Towards Modelling of an Industrial *Aspergillus oryzae* Aerobic Fed-batch Fermentation Process – Process Characterization Across Scales

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Abstract

In well-established fermentation processes, meaningful improvements in process performance can be achieved through real-time simulation and optimization. Thus, modelling is an essential tool to transform process data towards relevant predictions of variables. This contribution shows the characterization of an *Aspergillus oryzae* fermentation process, marked by significant batch-to-batch variation, both at production and pilot scales. The aim is to understand the impact of process variables and parameters on fermentation process performance for model development. Particularly, since it is a filamentous fungi fermentation, knowledge of biomass concentration and rheology is crucial for successful simulations since the viscosity of the fermentation broth rises due to the organism’s morphology, leading to lower mass transfer as biomass concentration increases. Firstly, the biomass concentration and viscosity levels were analysed in the industrial-scale process. Furthermore, the on-line process data were analysed to identify potential sources of batch-to-batch variation in production. Additionally, the process has been scaled down to pilot scale for detailed investigation of important process parameters regarding feeding settings and agitation power. Overall, the data collection and analysis done thus far provides the basis for model development and testing.

**Keywords**: scale-down, fermentation, *Aspergillus oryzae*, rheology, industrial scale

* 1. Introduction

The industrial production of enzymes by filamentous fungi (e.g., *Aspergillus oryzae*) is common, due to their highly efficient protein secretion (McIntyre et al., 2001). Protein secretion is associated with hyphal growth, which can influence the broth’s rheology by increasing viscosity, causing a negative impact on mixing and mass transfer (McIntyre et al., 2001). For these reasons, understanding the morphology is essential for process productivity; hence the importance of having cell concentration measurements and knowledge of the viscosity of the fermentation broth. Through the years there has been significant effort in characterizing and modelling filamentous fungi fermentation processes, for example by morphological characterization (Spohr et al. 1997), by evaluation of the effects of shear stress on productivity (Amanullah et al. 2002), by modelling of rheological behaviour from biomass concentration (Olsvik and Kristiansen 1994) and by enzyme production with differing agitation and aeration parameters (Albæk et al. 2011).Furthermore, when studying industrial scale processes, a meaningful way to gain process understanding for optimization is through scale-down studies. Running experiments on production scale is not economically feasible, but with an adequate scale-down model, it is possible to study the large-scale process and find leads for improved process performance (Noorman 2011). With a scale-down model, it is also possible to develop relevant process models that can accurately predict process variables from the available data.

* 1. Methodology
     1. Sampling at industrial scale

The sampling at industrial scale was done in seven batches that were running simultaneously of the product of interest, all under the same recipe. They were at different stages of the fermentation process. When presenting the data for biomass concentration and apparent viscosity, the data points from the different batches were grouped together to get an overview of the whole fermentation duration.

* + 1. Biomass analysis

The biomass content (cell dry weight per litre medium) of the fermentation broth was determined as described in Albæk et al. 2011.

* + 1. Rheology

The rheological measurements and description of rheological behaviour were performed as described in Albæk et al. 2012.

* + 1. Enzyme activity essay

Enzyme activity was determined using a proprietary enzyme activity assay, and results are reported as arbitrary units per liter of fermentation broth.

* + 1. Scale-down experimental plan

For the scale-down experiment, five batches were executed. The fermentation processes were run as fed-batch in 550L fermenters in a process very similar to the one described in Albæk et al. 2011. Batches 1 and 2 had the same inoculation material, while batches 3, 4 and 5 were inoculated from a different seed fermentation process. The seed transfer criterium for batches 1 and 2 was based on seed fermentation duration, and for batches 3, 4 and 5, on a pH drop below a defined threshold. Table 1 summarizes the experimental plan.

Table 1 – Summary of scale-down experimental plan.

|  |  |  |
| --- | --- | --- |
| **Batch number** | **Seed material** | **Details** |
| 1 | A | Reference |
| 2 | A | Test of the nutrient feed settings |
| 3 | B | Test of pH and temperature settings |
| 4 | B | Reference |
| 5 | B | Test of higher agitation power |

* + 1. Statistical data analysis

The statistical data analysis was conducted for 44 production batches executed with the same recipe, using the software SAS JMP 17. An analysis of variation (ANOVA) test was performed of the titre and the product yield on substrate from grouping by tank type and fermenter unit. In the cases where the ANOVA test calculated a significant p-value, a post hoc Tukey’s test was performed to identify which groups had statistically different means. The result is a connecting letters report, where groups identified by the same letter, have the same statistical mean.

* 1. Results and discussion
     1. Industrial scale enzyme activity

The activity titre evolution throughout fermentation time was plotted for 44 batches to evaluate the variation. This is shown in Figure 1, where a range of approximately 35% variation is observed for the final activity titre. The coefficient of variation (CV) for this performance metric is 9%.

