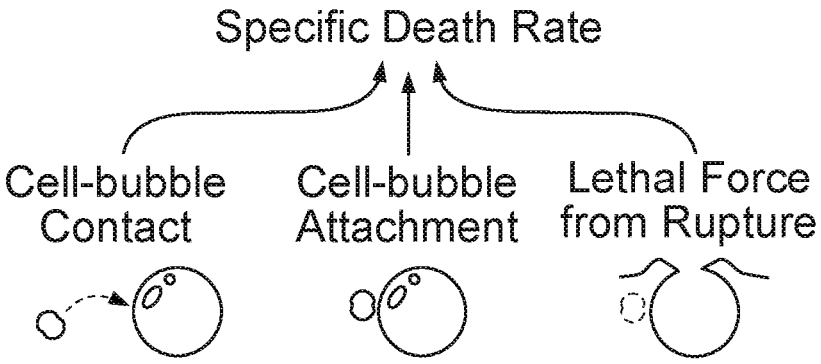

Specific Death Rate
Cell-bubble Contact
Cell-bubble Attachment
Lethal Force from Rupture

FIG. 1

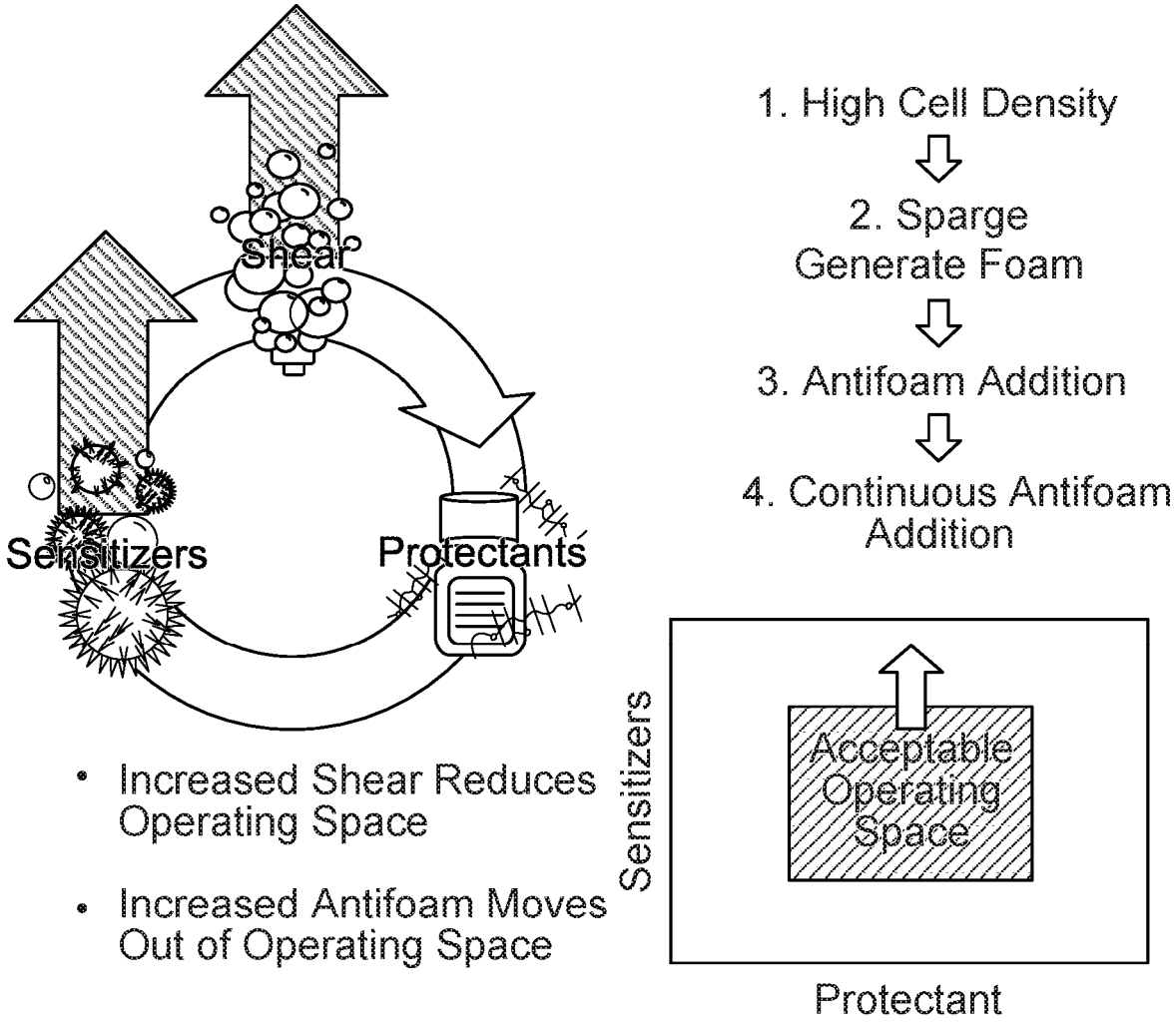

Shear
Sensitizers
Protectants
1. High Cell Density
2. Sparge Generate Foam
3. Antifoam Addition
4. Continuous Antifoam Addition
Sensitizers
Acceptable Operating Space
Protectant
• Increased Shear Reduces Operating Space
• Increased Antifoam Moves Out of Operating Space

