Asian Journal of Water, Environment and Pollution, Vol. 16, No. 4 (2019), pp. 1–6.
DOI 10.3233/AJW190042

Decolourization and Degradation of Reactive Textile Dyes by Isolated Strain *Proteus mirabilis*

H.G. Madhushika*, Thilini U. Ariyadasa and S.H.P. Gunawardena
Department of Chemical and Process Engineering, University of Moratuwa, Sri Lanka
✉ gimhani.madhushika11@gmail.com

Received July 26, 2017; revised and accepted June 17, 2019

Abstract: Dyes used in textile industries are considered to be toxic and create pollution problems when released to the environment without proper treatments. Biological effluent decolourization techniques can be suggested as a remediation for this pollution problem. In the present study, a bacterial strain was isolated from an effluent treatment plant of a textile industry. This strain was able to decolourize 50 ppm dye solutions of Sumifix Supra Yellow EXF, Sumifix Supra Red EXF, Sumifix Supra Blue EXF and Cibacron Black WNN up to 96%, 94%, 83% and 95% respectively under static conditions at 35°C within 72 h of treatment. Further, the potential of this strain to decolourize mixture of these four dyes was evaluated under shaking and static conditions. Decolourization of the dyes was confirmed by UV–VIS analysis results and the strain was identified as Proteus mirabilis by using 16srRNA gene sequencing analysis.

Key words: Biodegradation, decolourization, dyes, effluent.

Introduction

Textile processing industries including textile dyeing and washing plants consumes large quantities of water and has become the second biggest polluter of clean water globally (Valli Nachiyar et al., 2014). Approximately 10% of the textile dyes used during textile dyeing process remain unfixed and enter the process water resulting in colourful effluent (Singh and Arora, 2011). This colourful effluent reduces light penetration in rivers and thus affects the photosynthetic activities of aquatic flora, thereby severely affecting the food source of aquatic organisms (Pereira and Alves, 2012).

Therefore, textile industries use different effluent treatment methods especially for decolourization of effluent. These methods can be classified as physical, chemical and biological. Out of these methods, physical and chemical treatments such as coagulation–flocculation, filtration, adsorption, ozonation, ion exchange and Fenton's method are most widely used in industry. However, these treatment methods have several drawbacks such as high cost, generation of sludge and other pollutants, and hence there is a need for sophisticated technologies (Pereira and Alves, 2012). Conversely, biological treatment methods are environmentally friendly, produce less sludge and are relatively inexpensive as the running cost is low (Solís et al., 2012).

Biological dye decolourization can be due to biosorption, biodegradation or a combination of both (Solís et al., 2012). Decolourization mechanism will vary depending on the structure of the dye and the microbial species. Different micro-organism types such as filementaous fungi (Senthilkumar et al., 2014), bacteria (Deng et al., 2008), algae (Jadhav et al., 2007) and yeast (El-Sheekh et al., 2009) that are capable of decolourizing textile dyes are reported in literature.

*Corresponding Author

Textile dyes used in industry can be categorized depending on the dye structure (azo, anthraquinone, sulphur, indigoid, triphenylmethyl (trityl), and phthalocyanine derivatives (Forgacs et al., 2004)) or application (reactive, acid, direct, basic, mordant, disperse, sulphur, and vat dyes (Popli and Patel, 2014)). Reactives are the most important class of colours for the dyeing of cotton (S.K. and Vijay Kumar, 2013) and widely used in textile industries. They are highly water soluble and therefore difficult to be removed from effluent using most widely practiced dye decolourization techniques such as coagulation-flocculation (Saratale et al., 2011).

This study was carried out to isolate microorganisms from the local environment that are capable of decolourizing reactive dyes used in textile industries and to identify the most effective species in colour removal under different culture conditions.

Materials and Methods

Effluent Sampling

A sludge containing effluent sample was collected from an oxidation ditch of a local textile processing facility located in Colombo, Sri Lanka.

Dyes and Reagents

Commercial grade Sumifix Supra Yellow EXF (Yellow), Sumifix Supra Red EXF (Red), Sumifix Supra Blue EXF (Blue) and Cibacron Black WNN (Black) dyes were provided by a local textile dyeing industry (Each of these dye is a mixture of reactive dyes; structures of dyes except black dye are not revealed due to trade secrets). All the other chemical reagents used were of analytical grade.

