Introduction

Potable reuse is one of the alternatives to approach water sustainability and it is gaining widespread acceptance all over the world due to increasing stress on water resources (Rodriguez et al. 2009). As water reclamation and potable reuse is a viable option, constituents that might threaten human health must be considered seriously. Endotoxins, interchangeably named Lipo-polysaccharide (LPS), are among the constituents of concern. Unlike other pollutants and being associated to the biological reaction it is impossible to control the LPS endotoxins at the source. In several studies, reclaimed wastewater toxicity has been linked to the existence of the LPS endotoxin in treated water. While toxicity of reclaimed wastewater have been investigated previously, the current knowledge on the endotoxin problem is still limited, particularly regarding the amount of endotoxin in treated wastewater, the removal alternatives of these chemicals from water and their role in water toxicity. Thus the aim of this research was to gain understanding of the control and fate of endotoxin during wastewater reclamation processes. Ohkushi 2007 reports that the increase of endotoxicity in river water was associated with high endotoxicity in the effluent of treatment plants discharged in the river. However, no studies were reported investigating endotoxin in treatment processes.

In Chapter 1, background and objectives of the study were described. The trends of potable reuse of reclaimed wastewater were presented. Moreover, the lipo-polysaccharide endotoxin as a constituent of concern and treated wastewater toxicity are described in this section. The knowledge gaps related to endotoxin in reclaimed wastewater are also discussed. As far as we know, no studies investigated endotoxin during wastewater reclamation (Figure 1).

The research route map is shown in Figure 2. First a survey of endotoxicity in wastewater treatment plants and sludge treatment facility will be conducted. This section will focus on the control and fate of endotoxicity during biological wastewater treatment. Then, a characterization of organic matter showing endotoxicity, in domestic wastewater will be carried on. Based on characterization finding findings, several removal alternatives will be discussed. Finally, an annexe chapter will deals with the stress response and Heat shock protein HSP 47 release due to exposure to water contaminated with endotoxin (before and after treatment).
Fate of endotoxin during biological treatment of wastewater

In this thesis, control and fate of endotoxic active material were studied during wastewater reclamation processes. The investigation presented in chapter 2 included a field survey and lab scale experiments to assess endotoxin in wastewater treatment plants as well as in rejected water from sludge treatment facility and examine the fate of endotoxin during biological reaction.

Assessment of endotoxin activity in wastewater treatment plants

A field survey of endotoxic activity in 3 different treatment plants (A, B and C) was carried out. The three plants are operated using activated sludge process. While plant A and B receive only domestic wastewater, plant C receives domestic wastewater and rejected water from sludge treatment facility. Figure 3 shows that significant decrease of COD was observed in three different treatment plants. However, endotoxin decreased after treatment, but not significantly. This has lead to an increase in the ratio of endotoxin to COD in the effluents of these plants.

A batch test, reproducing the operation of treatment plants under controlled conditions and consisting of sewage aeration for 12 hours at 1500mg/l mixed liquor suspend solids, confirmed the observed trends. Indeed, Figure 4 shows how COD was decreasing significantly during the observed period of time (12 hours), while endotoxin is decreasing but not at the ratio as COD do. This leads to higher ratio of endotoxin to COD after 12 hours aeration. It clearly, reflects that domestic sewage contains organic matter showing endotoxicity, but significant part of it remains after biological treatment. This might explained by two facts. 1) Endotoxic active materials are not biodegradable but remaining in the effluent of treatment plants; 2) endotoxin is released during biological reaction.

Assumptions of endotoxin release during biological reaction, was confirmed using decay test, as shown in Figure 5. An increase in the endotoxicity was produced during the first 5 hours of aeration. This was followed by a decrease in the endotoxicity. Therefore, endotoxin is produced during decay test. However the organic matter showing endotoxicity is partly biodegradable.

No production occurs during growth phase as illustrated in figure 6. In addition, estimation of production and degradation proportions indicates that the initially existing endotoxin and the produced ones are partially non biodegradable.