FIG. 2

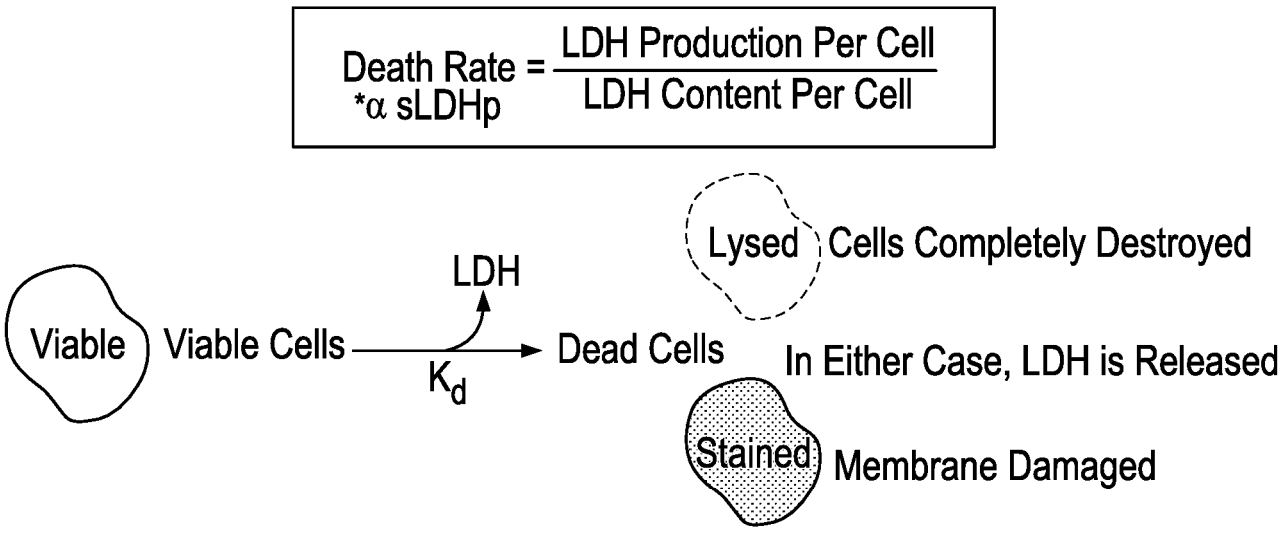

Death Rate = LDH Production Per Cell / LDH Content Per Cell
*α sLDHp
Lysed
Cells Completely Destroyed
LDH
Viable
Viable Cells
Dead Cells
In Either Case, LDH is Released
K_d
Stained
Membrane Damaged

FIG. 3

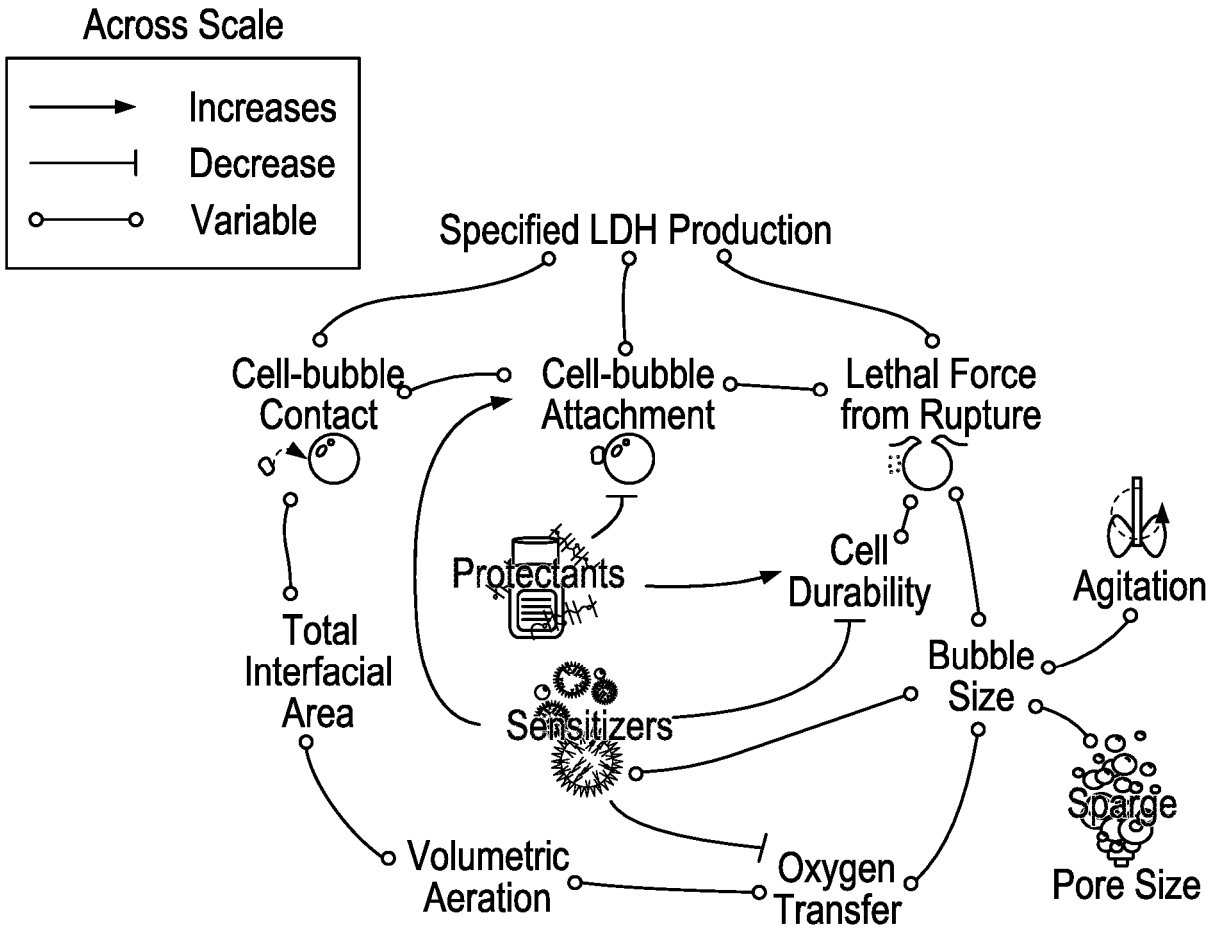

Across Scale
Increases
Decrease
Variable
Specified LDH Production
Cell-bubble Contact
Cell-bubble Attachment
Lethal Force from Rupture
Protectants
Cell Durability
Agitation
Total Interfacial Area
Sensitizers
Bubble Size
Sparge
Pore Size
Volumetric Aeration
Oxygen Transfer

