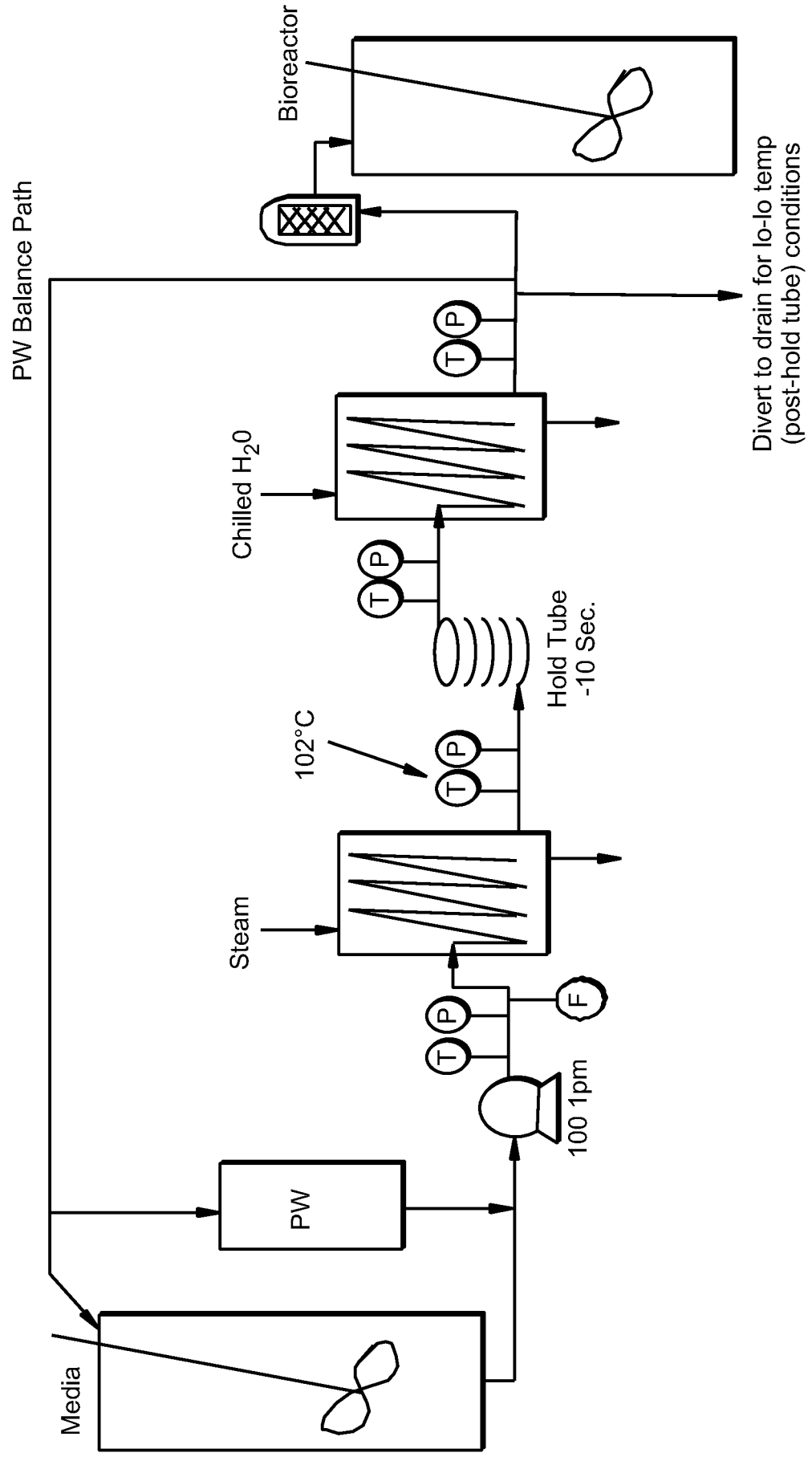

Bioreactor
PW Balance Path
Divert to drain for lo-lo temp
(post-hold tube) conditions
Chilled H_2O
Hold Tube
-10 Sec.
102°C
Steam
100 1pm
PW
Media
T
P
F

FIG. 1

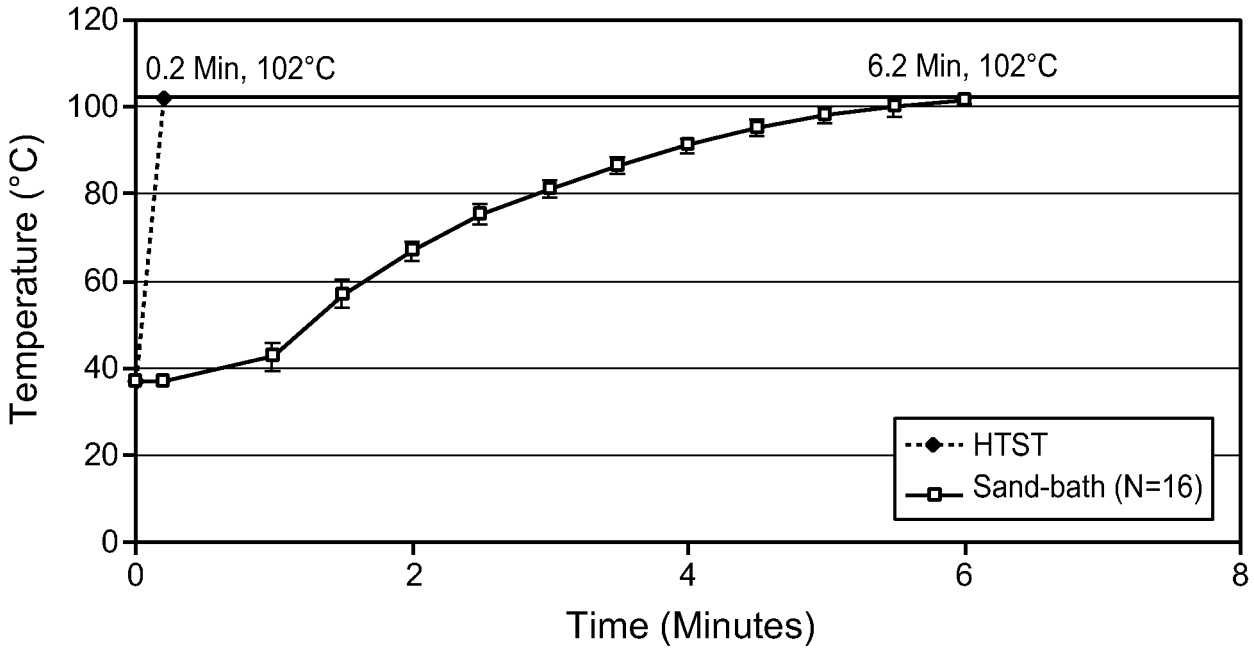

120
100
80
60
40
20
0
0.2 Min, 102°C
6.2 Min, 102°C
Temperature (°C)
HTST
Sand-bath (N=16)
0
2
4
6
8
Time (Minutes)

FIG. 2

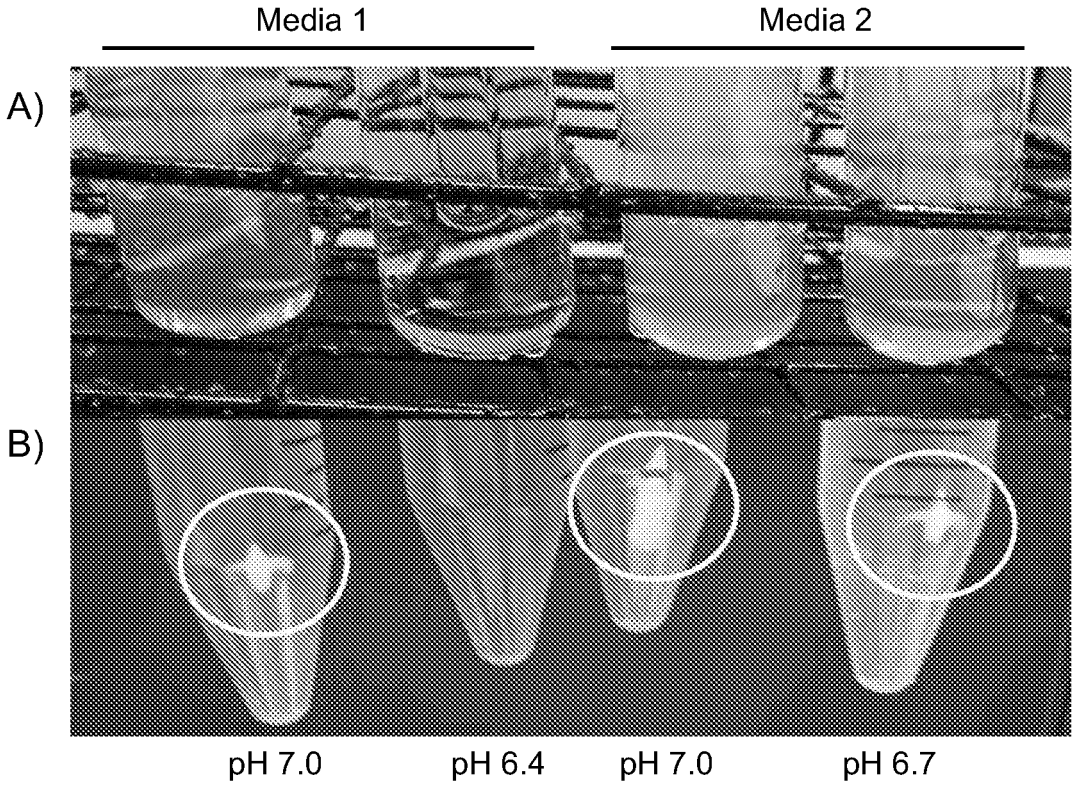

Media 1
Media 2
A)
B)
pH 7.0
pH 6.4
pH 7.0
pH 6.7

FIG. 3

Sorted Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
[PO4] mM*[Ca] mM	28.678271	2.413001	11.88	<.0001*
[Ca] mM*pH	28.174984	5.263934	5.35	0.0002*
[PO4] mM(0.1,5.9)	19.481343	5.449794	3.57	0.0038*
[PO4] mM*pH	11.959851	6.270648	1.91	0.0807
[Ca] mM*[Ca] mM	8.7582743	4.753139	1.84	0.0902
[Ca] mM(0.1,2.9)	7.0752298	4.027581	1.76	0.1044
pH(6,7.2)	12.889804	15.75033	0.82	0.4291
[PO4] mM*[PO4] mM	3.8623212	4.870252	0.79	0.4432
pH*pH	7.699752	12.35742	0.62	0.5449

