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Aims and Scope

Journal of Water Reuse and Desalination is an international journal publishing peer-reviewed papers on the science and technology, policy, regulation, social and economic aspects and applications of sustainable sources of water to cope with water scarcity, including new sources of non-conventional water. *Journal of Water Reuse and Desalination* publishes review articles, theoretical and experimental research papers, new findings and issues of unplanned and planned reuse. The journal welcomes contributions from developing and developed countries.

The journal includes, but is not limited to, the following topics:

- Wastewater, greywater, stormwater treatment and reuse
- Municipal, industrial, agricultural and environmental applications
- Desalination technology for seawater and brackish water
- Brine management and technology
- Quality aspects
- Environmental impacts
- Health considerations
- Risk Assessment
- Design and application of water reuse systems
- Economic, social and policy issues
- Augmentation of surface and ground water sources

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All manuscripts should be submitted electronically through our Online Submission and Peer Review System: https://www.editorial-manager.com/jwrd. Authors should prepare their papers in accordance with the current *Instructions for Authors* printed at the back of this journal, and also available at **www.iwaponline.com** or from the Editorial Office at the address given inside the back cover.

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Contents

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Editorial

It is a great honor to introduce this first issue of the *Journal of Water Reuse and Desalination* for three reasons:

- It is now, and certainly will be in the future, another important contribution from the International Water Association in fostering knowledge and increasing the exchange of international experiences for the sector.
- It addresses one of the major challenges of the 21st century: to provide additional sources of safe water to address increasing demand originating from population growth, economic improvements and the impacts of climate change.
- The journal has an ample and sound scope to allow it to thoroughly cover the subjects at which it is aimed. It will review theoretical and experimental research papers, new technical findings, socio-economic issues and unplanned and planned reuse study cases covering the needs and interests of developing and developed countries.

It is estimated that at the beginning of the 21st century century at least one third of the world's population is living in areas suffering from water stress, and that by the year 2025 this will rise to two thirds. In some regions, such as the Middle East, people are actually living well below the absolute water stress value of 500 m^3 of water per inhabitant per year, and by the year 2050 these conditions will decline to the minimum survival level of 100 m^3 per inhabitant per year. As a result of lack of water in several parts of the world, there are examples of water reuse and desalination which have remained relatively unknown at an international level. These practices have gained momentum, giving the impression that the formal scientific, social and political recognition of their importance have been left far behind. Some examples can be found in *Water Reuse: An International Survey of current practice, issues and needs* (B. Jiménez & T. Asano, 2008, IWA Publishing, London):

- The pioneering and unique example of the direct reuse of used water for human consumption in Windhoek, Namibia for more than 35 years, with no impacts on health or rejection by society.
- The large-scale implementation of water reclamation from used water for direct non-potable and indirect potable use such as the Groundwater Replenishment System in South California, USA and the NEWater in Singapore.
- The importance of reuse for irrigation of agriculture in almost all semi-arid and arid regions to produce food. Unplanned reuse is thought to be ten times more prevalent than planned reuse.
- The several cases of reuse and recycling in industry that are simply unknown outside private companies, due to the lack of motivation to socially recognize it as a beneficial activity.
- The sharp increase in the number of desalination projects to supply water. This rose from 326 m^3/day in 1945, to over 5,000,000 m^3/day in 1980 to more than 35,000,000 m^3/day in 2004 (http://www.worldwater.org/data20062007/Table22.pdf), and annual new capacity has risen exponentially since 2004 to reach 68,000,000 m^3/day by the end of 2010. Countries such as Israel and Singapore plan to have 30% or more of their water supplies from seawater desalination, while other Middle Eastern countries such as Saudi Arabia receive more than 70% of their water supplies from desalination.

In this first issue, the contributions of authors from different regions and on different subjects illustrate the aims of this journal: to provide a forum to exchange ideas to build a new and better future in which water is provided to all in a safer and more reliable way.

Blanca Jiménez
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Direct potable reuse: a future imperative

Harold L. Leverenz, George Tchobanoglous and Takashi Asano

ABSTRACT

As a result of population growth, urbanization, and climate change, public water supplies are becoming stressed, and the chances of tapping new water supplies for metropolitan areas are getting more difficult, if not impossible. As a consequence, existing water supplies must go further. One way to achieve this objective is by increased water reuse, particularly in supplementing municipal water supplies. Although water reuse offers many opportunities it also involves a number of problems. A significant cost for nonpotable water reuse in urban areas is associated with the need to provide separate piping and storage systems for reclaimed water. In most situations, the cost of a dual distribution system has been prohibitive and thus, has limited implementation for water reuse programs. The solution to the problem of distribution is to implement direct potable reuse (DPR) of purified water in the existing water distribution system. The purpose of this paper is to consider (a) a future in which DPR will be the norm and (b) the steps that will need to be taken to make this a reality. Following an overview, the rationale for DPR, some examples of DPR projects, technological and implementation issues, and future expectations are examined.

Key words | direct potable reuse, engineered storage buffer, potable reuse, water reuse

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DIRECT POTABLE REUSE: AN OVERVIEW

Direct potable reuse (DPR) refers to the introduction of purified water, derived from municipal wastewater after extensive treatment and monitoring to assure that strict water quality requirements are met at all times, directly into a municipal water supply system. The resultant purified water could be blended with source water for further water treatment or even direct pipe-to-pipe blending of purified water and potable water. DPR offers the opportunity to significantly reduce the distance that purified water would need to be pumped and significantly reduce the head against which it must be pumped, thereby reducing costs. The other significant advantage of DPR is that it has the potential to allow for full reuse of available purified water in metropolitan areas, using the existing water distribution infrastructure.

A general flow diagram for alternative potable reuse strategies is shown on Figure 1. As shown, two DPR options are available. In the first option (heavy solid black line), purified water is first placed in an engineered storage buffer (ESB). From the ESB, purified water can either be blended with the water supply source prior to water treatment or can be blended directly with treated potable water. In the second option (heavy dashed back line) purified water, without the use of an ESB, can be blended in either of the two locations discussed for option 1. As will be discussed later, implementation of option 2 would entail more extensive reliability measures and effective on-line continuous monitoring. The concept and role of the ESB is considered in the following discussion.

Engineered storage buffers for quality assurance

An important element of a DPR system is the ability to provide water of a specified quality reliably all the time. Because of the past limitations in providing this level of quality control in real-time and the large number of unknown factors, there was a preference for indirect potable reuse (IPR) projects instead of DPR projects. IPR systems make use of an environmental buffer, such as a surface reservoir or groundwater basin, to store water and ostensibly provide enhanced

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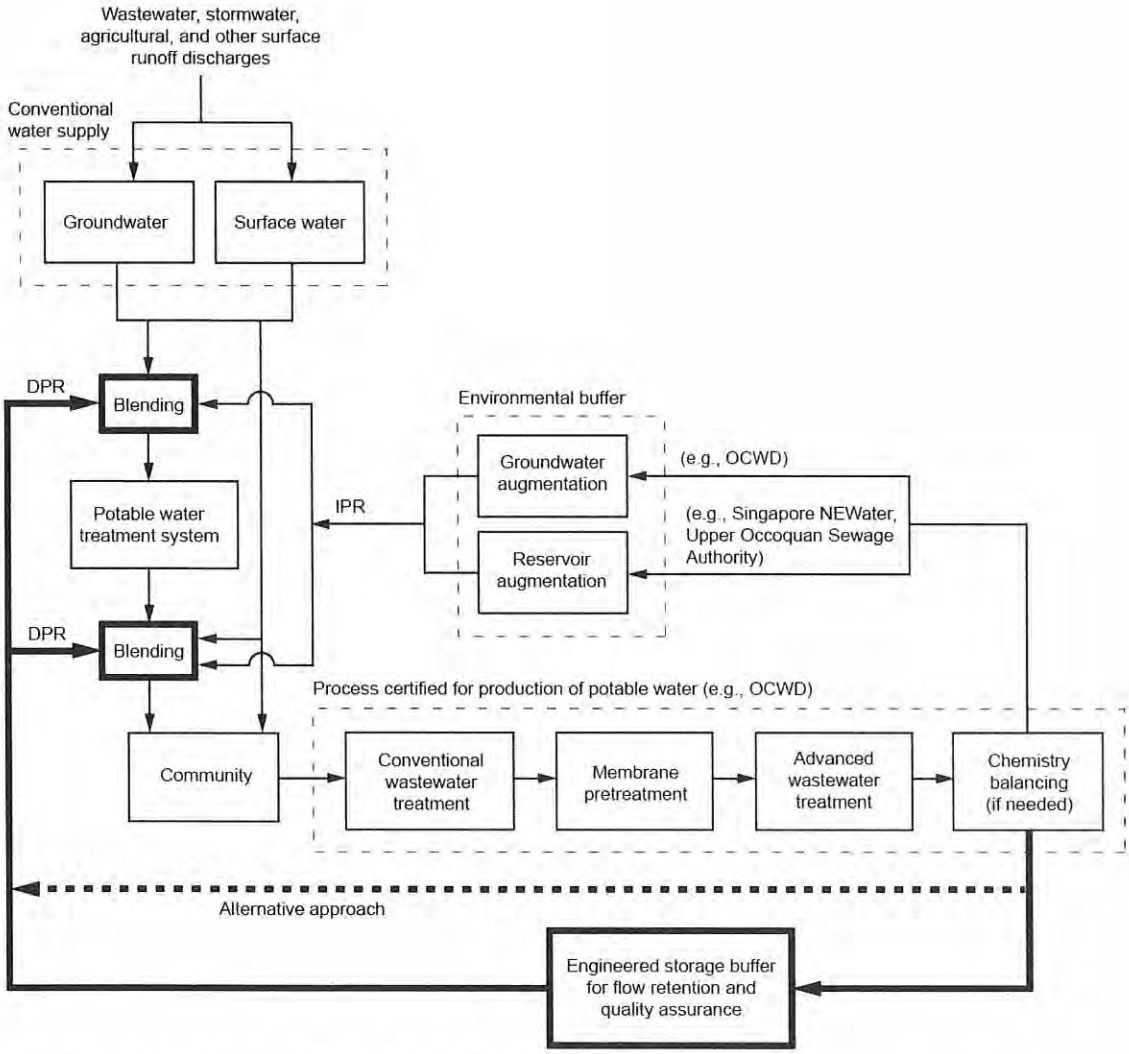

Figure 1 | Flow diagram for alternative direct potable reuse schemes (Tchobanoglous *et al.* 2011).

quality. In early IPR projects where the product water was not of the highest quality, the environmental buffer was thought to have provided a level of *in situ* advanced treatment. Further, the environmental buffer was presumed to provide loss of water identity and a measure of safety, in that it provided time to correct issues in the event that off-spec product water was detected.

However, when water is treated to a high level of purity, placement into an environmental system may not result in improved water quality, and can instead expose the purified water to potential environmental contaminants. Thus, when purified water can be produced using a system with proven performance and reliability and the quality can be validated rapidly with extensive monitoring systems, a relatively small ESB, if any, may be sufficient for use prior to blending into the potable water system.

An additional implication of the ESB concept is that, with some additional infrastructure, an existing IPR system could blend the purified product water directly with the area's general water supply system, allowing for greater flexibility in system operation. For example, when there are periods of purified water production in excess of the immediate potable demand, purified water could be placed into long-term environmental storage, such as aquifer recharge. Additional discussion on ESBs is presented in the 'Technical issues' section of this paper.

Water is water

Understandably, DPR may be the most difficult category of water reuse applications for the community to accept. One of the dilemmas in considering DPR has been the perception, even among water professionals, that nearly any water obtained from the environment, i.e., natural, is pure and better (Lohman 1988). However, the distinction that natural water is pure and better is no longer valid in many areas, mostly due to intentional and unintentional discharges of wastewater and agricultural and urban runoff. As a result, much of the research that originally addressed potable reuse has become of equal relevance to drinking water supplies taken from most water bodies. Thus, the sage words of Dr Lucas van Vuuren have successfully withstood the test of time over 40 years: 'Water should not be judged by its history, but by its quality' (Haarhoff & van der Merwe 1996).

A future imperative

It is inevitable that purified water will be used as a source of potable water supply in the future. Implementation of DPR will require a confidence in, and reliance on, the applied technology to always produce water that is safe and acceptable to consume. Designing interconnected water supply, collection, treatment, purification, and distribution systems has the benefit of providing maximum flexibility in the event of expected or unexpected shortages of natural water supply. Once a decision has been made to augment an existing water supply with purified water, the technical and implementation issues introduced in this paper must be considered. Further, the concepts described in this paper can also be applied in developing countries when provisions are made for reliable power supply and operation and maintenance for their vital water supplies.

RATIONALE FOR DIRECT POTABLE REUSE

In the past, it has been standard practice that whenever additional sources of water supply are necessary but not readily available, nonpotable water reuse options have been explored using recycled water. For example, nonpotable water reuse applications, such as agricultural and landscape irrigation, are major options for planned reuse. As a result of the preference for nonpotable reuse, water reuse applications in the United States, in order of descending water volume, are: (1) agricultural irrigation; (2) industrial recycling and reuse; (3) landscape irrigation; (4) groundwater recharge; (5) recreational and environmental uses; (6) nonpotable urban uses; and finally, (7) potable reuse (Asano 1991; Asano *et al.* 2007). However, most of the economically viable nonpotable reuse opportunities have been exploited. For example, the typical cost for parallel distribution of tertiary-treated recycled water is 0.3 to \$1.7/m^3 whereas the typical cost for purified water, which could be added directly to the distribution system, is 0.6 to \$1.0/m^3 (Tchobanoglous *et al.* 2011).

Indirect planned and unplanned potable reuse

Planned IPR includes groundwater recharge operations, such as Orange County Water District in California and the Occoquan Reservoir in northern Virginia (Asano *et al.* 2007). Planned IPR will continue to be of great importance in supplementing water supplies in the United States and elsewhere in the world. *Unplanned* IPR, in the cities and towns along the Colorado River as an example, occurs when treated wastewater is discharged to surface and groundwater that is subsequently used for municipal water supply. Thus, much of the research that originally addressed potable reuse is becoming of equal relevance to drinking water supplies taken from water bodies used for discharge of wastewater and runoff.

Factors limiting nonpotable and indirect potable water reuse

While there has been a clear preference for nonpotable and IPR applications, a number of factors are making it less feasible to further increase water reuse in these applications. Important limiting factors for agricultural and landscape irrigation, and IPR are listed in Table 1. Although agricultural irrigation is currently the largest user of recycled water, it is expected that this will change with the world-wide trend towards urbanization, especially near coastal areas. For example, the City of Los Angeles currently discharges about 1.5 Mm^3/d (400 Mgal/d) of treated wastewater to the Pacific

Ocean. Further, the energy to provide water supply to some areas is excessive compared to the energy to purify water. For example, the energy required to provide 1,234 m^3 (1 ac-ft) to an Orange County water system is: ocean desalination = 3,700 kWh (kilowatt-hour); State Project water = 3,500 kWh; Colorado River water = 2,500 kWh; purified water = 800 to 1,500 kWh (Tchobanoglous *et al.* 2011).

Factors favoring direct potable reuse

In addition to the limiting factors identified in Table 1, there are a number of factors that support the implementation of DPR in the future. For example, drought events are expected to become more extreme due to climate change and the potential use of purified water for potable supply offers improved overall water supply reliability in coastal metropolitan areas. Another consideration is that as the reality of unplanned IPR and concern about the quality of existing water supplies becomes more transparent and understandable to the public, there will be increased pressure to provide water of the highest quality for public consumption. Advances in treatment technology over the last decade have made it possible to produce high quality purified water with advanced water treatment processes. Additional considerations that support DPR are summarized in Table 2. Given the factors presented in Tables 1 and 2, it is clear that there is a need in some regions to consider alternatives to conventional water supply and nonpotable water reuse applications.

REVIEW OF DPR SYSTEMS

Some DPR systems that are currently in operation and/or under construction are highlighted in this section. These example projects are important because 'the treatment process flow diagrams and treatment technologies employed have been accepted by various regulatory authorities as being able to produce safe potable drinking water, and ... the implementation of these projects has been accepted by the public' (Tchobanoglous *et al.* 2011). Therefore, the focus of this section is primarily on treatment technologies and not the removal of specific constituents.

Typical flow diagrams for DPR

Representative treatment process flow diagrams from (1) Windhoek, Namibia; (2) Big Springs, Texas; (3) Cloudcroft, New Mexico; and (4) Orange County Water District (OCWD) Groundwater Replenishment System (GWRS), Fountain Valley, California for potable reuse are presented on Figure 2. The Windhoek, Namibia DPR facility, shown

Table 1 | Factors that have limited nonpotable and indirect potable reuse

Agricultural irrigation
• The long distance between the municipal recycled water supplies and the major agricultural demand areas. • The cost and disruption to construct pipe systems to convey recycled water. • The need to provide winter recycled water storage facilities further limits agricultural reuse. • Historically, the value of water from surface and groundwater supply sources has not reflected the true costs of providing the supply, resulting in a distinct economic disadvantage for the production of recycled water.
Urban landscape irrigation
• Landscape irrigation may not be economically feasible due to the dispersed nature of the demand. • The cost of providing parallel distribution of recycled water supply is high due to the fact that the distance between large users in most communities is great. Further, most of the water is consumed by small users that cannot be served efficiently and or economically.
Indirect potable reuse (IPR) projects
• Communities that lack suitable hydrogeology for groundwater recharge may not be able to implement IPR projects. • For surface water augmentation, blending and residence time requirements may limit IPR applications to large reservoirs (which are not available to many communities).

Table 2 | Factors that favor direct potable reuse

- Need for a separate recycled water distribution system is avoided.
- Alternative sources of water supply are often either of poor quality or prohibitively expensive.
- Traditional sources of surface water and groundwater supply are being limited.
- With advanced treatment technology it is now possible to remove contaminants effectively and reliably to extremely low levels that have no known health concerns.
- Purified water is a reliable source of supply which exists in close proximity to the demand.
- Communities that lack suitable hydrogeology for groundwater recharge cannot implement IPR projects.
- DPR with purified water is potentially less costly than the use of tertiary-treated recycled water for irrigation.
- DPR may require less energy than is required for other water supply sources.
- DPR avoids potential water quality issues associated with groundwater and surface water sources.
- Current technology is sufficient to replace the environmental buffer with an engineered storage buffer through a combination of monitoring, storage, and treatment reliability measures.

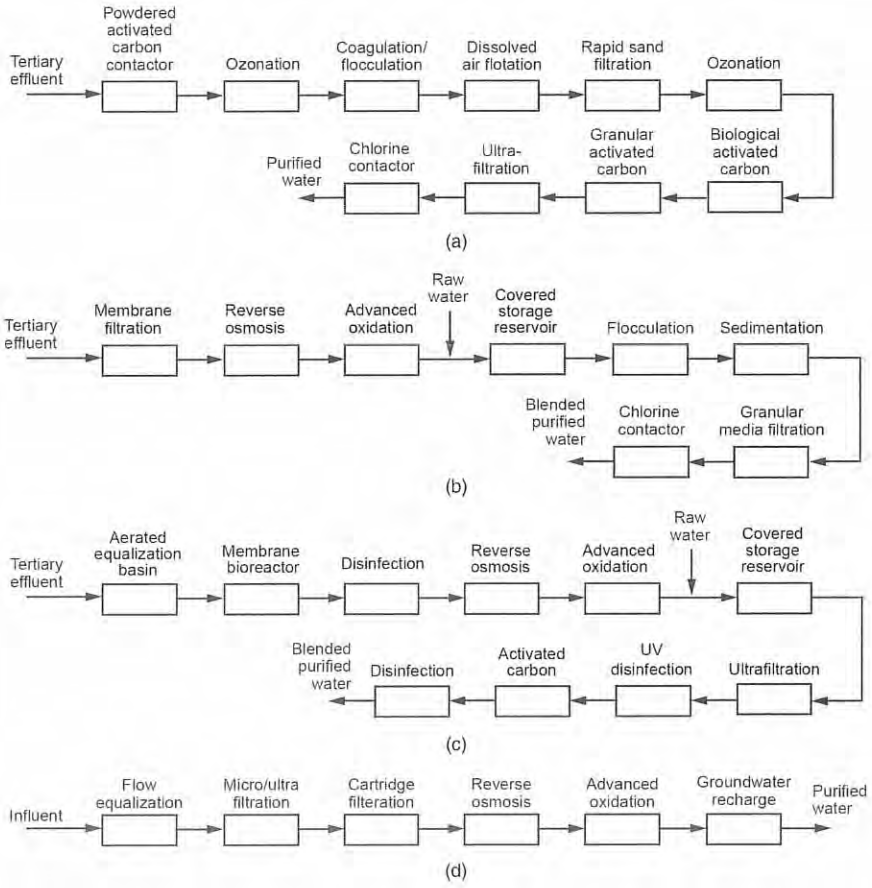

Figure 2 | Representative treatment process flow diagrams for potable reuse: (a) Windhoek, Namibia; (b) Big Springs, Texas; (c) Cloudcroft, New Mexico; and (d) Orange County Water District (OCWD) Groundwater Replenishment System (GWRS), Fountain Valley, California.

on Figure 2(a), has been in operation since 1997 and replaced the previous treatment facility, which had been in operation since 1968. It should be noted that all of the flow diagrams in Figure 2, with the exception of Figure 2(d), are consistent with the generalized conceptual DPR flow diagram given on Figure 1. Although the purified water from the GWRS

system, shown on Figure 2(d), is used for groundwater recharge, the treatment process flow diagram is included as a benchmark for water quality, as the water has been determined to be safe for direct potable reuse (Burris 2010).

Assessment of flow diagrams for DPR

In reviewing the flow diagrams presented in Figure 2, it is interesting to note that a number of different unit processes have been employed for the removal of the constituents of concern in wastewater. For the near future, it is anticipated that the treatment processes employed in these flow diagrams will serve as a benchmark for the development of alternative process flow diagrams for DPR. As new treatment process flow diagrams are developed it will be important to assess the need for and size of the ESB, based on system reliability and the use of appropriate monitoring equipment and analytical techniques.

TECHNICAL ISSUES IN DPR

The technology required for advanced wastewater treatment, capable of producing an effluent of sufficient quality that is suitable for potable reuse, has been a reality for more than 40 years. However, over the last decade, the ability to produce purified water reliably from tertiary and advanced effluent at the municipal scale has become technically and economically feasible. As more communities and water agencies begin to explore the feasibility of DPR, some of the technical issues that must be addressed include appropriate treatment process configurations, features of ESBs, process reliability, and monitoring requirements. These topics are considered below along with some research needs.

Treatment process configurations for purified water production

The combination of improved technology and analytical capabilities has made it possible to validate the concept that water can be purified using several alternative process flow schemes. The basic system used to purify water consists of several processes collectively referred to as advanced treatment. The current advanced treatment scheme has evolved over time, and now commonly includes microfiltration, reverse osmosis, and advanced oxidation, as shown on the flow diagrams presented in Figure 2. Major innovations in the future are expected to include improvements in overall process cost and efficiency, such as demineralization processes that minimize brine formation and operate with reduced energy input.

Features of ESBs

ESB designs can be stand-alone facilities or incorporated into the transport and distribution system, depending on site-specific factors and needs. Stand-alone storage buffers may take a variety of forms varying from well-defined engineering structures to natural or constructed confined groundwater aquifers. The specific design of the ESB will be a function of several factors, including: (1) site-specific constraints; (2) capabilities of the monitoring and constituent detection system; (3) flow rate and degree of flow equalization required; and (4) safety factors. Important features of the ESB include:

- fully controlled environment,
- contained to prevent contamination and evaporative losses,
- no source of contaminants from within the buffer itself,
- ability to divert flow out of the buffer as needed,
- accommodation of monitoring and sampling equipment,
- well-characterized and optimized hydraulics, and
- high level of security.

In general, the storage requirements will be controlled by the time required for constituent analysis and overall reliability of the monitoring system. Purified water must be retained in the ESB for sufficient time to validate the quality of the water for specified constituents and surrogate measures prior to blending into a potable water supply for consumption.

