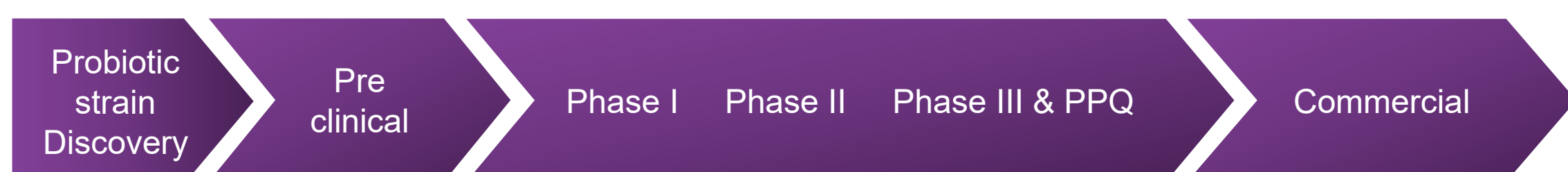


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Biose Industrie's NGP Challenge

Biose Industrie supports clients from early development to clinical supply by providing process development, analytical development, GMP manufacturing, and finished drug product services for complex Next-Generation Probiotics and Live Biotherapeutic Products. With experience handling more than 800 challenging bacterial strains, Biose has built strong expertise in developing and manufacturing difficult-to-grow microorganisms across the full product lifecycle, from strain discovery to patient use.



However, one key analytical step remained a bottleneck: traditional CFU counting. While reliable, CFU enumeration is slow, labor-intensive, and not well suited to real-time decision-making during process development and manufacturing.

For CDMO customers working under tight development timelines, a faster and more precise approach was needed to monitor viability, accelerate decisions, and improve process control.

Quality at BioForm Solutions

The commercialization of NGPs is currently bottlenecked by the extreme physiological sensitivities of species like *Akkermansia muciniphila*. As obligate anaerobes, these organisms are highly susceptible to oxygen exposure and the mechanical stresses of downstream processing, such as lyophilization and milling.

Traditional Colony Forming Unit (CFU) methods often fail to capture the "true" state of these populations, as they cannot account for injured or viable-but-non-culturable (VBNC) cells. To overcome these limitations, this collaboration demonstrates a specialized CDMO workflow by **Biose Industrie** that integrates strict anaerobic manufacturing with advanced flow cytometric validation. By using flow cytometry as a real-time decision-making tool at every production stage, we ensure high-potency NGP products are maintained from pilot scale to the commercial shelf.

Flow cytometry evaluation of cell health

To accurately assess microbial health, we utilize a dual-staining approach with cell-permeant and cell-impermeant DNA-binding dyes:

Cell-Permeant Dyes: Penetrate all cells to establish the total microbial population.

Cell-Impermeant Dyes: Selectively enter only those cells with compromised membranes—typically identifying the injured or dead subpopulations.

Throughout production, packaging, and storage, various environmental stressors can compromise membrane integrity. This staining strategy allows for the precise quantification of multiple microbial subpopulations, providing a detailed, high-resolution snapshot of overall product health that traditional plating simply cannot match.

