2014 ASBC Annual Meeting

Microbiology in Process Control Made Easy – Is PCR Finally Available for Everybody?

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Introduction of PCR into a production lab

PCR is today an acknowledged state-of-the art analysis method in brewery analytics, but for a long time was regarded to be too complicated to find its way into routine microbiology in a standard brewer's lab. With the latest instrumentation and ready-to-use kits, PCR analysis method now has become very robust and also easy to use.

Mainly because more practical technical equipment recently has come to market, this technique now can be applied even in small breweries.

We want to give a basic insight in what is needed and what comes out when PCR is used for microbiology analysis in a brewer's lab.

Preliminary enrichment is usually done as often the samples contain only traces of microorganisms., and often it is not yet decided if a sample will later go into PCR analysis or will have time to wait for conventional visual inspection.

Real Time PCR Skills

Instrumentation

The first commercially available PCR instrumentation was intended to be used by researchers only - therefore it was built to allow as many variations and settings as possible.

Different channels and incubation times, additional melting curves analyses etc. allowed the user even to make changes within a running program which was already started.

On the other side, these instruments did not fulfill the basic requirements which a production control laboratory needs to have in routine use:

1. **Stability:** Apply one method to different sample types, even under non-optimal conditions, and get a readable and constant result
2. **Ease of use:** Allow different people to work without intensive training
3. **Constancy:** Outcome/Result has to be read by everybody with the same result

Today we have a choice of instrument and kit manufacturers who have designed their products according to the needs in a brewer's quality control laboratory.

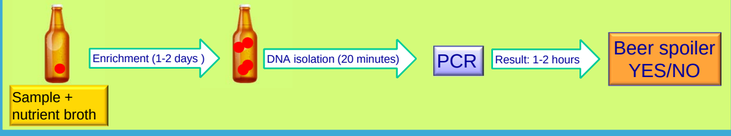
People

From users we know that a laboratory technician or other person who is doing microbiology can also do PCR after a basic training.

Sample Handling

Sampling is the same as conventional, but the time point of sampling may be changed due to faster results and lower detection limits of PCR (ref. center graph).

Choice of enrichment medium influences the range of growing microorganisms and therefore the spectrum of detectable spoilers. As differentiation between spoilers and non-spoilers is achieved by specific PCR tests, it is advisable to use enrichment media pre PCR which allow growth of a broader range of microorganisms – usually they do proliferate faster than in selective media.



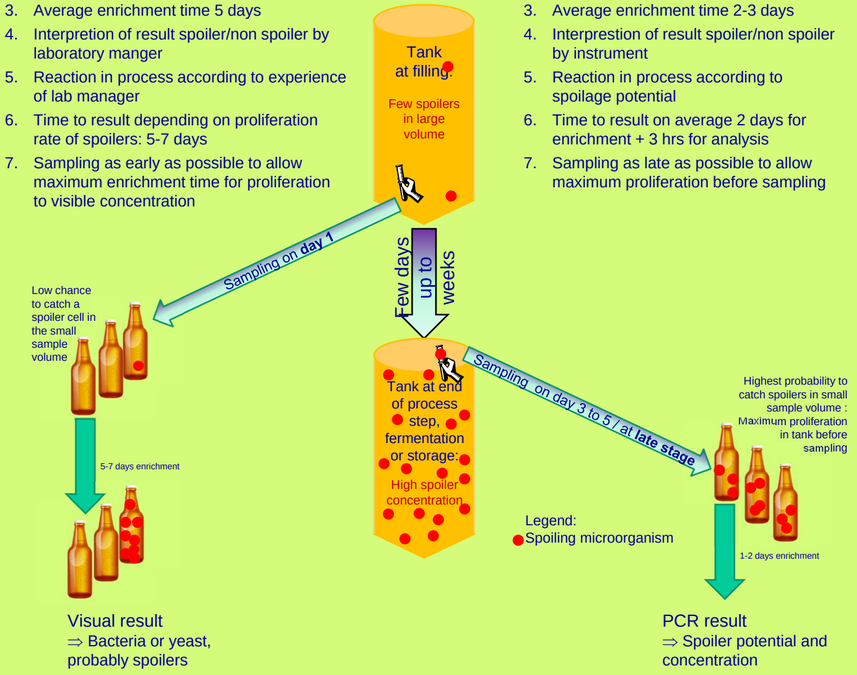
PCR improves the probability to catch spoilers in the process

Conventional

1. Detection by turbidity
2. Detection limit at 10e6 to 10e7 cells/ml
3. Average enrichment time 5 days
4. Interpretion of result spoiler/non spoiler by laboratory manger
5. Reaction in process according to experience of lab manager
6. Time to result depending on proliferation rate of spoilers: 5-7 days
7. Sampling as early as possible to allow maximum enrichment time for proliferation to visible concentration

PCR

1. Detection with sensitive instrument
2. Detection limit at 1,000 cells/ml
3. Average enrichment time 2-3 days
4. Interpretion of result spoiler/non spoiler by instrument
5. Reaction in process according to spoilage potential
6. Time to result on average 2 days for enrichment + 3 hrs for analysis
7. Sampling as late as possible to allow maximum proliferation before sampling



Points of use for PCR in a Medium Size Brewery

Aim

Use of PCR has to strictly follow the ratio cost : benefit so only segments of the process are monitored in routine.

Decision making

Q1: How do you decide which method to use – conventional enrichment only, or PCR?
A1: We are an independent brewery, so decide depending on situation. The following samples are always PCR tested in routine:

- Yeast propagation tanks
- Harvest yeast from fermentation tanks for re-pitching - result needed within 2 days
- Continuous sampling bottles at filler
- Fermenters with top fermenting beers – risk of fast growth of spoilers due to high temperature
- Positives from conventional microscopic analyses - to verify results

Q2: What are the follow-on steps in case of positive results?
A2: The aim is not always to know the spoiler species, but to find out the contamination source and to keep an eye on the hygiene status.

- Yeast propagation and harvest yeast: No re-pitching to avoid spread-out into other tanks and product lines
- Try to eliminate spoilers in product, e.g. do flash pasteurisation
- Do extensive cleaning in positive line to start safe with following production streams

Q3: Who works with PCR in the lab?
A3: Due to recent developments in instrumentation and kits, every experienced laboratory technician can do PCR.

Q4: What is the equipment needed, what are the average costs?
A4: Besides a thermocycler instrument, basics include a small centrifuge, microliter pipettes, and a heating block. Total investment costs start at approximately 15,000 US $

Minimum instrumentation and bench space needed to do PCR analysis

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