Isolation and Identification of Dye Decolourizing Microorganism

Luria–Bertani (LB) medium with 100 mg/l of each dye was added into test tubes and inoculated with 1 ml of textile effluent sample. After 24 h incubation at 35°C, 1 ml samples were withdrawn from each test tube and serial dilutions of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} concentrations were prepared. Samples of each dilutions were spread on 100 mg/l dye containing agar plates, incubated at 35°C for 24 h and colonies were isolated by sub culturing by streak plate method. Isolated micro-organisms were then screened to check their potential to decolourize textile dyes in test tubes containing LB media and 100 mg/l of each dye. Out of the screened micro-organisms, the isolate that exhibited high level of colour removal was selected for further studies.

Strain identification was done considering morphology characters, conducting biochemical tests and sequencing analysis of 16S rRNA gene. Universal primers 27F 5′ (AGA GTT TGA TCM TGG CTC AG) 3′ and 1492R 5′ (TAC GGY TAC CTT GTT ACG ACT T) 3′ were used for the amplification of 16S rRNA gene.

Decolourization Experiments

100 ml of media containing 12.8 g/1 $Na_2HPO_4.7H_2O$, 3 g/1 KH_2PO_4, 0.5 g/1 NaCl, 1.0 g/l NH_4Cl and 5.0 g/l yeast extracts with 50 mg/l of each dye solution were used in decolourization studies.

In order to investigate the decolourization effect of dye mixture, equal proportions of yellow, red, blue and black dyes were mixed with sterilized decolourization medium so that the final concentration of dye mixture was 50 mg/l.

Bacterial isolate precultured in LB medium and incubated at 35°C for 24 h was used as the inoculum. Sterilized decolourization media containing a single dye was inoculated with 2% (v/v) inoculums (approximately 0.25 mg dry cell weight) and incubated at 35°C under static (anoxic) conditions. Decolourization media containing dye mixture were inoculated with 2, 5 and 10% (v/v) inoculum and incubated at 35°C under static (anoxic) conditions. In addition, a flask containing the dye mixture was inoculated with 2% (v/v) inoculum and incubated at 35°C under shaking conditions (120 rpm).

5 ml samples were withdrawn from decolourization medium at pre-defined intervals, centrifuged at 10,000 g (12000 rpm) at 28°C for 10 minutes, and the supernatant was scanned at λ_{max} of each dye under visible light in a spectrophotometer (UV-VIS RS spectrophotometer). Uninoculated dye-free medium was used as the blank. All assays were performed in duplicate and compared with the control.

Determination of Cell Growth and Decolourization Percentages

Samples withdrawn from the decolourization media were analyzed to quantify the cell concentration (equations 1 and 2) and the dye concentration (equation 3) (Chen, 2002; Silveira et al., 2009).

1. OD_{600nm} of the sample mixtures without centrifugation:

$$OD^{X+dye}_{600nm} = OD^{dye}_{600nm} + OD^{X}_{600nm} \quad (1)$$

2. OD_{600nm} of sample supernatant (sup) after centrifugation for 10 minutes at 10,000 g (12000 rpm):

$$OD^{sup}_{600nm} = OD^{dye}_{600nm} \quad (2)$$

3. $OD_{\lambda max}$ of sample supernatant after centrifugation:

$$OD^{sup}_{\lambda max} = OD^{dye}_{\lambda max} \quad (3)$$

where OD^{X} is optical density due to microbial cells and $OD^{dye}_{\lambda max}$ is optical density at the maximum wavelength of the dye.

Cell mass was calculated considering the difference of the values of equations (1) and (2). Percentage dye decolourization was calculated using equation (4).

$$\text{Percentage decolourization} = \frac{(A_{initial} - A_{final}) \times 100\%}{A_{initial}} \quad (4)$$

where $A_{initial}$ is the absorbance before decolourization and A_{final} is the absorbance after a certain time of decolourization. Each decolourization value is the mean of two parallel experiments.

Results and Discussion

Identification of Dye Decolourizing Microorganisms

Out of the isolates exhibited decolourization ability, one strain capable in decolourizing all four dyes used in this study was selected for further work. This strain has comparably different colony morphology on agar plate with concentric rings; exhibited rod-shaped, gram-negative, catalase positive characteristics. The nucleotide sequences of 16s rRNA gene of this strain exhibited 99% similarity to *Proteus mirabilis* according to data available in GenBank.

