Desalination and Water Treatment
www.deswater.com

doi: 10.5004/dwt.2020.26209

203 (2020) 104–111
November

Profiling the susceptibility of the autochthonous bacterial community in raw wastewater to chlorine dioxide with denaturing gradient gel electrophoresis

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Received 5 September 2019; Accepted 6 June 2020

ABSTRACT

Bacteria populations in wastewater systems are diverse and exhibit different responses to water disinfectants. We investigated the susceptibilities of the autochthonous bacteria community from typical municipal wastewaters toward various concentrations of chlorine dioxide (0.5–5.0 mg/L) by 16S rRNA gene-directed polymerase chain reaction based denaturing gradient gel electrophoresis (PCR-DGGE) as a culture-independent technique. We compared the results with the classical heterotrophic plate count culture-based method. Pre-treatment of chlorine dioxide disinfected bacteria with propidium monoazide was utilized to selectively exclude the DNA of membrane-compromised cells from viable cells in the amplification stage by PCR. The bacterial susceptibilities varied between the two approaches. A 3.0 mg/L chlorine dioxide dose sufficiently eliminated the heterotrophic bacteria population to achieve an approximate 4.0 ± 1 log reduction in just 30 s from the culture-based protocol. In contrast, the PCR-DGGE profile showed that 1.0 mg/L was adequate to inactivate three predominant species identified as *Arcobacter suis* F41, *Pseudomonas* sp. strain QBA5 and *Pseudomonas* sp. B-AS-44. However, a significant population of other species such as *Pseudomonas* sp. CCI2E presumably remained viable to 5.0 mg/L chlorine dioxide. The results of this study could broadly influence the monitoring strategies used for assessing the dose-response effect of a disinfection regime and prospecting for potential organisms that might be resistant toward water disinfectants.

Keywords: PCR-DGGE; Propidium monoazide; Chlorine dioxide; Wastewater; Disinfection; Bacterial community

1. Introduction

A growing number of research findings in recent times have shown that municipal wastewater plants are hotspots for the development and transfer of clinically relevant antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARG) [1–4]. These have partly been attributed to inadequate disinfection of the reclaimed water during the treatment process before being supplied into the distribution system or discharged into the receptor water bodies [5]. The deficient disinfection regimes usually emanate from the exposure of the microbial agents to sub-inhibitory doses of conventional disinfection technologies including chlorination or UV irradiation which consequently lead to the triggering of biochemical stress responses [6,7]. Due to the complexities of ARB and ARG as emerging contaminants in wastewater and the potential adverse effects on public health associated thereof, considerable attention has been

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focused currently on ensuring the application of adequate dosages of a disinfectant in the wastewater treatment process and the validation of its adequacy for preventive and corrective actions.

Conventionally, indicator organisms, including total coliforms, fecal coliforms and coliphages are used to validate the reliability of a disinfection performance at a water treatment plant [8–10]. Such monitoring processes commonly utilize culture-dependent techniques, including the heterotrophic plate count (HPC) to assess microbial viability and to determine the effectiveness of the disinfection activity. Culture-based methods are relatively simple and cost-effective in terms of equipment and other logistics, do not usually require high technical expertise and are more suitable for routine monitoring. However, reportedly more than 99% of the bacterial diversity in oligotrophic habitats like disinfected wastewater may be non-cultivable or present in viable but non-culturable (VBNC) states [11–13]. It follows therefore that considerable details about most of the diverse bacterial community and its susceptibility to disinfectants in the water system may not be known, thus underestimating the number of pathogenic microbes that remain viable after disinfection [14]. Another limitation is the inability of microbial indicators to predict the presence of some pathogens adequately. For instance, coliform bacteria are relatively more susceptible to chemical disinfection. Hence they may not adequately reflect the occurrence of pathogens in disinfected reclaimed water containing protozoan parasites such as *Cryptosporidium* and enteric viruses [15].

Molecular bacterial identification or culture-independent techniques overcome the limitations of conventional culture-based methods. Presently, the revolutionary high-throughput next-generation sequencing (NGS) technique is at the forefront of the molecular-based approaches employed in the study of the microbial community structure in a microbiome like wastewater. It can identify a large number of bacteria at a time from various sample sources and has been utilized in several studies to monitor the community dynamics of microorganisms in water after disinfection [16,17]. However, its complexity and the cost are quite high and also require a higher level of expertise for analysis which may not be readily available for routine practice at the treatment plant.