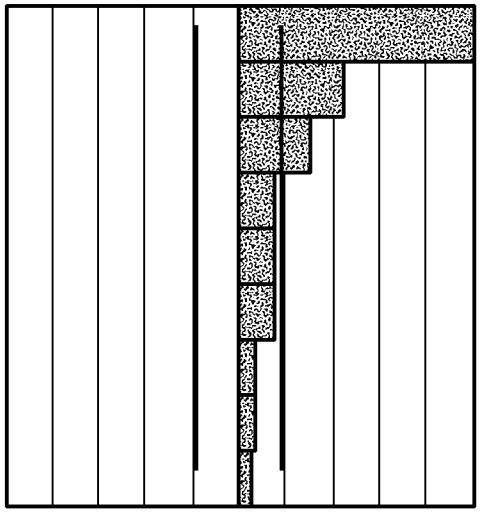


FIG. 4

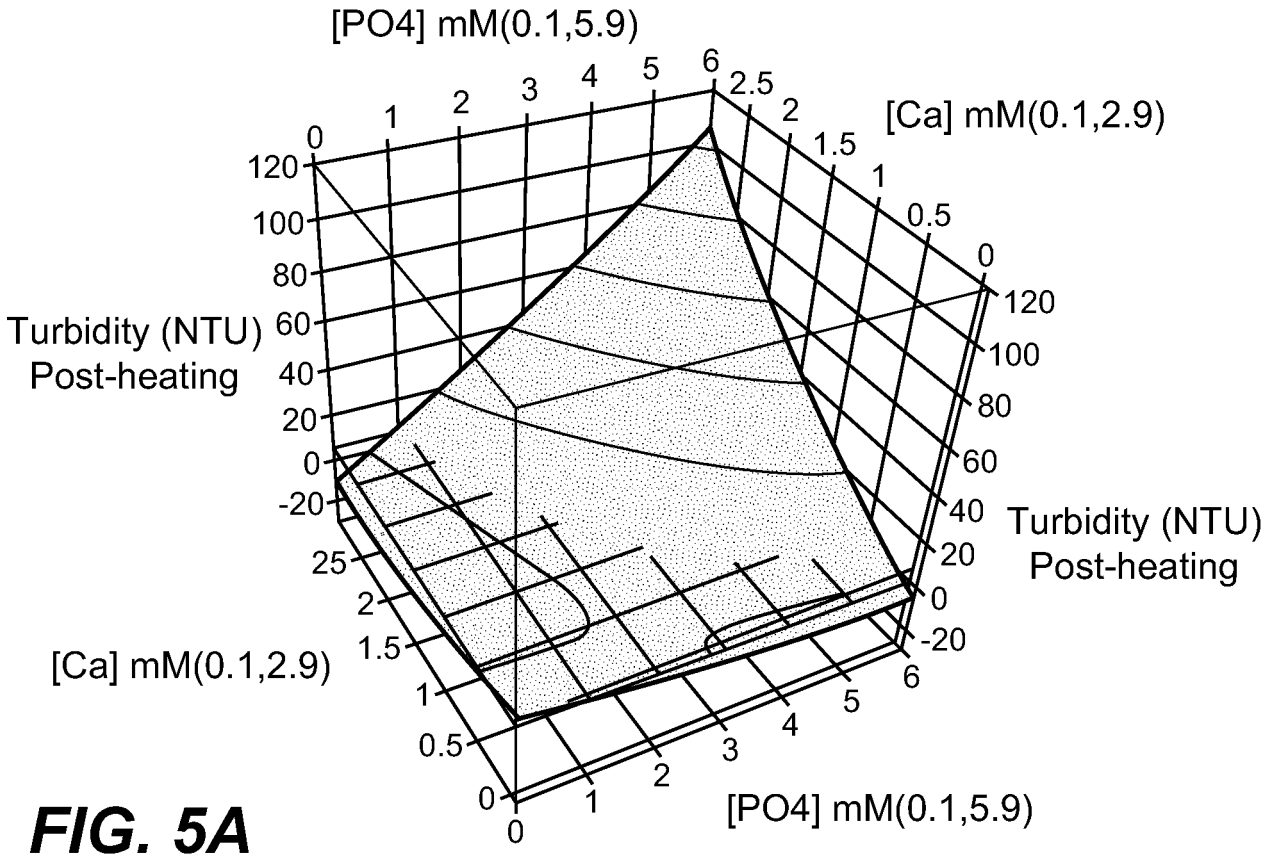

[PO4] mM(0.1,5.9)
[Ca] mM(0.1,2.9)
Turbidity (NTU)
Post-heating
Turbidity (NTU)
Post-heating
[Ca] mM(0.1,2.9)
[PO4] mM(0.1,5.9)

FIG. 5A

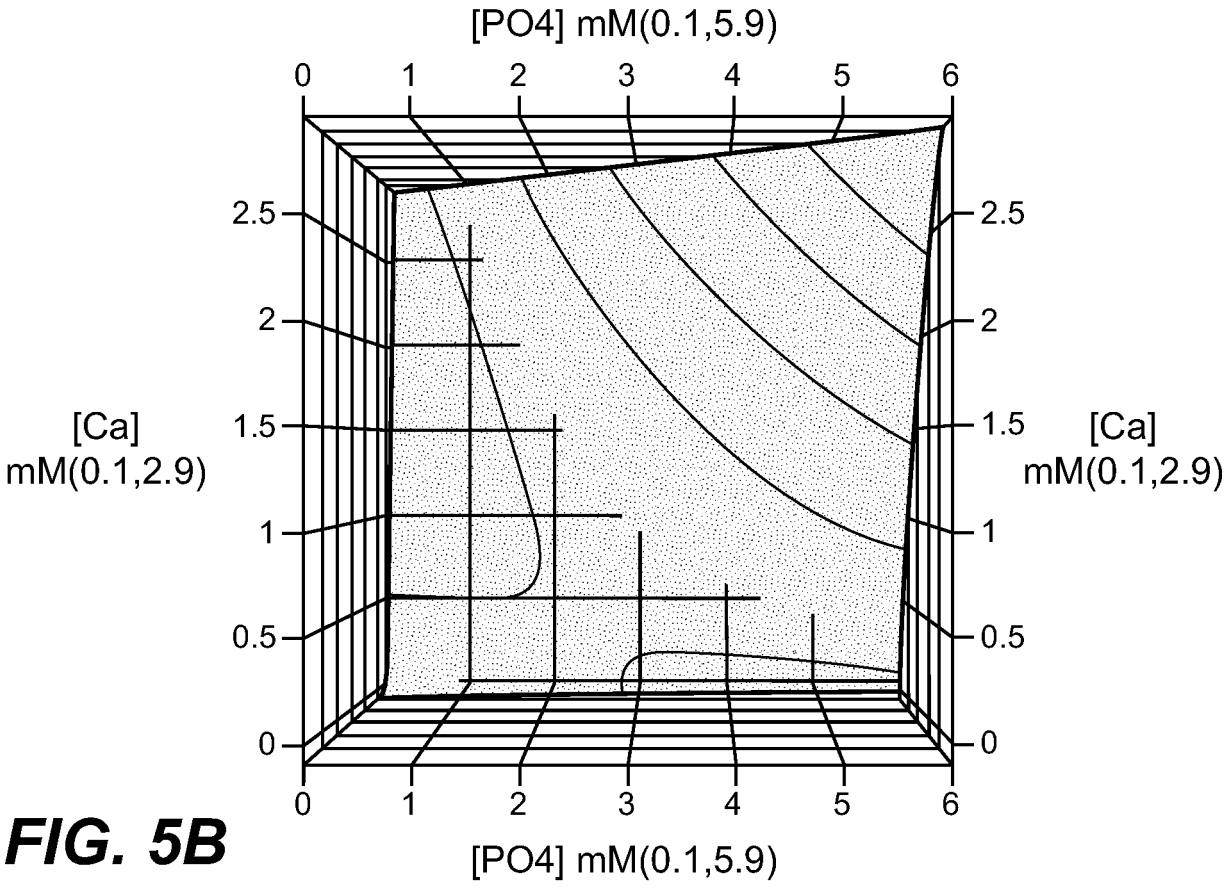

[PO4] mM(0.1,5.9)
[Ca]
mM(0.1,2.9)
[Ca]
mM(0.1,2.9)
[PO4] mM(0.1,5.9)