Determination of Cell Growth and Decolourization

Percentage decolourization with time for four dyes used in this study by *Proteus mirabilis* are shown in Figure 1. Decolourization rates were high in the first 24 h after inoculation and then the rate gradually decreased. After 72 h of treatment, colour removal from yellow, red, blue and black dyes were 96, 94, 83 and 95% respectively. Figure 2 shows the decolourized dye samples and their controls at 72 h.

After the supernatant was separated by centrifugation, it was clearly seen that the microbial cells in the flask containing blue dye were coloured (blue). From this observation, it can assume that the colour of the blue dye has been adsorbed by the microbial cells (biosorption). However, microbial cells separated from the remaining flasks were not coloured and hence, it can be considered that the colour removal from the contents in these flasks is not due to biosorption. Hence, there is a strong possibility that the colour giving compounds have undergone a structural change due to microbial activity (biodegradation) and the degraded compounds do not show the original colour.

Figure 3 shows the UV-Visible spectra of four dyes before and after decolourization (72 h of treatment). Yellow dye solution indicated a peak at 422 nm before the treatment; however, with biological treatment this peak has disappeared and a new peak has formed at 364 nm (Figure 3-A). Red dye displayed two peaks at 522 nm and 544 nm but these peaks have disappeared with incubation, forming a new peak at 439 nm (Figure 3-B).

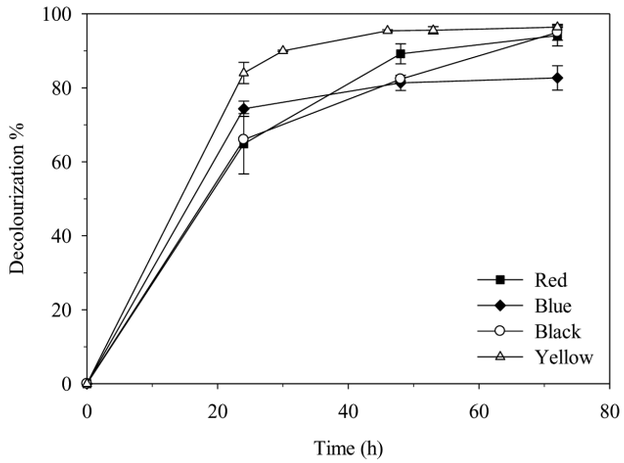

Figure 1: Decolourization of Yellow, Red, Blue and Black dyes by *Proteus mirabilis*.

Figure 2: Controls and 72 h biologically treated dye samples: A – Yellow, B – Red, C – Blue and D – Black.

Blue dye solution indicated the maximum absorbency peak at 606.5 nm and with biological treatments this peak has gradually reduced and after 72 h incubation minor peak has formed at 401 nm (Figure 3-C). Maximum absorbency peak of black dye solution can be seen at 598 nm and after decolourization with *P. mirabilis*; a slightly visible peak is visible at 405 nm (Figure 3-D). If decolourization was due to biosorption, absorption peaks decrease proportionately, whereas in biodegradation, either the major peak in visible region completely disappears or gives rise to a new peak (Pokharia and Ahluwalia, 2016) as a result of formation of degradation products.

In biosorption, dye structures do not change and hence formation of new products (peaks) is not visible in UV-visible spectrum. However, entrapment of dyes into the microbial cells may lower the colour in the supernatant resulting in reduction in absorption values. Disappearance of major peaks and formation of new peaks in the visible region are observed with yellow, red and black dyes indicating biodegradation of these dyes. Proportional reduction of the peak, without forming new peaks within initial 24 h incubation, agrees with initial biosorption of blue dye (Figure 3-C).

Most of the reported dye decolourization studies have been carried out with primary dyes with known chemical compositions and structures; however, their application in industry is limited. All the dyes used in this study are currently employed in textile industries but biological decolourization studies for these dyes are not reported in literature.