FIG. 4

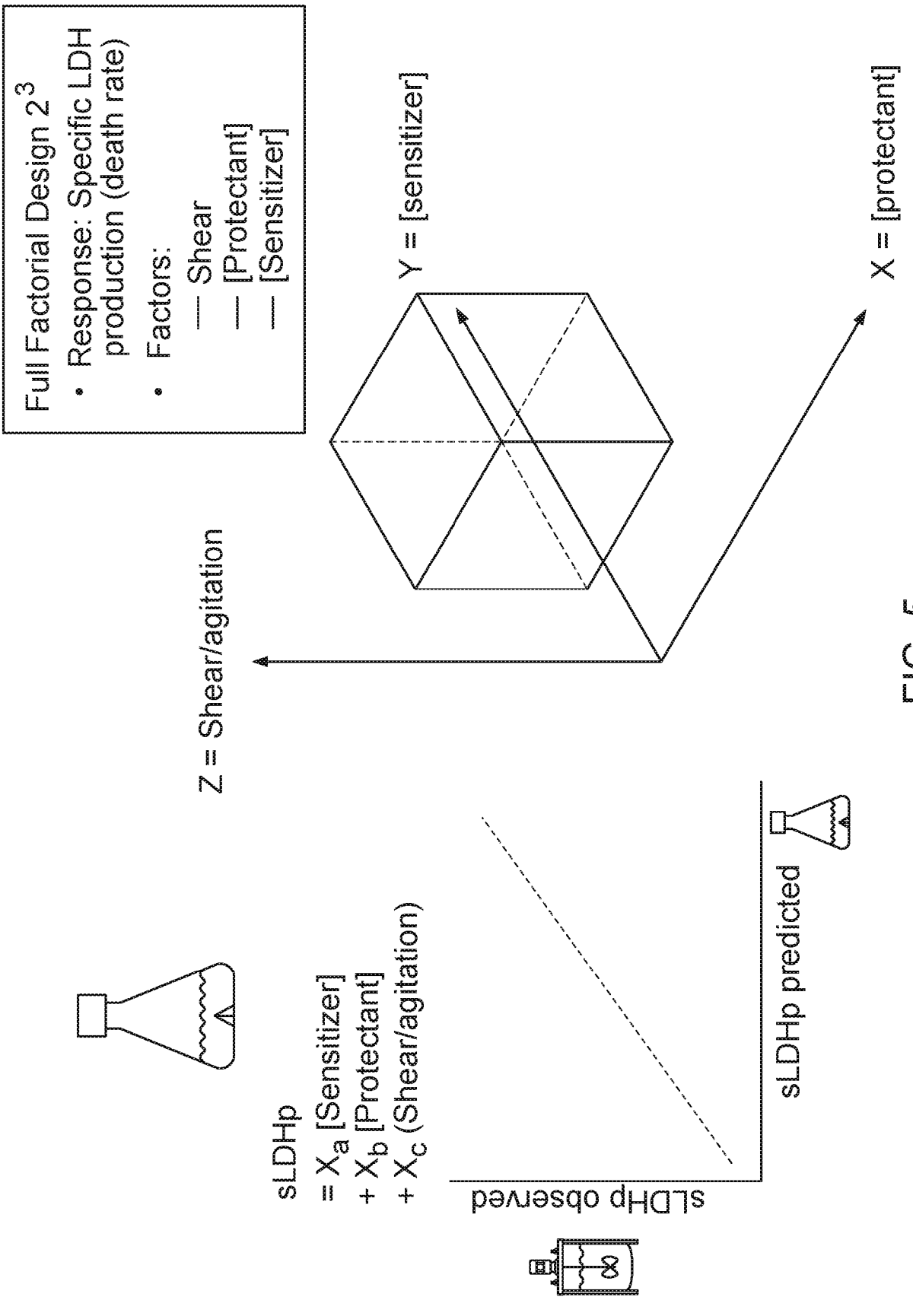

Full Factorial Design 2^3
• Response: Specific LDH production (death rate)
• Factors:
— Shear
— [Protectant]
— [Sensitizer]
Z = Shear/agitation
Y = [sensitizer]
X = [protectant]
sLDHp
= X_a [Sensitizer]
+ X_b [Protectant]
+ X_c (Shear/agitation)
sLDHp observed
sLDHp predicted

