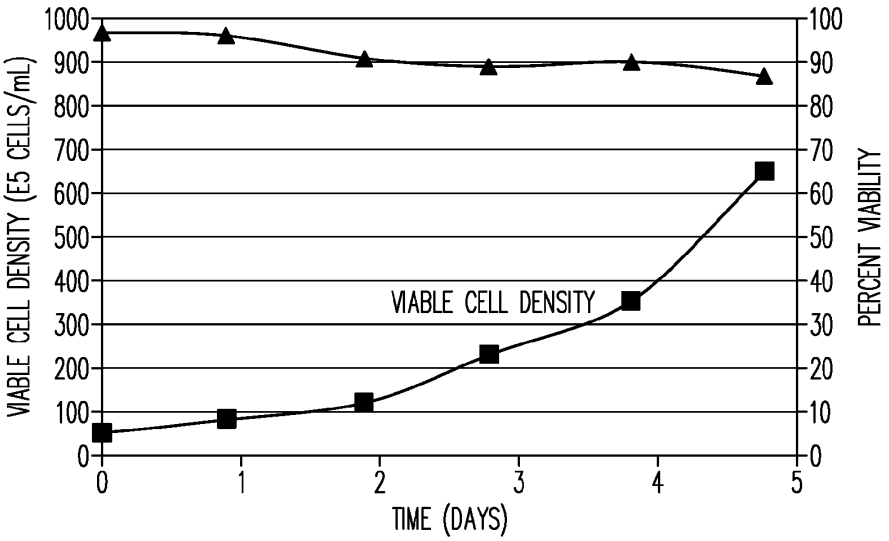

VIABLE CELL DENSITY (E5 CELLS/mL)
PERCENT VIABILITY
VIABLE CELL DENSITY
TIME (DAYS)
0
100
200
300
400
500
600
700
800
900
1000
10
20
30
40
50
60
70
80
90
100
1
2
3
4
5

