# Characterization of wastewater with regard to microbial and physicochemical parameters

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## Abstract

The microbiological quality of Norwegian municipal wastewaters, both from combined and source-separated systems, is of interest today. Variation in faecal indicator bacteria concentrations in municipal wastewater is an essential data.It is used in modelling of the impact of discharges from combined sewer overflows during intense rainfall on the hygienic bathing water qualityon the territory of inner Oslofjord. E.coli and Intestinal Enterococci are used as the main parameters of water quality evaluation according to EU Directive 2006/7/EC.

It is determined that high concentration E.coli and Total coliforms in greywater from Kaja dormitories in NMBU does not caused by high faecal contamination, but most likely comes from kitchen sinks.

**Keywords:** enzyme activity, faecal indicator bacteria, greywater, municipal wastewater.

## Introduction

Sewerage system in Norway was constructed many years ago, e.g. system in Oslo is more than 150 years old. Wastewater treatment plants (WWTP) were built to decrease concentrations of organic matter, suspended solids and nutrients (phosphorus and nitrogen), avoiding water eutrophication.

Now it is not enough to take into consideration only physical and chemical parameters of wastewater. Big amount of pathogenic microorganisms from feaces can cause human diseases in case of different accidents.

WaterQualityTools project makes modelling of the impact of discharges from combined sewer overflows (CSO) during intense rainfall on the local hygienic bathing water quality of the inner Oslofjord. It requires data about the variations in concentrations of the faecal indicator bacteria (FIB) in WWTP raw influents both within short (during the day) and long (day-to-day) periods. According to EU bathing water quality directive controlled parameters are E.coli and Intestinal enterococci (EU Directive 2006/7/EC)

It is expected, that concentration of both these parameters should be low in the morning and higher in the afternoon. In the evening their concentration should be lower, than in the afternoon. FIB peakis caused by morning defecation and time of its observation depends on residence time in the system and sampling place (Lucas, 2014).

An important role in Norwegian sewers belongs to source-separated systems. They seems to be perspective using in small separate settlements, in the railway stations, in dormitories etc. Due to separation of fecal matter as a blackwater, most of pathogenic microorganisms should be concentrated there. Then greywater (all household water except toilet) considered being safer, than municipal wastewater from combined system.

Nevertheless, microbiological analysis of greywater from Kaja dormitories in Norwegian University of Life Sciences (NMBU) shows the high concentrations of total Coliform bacteria

(TCB) 2.98·105 – 2.42·107/100 ml, and E.coli 1.10·105 – 4.10·106/100 ml. It can be caused by faecal contamination, bacterial growth in pipelines or habits of tenants. (Ecomotive, 2014)

Literature survey shows, that number of bacteria in greywater significantly depends on the source of wastewater. According to this greywater can be devided into “light grey” – wastewater from bathroom sinks, baths and showers – and “dark grey” – more contaminated waste from laundry facilities, dishwasher sand, in some instances, kitchen sinks. “Light” greywater can contain up to 2.4·106/100 ml of E.coli and 2·104/100 ml for Intestinal Enterococci (Birks, 2007).

Especially high concentrations of FIB are shown for kitchen greywater: up to 7.4 log10/100 ml for E.coli and 7.7 log10/100 ml for Enterococci (Ottosson J, 2003). E.coli meanings can reach even 2.5·108/100 ml (Eriksson, 2002).

During the day FIB concentration can change significantly, as people use bath, shower and wash basin mainly in the morning and in the evening. While kitchen devices are mainly used during the dinnertime (Matos, 2004).

Analyzing fecal contamination, very often essential to do it as quick as possible. Minimizing detection time provides real-time water quality evaluation, i.e. prevention of epidemiological accidents.

Rapid method of coliforms detection and enumeration based on the direct measurement of enzymatic activity: β-D-galactosidase (GALase) for coliforms and β-D-glucuronidase (GLUase) for E.coli. This method is simple, fast (1-2 hours in comparison with 18-24 hours for standard methods), but has low specificity. Even so, it can be useful for approximate evaluation of FIB concentrations (Fiksdal, 2008).

The objectives of the research:

- investigate within-day and day-to-day variations of fecal indicator bacteria (FIB) in municipal wastewater
- discover reasons of high FIB level in greywater from Kaja dormitories
- check the relation between enzyme activity and FIB concentration in wastewaters.

## Materials and methods

### Sample collection

All the wastewater samples were collected and analyzed in Norwegian University of Life Sciences (NMBU).

