

Implementation of Quality by Design Concepts to Fermentation Process Development

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Presentation Outline

- How we approached our mammalian cell process development circa 2008
- How have we altered the 2008 process to enable us to implement a QbD approach to process development
- What further scope is there for modifying our approach

2008 Resources

- Equipment Resources
 - 16 × 5L Reactors (as two banks of 8 reactors)
 - 3 × 20L Reactors
 - 1 × 50L Reactor
 - Scale-Up and GMP Production hardware incorporating 5L Reactor, 60L Reactor & 500L Reactor

2008 Structure

- Development
 - Utilise flasks and reactors up to (and including) 50 litres
 - Identify acceptable media
 - Define appropriate fermentation regime
 - Develop acceptable midstream process (MSP)
 - Perform material supply for downstream functions
- Scale-Up and GMP
 - Scale-up defined regime, adapting as appropriate for GMP manufacture
 - Produce material for Pre-clinical and Clinical studies

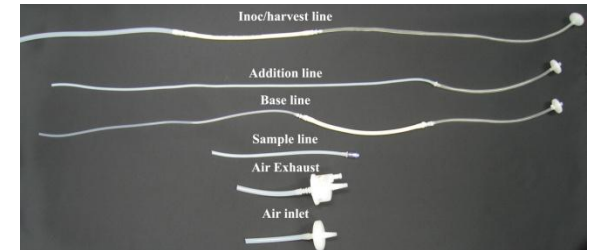
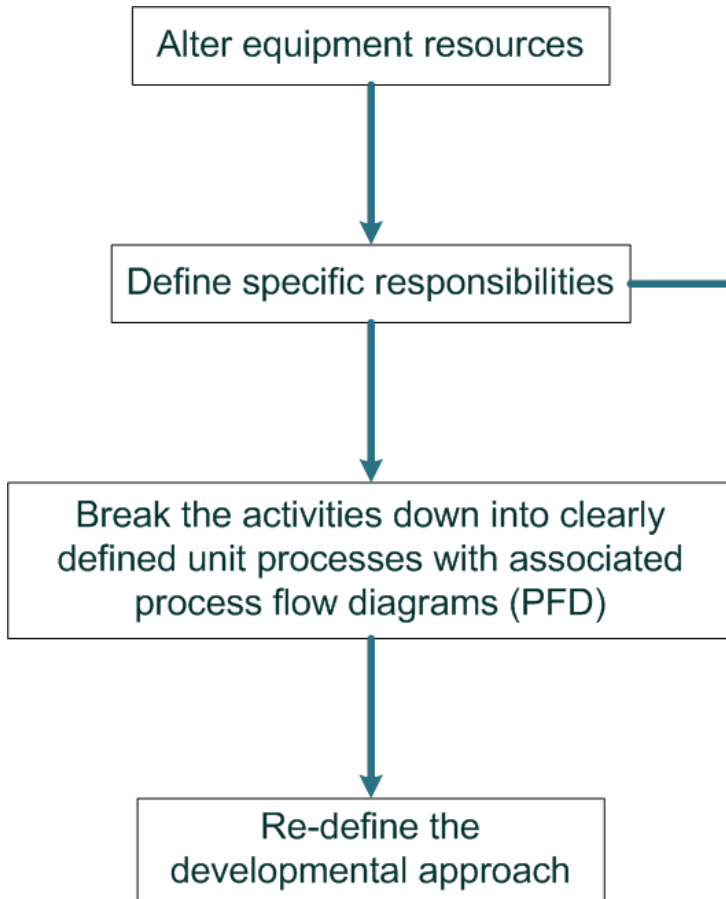
2008 Development Approach

- Hypothesis for evaluation
 - Define the experiment for fermentation and midstream processing development
 - Perform fermentation using a bank of eight reactors
 - Set-up each fermentation condition in duplicate
 - Cultivate each reactor discretely until viability is $\leq 30\%$
 - Harvest and utilise for MSP evaluation
 - Assay in-process samples
 - Analyse data – essentially a one-factor-at-a-time (OFAT) approach
 - Determine subsequent studies

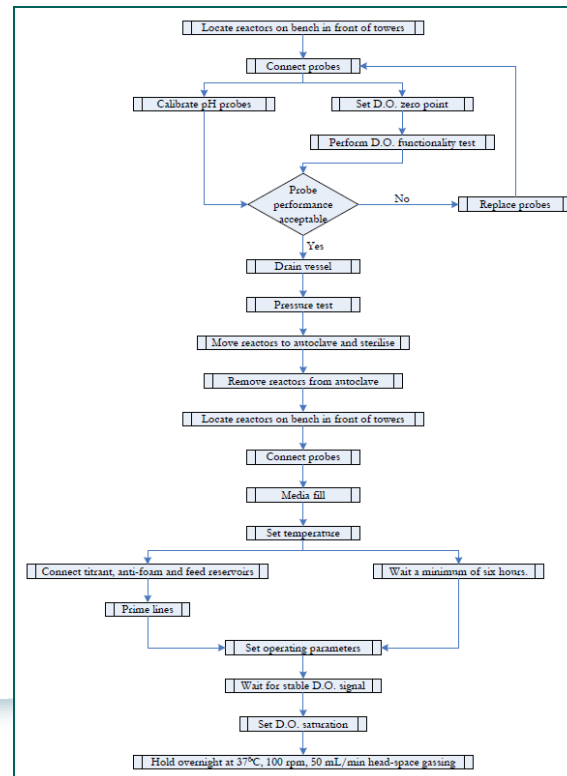
Current Structure

- Three distinct streams with distinct focus
 - Flask development
 - Small scale reactor development
 - Scale-Up (no longer GMP)
- QbD approach is currently applied to the first two streams
- Focus on the small scale reactor stream and how it had to be restructured in order to implement a QbD work practice

