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Toxicity analysis of coke wastewater treated in a rotating biological contactor and a membrane bioreactor

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ABSTRACT

To investigate the effectiveness of a rotating biological contactor (RBC) and a two-stage membrane bioreactor (MBR) for the treatment of coke wastewater, samples were collected three times (Batches I, II, III) from the "Jadwiga" coke plant in Zabrze, Poland at two-week intervals. The wastewater was then diluted with tap water (1:3 ratio, wastewater: tap water) and then treated at retention times of 4.1 days (RBC) and 7 days (MBR). For phytotoxicity and genotoxicity tests, the wastewater was sampled from various points in the treatment systems and further diluted to produce a range of concentrations. In the phytotoxicity tests (growth inhibition), *Lemna minor* and *Vicia faba* were used. A low concentration of wastewater (6.25%) often stimulated growth. Higher concentrations, however, inhibited *L. minor* growth completely. These tests indicated that the MBR generally reduced growth inhibition more effectively than the RBC. In the genotoxicity tests (chromosome aberrations and micronuclei formation) root meristem cells of *V. faba* were examined. The genotoxicity of the different batches varied, and neither system was particularly effective for reducing genotoxicity. The results of this study indicate that, because its composition is so variable, coke wastewater should be constantly monitored. Also, because of its potentially high genotoxicity, the ecotoxicological characteristics of coke wastewater should be monitored in addition to basic indicators of wastewater quality, such as COD, BOD, and content of nitrogen compounds.

ABBREVIATIONS

CA	chromosome aberration
EC_{50}	half maximal effective concentration
E_{mbr}	effluent, after wastewater treatment in MBR
E_{rbc}	effluent, after wastewater treatment in RBC
F_{mbr}	flow between two units of MBR reactor
INF	influent, wastewater after preliminary treatment, diluted four times with tap water before biological treatment
MBR	two-stage membrane bioreactor
MI	mitotic index
MN	micronucleus

NC negative control
PC positive control
RBC rotating biological contactor
U undiluted wastewater after preliminary treatment

INTRODUCTION

Because coke is a commonly used fuel in heavy industry, the treatment of coke wastewater is an important issue. This is particularly true in Poland, which is one of the largest producers of coke in the world (Machowska 2011). The Silesian region of Poland, in particular, must deal with large amounts of coke wastewater due to the massive production of coke in this area.

The composition of coke wastewater makes it difficult to treat, and even after treatment it may threaten the environment and wildlife when introduced into water bodies (Kumar et al. 2015). Coke wastewater is characterized by a temperature of 30-92°C, pH 7.1-8.8, 64-2600g·m^{-3} of biological oxygen demand (BOD), 525-6500g·m^{-3} of chemical oxygen demand (COD), 50-1200g·m^{-3} of phenols, 50-1100g·m^{-3} of total Kjehldahl nitrogen, 265-465g·m^{-3} of thiocyanates and 15-80g·m^{-3} of cyanides (Pal and Kumar 2014). It also contains suspended solids, tarry substances, polycyclic aromatic hydrocarbons, phenols, ammonia, thiosulfates, and hydrogen sulfide (Zhao et al. 2009). Some components of coke wastewater are carcinogenic (Dong and Zhang 2010).

The biological treatment of coke wastewater is a very slow and sensitive process because the toxic compounds that are present can be harmful to the microorganisms involved. To minimize these adverse effects, physicochemical pre-treatment is used, such as settling, coagulation, aeration, steam stripping or separation of oil and phenols (Pal and Kumar 2014).

In the present study, two technological systems were tested: a rotating biological contactor (RBC) and a two-stage membrane bioreactor (MBR). The advantages of the RBC are that no recirculation is required, the biomass concentration is sufficient, and bacteria are protected by the biofilm. The advantages of the MBR are that the biomass concentration and the loading rate are high, no recirculation is required, and the separating properties of the biomass are good. Both systems can be successfully used in coke wastewater treatment (Qi et al. 2007; Zhao et al. 2009), but membrane systems are potentially more attractive (Pal and Kumar 2014). Recent reports indicate that nanofiltration membranes enable efficient separation of cyanides and phenols (Kumar and Pal 2014). Thus, a membrane-integrated hybrid system could provide efficient and low-cost coke wastewater treatment (Kumar et al. 2015).

