Short Communication

Microbial Contamination of Screenings from Wastewater Treatment Plants

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Received: 19 October 2011
Accepted: 15 May 2012

Abstract

Screenings represent the type of waste resulting from the primary treatment of wastewater. Their content varies significantly and it depends on numerous factors. The microbial contamination of screenings has not yet been monitored in detail; therefore, information in this area is either insufficient or unavailable. Our objective was to analyze the indicative microorganisms present in the screenings from wastewater treatment plants. Faecal thermo-tolerant coliform bacteria, coliform bacteria, and enterococci were monitored during microbial analyses. All tested samples proved the presence of the aforementioned microorganisms (in different concentrations). Furthermore, some samples confirmed the presence of *Salmonella* sp. bacteria, which originate from the faecal solids getting to the trash screens of the wastewater treatment plants together with contaminated water.

Keywords: wastewater treatment, primary treatment, trash screens, screenings, microbial contamination

Introduction

Wastewater treatment is related to the numerous physical, biological, and chemical processes used for the removal of substances getting into water as a result of people's activities. The first stage is the primary treatment focused on the gradual removal of inorganic solids from wastewater. This stage may decrease the risk of damage to the equipment and machinery used in subsequent stages of the treatment, as well as contingently preventing decrease of efficient volume of the tanks.

The trash screens are one piece of equipment commonly used for the primary treatment of wastewater. The trash screens serve the purpose of catching suspended trash, which is generally referred as screenings. They most commonly contain a mixture of the various materials – mainly plastics, paper, textiles, food residues, and excrement [1, 2]. The production of the screenings converted to 1-person equivalent represents 4-6 kg per person per year [1, 2]. Regarding screening removal from the wastewater, the screenings are generally disposed of in accordance with the valid legislation. The screenings are usually stored at a dump or are incinerated [3, 4]. Considering the origin of the screenings, their contamination by pathogenic microorganisms may be anticipated [5]. However, a detailed description of screenings is not presented in the available scientific journals – primarily due to the fact that it is highly complicated to unify the content and properties of the screenings with respect to, mainly, their variability. The variability of the screenings depends primarily on the size of the respective populated area, amount of the industrial wastewater and other factors that might affect both the quality and quantity properties. The objective of this paper is to describe the fundamental properties of screenings from the selected wastewater treatment plants by means of determining their solid content, ash-free dry mass, and cultivation determination of the indicator groups of contaminating microorganisms. The screenings separated in the waste-

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Table 1. Description of WWTPs.

WWTP	No. of p.e.	Sewage system type	Trash screen type
1	1500	Combined	Self-cleaning, mechanical
2	2400	Combined	Self-cleaning, mechanical
3	3048	Combined	Self-cleaning, mechanical
4	8000	Combined	Self-cleaning, mechanical
5	30000	Combined	Self-cleaning, mechanical
6	13060	Combined	Self-cleaning, mechanical
7	3950	Combined	Self-cleaning, mechanical

water treatment process may contain pathogenic germs of those microorganisms that would – in high concentrations – represent a risk pertaining to its subsequent treatment [5].

Material and Methods

The samples of screenings were collected at 7 wastewater treatment plants (WWTP) in the South Moravia Region; for the basic descriptions of the individual wastewater treatment plants see Table 1.

On average, 40 samples were collected at each WWTP in the time period January through December 2010. The collection of samples was based on ČSN-ISO Standard No. 10381-6:1998 Soil Quality – Sampling – Section 6. On the days of collection the respective samples were transported to a laboratory in the sterile sample containers (at a temperature not exceeding 5ºC), thus preventing their secondary contamination. The samples were weighed immediately after receipt, the content of solids and ash-free dry mass were determined and a microbiological analysis was conducted. The methodology applicable to physical and chemical analysis of the screenings, i.e. determination of the total content of solids annealing residue and ash-free dry mass is stipulated in ČSN Standard No. 83 0550 (Section 3). Total solid content and ash-free dry mass in screening samples were determined using the electric muffle furnace LMH 07/12, which is designed to measure the incineration processes, drying, degradation, re-heating, thermal treatments etc. Analytical laboratory balances Radwag AS 220/X were used for precise weighing, readability to 0.0001g. A well-mixed sample (10 g) of screenings was evaporated in a weighed dish and dried to a constant weight in an electric muffle furnace at 105ºC±2ºC. The increase in weight over the empty dish weight represents the total solids [%]. After total solid assessment the dish with sample is put back to electric muffle furnace at 550ºC±2ºC. The difference in weight over the dish after total solid assessment represents the ash–free dry mass [%].