Samples of municipal wastewater were taken from the collecting pipe near the Sørhellinga building.

Greywater samples were taken from two places: from the collecting pipe after Kaja-dormitories before first collecting tank and from the second collecting tank in Fløy 4.Blackwater samples were taken from the collecting tank in Fløy 4.

To investigate the variation samples were collected three times during the day: morning, afternoon and evening during several days. To explore relation between enzyme activity and FIB concentration samples were taken 3 times a day with minimum interval 1 hour.

### Microbial analyses

The water samples were analysed for total coliforms (TCB) and E.coli using the Colilert 18 QuantiTray method (ISO 9308-2) ISO 7899-2, 2012. The results are given as Most Probable Number (MPN) per 100 ml.

Intestinal enterococci and faecal coliforms (FCB)in the water samples were quantified after membrane filtration using method from ISO 7899-2 (ISO 7899-2, 2000) and NS 4792 (NS 4792, 1990). The results are given as colony forming units (cfu) per 100 ml.

Enzyme activity in samples was measured using Colifast method according to the manufacturer guideline.

### Chemical analyses

To get the whole characteristic of wastewater there was measured a set of physicochemical parameters: electrical conductivity (EC), pH, total phosphorus (TP), chemical oxygen demand (COD), total suspended solids (TSS), turbidity and color.

TSS were determined according to standard methods using glass fiber filter disksGF/C 1.2 µm (APHA, 2005; SINTEF, 2004).The volume of filtered sample varied from 5 to 50 ml depending on visual observations.

TP was determined in the unfiltered samples using Hach Lange cuvette tests LCK350 and LCK349 according to standard methods (APHA, 2005). Highly concentrated samples were diluted up to 10 times.

COD was determined in the unfiltered samples using Hach Lange cuvette tests LCK114and LCK314according to standard methods (ISO 15705:2002).

EC, pH, turbidity and color were measured according to standard methods (APHA, 2005).

## Results

### Municipal wastewater

Samples of MWW were collected within one day and the variation in concentration of FIB is shown on the Fig. 1. As expected, concentration of FIB is lower in the morning, due to the dilution by shower water, although concentration of Enterococci is higher in the morning.

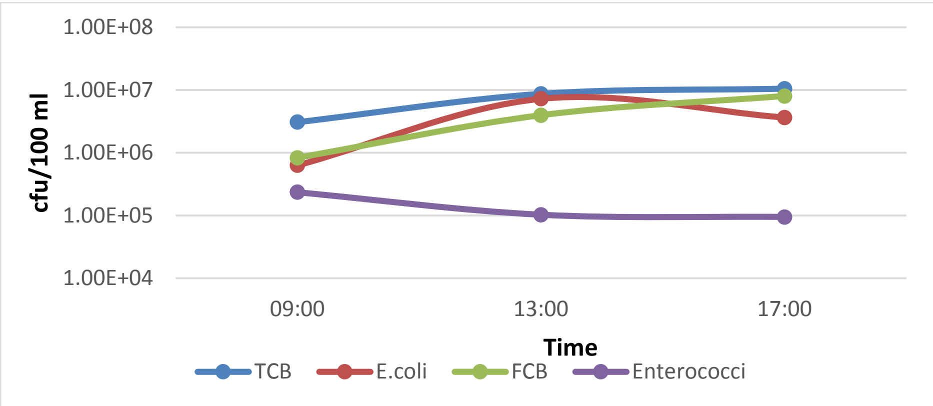


**Figure1** within day variation in concentration of FIB in municipal wastewater from NMBU.

Physico-chemical parameters of samples match those of typical Norwegian municipal wastewater (Table 1).

**Table 1** Physicochemical and microbiological parameters of municipal wastewater in NMBU.

| Chemical parameters | | | | Microbiological parameters / 100 ml | | | |
|---|---|---|---|---|---|---|---|
| **Time** | 9:00 | 13:00 | 17:00 | **Time** | 9:00 | 13:00 | 17:00 |
| **Ec, µS/cm** | 322 | 830 | 820 | **TCB** | 3.08E+06 | 8.66E+06 | 1.05E+07 |
| **pH** | 8.71 | 9.17 | 9.08 | **E.coli** | 6.31E+05 | 7.27E+06 | 3.65E+06 |
| **TP, mg P/l** | 7.76 | 13.8 | 17.4 | **FCB** | 8.33E+05 | 3.97E+06 | 8.03E+06 |
| **COD, mg/l** | 480 | 2261 | 1343 | **St. deviation** | 4.16E+05 | 4.51E+05 | 1.67E+06 |
| **SS, g/l** | 0.21 | 0.84 | 0.58 | **Enterococci** | 2.35E+05 | 1.03E+05 | 9.45E+04 |
| **Turbidity** | 172 | 971 | 581 | **St deviation** | | 6.01E+04 | 7.07E+02 |
| **Color, mg/l Pt** | 157 | 467 | 302 | | | | |

