Appendix I Test Methods and Organisms Addition

The objective of the test is to verify the disinfection efficiency for aquatic organisms including microorganism, zooplankton, phytoplankton, zoobenthos larvae and nekton larvae of the OceanDoctor BWMS developed by Jiujiang Precision Measuring Technology Research Institute. In consideration of test requirements such as equipment space and water volume, the test will be carried out in the Ballast Water Detecting Laboratory of Shanghai Ocean University Land-Based Testing Base. The test will be conducted form July 2012 to October 2012. During this period, the installation and maintenance of treatment equipment will be undertaken by the engineers of Jiujiang Precision Measuring Technology Research Institute.

There will be ten test cycles. During each test cycle, water samples of influent water, control water, treated water, treated water and control water after a120h hold, are collected; triplicate samples will be collected also for each sample. After some necessary pretreatments, samples will be brought back to laboratory for further analysis; meanwhile some parameters of temperature, salinity and total residual oxidants (TRO) will be tested on site.

A. Test Methods of Environmental Parameters

1. pH

The testing method use Switzerland METTLER Desktop pH meter to determine pH value of water sample in accordance with The Specification for Marine Monitoring - Part 4: Seawater Analysis (GB17378.4-2007/26).

1.1 Scope and Application

This method is applicable to pH value determination of seawater in ocean and offshore area. Determination should be completed within 6 hours after sampling. If one drop of $HgCl_2$ solution is added into the water sample and the bottle stopper is sealed, this sample can be preserved for 2 days. Factors such as water chromaticity, turbidity, colloidal particles, free chlorine, oxidants, reducing agents and high salinity all have small

interference on pH value determination. However, when pH is higher than 9.5, the considerable Na^+ will cause a big error and make the reading lower.

1.2 Principle of the Method

Insert Glass-Calomel electrode pair into the water sample to form the battery. Then, pH of the water sample and EMF (E) of the battery have the following linear relationship:

$$pH_x = A + \frac{E_x}{2.3026RT/F}$$

When Glass-Calomel electrode pair is inserted into the standard buffer solution, the relationship is as follows:

$$A = pH_s - \frac{E_x}{2.3026RT/F}$$

At the same temperature, measure EMF of the same electrode pair both in standard buffer solution and the water sample. The pH value of the water sample is obtained by the following equation:

$$pH_x = pH_s + \frac{E_x \times E_s}{2.3026RT/F}$$

Where: pH_x——pH value of the water sample;

pH_s——pH value of the standard buffer solution;

E_x——EMF of Glass-Calomel electrode pair in the water sample;

E_s——EMF of Glass-Calomel electrode pair in the standard buffer solution;

R——gas constant;

F——Faraday constant;

T——absolute temperature K.

1.3 Reagents and Preparation

1.3.1 Standard buffer solution (preparing by using pH standard buffer substance)

1.3.1.1 Potassium hydrogen phthalate standard buffer solution: c ($KHC_8H_4O_4$) = 0.05 mol/L (pH_s = 4.003 at 25°C);

1.3.1.2 Mixed standard buffer solution (pH_s = 6.864 at 25°C): 0.025 mol/L potassium dihydrogen phosphate (KH_2PO_4) and 0.025 mol/L disodium hydrogen phosphate (Na_2HPO_4);

1.3.1.3 Mixed standard buffer solution (pH_s = 7.413 at 25°C): 0.008695 mol/L potassium dihydrogen phosphate (KH_2PO_4) and 0.03043mol/L disodium hydrogen phosphate (Na_2HPO_4);

1.3.1.4 Borax solution standard buffer solution (pH_s = 9.182 at 25°C): c ($Na_2B_4O_7 \cdot 10H_2O$) = 0.0l0mol/L;

1.3.2 Saturated potassium chloride solution: Weigh 40g potassium chloride (KCl). Mix with 100 mL water thoroughly. Then, store the solution in reagent bottle. (The solution should be co-existed with solid KCl).

1.3.3 Mercuric chloride solution (25 g/L): Weigh 2.5g mercuric chloride ($HgCl_2$). Dissolve it into water and dilute to 100ml. Mix thoroughly. Then, store it in brown reagent bottle.

1.4 Instruments and Equipment

pH meter: Accuracy of 0.01, with a glass electrode and calomel electrode;

Polyethylene wash bottle: Capacity of 500ml;

Thermometer: (0～60) °C;

Beaker: Capacity of 150ml;

Brown drop bottle: Capacity of 60ml;

HDPE bottle: Capacity of 100ml, 500ml, 1000ml;

Wide-mouth bottle: Capacity of 50ml;

Brown reagent bottle: Capacity of 250ml, 50ml, 1000ml;

Volumetric flask: Capacity of 250ml, 50ml, 1000ml;

Common used instruments and equipment in laboratory.

1.5 Procedure

1.5.1 Start up and preheat the instrument for 20 minutes. Shift pH-mV selector switch to "pH".

1.5.2 Place the beaker holder, electrode clip, etc. Fix the glass electrode and calomel electrode on the clip (the bottom of the calomel electrode should be slightly lower than that of the glass electrode). Insert and connect the electrode to the corresponding jacks and terminals.

1.5.3 Wash electrode with water (there should be potassium chloride crystals in calomel

electrode and remove the rubber plug on the bottom of the electrode). Dry it with filter paper, and then insert the electrode to the calibrated standard buffer solution.

1.5.4 Calibration

Before testing, use standard buffer solution for calibration. The standard buffer solution which has the similar pH value with that of the test solution would be selected as the calibration solution. If the pH range of the test solution is unknown, choose the phosphate standard buffer solution. The calibration procedure is as follows:

1.5.4.1 Keep the scale of "temperature compensator" of the instrument the same with the temperature of the solution.

1.5.4.2 Zero setting, to make the instrument reading between ± 0.

1.5.4.3 Press the "Reading" switch and adjust the "Calibration" switch to read the pH value at present temperature. Note that electrode potential must be kept fully balanced and stable when calibration.

1.5.4.4 After calibration, release the "Reading" switch. The "Calibration" switch should not be rotated casually any more, otherwise the instrument should be re-calibrated.

1.5.5 Sample Determination

1.5.5.1 Take out electrode and wash the bottom of the electrode with distilled water. After that, dry it with filter paper and insert the electrode to the test solution. Rotate the beaker containing the solution from time to time to keep the electrode potential fully balanced.

1.5.5.2 Keep the scale of "temperature compensator" of the instrument the same with the temperature of the solution.

1.5.5.3 Zero setting. Press the "Reading" switch and read pH value of sample. Then release the "Reading" switch and write down the data on the datasheet.

1.5.5.4 The instrument should be re-calibrated if it is used for 2-3 hours or the temperature changes more than 2°C.

1.5.5.5 After determination, take out the electrode and wash it with distilled water until it is clean. Cap the rubber plug of calomel electrode and store it in electrode box. Glass electrode is soaked in distilled water.

1.6 Computing and Reporting

Depending on the requirements of datasheet, fill in the datasheet and complete the data calculation.

Convert the pH value measured in laboratory to that of onsite sample. Calibrate temperature and pressure according to the following formula:

$$pH_w = pH_m + \alpha(t_m - t_w) - \beta \times d$$

Where: pH_w, pH_m——pH value on the scene and in the laboratory respectively;

t_w, t_m——water temperature on the scene and in the laboratory respectively, the unit is Degrees Celsius (°C);

d——the depth of water sample, the unit is meter (m);

α——temperature correction factor;

β——pressure correction factor;

$\alpha(t_m - t_w)$ value can be found in temperature correction table and pressure correction coefficient method.

1.7 Attentions

Unless otherwise stated, all reagents used in this method are of analytical reagent (AR) grade and all water used is de-ionized water or equivalent pure water;

The "reading" switch, glass electrode jack, calomel electrode, terminal, etc. should be kept dry and clean;

Common faults and solutions:

a) If the deterioration of buffer solution leads to pH value change (floccule appears, etc.), the buffer solution should be replaced. To prevent deterioration of the buffer solution, it is better to add a few small thymol crystals to the solution beforehand;

b) If the passivation of glass electrode results in poor response of pH value. The pH meter can be soaked in 6 mol/L hydrochloric acid or 20% ammonium hydrogen fluoride (NH_4HF_2). If no improvement still, replace the glass electrode.

c) If the bubbles in potassium chloride solution inside calomel electrode lead to open circuit or test instability, the bubbles in the liquid column should be removed and fill the liquid column with saturated potassium chloride solution.

d) If electrode wiring doesn't connect well or the instrument is affected with damp which

leading to meter hand is unstable, the wiring should be reconnected or dry the instrument.

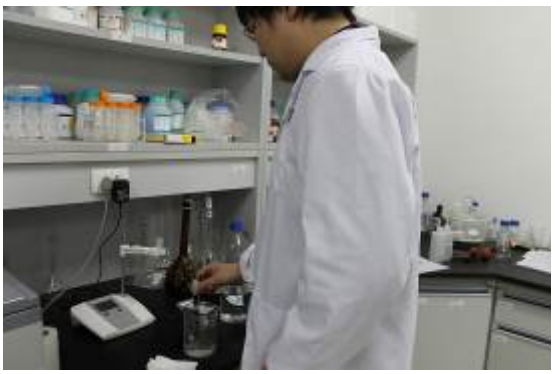
the electrode must be immersed in the solution during measurement, otherwise it is likely to cause open circuit and break the instrument;

before measuring of each batch of water samples, the instrument must be calibrated with phosphate buffer solution (refer to 3.1.2). If the deviation between measured value and reference value is more than ±0.01, the instrument should be re-calibrated;

the bulb of glass electrode is very thin, so not to collide with hard objects to make it broken. Calomel electrode should be slightly lower than the glass electrode when in use;

new glass electrode should be soaked in water for 1-2 days before use;

after determination of turbid water, the electrode should be washed immediately



2. Turbidity Unit (NTU)

The testing method use Shanghai Xinrui Photoelectric Turbidimeter to determine turbidity of water sample in accordance with the Specification for Marine Monitoring - Part 4: Seawater Analysis (GB17378.4-2007/30.1).

2.1 Scope and Application

This method is applicable to turbidity unit determination of seawater in ocean and offshore area. The turbidity of 1L pure water containing 1mg Kaolin is defined as 1° in this method. Both the rapid sinking debris and thick sediment in water sample can be determined as turbidity.

2.2 Principle of the Method

Turbidity can be determined by comparing the transmitted light intensity of the water sample with that of the turbidity-free water, when certain light beam irradiate water sample.

2.3 Reagent and Preparation

2.3.1 Turbidity-free water: Filtrate distilled water or deionized water through 0.2μm membrane and store the water in polyethylene bucket. Ish the polyethylene bucket by the filtered water twice, and then abandon the 200ml initial filtered water. Prepare the turbidity-free water on the sampling day.

2.3.2 Mercury dichloride ($HgCl_2$) solution (50g/L): weigh 5.0g mercury dichloride ($HgCl_2$). Dissolve it into little water and dilute to 100ml. Store it in brown reagent bottle.

2.4 Instruments and Equipment

Instruments and equipment are as follows:

——Photoelectric turbidimeter;

——Reagent bottle with rubber stopper;

——Common instruments and equipments in general laboratory.

2.5 Procedure

2.5.1 Start up and preheat the turbidimeter for 15~30 minutes.

2.5.2 Use long test-cell to determine the water sample having low turbidity (0°~30°). The procedure is as follows:

a) Zero setting: Pour the turbidity-free water into the cell and face the numbered side of

the cell with the right side of the tank. Close the cap. Rotate the vernier adjustment knob slowly and adjust the hand to zero on the right side of dial. Take out the turbidity-free water from the cell.

b) Water sample determination: Pour the sample water into the cell to the standard line. Put the cell back to the tank and face the numbered side of the cell with the right side of the tank. Close the cap. Record the reading directly.