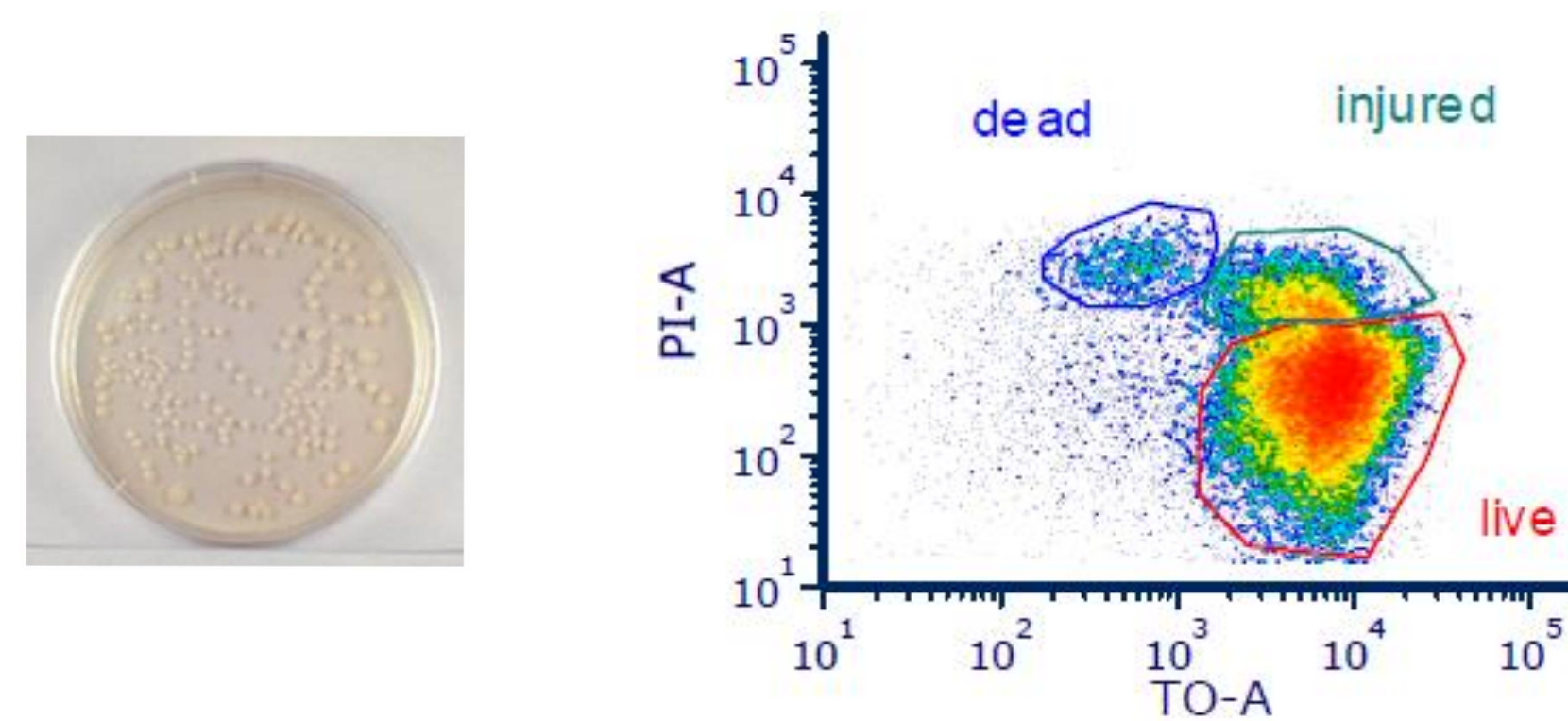
From Isolation to Patient, Speed and Accuracy of Quality Metrics Matter



AFU fits naturally across three manufacturing phases:

