Detection of Reverse Osmosis Filters Efficiency in Drinking Water

Likaa Mahdi Serheed &Yaaroub Faleh Al- Fatlawy

Biology department- cologe of science- university of Baghdad

srhydlylqamhyd @ gmail.com

Abstract

The wide application of chlorine disinfectant for drinking water treatment has led to the appearance of chlorine-resistant bacteria, which pose a severe threat to public health. This study was performed to search for the relationship of the presence of bacteria with the purity of drinking water or the relationship between growth of bacteria on efficiency of R.o. water and its effects on the purification of drinking water that used by human in my country , Its serious attempts to find acommon problem and find persuasive solutions.
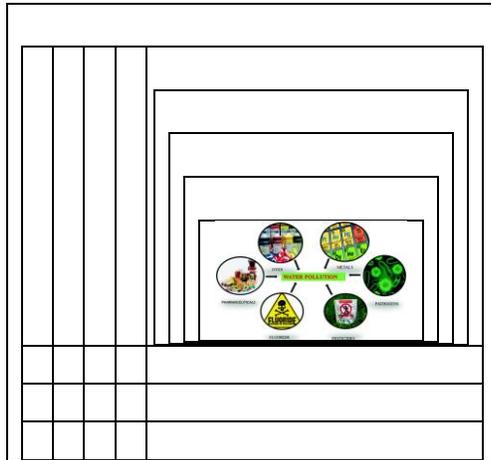
The samples of raw and RO of drinking water were collected from the study sites , the number of samples were (25) samples before and after Ro in three replication, finally were collected (10) samples of household Ro filters from (karkh and Rusafa) as acomparison the best type of efficiency in purification of drinking water.samples were collected in bottles prepared in paragraph 2-1-5 and closed tightly and taken to the laboratory within 2-3 hours in cooling box to conduct the physiochemical tests .

The results of bacteriological tests show that the Ro filter was able to reduce and remove the bacteriological contamination in water and (Ricardo Franci Gonçalves a, Laila de Oliveira Vaz a, Mario Peres a , Solange Sarnaglia Merlo, 2021) and

(j.L.BANCH;H.j.van der Fels.klerx, ,j food prot 2020)

Exept some samples that were diagnose as containing bacterial growth from .Several type of bacteria .it shows the Ability of bacteria to to form biofilm in R.O filter(ELSEVIER P Kumar, K Hegde, SK Brar and M Cledon,2020 - Chemical Engineering)

… This experiment provided more information to assess the **ability** of the **bacterial** strain to perhaps because filtes were not replaced and it has became out of date.(EF Haney, MJ Trimble, REW Hancock - Nature Protocols, 2021)

Introduction)

The project work gives current analysis of drinking water samples and filtered water based on the literature of improvised purification technique from candle filter to RO filter The samples were collected in cans and sterilized DO bottles. The samples were examined for microbiological parameters. the analysis was conducted at central environmental laboratory of ministry of health and environment ,Baghdad university at biology and chemistry departments and acentral health laboratory.samples.were collected from Baghdad in both sides (karkh & Rusafa) from 11/9/2020 until 18/1/2021 , Drinking water should contain minimum levels of certain minerals such as calcium and magnesium, but importance has been given to the protective or beneficial effects of drinking water substances. The main interest has been given to harmful properties of contaminants. Studies has been conducted to describe the minimum content of essential elements, drinking water, and several countries have included guidelines to regulate certain mineral content in the drinking water. The issue is applicable not only where drinking water is obtained by desalination, but also where water purifiers removes the significant amount of important minerals and low-mineral water is consumed. Dematerialized water which has not been rematerialized, which has low-mineral content is not considered ideal for drinking, hence regular consumption of low mineral water do not provide adequate levels of beneficial nutrients,. In recent years the application of reverse osmosis technique is used in the removal of inorganic and organic pollutants simultaneously.

RO can effectively remove various suspended matters, colloids, and microorganisms from water, thus is commonly used in the HWPs (Yang et al., 2019). Recent studies also found that RO is equally effective in eliminating ARGs from water (Slipko et al., 2019; Lan et al., 2019). For example, > 99.8 % of extracellular free DNA can be removed from the distilled water and wastewater treatment plant effluent by the RO membrane (Slipko et al., 2019). Therefore, RO filter in the RO-based HWPs could be an unheeded reservoir of antibiotic resistance due to the accumulation of the ARGs and ARB from the tap water.
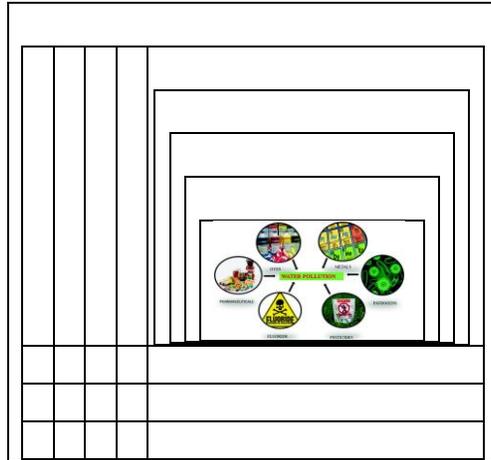
Material and Methods