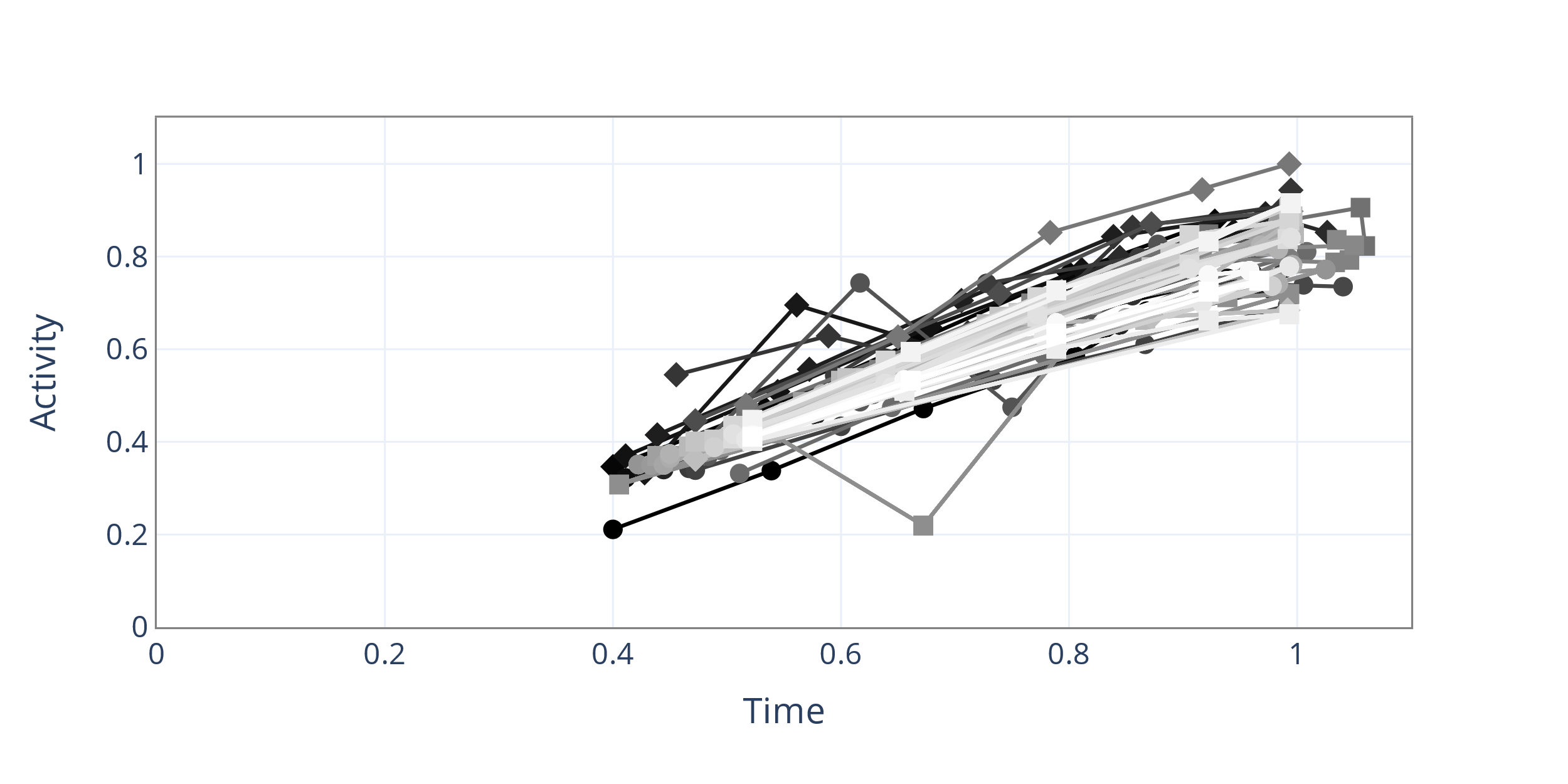
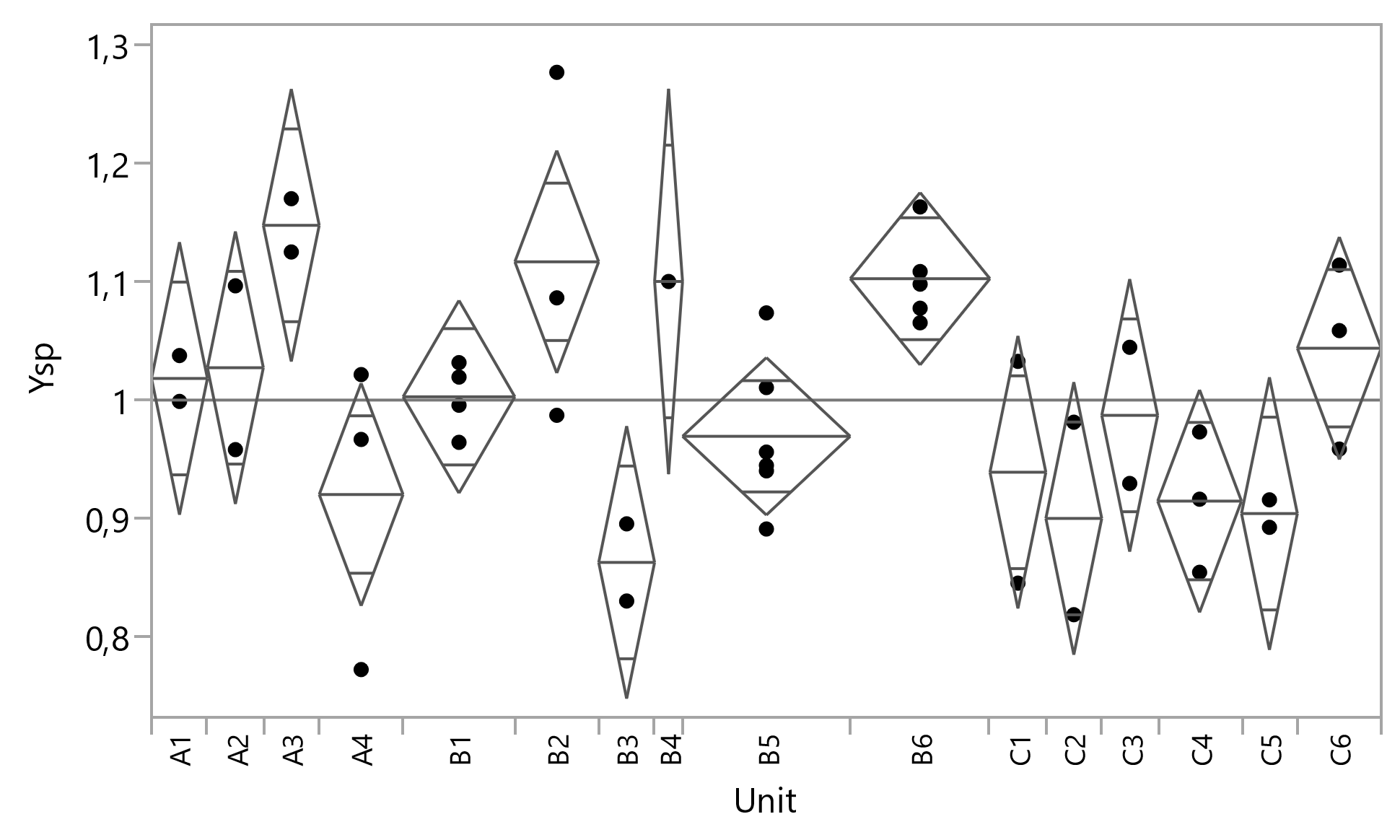