Measures to enhance reliability

The pretreatment processes used for production of the feed water to advanced treatment and purification processes

must be refined to achieve the highest level of reliability possible. Optimizations of existing processes as well as incorporation of new facilities, such as full flow equalization, are needed to produce a consistent and stable input. Measures that can be taken to enhance the reliability of a DPR system include:

- enhanced source control,
- enhanced fine screening,
- elimination of untreated return flows,
- flow equalization,
- operational mode for biological treatment,
- improved performance monitoring,
- ongoing pilot testing and
- reformulation of consumer products for improved biodegradability.

The discharge of substances known to be difficult to treat can be reduced or eliminated with enhanced source control programs. Enhanced fine screening improves the performance of biological treatment processes. The elimination of return flows is significant with respect to achieving effective nitrogen removal. Flow equalization, coupled with operational mode of the biological treatment process, is effective in the treatment of trace organics. Improved process monitoring will enhance overall process performance. Pilot testing is used to keep abreast of the latest technological developments. Elimination of consumer products that end up in wastewater that are not amenable to treatment is the long-term goal.

Monitoring and constituent detection

While there have been a number of recent improvements in online monitoring and constituent detection, it is not, at present, feasible to provide real-time monitoring of all constituents of concern. However, the identification of surrogate and indicator constituents that can be used to assess performance reliability of key unit processes can be used in place of direct measurements for all constituents of interest. The use of indicators and surrogates is somewhat site specific and will need to be established for individual treatment operations (Drewes *et al.* 2010). However, after these parameters are established they can be used to enhance the monitoring program through rapid detection programs. The ability to detect constituents of concern rapidly will reduce the overall size of the ESB facilities that are used for quality assurance.

Monitoring at specific locations is used: (1) to assess process performance and reliability; (2) for process control; and (3) to verify compliance with public health or other regulatory requirements. As described previously, the ESB is a key monitoring location because it may be the final safeguard prior to distribution in the potable water system. Thus, the development of the monitoring program needs to be planned carefully to ensure that all constituents of importance can be assessed in the product water with sufficient speed and accuracy to justify the size and design of the ESB facilities. It is at this point that off-spec water would be diverted to an alternate location, such as the wastewater treatment facility or a specified point in the purification process.

Research needs

Although the technical feasibility of DPR is well established and will only improve in the future, areas of technical research that will enhance and hasten the adoption of DPR include (1) development of sizing criteria for ESBs; (2) treatment train reliability; (3) blending requirements; (4) enhanced monitoring techniques and methods; and (5) effectiveness of equivalent advanced treatment trains. Research on public acceptance will also be an important adjunct to and will be complementary to the technical areas of research discussed in this paper.

FUTURE TECHNICAL DEVELOPMENTS

Future technical developments that will impact DPR include the need for enhanced wastewater treatment, the development of alternative treatment processes, and integrated wastewater treatment plant design for DPR.

Enhanced wastewater treatment

It is important to consider that all water discharged to the surface and groundwater, from point and non-point sources, is basically a form of IPR. In recent surveys of

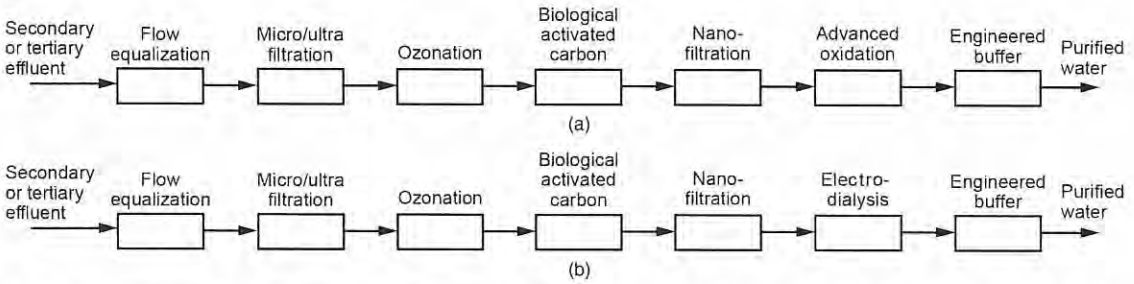

Figure 3 | Alternative advanced treatment flow diagrams with trace organic removal by (a) ozonation, biological activated carbon, nanofiltration, and advanced oxidation and (b) ozonation, biological activated carbon, nanofiltration, and electrodialysis.

surface and groundwater quality by the US Geological Survey (Kolpin *et al.* 2002; Barnes *et al.* 2008), it was concluded that essentially all surface and groundwater are contaminated with chemicals commonly associated with wastewater, such as pharmaceuticals. In the future, it is anticipated that surface and groundwater discharges will need to comply with much more stringent discharge requirements to protect sensitive environmental species and ecosystems. The level of treatment needed to protect environmental species and ecosystems may, in some cases, be higher than that needed for DPR. Thus, the implementation of DPR may make more sense environmentally than the discharge of purified water to the aquatic environment.

Alternative treatment processes for direct potable reuse

One of the major problems with most common DPR treatment schemes employing reverse osmosis is the management of brine, especially in inland locations. To deal with this issue, a variety of new advanced treatment processes are currently under development for the oxidation of trace organics, without the removal of dissolved solids. An example of such a system is shown on Figure 3(a). Another issue with DPR schemes employing reverse osmosis is the high energy usage required for treatment. An alternative treatment approach involves the use of electrodialysis as illustrated on Figure 3(b). New and enhanced biological treatment systems are also under development. As new technologies become available in the future, it is anticipated that constituent removal effectiveness will improve with a concomitant reduction in energy and resource usage.

Integrated DPR treatment designs

The current trend in water and wastewater systems design can best be described as incrementalism. In examining the treatment process flow diagrams for DPR presented previously in Figures 2 and 3, it can be concluded that the production of purified water for DPR was an afterthought. Basically additional unit processes were tacked on to the end of existing secondary treatment process flow diagrams to remove specific compounds. However, at some point in the future there will need to be a complete rethinking of urban infrastructure to obtain the highest levels of performance and reliability. For water and wastewater systems, the advanced infrastructure model will likely include decentralization, remote management, resource recovery, source separated waste streams, and application of specific optimization of water quality. What is needed is the development of integrated water management systems in which new wastewater treatment plants are planned and designed from the ground up to optimize treatment performance with respect to the production of purified water, along with the recovery of energy and resources.

SUMMARY

Because it is inevitable that DPR will become part of the water management portfolio for the reasons cited in this paper, it is important that water agencies begin to develop the necessary information that will allow DPR to become a reality. The technical feasibility of DPR is well established and will only get better in the future. In planning for wastewater treatment upgrades or

new plants that will be used to produce purified water, it is imperative that the incrementalism of the past be replaced with new integrated designs that will produce purified water along with the recovery of energy and resources.

ACKNOWLEDGEMENTS

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A systematic approach to determine herbicide removals in constructed wetlands using time integrated passive samplers

Declan W. Page, Stuart J. Khan and Konrad Miotlinski

ABSTRACT

Natural water treatment systems such as wetlands are increasingly being recognised for their role as part of a multi-barrier system for water recycling. Natural wetland systems have the ability to provide effective treatment for a wide range of organic chemicals. However, techniques are required to validate the performance of these treatment processes in the field. This paper provides a new method for evaluating wetland systems using passive samplers and applies a statistical method for use in advanced water treatment processes. Three years of stormwater quality passive sampler data for diuron, simazine and atrazine is provided to determine herbicide removal between the inlet and outlet regions of a constructed wetland. Mean removal rates over the three year period for diuron, simazine and atrazine were 43, 54 and 50% respectively. The results show that this method coupled with passive samplers is amenable to wetland system barrier characterisation where opportunities for process validation is not feasible.

Key words | atrazine, constructed wetlands, diuron, passive samplers, simazine, stormwater harvesting

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INTRODUCTION

Wetlands are increasingly being constructed in urban areas to detain and improve the quality of stormwater (Terzakis *et al.* 2008; Imfeld *et al.* 2009; Janzen *et al.* 2009). This captured stormwater is a potentially important resource and is increasingly being used as an alternative water source.

Despite growing interest in the use of urban stormwater runoff, water quality hazards including herbicides may limit the suitability for some applications (Kohler *et al.* 2004; Imfeld *et al.* 2009; Page *et al.* 2010a). Monitoring the concentrations of herbicides in stormwater presents a challenge because many occur at trace levels that are very difficult to detect and quantify. These analytical difficulties, coupled with the highly variable nature of stormwater, result in limitations in the ability to quantify and assess the efficiency of urban stormwater treatment systems such as constructed wetlands (Page *et al.* 2010a).

To address these difficulties, time-integrated passive sampling techniques have been deployed in urban stormwater wetland harvesting systems (e.g. Page *et al.* 2010b). These techniques are based on the diffusion of chemicals from the aqueous phase onto a solid phase that has a relatively high capacity for the herbicides of interest. When deployed for a period of time (e.g. a week or a month), the passive samplers provide for easier detection of herbicides than conventional monitoring techniques. Passive sampling techniques provide time-weighted average water concentrations during the period of passive sampler deployment. The concentrations are calculated from the amount of chemical sequestered in the sampler using sampling rates determined either by calibrations conducted in the laboratory or via field deployments (Shaw *et al.* 2009).

The aims of this paper were to calculate the removal efficiencies of three selected herbicides in a constructed

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wetland over a three year period. Passive samplers and a probabilistic modelling approach incorporating the use of probability density functions (PDFs) are presented as a new technique to assess the herbicide removal capabilities of natural wetland systems. The probabilistic approach accounts for variable concentrations of herbicides in the stormwater and variable performances of the constructed wetland, as well as incorporating the underlying uncertainty in the analytical measurements. This method is consistent with some of the probabilistic techniques that have been used for assessing the performance of multiple-barrier water recycling schemes.

METHODS

Site description

The Parafield stormwater harvesting system is located on the Parafield airport in the city of Adelaide, Australia. Stormwater from a 16.2 km^{-2} mixed light industrial and residential catchment drains into the system which is designed to provide treatment for an average annual supply of 1,100,000 m^3 y^{-1} (Swierc *et al.* 2005). The system is currently configured to provide water for non-potable uses, including public green space irrigation and use by a wool processing plant.

A weir diverts water from the Parafield drain into the in-stream basin (50,000 m^3), which is the first of three stages of the stormwater harvesting system. The in-stream basin serves as an initial settling basin for sediments. Water flows into the in-stream basin during a storm event and is pumped at ~3,000 m^3 h^{-1} to the holding storage until capacity (50,000 m^3) is reached or the in-stream basin is drained. Water flows by gravity from the holding storage into the constructed wetland (25,000 m^3). The wetland is designed to achieve a minimum holding time of 7 days. Water flows and water levels were measured in real time at the wetland outlet.

The wetland is rhomboid shaped with the inlet and outlet at the apexes, a total land area of 0.11 km^{-2}, standing water depth of 30–60 cm and has been vegetated with seven different species of wetland plants (*Phragmites australis*, *Eleocharis sphacelata*, *Schoenoplectus validus*, *Baumea articulate* and *Typha orientalis*), that were planted in parallel rows that are perpendicular to flow (Marks *et al.* 2005).

Probabilistic approach to characterise natural treatment barriers

Assessment of wetland treatment performance can be challenging as the concentrations of herbicides in stormwater are highly variable over time and the performance of the wetland treatment also varies depending on a range of factors including hydraulic flow rate, water quality and season. As a result, final concentrations of herbicides in water are stochastic (rather than deterministic) variables and therefore are appropriately described by probability density functions (PDFs) rather than point values. Hence an alternative approach to determine the treatment performance is to determine a source water concentration in terms of an 'inlet' PDF and a post wetland treatment concentration in terms of an 'outlet' PDF. These inlet and outlet PDFs can then be used to derive theoretical wetland treatment efficiency PDF for each specific pesticide. The method adopted is one that has recently attracted some interest in Australia for the probabilistic assessment of advanced water treatment plants (Khan 2010; Khan & McDonald 2010).

Water quality data is commonly well described by lognormal distributions (Richards & Baker 1993; Eisenberg *et al.* 2001; Vogel *et al.* 2005). Accordingly, the variability of pesticide concentrations has been characterised by fitting observed data to lognormal PDFs. Removal efficiency (RE) can be obtained by the following:

$$RE = 1 - PDF_{inlet}/PDF_{outlet} \quad (1)$$

where RE is the removal efficiency and the PDF_{inlet} and PDF_{outlet} are the inlet and outlet fitted PDFs.

This technique has not been previously reported for natural treatment systems performance assessment because of the high cost of determining PDFs. An advantage of this method is that such plots can be prepared even when some of the data is below the limit of detection (LOD) since the PDF can be extrapolated from the available (higher) percentile data. This paper presents a method which overcomes the problem of conventional monitoring

of stormwater contaminants by use of passive samplers to estimate a treatment efficiency for a stormwater harvesting system.

Passive samplers

The monitoring of polar chemicals such as herbicides by the 'Chemcatcher' passive sampler employs use of a high surface area adsorptive sequestering phase. The Chemcatcher Empore disk passive samplers have been previously well documented in the literature (Shaw *et al.* 2009; Page *et al.* 2010b). Passive samplers have been more widely used in the past decade. Validation of the use of performance reference compounds (Shaw *et al.* 2009) introduced into the sampler to enable adjustment of field data from samplers with kinetic data from the laboratory has increased confidence in these techniques. While some uncertainty factors with the use of the passive samplers remain such as issues relating to biofouling (Page *et al.* 2010b) and nonlinear uptake, the utility of this approach reflects the limitations of more conventional grab sampling. The determination of removal efficiencies using passive samplers with the approach described in this study is largely independent of such issues unless there are systematic differences between passive sampler performance at the inlet and outlet of the wetland.

In this study, passive samplers with membranes were deployed in triplicate in winter at the inlet and outlet of the constructed wetland for variable durations of 28–34 days. Passive samplers without membranes were deployed in triplicate in the same sampling locations for periods of 7 days. Complete details of the monitoring procedure, analytical methodology (Page *et al.* 2010b) and laboratory calibration, use of reference compounds and determination of sampling rates, calculation of time weighted average concentrations and limitations of the approach (Shaw *et al.* 2009) have been previously reported.

RESULTS AND DISCUSSION

Passive sampler water quality monitoring

The results of the deployment of passive samplers and estimated time weighted average concentrations for each deployment of the herbicides diuron, simazine and atrazine are presented in Table 1.

Table 1 | Chemcatcher passive sampler time weighted average concentrations (ng L^{-1}) for deployment periods during 2006–2008

Year	Deployment duration (d)	Diuron Inlet (ng L^{-1})	Outlet (ng L^{-1})	Simazine Inlet (ng L^{-1})	Outlet (ng L^{-1})	Atrazine Inlet (ng L^{-1})	Outlet (ng L^{-1})
2006	34	64	25	39	19	15	21
	34	64	26	42	19	16	18
2007	7	100	39	41	14	2.7	1
	7	72	42	20	13	<0.3	0.9
	7	61	72	11	23	1.3	1
	7	95	36	25	7	1.6	<0.3
	28	130	86	80	34	4.4	3.5
	28	240	99	150	51	8	4.2
	28	210	95	130	55	11	3.3
	28	NA	97	NA	56	NA	<0.3
2008	7	17	10	6	3	1	1
	7	20	8	8	3	1	1
	7	NA	9	NA	9	NA	0.5
	7	25	10	84	31	1	0.5
	28	36	12	68	22	2	1
	28	36	10	66	22	2	1
	28	31	15	169	29	3	1
	28	NA	11	NA	23	NA	1

NA, not available.

Lognormal probability plots for the inlet and outlet diuron, simazine and atrazine concentrations over the 14–18 sampling periods of either 7 or 28 days are presented in Figures 1–3 using the method described by Khan (2010).

Figure 1 shows atrazine concentrations in the untreated stormwater could be expected to range between <0.3–16 ng/L. Figure 2 shows diuron concentrations of 17–240 ng/L and Figure 3 shows simazine concentrations of 6–169 ng/L with the 7 day and 28–34 day sampler data separated (Figure 4). A lognormal best fit line is shown in each of the Figures 1–4. For atrazine the lognormal fit was quite poor, as highlighted by the cluster of data points at

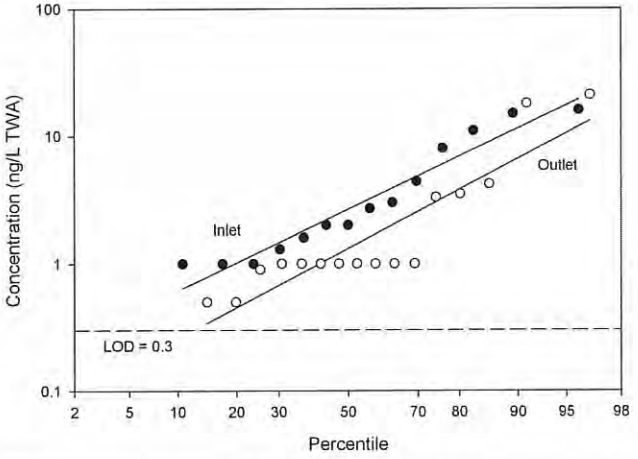

Figure 1 | Lognormal cumulative probability plot for diuron during passage through the wetland treatment process. Closed circles represent the inlet concentration, open circles represent the outlet concentration.

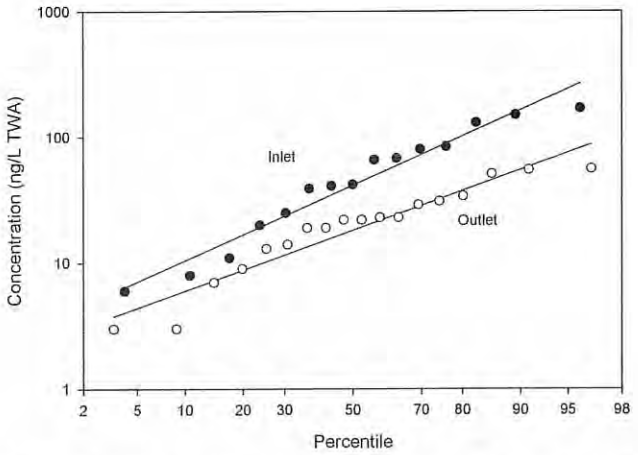

Figure 2 | Lognormal cumulative probability plot for simazine during passage through the wetland treatment process. Closed circles represent the inlet concentration, open circles represent the outlet concentration.

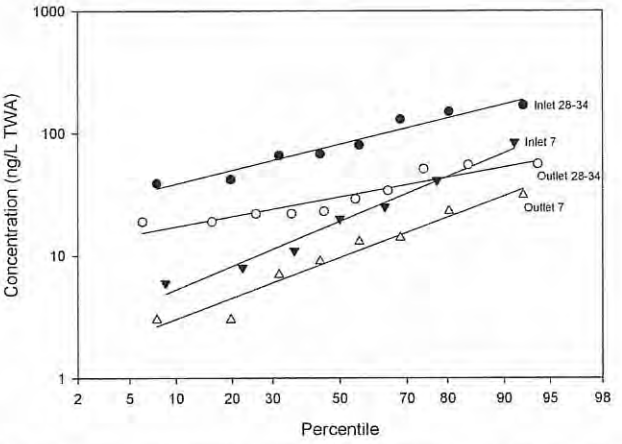

Figure 3 | Lognormal cumulative probability plot for simazine (with 7 day passive sampler data separated) during passage through the wetland treatment process. Closed circles represent the inlet concentration, open circles represent the outlet concentration, for the 28–34 day deployment. Closed triangles represent the inlet concentration, open triangles represent the outlet concentration, for the 7 day deployment.

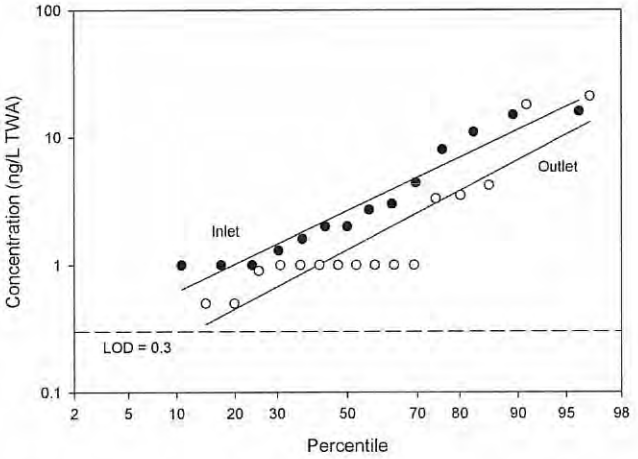

Figure 4 | Lognormal cumulative probability plot for atrazine during passage through the wetland treatment process. Closed circles represent the inlet concentration, open circles represent the outlet concentration. LOD is the limit of detection.

1 ng/L, the quality of the fitted PDF are discussed later. Similarly, concentrations at the wetland outlet ranged from <0.3–21 ng/L for atrazine (Figure 1), 8–99 ng/L for diuron (Figure 2), 3–56 ng/L for simazine (Figures 3–4).

Probability distribution functions of passive sampler data

The diuron, simazine and atrazine data in Table 1 was used to fit lognormal PDFs. Fitting was performed on the entire

data set in Table 1 as well as subsets containing only the 7 day deployments and 28–34 day deployments. These fitted PDFs are summarised in Table 2.

For diuron and atrazine the lowest root mean square fitted PDFs were obtained using all the passive sampler data. For simazine, the lowest root mean square error was obtained by using only the 7 day passive sampler deployment data. The fitted PDFs presented here are derived from real data and while they may be fitted to a lognormal distribution, they do not conform perfectly (e.g. atrazine, Figure 4) and the estimation of the removal efficiency PDF may not be possible using this approach if the data does not support it. However, the general features of these PDFs may be statistically summarised as presented in Table 2. Some of the PDFs can have an improved fit by filtering the data; for example simazine (Figure 3), where separating the 7 day and 28–34 day passive sampler data leads to an improved PDF fit and lower root mean square error (Table 2). Other data sets such as atrazine were not so easily improved and a better fitting PDF can only be obtained by collecting more data.

Table 2 | Summary of inlet and outlet fitted PDFs

	Mean	Standard deviation	Shift	RMS error
Diuron inlet (all)	85.3	106.5	4.15	0.0014
Diuron inlet (28–34 days)	178.7	922.9	27.5	0.0035
Diuron inlet (7 days)	20,637	43	−20,581	0.0049
Simazine inlet (all)	88.1	75.0	−18.7	0.0012
Simazine inlet (28–34 days)	90.4	86.9	14.3	0.0031
Simazine inlet (7 days)	35.4	85.5	3.7	0.0007
Atrazine inlet (all)	7.6	39.9	0.75	0.0014
Atrazine inlet (28–34 days)	10.1	15.4	0.2	0.0034
Atrazine inlet (7 days)	0.70	1.4	0.8	0.0064
Diuron outlet (all)	86.9	558.3	7.44	0.0032
Diuron outlet (28–34 days)	174.0	2,123.1	9.5	0.0048
Diuron outlet (7 days)	45.1	37.7	−11.9	0.0098
Simazine outlet (all)	42.1	17.1	−17.9	0.0017
Simazine outlet (28–34 days)	25.6	70.9	17.6	0.0032
Simazine outlet (7 days)	18.1	12.7	−4.3	0.0016
Atrazine outlet (all)	3.7	19.2	0.39	0.0108
Atrazine outlet (28–34 days)	10.0	61.3	0.4	0.0069
Atrazine outlet (7 days)	0.28	0.13	0.66	0.0304

RMS, root mean square.

Calculation of wetland removal efficiency PDFs

The PDFs from Table 2 were then used to calculate the percentage removal of each herbicide by the wetland treatment process according to Equation (1). The results of the removal efficiency simulations are shown in Table 3. An example of the calculated removal efficiency PDF for simazine using the 28–34 day sampler data is shown in Figure 5.

