Examining Magnetically Driven Agitation Decoupling Events in Benchtop Microbial Stir-Tank Reactor Systems

Justin Cesmat , Greg Kauffman and Jake McAndrew, MSc
Distek, Inc. | North Brunswick, NJ
Contact: bione@distekinc.com

BIOPROCESSING

Abstract

The agitator drive assembly is an essential part of all stir-tank reactor (STR) systems. This component supports the rotation of the internal impellers and is critical for maintaining suitable mass and thermal transfer within the system. To drive impeller rotation, a coupling mechanism is needed to link the external motor and the internally sealed agitator shaft. Both mechanical and magnetic coupling mechanisms are available as solutions to create this linkage. Magnetically coupled drive systems offer several advantages over mechanically coupled systems, including a more robust sanitary barrier, and decreased preventative maintenance requirements.

One of the potential disadvantages of utilizing a magnetically coupled agitation drive system is the risk of high agitation rate decoupling events occurring during processing. The possibility of such events increases for highly viscous bioprocesses, such as some fermentation applications. During magnetic decoupling events, the connection between the motor and the impeller shaft is lost, and system agitation stops until the issue is manually resolved. To reduce the risk of magnetic decoupling events, STR systems can be characterized to define the magnetic decoupling agitation speed thresholds for specific process conditions. Agitation setpoints defined under the decoupling agitation rate threshold would allow end users to operate the system with minimal risk of decoupling events.

In this work, we characterized decoupling agitation speed thresholds for a 5-L benchtop fermentation system which was operated under a variety of different media viscosity and bottom air sparge rate process conditions. Results demonstrated that viscosities of ≥ 10 cP were able to decrease the agitation threshold speeds required to induce decoupling events in the system. It was also shown that aeration rates of ≥ 1.5 vvm were able to support near maximum agitation rates, even when the system contained a highly viscous medium (2820 cP). Overall, the data generated in this study provides a robust framework that demonstrates how thorough system characterization can support agitation strategies that are highly suitable for use with magnetically coupled drive systems across a variety of upstream bioprocess applications.

Introduction

Stir-Tank Reactors (STRs) are some of the most common types of bioreactor and fermentation systems used across upstream biopharmaceutical applications. STR systems traditionally use either single or multiple internal impellers to drive mass and thermal transfer to support the process needs of expanding cultures.[1] To drive the internal impellers, STR systems require a linkage to transfer torque from the external motor to the internal agitator shaft.[2] For many systems, this requirement is often supported through the integration of either mechanical or magnetic drive shaft coupling elements.[3]

Mechanical drive shaft coupling elements traditionally utilize an interlocking spline mechanism to transfer rotation force from the motor to the impeller shaft. For these types of systems, a mechanical seal is required to create a sanitary barrier. As mechanical seals wear through regular use, this type of design can introduce a potential vector for contaminants to breach the system.[4] Therefore, it is essential that mechanical

agitator seals are examined and replaced on regular preventative maintenance (PM) intervals. This PM requirement is often a disadvantage associated with mechanical drive shaft systems, due to maintenance costs, equipment downtime, and personnel allocation.

Magnetic drive shaft coupling elements represent a viable alternative to mechanical drive systems. Magnetic drive shaft bioreactors and fermenters have been demonstrated to be suitable options for laboratory teams who either do not have the resource bandwidth to support frequent PM tasks, cannot afford extended equipment downtime, or have elevated concerns regarding the potential for process contamination. Magnetic drive shaft agitation supports the creation of a complete barrier between the non-sanitary and sanitary sides of the system.[5] A drive magnet, directly connected to the motor, is attracted to the dipole of a second magnet, located on the internal impeller shaft. As the motor turns the drive magnet, its interaction with the shaft magnet causes the internal impeller to rotate. This magnetic drive mechanism supports aseptic agitation within the bioreactor system without the need for a mechanical seal.