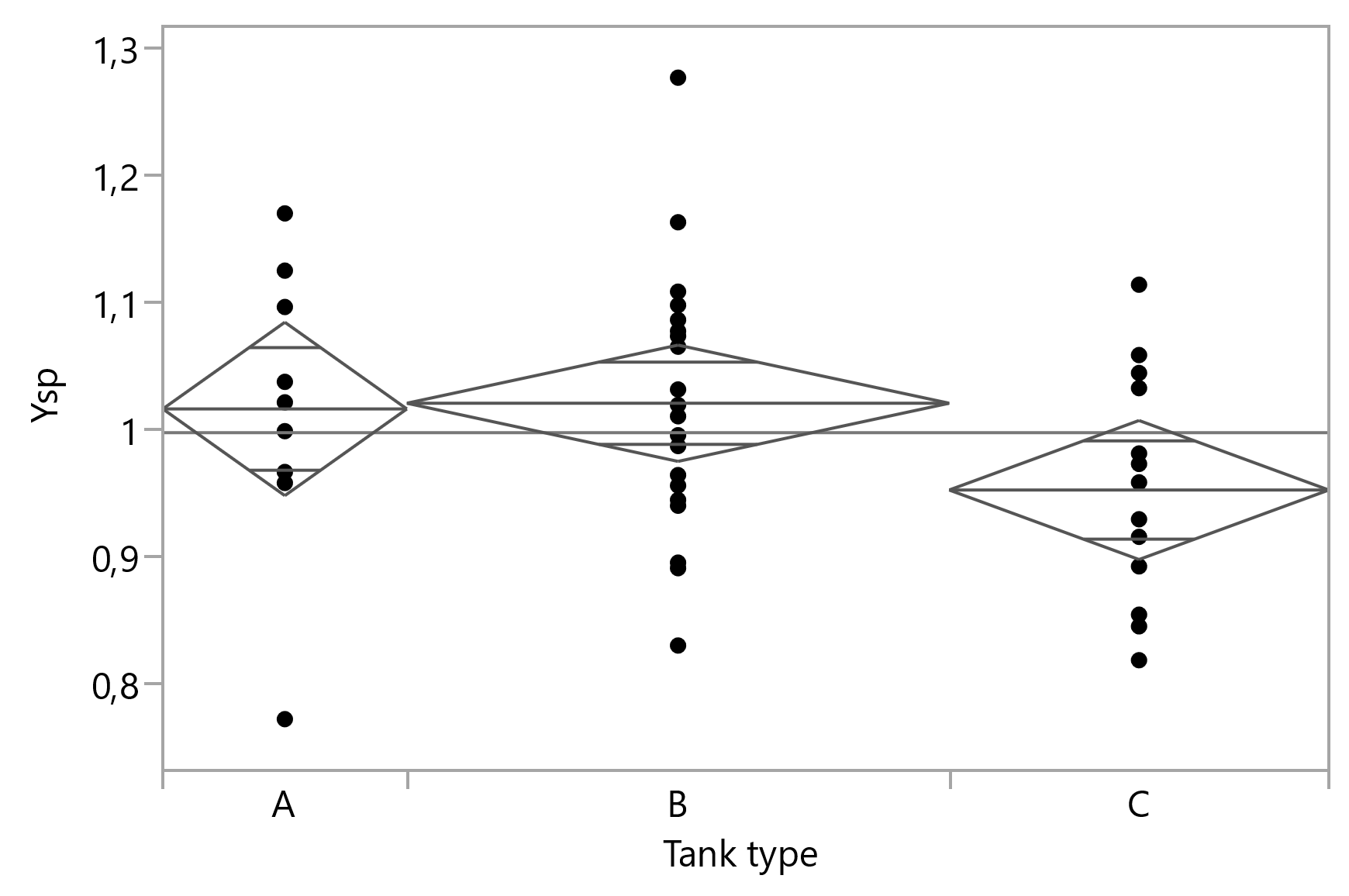