1--Microbial tests

These tests were conducted to examine the validity of water for drinking by detecting bacteria that may contaminate the water at the Central Environmental Laboratory/ Ministry of Environment, following the Standard Methods .

Water sampling

The samples of raw and RO of drinking water were collected from the study sites , the number of semples were (25) samples before and after Ro in three replication, finaly were collected (10) samples of household Ro filters from (karkh and Rusafa) as acomparison the best type of effienciency in purafication of drinking water.Samples were collected in bottles prepared in paragraph 2-1-5 and closed tightly and taken to the laboratory within 2-3 hours in cooling box to conduct the physiochemical tests (APHA, 2012).

1-**Aerobic Plate Count (APC)**   - 1
-Microbial tests

These tests were conducted to examine the validity of water for drinking by detecting bacteria that may contaminate the water at the Central Environmental Laboratory/ Ministry of Environment, following the Standard Methods (APHA, 2012).

- Aerobic Plate Count (APC). 1

Pour plate was the method that followed in the counting of bacteria, 1 ml of each sample of drinking water was taken after shaken well, placed in a sterilized petri dish, then added to it 12 – 15 ml of N Agar (44.5°C) culture media that was prepared in paragraph 2-1-4-1, the dish was moved in a circular and in opposite directions to ensure blending the sample with the culture media very well, incubated after solidifying upturned in the incubator at $35 \pm 0.5°C$ for 24 – 48 hours, the positive dishes were read by observing and counting the colonies by the Colony Counter device (APHA, 2012).

Total, Faecal Coliform and Escherichia coli (T.C/100ml), (F.C/100ml).

(E.coli/100ml)

Lauryl Treptose broth (douple strength) used with RO Water to activated bacteria that weak in R0 water , but in raw water always used single strength.

The Most Probable Number :-

1-Presumptive stage

Ten test tubes were used for each water sample and each tube was filled with 10ml of double strength LSB medium with inverted dirham tube .

Water sample was shaking very well to ensure a homogeneous

distribution of bacteria, then transferred 10 ml to each test tube in the

previous step by sterile pipette.

Test tubes were incubated at $35 \pm 0.5°C$ for 24 ± 2 hours. If no gas or

color change was present, the samples were reincubated, and read again

at the end of 48 ± 4 h.

Test tubes were examined and results were recorded, positive results

were known by forming gas or acid, that could be noticed by the color

changing from purple due to promo cresol purple, stain was added to the culture media at prepared, to culture media at prepared, to The positive tube number was recorded and prepared to the confirme stage.

The positive tube number was recorded and prepared to the confirme stage

2- Confirmed stage

-Submiting all presumptive tubes showing growth, any amount of gas, or

acidic reaction within 24 ± 2 h or 48 ± 4 h of incubation to the confirmed

phase.

- Tubes showing gas or acidic growth were gently shaked or rotated

p- With a sterile loop 3.0 to 3.5 mm in diameter, one or more loopfulls of

culture were transfered to a fermentation tube containing 10ml BGBL broth..

. The BGBL broth tube was incubated at $35 \pm 0.5°C$ for 48 ± 3 hours-

-Formation of gas in any amount in the inverted vial of the BGBL broth tube within 48 ± 3 h constitutes a positive confirmed phase and prepared to the complet phase.

- Calculation the MPN of T.C/100ml by comparing the positive tubes number as in appendix (1).resumptive to resuspend the organisms.

3-Completed phase

- Submiting all comfirmed fermentation tubes showing any amount of gas within 48 h of incubation to the FC test and E. coli test .

- Comfirmed tubes showing gas were gently shaked or rotated to resuspend the organisms. - With a sterile loop 3.0 to 3.5 mm in diameter, one or more loopfuls of culture were transfere to a fermentation tube containing 10ml of E.C broth (FC test) and the same to a fermentation tube containing 10ml of E.C MUG broth (E. coli test) for each positive tube in the previous stage.

