Rapid Bioburden of Pharma Tablet Binder

Introduction

Binders used for tablet are highly susceptible to contaminations as no biocides can be added. As most binders are a mixture of natural ingredients such as cellulose, starch, gelatin, and other polysaccharides, they serve as excellent growth medium for a broad range of microorganisms. The instability of the binder material emphasizes the importance of fast results when analyzing the microbial quality of the binder, as the concentration will increase over time. IntuGrow is a method where the microbial quality of binder material can be analyzed in less than 24 hours instead of the 5 days currently specified in USP61/ ph eur 2.6.12.

Materials and Methods

IntuBio received a test sample of binder used for pharmaceutical tablets. For dilution series sterile 15 ml tubes where used. Dilution series were performed using H_2O 0,9% NaCl. For test 1 100x diluted binder was used. For test 2 both 50x and 100x diluted binder was used. All cultivations were performed using tryptic soy agar (TSA) at 30°C for 24 hours. For up-concentration of the microorganisms present in the sample IntuGrow sterile filters with a pore size of 0,45 µm were used together with the IntuGrow filtration device. The image acquisition was performed using the oCelloScope 2.0 and the CFU concentrations were calculated using the IntuGrow software package.

Results

Test 1 - Initial concentration

The aim of test 1 was to determine the sample preparation for the binder and the bacterial concentration upon arrival. Two different volumes were tested to establish the best suitable amount of sample passed through the filter. After 18 hours the final concentration was observed (Table 1). The population of microorganism were highly uniform, and one colony morphology was identified (Figure 1).

Table 1 Overview of the results from Test 1 after 18 hours. LOD represents the calculated concentration if one CFU was detected in the sample. CFU count is the count of colonies on the filters after incubation. CFU/ml is calculated as: (CFU count x dilution)/volume.

Dilution	Volume	LOD	CFU count	CFU/ml
100xdiluted	5 ml	20 CFU/ml	329	6580
100xdiluted	2 ml	50 CFU/ml	130	6500

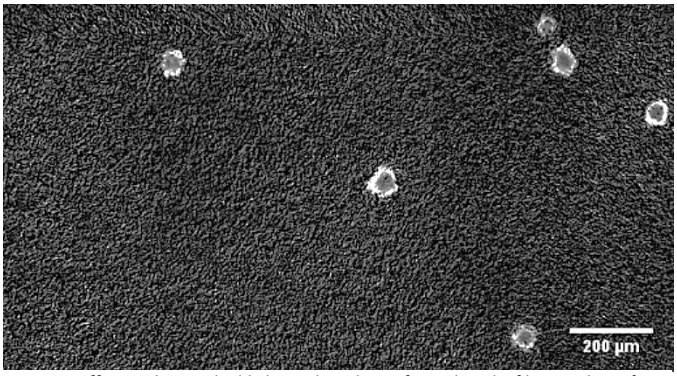

Figure 1: Differential image highlighting the colonies formed on the filter in white after 18 hours.

ID

It was arranged to have ID performed on the colonies from the binder. The entire filter was removed from the plate and vortexed for 5 mins in a 50 ml sterile tube with 10 ml H_2O 0,9% NaCl. Three different volumes were plated on TSA 90 mm petri dishes to ensure isolated colonies. After 24-hour incubation, an isolated colony picked and streaked on a new TSA plate. This plate was sent to an external laboratory for ID using DNA based methods.

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The bacterium identified (score 2.378) was *Microbacterium oxydans.* Notably *M. oxydans* has a standard cultivation time of 10-14 days (DSMZ).

Test 2 - Bacterial proliferation over time and Time-to-Result (TTR)

Test 2 was performed to assess the performance using 50x dilution as well as 100x dilution. Furthermore, due to the microbiological instability of the binder, Test 2 was performed to examine the extent of bacterial proliferation during the time in-between Test 1 and Test 2 (48 hours). Test 2 was analyzed continuously for 24 hours.

