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Sterols indicate water quality and wastewater treatment efficiency

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ABSTRACT

As the world's population continues to grow, water pollution is presenting one of the biggest challenges worldwide. More wastewater is being generated and the demand for clean water is increasing. To ensure the safety and health of humans and the environment, highly efficient wastewater treatment systems, and a reliable assessment of water quality and pollutants are required. The advance of holistic approaches to water quality management and the increasing use of ecological water treatment technologies, such as constructed wetlands and waste stabilisation ponds (WSPs), challenge the appropriateness of commonly used water quality indicators. Instead, additional indicators, which are direct measures of the processes involved in the stabilisation of human waste, have to be established to provide an in-depth understanding of system performance. In this study we identified the sterol composition of wastewater treated in WSPs and assessed the suitability of human sterol levels as a bioindicator of treatment efficiency of wastewater in WSPs. As treatment progressed in WSPs, the relative abundance of human faecal sterols, such as coprostanol, epicoprostanol, 24-ethylcoprostanol, and sitostanol decreased significantly and the sterol composition in wastewater changed significantly. Furthermore, sterol levels were found to be correlated with commonly used wastewater quality indicators, such as BOD, TSS and *E. coli*. Three of the seven sterol ratios that have previously been used to track sewage pollution in the environment, detected a faecal signal in the effluent of WSPs, however, the others were influenced by high prevalence of sterols originating from algal and fungal activities. This finding poses a concern for environmental assessment studies, because environmental pollution from waste stabilisation ponds can go unnoticed. In conclusion, faecal sterols and their ratios can be used as reliable indicators of treatment efficiency and water quality during wastewater treatment in WSPs. They can complement the use of commonly used indicators of water quality, to provide essential information on the overall performance of ponds and whether a pond is underperforming in terms of stabilising human waste. Such a holistic understanding is essential when the aim is to improve the performance of a treatment plant, build new plants or expand existing infrastructure. Future work should aim at further establishing the use of sterols as reliable water quality indicators on a broader scale across natural and engineered systems.

1. Introduction

Declining water quality has become a major global issue (UN-Water, 2011) and the supply of water represents a major challenge for future generations (Vörösmarty et al., 2010). Land-use changes related to agriculture, husbandry and urbanisation contribute to the deterioration of water quality worldwide (Foley et al., 2005). Run-off from these areas contains high concentrations of nutrients, pathogens, metals and other micropollutants, which can contaminate groundwater and surface water. In addition, an increasing volume of sewage is produced by the growing human population and this requires adequate treatment before being discharged into the environment or re-used. Constructed wetlands and waste stabilisation ponds (WSP) have been established as sustainable treatment technologies that harness the natural self-purification ability of water bodies (Hench et al., 2003; Mara, 2004; Mitchell et al., 1995; Nelson et al., 2004; Wu et al., 2016) and are increasingly used on a global scale. Although used extensively worldwide for decades, WSPs are known to suffer from

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differing levels of efficiency, partially due to the accumulation of sludge at the bottom (Chua et al., 2013). A low efficiency will lead to low quality effluent and potentially the presence of environmental risks such as the development of toxic cyanobacterial blooms (Barrington et al., 2013, 2015) or greenhouse gas emissions (Glaz et al., 2016). Low water quality and the presence of toxic cyanobacteria pose a direct risk to humans (Barrington et al., 2014; Reichwaldt et al., 2016) and the environment through eutrophication (Ghadouani and Coggins, 2011) or cyanotoxins presence (Parker et al., 2016; Sinang et al., 2015; Song et al., 2015) emphasising the importance to accurately quantify treatment efficiency. However assessing the efficiency of the treatment process is currently difficult due to a lack of reliable, stable and meaningful water quality indicators.

Contamination of water resources through sewage is an ongoing problem worldwide (Deblonde et al., 2011; Pal et al., 2010, 2014; Wang et al., 2015). Commonly used indicators for assessing the quality of wastewater after treatment and water contaminated by wastewater include nutrients, biological oxygen demand (BOD), total suspended solids (TSS) and *Escherichia coli* (e.g., ANZECC, 2000). However, there are several problems related to the use and quantification of some of these indicators, resulting in an inadequate assessment of wastewater quality and identification of sewage pollution of surface water. These problems are mainly related to difficulties with methods (e.g., BOD, *E. coli*; see Augustin and Carlier, 2006; Corry et al., 2007; Gray, 2004; Servais et al., 2009), the indicators' drastic response towards changes of environmental conditions (e.g., *E. coli*, BOD, TSS: see Gray, 2004), a lack of comparability with other human contaminants (*E. coli*: see Maiga et al., 2009), or their general relevance (e.g., TSS: Maynard et al., 1999). Therefore, to reliably identify human pollution and quality of water impaired by humans, indicators specific to human contamination that are stable under varying environmental conditions must be established.

