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EFFECTIVENESS OF INDICATOR BACTERIA REMOVAL IN VERTICAL FLOW FILTERS FILLED WITH NATURAL MATERIALS

The effectiveness of removing indicator bacteria from domestic sewage using a vertical flow filter filled with natural materials has been analysed. The count of *Escherichia coli* and coliform bacteria was measured in raw and treated sewage and effectiveness of their removal under variable conditions of hydraulic load and depending on the filter filling (type, size and filling proportions) was assessed. A single-layer sand or zeolite filter was the most effective in reducing bacterial contamination. The sand-filled filter allowed the removal of 99.993–99.997% of *Escherichia coli* and the one filled with fine zeolite of 99.995%. The principal component analysis was used to comprehensively evaluate the filter performance. Three principal components were identified and they were responsible for 81% of raw data variability. The effectiveness of indicator bacteria removal depended mainly on the filling grain size and did not on filling proportions or its type.

1. INTRODUCTION

Recently the idea of introducing an additional step of domestic wastewater treatment in the form of its final disinfection has been gaining popularity. Water from a typical wastewater treatment plant may still include from a few dozen thousand to around a million of coliform bacteria [1–4]. Treated wastewater is discharged mainly to surface rivers and water reservoirs. When the water leaving a wastewater treatment plant is not disinfected, bacteria, including pathogenic ones, are introduced into the receiving water bodies, deteriorate their sanitary status and may even cause their degradation.

This problem involves not only the wastewater from municipal wastewater treatment plants (WTP) but also treated sewage discharged from on-site sewage treatment facilities. Research shows that the removal of indicator bacteria in a septic tank/leach

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drains or soil-plant system is insufficient as evidenced by the presence of *Escherichia coli* and coliform bacteria in the wastewater treated in these systems [3, 5].

The collective name *Escherichia coli* comprises a few hundreds of different coliform strains. They form the colonic bacterial flora in humans and warm-blooded animals. One gram of faeces usually contains from 10^6 to 10^9 of these microorganisms [3]. The presence of allochthonous *Escherichia coli* in surface waters is a popular indicator of their contamination with secretions and faeces. Other coliform bacteria, e.g., *Klebsiella* sp., *Enterobacter* sp., *Citrobacter* sp. or *Proteus* sp. are also indicators of faecal contamination. Their presence indicates possible contamination with much more dangerous pathogenic bacteria of *Salmonella* sp. or *Shigella* sp. [6].

To reduce the count of pathogenic microorganisms entering the environment from on-site sewage treatment facilities, a final step involving disinfection must be introduced. This may be achieved by using a technology employed for bacteria removal from water, that is a slow filtration through a vertical flow sand bed. Langenbach et al. [7, 8] demonstrated the usefulness of such a filter as the third step of wastewater purification that allows the removal of faecal bacteria. The filters working for 59–148 days managed to reduce the count of *Escherichia coli* by (1.9–2.6) logarithmic units and to achieve mean count <100 MPN/100 cm^3. Such a good performance of the filtration beds depended mostly on the sand surface, determined by grain size distribution and filter height, and on a biofilm layer. This layer, called *schmutzdecke*, is formed on the border of sand and water and it is composed of not only of microorganisms but also of various organic and inorganic substances.

Bellamy et al. [9] demonstrated that the bacteria were removed due to their absorption on the surface of the biofilm that adhered to sand grains. According to Jenkins et al. [10], grain size is the key factor determining the performance of sand filters. Within ten weeks of their study, the removal of faecal coliforms was on average 1.4 logarithmic units, and for the most effective operation parameters of a filter filled with fine sand, the removal effectiveness was on average 1.8 logarithmic units, i.e., 98.5%.