FIG. 5A

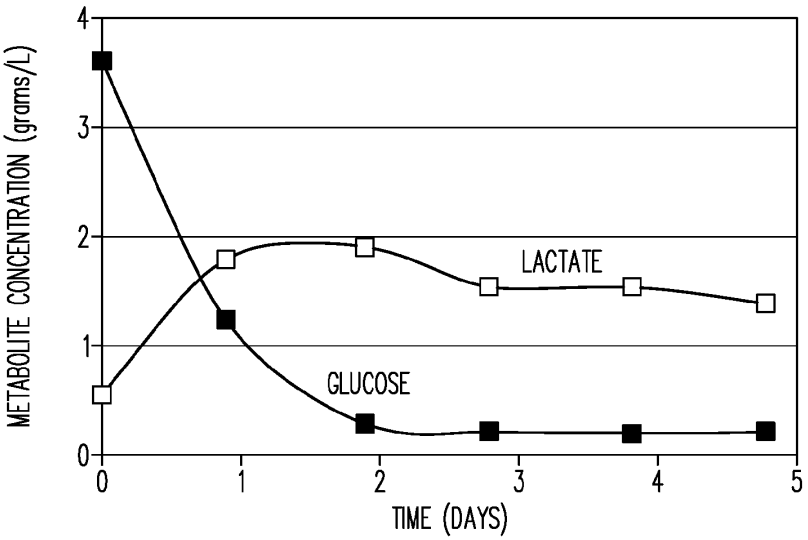

METABOLITE CONCENTRATION (grams/L)
LACTATE
GLUCOSE
TIME (DAYS)
0
1
2
3
4
5

FIG. 5B

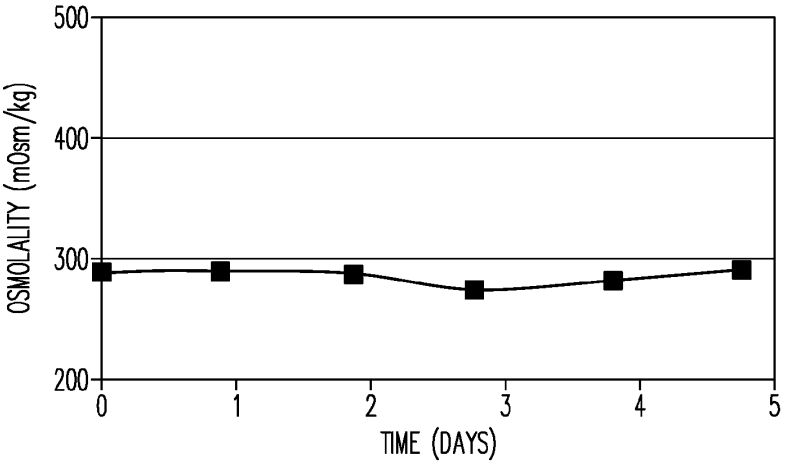


FIG. 5C

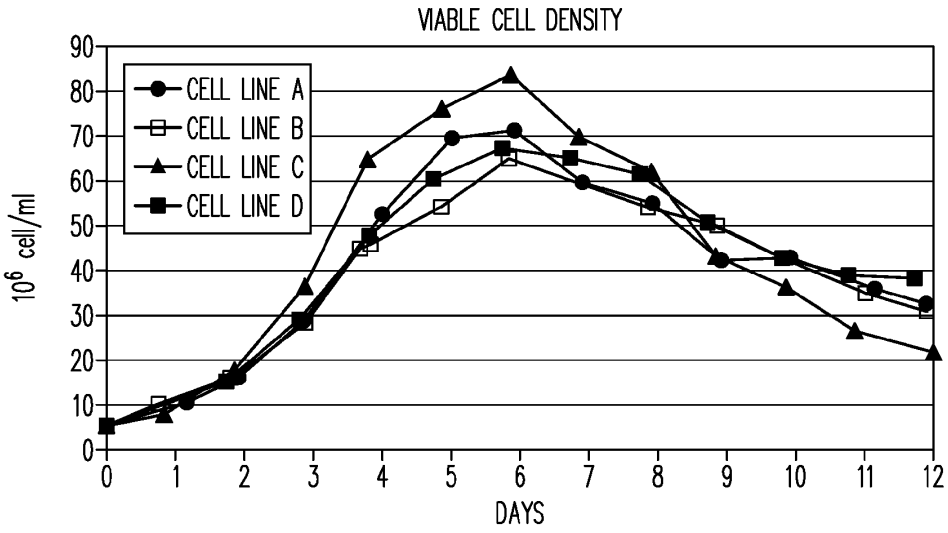

VIABLE CELL DENSITY
10^6 cell/ml
DAYS
CELL LINE A
CELL LINE B
CELL LINE C
CELL LINE D
0
10
20
30
40
50
60
70
80
90
0
1
2
3
4
5
6
7
8
9
10
11
12

FIG. 6A

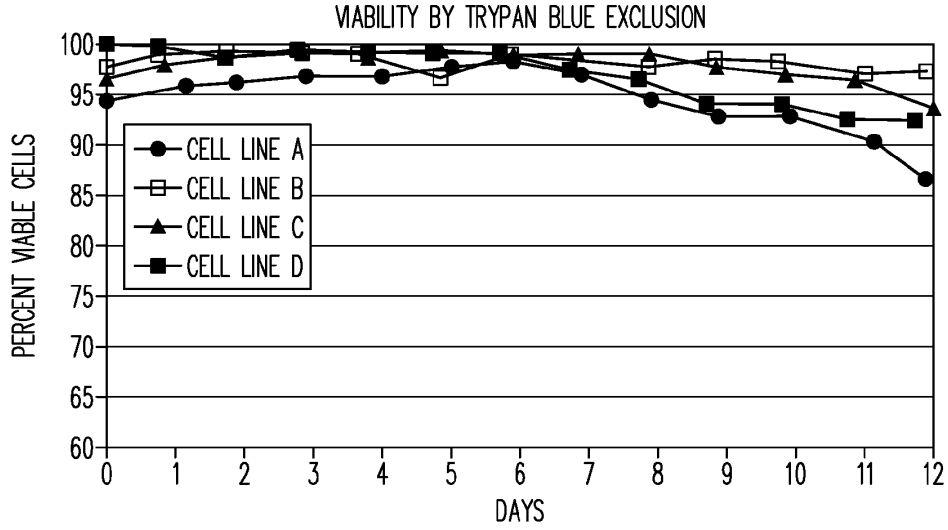

VIABILITY BY TRYPAN BLUE EXCLUSION
PERCENT VIABLE CELLS
DAYS
CELL LINE A
CELL LINE B
CELL LINE C
CELL LINE D
60
65
70
75
80
85
90
95
100
0
1
2
3
4
5
6
7
8
9
10
11
12