## Greywater

It was assumed that high level of FIB is caused by bacterial growth in collecting tanks. Water samples from both Kaja and Fløy 4 have been taken in the morning during 3 days. Comparison of FIB concentrations shows that number of coliform bacteria in greywater decreases after it gets into tank, while number of Enterococci increases (Fig. 2).

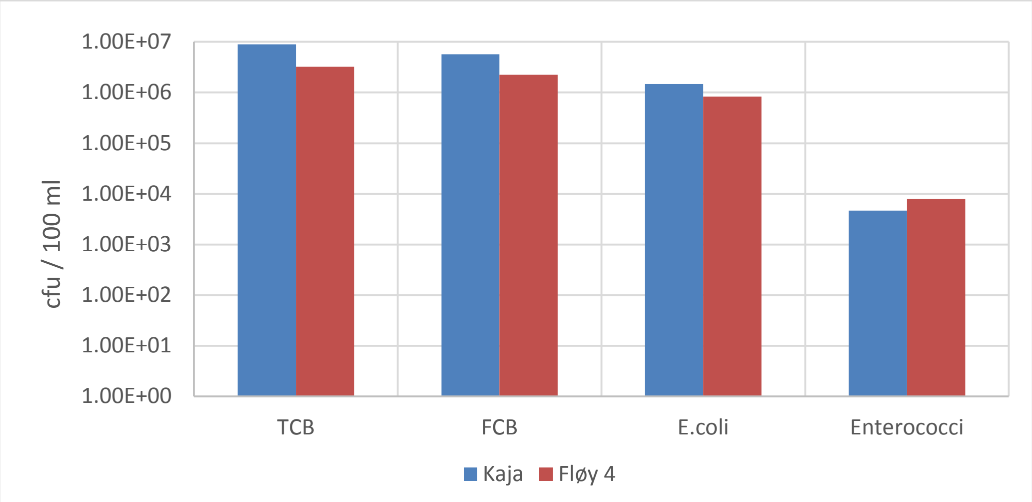


**Figure 2** FIB concentrations in greywater from Kaja and Fløy 4.

If to talk about physicochemical parameters pH and TP concentration of “fresh” greywater from Kaja are lower, than that of wastewater samples from Fløy 4 (Table 2). Whereas COD concentration is higher in Kaja samples.

**Table 2** Chemical and microbiological parameters of greywater from NMBU.

| **Chemical parameters** | **Kaia** | **Fløy 4** | **Microbial parameters, cfu/100 ml** | **Kaia** | | **Fløy 4** | |
|---|---|---|---|---|---|---|---|
| | | | | Average | St. dev. | Average | St. dev. |
| **Ec, µS/cm** | 270±23 | 259±17 | **TCB** | 9.01E+06 | 3.12E+06 | 3.22E+06 | 4.50E+05 |
| **pH** | 6.82±0.20 | 7.23±0.09 | **E.coli** | 1.46E+06 | 6.98E+05 | 8.38E+05 | 5.05E+05 |
| **TP, mg P/l** | 1.16±0.16 | 1.44±0.24 | **FCB** | 5.69E+06 | 4.58E+06 | 2.24E+06 | 1.06E+06 |
| **COD, mg/l** | 262±75 | 231±53 | **Enterococci** | 4.65E+03 | 2.56E+03 | 7.85E+03 | 5.08E+03 |
| **SS, mg/l** | 61±17 | 131±91 | | | | | |
| **Turbidity, FNU** | 114±59 | 122±64 | | | | | |
| **Colour, mg/l Pt** | 193±132 | 97±59 | | | | | |

As first assumption was not confirmed, it was decided to check the origin of contamination. To find the source of bacteria there were conducted growth potential test and qPCR analysis for human specific Bacteroides and Enterococci.

Results of growth potential test match the expectations, i.e. at room temperature number of bacteria rises during the first day and then begin to decrease (Fig. 3). Number of Enterococci decreases from the first day. The initial amount of bacteria is very high, so most probably FIB have non-faecal origin.