A risk associated with the use of a magnetic drive coupling mechanism is the potential for decoupling events to occur during processing. During a decoupling event, the magnetic connection between the external motor and the internal impeller shaft is broken. Vessel agitation in the system will stop, despite the motor still operating at the defined setpoint. This loss of vessel agitation, even if brief, can result in the development of thermal and chemical gradients, the emergence of localized hypoxic conditions, and the settling of the culture out of the suspension medium. All such outcomes have the potential to unfavorably effect culture growth, productivity, and overall product quality.

The risk of magnetic decoupling is elevated during high-density aerobic microbial and fungal processes.[6] These processes can be quite viscous due to the high cell densities achieved during the logarithmic growth phases. Additionally, such processes typically require relatively higher agitation rates to maintain both overall homogeneity and sufficient oxygen mass transfer. The combination of these factors increases the risk of magnetic drive decoupling events.

One of the major concerns associated with magnetic decoupling is the inability to effectively detect and correct such events before they cause irreversible harm to the process. Agitation alarms are typically defined based upon the system controller maintaining a defined motor speed. During out-of-specification (OOS) agitation events, the system controller detects that the current motor speed has deviated from a specified operational range, and it subsequently alarms to alert the end-user. During a decoupling event, the motor maintains operation at the defined setpoint, despite it no longer turning the impeller. Therefore, during these decoupling events, standard agitation alarms will not be activated, and the end-user may be left unaware of the issue.

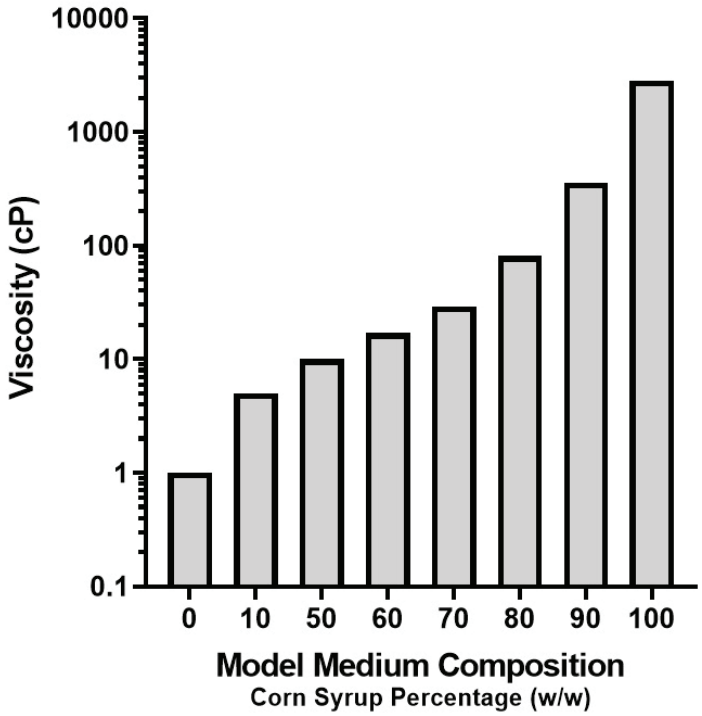
Due to the challenges of real-time detection of magnetic decoupling, bioreactor and fermentation systems should be properly characterized so that the agitation speed thresholds for triggering such events are well-understood. Key process inputs should be analyzed in regard to how they impact the agitation speed threshold that is required to induce a decoupling event in the system.

In this work, we characterized the agitation speed thresholds required to induce decoupling events under a variety of different operational conditions. Both medium viscosity and bottom air sparge rate were investigated as process inputs that had the potential to affect magnetic decoupling within the system. Results demonstrated that magnetic decoupling was induced at lower agitation rates as the medium viscosity was increased. However, it was also shown that increasing the system bottom air sparge rate could successfully mitigate the effects of media viscosity and support higher agitation rates in the system. Overall, results demonstrated that suitable operational parameters could be defined for the magnetically coupled agitator of the STR system, even for highly viscous bioprocess environments.

Materials and Methods

To perform the decoupling threshold characterization study, a series of different testing media were prepared to model upstream bioprocess environments of varying viscosities. Individual lots of testing media were batched at concentrations of 0% to 100% (w/w) light corn-syrup (Karo) to water. The media viscosities for individual testing media were measured prior to each testing iteration (NDJ-5S Rotary Viscosimeter, CGOLDENWALL). The viscosities of the model media used during this study ranged from 1 cP to 2820 cP.

