

Application Note AN-PAN-1057

Inline monitoring of fermentation processes

Determination of multiple parameters in a fermentation broth for bioethanol production

The concern for the development of alternative and renewable fuels has increased over the past several years. Bioethanol is a good replacement for fossil fuels. It can be made from items like sugar, starch, or lignocellulosic biomass, such as kernel corn.

Global ethanol production exceeded 28 billion gallons in 2022 [1]. Ethanol is mainly produced via the process of fermentation. Fermentation transforms sugars within the biomass into ethanol through the use of yeast.

It is well known that the quality of the feedstock can vary from season to season which requires ethanol producers to adapt to each batch. With the use of inline near-infrared spectroscopy (NIRS), several fermentation quality parameters can be monitored simultaneously directly in the tank, as shown in this Process Application Note.

INTRODUCTION

To guarantee high yield and top-quality ethanol, many parameters should be monitored during ethanol production. Traditionally, the amount of reactants, products, and byproducts are measured in the laboratory after taking a sample out of the process. However, manual laboratory methods can result in long response times in case of process changes (e.g., temperature, reaction mixture, moisture levels), and sample preparation (dilution, filtration, pipetting) can introduce errors altering the precision of the analysis.

Additionally, it can be quite cumbersome since multiple techniques and/or operating methods are required to analyze the following parameters: ethanol, dextrin (DP4), maltotriose (DP3), maltose, glucose, lactic acid, glycerol, and acetic acid (Table 1), along with moisture and solids (enzymes).

In any chemical process, «real-time» monitoring allows for optimal process modeling and control, which means enhanced throughput, reproducibility, and productivity.

For instance, tight monitoring and control over the various sugars present (glucose, maltose, DP3, and DP4) throughout the fermentation process is necessary to understand the breakdown pathway of the starch (glucose generation) present in the mash and optimize ethanol production [2]. Understanding the sugar pathway enables the right dosing of «enzymatic mix» and «yeast blend» to the mash in the slurry tanks to accelerate breakdown [3].

Therefore, optimizing the enzyme and yeast blend is crucial for this process. These are the highest consumable costs for ethanol production and significantly affect the rate of production and final yield of ethanol.

Inline analysis provides «real-time» process data. This data helps producers determine the optimal fermentation time (Figure 1). It also allows them to adjust the impeller spin rate and tank temperatures. These adjustments can increase ethanol production using the same materials. A reduced fermentation time means being able to carry out more daily fermentation batches, which results in more profits.

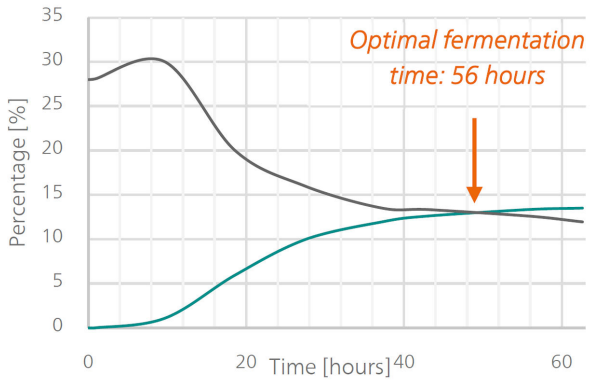


Figure 1. Trend chart for an ethanol fermentation process (green: ethanol, grey: solids [enzymes]).

For optimal fermentation, multiple parameters must be monitored in a safer, more efficient, and faster manner, which is possible via inline analysis with reagent-free near-infrared spectroscopy (NIRS) (Figure 2). Metrohm Process Analytics offers the 2060 *The* NIR Analyzer (Figure 3) which enables direct comparison of «real time» spectral data from the process to a reference method (e.g., HPLC) to create a simple yet indispensable calibration model used to produce quantitative results during the fermentation process.

