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ORIGINAL ARTICLE

Industrial wastewaters harbor a unique diversity of bacterial communities revealed by high-throughput amplicon analysis

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Abstract

Industrial wastewater effluents present a major source of water pollution, and can potentially alter the microbial ecological landscape. While there are numerous reports on the microbial quality of domestic municipal effluents and their perceived environmental effects, there are limited reports devoted to the study of bacterial diversity of effluents from individual industries before they are mixed up with other sources. This study analyzed both the physicochemical parameters and bacterial community structures of different industrial wastewaters using Illumina high-throughput sequencing platform. Industrial wastewater with temperature ranging from 18.9 to 21.5 °C, and total dissolved solid (TDS) levels at up to 4611 mg/L, appeared to be predominated by *Proteobacteria* (44.44–75.86%) with the exception of the Capegate sample where *Actinobacteria* (39.66%) were the highest. Sulfur levels were significantly higher ($p < 0.05$) in Dixon wastewater constituting higher populations of sulfur reducing bacteria (SRB) compared to the other sites. Diversity index (Shannon-H index) and richness estimator (Chao1 index) ranged from 974 (Capegate) to 4552 (Dixon) and 6.04 (Dixon) to 4.15 (CWI), respectively. Multivariate analysis results highlighted that the bacterial communities were strongly shaped by physicochemical variables. The top 10 operational taxonomic units (OTUs) of each industrial sample had the potential to play important roles in the bioremediation and biodegradation of pollutants. Dominant OTUs belonging to the phyla *Planctomyces* from the Chemreem sample could not be classified to any genera and are likely to represent novel species.

Keywords Bacteria · Wastewater · Microbial diversity · 16S rRNA, Illumina sequencing

Introduction

Industrial wastewaters are generated with a complex and variable composition of organic and inorganic chemical load representing a major source of surface and underground water pollution (Bassin et al. 2017). Though some industries are important contributors to the economy and earn considerable amounts of valuable foreign exchange, their effluents still pose a significant threat to natural ecosystems. Industries manufacturing batteries, steel wires, electrical products, and commercial vehicle washers produce effluents that may contain contaminants such as strong acids, metallic ions, polyaromatic hydrocarbons (PAHs), among other pollutants (Orisakwe et al. 2004; Tekere et al. 2016). While these pollutants tend to be toxic to most forms of life from prokaryotic to eukaryotic, a number of microorganisms have been found to thrive in such environments (Kamika et al. 2016) either directly by utilizing the pollutants as sources of carbon or indirectly by biotransformation of the organic and inorganic compounds (Bassin et al. 2017). Most importantly, however, these

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pollutants have potential to exert selective pressure on microbial communities of receiving environments. Microbial community composition and diversity in aquatic environments are influenced by a combination of environmental parameters such as pH, dissolved oxygen (DO) and salinity, availability of nutrients (Lee et al. 2017), heavy metals (Yao et al. 2017), and anthropogenic land use (Ranjard et al. 2003; Somboonna et al. 2012), especially the introduction of chemical contaminants (Quero et al. 2015). The presence of certain contaminants in the environment may contribute to large alterations in indigenous microbial community structure, which is reflected as changes in species richness often accompanied by shifts in dominance/evenness (Lee et al. 2017), depending on the nature of the pollutants and their impacts on the resident microbes.

Since there is considerable evidence that microorganisms can rapidly adapt to toxic substances in their environment (Ford 1994), researchers have always found it needful to know the microbial composition of any environment, pristine or otherwise. In this regard, culture independent technologies like next-generation sequencing (NGS) are widely used to determine the taxonomic fingerprint of microbial populations in different environments (Paul et al. 2016). Using this metagenomic approach, bacterial communities have been extensively studied in different environments including thermal springs (Magnabosco et al. 2014; Selvarajan et al. 2017a), saltpans (Selvarajan et al. 2017b), inland deserts (Abed et al. 2015), acid mine drainage (Kamika et al. 2016), hydrothermal vents (Xie et al. 2011), ice glaciers (Peter and Sommaruga 2016) and also in different waste water treatment plants (Shchegolkova et al. 2016; Meerbergen et al. 2017) and water reclamation plants (Sekar et al. 2014). The nature of influents plays a role in determining the composition and structure of microbial communities found in wastewater treatment plants (WWTPs), with *Proteobacteria*, *Bacteroidetes* and *Actinobacteria* dominating the microbiota of domestic influents (Wang et al. 2012; Yu and Zhang 2012; Zhang et al. 2012; Gao et al. 2016) while textile influents are dominated by *Planctomycetes, Chloroflexi, Acidobacteria,* and *Chlorobi* (Meerbergen et al. 2017). Bassin et al. (2017) also revealed that the bacterial profile from the effluents of a pesticide chemical industry were dominated by *Proteobacteria, Bacteroidetes, Planctomycetes, Actinobacteria, Verrucomicrobia, Deinococcus-Thermus,* and *Firmicutes*. Based on these preceding findings, one might expect the microbial community structure and composition of wastewater treatment plants treating effluents from different domestic and industries to be different.

Indigenous microbial communities in different effluents play an important role in wastewater treatment all over the world (Yan et al. 2015). For example, nitrogen removal in wastewater is conventionally accomplished by resident nitrifying and denitrifying bacteria (Bassin et al. 2017). Similarly, the activities of sulfur reducing bacteria (SRB) (van den Brand et al. 2015), phosphate accumulating bacteria (PAB) (Meerbergen et al. 2017) and iron oxidizing bacteria (IOB) (Marchenko et al. 2016) are important contributors in the treatment of domestic and industrial waste waters. Moreover, these heterotrophic and chemolithoautotrophic bacteria play important roles in biogeochemical cycles, such as nitrogen, sulfur and carbon cycles (Meerbergen et al. 2017), and pollutant degradation process (Abed et al. 2002), making them suitable candidates for optimal in situ bioremediation strategies. Therefore, it is important to investigate these potentially diverse groups of microbes for efficient biodegradation of organic and recalcitrant compounds in industrial streams (Nzila et al. 2016).

Currently, there has been a spike in the number of studies targeting the microbial diversity of wastewater treatment plants treating composite effluents using high-throughput sequencing (Rani et al. 2008; Sekar et al. 2014; Meerbergen et al. 2017). However, there are limited studies on the microbial diversity of effluents from individual industries. Essentially, there is a lack of detailed information on the microbial diversity of wastewaters generated from different industries before they are mixed with wastewaters from other sources. Hence, this study was aimed at exploring the complete bacterial diversity along with potential functional OTUs of five different industrial effluents based on Illumina next-generation sequencing (NGS) platform as well as determining the effect of physicochemical factors on microbial diversity of each individual sample using redundancy analysis (RDA). Additionally, the study investigated bacteria that are potentially actively involved in wastewater treatment processes.

Materials and methods

Sample collection and physicochemical characteristic analysis

In the present study, samples were collected from five different industrial plants located in Gauteng province, South Africa, which was involved in the production of automotive batteries (Dixon), high-tensile fencing and barbed wire (CWI), steel wire and its related products (Capegate), coating of steel in zinc (Ford), and washing of petrol and chemical tank trucks (Chemreem). These industries produce large amount of effluents which contain toxic waste and high loads of organic matter, which is treated by a waste water treatment plant before their final discharge to Vaal River. Mixed liquor wastewater samples were collected separately using pre-sterilized glass bottles from the above said five industries. The collected samples were immediately kept at 4 °C in a cooler box and transported to the laboratory at the University of South Africa (UNISA) Florida Campus, South Africa.