The dilution strategy of the model media was defined to support thorough testing across the range of viscosities that have been reported for biopharmaceutical, agricultural, and other industrial upstream bioprocesses. Biopharmaceutical processes have reported viscosities in the 1 cP to 100 cP range, while industrial and agricultural processes have described viscosities of approximately 30 to 3000 cP.[7,8,9,10,11,12] These previously reported data suggested that our 1 cP to 2820 cP range would be highly suitable for modeling a wide array of upstream applications. An overview of the dilution strategy used to prepare the model media, and the associated viscosities, are presented in **Figure 1**.



Model Medium No.	Percent Corn Syrup (w/w)	Viscosity (cP)
1.	0%	1
2.	10%	5
3.	50%	10
4.	60%	17
5.	70%	29
6.	80%	81
7.	90%	358
8.	100%	2820

Figure 1: ***Overview of the model medium strategy using during the magnetic decoupling characterization.*** *Viscosity range was determined based on reported values from biopharmaceutical, agricultural, and industrial upstream processes.*

During the magnetic decoupling characterization, each model media formulation was tested in a 5-L working volume autoclavable microbial fermentation system (Distek, Inc.). This autoclavable system utilized two Rushton impellers, three baffles, and a drilled hole sparger (7 × 1.0 mm holes). The system was operated using the BIOne 1250 Bioprocess Control Station (Distek, Inc.).

During testing, the agitation rate of the system was gradually increased in stepwise increments of five revolutions per minute (rpm). After each incremental increase in agitation rate, the system was allowed to stabilize, then the rate was increased again. This process was repeated until a magnetic decoupling event occurred within the system or until the system reached the maximum manufacturer-defined operational speed of 1,250 rpm. If a magnetic decoupling event occurred, the agitation speed at which the event was triggered was recorded.

The described magnetic decoupling testing was completed with vessel filled to 5-L and five different aeration conditions, each defined by a different bottom air sparge rate. Aeration conditions of 0.0, 0.5, 1.0, 1.5, and 2.0 vessel volumes per minute (vvm) were tested for each model media formulation. Decoupling trials were performed in triplicate for each individual set of testing conditions.

Results and Discussion

The overall results of the magnetic decoupling characterization testing are shown in **Figure 2**. These data clearly demonstrate the agitation rates needed to trigger magnetic decoupling events decrease as the viscosity of the process media increases. Additionally, the results show that media viscosities ≥ 10 cP were able to decrease the agitation speed threshold required to induce a decoupling event in the system. The results also suggest that increasing the bottom air sparge rate can be a viable option to increase the magnetic decoupling agitation speed threshold, especially when mixing highly viscous media formulations.