- The E.C broth tube was incubated and the E.C MUG broth tube in the water path at 44.5 ± 0.2 °C for 24 ± 2 h. All E.C tubes were placed in water bath within 30 min after inoculation. A sufficient water depth in water bath incubator were maintain to immerse tubes to upper level of the medium.

- The positive result for F.C was gas forming should be seen in E.C broth tubes.

- E.C MUG broth tubes were examined under UV lamp container and the positive result depending on florescent tubes.

-Calculation the MPN of T.C/100ml and MPN of E. coli/100ml

Presumptive and Confirmed Test for Faecal Streptococcus Bacteria

-Presumptive test for Faecal Streptococcus bacteria

The MPN was the method that used for faecal Streptococcus test by using a double strength azide glucose broth in as five tubes for each drinking water sample. The method was made with the same technique as the previous paragraph, the differences were only in the absence of dirham tube. Tubes were incubated at 37 °C for 24 ± 2 hours. If no definite turbidity was present, the sample should reincubate, and examined again at the end of 48 ± 4 h. The positive test tubes were observed via the deposit formed in the bottom of the tube with turbidity appearance when the tube was shaken

Confirmed test for Faecal Streptococcus bacteria-

Each positive tubes that observed in total Streptococcus bacteria test, was planted on a Pfizer Agar Petri dish by transferring a loopfull from the tube to the petridish and spread it homogeneously, and incubated at 37°C for 24 ± 2 hours.

The positive dishes were tested by observing the growing black colonies on the agar and results of drinking water sample calculating by comparing the positive

dishs number as in appendix (1).

انتائج الفحص البكتريولوجي لعينات الماء المأخوذة من الفلاتر التناضحية العكسية

رقم النموذج	التشخيص البكتيري بالتردد الاول	التشخيص البكتيري بالتردد الثاني	التشخيص البكتيري بالتردد الثالث
1	*Pseudomonus auroginosa*	*Pseudomonus auroginosa*	*Acinetobacter lwoffii*
2	No growth of bacteria	No growth of bacteria	No growth of bacteria
3	*Pseudomonus auroginosa*	*Pseudomonus auroginosa*	*Pseudomonus auroginosa*
4	No growth of bacteria	No growth of bacteria	No growth of bacteria
5	*Pseudomonus auroginosa*	*Pseudomonus auroginosa*	*Pseudomonus auroginosa*
6	No growth of bacteria	No growth of bacteria	No growth of bacteria
7	No growth of bacteria	No growth of bacteria	No growth of bacteria
8	No growth of bacteria	No growth of bacteria	No growth of bacteria
9	*Pseudomonus auroginosa*	*Pseudomonus auroginosa*	*Pseudomonus auroginosa*
10	No growth of bacteria	No growth of bacteria	No growth of bacteria
11	No growth of bacteria	No growth of bacteria	No growth of bacteria
12	No growth of bacteria	No growth of bacteria	No growth of bacteria
13	*Pseudomonus auroginosa*	*Pseudomonus auroginosa*	*Pseudomonus auroginosa*
14	*Pseudomonus auroginosa*	*Pseudomonus auroginosa*	*Pseudomonus auroginosa*
15	No growth of bacteria	No growth of bacteria	No growth of bacteria
16	*Shewanella putrefaciens*	*Ralstonia mannitolilytica*	*Ralstonia mannitolilytica*
17	*Cupriavidus pauculus*	*Cupriavidus pauculus*	*Cupriavidus pauculus*
18	No growth of bacteria	No growth of bacteria	No growth of bacteria
19	No growth of bacteria	No growth of bacteria	No growth of bacteria
20	*Pseudomonus auroginosa*	*Pseudomonus auroginosa*	*Pseudomonus auroginosa*

21	No growth of bacteria	No growth of bacteria	No growth of bacteria
22	No growth of bacteria	No growth of bacteria	No growth of bacteria
23	*Pseudomonus auroginosa*	*Pseudomonus auroginosa*	*Pseudomonus auroginosa*
24	No growth of bacteria	No growth of bacteria	No growth of bacteria
25	*Cupriavidus pauculus - Enterobacter asburiae - - Acinetobacter baumannii* **co**mplex	*Cupriavidus pauculus - Enterobacter asburiae - Acinetobacter baumannii - complex*	*Cupriavidus pauculus - Enterobacter asburiae - Acinetobacter baumannii - complex*

Results and discussion

The results suggest that chemical treatment facilitates initiation and subsequent maturation of biofilm structures on the RO membrane and feed-side spacer surfaces. Biofouling control might be possible only if the cleaning procedures are adapted to effectively remove the (dead) biomass from the RO modules after chemical treatment.

