Optimization of therapeutic protein production in an aquatic plant expression system using DOE

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Outline of talk

- Description of Lemna Expression (LEX) System^{SM} Technology
- Description of LEX System^{SM} Manufacturing
- An example of how we use Custom Design DOE in JMP 9.0 to optimize the yield of a vaccine antigen
- Conclusions

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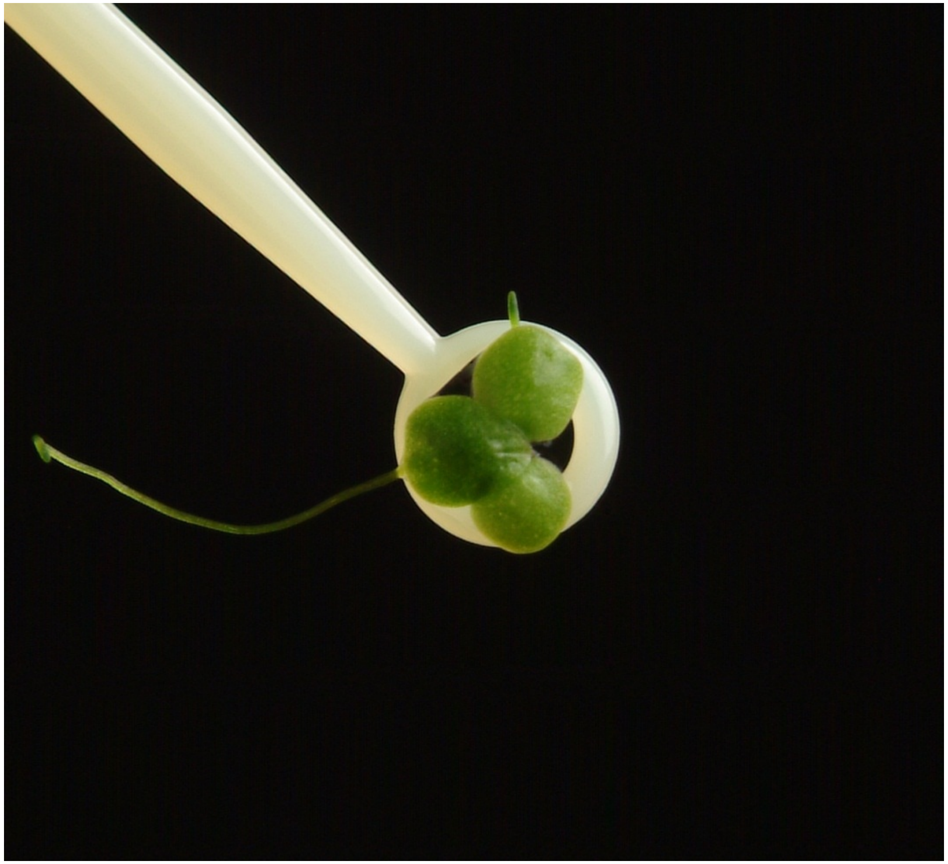
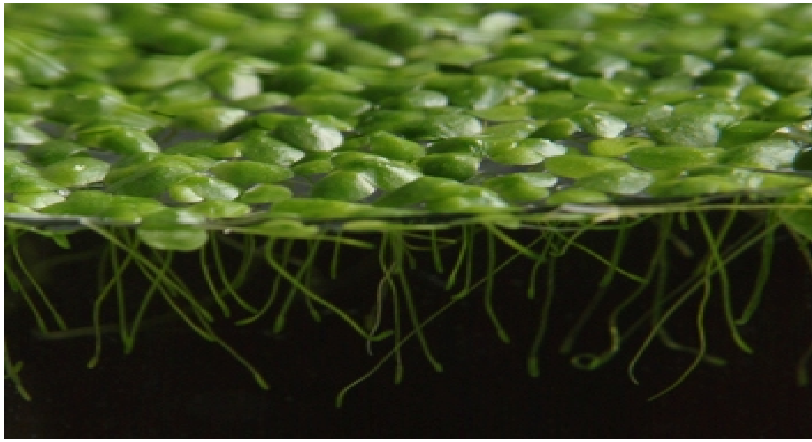
LEX System^{SM} Technology

The LEX System^{SM} uses the higher aquatic plant *Lemna* (duckweed)





One *Lemna* plant consists of:

- Three frond cluster (three leaves)
- A single root

The LEX System^{SM}: Ideal for expression of therapeutic proteins

- Mammalian cell culture-like features:
  - Clonal
  - Lemna easily transformed with DNA to give transgenic Lemna line making recombinant protein of choice (e.g. vaccine antigen, antibodies)
  - Doubles every 36 hours
  - High protein content
  - Makes small and large recombinant proteins
  - Complex post-translational processing
  - Secretes recombinant protein
- **Added *Lemna* advantages**
  - Simple salts media, animal derived component free
  - No animal viruses
  - Manufacturing in a contained, aseptic, animal free disposable upstream bag and harvesting production system in controlled cGMP facilities
  - Lower capital cost

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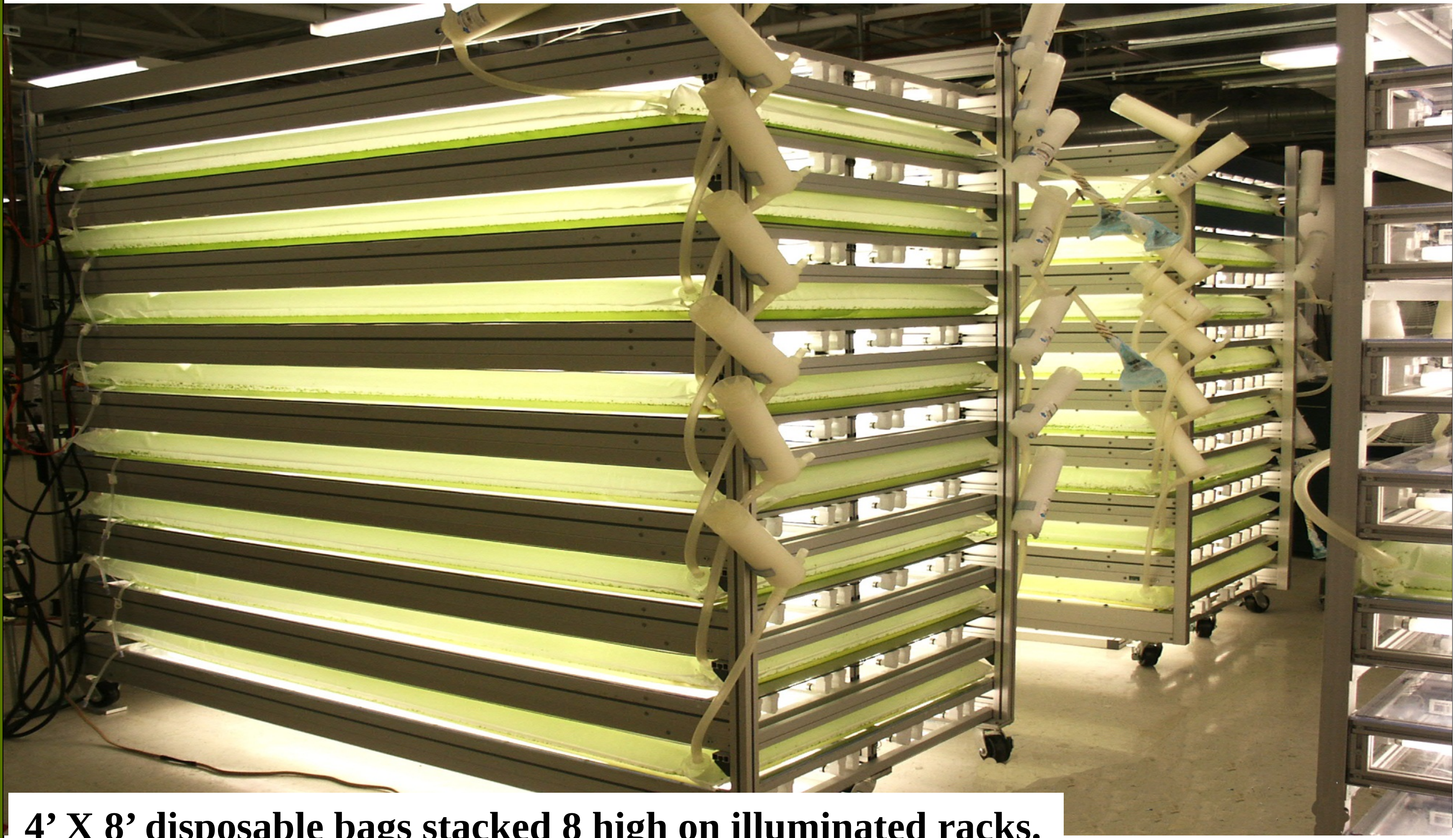
LEX System℠ Manufacturing