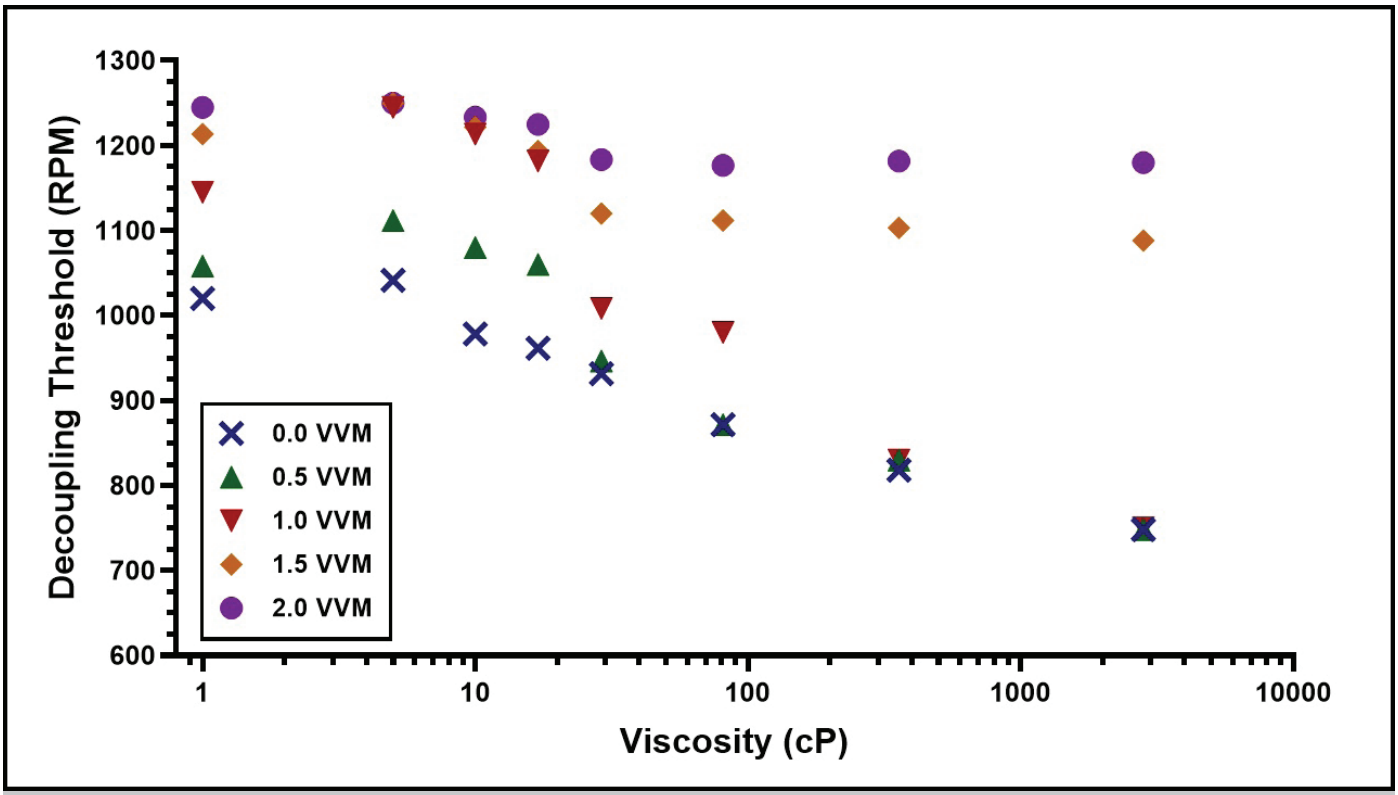


Figure 2: ***Results of magnetic decoupling characterization study.*** *Results demonstrate that magnetic decoupling agitation rate threshold decreases with an increase in system media viscosity. Data also show how increases bottom air aeration rate can potentially mitigate the effects of high viscosity media and increase the magnetic decoupling agitation threshold of the system.*

To further examine the effects of the bottom air sparge rate on the magnetic decoupling agitation speed threshold within the 5-L autoclavable fermentation system, the data were normalized to the maximum mean agitation speed observed for each specified bottom air sparge flow rate. These data, shown in **Figure 3**, demonstrate that total volumetric sparge air flow rates of ≥ 1.5 vvm appear to have the potential to considerably mitigate the effects of high media viscosity, allowing the system to maintain agitation speeds much closer to the maximum possible rate. For the highest media viscosity condition of 2820 cP, the magnetic decoupling agitation speed was 87% and 94% of the maximum recorded speeds at the 1.5 vvm and 2.0 vvm aeration conditions, respectively. In contrast, the magnetic decoupling agitation speed was 72%, 67%, and 60% of the maximum recorded speeds at the 0.0 vvm, 0.5 vvm, and 1.0 vvm aeration conditions, respectively. This response is likely due to the increased system aeration rates decreasing the overall density of the media under these operational conditions.