Rapid re-growth of biofouling layers The results indicate that microbial colonization of the collapsed biofilm layers starts directly after chemical cleaning. Two clearly different features were hereby observed: The observed biofilm removal failure and subsequent rapid biofilm layer re-growth were observed after each scheduled treatment. From a microbiological point of view, the regrowth process remains the same, with some small shifts in the structure and composition of the involved microbial community, more related to seasonal changes than to the operating and cleaning procedures.The reason of growthing *pseudomonas aurogenosa* in filter R.O beause of aging filter and ahuge ability of this bacteria to forming biofilm this bactaia can infected childs,olderly and patients with acquired immunodeficiency

Conclusions

Relationships between common water bacteria and pathogens in drinking-water To perform a risk analysis for pathogens in drinking-water, it is necessary, on the one hand, to promote epidemiological studies, such as prospective cohort and case–control studies. It is also appropriate, on the other hand, to better understand the ecology of these microorganisms, especially in analysing in detail the interactions between common water bacteria and pathogens in such diverse habitats as free water and biofilms. It appears essential to distinguish two categories of drinking-water sources: surface water and groundwater under the direct influence of surface water (2003 World Health Organization (WHO). Heterotrophic Plate Counts and Drinking-water Safety. Edited by J. Bartram, J. Cotruvo, M. Exner, C. Fricker, A. Glasmacher. Published by IWA Publishing, London, UK. ISBN: 1 84339 025 6)

HETEROTROPHIC BACTERIA AS INHABITANTS OF A DRINKING-WATER ECOSYSTEM

established that the cells of Pseudomonas spp., which are ubiquitous bacterial species, respond to favourable nutrient conditions by adhering to available organic or inorganic surfaces and by binary fission and exopolymer production to develop mature biofilms. These rod-shaped Gram negative cells grow predominantly in this matrix-enclosed sessile mode, in which they are protected from adverse environmental conditions and chemical antibacterial agents. Thus, the majority of microorganisms persist attached to a surface with a structured biofilm ecosystem and not as free-floating cells. The most striking studies with P. aeruginosa species (Costerton et al. 1995) have shown that the planktonic biofilm transformation is controlled by a σ factor that is similar to that which controls sporulation in Gram-positive bacteria. Biofilm bacteria could be the product of a σ factor-directed phenotypic change in a large cassette of genes.

Biofilm

Pseudomonas aurogonosa, which allow the examination of fully hydrated samples, has revealed the elaborate three dimensional structure of biofilms that agree with (Xu Teng, [a] Feng Li [a] and Chao Lu, 2020,)_and ,(Enrica Passione,2021). It has become widely recognized that bacteria as colonial organisms in biofilms elaborate systems of intercellular communication to facilitate their adaptation to changing environmental conditions (Wimpenny et al. 2000). In environmental habitats, bacteria within biofilms are notably resistant to bacteriophages, seudomonas aeruginosa is generally described as ubiquitous in natural settings, such as soil and water. However, because anecdotal observations and published reports have questioned whether or not this description is true, we undertook a rigorous study using three methods to investigate the occurrence of P. aeruginosa: We investigated environmental samples, analyzed 16S rRNA data, and undertook a systematic review and meta-analysis of published data. The environmental sample screening identified P. aeruginosa as significantly associated with hydrocarbon and pesticide-contaminated environments and feces, as compared to uncontaminated environments in which its prevalence was relatively low. The 16S rRNA data analysis showed that P. aeruginosa sequences were present in all habitats but were most abundant in samples from human and animals. Similarly, the meta-analysis revealed that samples obtained from environments with intense human contact had a higher prevalence of P. aeruginosa compared to those with less human contact. Thus, we found a clear tendency of P. aeruginosa to be present in places closely linked with human activity. Although P. aeruginosa may be ubiquitous in nature, it is usually scarce in pristine environments. Thus, we suggest that same result with (P. Matthew Malone , Mette H Nicolaisen , Esteban Martínez-García Catalina Rojas-Acosta , Maria Catalina Gomez-Puerto , Henrik Calum Marvin Whiteley, Roberto Kolter Thomas Bjarnsholt ˑ,2020).