Large bag: Manufacturing bioproduction format



4’ X 8’ disposable bags stacked 8 high on illuminated racks. Totally enclosed disposable manufacturing system

Large bag: Manufacturing bioproduction format-easily scalable

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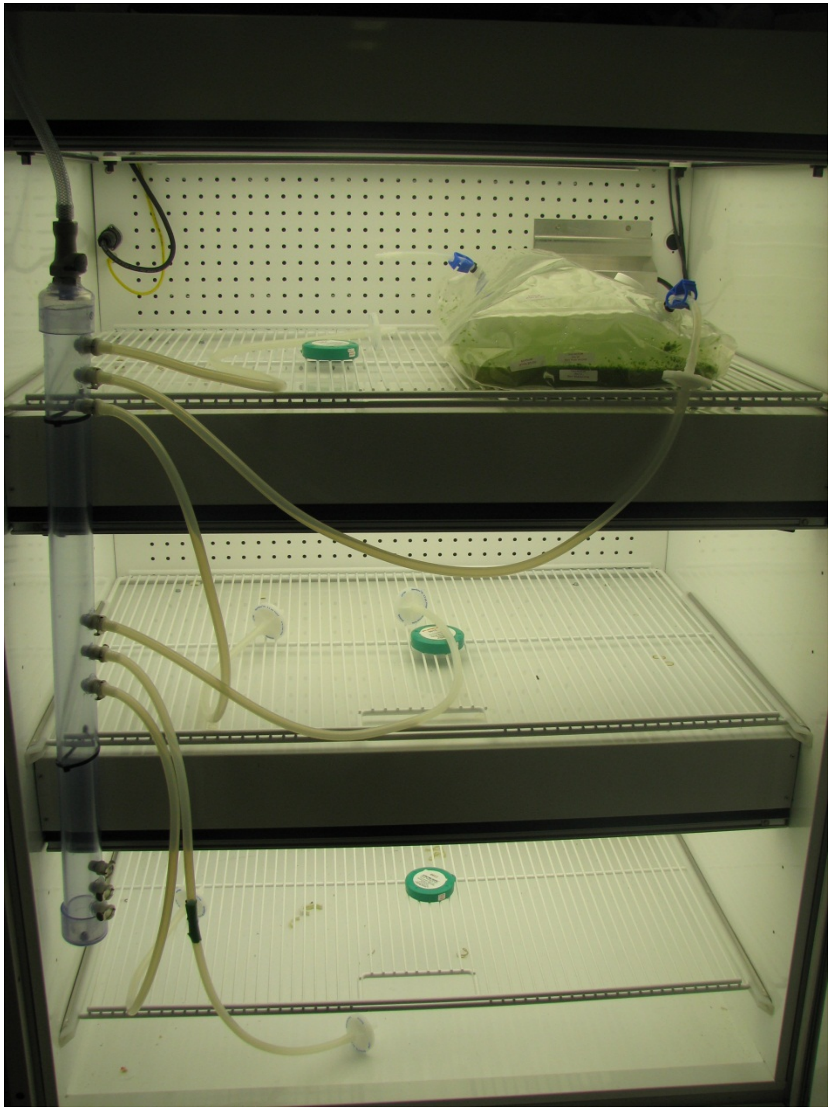
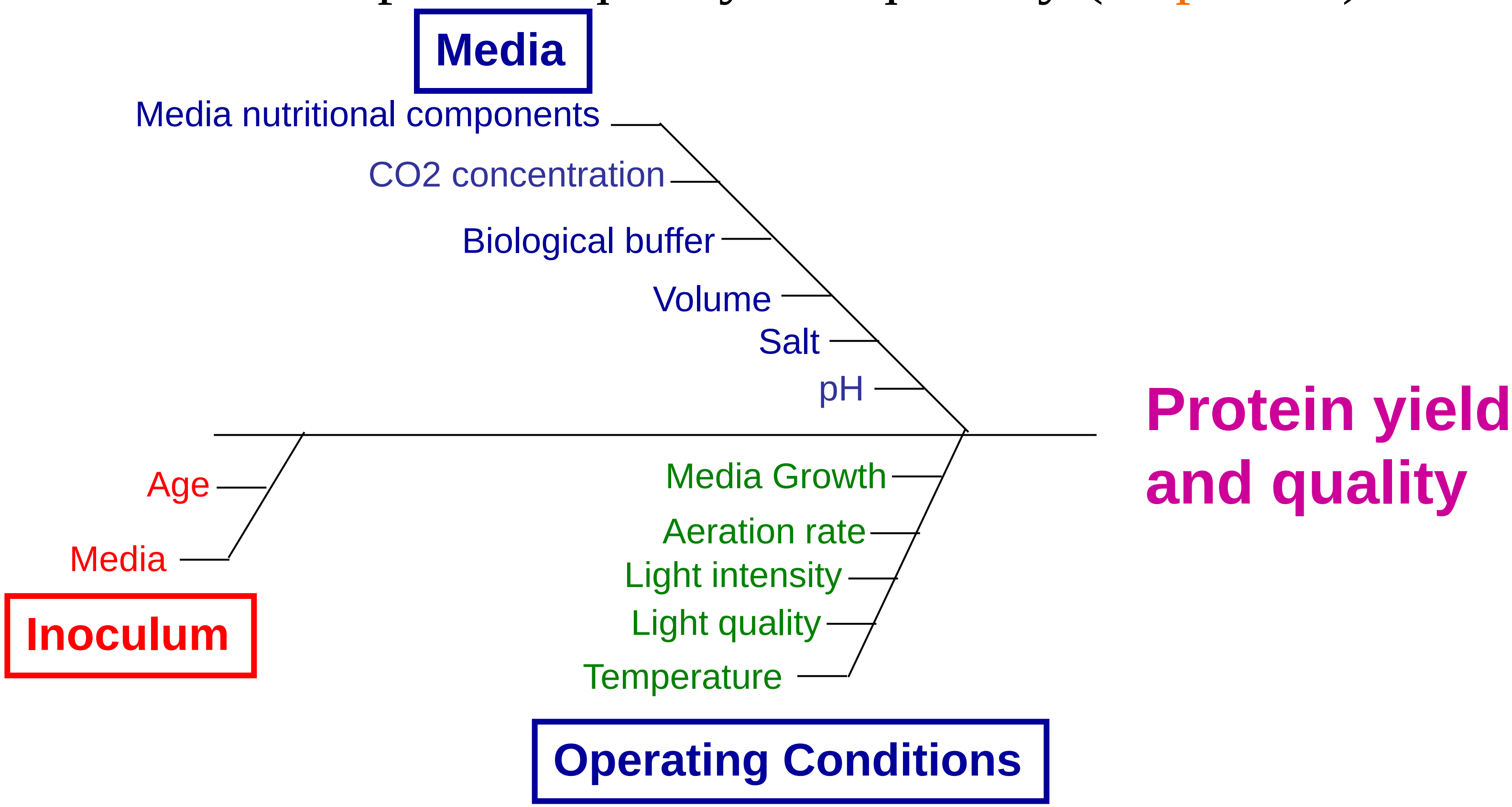
Use of Design of Experiment (DOE) in optimization of a vaccine antigen production