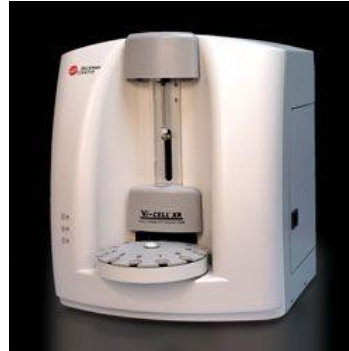
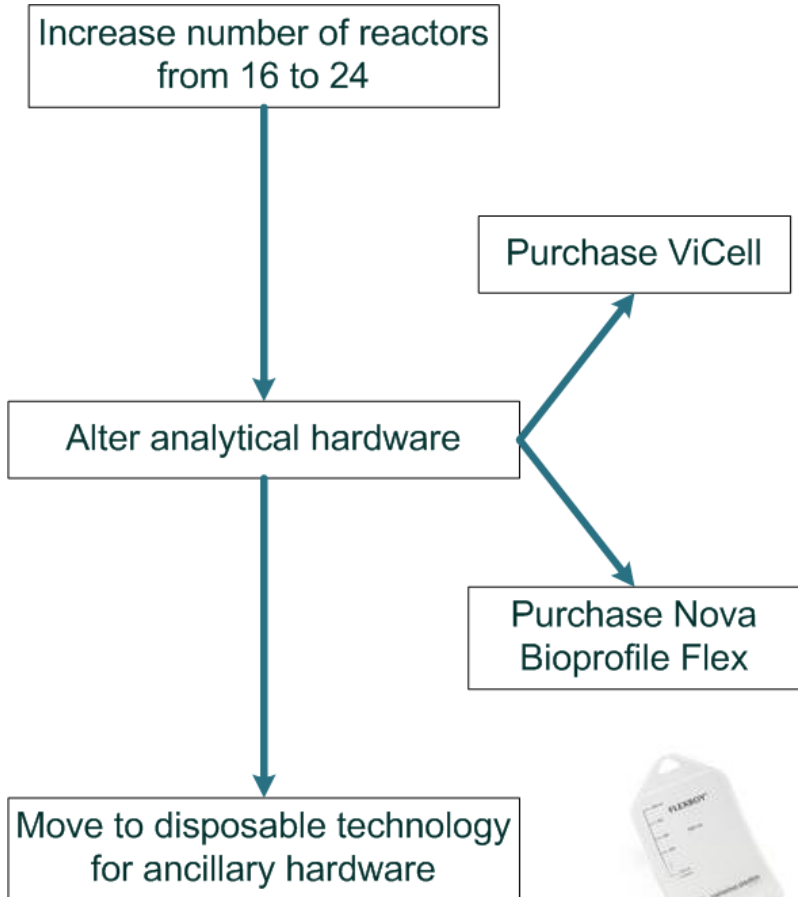
Small Scale Development Structure



Remove responsibility for activities that are "non-core"



Different Equipment Resources



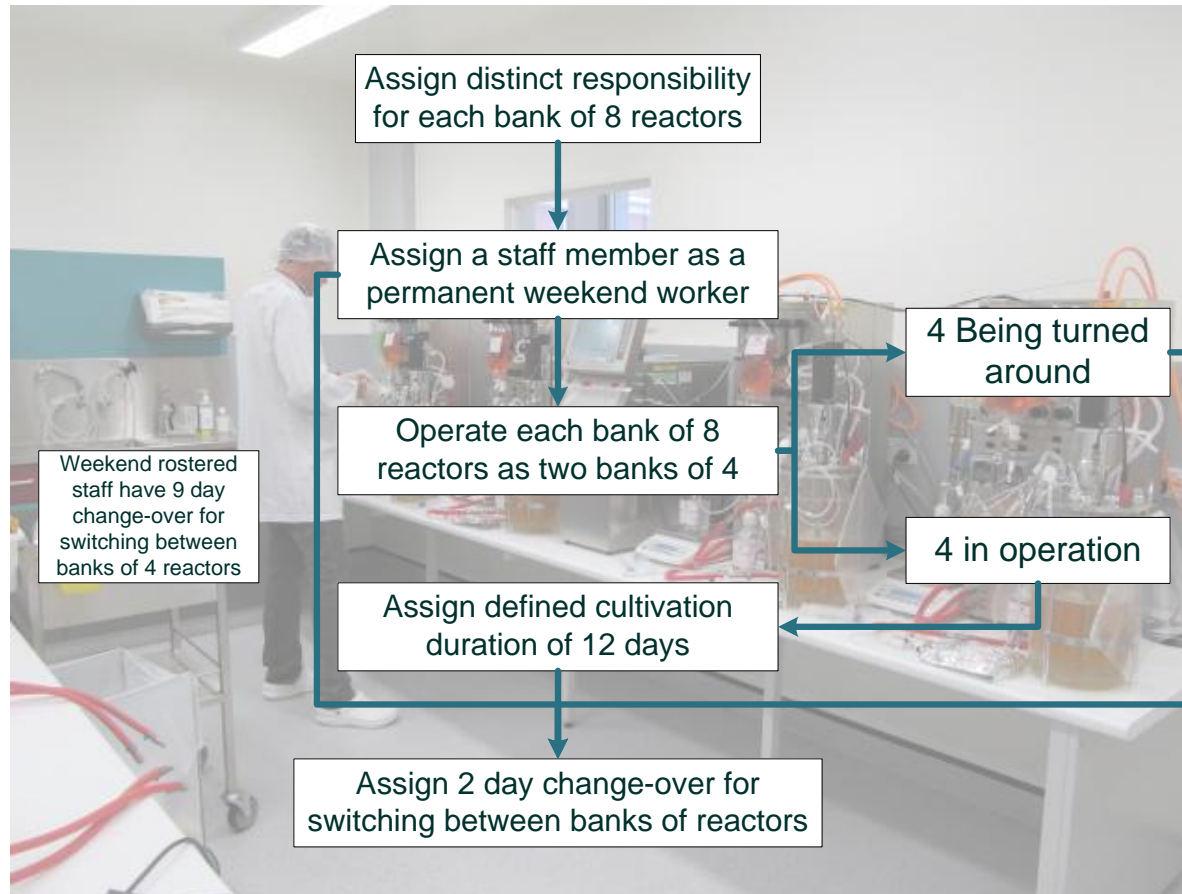
Specific Responsibilities

- Product quantity/quality assays responsibility re-assigned to a discrete Bioanalytical team
- Equipment preparation activities re-assigned to a discrete service department
- Media/solution preparation consolidated
- Seed train maintenance consolidated
- Media optimisation performed at flask scale
- MSP development and material supply moved to scale-up stream

Unit Processes

- Process flow diagrams were bedded down for the following unit processes
 - Reactor configuration
 - Reactor preparation
 - Media preparation
 - Vial thaw
 - Inoculum generation
 - Reactor operation
 - Sampling
 - Harvest
 - Reactor termination
 - Decontamination
 - Cleaning

