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Control of disinfection resistance microbes in drinking water treatment plant abstract its water from the downstream canal

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Abstract
Drinking water treatment plants (DWTPs) are supposed to be one of the most important operation challenges. For that, raw water resources must be selected and controlled. DWTPs facilities must be qualified and be efficient to give good and healthy quality. In this view, assessments were focused on nitrogenous compounds and microbial load of raw water of thedownstream canal and its relationship. The comprehensive removal efficiency of physicochemical and biological contaminants during thetreatment process was followed up. Raw water during the period of study which extended for a full annual cycle had levels of contaminants that were not suitable for proceeding through different stages of treatment (physically, chemically, and biologically). Raw water ammonia-N ranged between 0.23 ppm and 11.06 ppm and the mean value was 0.53 ppm. On the other side, biological parameters had more interest in which tests were applied on raw, clarified, filtered, and treated. The result of treated water was accepted and good where there is the removal of contaminants step by step during the treatment process. At high ammonia concentration, the turbidity has a negative correlation with the removal of microorganisms after injection of initial chlorine.

Keywords: Drinking water; downstream canal; microbial load; Ammonia; water quality

1. Introduction

In recent years, the increased use of water resources with the environmental pollution that accompanies it has attracted widespread attention. For, the removal of chemical and microbiological contaminants, drinking water treatment plants (DWTPs) operate with a combination of different processes (Bruno et al., 2018). The role and the impact of the treatment process on such microorganisms inhabiting downstream canal ecosystems and DWTP has not yet been fully elucidated.

Limiting microbial growth during drinking water operation systems is achieved by maintaining a disinfectant residual. The impact of these contrasting approaches on the drinking water microbiome is not systematically understood (Dai et al., 2020). While bacteria were the abundant domains, Archaea and Eukaryote were more abundant in non-disinfected and disinfected systems, respectively (Garner et al., 2018). Community-level differences in functional potential were driven by the enrichment of genes associated with carbon and nitrogen fixation in non-disinfected systems (Jia et al., 2015).

DWTPs, have some key issues associated with them. These include esthetic and corrosion-related problems (Rosario-Ortiz et al., 2016; Kooij et al., 2013; Kooij and Wielen, 2013) but more importantly the formation of harmful disinfection byproducts (DBPs) (Richardson, 2003; Sedlak and von Gunten, 2011; Li and Mitch 2018), which are also regulated. Further, there is an increasing recognition that the disinfectant residuals may be associated with the limitation of microbial number (Wanget al., 2013; Falkinham et al., 2013) that has coagulation properties in drinking water treatment (Zhang et al., 2019; Shi et al., 2013; Sevillano et al., 2019).

The problems of increasing ammonia in water resources lead to a suitable water shortage for human populations in certain areas (Rashida et al., 2015). In addition, microbial contaminants and organic contaminants were found in raw downstream water resources which have added constraints on the traditional drinking water treatment techniques and have led to the formation of chlorine by-products. These compounds have a carcinogenic effect. The reports on the mechanism of disinfectant resistance are not comprehensive enough, and the summary of the spread of disinfectant resistance is rarely mentioned. Therefore, comprehensive studies of the disinfectant resistance mechanisms and understanding of the resistance influencing factors are essential to solve the disinfectant resistance problem (Tong et al., 2021).

DWTP may be shut down whenever it receives exceeded allowable ammonia level in water resources. This study was designed for screening downstream raw water to know the safety and assurance of current water and how this water follows raw water treating guidelines. Effects of the interest of nitrogen salts during the treatment process (raw, clarified, filtered, and treated) were investigated. Screening changes in bacteriological parameters reacted with chlorine doses during the treatment process in sedimentation basins. Screening changes in the bacterial and algal count during the treatment process in raw and treated processes were monitored.

2. Materials and methods

All chemicals used in this study were analytical grade. Deionized water, specific resistivity of 18.2 MΩ cm, was obtained from a Veolia water system (UK) through filtering distilled water and was used in all the experiments.

2.1. Water samples

Water samples were collected from a drinking water treatment plant, Kafr Elshikh, Egypt, which has abstracted its raw water from downstream canal one. Other samples such as clarified, filtered, and treated water samples were collected from treatment steps of the drinking water treatment plant for a full 4 seasons between March 2017 and March 2018. All samples were preserved at 4 ^{0}C and all testes were measured at 25 ^{0}C. All the analyses were in duplicates and determined by the procedures recommended in the standard methods for the examination of water and wastewater (APHA, 2017).