Scaled down model: Growth chambers with small forced air disposable bags used for upstream DOE



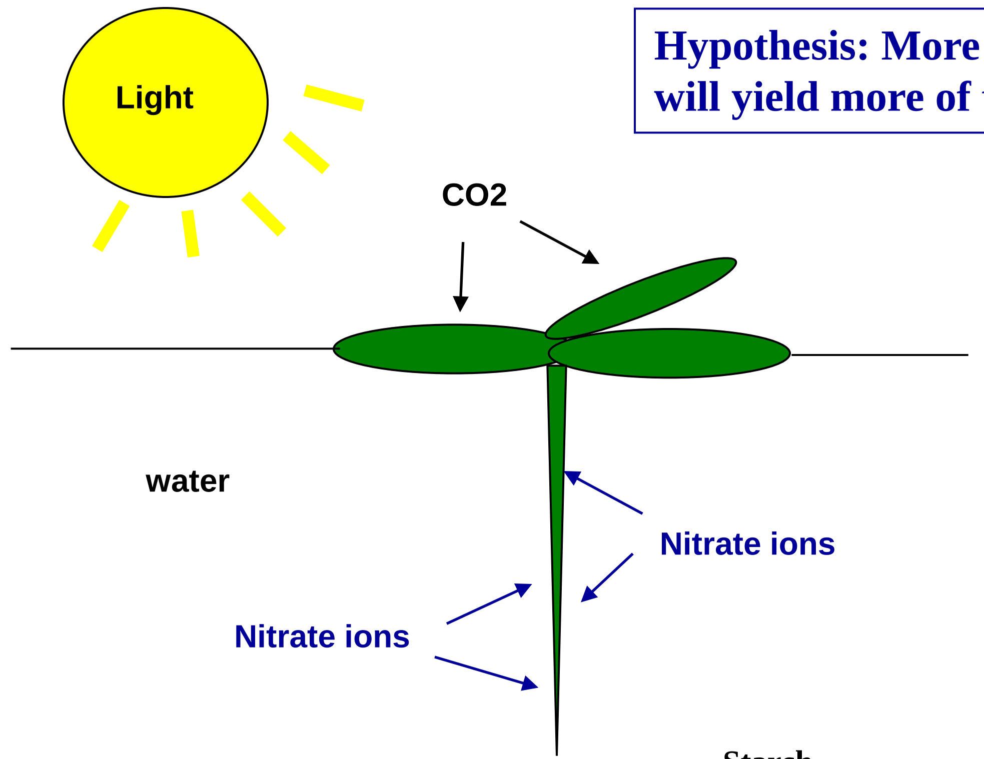
Use of DOE in optimization of vaccine antigen production

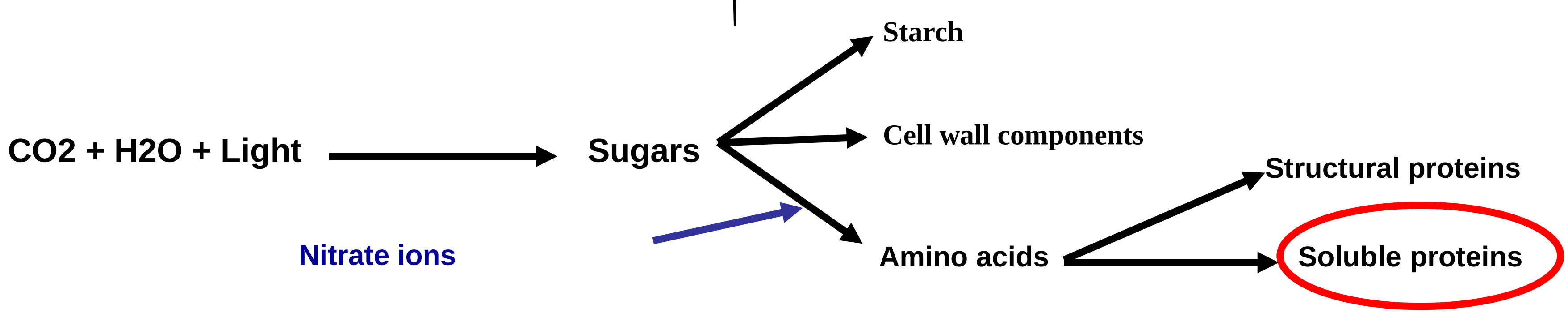
From our experience, process knowledge and preliminary experiments we identified potential critical factors that may affect our vaccine product quality and quantity (responses)



DOE experiment to examine the impact of light, carbon dioxide and nitrogen on vaccine antigen production

Hypothesis: More light, C02 and nitrate will yield more of the vaccine antigen





I will now use JMP 9 to show how to set up a Custom Design DOE and then analyze the data from one experiment used for optimizing the yield of a vaccine antigen in a transgenic *Lemna* line

