qPCR vs MCA Tests

Which is best for your needs

Summary
While quantitative Polymerase Chain Reaction (qPCR) and Microbial Community Analysis (MCA) tests both fall under the molecular testing umbrella, they are very different in what they are measuring and how the data can be used in wastewater applications. This whitepaper gives you information on the tests and which one best fits your needs.

Molecular Testing

In wastewater, molecular testing, also known as molecular diagnostics, involves analyzing genetic material (DNA or RNA) to identify organisms and metabolic pathways present in the biomass.

Quantitative PCR (qPCR)

qPCR is used to quantify specific DNA or RNA sequences in a sample. It provides precise, quantitative data on the abundance of target genes. The test involves amplification of DNA with real-time monitoring using fluorescent dyes or probes. The fluorescence intensity correlates with the amount of target DNA in the initial sample.

Advantages:

- High sensitivity and specificity
- Quantitative results
- Rapid and suitable for high-throughput analysis – results in hours

Limitations:

- Requires prior knowledge of target sequences. Your results depend completely on how well your primers fit your target.
- Limited to known organisms or genes – you must know exactly what you are looking for!

Example of using qPCR to quantify nitrifiers in an industrial wastewater system

Systems with fluctuating influents benefit from rapid testing for impact on Ammonia Oxidizing Bacteria (AOB) and Nitrite Oxidizing Bacteria (NOB) – both are slow growing organisms that are susceptible to upset conditions. Previous work with MCA testing allowed Aster Bio to customize the primers for industrial activated sludge MLSS. With a turnaround time in hours versus days, the facility can make adjustments before experiencing a nitrifier population decline and increased effluent ammonia. qPCR numbers below are presented as a percentage, relative to total 16S rRNA abundance of all bacteria.

MLSS	% AOB	% NOB
09-24	5.8%	0.2%
10-19	4.2%	0.8%
11-11	3.1%	1.7%
12-09	1.4%	3.3%
12-17	1.1%	3.2%
12-22	0.8%	1.8%
02-09	1.6%	4.5%
02-23	4.2%	2.9%

Microbial Community Analysis

This approach profiles the entire microbial community within a sample, providing insights into the diversity and relative abundance of different microorganisms.

- Uses next-generation sequencing (NGS) techniques such as 16S rRNA gene sequencing, metagenomics, or metatranscriptomics to analyze the genetic material of all microbes in a sample. For wastewater bacteria and archaea, Aster Bio targets the 16S rRNA gene as a microbial barcode for identification.
- Used to study microbial diversity, community structure, and functional potential in both soil and water.

Advantages:

- Comprehensive profiling of microbial communities
- Can identify both known and unknown organisms
- Provides insights into microbial interactions and ecosystem functions

Limitations:

- More complex and time consuming than qPCR – usually takes 4–6 business days.
- Provides relative abundance – results are reported as % of reads

Example of Functional Groups Summary from MCA report

Date	AOB	NOB	NRB	SOB	SRB	PAO	GAO	EPS	FOA	FIL	C1s
8/22	2.4	14.6	10.8	4.2	0	13.7	13.2	0.1	1.8	5.6	1.5
7/25	1.8	10.5	10.8	4.4	0	10.9	8.9	0.1	3.4	7.9	1.6
6/20	1.6	10.1	10.7	3.1	0	8.3	17.8	0.1	1.5	6.7	1.7

The functional group summary table was designed to give a quick summary of top ecological groups in wastewater samples. The functional groups present are those most important to wastewater treatment operations.

Which test to use?

By sequencing all bacteria in a sample, the MCA provides significantly more data than a single qPCR test. While the large amount of data in a total bacterial census can be summarized by the functional groups table, the data out also allows for a deep dive into the various genera in the sample and more information on the interaction among organisms in the biomass. Additionally, data from the MCA can be used to develop highly specific qPCR tests. qPCR is useful when you need rapid results and know your organisms of interest (and have correct primers). The MCA test is good for routine monitoring, while qPCR can be very useful for quick insights during upset conditions or when changing operational parameters.

How often to run molecular tests

Molecular testing is not a “daily” test. To make MCA testing most useful, baseline data for populations during normal operations is a good start. While we can compare data from similar systems, it is best to have information on the system to detect potential drifts in populations. For getting baseline data, we encourage users to test monthly or quarterly depending upon amount of variation. Another aspect of molecular testing is becoming familiar with the information provided by the MCA (qPCR is more direct without much need for interpretation). To understand the data and identify opportunities to improve treatment efficiency, it is good to have technical support, which is why Aster Bio likes to discuss results with our clients during initial phases of testing implementation. As we go along, the online interactive database allows both the user and Aster Bio to watch for changes in biomass composition.

For more information on Environmental Genomics™ Testing including qPCR & MCA, contact Aster Bio.

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