2.2. Treating process

Jar test by JLT6, Leaching test Jar test, (VELP SCIENTIFICA, EUROPE, ITALY), and breakpoint test were applied to detect the best dose of alum and chlorine.

2.3. Water analyses

All the physicochemical analyses were in duplicates and determined by the procedures recommended in the standard methods for the examination of water and wastewater (APHA, 2017). The quality of resource water samples was determined after some measurements such as pH (2510 platinum electrode), turbidity as Nephelometric turbidity units (NTU) (2130), chloride (ppm) (4500 Argentometeric methods), total alkalinity(ppm) (2320B titration method), total Hardness (ppm) (2340B EDTA titration), silicate (ppm) (4500C Molybdosilicate method), phosphate(ppm) (4500D Stannous chloride method), sulfate (ppm) (4500E Turbidimetric method). Nitrogen forms as free ammonia (ppm) (4500C Sodium Nitroprossiude method), and Nitrite (4500B colorimetric method) were measured. Total Nitrogen species (NH3, NO_2, and NO_3) were determined through Kjeldahl methods (Behr, Germany). Certified reference standards solution (1000 ppm) (Merck, Darmstadt, Germany) was used in order to prepare the working standard solutions ranged 10.0–1000.0 ppb.

2.4. Biological analyses of water samples

Nutrient agar was used for the total bacteria count and presented as CFU/ml. Detection of total and fecal coliform groups was based on growth on M-Endo broth and m–FC broth (Difco), respectively, and presented as CFU/100ml. The membrane filter technique was used for detection according to standard methods. Algal count and types were detected through a direct slide under a light microscope (APHA, 2017).

2.5. Statistical analysis

The data were analyzed by using statistical software (SPSS Version 17, SPSS INC, Chicago, IL, USA). Initially, the descriptive statistics were computed. One-way ANOVA was used followed by Duncan's post hoc test (α 0.05). In all tests, p values smaller than 5% were considered statistically significant.

3. Results and Discussion

Water quality through different treatment processes was assessed to know the ability to remove, eliminate or reduce physical, chemical, bacterial and algal content to reach as possible as an outlet. The quality of raw water samples collected from downstream sources was determined as mentioned in Table (1) and the mean results were recorded during the period of March 2017 to March 2018 as (turbidity 17.5 NTU), (temperature, 22.5 ^{o}C), (electrical conductivity, 622.5 µS/cm), (total dissolved solids, 398.4 ppm), (pH, 7.9), (chlorides, 29.8 ppm), (total alkalinity, 139.5 ppm), (total hardness, 139.3 ppm), (free $CO_{2,}$ 3.6 ppm), (total iron, 0.86 ppm), (manganese, 0.8 ppm), (aluminum, 0.03 ppm), (phosphate-P, 0.09 ppm), (sulphate, 60.01 ppm) and (dissolved oxygen, 5.76 ppm). Raw water during the period of study which extended for an annual cycle (except for little times in some categories), had a level of water that was suitable for abstracting without contaminants and proceeded through different stages of treatment (physically, chemically, and biologically).

The average removal of turbidity is 98.3% which is the key function of the treatment water process because there is a good relationship between turbidity and attached microbes. The turbidity ranged between the minimum value of 0.1 NTU recorded in winter and spring and the maximum value of 0.5 NTU recorded in autumn and the mean value was 0.3 NTU as in Table (1). Also, the temperature ranged between the minimum value of 15.4°C recorded in winter and the maximum value of 29.9°C recorded in summer, and the mean value was 23°C. Raw water DO was ranged between the minimum value of 4 ppm recorded in autumn and the maximum value of 7.8 ppm recorded in autumn and the mean value was 5.76 ppm, while the Treated water DO was ranged between the minimum value of 7.4 ppm recorded in winter and the maximum value 10 ppm recorded in spring, summer and autumn, and the mean value was 8.93 ppm as shown in Table (1).

Table 1. Statistics analyses of physical and chemical parameters of raw and treated water in DWTP.

