MUNICH 2020 DISCOVERY SUMMIT

Industrial Biotech Case Study – Customized DOE for Process Optimization in Small-Scale Bioreactors

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Lonza LSI & LPBN

Abstract

- Design of Experiments (DOE) is frequently used in industrial biotechnology for optimizing microbial cultivation process parameters; one major goal: increasing final product concentration, e.g. titer, or yield
- Standard DOE approaches facilitate the transferability to customers as well as the comparability between similar projects
- This case study shows a general framework for the optimization of process parameters in small-scale bioreactors (0.25L), including a subsequent DOE model validation in lab-scale (2L) and pre-pilot scale (30L)
- JMP Custom Design tool was applied to generate an I-optimal Response Surface Design with four continuous factors and three center points
- The generated model was able to accurately predict the output variable within the 95% confidence interval
- JMP Data Exploration tools enabled a fast evaluation of the data quality

Data Collection → Validation & Scale-Up → Pilot Run

0.25L scale

2L scale

30L scale

1500L scale

Conclusions & Outlook

- Successful process optimization from small-scale to pre-pilot scale bioreactors using JMP's Custom Design tool
- 1.9 fold improvement of product titer compared to initial process
- Multiple measurements of product titer at several timepoints enabled time-dependent evaluations using the JMP Prediction Profiler
- A subsequent augmented design can be performed in order to enhance process understanding and examine optimal factor settings
- Tailor-made DOEs contribute to process validations by identifying relevant process parameters already at development phase

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Case Study – Customized Design Approach

planning phase

1. Describe

Identification of experimental goals, responses and factors

Goals:
- Identification of relevant process parameters
- Product titer enhancement

Responses:
- Product titer, space-time yield

Factors:
- Feed rate, yeast extract, temperature, pH

2. Specify

Model determination & reactor system selection

- Model determination:

DOE → Screening → Fractional, DSD
DOE → Optimization → Custom Design, RSM

→ I-optimal response surface design with 4 continuous factors and 3 center points

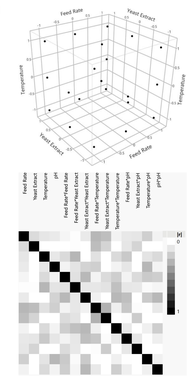
- DOE runs: small-scale bioreactors (0.25L)
  → 2 × 12 = 24 possible runs
- Validation & scale-up: 2L & 30L bioreactors

3. Design

Design generation and evaluation

Table: Response Surface Design Table

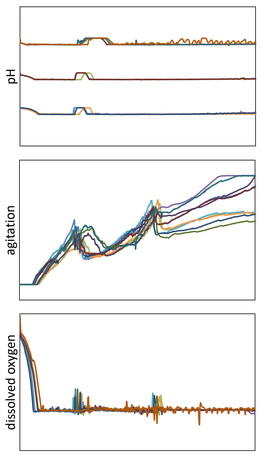
Run	Feed Rate	Yeast Extract	Temperature	pH
1	-0.1	1	1	-1
2	0	0	1	1
3	-1	0	0	0
4	0	1	-1	0
5	-1	-1	-1	1
6	1	-1	-1	0
7	1	1	1	0
8	0	0	0	0
9	0	-1	0	1
10	0	-1	-1	-1
11	1	-1	0	-1
12	1	0	1	-1
13	0	0	0	0
14	-1	1	1	1
15	-1	-1	1	0
16	1	1	-1	-1
17	-1	1	0	-1
18	-1	-1	1	-1
19	-1	0	-1	-1
20	0	0	0	0
21	-1	-1	-1	1
22	1	0	-1	1
23	1	1	0	1
24	1	-1	1	1



experimental phase

4. Collect Data

DOE execution in small-scale bioreactors



5. Fit & Predict

Model fitting & prediction of optimal factor settings

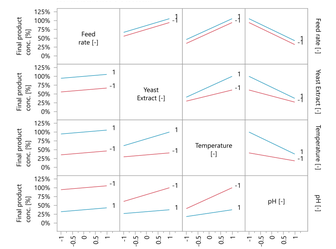
- Determine: model accuracy and significant effects