FIG. 5B

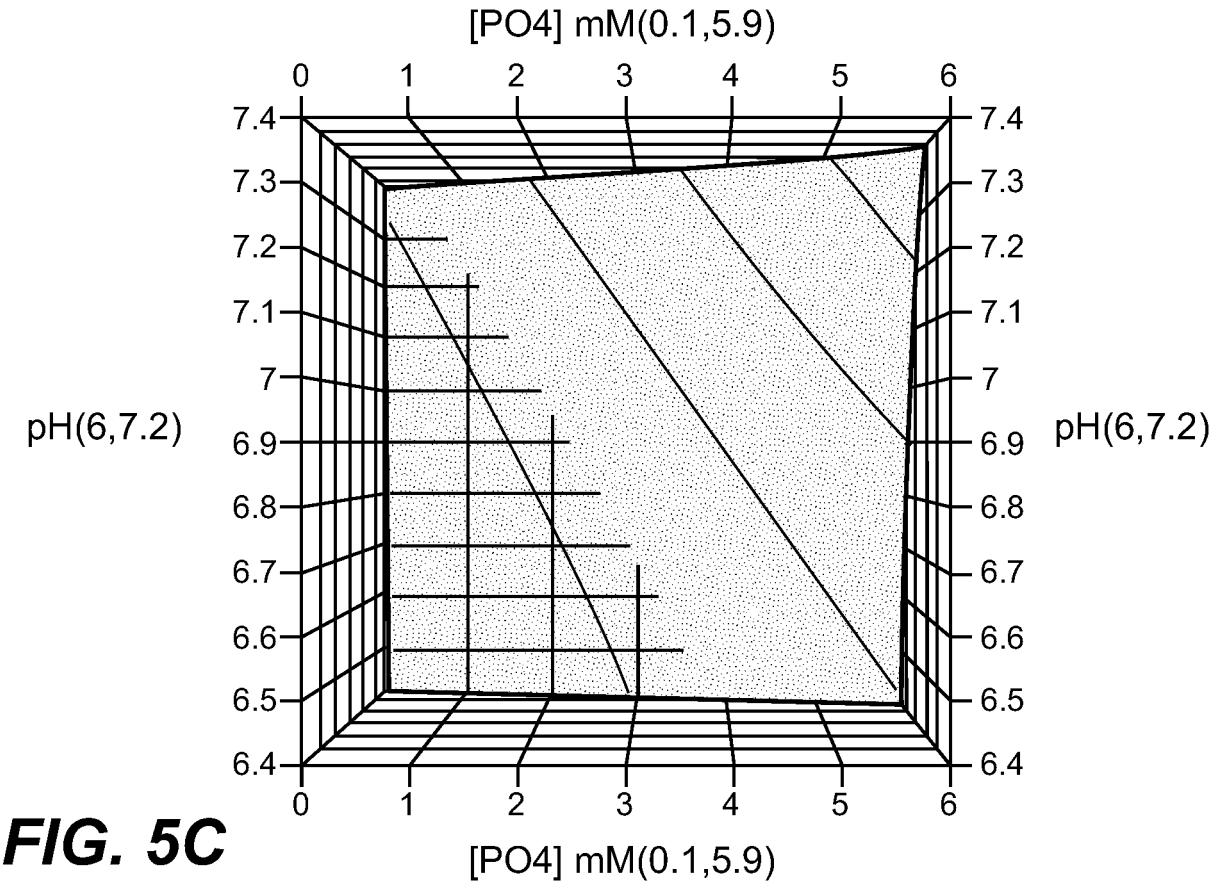

[PO4] mM(0.1,5.9)
0 1 2 3 4 5 6
7.4 7.3 7.2 7.1 7 6.9 6.8 6.7 6.6 6.5 6.4
pH(6,7.2)
pH(6,7.2)
0 1 2 3 4 5 6
[PO4] mM(0.1,5.9)

FIG. 5C

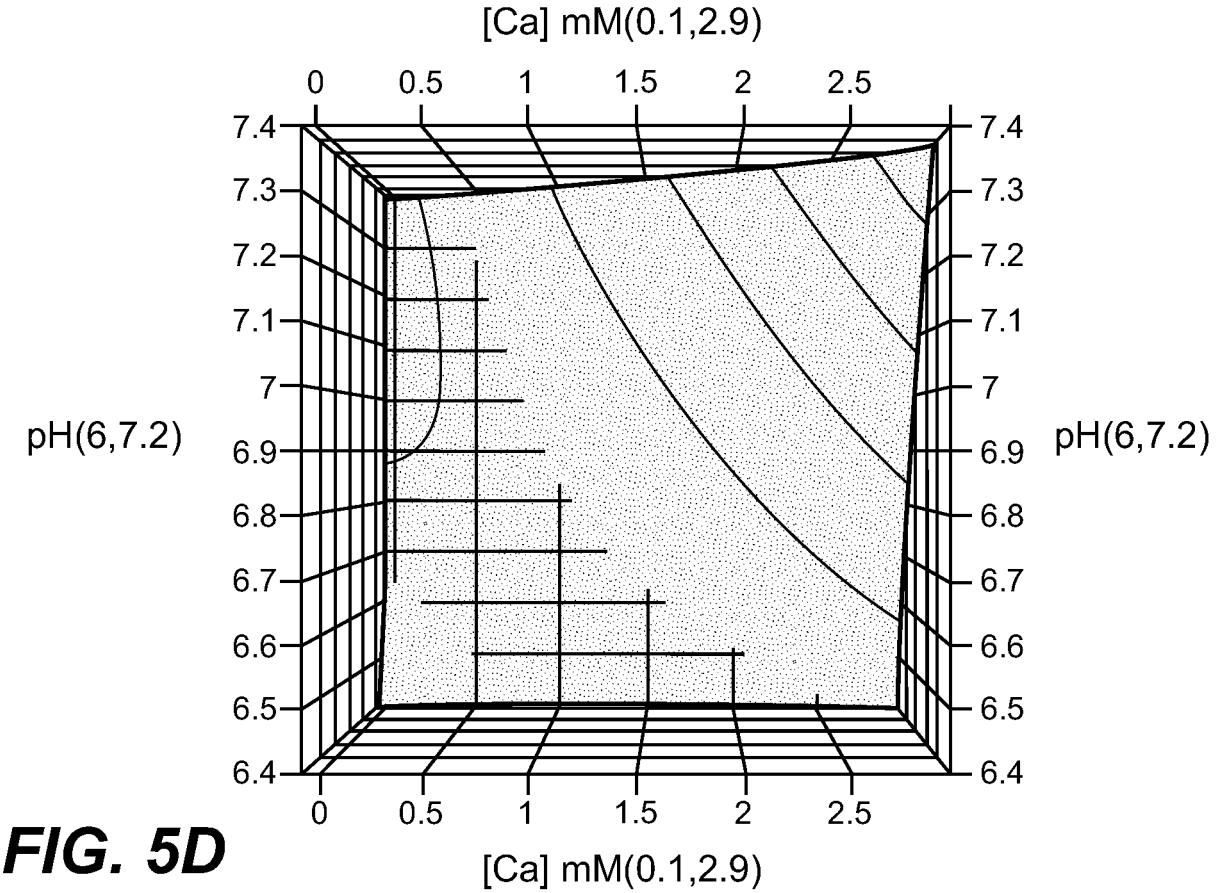

[Ca] mM(0.1,2.9)
0 0.5 1 1.5 2 2.5
7.4 7.3 7.2 7.1 7 6.9 6.8 6.7 6.6 6.5 6.4
pH(6,7.2)
pH(6,7.2)
0 0.5 1 1.5 2 2.5
[Ca] mM(0.1,2.9)