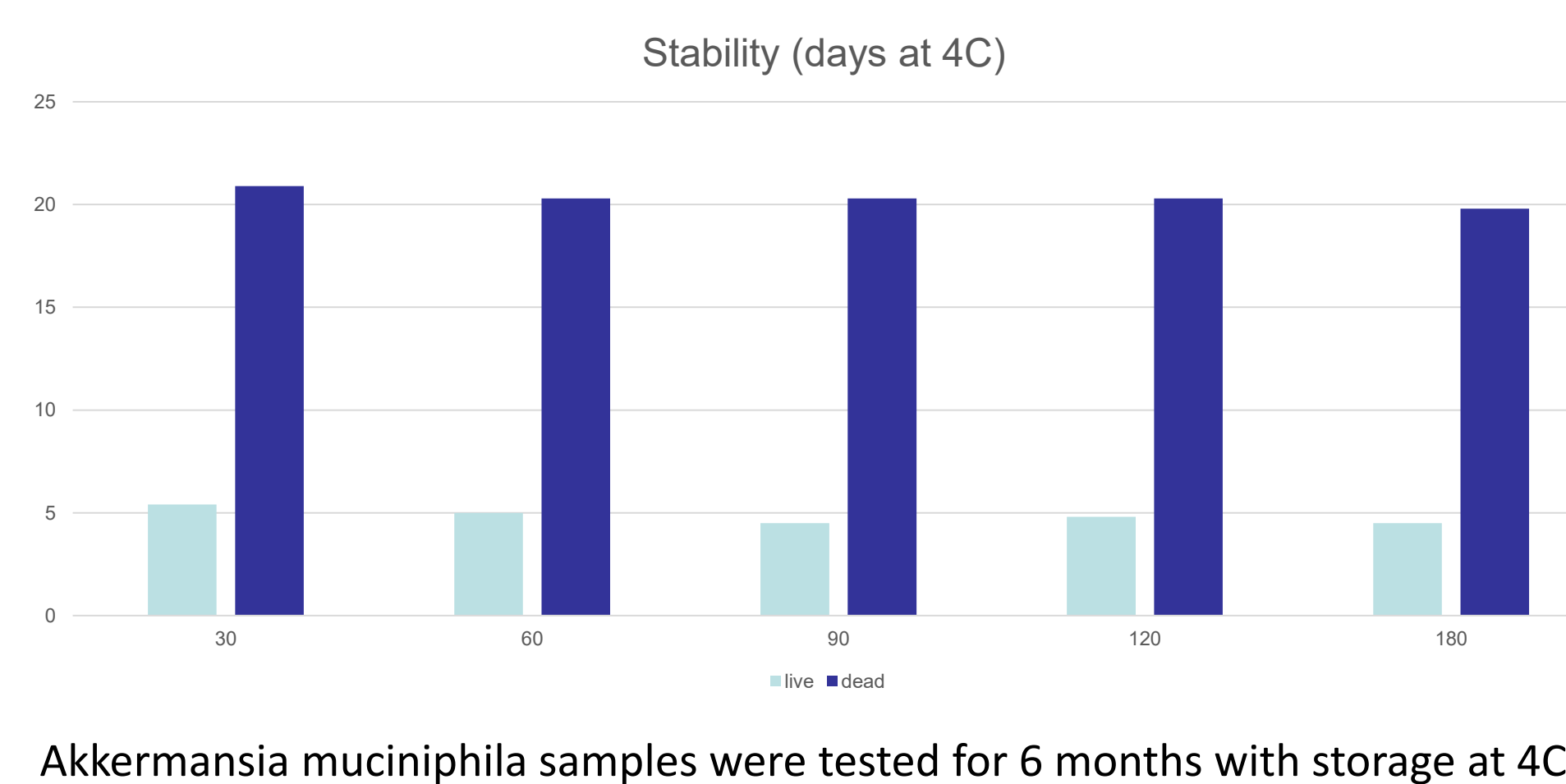
- Upstream growth & scale-up: providing rapid confirmation that concentration and population "fitness" are on track to continue with the seed train process.
- Downstream recovery & concentration: bacterial collection and concentration are known to severely affect bacterial viability and integrity. Applying AFU-based enumeration between the independent steps is paramount when identifying potential bottlenecks in optimization processes, improving overall bacterial recovery rates.
- Formulation & finishing: using AFU enumeration to track how the active fraction survives the drying stage and evolves during storage allows for the improvement of shelf-life and process robustness.

Advantages of flow cytometry over plating



Feature	Colony Forming Units (CFU)	Flow Cytometry Assay (AFU)
Population Depth	Measures only culturable cells; misses the "True" population.	Comprehensive enumeration of Live, Injured, and Dead subpopulations.
Anaerobic Reach	Growing obligate anaerobes on plates is technically challenging and inconsistent.	Direct measurement; can detect VBNC (Viable-But-Non-Culturable) cells.
Cellular Resolution	Limited to visible colony formation.	Distinguishes between dormant spores (haploid) and sporulated (diploid) cells.
Precision	High variability; results often underestimate actual enumeration.	Highly reproducible with <10% CV.
Assay Time	3-Day Assay (Retrospective testing).	15-Minute Assay (Real-time decision making).

6 month Stability Data



Monitoring cell health at each decision point

Why this matters for NGPs:

Traditional plating provides a "delayed snapshot," often resulting in a 72-hour feedback loop that is too slow for active manufacturing adjustments. By utilizing Flow Cytometry, we transform quality control from a final check into a **Real-Time Process Analytical Technology (PAT)**. This allows Biose to make critical decisions at the bioreactor or lyophilizer within minutes, ensuring that delicate strains like *A. muciniphila* are never left at risk during manufacturing.

Gate 1: Post-Fermentation. Are the cells healthy enough to survive the next step? (Check membrane integrity before harvesting).

Gate 2: Post-Lyophilization. Did the freeze-drying cycle cause significant damage? (Adjust cryoprotectants in real-time).

Gate 3: Post-Packaging. Does the final format (capsule/sachet) maintain the anaerobic seal?

AFU: Accepted in Commercial Product Quality

- Bifidobacterium infantis*
- Akkermansia muciniphila*
- Anaerostipes caccae*

CONCLUSIONS

Manufacturing LBPs and NGPs requires precise conditions to maintain efficacy over time. Success with Next-Gen Probiotics (NGPs) requires a shift from 'growth-based' metrics to 'physiology-based' metrics. By integrating advanced flow cytometry into the CDMO workflow, we ensure that the final product doesn't just meet a label claim—it arrives in the consumer's hands in peak physiological condition.