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# Potential of bacterial consortium for removal of cephalexin from aqueous solution

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**Abstract** Antibiotics represent a global environmental problem due to their role in the increasing of antimicrobial resistance. Therefore, the removal of antibiotics from wastewater has received unrivalled attention in the recent years. Several technologies including the biodegradation process have been applied for this purpose. However, the potential of bacterial biomass in the biosorption of antibiotics has limited studies. The present study investigated cephalexin removal from aqueous solution by consortium bacterial cells (living and dead) which are tolerant for antibiotics. The factors including cephalexin, biomass, pH, temperature as well as presence of heavy metal ions were tested. The maximum biosorption efficiency was recorded at 0.4 mg $L^{-1}$ (94.73% *vs.* 92.98% for living and dead cells respectively), dead cells exhibited more efficiency compared to living cells at 5 mg $L^{-1}$ (82.36% *vs.* 46.66% respectively). Living cells are more effective at pH value between pH 4 and 6 (71.95–68.90%). The maximum removal of living cells was highest at 30 °C (80.26%), while was at 25 °C of dead cell biomass (63.81%). Remarkable percentage for cephalexin biosorption by living cells was recorded in the presence low concentrations of $Ni^{2+}$ (0.21 mg $L^{-1}$, 40% *vs.* 30% of living and dead cells, respectively). Living cells exhibited 27.42% and 25% of the removal with $Cu^{2+}$ (1 mg $L^{-1}$) and $Pb^{2+}$ (0.4 mg $L^{-1}$) respectively. In conclusion the bacterial cells biomass has a potential to remove cephalexin with some negative effects of heavy metals which can be overcome by the removal of these metal ions first and then removal of antibiotics in a second cycle.



## 1. Introduction

The efficiency of treatment processes to remove antibiotic compounds is limited due to the ability of these compounds to persist the degradation process (Li et al., 2013). Therefore, the generated secondary effluents still contain detectable amounts of these antibiotics. Furthermore, antibiotics in the effluents even at minimum inhibition concentrations (MIC) may contribute into increasing the antimicrobial resistance due to transfer of resistance gene (Klavarioti et al., 2009). Hence, further removal of antibiotics from secondary effluents is needed to protect human health.

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Many advanced treatment processes have been evaluated for removal of antibiotic from sewage effluents such as oxidation (Lee et al., 2006), ozonation (Lee et al., 2014), ultrasonic irradiation (Naddeo et al., 2009), UV irradiation and activated carbon adsorption (Aksu and Tunc, 2005; Rahim and Garba, 2015; Jodeh et al., 2015). However, the limitation of ozone application lies in the toxic by-products forms.

The removal of antibiotics by the biosorption process using bacteria has not been studied in depth yet. However, the biodegradation of antibiotics process has been reported in the literature (Al-Gheethi and Norli, 2014). The biodegradation process depends on the ability of enzymes to degrade the structure of the active antibiotics to be inactive as in the case β-lactamase (Al-Gheethi and Norli, 2014). In contrast, the biosorption process depends on the simple diffusion (passive uptake) of antibiotics through the bacterial cell membrane (Al-Gheethi et al., 2015). It has been demonstrated that the microorganism cells have high potential for biosorption of heavy metals as well as the compounds such as dyes (Hanif and Bhatti, 2015; Mushtaq et al., 2016; Tahir et al., 2016; Rashid et al., 2016). Some of the authors focused on the nanotechnology application such as utilization of black phosphorus to remove the pollutants from the environments (Sun et al., 2015; Guo et al., 2015).

In previous work, the potential of β-lactamase produced from *Bacillus subtilis* strain to biodegrade of cephalexin was investigated, the maximum degradation was 25% (Al-Gheethi and Norli, 2014). The biodegradation of cephalexin has increased at low concentrations of $Ni^{2+}$, $Cu^{2+}$ and $Zn^{2+}$ but not in the presence of $Cd^{2+}$ and $Pb^{2+}$. However, the limitation of this technology lies in the efficiency where it is insufficient to totally remove the antibiotics. Therefore, this study focuses on the potential of bacterial cells biomass (living or dead) to removal of β-lactam antibiotics by biosorption process.