Last assumption was confirmed by PCR analysis. It was conducted in Norwegian Institute for Water Research (NIVA).There were taken 3 parallels of black, grey and municipal wastewater, which also were analyzed for total coliforms and E.coli (Table 3). While concentration of FIB is very high in greywater, amount of Bacteroides gene copies is 5 logs lower than in municipal wastewater, and number of Enterococci is 1 log lower.

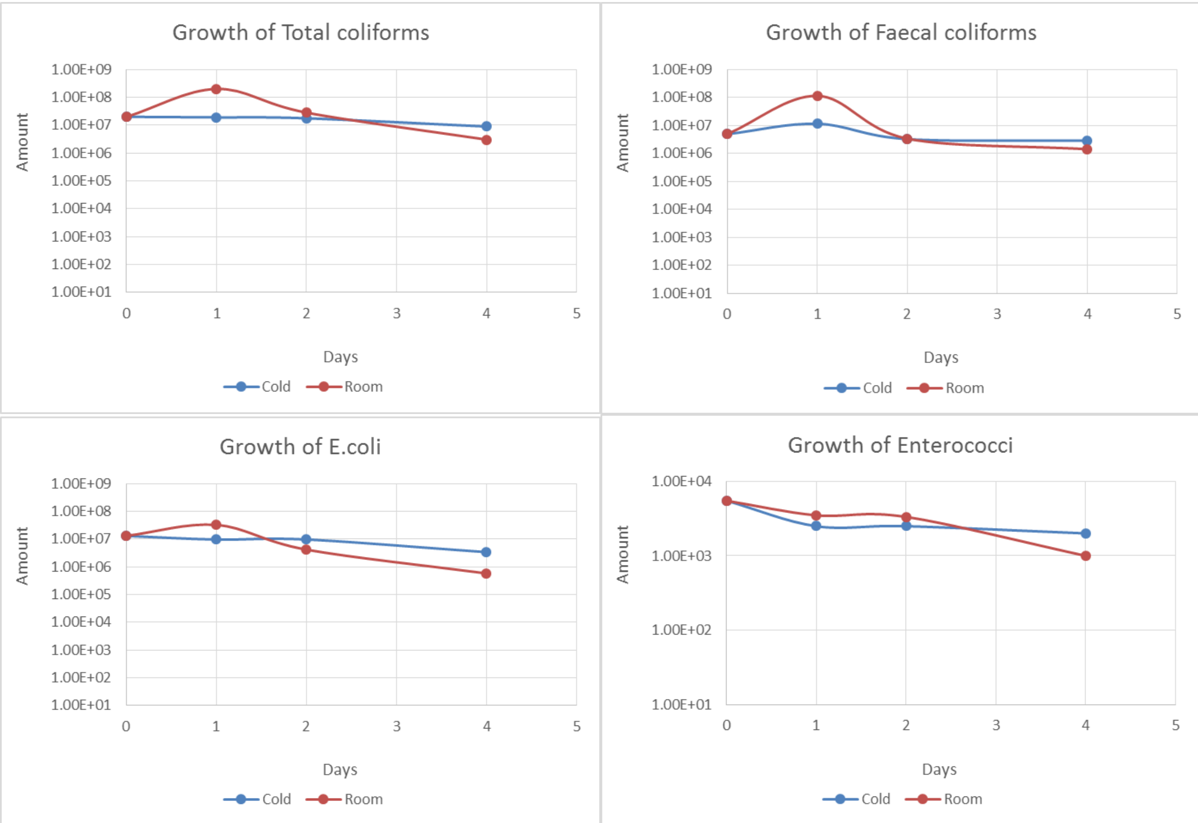


**Figure 3** Results of growth potential test for greywater from NMBU

**Table 3** Results of qPCR analysis for humanspecific Bacteroides and Enterococci.

| **Parameters** | **Blackwater** | | **Municipal wastewater** | | **Greywater** | |
|---|---|---|---|---|---|---|
| | Average | s | Average | s | Average | s |
| **TCB/100 ml** | 4.78E+07 | 9.6E+06 | 8.77E+06 | 6.3E+06 | 1.43E+07 | 9.7E+06 |
| **E.coli / 100 ml** | 8.99E+06 | 1.3E+06 | 4.49E+06 | 3.1E+06 | 1.55E+06 | 3.8E+05 |
| **Bacteroides Gene copies/ml** | 1.20E+09 | 1.1E+08 | 1.10E+09 | 1.1E+09 | 1.30E+04 | 4.8E+03 |
| **Enterococci relative CFU/ml** | 1.87E+08 | 5.5E+07 | 8.20E+06 | 3.0E+06 | 3.70E+05 | 5.6E+05 |