FIG. 5

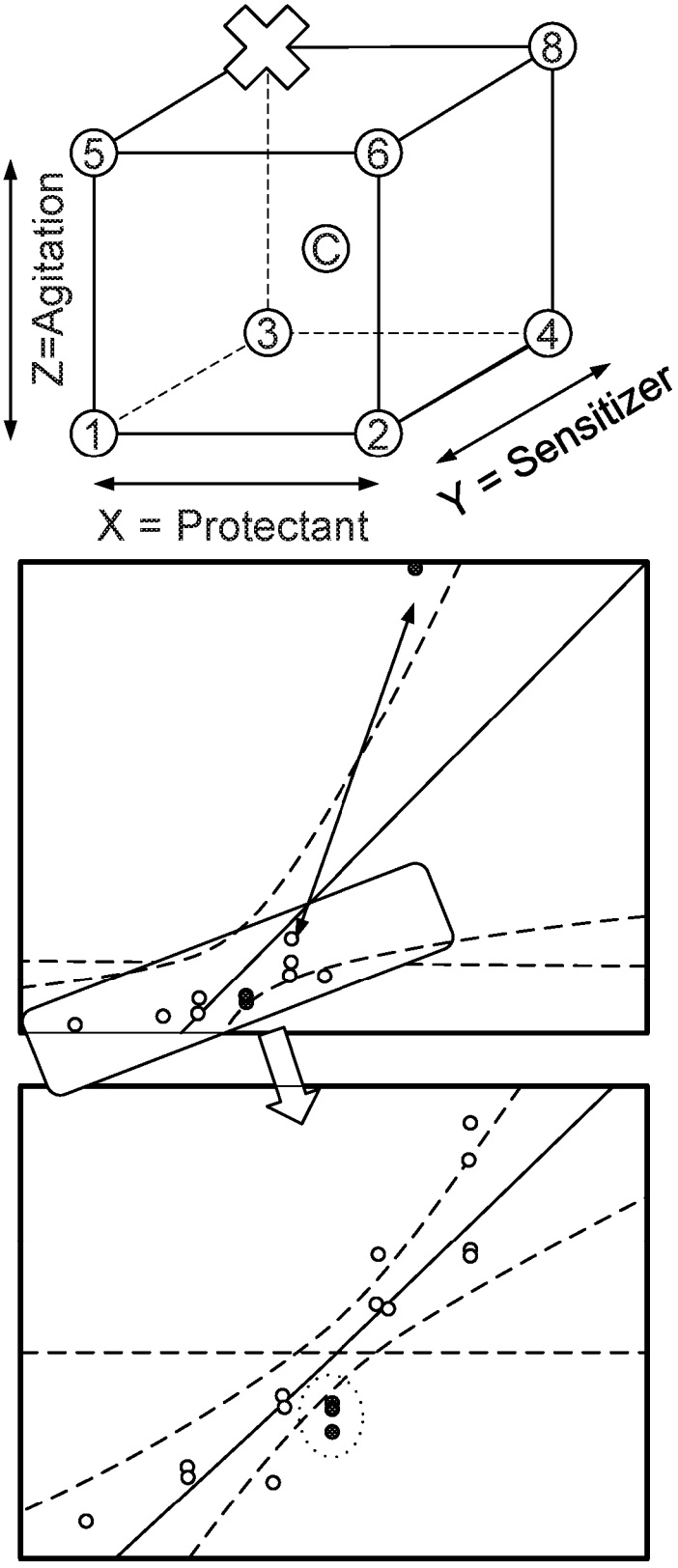
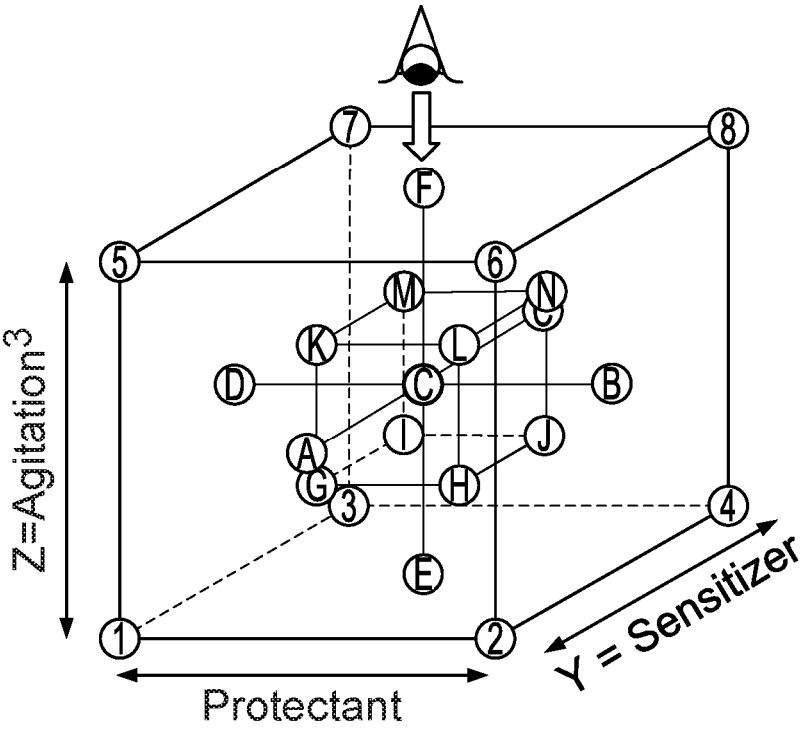


FIG. 6



RPM set to the 3rd power

FIG. 7

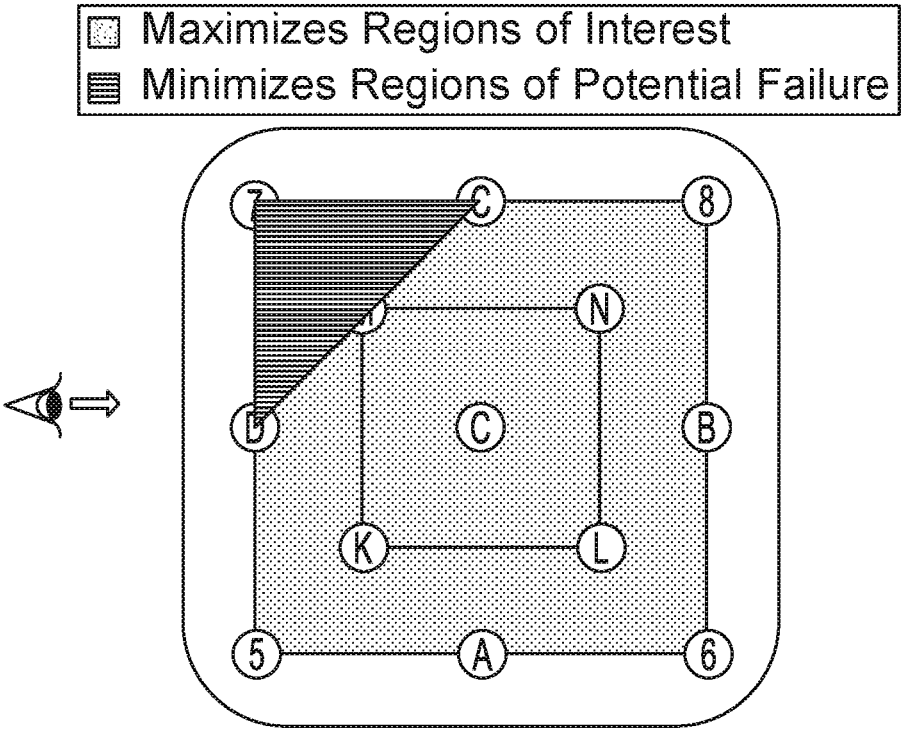
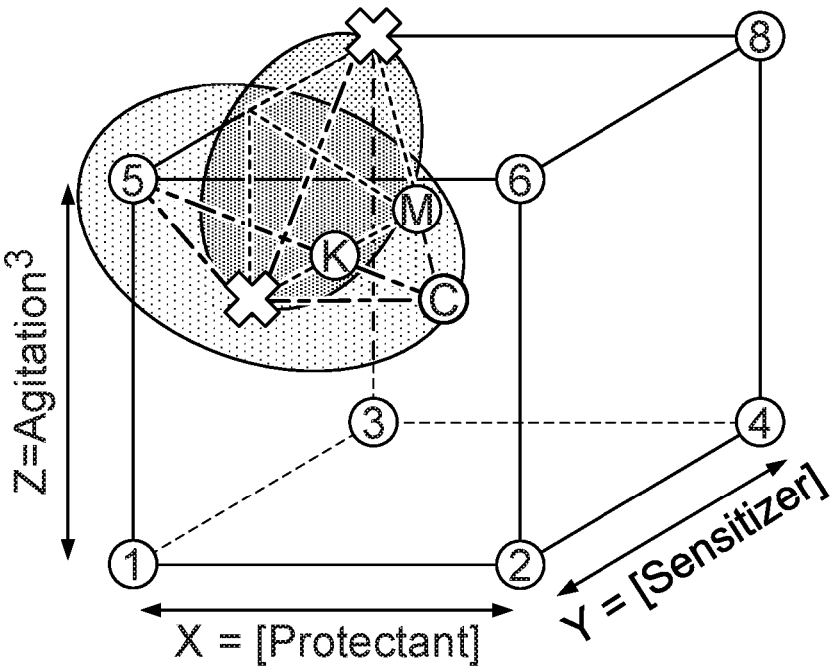
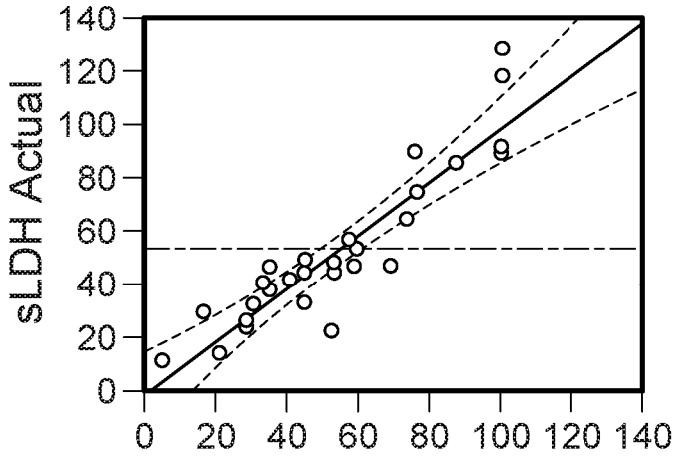
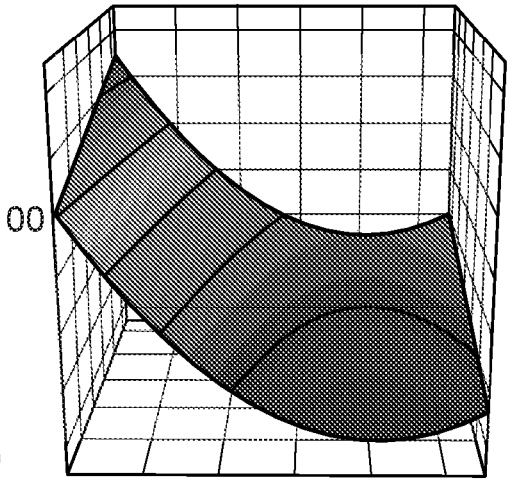