Percentage colour removal of dye mixture under static and shaking conditions is shown in Figure 4. It can be seen that the colour removal is more effective under static conditions compared to shaking conditions. There was 65% colour reduction under static conditions after 22 h incubation whereas it was only 10% reduction under shaking conditions. However, the cell growth was doubled under shaking conditions when compared to static conditions (data not shown) showing that the dye decolourization is not only dependent on cell concentration but has influence of other factors. Similar observation has been reported in literature for azo dye decolourization with *Micrococcus glutamicus* (Saratale

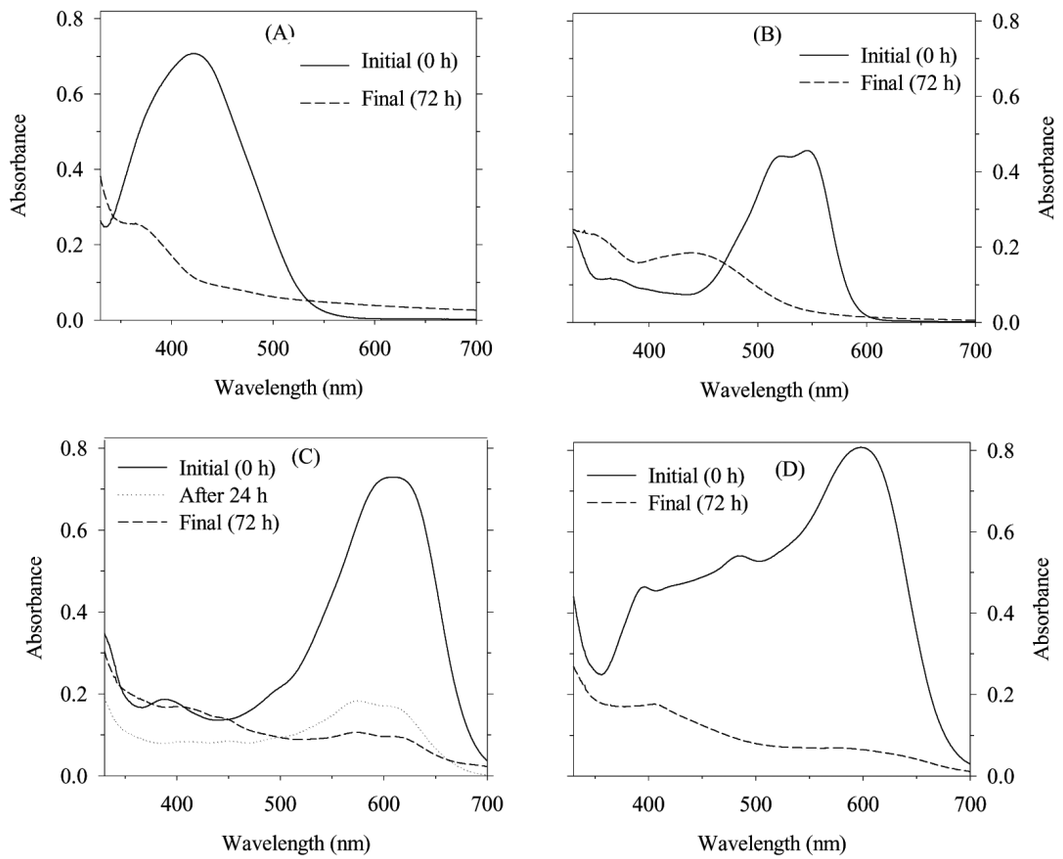

Figure 3: Variation of UV-visible spectra of dye solutions decolourized with *P. mirabilis*. A – Yellow, B – Red, C – Blue, D – Black.

et al., 2009), and for *P. mirabilis* (Chen et al., 1999; Madhushika et al., 2018).

Although aromatic compounds can be degraded under both aerobic and anaerobic conditions, nitro and sulphonic containing substituents in azo dyes are quite recalcitrant to aerobic bacterial degradation. This is due to electron accepting nature of the azo bond and their resistance to oxygenases attack (dos Santos et al., 2007). When oxygen is available in the decolourization medium, oxygen has preference for reducing equivalents as it is a more effective electron acceptor than azo dyes (dos Santos et al., 2007). Hence, in shaking conditions where the concentration of oxygen in the decolourization medium is high, higher percentage decolourization of azo dyes is not observed as in static conditions.

As Figure 5 shows, there was no increase in percentages decolourization with the increase of the size of the inoculum. Maximum decolourization was obtained when the flasks were inoculated with 2% (v/v). After 68 h incubation, highest cell concentration was observed in flasks with 5% inoculum and lowest in 10% inoculum. When the inoculum size increased, competition for nutrients may increase resulting early depletion of nutrients in the decolourization medium.

