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学 位 論 文 題 名

Control of endotoxins and their fate during wastewater reclamation

（排水再生処理過程におけるエンドトキシンの挙動とその制御）

学位論文内容の要旨

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. As water reclamation and potable reuse is a viable option, constituents that might threaten human health must be considered seriously. Endotoxins, interchangeably named Lipo-polisccharide (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.

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.

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. 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 is 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 endotoxicity in the treatment plants.

The characterization of organic matter showing endotoxicity is the topic of chapter 3. Indeed, a characterization of organic matter showing endotoxicity was carried out helping in selecting removal alternatives to control endotoxin in treated water. This characterization revealed that: 1) Decay of bacteria release endotoxic active material such as LPS; 2) Organic matter with larger size (100KDa 0.1μm) exhibited higher endotoxin concentration. 3) Hydrophobic fraction is higher than the hydrophilic portion. It represents about 60% of the total organic matter of the secondary effluents.

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.

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 No1 contains sand with a specific diameter ranging from 0.85-1.4mm. Column No2 is packed with 0.45-0.85mm sand. Column No3 and No4 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 infiltration rates showed that it ranges from 80 to 110ml/hr.Within the observed period of operation (six 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 vadose zone, while DOC concentrations showed a gradual decrease. Effectiveness of top layers in removing endotoxins has decreased over time and did not stabilize during the observed period of time. 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.

Membrane filtration system consisted of a pilot unit of MBR followed by a parallel set of NF/RO. 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.

Results form coagulation showed that Coagulation can, at a certain pH range, remove endotoxins. Coagulation test have shown that this treatment is efficient and removes endotoxicity of about 60% at its maximum efficiency at a pH ranging from 6.4 to 6.9. As a cheap treatment alternative, it can be considered as an effective first barrier for endotoxin reduction from reclaimed wastewater.

A comparison between the aforementioned treatment alternatives showed that all of these systems decrease water endotoxicity to a level higher that that of tap water. The most efficient one is the set of MB-NF/RO. Dilution and water storage can be considered to safely reuse wastewater. Therefore, futures research need to investigate the effect of retention time of the recycled water in the raw water supply if it allows any remaining endotoxins to be degraded by physical processes (e.g. natural ultraviolet light) or biological processes (e.g. native micro-organisms) is imperative. Furthermore, Storage of the recycled water for a period of time before consumption can provide an interval of time in which to either stop delivery of water or to apply corrective actions in the event of a treatment failure.

Toxicity assessment of reclaimed wastewater was also performed to assess the stress response induced by water contaminated with LPS endotoxin. Chapter 5 gives the results of stress response analysis using heat shock protein 47 assay. 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. We applied a microbial assay to detect the expression of heat shock protein HSP47. The HSP47 was detected in samples where transfected cells were exposed to secondary treated water. However, CHO cells exposed to water samples from the advanced treatment alternatives, investigated in this thesis, 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.

In Chapter 6, important results of the present study were summarized and recommendations for future study are presented.

学位論文審査の要旨

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水資源の不足に伴い，排水を再利用することが世界で広く実施されている．特に，飲用排水再利用は乾燥域，半乾燥域においてその重要が強く認識されている．排水の飲用再利用においては，再生中の毒性物質の制御が重要である．再生中に含まれる毒性物質は,(1) 排水中に存在し，処理できない物質,(2) 処理過程で使用される薬品の不純物として投入される物質,(3) 処理過程で生成する物質に大別される．本研究はこれらの毒性物質のうち，これまで，詳細に検討されることが少なかった生物処理過程での生成が避けられないエンドトキシンに注目したものである．すなわち，グラム陰性菌の細胞膜構成物質の一つである Lipo-polischaride (LPS) がエンドトキシン活性を有することに注目し，この物質の処理性，処理過程内での挙動，再生処理における除去性の検討を目的としている．

本論文は以下の 6 章より成る．

第 1 章では本研究の背景，目的を議論している．また，再生水の飲用利用に関する最近の動向を整理し，再生水の飲用利用における毒性物質管理の重要性について議論している．また，再生中に含まれる毒性物質のうち，エンドトキシン活性を示す，LPS の重要性とその研究の現状について整理し，排水再利用の分野において十分な検討がなされていないことを指摘している．