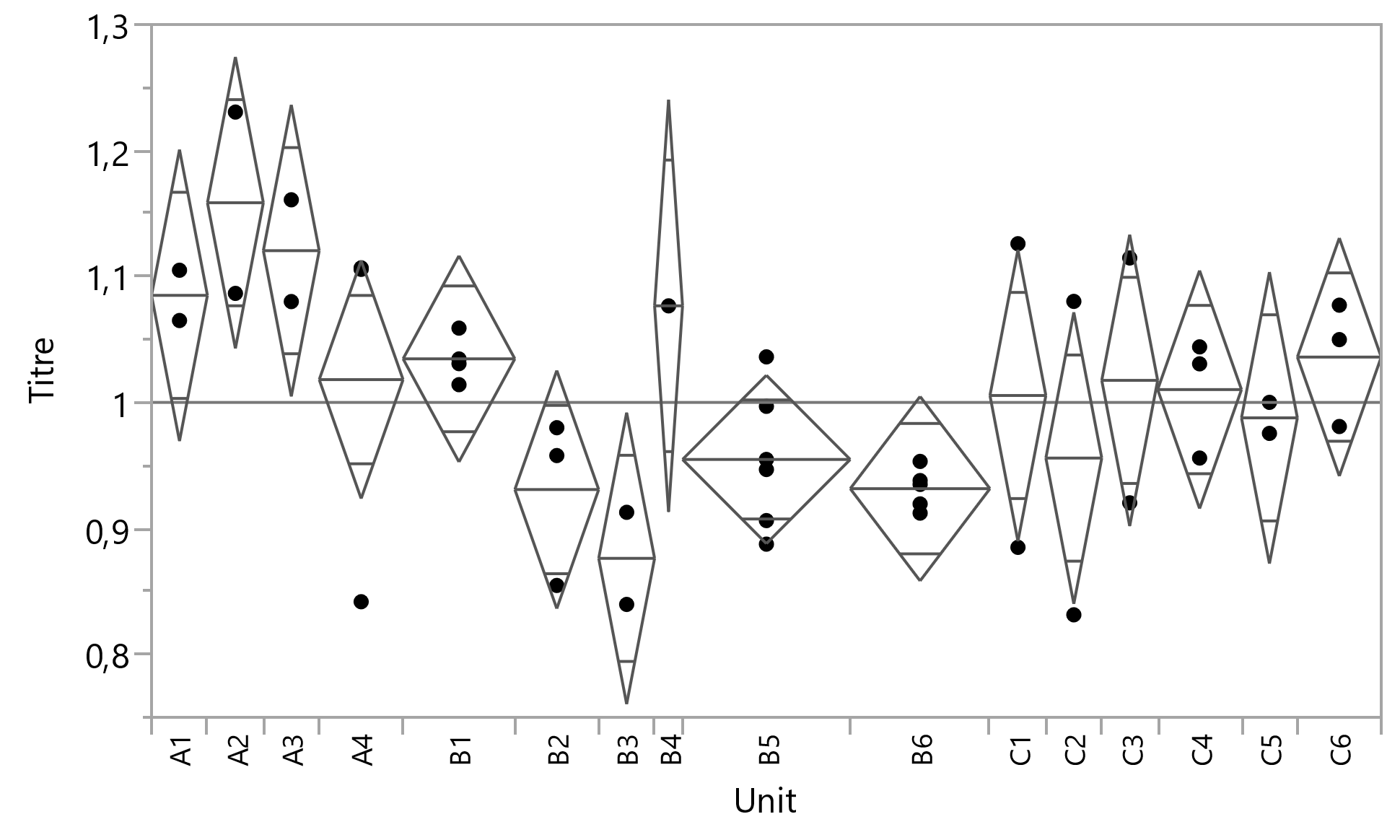
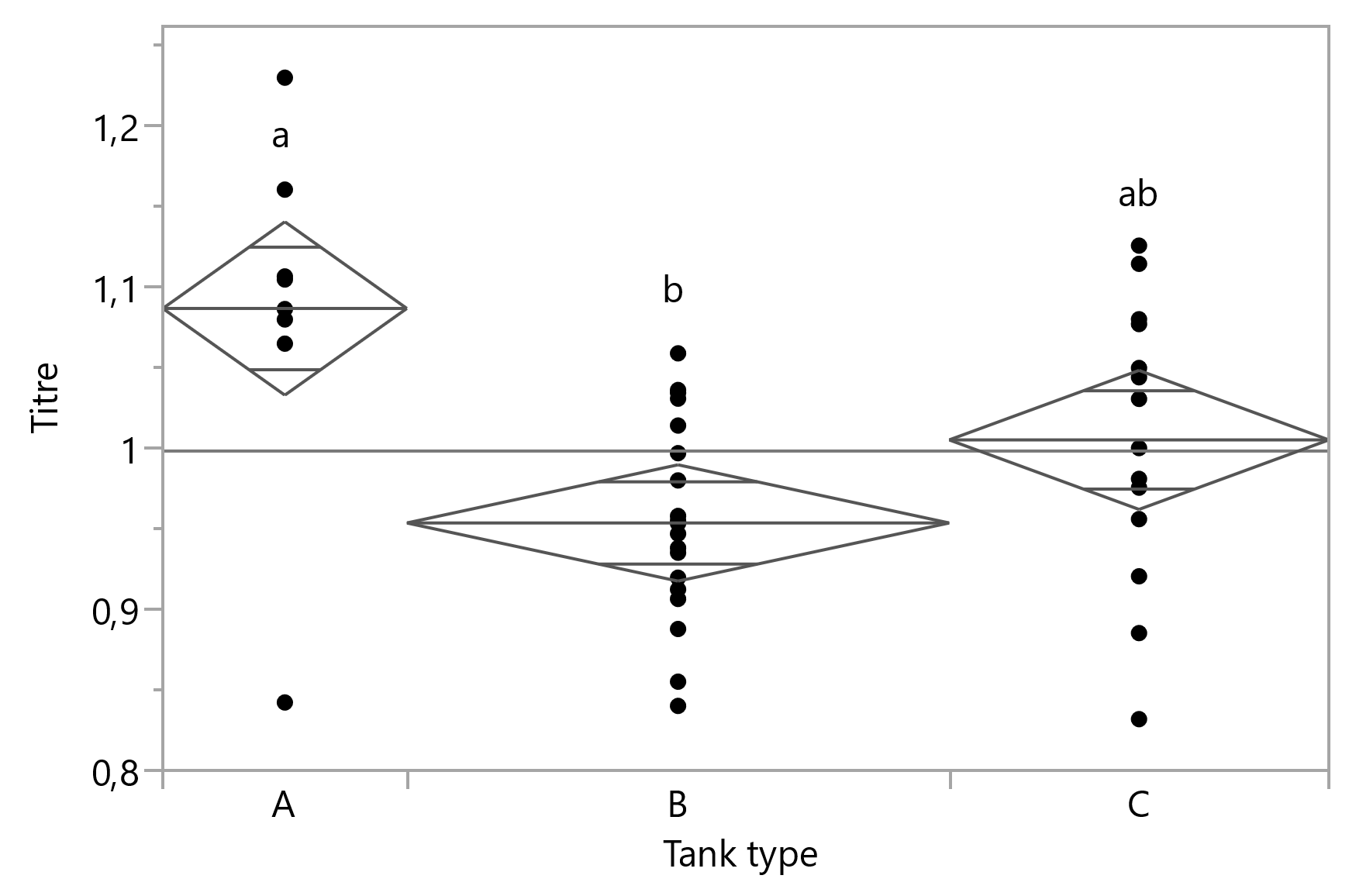