## Enzyme activity

There was checked the relation between enzyme activity in wastewaters and FIB concentrations. It is seen that this parameter can be used for previous evaluation of FIB concentration in different types of wastewater: blackwater, greywater and municipal wastewater. (Fig. 4)

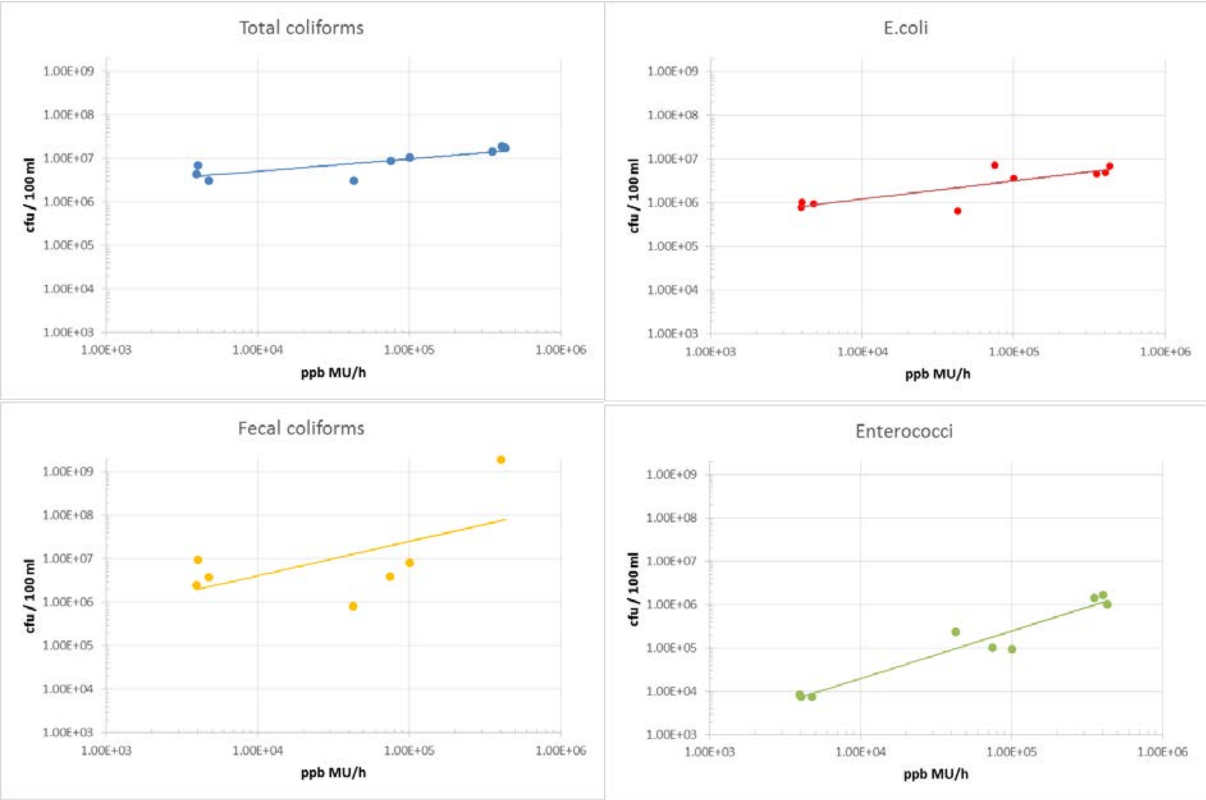


**Figure 4** Relation between FIB concentrations and enzyme activity.

## Conclusions

Parameters of municipal wastewater from combined sewer are common for this type of water. Concentration of bacteria is lower in the morning and increases during the day. The opposite picture observed for Enterococci.

According to regrowth test greywater contains enough nutrients to promote the Coliforms and E.coli growth during one day.

Greywater from Kaja dormitories contains high concentrations of Total Coliforms and E.coli, which correspond to previous measurements. At the same time only small part of them have fecal origin, whereas others more likely come from kitchen.

Rapid enzyme method can be used for preliminary evaluation of FIB concentration in different types of wastewater.

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