Immobilization of pathogenic bacteria present in the wastewater that flows through a porous media is achieved via two mechanisms – filtration and adsorption [8, 11]. Filtration is to a high degree controlled by the size of the filter filling. The effectiveness of bacteria retention due to filtration is inversely proportional to the grain size of a filtration material [11]. Adsorption is the main mechanism of bacteria retention in porous media with the pore diameter larger than the bacteria. As the microorganisms are retained not only on the surface but also inside the pores, the resulting biofilm may serve as an additional sorbent and increase adhesion of the contaminants.

Elliott et al. [12] claimed filtration to be the most probably mechanism of *Escherichia coli* removal on schmutzdecke layer. The biofilm may grow and reach from a few to a few dozen centimeters inside the bed. An eight-week study with silica sand Accusand and crushed granite showed that the growth of schmutzdecke layer was the most important factor enhancing *Escherichia coli* removal up to 5 logarithmic units. Bacteria retention rate did not differ significantly for individual fillings, despite differences in their chemical composition and grain size distribution. The granite used in the study was characterized by a much wider range of particle size and had two orders of magnitude more Al and Fe on its surface than Accusand. Aluminum and iron oxides have a positive charge, which is why they are capable of attracting Gram-negative bacteria such as *Escherichia coli* [13, 14]. However, due to the qualitative composition of water, this mechanism of bacteria removal is a short-term one. Foppen et al. [15] proved that dissolved organic matter blocks the sites on the surface of metal oxides to which *Escherichia coli* may be attracted.

New solutions based on biological beds filled with porous materials often increase the efficiency of wastewater treatment but they may be inadequate in terms of their microbiological quality. Therefore, supplementation of the porous materials with a layer of quartz sand seems important. Quartz sand is both inexpensive and provides an effective barrier for the indicator bacteria. Properly designed vertical flow sand filters ensure substantial removal (up to several orders of magnitude) of the pathogenic bacteria. Sewage treated with those filters may be purified to the mean level of 10^2–10^4 CFU for *Escherichia coli* and 10 CFU for *Salmonella* sp. and *Shigella* sp. [16].

In the paper, the possibility of using advanced statistical methods, i.e., principal component analysis (PCA) has been analyzed to determine the effect of the type, size and filling proportions of the materials filling a vertical flow filter on the effectiveness of indicator bacteria removal.

2. MATERIAL AND METHODS

A semi-technical installation comprising two model columns preceded by a septic tank was constructed. The installation contained a few identical, PVC, 1000 cm high columns, 200 mm (model 1) or 150 mm (model 2) in diameter (Fig. 1). They were filled with two types of porous materials and sand. Quartz sand, the most popular filling of vertical flow filters, had an equivalent diameter of d_{10} = 0.32 mm. Two other natural porous fillings characterized by high total pore volume were a natural zeolite (aluminosilicate with SiO_2 content up to 67%, Al_2O_3 content reaching 12%, and 1% Fe_2O_3) and expanded clay. Three size ranges of the filling were analyzed, further referred to as coarse (>4.0 mm), medium (2.5–5.0 mm), and fine (<2.5 mm). In model 1, the columns were filled with 700 mm layer of the porous material or sand (single layer filters). In model 2, four columns were filled with fine zeolite and sand in various proportions: (100, 25, 50, and 75% of zeolite, double-layer filters). Each filter was supplied with the same amount of sewage of similar quality pretreated in a septic tank. The sewage was supplied periodically (24 doses per day). The analysis was performed for the previously developed models that continuously operated at variable hydraulic conditions for 10–11 months. Hydraulic load of the filter surface varied throughout the study from 16 to 64 mm·day^{-1}.

