Temperature, Oxygen Solubility and OUR

- Oxygen depletion is an identified and documented route cause of dissociation
  - Rates of depletion decrease with temperature
  - O_2 solubility increases inversely with temp
- ∴ cooling bioreactors/samples can help prevent dissociation

	37°C	15°C	5°C
O_2 Solubility (µg/ml)	6.1	9.0	11.4
O_2 uptake rate $20x10^6$ cells (µg/h)	200	50*	25*
O_2 at 40% DOT (µg/ml)	2.4	3.6	4.6
O_2 depletion at 40% DOT in: (min)	0.7	4	11
O_2 depletion at 100% DOT in: (min)	1.8	11	27

- * Assumption is that OUR decreases 2-fold with every drop of 10°C
- In truth the effect is probably higher

FIGURE 1

Anoxia through Harvest and Storage - Mitigation Strategy
Bioreactor
Primary recovery
(note 2000L differs from other proces flows, centrifuge plus intermediate hold tank V9H)
Storage
Bag or Bottle
Potential causes of anoxia:
• High viable cell concentration (high oxygen consumption rate at working temperature 36.5 °C)
• Inadequate air/O_2 sparge
• Inadequate agitation
Mitigation
• Chill culture (reduces oxygen consumption rate by cells ~10x)
• Maintain oxygenation, at DOT set point or greater
• Maintain air/O_2 sparge
Potential causes of anoxia:
• Cells continue to consume oxygen.
• Residence time in e.g. Pod filters is ~10 minutes **BUT** oxygen exhaustion time by cells is 1 to 2 minutes
• Filter blockage increases processing time
Mitigation
• Chill culture to reduce oxygen consumption rate
• Maximise filter area to avoid filter blockage and excessive retention time
Potential causes of anoxia:
• Process stream from primary recovery is already depleted of oxygen
• Oxygen is consumed even in the absence of cells
Mitigation
• Ensure process stream from primary recovery is oxygenated (need to measure DOT)
• Include air headspace in storage vessel to allow for oxygen consumption.