FIG. 5D

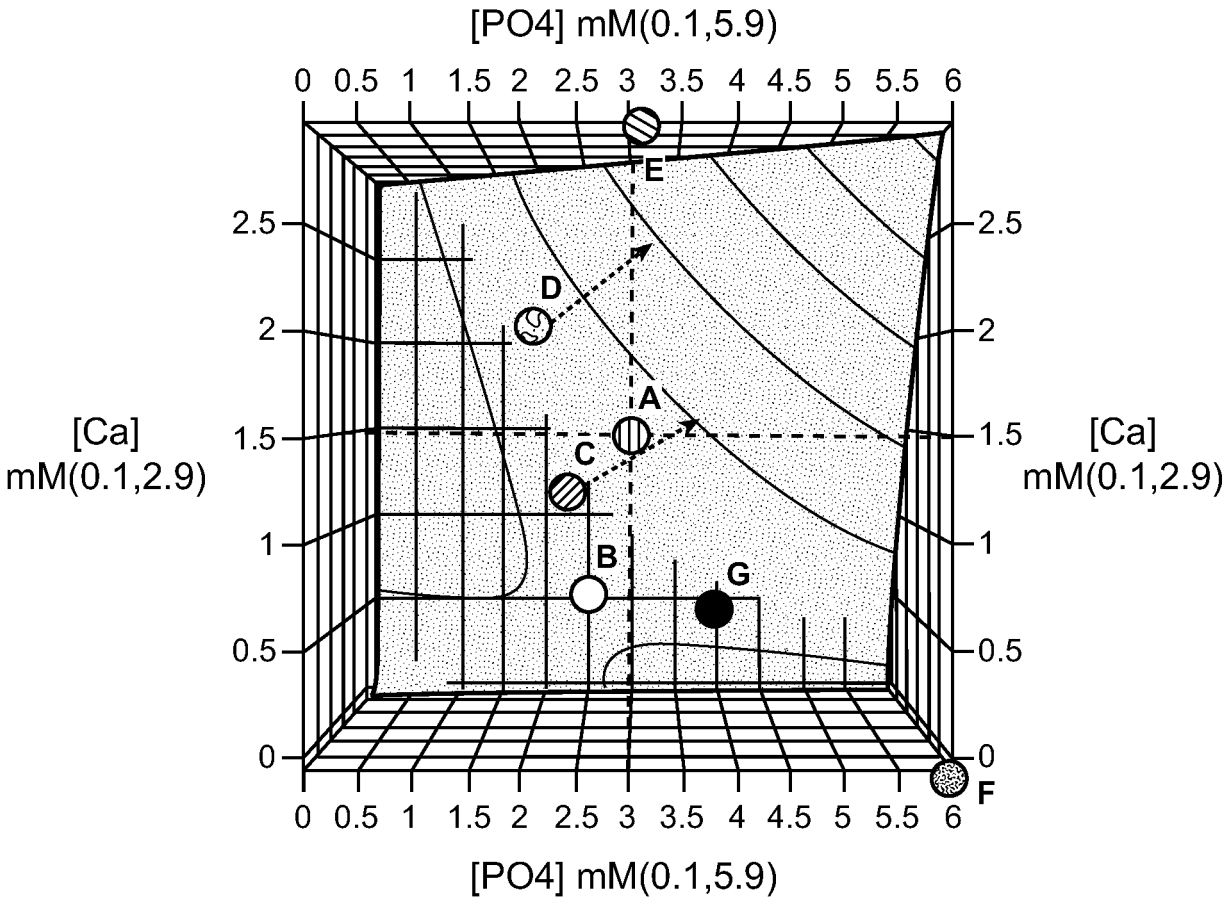

[PO4] mM(0.1,5.9)
0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6
[Ca] mM(0.1,2.9)
0 0.5 1 1.5 2 2.5
A
B
C
D
E
F
G
[PO4] mM(0.1,5.9)
[Ca] mM(0.1,2.9)
A = Media 3 and Media 4
B = Media 6
C = Media 11
D = Media 12
E = Media 13
F = Media 8 or Media 9
G = Media 7

FIG. 6

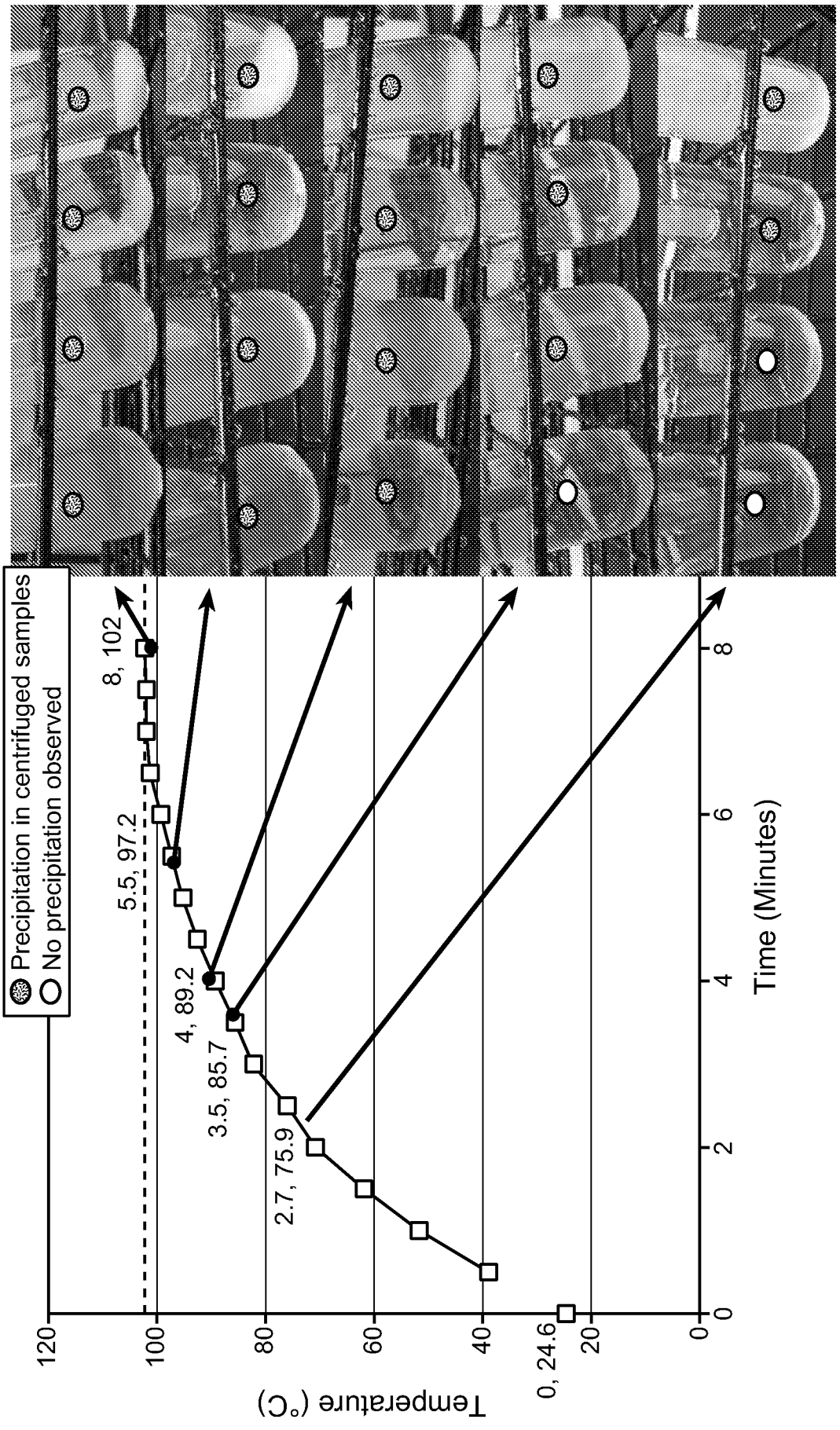

Media 1
Media 2
Media 4
Media 10
Precipitation in centrifuged samples
No precipitation observed
8, 102
5.5, 97.2
4, 89.2
3.5, 85.7
2.7, 75.9
0, 24.6
Temperature (°C)
Time (Minutes)
120
100
80
60
40
20
0
0
2
4
6
8

FIG. 7

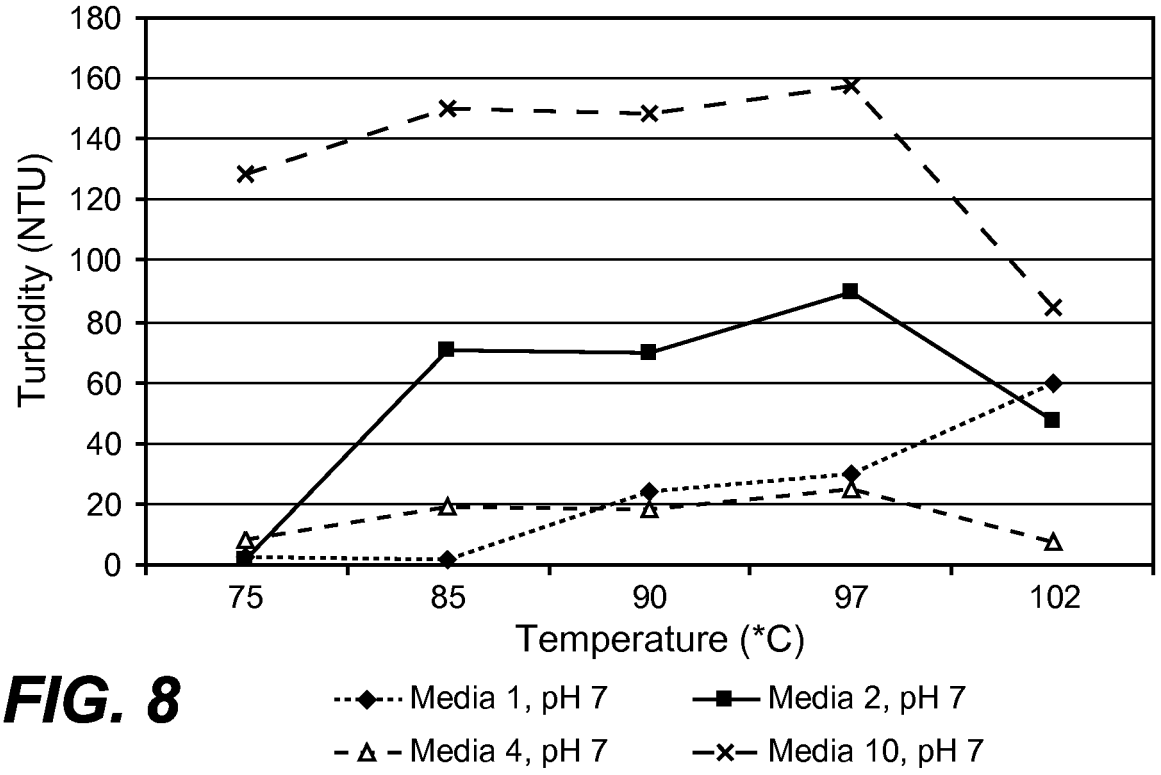

180
160
140
120
100
80
60
40
20
0
Turbidity (NTU)
75
85
90
97
102
Temperature (*C)
Media 1, pH 7
Media 2, pH 7
Media 4, pH 7
Media 10, pH 7

