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Sampling strategies for Assessing Wastewater Treatment Plant Efficiency - Effect of storage and transport times on the stability of microbiological parameters

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Highlights:

- **24-hour composite sampling enhances wastewater characterization, mitigating concentration variations, and diurnal fluctuations, improving data accuracy**
- **Manual and automated sampling methods show minimal differences in microbiological parameter results, ensuring methodological consistency in wastewater analysis.**
- **Longer storage times for composite samples (up to 48 hours) have negligible impact on microbiological parameter stability under refrigerated conditions.**

Keywords: Microbiological sampling; Composite sampling; Stability

INTRODUCTION

To assess the effectiveness of full-scale wastewater treatment processes in removing substances or microorganisms, removal factors are calculated based on influent and effluent concentrations of various parameters. To this end, it is necessary to collect datasets, which are representative for the influent and effluent concentrations. For microbiological parameters, random samples or qualified samples are often taken, as international norms like ISO 19458:2006 include recommended and acceptable durations between sample collection and analysis (see Table 1).

Tab. 1: Recommended and acceptable duration (in hours) between sample collection and laboratory analysis of different fecal indicator organisms according to ISO 19458:2006.

Microorganism	Recommended duration [h]	Acceptable duration [h]
E. coli	12	18
Spores	24	72
Phages	48	72

However, the norm also acknowledges that there is conflicting evidence regarding the exact duration a sample can be regarded as representative. Drawing 24-hour composite samples from wastewater treatment facilities confers distinct advantages over random grab samples. It ensures a more comprehensive representation of wastewater characteristics over time, accommodating fluctuations in influent composition, including population diurnal patterns and industrial

discharges, it minimizes the impact of momentary spikes or concentration variations that might be overlooked in individual grab samples, thereby enhancing the overall accuracy of data. Utilizing 24-hour grab samples and transportation to the responsible laboratory inevitably takes longer than the specified acceptable stability and delivery times (ISO 19458:2006), especially for the parameter *Escherichia coli*. In addition to the question of the stability of the microbiological parameters, it is often questioned whether the automated sampling devices have a direct influence on the microbiological quality of a sample, as not all components can be disinfected. Against this background, the aim of the present study was to examine the consistency of microbiological results over a period of 72 hours, specifically focusing on the stability of a microorganism over time for various relevant microbiological indicators, such as intestinal enterococci, *E. coli*, *Clostridium* spp. and somatic coliphages.

METHODOLOGY

Samples were collected from a wastewater treatment plant (WWTP) situated in Rheinbach, Germany. A specialized sampling protocol was computed at an existing automated sampling device located at the effluent. Using this protocol, effluent water was automatically collected from the wastewater effluent shaft at 5-minute intervals over the course of one hour, with each sample volume set at 25 mL. The samples were automatically combined in a sterile sampling bottle within the device to form a composite sample and stored at 5 °C until further processing. Since the automated sampling device has been under constant use at the wastewater treatment facility, and a complete disinfection of all components was not possible prior to each sampling event, methodical controls were included to either eliminate or account for potential sample contaminations resulting from the sampling technique and the colonized instrument parts. Comparative experiments were conducted by also drawing samples manually with sterile equipment, analogously and simultaneously to the automated sampling device (5-minute intervals over one hour, sample volume 25 mL each). This procedure was repeated three times. Samples were kept refrigerated during transport to the laboratory and processed within 2 hours of sampling (T_0). Samples were again analysed after being stored for 24 hours (T_{24}), 48 hours (T_{48}) and 72 hours (T72) at 4-6°C in the dark, mirroring the conditions within the automatic samplers. All samples underwent analysis for intestinal enterococci (ISO 7899-1), E. coli (ISO 9308-3), Clostridium spp. (ISO 14189) and somatic coliphages (ISO 10705-2). For the parameters intestinal enterococci and E. coli data obtained in a previous test was included in the results.