The Sample Preparation for the Estimation of Microbiological Parameters

The suspension was prepared by homogenizing a 10 g sample of screenings in 150 ml of sterile quarter-strength Ringer solution in a blender for 20 minutes. After filtration (Whatman Gr.1, Merci, CZE) the suspension was used for all following microbiological tests.

Thermo-Tolerant Coliform Bacteria

A standard method according to ČSN ISO 4832:1995 was used for the detection and identification of thermo-tolerant coliform bacteria in the screening samples. The dilution of suspension was made according to ČSN ISO 6887-1. Petri dishes with m-FC agar (Merck, Germany) were inoculated with 100 µl of the sample and consequently incubated at 44ºC±1ºC for 18-24 hours. Thermo-tolerant coliform bacteria were indicated by the presence of dark blue colonies on agar.

Coliform Bacteria

A standard method according to ČSN ISO 4832:1995 was used for the detection of all coliform bacteria in the screening samples. The dilution of suspension was made according to ČSN ISO 6887-1. Petri dishes with ENDO agar (Merck, Germany) were inoculated with 100 µl of the sample and consequently incubated at 37ºC±1ºC for 24-48 hours. Coliform bacteria were indicated by the presence of white or red colonies on agar.

Enterococci

A standard method according to ČSN EN ISO 7899-2:2001 was used for the detection and identification of intestinal enterococci in the screening samples. Petri dishes with m- Enterococcus selective agar according to Slanetz and Bartley (Merck, Germany) were inoculated with 100 µl of the sample. The dishes with sample were incubated at 37ºC±1ºC for 4 hours and consequently at 44ºC±0.5ºC for 20-44 hours. Enterococci were indicated by the presence of pink to maroon colonies on agar.

Salmonella sp. – Over-Propagation Method

Buffer peptone soil (Merck, Germany) was used as a diluent for preparation of the base suspension. The suspension was incubated at 37ºC for 16-20 h. The obtained culture was inoculated into a liquid culture medium in accordance with Rappaport and Vassiliadis (41.5ºC – 48 h) (Merck, Germany), and subsequently into soil containing selenite and cystine (37ºC – 48 h) (Merck, Germany). Each of the obtained cultures was inoculated into two solid selective soils in Petri dishes, agar with phenol red and brilliant green (Merck, Germany), and xylosole-lysine-deoxycholate agar (XLD agar, Merck, Germany). Following 24 h of incubation at 37ºC, the presence of suspect colonies of *Salmonella* sp. bacteria was determined. The growth of typical colonies in phenol red and brilliant green soils results in a change of the soil color from pink to red. As regards the XLD agar soil, red and black to black colonies prove by the growth in the case of a positive finding.

Salmonella sp. – Confirmation

It is essential to confirm the presence of the suspect colonies of *Salmonella* sp. bacteria at least using suitable biochemical tests, for example Enterotest 24 (Erba Lachema, CZE). The results of the colour responses were evaluated using TNW identification software (Erba Lachema, CZE).

Results

The samples of screenings collected at various WWTPs contained the different amounts of organic content. The determined content of dry solids ranged, as regards individual samples, between 14% and 50%. The determined ash-free dry mass values of 40 samples of each WWTP ranged, between 25% and 90%. Arithmetic means of ash-free dry mass content in 40 samples of screenings from each WWTP is shown in Fig. 1. Other authors [1, 2] state in their works similar rates of dry matter and ash-free dry mass of the samples of the screenings from the wastewater treatment plants.

All the collected samples also were subject to a microbiological analysis focused on the indicator groups of microorganisms that are commonly determined in wastewater treatment sludge [6, 7] prior to its contingent use on agricultural land [8]. The scope covers coliform bacteria including *Escherichia coli*, enterococci, and faecal coliform bacteria (Figs. 2-4). The figures present arithmetic means of 40 samples tested on each WWTP. Additive statistical values are given in Table 2.