The calculated mean (43–55%, 52–60%) and median (56–62%, 49–63%) removal efficiency for diuron and simazine respectively did not vary substantially with the data sets used. Generally these data sets were lognormally distributed and agreed with previous mass balance approaches at assessing the same wetland efficiency (Page *et al.* 2010b). Atrazine had a more variable mean (24–50%) and median (17–60%) removal efficiency, influenced by the atrazine outlet concentration PDF having the highest root mean square error (Table 2). There was generally greater convergence for the 95th percentile removal efficiencies for all the herbicides.

Table 3 | shows the calculated mean, median and 95th percentile removal efficiency (% reduction) of diuron, simazine and atrazine as a result of passage through the wetland

	Mean	50th	95th
Diuron (all)	43	58	70
Diuron (28–34)	53	62	69
Diuron (7 days)	55	56	91
Simazine (all)	54	58	76
Simazine (28–34)	60	63	71
Simazine (7 days)	52	49	85
Atrazine (all)	50	50	64
Atrazine (28–34)	48	61	74
Atrazine (7 days)	24	17	64

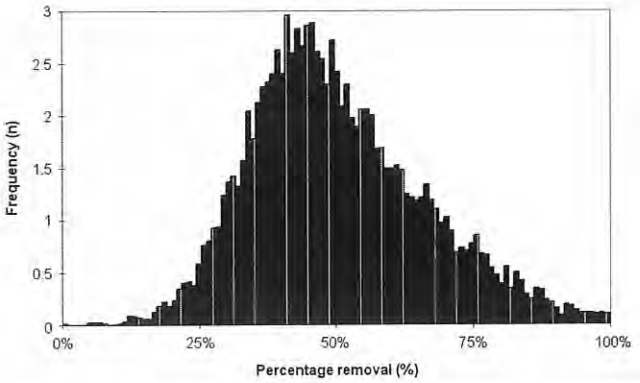

Figure 5 | Simazine removal efficiency PDF for the constructed wetland using 28–34 day sampler data.

The calculation of a removal efficiency PDF approach yielded similar results to the mass balance approach used in determining wetland treatment efficiency by conventional water quality monitoring and passive samplers (Page *et al.* 2010b). In that study, removal efficiencies for diuron and simazine ranged from 33–51% and 20–60% respectively. The ranges of removal efficiencies were in broad agreement for both studies. Atrazine however, had a much poorer fitted PDF, the highest root mean square error (Table 2) and as a result the highest variability in the reported removal efficiency (Table 3). This resulted in negative removal efficiencies for atrazine at the low percentiles ($<5^{th}$ percentile) of the removal efficiency PDF. However this described method is still robust in calculating the mean and median removal efficiencies for the herbicides. Other specific factors may also be operating such as systematic errors, for example biofouling of the passive samplers. In such cases as for atrazine, further data should be collected so that these PDFs could be better described and fitted with an improved lognormal distribution.

The stormwater harvesting wetland mean removal efficiencies reported in this study for diuron and simazine were generally higher than the ranges previously reported in literature. Matamoros *et al.* (2007) reported removal efficiencies for diuron and simazine of 0% and 25% respectively for a predominantly anaerobic subsurface flow gravel bed constructed wetland with an average water depth of 0.3 m, a surface area of 55 m^2 and a hydraulic residence time of 4.8 days. However, Stearman *et al.* (2003) reported a substantially greater removal efficiency of 64% for simazine in gravel subsurface flow constructed wetland planted with the common bulrush (*Scirpus validus*) and a hydraulic residence time of 2.3 days. Differences in wetland design, the longer residence time of the current study, vegetation types, flow (surface or sub-surface) as well as redox potential of the wetland are likely to result in these differences. Wetlands that are predominately aerobic are likely to be more effective in degrading triazines such as simazine (Moore *et al.* 2000).

CONCLUSIONS

Passive samplers designed for the determination of aqueous concentrations of a wide range of herbicides provide the basis of a new approach to characterise the treatment efficiency of natural wetland systems. A series of passive sampler data of time weighted average concentration collected from the inlet and outlet of a constructed wetland were used to determine the PDFs that describe the wetland treatment barrier. Mean removal rates over the three year period for diuron, simazine and atrazine were 43%, 54% and 50% respectively. The use of passive samplers was found to be suitable for the calculation of removal efficiencies for a constructed wetland with temporally variable concentrations of herbicides for diuron and simazine. For atrazine, more data would be required to better characterise the input and output PDFs prior to application of this technique. It is recommended that the techniques described in this paper be more widely and routinely applied to characterise natural system treatment barriers.

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Decolorization of industrial azo dye in an anoxic reactor by PUF immobilized *Pseudomonas oleovorans*

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ABSTRACT

This work reported the full degradation of an azo dye in a synthetic effluent by *Pseudomonas oleovorans* immobilized in polyurethane foam (PUF). For each fed-batch experiment, a screw-top vessel containing 160 mL of nutrient broth was inoculated with 0.16 g L^{-1} of fresh culture, incubated at 32 °C and supplemented with 50 mg L^{-1} of dye every 24 hours. Afterwards, the *P. oleovorans* were immobilized in PUF and inoculated in an anoxic reactor. The results showed that at fed-batch conditions, *P. oleovorans* was capable of removing 50 mg of dye in 192 hours. However, when the decolorization was performed in an anoxic reactor, it was capable of fully degrading 25 mg of dye in only 24 hours.

Key words | anoxic reactor, azo dye, immobilization, polyurethane foam, *Pseudomonas*

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INTRODUCTION

There are more than 100,000 commercially available dyes with more than 7×10^7 tons of dyestuff produced annually worldwide (Robinson *et al.* 2001; Akhtar *et al.* 2005). Its production in Brazil reaches 26,500 tons per year (Ulson de Souza *et al.* 2007; Silveira *et al.* 2009b). These dyes are widely used in a number of industries, such as textiles, food, cosmetics and paper printing, with the textile industry being the largest consumer of dyes (Pandey *et al.* 2007).

Considerable attention has been given to issues associated with the presence of coloured compounds in aqueous wastewater generated from textile industries, since water is the only efficient carrier for dyes and other compounds that are used in the dyeing and finishing processes. An average textile finishing company uses 100,000–150,000 litres of water per ton of textile material treated (Li & Guthrie 2010).

Colour is the first contaminant to be recognized in wastewater; however, in addition to the aesthetic problem, dyes obstruct light penetration and oxygen transfer in water bodies. Without an adequate treatment, dyes are stable and can remain in the environment for an extended period of time (Silveira *et al.* 2009b).

Currently, the most popular methods of colour removal from wastewater involve physical and chemical processes that can be costly and usually include the formation of a concentrated sludge that creates a secondary, highly significant disposal problem (Chang & Kuo 2000). Although integrated chemical methods seem to be feasible for the treatment of such wastewater, biological methods should be used preferably considering cost and technical advantages (Yu *et al.* 2010).

Environmental biotechnology is constantly expanding efforts in the biological treatment of dye-contaminated wastewater. Although numerous microorganisms are capable of decolorizing dyes, only a few are able to mineralize these compounds into CO_2 and H_2O (Junghanns *et al.* 2008). Under aerobic conditions, azo dyes are not easily metabolized by bacteria (Robinson *et al.* 2001); however, under anaerobic conditions, several bacterial strains,

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including *Pseudomonas oleovorans*, can enzymatically reduce the azo bonds in the dye molecule to produce colourless by-products (Silveira *et al.* 2009b).

Several research papers have reported the results of combined anaerobic–aerobic bioreactor treatment of azo dye-containing wastewater (O'Neill *et al.* 1999; Sponza & Isik 2002; Kapdan *et al.* 2003; Khehra *et al.* 2006; Isik & Sponza 2008). However, the colour removal is mainly associated with the anaerobic stage, whereas further decolorization in the aerobic stage is usually limited to a few extra percent (van der Zee & Villaverde 2005). According to Minke & Rott (2002), a two-stage anaerobic–aerobic colour removal process achieved 70% higher decolorization than that of a one-stage aerobic treatment process. Therefore, it is reasonable to assume that anaerobic colour removal is mainly due to azo dye reduction.

This work reports the decolorization of an industrial azo dye by PUF immobilized *P. oleovorans* using an anoxic reactor.

EXPERIMENTAL

Microorganisms

The microorganism was obtained from the Brazilian Collection of Environmental and Industrial Microorganisms (CBMAI) of the University of Campinas, previously identified as *P. oleovorans* (CBMAI 703).

The microorganisms were preserved in cryotubes containing glass beads and 50% glycerol (v/v). Each cryotube was loaded from the same initial culture and had an average of 30 beads. Thus, it was possible to use the same cell generation for all experiments (Silveira *et al.* 2009b).

Dyes and reagents

Textile dye was obtained with the kind permission of Clariant of Brazil (Sao Paulo, Brazil). As the dye is for commercial use, the commercial name has been omitted in this study, receiving the following codename: B15 (C.I. 13390). The dye was filter-sterilized with 0.2 μm filter (Millipore, USA), prior to addition to the sterile culture medium. All other reagents were of analytical grade.

Pre-culture conditions

For each experiment, an Erlenmeyer flask containing 20 mL of nutrient broth (3 g L^{-1} meat extract and 5 g L^{-1} peptone) was inoculated with a single glass bead from the same cryotube and incubated at 28 °C for 24 hours, when an early stationary or a final exponential phase was reached.

Dye decolorizing cultures

Decolorization was performed, under static conditions, using screw-top bottles (500 mL) containing 125 mg of nutrient broth (adjusted pH = 8.5), supplemented with 50 mg L^{-1} of dye, and inoculated with 10 mL of fresh 24-hour-old cultures (approximately 0.16 g L^{-1} of dry weight of cells). The bottles were incubated under static anoxic conditions away from light at 32 °C for 36 hours. Control experiments were performed using the same medium without microorganisms or dyes (Silveira *et al.* 2009a).

Decolorization in fed-batch cultures

After the decolorization, the cultures were fed with a concentrated dye solution, aiming to regain the initial dye concentration. The feed was performed every time the dye concentration reached values below 3.6 mg L^{-1}.

Determination of cell growth and decolorization

The samples from decolorization cultures were collected and analysed according to the methodology described previously (Silveira *et al.* 2009b). As all samples contained biomass and dye, biomass concentration (first and second steps) and dye (third step) were evaluated as follows:

1. $OD_{600\,nm}$ of sample mixtures without centrifugation: $OD^{X+dye}_{600\,nm} = OD^{dye}_{600\,nm} + OD^{X}_{600\,nm}$;
2. $OD_{600\,nm}$ of sample supernatant (sup) after centrifugation for 10 min at 10,000 *g*: $OD^{sup}_{600\,nm} = OD^{dye}_{600\,nm}$; and
3. $OD_{609\,nm}$ of sample supernatant after centrifugation: $OD^{sup}_{609\,nm} = OD^{dye}_{609\,nm}$.

The biomass produced was determined by subtracting the value obtained in the first step from the value obtained in the second. Color removal efficiency was determined by

the following equation:

$$\text{Decolorization} = \frac{A_{\lambda\text{initial}} - A_{\lambda\text{final}}}{A_{\lambda\text{initial}}} \quad (1)$$

in which $A_{\lambda\text{initial}}$ represented the absorbance before the decolorization process and $A_{\lambda\text{final}}$ the value obtained during the third step. Each decolorization value was a mean of three parallel experiments.

Decolorization was also determined by wavescan, performed in an Ultrospec 3000 (GE Healthcare, USA). Samples were scanned from 240 to 790 nm to measure the color removal and aromatic compound degradation by *P. oleovorans*; culture medium without industrial dye was used as blank.

Cell immobilization on PUF

P. oleovorans cells from an early stationary or a final exponential phase were centrifuged at 10,000 *g* for 10 min and then immobilized in polyurethane foam (PUF). The PUF was cut in 1-cm^3 cubes, washed in distilled water, autoclaved twice and oven-dried at 37 °C. Approximately 9×10^9 cells in culture medium were added to screw-top bottles containing PUF cubes.

The content of the bottles was mixed on a magnetic stirrer for two hours. The bottles were incubated on an orbital shaker for one hour at 140 RPM, and then incubated under static conditions for another two hours. Finally, the dye was added to the culture medium and then incubated at the conditions described elsewhere (Silveira *et al.* 2009a).

Anoxic reactor

A jacketed glass column with an effective volume of 140 mL, with an internal diameter of 3 cm and a height of 20 cm was used as the reactor (Figure 1). The column was autoclaved and dried at 37 °C before each run.

Rubber corks were perforated for silicon tubing passage used for the column's inlet and outlet. Temperature was controlled using a water bath with water cycle. The column was packed with a PUF cylinder with a main diameter of 3 and 19 cm height.

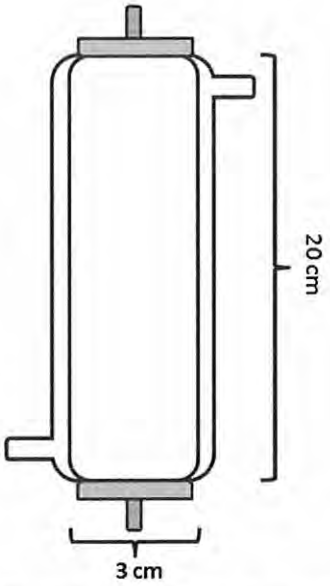

Figure 1 | A schematic of the column used for industrial dye decolorization.

Reactor operation

The experiments were conducted after the immobilization of *P. oleovorans* on PUF. The immobilization within the column was performed after 20 mL inoculation of fresh culture (approximately 9×10^9 cells) diluted in 100 mL of nutrient broth at 200 cm h^{-1} and sealed overnight at 32 °C.

The reactor was fed with synthetic effluent at a linear velocity of 15 cm h^{-1}, and at this rate no cell washout was observed. At given linear velocity, the hydraulic residence time (HRT) was approximately 2 hours. The reactor outlet was connected with the feed vessel to provide the dilution of the feed along the process time. The feed was stocked in a 1 litre bottle.

Synthetic effluent

The composition of the synthetic effluent was B15 industrial dye 25 mg L^{-1}; soluble starch 1,000 mg L^{-1}; acetic acid 150 mg L^{-1}; $(NH_4)_2SO_4$ 280 mg L^{-1}; NH_4Cl 230 mg L^{-1}; KH_2PO_4 67 mg L^{-1}; $MgSO_4 \cdot 7H_2O$ 40 mg L^{-1}; $CaCl_2 \cdot 2H_2O$ 22 mg L^{-1}; $FeCl_3 \cdot 6H_2O$ 5 mg L^{-1}; yeast extract 200 mg L^{-1}; NaCl 150 mg L^{-1}; $NaHCO_3$ 1,000 mg L^{-1}; and 1 mL L^{-1} of trace elements solution ($ZnSO_4 \cdot 7H_2O$ 10 mg L^{-1}; $MnCl_2 \cdot 4H_2O$ 100 mg L^{-1}; $CuSO_4 \cdot 5H_2O$ 392 mg L^{-1};

$CoCl_2 \cdot 6H_2O$ 248 mg L^{-1}; $NaB_4O_7 \cdot 10H_2O$ 177 mg L^{-1}; and $NiCl \cdot 6H_2O$ 20 mg L^{-1}).

Scanning electron microscopy

Samples of PUF before and after *P. oleovorans* immobilization were fixed in 2% glutaraldehyde (v/v) and 4% formaldehyde (v/v) at room temperature. The samples were then postfixed with 1% OsO_4 plus 0.8% $K_4[Fe(CN)_6]$ and 1% tannic acid, dehydrated in graded ethanol, critical point-dried with CO_2. The samples were then dehydrated in an ethanol series, transferred to propylene oxide, air-dried, mounted on aluminium stubs with double-sided tape (Scotch™), coated with gold in sputter system in a high vacuum chamber and examined in a Jeol JSM 5600LV at an acceleration voltage of 8 kV and a working distance of 8 mm.

RESULTS

Time-dependent and fed-batch cultures

The fed-batch decolorization was carried out for 216 hours, and the result is shown in Figure 2, which describes the decolorization of B15 dye in fed-batch. It was also observed that the biomass reached equilibrium after 42 hours. After 192 hours, *P. oleovorans* was able to decolorize up to 50 mg of dye; during this time, bacterial fatigue was not observed.

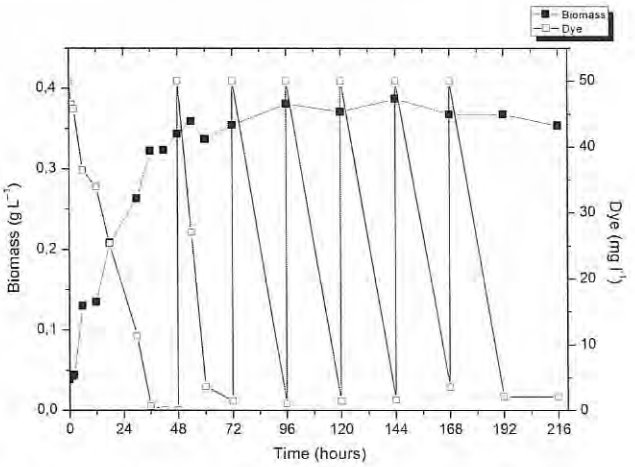

Figure 2 | Decolorization of B15 dye in fed-batch.

It was also observed a total decolorization of B15 dye, performed in only 36 hours in the first run, and substantial cell growth occurred along this first run. However, the cells did not seem to absorb the dye, since the centrifuged biomass showed no characteristics of absorption, remaining creamy white during the entire process.

It was also observed that there were two distinct behaviour patterns onto the fed-batch decolorization process of *P. oleovorans*. The first one was related to the cell growth, with a low decolorization rate (approximately 1.39 mg L^{-1} h^{-1}). The second one was presented along with the reactor feeding, with a raised decolorization rate (approximately 2.08 mg L^{-1} h^{-1}).

Immobilization of *P. oleovorans* on PUF

P. oleovorans was firstly immobilized on PUF cubes of 1 cm^3 and, after the immobilization process, the bacterial decolorization capacity was measured. Figure 3 shows the decolorization pattern of non-immobilized and immobilized cells with recycles.

Along with the decolorization process, and also at the beginning of each decolorization cycle, it was possible to observe some colour absorption by the foam. However, at the end of each run the support regained its original colour. It was also observed that after the first run, the decolorization process time was reduced to 30 hours on the following runs.

Figure 4 shows the scanning electron microscopy (SEM) of polyurethane foam (PUF) containing *P. oleovorans* cells.

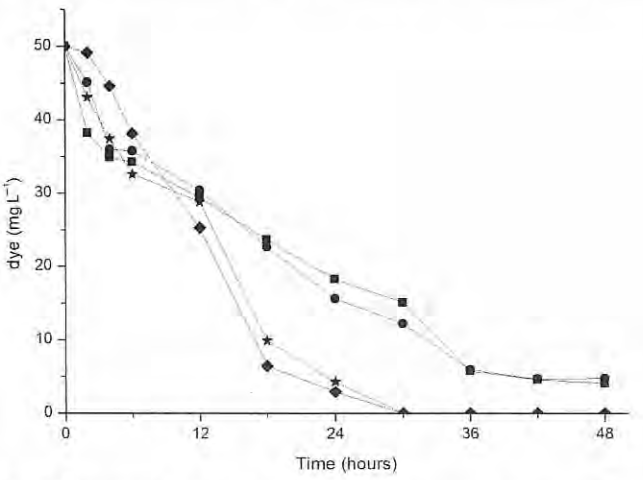

Figure 3 | Decolorization of B15 dye by: ▪ – Non-immobilized cells; and Immobilized cells • – 1st run; ◆ – 2nd run; and ★ – 3rd run.

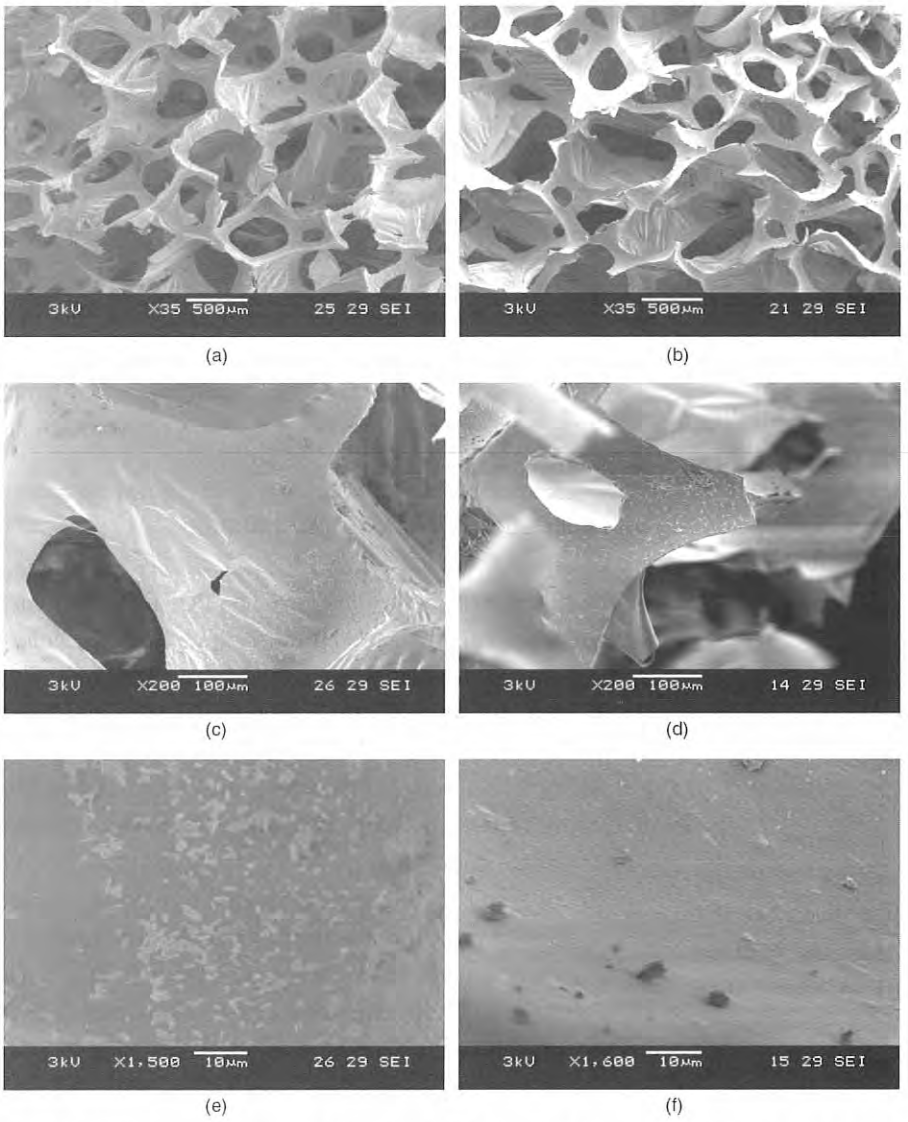

Figure 4 | Scanning electronic microscopy of immobilized cells in polyurethane foam (PUF). (a) PUF cross section after immobilization; (b) PUF cross section before immobilization; (c) PUF surface after immobilization; (d) PUF surface before immobilization; (e) PUF cross section surface containing immobilized with *P. oleovorans*; (f) PUF cross section surface before immobilization with *P. oleovorans*.

PUF was chosen as support due to its high porosity, inert nature, easy access and low cost. The PUF polyhedral structure can be observed in Figures 4(a) and (b), which provide a considerable large surface area to *P. oleovorans* biofilm development (Figure 4(e)).

Reactor decolorization process

Column packing with PUF cubes gave a non-ideal behaviour, in which was observed formation of channelling and stagnant zones within the reactor. Based on that, the reactor was packed with a PUF cylinder, which could fill all its inner space, thus giving it an ideal behaviour. Figure 5 shows the reactor before and after the decolorization process, and Figure 6 shows the decolorization pattern along the process time.

It was observed that in addition to using a lower concentration than the ones used in batch and fed-batch reactors, 25 and 50 mg L^{-1}, respectively, the reactor was able to decolorize a higher mass of dye per cycle. Therefore, the reactor could process 1 litre of effluent and the process time was reduced 6-fold.