2.5.3 Use short cell to determine the water sample having high turbidity (20°~100°). The procedure is as follows:

a) Pour the turbidity-free water into the high turbidity measuring cell to the standard line. Insert the 20° base plate to the numbered side of the cell and put the cell into the tank with numbered side of the cell facing one side of the tank.

b) Close the cap of the tank. Rotate the vernier adjustment knob slowly and adjust the hand to 20° scale value on the right side of dial.

c) Take out the 20° base plate and pour the sample water into the cell to the standard line. Put the cell into the tank and close the cap. Record the reading directly.

d) When the turbidity of water sample exceed l00°, it can be diluted by turbidity-free water before determination.

2.6 Computing and Reporting

Record the data in datasheet. If the water sample is diluted, calculate its concentration according to the following formula:

$$T_u = \frac{F \times (A + C)}{C}$$

Where: T_u Turbidity unit of the water sample, the unit is (°);

F Turbidity unit calculated according to working curve, the unit is degree (°);

A Volume of turbidity-free water, the unit is milliliter (mL);

C Volume of original water sample, the unit is milliliter (mL);

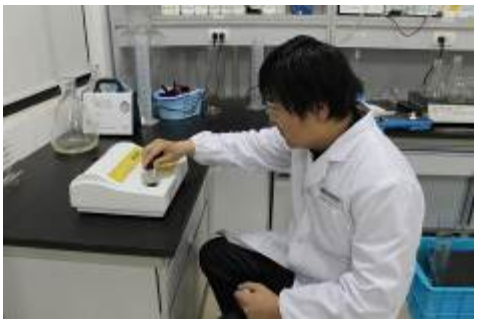
2.7 Attentions

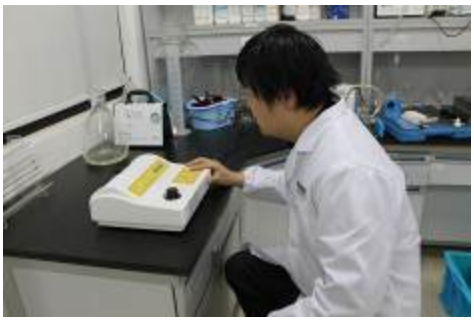
The following items should be paid attentions to:

——Unless otherwise stated, all reagents used in this method are of analytical reagent (AR) grade and all water used is turbidity-free water or equivalent pure water;

——Turbidity determination should be quick. The whole period from pouring the mixed water sample or standard sample to the cell to recording the reading should be within 3 minutes;

——The water sample should be determined on the sampling day. If the sample needs to be kept for longer time, it should be stored in dark place for 24 hours. If 0.5 g/L $HgCl_2$ fixative is added into the sample, it can be kept for 22 days.





3. Salinity (PSU)

The testing method use American YSI 85-25 Portable Multi-parameters Water Quality Meter to determine salinity of water sample in accordance with the Specification for Marine Monitoring - Part 4: Seawater Analysis (GB17378.4-2007/29.1).

3.1 Scope and Application

This method is applicable to salinity determination of seawater on land or in the laboratory on board.

3.2 Principle of the Method

Use inductive salinity meter for determination.

3.3 Reagent and Preparation

Standard seawater.

3.4 Instruments and Equipment

Main technical indicators of inductive salinity meter: conductivity ratio 0.07~1.2; measurement accuracy is 0.01; measurement precision is 0.003; salinity resolution is 0.001; accuracy of temperature measuring of electrical bridge is 0.5°C.

3.5 Procedure

3.5.1 Preparation: Place the water sample until the temperature difference between the sample and standard seawater is within ±2°C to prepare for measurement.

3.5.2 Temperature and salinity measurement inspection

3.5.2.1 Turn the temperature-salinity switch to “temperature measurement”. Compare the temperature reading with the room temperature. If the deviation is lower than ±1°C, the temperature bridge operates normally.

3.5.2.2 Tighten the drainage knob below the water cup. Place the seawater whose salinity is known to the inlet below the conductivity cell and turn the cock of conductivity cell to “inlet”. Open the air pump switch. Press the gas hole on the water cap tightly with the middle finger of the left hand, and at that time, the seawater will flow into the conductivity cell slowly. When a little seawater overflow from the outlet of conductivity cell to the water cup, turn off the cock of conductivity cell immediately, release the finger and close the air pump. The conductivity cell is filled with the seawater at that time.

3.5.3 Calibration

3.5.3.1 Fill the conductivity cell with the standard seawater slowly. Ish the cell for 1~2 times, and then measure the temperature of standard seawater and write down the data on datasheet.

3.5.3.2 Look up the corresponding R_2 value according to the temperature conversion table on the instrument panel. Rotate R_2 knob to this value and record R_2 value.

3.5.3.3 Shift the temperature-salinity switch to “salinity measurement” and adjust R1 knob to make zero-header point at “zero”. Stop stirring and drain water. Repeat filling adjustment until the reading repeats, then the calibration is completed. Record the R1 value.

3.5.4 Water sample measurement

Start up the air pump. Pipette the water sample into the conductivity cell and ish for 1~2 times. When the water sample overflows from the conductivity cell, turn off the inlet cock of conductivity cell immediately, cut down the power of the air pump and start mixing. Turn temperature-salinity switch to “temperature measurement”. Measure the temperature of the seawater sample and write down the data in the datasheet. Shift temperature-salinity switch to “salinity measurement” and adjust Rt knob to make zero-header point at “zero”. Stop stirring and drain the water sample in the conductivity cell. If the change of last digits of conductivity ratio knob in two measurements is less than 6, these two measurements are regarded as repeat. Record the conductivity ratio R_1 value of the seawater sample.

3.6 Computing and Reporting

According to the formula, programming should be relied on for calculation. The result should display three digits after the decimal point.

3.7 Attentions

Pay attention to the following items:

250mL sampling bottle and the plug should be washed for 3 times with the same water sample before use. The used sampling bottle should be filled with a little seawater but drained the water at next sampling time；

when fill the conductivity cell with the seawater sample, avoiding the bubbles in the

conductivity cell. If there are bubbles, the reading will be lower in general. At that time, the sample should be re-filled and measured again；

when fill the conductivity cell with the seawater sample, drain the residual water sample inside inflow tube and dry the tube. After that, measure the sample according to the steps described. Otherwise, the residual water will pollute the water sample.

4. Temperature (℃)

The testing method use Surface Water Thermometer to determine temperature of water sample in accordance with the Specification for Marine Monitoring - Part 4: Seawater Analysis (GB17378.4-2007/25.2).

4.1 Measuring Method

When using the surface water thermometer, tie the haul loop on top of the metal pipe with rope firstly. Use bucket to collect water sample. Put the surface water thermometer into the bucket during measurement. After responding for 1-2 minutes, empty the water in the bucket and thermometer tube. Collect the water sample and put the thermometer into the bucket again. Read the temperature after responding for 3 minutes and read the temperature again after another 1 minute. When the air temperature is higher than the water temperature, calibrate the smaller one of the readings to get the measure value of surface water temperature. On the contrary, calibrate the bigger one of the readings to get the measure value of surface water temperature.

4.2 Attentions

——when reading the data, the sight line and the top of the capillary of the surface water thermometer should be at the same level. Direct sunlight should be avoided.

——the bucket is better to be made of heat-insulation materials. The capacity is about 5L-10L.

——Surface water thermometer must have periodic verifications according to the specification.

5. Determination of Dissolved Oxygen (mg/L)（iodimetry）

Iodometric method is adopted to measure dissolved oxygen in accordance with the Specification for Marine Monitoring - Part 4: Seawater Analysis (GB17378.4-2007/31).

5.1 Application Scope and Fields

This method is applicable to dissolved oxygen determination of seawater in ocean and offshore area and river water in river and river mouth.

5.2 Principle of the Method

Dissolved oxygen in water sample reacts with manganese chloride and sodium hydroxide to generate brown precipitate of higher valency manganese. In the presence of iodide ions in an acidic solution, liberation of iodine is released which is equivalent to original DO content. Liberation of iodine is then titrated with a standard solution of sodium thiosulfate. DO content can be obtained by conversion.

5.3 Reagent and Preparation

5.3.1 Manganese chloride solution: weigh 210g manganese chloride ($MnCl_2 \cdot 4H_2O$). Dissolve it into water and dilute to 500ml.

5.3.2 Alkaline potassium iodide solution: weigh 250g sodium hydroxide (NaOH). Dissolve it into 250ml water along with stirring. After it cools down, add 75g potassium iodide (refer to 3.6) and dilute to 500ml. Store it in brown reagent bottle with rubber plug.

5.3.3 Sulfuric acid solution (1+1): Carefully add the same volume of concentrated sulfuric acid (H_2SO_4: 1.84 g/mL) to the same volume of water while stirring and make it mixed. Store it in reagent bottle.

5.3.4 Sodium thiosulfate solution [c ($Na_2S_2O_3 \cdot 5H_2O$) = 0.01 mol/L].

5.3.5 Starch solution (5 g/L).

5.3.6 Potassium iodide (KI): chemical pure.

5.3.7 Standard potassium iodate solution [c ($1/6KIO_3$) = 0.0100 moI/L]: weigh 3.567g potassium iodate (KIO_3, GR, pre-bake for 2 hours at 120°C and cool in the silica gel drier). Dissolve it into water and shift it to 1000ml volumetric flask totally. Fix the volume by adding water and mix the solution. Store it in cold and dark place. Period of validity is one month. Measure 10.00ml and dilute to 100ml with water when it is used.

5.4 Instruments and Equipment

Water sample bottle: Capacity of 125ml. Brown ground glass bottles with tapered cork. Grinding mouth should be tight and the capacity needs to be correction.

Glass tube: Diameter of 5mm~6mm. Length of 12cm.

Latex tube: Same diameter with the glass tube. Length of 20cm - 30cm.

Dissolved oxygen burette: Capacity of 25ml. Scale graduation of 0.05mL;

Magnetic stirrer: Rotational speed can be adjusted to (150-400) r/min;

Glass magnetic rotor: Diameter of about 3mm~5mm. Length of 25mm;

Conical flask: Capacity of 250mL;

Iodine bottle: Capacity of 250mL;

Graduated cylinder: Capacity of 100mL;

Beaker: Capacity of 500ml, 1000mL;

Double inflating ball;

Brown reagent bottle: Capacity of 500ml, 2500mL;

Bottle- top dispenser: Capacity of 5mL;

Transfer pipet: Capacity of 20mL;

Common instruments and equipments in general laboratory

5.5 Procedure

5.5.1 Water sample fixing: Open the cork of water sample, and immediately add 1.0 ml manganese chloride solution and 1.0 ml alkaline potassium iodide solution orderly to water sample using bottle- top dispenser (tube tip is inserted under liquid surface). Tie the cork tightly (bubble is forbidden in the bottle) and hold it. Turn the bottle upside down for no less than 20 times.

5.5.2 Determination steps

a) wait for about 1 hour or precipitation is produced completely, titration can be started;
b) Pour the supernatant of sample bottle into the 250 ml conical flask, and add 1.0 ml sulfuric acid solution immediately. Tie the cork tightly and shake the bottle until precipitates are dissolved totally.
c) Pure the whole solution in the sample bottle into the conical flask. Place it on the magnetic stirrer, and stir immediately. Titrate with the calibrated sodium thiosulfate

solution;

d) When the test solution looks yellow, add 1 ml starch solution. Continue to titrate until blue has just faded. Swing ish original water sample bottle with few test solution in conical flask, and then pour it back to the conical flask. Continue to titrate until colorless. Wait for 20 seconds. If the test solution doesn't look light blue, titration endpoint is reached. Record the volume of sodium thiosulfate solution for titration in datasheet.

5.5.3 Blank test

Measure 100mL seawater and mix the water with 1.0 ml sulfuric acid solution, 1.0 ml alkaline potassium iodide solution and 1.0 ml manganese chloride solution. Place the solution aside for 10 minutes. Add 1 ml starch solution. Mix thoroughly. Meanwhile, if the solution looks light blue, continue to titrate with sodium thiosulfate solution. If the used amount of sodium thiosulfate solution is more than 0.1mL, check the reliability of potassium iodide and manganese chloride reagent and prepare the reagent again. If the used amount of sodium thiosulfate solution is no more than 0.1mL or the solution doesn't look light blue after adding starch solution, and the solution looks blue immediately once 1 drop of standard potassium iodate solution is added, reagent blank can be ignored.

