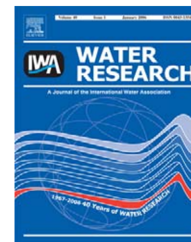
WATER RESEARCH 40 (2006) 541–548

Available at www.sciencedirect.com

SCIENCE DIRECT®

journal homepage: www.elsevier.com/locate/watres



Applying fluorescence based technology to the recovery and isolation of *Cryptosporidium* and *Giardia* from industrial wastewater streams

B.C. Ferrari[a,*], *K. Stoner*[a], *P.L. Bergquist*[a,b]

[a]*Department of Chemistry and Biomolecular Sciences, Environmental Biotechnology CRC and Biotechnology Research Institute, Macquarie University Sydney, NSW 2109, Australia*

[b]*Department of Molecular Medicine & Pathology, University of Auckland Medical School, Auckland, New Zealand*

ARTICLE INFO

Article history:
Received 18 August 2005
Received in revised form
21 November 2005
Accepted 25 November 2005

Keywords:
Detection
Industrial
Wastewater
Cryptosporidium
Giardia
Fluorescence

ABSTRACT

As increasing water shortages continue, water re-use is posing new challenges with treated wastewater becoming a significant source of non-potable water. Rapid detection strategies that target waterborne pathogens of concern to industry are gaining importance in the assessment of water quality. This study reports on the ability to recover spiked *Cryptosporidium* and *Giardia* from a variety of industrial wastewater streams of varied water quality. Incorporation of an internal quality control used commonly in finished water-enabled quantitative assessments of pathogen loads and we describe successful analysis of pre- and part-treated wastewater samples from four industrial sites. The method used combined calcium carbonate flocculation followed by flow cytometry and epifluorescence microscopy. Our focus will now aim at characterising the ambient parasites isolated from industrial wastewater with the objective of developing a suite of highly specific platform detection technologies targeted to industrial needs.

© 2005 Elsevier Ltd. All rights reserved.

1. Introduction

Sustainability of water supplies is fast becoming the major priority for water utilities worldwide. In Australia, extensive drought periods coupled with increasing urban populations are highlighting the need to obtain alternative sources of potable water (Anonymous, 2003, 2004). In order to successfully manage future water supplies, the current assumption that water is an endless resource needs to change and alternative sources of non-potable water need rapid investigation. Recent government initiatives recognise the need for change, with regulations now aimed at reducing water consumption per person while increasing the reuse or recycling of wastewater (Anonymous, 2003, 2004). However, the safe management of recycled or re-use water is a pressing concern for non-traditional sources to be acceptable by the community.

In terms of the food industry, water use is expressed per ton of food product (Casani et al., 2005). The ability to re-use processed water will significantly reduce water consumption as it is used extensively in many industrial applications such as the operation of boiler equipment or in cleaning purposes for reaching adequate food safety levels. Increased pressure to reduce water consumption within the manufacturing industry is widespread with food processors actively pursuing opportunities for safe recycling and re-use of water (Pagan and Prasad, 2005). While the majority of industry water re-use to date is on agricultural land, there is also the potential to increase the re-use of processed water within the same or a new manufacturing operation (Angelakis et al., 1999; Pagan

*Corresponding author. Tel.: +612 9850 9252; fax: +612 9850 8253.
E-mail address: bferrari@rna.bio.mq.edu.au (B.C. Ferrari).
0043-1354/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.watres.2005.11.037

and Prasad, 2005). To ensure the quality and safe management of water and food product, these re-use possibilities mean that there are increasing requirements for real-time monitoring of wastewater and commercial products for the presence of potential pathogens.

In many cases, current culture methods are too slow and expensive for routine use, and detection methods need to be extremely sensitive (Anonymous, 1999; Casani et al., 2005; Okhuysen et al., 1999). Challenges to the industry include a lack of reliable water quality or risk assessment methods directed to specific pathogens rather than to standard microbial indicators. What is required is the development of rapid, quantitative assessment methods and online systems (Casani et al., 2005). Pathogens of public health interest include the parasites *Cryptosporidium* and *Giardia* as they are of major concern to water utilities worldwide, while emerging pathogens include *Toxoplama gondii* and *Microsporidia*. *Cryptosporidium* is environmentally robust, it is capable of surviving standard chlorine disinfection practices and wastewater effluents are potential reservoirs for transmission of the infectious organism (Clancy et al., 2004; McCuin and Clancy, 2005; McEvoy et al., 2005). While few studies have focused on the detection and characterisation of pathogens in industrial wastewater, both *Cryptosporidium* spp. and *Giardia* have been reported in raw faecal waste in animals entering slaughterhouses and in wastewater influent (Cancy et al., 2004; Kaneta and Nakai, 1998; Pepperell et al., 2003). More recently, *Cryptosporidium parvum* and *C. andersoni*. have been isolated from faecal samples of cattle at slaughter (Koyama et al., 2005; Moriarty et al., 2005). In the future, the re-use of wastewater from processing industries may need to be assessed for the potential of these waters to act as a reservoir for known and emerging zoonotic pathogens.

