1. INTRODUCTION

Nitrogen is important in life as it is the fourth most abundant element in living organisms after carbon, oxygen and hydrogen. In fact, nitrogen is used by living organisms to produce a number of complex organic molecules such as amino acids, proteins and nucleic acids. Even though nitrogen is largely found in air, it appears as molecular nitrogen gas which is inert and is unavailable for many living organisms. Only a very small fraction of nitrogen gas is directly incorporated into terrestrial or marine ecosystems by natural processes; as a result, nitrogen bioavailability has been the most important limiting factor for biomass production on a global scale. In order to guarantee nitrogen supply for intensive agriculture and animal production systems, the industrial production of fertilisers by chemical nitrogen fixation from the atmosphere into ammonia has played a decisive role since the 1960s. As a consequence, manmade nitrogen fertilizers have contributed significantly to the sustained growth of world population over the past 50 years (Gijzen and Mulder, 2001).

1.1 THE CURRENT GLOBAL NITROGEN MASS BALANCE

The world's annual consumption of nitrogen fertilizer has increased ninefold from 10 Mt N in 1960 to 91 Mt N in 2006 (IFA, 2007). However, nitrogen is still poorly incorporated into plant protein (only some 10−15%) and therefore crops are for most of the time overdosed with N. Moreover, once plant protein has been consumed as a human food or animal feed, a major part of it returns to the environment in domestic wastewater and manure. The amount of nitrogen entering into the biosphere by means of industrial production of nitrogen fertilizers and natural fixation is currently 57 percent greater than the amount of nitrogen returning to the atmosphere via the denitrification process (210 Mt N/year) (Gijzen, 2001). Therefore, the net content of nitrogen in terrestrial and marine ecosystems is constantly rising on a global basis.

The excessive concentration of nutrients such as phosphorus and reactive nitrogen species, in surface water bodies, can introduce changes in ecosystem function and diversity. It may cause fish kills due to large diurnal variations in dissolved oxygen levels which are associated with greatly increased primary productivity due to excessive algal growth. Indeed, nutrients enhance the growth of all aquatic plants from the microscopic (algae) to the macroscopic (macrophytes) and this enhanced growth, which generally results in eutrophication, commonly interferes with desirable water uses. Eutrophication has been a serious environmental concern in the developed world over the last 30 years or so and is now a major global concern. In addition, high concentrations of ammonium nitrogen, in conjunction with high pH values, promote the presence of free ammonia which is toxic at low concentrations (>0.05 mg NH_3-N/L) to fish and may become lethal (>2.0 mg NH_3-N/L); it is well known that pH values rise under high primary productivity conditions. Furthermore, the presence of nitrate (>10 mg N/L) in raw waters is not desirable as conventional water treatment works are not able to remove nitrate and it may cause methaemoglobinemia in infants.

1.2 NITROGEN CONTROL IN WASTEWATER TREATMENT PLANTS

Domestic wastewater discharge is one of the primary sources of nitrogen in watersheds with low agricultural activity. This may become a major environmental problem due to many of the existing domestic wastewater treatment plants not being equipped to control nutrients (tertiary treatment). At the present time only 5 percent of the total volume of wastewater receives tertiary treatment on a global scale (Gijzen and Mulder, 2001). Accordingly, discharge consents for large wastewater treatment plants are being adjusted worldwide to achieve a greater degree of sustainable nutrient control.

In Member States of the European Union, for instance, the Urban Waste Water Treatment Directive (UWWTD) (91/271/EEC) and the Water Framework Directive (2000/60/EC) (CEC, 1991, 2000) require that the minimum percentage of reduction of the overall load[1] entering all large wastewater treatment plants (WWTP) (>10,000 p.e.[2]) located in areas with "sensitive" waters is at least 75 percent total nitrogen and total phosphorus. On the other hand, very few small works (<2,000 p.e.) are required by their local environmental regulator to remove nutrients. Water bodies can be identified as sensitive areas on three grounds: (a) where they are found to be eutrophic or where they may become eutrophic in the near future if protective action is not taken; (b) where they exceed or could exceed a specified concentration of nitrate (to protect water supply sources and/or the environment); and (c) where discharges affecting them are subject to more than secondary treatment to comply with the standards of other Directives. Currently, there

[1] Reduction in relation to the load of the influent = influent flow multiplied by analyte concentration.

[2] 1 p.e. (population equivalent) means the organic biodegradable load having a five-day biochemical oxygen demand (BOD_5) of 60 g of oxygen per day.

are 525 sensitive areas identified in the United Kingdom, of which 234 match criterion (a) in previous page (Defra, 2007).