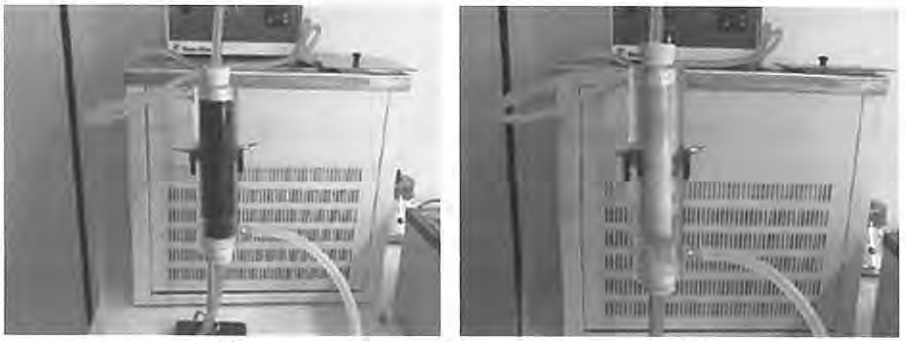

Figure 5 | Anoxic reactor packed with PUF cylinder. (a) Before and (b) after the decolorization.

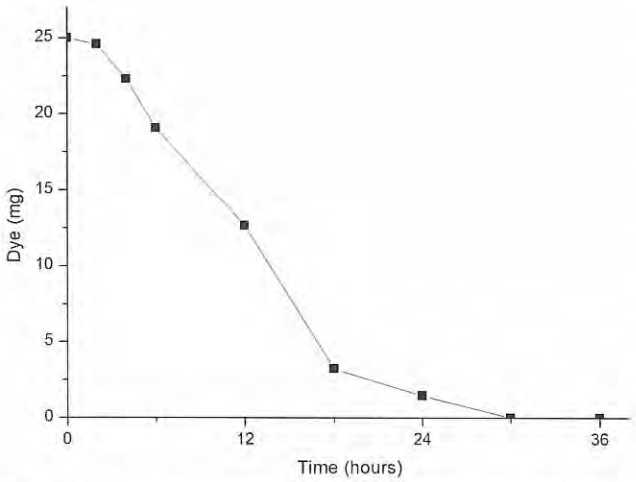

Figure 6 | B15 dye degradation pattern by *P. oleovorans* immobilized in PUF in an anoxic reactor.

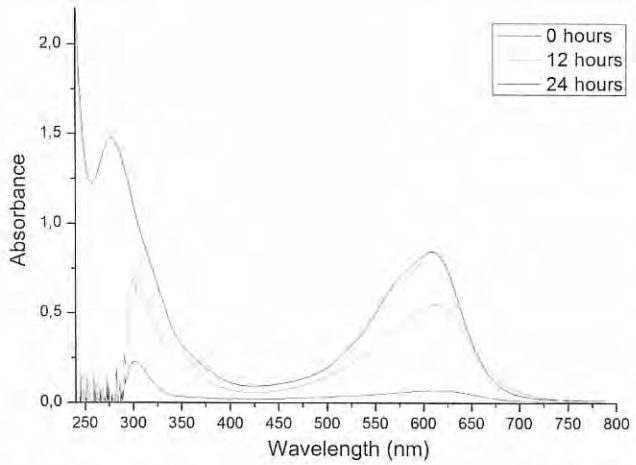

Figure 7 | Synthetic effluent wavescan along the decolorization process in the anoxic reactor.

Figure 7 shows the wavescan in several times along the decolorization of the effluent treated by the anoxic reactor packed with *P. oleovorans* immobilized on PUF. It was observed that apart from the colour reduction in the wavelength between 400 and 790 nm, there was a drastic reduction in the range of 200–350 nm.

DISCUSSION

Azo dye decolorization under anaerobic conditions has been described extensively (Carlieel *et al.* 1996; Chinwetkitvanich *et al.* 2000; Guo *et al.* 2006; Kim *et al.* 2008); however, little is described about total dye degradation. The behaviour present with the fed-batch decolorization suggests that cells were adapting during the first cycle, in which the cell machinery was adapting to consume an alternative and more recalcitrant carbon source. From the second cycle onwards, where all the cell machinery is already ready for the process, it was simpler to the cell to consume the dye as sole carbon source.

Zhang *et al.* (1999) described the use of a fed-batch reactor in a fluidized bed containing white-rot fungus, where the fungus was reused during nine cycles of decolorization. In addition, Chang & Lin (2000) described the decolorization of Reactive Red 22 by a *Pseudomonas luteola* strain in fed-batch, in which the dye was fed in the reactor without the total elimination of the initial dye.

This strategy resulted in a higher decolorization rate by *P. luteola*; however, the dye decolorization should undergo substrate inhibition, leading to an incomplete decolorization

at the final process. Nevertheless, the only advantage presented by the processes described previously was the increase of dye concentration in the effluent. The decolorization performed by *P. oleovorans* showed a significant increase in the colour removal rate, making it possible to achieve total colour removal.

Several researches describe the immobilization of microorganisms on polyurethane foam (PUF), but highlighted the immobilization of bacterial consortium (Khehra *et al.* 2006), semi-solid fungal fermentation (Susla *et al.* 2007) and fungi in submersed cultures (Casieri *et al.* 2008). Khehra *et al.* (2006) described an anoxic–aerobic sequential bioreactor using a bacterial consortium immobilized on PUF, where it was capable of reaching complete decolorization of a synthetic effluent containing 100 mg L^{-1} of Acid Red 88 dye.

Decolorization of much more mass of dye diluted in medium by *P. oleovorans* is possible provided the period of process time is increased (Silveira *et al.* 2009b). Furthermore, the microscopy results diverge from the ones described by Kim *et al.* (2003) and by Khehra *et al.* (2006) on the biofilm formation, probably due to the treatment prior to the microscopy.

According to Sharma *et al.* (2004), a plug flow reactor is capable of decolorizing synthetic effluent contaminated with dyes with a HRT of 23 hours. However, the results obtained showed that, with a decreased HRT of approximately 2 hours, it was possible to decolorize dyes, since the reactor is connected with the recycle.

The recycle, in other words the outlet flow return to the feed inlet, should be done for two reasons: (a) to preserve the media while it was not totally converted into the desired product; and/or (b) to increase the process yield (Missen *et al.* 1999). With the recycle, it was possible to reach a decolorization rate higher than 95%, without the need for a sequential reactor.

Khehra *et al.* (2006) described the use of an anoxic-aerobic sequential bioreactor, in which full decolorization of acid red 88 was achieved, however, it used a bacterial consortium (*Bacillus cerus*, *Pseudomonas putida*, *Pseudomonas fluorescens* and *Stenotrophomonas acidaminiphila*) in the process, making it necessary to use a second reactor to reach full dye degradation and its by-products.

The colour removal in several sequential reactors is basically associated with the anaerobic stage, where the further decolorization in the aerobic stage is normally limited to a few percent (van der Zee & Villaverde 2005). Minke & Rott (2002) described that two-stage decolorization (anaerobic and aerobic) was 70% higher than a single aerobic stage. The results showed that a second aerobic stage is not necessary, since *P. oleovorans* was capable of decolonizing the synthetic effluent in only one stage.

According to Pearce *et al.* (2008), the dye reduction capacity consists basically in the cell's ability to reduce azo bonds (–N=N–) or its intermediary ketohydrazone (–N–NH–). The results suggest that *P. oleovorans* is not only capable of reducing azo bonds, but also of degrading aromatic compounds present in the effluent.

According to Brand & Eglinton (1965), the strong absorbance at 200–300 nm of a solution is consistent with aromatic amines. The formation of aromatic amines resulting from decolorization is a common problem originating from biological treatment of dyes (Ulson de Souza *et al.* 2007). Pearce *et al.* (2008) described the decolorization of pigments by *Shewanella* strain J18 143, in which there was a reduction in its μ_{max} and an increase in the UV spectrum, suggesting the formation of high levels of aromatic amines.

Ulson de Souza *et al.* (2007) suggested that biological treatment should be considered as a pre-treatment, requiring a post-treatment to remove organic compounds (i.e. aromatic amines) remaining in the effluent. This should not be a concern with *P. oleovorans* degradation of industrial dyes, since an almost complete removal of UV spectrum was observed.

CONCLUSION

The ever-increasing legislation restrictions regarding effluent disposal combined with their toxicity, carcinogenicity and mutagenicity makes dye contamination both an environmental problem and a public health problem. The immobilization of *P. oleovorans* along with its use in fed-batch reactors, as well as in an anoxic reactor, is a promising green technology to the decolorization of dye-contaminated effluents, since it was possible to achieve full degradation of

industrial dye in a short period of time. Further work should be performed aimed at the scale-up of this process.

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Nitrogen-removal efficiency in an upflow partially packed biological aerated filter (BAF) without backwashing process

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ABSTRACT

An upflow, partially packed biological aerated filter (BAF) reactor was used to remove nitrogen in the form of ammonia ions by a nitrification process that involves physical, chemical and biological phenomena governed by a variety of parameters such as dissolved oxygen concentration, pH and alkalinity. Dissolved oxygen (DO) and pH were shown to have effects on the nitrification process in this study. Three C:N ratios i.e., 10, 4 and 1 were compared during this study by varying the nitrogen loading while the carbon loading was kept constant at 0.405 ± 0.015 kg chemical oxygen demand m^{-3} d^{-1}. The removal efficiencies of ammonia linearly increase with a rise of the initial concentration of ammonia-nitrogen. The results of the 115 days' operation of the BAF system showed that its overall NH_3-N performance was good, where a removal efficiency of 87.0 ± 2.9%, 89.2 ± 1.38% and 91.1 ± 0.7% and COD removal of 87.6 ± 2.9%, 86.4 ± 2.1% and 89.5 ± 2.6% were achieved for the C:N ratios of 10, 4 and 1, respectively on average, over 6 h hydraulic retention time (HRT). No clogging occurred throughout the period although backwashing was eliminated. It was concluded that the BAF system proposed in this study removed nitrogen by the nitrification process extremely well.

Key words | alkalinity, biological aerated filter (BAF), C:N ratio, DO, nitrification, pH

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INTRODUCTION

Carbon and nitrogen are major pollution sources that contribute to environmental quality problems all over the world, especially those that mainly cater to treatment of wastewater. The most adverse environmental impacts associated with improper discharge of municipal wastewater containing significant amounts of organic matter (chemical oxygen demand, COD), nitrogen (N) and phosphorus (P) include promotion of eutrophication, toxicity to aquatic organisms and depletion of dissolved oxygen receiving streams (Klees & Silverstein 1992; Moosavi *et al.* 2005). Nitrate- and nitrite-contaminated water supplies are also related to several diseases such as methemoglobinemia in infants, also known as 'blue baby disease' (Doyle *et al.* 1985; Gálvez *et al.* 2003). Due to the adverse impacts, completed treatment of municipal wastewater before discharge is increasingly necessary.

Although conventional biological treatment processes are mostly reliable, well designed and tested, they present a number of drawbacks in terms of treatment capacity, efficiencies, stability and space requirements. Advanced biological treatment processes, developed to overcome these deficiencies, are now in increasing demand. One of the systems, the biological aerated filter (BAF), appears to be promising.

The application of a BAF in municipal and industrial wastewater treatment has increased significantly, first in Western Europe and then worldwide, as a novel wastewater-treatment system due to its advantages over other systems (Mann *et al.* 1998; Ryu *et al.* 2008). Due to the number of advantages of the BAF process, a space-saving layout that takes up only one-third of the footprint size of an activated sludge process can be achieved (Han *et al.* 2009).

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BAF systems have also been shown to operate successfully at higher hydraulic and organic loading rates (OLR) than activated sludge systems (Mendoza-Espinosa & Stephenson 1999; Ryu *et al.* 2008). Peladan *et al.* (1997) reported OLR up to 18 kg COD m^{-3} day^{-1} in high-water-velocity BAFs. Although the BAF system has many advantages, it has been difficult to apply for the treatment of raw wastewater, which contains a high concentration of suspended solids. Several researchers have tested the BAF for ammonia and nitrogen removal (Tay *et al.* 2003; Lee *et al.* 2005). A pilot scale fixed-film bioreactor system demonstrates greater waste degradation than traditional technology, e.g., the removal rates for COD were consistently between 80% and 90% at an empty bed HRT of 8 hours for the entire system (Jou & Huang 2003). In addition, successful nitrogen removal in high-strength wastewater was also investigated by Tay *et al.* (2003) using a single fixed-bed filter with anaerobic, anoxic and aerobic zones.

A BAF consists of a medium that provides a large surface area per unit volume for biofilm development. The filter media play a significant role in wastewater treatment. The characteristics of the media are not only related to the initial capital outlay, process design and operation mode of BAFs, but also affect daily running costs like backwashing and air influx (Rozic *et al.* 2000; He *et al.* 2007). For the development of biofilm technologies, the BAF has been considered to be a system capable of enhanced biological carbon and nitrogen removal. But the average nitrification rate was reduced when weekly backwashing was applied due to a loss of autotrophic bacteria (Elenter *et al.* 2007). Most of the cost problems with the BAF are related to backwashing, media, aeration and sludge handling. A partially packed-bed BAF has a lower media cost, saves energy during aeration, requires a lower capital investment for pumping facilities and has a thinner and denser biofilm with better attachment of nitrifiers (Fatihah 2004). Studies on the effects of low C:N ratios on carbon and nitrogen removal in BAFs have been done to better understand removal behaviour as the ratio is decreased (Ryu *et al.* 2008). Fixed COD loading was applied to stimulate the growth of autotrophs as autotrophs decrease with the incremental increase of influent COD (Ni *et al.* 2008).

An understanding of the impact of C:N ratio on nitrogen removal wastewater is imperative for optimizing the biofilm reactor. A study by Fatihah (2004), at a C:N ratio of 24:1, showed that partial nitrogen removal occurred most probably because of a very low concentration of limiting carbon source due to high total organic carbon (TOC) removal efficiency of $92.1 \pm 6.5\%$ for full-bed BAF and $90.2 \pm 6.3\%$ in partial bed. However, information on the performance of the BAF under various operating and environmental conditions is still lacking.

For this purpose, the studies propose a partially packed upflow BAF process, within which nitrification steps were investigated. The main objective of this paper was to evaluate the effect of different low carbon–nitrogen ratios in synthetic wastewater to remove carbon and nitrogen using a partially packed biological aerated filter without any backwashing process at 30 ± 2 °C and to investigate ammonia-nitrogen removal impacting nitrification parameters.

MATERIALS AND METHODS

Synthetic wastewater

A synthetic wastewater prepared in the laboratory was used to provide a consistent organic substrate for loading. The synthetic feed was formulated by considering the major nutritional requirements for microbial growth including sources of carbon, sources of energy, electron acceptors, nitrogen sources and sources of other major mineral nutrients like sulfur, phosphate, potassium, magnesium, calcium and trace-metal requirements. The composition of the synthetic wastewater, prepared with tap water, is shown in Table 1.

Operation of BAF and experimental setup

The BAF reactor used in this study was cylinders 1.5 m in height (0.65 m in attached growth zone and 0.85 m in suspended growth zone) and 0.15 m in diameter with a working volume of approximately 26 L. The reactor was partially packed with plastic media (Kaldnes K1, Sweden) and the system was operated at 30 ± 2 °C. According to the mechanical and biological tests performed, plastic has the lowest weight loss and it can be considered as the most suitable medium for application in the pilot scale BAF

Table 1 | Synthetic wastewater composition

Source	Composition	Concentration (mg L^{-1})	Total concentration (mg) C:N 1	C:N 4	C:N 10
COD	Glucose	1,000	13,000	13,000	13,000
Nitrogen	NH_4Cl	600	13,000	3,250	1,296
	KNO_3	50	650	650	650
Phosphorus	KH_2PO_4	7.5	1	1	1
	Na_2HPO_4	944	129	129	129
Nutrient	$MgCl_2$ $6H_2O$	100	1,300	1,300	1,300
	$MnCl_2$ $4H_2O$	0.5	6.5	6.5	6.5
	$FeCl_2$ $6H_2O$	0.5	6.5	6.5	6.5
	$CaCl_2$ $2H_2O$	7.5	97.5	97.5	97.5

(Farabegoli *et al.* 2003). The experimental equipment consisted of the partial-bed reactor, one feed tank, one effluent tank, an interconnecting pipe network, pumping facilities, diffuser and the sensors for ORP (RD1R5, Hach, USA), pH (PD1R1, Hach, USA) and DO (5540DOA, Hach, USA). Masterflex peristaltic pump (77200-60, USA) was used to supply the feed. A polypropylene mesh was positioned in between the suspended growth and attached growth zones to keep the medium in place. A schematic of the experimental set-up is shown in Figure 1.

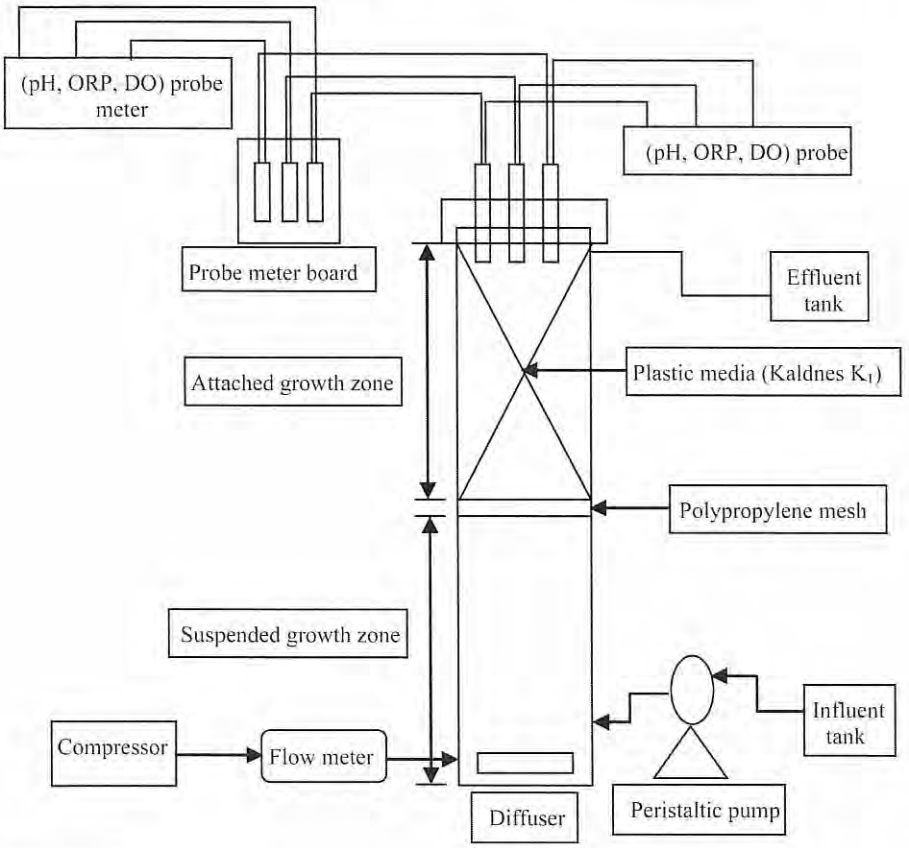

Figure 1 | BAF reactor.

The pH, DO and ORP data were taken from pH (P33, Hach, USA), DO (D33, Hach, USA) and ORP (P33, Hach, USA) meters, respectively, during the off-line monitoring period of the BAF reactor. Due to the lack of on-line monitoring, pH, ORP and DO data were taken once a day. Aeration was supplied by a compressor into the air inlet pipe to the reactor. The air pump was connected to the air diffuser, which was placed at the bottom of the column to provide air to the reactor. The flow rate for the continuous aeration was determined by the air control meter (Model No 0Z0395F, Japan). During this study, air flow was set at 2.5 L min^{-1}. Air delivered through this system had to provide adequate air supply for biological activity and for mixing within the reactor. Activated sludge from a biological nutrient-removal municipal wastewater-treatment plant was used as a seeding culture, in which the mixed-liquor suspended-solids (MLSS) value was approximately 2,500 mg L^{-1}. Activated sludge was used for the seeding process because of its high suspended biomass concentration, which leads to rapid biofilm formation (Mann *et al.* 1999). In this seeding process, the activated sludge was fed daily batchwise with 0.405 ± 0.015 kg COD m^{-3} d^{-1} of synthetic wastewater until a high concentration of biomass was obtained and a biofilm was formed on the plastic particles. During the study period, the COD load was set at 0.405 ± 0.015 kg COD m^{-3} d^{-1} and the loads of ammonia were investigated based on different C:N ratios as shown in Table 2.

ANALYSIS

Influent and effluent samples were collected each day from the influent tank and effluent pipe. The samples were analyzed for COD, NH_3-N, NO_3-N, alkalinity, suspended solids and MLSS. Chemical analyses were carried out according to standard methods (APHA 1998). DO, pH and ORP were measured using a DO probe meter, pH meter and ORP probe meter, respectively. All samples were analysed after being filtered through 0.45-μm pore size filter paper.

Table 2 | Applied loads for a partially packed BAF according to the C:N ratio

Load	C:N 1	C:N 4	C:N 10
COD (kg m^{-3} d^{-1})	0.405 ± 0.015	0.405 ± 0.015	0.405 ± 0.015
NH_3-N (kg m^{-3} d^{-1})	0.184 ± 0.001	0.046 ± 0.001	0.018 ± 0.001

RESULTS AND DISCUSSION

COD-removal performance

Organic matter, in terms of COD, is one concern in the treatment of wastewater. Therefore, its removal is the focus of a wastewater treatment facility. The removal process was carried out by the microorganisms that grow attached to the filter-packed media. In this study, the COD removal pattern is quite consistent with the loadings and COD-removal efficiency was high at 87.6 ± 2.9%, 86.4 ± 2.1% and 89.5 ± 2.6% with different C:N of 10, 4 and 1, respectively, at 6 h HRT. At a C:N ratio of 24, the carbon removal was also known to be high (TOC removal efficiency of 90.2 ± 6.3% in partial bed) (Fatihah 2004). For different C:N ratios, the COD removal efficiency did not vary significantly based on changes of the ratio. Sales & Shieh (2006) reported that for C:N ratios of 50 and 100 the COD removal efficiency (above 90%) was similar for both ratios for an A/O hybrid bioreactor. However, the COD removal was almost stable during the experimental period. This trend occurred because of the fully developed biofilm structure inside the reactor on the plastic media.

The high removal efficiency in the experiment was due to the efficient utilization of organic compounds in the aerobic process. In addition, the high removal rate could also be attributed to the complete particulate retention of suspended COD and BOD, high-molecular-weight organics and biomass (Stephenson *et al.* 2000). In this study, suspended solids was found to be high and this resulted in the depletion of COD and nitrogen removal due to competition for oxygen inside the reactor. The attached-growth system does not possess good settling characteristics. According to Ong *et al.* (2004), a shorter HRT would lead to more dispersed growth and therefore poorer suspended solids settling in the treated effluent. Furthermore, there was no biomass separation step in the BAF reactor. Most of the suspended solids, mainly from biofilm detachment, accumulated at the bottom of the reactor because of a lack of backwashing in this experiment. The nitrification process typically requires an MLSS concentration between 2,000

and 3,500 mg L^{-1}. It was indicated that low MLSS concentrations do not provide for the establishment of an adequate population of nitrifying organisms to perform nitrification. High MLSS concentrations can result in unacceptable suspended solids concentrations in the effluent. The COD removal rate for BAF reactors was very high throughout the operation, and was unaffected by changes in the MLSS concentration. The results show that the partially packed upflow BAF reactor without backwashing was generally reliable and it had a better treatment capacity at a low C:N ratio with less HRT. Clogging did not occur also because of the low influent load and low sludge production during the operating period. Carbon-removal performance in this type of system showed improved carbon removal although the C:N ratio was low.