FIGURE 2

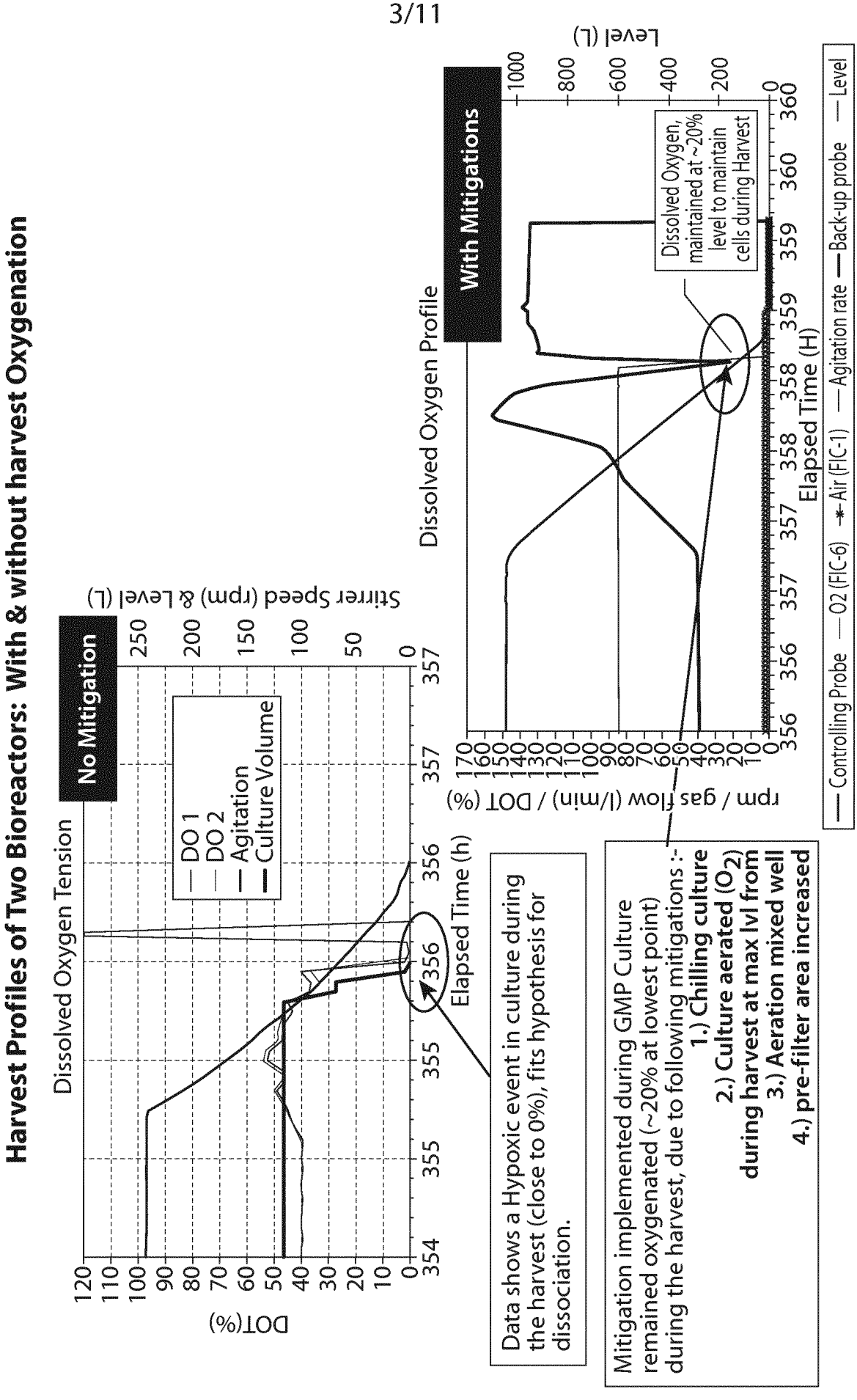

Harvest Profiles of Two Bioreactors: With & without harvest Oxygenation
No Mitigation
Dissolved Oxygen Tension
DOT(%)
Stirrer Speed (rpm) & Level (L)
DO 1
DO 2
Agitation
Culture Volume
Elapsed Time (h)
Data shows a Hypoxic event in culture during the harvest (close to 0%), fits hypothesis for dissociation.
Mitigation implemented during GMP Culture remained oxygenated (~20% at lowest point) during the harvest, due to following mitigations :-
1.) Chilling culture
2.) Culture aerated (O_2) during harvest at max lvl from
3.) Aeration mixed well
4.) pre-filter area increased
With Mitigations
Dissolved Oxygen Profile
rpm / gas flow (l/min) / DOT (%)
Level (L)
Dissolved Oxygen, maintained at ~20% level to maintain cells during Harvest
Elapsed Time (H)
Controlling Probe
O2 (FIC-6)
Air (FIC-1)
Agitation rate
Back-up probe
Level