As a summary, sewage and treated waste show high endotoxicity. Biological reaction contributes to the increase of endotoxicity. In addition the initially existing endotoxin and the produced ones are partially biodegradable.
Survey of LPS endotoxin in rejected water from sludge treatment facility

Field survey of endotoxin in treatment plants showed that plant C has always the highest endotoxin concentration (Figure 3). As plant C receives, in addition to domestic sewage, water from sludge treatment facility, a field survey of endotoxin in water form sludge treatment units was carried out. It was revealed that high endotoxinity in the influent of plant C was caused by water from sludge treatment units mainly dewatering and thickening processes. Indeed, endotoxinity in the sewage increased after receiving rejected water form sludge treatment units. The highest endotoxin loading is supplied from water from dewatering and thickening. Advanced analysis, using batch tests showed that these waters increase, also, the endotoxinity in the effluent of plant C as they contain organic matter showing endotoxinity which is not biodegradable. Mixture of sewage and rejected water are the main contributors of this endotoxinity in the effluent also. Figure 8a illustrates the high biodegradation of COD of both sewage and the mixture of sewage and rejected waters. However, Figure 8b clearly shows that higher biodegradation of organic matter showing endotoxinity is observed in case of sewage only. Thus after 12 hours aeration, higher endotoxinity remains in the reactor where sewage and rejected water are aerated.

Similar analysis was carried out to know which water has most of non biodegradable organic matter having endotoxinity. As shown in Figure 9a and b, water from dewatering and thickening are the main contributors of this endotoxinity in the effluent also.

In brief, the results of these investigations indicated that LPS endotoxins are uncontrollable in activated sludge system. This is worsening when treatment plant is receiving return flow from sludge treatment facility.

This is because: 1) These chemicals are produced during biological reaction, mainly decay process; 2) A significant amount of these chemicals are non biodegradable and can be found in the secondary effluent; 3) Endotoxin supplied from sludge and rejected water from sludge treatment facility, especially water from thickening and dewatering processes, have low biodegradability. Thus, the rejected water contributes to the increase of endotoxinity in the treatment plants.

Characterization of organic matter showing endotoxinity

The characterization of organic matter showing endotoxinity is the topic of chapter 3. First, using a decay test we investigated the endotoxicy organic matter released and its relationship with Lipo-polysaccharide (LPS). Then, water samples from raw wastewater and secondary treated water and showing endotoxicy wer characterized in terms of molecular weight and...
hydrophobic/hydrophilic structure using C18 sep pack cartridge and micro-filters and ultra-filters. This characterization revealed that: 1) Decay of bacteria release endotoxic active material such as LPS (Figure 10); 2) Organic matter with larger size (100KDa–0.1μm) exhibited higher endotoxin concentration (Figure 11). 3) Hydrophobic fraction is higher than the hydrophilic portion. It represents about 60% to 80% of the total organic matter of the secondary effluents (Figure 12).

Indeed, a characterization of organic matter showing endotoxicity was carried out helping in selecting removal alternatives to control endotoxin in treated water.

As organic matter showing endotoxicity is mainly large molecules, representing up to 80% of the total organic matter, it can be concluded that size exclusion (ultra-filtration and soil treatment) and coagulation could be used to remove these large molecules. Furthermore, hydrophobic fractions could be removed through their attachment to colloids and particulate matter. Soil treatment and hydrophobic membranes are some alternatives to remove endotoxins.

Figure 10: COD, KDO endotoxin and ratio KDO to COD during decay test

Figure 11: Size characterization of organic matter having endotoxicity (Molecular weight distribution)

Figure 12: Structural characterization of organic matter having endotoxicity (Hydrophobic/Hydrophilic)
Endotoxin removal by the means of advanced treatment alternatives

**Soil aquifer system**

In this thesis, the aforementioned advanced treatment alternatives (soil columns, coagulation and membrane filtration) have been investigated. Chapter 4 presents the results of endotoxin removal from secondary treated wastewater using soil columns, a submerged MBR followed by a parallel set of nano-filter and reverse osmosis and a coagulation test.