Sterols and their ratios have long been used as indicators of sewage pollution in a variety of aquatic systems due to their resistance to environmental changes (de Castro Martins et al., 2007; Furtula et al., 2012a; Bujagić et al., 2016; McCalley et al., 1981; Walker et al., 1982). Sterols are naturally occurring lipid molecules produced by any organism. The high specificity of some sterols for their faecal source (Bull et al., 2002; Leeming et al., 1996; Sinton et al., 1998) makes them ideal indicators to trace human contamination in a variety of environmental systems (reviewed in Field and Samadpour, 2007) such as sediments of rivers (Ayebo et al., 2006; Writer et al., 1995) and estuaries (Carreira et al., 2004; Reeves and Patton, 2005), coastal marine water (Leeming et al., 1998), inner-shelf sediments (Leeming et al., 1997), and to estimate human populations (Daughton, 2012). Further, one study quantified changes in sterol compositions during secondary and tertiary wastewater treatment to propose new criteria for identification of human faecal contamination (Furtula et al., 2012a). However, so far there has been no attempt to use sterols as indicators of the treatment process.

Secondary wastewater treatment in waste stabilisation ponds (WSPs) relies on a mutualistic relationship between bacteria and algae to remove contaminants. In WSPs it can be expected that the relative abundance of human sterols decreases, while sterols originating from natural aquatic organisms such as non-faecal bacteria, phytoplankton, fungi and zooplankton increase (Fig. 1). The main removal mechanism of sterols from human faeces during wastewater treatment is through a physical removal of particulate matter to which the sterols are adsorbed; however bacterial degradation rates of sterols have been found to be system specific (Walker et al., 1982) and have been determined to range from being substantial (Teshima and Kanazawa, 1978) to minor (Daughton, 2012).

The main sterols that can be found in human faeces are coprostanol, 24-ethylcoprostanol, cholesterol, sitosterol and sitostanol (Bull et al., 2002; Leeming et al., 1996; Sinton et al., 1998; Walker et al., 1982). Cholesterol and sitosterol are both unsuitable to serve as strict indicators of faecal contamination, due to their ubiquitous nature and their origin from ingested plant material, respectively (Furtula et al., 2012a; Murtaugh and Bunch, 1967). Coprostanol on the other hand, as the major sterol in human faeces (Leeming et al., 1996) has been used as an indicator of faecal contamination in the environment (Mudge and Seguel, 1999; Peng et al., 2005; Reeves and Patton, 2005) even under conditions when pathogens (e.g., *E. coli)* have been destroyed by chlorination or heat (Devane et al., 2006; Goodfellow et al., 1977; Walker et al., 1982). However, because coprostanol is in small amounts ubiquitous as it can be produced in-situ under anoxic conditions (Grimalt et al., 1990; Nishimura, 1982; Nishimura and Koyama, 1977), and because correlations between organic carbon and coprostanol have been found (Chan et al., 1998), ratios between sterols have been suggested as indicators of sewage pollution in environmental systems (Carreira et al., 2004; Devane et al., 2015; Froehner and Sanez, 2013; Furtula et al., 2012a, 2012b; Reeves and Patton, 2005) (Table 1). Sterol ratios that are commonly used include $5\beta/(5\alpha+5\beta)$ [Coprostanol/(Coprostanol + Cholestanol)], which identifies if the source of the sterols is biogenic or from sewage (Grimalt et al., 1990), Coprostanol/Epicoprostanol which discriminates between human and non-human pollution (Reeves and Patton, 2005), Coprostanol/Cholestanol, which discriminates between human and algal sterols (Devane et al., 2006; Leeming et al., 1997, 1998), and Coprostanol/Cholesterol, which distinguishes between biogenic sources e.g., human versus phytoplankton and zooplankton (Fattore et al., 1996). Further, under natural conditions, coprostanol is biosynthesised to epicoprostanol by microbes, making it possible to use the ratio Epicoprostanol/Coprostanol to discriminate between untreated and treated sewage (de Castro Martins et al., 2007; Mudge and Seguel, 1999) and to determine the age of faecal matter (Adnan et al., 2012; Mudge and Duce, 2005; Mudge and Seguel, 1999). The threshold for each ratio to identify contamination differs between the published literature, which might be due to system specific differences in biophysicochemical processes, including the temperature dependent microbial activity (Carreira et al., 2004). The development of these thresholds has largely been based on studies investigating sewage contamination from advanced treatment plants (Furtula et al., 2012a; Glassmeyer et al., 2005; Venkatesan and Kaplan, 1990; Walker et al., 1982). However, these differ in their treatment processes from waste stabilisation ponds, which rely more on a healthy bacteria-algae mutualism to stabilise the waste (Mara, 2004). To establish the use of sterol ratios as reliable, universal indicators of sewage pollution in the environment, it is therefore essential to identify sterol fingerprints during the treatment of wastewater in a range of sewage treatment technologies. As such, this study focusses on changes in the sterol composition during the treatment of wastewater through waste stabilisation ponds (WSP) as a first step towards an integrative approach that uses sterols as indicators of pollution.

The overall aim of this study was to evaluate the suitability of human sterol levels as an indicator for treatment efficiency of wastewater in waste stabilisation ponds and to assess the use of sterols as a biomarker of the relative importance of human waste within waste stabilisation ponds. More specifically, the objectives of this study are i) to quantify the change in the relative contribution of faecal sterols to the total sterols during the wastewater treatment process, ii) to investigate the dynamics of sterol fingerprints as a function of treatment, iii) to assess the suitability of sterol ratios to detect faecal signals in a continuum of treated wastewater, and iv) to identify the relationship between sterols and common water

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