The relationship between the COD loading rate and COD removal is shown in Figure 2. A linear regression of the data indicated that COD removal rate had a linear relationship with loading rate (R^2) of 0.85. According to Farabegoli *et al.* (2003), a linear relationship between the COD loading rate and its removal efficiency was investigated in a downflow-submerged BAF with an R^2 of 0.6192. Westerman *et al.* (2000) also similarly obtained a linear relationship between the COD loading rate and its removal in an up-flow biological aerated filter with an R^2 of 0.92.

Nitrogen-removal performance

The variation of ammonium-nitrogen (NH_3-N) in BAF was observed according to the variation of influent of ammonium during the operation period. The average influent loading of ammonium was 0.182 kg NH_3-N m^{-3} d^{-1}, 0.046 kg NH_3-N m^{-3} d^{-1} and 0.018 kg NH_3-N m^{-3} d^{-1} with a C:N of 10, 4 and 1, respectively. In this study, the removal of NH_3-N was 87.0 ± 2.9%, 89.2 ± 1.3% and 91.1 ± 0.7% with a C:N of 10, 4 and 1, respectively. The study done by Ryu *et al.* (2008) proposed a four-stage biological aerated filter system to treat low C:N ratio wastewater with a nitrogen removal of more than 95% for a ratio of TCOD:TKN of 4.3 ± 1.1. NH_3-N removal was relatively high over this time but stability of the removal rate was only achieved at a C:N of 1 during the study period, which may be due to variation of alkalinity consumption. The relationship between the ammonia loading rate and ammonia removal loading rate was linear with an R^2 of 0.97 (figure not shown here). On the other hand, there was no backwashing involved in this operation.

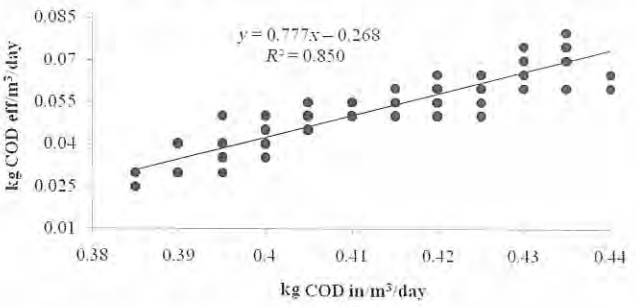

Figure 2 | Relationship between COD loading and removal.

It was observed that ammonia decreased over time under aerobic conditions, and there was a corresponding increase of the nitrate concentration over time as ammonia was converted to nitrate through nitrification. Nitrate concentration rose as the aeration continued, indicating that the nitrite-oxidizers were present in the reactor, and that they were adjusting to the aerobic environment. The nitrification rate may have been limited by the biodegradable organic matter concentration or by the variation in oxygen concentration. Nitrate removal occurred at more than 70% when the DO was below 0.6 mg L^{-1} and the anoxic phase was available at C:N 10 (Figure 3). If oxygen is properly supplied to the reactor with the inlet wastewater, biodegradable organic matter will be consumed in the process of oxygen respiration and thus reduce the amount available for denitrification. But under low DO conditions in the biofilm layer, nitrate also produced by the nitrification process was converted via nitrite to nitrogen gas, which was then evolved from the reactor. Therefore, removal of nitrate can be found in the process (Figure 3).

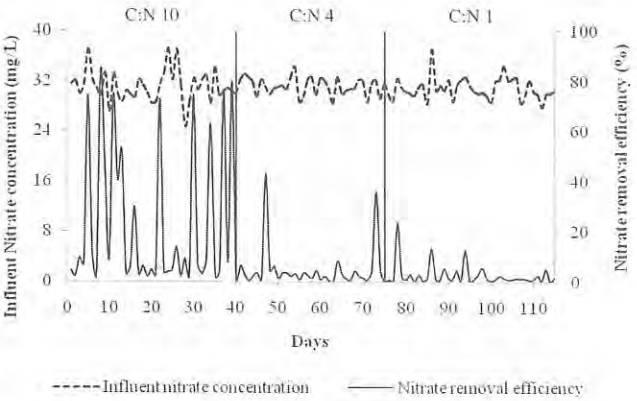

Figure 3 | Nitrate removal at different C/N.

The removal efficiency of ammonia-nitrogen linearly increased with the decrease of COD/NH_3-N. The study shows that the NH_3-N removal percentages of 91.1, 89.2 and 87.0% were achieved for the COD/NH_3-N of 2.2, 8.8 and 22.5, respectively (Figure 4). Ahmed *et al.* (2007) also found that for the influent COD/NH_4 of 7.2, 9.9 and 14.7, the removal efficiencies in the combined system were 88, 80 and 69%, respectively. Therefore, the results suggest that the nitrification efficiency may have also been inhibited by substrate (ammonia) concentration, because an increase in ammonia loading led to an increase in nitrification rate.

Free ammonia concentration

pH, temperature and ammonium concentration are the most important parameters that can influence the equilibrium controlling the free ammonia concentration (Farabegoli *et al.* 2004). Temperature influences bacterial kinetics of the nitrification process in the biofilter operation. The study was only performed at 30 ± 2 °C and the NH_3-N removal was 87.0, 89.2 and 91.1% with the C:N ratios of 10, 4 and 1, respectively. When experimental temperature varied between 21 °C and 31 °C, higher temperature greatly improved the nitrification and COD reduction gradually went up from 73 to 86% (Li & Zheng 2004).

Figure 5 shows the concentration of the free ammonia in the BAF reactor after the start-up, calculated by Equations (1) and (2), was introduced in this study. The maximum levels of free ammonia are found at the bottom of the reactor since they decrease as the height increases (Fdz-Polanco *et al.* 1994). Figure 5 shows that at the bottom of the filter pH increase brings about an increase of free ammonia concentration at 30 ± 2 °C according to Equation (1). Values of

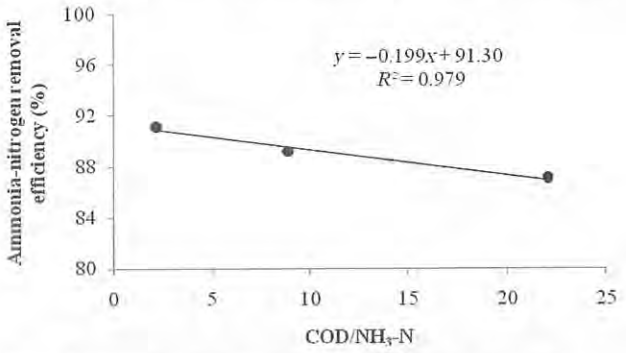

Figure 4 | Relationship between ammonia-nitrogen removal and COD/NH_3-N.

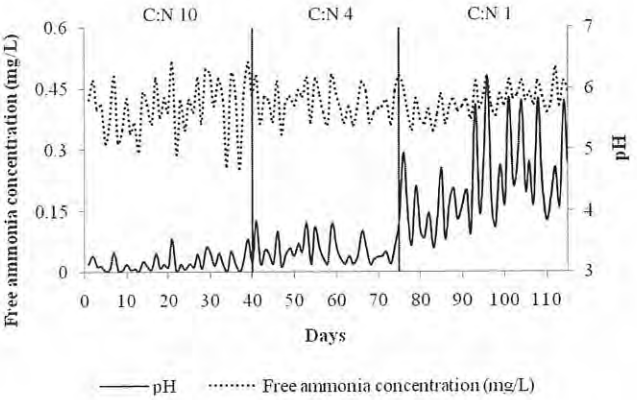

Figure 5 | Free ammonia concentrations at different C/N.

free ammonia concentration were 0.02 mg L^{-1}, 0.124 mg L^{-1} and 0.176 mg L^{-1} with respect to the C:N ratios of 10, 4 and 1, respectively. The free ammonia concentration was always in the range of 0.1–1 mg NH_{3free}-N L^{-1} from 55 cm to the bottom of the filter (Farabegoli *et al.* 2004). High free ammonia (NH_3-N) inhibited not only nitrite oxidizing bacteria (NOB) but also ammonia oxidizing bacteria (AOB) (Kim *et al.* 2006). Furthermore, very little is known about the effects of different growth environments on nitrifying bacteria communities. Finally, the discussion of the concentration of free ammonia was carried out based on results from the lower part of the BAF reactor.

Free ammonia concentration depends heavily on pH based on the following equation:

$$[NH_3\text{-}N]_{free} = \frac{[NH_4\text{-}N] \times 10^{pH}}{(K_a/K_w) + 10^{pH}} \quad (1)$$

where K_a is the ammonia constant and K_w is the water ionization constant. The hydrolysis of ammonia reaction constant is dependent on temperature:

$$K_a/K_w = \exp\left[\frac{6,334}{273 + T}\right] \quad (2)$$

Effect of DO, pH and alkalinity on nitrification

DO profile

DO concentrations have a direct effect on the growth rates of nitrifying bacteria. In the presence of low dissolved oxygen,

incomplete nitrification occurred, which led to a build-up of ammonium within the BAF (due to the insufficient aeration time to convert the ammonia to nitrate). Zhu & Chen (2002) reported that it was more important to maintain sufficient DO in the fixed film process than in the suspended growth processes due to the nature of diffusion transport with fixed film.

Figure 6 shows that the nitrification rate increased along with a rise of DO concentration. The influence of DO on the nitrification process indicated that for 1 mg L^{-1} changes in DO in an aerobic reactor 10% removal of ammonia-nitrogen was achieved when the DO range was 0.48–0.98 mg L^{-1} in this study. According to the results, the nitrification rate improved with an increase of DO up to 3.7 mg L^{-1} and decreased above 4.0 mg L^{-1}, which shows that over-aeration leads to a reduction in the nitrification efficiency because of detachment of the biofilm from the plastic media. In order to achieve an NH_4-N removal of above 60%, the dissolved oxygen concentration in the aerobic system should be maintained above 1 mg L^{-1} (Hsu & Chiang 1997). It is generally known that a DO concentration above 1 mg L^{-1} is essential for nitrification; if the DO level is lower, oxygen becomes the limiting factor and nitrification slows. Since DO acts as an electron acceptor in the biochemical reaction, its concentration is necessary in the reactor at the time of the nitrification process. Based on the available information, a suitable range of DO concentrations required to reliably achieve nitrification is between 2 and 4 mg L^{-1} in order to prevent the possibility of oxygen limitation and to enhance ammonia removal.

Alkalinity and pH profile

Alkalinity is important not only for nitrification but also to indicate system stability. The decrease in pH is caused by the removal of ammonia from the system, and ammonia was also strongly correlated to the alkalinity of the wastewater. The end of alkalinity consumption in the wastewater was indicated by the complete removal of ammonia. The relationship between alkalinity (total alkalinity, in $CaCO_3$) and the NH_3-N removal efficiency is shown in Figure 7. It can be seen that the nitrogen removal rate was enhanced with an increase of alkalinity. The alkalinity affecting nitrification and the nitrogen removal rate improved with an increase of alkalinity when the wastewater's alkalinity to NH_3-N ratio is less than 8.85 (Sakairi *et al.* 1996); however, this study shows the average ratio to be 2.10. Gujer & Boller (1986) also reported that in nitrifying biofilters used in municipal wastewater treatment, an alkalinity level of at least 75 mg L^{-1} was needed to maintain maximum nitrification rate. The result shows that the average alkalinity concentration was 93 mg L^{-1} during the entire period of this study. Therefore, sufficient alkalinity must be available in order to achieve complete nitrification, and operators must continuously monitor this parameter to achieve at least 100 mg L^{-1} of alkalinity in the wastewater effluent.

The impact of alkalinity on the nitrification rate is related to the pH. It has been reported that the pH of wastewater should be maintained between 6 and 9 to protect organisms (Akpor *et al.* 2008). In a study on the pH effect upon the nitrification efficiency in an upflow biofilter, it was reported that nitrification efficiency showed a linear increase of 13% per unit pH increase from pH 5.0 to 8.5 (Villaverde *et al.* 1997). The study also showed that the ammonia-nitrogen removal increased linearly with the raise of pH value with an R^2 of 0.72 (data not shown here). Figure 8 shows the NH_3-N removal increasing from

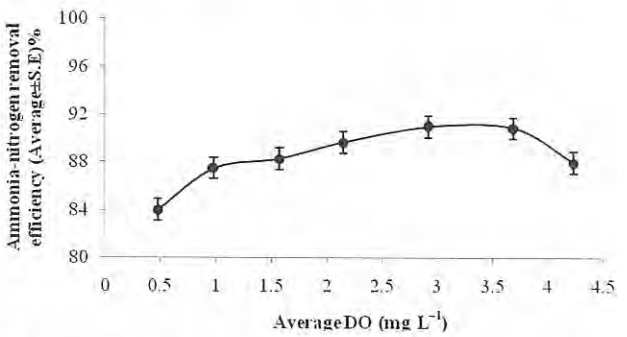

Figure 6 | Relationship between DO and ammonia-nitrogen removal efficiency.

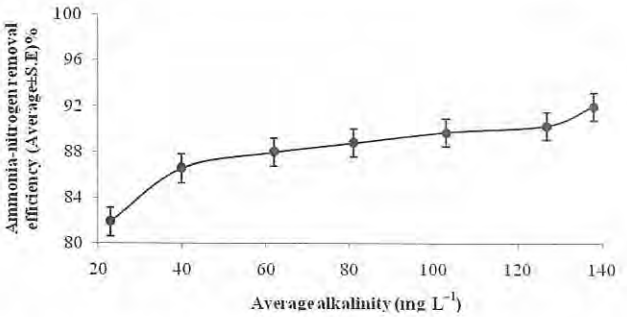

Figure 7 | Relationship between alkalinity and ammonia-nitrogen removal efficiency.

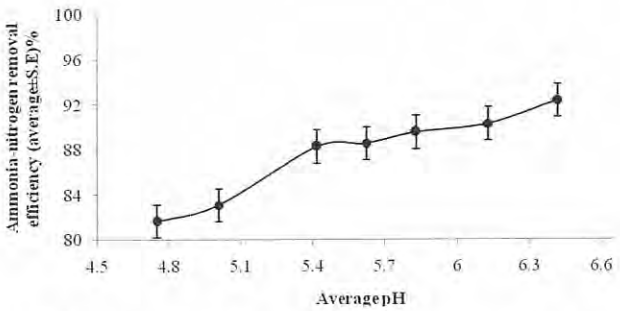

Figure 8 | Relationship between pH and ammonia-nitrogen removal efficiency.

81.6 to 92.3% when there is a change of pH value from 4.7 to 6.4. While it was indicated that pH 6.45 to 8.95 had no effect on nitrification, a pH lower than 6.45 and over 8.95 completely inhibited the nitrification process (Jianlong & Ning 2004). After all, nitrification was not consistent due to the instability of the pH of the system. Thus, pH control was required for the reactor to complete nitrification and enhance nitrogen removal.

CONCLUSIONS

The up-flow partially packed BAF system with plastic media was evaluated for enhancing nitrogen removal in the treatment of low C:N ratio synthetic wastewaters. The study shows that NH_3-N removal efficiencies increased as the carbon-nitrogen ratio decreased but COD removal was not really affected by the C:N ratio. It was highly affected by the pH and dissolved oxygen (DO) on the nitrification process. The nitrification rate improved with an increase of DO up to 3.7 mg L^{-1} and decreased at above 4.0 mg L^{-1}, while the ammonia-nitrogen removal increased from 81.6 to 92.3% when there was a change of pH value from 4.7 to 6.4. The results demonstrated that an up-flow partially packed BAF can be operated at an HRT of 6 h, where NH_3-N removal efficiency of 87.0 ± 2.9%, 89.2 ± 1.3% and 91.1 ± 0.78% and COD removal of 87.6 ± 2.9%, 86.4 ± 2.1% and 89.5 ± 2.6% were achieved for the C:N ratios of 10, 4 and 1, respectively. This study demonstrates that partially aerated BAF systems can be operated at a low HRT and can be used as a compact system for small communities to treat wastewater for NH_3-N removal. Future work will be directed at online control and monitoring of the biological aerated filter to enhance nitrogen removal.

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Impact on Gaza aquifer from recharge with partially treated wastewater

Sami M. Hamdan, Abdelmajid Nassar and Uwe Troeger

ABSTRACT

The Gaza Strip suffers from high pressure imposed on its water resources. There is a deficit of about 50 mm^3 every year, which has led to a declination of groundwater level and deterioration of groundwater quality. New water resources are sought to fulfil the water deficit; among them is the artificial recharge of treated wastewater to groundwater. The impact of recharging partially treated wastewater in Gaza was tested through a pilot project implemented east of the existing wastewater treatment plant. The daily application of about 10,000 m^3 of effluent to infiltration basins had an effect on the aquifer, which was monitored through the surrounding operating water wells over five years from 2000 until 2005. Although the monitored wells are operated for irrigation by farmers, impacts were clearly noticed. Groundwater levels improved and an increase in some areas of 0.6 m within three years was observed. The nitrate ion concentration also decreased in the groundwater due to nitrification processes. However, chloride ion, which indicates salinity, increased because the effluent has high chloride concentration. Boron levels increased in some areas to 0.5 mg/l, which could affect sensitive crops grown in the area.

Key words | effluent, groundwater, pollution, recharge, reuse, water quality

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INTRODUCTION

The increasing water demand and limited water resources in Palestine in general, and in the Gaza Strip in particular, have led to the depletion of the water systems quantitatively and qualitatively. The aquifer in the coastal region, i.e., Gaza Strip, suffers from high pressure imposed by supplying domestic and agricultural needs. The overall water use is 164 mm^3 per year, where the overall supply is only 122 mm^3 per year (PWA 2007). This means that there is a deficit of about 42 mm^3 every year. The deficit has led to a continuous declination of groundwater level and deterioration of groundwater quality.

The policy of water resources management is to use non-conventional water resources such as seawater desalination and artificial recharge of groundwater from storm water and treated wastewater. The agricultural demand is almost constant since the agricultural areas are limited or even decreasing. However, the domestic demand increases due to the rapid growth of the population. This increases the amount of wastewater produced and the treated effluent becomes a significant resource of water that could improve the water balance in the region. The reuse of this effluent could be accomplished in two ways: either by direct irrigation to farms and/or through artificial recharge to groundwater, which is then pumped to irrigate farms with reclaimed wastewater.

Water demand is continuously increasing due to economic development and population increase, due to natural growth and returnees, while the water resources are constant or even decreasing due to urban development (CAMP 2000). The Gaza Strip is classified as a semi-arid region and suffers from water scarcity. The renewable amount of water that replenishes the groundwater system is much less than the demanded amount, and this has resulted in deterioration of the groundwater system in both quantitative and qualitative aspects (PWA 2000a). The Palestinian Water Authority seeks other resources to fill

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the water gap between the supply and the demand and to attain sustainable water resources management. There are large quantities of wastewater estimated at 40 mm^3 every year (CMWU 2007) that are produced by the municipal sewerage systems and the treated effluents are disposed to the sea or flooded without good treatment or control to the surrounding areas and underground aquifer. Biological oxygen demand (BOD) is reduced from 440 to 140 mg/l, while chemical oxygen demand (COD) is reduced from 900 to 300 mg/l through the poor treatment at Gaza plant (CMWU 2007). For direct reuse of wastewater, more treatment is needed to reach the Palestinian standards for direct reuse in agriculture.

Some projects adopted by the Palestinian Water Authority were started with the reuse of treated wastewater obtained from the Gaza Wastewater Treatment Plant (GWWTP), which is the case study in this paper. An amount of about 10,000 m^3 is diverted to infiltration basins of an area of four hectares every day (PWA 2004). The crops grown in this area are mostly citrus and olives. The water wells that recover the reclaimed wastewater mixed with native groundwater were monitored for groundwater level fluctuations and chemical analyses of their pumped water.

The quality of the native groundwater in the zone of the pilot project showed high values of nitrate ranging from 39 to 177 mg/l with an average of 118 mg/l as shown in Table 1. The high value of nitrate concentration comes from intensive application of chemical fertilizers in the agricultural activities in the area. The salinity of the native groundwater is expressed in the form of chloride ion ranging from 217 to 607 mg/l so this part of the aquifer is relatively good compared to other regions in the Gaza Strip. Any application of treated wastewater to the aquifer through artificial recharge should be recovered from well-designed recovery wells in addition to continuous monitoring of the groundwater to predict any pollution that may occur. At the same time, the project is at least two kilometres away from public water supply wells that are used for drinking purposes.

Table 1 | Quality of native groundwater in the zone of the pilot project

Well No.	Sampling	EC (μS/cm)	Cl^- (mg/l)	NO_3 (mg/l)
R/135	25 July 1999	1,465	239	162
R/141	31 March 1999	2,568	607	177
R/255	12 July 2000	1,412	217	144
R/112	30 October 2000	1,680	322	67
R/254	30 October 2000	2,038	392	39
Average		1,833	355	118

According to the Palestinian strategy, a minimal amount of wastewater will be used for agricultural purposes such as soil flushing and irrigation of high-value crops. It is planned that wastewater reuse will be 34 mm^3 in 2010, increasing to 63 mm^3 in 2020 (PWA 2000a). Part of the reused amount will be diverted directly to the farms, and the rest will be recharged artificially through infiltration basins and other schemes to undergo soil aquifer treatment (SAT) processes that purify the effluent. From previous studies on the biological impact on groundwater, it was determined that SAT was efficient in removing faecal coliforms and faecal streptococci, and removed 85% of total BOD and COD applied in the effluent (Abushbak & Al Banna 2005).

Conventional water resources

The Gaza Strip depends mainly on conventional water resources coming from natural infiltration of rainfall that feeds the Pleistocene sandstone aquifer. Average annual rainfall fluctuates from 200 mm in south Gaza to 400 mm in the north, giving a bulk amount of water of about 115 mm^3, from which only 42 mm^3 infiltrate to the aquifer and the rest either evaporates or floods and runs off to the sea. The total supply was 120 mm^3/year, and the total demand was 165 mm^3, which led to a total deficit of about 45 mm^3 (CAMP 2000) and this deficit increases with time. The population in the Gaza Strip was estimated at 1,443,814 in 2006 leading to a total domestic demand of 79 mm^3 and the total agricultural consumption of 85.5, giving a total water demand in the Gaza Strip of 165 mm^3 (PWA 2007). Therefore, there is an annual deficit in the water budget of about 50 mm^3.

Non-conventional water resources

Due to the increasing demand and fixed supply of the groundwater system in Gaza, it became urgent to allocate new non-conventional water resources in order to fill the gap in the water budget. The potential resources that could

be used are seawater desalination, wastewater reuse and storm water harvesting. According to a Coastal Aquifer Management Program (CAMP) study in 2000, it was planned that the amounts of treated wastewater that will be reused in 2020 will reach about 60 mm^3 every year and another 55 mm^3 will come from seawater desalination.

Some wastewater reuse projects are seen in the PWA area, in the north, middle and south of the Gaza Strip. In North Gaza the effluent is already flooded to the surrounding are of the wastewater treatment plant without control. According to the Sogreah (2001) feasibility study, it was found that the most feasible solution of this flooding effluent is to use controlled infiltration basins if compared with other solutions such as pumping the effluent to the sea or to the future treatment plant in the east of Northern governorate. The Swedish financed study proposed an area of 3,600 ha to be irrigated with treated wastewater (World Bank 2004a). In Rafah City, in the south of the Gaza Strip, the existing wastewater treatment plant is efficient and needs upgrading. However 10 ha close to the plant are proposed if the effluent is improved and reached WHO guidelines for irrigation (World Bank 2004a). The local people showed acceptance to use reclaimed wastewater. About 60% of the local people in the Gaza Strip are highly willing to use treated reclaimed wastewater for irrigation use, and about 22% are highly willing to use the reclaimed wastewater for domestic uses such as toilet flushing, washing, etc. (World Bank 2004b).