			Raw water			Treated water		
Parameter	**Unit**	**N**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**
Turbidity	**NTU**	**52**	5	41	17.5	0.1	0.5	0.3
R. Chlorine	**ppm**	**52**	0	0	0	1.5	2.5	1.9
Temp.	**°C**	**52**	15	29.5	22.5	15.4	29.9	23.0
EC	**µS cm^{-1}**	**52**	523.4	715.6	622.5	382	547	419.3
TDS	**ppm**	**52**	335	458	398.4	229	282	251.8
pH	**Unit**	**52**	7.6	8.3	7.9	7.28	7.78	7.51
Chlorides	**ppm**	**52**	20	48	29.8	24	53	34.8
T. Alkalinity	**mg $CaCO_3$/l**	**52**	124.2	156.6	139.5	108	145.8	127.8
T. Hardness	**mg $CaCO_3$/l**	**52**	122	164	139.3	122	164	131.3
Ca Hardness	**mg $CaCO_3$ /l**	**52**	72	100	83.3	72	100	78.3
Mg Hardness	**mg $CaCO_3$/l**	**52**	40	78	55.1	40	78	52.2
Ammonia	**ppm**	**52**	0.45	11.06	3.60	0.14	0.81	0.35
Fe	**ppm**	**52**	0.09	3.50	0.86	0.027	0.72	0.08
Mn	**ppm**	**52**	0.13	2.64	0.80	ND	0.50	0.05
Al	**ppm**	**52**	0.007	0.06	0.03	0.03	0.20	0.09
PO_4-P	**ppm**	**52**	ND	0.90	0.09	ND	0.10	0.01
SO_4	**ppm**	**52**	16.10	64.80	40.01	32.60	78.17	52.33
DO	**ppm**	**52**	4.00	7.80	5.76	7.40	10.00	8.93

N* = number of samples.
Min* = minimum annual value.
Mean* = mean annual value
Max*= maximum annual value.
ND* = not detected by this method

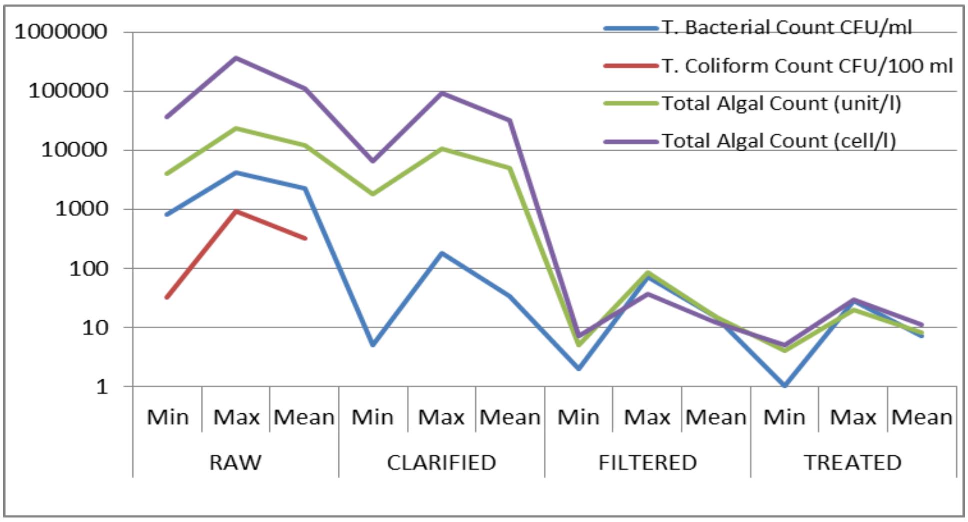

Figure 1. logarithmic scale of seasonal variation of biological parameters of water collected from raw, clarified, filtered, and treated positions of DWTP.

For total coliform count, weekly variations in raw, clarified, filtered and treated water obtained from DWTP were shown in Fig. (1). For Raw water, the total coliform count of raw water ranged between the minimum value of 32 CFU/100 ml recorded in winter and the maximum value of 940 CFU/100 ml recorded in spring and the mean value was 324 CFU/100 ml. Total bacterial count of raw water showed strong positive correlations with total coliform count (r = 0.9), total algal count unit/l (r = 0.5), total algal count cell/l (r = 0.7), alum dose (r = 0.5) and initial dose of chlorine (r = 0.6). While, total coliform count of raw water showed strong positive correlations with total algal count cell/l (r = 0.6), alum dose (r = 0.6) and initial dose of chlorine (r = 0.6).