FIGURE 3

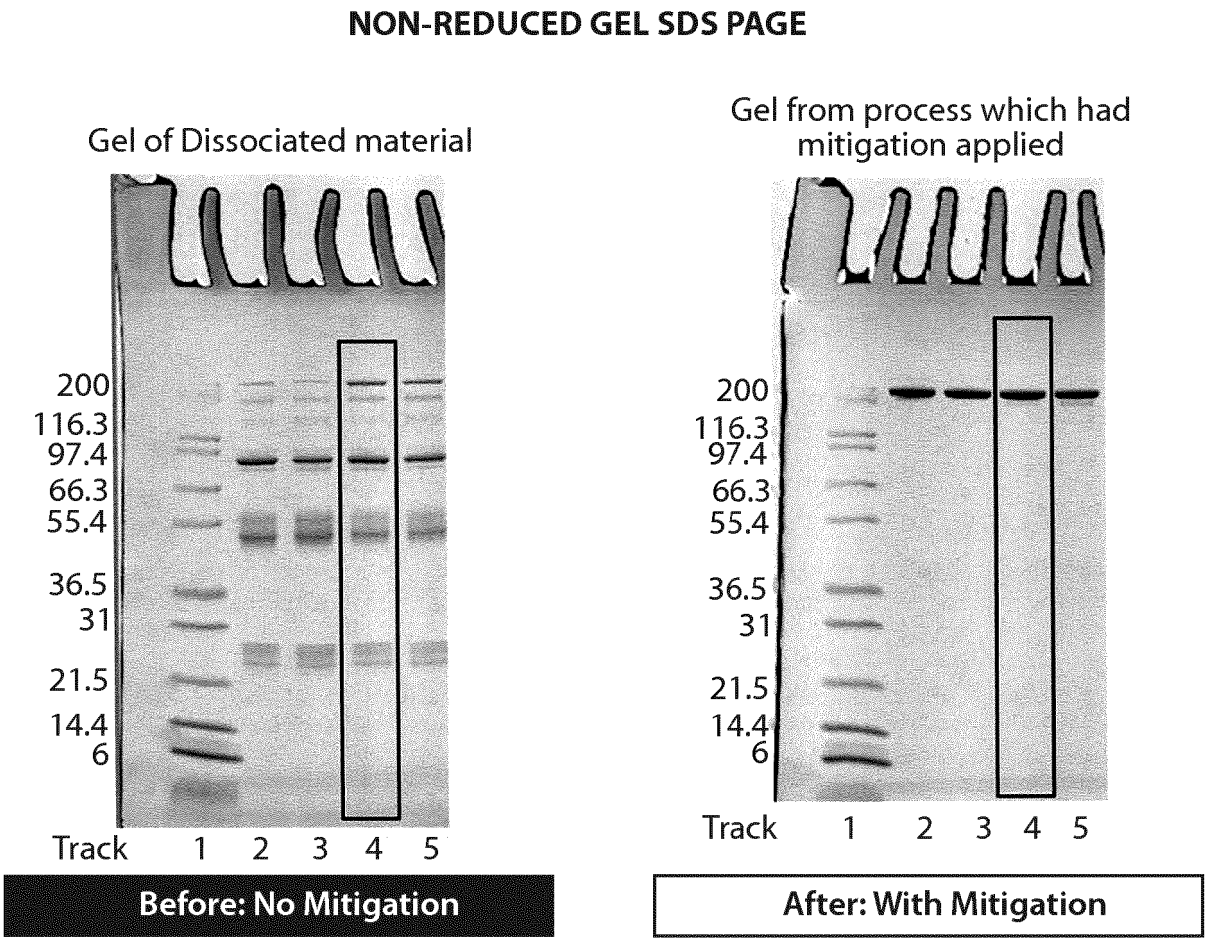

NON-REDUCED GEL SDS PAGE
Gel of Dissociated material
Gel from process which had mitigation applied
200
116.3
97.4
66.3
55.4
36.5
31
21.5
14.4
6
Track 1 2 3 4 5
Before: No Mitigation
After: With Mitigation