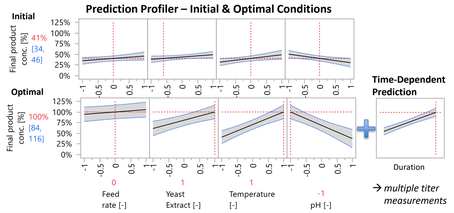
ANOVA

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	7	0.70204363	0.100292	10.6420
Error	13	0.12251404	0.009424	Prob > F
C. Total	20	0.82455767		0.0002 *

Effect Summary

Source	LogWorth	PValue
pH [-]	3.582	0.00026
Temperature [-]	3.273	0.00053
Temperature [-]*pH [-]	1.853	0.01403
Yeast Extract [-]*pH [-]	1.284	0.05195
Yeast Extract [-]*Temperature [-]	1.194	0.06405
Yeast Extract [-]	1.148	0.07114
Feed rate [-]	1.038	0.09156

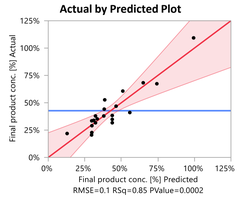




Time-Dependent Prediction

Duration

→ *multiple titer measurements*



6. Validate & Scale-Up

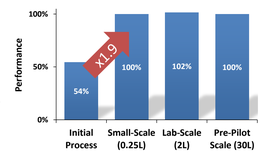
DOE model validation in pre-pilot scale

- Successful DOE model validation & scale-up

Setup							Analytics	DOE Model	
No.	Scale	Feed Rate	Yeast Extract	Temp.	pH	Remark	Titer [%]	Titer [%]	Confidence Interval [%]
1	2 L	0	1	1	-1	Validation	102	100	84 – 116
2	2 L	0	-0.6	1	-1	Validation	82	69	54 - 84
3	30 L	0	1	1	-1	Scale-Up	100	100	84 – 116

valid model *- measured titer in line with predicted mean or 95% confidence interval*

- Performance 1.9 fold higher compared to initial process
- Process transfer from 0.25L to 30L



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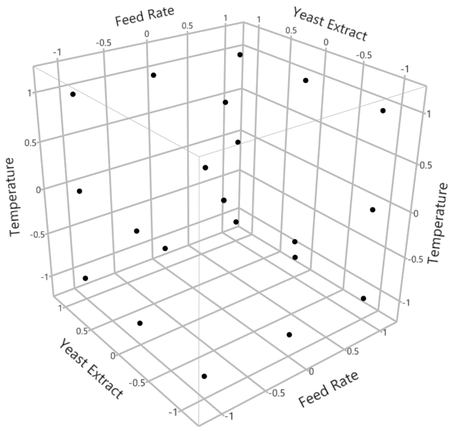

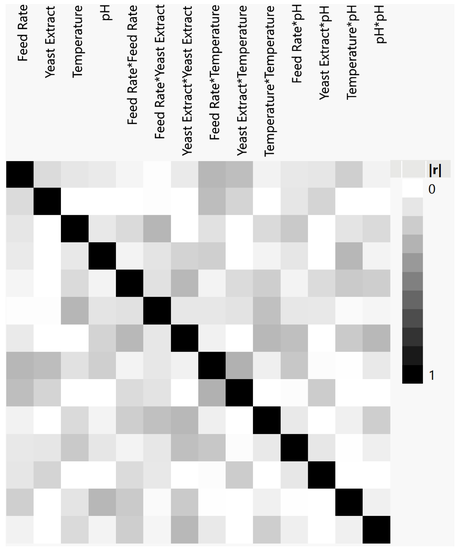

Design

The generated design has to be consistent with the chosen model and selected factors, ensuring the achievement of experimental goals.

Evaluation

The Design Evaluation or Design Diagnostics outline in the JMP design generation platform gives insights about the design's strengths and limitations.