We are developing a suite of platform technologies modeled on *Cryptosporidium* and *Giardia* detection using fluorescence and flow cytometry (Ferrari and Veal, 2003; Veal et al., 2001). These approaches are aimed at the rapid and sensitive detection of specific microorganisms from the processing environment, initially to enable their further characterisation. We have modified methods for simultaneous detection of *Cryptosporidium* and *Giardia* from various types of industrial wastewater streams. The method combining calcium carbonate flocculation for concentration and immunofluorescence and flow cytometry for isolation produced variable recovery frequencies which highlighted the need for internal controls (Francy et al., 2004; McCuin and Clancy, 2005). We present quantitative results on the recovery efficiency of the analysis of wastewater following the incorporation of Colorseed™, a colour modified *Cryptosporidium* and *Giardia* spike which is easily distinguished from naturally occurring organisms (Ferguson et al., 2004; Francy et al., 2004; Warnecke et al., 2003). This initial study will be now be used for the development of a suite of platform technologies which are appropriate to field situations, that will be more specific, ranging from simple 'dipstick' field tests for field deployment to more complex analyses using fluorescence-based detection methods and online systems. Identification of the potential sources of, and quantitative measures of, pathogen loads will provide reliable information for industrial operators in terms of the potential for future wastewater re-use.

2. Materials and methods

2.1. *Cryptosporidium oocysts and Giardia cysts*

C. parvum oocysts (Camden strain), were isolated from naturally infected calves using a modified sucrose-percoll floatation method (Truong, Pers. commun.). Sieved faecal samples were centrifuged and resuspended in 30 ml sucrose (Univar, Sydney, Australia) and overlaid with 10 ml water. After centrifugation at $3500 \times g$ for 30 min, oocysts were collected from the interface and were placed through a series of sucrose gradients. Finally, 1 ml aliquots of oocysts were laid over a 30%/20%/15% Percoll™ gradient (Amersham Biosciences, Castle Hill, Australia) and centrifuged. Oocysts were collected between the 30%/20% interface and washed. Oocysts were stored live in 0.01 M phosphate buffered saline (150 mM NaCl, 15 mM KH_2PO_4, 20 mM Na_2HPO_4, 27 mM KCL, pH 7.4 ± 0.2) (PBS), (Oxoid, Sydney, Australia) at a concentration of 1×10^6 oocysts ml^{-1} and stored at 4 °C. *Giardia lamblia* cysts strain HS reduced by passaging through gerbils were supplied by Waterborne Ltd (New Orleans, USA) live in phosphate buffered saline (PBS). Cysts were stored in PBS at a concentration of 1×10^6 cysts ml^{-1} at 4 °C. A high seed load was used for setting up instruments and confirming recovery of viable oocysts and cysts. Viable spike samples consisting of 100 (oo)cysts were prepared for recovery determinations using fluorescence activated cell sorting as described previously (Bennett et al., 1999).

2.2. *Wastewater sample preparation*

Twenty two $\times$ 100 ml industrial wastewater samples were obtained from three cattle and one sheep abattoir sites within Australia. Both 'pre' and 'part' treated wastewater samples were obtained for the study. Each wastewater sample was placed into a sterile container and stored at 4 °C until analysis. Due to the unavailability of a small volume nephelometer, turbidity indicator measurements were taken by measuring the sample absorbance at 600 nm using an Eppendorf biophotometer. Reagent water control samples (distilled water) were seeded with viable spikes consisting of 100 ± 2.5 *Cryptosporidium* (Camden strain) and *Giardia* (Waterborne) (oo)cysts isolated by cell sorting. Reagent water samples and all wastewater samples were then seeded with one vial of Colorseed™ (BTF Pty Ltd, North Ryde, Australia) prior to processing. Two Colorseed™ batches were used and include batch numbers CS-CG100-64 and CS-CG100-66. Colorseed™ vials were supplied with 100 or 99 oocysts and 99 cysts per vial and standard deviations of each batch were within strict requirements of 100 ± 2.5 (Flecknoe, Pers. commun.). Colorseed™ contains modified oocysts labelled permanently with the fluorescent dye Texas-red. The modified oocysts do not interfere with flow cytometric or microscopic analysis of FITC-stained organisms. In order to overcome poor *Giardia* recovery efficiency, a further two batches of *Giardia* Colorseed™ (CS-CG100-67 and CS-CG100-68) were analysed.

Download English Version:

https://daneshyari.com/en/article/4484683

Download Persian Version:

https://daneshyari.com/article/4484683

Daneshyari.com