Revised Operational Approach



Revised Operational Approach

• Excel based protocol templates drafted that encompasses defined unit processes	Generation	Cell Age	VCD	Target Density	Media Volume	Media Batch	Inoc. Vol. Required	Inoc. Vol. Actual	Reactor Volume
	35	0.35	2.345	ACM-R1.13-2011-0163	473.51	0.47	2.82		

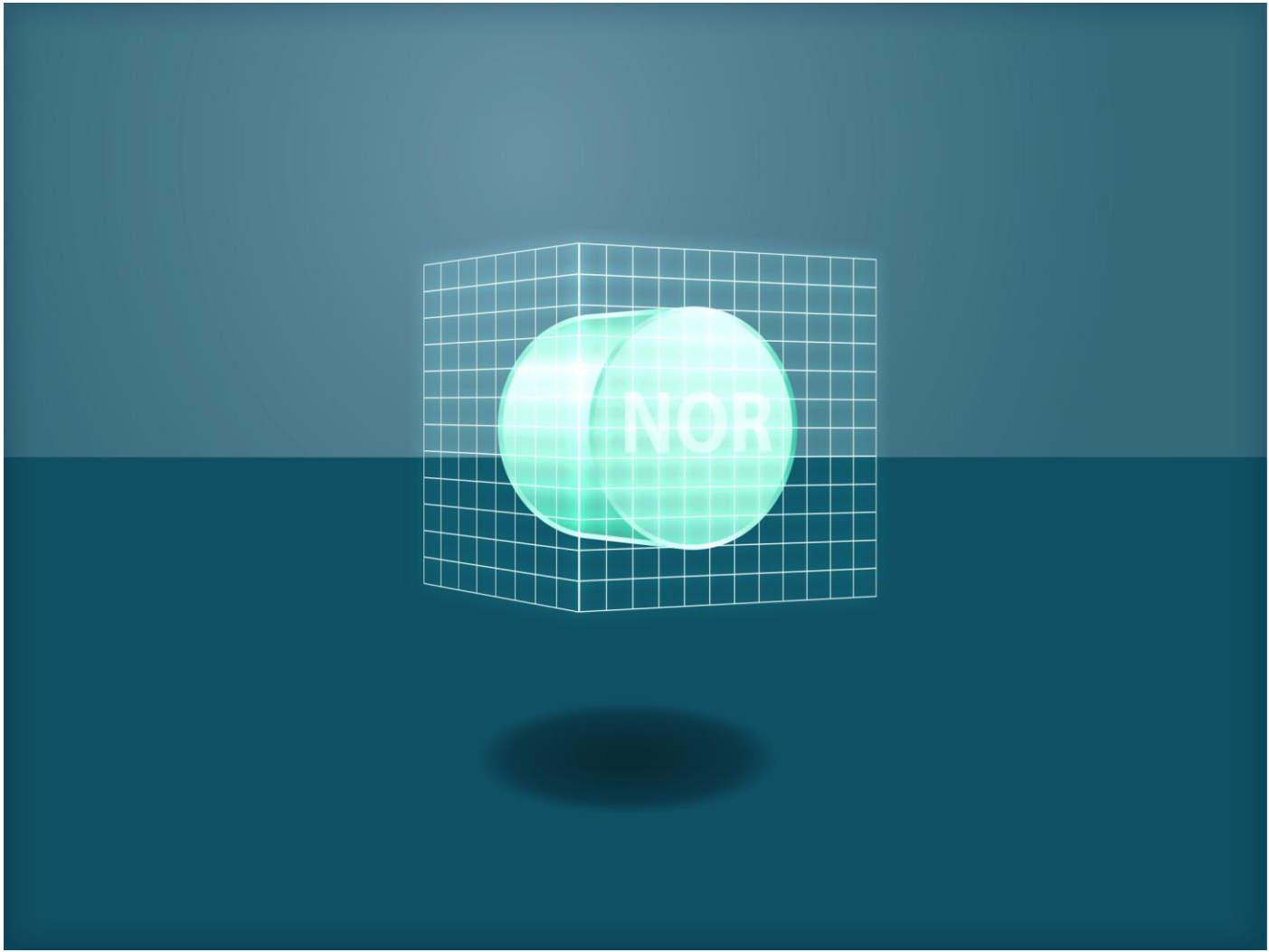
• Data record	Fermentation Strategy																									
• Consistency of function	Set-Point	±	P	I	D	Pre	WD0	WD1	WD2	WD3	WD4	WD5	WD6	WD7	WD8	WD9	WD10	WD11	WD12	WD13	WD14	WD15	WD16	WD17	WD18	
• Excel based comparison templates drafted to monitor in-process performance	pH	7.1	0.71	50	30	0	21/04/2011	21/04/2011	22/04/2011	23/04/2011	24/04/2011	25/04/2011	26/04/2011	27/04/2011	28/04/2011	29/04/2011	30/04/2011	01/05/2011	02/05/2011	03/05/2011	04/05/2011	05/05/2011	06/05/2011	07/05/2011	08/05/2011	09/05/2011
• Utilises VBA code to separate the interpretational activities from the operational activities	Dissolved Oxygen	40	4																							
	AirSP	5	0.5	#N/A	#N/A	#N/A																				
	O2SP	Cascade	#N/A	700	999	0																				
	200 g/Kg Glucose solution	lot:		BU-R1.13-2010-0570																						
	200 mM Glutamine solution	lot:		BU-R1.12-2011-0087																						
	Custom SuperCell	lot:		ACM-R1.13-2011-02345																						