Discussion of Results

Totally missing information about the microbial contamination of the screenings from the wastewater treatment plants led us to develop a unique work not yet published in

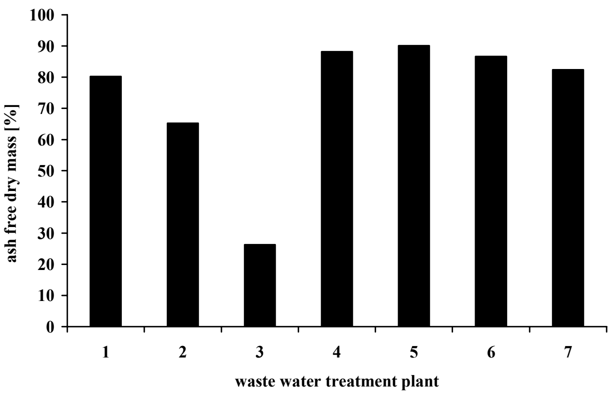

Fig. 1. Arithmetic mean of ash-free dry mass of samples of screenings from various WWTPs.

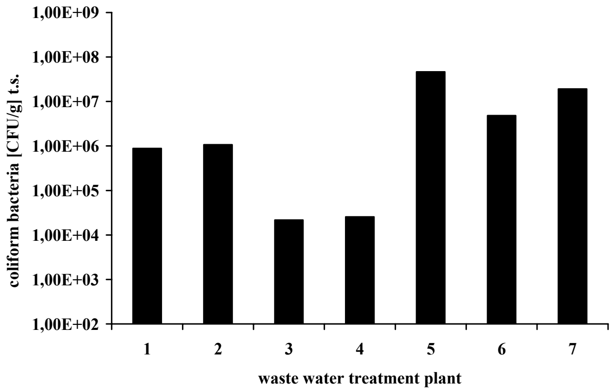

Fig. 2. Arithmetic mean of content of coliform bacteria in samples of screenings from various WWTPs.

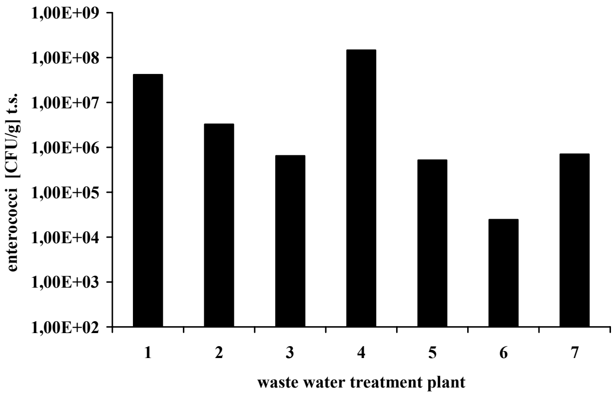

Fig. 3. Arithmetic mean of content of enterococci in samples of screenings from various WWTPs.

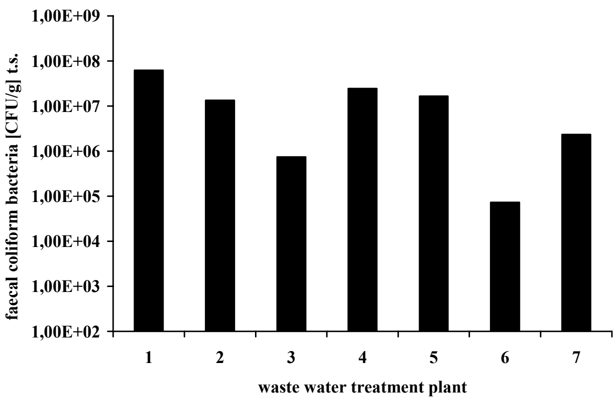

Fig. 4. Arithmetic mean of content of faecal coliform bacteria in samples of screenings from various WWTPs.

scientific journals. The microbial contamination of the screenings from the selected wastewater treatment plants is evident from submitted results. For a statistical evaluation it will be necessary to extend the data and perform further analyses to confirm the results obtained in the conducted tests. Samples collected gradually – in the course of 12 months – sometimes proved to feature similar parameters (WWTP No. 1), while the values determined at other

Table 2. Statistical evaluation of tested samples.