One set of samples were used for the analysis of chemical oxygen demand (COD), nitrate (NO_3), phosphate (PO_4) while the second set of samples meant for trace metal analysis was collected into pre-washed acid (HNO_3) glass containers. Physicochemical parameters such as temperature, pH, conductivity, salinity, total dissolved solids (TDS) and dissolved oxygen (DO) were measured and recorded *on site* using a Multi-Parameter Meter (Hanna Instruments PTY LTD, Johannesburg, RSA). In the laboratory, collected water samples were homogenized using homogenizer and then filtered using no. 1 filter paper (Whatman, USA) prior to the analysis of COD, NO_3, PO_4 and metal contents. The closed reflux method was used to quantify the COD concentration following the methodology of APHA (APHA 2001) while the biochemical oxygen demand (BOD) was determined electrochemically. The concentrations of NO_3 and PO_4 were determined calorimetrically following a method described (Dickman and Bray 1940; Pappenhagen 1958), while heavy metals and metalloids were determined using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (PerkinElmer Optima 5300 DV).

Genomic DNA extraction and Illumina sequencing

Samples collected for metagenomic analysis were initially filtered using 1.6 μm pore-sized GF/A filters to remove solid impurities, followed by filtering through 0.22 μm pore-sized polyethersulfone membrane filters (Millipore, USA) using a peristaltic pump as required to concentrate the microbial cells. After filtration, the membrane filters were suspended in 50 mL of phosphate saline buffer (PBS) and centrifuged at 12,000 RPM for 5 min at 4 °C. Cell pellets were collected and resuspended in Tris-EDTA (TE) buffer (pH 8.0) and subjected to total DNA extraction using the Quick *g*-DNA Extraction Kit™ (Zymo Research Corporation, USA) according to the manufacturer's protocol. The eluted DNA was assessed for purity on 1.0% agarose gel and then quantified using a NanoDrop spectrophotometer (Nanodrop 2000, Thermo Scientific, Japan). Polymerase chain reaction (PCR) was performed on the extracted DNA samples using the universal bacterial primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) (Saiki et al. 1988) and 518R (5′-GTATTACCGCGGCT GCTG G-3′) (Muyzer et al. 1993) targeting the variable region V1-V3 of the 16S ribosomal DNA. PCR reactions were prepared using 25 μL of one *Taq* 2X Master Mix, 22 μL of nuclease-free water, 1.5 μL of both forward and reverse primers at a concentration of 0.2 μM and 2 μL of extracted DNA (50–100 ng μL^{-1}). Following thermal cycler program was used for the 16 s rRNA gene amplification; initial denaturation step at 95 °C for 10 min, followed by 32 cycles of denaturation at 95 °C for 30 s; annealing at 55 °C for 30 s; extension at 72 °C for 1 min; final extension at 72 °C for 10 min. PCR amplicons were purified using a DNA Clean & Concentrator Kit (Zymo Research Corporation, USA) according to the manufacturer instructions. The purified PCR products were then sequenced along with its multiplex sample identifiers on the Illumina Mi-Seq platform by Inqaba Biotechnology (Pretoria, South Africa).

Sequence data analysis

The obtained raw sequence datasets were initially scrutinized for PCR artifacts and low-quality reads using *ngsShoRT* (next-generation sequencing Short Reads) trimmer as described by Chen et al. (2014). Following the initial screening process, all the sequence data sets were processed by the using Mothur pipeline (Schloss et al. 2009). Sequence reads containing less than 50 nucleotides, reads with more than 2% of ambiguities or 7% of homopolymers were excluded during the course of analysis. Similarly, sequences that belong to the mitochondrial and chloroplast origins were also excluded from the analysis and the chimeric sequences were removed by using UCHIME algorithm according to the de novo method (Edgar et al. 2011). Non-chimeric rRNA (490 nt) reads were later aligned against the SILVA 16S rRNA gene database (version 128) and a pairwise distance matrix was created from the curated aligned datasets to group sequences into Operational Taxonomic Units (OTUs) at a confidence threshold of 97%. Further classification of reads were done to genus level using the Naïve Bayesian classifier algorithm against the SILVA 16S rRNA gene database with a confidence threshold of 80% to assign taxonomic identity (Wang et al. 2007; Quast et al. 2013). Dominant bacterial OTUs were further subjected to BLAST analysis to compare their identity using the NCBI-BLAST tool (Johnson et al. 2008). Phylogenetic analysis was done using Molecular Evolutionary Genetic Analysis v6 (MEGA6) software (Tamura et al. 2013). The nonparametric diversity indices including Shannon–Weaver index and the Chao1 richness estimator were calculated at the genetic distance of 0.03 to measure the diversity of bacterial species among the data sets. Coverage value was calculated by using Good's formula. The percentage of relative abundance of individual taxa within each community was estimated by comparing the number of sequences assigned to a specific taxon against the number of total sequences obtained for that sample. The top OTUs at genus level were used to generate a phylogenetic heatmap and to visualize the pattern of industrial wastewater bacterial community variation and distribution along with the measured environmental variables, redundancy analysis (RDA) was plotted using XLSTAT (Addinsoft, USA). This method was chosen chiefly because it is a direct gradient analysis technique which summarizes linear relationships between components of response variables that are explained by/redundant with a set of explanatory variables. All the statistical analyses were performed by using PAST software package (Hammer et al. 2001).

Availability of data and material The sequence data sets were submitted to NCBI Sequence Read Archive (SRA) under the accession number (SRP117902) for public access.

Results

Physicochemical quality of industrial wastewater

The physicochemical profiles of the different industrial wastewater samples are presented in Table 1. Wastewater temperature ranged from 18.9 to 21.5 °C. Four industrial wastewater samples had slight to high alkaline pH ranging from 8.5 (Ford) to 12.8 (Chemreem) while Dixon wastewater had highly acidic (4.6) pH. The concentration of TDS was high in Capegate (4611 mg/L), while it ranged from 1553 to 2487 mg/L for other industrial wastewater samples. Dissolved oxygen was high in Chemreem (2.95 mg/L) and low in Ford (1.26 mg/L), while it ranged from 1.85 to 2.43 mg/L in other samples. Among the nutrients, concentration of sulfur was significantly higher ($p < 0.05$) in Dixon wastewater (450 mg/L) compared to the other industrial wastewaters where it ranged from 7.13 to 27.7 mg/L. Similarly, the concentration of phosphate was relatively higher than nitrate in four industrial effluents except Ford sample. Heavy metals like magnesium, aluminum, silicon, and zinc were detected in considerably higher concentrations, while boron, lead, and strontium were present at relatively lower concentrations in collected wastewater. However, the concentrations of barium for three industrial samples were under the detection limit.

Microbial diversity

A total of 90,924 quality filtered reads were obtained from the five industrial wastewater samples after removal of PCR artifacts, and chimeric sequences were used further in the present study. About 70 quality reads were classified under archaea, in which 94.28% were closely related to *Euryarchaeota* and 5.72% represented *Woesearchaeota* (data not shown). As for bacterial diversity, the result showed 41 phyla, 111 classes, 198 orders, 380 families and 856 genera in all industrial wastewater samples (S. Table 1). The quality reads of bacteria distributed into 8793 OTUs from all industrial wastewater were as follows: CWI 9.76%, Capegate 18.04%, Ford 22.10%, Chemreem 23.08% and Dixon 27.01%. Chao1 index considered as expected OTU richness estimator showed the

Table 1 Physicochemical parameters of collected wastewater samples from five different industries