METHODOLOGY

A review of the strategic plans of the wastewater reuse were carried out and interpreted. Part of the treated effluent (10,000 m^3) from the existing wastewater treatment plant in Gaza was diverted to three spread infiltration basins with a total base area of 3.7 hectares (ha) distributed to three ponds: pond 1 with an area of 1.1 ha, pond 2 of 1.3 ha and pond 3 of 1.3 ha (CAMP 2001a) as shown in Figure 1. The three ponds were undergoing one day wetting and two days drying periods. The impact on groundwater levels and chemical quality was evaluated based on previous monitoring of the surrounding groundwater wells in different directions, where the water samples were analysed in the laboratory of the Palestinian Ministry of Agriculture according to the American Standard Method Manual. The samples were analysed for boron, Cl, NO_3, detergents and other ions, of which Cl, NO_3 and boron are interpreted in this paper. Due to different political, financial and social constraints, it was not possible to drill monitoring wells beside the infiltration basins. However, the existing operating wells were sufficient at this stage, and water samples were taken from them.

The infiltration areas located east of the current Gaza Waste Water Treatment Plant (GWWTP) is considered here. This project started in 2000 with the help of USAID through the CAMP project. The treatment plant receives about 40,000 m^3 every day and all of the effluent was pumped to the sea before the construction of the infiltration facilities. In 2000, about 10,000 m^3 were pumped to the infiltration basins.

RESULTS AND DISCUSSION

The infiltration basins are set on an area with groundwater of medium quality between fresh and brackish, where chloride concentration in the area fluctuates between 250 and 500 mg/l and nitrate concentration fluctuates between 50 and 200 mg/l (PWA 2008). The quality of the treated effluent was monitored in the period from January 2002 to November 2004. This showed a range of chloride level between 400 and 600 mg/l, which is slightly greater than that in native groundwater. However, the nitrate level in the treated effluent ranged between 20 and 30 mg/l in the same period, and this will dilute the nitrate concentration in the native groundwater. The Palestinian standards of effluent recharge are set at 600 for chloride and 20 mg/l for nitrate (KfW 2005). The reclaimed wastewater was planned to be pumped from six recovery wells, and the effect of the infiltration process was to be monitored in ten surrounding wells (CAMP 2001b). Due to local political conditions, the monitoring wells were not constructed and the monitoring itself was done in the existing operating wells owned by the farmers.

Impact of infiltration on groundwater levels

The ground elevation at the zone of the pilot projects ranges from 30 to 40 m above sea level, where two clayey

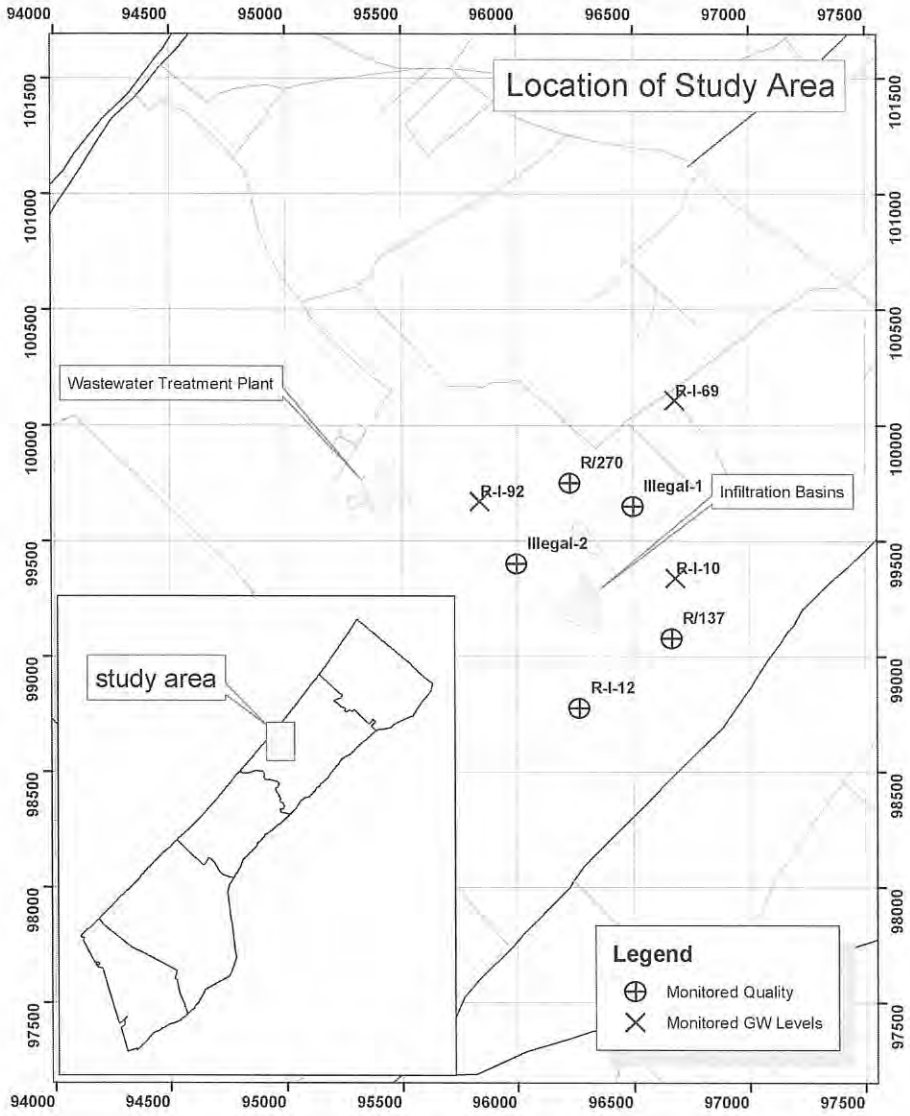

Figure 1 | Location map of infiltration area and monitored wells.

sand layers alternating with sand and sandstone are found at depths of 20 to 30 m below the ground surface (PWA 2001). For recovery well (R/137) ground surface is 40 m and the total depth of the well is 47.62 m and groundwater elevation of 4.0 m a.m.s.l. (PWA 2000b). According to the regional monitoring of groundwater levels, groundwater flows from the east to the sea in the west. However, due to the water mound created by the artificial recharge of wastewater in the study zone, the direction of groundwater flow becomes radial outward from the infiltration basins.

The area is surrounded by irrigation wells and monitoring the water levels in these gives an approximate indication of the influence of infiltration on the groundwater levels. Three operating water wells were monitored after the application of treated wastewater infiltration in the allocated basins. In well R-I-10, which is about 500 m east from the infiltration basin, there was an increase in water level of about 0.6 m by the end of 2003, almost constant during the whole period of infiltration since 2000 (Figure 2). The other monitored wells, which are R-I-69 (1,500 m north-east from the basins) and R-I-92 (1,000 m north-west

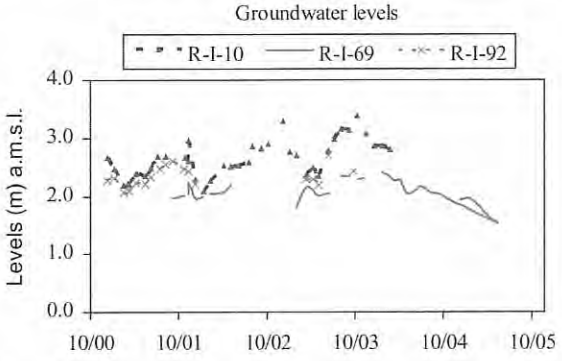

Figure 2 | Groundwater levels in wells around infiltration basins.

from the basins), showed slight decreases in the groundwater levels. No doubt that there was input to the groundwater system from the application of infiltration but the continuous abstraction through irrigation wells in the area hides the positive influence on the groundwater levels.

Impact of infiltration on groundwater quality

Five operating water wells in addition to the effluent recharge basins were selected to study the influence of effluent infiltration on the native groundwater quality. There was a clear increase in the chloride ion concentration in the monitored wells since the concentration level in the effluent is more than that of the native groundwater (Figure 3). The chloride concentrations in the study area range from 200 to 700 mg/l, depending on the layer from which water is pumped. Most of the water supplied through the municipal pipe networks has a chloride level of over 500 mg/l. Consequently, the sewage has naturally almost the same chloride level as this is not affected by the treatment processes in the wastewater treatment plant.

From the chemical analyses of effluent, the chloride level was found to be in the range from 400 to 600 mg/l. According to (Icekson-Tal & Blanc 1998), chloride in applied effluent in the Dan Region was 289 mg/l and after SAT processes it was observed to be 266 mg/l. In Gaza, effluent infiltration has negatively affected the salinity (chloride level) in the native groundwater in the area since chloride came from the high concentration effluent and without removal through SAT. This is considered as a threat to artificial recharge using effluent.

Figure 4 shows that the nitrate level in the effluent is much less than the nitrate level in all of the surrounding monitored wells. A slight decrease in nitrate concentrations was observed in all monitored wells, especially in well R-137, which is the closest to the infiltration basins (about 300 m east of the infiltration basins). In this case the infiltration has improved the quality of the groundwater in terms of nitrate level, from which most of the water wells in the Gaza Strip suffer. In the Dan Region case, the same conclusion was reached where the total nitrogen in the applied effluent decreased from 10.8 to 3.19 mg/l through SAT processes, i.e., removal was 70.5% (Icekson-Tal & Blanc 1998).

From an agricultural aspect, even though boron is an essential micronutrient for plants it may cause toxicity to sensitive crops when concentrations in irrigation water exceed 0.5 mg/l (FAO 2000). Boron concentrations monitored in the infiltrated effluent for 2002 until 2005 fluctuate from about 0.4 to 1.0 mg/l. This has negatively affected water quality in the neighbouring wells, most

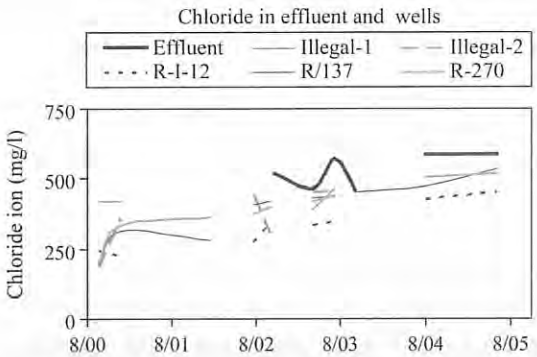

Figure 3 | Chloride levels in effluent and surrounding wells.

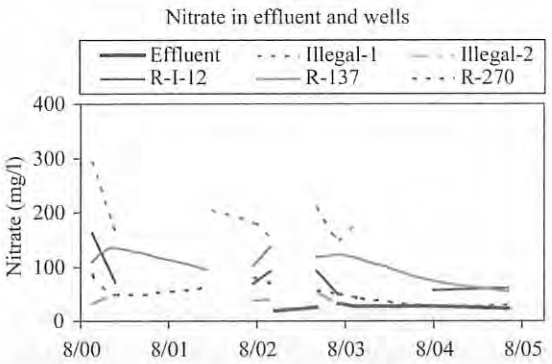

Figure 4 | Nitrate levels in effluent and surrounding wells.

obviously in the well closest to the basins. The boron concentration was 0.234 mg/l in January 2002 and increased to 0.61 mg/l in June 2005 (Figure 5). In other wells, there was a clear increase in boron concentrations. In well R-270, boron increased from 0.232 mg/l in January 2002 to 0.482 mg/l in July 2003 and then decreased to 0.24 mg/l in June 2005. In well R-I-12, boron increased from 0.29 mg/l in January 2001 to 0.635 mg/l in April 2003 and decreased to 0.2 mg/l in June 2005. The latter well results indicate clear influence on native groundwater on boron as SAT is not efficient in removing boron from the infiltrated water. The analyses of more chemical parameters carried out in June 2005 of the effluent and water from surrounding water wells are shown in Table 2 according to PWA (2008).

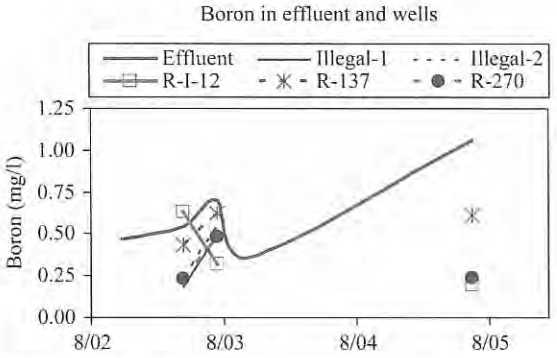

Figure 5 | Boron levels in effluent and surrounding wells.

In a similar area in the Dan Region, boron was removed during percolation in the early stage of the project. However, after several months, boron increased gradually in the recovery wells until it reached the same concentration as the effluent (Idelovitch & Michail 1985; Icekson-Tal & Blanc 1998), and SAT efficiency decreased. Boron removal was minimal (1.8%) as its concentration was 0.54 mg/l in the applied effluent and was observed to be 0.54 mg/l in the groundwater (Icekson-Tal & Blanc 1998).

Locally, in the Gaza Strip, boron compounds are reduced under high pH depending on the process; pH precipitation is likely indicated and advisable. There are some ion exchange compounds that can achieve the desired level, again subject to objectives. For example, a zeolite process with caustic soda may raise the pH to 9.5 or 10. This may precipitate elemental boron by 60%.

Socioeconomic impact

From the economic point of view, a cost estimation was carried out by SWECO (2003) for the infiltration system on infiltration of treated wastewater of the North Gaza governorate. The expected wastewater production for 2012 is 35,600 m^3 every day and it needs 8 ha for infiltration basins. The initial investment cost was 4.58 M USD including infiltration basins and construction of recovery wells

Table 2 | Water quality of effluent and water wells surrounding the infiltration basins

			Water from recovery wells							
Parameter	**Unit**	**Effluent water**	**R-270**	**R-137**	**R-I-54**	**R-I-69**	**R-I-92**	**R-139**	**R-I-10**	**R-I-12**
pH		8.0	7.6	7.2	7.0	7.3	7.8	8.6	7.4	7.4
TDS	mg/l	2,173	1,720	1,860	1,773	1,085	937	664	1,360	1,560
NO_3^{-1}	mg/l	23	30	55	483	265	63	33	101	60
Cl^{-1}	mg/l	587	516	535	376	197	269	120	384	454
B	mg/l	1.1	0.2	0.6	0.2	0.0	0.1	0.2	0.2	0.2
Deterg.	mg/l	0.9	0.1	0.2	0.0	0.0	0.0	0.0	0.1	0.1
Ca^{2+}	mg/l	109	96	104	125	119	55	29	86	80
Mg^{2+}	mg/l	57	79	79	90	68	51	17	73	101
K^+	mg/l	37.5	4.5	8.0	2.3	2.5	3.0	2.0	3.8	6.0
Na^+	mg/l	406	311	350	298	109	163	157	227	250
TOC	mg/l	11.9	2.0	2.7	1.8	0.2	0.4	0.4	2.4	1.9
BOD	mg/l	45.0	5.0	7.0	2.2	4.6	3.2	3.5	1.3	2.3
COD	mg/l	135	10.0	11.0	10.0	0.0	10.0	5.0	10.0	0.0

and pipes, while the operational cost was 0.14 M USD per year. Assuming that the investment system will operate for 20 years to infiltrate 35,600 m^3 per day i.e., 12.3 mm^3 per year, this will give an initial investment cost of 0.019 USD for each cubic metre infiltrated. The operational and maintenance costs for each cubic metre will be 0.018 USD. The total cost for each cubic metre is 0.037 USD for every cubic meter, which has been also reached by (Nassar *et al.* 2009), where 0.04 USD per cubic metre was estimated for operational costs. This cost is acceptable for Gaza, which is considered as a scarce water region. At the same time, the farmers pay 0.5 USD for each cubic metre pumped for irrigating their crops (PWA 2006). According to the survey conducted in a later study (PWA 2006), the farmers showed interest and willingness to pay 0.14 USD for each cubic meter of reclaimed effluent, where 68% of farmers in north Gaza and 91% of farmers in south Gaza are willing to use reclaimed wastewater either as direct reuse or from recovery wells (Nassar *et al.* 2009).

To convince the users, the reclaimed wastewater should be treated through the SAT in addition to preventing technical problems occurring in the distribution system and establishing an appropriate institutional framework to operate the system. The quality levels of reclaimed wastewater for irrigation should be managed well in terms of suspended solids to avoid blockage of the irrigation system, nutrients to adjust fertilization, salinity to estimate soil leaching requirement and control of pathogens to protect public health. The infiltration system itself needs to be properly assessed environmentally to prevent hazards to the neighbouring residents. From the other side, the public should be aware of the advantages of the new water sources, together with economic incentives to reclaim wastewater with a lower price than well water.

The current wastewater production is 32.7 mm^3 per year for the partial coverage of wastewater networks and it is estimated at 51.6 mm^3 if full coverage of wastewater services is achieved, as shown in Table 3. With a population growth rate of 3.5%, the total wastewater production will increase to 80 mm^3 per year by 2020. According to the National Water Plan (PWA 2000a), this will provide an input to water resources of about 60 mm^3 per annum by 2020.

Table 3 | Wastewater production in Gaza governorates[a]

Governorate	Population (capita)	Coverage percent (%)	Wastewater production (m^3/day)	Production with full coverage (m^3/day)
North Area	298,125	68.51	16,341	23,851
Gaza	546,959	79	48,243	61,067
Middle area	223,679	64	11,420	17,843
Khanyounis	299,918	20.60	4,942	23,988
Rafah	183,649	59.79	8,784	14,691
			89,730	141,440

Total production = 32.7 million m^3/year and 51.6 million m^3/year for full coverage, and based on average per capita of 80 l/day.
[a]CMWU (2007).

CONCLUSION AND RECOMMENDATIONS

Like other scarce water countries in the region, there is an urgent need to look for new non-conventional water resources such as reuse of reclaimed wastewater. The policy of the Palestinian Water Authority is to reduce the amount of fresh water to be used for irrigation (83 mm^3/year) by replacement with reclaimed wastewater after ensuring sufficient treatment. This new water resource will play an important role together with other resources, e.g., seawater desalination and harvesting of storm water, in the sustainability of the water resources in the Gaza Strip. Potentially, about 63 mm^3 of treated wastewater (22% of total water demand) could be available for reuse by 2020 (CAMP 2000).

Although the quantity of effluent infiltrated to the aquifer is currently small compared to the strategic planned amounts, it has had a slight positive impact on improving the continuous declined water table, which rose 0.6 m. A positive decrease in the nitrate concentrations in the recipient aquifer was observed. However, the trend of boron concentrations is a concern as concentrations in the aquifer exceed the WHO recommended value of 0.5 mg/l.

Chloride concentration in the public water supply is high in most of the areas in the Gaza Strip, and consequently the chloride level will be high in wastewater and treated effluent since this is not removed by wastewater

treatment. Consequently, recharged effluent had negative impact on the chloride concentrations in the aquifer and is a challenge for artificial recharge of groundwater under the local conditions. It is recommended to reduce the salinity of the public water supply to reduce the level of chloride in the treated wastewater so that effluent becomes suitable for infiltration.

Previous studies have shown that infiltration of effluent through soil layers removed microorganisms and a large part of organic matter. In areas with a high boron level in effluent, it is recommended to use conventional treatment technologies (metal hydroxide precipitation) to reduce the boron level. Reverse osmosis (RO) is another recommended technology for boron reduction.

From the economic aspect, reuse through infiltration of effluent is feasible. The total cost of infiltrated effluent is 0.035 USD per cubic metre. However, more efforts are still needed on the socioeconomic and technical aspects. On the technical dimension, the applied effluent should be treated well in the treatment plant so that its constituents do not exceed the standards adopted by the Palestinian Water Authority based on WHO standards, in addition to the well-control on the management of infiltration spread basins. On the socioeconomic dimension, the public should be prepared to accept the idea of replacing their well water with distributed reclaimed wastewater for irrigation, and they should be economically encouraged through the pricing of the received water.

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Decoloration of methylene blue simulated wastewater using a UV-H_2O_2 combined system

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ABSTRACT

Methylene blue simulated wastewater was treated with a UV-H_2O_2 combined system. Influences of factors such as reaction time, initial pH value and H_2O_2 dosage were investigated, and the reaction kinetics of the process was explored. Results showed that the degradation of methylene blue happened only in the presence of both conditions: UV irradiation and H_2O_2 addition. Initial pH and H_2O_2 dosage had a remarkable influence on the degradation efficiency. Through several groups of univariate experiments, the optimum pH and H_2O_2 dosage of the photolysis process were found to be 4–5 and 0.165 mL 30% H_2O_2 per milligram of methylene blue, respectively. The photolysis process was relatively fast at the initial stage and, 20 min later, it was approximately in accordance with the first-order kinetic equation.

Key words | advanced oxidation process, kinetics, photolysis, reactive dye

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INTRODUCTION

With the rapid development of the printing and dyeing industry in China, its effluents have become one of the most important water pollution sources. The printing and dyeing effluents are very complex, usually containing some remaining dyes, some sizing agents, some surfactants and some accessory ingredients amongst others. Besides, many dyes are very toxic and can cause serious damage to human beings even at very low concentration. China is a major manufacturing country of dyes; dye production accounts for almost 60% of the total output of the world. In summary, it is very important and very urgent for China to explore effective wastewater treatments for printing and dyeing effluents (Dai *et al.* 2000; Liu 2007; Du & Ma 2009)

Traditional wastewater treatments for printing and dyeing effluents are mainly physical, chemical and biochemical methods (Wei *et al.* 2002; Wu *et al.* 2007; Sun & Huang 2009). Yet, considering the large quantities of benzene rings, naphthalene nuclei, amino groups and azo groups, among others, which are very common in dye structures, it is very difficult to dispose of them efficiently and completely. Moreover, with the rapid development of the textile industry in recent years, more and more new types of dye have been produced. Compared with traditional dyes, the newly developed ones are usually more resistant to photolysis, oxidation and biodegradation. This tremendously raises the level of difficulty in disposing of the dyeing wastewater. Traditional disposal methods are facing serious challenge, and ideal treatment efficiency is hard to achieve (Ali *et al.* 2005; Laila *et al.* 2007; Zhao *et al.* 2008; Zheng *et al.* 2009).

The advanced oxidation process (AOP) is a new type of chemical oxidation technology, which has been proven to be very efficient in treating the anti-degradation organic wastewater. Through the forming of strong oxidant ·OH free radical, AOP can break the unsaturated double bonds in the chromophoric group of dye molecules, and decompose them into low-molecular-mass organics or inorganics, thus the coloration of the wastewater is removed (Mehmet & Ibrahim 2007; Shaoqin *et al.* 2008; Chu *et al.* 2009). Because of the prominent advantages such as simple equipment, low cost, strong oxidation capacity, and complete disposal without secondary pollution, the application of AOP in the organic wastewater treatment field is very promising. In recent years, AOPs such as Fenton oxidation, catalytic wet

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oxidation, and semi-conductor catalysed photolysis have been popularly used in all kinds of industrial wastewater. The UV-H_2O_2 combined system is a new and promising type of AOP, the possible mechanism of which includes two aspects: on the one hand, H_2O_2 molecules absorb the energy of light quanta and are decomposed into much stronger oxidant $^{\cdot}OH$ free radicals, which would act as the leading role in oxidizing the pollutants; on the other hand, the unsaturated chemical groups in dye molecules can also absorb the energy of light quanta and become more active in the subsequent oxidation processes. On the basis of the above, the oxidation process of the UV-H_2O_2 combined system is very fast, efficient and clean. Taking into consideration the low price and the extensive sources of UV-H_2O_2 reagents, there is no doubt it would attract more and more attention in the wastewater treatment field (Xu *et al.* 2007; Guo *et al.* 2009; Mark *et al.* 2009).

In this study, methylene blue simulated wastewater was treated with the UV-H_2O_2 combined system. Influences of factors such as reaction time, initial pH and H_2O_2 dosage were investigated, and the reaction kinetics were explored.

EXPERIMENTS

Experimental object

Methylene blue, a cationic dye, is usually used in the dyeing of paper, linen and silk textiles, or in the painting of bamboo, wood and so on. It can also be used in the manufacturing of ink, lake colour, or in the staining of organisms and bacteria tissues, among other purposes.