To test the toxicity of coke wastewater after treatment ecotoxicological indicators can be used, such as *Lemna minor* and *Vicia faba*. *L. minor* is highly sensitive to ammonia and phenols (Cayuela et al. 2007; Wang 1990), whereas *V. faba* is particularly useful in genotoxicity tests (Dong and Zhang 2010; Roccotiello et al. 2011).

The aim of this study was to determine how efficiently an RBC and a two-stage MBR reduce the phytotoxicity (growth inhibition) and genotoxicity (frequency of micronuclei and aberrant mitotic divisions) of coke wastewater. Before these biological treatments, the coke wastewater was mechanically-chemically treated. To test the wastewater's phytotoxicity after biological treatment, *L. minor* was used in growth inhibition tests, and *V. faba* was used in growth inhibition and genotoxicity tests.

MATERIALS AND METHODS

Test of respiration activity of activated sludge

The aim of this test was to determine the concentration of coke wastewater that would be safe for activated sludge and biofilm microorganisms. Samples of activated sludge from a MBR reactor were centrifuged (1500 rpm, 10 minutes) and test suspensions were prepared by mixing volumes of activated sludge, centrifuged sludge supernatant, coke wastewater in proportions of 6:6:0, 6:5:1, 6:3:3, 6:1:5, and 6:0:6, respectively.

To measure oxygen consumption, test suspensions were placed in 120mL bottles and covered with airtight lids equipped with oxygen sensors (N5221 Elwro). The bottles were placed on magnetic stirrers and the oxygen sensors were connected with an oxygen level recorder (Line Recorder T2 4620). The recorder drew oxygen concentration curves over time, which allowed determination of the respiration activity of the suspensions, expressed as $g_{O2}\cdot m^{-3}\cdot h^{-1}$. On the basis of these results the wastewater was diluted four times (3 volumes of tap water: 1 volume of coke wastewater) before its further use in the experimental systems.

Coke wastewater characteristics

Coke wastewater was collected three times at two-week intervals (batch test) from the "Jadwiga" coke plant, which is part of JSW KOKS S.A. in Zabrze, Poland. Before collection, coke wastewater was pre-treated in the coke plant by separation of oil and phenols, and removal of ammonia and volatile acids. As the preliminary treatment and dilution had reduced the ammonium nitrogen concentration in the coke wastewater, ammonium chloride

was added ($1500g_{NH4Cl}·m^{-3}$) as a substrate for activated sludge before the start of the MBR and RBC processes. Coke wastewater was sampled at the following points in the treatment process:

- U (undiluted wastewater after preliminary treatment),
- INF (influent for biological treatment diluted in volumetric proportions of 1 part of undiluted coke wastewater per 3 parts of tap water),
- F_{mbr} (flow between two units of MBR reactor),
- E_{mbr} (effluent from MBR reactor), and
- E_{rbc} (effluent from RBC).
- The chemical characteristics of INF were pH 7.5-8.4, 397-$595g_{N\text{-}NH4+}·m^{-3}$ of ammonium nitrogen, 440-$1148g_{O2}·m^{-3}$ of COD, 20-$340g·m^{-3}$ of phenols, 2-$42g·m^{-3}$ of cyanides and 12-$14g·m^{-3}$ of thiocyanates.

Biological treatment systems and sampling points

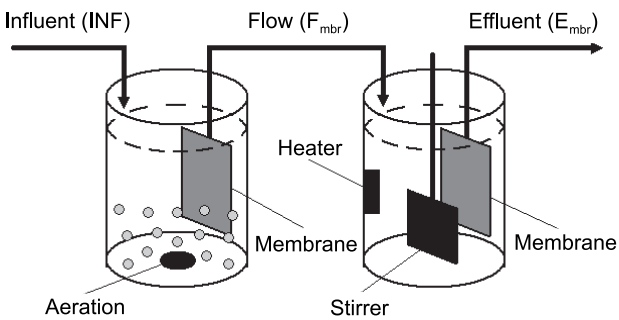

Figure 1. Schematic diagram of membrane bioreactor (MBR) system.

The MBR (Figure 1) consisted of two membrane bioreactors in series. The first reactor was aerobic with a temperature of about 20°C, while the second reactor was anoxic with a constant temperature of 30°C. The diameter of the pores in the A4 membrane (Kubota System) was 0.4μm. The total retention time in the MBR was 7 days.