FIG. 6B

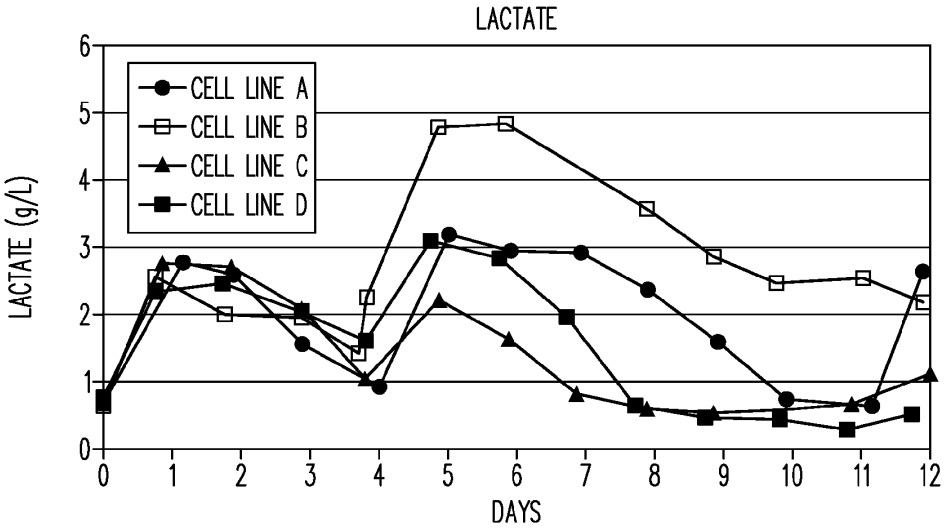

LACTATE
LACTATE (g/L)
DAYS
CELL LINE A
CELL LINE B
CELL LINE C
CELL LINE D
0
1
2
3
4
5
6
7
8
9
10
11
12

FIG. 6C

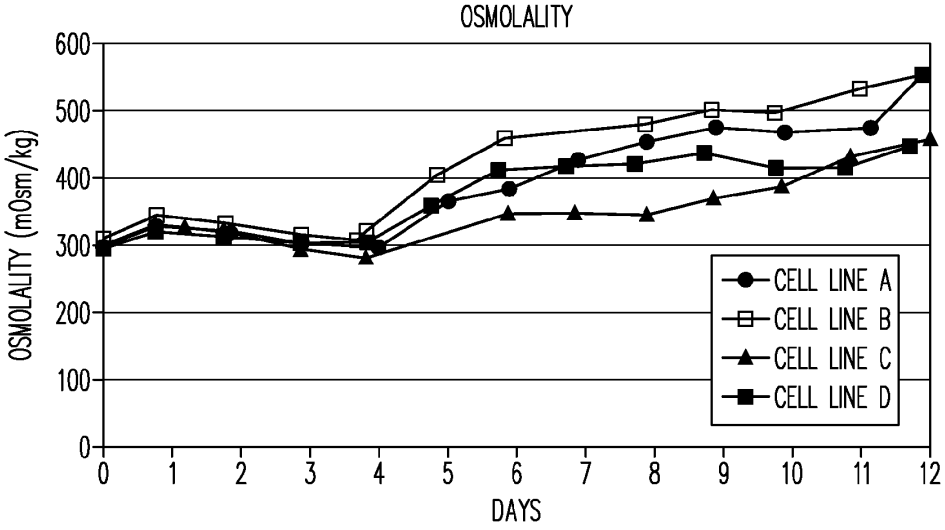

OSMOLALITY
OSMOLALITY (mOsm/kg)
DAYS
CELL LINE A
CELL LINE B
CELL LINE C
CELL LINE D
0
100
200
300
400
500
600
0
1
2
3
4
5
6
7
8
9
10
11
12

FIG. 6D

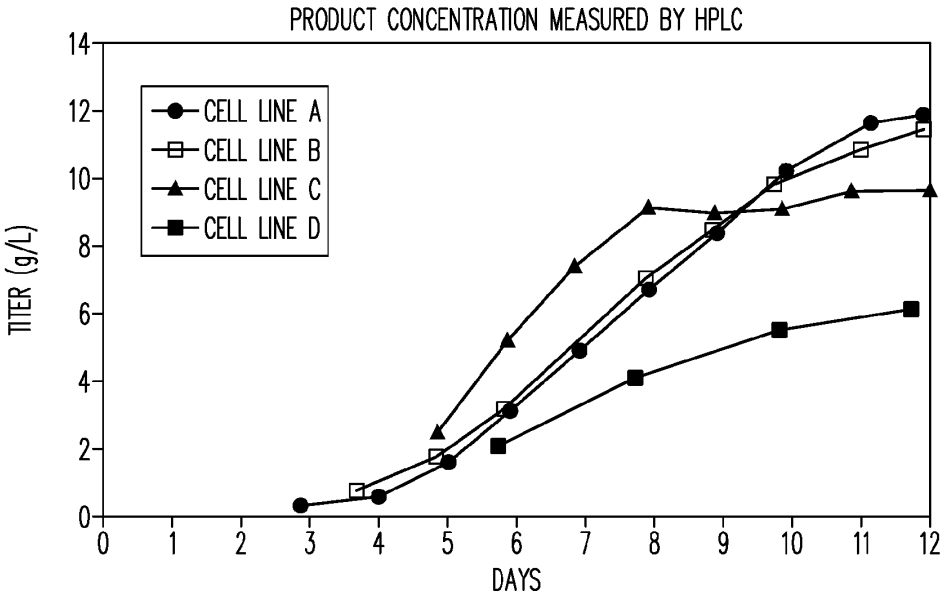

PRODUCT CONCENTRATION MEASURED BY HPLC
TITER (g/L)
DAYS
CELL LINE A
CELL LINE B
CELL LINE C
CELL LINE D
0
2
4
6
8
10
12
14
0
1
2
3
4
5
6
7
8
9
10
11
12

FIG. 6E

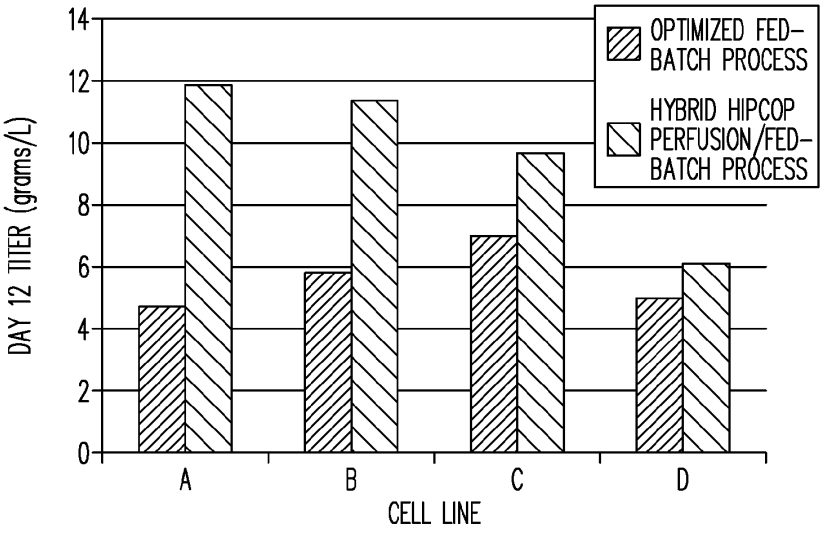

14
12
10
8
6
4
2
0
DAY 12 TITER (grams/L)
A
B
C
D
CELL LINE
OPTIMIZED FED-BATCH PROCESS
HYBRID HIPCOP PERFUSION/FED-BATCH PROCESS

FIG. 7

CELL LINE	TOTAL VOL. USED DURING PERFUSION (REACTOR VOLUMES USED FROM DAYS 0-4)	VOL. OF FEED DURING FED-BATCH (REACTOR VOLUMES)	MAXIMUM VOLUMETRIC PRODUCTIVITY ACHIEVED (grams/L/day)
A	1.6	0.4	1.04
B	1.4	0.4	1.00
C	1.4	0.4	1.16
D	1.8	0.3	0.56