Figure 1 – Enzyme activity evolution over time for 44 industrial batches for an industrial *A. oryzae* aerobic fed-batch fermentation process. The data are normalized.

To understand the sources of variation, the final activity titre and specific yield of product on substrate for each batch were grouped by tank type and fermenter unit. There are three different tank types, A, B and C. There are four fermenter units of type A, and 6 for both types B and C. A statistical analysis was conducted to assess if these variables could explain the variation. The results are presented in Figure 2. Unit B4 was excluded from the analysis, as the group only had one batch.

b)



d)

c)

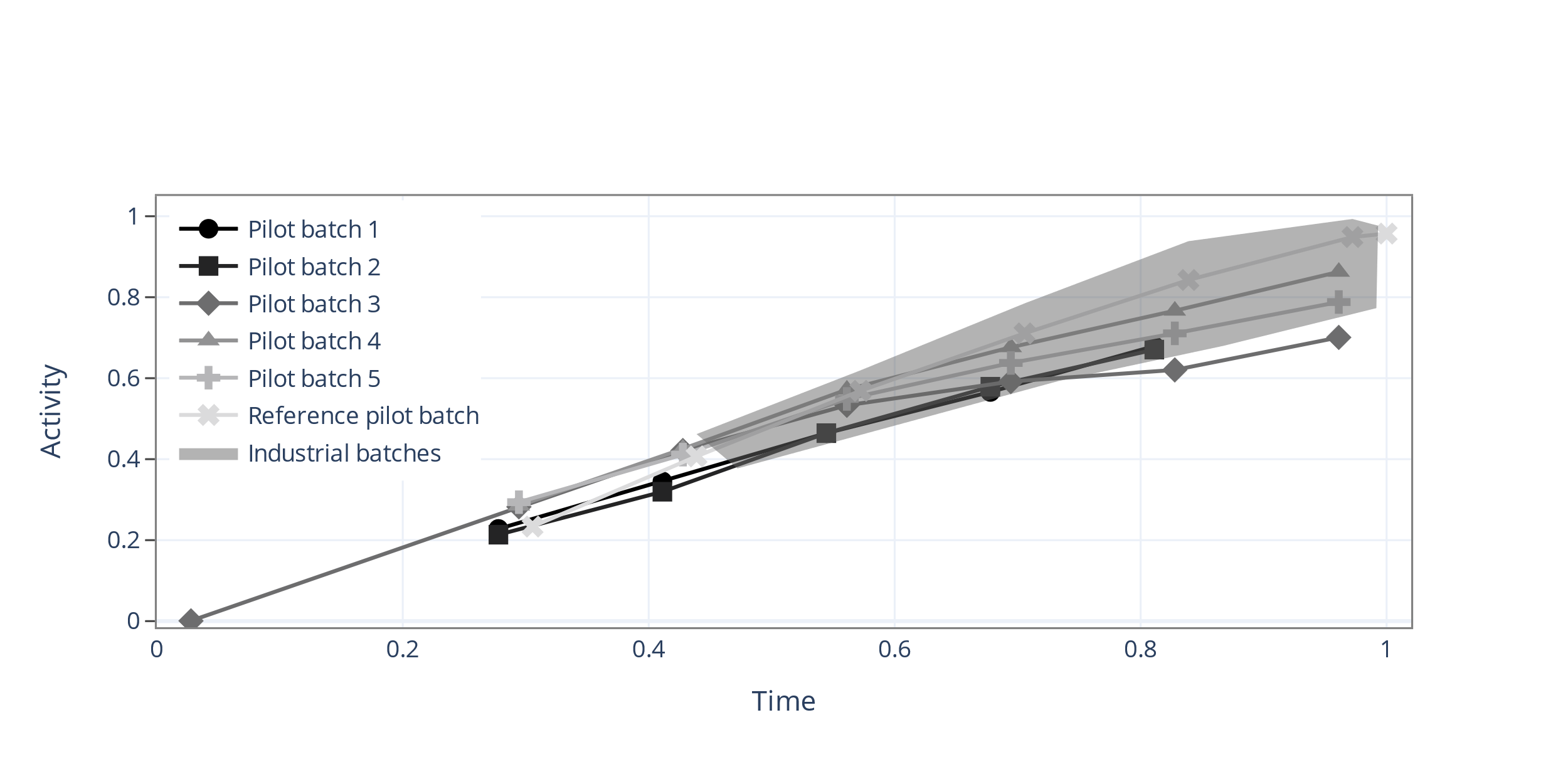
a)

Figure 2 – ANOVA test of a), b) activity titre and c), d) product yield on substrate (Ysp), by tank type (a and c) and fermenter unit (b and c). p-value of the ANOVA test: a) 0.013; b) 0.0444; c) 0.1132; d) 0.0051. A p-value lower than 0.05 indicates that there is a statistically significant difference between group means. The non-capital letters in figure a) refer to the post hoc Tukey’s test, for which groups classified with the same letter, have statistically equal means. The data are normalized. The scaling of the data was done by attributing the value 1 to the overall mean.

Regarding titre, both the grouping by tank type and by fermenter unit correspond to a relevant p-value, indicating that both factors are relevant for titre variation. When grouped by fermenter unit, the p-value is higher and thus less significant. Tukey’s test shows that tank types A and B have statistically significant different means, whereas type C has the same statistic mean as the other groups. Concerning the grouping by fermenter unit, even though a significant p-value was calculated, Tukey’s test did not reveal any pairwise comparison that is significantly different. For this test, the more pairwise comparisons made, the lower the individual significance value, so even if a difference between groups is present, the test might not be able to identify it. On the other hand, the product on substrate yield variation is not explained by tank type, but by fermenter unit, where a relevant p-value is calculated. Once more, the post hoc test did not identify any significantly different pairwise comparison. Summarizing, this analysis indicates that the tank type and unit are relevant for the observed variation, and titre and yield are affected differently. A bigger sample size would provide a more statistically significant conclusion, nonetheless, the analysed data shows a trend. Furthermore, it will be relevant to further investigate the differences between the tanks (e.g., variation in geometry and oxygen transfer among others).