Nitrogenous compounds effects

Simple nitrogenous compounds as the main bye products of biological process sources have important effects on the water quality. Raw water ammonia concentrations ranged between the minimum value of 0.45 ppm recorded in summer and the maximum value of 11.06 ppm recorded in autumn and the mean value was 3.6 ppm. The clarified water ammonia-N was decreased as a result of chlorine pre-disinfection and the minimum values were recorded in winter, spring, and summer, and the maximum value of 0.99 ppm was recorded in winter and the mean value was 0.72 ppm. Finally, the filtered water ammonia-N ranged between 0.44and 0.75 which shows the bioconversion through filtration processes. The minimum value was detected and recorded in all seasons and the average value was 0.35 ppm recorded in winter and the max. value was 0.81 ppm as in Tables (3). At the same time, the chemical conversion of ammonia resulted increasing in nitrate concentration, while, the concentrations of nitrite were fixed through all treatment steps.

Final treated water after post-disinfection, ammonia-N ranged between 0.14 and 0.81 ppm. The minimum value of ammonia was recorded in autumn and the mean value was 0.35 ppm as in Tables (3). Treated water ammonia-N showed positive correlations with nitrite (r = 0.5), and Turbidity (r = 0.56). The treated water ammonia-N has a negative correlation with an initial dose of chlorine (r = -0.6). The γ-aminobutyrate metabolism in disinfected systems is likely associated with the recycling of amino acids (Mook et al., 2012). Genome-level analyses for a subset of phylogenetically-related microorganisms suggest that disinfection selects for microorganisms capable of using fatty acids, presumably from microbial decay products, via the glyoxylate cycle (Bruno et al., 2018).

Figure 2 shows the results of experiments that deal with the correlation between ammonia concentration and turbidity. At the same time, the effect of chlorine addition on the count of total bacteria was studied. There are positive correlations between the existence of ammonia and the water turbidity degrees while there are negative correlations between doses of chlorine and the total count of bacteria. In addition, the filtration process has a significant effect on both nitrogenous compounds and microbial loads (El-Masry et al., 1995).

Finally, the survival bacteria in high doses of chlorine may produce resistance bacteria to disinfection. So, from these data, the selection of water sources with a low concentration of nitrogenous forms is a must for drinking water purposes. The emergence of disinfectant resistance has become a severe threat to the safety of life and health and the rational allocation of resources due to the reduced disinfectant effectiveness (Li et al., 2018; Tong et al., 2021).

However, the lack of scientific management and proper planning leads to the overuse and abuse of disinfectants, thereby increasing the abundance of antibiotic resistance genes (ARGs) (Kim et al., 2018). The abundances of extracellular ARGs (eARGs) and intracellular ARGs (iARGs) significantly increase in the chlorinated sewage treatment plants and greenhouse soils with fungicides (Bai et al., 2015; Zhang et al., 2020). Moreover, the diluted and remaining disinfectants in the environment lead to increase bacterial tolerance gene mutation, and horizontal gene transfer through phenotypic adaptation, (Cloete, 2003). Besides, the continuous exposure of bacteria to chlorine disinfectant develops disinfectant adaptability and tolerance (Tong et al., 2021).

In conclusion, based on bacterial and algal removal efficiency, conventional drinking water treatment protocol provide potable water with properties largely complying with the standard guidelines of Egyptian (458/2007) and WHO (2008) of drinking water with acceptable quality.

In the end, this study discussed the chemical oxidation process for ammonia removal proposing to find more technology to deal with it as an infield solution of ammonia to increase water resources. This study also recommends proceeding with the research and further investigation for finding an effective and efficient solution for ammonia problems in raw water resources. The development and enhancement of biological processes to control high ammonia concentration and make it possible to be applied in raw water resources are critical issues.

Table 2. Mean seasonal variations of biological parameters for filtered water in DWTP

		Spring			Summer			Autumn			Winter		
Parameter	N	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
T. Bacterial Count CFU/ml	13	6	53	16	3	41	17	2	21	8	8	72	19
T. Coliform Count CFU/100ml	13	0	0	0	0	0	0	0	0	0	0	0	0
Total Algal Count (unit/l)	13	8	45	26	8	38	19	8	25	14	9	32	16
Total Algal Count (cell/l)	13	16	360	167	8	127	39	8	56	26	16	62	36

Table 3. Statistics analysis of Nitrogen forms in raw, clarified, filtered and treated water of DWTP.