The 16S rRNA gene-directed polymerase chain reaction (PCR) based denaturing gradient gel electrophoresis (DGGE) also presents an invaluable tool to study the dynamics of unknown diverse bacterial populations and their responses to a disinfectant. DGGE is a molecular fingerprinting technique that separates double-stranded DNA (dsDNA) fragments from PCR products of similar length but of different base pair sequences [18]. It is relatively simple, less expensive to conduct and remains widely popular for assessing changes in bacterial community shifts [19]. Previous applications of the DGGE technique in water treatment processes focussed mainly on monitoring the long-term changes in bacterial community structures of either an environmental water body [20] or a drinking water distribution system [11,21]. Moreover, the limited reports in the literature regarding the use of culture-independent methods to assess inactivation efficiencies of water disinfectants aim at the specific target organisms [14,22]. However, in a complex system such as municipal wastewaters, diverse bacterial species with different susceptibilities or responses toward a disinfectant may exist. Profiling such a community of bacterial species in terms of their specific susceptibilities to a disinfectant is thus necessary, to determine the appropriate disinfectant dosages for treating the reclaimed water, and then to look for potential resistant bacterial species towards that disinfectant. Very little work is available using the PCR- DGGE technique.

Chlorine dioxide (ClO_2) is one of the chemical alternatives to conventional chlorine-based disinfectants including free chlorine and monochloramine for the disinfection of wastewater effluents [23]. It has broad-spectrum biocidal activity against a range of microorganisms, including bacteria, viruses and protozoa [24].

In this study, we explored for the first time the PCR based DGGE as a culture-independent technique to investigate the susceptibilities of the autochthonous bacterial community in urban wastewater treatment plants to varying concentrations of ClO_2 and compared with the susceptibilities observed from the HPC method.

2. Materials and methods

2.1. Preparation of chlorine dioxide

ClO_2 solutions were prepared following the procedure as described previously [10] by oxidizing approximately 25% (w/v) solution of sodium chlorite ($NaClO_2$) in a gas generating bottle with a dilute solution of sulphuric acid (H_2SO_4, 2 M). The generated gas was harvested through a stream of compressed air into a connecting chlorine scrubber system which contained a saturated solution of sodium chloride (10% w/v) to scrub contaminants such as chlorine gas. Chlorine dioxide gas was collected in a connecting bottle of demand free deionized water, and the concentrations of the prepared stock solutions were analyzed by the iodometric method. In contrast, the residual concentrations were determined by the N,N-diethyl-*p*-phenylenediamine method [10].

2.2. Water sampling and determination of physicochemical parameters

Samples of untreated influent wastewater were collected from the eThekwini wastewater treatment plant in Durban, South Africa according to common sampling protocols [10], kept on ice and immediately transported to the laboratory for further analysis. It is important to emphasize that in practice, disinfection is mostly performed in the effluent wastewater. However, in the present study, influent wastewater samples were used to target higher and diverse bacterial populations [25]. Nonetheless, the assay could also apply to related studies involving effluent samples. In any experimental event, 5.0 L composite samples were prepared by mixing the contents of the sampling bottles to obtain a uniform homogeneous matrix. Samples were filtered with Whatman No-1 filter papers (pore size 11 μm) to remove particulate matter and subsequently to determine the pH (Beckman pH meter, CA, USA), total dissolved solids and electrical conductivity (EC) using the CD401 probe fitted

onto HQ40d multimeter (HACH, Co., USA). The chemical oxygen demand and total suspended solids were determined according to standard methods for the examination of water and wastewater [10].

2.3. Disinfection of water samples with chlorine dioxide

Approximately 500 mL portions of the filtered samples were exposed to different concentrations (0.5 to 5.0 mg/L) of chlorine dioxide at 22°C ± 2°C in 1.0 L batch reactors under constant agitation on a magnetic stirrer. These concentrations were chosen after preliminary trials of wastewater samples exposed to ClO_2 using the HPC method and as suggested by similar studies for water disinfection [26,27]. The inactivation kinetics of the autochthonous bacteria was monitored by withdrawing 5.0 mL of aliquots from the reactor at predetermined times (0–60 min) into tubes containing 2 mL of 10 mM sodium thiosulphate, to quench the reaction. Total heterotrophic cultivable bacteria populations were subsequently enumerated in triplicate on tryptone soy agar (TSA) by the spread plate count technique (upon appropriate dilution) following incubation at 37°C for 18–24 h. Processing of each sampled water was conducted within 24 h after collection. Control samples were identically treated except that no chlorine dioxide was added.