FIGURE 4

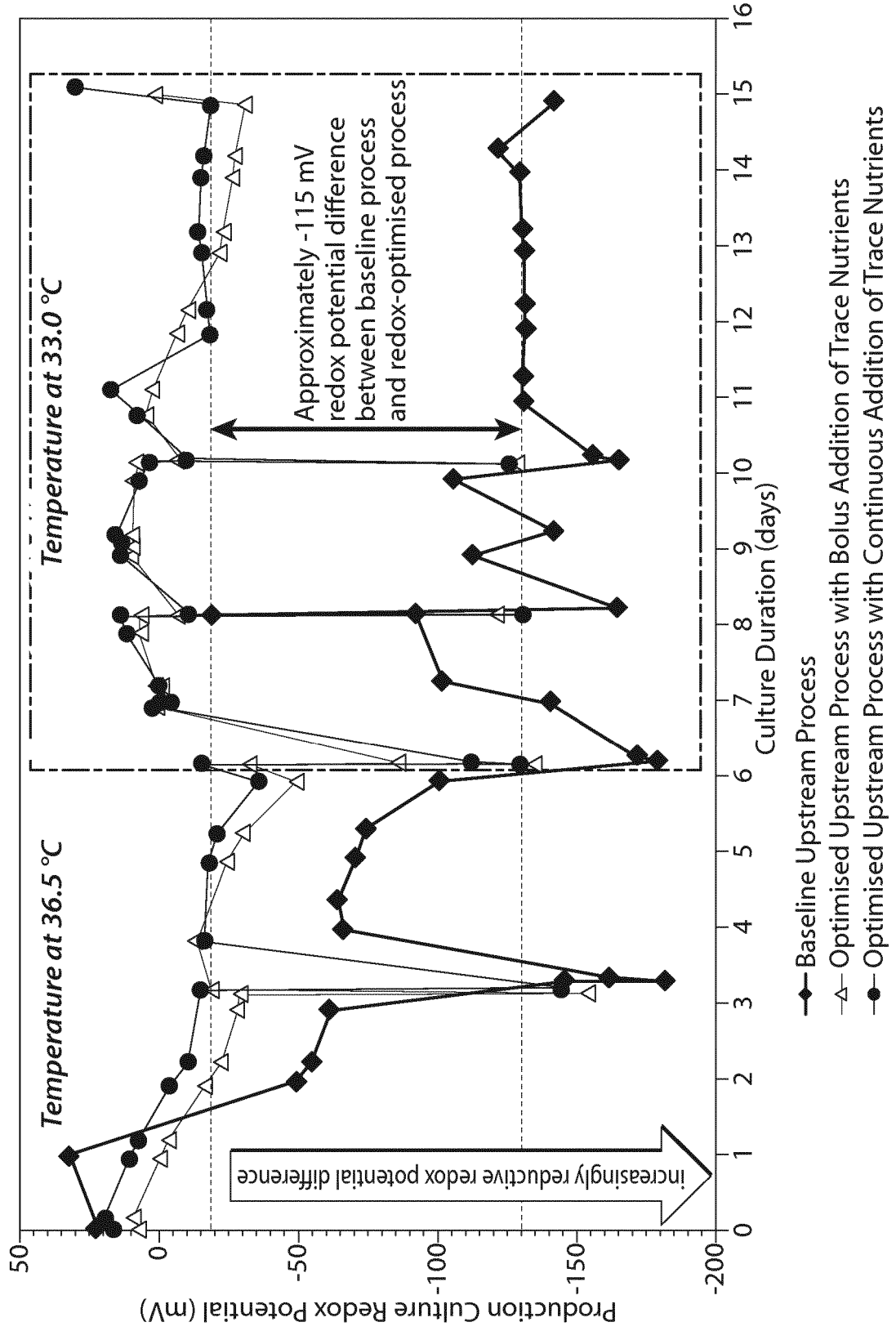

Temperature at 36.5 °C
Temperature at 33.0 °C
Approximately -115 mV redox potential difference between baseline process and redox-optimised process
increasingly reductive redox potential difference
Production Culture Redox Potential (mV)
50
0
-50
-100
-150
-200
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Culture Duration (days)
Baseline Upstream Process
Optimised Upstream Process with Bolus Addition of Trace Nutrients
Optimised Upstream Process with Continuous Addition of Trace Nutrients