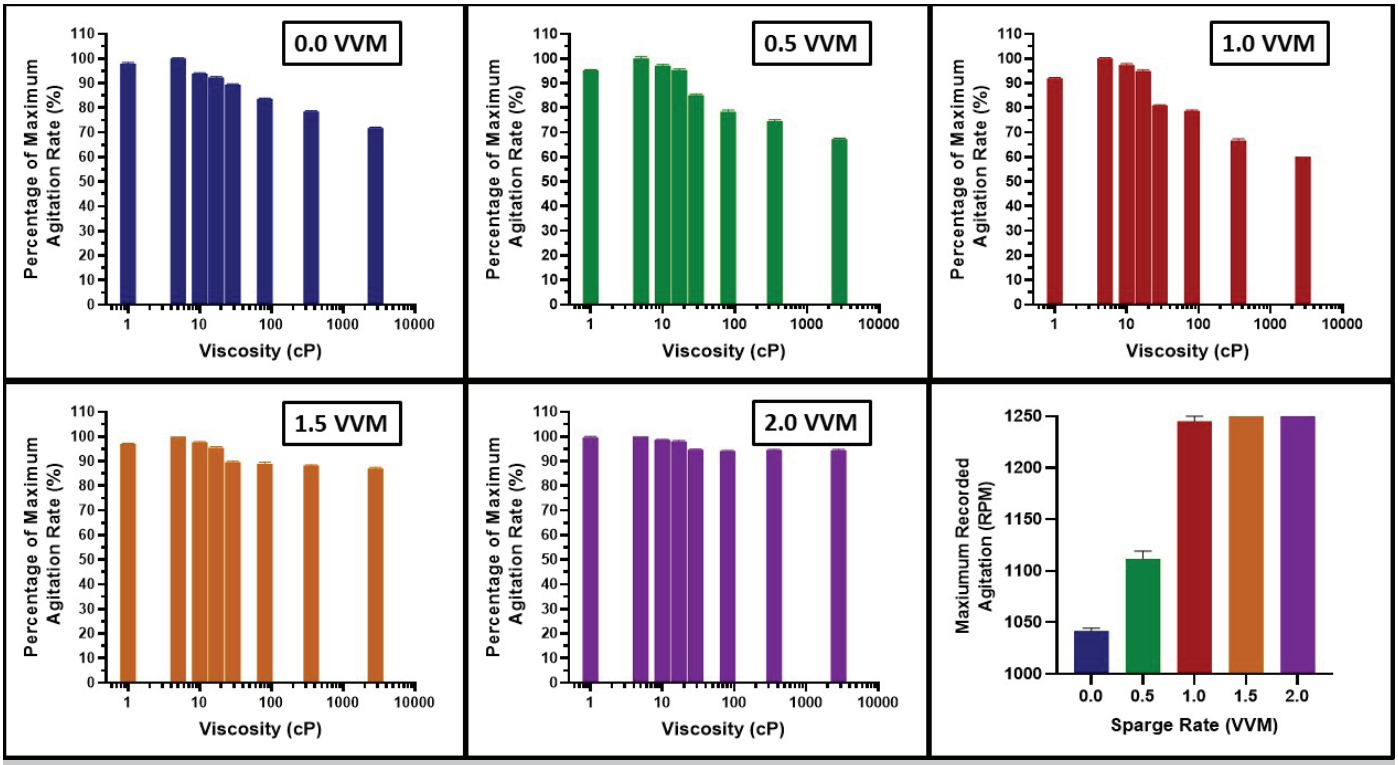


Figure 3: ***Normalized viscosity testing data across different aeration strategies.*** *Results demonstrate that sparged air flow rates of 1.5 and 2.0 vvm can effectively mitigate some of the effects of higher media viscosities to support higher agitation rates in magnetically coupled agitation systems.*

Conclusions

Magnetically coupled agitation drive designs have been successfully integrated into both bioreactor and fermentation upstream bioprocess systems. Magnetically coupled systems offer several benefits in comparison to more traditional mechanically coupled systems. These benefits include both increased sanitary protection and decreased system maintenance. One disadvantage that has been reported with magnetically coupled systems is the risk of magnetic decoupling events occurring during processing.

