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Total cell count as an alternative to the total number of microorganisms for technological analysis of water treatment processes

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ABSTRACT

This article presents the results of a comparative study of culture and cytometric methods of determining microorganism numbers in water after subsequent unit processes of surface water treatment. This study aimed to find a relationship between the two results and to evaluate the possibility of using this method in the operation of water treatment processes. These studies have shown, that the number of cells found in cytometric measurements is many times greater than the total psychrophilic and mesophilic organism counts. Rapid information concerning intense increases of algae cells in water may be used for controlling the coagulation process. The effectiveness found in reducing the number of microorganisms or cells in unit water treatment processes indicates a different direction of changes. The relationships between the number of microorganisms and the number of cells are different for different water treatment stages, and therefore, this method must be calibrated prior to use for each type of water (after every unit process).

Keywords: Flow cytometry; Psychrophilic microorganisms; Mesophilic microorganisms; Total cell count

1. Introduction

The increase in the contamination of source waters results not only in the use of a more complex treatment process trial [1,2] but also in the use of modern and precise analytical methods for monitoring water quality and the variability in water composition. This allows for the possibility of evaluating hazards related to water consumption connected with the presence of harmful or toxic contaminants. Generally, determining the number of chemical contaminants that are subject to obligatory monitoring is not problematic and does not require a long period of time before the analysis results are available. Microbiological analysis, on the other hand, whose methods are also well known, is time-consuming, and therefore the detection of microbiological contamination and the technological reaction usually occurs with a significant delay. Due to the very large hazard to health connected with the presence of microorganisms, including pathological ones [3], studies are conducted worldwide concerning microbiological sensors [4] or creating new methods of microbiological analysis [5,6]. This problem concerns not only the source water and output water introduced into the distribution network but also water after unit treatment processes.

Therefore, in the last few years, studies have been conducted concerning the use of flow cytometry for determining the number of microorganisms in water [7,8]. This method allows for determining the total cell count (TCC) [9,10], and not only the number of colony-forming organisms. Due to this, the numbers obtained from flow cytometry

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are greatly larger than those obtained by culture methods [11]. This causes the fact that in the majority of European countries, flow cytometry is not a legally recommended method for determining the total number of microorganisms or the number of indicator bacteria. It is possible, using the characteristics of individual strains, to determine the number of, for example, *Escherichia coli* bacteria. However, this requires the use of flow cytometry together with adenosine triphosphate or polymerase chain reaction analysis.

Therefore, this method has not yet been widely used in water treatment technology [12]. Additionally, the large sensitivity and large analytical error of this analysis cause the fact that this method is not commonly used in water analyses. An indisputable advantage of this method is, however, obtaining a result in several minutes [13].

In some European countries, this method is used for the on-line monitoring of water contamination in the distribution system [14,15]. It is not a method, however, that is widely recommended. Some countries use this method for monitoring water microbiological quality in distribution systems, but not for assessment of hazards related to water consumption. The colony methods are used for microbiological pollution assessment because most of the pathogenic bacteria can form colonies. On the other hand the analysis error for the flow cytometry method is too high for it to be an indicator of microbiological hazards. Every water has a specific number of cells, therefore observation of their changes is used to monitor changes in the distribution system for example biofilm development.

This article presents the results of a comparative study of culture and cytometric methods of determining microorganism numbers in water after subsequent unit processes of surface water treatment.

2. Subject and methods of study

The studies were performed in a pilot surface water treatment system with a throughput of 1 m^3/h, consisting of the following processes: volume coagulation with a pre-hydrolyzed aluminum coagulant (PAX-XL3), sedimentation, filtration, adsorption on micropores formed activated carbon (WG12) bed and disinfection by sodium hypochlorite. The object of this study was water samples taken before and after each unit process over the course of 5 months, that is, between August and December. Such a study period allowed for an evaluation of the utility of flow cytometry at different water contamination levels, especially since the study period included the intense algae growth that takes place in autumn.