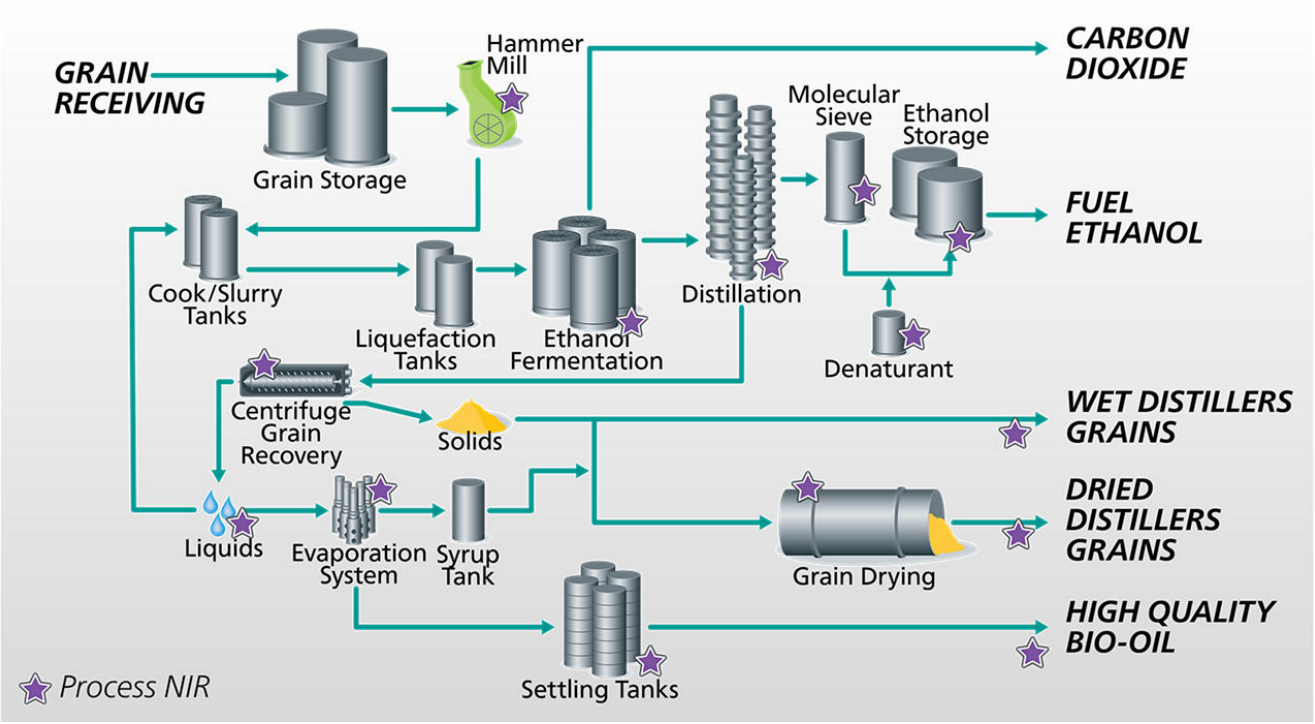


Figure 2. Illustration of a typical dry-grind ethanol process from grains with purple stars noting suggested process NIR analysis points.



Figure 3. 2060 The NIR Analyzer from Metrohm Process Analytics.

APPLICATION

Measurements can be performed directly inline thanks to a dedicated immersion probe (Table 2) coupled to microbundle fibers. Such a combination allows the NIR measurement of samples with suspended solids and the presence of bubbles, without requiring filter screens around the probe that may become clogged during the fermentation. Where a bypass or fast loop is available, using a flow cell is recommended so that solid matter can be removed prior to measurements.

Table 2. Dedicated solutions for your NIRS sampling needs.