Sample	Indicator	Minimum [CFU/g]	Maximum [CFU/g]	Median [CFU/g]	Standard deviation [CFU/g]	Variance [CFU/g]
WWTP1	coliform bacteria	1.83E+05	1.24E+06	1.23E+06	4.96E+05	2.46E+11
	enterococci	2.06E+07	6.85E+07	3.56E+07	2.00E+07	4.00E+14
	feacal coliform bacteria	2.36E+07	8.26E+07	7.96E+07	2.71E+07	4.00E+14
WWTP2	coliform bacteria	5.36E+05	1.44E+06	1.23E+06	3.87E+05	1.50E+11
	enterococci	1.18E+06	6.35E+06	2.28E+06	2.22E+06	4.95E+12
	feacal coliform bacteria	8.56E+06	1.98E+07	1.23E+07	4.65E+06	2.17E+13
WWTP3	coliform bacteria	3.26E+03	5.72E+04	4.61E+03	2.51E+04	6.31E+08
	enterococci	4.92E+05	8.23E+05	6.15E+05	1.37E+05	1.87E+10
	feacal coliform bacteria	4.82E+05	9.75E+05	7.68E+05	2.02E+05	4.09E+10
WWTP4	coliform bacteria	5.60E+03	6.30E+04	7.78E+03	2.66E+04	7.05E+08
	enterococci	9.90E+07	2.30E+08	1.08E+08	5.98E+07	3.58E+15
	feacal coliform bacteria	8.90E+06	3.89E+07	2.56E+07	1.23E+07	1.51E+14
WWTP5	coliform bacteria	2.25E+04	9.55E+07	4.39E+07	3.90E+07	1.52E+15
	enterococci	1.38E+05	9.56E+05	4.67E+05	3.36E+05	1.13E+11
	feacal coliform bacteria	1.59E+05	3.68E+07	1.31E+07	1.52E+07	2.30E+14
WWTP6	coliform bacteria	2.80E+06	7.34E+06	4.33E+06	1.89E+06	3.56E+12
	enterococci	1.13E+04	4.36E+04	1.89E+04	1.38E+04	1.91E+08
	feacal coliform bacteria	4.10E+04	1.30E+05	4.92E+04	4.02E+04	1.62E+09
WWTP7	coliform bacteria	8.83E+04	5.12E+07	6.53E+06	2.27E+07	5.17E+14
	enterococci	6.75E+05	7.25E+05	6.98E+05	2.05E+04	4.20E+08
	feacal coliform bacteria	1.82E+06	3.11E+06	2.08E+06	5.60E+05	3.13E+11

WWTPs featured major fluctuations. For example, at WWTP No. 5 the difference between individual samples represented a multiple of 4,000 in the parameter of coliform bacteria as well as a multiple of 200 in the parameter of faecal coliform bacteria (Figs. 3 and 5). The respective results reflect the technological equipment at individual WWTPs, rainfall prior to collection of samples as well as numerous other factors that might affect the quality of inflowing wastewater, thus affecting its microbial contamination as well. Certainly, it is possible to confirm microbial contamination in all tested samples of screenings with a rather different content of microorganisms, which is mainly due to the different content of organic material in the individual samples of screenings. Biochemical testing proved the presence of *Salmonella* sp., specifically *Salmonella* subgroup 1 (WWTP No.7 and No.5) in only two tested samples of screenings. Other samples contained mainly other representatives of *Enterobacteriaceae* (*Klebsiella ornithinolytica* or *K. oxytoca*, *Enterobacter kobei*, *Proteus mirabilis,* and *Citrobacter braakii*). *Enterobacter* sp. bacteria represent typical faecal coliform microorganisms. WHO classifies all the aforementioned (mainly enteric) bacteria as Class 2 pathogenic microorganisms. They often originate from the intestines of humans or animals, and they are isolated from the environment – primarily from soil. Human clinical material represents the source of these bacteria only rarely.

Conclusion

The origin of screenings is related mainly to people's activities – they comprise mainly the faecal solids, hair, plastics, paper, etc. These materials are subsequently transported through the sewage system to a wastewater treatment plant. Our objective was to evaluate the microbial content of the screenings from the various wastewater treatment plants. The presence of potentially pathogenic microorganisms (faecal thermo-tolerant coliform bacteria, coliform bacteria, and enterococci) was proven in all tested samples. Furthermore, some samples proved the presence of *salmonella*, which originate (like all other identified enterobacteria) mainly from contaminated excrement.

Acknowledgements

Our study was financed by Research plan No. MSM6215648905 "Biological and technological aspects of sustainability of controlled ecosystems and their adaptability to climate change," financed by the Ministry of Education, Youth and Sports of the Czech Republic.

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