The process parameters of the unit treatment processes are presented in Table 1.

Samples were taken weekly and, apart from indicators of physicochemical composition, the total number of psychrophilic and mesophilic microorganisms and the TCC were determined, along with the number of damaged cells, which allowed for a determination of the intact cell count (ICC). For the cytometric analysis, the following DNA dyes were used SYBR Green (SG) – TCC determination and pyridine iodine – analysis of damaged cell numbers [16]. The cytometric analysis was performed with a BD C6 cytometer.

The total psychrophilic and mesophilic organism count analysis was performed by culture methods in accordance with current Polish standards (PN-EN ISO 6222).

Water analysis by both methods came from the same samples taken into sterile dark containers and was performed just after sampling.

This study aimed to compare the two microbiological water analysis methods and to determine the possibility of using flow cytometry to control water treatment processes, therefore evaluating flow cytometry as a process monitoring tool.

3. Results and discussion

The sourced surface water was characterized by high variability in the contamination level, and therefore by varied numbers of microorganisms and cells (Table 2).

Variation in water quality, as the effect of seasonal changes, characterized raw water. The most variation concerns the turbidity, temperature, and watercolor. It can be the effect of algae growth in raw water, but the correlation between the number of microorganisms and chemical parameters was not observed.

During the initial part of the study (August/September) the presence of a large number of algae was found in the source water (Fig. 1). This fact may be confirmed based on the cytometric plot of the water, as algae exhibit autofluorescence [17,18].

All water samples were characterized by the concentration of biodegradable dissolvet organic carbon (Table 2) higher than the limit of bacteria growth [19], also it relates to inorganic nutrients substrates. The concentration of analyzed water chemical quality parameters decreased in every unit

Table 1
Unit treatment process parameters

No.	Unit process	Used dosage	Retention time (min)	
1.	Coagulation with sedimentation (pre-hydrolyzed aluminum coagulant; basicity 70%)	1.287–4.700 g Al/m^3	Speed mixing	6
			Floculation	60
			Sedimentation	360
2.	Filtration	–	30	
3.	Adsorption (formed active carbon-WG-12, specific surface area – 976 m^2/g)	–	50	
4.	Disinfection	0.5–1.5 g Cl_2/m^3	–	

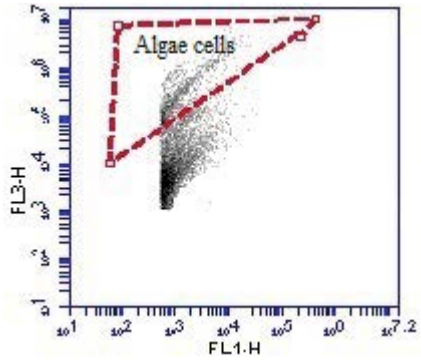

Fig. 1. Algae autofluorescence in raw water in the cytometric image during the time the intense algae growth (FL1 – green fluorescence and FL3 – blue fluorescence).

processes of water treatment.However, changes in chemical parameters value were not correlated with microbiological ones. It means that chemical water quality can stimulate the regrowth of microorganisms for example on the surface of filtrate beds.

The presence of algae in conventional microbiological analyses may be determined visually – during algal blooms the water color changes [20,21] or under a microscope [22]. Such studies are usually only performed during intense algal blooms. Furthermore, cytometric methods allow not only for determining algae presence but also for their number, which is much more difficult in classic methods. Rapid information concerning intense increases of algae cells in water may be used for controlling the coagulation process – increasing the coagulant dosage and the maximum water post-coagulation sedimentation time. Removing algae from water during the coagulation and sedimentation processes requires increased coagulant dosages [23,24], which results from the need to agglomerate these cells in the post-coagulation suspension. In natural conditions, without additional processes, these cells float and do not sediment.