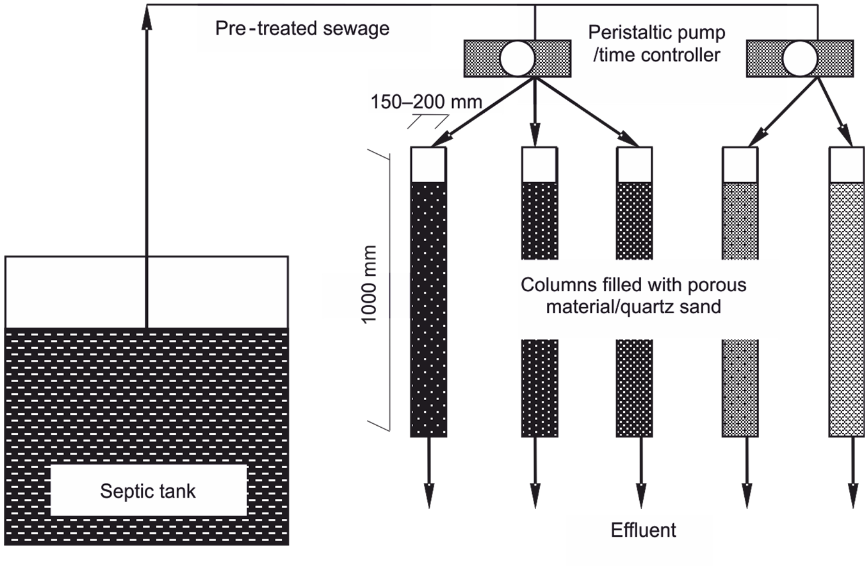

Fig. 1. Schematic model of vertical flow filters (dimensions in mm)

Exudate samples were collected every week in a sterile way. They were then subjected to bacteriological analysis involving the determination of two types of indicator bacteria. The analyses of *Escherichia coli* and coliform bacteria employed two methods. In the raw sewage entering the filters, the bacteria were determined by means of serial dilution and surface plating and in the treated sewage by means of membrane filtration. In both cases, Endo media manufactured by BioMérieux were used. The effectiveness of indicator bacteria count reduction in individual columns was assessed based on their percentage removal rate and logCFU removal calculated as (log of the count in the raw sewage) – (log of the count in the treated sewage).

The results were statistically analyzed using STATISTICA 12 software. For both models, the arithmetic mean, minimum and maximum value, standard deviation and coefficient of variation of those contamination indicators were determined. The second stage of statistical processing was the use of principal component analysis (PCA) to identify the relationships in a data set comprising seven factors describing the filling and the indicator bacteria. PCA involves a reduction of the number of correlated principal variables and replacing them with components that significantly explain their variability. Therefore, this analysis makes interpretation of the results easier but still retains maximum information about the process [17–19]. Scree criterion was used to decide on the number of principal components to be used in PCA method. The last step involved identification of the correlations between factorial variables based on a two-dimensional plot of their coordinates.

3. RESULTS AND DISCUSSION

The statistical analysis revealed that the number of bacteria in the sewage pre-treated in the septic tank and then entering the columns was similar to that reported by other authors [3, 5]. The value >0.5 of the ratio of BOD_5/COD_{Cr} in the wastewater flowing to the pre-treated columns indicates the presence of the easily degradable organic substances in biochemical processes [20, 21] (Table 1).

Table 1

Characteristics of the pre-treated sewage entering the columns in models 1 and 2

Parameter Indicator bacteria	Descriptive statistics				
	Mean value	Minimum value	Maximum value	Standard deviation	Coefficient of variation
Physical and chemical characteristics					
pH	8.51	8.36	8.72	0.15	0.02
BOD_5, mg $O_2 \cdot dm^{-3}$	196.4	40.0	400.0	90.8	0.46
COD_{Cr}, mg $O_2 \cdot dm^{-3}$	366.4	131.0	536.0	106.9	0.29
Bacteriological characteristics					
Escherichia coli, CFU/100 cm^3	$1.29 \cdot 10^8$	$4.00 \cdot 10^5$	$5.00 \cdot 10^8$	$1.85 \cdot 10^8$	1.44
Coliform bacteria, CFU/100 cm^3	$3.12 \cdot 10^8$	$6.80 \cdot 10^6$	$1.10 \cdot 10^9$	$3.01 \cdot 10^8$	0.97

The first stage of the study focused on a comparison of the indicator bacteria removal in a single layer filter under variable hydraulic load. The results are shown in Table 2.

