Multivariate analysis for increased understanding of the USP process of MeiraGTx's products

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Introduction

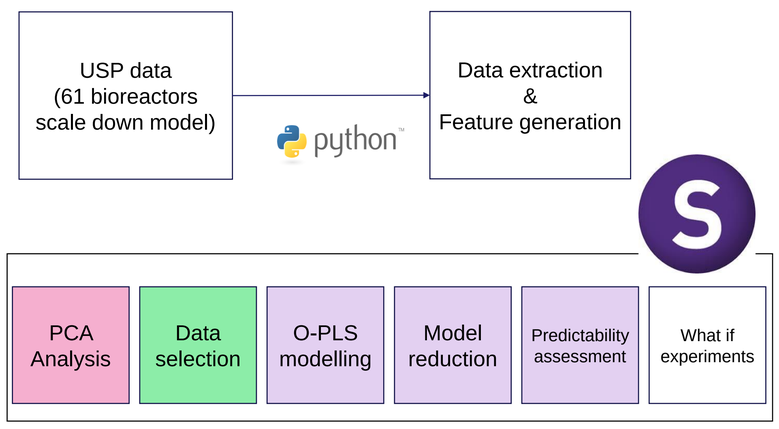
Orthogonal Partial least squares (O-PLS) is a multivariate statistical modelling technique which:

- deals with multicollinearity and considers data structure.
- provides visual results to understand interactions between variables and distinguish groups.
- models several response variables or outputs while considering their structure.
- alongside with PLS it has been used extensively in the bioprocessing industry to model mAb titres and levels of glycosylation [1,2].

In this study an O-PLS model was used to predict VG titre and F:E ratio for a specific product. The model was used to understand edges of failure of the transfection parameter design space.

Methods

- 61 small and intermediate scale bioreactors were used to generate an O-PLS model in SIMCA 17.
- Generated features include specific consumption of metabolites.



Type	Parameter name	Acronym	Number of points/Phase
X	Viable cell concentration (cells/mL)	VCC	Expansion, Transfection, Production
	Glucose (mM)	G	
	Lactate (mM)	L	
	Alanine-Glutamine (mM)	ALA	
	Ammonia (mM)	AM	
	Lactate Dehydrogenase Activity (U/mL)	LDH	
	pH	PH	Expansion and Production
	DO	DO	
	TFX variable 1	TFX_1	-
	TFX variable 2	TFX_2	-
	TFX variable 3	TFX_3	-
	TFX variable 4	TFX_4	-
	TFX variable 5	TFX_5	-
	TFX variable 6	TFX_6	-
Y	VG titre (VG/mL)	VG	-
	Full:Empty ratio (%) (VG/VP)	F:E	-

Conclusions

- Multivariate analysis supports decision making for complex multi parameter processes. A model was created that enables the prediction of VG titre and F:E ratio 48 hours before harvest and allows for hypothesis testing.
- The model supported the analysis of results of the scale down model to infer optimized transfection conditions.
- Future work includes the addition of large-scale experiments to refine the model and adaptation of the model to new products.

References

[1] Sokolov, M., Ritscher, J., MacKinnon, N., Souquet, J., Broly, H., Morbidelli, M. and Butté, A., 2017. Enhanced process understanding and multivariate prediction of the relationship between cell culture process and monoclonal antibody quality. *Biotechnology Progress*, 33(5), pp.1368-1380.

[2] Green, A. and Glassey, J., 2014. Multivariate analysis of the effect of operating conditions on hybridoma cell metabolism and glycosylation of produced antibody. *Journal of Chemical Technology, Biotechnology*, 90(2), pp.303-313.

[3] COX, I., 2019. *Discovering partial least squares with jmp*. Cary, NC, USA: SAS Institute.

