Methods in microbial ecology

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AQ4000 Colorimetric tests (phosphate and chlorine dioxide)

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&

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Introduction

- Purpose
- Procedure

Purpose

- Measure the **concentration of solutes** (chlorine dioxide and phosphate) **in water**

Basic principle

- Produce **colorful compounds** in quantities **proportional to the compound of interest** thanks to chemical reactions
- **Measure the color**

A two-part system

Ampoules

- Thermo Scientific Orion AQUAfast AC4095 **Phosphate** Ampoules
- Thermo Scientific Orion AQUAfast AC4099 **Chlorine Dioxide** Ampoules

Colorimeter

- Thermo Scientific Orion AQ4000 AQUAfast IV colorimeter

- AQ4000 Colorimetric tests (phosphate and chlorine dioxide)

Procedure – before measurement

- Before each series of measurements, **zero** the colorimeter
  - If samples to measure are **colorless** -> zero with **distilled water**
  - If samples are **turbid** -> zero with **unreacted sample**

Procedure

1. **Sample water** to sample cup (15 mL for chlorine dioxide samples // 25 mL for phosphate samples)
2. **Add drops** (6 // 2) of Chlorine Dioxide Neutralizer solution // Phosphate Activator solution
3. Close the cup and **mix**
4. Place the **Auto-Test ampoule in the cup and snap the tip** to fill it up with the sample
5. **Mix** the contents in the ampoule by inverting it and wipe the exterior
6. Insert the ampoule **in the colorimeter**, aligning the signs
7. **Cover** the ampoule with the light cover
8. **Wait** for 1 // 3 minutes and **read the concentration** measurement

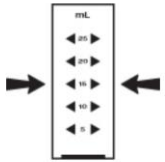


Figure 1:

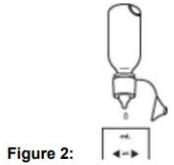


Figure 2:

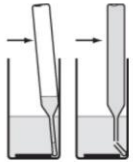


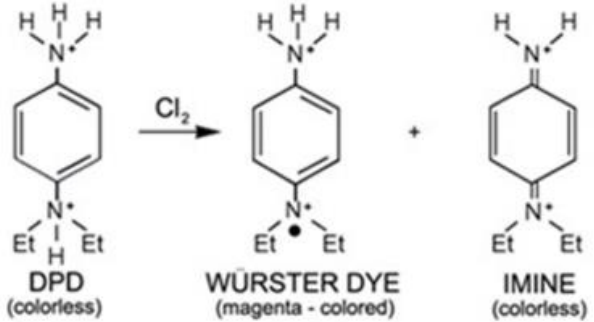
Figure 3:

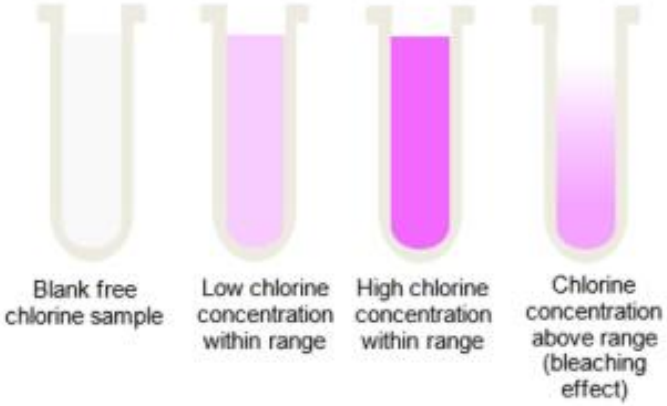
Mechanism

- Ampoules’ chemistry
- Colorimeter

Chlorine Dioxide Ampoule

Chlorine dioxide (ClO_2) + DPD *(N,N*-diethyl-*p*-phenylenediamine) → **pink colored** species





To keep in mind:

- Added glycine to prevent interference from free chlorine (Cl_2) (goes to chloroaminoacetic acid instead)
- High test results are produced with bromine, iodine, ozone and halogenating agents
- If ClO_2 > 500 ppm, may inhibit color development