第 2 章では実処理施設におけるエンドトキシン活性物質の測定結果，実験室規模の実験，ならびに汚泥処理施設からの返流水中エンドトキシン測定結果について議論している．これらの検討から,(1) 通常の活性汚泥処理プロセスでは，単位有機物あたりのエンドトキシン活性は流入水よりも処理水で増加すること,(2) 活性汚泥処理プロセスではエンドトキシン活性物質のコントロールは容易でないことを結論付けている．また,(3) 汚泥処理施設返流水が処理水のエンドトキシン活性に影響することを見出し，その理由として (a) エンドトキシン活性物質が汚泥の自己分解過程で生成されること,(b) そして，これらの物質の多くが難生物分解性であることを明らかにし，特に汚泥濃縮層上澄水，脱水ろ液の寄与が高いことを示している．

第 3 章では，エンドトキシン活性物質処理の基礎知見を得るために実施したエンドトキシン活性を有する有機物の特性解析の結果を記載している．本章で得られた主要な結果を整理すると,(1) 汚泥の分解過程で LPS が放出され，その結果エンドトキシン活性が増加する,(2) 分子量 100kDa 以上の有機物のエンドトキシン活性が高い,(3) 疎水性の強い有機物のエンドトキシン活性が高い．そして，エンドトキシン活性物質の処理には膜処理，土壌処理，凝集処理等が適していることを見出し，以

後の章において, これらのエンドトキシン除去特性を検討している.

第 4 章では二次処理水を土壌処理, 凝集処理した場合, ならびに二次処理として膜分離活性汚泥法 (MBR) を用い, その処理水を NF 膜,RO 膜で処理した場合について, エンドトキシンの処理性について検討している. まず. 土壌処理については, 粒径の異なる 3 種類の砂粒子, ならびにシルト粒子を充填したカラムによる処理実験結果を比較している. 得られた結果は (1) 土壌の不飽和層においてエンドトキシンは除去され, その除去率は 64 から 86 パーセントの範囲にある,(2) 粒径の小さい砂, シルトの除去率が最も高い, (3) 不飽和層において, エンドトキシン濃度は指数関数的に上層において減少するのに対し, 有機物濃度は全層にわたって緩やかに減少する,(4) 吸着試験の結果もあわせて考察すると, 除去の主要な機構は吸着と考えられる,(5) 土壌処理は再生水の地下水涵養過程において, 不飽和層において生じる処理であり, エネルギー消費の少ないプロセスである. 次に, 膜分離活性汚泥法 (MBR) を用いた場合の結果について, 次のように整理している.(1) 精密ろ過膜 (MF 膜) により, エンドトキシンは分離され, 処理水中には通常の活性汚泥法の場合と比較して低い濃度となる,(2) ただし, エンドトキシンの分子量に比較して MF 膜は大きな孔径となっており, エンドトキシン活性を有する有機物の疎水性が分離の主要因子である,(3) 膜分離活性汚泥法の処理水に残存するエンドトキシンは NF 膜または RO 膜により除去される,(4)MBR ならびに NF/RO 膜においてファウリングが進行するにつれ, エンドトキシン除去性の低下がみられる, これはファウリングの要因の一つである生物膜の形成に関連すると推察される. 最後に凝集処理のエンドトキシン除去特性を検討した結果を次のように整理している.(1) 二次処理水に対する凝集処理のエンドトキシン活性の除去率は約 60 パーセント程度である,(2) 最適 pH の範囲は 6.4 から 6.9 である. 以上,3 種の処理プロセスのエンドトキシンの除去性を比較すると,MBR 処理水を NF 膜または RO 膜によって処理する場合が最も除去性が高いが, 依然として水道水中エンドトキシン濃度よりは高い値となっており, 排水の再利用においては, 希釈と貯水池等においての貯蔵が必要であると判断されるとしている. 今後の課題として, 貯留時におけるエンドトキシンの挙動について知見を得ることが必要としている.

第 5 章では heat shock protein47 を用いたバイオアッセイ結果とエンドトキシン活性との関連について検討した結果が記されている. 得られた知見は次のようである:(1)NF 膜や RO 膜で高度に処理した場合には heat shock protein47 よる応答が観察されない場合においても, エンドトキシンの活性は観察される,(2) heat shock protein47 による応答とエンドトキシン活性の間には明確な相関関係は存在しない.

第 6 章には本研究で得られた主要な成果が整理され, 将来の展望が述べられている.

これを要するに, 著者は排水の飲用再利用において, その安全性を担保する際に重要と判断される物質の一つであるエンドトキシン活性物質について, その処理プロセスにおける挙動, 分子量と処理性, 再生処理プロセスにおける除去性について新知見を与えており, 排水再利用学に対して貢献するところ大なるものがある. よって, 著者は, 北海道大学博士 (工学) の学位を授与される資格あるものと認める.