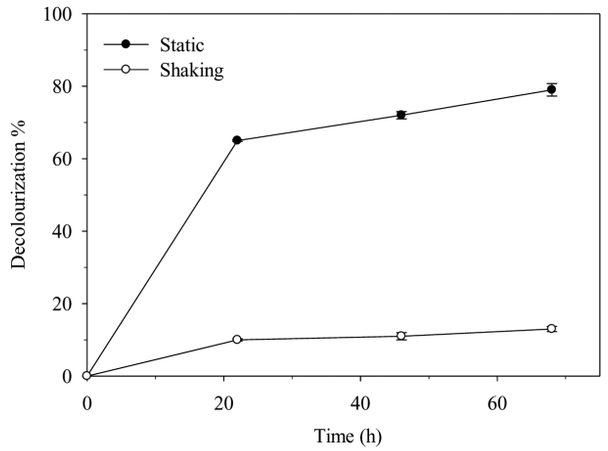

Figure 4: Decolourization of dye mixture by *Proteus mirabilis* under static and shaking conditions.

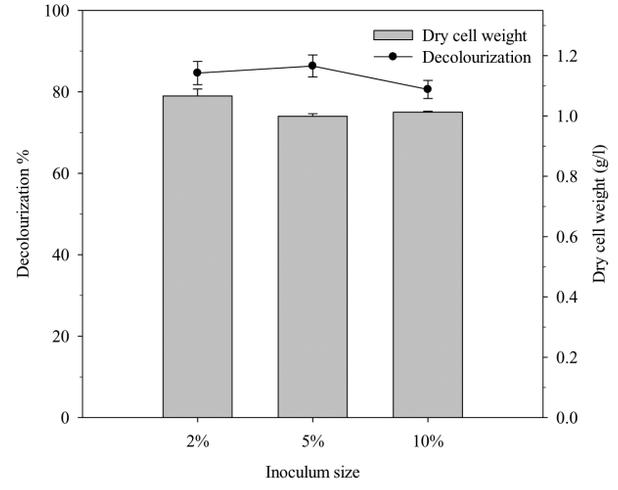

Figure 5: Percentage dye decolourization and dry cell weight variation with different inoculum sizes after 68 h incubation.

Conclusions

Bacterial strain, *Proteus mirabilis* isolated from textile industry effluent treatment plant exhibited dye decolourization potential. It was able to decolourize more than 80% of four different textile dyes within 72 h of treatment and also the mixture of these dyes. Results show that, this strain can effectively be used for further studies in biological decolourization of textile effluent in large scale treatment processes.

Acknowledgements

This work was supported by Senate Research Committee grant (SRC/CAP/15/03), University of Moratuwa, Sri Lanka.

References

Chen, B.-Y. (2002). Understanding decolorization characteristics of reactive azo dyes by Pseudomonas luteola: Toxicity and kinetics. *Process Biochem.*, **38:** 437– 446. https://doi.org/10.1016/S0032-9592(02)00151-6

Chen, K.-C., Huang, W.-T., Wu, J.-Y. and J.-Y. Houng (1999). Microbial decolorization of azo dyes by Proteus mirabilis. *J. Ind. Microbiol. Biotechnol.*, **23:** 686–690. https://doi.org/10.1038/sj.jim.2900689

Chinta, S.K. and S. Vijay Kumar (2013). Technical facts & figures of reactive dyes used in textiles. *Int. J. Eng. Manag. Sci.*, **4:** 308–312.

Deng, D., Guo, J., Zeng, G. and G. Sun (2008). Decolorization of anthraquinone, triphenylmethane and azo dyes by a new isolated Bacillus cereus strain DC11. *Int. Biodeterior. Biodegrad.*, **62:** 263–269. https://doi.org/10.1016/j.ibiod.2008.01.017

dos Santos, A.B., Cervantes, F.J. and J.B. van Lier (2007). Review paper on current technologies for decolourisation of textile wastewaters: Perspectives for anaerobic biotechnology. *Bioresour. Technol.*, **98:** 2369–2385. https://doi.org/10.1016/j.biortech.2006.11.013