The RBC (Figure 2) consisted of three chambers arranged in series, and the system's retention time was 4.1 days. Each chamber had four disks that were installed on a common rotation axis. Disks were covered with biofilm, 41% of each disk was submerged, and the area of each disk that was available for bacterial growth was $0.87m^2$. Untreated coke wastewater at about 20°C was continuously supplied by peristaltic pump to the first chamber of the set. Rotation of disks allowed the biofilm to alternate contact with wastewater and air, for feeding and aeration of the biofilm.

Figure 1 and Figure 2 show sampling points in the MBR and RBC, respectively.

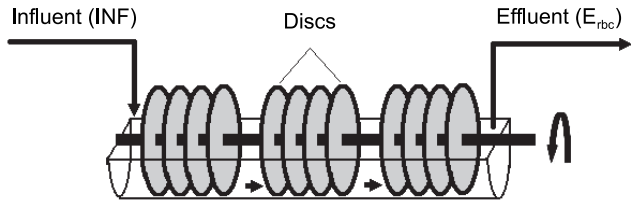

Figure 2. Schematic diagram of rotating biological contactor (RBC) system.

Phytotoxicity (growth inhibition) tests

The first batch of coke wastewater was used in the growth inhibition tests, which were performed in triplicate for each dilution. In these tests both *L. minor* and *V. faba* were used.

In the *L. minor* tests, the concentrations of each sample (U, INF, F_{mbr}, E_{mbr}, E_{rbc}) were 6.25%, 12.5%, 25%, 50%, and 100%. As a negative control (NC), standard growth medium for macrophytes was used, which was prepared according to the OECD Test No. 221 (2006). *L. minor* plants, which together had a minimum of 12 leaves, were placed in plastic Petri dishes containing 20mL of test solution and incubated for 13 days at room temperature under natural photoperiod. At the end of the test, the leaves were counted, the percentage of growth inhibition was determined, and EC_{50} values (concentration of wastewater at which plant growth was 50% of that observed in a negative control) were calculated with a probit model.

In the *V. faba* tests, the concentrations of each sample (INF, F_{mbr}, E_{mbr}, E_{rbc}) were also 6.25%, 12.5%, 25%, 50%, and 100%. Tap water was used as a negative control. *V. faba* seeds were soaked in tap water for 48 hours and then transferred to a moistened paper towel until the roots germinated. Seven seeds with a maximum root length of about 3.5cm were placed in containers with 200mL of the test solutions described above. Seeds were incubated for 7 days at room temperature and under natural photoperiod. At the end of the test, the length of the roots was measured, the percentage of growth inhibition was determined, and EC_{50} values were calculated with a probit model.

Genotoxicity tests

Each of the three batches of coke wastewater were sampled at the points mentioned above. Each of the samples was then diluted with tap water to obtain several concentrations. For INF these concentrations were 12.5%, 25%, 50%, 75%, and 100%; for F_{mbr}, they were 3.13%, 6.25%, 12.5%, 25%, and 50%; for E_{mbr}, 6.25%, 12.5%, 25%, 50%, and 75%; and for E_{rbc}, 3.13%, 6.25%, 12.5%, 25%, and 50%. As a negative control (NC), tap water was used, and as a positive control (PC), a $5g·m^{-3}$ solution of maleic hydrazide.

V. faba seeds were soaked in water for 48 hours and then transferred to a moistened paper towel until the roots germinated. A test container was prepared with 200mL of each of the test concentrations described above; thus, with INF at a concentration of 12.5%, there was one test container for each batch of wastewater. Seven *V. faba* seeds were placed into each of the test containers. Seeds were incubated for 44 hours at room temperature and under natural photoperiod. To arrest mitotic division of cells after incubation, roots were placed in a solution of 70% ethanol and glacial acetic acid mixed in a 3:1 ratio. In this solution, roots were incubated in darkness at 4°C for 24 hours. Then the roots were transferred to 70% ethanol and refrigerated until microscopic examination (Ma et al. 1995; Rank and Nielsen 1993).