FIG. 8

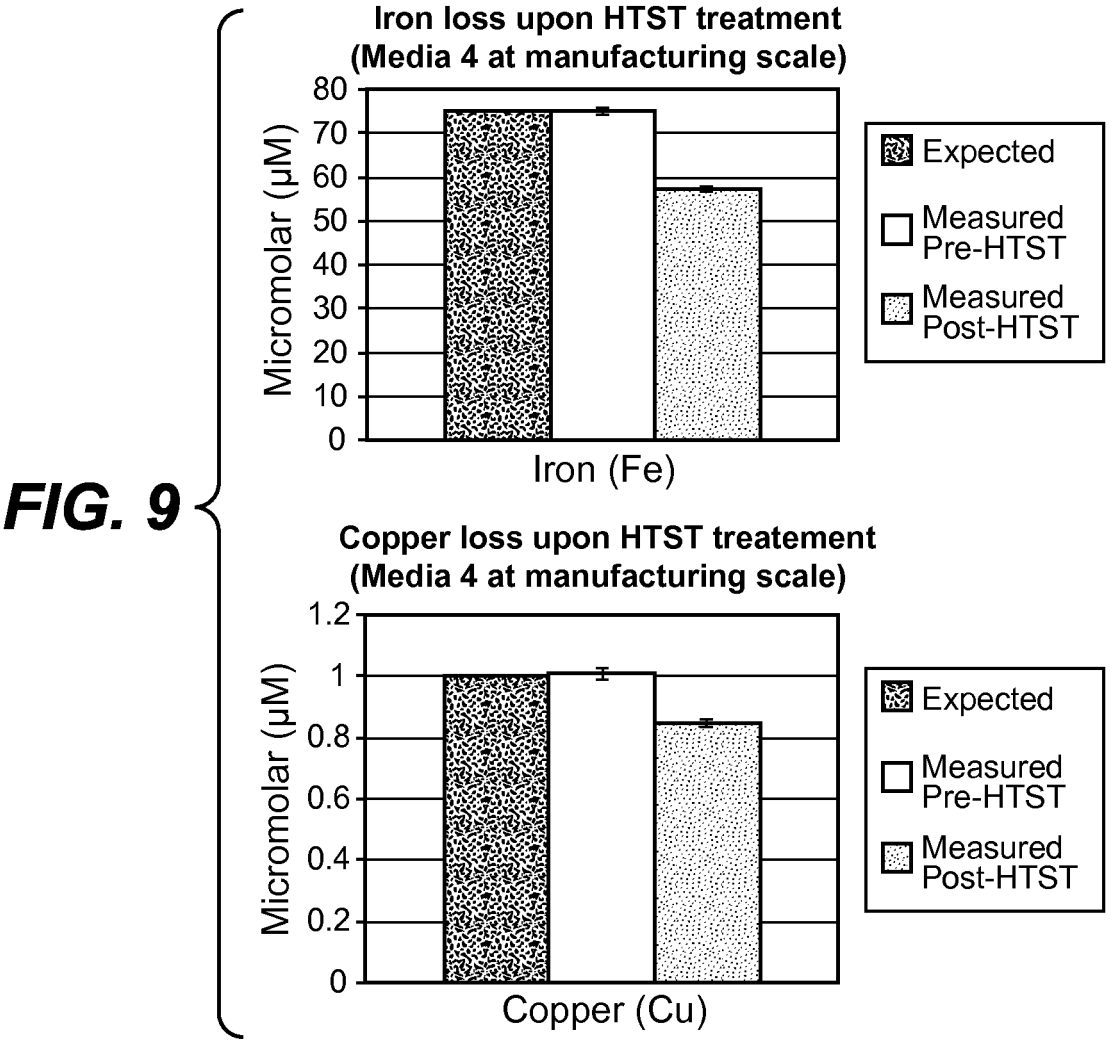

Iron loss upon HTST treatment
(Media 4 at manufacturing scale)
Micromolar (µM)
80
70
60
50
40
30
20
10
0
Iron (Fe)
Expected
Measured Pre-HTST
Measured Post-HTST
Copper loss upon HTST treatement
(Media 4 at manufacturing scale)
Micromolar (µM)
1.2
1
0.8
0.6
0.4
0.2
0
Copper (Cu)
Expected
Measured Pre-HTST
Measured Post-HTST