Blank test should be done one time for each batch of new prepared reagent.

5.6 Computing and Reporting

5.6.1 Concentration of dissolved oxygen in water sample can be calculated as follows:

$$\rho_{O_2}=\frac{c\times V\times 8}{V_0}\times 1000$$

Where: ρ_{O2}——concentration dissolved oxygen in water sample, the unit is mole per liter (mg/L);

c——concentration of sodium thiosulfate solution, the unit is mole per liter (mg/L);

V——volume of sodium thiosulfate solution used for titration of the sample, the unit is milliliter (mL);

V_0——actual volume of the water sample used for titration (=capacity of the water sample bottle – fixative to fix the water sample), the unit is milliliter (mL);

5.6.2 Dissolved oxygen saturation can be calculated as follows:

$$\text{Oxygen saturation}(\%) = \frac{\rho_{O_2}}{\rho_{O_2}'} \times 100$$

Where: ρ_{O2}——measured oxygen content, the unit is milligrams per liter (mg/L);

ρ_{O2}'——oxygen saturation concentration in seawater at water temperature and salinity on the scene, the unit is milligrams per liter (mg/L).

5.7 Attentions

Unless otherwise stated, all reagents used in this method are of analytical reagent (AR) grade and all water used is distilled water or equivalent pure water;

Dissolved water sample bottle needs capacity calibration: Fill the water sample bottle with full of distilled water. Tie the cork, dry the bottle and then weigh it. This weight minus the weight of dry empty bottle and is divided by the density of distilled water, and then the capacity of the bottle can be achieved. Record the number of the bottle and the corresponding measurement results of capacity for future reference;

When endpoint is near, the titration speed should not be too slow, otherwise color changes would be insensitive at the endpoint. If the solution looks amaranth before reaching the endpoint, it shows the starch solution has been deteriorated and needs to be prepared again.

If water sample contains oxidizing substances, it can separate iodine so as to produce the positive interference. If it contains reducing substances, it can consume iodine so as to produce the negative interference.

6. Determination of Total Suspended Solids - Gravimetric method

6.1 Scope and Application

This method is applicable to suspended matter measurement of water in river mouth, harbor and ocean.

6.2 Principle of the Method

When the certain amount of water pass through 0.45μm filter membrane, weigh the suspended matter which left on the filter membrane to calculate the concentration of suspended matter in water.

6.3 Instruments and Equipment

Instruments and equipment is as follows:

Balance: senses of balance are 0.1 mg and 0.01 mg;

Water sampler;

Organic glass screw mouth filter: 60 mm diameter and is fit for high concentration water body in river mouth or shallow sea;

Glass clamp type filter: 47 mm diameter and is fit for low concentration water body;

Vacuum pump: sucking rate is 30 L/min;

Measuring cylinder: 250 ml, 500 ml and 1000 ml;

Filter membrane: 0.45μm pore size and 47 mm or 60 mm diameter;

Filter membrane box: 50 mm or 63 mm diameter;

Conical flask, washing bottle, rubber tube, bucket, gas pressure meter, sample box, etc.

Stainless steel tweezers;

Common used instruments and equipment in laboratory.

6.4 Procedure

6.4.1 Filter membranes are baked at 40-50℃ and keep them at constant temperature for 6 – 8 h. After that, take them into the silica gel drier for 6 – 8 h.

6.4.2 Count the number of blank calibrated membranes and point color dot on membrane to distinguish the filter membranes of water samples.

6.4.3 Weigh filter membranes and put them into the numbered filter membrane boxes.

6.4.4 Use stainless steel tweezers to put the weighed water sample filter membrane (W2)

on the weighed blank calibrated membranes (Wb), and place both of them into filter.

6.4.5 Mix water sample thoroughly. Use measuring cylinder to take a certain volume of water sample (If the concentration of suspended matter is greater than 1000 mg/L, take 50 – 100 ml of water sample; if the concentration of suspended matter is less than 100 mg/L, take 1 – 5 L of water sample).

6.4.6 Turn on vacuum pump. Pour water sample into the filter. Use distilled water to wash the measuring cylinder and the washing water is also poured into the filter. In order to wash away salinity, after pumping water out at the first time, use distilled water to wash filter membrane for three times (50 ml at a time), and then pump water out again.

6.4.7 Use stainless steel tweezers to take the filter membranes back to their original filter membrane boxes. Either place the boxes into oven and dry them at 50℃, or dry them by air drying in natural environment. Cap the filter membrane box and preserve them in order. Bring these boxes to laboratory.

6.4.8 Drying: place filter membranes into the electro-thermal constant temperature dry box (40~50℃) to dewater filter membranes at constant temperature for 6 – 8 h. Thereafter, take filter membranes out and place them into the silica gel drier. After 6 – 8 h, weigh them.

6.4.9 Weighing: select the sense of analytical balance on basis of the quality of suspended matter. If the quality is less than 50 mg, select the one hundred thousandth balance; if the quality is greater than 50 mg, select the one ten thousandth balance. Weighing should be quickly. Two times of weigh, before and after filtration, should keep the similar conditions in terms of temperature and humidity in balance room.

6.4.10 Blank calibration of filter membrane

During filtration, the glass fiber filter membrane might be dissolved leading to weight loss. Therefore, it must carefully deal with the weighing work of blank calibration sample.

6.5 Computing and Reporting

$$\rho = \frac{W_1 - W_2 - \Delta W}{V}$$

$$\Delta W = \frac{1}{n} \times \sum^{n} (W_n - W_b)$$

where:

ρ —— the concentration of suspended matter, (mg/L);

W_1—— the total weight of suspended matter, water sample and filter membrane, (mg);

W_2—— the weight of water sample and filter membrane, (mg);

ΔW —— the calibration value of blank calibration sample, (mg);

V—— the volume of water sample, (L).

The calibration value of blank calibration sample can be calculated by the following formula:

$$\Delta W = \frac{1}{n} \times \sum^{n} (W_n - W_b)$$

where:

W_n—— the weight of the blank calibration filter membrane after filtration, (mg);

W_b—— the weight of the blank calibration filter membrane before filtration, (mg);

n—— the number of the blank calibration filter membrane;

ΔW should be negative number.

7. Determination of Dissolved Organic Carbon (DOC)

The determination of dissolved organic carbon adopts the total organic carbon instrument method in accordance with the Specification for Marine Monitoring - Part 4: Seawater Analysis (GB17378.4-2007/34.1).

7.1 Scope and Application

This method is applicable to measure total organic carbon (TOC) and dissolved organic carbon (DOC) in seawater.

7.2 Principle of the Method

The seawater sample accessed to the sample injector would automatically enter into the total carbon (TC) combustion tube which has platinum catalyst. The combustion temperature is 680℃. Feed high purity air to oxidize carbonaceous organic material to carbon dioxide, and then total carbon is quantified by the non-dispersive infrared analyzer. After that, the same sample is automatically inputted into inorganic carbon (IC) reactor which has 25% phosphoric acid solution inside. At the room temperature, inorganic carbonate is acidized to carbon dioxide, and then total carbon is quantified by the non-dispersive infrared analyzer. The quantity of TOC would be achieved by TC minus IC.

In addition, a 2 mol/L of hydrochloric acid is used to acidize the seawater sample beforehand and air pump bubbles the water sample for 5 – 10 min to remove IC, the measure value of TC is equal to TOC.

The collected sample should be filtered and analyzed immediately by the preheat (450℃, 5h) Whatman GF/C glass fiber filter membrane. If the sample cannot be analyzed immediately, add some mercury chloride to the sample and place the sample into the freezer for preservation. Other procedures are same with TOC test. The measure value of TOC is equal to DOC.

7.3 Reagents and Preparation

7.3.1 Potassium peroxydisulfate ($K_2S_2O_8$).

7.3.2 Phosphoric acid (H_3PO_4).

7.3.3 Sodium carbonate (Na_2CO_3).

7.3.4 Sodium bicarbonate ($NaHCO_3$).

7.3.5 Hydrochloric acide (HCl).

7.3.6 Potassium hydrogen phthalate.

7.3.7 Anhydrous sodium sulfate.

7.3.8 Carbon-free water: distilled water is imported into the glass reflux equipment, and 10 g potassium sulphate (K_2SO_4) and 2 ml phosphoric acid are added to 1 L water. Besides, add some zeolite. After heating and refluxing for 4 h, use ground glass receiving device to collect the fractionated water. When the water cools down to the room temperature, use it immediately.

7.3.9 Sodium carbonate: prebake sodium carbonate at 500℃ for 30 min, and then put it into the desiccator which has anhydrous sodium sulfate inside. Store it for later use.

7.3.10 Hydrochloric acid solution: 2mol/L.

7.3.11 Phosphoric acid solution (25%): use water to dilute 25 ml phosphoric acid solution to 100 ml.

7.3.12 Standard stock solution of total carbon (1000 mg/L): dissolve 2.1250 g phthalic acid which is prebaked at 115℃ for 2 h into water. Move the solution into 1000 ml volumetric flask and fix the volume by adding water. Mix thoroughly.

7.3.13 Standard stock solution of inorganic carbon (1000 mg/L): dissolve 4.4100 g sodium carbonate and 3.5000 g sodium bicarbonate into water. Move the solution into 1000 ml volumetric flask and fix the volume by adding water. Mix thoroughly.

7.3.14 Standard solution of total carbon: pipette 0.00 ml, 5.00 ml, 10.00 ml and 15.00 ml of stock solution of total carbon into four 250 ml volumetric flasks, respectively. Fix the volume by adding the no-carbon water. The corresponding concentration of standard solution is 0.00mg/L, 20.00mg/L, 40.00mg/L and 60.00mg/L, respectively. Prepare this solution just when using.

7.3.15 Standard solution of inorganic carbon: pipette 0.00 ml, 5.00 ml, 10.00 ml and 15.00 ml of stock solution of inorganic carbon into four 250 ml volumetric flasks, respectively. Fix the volume by adding the no-carbon water. The corresponding concentration of standard solution is 0.00mg/L, 20.00mg/L, 40.00mg/L and 60.00mg/L, respectively. Prepare this solution right before using.

7.4 Procedure

7.4.1 Calibration curve

Under optimal technical parameters of instrument, inject 0mg/L, 20.00mg/L, 40.00mg/L and 60.00mg/L of the standard solutions of total carbon (TC). The instrument would draw the working curve of TC. Similarly, input 0mg/L, 20.00mg/L, 40.00mg/L and 60.00mg/L of the standard solutions of inorganic carbon (IC). The instrument would draw the working curve of IC. In general, making parallel determination for 2 – 3 times is necessary. Relying on the zero point transfer function of the instrument, move calibration curve to the origin point and save the results.

7.4.2 Sample testing

Wait for stabilization of instrument and start testing. First test TC value of sample and later test IC value of sample.

7.5 Computing and Reporting

7.6 Precision and Accuracy

When carbon content is 3.00mg/L, the relative standard deviation of repeatability is 2%; the relative standard deviation of reproducibility is 2%; relative error is 1%.

8. Determination of Particulate Organic Carbon (POC)

Spectrophotometry is adopted as the testing method in accordance with Gulf ecosystem observation methods (China Environmental Science Press, 2005/4.5.14.1).

8.1 Principle of the Method

Spectrophotometry measures the decreased absorbance of yellow dichromate solution to determine the amount of oxidizable carbon. The reaction is wet oxidation between acidic dichromate and carbon. The measurement range (measured as C) is 10~4000μg/L.

8.2 Instruments and Equipment

8.2.1 Spectrophotometer

8.2.2 Filter membrane: 0.45μm of pore size; GF/C glass fiber filter membrane (diameter is 47 mm) and standard micropore filter equipment. Filter membrane should be packed inside aluminum foil and placed into muffle furnace. 24 h heating at 450~500℃ could remove oxidizable substances (the temperature should be not more than 500℃, otherwise the filtration ability would be changed).