To prepare a microscope slide, the root was immersed in 1M HCl and kept in a water bath at 55-60°C for 6-8 minutes. Then it was flushed with distilled water and 1mm of the meristematic part of the root tip was crushed with a scalpel. Root cells were stained with drops of 2% orcein, covered with a cover slip, and examined with a light microscope at 1000X magnification. Approximately 600 to 1000 cells were examined from each root, for a total of 6000 cells. The results were expressed in the following manner:

- mitotic index (MI) (percentage of cells undergoing mitotic division),
- frequency of micronuclei (MN) (percentage of cells with visible micronuclei),
- frequency of chromosomal aberrations (CA) (percentage of aberrant mitotic divisions in all examined cells undergoing mitosis).

RESULTS AND DISCUSSION

Phytotoxicity (growth inhibition) tests

Table 1. Inhibition of growth (%) of *Lemna minor* exposed to coke wastewater for 13 days and of *Vicia faba* exposed to coke wastewater for 7 days, and EC50 values of the coke wastewater.

Wastewater sample	Coke wastewater concentration [%]					EC_{50}
	6.25	12.5	25	50	100	
Lemna minor						
U	25.0	4.2	91.7*	100.0*	100.0*	8.0
INF	46.4	54.8	0.0	-27.8	63.4*	out of range
F_{mbr}	-59.7*	46.4	90.5*	100.0*	100.0*	11.3
E_{mbr}	-89.0*	22.4	100.0*	100.0*	100.0*	10.2
E_{rbc}	-5.4	61.4*	100.0*	90.0*	100.0*	4.6
Vicia faba						
INF	-43.7	0.4	-2.9	23.5	44.1	out of range
F_{mbr}	-6.2	20.8	37.8	53.8*	63.7*	47.3
E_{mbr}	35.1	35.6	34.5	40.0	60.9*	72.7
E_{rbc}	13.6	23.0	60.4*	57.1*	81.3*	29.0

* significantly different ($p<0.05$) from negative control (for *L. minor*, standard growth medium for macrophytes; for *V. faba*, tap water)
U undiluted wastewater after preliminary treatment
INF influent for biological treatment diluted in volumetric proportions of 1 part of undiluted coke wastewater per 3 parts of tap water
F_{mbr} flow between the two chambers of the MBR reactor
E_{mbr} effluent from the MBR reactor
E_{rbc} effluent from the RBC reactor

In the growth inhibition tests, the MBR generally outperformed the RBC (Table 1). With both *V. faba* and *L. minor*, the EC_{50} of E_{mbr} was higher than that of E_{rbc}, indicating that E_{mbr} was less toxic. Although low concentrations of E_{mbr} (6.25 and 12.5%) inhibited *V. faba* growth more than the same concentrations of E_{rbc}, high concentrations of E_{mbr} inhibited its growth less than high concentrations of E_{rbc}. With *L. minor*, in contrast, 6.25 and 12.5% concentrations of E_{mbr} inhibited growth less than the same concentrations of E_{rbc}. Higher concentrations of both effluents inhibited *L. minor* growth completely, with one exception (90% inhibition by E_{rbc} at 50% concentration).

The calculated EC_{50} values of INF with both *L. minor* and *V. faba* were out of range, suggesting that even undiluted INF could not cause 50% inhibition of growth. However, this is contradicted by the experimental results showing more than 50% inhibition of *L. minor* growth at 12.5 and 100% concentrations of INF. It appears that the large fluctuations in the results made it impossible to accurately estimate the EC_{50}. We can only postulate that the reasons for these unexpected results were either some methodological mistake or unknown interactions between various components of the wastewater samples (Dong and Zhang 2010).

The results for inhibition of *L. minor* growth by F_{mbr} and E_{mbr} were similar: a 6.25% concentration stimulated growth, whereas all higher concentrations strongly inhibited growth. There are several possible reasons why growth was stimulated at a 6.25% concentration. It may simply have been that the concentrations of toxicants were low enough that they had little or no effect on *L. minor* growth, and other substances in the coke wastewater stimulated growth. It is also possible that low concentrations of the toxicants themselves may have stimulated growth. For example, $N\text{-}NH_4^+$ was present in INF at concentrations from 397 to 595$g_{N\text{-}NH4+}\cdot m^{-3}$. *L. minor* can remove $N\text{-}NH_4^+$, and at this low concentration of coke wastewater, its removal capacity may not have been exceeded by any remaining $N\text{-}NH_4^+$ in F_{mbr} and E_{mbr}. Also, although the heavy metal content of the wastewater was not studied here, low concentrations of heavy metals have been reported in coke wastewater (Vazquez et al. 2007). Such low concentrations of heavy metals, if present, may have been beneficial to *L. minor*: low doses of certain heavy metals (e.g. Cd, Cr, Zn) may help organisms to defend themselves against certain chemical mutagens (Burkart and Ogorek 1986; De Marco et al. 1999; Rieger et al. 1990).