FIG. 9

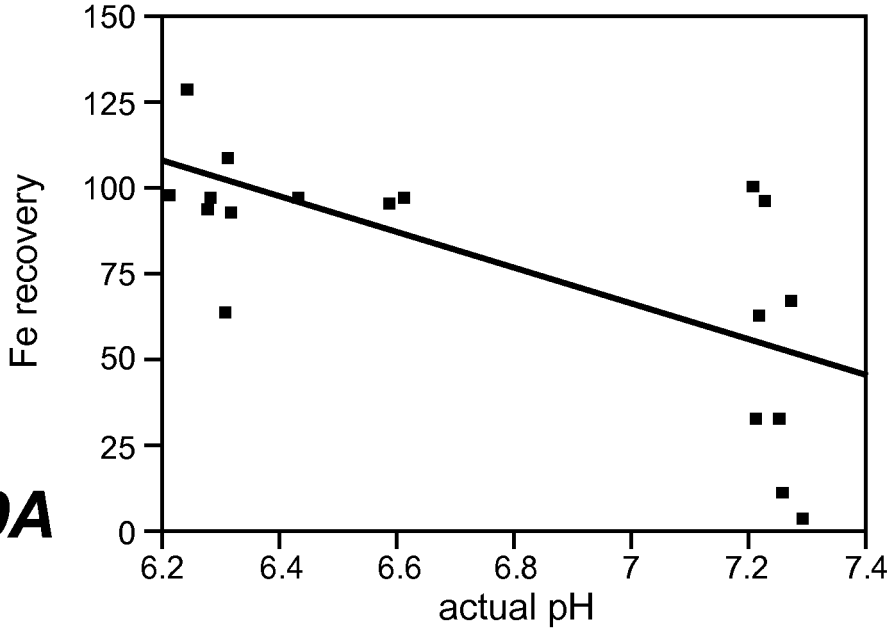


FIG. 10A

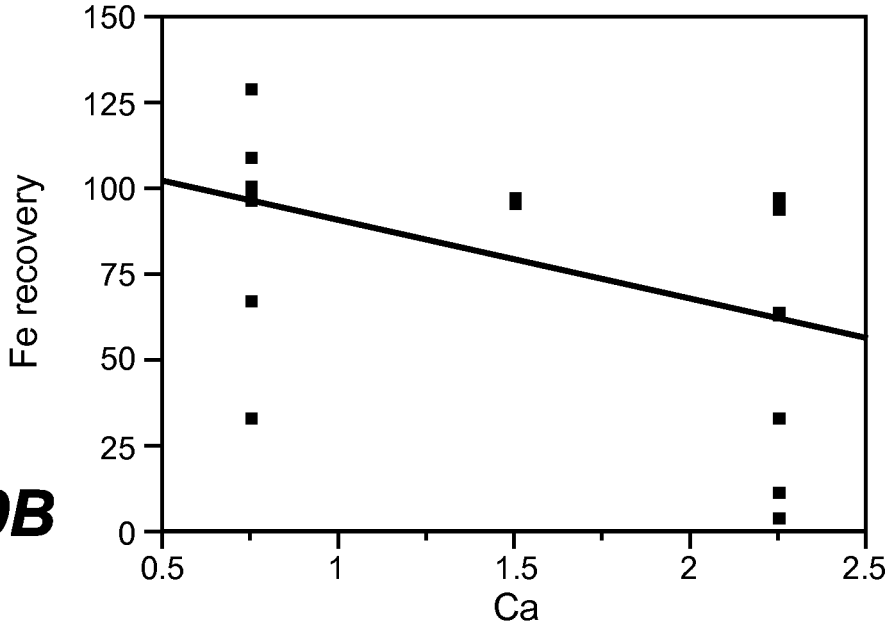


FIG. 10B

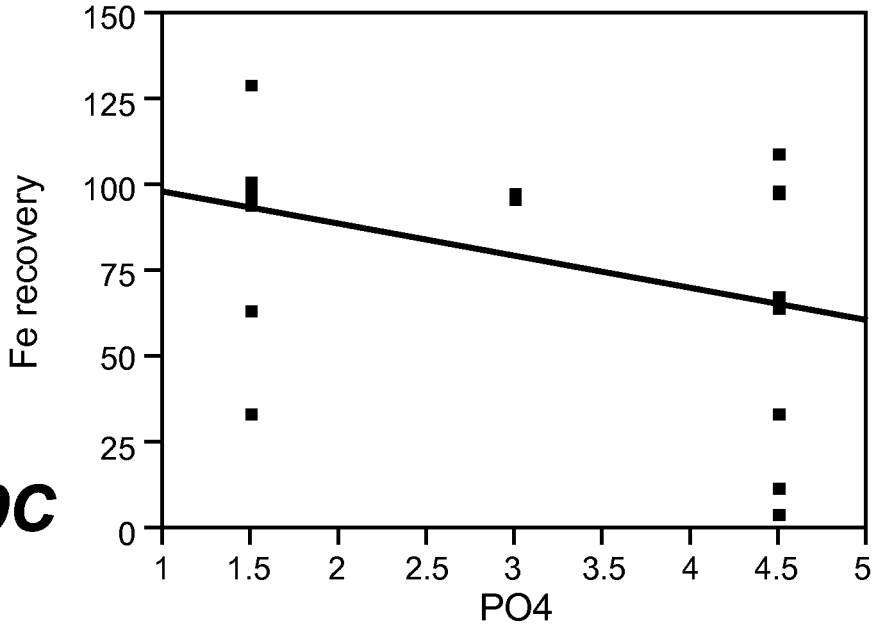


FIG. 10C

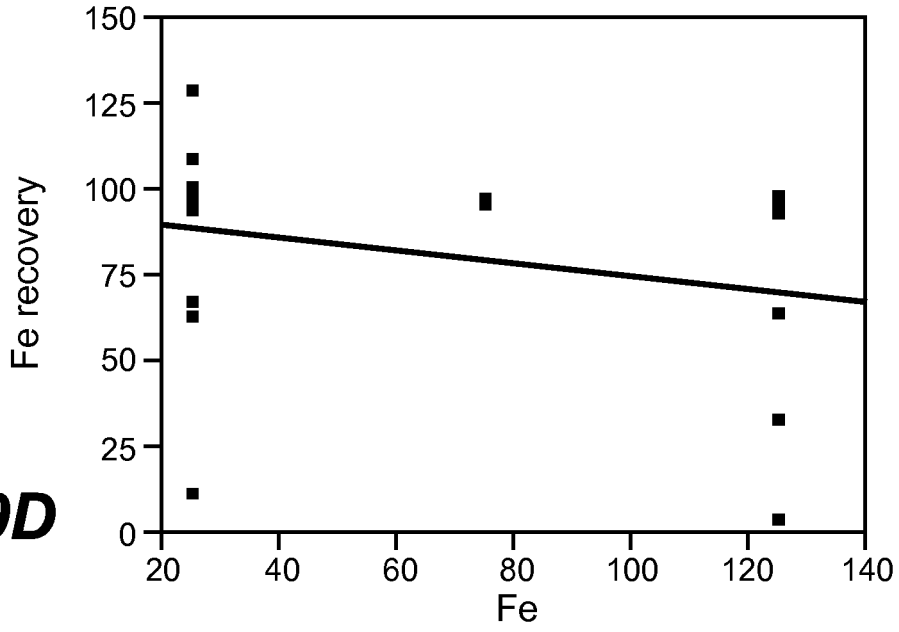


FIG. 10D

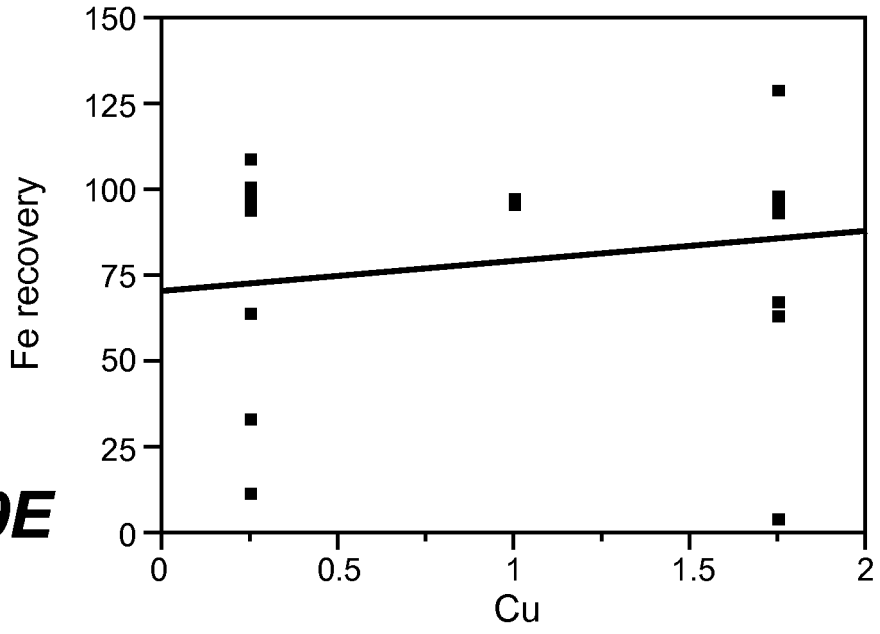


FIG. 10E

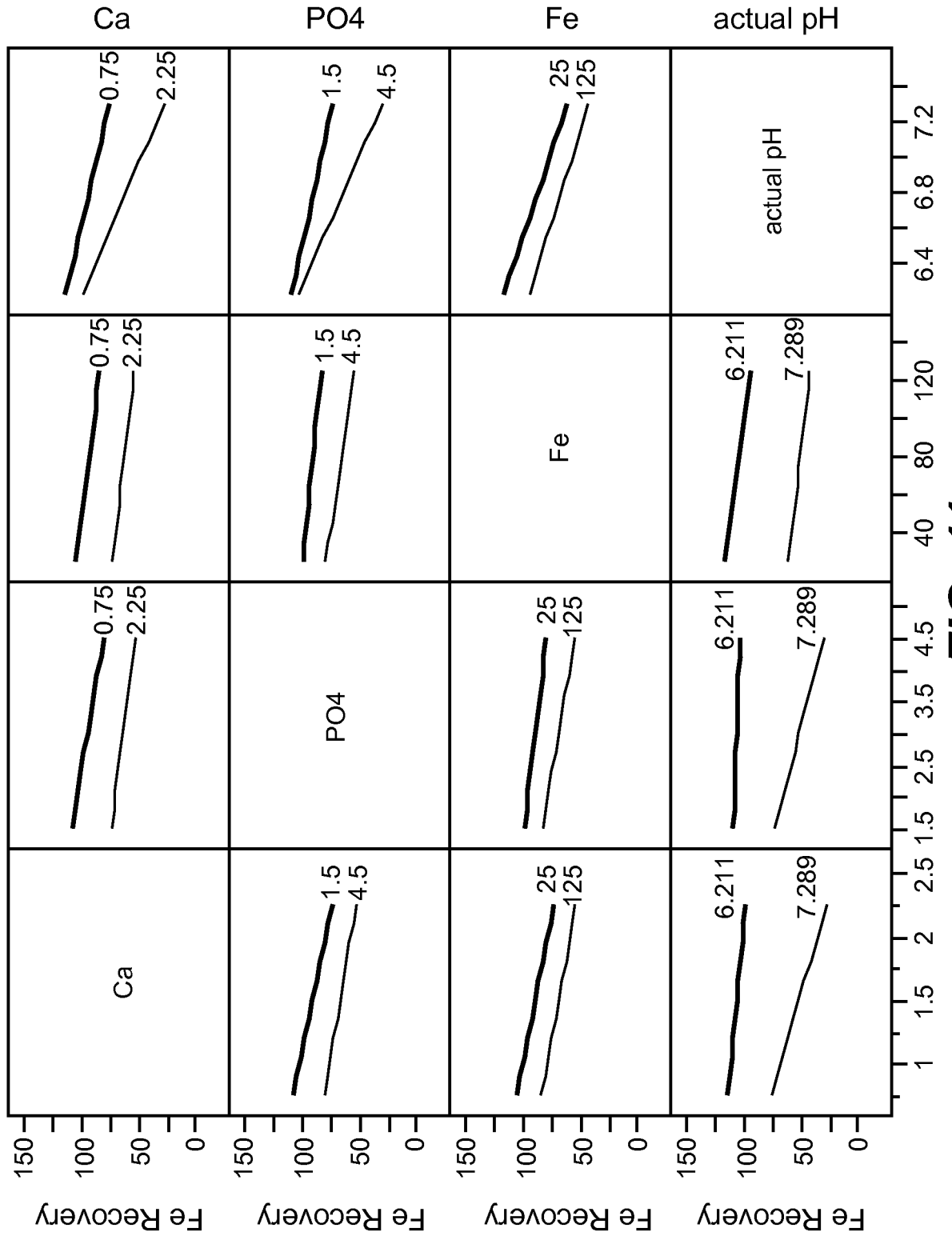

Ca
PO4
Fe
actual pH
Fe Recovery
0.75
2.25
1.5
4.5
25
125
6.211
7.289
1
1.5
2
2.5
3.5
4.5
40
80
120
6.4
6.8
7.2
150
100
50
0