Parameters	Units	Capegate	Chemreem	CWI	Dixon	Ford
Temperature	°C	21.5 ± 0.44	19.39 ± 0.01	19.8 ± 0.02	20.4 ± 0.48	18.9 ± 0.50
pH	–	11.5 ± 0.07	12.8 ± 0.56	10.4 ± 0.04	4.6 ± 0.52	8.5 ± 0.01
Dissolved oxygen	mg/L	1.85 ± 0.01	2.95 ± 0.02	2.06 ± 0.01	2.43 ± 0.24	1.26 ± 0.12
Conductivity	μS/cm	9224 ± 33.6	4979 ± 43.5	3555 ± 21.02	2703 ± 13.9	3921 ± 24.2
Salinity	ppm	5.17 ± 0.07	0.82 ± 0.01	22.81 ± 0.37	16.62 ± 0.02	2.05 ± 0.01
TDS	mg/L	4611 ± 141.4	2487 ± 58.20	1801 ± 37.47	1553 ± 142.83	1964 ± 147.78
Nitrate	mg/L	1.91 ± 0.01	3.93 ± 0.05	2.63 ± 0.01	8.77 ± 0.02	6.29 ± 0.04
Phosphate	mg/L	3.28 ± 0.01	7.76 ± 0.14	5.98 ± 0.13	9.25 ± 0.88	1.16 ± 0.02
Total sulfur	mg/L	7.13 ± 1.69	15.7 ± 3.46	22.3 ± 1.13	450 ± 84.14	27.7 ± 1.55
Silicon	mg/L	3.66 ± 0.03	4.8 ± 1.5	0.2 ± 0.01	18.7 ± 0.65	3.65 ± 0.2
Calcium	mg/L	44.8 ± 13.93	324 ± 34.64	337 ± 78.48	19.2 ± 0.98	44.8 ± 9.75
Potassium	mg/L	12.1 ± 1.06	5.94 ± 0.77	53.3 ± 8.48	9.19 ± 1.90	6.44 ± 0.60
Magnesium	mg/L	14.5 ± 0.49	32.6 ± 3.50	94.6 ± 6.35	110 ± 2.12	4.55 ± 0.44
Sodium	mg/L	474 ± 107.83	299 ± 34.5	54.3 ± 1.27	264 ± 26.16	321.5 ± 16.61
Aluminum	mg/L	20.31 ± 2.18	19.4 ± 3.29	16.1 ± 0.18	16.2 ± 2.43	6.75 ± 0.22
Boron	mg/L	12.3 ± 0.35	22.5 ± 0.60	9.25 ± 1.05	0.2 ± 0.01	7.81 ± 0.05
Barium	mg/L	Trace	0.24 ± 0.02	0.2 ± 0.01	Trace	Trace
Lead	mg/L	0.2 ± 0.01	0.2 ± 0.01	0.43 ± 0.02	5.06 ± 0.05	0.2 ± 0.01
Strontium	mg/L	0.23 ± 0.01	0.61 ± 1.13	1.34 ± 0.09	6.61 ± 0.99	6.56 ± 2.96
Zinc	mg/L	0.78 ± 0.02	5.28 ± 0.39	8.51 ± 2.28	50.6 ± 3.50	207 ± 17.21
BOD	ppm	1.55 ± 0.12	1.39 ± 0.09	1.53 ± 0.12	1.83 ± 0.67	1.47 ± 0.13
COD	ppm	295.4 ± 12.72	948.6 ± 93.16	893.6 ± 57.34	534 ± 72.83	491.6 ± 60.35

Measurable quantities of trace elements lower than < 0.2 mg/L considered as "Trace"

TDS, total dissolved solids; BOD, biological oxygen demand; COD, chemical oxygen demand

lowest number of OTUs richness in Capegate wastewater (974 OTUs) and the highest in Dixon effluent (4552 OTUs), respectively. Based on Goods formula, the coverage of the samples ranged between 81.96 and 90.86%, suggesting that the most dominant bacterial communities were covered in the present study. Similarly, to Chao1, the diversity index was estimated by the Shannon-H index, showed highest in Dixon wastewater (6.04) and lowest in CWI wastewater (4.15). Detailed information concerning the recovered OTUs, percentage of coverage, Chao1 and Shannon indices is shown in Table 2.

Phylogenetic classification revealed the distribution of 41 phyla across all industrial effluent samples. Of these, the four most dominant phyla were, in order of magnitude of dominance; *Proteobacteria* whose relative abundance ranged from 17.14% in Capegate to 75.87% in Dixon, *Actinobacteria* (1.95% in Dixon to 39.66% in Capegate), *Firmicutes* (2.05% in Dixon to 34.74% in Capegate) and *Planctomycetes* (1.06% in CWI to 25.21% in Chemreem). Further, substantial reads belonging to the phyla *Bacteroidetes* (0.01–6.06%), *Chlamydiae* (0.2–4.79%) and *Cyanobacteria* (0.01–4.9%) were also identified among all wastewater samples. However, the phylum *Verrucomicrobia* was not recorded in Ford sample; it was, in other samples, represented by 0.16–5.09% of the total 16S rRNA gene sequences. In addition, sequences belonging to some minor phyla with lower frequencies were also found. The distribution of the bacterial phyla obtained from five different industrial effluents is given in Fig. 1.

Further breakdown of phylogenetic classification from phyla into family level showed a total of 380 families were present among all wastewater samples. Within the 380 families detected among all the groups, 26 families were detected with a relative abundance greater than 2% in at least one group. Family *Bifidobacteriaceae* was dominant across all industrial effluents, occurred at a relative abundance of between 0.05% (Dixon) and 35.79% (Capegate) followed by *Rhodobacteraceae* (0.32% in Dixon and 46.01% in CWI). Sequences representing the family *Lactobacillaceae* were high in Capegate (29.62%) and CWI (5.55%), respectively. The fourth most dominant family was *Comamonadaceae*, with a relative abundance in the range 8.36% (Ford) to 17.27% (Dixon) followed by *Phycisphaeraceae* (0.15 to 21.62%), *Enterobacteriaceae* (2.09 to 10.99%), *Moraxellaceae* (0.07 to 15.73%), *Bradyrhizobiaceae* (0.25 to 6.84%), *Methylobacteriaceae* (0.05 to 12.06%), *Sphingomonadaceae* (0.9 to 5.13%), *Alcaligenaceae* (0.17 to 10.05%) and *Rhodocyclaceae* (0.10 to 10.12%). More detailed information on the distribution and relative abundance of bacterial families across the five industrial wastewater samples is given in Fig. 2.

For comprehensive and detailed scrutiny, we restricted in-depth analysis of the sequence data to the 10 OTUs displaying the highest richness in each sampling site. A total of 43 different OTUs were among the top 10, considering the five sampling sites. The top 10 OTUs identified in CWI effluent sample comprised of 57.91% of the total OTUs observed in this site. Similarly, the top 10 OTUs for the other sampling sites constituted 39.93%, 32.55%, 30.85 and 29.48% of the total OTUs recovered in Capegate, Ford, Chemreem and Dixon wastewater samples respectively (S. Fig. 1). To identify the closest relatives of the top 10 OTUs, a phylogenetic tree was drawn (Fig. 3) using maximum-likelihood analysis. Twenty-five (25) of the 43 OTUs were closely related to *Gammaproteobacteria* (14) and *Alphaproteobacteria* (11), followed by *Actinobacteria* (7), *Planctomycetes* (5), and *Firmicutes* (4). OTUs belong to phylogenic clade *Bacteroidetes* and *Chlamydiae* occurred in very low percentages. At the genus level, the distribution of OTUs in the different sampling sites was as follows: Dixon (*Acinetobacter*, 6.78%; *Comamonas*, 5.09%; *Sphingobium*, 3.52%; *Propionivibrio*, 2.70%; *Rhizobium*, 2.20% and *Enterobacter*, 1.50%), CWI (*Seohaeicola*, 24.97%; *Sphingopyxis*, 4.84%; *Rhizobium*, 1.87% and *Terasakiella*, 0.96%), and Ford (*Methylobacterium*, 9.45%; *Achromobacter*, 4.62%; *Bradyrhizobium*, 3.75%; *Herbaspirillum*, 3.48%; *Ralstonia*, 2.12% and *Enterobacter*, 1.67%). Only one genus each was recorded in the samples Capegate (Uncultured_gammaproteobacteria, 1.15%) and Chemreem (*Legionella*, 5.56%), both of which belonged to the phylum *Proteobacteria*.