8.2.3 50 ml cylinder with stopper and 1000 ml volumetric flask

8.3 Reagents and Preparation

8.3.1 Sulfuric acid – dichromate oxidation: dissolve 4.84 g potassium dichromate ($K_2Cr_2O_7$) in 20 ml distilled water, and put this solution slowly into 1000 ml volumetric flask which has 500 ml concentrated sulfuric acid (AR). After the mixed solution cools down, fix volume of 1000 ml volumetric flask by adding concentrated sulfuric acid, and then preserve this solution in glass bottle with stopper. This solution is stable.

8.3.2 Phosphoric acid (H_3PO_4, AR): 70%.

8.3.3 Sodium sulfate solution: dissolve 45 g sodium sulfate (Na_2SO_4) in 1000 ml distilled water.

8.4 Procedure

8.4.1 Put the pretreated glass fiber filter membrane in the standard micropore filter equipment. Connect filter equipment with vacuum pump that can control the vacuum degree. Pipette some seawater sample (0.5 L – 2 L in general) to filter equipment. After filtration, the air suction of filter membrane lasts for 1 min. Add 2 ml sodium sulfate solution and then repeat air suction. Add 2 ml sodium sulfate solution again

and take out filter membrane during air suction.

8.4.2 Put the filter membrane into a 30 ml beaker. Add 1.0 ml phosphoric acid and 1.0 ml distilled water into the beaker. Mix thoroughly. Put the beaker on heating plate for heating 30 min at 100-110℃. Watch glass should be covered when heating.

8.4.3 Add some sulfuric acid – dichromate oxidation and distilled water. The volume of additions can be determined by the expected carbon quantity. As shown in the following table:

Table 1 evaluation of the dosage of oxidation

Expected carbon quantity/μg	Oxidation/ml	Distilled water/ml	Final volume/ml	Length of cuvette/cm
- 300	2.00	0.8	100	10
300 - 700	4.00	1.6	50	2.5
700 - 2000	10.00	4.0	50	1

8.4.4 Mix thoroughly. Every beaker should be covered by watch glass and be heated at 100~110℃ for 60 min.

8.4.5 After mixed substances cools down, move the solution and glass fiber filter membrane to the proper measuring cylinder which is mentioned in Step C. Distilled water is used to wash beaker wall and pour the wash water into the measuring cylinder. Plug the bottle tightly and mix upside down thoroughly. Waiting for the solution to cool down at room temperature and the filter membrane would sink to the bottom of the measuring cylinder.

8.4.6 At the wavelength of 440 nm, measure the decreased absorbance of blank solution using practical water sample as the reference solution.

8.4.7 Because Cr III absorbs the decreased light, the measured absorbance should be calibrated by the following formula:

$$E = 1.1 \times E_f$$

Where:

E_f— the difference of decreased absorbance between practical water sample and blank solution in Step F.

POC (measured as C) could be computed by the following formula, μg/L:

$$\text{POC} = \frac{E \times F \times v}{V}$$

where:

V— the volume of the filter seawater, L;

v— the required volume of oxidation;

F— see the Calibration 8.6.

8.5 Blank Test

Every unknown sample should carry out the blank determination using the filter membrane and oxidation. Using distilled water as reference solution, the decreased absorbance of blank should be in the range of 1 – 1.1.

8.6 Calibrations

8.6.1 Standard solution of glucose: dissolve 7.50 g glucose and some quantities of mercuric chloride crystal ($HgCl_2$) to form 100 ml solution. The stability of the solution could be preserved in freezer for several months. If the solution turns up turbidity, it cannot continue to be used.

Add distilled water to dilute the solution from 10 ml to 1 L so that 1.00 ml solution has 300μg C.

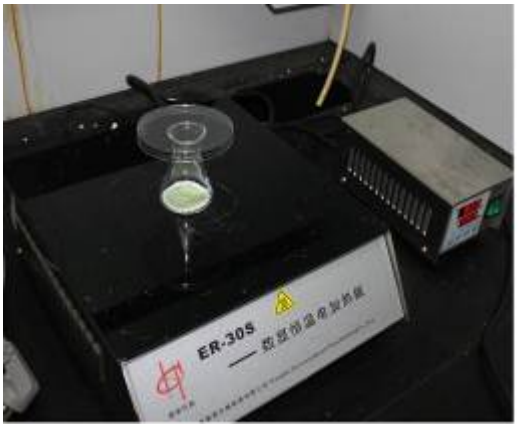
8.6.2 Steps: put one piece of glass fiber filter membrane and 1 ml phosphoric acid into a 30 ml beaker. Heat them at 100 – 110℃ for 30 min. Add 10 ml oxidation and 4 ml dilute glucose solution into every beaker. Following the Step D – G in 4, factor F could be computed as follows:

$$F = \frac{120}{E_3}$$

where:

E_3— the average decreased absorbance of the calibrated Cr III at 440 nm wavelength.

The computed result of F is about 274.


ER-30S

0396

9. Determination of Total residual oxidants (TRO)

The testing method is in accordance with Water quality - Determination of free chlorine and total chlorine - Spectrophotometric method using N, N – diethyl - 1, 4 – phenylenediamine (HJ 586-2010).

9.1 Principle of the Method

When pH value is in the range of 6.2-6.5 and having excess potassium iodide, chlorine, hypochlorous, pypocholoride and chloramine would react with DPD to produce red compound. The absorption measurements of this color could be measured by spectrophotometry at wavelength of 515 nm to measure the total residual oxidants.

Unless otherwise stated, analytical reagent (AR) which meets the national standards should be used in the following analysis.

9.2 Reagents and Preparation

9.2.1 The water used for experiment should be deionized water or double distilled water that does not contain chlorine and reducing substances. The water should be tested before use.

Test method: Mix 100 ml of the water under test and 1.0 g KI in 250 ml conical flask, and after 1 min, add 5.0 ml buffer solution and 5.0 ml DPD solution; meanwhile mix 100 ml of the water under test and 2 droppers of NaClO solution in another 250 ml conical flask, and after 2 min, add 5.0 ml buffer solution and 5.0 ml DPD solution.

The first flask should show colorless and the second flask should show pink. Otherwise, the water used for experiment should be pretreated by activated charcoal column to remove chlorine, and thereafter follow above test procedures until the water quality meet the demand.

9.2.2 Concentrated sulfuric acid: $\rho = 1.84$ g/ml.

9.2.3 Potassium iodide (KI): crystal.

9.2.4 NaClO solution: ρ (Cl_2) ≈ 0.1 g/L, dilute the concentrated NaClO solution to obtain.

9.2.5 Sulfuric acid solution: c (H2SO4) = 1.0 mol/L. Carefully add 54.0 ml of the concentrated sulfuric acid into 800 ml water and keep stirring simultaneously. When the solution cools down, move it into 1000 ml volumetric flask and fix the volume by adding water. Mix thoroughly.

9.2.6 NaOH solution: c (NaOH) = 2.0 mol/L

Weigh 80 g NaOH and mix with 800 ml water. After the solution cools down, move it into 1000 ml volumetric flask and fix the volume by adding water. Mix thoroughly.

9.2.7 NaOH solution: c (NaOH) = 1.0 mol/L

Weigh 40 g NaOH and mix with 500 ml water. After the solution cools down, move it into 1000 ml volumetric flask and fix the volume by adding water. Mix thoroughly.

9.2.8 Stock solution of potassium iodate: ρ (KIO_3) = 1.006 g/L

Weigh 1.006 g potassium iodate (GR) which should be prebaked at 120℃~140 ℃ for 2 h. Dissolve it in water and move it into 1000 ml volumetric flask and fix the volume by adding water. Mix thoroughly.

9.2.9 Standard solution of potassium iodate I: ρ (KIO_3) = 10.06 mg/L

Pipette 10.0 ml of stock solution of potassium iodate into 1000 ml brown volumetric flask. Add 1 g KI to the flask and fix the volume by adding water. Mix thoroughly. Prepare this solution just when using. 1.00 ml Standard solution of potassium iodate has 10.06 μg KIO_3 that is equal to 0.141 μmol (10.0 μg) Cl_2.

9.2.10 Standard solution of potassium iodate II: ρ (KIO_3) = 1.006 mg/L

Pipette 10.0 ml of stock solution of potassium iodate into 100 ml brown volumetric flask. Fix the volume by adding water. Mix thoroughly. Prepare this solution just when using. 1.00 ml Standard solution of potassium iodate has 1.006 μg KIO_3 that is equal to 0.0141 μmol (1.0 μg) Cl_2.

9.2.11 Phosphate buffered solution: pH = 6.5

Weigh 24.0 g Na_2HPO_4 or 60.5 g $Na_2HPO_4 \cdot 12H_2O$ and 46.0 KH_2PO_4. Dissolve them in water successively. Moreover, add 100 ml $C_{10}H_{14}N_2O_8Na_2 \cdot 2H_2O$ (c = 8.0 g/L) or 0.8 g EDTA and move this solution into 1000 ml volumetric flask and fix the volume by adding water. Mix thoroughly. On occasion, it could add 0.02 g mercuric chloride to the solution to avoid mycete reproduction and the influence of trace iodide on free chlorine test.

9.2.12 N, N – diethyl - 1, 4 – benzene 2 amine sulfate solution (DPD): ρ [NH_2 - C_6H_4 – N $(C_2H_5)_2 \cdot H_2SO_4$] = 1.1 g/L

Add 2.0 ml sulfuric acid solution and 25 ml $C_{10}H_{14}N_2O_8Na_2 \cdot 2H_2O$ (c = 8.0 g/L) or 0.2 g EDTA into 250 ml water to form the mixed solution. Besides, add 1.1 g anhydrous DPD sulfate or

1.5 g compound with $5H_2O$ into the solution. Move this solution into 1000 ml brown volumetric flask and fix the volume by adding water. Mix thoroughly. this solution should be preserved in reagent bottle at 4℃. After the long time preservation, if the solution color changed, it should be re-prepared again.

Note1: it is possible to use 1.1 g DPD oxalate or 1.0 DPD hydrochloride to replace DPD sulfate.

9.2.13 Sodium arsenite solution or thioacetamide solution: ρ ($NaAsO_2$) = 2.0 g/L, ρ (CH_3CSNH_2) = 2.5 g/L.

9.3 Instruments and Equipment

9.3.1 Visible infrared spectrometer: having 10 mm and 50 mm cuvettes.

9.3.2 Balance: precision is 0.1 g and 0.1 mg respectively.

9.3.3 Common used instruments and equipment in laboratory.

Note2: glassware used in experiment should soak in sodium hypochlorite solution for 1 h, and then ish them adequately using water.

9.4 Sample

9.4.1 Sampling

Free chlorine and total chlorine are not stable, so samples should be test on site as far as possible. Otherwise, the samples should be added fixing agent for preservation. It is better to add NaOH solution (1% volume of sample) to brown glass bottle beforehand. When the glass bottle is full of sample, plug the bottle tightly and seal the mouth to avoid contacting with air. If the sample is acidic solution, it should add more NaOH solution to ensure the pH value is greater than 12.

9.4.2 Preservation

Use freezer to transport water sample to laboratory. The water sample should be preserved in dark place at 4℃ and should be tested in 5 days.

9.5 Procedure

9.5.1 Calibration curve

9.5.1.1 Calibration curve for high concentration samples

Pipette 0 ml, 1.00 ml, 2.00 ml, 3.00 ml, 5.00 ml, 10.0 ml and 15.0 ml of standard solution of potassium iodate I (KIO_3) into 100 ml volumetric flask, respectively. Add 50 ml

water and 1.0 ml sulfuric acid solution (H_2SO_4) to every volumetric flask. After 1 min, add 1 ml NaOH solution to every volumetric flask and fix the volume by adding water. The mass concentration of chlorine ρ (Cl_2) in each volumetric flask is 0.00, 0.10, 0.20, 0.30, 0.50, 1.00 and 1.50 mg/L, respectively.

Add 15.0 ml buffer solution and 5.0 ml DPD into a 250 ml conical flask. In 1 min, add above series of prepared solutions into the conical flask. Mix thoroughly. Use 515 nm of wavelength and 10 mm cuvette to measure absorbance of every solution. It should finish colorimetric analysis in 60 min.