2.4. Propidium monoazide treatment and genomic DNA extraction

A protocol involving propidium monoazide (PMA) [Phenanthridiun, 3-amino-8-azido-5-[3-(diethylmethylammonio) propyl]-6-phenyl dichloride] with appropriate modifications was employed, [28,29] to distinct the DNA of viable cells from the dead cells after chlorine dioxide treatment. ClO_2 disinfected wastewater samples corresponding to each applied concentration in the reactor were quenched with 10.0 mL of 10 mM sodium thiosulphate after incubating for 1 h. Samples were centrifuged in 250 mL centrifuge bottles at 10,000 x g, and the pellets washed and concentrated to a total of 1.0 mL in microcentrifuge tubes with autoclaved deionized water. Desired quantities of PMA (Biotium, Inc., Hayward, CA, USA) sufficient to give an approximate final concentration of 50 μmol/L from a prepared stock of 2.0 mmol/L were added to each of the aliquots in the tubes, mixed thoroughly and incubated in the dark for 20 min. Subsequently, samples were placed horizontally on ice and exposed to light from a halogen lamp (26W, OSRAM DULUX, China) from an approximate distance of 30 cm for 10 min, vortexed thoroughly and the contents transferred directly into the 5.0 mL bead beating tubes provided in the PowerWater DNA Isolation kit (MO BIO Laboratories, USA). The exposure to halogen light enhances the covalent cross-linkage of PMA molecules to DNA through its light-activatable azide group to inhibit subsequent PCR amplification [30]. The total genomic DNA was extracted according to the manufacturer's instructions and stored at –20°C for further molecular analysis. The quality of purified DNA extracts was assessed by agarose gel electrophoresis [31] while the quantity and quality were further verified with a NanoDrop 2000C Spectrophotometer (Thermo Scientific, USA).

2.5. Amplification of 16S rRNA gene by PCR using DGGE primers

A PCR was performed to amplify a 586 bp universal 16S rRNA gene fragment using the universal forward primer 341F (5′-CCT ACG GGA GGC AGC AG-3′) and the reverse primer 907R (5′- CCG TCA ATT CMT TTG AGT TT-3′). A GC – clamp (CGC CCG CCG CGC CCC GCG CCC GTC CCC GCC CCC GCCC) which has a high melting domain was attached to the 5′ end to prevent a complete denaturation of DNA fragments and hence to enable the detection of the corresponding PCR product during the DGGE [32]. The PCR mixture (50 μL) composed of 25 μL of 2 × Phusion flash high-fidelity PCR master mix (Thermo Scientific, USA), 0.4 μM of each primer and about 10–30 ng/μL of genomic DNA. The reaction was conducted in an automated thermal cycler (T100™ Bio-Rad, USA) under a touchdown PCR program as follows: initial denaturation at 94°C for 5 min, followed by 20 cycles of 94°C for 1 min, 65°C for primer annealing for 1 min and 72°C for 1 min. The annealing temperature was decreased by 0.5°C per cycle until a touchdown at 55°C. The same procedure was followed by another 15 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 3 min and a final extension at 72°C for 7 min before holding at 4°C [18]. Negative control reactions in the absence of the DNA template were run simultaneously.

2.6. Denaturing gradient gel electrophoresis

DGGE was carried out on a DCode™ Universal Mutation Detection System (Bio-Rad Laboratories, USA) following the manufacturer's instructions. In this protocol, approximately 1,000 ng of PCR amplicons were loaded onto a vertical polyacrylamide gel (6% w/v) in a 1 x TAE buffer using a denaturing gradient ranging from 40% to 60% (100% denaturant solution contained 7 M urea and 40% deionized formamide [11]. Electrophoresis conditions were set at 60 V to run for 16 h, following which the gel was stained with ethidium bromide for 30 min and de-stained in 1 × TAE buffer for 15 min. The gel was subsequently visualized and imaged under UV light with a Gel Doc system (Syngene, UK).