El-Sheekh, M.M., Gharieb, M.M. and G.W. Abou-El-Souod (2009). Biodegradation of dyes by some green algae and

cyanobacteria. *Int. Biodeterior. Biodegrad.*, **63:** 699–704. https://doi.org/10.1016/j.ibiod.2009.04.010

Forgacs, E., Cserháti, T. and G. Oros (2004). Removal of synthetic dyes from wastewaters: A review. *Environ. Int.*, **30:** 953–971. https://doi.org/10.1016/j.envint.2004.02.001

Jadhav, J.P., Parshetti, G.K., Kalme, S.D. and S.P. Govindwar (2007). Decolourization of azo dye methyl red by Saccharomyces cerevisiae MTCC 463. *Chemosphere*, **68:** 394–400. https://doi.org/10.1016/j.chemosphere.2006.12.087

Madhushika, H.G., Ariyadasa, T.U. and S.H.P. Gunawardena (2018). Decolourization of Reactive Red EXF Dye by Isolated Strain *Proteus Mirabilis*. *In:* 2018 Moratuwa Engineering Research Conference (MERCon). pp. 231–234. https://doi.org/10.1109/MERCon.2018.8421983

Pereira, L. and M. Alves (2012). Dyes—Environmental Impact and Remediation. *In:* Malik, A. and Grohmann, E. (eds), Environmental Protection Strategies for Sustainable Development. Springer Netherlands, Dordrecht, pp. 111–162. https://doi.org/10.1007/978-94-007-1591-2_4

Pokharia, A. and S.S. Ahluwalia (2016). Decolorization of Xenobiotic Azo Dye Black WNN by Immobilized *Paenibacillus alvei* MTCC 10625. *Int. J. Environ. Bioremediation Biodegrad.*, **4:** 35–46. https://doi.org/10.12691/ijebb-4-2-2

Popli, S. and U.D. Patel (2014). Destruction of azo dyes by anaerobic–aerobic sequential biological treatment: A review. *Int. J. Environ. Sci. Technol.*, **12:** 405–420. https://doi.org/10.1007/s13762-014-0499-x

Saratale, R.G., Saratale, G.D., Chang, J.S. and S.P. Govindwar (2011). Bacterial decolorization and degradation of azo dyes: A review. *J. Taiwan Inst. Chem. Eng.*, **42:** 138–157. https://doi.org/10.1016/j.jtice.2010.06.006

Saratale, R.G., Saratale, G.D., Chang, J.S. and S.P. Govindwar (2009). Ecofriendly degradation of sulfonated diazo dye C.I. Reactive Green 19A using Micrococcus glutamicus NCIM-2168. *Bioresour. Technol.*, **100:** 3897–3905. https://doi.org/10.1016/j.biortech.2009.03.051

Senthilkumar, S., Perumalsamy, M. and H. Janardhana Prabhu (2014). Decolourization potential of white-rot fungus Phanerochaete chrysosporium on synthetic dye bath effluent containing Amido black 10B. *J. Saudi Chem. Soc.*, **18:** 845–853. https://doi.org/10.1016/j.jscs.2011.10.010

Silveira, E., Marques, P.P., Silva, S.S., Lima-Filho, J.L., Porto, A.L.F. and E.B. Tambourgi (2009). Selection of Pseudomonas for industrial textile dyes decolourization. *Int. Biodeterior. Biodegrad.*, **63:** 230–235. https://doi.org/10.1016/j.ibiod.2008.09.007

Singh, K. and S. Arora (2011). Removal of synthetic textile dyes from wastewaters: A critical review on present treatment technologies. *Crit. Rev. Environ. Sci. Technol.*, **41:** 807–878. https://doi.org/10.1080/10643380903218376

Solís, M., Solís, A., Pérez, H.I., Manjarrez, N. and M. Flores (2012). Microbial decolouration of azo dyes: A review. *Process Biochem.*, **47:** 1723–1748. https://doi.org/10.1016/j.procbio.2012.08.014

Valli Nachiyar, C., Namasivayam, S.K.R., Kumar, R.R. and M. Sowjanya (2014). Bioremediation of textile effluent containing Mordant Black 17 by bacterial consortium CN-1. *J. Water Process Eng.*, **4:** 196–200. https://doi.org/10.1016/j.jwpe.2014.10.003