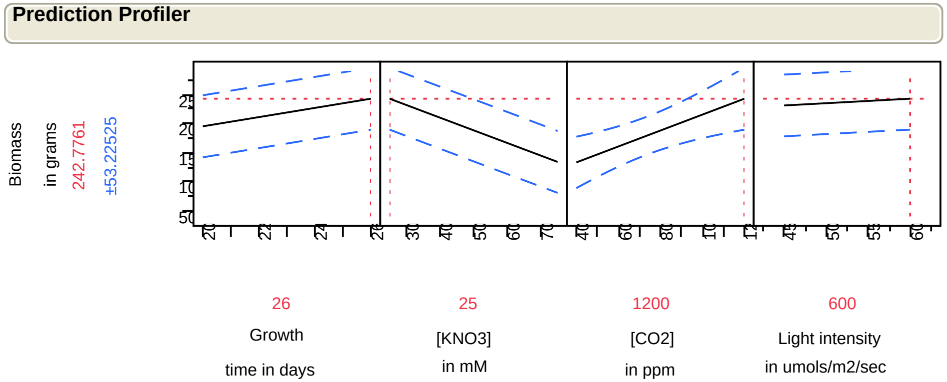
Effects of critical factors on the biomass yield

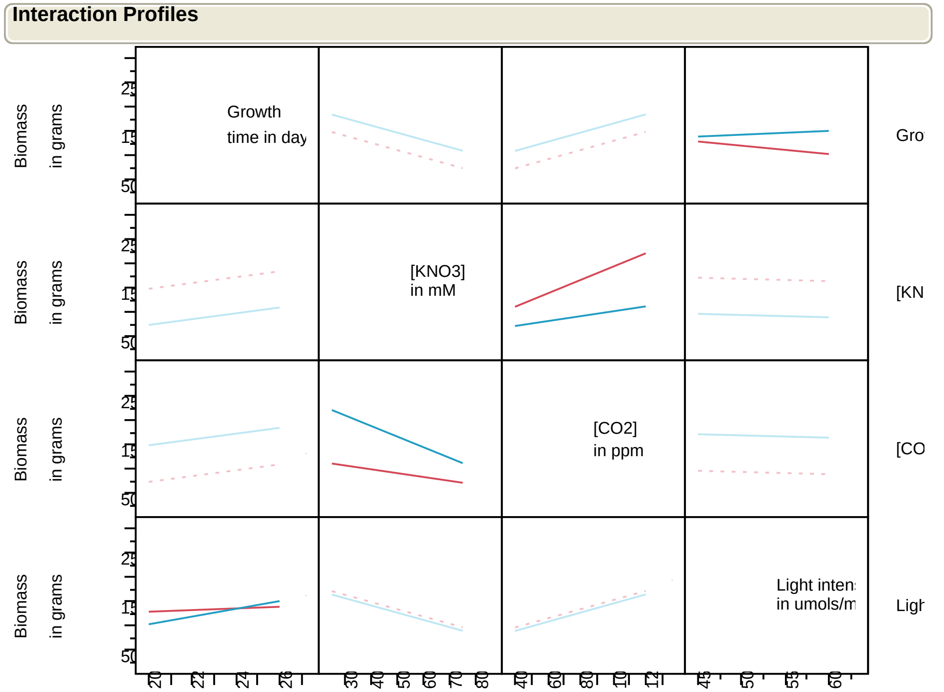
Summary of Fit

RSquare	0.982451
RSquare Adj	0.973677
Root Mean Square Error	10.14895
Mean of Response	126.3368
Observations (or Sum Wgts)	19

Parameter Estimates

Term	Prob>|t|
Intercept	0.0025*
Growth time in days(20,26)	0.0009*
[KNO3] in mM(25,75)	<.0001*
[CO2] in ppm(400,1200)	0.0472*
Light intensity in umols/m2/sec	0.5116
[KNO3] in mM*[CO2] in ppm	0.0006*
Growth time in days*Light intensity	0.0202*





Effects of critical factors on the total soluble protein per gram of biomass

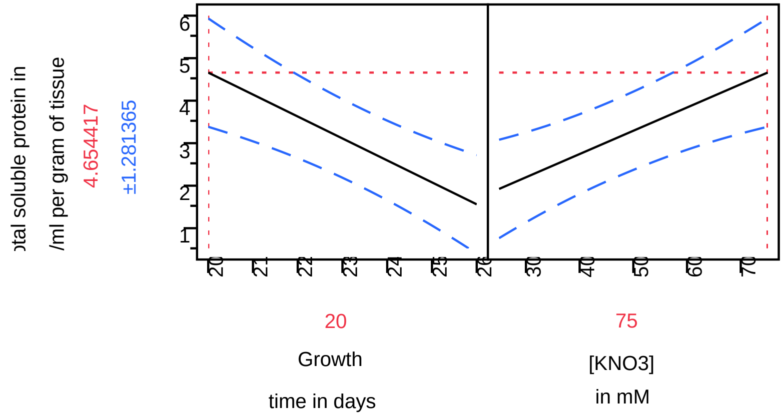
Summary of Fit

RSquare	0.74597
RSquare Adj	0.695163
Root Mean Square Error	0.845507
Mean of Response	2.737368
Observations (or Sum Wgts)	19