Despite a high coefficient of variation, the lowest count of *Escherichia coli* (10^2–10^3 CFU) was detected in the effluents from the columns filled with fine sand, zeolite and expanded clay and medium zeolite. The bacteria removal rate was 4.1–4.8 logarithmic units. These values were similar to the results obtained by Elliott et al. [12]. The count of coliform bacteria in the wastewater filtered through fine expanded clay and zeolite of three grain sizes was similar (10^3–10^4 CFU). The bacteria removal rate in these filters was 3.5–4.6 logarithmic units. The increase in the effectiveness of removal of Gram-negative *Escherichia coli* due to electrostatic attraction to the zeolite surface (as a result of the adsorption process) was not observed. Due to the presence of dissolved organic matter in the raw sewage, the surface of zeolite was blocked as Foppen et al. [15] suggested in their research.

Table 2

The count of *Escherichia coli* and coliform bacteria in the effluents from model 1 columns depending on the type and size of the filling

Filling type/size	Descriptive statistics ($n = 8$)	Fine	Medium	Coarse
Escherichia coli [CFU/100 cm^3]				
Sand	mean	6.05×0^3	–	–
	median	2.05×10^3	–	–
	minimum	1.00×10^2	–	–
	maximum	2.00×10^4	–	–
	coefficient of variation	1.57	–	–
Zeolite	mean	5.75×10^2	2.50×10^3	8.67×10^3
	median	1.00×10^2	1.40×10^3	7.50×10^3
	minimum	1.00×10^2	2.00×10^2	1.00×10^3
	maximum	2.00×10^3	6.00×10^3	2.00×10^4
	coefficient of variation	1.65	1.12	0.84
Expanded clay	mean	3.08×10^3	2.93×10^5	3.45×10^5
	median	1.00×10^3	8.50×10^4	3.00×10^5
	minimum	1.00×10^2	2.00×10^4	8.00×10^4
	maximum	1.20×10^4	1.00×10^6	7.00×10^5
	coefficient of variation	1.93	1.62	0.79
Coliform bacteria [CFU/100 cm^3]				
Sand	mean	1.09×10^5	–	–
	median	5.40×10^4	–	–
	minimum	2.60×10^4	–	–
	maximum	3.00×10^5	–	–
	coefficient of variation	1.20	–	–
Zeolite	mean	1.85×10^4	1.15×10^4	3.47×10^4
	median	6.00×10^3	9.00×10^3	2.50×10^4
	minimum	2.00×10^3	2.00×10^3	8.00×10^3
	maximum	6.00×10^4	2.80×10^4	1.00×10^5
	coefficient of variation	1.50	0.78	0.95
Expanded clay	mean	5.05×10^4	6.25×10^5	5.90×10^5
	median	3.50×10^4	5.70×10^5	5.80×10^5
	minimum	2.00×10^3	1.60×10^5	3.00×10^5
	maximum	1.30×10^5	1.20×10^6	9.00×10^5
	coefficient of variation	1.15	0.75	0.42

A single-layer sand or zeolite filter was the most effective in reducing bacterial contamination. The sand-filled filter allowed for the removal of 99.993–99.997% of *Escherichia coli* and the one filled with fine zeolite of 99.995% (Table 3). The effectiveness of zeolite-filled filters encouraged the authors to test sand filters with an additional layer of zeolite on the top (model 2). Mean removal rate was definitely the highest in the double-layer filter containing 75% of sand and 25% of fine zeolite. It allowed the removal of 99.993% of *Escherichia coli* and 99.953% of coliforms.