Take the absorbance value of corrected blank sample as vertical coordinates and take the corresponding mass concentrations of chlorine $\rho(Cl_2)$ as abscissa to draw the calibration curve.

9.5.1.2 Calibration curve for low concentration samples

Pipette 0 ml, 2.00 ml, 4.00 ml, 8.00 ml, 12.00 ml, 16.0 ml and 20.0 ml of standard solution of potassium iodate II (KIO_3) into 100 ml volumetric flask, respectively. Add 50 ml water and 1.0 ml sulfuric acid solution (H_2SO_4) to every volumetric flask. After 1 min, add 1 ml NaOH solution to every volumetric flask and fix the volume by adding water. The mass concentration of chlorine ρ (Cl_2) in each volumetric flask is 0.00, 0.02, 0.04, 0.08, 0.12, 0.16 and 0.20 mg/L, respectively.

Add 15.0 ml buffer solution and 1.0 ml DPD into a 250 ml conical flask. In 1 min, add above series of prepared solutions into the conical flask. Mix thoroughly. Use 515 nm of wavelength and 10 mm cuvette to measure absorbance of every solution. It should finish colorimetric analysis in 60 min.

Take the absorbance value of corrected blank sample as vertical coordinates and take the corresponding mass concentrations of chlorine $\rho(Cl_2)$ as abscissa to draw the calibration curve.

9.5.2 Total chlorine test

Add 15.0 ml phosphate buffered solution, 5.0 ml DPD solution, 100 ml water (or the diluted water sample) and 1.0 g KI into 250 ml conical flask. Mix thoroughly. Under the same condition with that of drawing calibration curve, measure the absorbance. Use the absorbance value of corrected blank sample to compute the corresponding mass

concentration of chlorine (ρ_2).

Note 4: when measuring the low concentration sample, it should add 1.0 ml DPD solution.

9.5.3 Blank test

Use experimental water to replace practical water samples. Following the same procedure of 9.5.2, measure the blank samples at the same time.

9.6 Computing and Reporting

9.6.1 Computational formula

The mass concentration of total chlorine is computed by the following formula:

$$\rho(Cl_2) = (\rho_2 - \rho_3) \times f$$

where:

$\rho(Cl_2)$—— the mass concentration of total chlorine in water sample, mg/L;

ρ_2—— the mass concentration of total chlorine in test sample mg/L;

ρ_3 —— the equivalent mass concentration of chlorine when measuring the interference of manganese oxide and Cr VI, mg/L; if there is not manganese oxide and Cr VI in sample, the ρ3=0 mg/L;

f—— the dilution factor of test sample.

9.6.2 Reporting results

If the result is less than 0.01 mg/L, it should be accurate to three decimal places; if the result is greater than or equal to 0.01 mg/L and less than 10 mg/L, it should be accurate to two decimal places; if the result is greater than 10 mg/L, it should present three significant figures.

9.7 Quality Control

9.7.1 The correlation coefficient of regression equation of calibration curve should be greater than 0.999.

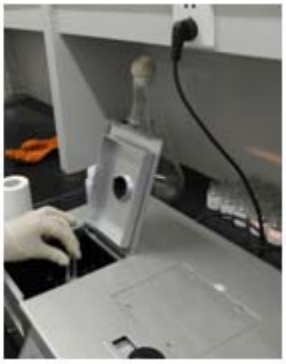
9.7.2 Each group of samples should have one middle check point. The relative error between the measure result of middle check point and the corresponding concentration in calibration curve should be more than 15%.

9.8 Attentions

9.8.1 When measuring samples on site, if the sample is too acidic, too alkaline or has too

high salinity, the dosage of buffer solution should be increased to ensure the pH value in the range of 6.2-6.5. In the process of testing, the sample should be avoided bright light, shake and moist heat.

9.8.2 If the sample is much acidic solution, it should add more NaOH fixing agent to ensure the pH value is greater than 12; if dosage of NaOH solution is more than 1% of sample volume, the volume of sample should be calibrated; for the much alkaline samples (pH>12), it is no need to add the fixing agent but to increase the dosage of buffer solution to adjust the pH value in the range of 6.2 - 6.5; for the high saline samples having fixing agent, it also should adjust the dosage of buffer solution when testing to adjust the pH value in the range of 6.2 - 6.5.



B. Test Methods for Organisms

1. Organism Size: 10-50μm

1.1 Algal Cultivation

1.1.1 Algal community preparation

Prepare five algal species of three phyla for the artificial culture. Except for the initial algal species, others could be achieved by purchase or separation.

1.1.2 Algal purification

The water sample for separation could be derived from the port, or could use plankton net to gain the algal species with size (10-50μm) from seawater. After sampling, the first step is preparation cultivation. When the density of target algal species reaches the required value, separation could be started.

Before the preparation cultivation, the water should be filtered by fine sieve to remove big algal species, and then add some NH_4NO_3 (10-25ppm) and KH_2PO_4 (1-2ppm). For some algal species which is difficult to cultivated, the addition of organic matter including the soil extraction liquid, sea mud extraction liquid, glucose and peptone is commonly used.

The triangle flask or culture tube is used as container for preparation cultivation. Put 100ml culture solution into container and add the filtered seawater, and then place the container into the illumination incubator to have the appropriate condition of cultivation.

During the preparation cultivation process, certain amount of nutrients could be supplied every half month or one month. According to the different character of target algal species, container shake may not be necessary. Because of the different algal species, the special algal community could be formed insider container. When one algal community is transferred to other culture solution, there is possible to get more pure algal species or reduce other unwanted algal species so as to make the algal separation easier.

After some periods of cultivation, there appear to have some colors in water which shows the rapid reproduction and growth of algal have been inside container. If the target algal species can be observed by microscopic observation, it shows the separation could be processed. Micropipette separation method could be adopted.

1.1.3. Expansion cultivation

Prepare F/2 liquid medium, and transfer the algal species which is in exponential growth phase to incubator for step by step expansion. There is necessary to add $NaSiO_4$ to culture solution when cultivating algal species of bacillariophyta.

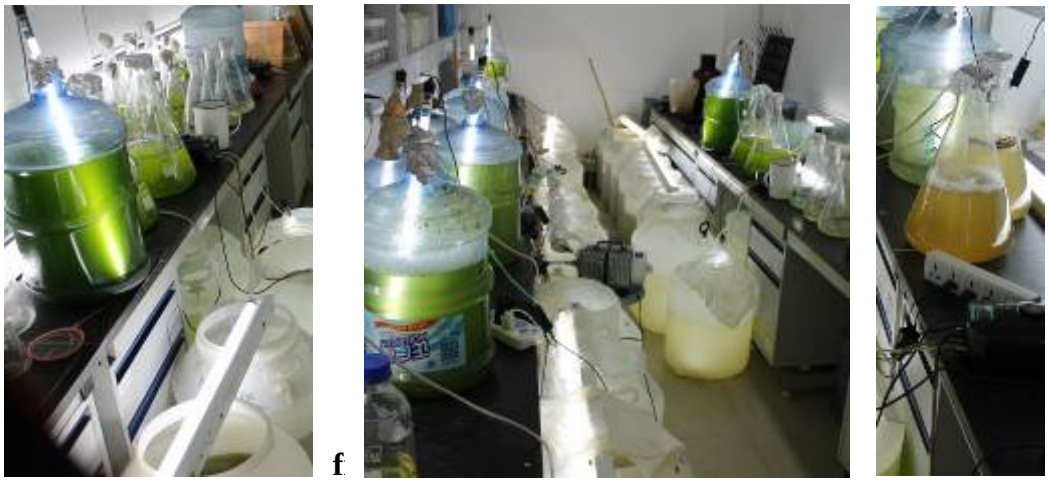
1.1.4 Cultivation for experimental use

When expansion cultivation arrive a certain scale, through controlling temperature in thermostatic chamber the big scale of algal cultivation for experimental use could be done.

1.1.5 Growth testing

In the process of above experiments, it should regularly test algal species growth speed to determine the optimal algal growth period to identify the proper time of filter experiment.

In the big scale of algal expansion cultivation, it should properly and timely add nutrients in order to avoid algal decaying, to maintain algal healthy growth and to prevent algal pollution.



1.2.1 Phytoplankton addition

Add *Nitzschia frustulum* (Bacillariophyta), *Platymonas* sp. (Chlorophyta), *Pyramimonas* sp. (Chlorophyta), *Amphora coffeaeformis* (Bacillariophyta) and *Pyrodinium* bahamense (Pyrrophyta), which are in exponential growth period, into water sample to ensure the density of plankton (10μm-50μm) in water are in the range of 10^3-10^4 cells/ml.

1.2.2 Sampling and processing methods

Collect water sample from raw water, treated water, control water sample, 5-days later of the control water sample, 2-days later of the treated water and 5-days later of the treated water. The sizes of samples are 1 L, 10 L, 1 L, 10 L, 10 L and 10 L respectively. For 10L water sample, special water sampler and 10 μm plankton net are used for the collection and filtration, and the concentrated water sample is putted into a 60 ml specimen bottle. Five drops stains are used to dye water in 15 min at first, and then use formalin to fix water sample. For 1 L water sample, 2 ml stains are used to dye water in 15min and also use formalin to fix water sample. Moreover, after 24 hours precipitation, extract the supernatant into 60 ml specimen bottle.

1.2.3 Sample identification and assessing the removal efficiency of system

Equipments:

— Microscope

— Counting Cell: 0.1 ml

— Water sampler: 0.1 ml pipette

— Coverslip.

1.2.3.1 Algal species identification and count

Except for the special handling phytoplankton and special cultivated algal species, identifications of species should be more than 80% and identifications of genus should be more than 90% in principle. The microscopic observation time of every water sample should not be less than 100-200, and the microscopic observation time of net collection sample should not be less than 500.

1.2.3.2 Sample classification and abundance measurement

If the conditions permit, the method of identification should adopt combinations of living organism and fixed sample and combinations of net collection water sample and

normal water sample to obtain the exact information of plankton community in water. Quantitative count generally based on the value of normal water sample, but quantitative count in water column should base on the value of net collection water sample. The net collection water sample could be supplementary to species composition analysis and be the quantitative count for planktons which are larger than dragnet aperture.

1.2.2.3 Optical microscope count

Extraction count,

$$C = \frac{n \times V_1}{V_2 \times V_n}$$

where:

C - the number of specimens in unit volume seawater (cells/m^3);

n - count number (cells);

V1 - the volume of extracted water sample (ml);

V2 – the volume of filtered water (m^3)

Vn – the count volume of water sample (ml).

2. Organism Size: ≥50μm

2.1 Artemia Salina Cultivation

2.1.1 Separation and preservation of artemia cysts

Artemia cysts can be obtained through separation ourselves or purchase from other institutions. In the warm season (water temperature > 15°C), it is easy to separate artemia salina by microscopic observation of plankton samples which are collected by plankton net (200 mesh). Avoiding the mix of other zooplankters, it is better to observe artemia salina on glass slide firstly and then move sample into small erlenmeyer flask or beaker to ensure the purity of artemia salina. Moreover, several parallel cultivations of pure artemia salina are necessary to avoid the possible pollution and some other accidents. Following above procedures, the abundant artemia cysts could be stored.

The separated artemia salina could be used for continuous cultivation or stored as winter egg. When the cultivation circumstance becomes bad, artemia salina could turn into mictic female and its spawn is called winter egg. After fertilization of winter egg, a thick egg shell is secreted to resist bad circumstance and finally a resting egg. Each mictic female could simultaneously or continuously spawn resting eggs or winter eggs which can hatch males. The weight of resting egg is relative heavy and generally sinks to the bottom, and some of them adhering to parent's body sink to bottom.

This kind of eggs could afford the circumstances of dry, high temperature, freeze and dramatic change of water quality. Because artemia salina mainly relies on winter egg to go through winter, winter egg is a good preservation way for artemia salina.

2.1.2 Winter egg hatch

The water used for hatch and cultivation of winter egg should be disinfected by 8ppm bleaching powder in advance and then be filter by 250 sieve mesh. Artemia salina can be obtained after 2-3 days cultivation of winter eggs in suitable temperatures (20°C±1).