OTUs for the phylum *Actinobacteria* were the most abundant in Capegate and least in CWI and Ford. Within this phylum, the genus *Bifidobacterium* was dominant in Capegate

Table 2 Summary of diversity indices for industrial wastewater samples

Sampling sites	Raw reads	Processed reads	Total OTUs	Chao1	Coverage %	Shannon (H)
Capegate	49,158	16,404	885	974.4	90.86	4.82
Chemreem	67,343	20,992	1916	2207	86.81	5.55
CWI	27,702	8878	1223	1453	84.17	4.15
Dixon	78,039	24,555	3731	4552	81.96	6.04
Ford	57,251	20,096	1038	1212	85.64	4.94

Chao1, community richness-higher number represents more richness; Shannon, community diversity-higher number represents more diversity; coverage, sampling depth; OTUs, operational taxonomic units

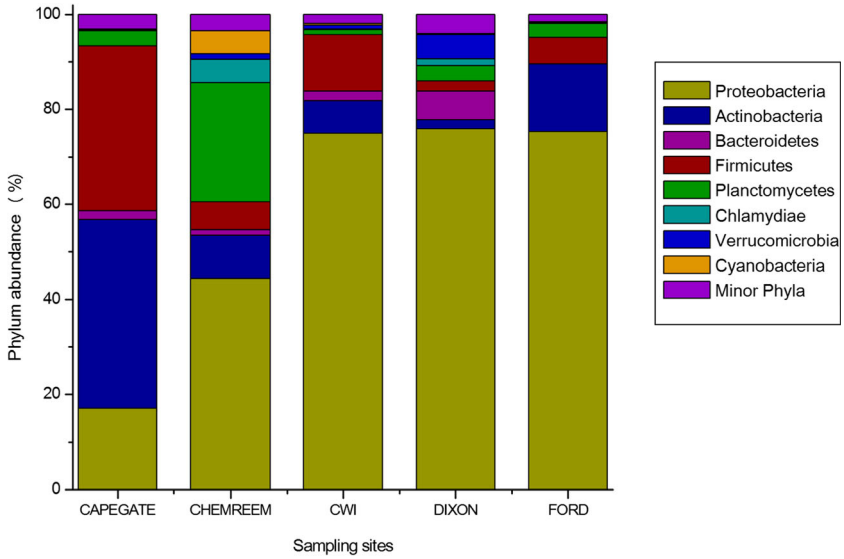

Fig. 1 Relative abundance of bacterial phyla obtained from collected industrial wastewater. (Members of minor phyla included other smaller phylum given in the supplementary)

(16.01%) and CWI (0.94%), while *Aeromicrobium* (1.79%) was recorded in Ford sample. OTUs sequences resembling those of the phylum *Planctomycetes* were only observed in Chemreem effluent sample with a relative abundance ranging from 5.56 to 1.54%. However, these OTUs formed a separate clade in a phylogenetic tree (Fig. 3) and the percentage of the closest similarities in GenBank was below 82% sequence identity (S. Table 4). OTUs of the genus *Lactobacillus* (8.76%) and *Clostridium* (1.15%) belonging to the phylum *Firmicutes* were found abundantly in Capegate sample, while sequences of OTUs corresponding to the phylum *Chlamydiae* and *Bacteroidetes* were relatively low in Chemreem and Dixon wastewater respectively (Fig. 4).

Functional OTUs

Categorization of the bacterial genera into possible functional OTUs formed six possible categories including sulfur

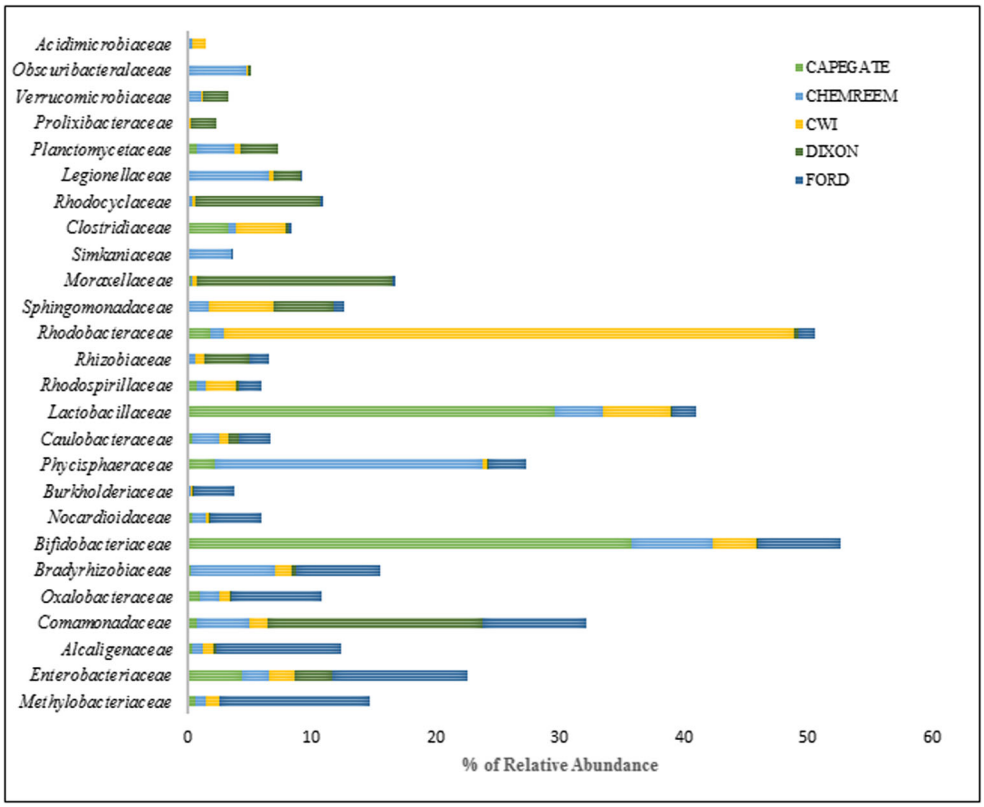

Fig. 2 Relative abundance of various families across the five industrial wastewater samples. (Members of minor families were given in supplementary)

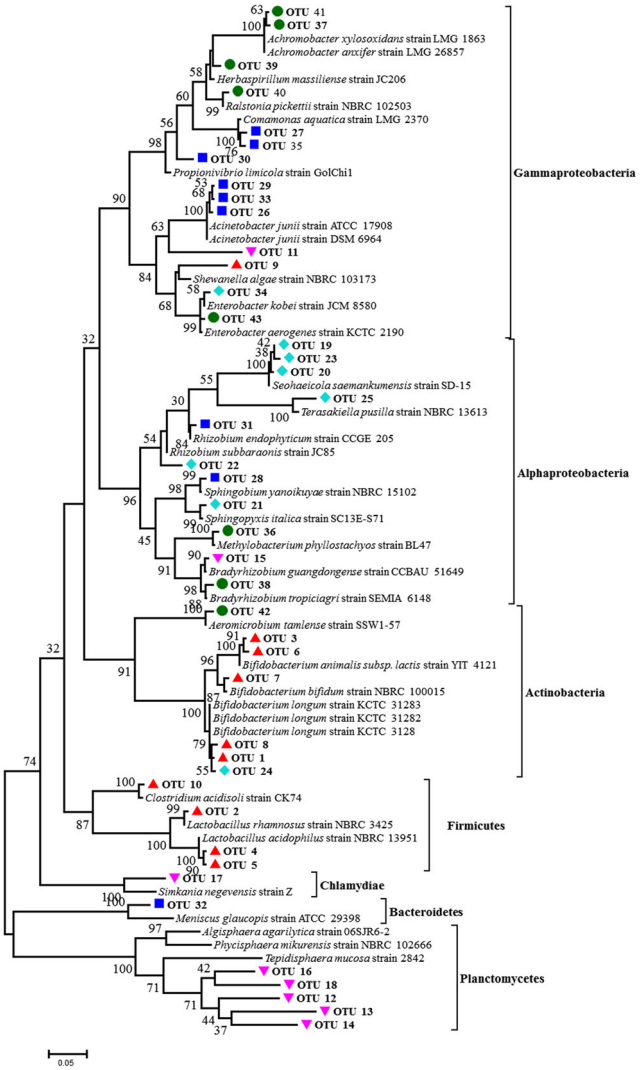

Fig. 3 Maximum-likelihood phylogenetic tree based on top 10 OTUs observed in five different industrial effluents. (Representative OTUs appear in different colors; green—Ford; dark blue—Dixon; cyan blue—CWI; pink—Chemreem, and red—Capegate)