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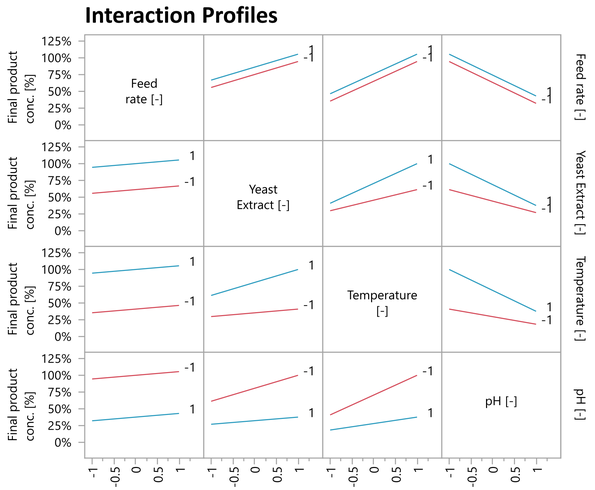

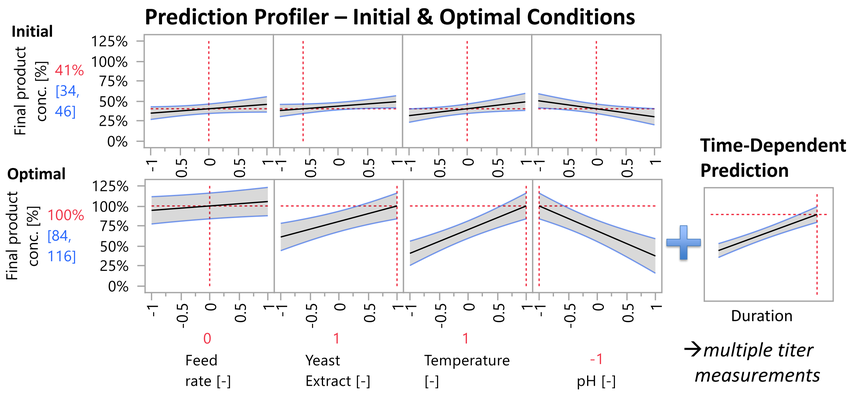

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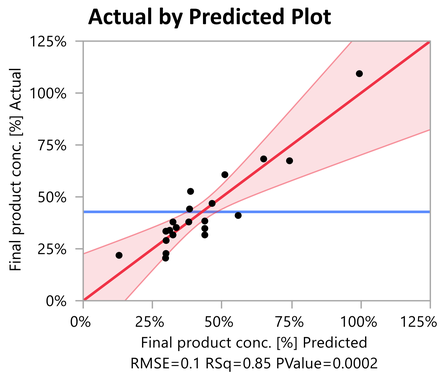

Fit

The assumed model has to be fitted to the collected data. The JMP modeling platforms can be used for this purpose, including model trimming (exclusion of inactive interaction effects, etc.). Augmenting the design with additional data and/or experiments can help to resolve uncertainties and intensify process knowledge.

Predict

The refined model can be used to address the experimental goals. Predictive models enable the determination of active effects in order to optimize responses, e.g. maximize final product concentration.

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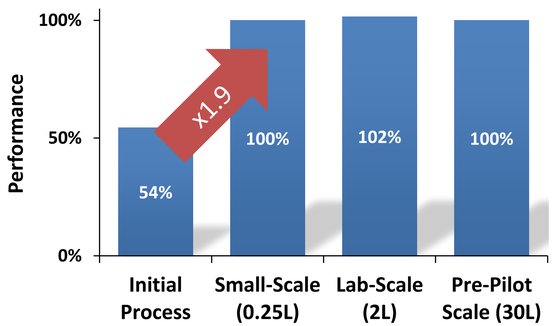

Validate

The prediction accuracy of the DOE model is validated by evaluating (new) factor settings in a follow-up experiment. Measured responses need to be in line with the model forecast, e.g. within the 95% confidence interval.

Scale-Up

After DOE model validation, a scale-up run can be performed, e.g. in 30L pre-pilot scale. Tailor-made DOE approaches can contribute to future process validations by identifying relevant process parameters already at the development phase.