FIG. 8



Identified Poor Region of Poor Performance/Failure





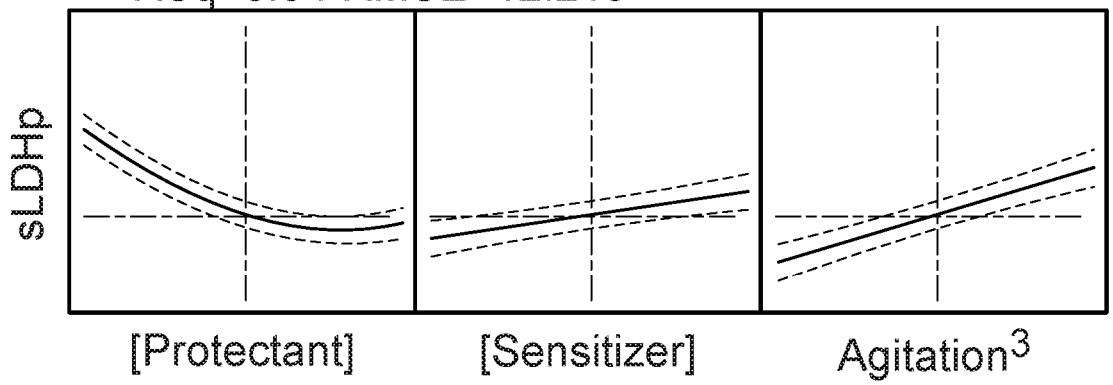
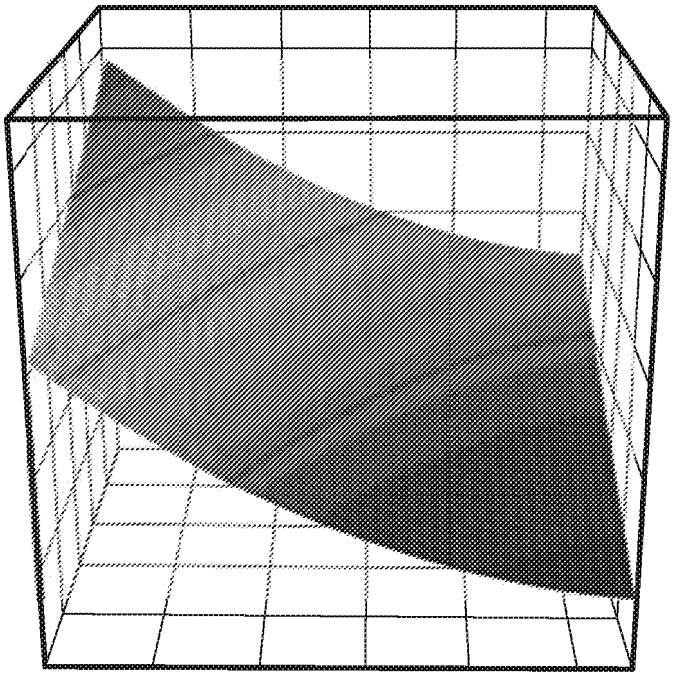
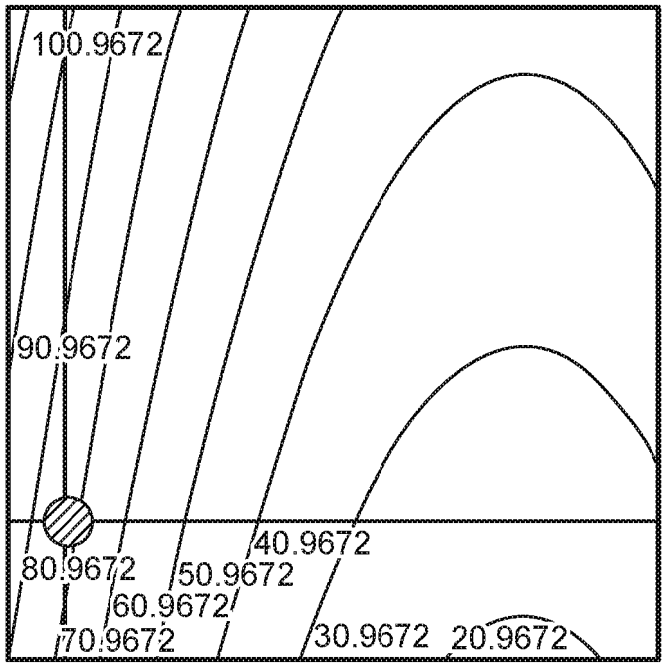