reducing bacteria (SRB), iron oxidizing bacteria (IOB), fermentative acidogenic bacteria (FAB), phosphate accumulating bacteria (PAB), nitrifying bacteria (NB) and denitrifying bacteria (DNB). Figure 5 shows that the sulfur reducing bacterial group constituted 3.10% of the entire bacterial population in the Dixon wastewater; the most dominant genera being *Desulfobulbus, Desulfomicrobium, Desulfovibrio, Desulforegula, Desulfatiferula* and *Desulfobacter* while the SRB in the sample CWI constituted 0.89% of the total bacterial population, the dominant of which were the genera *Desulfosarcina, Desulfovibrio* and *Desulfobacter.* The other three samples covered relatively low percentages of sulfur reducing OTUs, about 0.02–0.27% of the total bacterial communities. Among the iron oxidizers, OTUs of *Aquabacterium* were dominant in Ford (3.57%) and Chemreem (1.27%) while those of *Acidovorax* and *Azospira* were dominant in Dixon (0.85%) and Capegate (0.18%). Other IOB members including *Rhodobacter, Paracoccus* and *Geobacter* were unique to Chemreem and CWI, Capegate and Ford and Dixon effluents, respectively.

The genus *Clostridium* was the most dominant acidogenic bacteria in Capegate, CWI, Dixon and Chemreem. Separately, *Pseudomonas* and *Flavobacterium* were the second most dominant acidogenic bacteria in Dixon wastewater while *Micrococcus* was dominant in Capegate and *Enterobacter* in Chemreem and Ford. Bacteria of the genus *Rhodocyclus* (1.13%) dominated the phosphate accumulating group in Dixon wastewater. Additionally, members of the *Candidate* genera were unique to some samples; *Candidatus_Accumilibacter, Candidatus_Microthrix* and *Candidatus_Competibacter* were present only in Dixon, *Candidatus_Alysiosphaera* were found in Capegate and Dixon while the genus *Candidatus_Azambacteria* was present in Capegate, Chemreem and CWI, respectively.

Nitrifying and denitrifying bacterial members were also recovered in industrial effluents. The genus *Nitrosospira* was the dominant nitrifying bacteria in Chemreem sample, other genera including *Nitrosomonas* (Chemreem), *Nitrobacter* (Ford), *Candidatus_Anammoximicrobium* (CWI) and *Candidatus_Omnitrophus* (Dixon) were encountered in relatively low percentages. Similarly, the genera *Acinetobacter* (Dixon), *Achromobacter* (Ford), *Acidovorax* (Dixon), *Brevundimonas* (Ford) and *Comamonas* (Dixon) dominated among the denitrifying bacteria (Fig. 6). The graphical representation of the relationship between physicochemical variables and microbial diversity of each individual sample as determined by redundancy analysis (RDA) is shown in Fig. 7. RDA Axis 1 explained 49.01% of the variance while Axis 2 explained 35.30% of the variance in the bacteria-environmental parameters relationship. In the Dixon and Ford samples, the distribution of the phyla *Proteobacteria, Cyanobacteria,* and *Verrucomicrobia* was influenced by a combination of parameters such as DO, NO_3, BOD, Zn, Pb, PO_4, S and Mg concentrations. In Capegate samples, members of the phyla *Actinobacteria* and *Firmicutes* were correlated to pH, conductivity, Al and TDS whereas *Bacteroidetes* was correlated to Na, Fe and P. Finally, bacterial phyla distribution in Chemreem and CW1 samples followed an almost similar pattern where the phyla *Planctomycetes* and *Chlamydiae* were correlated to K, Ca, Mn, COD and salinity.

Discussion

Environmental discharge of untreated industrial effluents leads to the deterioration of environmental and ecological quality (Zhang et al. 2015). Microbes accommodated in different industrial wastewaters have the potential of remediating xenobiotic pollutants under various extreme conditions (pH,

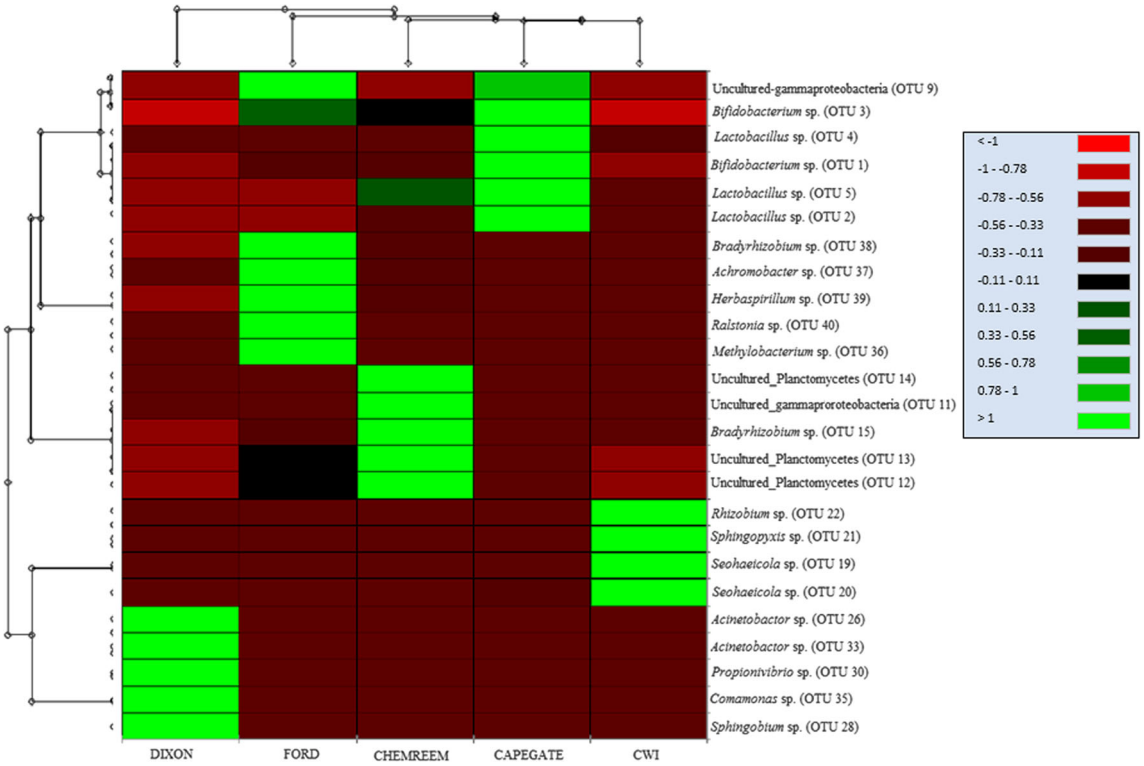

Fig. 4 Heat map indicating the clustering of top 10 OTUs representing genera from five different industrial effluents. The color indicates the relative abundance of OTUs in the samples

temperature or salinity). Such effluents carry a diverse population of microbes that convert organic and inorganic compounds to energy and other cellular components to adapt and survive indigenously. Therefore, it is very important to identify resident microorganisms of industrial effluents in order to better understand the ideal in situ bioremediation strategies and microbial food webs (Ben-Dov et al. 2008). As at present, this study is one of a few studies (Hülsen et al. 2018) to present a comprehensive comparative sequence analysis of bacterial community diversity in different industrial milieu such as battery industry, commercial vehicle washing industry, galvanizing industry and steel and wire industrial wastewater.