Bright representative bands were excised into sterile 1.5 mL Eppendorf tubes to elute the DNA in an elution buffer after being kept overnight at 4°C. Essentially, the eluted DNA samples were re-amplified with the primers previously described but without the G-C clamp under identical PCR conditions. The amplicons were sequenced (Inqaba Biotechnical Industries (Pty.) Ltd, Pretoria, South Africa) and the sequences edited with Chromas (Technelysium Pty. Ltd., Brisbane, Australia) and then compared against the NCBI non-redundant database using the basic local alignment search tool (BLAST) to reveal their identity.

2.7. Data analysis

The data from the inactivation of the heterotrophic bacteria count were described by the C_{avg} Hom model previously used for describing disinfection kinetics data. This model is often characterized by a tailing off behavior [33,34].

$$\log\left(\frac{N_t}{N_0}\right) = -kC_{avg}^{n}T^{m} \quad (1)$$

$$C_{avg} = \sqrt{\left(C_0 \times C_f\right)} \quad (2)$$

where C_0 and C_f are the initial and final disinfectant concentrations (mg/L) respectively. (N_t/N_0) is the survival ratio of the microorganisms (where N_t = number of organisms surviving at the time, t and N_0 at t = 0, (CFU/mL)), k is inactivation rate constant of the target organism, T is the contact time required to achieve a given level of inactivation, and n is an empirical factor called the coefficient of dilution, whilst m is an empirical constant.

3. Results and discussion

3.1. Inactivation kinetics of wastewater total heterotrophic bacteria

The present study aimed at determining the susceptibilities of wastewater bacterial community to different concentrations of chlorine dioxide applied as a disinfectant by using both culture-dependent methods and molecular-based culture-independent techniques such as PCR-DGGE. Fig. 1 is an illustration of the inactivation kinetics data of chlorine dioxide-initiated disinfection of typical municipal wastewater samples at different doses. Table 1 summarises the results of the measured physicochemical parameters of the wastewater samples. Total heterotrophic bacteria densities ranged from 10^4 to 10^5 CFU/mL. The application of 0.5 mg/L of chlorine dioxide resulted in ~0.3 log reduction of the bacterial population within 1 h of contact time while approximately 2.0 log reduction was achieved after treating the water samples to 1.0 mg/L. The increase of the chlorine dioxide concentration to 3.0 mg/L was sufficient to eradicate almost all the heterotrophic bacteria, to yield more than 4 log inactivation. This observation was consistent in almost all samplings and independent experimental events conducted and agreed well with the results of other similar studies [23,35,36].

The findings demonstrate the exceptional efficiency of chlorine dioxide as a disinfectant of choice for the treatment of wastewater. The efficiency can presumably be attributed to the fundamental selective reactivity of chlorine dioxide towards organic compounds in a typical water application [26,37]. Generally, when compared to chlorine, it is less reactive towards compounds containing olefinic double bonds, primary and secondary amines, aromatic hydrocarbons, aldehydes, ketones and carbohydrates [38,39] as well as humic and fulvic acids [40]. These result in a lesser disinfectant demand for the applied dose and eventually making it more available for microbial inactivation.

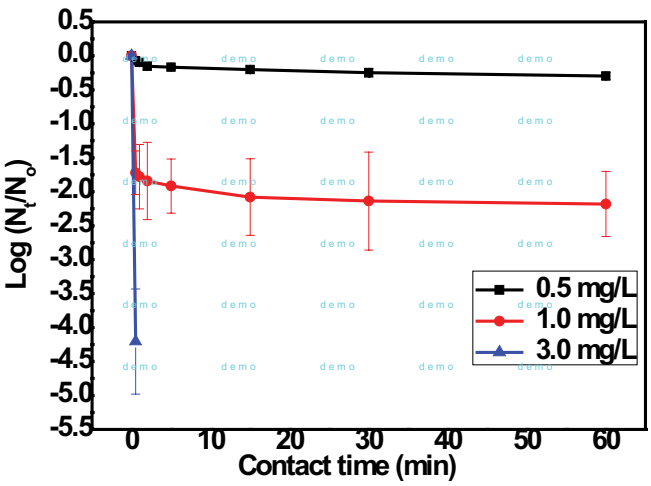

Fig. 1. Inactivation kinetics of heterotrophic bacteria from wastewater samples by chlorine dioxide applied at different concentrations.

3.2. PCR-DGGE profile of the bacterial community in wastewater exposed to chlorine dioxide

The amplified DGGE-PCR products of the extracted genomic DNA from the bacterial community in the sampled municipal wastewater after chlorine dioxide and PMA treatments are depicted in an agarose gel in Fig. 2.