FIG. 9



1. Match Correct Contour
Known:
[Sensitizer]
[Protectant]
Solve:
Shear



2. Predict ΔParameter
Known:
Shear
[Sensitizer]
Solve:
Δ[Protectant]

FIG. 10

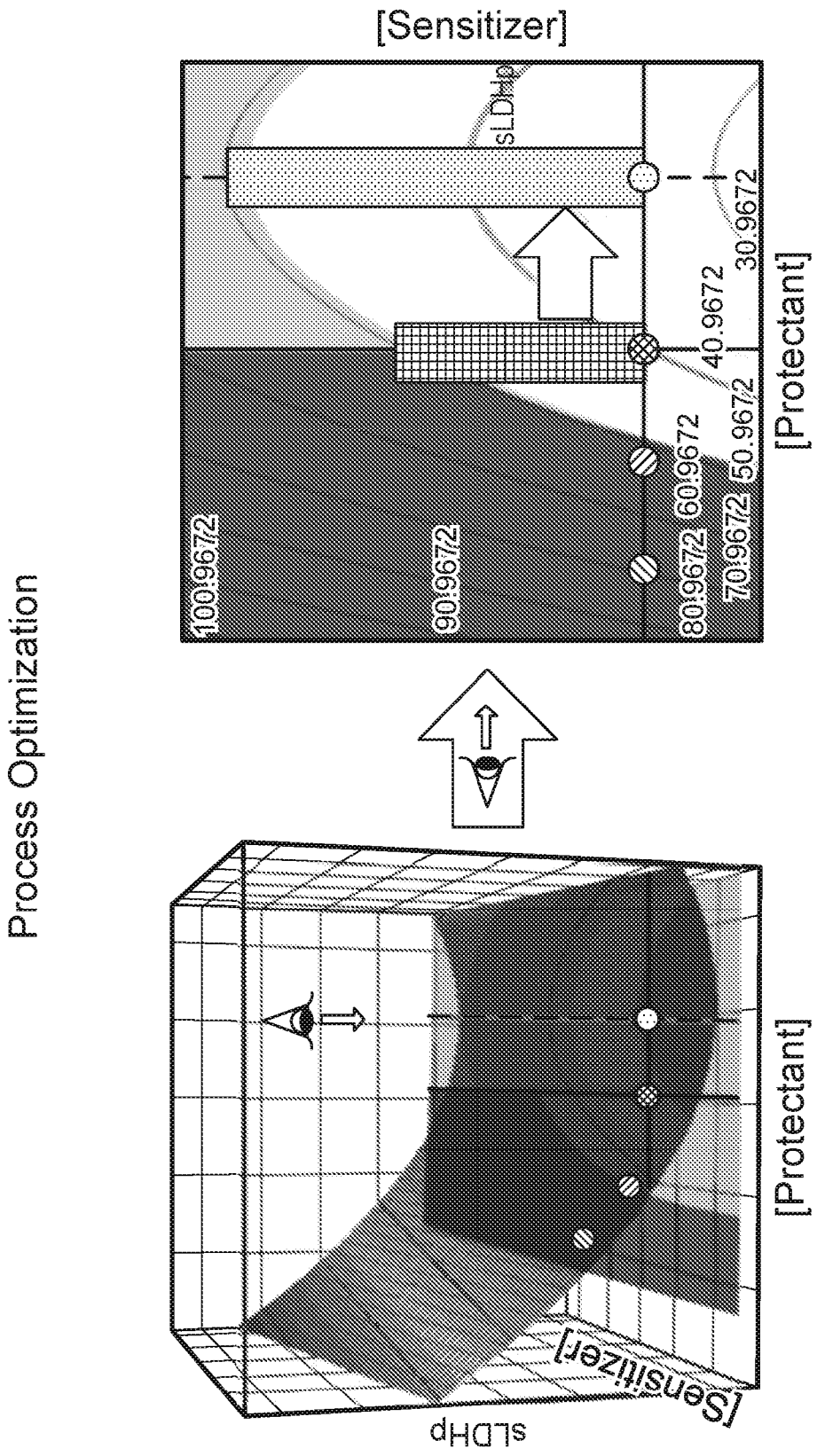

Process Optimization
[Sensitizer]
sLDHp
100.9672
90.9672
80.9672
70.9672
60.9672
50.9672
40.9672
30.9672
[Protectant]
[Sensitizer]
sLDHp
[Protectant]