FIG. 8

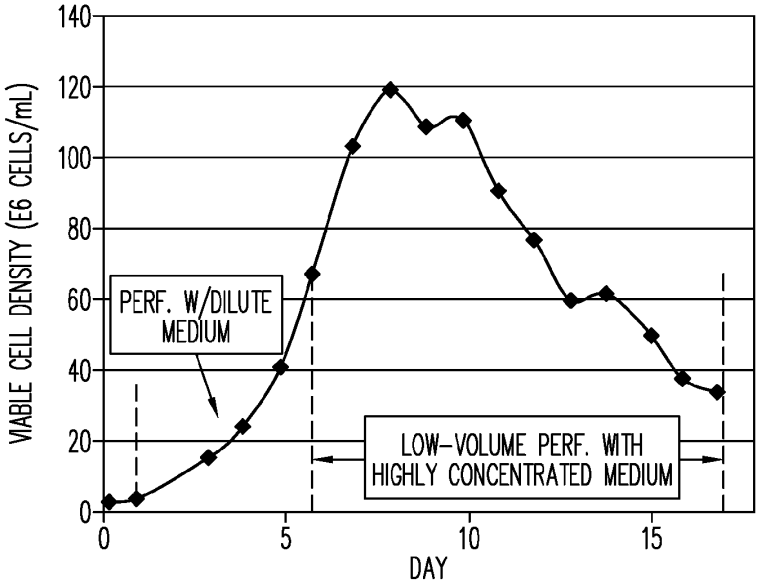

VIABLE CELL DENSITY (E6 CELLS/mL)
140
120
100
80
60
40
20
0
0
5
10
15
DAY
PERF. W/DILUTE MEDIUM
LOW-VOLUME PERF. WITH HIGHLY CONCENTRATED MEDIUM