Fig. 3 shows the DGGE profile of the bacterial community in the wastewater sample exposed to varying concentrations of chlorine dioxide. Each band on the profile is assumed to represent a different operational taxonomic unit (OTU) corresponding to a single species. Bands appearing to be common to each of the lanes also indicate the presence of common species in the analyzed samples. Moreover, under the same experimental conditions, the relative intensities of the bands are also assumed to correspond to the relative abundance of the diverse species present. Before the DNA extraction, cells were exposed to PMA to selectively separate the DNA of the dead cells from the viable cells in the essential downstream analysis [41]. PMA is a high-affinity photoreactive DNA binding dye, which does not permeate the intact cells. Still, it readily permeates through compromised cell membranes to intercalate with the inner or naked DNA found in the debris of lysed cells. This reaction forms an irreversibly modified DNA complex that inhibits the subsequent PCR amplification of the DNA templates of dead cells [29]. It implies, therefore, that; the visible bands on the gel could reliably indicate the bacterial cells that survived the doses of chlorine dioxide.

The control lane represents the original strength of the bacterial population in the wastewater samples before the treatment with ClO_2. Moreover, in Fig. 3, the bright representative bands of each class, which were excised and sequenced are labeled A–D. The OTU labeled A, appeared more intense in the control sample and at 0.5 mg/L ClO_2

Table 1
Selected physicochemical parameters determined from the influent wastewater samples of the eThekwini wastewater treatment plant, Durban

Parameter	Mean ± SD
pH	7.35 ± 0.4
EC, μS/cm	1,128 ± 31
Turbidity, NTU	32.8 ± 4.1
Chemical oxygen demand, mg/L	112 ± 8.7
Total suspended solids, mg/L	67.3 ± 5.4

Values are averages of triplicate measurements ± standard deviation (SD)

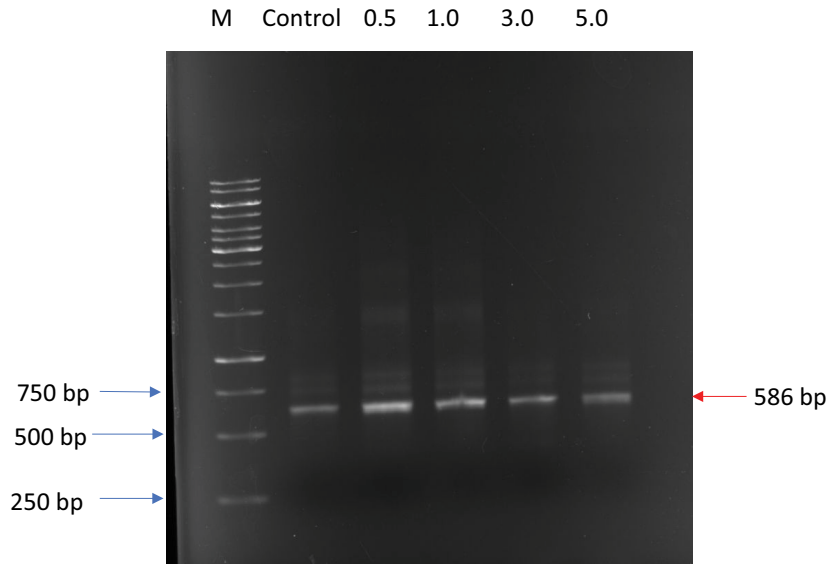

Fig. 2. Representative agarose gel showing the PCR amplicons of the extracted bacterial DNA from a wastewater sample using 341F-GC and 907R primers. M contains a 1 kb marker (Thermo Scientific, USA), the control represents the sample without ClO_2 treatment, and the rest depict the concentrations of chlorine dioxide (mg/L) applied to disinfect the water samples.

