Study on Bacterial Diversities in Stored Water under Different Storage Environments

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Abstract. Stored water is one kind of survival supply in emergency preparedness, which should be biologically safe during a certain storage period. Different storage environments including temperature and condition of sealing decide the microbial quality of water. An orthogonal test were conducted to discover the regulation of biological diversities in stored water for a period of 15 days. Growth-dose-response model were applied into the growth phase to simulate the bacterial growth. Statistical analysis revealed that: (1) nutrients including AOC determined the maximum values of HPC; (2) Temperature played its role through affect the growth rate of bacterium. (3) Under sealing conditions, water sample could keep biological stability for longer time, compared with unsealing surroundings.

1. Introduction
During a disaster or military conflicts, crash in the water treatment system prevent people from potable water. Stored water is one kind of survival supply in emergency preparedness. Clean water is crucial support to the survival of victims[1]. The US Federal Emergency Management Agency (FEMA) recommends that residents store tap water. Safe storage of drinking water are regarded immediate priorities for human health by The World Health Organization. Besides, water storage water has been an important element in sponge city and smart city construction. However, microbial quality deterioration including bacterial regrowth and pathogenic properties is the most vulnerable and unmanageable spot in water storage. Excessive growth of bacteria in drinking water led to hygienic, aesthetic and operational problems.

Different storage environments including temperature and condition of sealing decide the microbial quality of water. Compared with water distribution systems, stored water keeps in stagnation for long time[2] so that HPC can be up to 580-fold[3] higher than in flushed tap water. The growth of bacteria can be divided into four phases: lag phase, exponential phase, stationary phase and dead phase. To detect the impacts of environments on bacterial changes in stored water is beneficial to provide instruction to water treatment strategy.

Here we present an orthogonal test to explore the effect of storage environments on different drinking water. The research focuses on that (1) study the comprehensive impacts of temperature and condition of sealing on bacterial growth in stored drinking water; (2) explore the correlation among different factors and biological indexes; (3) reveal the diversity of biological changes for stored water.
2. Methods and materials

2.1 Preparation of materials
Tap water and bottled water were selected as experimental water samples. Water quality indexes of water samples were presented in Table 1.

| Number | Water sample     | Residual chlorine (mg/L) | TDS (mg/L) | AOC (µg Ac C/L) | HPC (cfu/ml) |
|--------|------------------|----------------------------|-------------|-----------------|---------------|
| 1      | Tap water (TW)   | 0.15±0.02                 | 0.45±0.01   | 90±15           | 89±5          |
| 2      | Bottled water A (BA) | 0                         | 0.27±0.02   | 72±10           | 5±2           |
| 3      | Bottled water B (BB) | 0                         | 0.18±0.01   | 0               | 16±4          |

Carbon-free glassware were prepared referring to the method of Hammes[4]. The sample bottles and triangular flask were soaked in 0.2N HCl overnight, rinsed with tap water, distilled water and ultrapure water three times in sequence, air dried and then carbonized in a muffle furnace at 550 °C for 6 h. Other glassware was autoclaved at 121 °C for 20 minutes and air dried before use.

2.2 Microbial index evaluation
Bacterial counts were determined through flat pouring method. After neutralizing the residual chlorine in the water sample to be tested, shaking the water sample, take 1 mL in the culture dish, and the water sample was poured in R2A medium under the condition of 25 °C for 7 days. HPC were obtain through counting the bacterial counts.

Assimilable organic carbon (AOC) is the primary nutrients in water, which can be directly utilized by bacterium. It is an important index to evaluate the nutrients in water. 40mL of the water sample was taken to be tested in a 50mL flask, the residual chlorine was neutralized, the bacteria was pasteurized and it was cooled to the room temperature. Pseudomonas fluorescens P17 was inoculated into water samples at the concentration of 10,000 cfu/mL and was cultured at 25°C in dark environment for 3 days to obtain bacterial counts. Subsequently, P17 in water samples were killed after pasteurization. Spirillum NOX were incubated in pasteurized samples and cultured for another 4 days to get bacterial counts. Total counts of P17 and NOX were converted to AOC value in µg Acetate C/L[5].

BRP indicates the maximum potential of nutrients in water to support bacterial growth. Indigenous bacteria of 1ml were inoculated into pasteurized water samples. Bacterial counts were the BRP value after incubation of 5 days.
2.3 Pilot test
As shown in Tab.1 and Fig.2, orthogonal test were conducted under different environments. Heating rod was set in the tank to create the constant temperature condition. The sealed groups were block using the 0.22µm filter to keep bacteria from entering water samples.

The initial AOC value and BRP value were determined according to the protocol depicted in previous section. HPC values of each group were evaluated every day from the initial day to the 14th day. Three parallel samples of each group were conducted to calculate the average value. The AOC value and BRP value were obtained again at the last day to predict the nutrients diversities in each group.

| Number | sample | temperature | Sealing | Number | sample | temperature | Sealing |
|--------|--------|-------------|---------|--------|--------|-------------|---------|
| OT1    | TW     | 20°C        | N       | OT7    | TW     | 20°C        | Y       |
| OT2    | BA     | 20°C        | N       | OT8    | BA     | 20°C        | Y       |
| OT3    | BB     | 20°C        | N       | OT9    | BB     | 20°C        | Y       |
| OT4    | TW     | 25°C        | N       | OT10   | TW     | 25°C        | Y       |
| OT5    | BA     | 25°C        | N       | OT11   | BA     | 25°C        | Y       |
| OT6    | BB     | 25°C        | N       | OT12   | BB     | 25°C        | Y       |

Figure 2. Orthogonal test of stored water in different storage environments

2.4 Statistical analysis
Bar charts and plots were generated from Origin 8.0. The principal component analysis based on Growth-dose-response was evaluated by SPSS 20.