Table 3

Mean effectiveness of indicator bacteria removal in single and double layer filters depending on the type and proportion of filling materials [%]

Single-layer filter				
Bacteria	Filling type/size	Fine	Medium	Coarse
Escherichia coli	sand	**99.997**	–	–
Coliform bacteria		**99.993**	–	–
Escherichia coli	zeolite	**99.995**	99.985	99.951
Coliform bacteria		99.977	99.964	99.94
Escherichia coli	expanded clay	99.979	97.991	97.017
Coliform bacteria		99.816	99.515	99.632
Double-layer filter				
	Filling type/proportion [%]	25%/75%	50%/50%	75%/25%
Escherichia coli	fine zeolite/sand	**99.993**	98.807	99.901
coliform bacteria		99.953	99.925	99.924

The principal component analysis was used to comprehensively evaluate the filter performance. A correlation matrix for seven variables selected for the analysis of indicator bacteria removal is presented in Table 4.

Table 4

Matrix of the correlation coefficients for the variables describing the process of the indicator bacteria removal

	Filling material	Filling size	Filling proportions	*Escherichia coli*	Coliform bacteria	log removal *E. coli*	log removal coliforms
Filling material	1.0000						
Filling size	–0.1984	1.0000					
Filling proportions	–0.8481	0.3329	1.0000				
Escherichia coli	–0.0072	0.3254	0.1470	1.0000			
Coliform bacteria	0.0252	0.3825	0.1770	**0.8262**	1.0000		
log removal *E. coli*	–0.0712	–0.3407	–0.0727	–0.5110	–0.4872	1.0000	
log removal coliforms	0.0573	–0.3349	–0.2054	–0.4307	–0.5594	0.6501	1.0000

Correlation coefficients between the variables describing the indicator bacteria and those describing the filling were low. Strong positive correlations marked in bold denote pairs of variables *Escherichia coli* and coliform bacteria. The greater the absolute values of linear correlation coefficients, the smaller number of major components carried the majority of information contained in the original set of variables was [18, 19].

Eigenvalues of the correlation matrix that reflect the variability of the raw data in the form of principal components are presented in Table 5. As required by the hierarchy of explaining the information resources of the raw data, the first column of the table shows the eigenvalues of consecutive principal components, which are also their variances. Total percentage indicated that the first principal component carried 44% of the information contained in the original variables, and the second component was responsible for 26.4% of the information. Following the criterion of eigenvalue, further analysis was based on the first three principal components. The last column that comprises cumulative percentage variance shows that the first three principal components were responsible for over 81% of the information contained in the original data.

Table 5

Values and cumulative eigenvalues of the principal components and percentages and cumulative percentages of the total variance

No.	Eigenvalue	% total – variance	Cumulated eigenvalue	Cumulated % variance
1	**3.082399**	44.03427	3.082399	44.0343
2	**1.850012**	26.42875	4.932411	70.4630
3	**0.747204**	10.67434	5.679615	**81.1374**
4	0.685217	9.78881	6.364832	90.9262
5	0.367621	5.25173	6.732453	96.1779
6	0.156976	2.24251	6.889429	98.4204
7	0.110571	1.57959	7.000000	100.0000

Table 6

Values of the principal component coefficients (factor coordinates of variables)

	Factor 1	Factor 2	Factor 3
Filling material	–0.215914	**0.928868**	0.038095
Filling size	**0.611547**	–0.201242	0.207355
Filling proportions	0.415477	**–0.863764**	–0.037860
Escherichia coli	**0.800502**	0.223909	–0.479724
Coliform bacteria	**0.842347**	0.225515	–0.385556
log removal *E. coli*	**–0.740583**	–0.287232	–0.402151
log removal coliforms	**–0.768344**	–0.130903	–0.401010

Table 6 presents an interpretation of factorial coordinate values for the principal components that were also the linear correlation coefficients between the input variables and the principal components. The first principal component represents the following variables: *Escherichia coli* and coliform bacteria, their removal and Filling size. The second principal component carries the data contained in the following variables: Filling type and Filling proportions.

To assess the contribution of the information contained in the input variables explained by three principal components selected for further analysis, the sum of squared coefficients of principal components, so called communality, was used (Table 7). The first three principal components carried together about 77% of the information on Filling size variable. For *Escherichia coli* and Coliform bacteria variables this value was about 93%.