In this work, we examined the minimum agitation rate thresholds needed to induce magnetic decoupling events in a 5-L autoclavable benchtop fermentation system. Multiple batches of a corn-syrup based model medium were prepared that ranged in viscosities from 1 cP to 2820 cP. The system was aerated with individual sparged air strategies that ranged from 0.0 to 2.0 vvm. Results demonstrated that media viscosities ≥ 10 cP were able to decrease the agitation speed threshold required to induce a decoupling event in the system. It was also shown that aeration rates of ≥ 1.5 vvm were able to support higher agitation rates in highly viscous environments. Overall, the results of this study demonstrate how properly characterized magnetically coupled drive systems can be successfully integrated in a variety of upstream bioprocesses, including even highly viscous microbial and fungal applications.

References

1. *Zhong, J.-J. (2011). Bioreactor Engineering. Comprehensive Biotechnology, 165–177. https://doi.org/10.1016/b978-0-08-088504-9.00097-0*
2. *Cronin, K., & Ring, D. (2022). Mixers and agitators. Encyclopedia of Dairy Sciences, 362–370. https://doi.org/10.1016/b978-0-12-818766-1.00123-9*
3. *Schirmer, C., Maschke, R. W., Pörtner, R., & Eibl, D. (2021). An overview of drive systems and sealing types in stirred bioreactors used in biotechnological processes. Applied Microbiology and Biotechnology, 105(6), 2225–2242. https://doi.org/10.1007/s00253-021-11180-7*
4. *Liu, S. (2017). Bioreactor Design Operation. Bioprocess Engineering, 1007–1058. https://doi.org/10.1016/b978-0-444-63783-3.00017-4*
5. *Matthews, G. (2009). Fermentation Equipment Selection: Laboratory Scale Bioreactor Design Considerations. In B. McNeil & L. Harvey (Eds.), Practical fermentation technology (pp. 3–36). essay, Wiley.*
6. *McNeil, B., & Harvey, L. M. (1993). Viscous fermentation products. Critical Reviews in Biotechnology, 13(4), 275–304. https://doi.org/10.3109/07388559309075699*
7. *Schelden, M., Lima, W., Doerr, E. W., Wunderlich, M., Rehmann, L., Büchs, J., & Regestein, L. (2016). Online measurement of viscosity for biological systems in stirred tank bioreactors. Biotechnology and Bioengineering, 114(5), 990–997. https://doi.org/10.1002/bit.26219*
8. *Aguirre-Ezkauriatza, E. J., Galarza-González, M. G., Uribe-Bujanda, A. I., Ríos-Licea, M., López-Pacheco, F., Hernández-Brenes, C. M., & Alvarez, M. M. (2008). Effect of mixing during fermentation in yogurt manufacturing. Journal of Dairy Science, 91(12), 4454–4465. https://doi.org/10.3168/jds.2008-1140*
9. *Newton, J. M., Schofield, D., Vlahopoulou, J., & Zhou, Y. (2016). Detecting cell lysis using viscosity monitoring inE. colifermentation to prevent product loss. Biotechnology Progress, 32(4), 1069–1076. https://doi.org/10.1002/btpr.2292*
10. *Shamala, T. R., & Prasad, M. S. (1995). Preliminary studies on the production of high and low viscosity dextran by Leuconostoc spp. Process Biochemistry, 30(3), 237–241. https://doi.org/10.1016/0032-9592(95)85004-x*
11. *Kong, S., Day, A. F., O'Kennedy, R. D., Shamlou, P. A., & Titchener-Hooker, N. J. (2009). Using viscosity-time plots of escherichia coli cells undergoing chemical lysis to measure the impact of physiological changes occurring during batch cell growth. Journal of Chemical Technology & Biotechnology, 84(5), 696–701. https://doi.org/10.1002/jctb.2101*
12. *Caşcaval, D., Galaction, A.-I., & Turnea, M. (2010). Comparative analysis of oxygen transfer rate distribution in stirred bioreactor for simulated and real fermentation broths. Journal of Industrial Microbiology & Biotechnology, 38(9), 1449–1466. https://doi.org/10.1007/s10295-010-0930-3 vb*

DISTEK

888.2.DISTEK

distekinc.com • info@distekinc.com

121 North Center Drive • North Brunswick, NJ 08902