FIG. 11

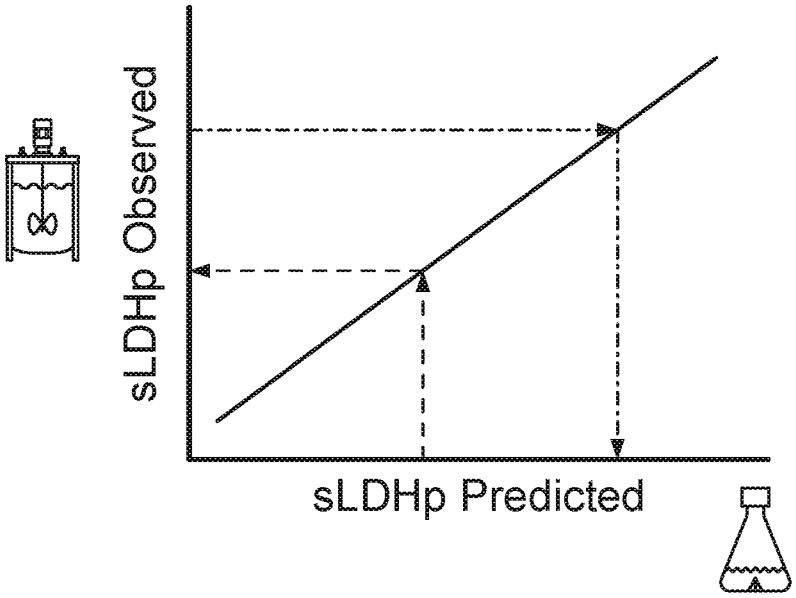


FIG. 12

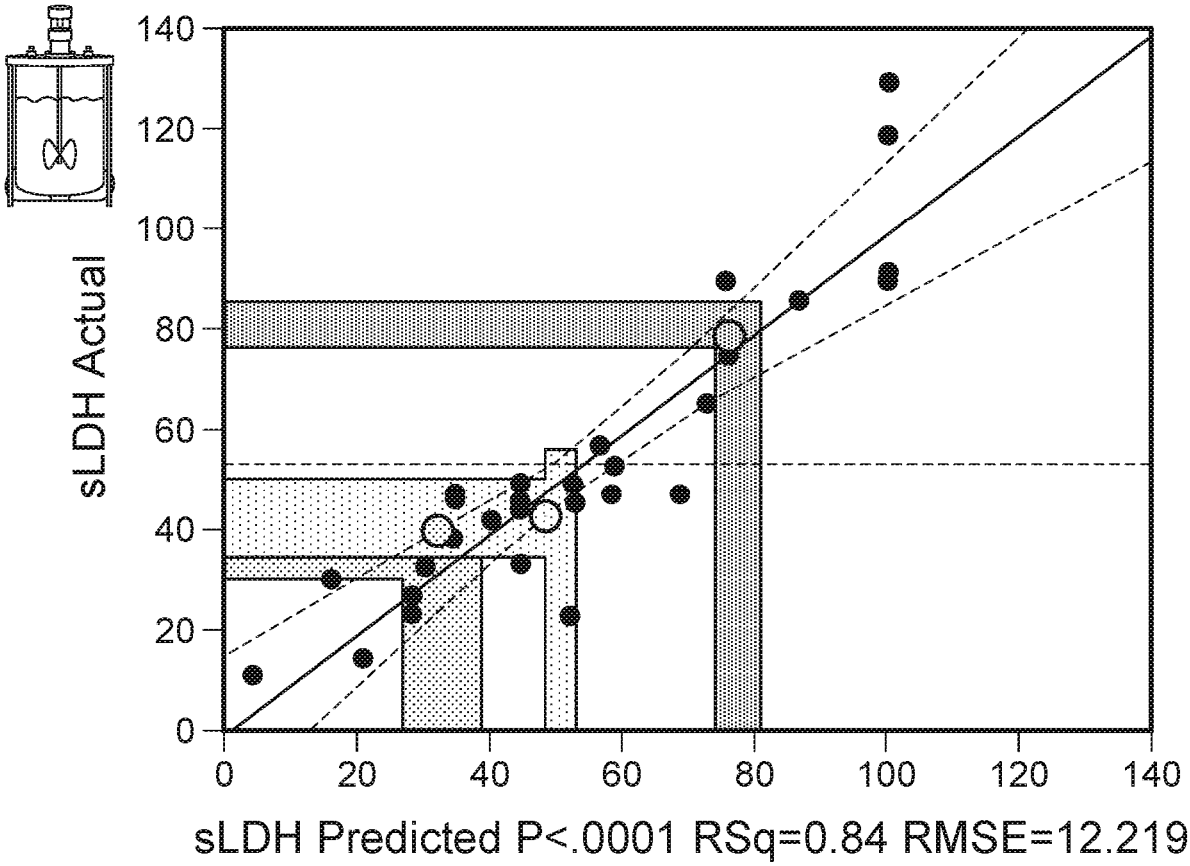


FIG. 13A

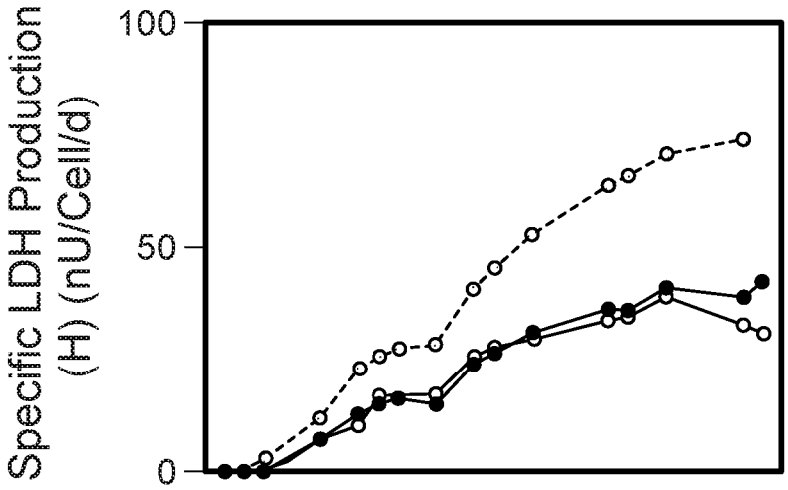
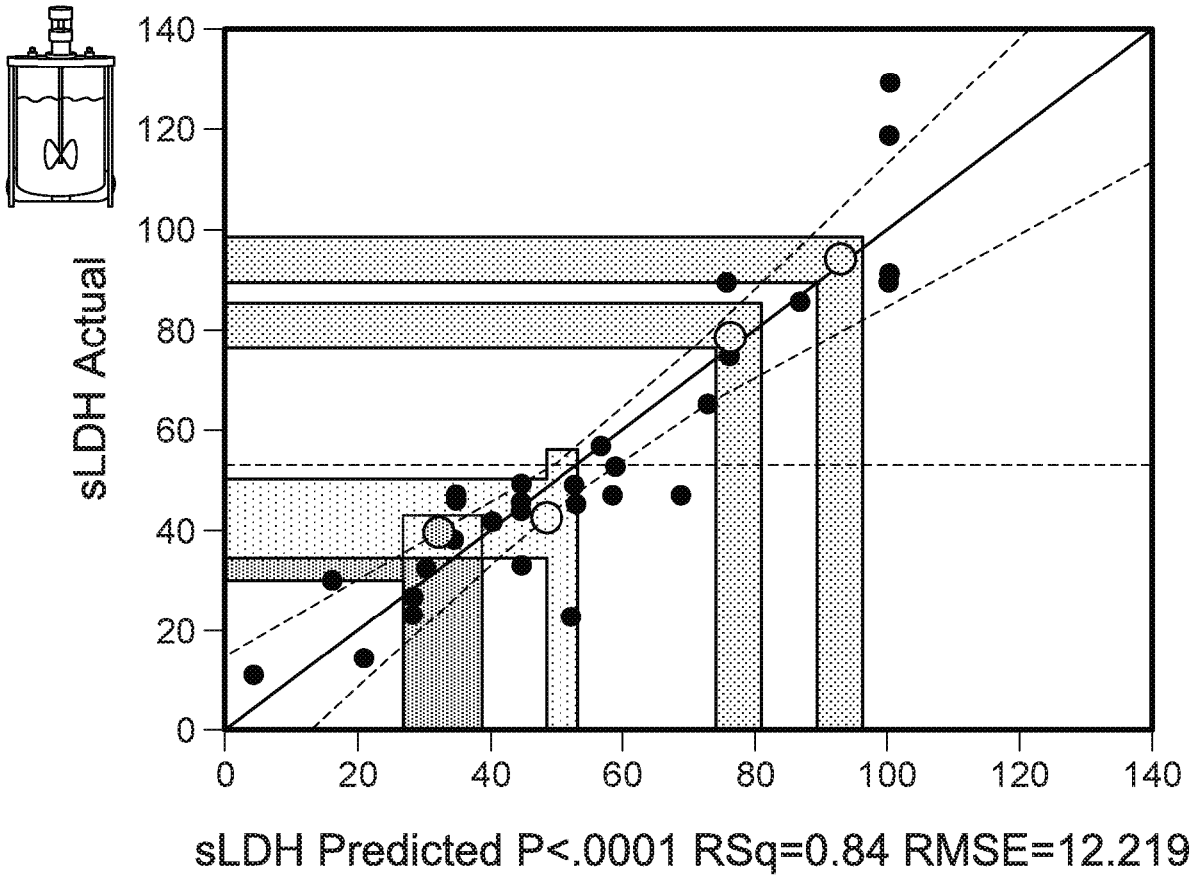