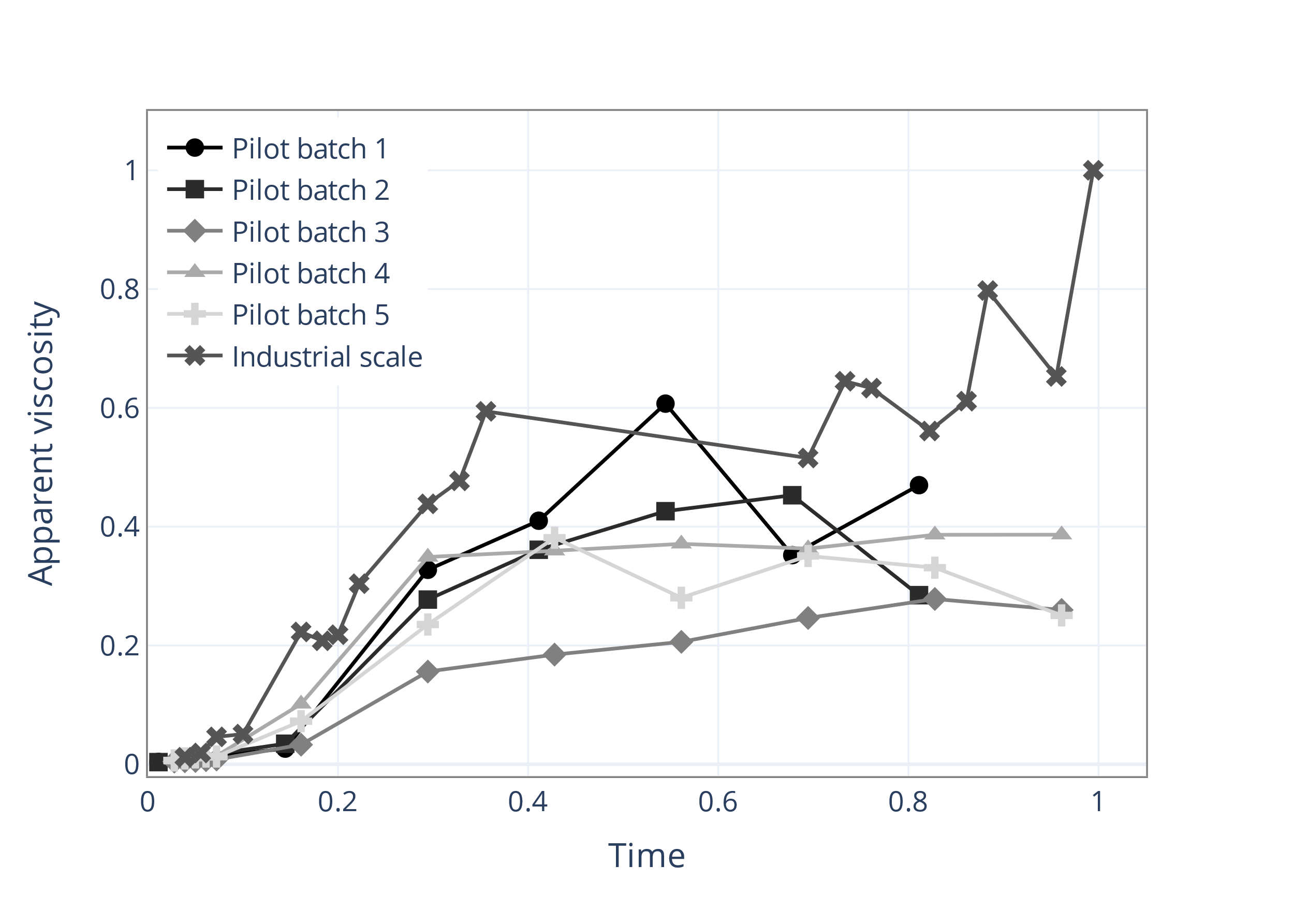
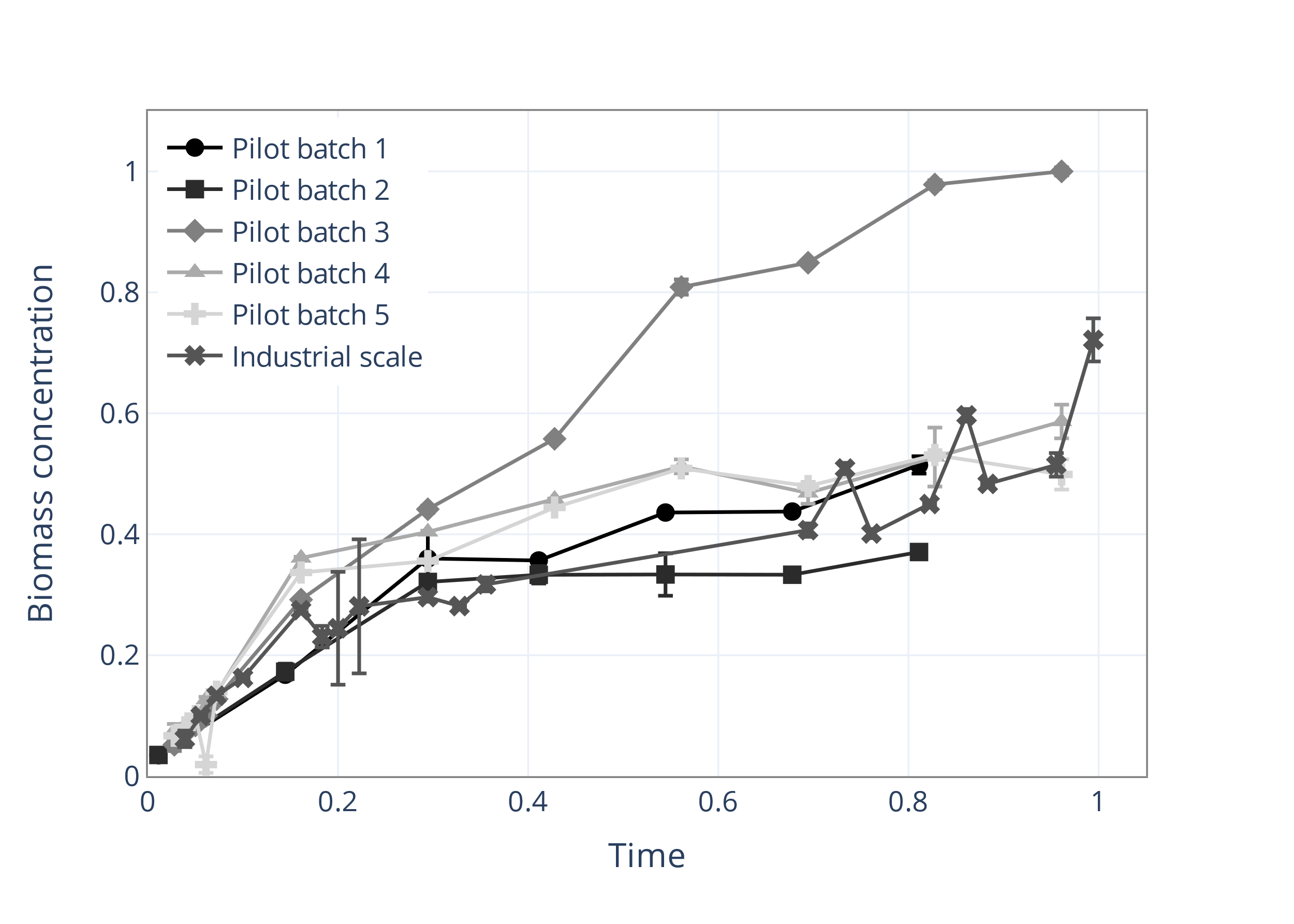
* + 1. Scale-down to pilot scale