Bacterial community analysis from the different wastewater effluents was achieved using an Illumina high-throughput sequencing platform. While classifying the sequences, about 70 sequences were classified under the archaeal domain, showing that 27F and 518R were universal 16S rRNA primers for a broad range of prokaryotic members, which could yield accurate phylogenetic information (Ma et al. 2015a). For archaea, the genus *Methanomicrobia* (phylum *Euryarchaeota*) was dominant in all samples, accounting for 0.05–0.8%, similar to the findings of previous studies of textile and municipal wastewater (Meerbergen et al. 2017). The remaining sequences (over 99.9%) were classified under the bacterial domain, which yielded 41 phyla, 111 class, 198 order, 380 families and 856 genera in all wastewater samples (S. Table 1). Results showed that OTU richness from different industrial wastewater ranged from 885 to 3731, which was higher than the average OTUs reported previously from gold and vanadium (1315 OTUs) wastewater (Keshri et al. 2015), acid mine (960 OTUs) wastewater (Kamika et al. 2016), textile (196 OTUs) and municipal (297 OTUs) wastewater (Meerbergen et al. 2017), activated sludge treatment plant (1063 OTUs) with different wastewater (Shchegolkova et al. 2016), biofilm reactor (640 OTUs) treating a chemical industrial effluents (Bassin et al. 2017) and lower than full-scale wastewater treatment plant (8652 OTUs) of different industrial effluents (Shu et al. 2015).

Furthermore, based on the number of OTUs, the community diversity (Shannon) and richness (Chao1) estimators were calculated along with Good's Coverage at the cut off level of 3% (Table 2). Good's coverage of the five wastewater samples ranged from 82 to 91%, indicating that the most dominant bacterial communities were covered in our study. The Shannon diversity values ranged from 4.82 to 6.04 and the highest was observed in Dixon wastewater. These values are in accordance with those reported from other industrial and mine wastewater (Keshri et al. 2015; Kamika et al. 2016; Meerbergen et al. 2017). However, another bacterial diversity study conducted from a coking wastewater treatment plant in the steel industry (Ma et al. 2015a) produced diversity indices between 3 and 4.40. In a different study, low diversity values ranging from 2.89 to 3.86 were obtained from an anoxic-aerobic moving-bed biofilm reactor system treating a

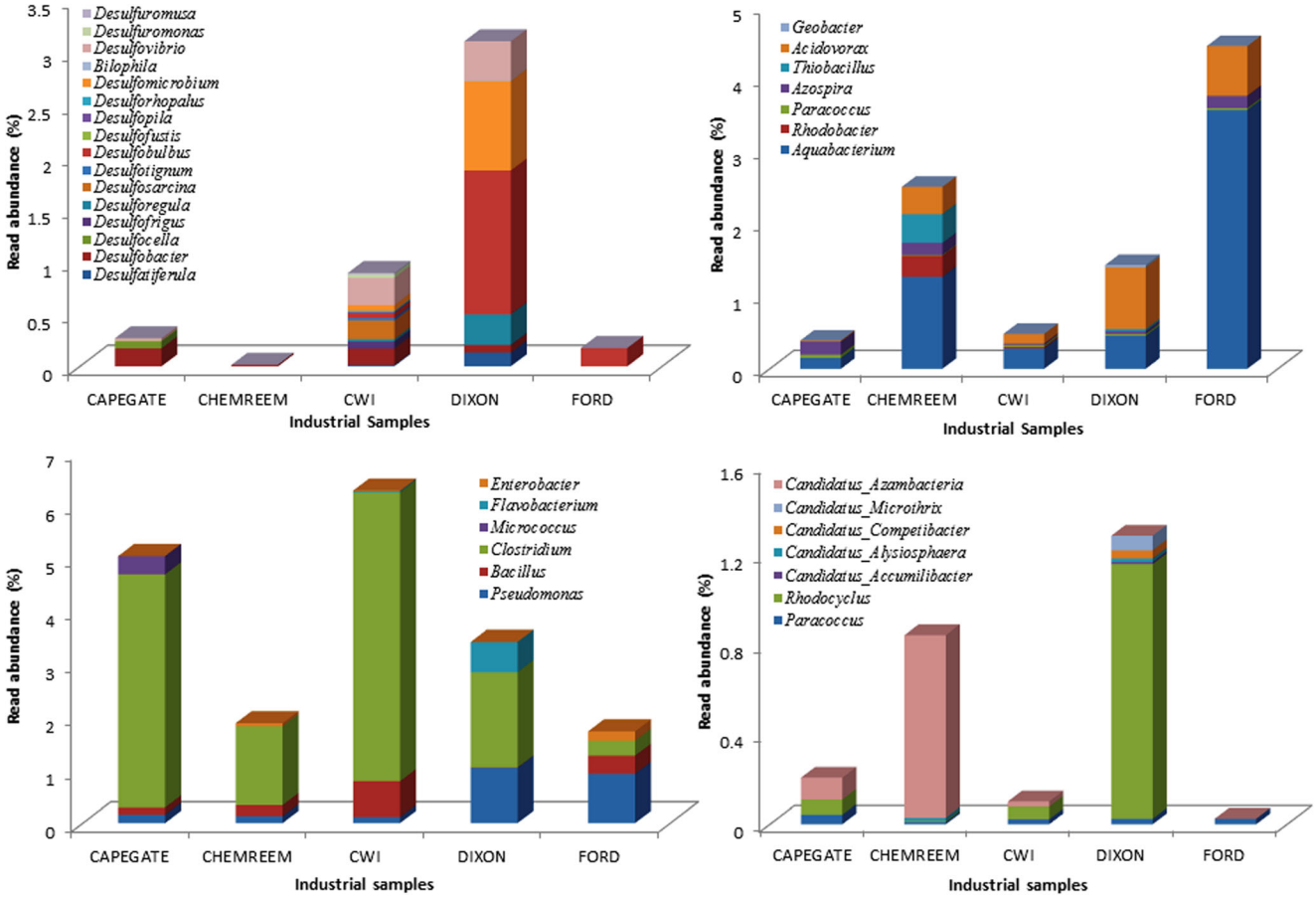

Fig. 5 Probable functional OTUs showing the distribution and abundance of (1) sulfur reducing bacteria, (2) iron oxidizing bacteria, (3) acidogenic bacteria, and (4) phosphate accumulating bacteria across industrial wastewater

chemical industry wastewater in Brazil (Bassin et al. 2017). Similarly, the community richness values ranged from 974 to 4552, which was higher than the values reported from textile and municipal WWTP (Meerbergen et al. 2017), coal mine wastewater (Ma et al. 2015b) and domestic and industrial WWTP (Ahmed et al. 2017). These results indicate that studied industrial wastewaters may harbor higher microbial diversity and species richness compared to other industrial streams.