2.1.3 Cultivation and management

2.1.3.1 Cultivation container

Common cultivation containers include: various types of glassware and aquarium.

Cultivation containers should be strictly sterilized before use. Due to the small size of

glassware, put them into boiler and boil them with water for 30min. As for the big size glassware and aquarium in mass cultivation, use general chemical disinfection method is enough. The common disinfect reagents are bleaching powder, potassium permanganate and so on.

Bleaching powder: Big containers or tools can be used after soaking and disinfecting with 20ppm bleaching powder for 1 hour and washing with clear water 3-4 times. For big cultivation pools such as aquarium, the high concentration starchiness liquid should be scattered on pool wall and after 24 hours that of big cultivation pools can be used to keep clear water.

Potassium permanganate: Scatter 10ppm liquid on pool wall in several times. To avoid the attachment of manganese oxide, wash container with clear water after being sterilized for 30min. This method is suitable for small size containers.

2.1.3.2 Water for cultivation

The water for cultivation should be strictly disinfected. In early cultivation, because the required volume of water is relative few, water disinfection could adopt the boiling method in high temperature. But for mass cultivation, the water should be disinfected by chemical reagents. The common used reagent is bleaching powder, which can destroy the most obnoxious microorganisms and algaes. The dosage is 8ppm. Due to the rapid wear of reagent effects, with sufficient aeration after 5-7 days, the water can be used. But before the formal cultivation, a little amount of artemia salina should be tested to make sure no adverse reaction to artemia salina and then inoculation can be started.

2.1.3.3 Inoculation

The reasonable inoculation intensity for artemia salina in general is 2-5 cells/ml. Under the appropriate conditions of temperature, baits, dissolved oxygen and illumination, it is proper to enlarge cultivation or harvest after 8-10 days

2.1.3.4 Baits feeding

The baits for artemia salina cultivation mainly are unicellular algae and organic scraps. On the suitable illumination condition, it is better to dose nutrients into the container to cultivate unicellular algae firstly, and when the color turns up to start inoculating artemia salina. In the cultivation process, to maintain the proper illumination is necessary. With the

growing density of artemia salina, the amount of unicellular algae and organic scraps cannot satisfy nutrient needs for artemia salina growth. Therefore, resupplying baits is necessary. At present, the favorite baits for artemia salina are unicellular algae, marine yeast, bread yeast, beer yeast and photosynthetic bacteria, etc.

Bread yeast should be grinded before use and filtered by 250 mesh sieve. The daily dosage is 0.01g per 10 thousand cells of artemia salina for twice. And also, the mix baits of photosynthetic bacteria and fresh yeast is good and the dosage is $5*10^6$~$10*10^6$ cells/ml. Photosynthetic bacteria can obviously prompt the growth of artemia salina.

In the small size of water body, to mix gently after dosing yeast can make yeast distributed evenly and increase dissolved oxygen in water. In the big size of water body, the continuous aeration is necessary to maintain the level of dissolved oxygen in water and to prevent precipitation of baits. But the aeration quantity should be controlled to be good for the rapid growth and reproduction of artemia salina.

2.1.3.5 Temperature control

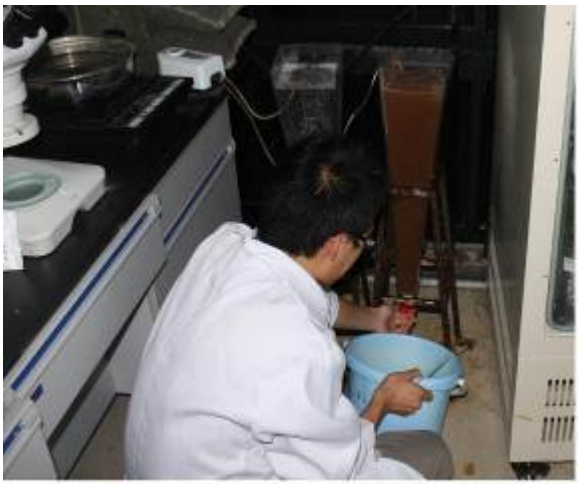
The adaptive temperature range of artemia salina is wide. The perfect growth temperature range is 25~30°C. To maintain the rapid growth speed of artemia salina, it should pay attention to keep and warm the growth temperature. It would be best to do the cultivation in illumination incubator.

2.1.3.6 Enlarge cultivation of artemia salina

Under the appropriate conditions of temperature, illumination, sufficient baits and water quality, relying on the reasonable cultivation method, when the density of artemia salina reach more than 200 cells/ml after about 10 days cultivation, the enlarge cultivation or harvest of artemia salina could be done.

2.1.4 Quantitative test of artemia salina before treatment

Take out of some culture solution and use formalin to fix the sample, after 24 hours' precipitation, microscopic examination of precipitate would be done to classify, identify and count artemia salina.



2.1.5 Quantitative test of artemia salina after treatment

Take 1 m^3 treated water sample and concentrate it by plankton net, and then use formaldehyde to fix the sample, after 24 hours' precipitation, microscopic examination of precipitate would be done to classify, identify and count artemia salina.

2.2 Addition, Identification and Count of Zooplankter

2.2.1 Addition of artemia salina

Add the experimental cultivated artemia salina to raw water to ensure the microorganism density reaches 10^5~10^6 cells/m^3.

2.2.2 Sampling method

Collect water sample from raw water, treated water, control water sample, 5-days later of the control water sample, 2-days later of the treated water and 5-days later of the treated water. The collect water quantities for these samples are 20L, 1m^3, 20L, 1m^3, 1m^3 and1m^3 respectively. Filter these samples by the plankton net with maximum oblique diameter of 50μm. The concentrated water sample is putted into a 60ml specimen bottle. Five drops stains are used to dye water in 30 min at first, and then use formalin to fix water sample.

2.2.3 Sample analysis

2.2.3.1 Equipment and materials:

— Stereo Microscope;

— Ordinary microscope;

— Counter;

— Tweezers;

— Dissecting needle;

— Counting Cell: 1 ml;

— Concentration sampling tube: use JF_{62} bolting silk to fix dropper mouth to make a concentration sampling tube. When sampling, the supernatant can be removed by the tube. The left sample would be mixed and taken to zooplankter count cell 1 ml at a time till finishing counting;

— Ordinary sampling tube: 3 ml plastic dropper.

2.2.3.2 Counting

Analysis of the concentrated sample demands that more than 90% species should be identified (except for larvae) and counted according to species.

Steps for counting:

— If the sample volume is greater than 30 ml, the sample should be placed on experiment table for 12 hours at first, and then use concentration sampling tube to remove supernatant to make a constant volume of sample at about 30 ml.

— Take the concentrated sample to a 50 ml centrifuge tube and use distilled water to wash the original sample bottle 3-4 times. The wash waters are also taken to the 50 ml centrifuge tube, and moreover, 1 ml formaldehyde should be added to fix the sample.

— The sample in the 50 ml centrifuge tube is placed on experiment table for 12 hours. Without stirring the precipitate, use the concentration sampling tube to take some supernatant to a 60 ml specimen bottle and mix the precipitation sample. Use the ordinary sampling tube to take 1 ml precipitation sample to counting cell for identification and count. The big size specimen (like jellyfish, shrimp, arrow worms, etc.) should be picked out and separately counted at first.

— The count result is recorded on datasheet.

— The precipitation sample should be repeated the above steps till counting the whole sample.

— After counting the precipitation sample, distilled water is used to wash 50 ml centrifuge tube for 3 times. Following above steps, all the wash waters should be counted.

— When finishing counting, take the sample in counting cell back to the 60 ml specimen

bottle.

2.2.3.3 Attentions

The following attentions should be noticed.

— Generally, organisms count is according to species; the dominant species and common species should be strived to identify at the level of species.

— References for identification:

a) Wenfeng Shen. Protozoology. Science press, 1999.

b) Zhong Zhen and Wenqing Cao. Marine cladocera biology. Xiamen university press, 1987.

c) Wenfang Su and Maosen Hans. China marine plankton map. Ocean press, 1993.

d) Wuchang Zhang and Nan Zhao. China marine copepoda plankton map. Science press, 1979.

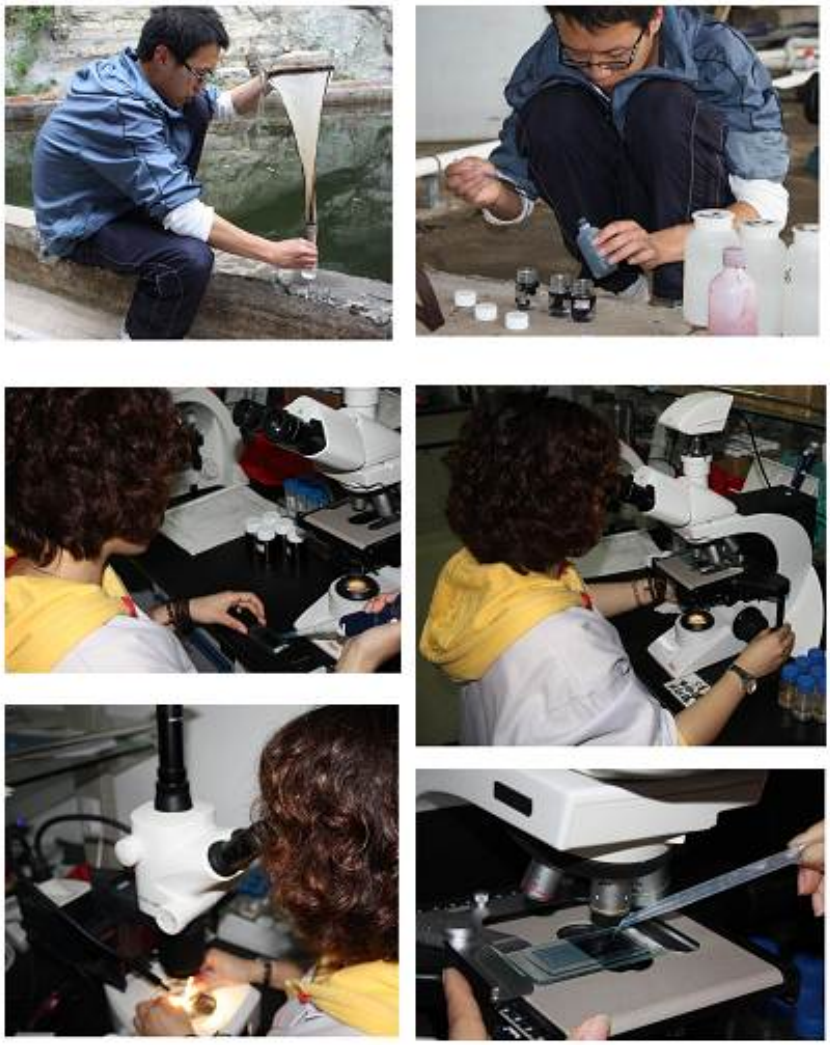
— For all the residual bodies of zooplankters, the count is according to the number of head.

— In the process of reporting and computing, the conversion of factors such as sample volume, counting results, diluted times and filter water volume should be attended to.

2.2.3.4 Sample preservation

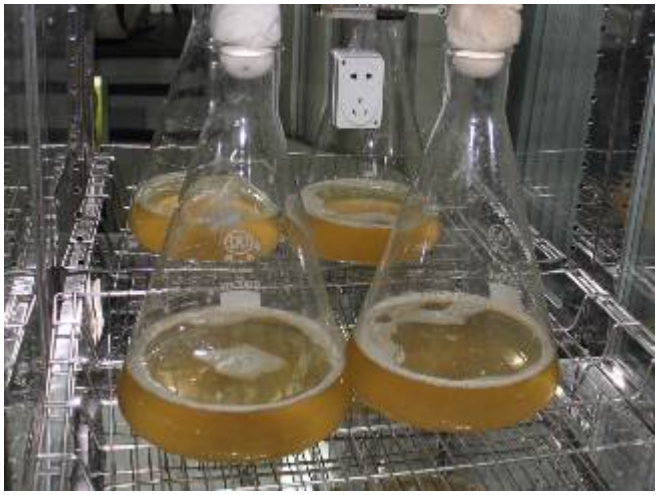
All the identified and counted samples should be taken back to original specimen bottles for preservation in case of reexamination and further analysis. The specimen bottles should have good seal and when necessary put on some olefin to conserve it. The labels of sample inside and outside bottle should be complete.