Cupriavidus pauculus is an emerging organism causing infections in immunocompromised and immunocompetent patients. We report a C.pauculus pneumonia case susceptible to cefepime in an infant with end-stageame renal failure. Cupriavidus pauculus, formerly CDC group IV c-2, is a Gram-negative mesophilic bacillus widely distributed in nature, especially in water and soil it consedard same result with [1,2]. It causes infections in immunocompromised patients with underlying diseases such as malignancies and AIDS as well as infections in otherwise healthy patients [2-4]. It can be an opportunistic pathogen in a hospital setting and can cause outbreaks,

especially in intensive care units [2,5]. Water, including tap and bottled water, has been suspected to be a potential source of contamination [2,5]. C. pauculus has been implicated in several types of infections, including bacteremia, pneumonia, meningitis, and septicemia [2,6,7]. Here, we report a case of pneumonia caused by C. pauculus in an infant in Saudi Arabia. To the best our knowledge, this is the first case of lower respiratory infection caused by C. pauculus in the Kingdom of Saudi Arabia and other Gulf States. Discussion Cupriavidus pauculus was previously classified as CDC group IV c-2, Ralstonia paucula and then as Wautersia paucula [1,8]. It is a ubiquitous environmental organism found mainly in soil, water, and on plants [1,2,8]. C. pauculus is a Gram-negative, motile, aerobic, non-spore-forming rod bacteria. It is catalase and oxidase positive, and a non-lactose fermenter on MacConkey agar [1,2]. C. pauculus can cause infections in hospitalized immunocompromised patients, especially in patients with hematologic malignancies, transplants, and AIDS patients. Examples of these infections include bacteremia, peritonitis, abscess, and septicemia caused by C. pauculus [2,9].

REFRENCES

1. Abdel-Rahman, M. A., and Sonomoto, K. (2016). Opportunities to overcome the current limitations and challenges for efficient microbial production of optically pure lactic acid. *J. Biotechnol.* 236, 176–192.
2. Balaban, N. Q., Merrin, J., Chait, R., Kowalik, L., and Leibler, S. (2004). Bacterial persistence as a phenotypic switch. *Science* 305, 1622–1625.
3. Bidlack, J. E., and Silverman, P. M. (2004). An active type IV secretion system encoded by the F plasmid sensitizes *Escherichia coli* to bile salts. *J. Bacteriol.* 186, 5202–5209.
4. Blair, K. M., Turner, L., Winkelman, J. T., Berg, H. C., and Kearns, D. B. (2008). A molecular clutch disables flagella in the *Bacillus subtilis* biofilm. *Science* 320, 1636–1638.
5. Boetius, A., Ravenschlag, K., Schubert, C. J., Rickert, D., Widdel, F., Gieseke, A., et al. (2000). A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature* 407, 623–626.
6. Bourret, R. B., and Stock, A. M. (2002). Molecular information processing: lessons from bacterial chemotaxis. *J. Biol. Chem.* 277, 9625–9628.
7. Butaité, E., Baumgartner, M., Wyder, S., and Kummerli, R. (2017). Siderophore cheating and cheating resistance shape competition for iron in soil and freshwater *Pseudomonas* communities. *Nat. Commun.* 8:414.
8. Cavicchioli, R., Ripple, W. J., Timmis, K. N., Azam, F., Bakken, L. R., Baylis, M., et al. (2019). Scientists' warning to humanity: microorganisms and climate change. *Nat. Rev. Microbiol.* 17, 569–586.
9. Cho, E. J., Trinh, L. T. P., Song, Y., Lee, Y. G., and Bae, H. J. (2020). Bioconversion of biomass waste into high value chemicals. *Bioresour. Technol.* 298:122386.
10. Clewell, D. B. (2011). Tales of conjugation and sex pheromones: a plasmid and *Enterococcal odyssey*. *Mobile Genet. Elements* 1, 38–54.

11. Cochrane, C. A., and Percival, S. L. (2007). Biofilms and their relevance to veterinary medicine. *Vet. Microbiol.* 121, 1–17.
12. Costerton, J. W., Lewandowski, Z., Caldwell, D. E., Korber, D. R., and Lappin-Scott, H. M. (1995). Microbial biofilms. *Ann. Rev. Microbiol.* 49, 711–745.
13. Cotter, P. D., Ross, R. P., and Hill, C. (2013). Bacteriocins—a viable alternative to antibiotics? *Nat. Rev. Microbiol.* 11, 95–105.
14. Dash, H. R., Mangwani, N., Chakraborty, J., Kumari, S., and Das, S. (2013). Marine bacteria: potential candidates for enhanced bioremediation. *Appl. Microbiol. Biotechnol.* 97, 561–571.
15. Deinema, M. H., Habets, L. H. A., Scholten, J., Turkstra, E., and Webers, H. A. A. M. (1980). The accumulation of polyphosphate in *Acinetobacter* spp. *FEMS Microbiol. Lett.* 9, 275–279.
16. Dumas, Z., Ross-Gillespie, A., and Kümmerli, R. (2013). Switching between apparently redundant iron-uptake mechanisms benefits bacteria in changeable environments. *Proc. R. Soc. B Biol. Sci.* 280:1055.