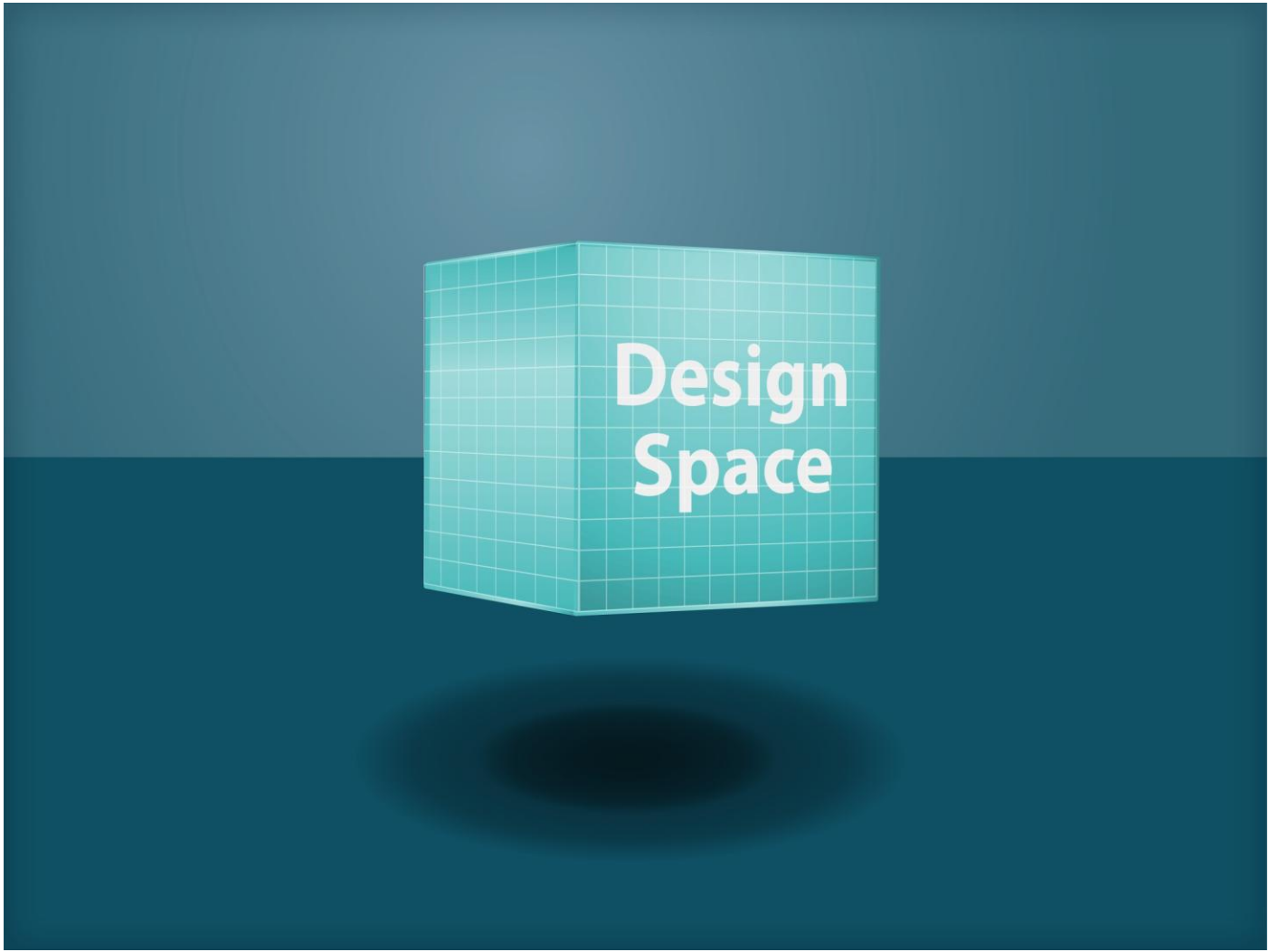
Initial	Operator	SD	Time	Value	Comments/Deviations
JB					
SO	24-Apr-11	3	68.54	pH shift to 6.8	