The regular check of the preserved sample is needed to avoid the stationary liquid dry up or specimen mildew.



3. Marine Microorganism Test and Addition

The addition of microorganism means adding the enlargement cultivated bacteria liquid to batching pool to ensure the total amount and density of bacteria in raw water reach the demand of G8 guideline. The enlargement cultivated bacteria is *Escherichia coli* which is separated from natural sea area by Hong Kong University of Science and Technology and is processed by the non-pollution genetic engineering. In the process of the enlargement cultivation, firstly prepare 4 L 2216E liquid nutrient medium following the preparation instruction of 2216E liquid nutrient medium; after that make the nutrient medium under the condition of high temperature and heavy pressure to disinfect bacteria for 15 min; when the sterilized nutrient medium cool down, inoculate *Escherichia coli* on the 2216E liquid nutrient medium and cultivate 1 day at 37℃. Until then the enlargement cultivation is finished.



3.1 Total Amount of Heterotrophic Bacteria

The plate count method is adopted in accordance with the national standard of the Specification for Marine Monitoring—Part 7: Ecological Survey for Offshore Pollution and Biological Monitoring (GB17378.7-2007/10.1).

3.1.1 Principle of the method

The single bacteria cultivated on plate nutrient medium could form the visible colonies. One colony represents one cell. Relying on counting the number of visible colonies, the plate count method could get the number of bacteria. The keys to count bacteria are: to separate bacteria on sample to single cell as far as possible; prepare diluents

of different concentrations and evenly inoculate certain volume of the diluents on Petri plate with solid nutrient medium (hereinafter to be referred as plate).

3.1.2 Preparation of reagent and nutrient medium

3.1.2.1 Reagent

NaOH solution (160 g/L): dissolve 160 g of solid NaOH into 1000 ml distilled water.

Tween-80 solution: mix 1 ml Tween-80 with 2000 ml distilled water.

3.1.2.2 Nutrient medium

The components of 2216E nutrient medium: 5 g peptone, 1 g yeast extract, 0.1 g ferric phosphate, 20 g agar; 1000 ml aged seawater.

Preparation: heat up above substances for dissolution and use NaOH solution (see 2.1.1) to adjust pH value to 7.6. Put the solution into conical flask and place them into autoclave sterilizer for disinfection under the condition of 121°C (about 105kpa) for 20 min. After that, pour nutrient medium into the disinfected plate and the volume of nutrient medium in every plate is about 15 ml. When the samples cooled and solidified, put them into refrigerator for perseveration.

3.1.3 Equipment and materials:

— Constant temperature incubator;
— Oven;
— Autoclave sterilizer;
— Petri dish: diameter is 9 cm;
— Pipet: 1 ml;
— Test tube: 12mm*150mm;
— Gauze and cotton: used for sealing test tube;
— Wild-mouth sampling bottle: 250 ml;
— Glass rod: used for inoculation;
— Conical flask;
— Kraft;
— Cotton ropes;
— Electric furnace: 2000 w;
— Precise pH test paper;

— Super clean bench;

— Common used instruments and equipment in laboratory.

3.1.4 Sampling

When open bottle stopper, kraft should be also moved away avoiding contamination. Flame sealing should be done to collect water sample. The bottles having the collected water sample should be tightly closed. Enough space in bottle (at least 2.5 cm in height) should be saved for sample shaking before test.

This experiment collect water sample from raw water, treated water, control water sample, 2-days later of the treated water, 5-days later of the treated water and 5-days later of the control water sample.

3.1.5 Test steps

3.1.5.1 On basis of water volume, add 1 ml Tween-80 solution to every 100 ml water sample. Shake well to separate bacteria in sample to single cell.

3.1.5.2 Under the sterile operation, 1 ml water sample is pipetted into the test tube which has 9 ml sterile aged seawater. And then mix the sample thoroughly. Following the same procedure, continuous dilution should be done until getting the required extent which depends on the amount of bacteria in water sample. The proper bacterial colony in every plate should in range of 30 - 300 cells. Each dilution extent should have three duplicate samples.

3.1.5.3 A volume of 0.1 ml diluted sample is pipetted into the prepared plate. Use the sterile glass rod to spread bacteria liquid evenly on the plate. Place the plate into super clean bench to stay for 20-30 min so as to make bacteria liquid infiltrated well into nutrient medium.

3.1.5.4 Place the plate into the 25°C constant temperature incubator for 7 days cultivation. Thereafter, take the plate out to count bacterial colony.

3.1.5.5 Bacterial colony count method

If there is large lawn on the plate, the count is invalid.

Select the plate which has the total number of colonies between 30 and 300. To obtain results, multiply the average number of colonies per plate by the dilution extent.

— If the average numbers of colonies in two dilution factors are in the range of 30- 300

colonies per plate, the count is determined by the ratio of two numbers. If the ratio is less than 2, the count takes the average number; if the ratio is greater than 2, the count takes the fewer colony number;

— If plates from all dilutions have more than 300 colonies, compute the count by the multiplying the average colony number of the least dilution factor plate (the highest concentration plate) by the dilution factor;

— If plates from all dilutions have less than 30 colonies, compute the count by the multiplying the average colony number of the highest dilution factor plate (the least concentration plate) by the dilution factor;

— No colonies – if plates from all dilutions of any sample have no colonies and found no contamination, report the count as less than one times the dilution factor. For example, if the minimum dilution factor is 1:100, report the count as less than 100.

3.1.6 Attentions

The following attentions should be noticed.

The bacteriological testing should strictly follow the sterile operation.

The collected sample should be tested in time. The allowed storage time is no more than 2 hours. Otherwise, the sample must be stored in ice bottle, but the storage time is not more than 6 hours.

Plates should be prepared in advance, or the water on plate would influence the testing results.

3.2 Dertermination of Coliform in Water

The collected sample should be transported at low temperature to laboratory immediately. The testing method is adopted the multiple-tube fermentation technique in accordance with 'The specification for marine monitoring—Part 7: Ecological survey for offshore pollution and biological monitoring (GB17378.7-2007/10.1) and 'Standard examination methods for drinking water-Microbiological parameters (GB/T 5750.12-2006).

Shake water sample vigorously 25 times before testing or dilution so as to make sample mixed well. Use the sterile pipet to put 10 ml water sample into the 90 ml sterile dilution solution to form the water sample having 10 of dilution factor. And then shake the sample well. Following the same procedure, the samples with different dilution factors such as 100 and 1000 can be obtained. In the process of above dilution, the sterile pipet needs to be replaced every time. Inoculate 10 ml sample into 10 ml double lactose peptone medium. Inoculate 1 ml sample into 10 ml single lactose peptone medium. Besides, inoculate 1 ml dilution samples with dilution factors of 10^{-1}、10^{-2}、10^{-3} into 10 ml single lactose peptone medium, respectively. Every dilution factor should be inoculated in five tubes. Incubate the inoculated tubes at 36±1°C for 24±2 h. If no gas or acidic reaction is evident, report negative results of the total coliform; if gas or acidic reaction is evident,

to carry out procedures as follow: inoculate the fermentation tubes into the plate having eosin methylene blue agar at 36±1°C for 18 to 24 h; Observe colonial morphology and select the required colonies as objects to do the experiments of gram staining, microscopic observation and demonstration. The required characteristics are dark purple black with a metallic surface sheen, purple black without or with little metallic surface sheen and pale purple pink with darker center. If the result of staining microscopic observation shows gram-negative budless bacillus and gas or acidic reaction is evident after inoculation of lactose peptone medium and incubation at 36±1°C for 24±2 h, it demonstrates the presence of the coliform group.

Test *Escherichia coli* in initial fermentation tubes of above multiple-tube fermentation technique. Use the sterile metal inoculation loop or sterile cotton swab to inoculate the liquid inside fermentation tube into the EC-MUG tube. Incubate inoculated EC-MUG tube in an incubator at 44.5±0.2 °C for 24±2 h. The cultivated EC-MUG tube is exposure to the wavelength 366nm ultraviolet (UV) light (6W). The presence of bright blue fluorescence is considered a positive response for *E. coli*. Calculate the number of positive EC-MUG tubes and look up the most possible number (MPN) table to get the MPN of *E. coli*. Report the result as MPN/100ml.

3.3 Determination of Enterococcus in Water

The test method is in accordance with Water quality - Detection and enumeration of intestinal enterococci - Part 2: Membrane filtration method (ISO 7899-2:2000).

The collected sample should be transported at low temperature to laboratory immediately. Shake water sample vigorously 25 times before testing so as to make sample

mixed well. Connect the sterile filter device to filter flask. Use the sterile tweezers to put filter membrane on the bottom of filter. Pipette some water sample into filter and open vacuum pump to start extraction filtration. After all the water passes through filter membrane, use 20 – 30 ml of sterile normal saline to ish the edge of filter at least two times, and then turn off the vacuum pump. Open the filter and use sterile tweezers to move the filter membrane to the surface of mEI agar medium (Slanetz and Bartley medium). Prevent bubbles production between filter membrane and medium. Invert and incubate the plate at 36°C±2°C for 44±4 h. After incubation, the colors of red, maroon or pink and concentrating on the center or spreading on the plate, all the colonies are defined as *typical*. If there are typical colonies, use sterile tweezers to move filter membrane and bacteria colonies to the plate of 44°C preheated bile esculin azide agar. Incubate the plate at 44°C±0.5°C for 2 h and then observe the plate immediately. The presence of brownish black around colonies is considered a positive response for enterococcus. Count the identified enterococcus and report the result as CFU/100ml. The calculation formula is as follow:

$$\text{CFU/100mL}=\frac{P\times 100}{T}$$

where:

P= number of positive results,

T= total volume of the filtered water, mL.

3.4 Determination of Vibrio choleraee in Water

The test method is in accordance with Diagnostic criteria of Vibrio cholerae (WS289-2008). The collected sample should be transported at low temperature to laboratory immediately and inoculated into nutrient medium directly. Every 50 ml of ten times concentration of alkaline peptone medium could be inoculated 450 ml water sample. Place the plates into incubator for enlargement cultivation at 37°C for 6 - 8 h. Take one inoculation loop of cultivated substance from lower surface of biofilm. Make streak inoculation into a strong nutrient medium (gentamycin agar, TCBS agar and No.4 agar) and a weak nutrient medium (alkaline nutrition agar), respectively, and then incubate the two nutrient mediums at 37°C for 18 - 24 h. Identify the typical colonies appeared in the strong and weak nutrient mediums through slide agglutination test, oxidase test and colonies reexamination.