FIGURE 5

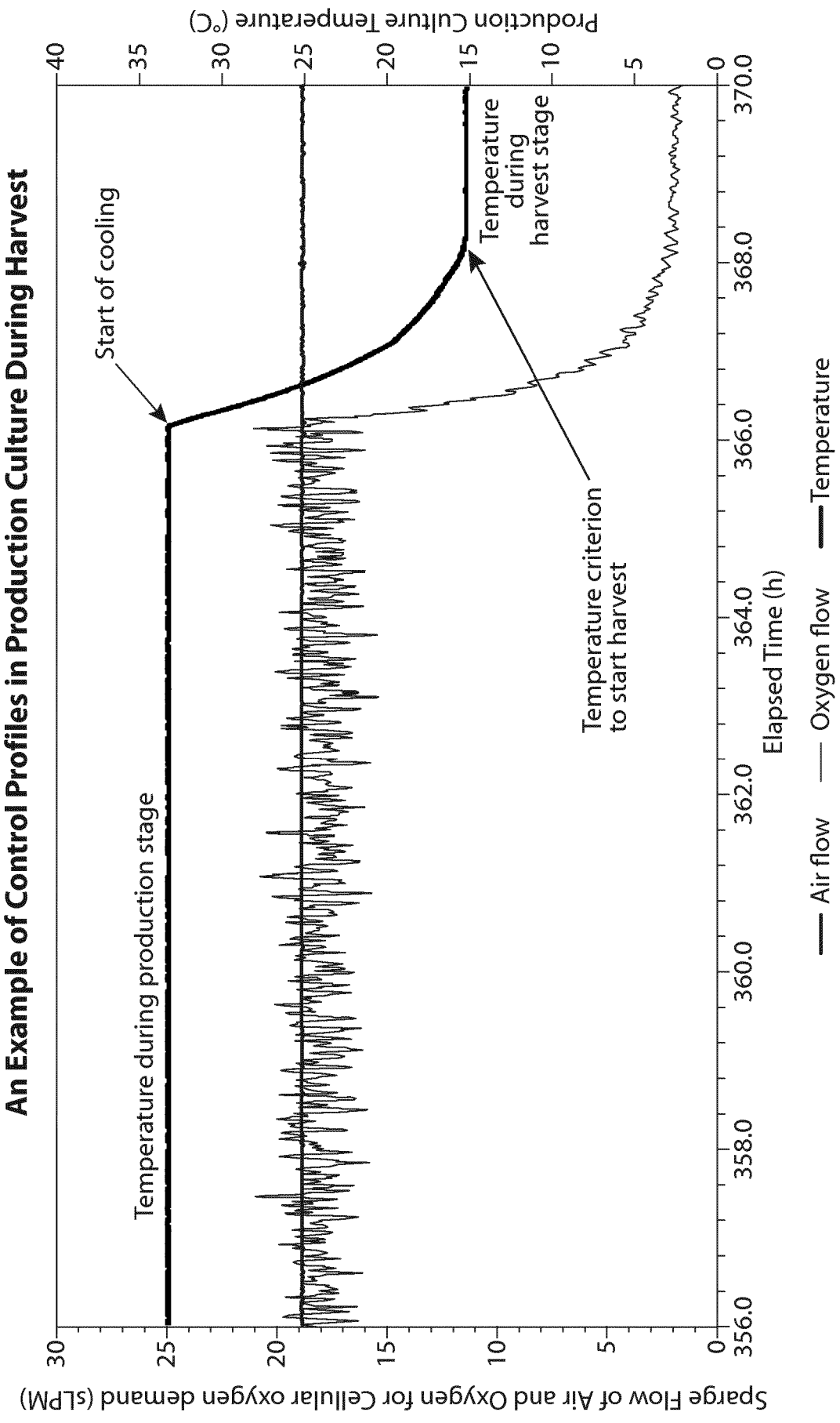

An Example of Control Profiles in Production Culture During Harvest
Sparge Flow of Air and Oxygen for Cellular oxygen demand (sLPM)
Production Culture Temperature (°C)
Elapsed Time (h)
Start of cooling
Temperature during production stage
Temperature during harvest stage
Temperature criterion to start harvest
Air flow
Oxygen flow
Temperature
0
5
10
15
20
25
30
35
40
356.0
358.0
360.0
362.0
364.0
366.0
368.0
370.0