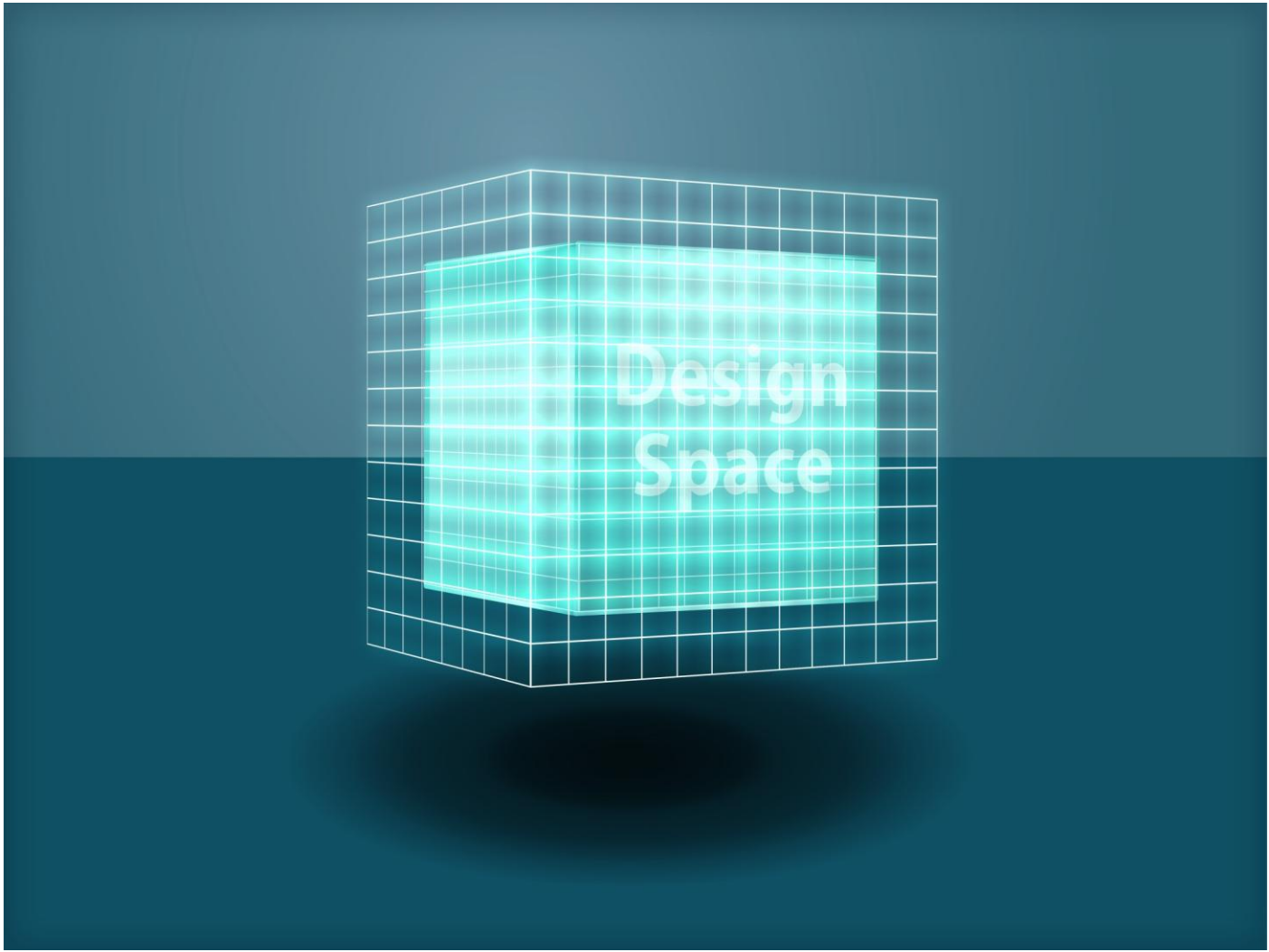
Revised Supervisory Approach

- Purchase specific statistics software for experimental definition and data analyses
 - JMP from SAS
 - JMP has a graphical approach to data interpretation that enables efficient analyses without dedicated bio-statistical support
- Use software under Design of Experimentation (DoE) to implement hypothesis testing with statistical weight
- **This component is integral to QbD**







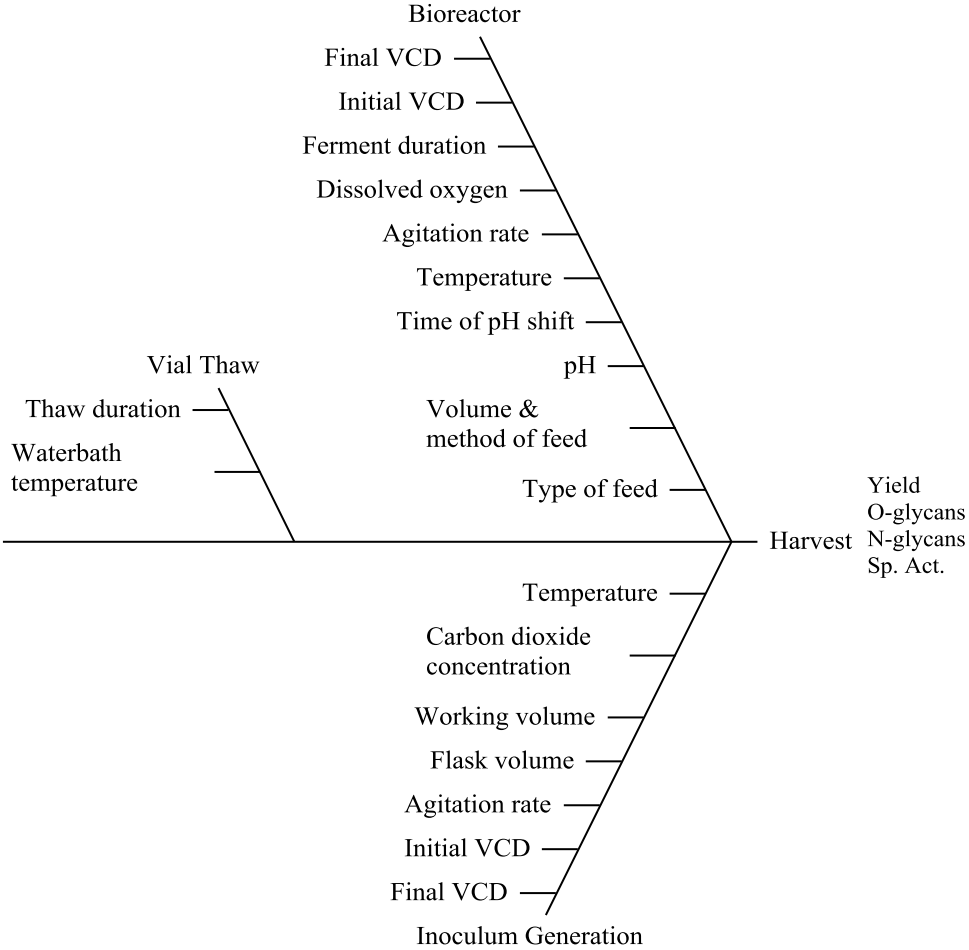




**Knowledge
Space**

Revised Supervisory Approach

Cause and Effect Diagram



Revised Supervisory Approach

Responses				
Add Response		Remove	Number of Responses...	
Response Name	Goal	Lower Limit	Upper Limit	Importance
Titre	Maximize	.	.	.
pcd	Maximize	.	.	.
Growth	Maximize	.	.	.
Full length	Maximize	.	.	.
Aggregates	Minimize	.	.	.
Fragments	Minimize	.	.	.
Clipped forms	Minimize	.	.	.
Light chain	None	NA	NA	NA
Peak lactate	Minimize	.	.	.
Peak ammonia	Minimize	.	.	.
Peak CO2	None	NA	NA	NA

Revised Supervisory Approach

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Add Response		Remove	Number of Responses...	
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Light chain	None	NA	NA	NA
Peak lactate	Minimize	.	.	.
Peak ammonia	Minimize	.	.	.
Peak CO2	None	NA	NA	NA

Factors				
Add Factor		Remove	Add N Factors	1
Name	Role	Changes	Values	
Seed density	Continuous	Easy	150000	350000
pH shift	Categorical	Easy	No	WD3 WD5
pH1	Continuous	Easy	7.1	7.4
Temperature shift	Categorical	Easy	No	WD3 WD5
Temperature 1	Continuous	Easy	35	37
Osmotic shock	Categorical	Easy	No	WD4 WD6
Feed method	Categorical	Easy	Bolus	Linear
Feed start	Continuous	Easy	3	5
Feed finish	Continuous	Easy	3	10
pH2	Continuous	Easy	6.8	7.1
Temperature 2	Continuous	Easy	35	37