To further study the process, it was scaled-down to pilot-scale. The focus was 1) to have an accurate scale-down model, that can be later used to study different process conditions (batches 1 and 4) and 2) to have an indication of which process conditions are relevant for performance (batches 2, 3 and 5). Table 1 in the Methodology section details the con­ditions of each batch.



b)

a)



c)

Figure 3 – a) Enzyme activity evolution over time for five pilot-scale batches executed (details in Table 1) and 7 industrial scale batches. The “reference pilot batch” refers to a historical pilot-scale scale-down fermentation process of the same industrial process previously conducted with remarkably high titres. b) Evolution of biomass concentration and c) apparent viscosity for the same batches as in a), except for the “reference pilot batch”. The data are normalized.

Batches 1 and 4 had different seed transfer criterium, which led to a titre improvement of approximately 10% for batch 4. Batch 1 had the same transfer criterium as the production batches, and as described in section 2.4, it was different for batch 5. Even though more data are needed to draw statistically significant conclusions, this finding suggests that the seed transfer criterium might be an important factor to investigate further for fermentation process optimization. Batch 3 was an exact copy of the historical reference pilot batch; however, the results were different. Batch 3 had the highest biomass concentration amongst all the analysed, as well as the lowest activity titre, meaning that the carbon was diverted more towards growth rather than enzyme production. Moreover, this batch also presented the lowest apparent viscosity, indicating the possibility of morphological differences from the other batches. Free hyphal growth is generally the preferred morphology when compared to a pelleted growth, as it is associated with higher productivity. However, it is also resulting in higher broth viscosity (Haack et al. 2006). Therefore, a bigger fraction of hyphal elements growing inside pellets could explain both the lower viscosity and titre of batch 3. Nevertheless, there is a lack of biomass and rheological data for the reference pilot batch, as well as no morphological data for either batch. So, it is not possible to conclude, if a different relationship between biomass concentration and rheological behaviour is the driver for performance disparity under the same process conditions.

* + - 1. Power law fit for a viscosity prediction model

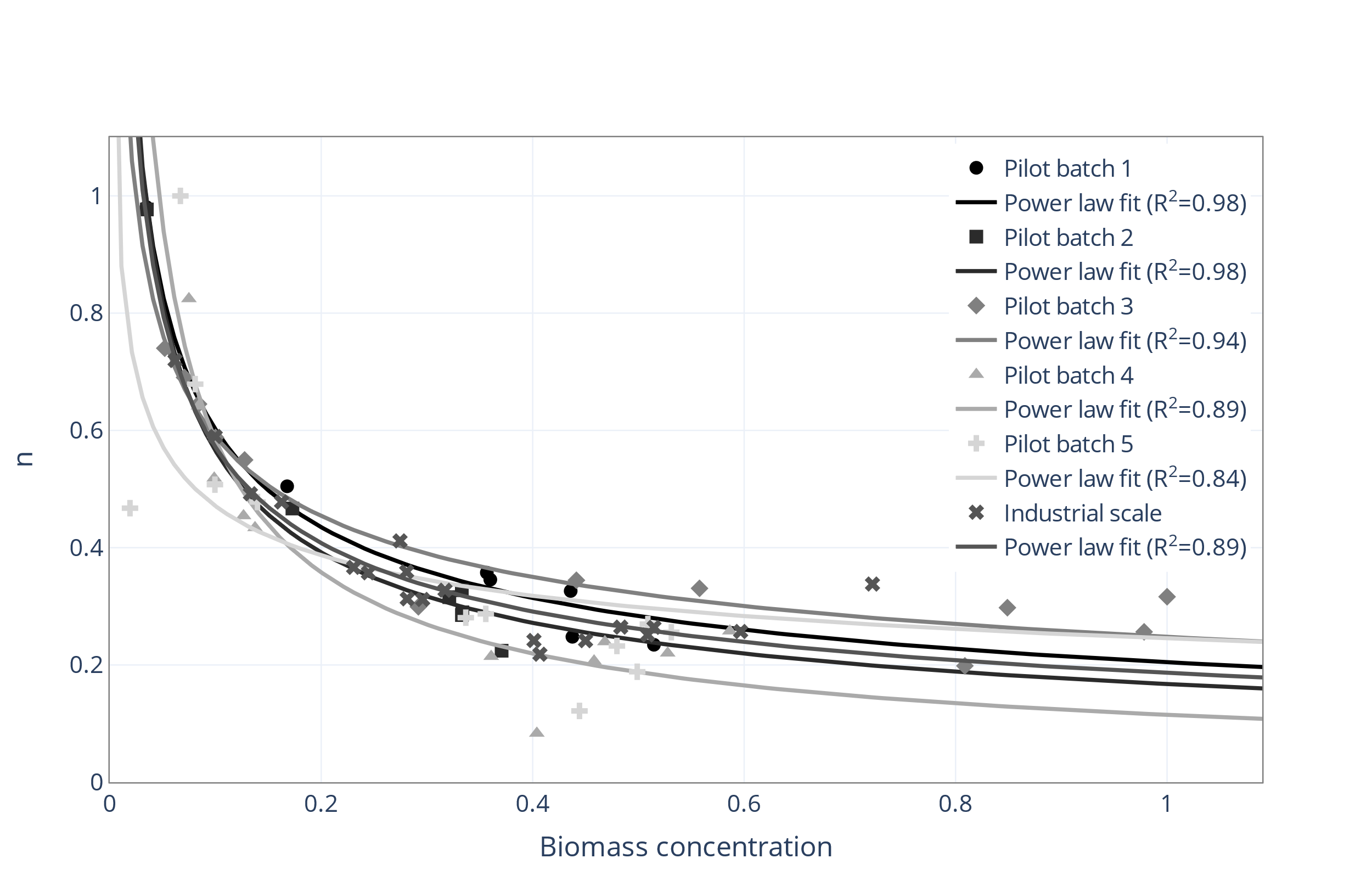
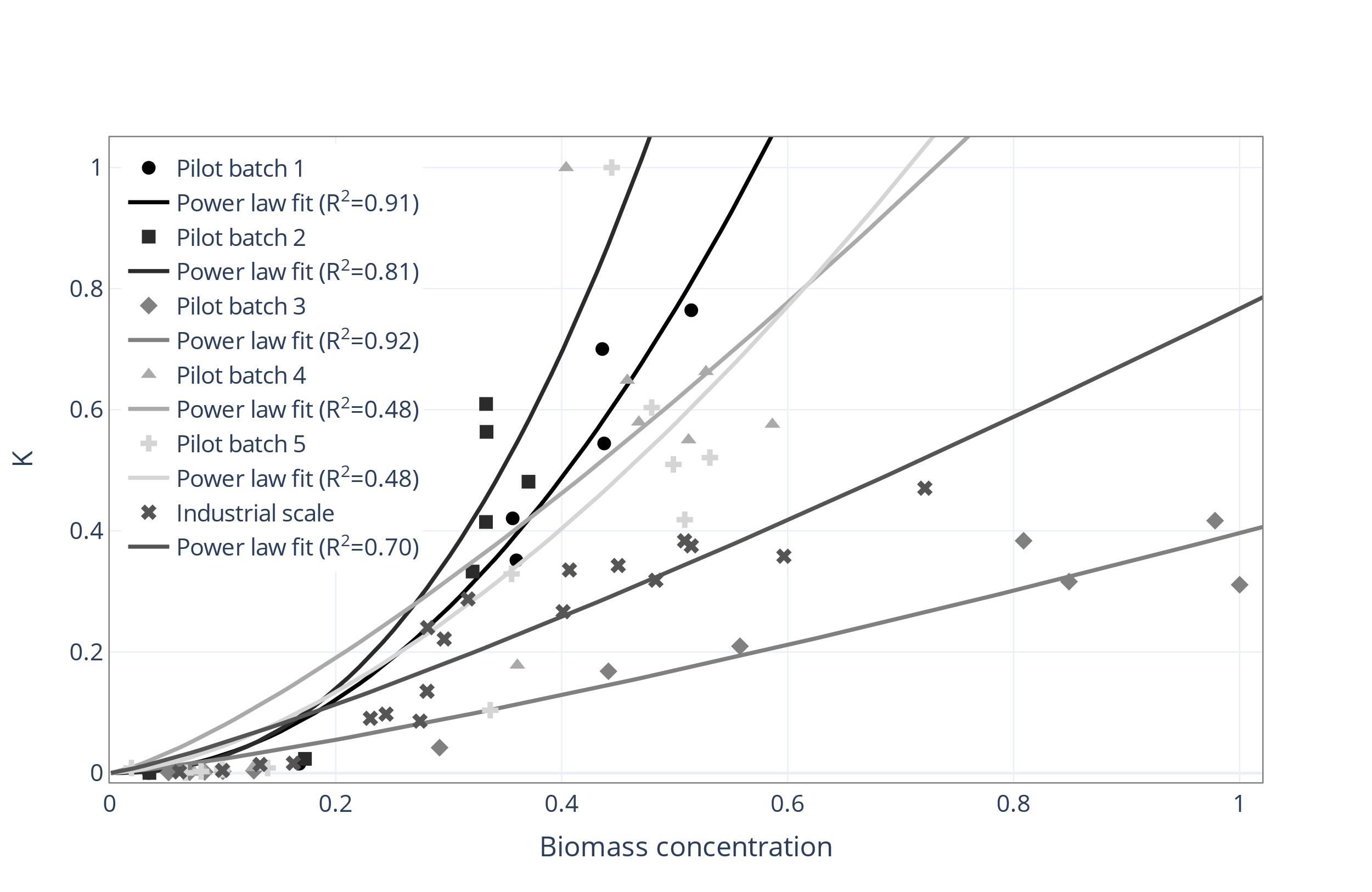
The rheological behaviour of each sample can be described by the power law model Eq. (1) (Nienow 1996).