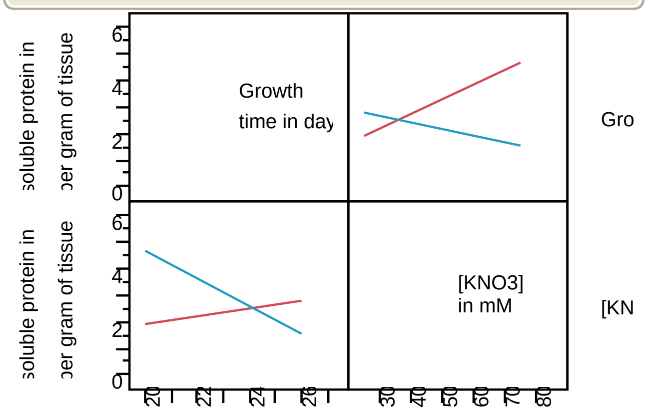
Parameter Estimates

Term	Prob>|t|
Intercept	0.0005*
Growth time in days(20,26)	0.0563
[KNO3] in mM(25,75)	0.1385
Growth time in days*[KNO3] in mM	0.0070*

Prediction Profiler



Interaction Profiles



Effects of critical factors on the vaccine antigen in units per gram of biomass (specific productivity)

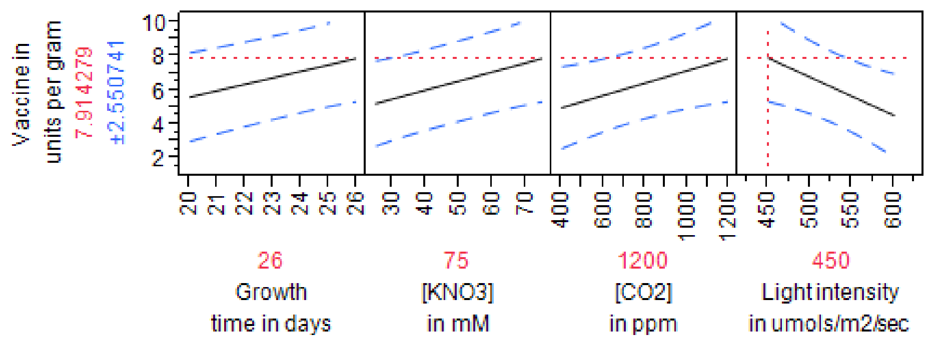
Summary of Fit

RSquare	0.73745
RSquare Adj	0.606175
Root Mean Square Error	1.172945
Mean of Response	4.8
Observations (or Sum Wgts)	19