Recent studies have revealed that the phylum compositions were different in different industrial and domestic wastewater, For instance, analysis of the bacterial community composition of a textile wastewater by Meerbergen et al. (2017) showed that *Proteobacteria* (44.3%) were the most dominant followed by *Bacteroidetes* (24.8%). In another study involving the bacterial community composition of an industrial wastewater reclamation plant in South Africa, the phyla *Proteobacteria, Firmicutes* and *Actinobacteria* were present in each treatment stage, whereas members of the *Bacteroidetes* were not recorded (Sekar et al. 2014). Elsewhere, four major phyla including *Proteobacteria, Bacteroidetes, Firmicutes* and *Chloroflexi* were detected in pharmaceutical (Tao et al. 2016), petroleum refinery (Silva et al. 2012), steel industry (Ma et al. 2015a), sewage and industrial wastewater treatment plants (WWTPs) (Ibarbalz et al. 2013) in different relative abundances. Consistent with previous studies, *Proteobacteria* was present in relatively higher percentages (44.44–75.86%) in four industrial effluents except Capegate sample (producer of mild steel wire and wire products), where *Actinobacteria* (39.66%) was dominant. After *Proteobacteria,* the phylum *Planctomycetes* (25.21%) was abundant in Chemreem; *Bacteroidetes* (6.06%) in Dixon, members of *Actinobacteria* (14.21%) and *Firmicutes* (11.87%) were dominated in Ford and CWI samples, respectively. In Capegate sample, *Firmicutes* represented the second most dominant phylum followed by *Proteobacteria*. Our study suggests that bacterial distribution between the industrial wastewater did not share the characteristic profile of high bacterial rank, which is commonly observed in domestic and municipal wastewater (Ibarbalz et al. 2013), but exhibited a unique composition of bacterial communities. Other bacterial phyla such as *Chlamydiae* and *Acidobacteria* (Chemreem and Dixon), *Cyanobacteria* (Chemreem), *Verrucomicrobia* (Dixon) were also present as major phyla. Members of these phyla have previously reported to be widespread in different wastewater treatment systems,

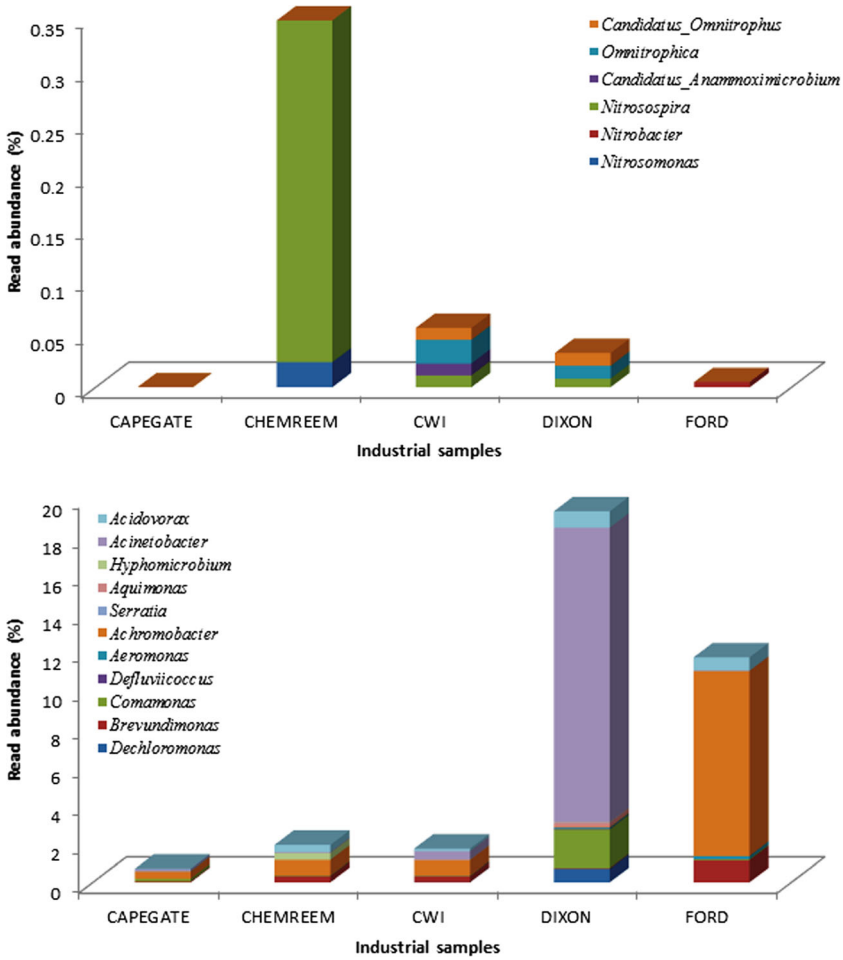

Fig. 6 Probable functional OTUs showing the distribution and abundance of nitrifying (top) and denitrifying bacteria (bottom)

suggesting these bacteria play the key roles in nutrient removal processes (Ma et al. 2015b).

To simplify the results, we selected the top 10 OTUs in each industrial wastewater for comparison. In total, 43 OTUs (S. Fig. 1) were obtained from five different industrial effluents. OTUs for the genus *Bifidobacterium* (16.01%) belonging to the family *Bifidobacteriaceae* was dominant in Capegate, and also in CWI (Fig. 2). Previously, *Bifidobacterium* was reported in sewage and wastewater from several animal slaughterhouses where its presence was mainly attributed to human and animal fecal matter (Ballesté and Blanch 2011). However, this is the first report detailing the presence of *Bifidobacterium* (*Actinobacteria*) in industrial wastewater and our RDA results indicate that its presence was influenced by high concentration of pH, TDS and some metals (Fig. 7). Coming second was the genus *Lactobacillus* (8.76%) belonging to the family *Lactobacillaceae* which is known to produce exopolysaccharides (EPS), and reported for the treatment of textile and olive mill wastewater (Bronze et al. 2008; Sayilgan and Cakmakci 2013). OTUs whose sequences resembled those of the genus *Acinetobacter* (OTU 26, 29, 33), *Sphingobium* (OTU 28), *Comamonas* (OTU 27) and *Propionivibrio* (OTU 30) were dominant in Dixon wastewater. However, these isolates play different roles in wastewater treatment process, for example, the genus *Acinetobacter* (*Gammaproteobacteria)* and *Comamonas (Betaproteobacteria)* were reported as major components in industrial WWTPs that are able to degrade aromatic compounds (phenols), polycyclic aromatic hydrocarbons and heterocyclic aromatics, such as indole, quinolone and carbazole (Felföldi et al. 2010; Poi et al. 2017). Similarly, bacteria of the genus *Sphingobium* and *Propionivibrio* play important roles in the bioremediation and biodegradation of pollutants such as carbamate pesticides (Yan et al. 2010) and removal of phosphorous from different wastewaters (Albertsen et al. 2016), respectively.

Seohaeicola members (OTU 19, 20, 23) were the most dominant genus in CWI effluent sample, these OTUs belonged to the family *Rhodobacteraceae (Alphaproteobacteria)* that are known to frequently thrive in marine environments. The presence of this genus was possibly influenced by the high saline conditions (22.81 ppm), which was also evidenced by our RDA results (Fig. 7). OTUs with sequences resembling