These studies have shown, as previous studies [25,26] have, that the number of cells found in cytometric measurements is many times greater than the total psychrophilic and mesophilic organism counts that have been obtained (Fig. 2).

During this study, it was also found that the number of mesophilic and psychrophilic organisms in the source water was directly proportional to the total number of cells found by dying with SG (Fig. 3). It must be noticed that this relationship is more significant for psychrophilic organisms than for mesophilic organisms. This is connected to the presence of a much smaller number of mesophilic organisms in natural waters.

The presence of such a relationship allows for an initial evaluation of the number of microorganisms in water and maybe the basis for making decisions with regards to coagulation process parameters, for example, the coagulant dosage.

The total number of psychrophilic organisms in raw water was also directly proportional to the ICC, which confirms the possibility of the technical utility of results obtained from cytometry.

Table 2
Ranges of water quality parameters

Parameter	Unit	Raw water		Settling outflow		Sand filter outflow		Carbon filter outflow		Purified water	
		Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
T	°C	12.8	24.5	14.0	24.4	12.9	24.5	14.3	24.3	13.8	24.6
pH		6.38	7.85	6.78	7.61	6.60	7.57	6.32	7.71	6.10	7.53
C_{340}	g Pt/m^3	5.91	10.35	3.85	6.93	3.73	6.05	2.18	3.87	1.77	2.86
Turbidity	NTU	2.14	11.70	0.15	3.83	0.01	0.28	0.01	0.49	0.01	1.86
Total organic carbon	g C/m^3	3.12	5.37	2.41	3.95	2.21	3.22	–	–	1.65	4.46
Dissolvent organic carbon	g C/m^3	3.02	4.70	2.30	3.72	2.27	3.14	1.69	3.05	1.58	4.37
Biodegradable dissolvet organic carbon	g C/m^3	0.34	0.96	0.27	0.79	0.22	0.68	0.27	0.98	0.15	0.40
NH_4^+	g NH_4^+/m^3	0.02	0.19	0.01	0.16	0.003	0.018	0.001	0.009	0.001	0.008
NO_3^-	g NO_3^-/m^3	0.22	9.91	0.15	9.94	0.21	10.42	0.23	10.29	0.21	10.22
PO_4^{-3}	g PO_4^{-3}/m^3	0.16	11.70	0.02	0.21	0.01	0.12	0.01	0.10	0.01	1.93
TCC	cell/mL	1,376	339,929	3,817	137,658	523	115,734	200	75,404	101	73,375
ICC	cell/mL	961	337,365	9,645	134,801	6,770	115,676	815	75,332	43	73,192
TNMpsych	cfu/mL	990	9,200	170	8,100	36	920	11	1,300	0	57
TNMmeso	cfu/mL	100	7,700	7	6,000	3	240	2	860	0	22