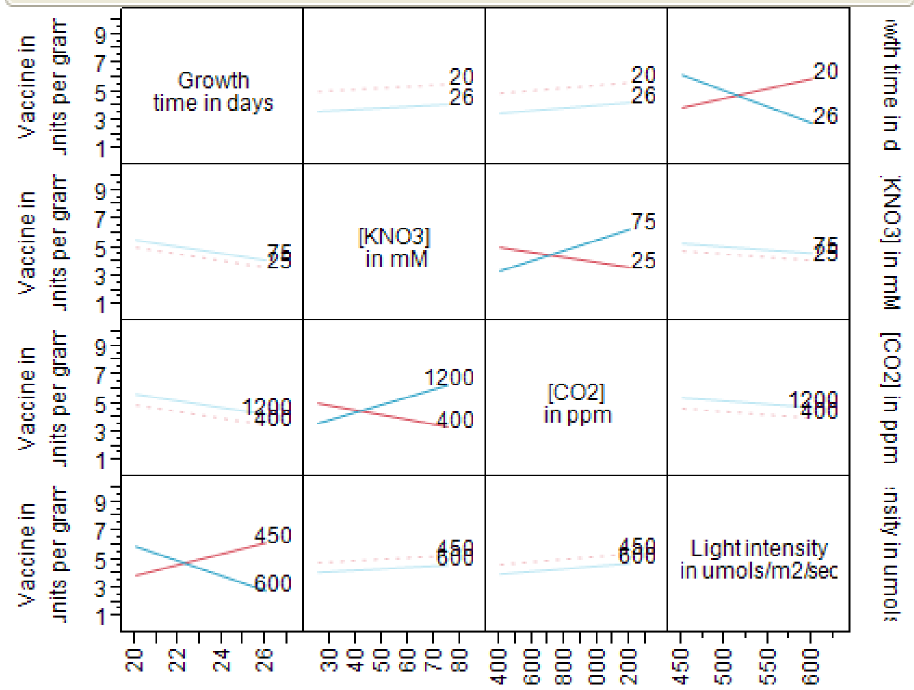
Parameter Estimates

Term	Prob>|t|
Intercept	0.1345
Growth time in days(20,26)	0.1306
[KNO3] in mM(25,75)	0.4881
[CO2] in ppm(400,1200)	0.4990
Light intensity in umols/m2/sec	0.5428
[KNO3] in mM*[CO2] in ppm	0.0218*
Growth time in days*Light intensity	0.0059*

Prediction Profiler



Interaction Profiles



Effects of critical factors on the vaccine antigen in units per growth bag

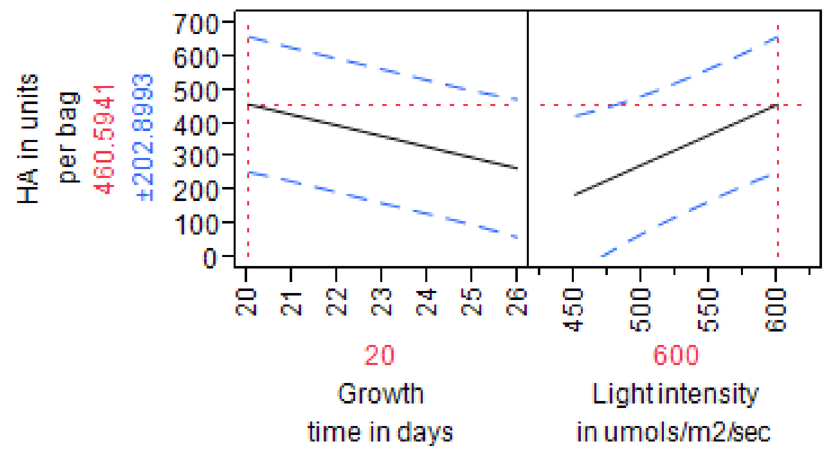
Summary of Fit

RSquare	0.809536
RSquare Adj	0.771443
Root Mean Square Error	90.83641
Mean of Response	370.9474
Observations (or Sum Wgts)	19

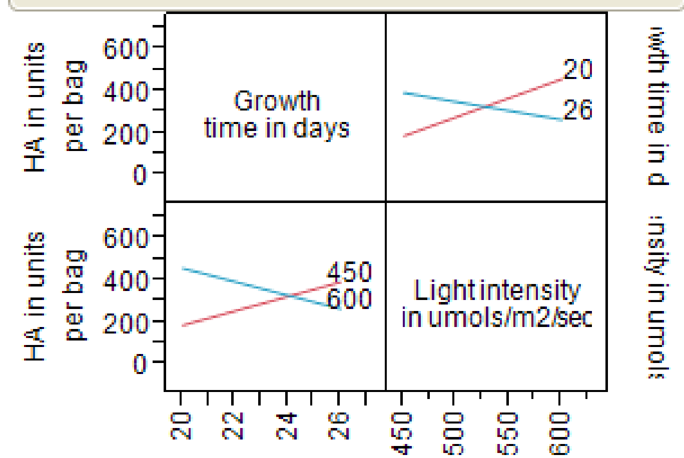
Parameter Estimates

Term	**Prob>|t|**
Intercept	0.8123
Growth time in days(20,26)	0.3186
Light intensity in umols/m2/sec	0.4110
Growth time in days*Light intensity	0.0064*

Prediction Profiler



Interaction Profiles



Conclusions

We used the Custom DOE format in SAS JMP® 9.0 software in Upstream Process Development:

- To identify critical factors that influence the yield and quality of recombinant proteins (e.g. vaccine antigens, antibodies)
- Optimization of the upstream process for the **responses** of recombinant protein yield and quality
- Failure mode effects analysis (FMEA)