Revised Supervisory Approach

Design

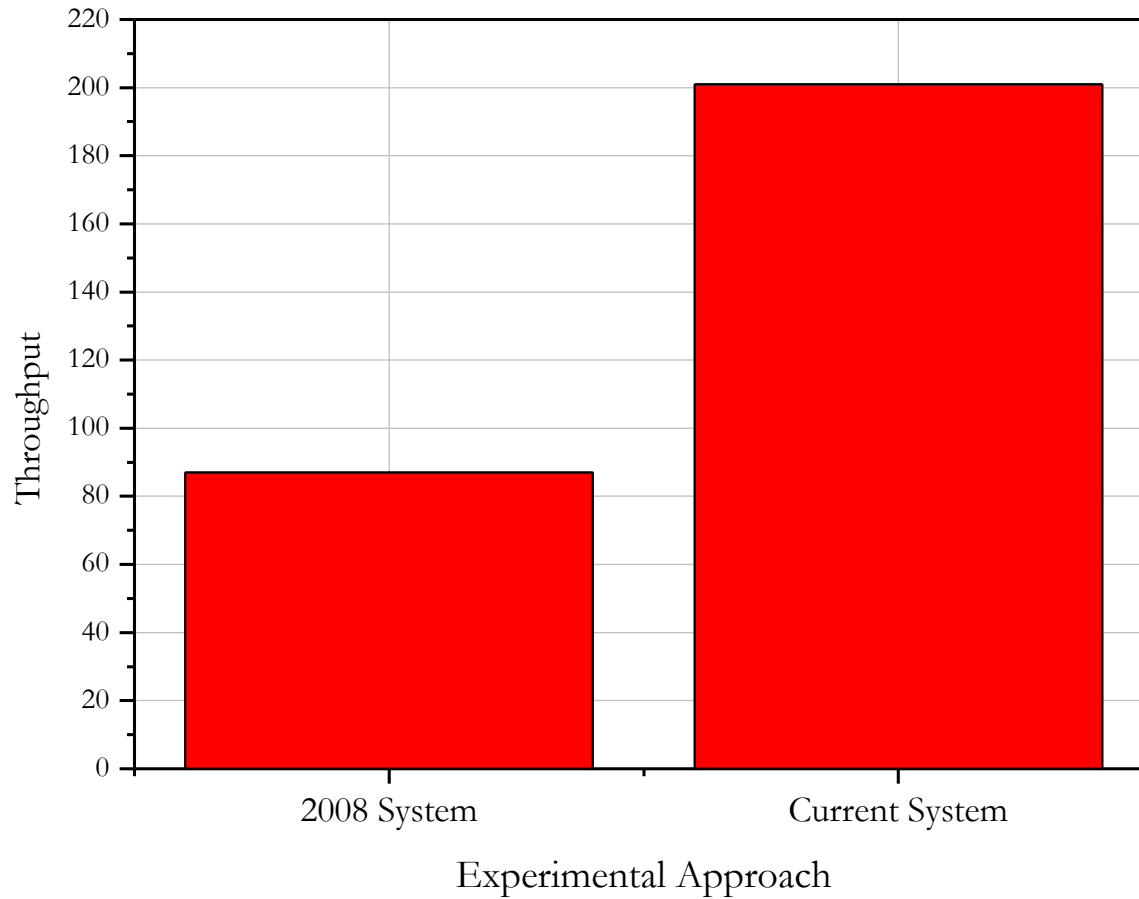
Run	Seed density	pH shift	Temperature		Osmotic		Feed method	Feed start	Feed finish	pH2	Temperature 2
			pH1	shift	Temperature 1	shock					
6	150000	WD3	7.4	WD5	35	WD6	Bolus	5	10	6.8	37
7	150000	No	7.4	WD5	35	WD4	Linear	5	3	7.1	37
8	350000	No	7.1	WD3	35	No	Bolus	5	10	7.1	35
9	350000	WD5	7.4	WD3	37	WD4	Linear	3	3	7.1	37
10	150000	No	7.4	WD3	37	WD4	Linear	3	10	7.1	35
11	150000	WD3	7.4	No	35	WD4	Bolus	3	10	7.1	35
12	150000	No	7.1	WD3	37	WD6	Bolus	5	10	6.8	35
13	350000	WD3	7.1	No	35	No	Bolus	5	3	6.8	37
14	150000	No	7.4	No	35	WD6	Linear	5	10	6.8	37
15	350000	WD5	7.1	No	37	WD4	Bolus	5	10	7.1	37
16	350000	No	7.1	No	35	WD4	Linear	3	3	7.1	37
17	150000	WD3	7.1	WD3	35	WD4	Linear	5	10	7.1	37
18	350000	No	7.1	WD5	37	No	Linear	3	10	6.8	37
19	350000	WD3	7.1	WD5	35	WD6	Linear	3	10	7.1	35
20	350000	No	7.4	WD3	35	No	Linear	5	10	6.8	35

Outcomes

- Increased throughput
- Increased efficiency
- Increased consistency
- Increased quality
 - DoE approach enables statistical weight to be built into the experiment up front
 - Provides clearer direction as to critical factors earlier
 - Factors identified evaluated in a timely and controlled manner