FIGURE 6

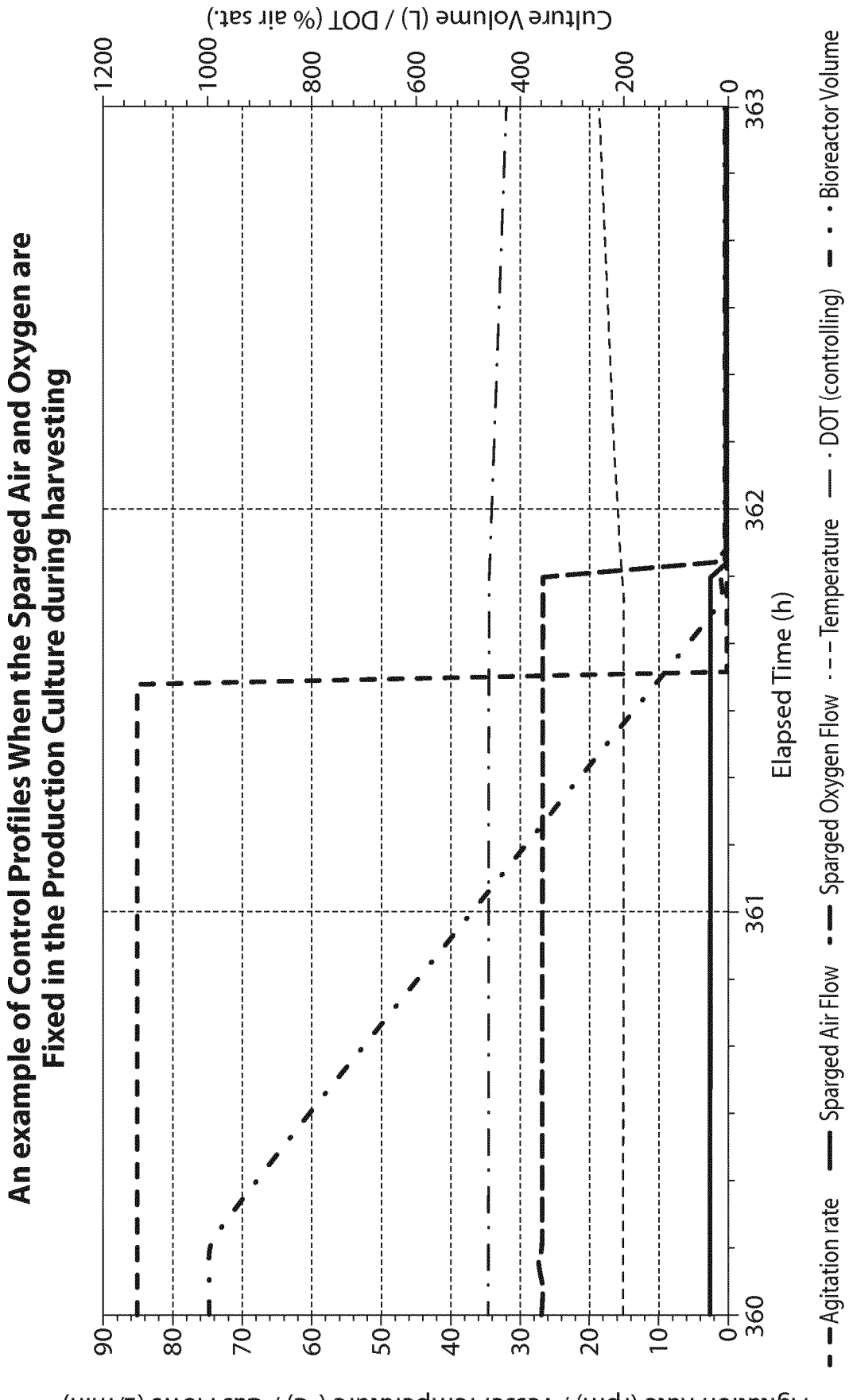

An example of Control Profiles When the Sparged Air and Oxygen are Fixed in the Production Culture during harvesting
Agitation Rate (rpm) / Vessel Temperature (°C) / Gas Flows (L/min)
Culture Volume (L) / DOT (% air sat.)
Elapsed Time (h)
Agitation rate
Sparged Air Flow
Sparged Oxygen Flow
Temperature
DOT (controlling)
Bioreactor Volume