			Raw			Clarified			Filtered			Treated		
Parameter	Unit	N	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Ammonia-N	ppm	52	0.45	11.1	3.6	0.41	0.99	0.72	0.44	0.75	0.32	0.14	0.81	0.35
Nitrite-N	ppm	52	0.03	0.18	0.09	0	0.05	0.02	0	0.05	0.02	0	0.03	0.01
Nitrate-N	ppm	52	0.15	7.30	5.16	1.25	8.70	5.62	0.31	3.80	1.90	4.50	6.00	5.09

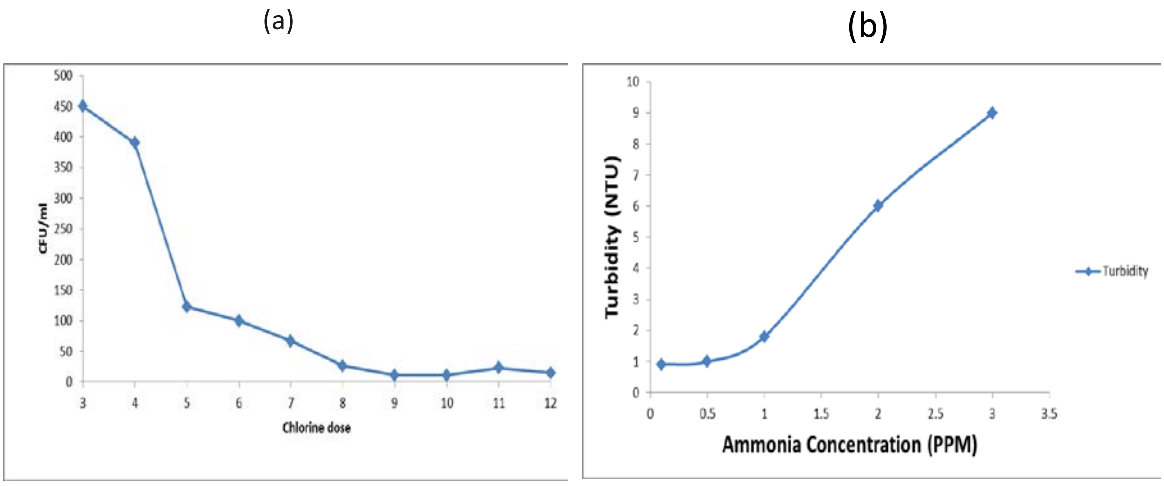

Figure 2. Correlation between the addition of chlorine doses (ppm) and the total bacterial counts (a) and ammonia concentrations and resulted turbidity (b).

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References

American Public Health Association (APHA) (2017). Standard methods for the examination of water and wastewater, 22nd ed. American Public Health Association, Washington, D.C.

Bai, X. Ma, X. Xu, F. Li, J. Zhang, H. Xiao, X. (2015) The drinking water treatment process as a potential source of affecting the bacterial antibiotic resistance. Sci. Total Environ., 533: 24-31.

Brown, C.T. Hug, L.A. Thomas, B.C. Sharon, I. Castelle, C.J. Singh, A. (2015) Unusual biology across a group comprising more than 15% of domain Bacteria. Nature 523, 208–211. doi: 10.1038/nature14486

Bruno, A. Sandionigi, A. Bernasconi, M. Panio, A. Labra M., Casiraghi, M. (2018). Changes in the Drinking Water Microbiome: effects of water Treatments along the flow of two drinking water treatment plants in an