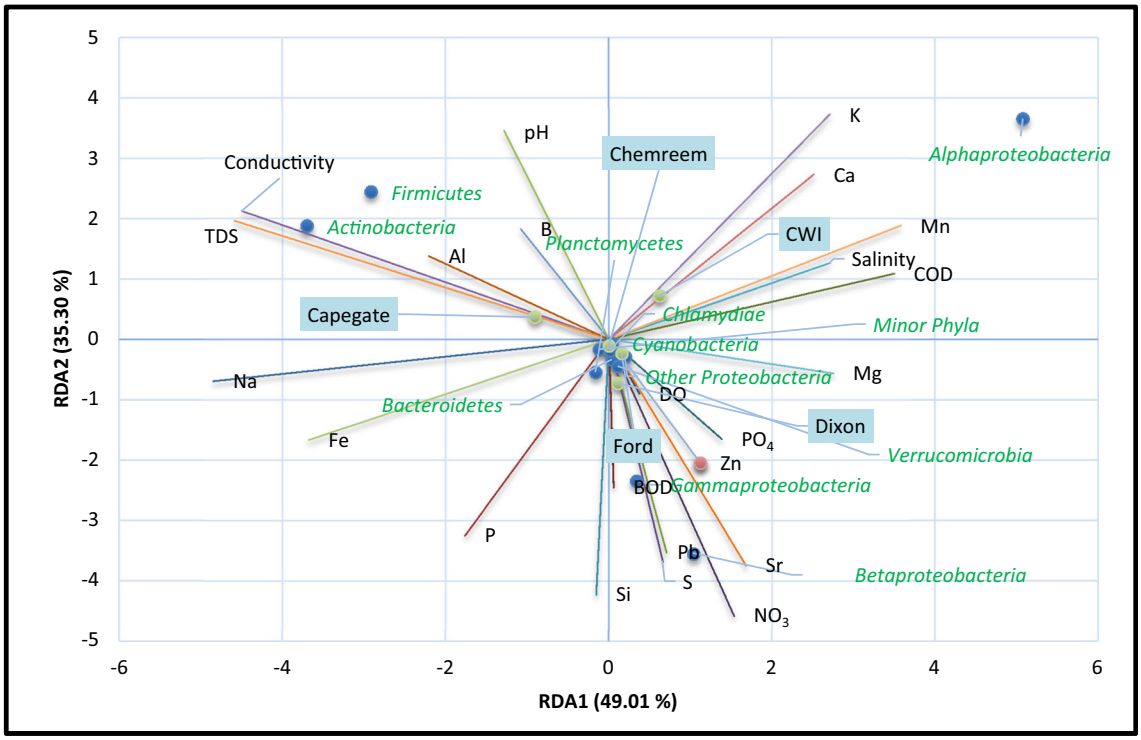

Fig. 7 Redundancy analyses (RDA) plot evaluating the effect of physicochemical parameters on bacterial community structures

the genus *Rhizobium* (OTU 22) and *Sphingopyxis* (OTU 21) were the second and third most abundant in this sample, respectively. Species belonging to these genera were previously reported to be able to degrade various xenobiotic substrates in oil polluted saline environments (Kim et al. 2014; Sellami et al. 2015). OTUs belonging to the families *Methylobacteriaceae* and *Ralstoniaceae* were predominantly detected in Ford sample (Fig. 2), with the most dominant genera being *Methylobacterium* (9.45%) and *Ralstonia* (2.12%). Previously, it was reported that the genera *Methylobacterium* and *Ralstonia* have the ability to degrade volatile toxic halogenated solvents (Muller et al. 2011) and petroleum hydrocarbons (Płaza et al. 2008), respectively, as well as tolerate high doses of heavy metals such as Boron (B) and Zinc (Zn) (Kunito et al. 1997), which were also abundant in this effluent sample. Other members such as *Achromobacter* (4.62%), *Bradyrhizobium* (3.75%) and *Herbaspirillum* (3.48%) were known denitrifying heterotrophic bacteria involved in denitrification during wastewater treatment (Kathiravan and Krishnani 2014). Interestingly, the dominant members (OTU 12, 13, 14, 16, 17, and 18) from the sample Chemreem were not assigned to any genera. Additionally, blast analysis showed that these OTUs were distantly related to any known bacterial genera using the 16S rRNA sequence database (closest relative similarity range 80–89%) (S Table 4). It was also confirmed through phylogenetic analysis where all these OTUs formed a separate clade under the phylum *Planctomycetes* (Fig. 3). These uncultured bacteria are likely to represent novel isolates from this industrial sample and might play important roles in wastewater treatment processes and required further comprehensive evaluations in future studies.

Besides the top 10 OTUs, possible functional OTUs were also identified (Figs. 5 and 6) in the industrial effluents. Zhao et al. (2008) stated that fermentative acidogenic bacteria (FAB) play a key role in breakdown of organic matter to yield hydrogen, ethanol and volatile fatty acids (VFAs). FAB members such as *Clostridium* (Capegate, Chemreem, CWI and Dixon) and *Pseudomonas* (Ford) convert monomers to VFAs such as acetate, propionate, butyrate, isobutyrate, valerate and isovalerate, which are then utilized by SRB members as electron donors for efficient reduction processes (Jabari et al. 2016). Additionally, *Pseudomonas* has the ability to degrade diverse chemical pollutants (Ma et al. 2015b). The higher in SRB populations in Dixon sample compared to other effluents samples could have been due to high concentration of sulfur accommodated in the sample (Table 1). The genus *Desulfobulbus, Desulfomicrobium and Desulfovibrio* were more abundant in the present study compared to their relative presence in textile and municipal wastewater as reported by Meerbergen et al. (2017). Other genera including *Desulforegula, Desulfatiferula* and *Desulfobacter* have not been reported elsewhere in industrial wastewater treatment systems. *Nitrosospira* (0.33%) and *Nitrosomonas* (0.02%) were two important ammonium oxidizing bacteria (AOB) dominant in Chemreem sample. Contrary to our findings, Ma et al. (2015a) reported that members of *Nitrosomonas* were dominant in steel industrial WWTPs compared to

Nitrosospira, which they attributed to its high growth rate. According to Bassin et al. (2017), high concentrations of COD could provide favorable conditions for potential nitrifiers, which is in agreement with our results. Similarly, members of the denitrifying bacteria were dominant in Dixon and Ford samples, respectively. Notably, sequences of the bacterial genus *Acinetobacter* (Dixon – 15.33%) and *Achromobacter* (Ford – 9.61%) were found to be relatively higher, which could play potentially significant roles in denitrification processes (Juretschko et al. 2002; Kathiravan and Krishnani 2014) in the wastewater. *Aquabacterium* and *Acidovorax* were the dominant iron oxidizers in this study. Bacteria of the genus *Rhodocyclus* were dominant among the phosphate accumulators, constituting 0.05–1.13% of the entire bacterial population across all sampling sites. They are well-known phototrophic acyltransferase containing bacteria involved in phosphate accumulation, and iron and sulfur-oxidizing processes (Ferrer et al. 2011) using nitrate as an electron acceptor. Though it was not found in relatively high abundance in the industrial wastewater in this study, the role played by this versatile genus should not be neglected. On other hand, RDA confirmed that the elements such as Mn, Pb, Zn or Sr are more relevant with respect to microbial diversity in wastewater. However, further investigations are needed to reveal the temporal dynamic relationship between the microbial community composition and physicochemical factors.

In conclusion, the present study reports the complete bacterial community analysis of five different industrial effluents using a high-throughput Illumina sequencing approach. To the best of our knowledge, this stands as the first report in South Africa providing an insight into the bacterial community structure from different industrial wastewater. In summary, the phylum *Proteobacteria* occurred in relatively higher percentages in the four industrial effluents except in Capegate sample where *Actinobacteria* was dominant. Our study suggests that bacterial distribution in the industrial wastewaters did not share the characteristic profile of high bacterial rank commonly observed in domestic and municipal wastewater, but exhibit a unique composition of bacterial communities. The estimated community diversity and richness indicated that studied industrial wastewater may harbor higher microbial diversity and species richness compared to other industrial streams. Dominant OTUs in each industrial wastewater sample were reported as potential strains for bioremediation processes. However, OTUs belonging to the phyla *Planctomyces* identified in Chemreem sample could not be classified to genus level, suggesting that these OTUs are likely to represent novel bacterial species. Further, analysis of RDA explained that physicochemical parameters play an important role in defining the bacterial community structure within the wastewater.

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Compliance with ethical standards

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest The authors declare that they have no competing interests.

Ethical approval This study does not contain any study with human participants or animals performed by any of the authors.

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