3. Results and discussions

3.1 Bacterial changes in different samples
Each group went through four phases of growth: lag phase, exponential phase, stationary phase and dead phase. As shown in Fig.3, HPC of all samples started at the value of no more than 100 cfu/ml and the terminate value were less than 3×10^5 cfu/ml. OT2 was the first group to get its own peak value of HPC while OT4 had the highest peak value of HPC. Under the same temperature and storage conditions, HPC values are tap water, bottled water A and bottled water B, respectively. This
phenomenon revealed that under the same environmental conditions, nutrients in water determined the BRP of water.

However, the growth rate of bacterial did not well consistent with nutrients such as AOC in water during the full growth phases. When the bacterial growth entered into exponential phase, nutrients were the first impacting factor on the bacterial growth rate. After the consumption of nutrients, the bacteria died and dissolved, and these substances were reused, which led to the repeated growth and decline of bacteria. Due to the difference between bacterial absorption and dissolution of AOC, HPC has a downward trend, as shown in Fig.4. In the indigenous flora, different bacteria have different growth and decline cycles. The dominant populations in each stage have changed, and some bacteria deformed and mutated. The changes could be reflected in the HPC plate counting process through observing morphology, size and colour of colony.
3.2 Impact of temperature

As shown in Fig.5, we separately took the group of tap water (OT1, OT4, OT7, OT10), bottled water A (OT2, OT5, OT8, OT11), bottled water B (OT3, OT6, OT9, OT12) to evaluate the impact of temperature. Temperature could affect the maximum value of bacterial and the time to reach this value. Besides, the growth rate at different temperature showed obvious differences. Conventionally, higher temperature would lead to high growth rate and bacterial value, yet in the group of bottled water A and bottled water B, values showed certain differences. At the condition of low nutrients, the peak values in the group of high value were lower than that of low temperature. The possible reason could be concluded: high temperature could promote the growth rate and endogenous respiratory depletion, and the competition would be more serious when the temperature was higher.

3.3 Impact of sealing condition

We separately compared the sealing groups with unsealing groups to explore the different results. As shown in Fig.5, colony counts in unsealing groups were obviously higher than that in sealing groups. Three possible reasons could explain the phenomenon. The first one was that more dissolved oxygen could promote the growth of bacteria in the open air. The other one was that consistent nutrients could cater for the sustainable growth for bacterial. Besides, bacterial in the air could replenish the microbial diversities in water so that the water could have high bacterial growth potential.

3.4 Impact of nutrients

In order to detect the effects of nutrients, the effects of protrophs and external nutrients should be considered. First, OT1, OT2, OT3 represented three different water samples with different original nutrients. The most obvious differences was the maximum values of HPC, especially under the sealing condition, as shown in Fig.5 (d).

Besides, when the water samples were exposed outside, the extra nutrients from surroundings could not be ignored. Here we regard under sealing conditions, the total organic carbon in water or in bacterial should follow conservative, so the difference between sealing groups and unsealing groups could reflect the extra nutrients. This could be well reflected in equation (1).

\[ \Delta \text{AOC} = \frac{\text{BRP}_0 - \text{BRP}_0 + \text{HPC}_0 - \text{HPC}_0}{Y_{\text{AOC}}} \]

In which, \( \text{BRP}_0 \) – initial BRP, \( \text{HPC}_0 \) – initial HPC, \( Y_{\text{AOC}} \) – yield values of AOC to bacterial colony counts.
3.5 Principle component analysis

In order to better reveal the influence of different factors, the Growth-dose-response model was applied to simulate bacterial growth at the growth stage. as it’s shown in Fig 6. (a) took equation (2) as an example \[6\].

\[ C(t) = \frac{C_1}{1+10^{p(t_0-t)}} + C_0 \]  

In which, \( C \) - bacteria concentration, \( t_0 \) – fastest growing time, \( p \)-growth coefficient.

Figure 5. Bacterial curves in water samples

Figure 6. Bacterial diversities in different phases (a) and PCA of different factors (b)

The growth values were simulated and calculated as shown in Tab.2.
Table 3. Bacterial growth values.

| Number | Cmax   | t_0  | P     | number | Cmax   | t_0  | p     |
|--------|--------|------|-------|--------|--------|------|-------|
| OT1    | 5.85×10^5 | 4.09 | 0.71  | OT7    | 5.70×10^5 | 6.30 | 0.55  |
| OT2    | 2.36×10^5 | 3.48 | 0.63  | OT8    | 1.70×10^5 | 2.77 | 1.26  |
| OT3    | 6.45×10^4 | 3.72 | 0.71  | OT9    | 3.28×10^4 | 6.25 | 0.25  |
| OT4    | 1.55×10^6 | 4.38 | 1.43  | OT10   | 5.00×10^5 | 5.40 | 1.26  |
| OT5    | 4.12×10^4 | 1.97 | 2.92  | OT11   | 1.36×10^5 | 2.96 | 2.15  |
| OT6    | 7.88×10^4 | 3.83 | 0.71  | OT12   | 1.85×10^5 | 4.86 | 1.07  |

Principle components analysis were shown in Fig.6 (b). It could be regarded that nutrients including AOC determined the maximum values of HPC. Temperature played its role through affect the growth rate of bacterium. Under sealing conditions, water sample could keep biological stability for longer time, compared with unsealing surroundings.

4. Conclusion

Safe storage water was an important emergency preparedness. Three factors including temperature, nutrients (AOC, as a primary index) and sealing conditions were studied through orthogonal test. Through mathematical model and analysis, the principle component analysis were conducted, which could provide better instruction for following water treatment strategy.

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