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Abstract BACKGROUND Clostridium pasteurianum is a well described strain for the conversion of glycerol into butanol. In general, cell growth kinetics depends on the type of glycerol used and the concentration of butanol in the fermentation broth. However, despite the numerous studies existing on the subject, there is limited information in the literature regarding growth inhibition kinetics due to the cytotoxic effect of butanol on the cell growth. This can be attributed to the difficulty of growing cells at high butanol concentration which renders the determination of inhibition kinetics a rather challenging task. RESULTS During this study a new approach for the determination of the butanol inhibition kinetics on the growth of C. pasteurianum was tested. Specifically, pulses of crude glycerol were applied when steady state was reached during continuous fermentation experiments with increasing butanol concentration in the feed. Combining pulse experiments with batch fermentation at low substrate concentration allowed for accurate determination of the kinetic constants for inhibited growth. This approach also minimised the correlation of the growth constants which often leads to poor identifiability. CONCLUSION In overall, the proposed experimental approach showed good identifiability of the kinetic parameters for butanol inhibition of the microbial growth and can be proven valuable for the determination of inhibitory effects of highly toxic compounds. This article is protected by copyright. All rights reserved.

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