Results

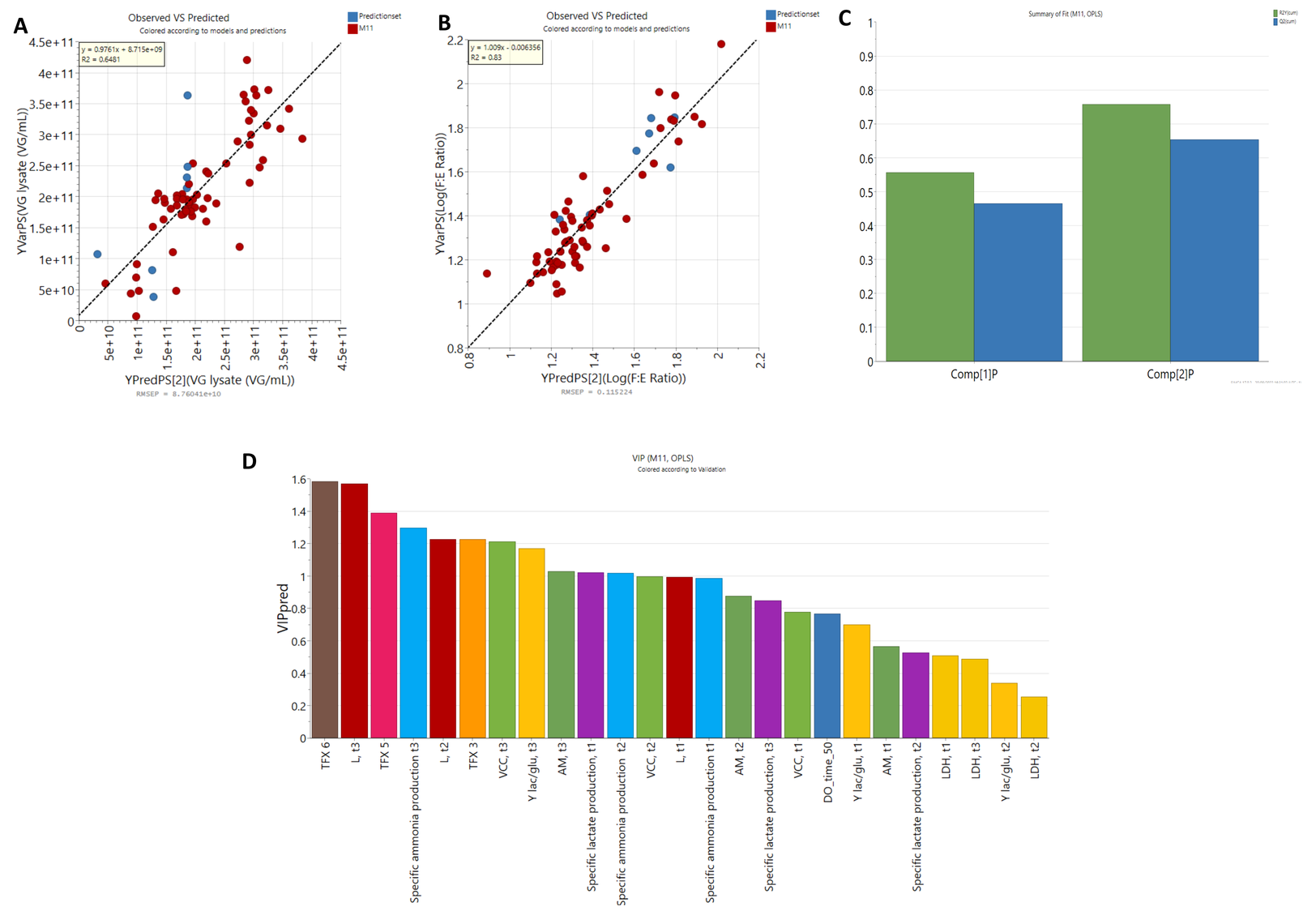


Figure 1. 2 factor O-PLS model performance. Cumulative Q2:0.65, Cumulative R2X 0.71, Cumulative R2Y: 0.76. (A) Predicted VG titre in the lysate versus actual VG titre in the lysate. **(B)** Predicted F:E (%) in the lysate versus actual F:E (%) in the lysate. Dots in blue represent predicted experiments. Dots in red represent experiments included in the model. **(C)** Summary of fit showing the contribution of each component for X and Y explanation of variation. **(D)** Variable importance plot (VIP) shows that viable cell density (VCC), TFX 3, TFX 5, and TFX 6 are included amongst the variables with higher importance in the model.

- Green dotted line in figure 2 (A) and 2 (B) denotes the desired area for the VG titre and F:E ratio.
- VCC, t1 is higher than the middle point in the design space considered for this variable within the desired region.
- The model indicates that the operation should yield better results in the lower edge of the design space for variables TFX 3, TFX 5, and TFX 6. However, will process reproducibility be impacted when transfection parameters are picked at the edge of the design space?
- Two conditions included in the region delineated in orange show that their predictions are within the model's desired region (green region), however their observed value is not within this region. This gives an indication that operating at the bottom edge of the design space for variables TFX 5 and TFX 6 is a risk.
- Furthermore, operating at the bottom edge of the design space for variable TFX 3 also constitutes a risk as the conditions inside of the red area have all failed (low VG titres).

