Dear Reviewer,

Thank you for your revision. We did not want to compare shake flask cultivations to bioreactor cultivations and achieve the same protein titers in shake flasks. Expression clones that may perform well in a micro-scale screening where constant feed is hardly possible and parameters such as cell density and oxygen are usually not monitored in high-throughput, might yield totally different results in following bioreactor cultivations where all parameters are defined and controlled. This cultivation method which was especially developed for the described de-repressed promoters displays a compromise between impossible control of parameters and throughput amount. Experiments in our lab showed in the past that the performance of respective clones (in relative comparison to each other) in shake flasks using this cultivation method is much more applicable to their later performance in bioreactors than batch or pulsed cultivations in shake flask scale as in this method also a limited feed strategy is applied by the slow carbon source release. For this kind of derepressed protein expression a good monitoring system even in shake flask scale is extremely useful to determine the optimal timepoint for fed-batch start. Of course, this cultivation method is not replacing bioreactor experiments and also derepressed protein expression with the mentioned promoters yields higher protein titers in bioreactors, but by employing this method one can limit the number of possible candidates for laborious large-scale cultivations by preselecting clones that perform well under simulated bioreactor-like conditions due to the more similar behavior of the strains compared to batch or pulsed feed cultivations.