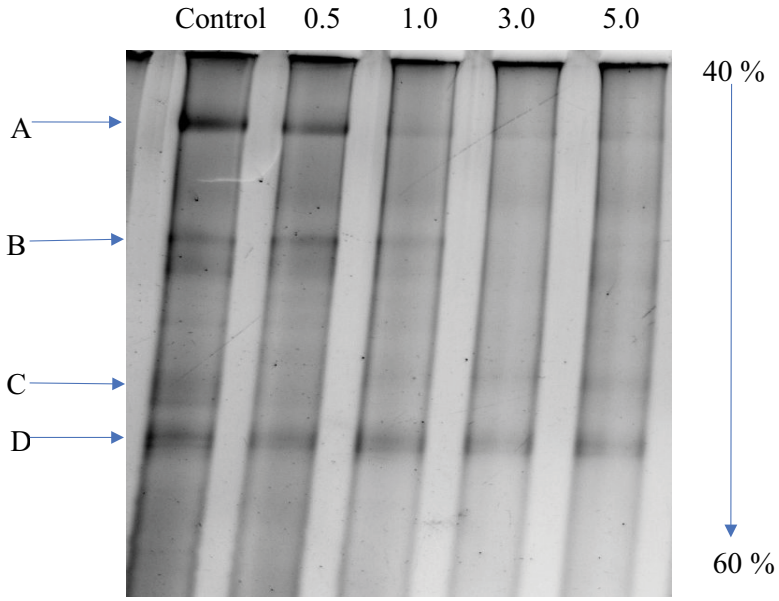

Fig. 3. A DGGE profile of the bacterial community in the sampled wastewater exposed to varying concentrations of chlorine dioxide. The control indicates the sample without ClO_2 treatment, while the other lane labels depict the concentrations of chlorine dioxide (mg/L) applied to disinfect the water. The A-D labeling shows the dominant OTUs excised for sequencing.

concentration. This intensity was observed to decline from 1.0 mg/L through to 5.0 mg/L, indicating a substantial reduction in the population of the viable bacteria species to below detection levels. Similar observations were also made for B, while the band class ascribed as C was only visible in the control sample. These observations suggest that a chlorine dioxide concentration of 1.0 mg/L was sufficient to inactivate the bacterial species in the wastewater samples represented by bands A, B, and C on the gel.

However, quite interestingly, relatively brighter or more intense bands were observed for the OTU labeled D in control and all the other samples subjected to the varying chlorine dioxide concentrations. These bands represent the dominant species of bacteria in the composite wastewater whose population could not be substantially degraded below detection in the gel even at 5.0 mg/L. It thus indicates that such species are relatively less susceptible to chlorine dioxide and their abundance in the composite water

samples substantially corresponds to the intensity of the bands in the gels.

It is important to note that the culture-based HPC technique (Fig. 1) showed all the cultivable bacteria on the TSA plates were effectively inactivated at 3.0 mg/L, within the initial 30 s. This result shows that the dominant species found in the OTU labeled D could not have been present as detectable colonies on the TSA plates at 3.0 mg/L or higher concentrations thereof. However, they could be detected on the DGGE fingerprint profile. Such species could be VBNC or non-viable but still, possess intact membranes [21]. Recently, it has been reported that exposure to low concentrations of oxidants such as chlorine, monochloramine and ozone, induced VBNC states to *E. coli*, *Salmonella*, and *Legionella* spp. and enhanced their reactivation and regrowth, and their persistence and resistance towards antibiotics [42–44].

A fundamental assumption underlying the PMA treatment stage in this analysis is that cells with compromised or injured cell membranes are assumed to have lost their viability [45]. However, in relying absolutely on the membrane integrity for viability and consequent efficiency of a disinfection system as in the current study, knowledge about the bactericidal mechanism of the disinfectant becomes necessary. In a recent work conducted by our research group [46], chlorine dioxide was found to principally inactivate *E. coli* by disrupting the integrity of the outer cell and the cytoplasmic membranes to release intracellular components without necessarily lysing the cells. On the contrary, the primary bacterial inactivation mechanism of UV- light involves the damage of the DNA without necessarily compromising the integrity of the outer cell membrane [21,47]. In such a case, membrane integrity becomes a poor indicator of cell viability.

3.3. Identities of dominant species from the DGGE profile

Table 2 summarises the results of the identified dominant species of bacteria based on the BLAST comparison of sequences against the NCBI Genbank database. The OTU labeled A was found to possess 99% similarity to the 16S rRNA sequence of *Arcobacter suis* F41. The *Arcobacter* species are highly abundant in sewage, raw (untreated) environmental waters, as well as secondary effluents where they are estimated to constitute approximately 5%–11% of the bacteria population in such habitats [48,49]. Even though no known resistance of the *Arcobacter* spp. to conventional chemical-based water disinfection technologies such as chlorination, chlorine dioxide, monochloramine or ozone, have been reported in the literature, some strains of clinical relevance such as *A. butzleria* have been identified to be resistant to ampicillin and cefotaxime [50].

