Bioreactor Cleaning Study

# Cleaning Characterization of Bioreactor Air-Liquid Interface (ALI) Residue

Confidential Client

## STUDY OVERVIEW

A client desired to perform small-scale studies to characterize the cleanability of residual soil in their bioreactors. The study was specifically focused on the cleanability of the residue at the air-liquid interface (ALI), which is known to represent a cleaning challenge for bioreactors. The client requested a study from Hyde to evaluate the cleanability of multiple soil residues representative of those present in the bioreactor to determine if combining an additive with the caustic step of the cleaning cycle would aid the removal of the ALI residue.

## PROJECT DESCRIPTION

The ALI residue created in bioreactors is known to be a cleaning challenge during cleaning activities. Bioreactors have a long incubation time (e.g., 3-10 days) and a high packed cell volume (PCV), which contributes to the residue buildup at the ALI and often represents a more difficult cleaning challenge than the bioreactor bulk liquid soil, i.e., bioreactor vessel surfaces that are submerged in soil during production. Therefore, any chemistry shown to be more effective at removing the bulk liquid bioreactor soil should be confirmed as also effective at removing the ALI residue.

The Hyde Analytical Laboratory was contracted to determine the effectiveness of using additives with the client's current caustic cleaning agent when cleaning bioreactor residual soil. The evaluation was performed by assessing the cleanability of two soil types from the bioreactor: 1) directly spiking bioreactor liquid soil onto a coupon, which represents bioreactor vessel surfaces that are submerged in soil during production; and 2) ALI residue, which was provided for the study via client-provided baffles from a pilot-scale bioreactor that had the ALI residue present on the surface of the baffle.

Due to a limited number of replicates, i.e., baffles, with the ALI residue, initial screening of additive efficacy was first performed on the bulk bioreactor soil in form of spiking liquid soil onto a coupon. This provided directional data as to which additive would likely be more effective in removing the ALI residue. Additionally, it provided a comparison of cleaning challenge for the bulk bioreactor soil and ALI residue to determine if the ALI residue truly represents a greater cleaning challenge for this bioreactor process.

**NOTE: Hyde has also performed these bioreactor studies by generating ALI residue on coupons at small-scale. Client-provided ALI residue (e.g., from pilot-scale baffles) is not required.**

## SCOPE AND DELIVERABLES

**Selection of Additive Chemistry:**
As a baseline for comparison, the caustic cleaning step of the current cleaning cycle was evaluated for its effectiveness in removing bioreactor residual soil that had been spiked onto small-scale coupons (representative of bioreactor vessel surfaces that are submerged in soil during production). Once the baseline cleaning for caustic alone was established, four additives to the caustic were evaluated to determine which additive most significantly increased the cleaning efficacy of the caustic-only cleaning cycle. The single most effective additive was selected for all subsequent testing.

**Caustic Alone vs. Caustic + Additive Comparison:**
The cleaning efficacy of the caustic alone was compared to that of the single most effective caustic + additive determined during the preceding testing. The comparison was performed for both bioreactor soil types: 1) directing spiking bioreactor liquid soil onto a coupon, which represents bioreactor vessel surfaces that are submerged in soil during production; and 2) ALI residue. This determined if both bioreactor soil types would be effectively cleaned using either cleaning chemistry selected for use at full scale.

**Cleaning Challenge Comparison:**
The cleaning challenge of the bulk liquid bioreactor soil, which represents bioreactor vessel surfaces that are submerged in soil during production, was compared to the cleaning challenge observed for the ALI residue. This provided an indication of any additional cleaning challenges to be expected with the ALI residue at full scale.

## SOLUTIONS, RESULTS AND ACCOMPLISHMENTS

- **Determined the most effective additive for use with the current caustic utilized at full scale.** A single most effective additive to the caustic was identified, which including optimizing the concentration, temperature, and exposure duration of the caustic + additive combination. This caustic + additive was selected for use in all subsequent phases of testing and recommended for full-scale use if an additive was desired

- **Provided valuable cleaning observations critical to full-scale visual inspections of clean equipment.** During execution of the standard observations made during Hyde Lab cleaning studies, it was captured that the tested soil is not visible on the cleaned surface when viewed under ambient light conditions after the simulated cleaning cycle. However, when the same surface is viewed under a flashlight, the soil residue is clearly visible. This is important knowledge for full scale inspection of equipment surfaces after a cleaning cycle, as viewing the equipment under ambient lighting could lead to false passing results, especially if the unseen residue builds up over time. This observation led to the recommendation that the client utilize flashlights whenever visually inspecting the bioreactor vessel.

- **Determined some residues are not visible at the limit of concern. Some soils evaluated in this study could not be detected at the limit of concern, even with the use of a visual aid such as a flashlight.** This indicates visual inspection cannot be used as a sampling method for these soils and other methods such as swabbing or rinsing must be used.

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