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New Tools and Method for Concentration of Microorganisms from American Lager Beers for Spoilage Detection

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Introduction

Beer spoilage organisms and contamination present a major risk for the brewing industry. As such, microbiological testing for these organisms is necessary throughout the brewing process. However, most laboratories still use conventional cultivation methods, which are time-consuming – requiring 3 to 5 days for beer to be released to the market. Rapid microbiological analytical methods offer great potential for increasing the reliability of spoilage detection in beer while reducing labor costs and product hold times; however, small analysis volumes limit the usefulness of these methods. In this study, InnovaPrep's Concentrating Pipette was investigated as a bridge to concentrate 12 ounces (355 mL) of beer into volumes more appropriate for rapid detection methods.

Background

The InnovaPrep Concentrating Pipette is an automated and rapid bioconcentrator. The system uses special consumable pipette tips with an internal filter of either flat membrane filter or hollow fiber membrane filters in various pore sizes to capture microorganisms from large liquid volumes. Following filtration, rapid recovery of the microorganisms is performed by a process termed 'Wet Foam Elution™' in which carbonated elution fluid is released from a canister, to tangentially flush the trapped particles into a final volume of a few hundred microliters. Following elution, the foam breaks down immediately to allow for rapid analysis. The process eliminates the need to use time-intensive enrichment steps prior to molecular detection.

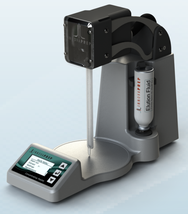
Degassing Jar

Be Flat™ Degassing Jar

The high level of carbonation in beer created a significant hurdle in applying the Concentrating Pipette to this application. During processing, significant quantities of carbon dioxide are released from the beer causing the hydrophilic membrane filter in the pipette tip to lock up. Though there are numerous decarbonation methods, none of these methods were able to reduce the amount of residual CO_2 that allowed an entire can of beer to be processed by the Concentrating Pipette.

A special degassing glassware container and a method were developed and patented by InnovaPrep for more efficient decarbonation. The glassware's interior is specially sandblasted to create a large surface area of nucleation points to help in the degassing process. To degas, twelve ounces (355 mL) of room temperature American lager beer was poured into the glass containers. The beer was incubated at 4°C for 10 minutes to increase the solubility of CO_2.

Method



InnovaPrep Concentrating Pipette

- Pour room temperature, beer into degassing jar from a height of 8-12 inches above the jar to achieve maximum foaming.
- Incubate beer for 10 minutes at 4ºC
- Concentrate sample using Concentrating Pipette using either a 0.45 µm hollow fiber Concentrating Pipette Tip or 0.2 µm Polycarbonate, Track-Etched Flat Membrane Tip.
- Capture and weigh elution volume.

For concentration of infected beer, the same protocol was used as above except:

- 1 mL of 100/mL CFU *Lactobacillus brevis* (WLP 672, White Labs) was dispensed into beer prior to 4ºC incubation.
- Elution volumes were plated onto MRS agar plates and incubated at 30ºC for 48 hours, at which time colonies were enumerated.

Results

Concentration of American Lager Beer from Three Different Breweries using the Concentrating Pipette

Sample	Volume of Beer Processed	Dwell time at 4°C (min)	Average Run Times for 25°C Beer (min)	Average Run Times for 37°C Beer (min)	Average Elution Volume (mL)	Standard Deviation
Lager brewery 1	355	20	14.50	6.53	0.279	0.011
Lager brewery 2	355	20	15.85	12.45	0.256	0.019
Lager brewery 3	355	20	17.26	10.80		

Prior to concentration, cans of beer were either incubated at room temperature or 37°C for at least 2 hours prior to pouring into Be Flat Degassing Jar and incubated at 4°C for 20 minutes. Each sample was processed using a 0.4 µm flat Concentrating Pipette Tip (polycarbonate, track-etched membrane).

Recovery of *Lactobacillus brevis* from Artificially Infected American Lager Beers using the Concentrating Pipette

American Lager from Brewery 2								
	Can 1	Can 2	Can 3	Can 4	Can 5	Can 6	Average	St. Dev.
Concentrate								
Recovery Efficiency	67.81%	74.45%	72.97%	58.79%	71.61%	80.65%	71.05%	6.69%
Recovery Volume (mL)	0.2408	0.2175	0.2187	0.1977	0.4157	0.457	0.2912	0.1041
Processing Time (min)	5.40	5.96	6.32	5.95	7.76	6.48	6.31	0.73
Second Elution								
Recovery Efficiency	2.21%	0.74%	5.16%	11.31%	7.54%	8.29%	5.87%	3.62%
Recovery Volume (mL)	0.402	0.3685	0.5071	0.3149	0.402	0.4259	0.4034	0.0582
Total Recovery Efficiency	70.02%	75.18%	78.13%	70.10%	79.15%	88.94%	76.92%	6.43%
Concentration Factor	**999.7**	**1215.1**	**1184.5**	**1055.7**	**611.5**	**626.5**	**948.9**	244.3

American Lager from Brewery 4						
	Can 1	Can 2	Can	Can 4	Average	St. Dev.
Concentrate						
Recovery Efficiency	85.10%	60.61%	73.47%	67.96%	73.06%	10.00%
Recovery Volume (mL)	0.7049	0.5326	0.5856	0.6609	0.6210	0.0665
Processing Time (min)	3.78	4.03	3.99	3.73	3.8808	0.1319
Second Elution						
Recovery Efficiency	1.22%	7.35%	1.22%	2.45%	3.06%	2.52%
Recovery Volume (mL)	0.5598	0.5883	0.5496	0.5326	0.5576	0.0202
Total Recovery Efficiency	86.33%	67.96%	74.69%	70.41%	74.85%	7.05%
Concentration Factor	**428.6**	**404.0**	**445.4**	**365.0**	**426.0**	**17.0**

Each beer was inoculated with 1 mL of ~100 CFU/mL *Lactobacillus brevis* prior to 10 minute incubation at 4ºC. Samples were then concentrated by using 0.45 µm hollow-fiber Concentrating pipette tips, eluted, weighed, then plated onto MRS agar plates, and incubated for 48 hours at 30ºC, at which time plates were enumerated and compared to spike dilution.

Conclusions

- Concentrating American Lager beer into volumes more readily compatible with molecular methods is possible using the Concentration Pipette and Be Flat degassing glass.
- Concentration factors between ranging from 365X – 1215X are achieved can be achieved using this process
- All recovery volumes are, on average, 620 µL and below, with average recovery efficiencies of *L brevis* being around 70%.

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