FIGURE 7A

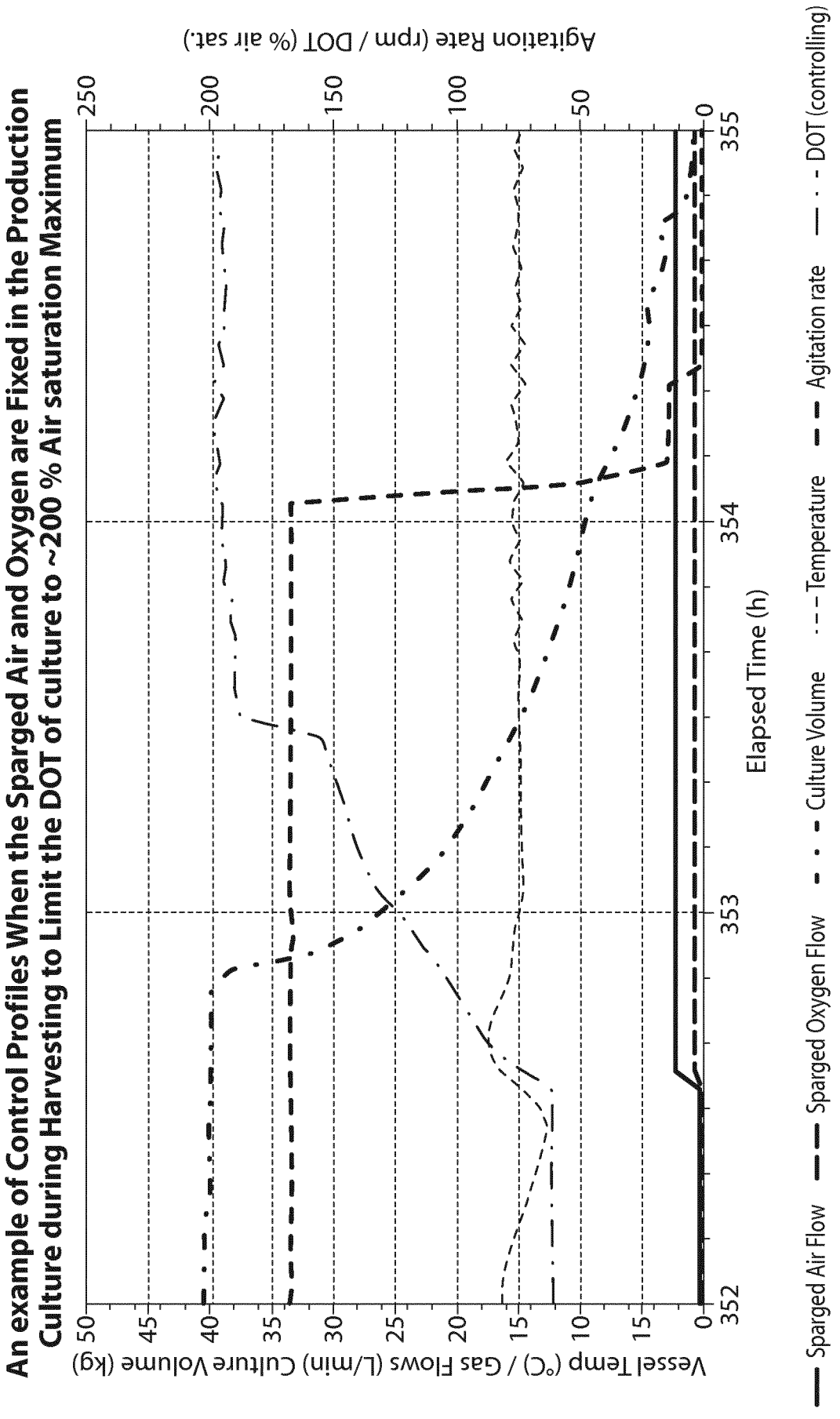

An example of Control Profiles When the Sparged Air and Oxygen are Fixed in the Production Culture during Harvesting to Limit the DOT of culture to ~200 % Air saturation Maximum
Vessel Temp (°C) / Gas Flows (L/min) Culture Volume (kg)
Agitation Rate (rpm / DOT (% air sat.)
Elapsed Time (h)
0
5
10
15
20
25
30
35
40
45
50
0
50
100
150
200
250
352
353
354
355
Sparged Air Flow
Sparged Oxygen Flow
Culture Volume
Temperature
Agitation rate
DOT (controlling)