In the case of *V. faba*, lower concentrations of wastewater appeared to inhibit growth to a lesser extent than higher concentrations, and the lowest concentration (6.25%) of INF and F_{mbr} may even have stimulated growth, although this effect was not significant. Wiszniowski et al. (2009) reported a similar result, which could be explained by the presence of elements that are essential for plant growth in the wastewater (Dane et al. 2006). This apparent trend might also be due to the fact that *V. faba* was able to detoxify low concentrations of toxic substances, as in Baranowska-Morek (2003).

Genotoxicity tests

Table 2. Mitotic index (MI), and frequency of chromosomal aberrations (CA) and micronuclei (MN) observed in roots of *Vicia faba* exposed for 7 days to three batches of coke wastewater (I, II, III). Data for positive controls (PC) were 1.9% for MI, 3.4% for CA, and 2.38% for MN.

Sampling site	Indicator [%]	Batch of coke wastewater																							
		I								II								III							
		NC	INF concentration [%]							NC	INF concentration [%]							NC	INF concentration [%]						
			3.13	6.25	12.5	25	50	75	100		3.13	6.25	12.5	25	50	75	100		3.13	6.25	12.5	25	50	75	100
INF	MI	63.1	nt	nt	50.8	64.0	63.0	42.7	37.5	75.7	nt	nt	64.4	62.3	66.4	61.4	54.2	66.4	nt	nt	57.1	57.1	57.1	42.9	36.4
	CA	0.6	nt	nt	2.6	8.1	10.4	27.4	33.1	0.0	nt	nt	4.0	9.2	11.4	8.6	9.7	1.0	nt	nt	7.2	8.6	16.4	29.9	36.5
	MN	0.07	nt	nt	0.00	0.03	0.20	0.57	1.80	0.00	nt	nt	0.00	0.03	0.10	0.00	0.03	0.30	nt	nt	2.80	4.50	4.67	7.10	12.73
F_{mbr}	MI	63.1	52.1	48.6	54.8	47.7	45.5	nt	nt	75.7	50.0	42.0	60.5	47.1	34.9	nt	nt	66.4	61.3	46.5	47.4	39.0	25.9	nt	nt
	CA	0.6	0.5	1.9	0.4	0.4	1.5	nt	nt	0.0	0.8	4.4	0.9	1.5	0.8	nt	nt	1.0	0.9	0.8	1.5	3.0	6.9	nt	nt
	MN	0.07	0.17	0.90	0.20	0.20	1.30	nt	nt	0.00	0.10	0.08	0.05	0.10	0.42	nt	nt	0.30	0.15	0.38	1.17	0.20	0.40	nt	nt
E_{mbr}	MI	63.1	nt	47.2	44.4	31.9	35.5	39.7	nt	75.7	nt	61.1	63.2	62.7	50.2	22.7	nt	66.4	nt	56.7	42.9	40.8	43.2	27.2	nt
	CA	0.6	nt	1.1	3.1	1.8	1.8	3.7	nt	0.0	nt	0.5	0.7	0.6	3.1	4.5	nt	1.0	nt	2.2	4.3	5.2	5.1	8.1	nt
	MN	0.07	nt	0.08	0.48	0.23	0.33	0.30	nt	0.00	nt	0.13	0.18	0.58	0.18	1.23	nt	0.30	nt	1.02	1.17	1.22	1.33	1.73	nt
E_{rbc}	MI	63.1	72.0	69.5	53.5	61.7	54.9	nt	nt	75.7	60.5	65.8	77.0	59.0	42.9	nt	nt	66.4	57.9	59.4	58.6	58.1	55.8	nt	nt
	CA	0.6	0.9	2.0	4.6	3.1	8.2	nt	nt	0.0	0.3	0.4	0.4	1.0	0.4	nt	nt	1.0	10.1	16.6	18.5	20.5	25.2	nt	nt
	MN	0.07	0.00	0.03	0.17	0.03	0.03	nt	nt	0.00	0.03	0.00	0.03	0.00	0.00	nt	nt	0.30	1.57	1.47	3.20	6.65	10.35	nt	nt

nt not tested
NC negative control (tap water)
PC positive control (5g·m^{-3} solution of maleic hydrazide)
INF influent for biological treatment diluted in volumetric proportions of 1 part of undiluted coke wastewater per 3 parts of tap water
F_{mbr} flow between the two chambers of the MBR reactor
E_{mbr} effluent from the MBR reactor
E_{rbc} effluent from the RBC reactor