FIG. 9A

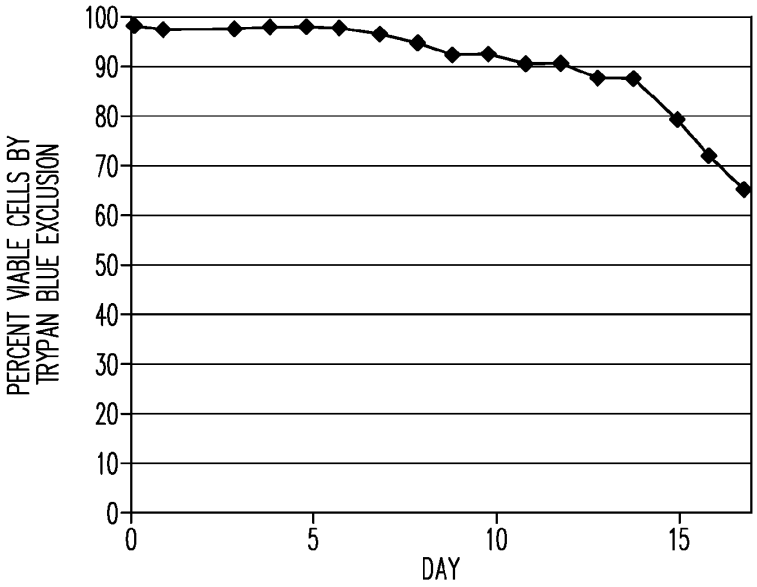

PERCENT VIABLE CELLS BY TRYPAN BLUE EXCLUSION
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
DAY

FIG. 9B

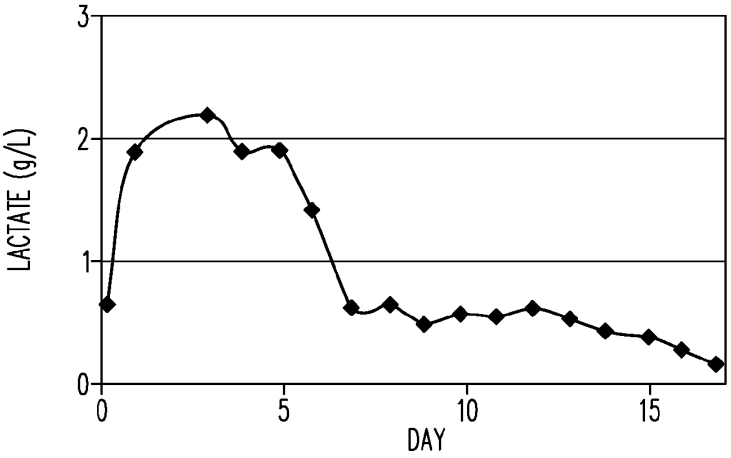

LACTATE (g/L)
3
2
1
0
0
5
10
15
DAY

FIG. 9C

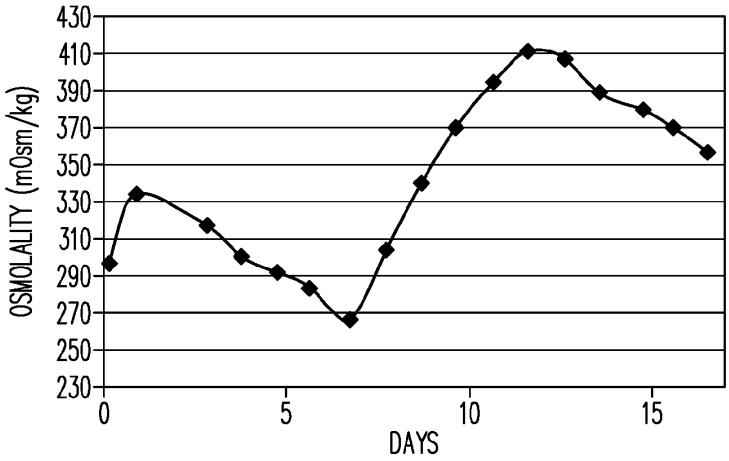

OSMOLALITY (mOsm/kg)
430
410
390
370
350
330
310
290
270
250
230
0
5
10
15
DAYS

FIG. 9D

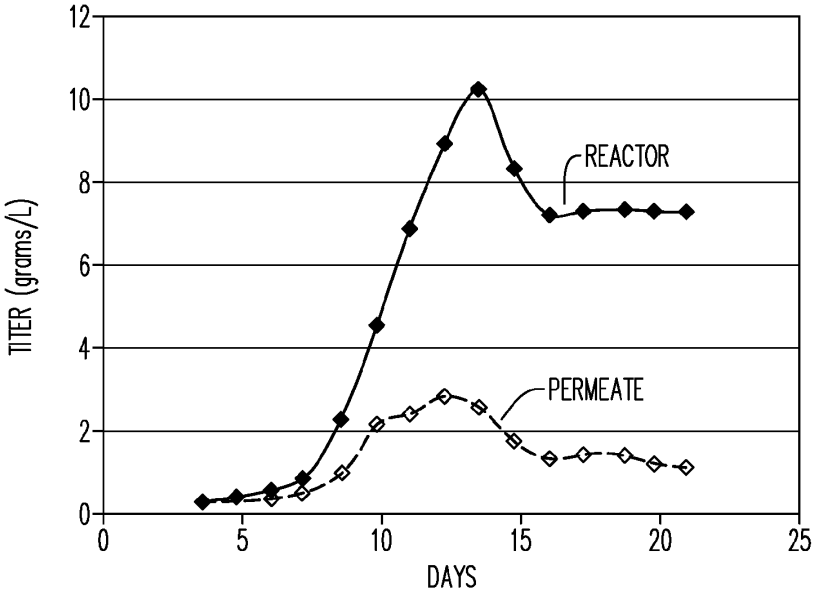

TITER (grams/L)
DAYS
REACTOR
PERMEATE
0
2
4
6
8
10
12
0
5
10
15
20
25

FIG. 9E

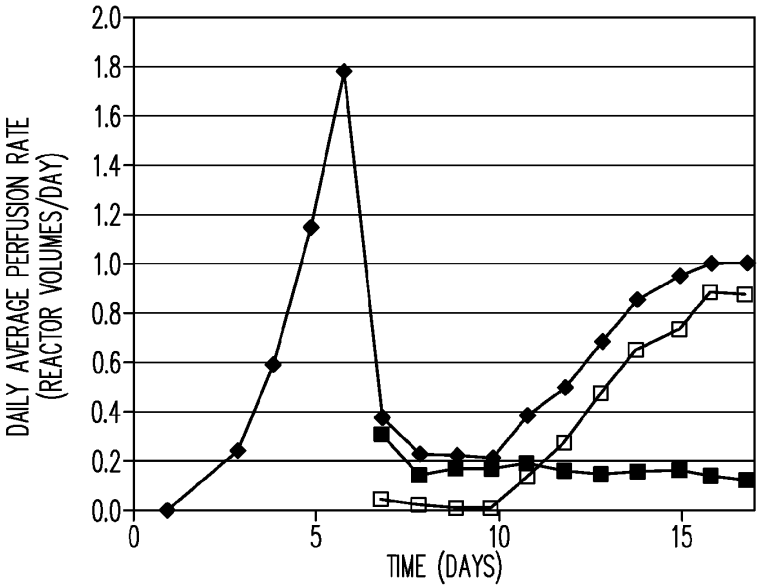

DAILY AVERAGE PERFUSION RATE
(REACTOR VOLUMES/DAY)
TIME (DAYS)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
0
5
10
15

FIG. 9F

OVERALL VOLUMETRIC PRODUCTIVITY OF PROCESS (grams/L/day)	TOTAL VOL. OF PERFUSION MEDIUM USED DURING HIPCOP PHASE OF PERFUSION-DAY 1-6 (REACTOR VOLUMES)	TOTAL VOL. OF HIGHLY CONCENTRATED PERFUSION MEDIUM USED FROM DAY 6-17 (REACTOR VOLUMES)	TOTAL VOL. OF SALINE DILUENT USED FROM DAY 6-17 (REACTOR VOLUMES)
1.51	3.8	1.73	4.0