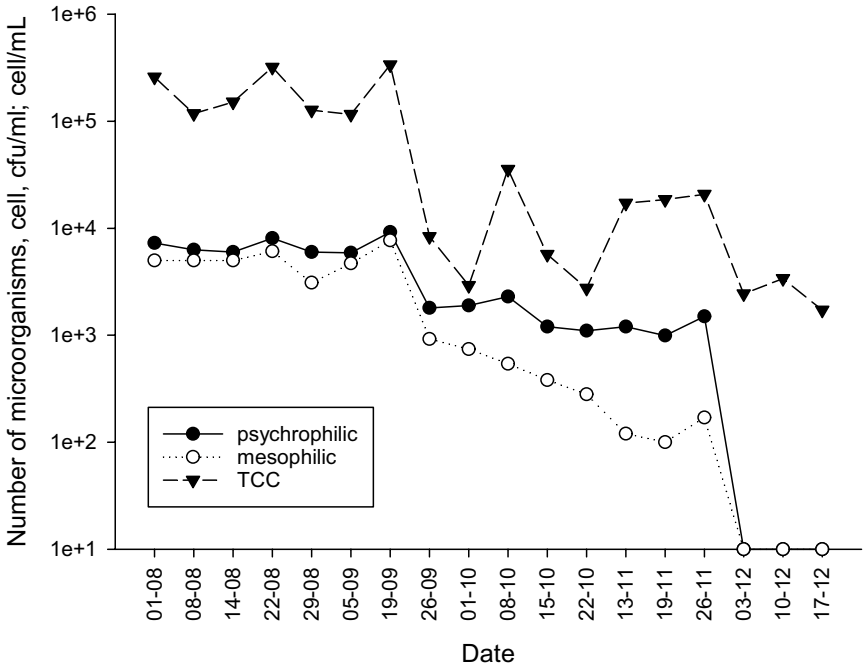

Fig. 2. Variation in the number of psychrophilic and mesophilic organisms and the total number of cells.

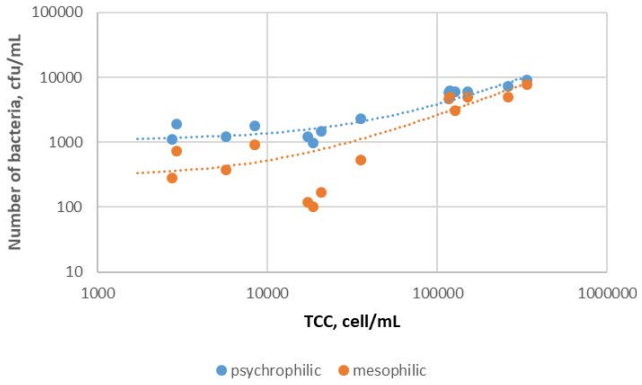

Fig. 3. Relationship between the total cell number of cells and the number of psychro- and mesophilic microorganisms in raw water.

The correlation between the total number of psychrophilic microorganisms and TCC was found for water after subsequent unit processes (Table 3), although the correlation coefficients were lower with lower numbers of microorganisms in the analyzed water.

The reduction in the correlation coefficient probably results from the large error of cytometric analyses, which causes significantly smaller precision, especially in the case of low-contaminated water.

This indicates a possibility of an initial evaluation of the effectiveness of removing microorganism cells in the first processes of treatment systems, that is, coagulation with sedimentation and rapid filtration, and the limited implementation potential in the final water treatment stages.

The information concerning the microorganism numbers in the filtrate allows for an evaluation of bed flushing effectiveness. Rapid information concerning the number of organisms may form the basis for determining the filter flushing time and may be used for determining the filtration cycle time (frequency of filtration as a function of input water quality)

In water after disinfection, the correlation between the number of psychrophilic microorganisms and the total number of cells was greatly reduced. This is probably due to the transformations that cells undergo during the oxidation process. This theory is confirmed by the correlation that was found between the total number of psychrophilic organisms and the total number of intact cells, or those which have not been destroyed during chlorination.

The effectiveness found in reducing the number of microorganisms or cells in unit water treatment processes indicates a different direction of changes in the number of cells found for culture and cytometric analyses (Table 4).

The total microorganism number (TNM) psychrophilic and mesophilic decrease during all unit water treatment processes except for adsorption by formed active carbon. The decrease of TNM psychrophilic and mesophilic microorganisms in unit processes caused by cell adsorption on the suspension surface (coagulation and filtration processes) or cells destroyed during chemical disinfection. The increase of TNM during adsorption is the effect of microorganisms being released from biofilm developed on the surface of activated carbon. This effect can be observed periodically in the intense microorganisms development period.

The changes in TCC and ICC had different trends, which can be caused by different analysis methods. During coagulation, process cells can be adsorbed on the coagulation suspension, similar to the TNM removal. The increase of TCC and ICC during coagulation takes place periodically only before sedimentation suspension removal. In that situation cells or parts of DNA are released to the treating water.