FIG. 13B

10L Benchtop		Shakeflask Model	
Observed LDH		Predicted LDH	
Avg	Std	Avg	Std
39.00	8.49	37.00	4.24
43.00	7.07	54.00	4.24
80.50	3.54	80.00	4.24
Min	Max	Min	Max
30.51	47.49	32.76	41.24
35.93	50.07	49.76	58.24
76.96	84.04	75.76	84.24

FIG. 13C



n=1 for Condition Changed

Antifoam Increased 30ppm to 80ppm			
Observed LDH Range		Predicted LDH Range	
94.74	99.36	90.19	96.38

FIG. 14

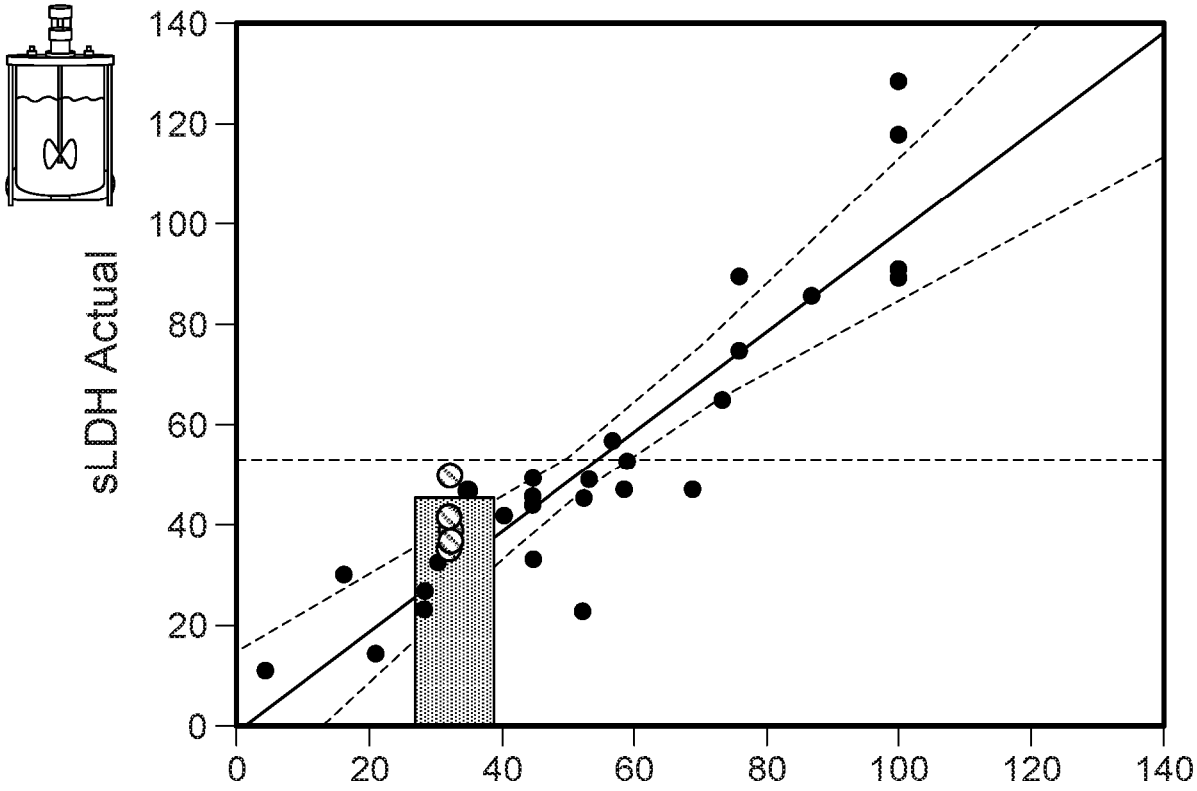


FIG. 15A

LDH		Predicted	
50L		Model	std
S16	39.72	37.00	4.24
S18	51.05	37.00	4.24
S19	39.6	37.00	4.24
100L		37.00	4.24
S27	37.61	37.00	4.24
S28	42.55	37.00	4.24

FIG. 15B