FIG. 11

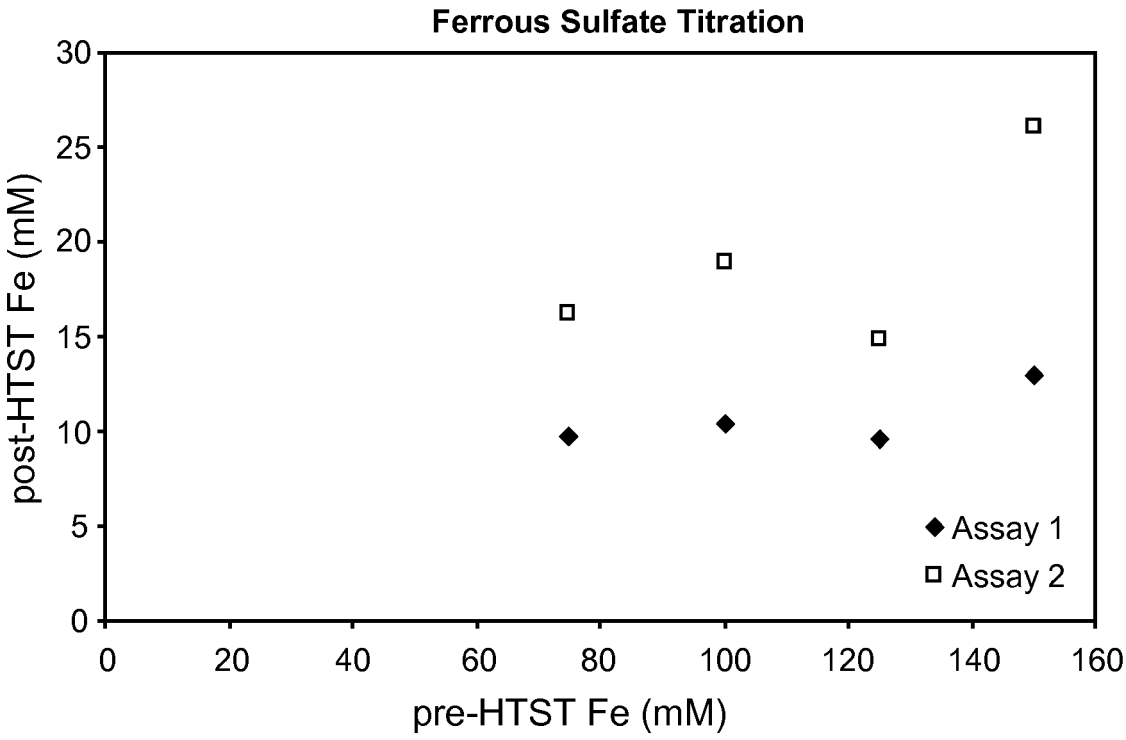

Ferrous Sulfate Titration
post-HTST Fe (mM)
pre-HTST Fe (mM)
Assay 1
Assay 2
0
5
10
15
20
25
30
0
20
40
60
80
100
120
140
160

FIG. 12

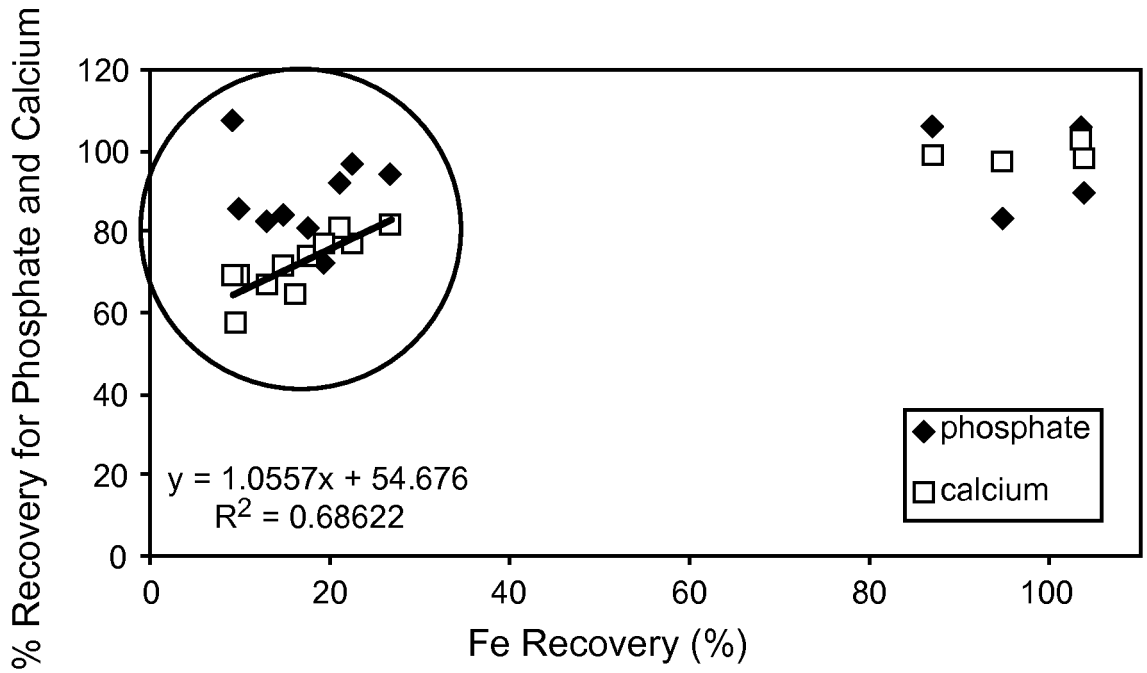

% Recovery for Phosphate and Calcium
Fe Recovery (%)
y = 1.0557x + 54.676
R2 = 0.68622
phosphate
calcium
0
20
40
60
80
100
120