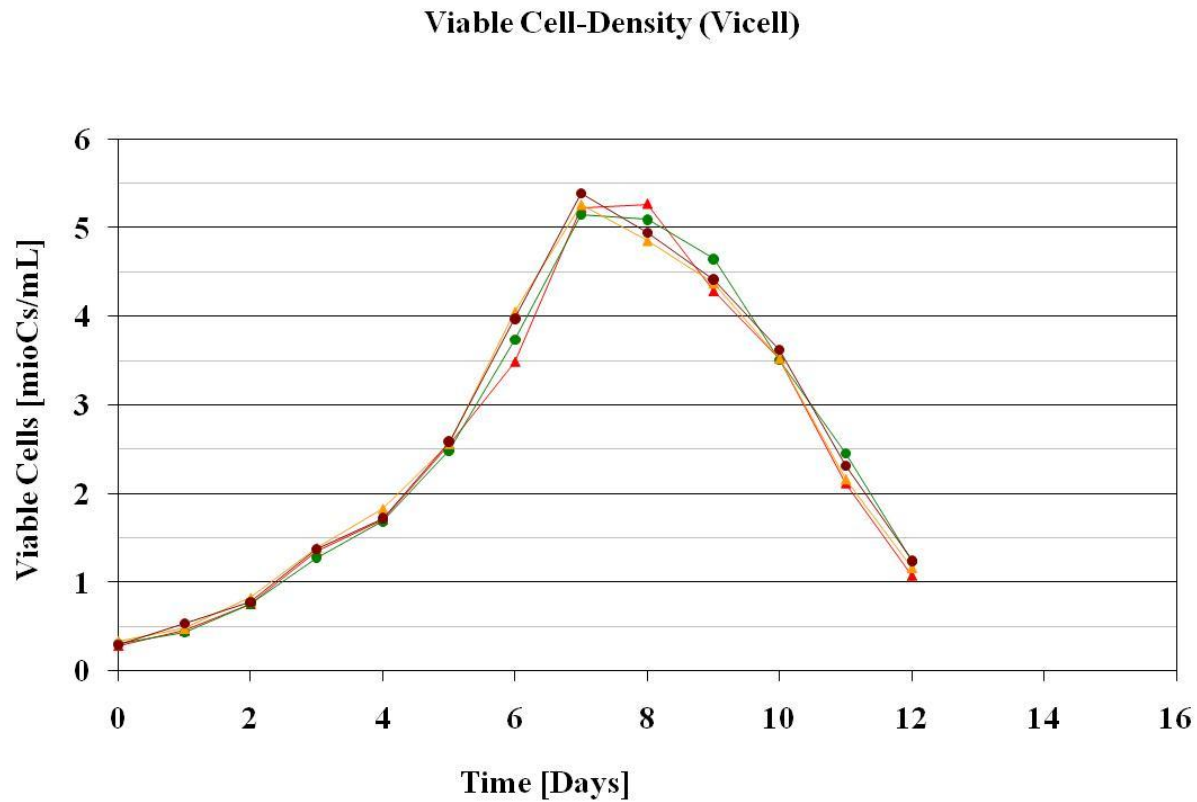
Outcomes

- Increased throughput



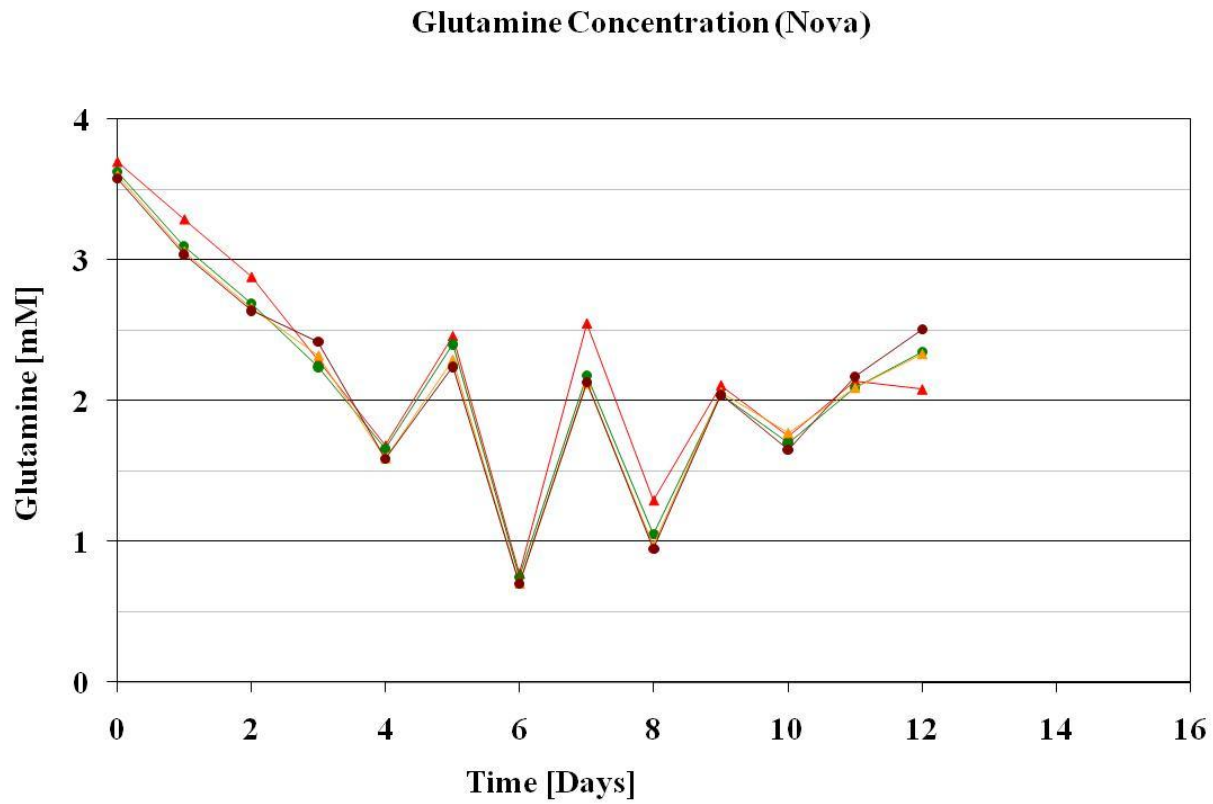
Outcomes

- Increased consistency



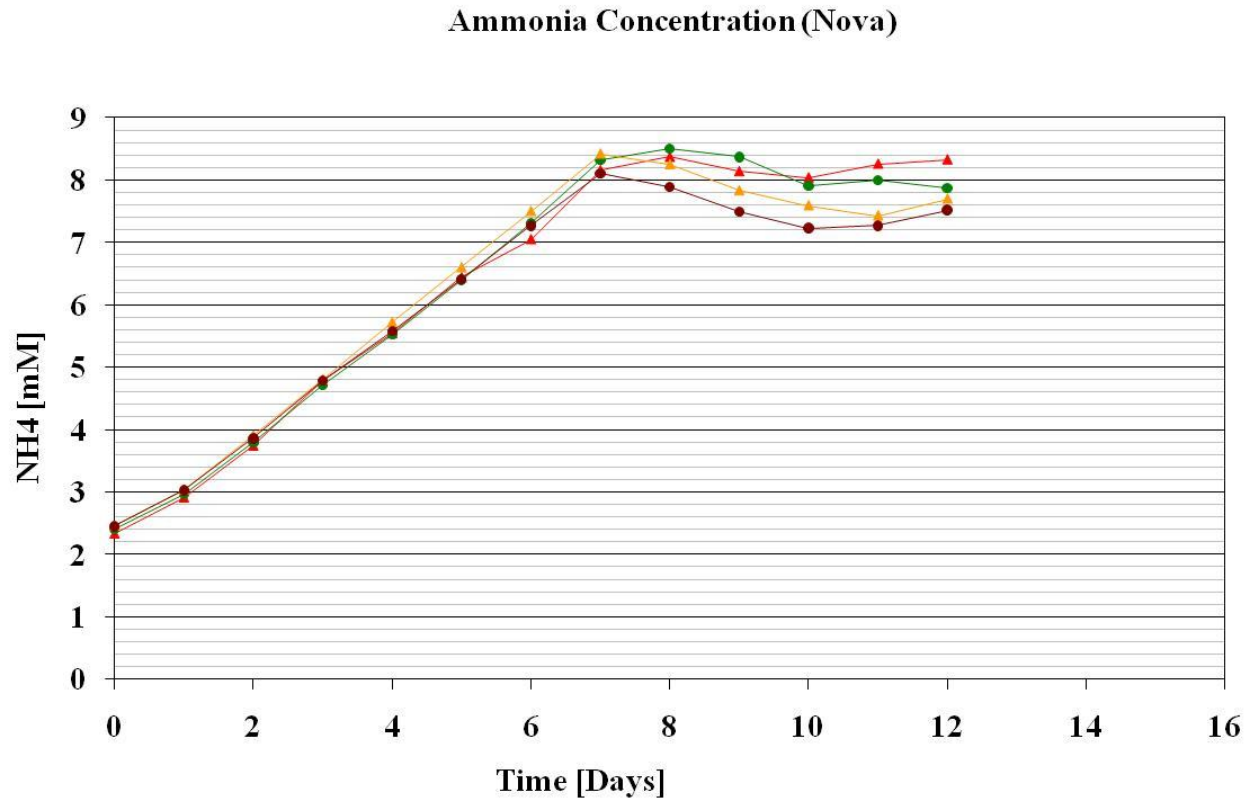
Outcomes

- Increased consistency



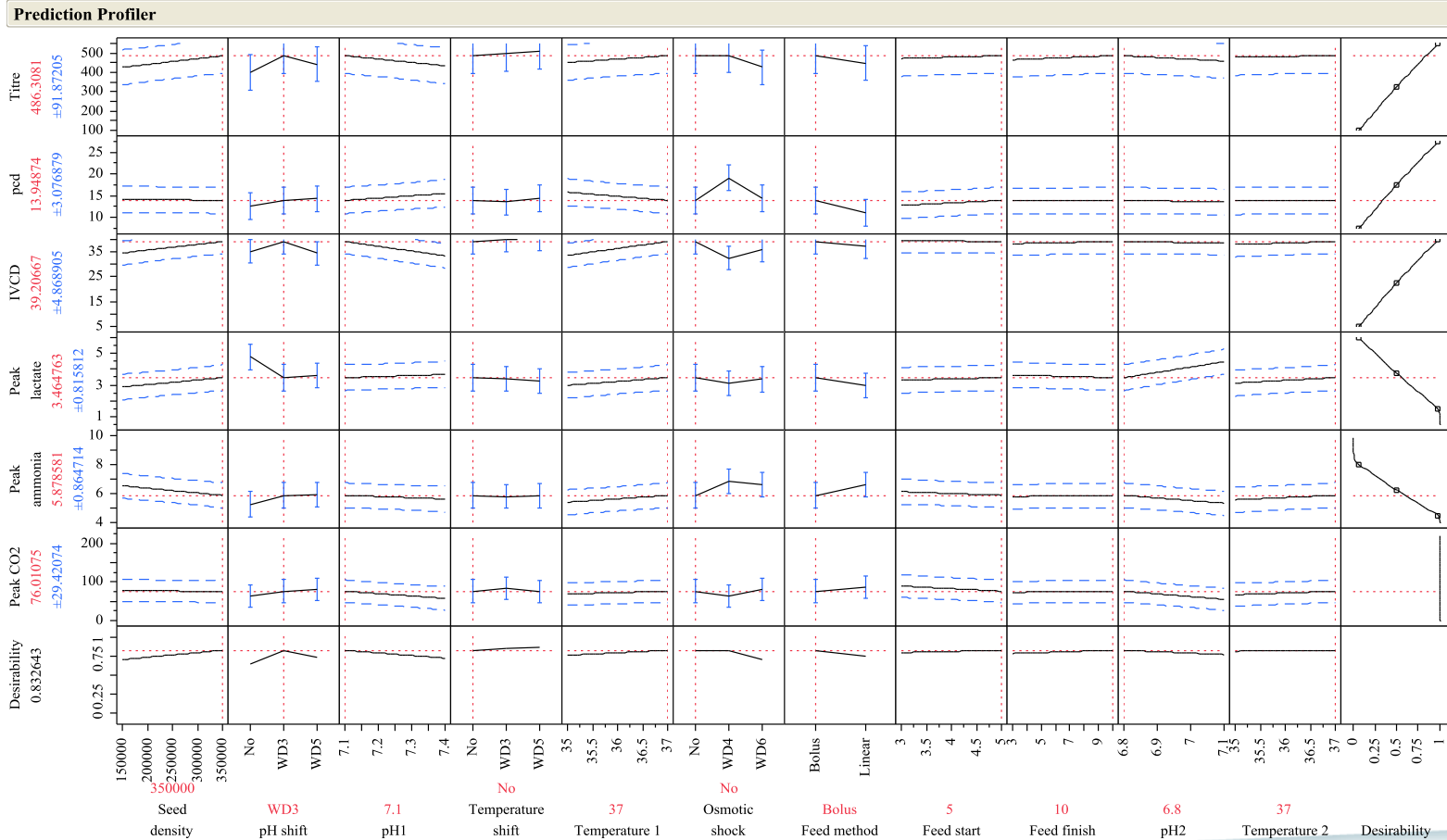
Outcomes

- Increased consistency



Outcomes

- Increased quality



Future Refinement

- Increase equipment resources
 - Purchase 24 new vessels without associated control equipment
- Alter operational approach
 - Operate each bank of 8 reactors as banks of 8 reactors
 - 8 reactors being turned around, 8 in operation
- Changes should yield an increase in capacity to 350 – 400 developmental activities per annum

Implications

- Increased burden on the analytical systems
 - Current system requires the implementation of a high throughput analytical approach
 - Further increases in capacity will have an even higher reliance on high throughput analytics
- Increase in human resources required to support expanded capacity

Questions