(1)

Where is the apparent viscosity (Pa.s), K the flow consistency index (Pa.sn), the average effective shear rate (s-1) and n the flow behavior index (dimensionless).

The coefficients K and n from Eq. (1)were plotted against the biomass concentration (X). The data were fitted with a power law to investigate the possibility of having a viscosity prediction model based on biomass concentration. This is shown in Figure 4.

b)



a)

Figure 4 – a) K and b) n coefficients from the viscosity power law (Eq. (1)) against biomass concentration, for the five pilot batches performed (Table 1) and for the 7 industrial batches that were sampled. For each batch, the data points were fitted with a power law, and the regression coefficient was calculated and shown in the legend. The data are normalized.

The regression coefficient value and the shape (values of α and β) of the fits for coefficient K, varied significantly from batch to batch, with the best and worst fits corresponding to batch 3 and 4-5, respectively. As indicated in Table 1, batch 3 had different temperature and pH settings, which could have an impact on the broth’s rheological behaviour and thus the fit of the proposed model. This indicates that 1) different equations, such as a logistic regression should be tested to improve the correlation factor (R2) (Goudar et al. 1999), and that 2) the evolution of coefficient K depends on process conditions. For coefficient n, all batches had very similar power law fit results. These results are in line with the observation by Olsvik and Kristiansen (1992) that K is more sensitive to process conditions than n, thus reflecting to a larger extend the different rheological behaviours. When comparing Fig. 3c) with Fig. 4a), it can be observed that, even though the industrial batches’ apparent viscosity values are higher than those of the pilot batches, the values for coefficient K are lower. This can be explained by the lower effective shear rate () inside the large-scale reactors since the impellers rotate at a lower speed.

* 1. Conclusions

This work aimed at studying and understanding the variation of a well-established industrial-scale fungal fermentation process. The main conclusions are the following:

* The use of different tank types/unit has an impact on process performance. Further data collection at industrial scale as well as using a scale-down model to mimic the different tank types should be done to further understand their impact on fermentation process performance.
* Pilot scale experiments highlighted the seed tank transfer criterium as a relevant optimisation parameter. Furthermore, the process conditions that were tested led to different relationships between biomass concentration and the broth’s rheological behaviour.
* High variation is observed at both production and pilot scale. Thus, deepening the biological understanding of the process is relevant to study how differences at the cell level can lead to different process performance.

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