The structure of methylene blue is relatively stable compared with other dyes, so the traditional treatment methods used for dyeing wastewater cannot degrade it effectively, which made it our experimental object.

The molecular and structural formulas of methylene blue are shown in Table 1.

Instruments and reagents

The main experimental instruments and reagents used in this study are exhibited in Tables 2 and 3, respectively.

Experimental equipment

The structure of the multifunctional photochemical reactor is shown in Figure 1.

The light source is a medium pressure mercury lamp of 300 W, whose spectrum distribution is shown in Figure 2, with dominant wavelength 365 nm. The mercury lamp is placed in a quartz sleeve, through which cooling water is circulated to remove excess heat emitted by the mercury lamp and keep the reaction at room temperature. The quartz sleeve is provided with an exterior ground joint, so it can be fitted tightly into the hardened glass reactor which has an interior ground joint and in which the reacting solution is loaded. Thus the reacting solutions can be directly irradiated

Table 1 | Molecular and structural formulae of methylene blue

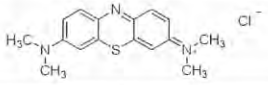
Common name	Structural formula	Molecular formula	Relative molecular mass	λ_{max} (nm)
Methylene blue		$C_{16}H_{18}ClN_3S{\cdot}3H_2O$	373.9	664

Table 2 | Main experimental instruments

Name of instruments	Type	Manufacturer
Multifunctional photochemical reactor	SGY-I	Nanjing Stoke Electrical Equipment Co., Ltd
Dual-beam UV–VIS spectrophotometer	TU-1901	Beijing Purkinje General Instrument Co., Ltd
Digital pH meter	pHS-3C	Shanghai Precisions & Scientific Instrument Co., Ltd
Electronic analytical balance	ALC310.3	German Sartorius Mechatronics

Table 3 | Main experimental reagents

Name of reagents	Purity grade	Manufacturer
Methylene blue	A.R	Tianjin Guangfu Institute of Fine Chemicals
NaOH	A.R	Beijing Chemical Reagent Factory
HCl	A.R	Beijing Chemical Reagent Factory
30% H_2O_2	A.R	Tianjin Chemical Reagent Factory

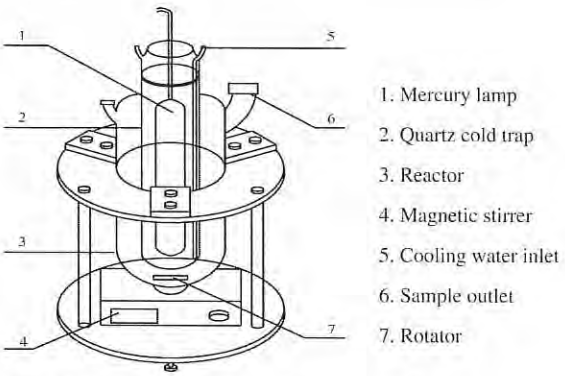


Figure 1 | SGY-I type of multifunctional photochemical reactor.

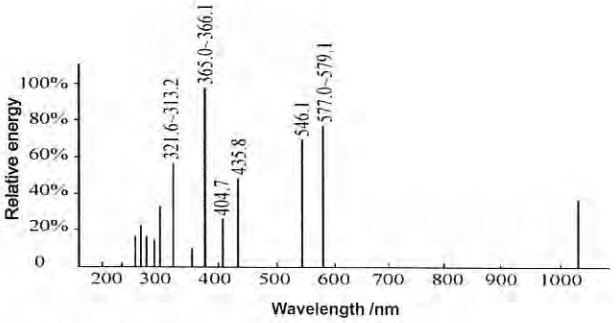


Figure 2 | Spectrum distribution of mercury lamp.

by the mercury lamp, as quartz does not block any part of the lamp's emission spectrum. The effective volume of the hard glass reactor is 500 mL, a sample outlet is placed in its upper part, and a magnetic stirring instrument is placed in the bottom to keep the reaction solutions uniform.

Experimental methods

Some methylene blue solutions with different concentrations were prepared and adjusted to a certain pH value using HCl or NaOH solutions. After the addition of a certain volume of H_2O_2, the mixed solution was poured into the multifunctional photochemical reactor immediately. First, cooling water circulation and magnetic stirring were started, then the UV lamp was switched on after stabilizing the temperature and the stirring rate; time counting began simultaneously. Small amounts of the reacting solution were withdrawn from the system every 10 min. The samples were used for absorbance measurement at the characteristic absorption wavelength of methylene blue, 664 nm. Decoloring ratio of the dye solution could be calculated using the absorbance before and after the reaction, which was used to represent the disposal efficiency in this study.

RESULTS AND DISCUSSION

Exploration of the decoloration conditions

To verify the synergetic effect between UV and H_2O_2, three sets of experiments were carried out. The first one was the experiment group, using the UV-H_2O_2 combined system to dispose of the methylene blue wastewater. The other two sets were, respectively, control group 1 using only H_2O_2 without UV irradiation, and control group 2 using only UV irradiation without H_2O_2. Other reaction conditions such as temperature and stirring rate were all the same for the three sets of experiments. Decoloring ratio of the methylene solution for the three sets of experiments are exhibited in Figure 3.

Figure 3 reveals that there was no degradation of methylene blue when it was only under UV irradiation with no H_2O_2 addition. When there was only H_2O_2 without UV, very little of the methylene blue was degraded. Yet under

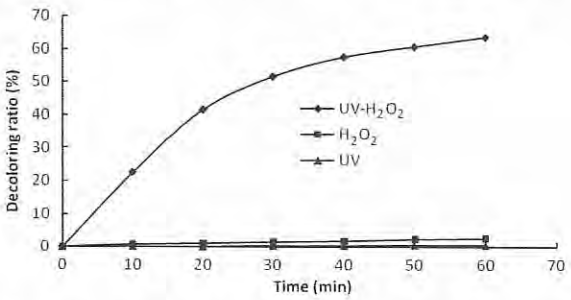


Figure 3 | Comparison of the disposal effect under different reaction conditions.

conditions of both UV irradiation and H_2O_2 addition, almost 70% of methylene blue was degraded within 60 min.

The presumed cause of this might be that, on the one hand, H_2O_2 molecules absorbed the energy of light quanta and decomposed into much stronger oxidant ·OH free radicals, which were the main oxidative species in the system; on the other hand, the unsaturated chemical groups in dye molecules also absorbed the energy of light quanta and became more active in the subsequent oxidation processes.

Summing up, methylene blue could be effectively degraded only in the presence of both conditions: UV irradiation and H_2O_2 addition. The following experiments were all carried out under these two conditions.

Influence of reaction time

A 300 mL methylene blue solution with the concentration of 30 mg/L was prepared. After being added into 3 mL 30% H_2O_2, the solution was poured into the multifunctional photochemical reactor. Experimental steps were as above, and the sample drawing interval was set to be 10 min. The relationship between decoloring ratio and reaction time is shown in Figure 4.

It is clear in Figure 4 that the decoloring ratio increased very rapidly during the initial stage of the reaction and, after 20 min, it began to slow down. The ultimate decoloring ratio was beyond 80% during the investigated reaction time of 150 min. To save time and ensure rapid progress in the investigation, the reaction time was chosen to be 30 min in all the following experiments in this study.

Influence of initial pH

Several groups of 30 mg/L methylene blue solutions with a volume of 100 mL were prepared and adjusted to different final pH values from 2 to 13 using different amounts of HCl or NaOH solutions. After adding of 1 mL 30% H_2O_2, the experimental solutions were poured into the photochemical reactor in sequence. All the experiments were carried out under the same reaction conditions, which were: UV light source of 300 W, reaction temperature of 20 °C, 30% H_2O_2 dosage of 1 mL/100 mL, reaction time of 30 min. The experimental results are shown in Figure 5.

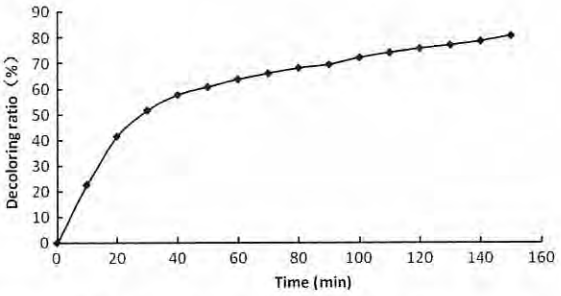


Figure 4 | Dependence of decoloring ratio on reaction time.

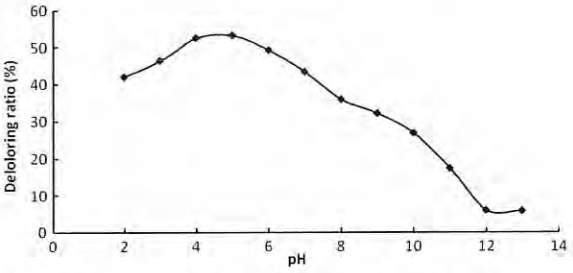


Figure 5 | Dependence of decoloring ratio on initial pH values of the wastewater.

Figure 5 suggests that, when the initial pH value of the wastewater was around 4–5, maximum decoloring ratio was achieved. A strong acid or alkaline environment was unfavourable for the degradation of methylene blue. This might be because a strong acid environment inhibits the process of ·OH forming from H_2O_2, and a strong alkaline environment induces the catalytic destruction of ·OH radicals by carbonate anions, which would both result in a decrease of the removal ratio (Peyton 1996).

Influence of H_2O_2 dosage

Several groups of 30 mg/L methylene blue solutions with the volume of 100 mL were prepared and adjusted to pH 4. After being added into different volumes of 30% H_2O_2, which was respectively 0 mL, 0.1 mL, 0.2 mL, 0.3 mL, 0.5 mL, 0.8 mL, 1.0 mL, 1.5 mL, 1.8 mL and 2.0 mL, the solutions were degraded by UV-H_2O_2 combined system in sequence. The other reaction conditions were all the same, UV light source of 300 W, reaction temperature of 20 °C, reaction time of 30 min. The experimental results are shown in Figure 6.

Figure 6 reveals that, when the dosage of 30% H_2O_2 was below 0.5 mL/100 mL, decoloring ratio of the dye solution

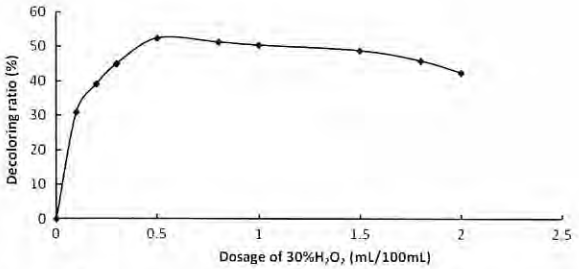


Figure 6 | Dependence of decoloring ratio on H_2O_2 dosage.

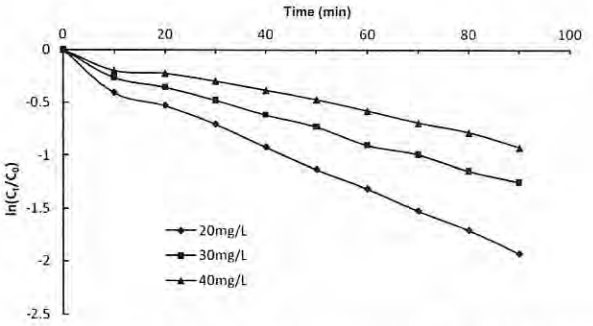


Figure 7 | $\ln(C_t/C_0) \sim t$ curves for different concentrations of methylene blue solution.

increased rapidly, yet when it exceeded 0.5 mL/100 mL, decoloring ratio began to decrease slowly with the H_2O_2 dosage. The presumed cause was that, when the dosage of H_2O_2 was excessive, the H_2O_2 molecule began to compete strongly in absorbing the light quantum energy, and the average distribution of the light energy led to the result that many H_2O_2 molecules could not absorb enough energy to induce their energy level transition, which would certainly reduce the yield of ·OH free radical, and in turn decrease the decoloring ratio of the methylene blue solutions.

The above experiments were repeated using 20 and 40 mg/L methylene blue solutions, and results showed that the optimum dosage of 30% H_2O_2 was 0.3 mL/100 mL and 0.7 mL/100 mL, respectively.

In summary, under the experimental conditions of this study (300 W mercury lamp, 20 °C), when using the UV-H_2O_2 combined system to treat methylene blue wastewater, the optimum dosage of 30% H_2O_2 was approximately 0.165 mL per milligram of methylene blue.

Photolysis kinetics of methylene blue

Three groups of methylene blue solutions, with concentrations of 20, 30 and 40 mg/L, respectively, were prepared and adjusted to pH 4. Then appropriate amounts of H_2O_2, calculated according to the above-mentioned optimum dosage, were added. The three experimental samples were put into the photochemical reactor one by one to be degraded under the same conditions (300 W mercury lamp, 20 °C) and a small amount of sample was withdrawn every 10 min. Absorbances of the samples at 664 nm were measured, and the remaining concentrations of methylene blue after different reaction times were calculated. The total time period investigated was 90 min. The $\ln(C_t/C_0) \sim t$ curves for the three experimental groups are all given in Figure 7.

It is clear in Figure 7 that, for the three methylene blue solutions with different concentrations, the $\ln(C_t/C_0) \sim t$ curves were all very steep at the beginning, and then the slopes tended to decrease with time. Yet after 20 min, the slopes of $\ln(C_t/C_0) \sim t$ curves remained almost unchanged, and the three curves could all be very well regressed to a straight line. The regression results are shown in Table 4.

According to the chemical reaction kinetics theory, for a reaction that is in accordance with the first-order kinetic equation, there must be:

$$-\frac{dC}{dt} = kC \tag{1}$$

The integral calculation of the above equation can lead to:

$$\ln\left(\frac{C_t}{C_0}\right) = -kt \tag{2}$$

where C_0 refers to the initial concentration of the reactant; C_t was the remaining concentration of the reactant at time t, and k was rate constant.

Table 4 | Linear regression results for 20–90 min in $\ln(C_t/C_0) \sim t$ curves

Initial concentration (mg/L)	Regression equations	R^2
20	$y = -0.0104x + 0.0338$	0.995
30	$y = -0.013x - 0.0918$	0.9965
40	$y = -0.02x - 0.113$	0.9994

According to the above analysis, for the reaction that is in accordance with the first-order kinetic equation, $\ln(C_t/C_0) \sim t$ curve should be a straight line, and vice versa.

Summing up the above, the degradation process of methylene blue by UV-H_2O_2 was in accordance with the first-order kinetic equation after 20 min.

At the beginning of the reaction, methylene blue was in high concentration, so the controlling factor of the oxidation reaction was the concentration of ·OH. Besides, during the initial stage of the reaction, light energy was adequately absorbed by large quantities of H_2O_2 and ·OH radical groups were produced rapidly. Thus, the oxidation reaction proceeded very quickly at the beginning.

According to the effective collision theory, chemical reaction happens only when the reacting particles collide with each other and the chemical bond is broken. If there is no collision, particles only jump from here to there in the system without hurting one another. In this study, as the reaction proceeds, the concentration of methylene blue decreased gradually, so the collision frequency of methylene blue molecules with ·OH free radicals began to depend on the concentration of methylene blue. That is, the rate of the oxidation process began to obey the first-order kinetic equation during the later period of the reaction.

CONCLUSIONS

Methylene blue wastewater could be effectively degraded only in the presence of both conditions: ultraviolet irradiation and H_2O_2 addition. A synergetic effect was produced between UV and H_2O_2, which was the key factor to oxidize the dye and decolorize it.

The optimum initial pH value of methylene blue wastewater treated by the UV-H_2O_2 combined system was around 4–5; a strong acid or alkaline environment was not beneficial to the oxidation reaction.

The optimum dosage of 30% H_2O_2 per milligram of methylene blue was 0.165 mL; lower or higher dosage would both decrease the disposal efficiency.

The degradation rate of methylene blue wastewater by the UV-H_2O_2 combined system was relatively rapid in the initial 20 min, then it began to obey the first-order kinetic equation.

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Application of immobilized peroxidase for the removal of *p*-bromophenol from polluted water in batch and continuous processes

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ABSTRACT

Concanavalin A layered calcium alginate-cellulose beads adsorbed and cross-linked peroxidase of *Momordica charantia* was employed for the treatment of *p*-bromophenol polluted water. Immobilized peroxidase showed remarkably higher storage stability and retained about 78% phenolic compound removal efficiency over a period of one-month's storage at 4 °C. After a fourth repeated use immobilized enzyme retained nearly 50% *p*-bromophenol removal efficiency. *p*-Bromophenol removal by immobilized enzyme was ~84% in the presence of 0.1 mM $HgCl_2$. A significantly higher concentration of *p*-bromophenol was removed by immobilized enzyme in the presence of water-miscible organic solvents as compared to free enzyme. In stirred batch processes nearly 91%, 94% and 83% of *p*-bromophenol was removed in 3 h at 30, 40 and 50 °C, respectively. Immobilized enzyme present in two different reactors and operated at flow rates of 10 and 20 ml h^{-1} retained 75 and 65% *p*-bromophenol removal efficiency even after one month of their continuous operation. Absorption spectra for treated and untreated *p*-bromophenol exhibited a marked difference in absorbance at various wavelengths. Hence, it is concluded that reactors filled with immobilized enzymes can successfully be operated for the treatment of huge volumes of effluent containing various types of aromatic pollutants.

Key words | batch process, *p*-bromophenol, peroxidase, polyethylene glycol, wastewater

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ABBREVIATIONS

BGP	bitter gourd peroxidase
DMSO	dimethylsulfoxide
SI-BGP	surface immobilized bitter gourd peroxidase
PEG	polyethylene glycol
p-BP	*p*-bromophenol
S-BGP	soluble bitter gourd peroxidase

INTRODUCTION

A large number of chemical pollutants is generated by various industries worldwide annually. Such compounds, even if present in very small quantities, can impart negative effects on both nature and human health (Bayramoglu & Arica 2008). Phenols form one of the important constituents of these industries and, henceforth, find their way into the environment through industrial effluents. Most phenolic compounds are not only phytotoxic but also mutagenic, antimicrobial, as well as carcinogenic (Zilly *et al.* 2002; Casa *et al.* 2003). Therefore, it is essential to remove them from industrial wastewater before their final discharge into the surroundings.

Several physicochemical and biological approaches have been employed for the removal of phenolic compounds from industrial effluents (Kumar *et al.* 2004; Lai & Lin 2005; Bodalo *et al.* 2008). However, conventional procedures have faced some serious drawbacks such as formation of hazardous by-products, incomplete purification, prohibitively high purification costs, low efficiency and applicability only to a limited concentration range (Husain 2006; Cohen

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et al. 2009). The focus on the microbial degradation of various phenolic compounds has resulted in the isolation, culture, adaptation and enrichment of a number of microorganisms that can grow on the compound as a sole carbon and energy source (Agarry *et al.* 2008). The biodegradation of phenolic compounds is not limited to the activity of a few microorganisms; it occurs frequently within bacteria, fungi, eukaryotic micro algae and higher plants (Semple & Cain 1997). However, microbial treatment has also exhibited some limitations such as high cost of production of microbial culture, limited mobility and metabolic inhibition (Husain 2010). Degradation of the aromatic compounds by bacteria, fungi and algae being attributed to secondary metabolic inhibition therefore requires appropriate growth conditions, which could be accomplished by additional loads of chemicals. Also the expression of the enzymes involved in the aromatic compound degradation is not constant with time but dependent on the growth phase of the organisms and is influenced by inhibitors that might be present in the effluent (Wesenberg *et al.* 2003). Moreover, algae and fungi take several days to detoxify the compounds present in wastewater (Sumathi & Manju 2000; Pinto *et al.* 2002). Henceforth, the use of peroxidases from plant and microbial sources has attracted the attention of enzymologists owing to their stable nature and relatively easy availability (Magri *et al.* 2005; Husain 2006; Husain *et al.* 2009).

Nevertheless, the use of soluble enzymes is limited due to their reusability, stability, sensitivity to various denaturants and applications in continuous reactors (Gomez *et al.* 2006; Husain & Husain 2008; Ashraf & Husain 2010). Such limitations can be overcome by using immobilized enzymes. Several supports like celite, sephadex and sepharose have been employed for the immobilization of peroxidases (Husain & Husain 2008; Husain *et al.* 2009; Husain 2010). Immobilization on calcium alginate-cellulose beads is straightforward and a large surface area is available for contact with the substrate (Matto & Husain 2009a, b; Ortega *et al.* 2009).

Oxidation of phenols by peroxidases in the presence of H_2O_2 resulted in the formation of free radicals, which might inactivate the enzyme. In this regard, use of various protective additives, such as borate, gelatin and polyethylene glycol (PEG), have been suggested to decrease enzyme inactivation, while being inexpensive, biodegradable and non-toxic (Bodalo *et al.* 2008; Ashraf & Husain 2009; Cohev-Yaniv & Dosoretz 2009). Studies have shown the addition of PEG is an effective procedure for protecting enzymatic activity and increasing aromatic compounds treatment efficiency (Yamada *et al.* 2010).

In this study, an attempt was made to employ bitter gourd peroxidase (BGP) immobilized on the surface of concanavalin A (Con A) layered calcium alginate-cellulose beads for the remediation of water contaminated with *p*-bromophenol (*p*-BP) in the presence of an additive, PEG. The operational stability of soluble and surface immobilized bitter gourd peroxidase (SI-BGP) for the treatment of *p*-BP has been compared in the presence of mercury (II) chloride ($HgCl_2$), *n*-propanol and dimethylsulfoxide (DMSO). *p*-BP removal reusability and storage stability of SI-BGP have been compared with its free form. SI-BGP was also used in stirred batch processes at various temperatures as well as in continuous packed-bed reactors for the removal of *p*-BP.

MATERIALS AND METHODS

Materials

o-Dianisidine-HCl was obtained from the Centre for Biochemical Technology, CSIR, India. Ammonium sulfate, PEG, *p*-BP, DMSO, $CaCl_2$ and cellulose were purchased from SRL Chemicals Pvt. Ltd (Mumbai, India). Glutaraldehyde was obtained from Thomas Baker Chemicals Pvt. Ltd (Mumbai, India). Sodium alginate was the product of Koch-Light Lab (Colnbrook, UK). Jack-bean meal was purchased from DIFCO (Detroit, USA). Bitter gourd was bought from a local vegetable market. All other chemicals and reagents employed in this study were of analytical reagent grade and were used without any further purification.

Ammonium sulfate fractionation of bitter gourd proteins

Bitter gourd (100 g) was homogenized in 200 ml of 0.1 M sodium acetate buffer at pH 5.5. The homogenate was filtered through four layers of cheesecloth. The filtrate was then centrifuged at a speed of $10,000 \times g$ on a Remi C-24 Cooling Centrifuge for 20 min. at 4 °C. The obtained clear supernatant was subjected to salt fractionation by adding 10–90% (w/v)

$(NH_4)_2SO_4$. The mixture was continuously stirred overnight at 4 °C for complete precipitation of proteins. The precipitate was collected by centrifugation at 10,000 × *g* on a Remi C-24 Cooling Centrifuge, redissolved in assay buffer and dialysed against the same buffer in order to remove traces of ammonium sulfate (Fatima & Husain 2008).

Preparation of jack-bean extract and layering of calcium alginate-cellulose beads by Con A present in extract

Jack-bean meal (10 g) was stirred in 100 ml of 0.1 M *tris*-HCl buffer, pH 6.2 overnight on a magnetic stirrer at room temperature. The clear extract obtained after centrifugation at 3,000 × *g* for 10 min. was used as a source of Con A for subsequent investigations (Matto & Husain 2009b).

A solution of sodium alginate (2.5%) and cellulose (2.5%) was prepared in 10 ml of 0.1 M sodium acetate buffer, pH 5.5. The mixture was slowly extruded as droplets through a 5.0 ml syringe with attached needle No. 20 into 0.2 M $CaCl_2$ solution to form calcium alginate-cellulose beads. The beads were further gently stirred for 2 h in $CaCl_2$ solution and washed by 0.1 M sodium acetate buffer, pH 5.5 and stored at 4 °C in the assay buffer for further use. Five hundred beads were incubated overnight with jack-bean extract (10 ml) at room temperature (30 ± 2 °C) and washed using the assay buffer.