Consequently, most large WWTP have opted for including either biological or chemical nutrient removal processes such as modified activated sludge or chemical precipitation processes for nutrient removal (nitrogen and phosphorus). However, such processes are very expensive in terms of energy consumption and sludge treatment and also require highly skilled operators. This approach may be reliable and reasonably cost-effective at large WWTP in industrialised countries, yet it is almost always financially and/or technically inappropriate for developing countries or small communities (<2,000 p.e.) since they are unable to afford the high capital and maintenance costs or achieve the same economies of scale that are achieved at large WWTP (Keplinger *et al*., 2004).

In the UK, for instance, WWTP serving populations of less than 2000 p.e. accounted for 78 percent of all treatment works in the year 2000 (Defra, 2002), but they treat only 4 percent of the wastewater produced. They present the greatest risk of non-compliance with effluent quality requirements, particularly when taking into account the fact that discharge permits are more restrictive than those in the UWWTD are generally imposed when final effluents are discharged to small water courses (Griffin and Pamplin, 1998). For that reason, it is expected that over the next few years many more small WWTP will be required to remove both nitrogen and phosphorus.

1.3. LOW-COST NITROGEN CONTROL FOR DOMESTIC WASTEWATERS

Natural wastewater treatment systems are specifically designed to obtain the intended waste treatment goal by utilizing natural responses to the maximum possible with the minimum use of electromechanical treatment processes (Crites *et al*., 2006). They are more appropriate for small communities and developing countries mainly due to their intrinsic simplicity and high efficiency. While they require greater areas of land than WWTP based on energy-intensive mechanical technologies (e.g., activated sludge), their lower cost of construction, operation and maintenance, as well as their much smaller production of sludge, make them an excellent and cost-effective option (Mara, 2004, 2006).

Natural wastewater treatment systems such as waste stabilisation pond (WSP) systems are extensively used in the United States (>7000 systems), France (>2500 systems) and Germany (>3000 systems), but hardly at all in the UK, which has only ~40 systems, all but two privately owned (Abis, 2002). WSP technology is based on bacterial waste degradation and the symbiotic relationship between algae and bacteria; it comprises a number of ponds connected in series, including anaerobic ponds, facultative ponds and maturation ponds. The reasons for the apparent unpopularity of WSP in the UK are not immediately obvious, but they are probably related to perceptions that land costs are too high, the climate is inappropriate, and the effluent quality requirements set by the environmental regulators are not easily attainable, especially for suspended solids (SS) as WSP effluents generally contain a high level of mainly algal SS (Johnson *et al.,* 2007).

Fortunately, most of these question marks have been answered in favour of WSP technology, due in no small part to projects such as Yorkshire Water's 'Ecological Wastewater Treatment Plant' at Scrayingham, North Yorkshire, which works extremely well and meets its consent (Long, 2006). Indeed WSP systems have several important advantages such as low capital costs, simple and inexpensive operation and maintenance, suitability for both large and small populations (from a few hundred to hundreds of thousands), ultra reliability, negligible sludge production, tolerance of peak and intermittent flows and the removal of faecal micro-organisms more effectively than achieved through conventional sewage treatment. Also, WSP systems can be designed easily to produce high-quality effluents suitable for restricted and unrestricted irrigation and for fish and aquatic vegetable culture or simply surface water pollution control (Mara, 2004).

Normally WSP are not considered a reliable technical option for nutrient removal from domestic wastewater. However, recent studies on WSP in the UK have shown that nitrogen is removed to low levels (<5 mg ammonium N per litre) in both winter and summer, but it is not yet known how to optimize WSP design criteria for nitrogen and ammonium removal (Abis and Mara, 2003). In a WSP system, maturation ponds are most commonly designed to reduce the number of pathogenic organisms in secondary effluents (facultative pond effluents), but they also make important improvements in physicochemical wastewater quality by reducing the concentrations of organic matter (BOD) and SS. Additionally, they can make a significant contribution to cumulative nitrogen and phosphorus removal in WSP systems (Mara and Pearson, 1986, 1998; Pearson *et al*., 1987a; Mara *et al*., 1992).

Feasible mechanisms and pathways by which nitrogen in its various forms can be transformed in and removed from WSP have been proposed and supported by research carried out in many parts of the world, including: (a) ammonia volatilisation (Pano and Middlebrooks, 1982; Soares *et al*., 1996; Rockne and Brezonik, 2006); (b) biological uptake (Digiano *et al*., 1982; Ferrara and Avci, 1982); (c) simultaneous nitrification-denitrification (Zimmo *et al*., 2003; Picot *et al*., 2005); and/or (d) sedimentation of dead biomass and accumulation of organic nitrogen in sludge layer after partial ammonification (Ferrara and Avci, 1982; Reed, 1985; Pearson *et al*., 1988).