Table 2: Overview of the results from Test 2 after 24 hours. LOD represents the calculated concentration if one CFU was detected in the sample. CFU count is the count of colonies on the filters after incubation. CFU/ml is calculated as: (CFU count x dilution)/volume.

Dilution	Volume	LOD	CFU count	CFU/ml
50xdiluted A	1 ml	50 CFU/ml	311	15.500
50xdiluted B	1 ml	50 CFU/ml	289	14.450
100xdiluted A	1 ml	100 CFU/ml	177	17.700
100xdiluted B	1 ml	100 CFU/ml	171	17.100

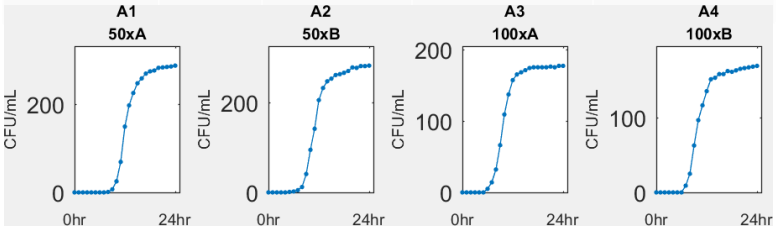

Figure 2: Growth curves of CFU concentrations calculated every hour for 24 hours.

The results of Test 2 showed significant bacterial proliferation since Test 1, highlighting the instability of the binder material even when stored refrigerated. The growth curves (Figure 2) showed that the final concentration was reached approximately after 18 hours. Like Test 1 the CFUs were of uniform morphology indicating that the proliferation was that of *M. oxydans (Figure 3).*

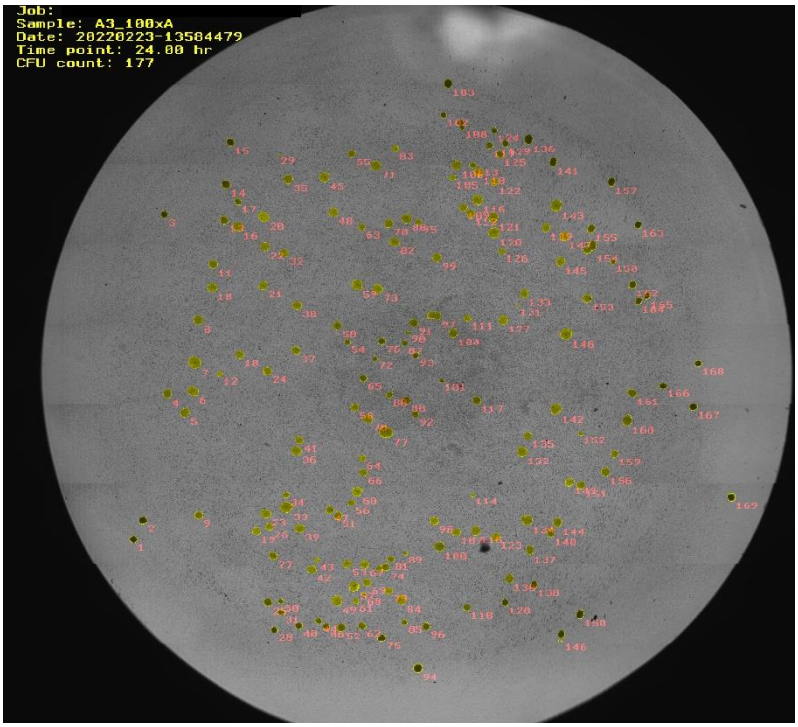

Figure 3: Tagged image showing each colony marked with yellow with corresponding ID number for colony traceability.

Conclusion

The results of the tests illustrate the ability of IntuGrow to quantify bacterial contaminants in complex matrices, as tested with tablet binder. Furthermore, a significant reduction in TTR from 5 days to 18-24 hours was observed. Notably *M. oxydans* identified by DNA analysis has long incubation times of 10-15 days using standard plating methods, and shorter TTR with IntuGrow should be expected with other types of microorganisms.