Table 7

Communality values

Shared variable based on the correlation			
	1 factor	2 factors	3 factors
Filling material type	0.021902	0.881406	**0.930601**
Filling size	0.260274	0.362258	**0.767181**
Filling proportions	0.106962	0.911890	**0.926709**
Escherichia coli	0.818860	0.846078	0.846919
Coliform bacteria	0.824812	0.846935	0.861162
% removal of *E. coli*	0.322577	0.368011	0.701144
% removal of coliform	0.754875	0.801753	0.801805

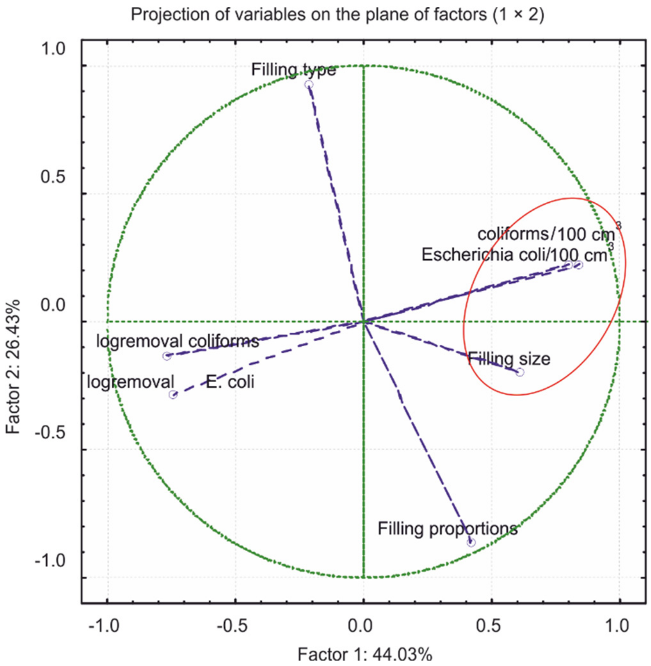

Fig. 2. Location of load vectors towards principal components (factors 1 and 2)

The relationships between seven input variables and three principal components are presented in Fig. 2. Location of three variables, i.e., *Escherichia coli*, Coliform bacteria and Filling size (inside ellipse) reflects their positive correlation. Two variables describing bacteria removal are in the close vicinity. Variables perpendicular to each other denote a lack of correlation. This type of relationship was observed for the variables describing the type of indicator bacteria and the type of filling.

Advanced statistical methods used made it possible to simplify the interpretation of seven factors describing the filling and the indicator bacteria. Principal component analysis showed that the effectiveness of indicator bacteria removal depended mainly on the filling grain size and not on the filling type or filling proportions. This means that – like in Elliott et al. research [12] – filtration was the primary mechanism of bacteria retention.

4. SUMMARY AND CONCLUSIONS

The count of *Escherichia coli* and coliform bacteria was measured in raw and treated sewage and effectiveness of their removal depending on the filter filling (type, size and filling proportions) was assessed.

The lowest count of *Escherichia coli* (10^2–10^3 CFU) was detected in the effluents from the columns filled with fine sand, zeolite and expanded clay and medium zeolite. The count of coliform bacteria in the wastewater filtered through fine expanded clay and zeolite of three grain sizes was similar (10^3–10^4 CFU). A single-layer sand or zeolite filter was the most effective in reducing bacterial contamination. The sand-filled filter allowed the removal of 99.993–99.997% of *Escherichia coli* and the one filled with fine zeolite of 99.995%.

The filter effectiveness depended not only on sand surface area determined by the grain size but also on the active height of the sand filter. Mean removal rate was definitely the highest in the double-layer filter containing 75% of sand and 25% of fine zeolite. It allowed the removal of 99.993% of *Escherichia coli* and 99.953% of coliforms.

Principal component analysis employed to assess the filtration performance allowed for identification of three primary components that explained over 81% of the raw data variability. The effectiveness of indicator bacteria removal depended mainly on the filling grain size and not filling type or filling proportions.

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