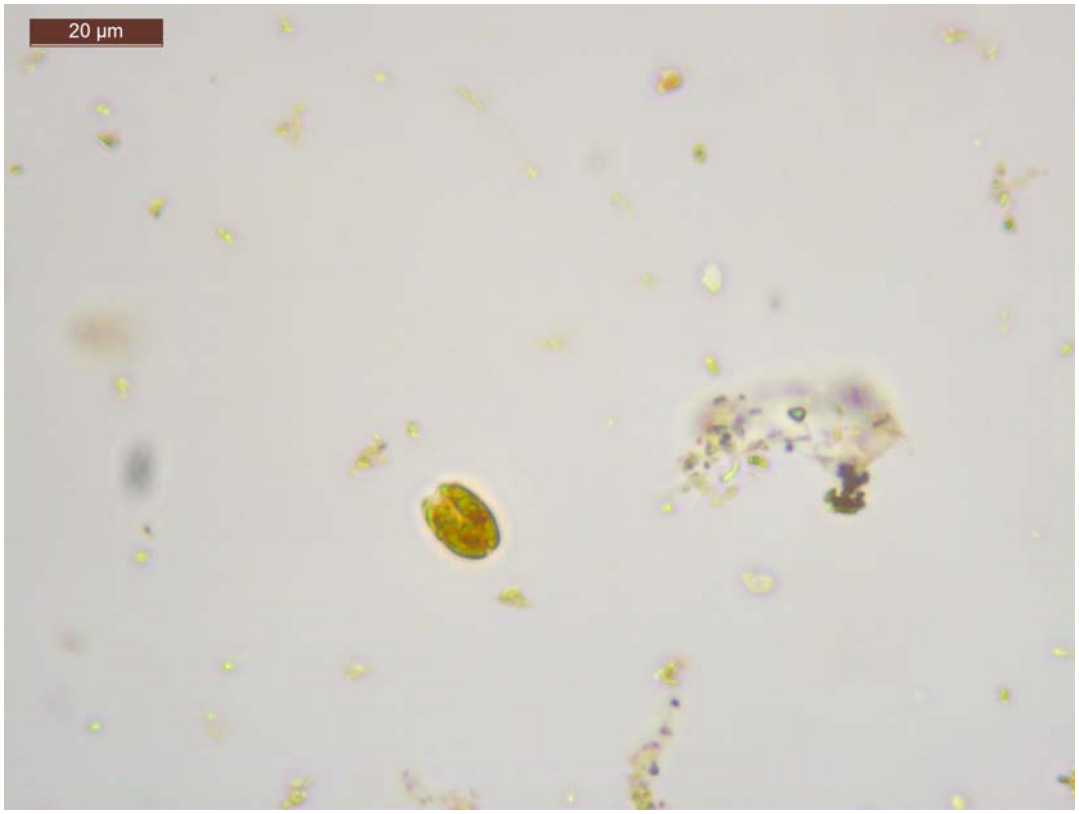
Vibrio cholerae in different isolation mediums presents different characteristics. In the common isolation medium with incubation of 37°C for 18 - 24 h, the characteristics of Vibrio cholerae is as follows:

(1) Alkaline nutrition agar: colorless, roundness, transparent or semitransparent, smooth surface, wet, flat or slight convex, neat edge and the diameter of colony is 2 mm in general.

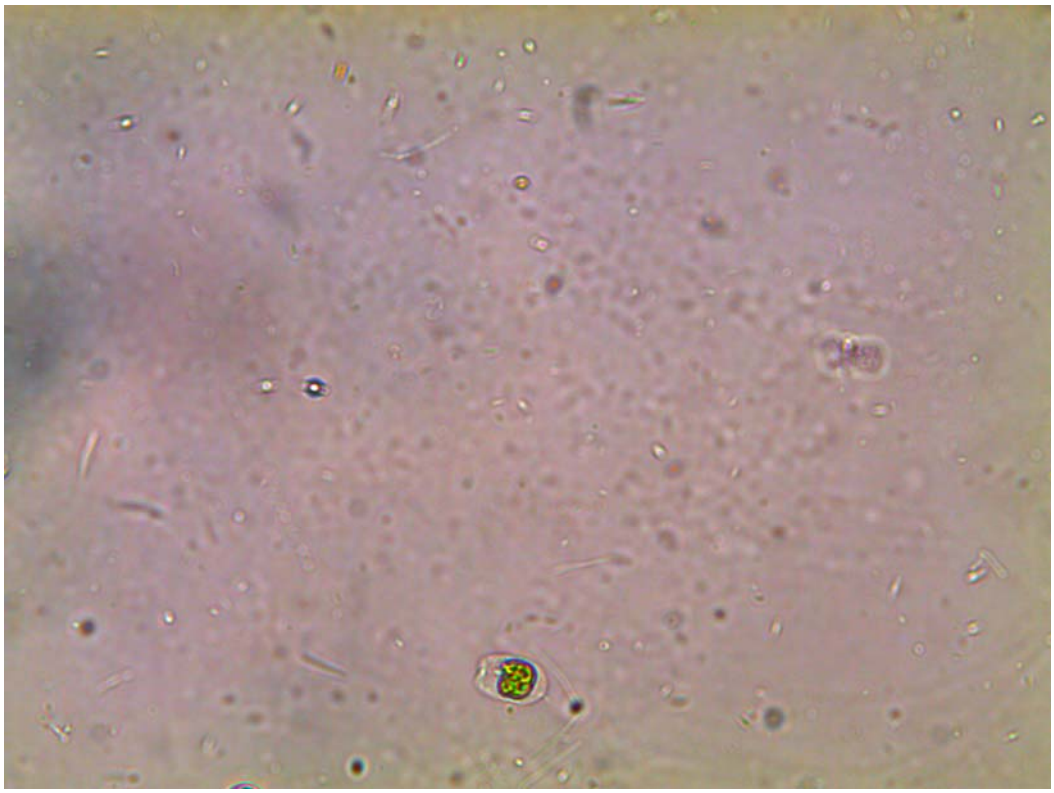
(2) Gentamycin agar and No.4 agar: the characteristics is similar with the colony in alkaline nutrition agar, but they have bad transparent and most of them show semitransparent; because these nutrient mediums contain tellurite, the center of colonies generally present grey or grey to black, and moreover the color become darker with the increase of cultivation time.

(3) TCBS agar: bright yellow, smooth surface, wet, slight convex and neat edge.

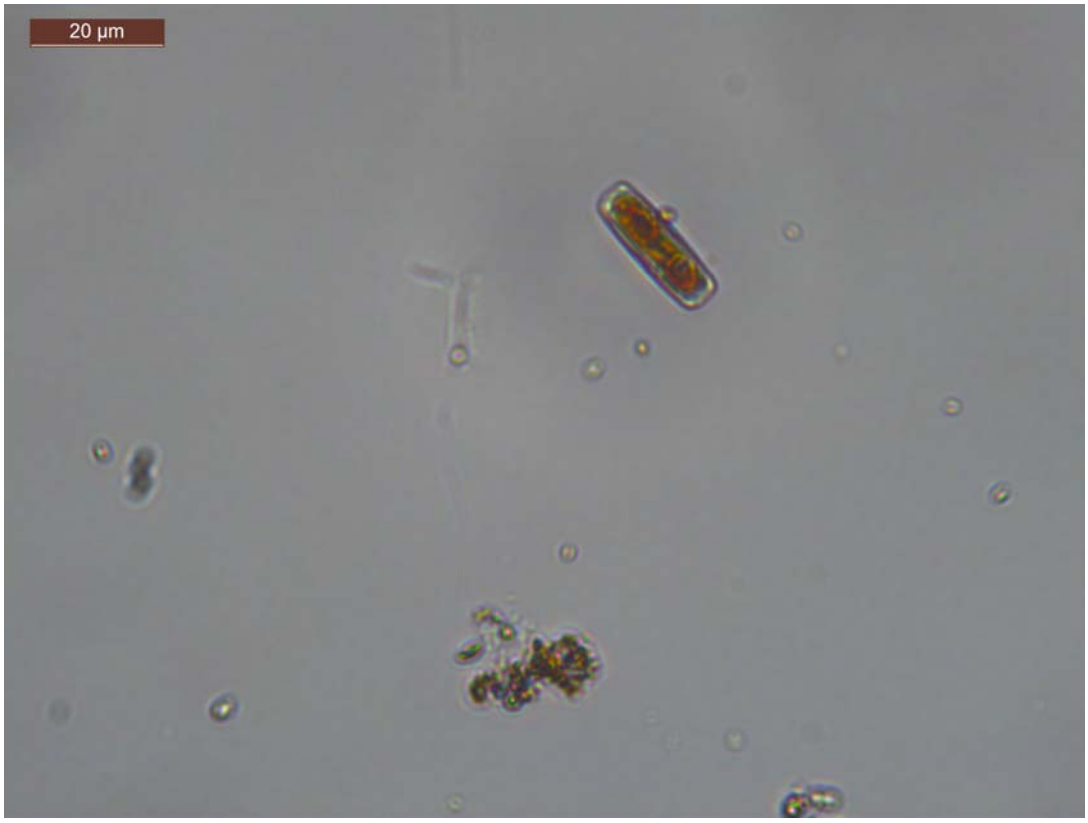
If finding suspicious colonies in serum agglutinate reaction, the sample should be transported to Shanghai Luwan District Disease Control Centre immediately to do subcontract detection.



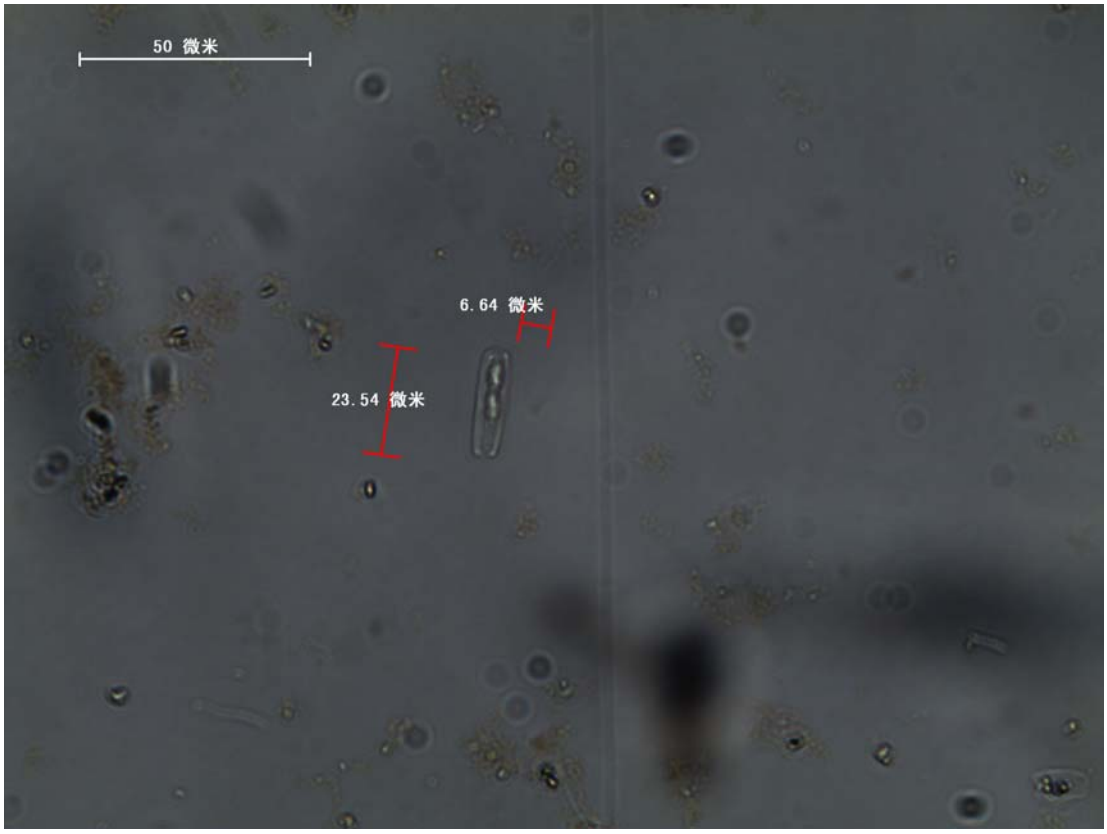
Platymonas sp. Stained



Platymonas sp. not stained



Small Navicula stained



Small navicula not stained

Appendix II References

1. The Specification for Marine Monitoring, Part 4: Seawater Analysis. GB17378.4-2007.
2. The Specification for Marine Monitoring, Part 7: Ecological Survey of Offshore Pollution and Biological Monitoring. GB17378.7-2007.
3. Standard Examination Methods for Drinking water – Microbiological Parameters. GB/T 5750.12-2006.
4. Diagnostic Criteria for Cholera. WS 289-2008.
5. Water Quality - Detection and Enumeration of Intestinal Enterococci in Surface and Iste water - Membrane Filtration Method. ISO 7899-2:2000
6. Water Quality – Determination of Free Chlorine and Total Chlorine – Spectrophptpnetric Method using N, N-diethy1-1, 4-phenylenediamine. HJ 586-2010.
7. Gulf Ecosystem Observation Methods. China Environmental Science Press, 2005.