FIG. 14

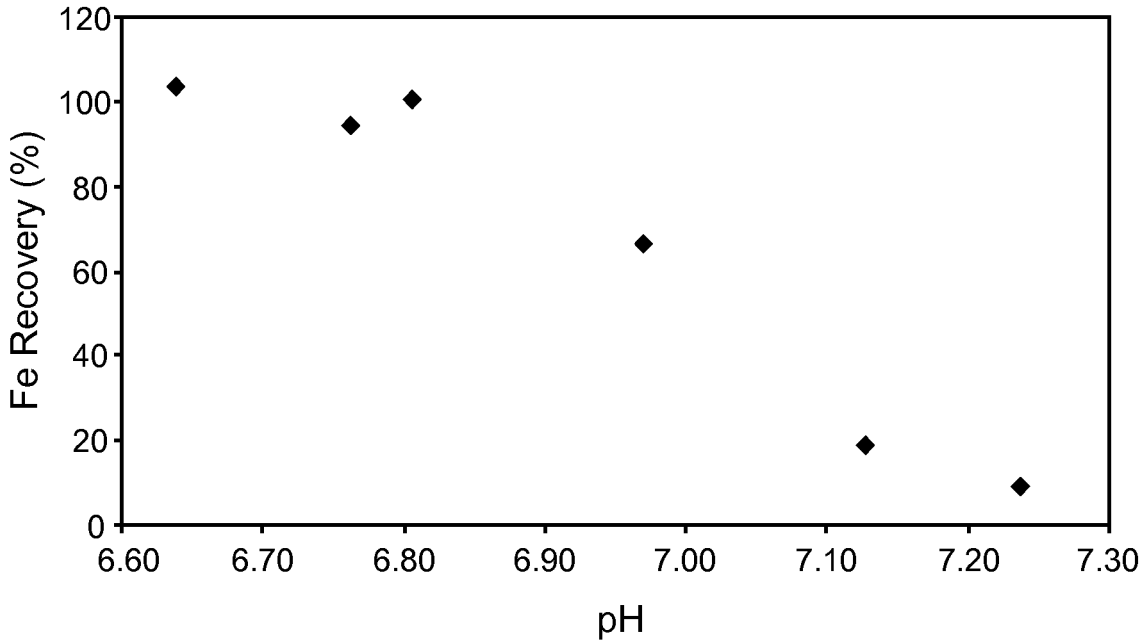


FIG. 13A

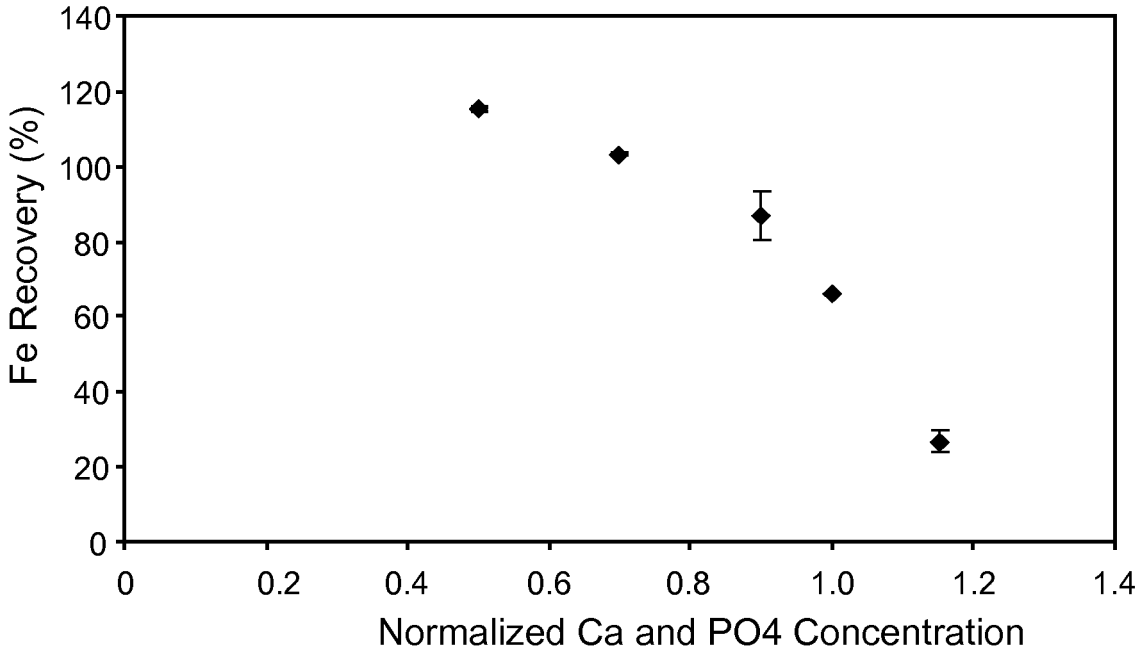


FIG. 13B

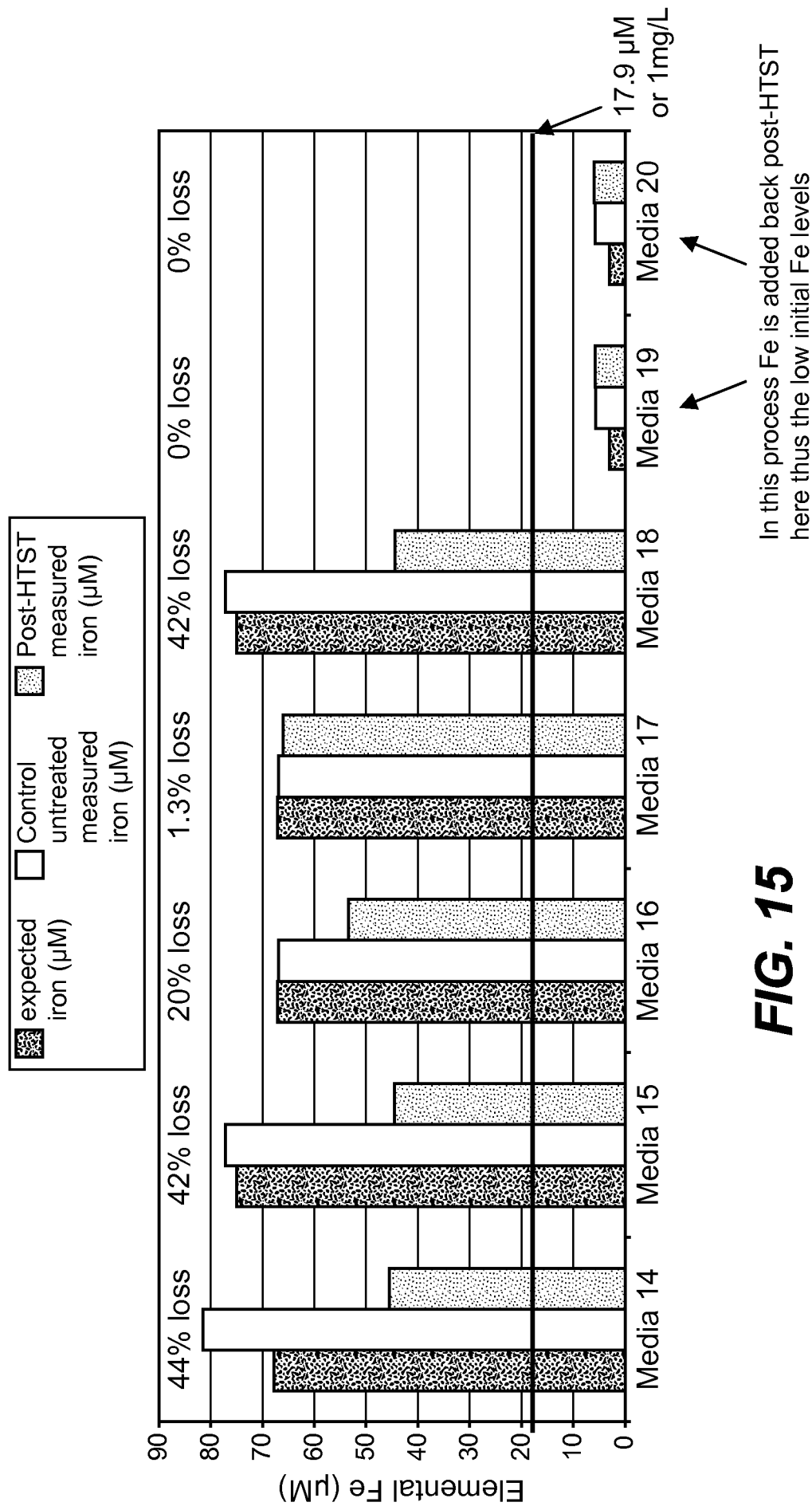

expected iron (µM)
Control untreated measured iron (µM)
Post-HTST measured iron (µM)
Elemental Fe (µM)
0
10
20
30
40
50
60
70
80
90
44% loss
42% loss
20% loss
1.3% loss
42% loss
0% loss
0% loss
Media 14
Media 15
Media 16
Media 17
Media 18
Media 19
Media 20
17.9 µM or 1mg/L
In this process Fe is added back post-HTST here thus the low initial Fe levels

FIG. 15

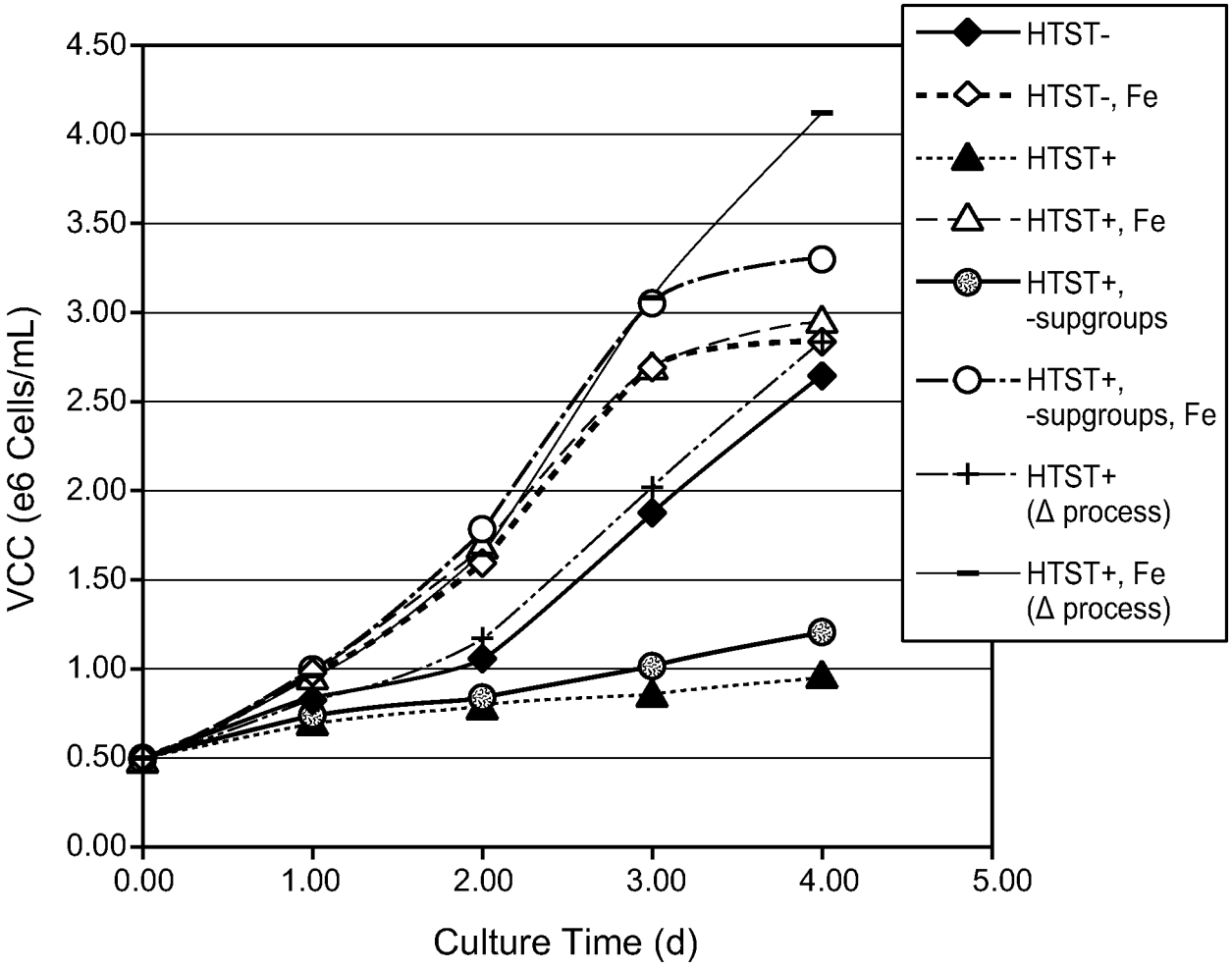

4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
VCC (e6 Cells/mL)
0.00
1.00
2.00
3.00
4.00
5.00
Culture Time (d)
HTST-
HTST-, Fe
HTST+
HTST+, Fe
HTST+, -supgroups
HTST+, -supgroups, Fe
HTST+ (Δ process)
HTST+, Fe (Δ process)

FIG. 16