Four different soil columns were operated. Each column was packed with a specific soil. Column N°1 contains sand with a specific diameter ranging from 0.85-1.4mm. Column N°2 is packed with 0.45-0.85mm sand. Column N°3 and N°4 were packed respectively with fine sand and silt (0.125-0.45mm). Prior to experiments, the soil columns were biologically acclimated by infiltration of secondary treated water for a period of 1 month. The system operated under gravity flow conditions. An estimation of flux rates showed that it ranges from 4.4 to 5.5ml/hr/cm². Within the observed period of operation (four months), we investigated the short term effectiveness of vadose zone in removing endotoxin from treated wastewater. Endotoxin removal ranged from 64.3% to greater than 86% during the study, with endotoxin levels averaging 307.6EU/ml, 211.6EU/ml, 194EU/ml and 114.5EU/ml for the SAT columns packed with different soils. Fine sand and silt were the most effective ones. Endotoxin concentrations exhibited exponential decline through the unsaturated zone, while DOC concentrations showed a gradual decrease (Figure 14). Effectiveness of top layers in removing endotoxins has decreased over time and did not stabilize during the observed period of time (Figure 15). Using adsorption test, it was found that adsorption plays an important role in reducing endotoxin concentration, mainly, through attachment of hydrophobic organic matter showing endotoxicity to soil particles. SAT and groundwater recharge would provide a high degree of endotoxin removal in an integrated low-tech wastewater reuse management strategy, especially for developing countries in arid regions of the world.

![Figure 13: SAT experimental set up](image)

![Figure 14: COD and endotoxin profiles in the unsaturated zone](image)

![Figure 14: decrease of efficiencies of upper layers of soil columns](image)

**MBR followed by a parallel set of NF and RO**

Membrane filtration system consisted of a pilot unit of MBR followed by a parallel set of NF/RO operated for six weeks without backwashing and chemical cleaning. MBR-NF/RO pilot unit results showed that: 1) MF using 0.1μm MBR removed a significant amount of the endotoxin, even though the membrane pore is larger than the molecular size of endotoxin meaning that a significant amount of endotoxin has a hydrophobic character.; 2) The NF and RO removed most of the endotoxin remained after MBR filtration; 3) A decrease in the MBR-NF/RO endotoxin removal efficiencies was associated with an increase of trans-membrane pressure (TMP). Development of bio-film on the membrane surface might be the cause of a decrease in the endotoxin removal efficiencies.
time in which to either stop delivery of water or to apply corrective actions in the event of a treatment failure.

**HSP47 production due to exposure of CHO cells to water contaminated with endotoxins**

Heat shock protein 47 assay was performed to assess the stress response induced by water contaminated with LPS endotoxin. Our objectives were achieved by utilizing the Chinese hamster ovary cells that were exposed to various concentrations of water samples. The samples were diluted with 0.1%, 1% and 10% fold dilution. The HSP47 was detected in secondary treated water samples. However, CHO cells exposed to water samples from the advanced treatment alternatives showed no response. In contrast, Endotoxicity was detected in these advanced treatments due to high sensitivity of LAL test used for endotoxin detection, which is not the case for bioassay. So far, no correlation has been found between LPS endotoxin and stress response. However, it is believed that we can reduce water toxicity through endotoxin reduction. This can be achieved, for instance, through removal of large molecules as these molecules are found to cause stress response and also endotoxicity.

**Conclusions**

Important results of the present study are the following:

- Biological treatment can not control endotoxicity, especially when receiving rejected water from sludge treatment facilities. The biological treatment can not produce an acceptable level of endotoxicity in the effluent of treatment plants.
- Biological treatment produces organic matter showing endotoxicity, such as LPS which mainly large and hydrophobic.
- Exploring, the aforementioned characteristics of endotoxic indicative material, advanced treatment alternatives such as soil treatment, MBR followed by NF/RO, coagulation can, significantly, reduce endotoxicity.
- However, even endotoxicity in RO permeate is higher than that of tap water.

**References:**