The majority of these studies are based on the measurements of nitrogen fractions (organic, ammonium, nitrite and nitrate) in water samples collected from both the pond influent and effluent. Occasionally, average in-pond characteristics such as ammonium, pH and temperature have been used as inputs into mathematical models to estimate nitrogen removal rates (especially ammonia volatilisation rates), regardless of their well known diurnal and seasonal variations. However, such approaches may in fact make understanding of the fate (or fates) of nitrogen in WSP particularly difficult in situations in which water quality changes are so small that they do not provide evidence about simultaneous processes such as, for instance, nitrification-denitrification or nitrification-biological nitrate uptake. A much better approach to understanding the fate(s) of nitrogen compounds in WSP may be based on using the stable nitrogen isotope ^{15}N, which has been largely used to illustrate the behaviour of nitrogen in aquatic ecosystems. In wastewater treatment, nitrogen transformations can be tracked and removal rates estimated by using two types of stable isotope studies: (a) isotope fractionation (Kanazawa and Urushigawa, 2007) and/or (b) tracer methods (Reddy, 1983).

1.4. SCOPE, AIM AND OBJECTIVES

The debate continues regarding the relative importance of the various pathways through which, and the mechanisms by which, nitrogen is transformed and removed in WSP. Therefore, in order to improve current understanding of the dynamics of inorganic and organic nitrogen species in WSP systems, this research work reports results from a series of experiments including, among others, tracer experiments with ^{15}N. These experiments were conducted to elucidate the important mechanism(s) and pathway(s) involved in nitrogen removal and transformations occurring in WSP, which will help engineers designing WSP systems to meet nitrogen discharge consents and consequently, to prevent negative environmental impacts associated to nitrogen discharges in receiving surface water bodies.

The aim of this project is to determine pathways and mechanisms involved in removal and transformations of nitrogen compounds to the interior of maturation ponds used as tertiary treatment in municipal wastewater treatment systems. The study seeks to determine under UK climatic conditions the relative importance of, and seasonal variation in, nitrogen removal through: (a) ammonium volatilisation through the pond surface and (b) sedimentation of organic nitrogen via biological uptake and subsequent accumulation of recalcitrant algal organic nitrogen in the pond sediments. The research results are then used to develop a descriptive model for understanding nitrogen transformation and removal in maturation ponds.

The specific objectives related to this research work are as follows:

- To evaluate the performance of maturation ponds in terms of nitrogen removal and its seasonal variation in a temperate-climate country.
- To determine the relative importance of ammonia volatilisation processes on ammonium and total nitrogen removal in maturation ponds.
- To establish the contribution to ammonium and total nitrogen removal of nitrogen algal uptake and its subsequent retention in the sludge layer in maturation ponds, previous sedimentation and partial hydrolysis of dead algal biomass.

1.5. STRUCTURE OF THESIS

The structure of this thesis comprises, apart from this introductory chapter, nine further chapters as follows: Chapter 2 – Literature Review, which is focused on the environmental significance of nitrogen compounds and fundamentals regarding to feasible pathways involved in nitrogen transformation in natural environments and removal mechanisms in domestic wastewater treatment systems. Also, there is an introduction to tracer experiments with stable nitrogen isotopes which are undertaken in this research. Chapter 3 – Materials and Methods: this describes in detail the methodology followed for all the experiments carried out, including tracer experiments with Rhodamine WT, design and testing of an apparatus to measure ammonia volatilisation rates from WSP, tracer experiments with ^{15}N stable isotopes, and monitoring for nitrogen removal and performance indicators from two pilot-scale maturation ponds working in series.

In chapters 4 to 8, the results from each experimental phase are reported, analysed and compared with previous research works. Chapter 4 – Physical and hydraulic characterization: this includes the determination of physical and hydraulic characteristics of the two maturation ponds under study. Chapter 5 – Maturation pond performance and nitrogen removal efficiency: it includes a study of the seasonal variation of the performance of maturation ponds, regarding operational parameters (biodegradable organic matter, suspended solids and pathogens removals) and nitrogen removal. Chapter 6 – Ammonia volatilisation in maturation ponds: this chapter includes the development of a reliable method to measure ammonia volatilisation rates from WSP in operation and the determination of the importance of ammonia losses to the atmosphere on permanent nitrogen removal.

Chapter 7 – Nitrogen removal by sedimentation of organic nitrogen: this investigates the role of organic nitrogen sedimentation, after biological nitrogen uptake, on nitrogen removal in WSP; and Chapter 8 – Tracer experiments with ^{15}N stable isotopes in maturation ponds: this reveals the fate of inorganic and organic nitrogen species in maturation WSP under summer and winter conditions, by using ^{15}N-labelled compounds as tracers.

Chapter 9 – General Discussion: this chapter summarises new evidence found as part of the research work undertaken which will contribute to improve our understanding about the fate of nitrogen compounds in WSP. Finally, Chapter 10 – Overall Conclusions and Recommendations: this chapter highlights the most relevant findings in this research project and recommends further research work in this area.