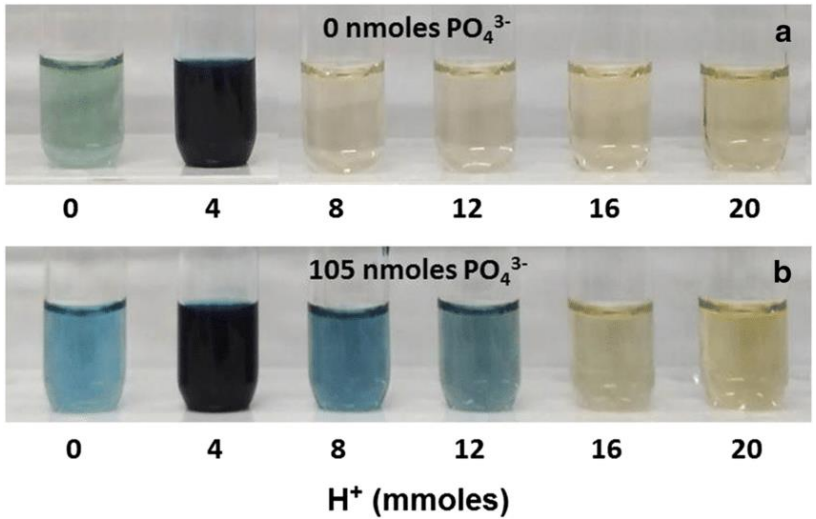
Phosphate Ampoule

Phosphate + Ammonium molybdate (in acidic solution) → Molybdophosphoric acid

$$PO_4^{3-} + 12(NH_4)_2MoO_4 + 27H^+ \rightleftharpoons H_3PO_4(MoO_3)_{12} + 24NH_4 + 12H_2O$$

Molybdophosphoric acid + Stannous chloride → **Molybdenum blue**

$$H_3PMo_{12}O_{40}^{3-} + SnCl_2 \rightleftharpoons 2Cl^- + SnH_nPMo_{12}O_{40}^{(7-n)-}$$



To keep in mind:

- Organic phosphate and condensed phosphate are not detected by this test
- Low test results are produced with sulfide, thiosulfate and thiocyanate

Mechanism - Colorimeter

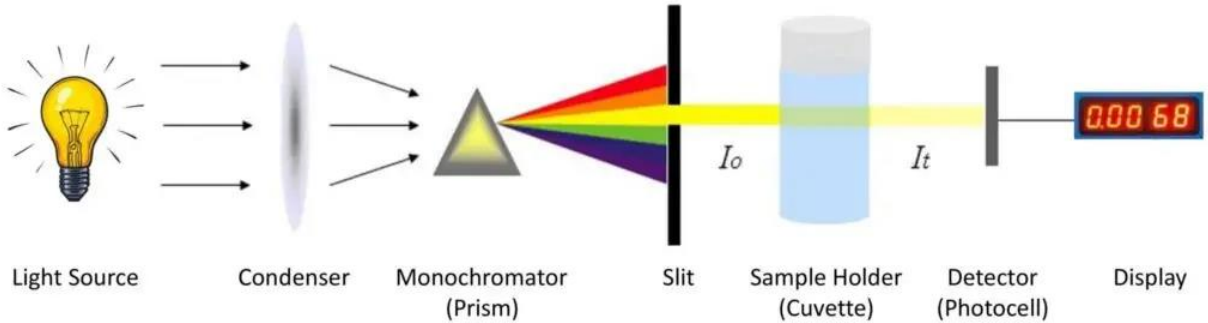
$$I_0 = I_r + I_t + I_a$$

I_0 = **intensity of entering** light

I_r = intensity of **reflected** light

I_t = intensity of **transmitted** light

I_a = intensity of **absorbed** light



Mechanism - Colorimeter

Beer's Law

"The amount of light **absorbed** is directly **proportional** to the **concentration** of the solute in the solution."

Lambert's Law

"The amount of light **absorbed** is directly **proportional** to the **length** and thickness of the solution."

Beer-Lambert's law

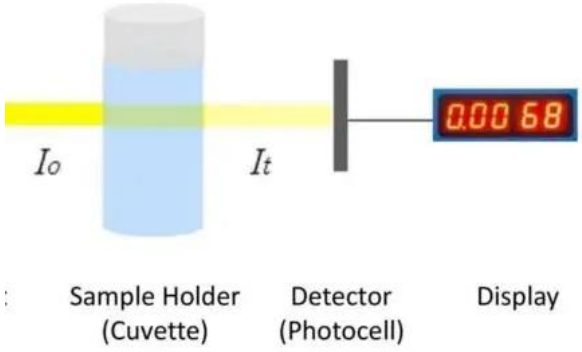
$$A = \text{Log}_{10} (I_0 / I_t) = \varepsilon\, c\, l$$

A = **absorbance** / optical density of the solution

ε = **molar absorptivity** (depends on solute and on wavelength)

c = **concentration** of the solute

l = **distance** travelled by light in solution



Analysed article

- Introduction and goal
- Methods
- Results

High cell density cultivation of the chemolithoautotrophic bacterium *Nitrosomonas europaea*

Benedek Papp [1] · Tibor Török [2] · Erzsébet Sándor [3] · Erzsébet Fekete [1] · Michel Flipphi [1] · Levente Karaffa [1]

Objective: Produce high cell density cultivation of *Nitrosomona europaea*

Why is this useful?