Mechanical separation of cells resulted in a decrease of TCC during filtration. The increase of ICC was below 7% and can perhaps be caused by the analytical error. The different direction of changes in TCC and ICC during adsorption was the effect of the phase of biofilm developing on the surface of beds, as showed by Gibert et al. [27].

The increase in the number of cells during disinfection is due to their damage and division of DNA. The increase in the number of intact cells that were found is insignificant and is caused by the analytical error of this method.

Different changes and their direction in both compared methods can be the effect of different mechanisms of analysis. The cytometry flow can show all parts of DNA, but the standard procedure only presents microorganisms which can form the colony. Sometime during unit water treatment process cells can be destroyed with causes a decrease of TNM and an increase of TCC (because it concerns more fragments of DNA). On the other hand the analytical error in cytometry methods is a few times larger than culture methods.

The mean effectiveness in removing microorganisms in the filtration process amounted to 77% and 76% for psychrophilic and mesophilic microorganisms respectively. The mean effectiveness of this process that was found with respect to TCC and ICC amounted 49% and 35% respectively. The effectiveness differences that were found for the disinfection process were smaller and amounted to 92%, 90%, 59%, and 82% for TNM_p, TNM_m, TCC, and ICC respectively. The significantly lower effectiveness in removing TCC is due to the fact that this number also contains damaged (deactivated) cells. This thesis is proven by the correlation between the reduction of the total number of psychrophilic bacteria and ICC in the disinfection process (Fig. 4).

On the other hand, the increase in the total number of cells during the coagulation process that was found for many water samples is probably caused by the release of cells from the sediment. These are mainly damaged cells.

However, during the adsorption process, flushing of microorganisms populating the bed into the flowing water occurs [28], which is why the increase that was found did not depend on the analysis method.

A significant correlation between the change in the number of psychrophilic microorganisms and the total cell number was found only for the filtration process. This means, that in the context of this process, flow cytometry may be used for evaluating changes in the number of microorganisms. In the context of other processes, it would be more reasonable to use the relationship between ICC and TNM, as the correlation coefficients for these parameters were larger than those found for TNM and TCC.

The relationships between the number of microorganisms and the number of cells are different for different water treatment stages, and therefore, this method must be calibrated prior to use for each type of water (after every unit process).

4. Conclusions

- The TCC found in the analyzed waters is many times larger than the total number of psychrophilic and especially mesophilic microorganisms.
- The number of cells in raw water was almost directly proportional to the total number of psychrophilic and mesophilic microorganisms.

Table 3
Relationship between TCC and the total number of psychrophilic organisms after subsequent unit processes

Water	Correlation equation	*R*
After sedimentation	TCC = 24.687 × Psych + 15,007	0.92
After filtration	TCC = 63.402 × Psych + 52,458	0.87
After adsorption	TCC = 80.12 × Psych + 20,831	0.86
After disinfection	TCC = 195.11 × Psych + 1,909	0.45

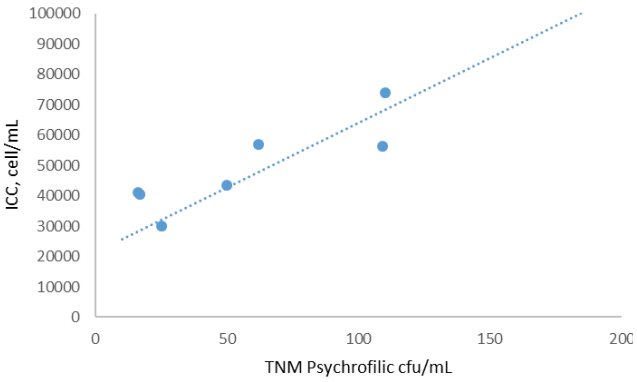

Fig. 4. Relationship between the total number of psychrophilic microorganisms and the total number of cells undamaged during the disinfection process.