FIGURE 7B

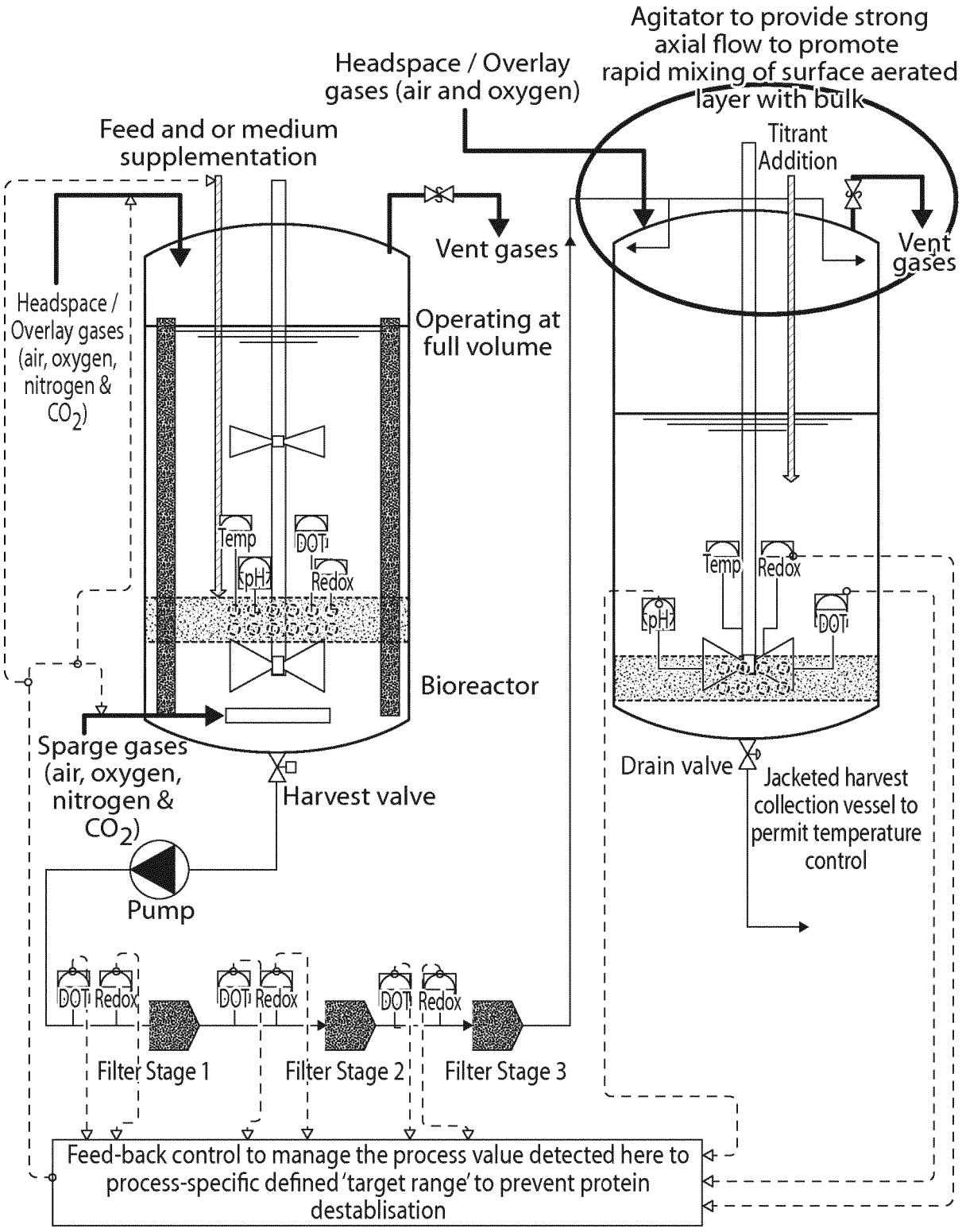

Agitator to provide strong axial flow to promote rapid mixing of surface aerated layer with bulk
Headspace / Overlay gases (air and oxygen)
Feed and or medium supplementation
Titrant Addition
Vent gases
Vent gases
Headspace / Overlay gases (air, oxygen, nitrogen & CO_2)
Operating at full volume
Temp
DOT
pH
Redox
Temp
Redox
pH
DOT
Bioreactor
Sparge gases (air, oxygen, nitrogen & CO_2)
Harvest valve
Drain valve
Jacketed harvest collection vessel to permit temperature control
Pump
DOT
Redox
DOT
Redox
DOT
Redox
Filter Stage 1
Filter Stage 2
Filter Stage 3
Feed-back control to manage the process value detected here to process-specific defined 'target range' to prevent protein destablisation

FIGURE 8A

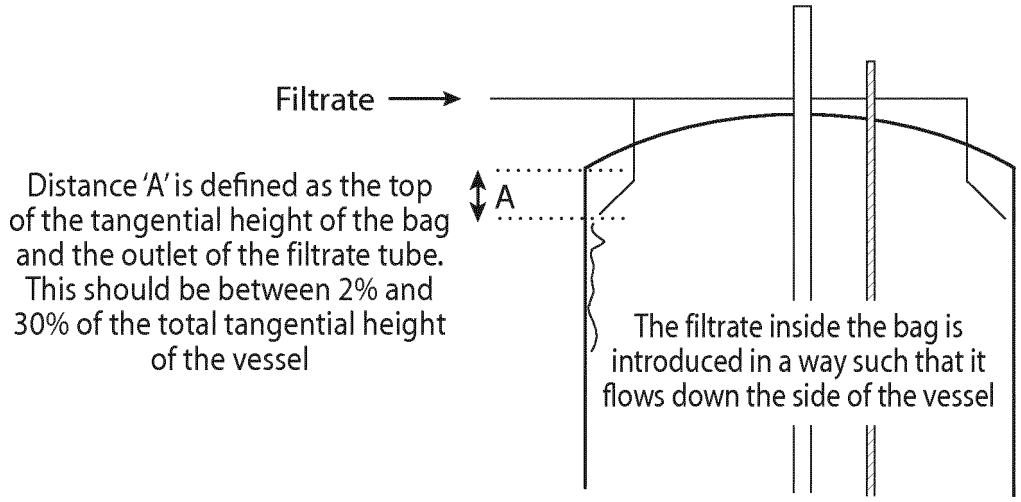

Filtrate
Distance 'A' is defined as the top of the tangential height of the bag and the outlet of the filtrate tube. This should be between 2% and 30% of the total tangential height of the vessel
A
The filtrate inside the bag is introduced in a way such that it flows down the side of the vessel

FIGURE 8B

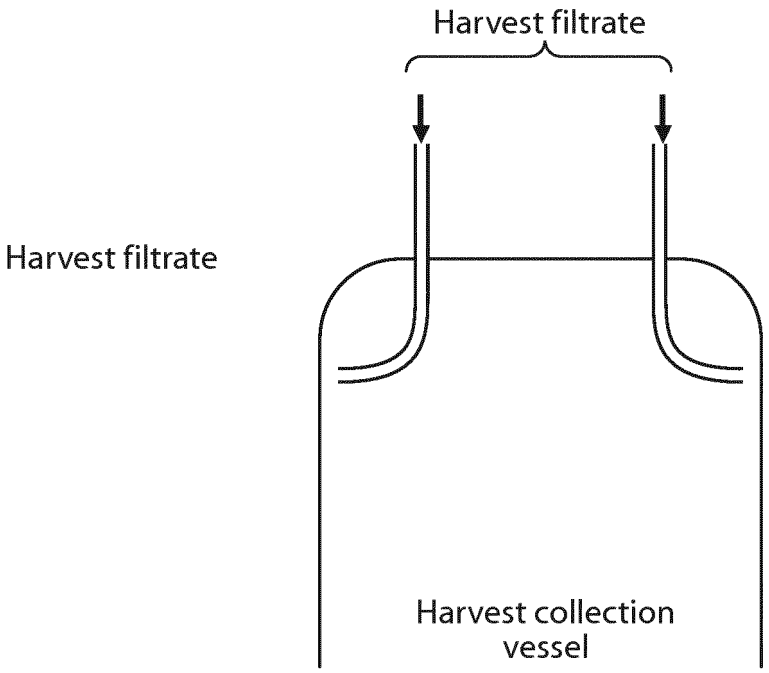

Harvest filtrate
Harvest filtrate
Harvest collection vessel

FIGURE 9