- Improves ammonia oxidation efficiency
  - ➢ Activated sludge in wastewater treatment systems

Nitrosomonas Europaea

- Chemolithoautotrophic
- Slow growth rate (0.4-1/day)
- Energy from the oxidation of ammonia to nitrite

Ammonia — Ammonia monooxygenase

$$2H^+ + 2e^- + NH_3 + O_2 \rightarrow NH_2OH + H_2O \quad (1)$$

$$NH_2OH + H_2O \rightarrow NO_2^- + 5H^+ + 4e^- \quad (2)$$

Nitrite

Difficulties

1. **Decrease in PH**

   PH control

2. **Inhibitory metabolite: nitrite**

   Ultrafiltration/microfiltration

   ➡ Membrane fouling

Proposition: Use a single vessel dialysis membrane bioreactor

Methodology – Cultivation Conditions

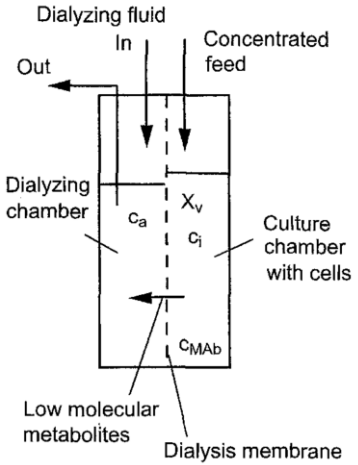
- ***Nitrosomonas europaea*** *Winogradsky* (ATCC 19718) is cultivated according to "**Nitrification network protocol**" (NNP)
  → Media preparation, shake flask culture, incubation

- All ongoing cultures were **protected from light**

- **LB agar-based count plates** : Look out for **contamination with heterotrophic bacteria**

Single vessel dialysis membrane bioreactor

- **Nitrite/ammonia can pass the membrane**
- Outer chamber is **fed by growth medium and drains old medium**



(Pörtner et al., 1996

Methodology – Analytical method

To check **environmental parameters**

- Ammo-N, nitrite ions and **phosphate ions** are measured using a colorimeter **(Orion AQUAfast IV AQ4000)**
- Presence of **struvite crystals** is verified, then collected and resolubilized in deionized water

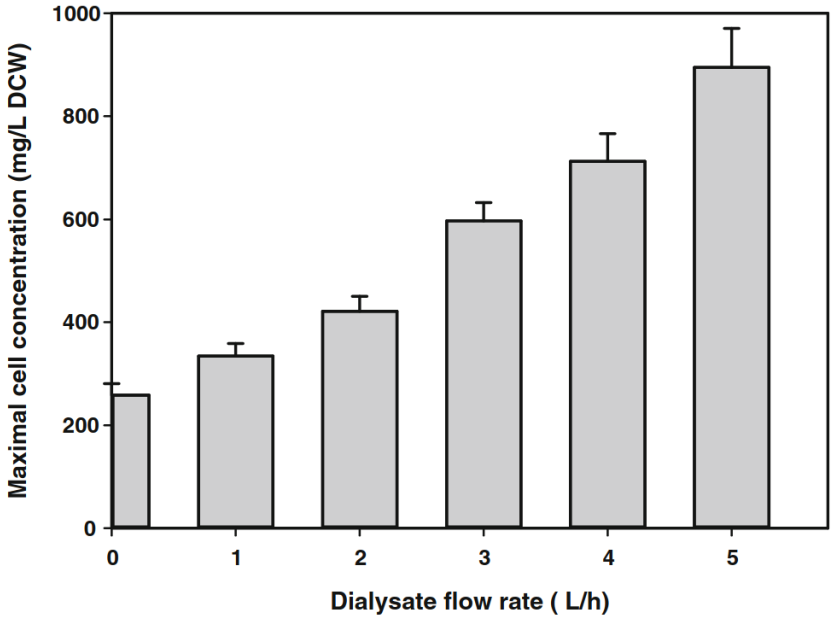
To estimate **microbial growth and activity**

- Total **cell number** (Bürker hemocytometer**)**
- **Dry cell mass** (filtering (0.2µm filter) + drying at 80°C)
- **Cell concentration** (absorbance at 600 nm)
- **Metabolic status** (acridine orange)

Results

Achievements

- **Specific growth rate** similar to conventional glass fermentators
- At a flow rate of **5 [L/h]** the mean biomass yield is **900 [mg/L] DCW**
  → **16 times more** as in conventional batches



- **Nitrite and ammonium** can pass the dialysis membrane
- Nitrite is **diluted** between inner and outer chamber
- Higher **flow rate**
  → More ammonium
  → Less nitrite

AQ4000 Colorimetric tests (phosphate and chlorine dioxide)

Thank you for your attention!

References

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