Probe Type	Applications	Processes	Installation
Micro interactance reflectance probe	Solids (e.g., powders, granules)	Bulk polymerization	Direct into process line
	Slurries with >15 % solids	Hot melt extrusion	Compression fitting or welded flange
Micro interactance immersion probe	Clear to scattering liquids	Solution phase	Direct into process line
	Slurries with <15% solids	Temperature- and pressure-controlled extrusion	Compression fitting or welded flange
Micro transmission probe pair	Clear to scattering liquids	Solution phase	Direct into process line or reactor
	Slurries with <15% solids	Temperature- and pressure-controlled extrusion	Into a side-stream loop
			Compression fitting or welded flange
Micro interactance reflectance probe with purge on collection tip	Solids (e.g., powders, granules)	Drying of granules and powders	Direct into the fluid bed dryer, reactor, or process line
	Environments where sample amount varies		Compression fitting or welded flange

Table 1. Key parameters to monitor with NIRS during ethanol production by fermentation.

Parameter	Range (%)
Ethanol	0–15
Glucose	0–8
Maltose	0–7
DP3 & DP4	0–15
Acetic acid	0–0.5
Glycerol	0–1
Lactic acid	0–0.25

REMARKS

An appropriate range of samples covering the fermentation process is needed to build a calibration model. These samples will be analyzed via NIRS and also via a primary reference method. The precision of the NIRS data is directly correlated to the precision of the reference method.

CONCLUSION

Traditional analysis methods do not provide sufficient «real-time» information about the fermentation process performance for bioethanol production. Inline analysis with NIRS can provide faster information about the fermentation process, which is ideal for rapid feedback (approximately every 30 seconds) and higher process throughput.

NIRS analysis allows for the comparison of real-time spectral data with a primary method (e.g., titration, Karl Fischer titration, HPLC, IC) to develop a straightforward yet essential model for meeting fermentation process needs. Enhance and improve production management using the Metrohm Process Analytics 2060 *The* NIR Analyzer, which grants even greater fermentation control by monitoring up to five process points per NIR cabinet with the multiplexer option.

REFERENCES

1. *Global ethanol production for fuel use 2022*. Statista. https://www.statista.com/statistics/274142/global-ethanol-production-since-2000/ (accessed 2023-10-04).
2. Chang, Y.-H.; Chang, K.-S.; Chen, C.-Y.; et al. Enhancement of the Efficiency of Bioethanol Production by Saccharomyces Cerevisiae via Gradually Batch-Wise and Fed-Batch Increasing the Glucose Concentration. *Fermentation* **2018**, *4* (2), 45. https://doi.org/10.3390/fermentation4020045.
3. Devantier, R.; Pedersen, S.; Olsson, L. Characterization of Very High Gravity Ethanol Fermentation of Corn Mash. Effect of Glucoamylase Dosage, Pre-Saccharification and Yeast Strain. *Appl Microbiol Biotechnol* **2005**, *68* (5), 622–629. https://doi.org/10.1007/s00253-005-1902-9.

BENEFITS FOR NIRS IN PROCESS

- **Safe production** due to «real-time» monitoring and no exposure of operator to chemical reagents.
- **More savings per measurement**, making results more cost-effective.
- **Increased product throughput**, reproducibility, production rates, and profitability (optimize fermentation time).



FURTHER READING

Real-time monitoring of hyaluronic acid fermentation by in situ transflectance spectroscopy

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CONFIGURATION



2060 The NIR Analyzer

The 2060 *The* NIR Analyzer is the next generation of process spectroscopy instruments from Metrohm Process Analytics. With its unique and proven design from the inside out, it delivers accurate results every *10 seconds*. It can provide non-destructive analysis of liquids and solids directly in the process line or in a reaction vessel by using fiber optics and contact probes. It has been designed to connect up to five (5) probes and/or flow-cells. All five channels can be configured independently from each other using our versatile embedded proprietary software.

As part of the 2060 Platform, the 2060 *The* NIR Analyzer has a modular concept and it is available in three other versions: the 2060 *The* NIR-R Analyzer, 2060 *The* NIR-Ex Analyzer, and 2060 *The* NIR-REx Analyzer.