FIG. 10

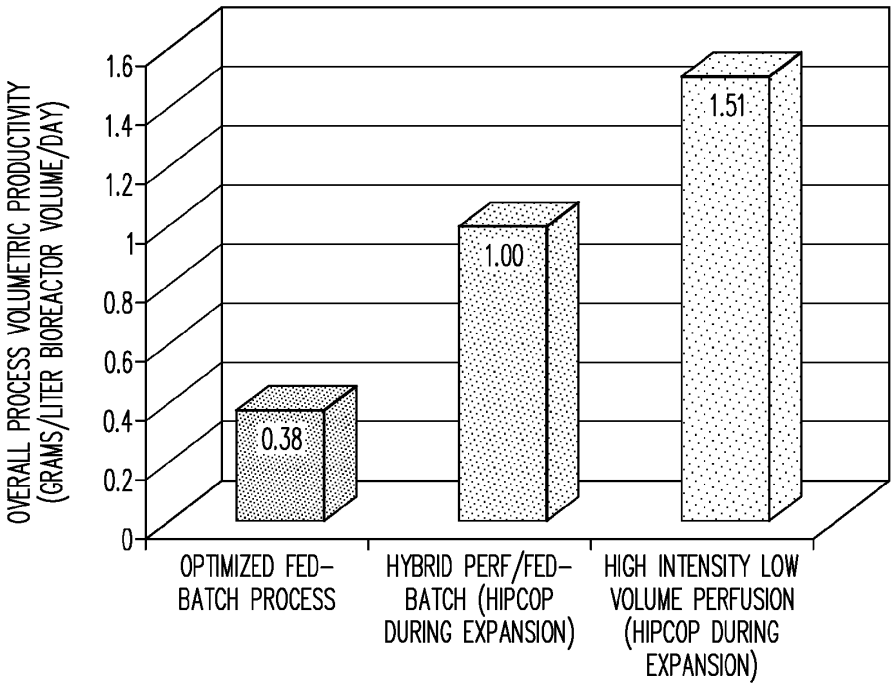


FIG. 11

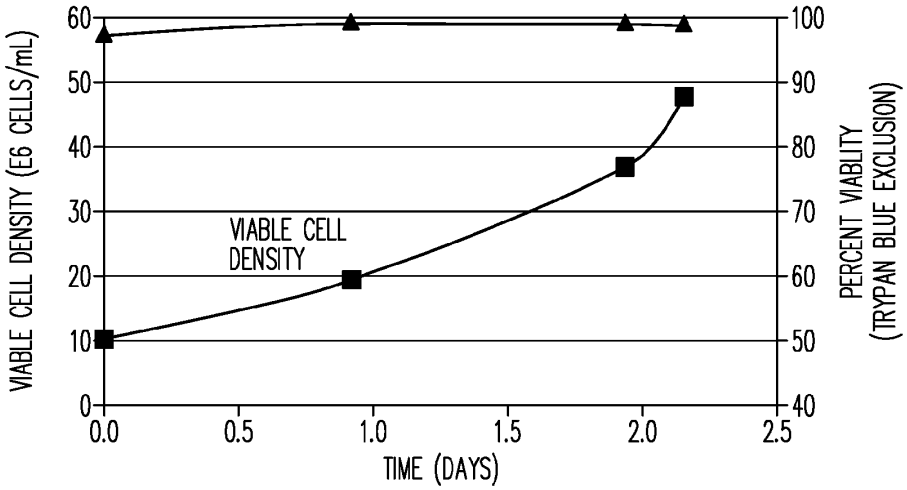


FIG. 12A

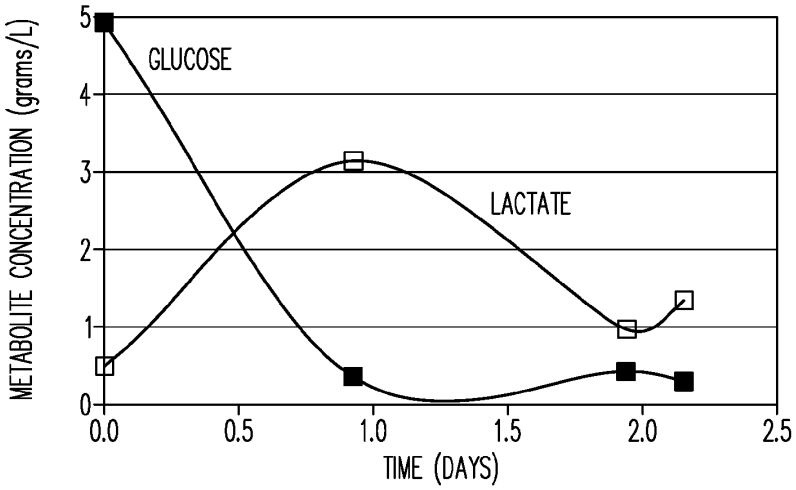


FIG. 12B

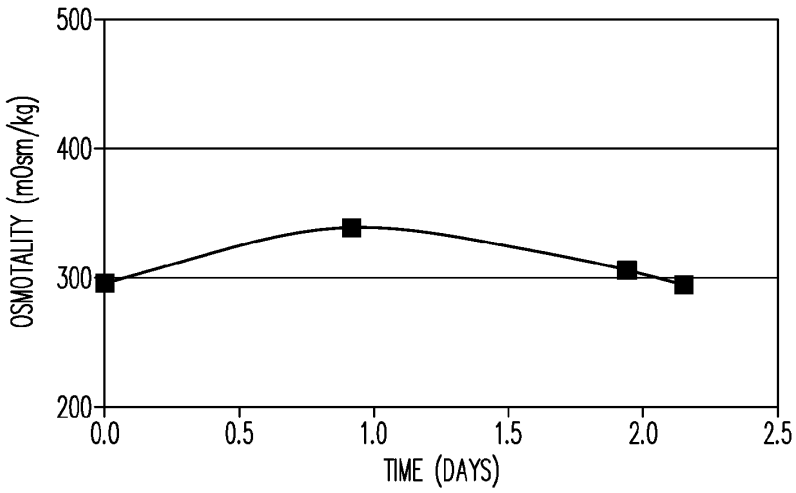


FIG. 12C

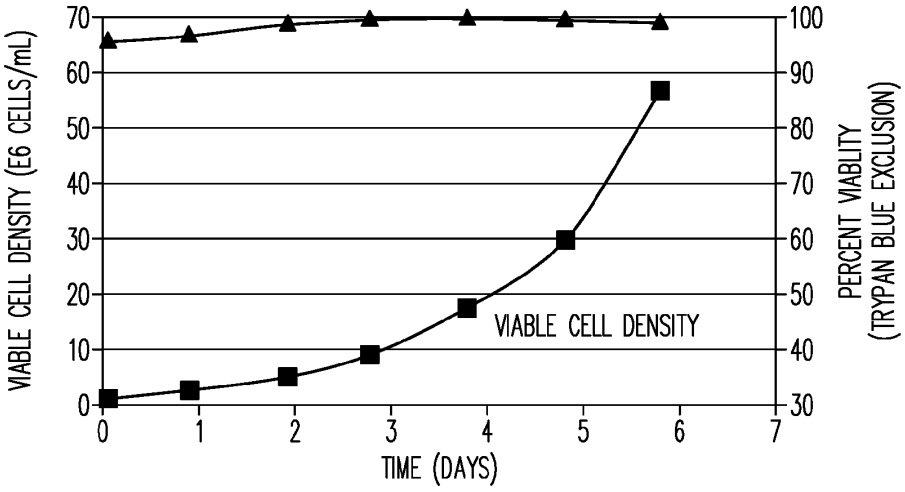


FIG. 13A