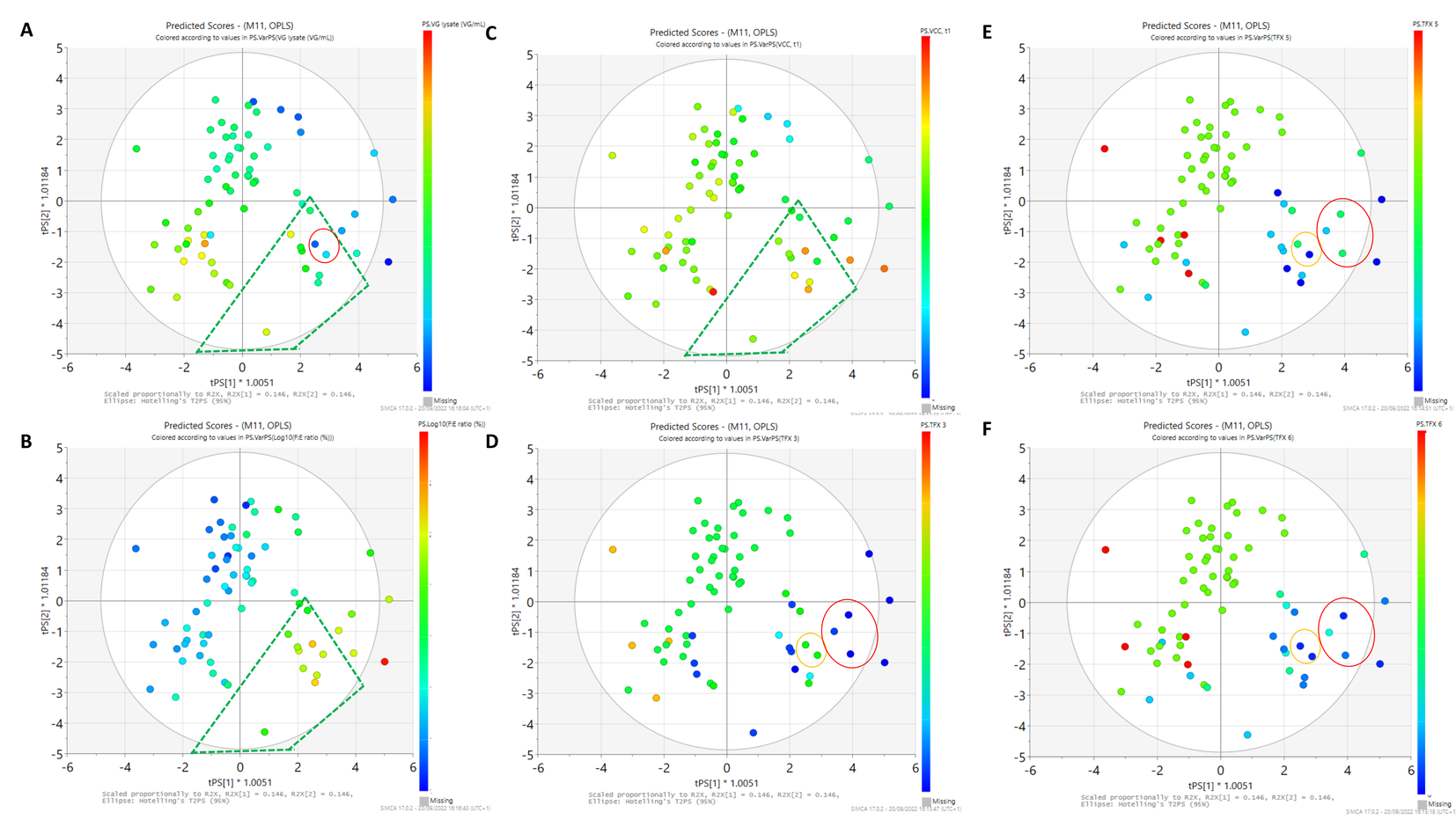


Figure 2. Y scores plots for factor 1 and factor 2 show an opposite trend for VG titre and F:E ratio (%). (A) Experiments coloured according to VG titre. **(B)** Experiments coloured according to F:E ratio (%). **(C)** Experiments coloured according to Viable cell density at transfection (cells/mL). **(D)** Experiments coloured against variable TFX 3. **(E)** Experiments coloured according to variable TFX 5. **(F)** Experiments coloured against variable TFX 6. Scale coloured from blue (low) to red (high).