The samples of coke wastewater, which were taken at two-week intervals, were highly toxic to the root cells of *V. faba*. The composition of the three samples for the batch tests varied, and overall, Batch III was the most toxic (Table 2). These differences in composition were reflected by differences in mitotic index, frequency of chromosomal aberrations and frequency of micronuclei in *V. faba* root cells exposed to each batch. These fluctuations indicate that the toxicity of coke wastewater should be continuously monitored.

The coke wastewater studied in this experiment contained rather high concentrations of phenols (20-340g·m^{-3}). It is known that the hydroxyl groups of phenols may initiate chains of reactions which can result in damage to DNA (El Hajjouji et al. 2007). Formation of chromosomal aberrations and frequency of micronuclei are related to the toxicity of wastewater (Żelazna et al. 2011). However, the frequency of micronuclei and chromosome aberrations depends not only on the concentration of the toxic agent, but also on the mitotic index, as the likelihood of genome damage during mitosis increases with increasing numbers of mitotic divisions (Dong and Zhang 2010).

The genotoxicity results presented in Table 2 indicate that the toxicity of the influent and of the effluent varied with the batch, and that neither treatment method was clearly superior.

When comparing the frequency of chromosomal aberrations at the concentrations at which the influent and both effluents were tested (12.5%, 25%, and 50%), and looking at the frequency of chromosomal aberrations, both E_{mbr} and E_{rbc} from Batches I and II were generally less toxic than INF. From Batch III, however, only E_{mbr} was less toxic than INF. In terms of frequency of micronuclei at these concentrations, E_{mbr} from Batches I and II was more toxic than INF, but E_{rbc} from these batches was generally similar to INF. In contrast, E_{mbr} from Batch III was less toxic than INF, whereas E_{rbc} from this batch was more toxic.

Chromosomal aberrations were observed much more frequently than micronuclei. There are at least two possible explanations for this observation. First, chromosomal aberrations may induce repair mechanisms that prevent micronuclei formation (Dong and Zhang 2010). Second, not all damage to chromosomes results in micronuclei formation in *V. faba* (Kalka et al. 2008; Wei et al. 2012).

Although typical indicators of effluent quality (BOD, COD, content of nitrogen compounds) were within acceptable ranges, the phytotoxicity (Table 1) and genotoxicity (Table 2) tests indicated that the treatments did not eliminate the toxicity of the coke wastewater. Indeed, numerous reports indicate that industrial wastewater can have acceptable levels of COD, BOD and nitrogen compounds, but still remain genotoxic (Wiszniowski et al. 2009). Thus, the results of this study support the assertions of Wei et al. (2012) and Zhu et al. (2013) that, when treating industrial wastewater, not only the typical indicators of wastewater quality should be considered, but also ecotoxicological indicators.

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REFERENCES

Baranowska-Morek, A. 2003. Mechanisms of plant tolerance to heavy metals. Kosmos. Problemy nauk biologicznych 52: 283-298 (in Polish).

Cayuela, M.L., P. Miller, J. Slovin, A. Roig. 2007. Duckweed (*Lemna ginna*) growth inhibition bioassay for evaluating the toxicity of olive mill wastes before and during composting. Chemosphere 68: 1985-1991.

Burkart, W., B. Ogorek. 1986. Genotoxic action of cadmium and mercury in cell cultures and modulation of radiation effects. Toxicological Environmental Chemistry 12: 173–183.

Dane, V., F. Ekici, Y.K. Aktac. 2006. The effect of wastewater on root growth and mitosis in onion (*Allium cepa*) root apical meristem. Asian Journal of Plant Sciences 28: 79-95.

De Marco, A., C. De Simone, C. D'Ambrosio, M. Owczarek. 1999. Buthionine sulfoximine prevents the reduction of the genotoxic activity of maleic hydrazide by soil humic substances in *Vicia faba* seedlings. Mutation Research 438: 89-95.