Table 4
The direction of changes in the number of microorganisms/cells in unit processes

Process		Psychrophilic	Mesophilic	TCC	ICC
Coagulation	Increase %	0	0	44.5	37.5
	Decrease, %	100	100	55.5	62.5
Filtration	Increase %	0	0	0	12.5
	Decrease, %	100	100	100	87.5
Adsorption	Increase %	22.3	27.8	55.5	62.5
	Decrease, %	77.7	72.2	44.5	37.5
Disinfection	Increase %	0	0	11.1	12.5
	Decrease, %	100	100	88.9	87.5

- The lack of correlation between chemical and microbiological parameters of water quality limited used the flow cytometry method to water treatment processes monitoring.
- The number of intact cells in waters after every unit treatment processes was directly proportional to the number of psychrophilic microorganisms. This correlation was not observed for TNM mesophilic.
- The TCC can be used for monitoring of water microbiological contamination but is useless in the assessment of changes of this contamination.
- The lack of one direction of change of the TCC in unit water treatment processes with the use of both methods testifies to the limited potential of using flow cytometry for process monitoring of changes in contamination in water treatment systems.
- The use of flow cytometry for water contamination monitoring requires calibration.

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References

[1] H.X. Wang, L. Ho, D.M. Lewis, J.D. Brookes, G. Newcombe, Discriminating and assessing adsorption and biodegradation removal mechanisms during granular activated carbon filtration of microcystin toxins, Water Res., 41 (2007) 4262–4270.
[2] J.K. Kim, B.S. Kang, DBPs removal in GAC filter-adsorber, Water Res., 42 (2008) 145–152.
[3] M. Tichoniuk, Electrochemical DNA Biosensor for the Detection of Pathogenic Microorganisms in Food, Scientific Papers, University of Economics in Poznan, Poznań, 183 (2011) 27–41.
[4] A. Kłos-Witkowska Microbiological Biosensors Based on Luminescence, Pomiary Automatyka i Robotyka, 21 (2017) 79-84 (in Polish).
[5] J. Zheng, J.F. Zhao, Y.Z. Tao, J.H. Wang, Y.J. Liu, J.J. Fu, Y. Jin, P. Gao, J.P. Zhang, Y.F. Bai, G.Y. Wang, Isolation and analysis of water stress induced genes in maize seedlings by subtractive PCR and cDNA macroarray, Plant Mol. Biol., 55 (2004) 807–823.
[6] R.A. Guy, P. Payment, U.J. Krull, P.A. Horgen, Real-time PCR for quantification of *Giardia* and *Cryptosporidium* in environmental water samples and sewage, Appl. Environ. Microbiol., 69 (2003) 5178–5185.
[7] D.A. Veal, D. Deere, B. Ferrari, J. Piper, P.V. Attfield, Fluorescence staining and flow cytometry for monitoring microbial cells, J. Immunol. Methods, 243 (2000) 191–210.
[8] D. Hoefel, W.L. Grooby, P.T. Monis, S. Andrews, C.P. Saint, Enumeration of water-borne bacteria using viability assays and flow cytometry: a comparison to culture-based techniques, J. Microbiol. Methods, 55 (2003) 585–597.
[9] M. Berney, F. Hammes, F. Bosshard, H.-U. Weilenmann, T. Egli, Assessment and interpretation of bacterial viability by using the LIVE/DEAD BacLight Kit in combination with flow cytometry, Appl. Environ. Microbiol., 73 (2007) 3283–3290.
[10] T. Falcioni, S. Papa, J.M. Gasol, Evaluating the flow-cytometric nucleic acid double-staining protocol in realistic situations of planktonic bacterial death, Appl. Environ. Microbiol., 74 (2008) 1767–1779.