Adsorption of BGP on Con A layered calcium alginate-cellulose beads and its cross-linking by glutaraldehyde

BGP (1,000 U) was incubated overnight with Con A layered calcium alginate-cellulose beads. The obtained immobilized enzyme was washed with the assay buffer and cross-linked with 0.1% (v/v) glutaraldehyde for 2 h at 4 °C. Ethanolamine was added to a final concentration of 0.01% (v/v) to stop further cross-linking. The solution was then allowed to stand for 90 min. at room temperature. The cross-linked beads were washed with the assay buffer and stored at 4 °C for further use (Matto & Husain 2009b).

Effect of heavy metal, $HgCl_2$, on *p*-BP oxidation

p-BP (0.4 mM, 5.0 ml) was independently treated by soluble and immobilized peroxidase (0.4 U ml^{-1}) in 0.1 M sodium acetate buffer in the presence of 0.75 mM H_2O_2, 0.1 mg ml^{-1} PEG and $HgCl_2$ (0.1–0.5 mM) for 2 h at 40 °C. The reaction was stopped by heating in boiling water for 5 min. The insoluble product was removed by centrifugation at 3,000 × *g* for 15 min. The oxidative degradation and removal of *p*-BP from polluted water was monitored at a wavelength of 280 nm in the UV. The percentage oxidation was calculated by taking untreated *p*-BP polluted water (containing all the reagents that were present in treated solution except the enzyme) as control (100%).

Effect of organic solvents on *p*-BP oxidation

p-BP (0.4 mM, 5.0 ml) was independently treated by soluble and immobilized BGP (0.4 U ml^{-1}) in the presence of increasing concentrations of water-miscible organic solvents: *n*-propanol/DMSO (2.0–30%, v/v) in 0.1 M sodium acetate buffer in the presence of 0.75 mM H_2O_2 and 0.1 mg ml^{-1} PEG for 2 h at 40 °C. Further reaction was stopped by heating in boiling water for 5 min. The insoluble product was removed by centrifugation at 3,000 × *g* for 15 min. The percentage oxidation was calculated by taking untreated *p*-BP polluted water as control.

Reusability of SI-BGP

p-BP polluted water (0.4 mM, 5.0 ml) was incubated with immobilized enzyme (0.4 U ml^{-1}) in sodium acetate buffer in the presence of 0.75 mM H_2O_2 and 0.1 mg ml^{-1} PEG for 2 h at 40 °C. After the reaction, enzyme was separated by centrifugation and stored in the assay buffer for over 12 h at 4 °C. The experiment was repeated four times with the same preparation of immobilized peroxidase but with an addition of a fresh batch of *p*-BP polluted water. The percentage removal was calculated by taking untreated compound as control (100%).

Storage stability of soluble and immobilized peroxidase

The soluble and immobilized enzyme preparations were stored at 4 °C in a refrigerator. Appropriate aliquots in triplicate of both the enzyme preparations were withdrawn over an interval of 5 days for a period of one month and were assayed for *p*-BP removal efficiency.

Treatment of *p*-BP in stirred batch processes

p-BP (0.4 mM, 100 ml) was treated by soluble and immobilized BGP (30 EU) independently in the presence of 0.75 mM H_2O_2 and 0.1 mg ml^{-1} PEG for 3 h at three different temperatures (30, 40 and 50 °C) in the presence of 0.1 M sodium acetate buffer, pH 5.5, with constant stirring. Aliquots were taken from the reaction mixtures at varying times and the reaction was stopped by heating in boiling water for 5 min. The insoluble product was removed by centrifugation at 3,000 × g for 15 min.

Treatment and removal of *p*-BP through a continuous reactor filled with immobilized enzyme

Two packed-bed reactors (10.0 × 2.0 cm) were filled with immobilized enzyme (500 U) and equilibrated with 0.1 M sodium acetate buffer, pH 5.5. The working volume of both the reactors was 12.6 ml. The *p*-BP polluted water (0.4 mM) containing 0.75 mM H_2O_2 and 0.1 mg ml^{-1} PEG was continuously passed through the reactors working at two different flow rates, 10 and 20 ml h^{-1} at room temperature with residence time of the former (when allowing for the effect of porosity on the working volume) maintained at 0.17 h and the latter at 0.08 h, respectively. Samples from the column outlet were collected at the interval of 5 days and were analysed using UV–visible spectrophotometry at 280 nm.

Determination of peroxidase activity and protein concentration

Peroxidase activity was measured by the change in the optical density at 460 nm and at 37 °C by estimating the initial rate of oxidation of 6.0 mM *o*-dianisidine HCl by 18.0 mM H_2O_2 (Matto & Husain 2009a). Immobilized enzyme was continuously stirred for the entire duration of assay. The assay was highly reproducible with immobilized enzyme.

One unit (1.0 U) of peroxidase activity was defined as the amount of enzyme protein that catalyses the oxidation of 1 μmol of *o*-dianisidine HCl in the presence of H_2O_2 per min at 37 °C into coloured product ($\varepsilon_m = 30{,}000\ M^{-1}\ cm^{-1}$).

The protein concentration was estimated using the procedure of Lowry *et al.* (1951), with bovine serum albumin as standard. One millilitre of a suitably diluted aliquot of protein sample was taken. To this, 5.0 ml of freshly prepared alkaline copper reagent was added. The alkaline copper reagent was prepared by mixing copper sulfate (1%, w/v), sodium potassium tartarate (2%, w/v) and sodium carbonate (2%, w/v) in 0.1 NaOH in the ratio of 1 : 1 : 100. After incubation for 10 min. at room temperature, 0.5 ml of 1.0 N Folin's reagent was added. The contents were mixed and colour intensity was read after 30 min. against the reagent blank at 660 nm.

The percentage removal of *p*-BP was defined as:

$$\frac{\text{Absorbance of untreated} - \text{Absorbance of treated}}{\text{Absorbance of untreated}} \times 100$$

Data analysis

Each value represents the arithmetic mean for three independent experiments performed in duplicate. The average standard deviation of all the obtained values for a particular experiment was less than 5%. The data expressed in various studies was plotted using Sigma Plot-10.0 and expressed as mean ± SD. Analysis was carried out by one-way ANOVA. *P*-values <0.05 were considered statistically significant.

RESULTS

Immobilization of BGP

In this study, Con A layered calcium alginate-cellulose beads were used as carrier for the immobilization of BGP. Ammonium sulfate fractionated and dialysed bitter gourd proteins were adsorbed onto the beads and cross-linked by glutaraldehyde. Con A layered calcium alginate-cellulose beads retained nearly 63% of the original peroxidase activity. However, a marginal loss of 6% activity was observed upon cross-linking (Table 1).

Effect of mercury and organic solvents

Figure 1 shows the effect of mercury on the removal of *p*-BP by soluble and immobilized enzyme. Immobilized peroxidase had successfully removed 69% of *p*-BP in the

Table 1 | Immobilization of BGP on Con A layered cellulose-alginate beads

Methods	Activity expressed (%)
BGP adsorbed on Con A layered calcium alginate-cellulose beads	63 ± 1.36
Cross-linked BGP adsorbed on Con A layered calcium alginate-cellulose beads	57 ± 1.21

Each value represents the mean for three independent experiments performed in duplicate, with average standard deviations <5%.

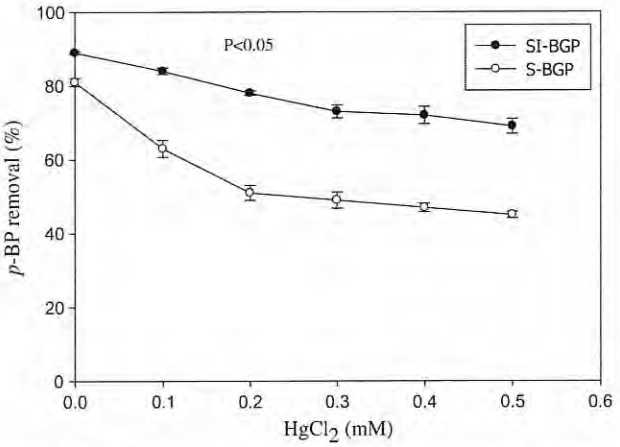

Figure 1 | Effect of $HgCl_2$ on BGP catalysed *p*-BP removal. *p*-BP (0.4 mM) was treated by peroxidase (0.4 U ml^{-1}) in the presence of 0.1 mg ml^{-1} PEG with increasing concentrations of $HgCl_2$ (0.1–0.5 mM), 0.75 mM H_2O_2 in 0.1 M sodium acetate buffer, pH5.5, at 40 °C for 2 h. Symbols indicate oxidative removal of *p*-BP by soluble (○) and immobilized (•) peroxidase.

presence of 0.5 mM $HgCl_2$ while its soluble counterpart oxidized only 45% of the compound.

The effect of organic solvents on the oxidation of *p*-BP by both enzyme preparations has been depicted in Table 2.

Immobilized peroxidase retained 37% *p*-BP removal efficiency in the presence of 30% (v/v) DMSO, whereas soluble bitter gourd peroxidase (S-BGP) removed only 18% of the compound. In the presence of 30% (v/v) of *n*-propanol, immobilized enzyme removed 43% *p*-BP while S-BGP showed a marginal removal of 14%.

Reusability studies and storage stability

Reusability of immobilized peroxidase was a necessary aspect to be studied to make its applicability more practical. The enzyme retained 50% *p*-BP removal efficiency even after its fourth repeated use (Figure 2).

Figure 3 shows the storage stability of soluble and immobilized enzyme in terms of its ability to catalyse the oxidation of *p*-BP over a period of one month when stored at 4 °C. Immobilized peroxidase was capable of removing 78% *p*-BP from polluted water even after 30 days' storage at 4 °C whereas the free enzyme retained only 56% of its removal efficiency.

Treatment of *p*-BP in stirred batch processes

The removal of *p*-BP was performed by soluble and immobilized peroxidase in stirred batch processes at three different temperatures (Table 3). It was observed that maximum *p*-BP removal by both enzyme preparations was at 40 °C. The removal of *p*-BP by soluble and immobilized enzyme preparations was 81 and 91% at 30 °C, respectively, at 180 min.

Table 2 | Effect of organic solvents on the removal of *p*-BP

	p-BP removal (%)			
	DMSO		*n*-propanol	
Organic solvent (%, v/v)	S-BGP	SI-BGP	S-BGP	SI-BGP
2.0	67 ± 1.45	78* ± 1.14	63 ± 1.11	82* ± 1.48
4.0	60 ± 1.02	73* ± 0.95	60 ± 1.34	76* ± 1.89
6.0	51 ± 1.56	68* ± 1.47	57 ± 1.57	69* ± 1.32
8.0	42 ± 1.84	59* ± 0.88	51 ± 0.97	64* ± 2.14
10.0	34 ± 2.14	51* ± 1.68	43 ± 2.27	59* ± 2.35
20.0	24 ± 2.32	44* ± 2.15	30 ± 1.83	51* ± 1.68
30.0	18 ± 1.64	37* ± 1.45	14 ± 2.21	43* ± 1.87

Each value represents the mean for three independent experiments performed in duplicate, with average standard deviations <5%. Analysis was carried out by one-way ANOVA where * denotes that values ($P < 0.05$) were statistically significant when compared with S-BGP with respect to DMSO and *n*-propanol.

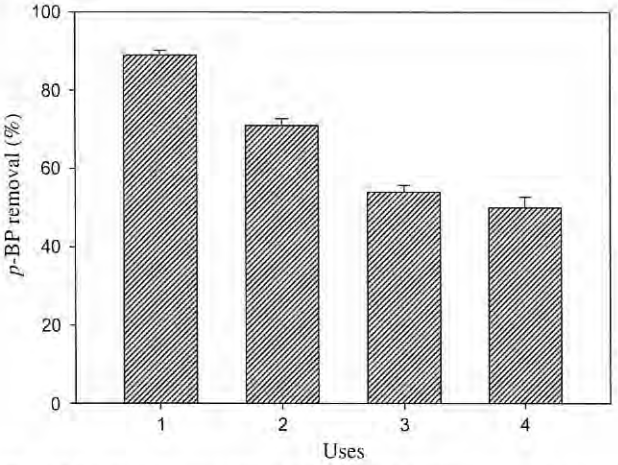

Figure 2 | *p*-BP removal reusability of SI-BGP. *p*-BP (0.4 mM) was treated by peroxidase (0.4 U ml^{-1}) in the presence of 0.1 mg ml^{-1} PEG, 0.75 mM H_2O_2 in 0.1 M sodium acetate buffer, pH 5.5, at 40 °C for 2 h. Immobilized enzyme was collected by centrifugation after the reaction and stored overnight in assay buffer at 4 °C. This procedure was repeated four successive times with the same immobilized enzyme preparation but with an addition of a fresh batch of *p*-BP polluted water.

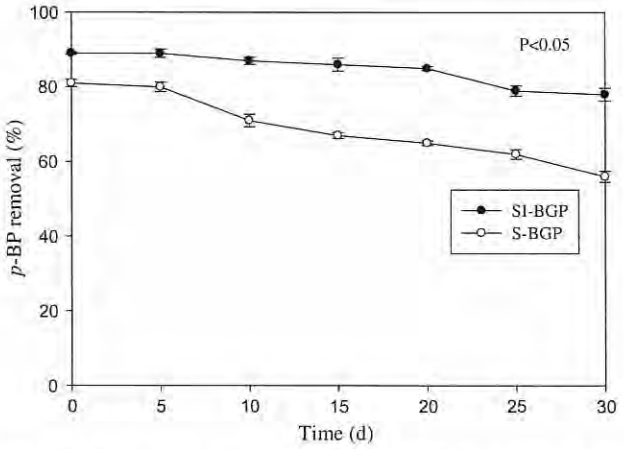

Figure 3 | Storage stability of soluble and immobilized BGP for the oxidation of *p*-BP. *p*-BP (0.4 mM, 5.0 ml) was assayed with 0.4 U ml^{-1} of the original activity of both the preparations of peroxidase over a gap of five days for a period of one month in the presence of 0.1 mg ml^{-1} PEG, 0.75 mM H_2O_2 in 0.1 M sodium acetate buffer, pH 5.5, at 40 °C for 2 h. Symbols indicate oxidative removal of *p*-BP by soluble (o) and immobilized (•) peroxidase.

However, *p*-BP removal by immobilized peroxidase was higher at all the investigated temperatures as compared to free enzyme.

Continuous oxidation and removal of *p*-BP in a packed-bed reactor

The oxidative polymerization and removal efficiency of *p*-BP by immobilized peroxidase present in packed-bed reactors was about 96 and 88% after 5 days of their continuous operation at the flow rates of 10 and 20 ml h^{-1}, respectively (Table 4). As the time of reactor operation increased, the oxidation of *p*-BP continuously decreased and after 30 days the corresponding *p*-BP reactor removal efficiencies correspondingly became 75% and 65%. Figure 4 demonstrates the absorption spectrum for both treated and untreated *p*-BP with respect to the number of days of reactor operation run at a flow rate of 10 ml h^{-1}. The diminution in absorbance peaks of enzymatically treated *p*-BP in the UV region confirmed significant removal of the compound.

Table 3 | Removal of *p*-BP in stirred batch processes by soluble and immobilized peroxidase

	***p*-BP removal (%)**					
	Temperature (C)					
	30		**40**		**50**	
Time (min)	**S-BGP**	**SI-BGP**	**S-BGP**	**SI-BGP**	**S-BGP**	**SI-BGP**
15	50 ± 0.66	46* ± 0.70	56 ± 1.87	53* ± 0.66	43 ± 1.76	41* ± 1.82
30	56 ± 1.39	61* ± 2.16	60 ± 1.62	69* ± 1.35	49 ± 1.17	55* ± 1.44
45	59 ± 1.85	67* ± 0.94	64 ± 0.75	74* ± 2.11	54 ± 1.33	63* ± 0.91
60	68 ± 2.58	80* ± 1.37	69 ± 1.90	81* ± 2.04	61 ± 1.78	71* ± 1.24
90	73 ± 1.93	84* ± 1.16	75 ± 1.41	87* ± 0.83	64 ± 0.91	76* ± 1.28
120	79 ± 0.65	88* ± 1.21	80 ± 1.87	91* ± 1.23	68 ± 1.18	79* ± 1.47
180	81 ± 1.28	91* ± 1.09	85 ± 0.73	94* ± 1.12	72 ± 2.12	83* ± 1.18

Each value represents the mean for three independent experiments performed in duplicate, with average standard deviations <5%. Analysis was carried out by one-way ANOVA where * denotes that values ($P < 0.05$) were statistically significant when compared with S-BGP at three different temperatures.

Table 4 | Removal of *p*-BP by immobilized peroxidase through a continuous reactor

Time (days)	*p*-BP removal (%) 10 ml h^{-1}	20 ml h^{-1}
5	96 ± 1.16	88 ± 1.25
10	92 ± 1.48	83 ± 1.63
15	86 ± 1.14	75 ± 2.10
20	83 ± 1.32	71 ± 1.76
25	79 ± 2.19	67 ± 2.34
30	75 ± 1.85	65 ± 1.78

Each value represents the mean for three independent experiments performed in duplicate, with average standard deviations <5%.

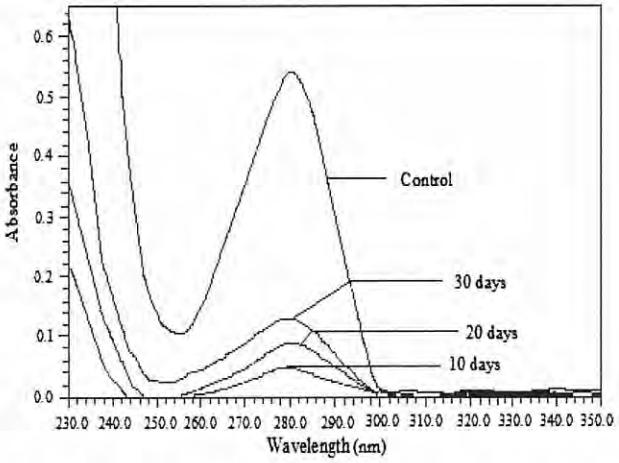

Figure 4 | UV spectra of *p*-BP contaminated wastewater. *p*-BP polluted water (0.4 mM) containing 0.1 mg ml^{-1} PEG and 0.75 mM H_2O_2 was passed continuously through the vertical-bed reactor (10.0 × 2.0 cm) filled with immobilized peroxidase (500 U) at room temperature. The flow rate of the column was maintained at 10 ml h^{-1}. Samples from the column outlet were collected after an interval of 5 days and analyzed spectrophotometrically and their spectra were also recorded after centrifugation.

DISCUSSION

Peroxidase-based treatment of aromatic compounds is gaining tremendous importance in comparison to other conventional methods due to their numerous advantages. However, easy availability, cost effectiveness and significantly higher stability in comparison to other peroxidases makes BGP a choice for the present study (Fatima *et al.* 2007; Fatima & Husain 2007, 2008).

Industrial wastewater is more complex with regard to the presence of other contaminated substances in addition to the aromatic compounds. It contains various types of heavy metals; therefore the oxidation of phenolic compounds by peroxidases in the presence of a heavy metal forms an important part of the study. Our findings showed that the inhibition of SI-BGP catalysed oxidation of phenolic compounds in the presence of $HgCl_2$ was quite low as compared to soluble enzyme (Figure 1). The improved stability against heavy metal inhibition in case of immobilized enzyme might be a consequence of structural changes introduced to the enzyme by immobilization whereas the interference in the structural integrity of soluble enzyme as a result of the binding of metal ion to its active site might have resulted in a decline in enzyme activity (Krajewska 1991; Coyle *et al.* 1999; Ashraf & Husain 2010).

Industrial wastewater may also contain various types of water-miscible organic solvents in addition to phenolic compounds, which might also interfere with the enzymatic removal efficacy. Our results revealed that immobilized peroxidase showed a greater efficacy in removing aromatic pollutants in the presence of organic solvents compared to its soluble counterpart (Table 2). A lower water requirement or enhanced rigidity to the enzyme structure was described by some earlier workers as a possible reason for the stabilization of immobilized enzymes against various forms of water-miscible organic solvents (Xin *et al.* 2005). The advantage of using immobilized enzyme also lies in increasing its stability and reusability. Unlike S-BGP, immobilized enzyme could be easily separated from the reaction mixture and reused. Studies showed that after four repeated cycles, the *p*-BP removal efficiency by immobilized enzyme decreased to 50% (Figure 2). The reaction products might have adsorbed onto the surface of immobilized enzyme and thus it resulted in a loss of catalytic activity (Bayramoglu & Arica 2008; Husain & Husain 2008). However, the data were significant in comparison to horseradish peroxidase immobilized on aluminium-pillared interlayered clay and white radish peroxidase immobilized on diethyl amino-ethyl cellulose, which only retained 4 and 37% removal efficiency after four and six repeated uses (Cheng *et al.* 2006; Ashraf & Husain 2010). The storage stability, among other factors, is an important character which determines the applicability of the immobilized enzyme on the industrial scale. *p*-BP removal efficiency of SI-BGP was decreased to 78% after a month of continuous storage at 4 °C. A significant decrease in the activity of immobilized horseradish peroxidase was also reported on a prolonged storage (Cheng *et al.* 2006).

The removal of *p*-BP in batch processes at various temperatures showed that immobilized enzyme was effective in removing a higher concentration of *p*-BP from wastewater (Table 3). Maximal removal of *p*-BP in a batch process by the enzyme was within a period of 3 h; however, no further increase in the removal of *p*-BP was reported on prolonged incubation. SI-BGP was more effective in removing a higher level of *p*-BP as compared to S-BGP (Table 3). This result was in agreement with several earlier studies where the immobilized enzymes have shown a remarkable removal of aromatic pollutant from polluted water (Husain 2006, 2010; Husain & Husain 2008; Husain *et al.* 2009). Shielding of reactable free amino groups in immobilized enzyme might have prevented its inactivation while in the case of soluble enzyme such groups were more exposed and hence more susceptible to product-mediated inactivation (Husain 2006; Bayramoglu & Arica 2008; Bodalo *et al.* 2008).

To evaluate the efficiency of SI-BGP on a large scale for the treatment of *p*-BP, vertical packed-bed reactor systems were designed and operated continuously with flow rates of 10 and 20 ml h^{-1}. The reactors were continuously operated without any operational problem and showed significant *p*-BP removal efficiency for the entire duration of operation. The extent of oxidation of phenolic compounds in the reactor varied inversely as the reactor flow rate. Several workers have shown a decrease in the degradation of phenolic compounds at a higher flow rate, which implied a lower residence time of phenolic compound inside the reactor (Bayramoglu & Arica 2008; Ashraf & Husain 2010). This decrease in removal rate at lower residence times might be due to insufficient contact time between the phenolic compound and the peroxidases. As shown in Table 4, a packed-bed reactor operated at a flow rate of 10 ml h^{-1} exhibited greater efficiency in terms of the removal of *p*-BP compared to the reactor operated with a flow rate of 20 ml h^{-1}. Confirmation of the oxidation of *p*-BP in the reactor was provided by a decrease in absorbance of the peaks in the UV region (Figure 4).

CONCLUSIONS

It is concluded that SI-BGP showed greater potential for the oxidative polymerization and subsequent removal of phenols from polluted water in comparison to S-BGP. Further studies showed that reactors constructed with such immobilized peroxidase preparations could be continuously used for the removal of phenolic compounds without any reactor blockage. This study has provided a relatively inexpensive technique for the large-scale removal of phenolic compounds from wastewater.

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Edited book: Yoshida, Z. 1963 Physical properties of *snow*. In: *Ice and Snow* (W. Kingery, ed.). MIT Press, Cambridge, Massachusetts, USA, pp. 124–148.

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