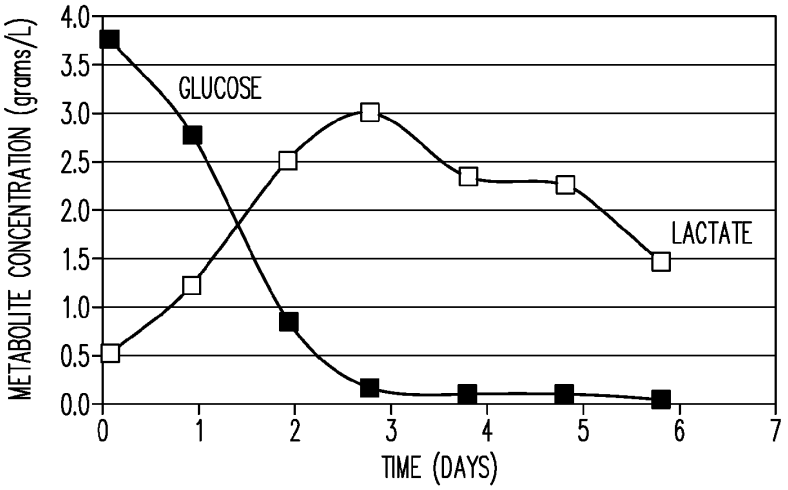


FIG. 13B

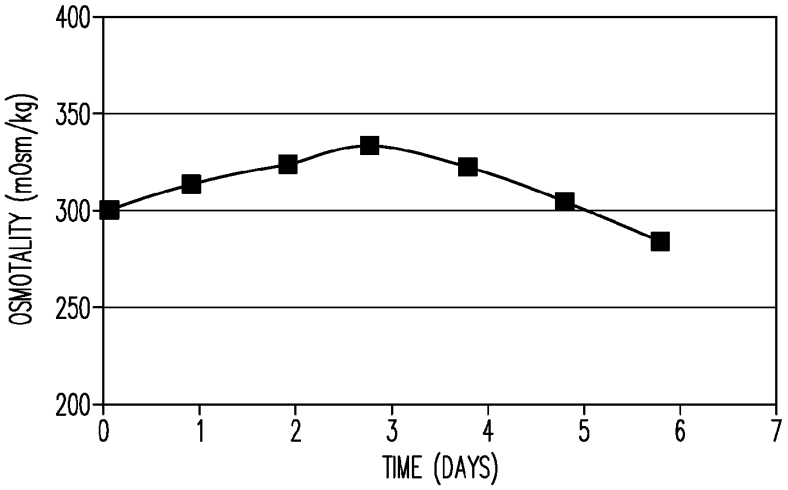


FIG. 13C

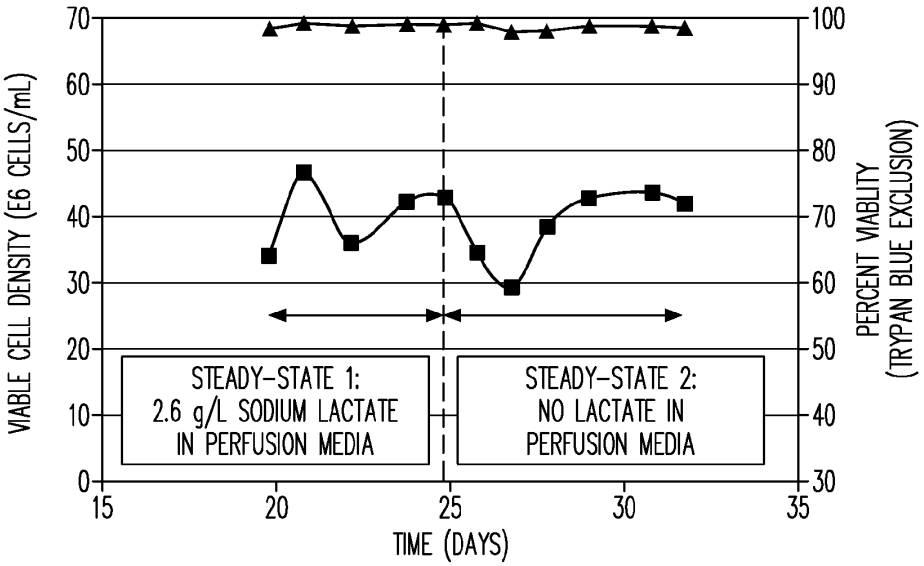


FIG. 14A

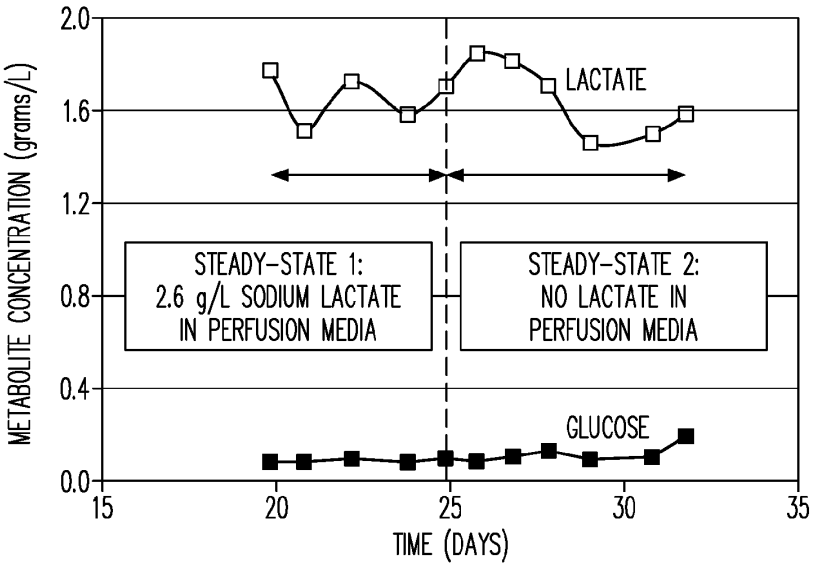


FIG. 14B

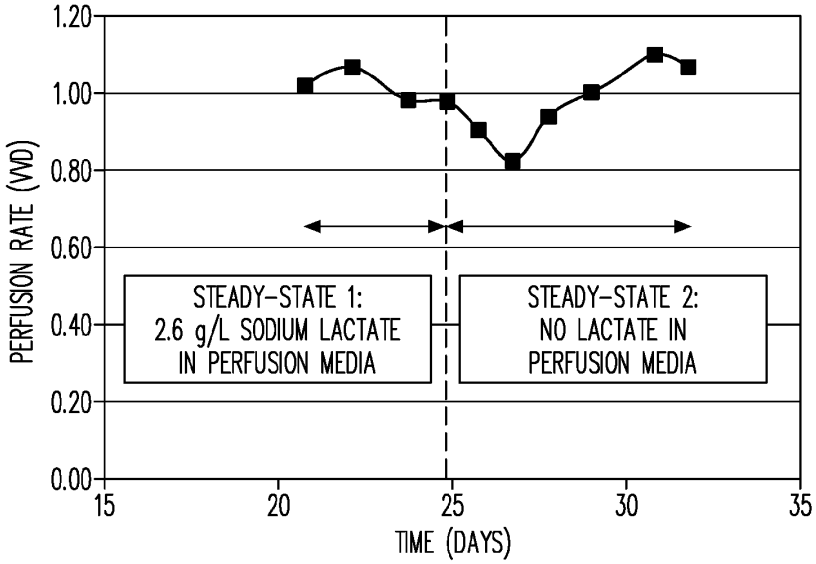


FIG. 14C