RESULTS AND CONCLUSIONS

The results depicted in Figure 1 illustrate the outcomes of individual parameters obtained from manual sampling and automated sampling devices. This evaluation exclusively utilized the T_0 samples to guarantee that any disparities in analysis values are solely attributable to differences in sampling methodology. The differences in individual parameter results averaged at 0.05 log-units for both intestinal enterococci and somatic coliphages, 0.19 log-units for *E. coli*, and 0.39 log-units for *Clostridium* spp. Considering fluctuations within each parameter at the same

sampling location obtained by monitoring the effluent over months, any bias introduced by automated sampling can be deemed inconsequential for the results presented in this study.

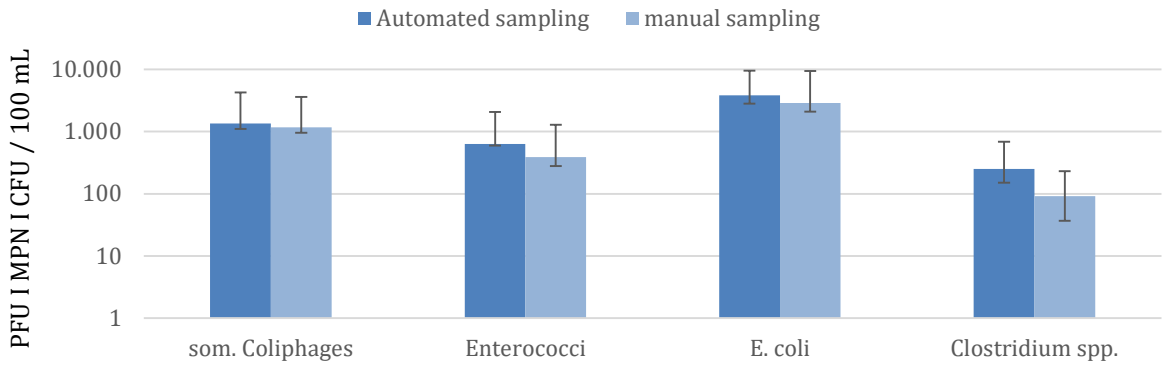

Fig. 1: Differences in microbiological findings when using automated samplers compared to manually drawn samples.

Figure 2 demonstrate that the analysed parameters remain stable for up to 48 hours' post-collection when appropriately stored under cold controlled conditions as described. Discrepancies between the results from T_0 to T_{48}, across biological triplicates, range from 0.02 to 0.3 log-units for *Clostridium* spp., 0.04 to 0.21 log-units for *E. coli* and 0.21 to 0.24 log-units for intestinal enterococci. Differences in stability over time for somatic coliphages range from 0.03 to 0.13 log-units. The impact of longer storage times as suggested in ISO 19458:2006, which inevitably occurs when conducting 24-hour composite samples can be considered negligible, if refrigerated conditions are maintained.

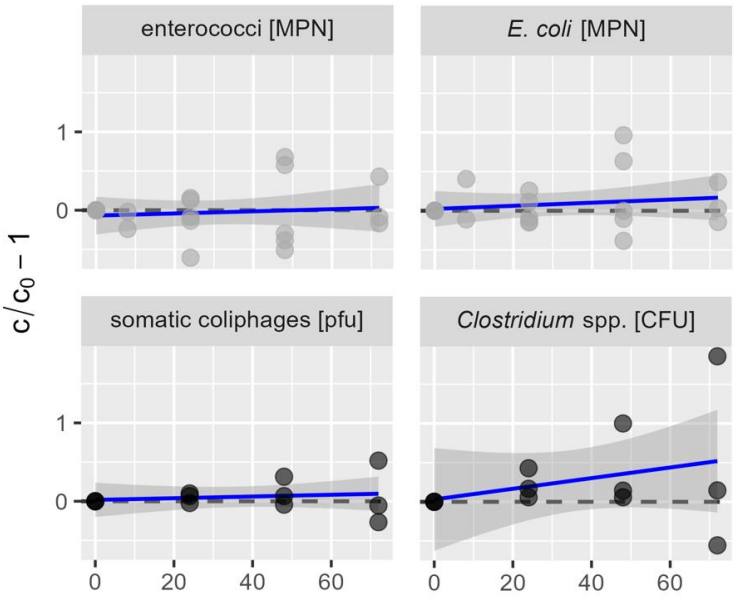

Fig. 2: Investigation of the influence of storage times on the concentrations of microbiological parameters in a sample.

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