[11] J.P. Taylor, B. Wilson, M.S. Mills, R.G. Burns, Comparison of microbial numbers and enzymatic activities in surface soils and subsoils using various techniques, Soil Biol. Biochem., 34 (2002) 387–401.
[12] M. Vital, M. Dignum, A. Magic-Knezev, P. Ross, L. Rietveld, F. Hammes, Flow cytometry and adenosine tri-phosphate analysis: alternative possibilities to evaluate major bacteriological changes in drinking water treatment and distribution systems, Water Res., 46 (2012) 4665–4676.
[13] G. Nebe-von-Caron, P.J. Stephens, C.J. Hewitt, J.R. Powell, R.A. Badley, Analysis of bacterial function by multi-colour fluorescence flow cytometry and single cell sorting, J. Microbiol. Methods, 42 (2000) 97–114.
[14] T. Broger, R.P. Odermatt, P. Huber, B. Sonnleitner, Real-time on-line flow cytometry for bioprocess monitoring, J. Biotechnol., 154 (2011) 240–247.
[15] M. Arnoldini, T. Heck, A. Blanco-Fernández, F. Hammes, Monitoring of dynamic microbiological processes using real-time flow cytometry, PLoS One, 8 (2013) 1–11.
[16] G. Widmer, T. Clancy, H.D. Ward, D. Miller, G.M. Batzer, C.B. Pearson, Z. Bukhari, Structural and biochemical alterations in Giardia Lamblia cysts exposed to ozone, J. Parasitol., 88 (2002) 1100–1106.
[17] T.Y. Suman, S.R.R. Rajasree, R. Kirubagaran, Evaluation of zinc oxide nanoparticles toxicity on marine algae *Chlorella vulgaris* through flow cytometric, cytotoxicity and oxidative stress analysis, Ecotoxicol. Environ. Saf., 113 (2015) 23–30.
[18] N.M. Franklin, J.L. Stauber, R.P. Lim, Development of flow cytometry-based algal bioassays for assessing toxicity of copper in natural waters, Environ. Toxicol. Chem., 20 (2001) 160–170.
[19] P. Niquette, P. Servais, R. Savoir, Bacterial dynamics in the drinking water distribution system of Brussels, Water Res., 35 (2001) 675–682.
[20] C.M. Hu, A novel ocean color index to detect floating algae in the global oceans, Remote Sens. Environ., 113 (2009) 2118–2129.
[21] K. Baith, R. Lindsay, G. Fu, C.R. McClain, Data analysis system developed for ocean color satellite sensors, EOS Trans. AGU, 82 (2001) 202–202.
[22] E. Flügel, Microfacies Analysis of Limestones, Springer Science & Business Media, New York, 2001.
[23] J. Ma, W. Liu, Effectiveness and mechanism of potassium ferrate(VI) preoxidation for algae removal by coagulation, Water Res., 36 (2001) 871–878.
[24] R.K. Henderson, S.A. Parsons, B. Jefferson, The impact of differing cell and algogenic organic matter (AOM) characteristics on the coagulation and flotation of algae, Water Res., 44 (2010) 3617–3624.
[25] S. Kern, H. Eichler, J. Stoeve, H. Klüter, K. Bieback, Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue, Stem Cells, 24 (2006) 1294–1301.
[26] F.Y. Ramírez-Castillo, A. Loera-Muro, M. Jacques, P. Garneau, F.J. Avelar-González, J. Harel, A.L. Guerrero-Barrera, Waterborne pathogens: detection methods and challenges, Pathogens, 4 (2015) 307–334.
[27] O. Gibert, B. Lefèvre, M. Fernández, X. Bernat, M. Paraira, M. Calderer, X. Martínez-Lladó, Characterising biofilm development on granular activated carbon used for drinking water production, Water Res., 47 (2013) 1101–1110.
[28] M. Wolska, J. Machi, S. Szerzyna, M. Mołczan, W. Adamski, J. Wiśniewski, Effect of ozonation on organic substance removal efficiency during adsorption, Desal. Water Treat., 117 (2018) 101–107.