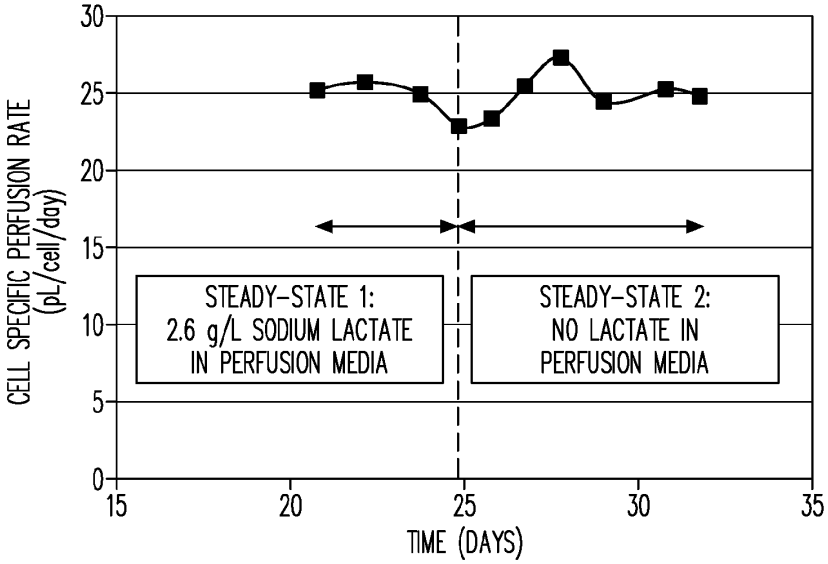


FIG. 14D

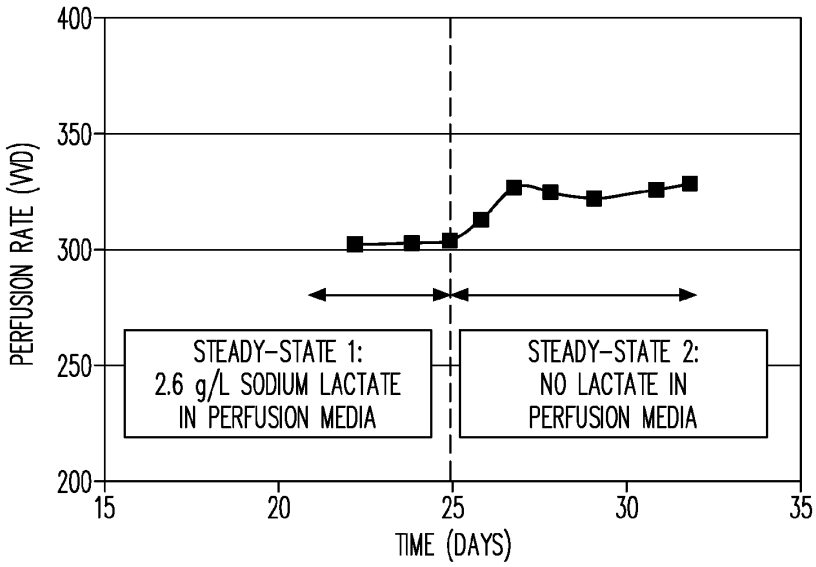

400
350
300
250
200
PERFUSION RATE (WD)
15
20
25
30
35
TIME (DAYS)
STEADY-STATE 1:
2.6 g/L SODIUM LACTATE
IN PERFUSION MEDIA
STEADY-STATE 2:
NO LACTATE IN
PERFUSION MEDIA

FIG. 14E