urbanized area, Milan (Italy). Frontiers of microbiology 9: article 2557.
Cloete, T.E. (2003) Resistance mechanisms of bacteria to antimicrobial compounds. Int. Biodeterior. Biodegrad., 51: 277-282
Dai, Z. Sevillano-Rivera, M.C. Calus, S. T. a Bautista-de Los Santos, Q. Eren, A M. van der Wielen, P.W Ijaz, U. Z Pinto, A.J (2020) Disinfection exhibits systematic impacts on the drinking water microbiome. Microbiome 20; 8(1):42; doi: 10.1186/s40168-020-00813-0.
El-Masry, M.H. Hassouna, M.S. El-Rakshy, N. Mousa, I. (1995) Bacterial populations in the biofilm and non-biofilm components of a sand filter used in water treatment. FEMS microbiology letters 131 (3) 263-269.
Falkinham, J.O. Pruden, A. Edwards, M. (2015) Opportunistic premise plumbing pathogens: increasingly important pathogens in drinking water. Pathogens 4:373–86.
Garner, E. McLain, J. Bowers, J. Engelthaler, D.M. Edwards, M.A. Pruden, A. (2018) Microbial ecology and water chemistry impact regrowth of opportunistic pathogens in full-scale reclaimed water distribution systems. Environmental Science & Technology.;52:9056–68.
Jia, S. Shi, P. Hu, Q. Li, B. Zhang, T. Zhang, X.X. (2015) Bacterial community shift drives antibiotic resistance promotion during drinking water chlorination. Environmental Science & Technology 49:12271–9.
Kim, M. Weigand, M.R. Oh, S. (2018) Widely used benzalkonium chloride disinfectants can promote antibiotic resistance. Appl. Environ. Microbiol., 84 ,7-19.
Kooij, D. Wielen, P. (2013) Microbial growth in drinkingwater supplies: problems, causes, control and research needs: IWA Publishing.
Kooij, D. Wielen, P. Rosso, D. Shaw, A. Borchardt, D. Ibisch, R. Apgar, D. Witherspoon, J. Toro, D. Paquin, P. (2013) Microbial Growth in Drinking Water Supplies. IWA Publishing.
Li, X-F. Mitch, W.A. (2018) Drinking water disinfection byproducts (DBPs) and human health effects: multidisciplinary challenges and opportunities. Environmental Science & Technology 52:1681–9.
Luef, B. Frischkorn, K.R. Wrighton, K.C. Holman, H.Y.N. Birarda, G. Thomas, B.C. (2015). Diverse uncultivated ultra-small bacterial cells in groundwater. Nat. Commun. 6:6372. doi: 10.1036/ncomms7372
Mook, W.T. Chakrabarti, M.H. Aroua, M.K. Khan, G.M.A. Ali, B.S. Islam, M.S. Abu Hassan, M.A. (2012) Removal of total ammonia nitrogen (TAN), nitrate and total organic carbon (TOC) from aquaculture wastewater using electrochemical technology: A review. Desalination 285: 1–13.
Mousa, I.E. (2016) Total petroleum hydrocarbon degradation by hybrid electrobiochemical reactor in oilfield produced water. Marine Pollution Bulletin 109 (2016) 356–360
Rashida, N. Praveenaa, S. Arisb, A. (2015) Drinking water assessment on ammonia exposure through tap water in Kampung Sungai Sekamat, Kajang. Procedia Environmental Sciences 30: 354 – 357
Richardson, S.D. (2003) Disinfection by-products and other emerging contaminants in drinking water. TrAC Trends in Analytical Chemistry.;22:666–84.
Rosario-Ortiz, F. Rose, J. Speight, V. Gunten, U. Schnoor, J. (2016) How do you like your tap water? Science (80-), 351:912–914
Sedlak, D.L. von Gunten, U. (2011) The Chlorine Dilemma. Science 331:42–3.
Sevillano, M. Dai, Z. Calus, S. Santos, QMB-dl, Eren, A.M. Wielen, P. Ijaz U.Z. Pinto, A.J. (2019) Disinfectant residuals in drinking water systems select for mycobacterial populations with intrinsic antimicrobial resistance. bioRxiv 675561.
Shi, P. Jia, S. Zhang, X.X. Zhang, T. Cheng, S. Li, A. (2013) Metagenomic insights into chlorination effects on microbial antibiotic resistance in drinking water. Water Research 47:111–20.
Tong, C. Hu, H. Chen, G. Li, Z. Li, A. Zhang, J. (2021) Disinfectant resistance in bacteria: Mechanisms, spread, and resolution strategies. Environmental Research 195, 110897
Wang, H. Pryor, M.A. Edwards, M.A. Falkinham, J.O. Pruden, A. (2013) Effect of GAC pretreatment and disinfectant on microbial community structure and opportunistic pathogen occurrence. Water Research.47:5760–72.
Zhang, H. Chang, F. Shi, P. Ye, L. Zhou, Q. Pan, Y. Li, A. (2019) Antibiotic resistome alteration by different disinfection strategies in a full-scale drinking water treatment plant deciphered by metagenomic assembly. Environmental Science & Technology 53:2141–50. 3
Zhang, H. Chen, S. Zhang, Q. Long, Z. Yu, Y. Fang, H. (2020) Fungicides enhanced the abundance of antibiotic resistance genes in greenhouse soil. Environ. Pollut., 259: 113877.