Dong, Y., J. Zhang. 2010. Testing the genotoxicity of coking wastewater using *Vicia faba* and *Hordeum vulgare* bioassays. Ecotoxicology and Environmental Safety 73: 944-948.

El Hajjouji, H., E. Pinelli, M. Guiresse, G. Merlina, J.-C. Revel, M. Hafidi. 2007. Assessment of the genotoxicity of olive mill waste water (OMWW) with the *Vicia faba* micronucleus test. Mutation Research 634: 25-31.

Kalka, J., A. Ośliźlok, J. Surmacz–Górska, S. Fudala–Książek. 2008. Assessing the genotoxicity of landfill leachate using micronucleus bioassay. Ochrona Środowiska 30: 15-17 (in Polish).

Kumar, R., S. Chakrabortty, P. Pal. 2015. Membrane-integrated physico-chemical treatment of coke-oven wastewater: transport modeling and economic evaluation. Environmental Science and Pollution Research 22: 6010-6023.

Kumar, R., P. Pal. 2014. Membrane-integrated hybrid system for the effective treatment of ammoniacal wastewater of coke-making plant: a volume reduction approach. Environmental Technology 35: 2018-2027.

Ma, T.H., Z. Xu, C. Xu, H. Mc Conell, E.V. Rabato, G.A. Arreola, H. Zhang. 1995. The improved *Allium*/*Vicia* root tip micronucleus assay for clastogenicity of environmental pollutants. Mutation Research 334: 185-195.

Machowska, H. 2011. Coke producing industry with relation to environmental protection. Proceedings of ECOpole 5: 269-274 (in Polish).

OECD Guidelines for the testing of chemicals. Test No. 221: Lemna sp. Growth inhibition test. 2006.

Pal, P., R. Kumar. 2014. Treatment of coke wastewater: a critical review for developing sustainable management strategies. Separation & Purification Reviews 43: 89-123.

Rank, J., M.N. Nielsen. 1993. A modified *Allium* test as a tool in the screening of genotoxicity of complex mixtures. Hereditas 118: 49-53.

Rieger, R.A., A. Michaelis, S. Takehisa. 1990. Involvement of phytochelatins in $NiCl_2$ – triggered protection against induction of chromatid aberrations by TEM and MH in *Vicia faba* root tips meristems. Mutation Research 244: 31–35.

Roccotiello, E., I. Viale, L. Cornara. 2011. Phytotoxicity tests with higher plants for environmental risk assessment. Journal of Biological Research 1: 45-47.

Qi, R., K. Yang, Z.-X. Yu. 2007. Treatment of coke plant wastewater by SND fixed biofilm hybrid system. Journal of Environmental Sciences 19: 153-159.

Vazquez, I., I. Rodriguez-Iglesias, E. Maranon, L. Castrillon, M. Alvarez. 2007. Removal of residual phenols from coke wastewater by adsorption. Journal of Hazardous Materials 147: 395-400.

Wang, W. 1990. Literature review on duckweed toxicity testing. Environmental Research 52: 7-22.

Wei, X., Z. Zhang, Q. Fan, X. Yuan, D. Guo. 2012. The effect of treatment stages on the coking wastewater hazardous compounds and their toxicity. Journal of Hazardous Materials 239-240: 135-141.

Wiszniowski, J., P. Sindera, I. Duquesnoy, J. Surmacz-Górska. 2009. Effect of biological treatment on genotoxicity of industrial wastewaters: root tips *Vicia faba* assay. Architecture Civil Engineering Environment 4: 137-144.

Zhao, W., X. Huang, D. Lee. 2009. Enhanced treatment of coke plant wastewater using an anaerobic–anoxic–oxic membrane bioreactor system. Separation and Purification Technology 66: 279–286.

Zhu, X., L. Chen, R. Liu, C. Liu, Z. Pan. 2013. Biotoxicity evaluation of coking wastewater treated with different technologies using Japanese medaka (*Oryzias latipes*). Environmental Science: Process & Impacts 15: 1391-1396.

Żelazna, K., K. Rudnicka, S. Tejs. 2011. *In vitro* micronucleus test assessment of polycyclic aromatic hydrocarbons. Environmental Biotechnology 7: 70-80.