Cephalexin is a zwitterion antibiotic contains both an acidic and a basic group and for this reason it presented in a dissolved form (Connor et al., 1994; Nitzan et al., 2002). Thes properties might explain the high potential of cephalexin to diffuse through cell membranes of bacteria and then develop resistance. Meanwhile, the properties of antibiotics might facilitate their removal based on the bacterial biomass as biosorbent. The utilization of bacterial cell biomass might be efficient in the antibiotics removal without developments for antibiotics resistance because no new generation is produced. Moreover, the tolerant bacteria might have more potential to biosorp antibiotics, because the cell accumulates the antibiotics by diffusion process (passive transport where no energy is require) and prevents formation of toxic compounds that lead to death (Lewis, 2008). Therefore, the bacterial cell plays as a store for antibiotics without effects on the cell. In biosorption process, pure or mixed biomass cells might be used. However, the utilization of bacterial consortium might provide high diversity of bacterial cell wall surface which might enhance the efficiency of cephalexin removal and this emphasize the novelty of the current work. Moreover, the effect of cephalexin, biomass concentration, time, temperature, pH, as well as heavy metals on biosorption of cephalexin was tested.

## 2. Materials and methods

### 2.1. Experimental set-up

The experimental-setup in the current work consists of isolation, purification, identification, screening for the resistance of antibiotics (cephalexin, amoxicillin, ampicillin, cefuroxime and ciprofloxacin) and heavy metal ions included nickel ($Ni^{2+}$), copper ($Cu^{2+}$), zinc ($Zn^{2+}$), cadmium ($Cd^{2+}$) and lead ($Pb^{2+}$). The flowchart of the experimental design is presented in Appendix A. The efficiency of living and dead biomass cells in the removal of cephalexin in a response for different factors was carried out. The effect of heavy metals on the biosorption efficiency was examined. Batch culture containing 100 mL of cephalexin solution was used to determine the removal efficiency by using living and dead cell biomass.

Factorial Complete Randomized Design (CRD) (3 * 5 * *x*) in triplicate was used to study the factors affecting biosorption process. Where: two (2) bacterial cell biomass (living and dead cells) and one (1) control for the biosorption process (cephalexin solution without inoculum) making a total of three (3) groups. Five (5) factors included cephalexin concentrations, biomass concentrations, time, pH and temperature, while *x* the values used for each factor (Appendix B). In order to examine the effect of heavy metals on the biosorption efficiency CRD (4 * 5 * *x*) was used. Where: two (2) bacterial cell biomass (living and dead cells) and two (2) control for the biosorption process (cephalexin solution without heavy metals, living and dead cells conducted at the optimal condition determined in this work) making a total of three (4) groups. Five (5) heavy metals ($Ni^{2+}$, $Cu^{2+}$, $Zn^{2+}$, $Cd^{2+}$ and $Pb^{2+}$), while *x* represent the concentration values for each heavy metals (Appendix C).

### 2.2. Selection of bacterial strains

The bacterial consortium used in this study was selected and identified as described in previous work (Al-Gheethi et al., 2014; Al-Gheethi and Norli, 2014). The pure bacterial isolates were subjected to the screening process to test the potential of these isolates to resist antibiotics (cephalexin, amoxicillin, ampicillin, cefuroxime and ciprofloxacin) and heavy metal ions ($Ni^{2+}$, $Cu^{2+}$, $Zn^{2+}$, $Cd^{2+}$ and $Pb^{2+}$) as described in previous work (Al-Gheethi et al., 2014). The bacterial isolates which have exhibited resistance for both antibiotics and heavy metals were selected and then used to prepare bacterial consortium biomass. These strains included *Burkholderia cepacia 103WTNC*, *B. cepacia 503WTNC*, *B. cepacia 903WTNC*, *Chryseomonas luteola 313WTNC*, *C. luteola 613WTNC*, *C. luteola 1113WTNC*, *Pseudomonas fluorescens 1353WTNC*, *B. subtilis 1556WTNC*, *B. subtilis 212WTNC*, *B. subtilis 1612WTNC*, *Bacillus megaterium 1558WTNC*, *Bacillus sterothermophilus 1050WTNC*, *Citrobacter freundii 1763WTNC* and *Kluyvera* spp. *736WTNC* as identified in previous work (Al-Gheethi and Norli, 2014).

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