On the other hand, the other OTUs, B, C, and D were predominantly similar in identity to different strains of *Pseudomonas* sp. with marked susceptibility differences toward chlorine dioxide. For instance, while 1.0 mg/L chlorine dioxide was sufficient to entirely reduce B (*Pseudomonas* sp. strain QBA5) and C (*Pseudomonas* sp. B-AS-44), a concentration of 5.0 mg/L was not enough to effectively remove D (*Pseudomonas* sp. CC12E). However, among the non-fermenting Gram-negative bacilli, *Pseudomonas aeruginosa* is the most prevalent species of clinical significance and thrives in diverse environments including soil, water and surfaces of medical equipment [51]. The identified species from this study are also consistent with other similar studies that used the advanced NGS technology which identified *Proteobacteria* such as *Pseudomonas* and *Arcobacter* as predominant genera in effluent municipal wastewater samples [16,52].

The concerns of interest in this circumstance lie with the potential of the presumable chlorine dioxide resistant gene elements embedded in strains such as *Pseudomonas* sp. CC12E to be horizontally transferred across into virulent strains of *P. aeruginosa* in wastewater systems [53] and potentially to other bacteria. The consequent health implications associated with their infections, most notably among immunocompromised patients, could be dire [54]. Other bacteria species such as *Burkholderia* sp. have also been reported to show resistance to monochloramine and chlorine in water [6].

Variations that exist in the responses of bacteria to different disinfectants or antiseptics are primarily due to the differences in cellular structure, composition, and physiology [55]. Bacteria susceptibility to disinfectants could be associated with a chromosomally controlled natural property of the organism (the intrinsic factor) or emanating from the genetic changes which develop from the acquisition of plasmids or transposons or by mutation (the acquired factor) [56,57]. Usually, an applied disinfectant inactivates bacteria by an initial interaction with the cell surface and subsequent penetration into the cell to reach its intracellular target sites. The cell outer surface membrane thus plays a significant role in determining the viability or susceptibility to a disinfecting agent. For instance, the differences in the lipopolysaccharides (LPS) composition and the cation content of the outer membranes of *Pseudomonas aeruginosa* account for its high resistance to several antimicrobial agents [57]. Furthermore, gram-negative bacteria are generally less susceptible to disinfectants than gram-positive

Table 2
Identities of the dominant bacterial species in the wastewater sample

OTU	Closest match from Genebank*	Accession number	Similarity (%)
A	*Arcobacter suis* F41	NR_116729.1	99
B	*Pseudomonas* sp. strain QBA5	MF782453.1	99
C	*Pseudomonas* sp. B-AS-44	JF901706.1	98
D	*Pseudomonas* sp. CCI2E	KM187145.1	100

*Based on the BLAST comparison of sequences to the NCBI database.

[55]. This, partly explains the reason why all dominant species that survived the chlorine dioxide treatment in the gel in this study are all gram-negative bacteria.

For multiple bacterial communities in a wastewater system, the DGGE based culture-independent technique could be used for simultaneously determining the responses of different species of bacteria to an applied disinfectant dose. Thus, indicating the potential resistant species, including the VBNC strains, which could not be determined by the classical HPC technique. However, it has a limitation of not being able to track the changes in the abundance of less dominant microbial groups and also in depicting the log reductions of bacterial populations at the different concentrations of disinfectant quantitatively.

4. Conclusion

Our study showed that DGGE could be utilized as a useful culture-independent technique for assessing the susceptibilities of diverse bacterial populations in municipal wastewater to a disinfectant. Three predominant bacterial species identified as *Arcobacter suis* F41, *Pseudomonas* sp. strain QBA5 and *Pseudomonas* sp. B-AS-44 were found to be more susceptible to ClO_2. In contrast, another species, *Pseudomonas* sp. CCI2E presumably remained viable to the highest applied dose (5.0 mg/L) of ClO_2. However, marked differences were observed between the bacterial susceptibilities depicted by the culture-based HPC technique and the culture-independent DGGE method. It is envisaged that the results of this study will broadly influence the monitoring strategies for verifying the efficiency of a disinfection regime and prospecting for potential organisms developing resistance towards water disinfectants.

Acknowledgement

The authors are grateful to the University of KwaZulu-Natal for providing the research facilities for the study.

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