4. Digestions

4.1 Microwave Digestions

4.1.5 Alkaline persulfate solution: Seston

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Suitability

Total phosphorus concentrations (TP) are the sum of atoms of this element, independent from matrix, binding from and availability for microorganisms. TP comprises available phosphate, dissolved organic P compounds, bound P in biomass, and P being sorbed to suspended particles - also in water samples.

Before measurement of TP all bound, dissolved and particulate P compounds have to be converted to phosphate. With an oxidative digestion all P-containing compounds are disintegrated in smallest component to release the whole phosphorus to phosphate. An oxidative digestion method at 90 °C exist but has to be incubated very long (Berthold et al. 2015). Besides this digestion method coupled (oxidative and UV) and UV digestion methods and can be used (chapter 4.1.5). The final phosphate is measured photometrically. The P analytics is based on DIN EN ISO 6878 (2004).

In many water bodies (lake, estuaries), P is the limiting factor for primary production. Since phosphorus is, especially during phytoplankton monitoring (spring, summer), bound in biomass and therefore only in traces measurable as plant available phosphate, TP is the proxy for P supply of the water body.

High nitrate concentrations (> 2 mmol l^{-1}, Hansen & Koroleff 1999) exclude extracts with nitric acid and a*qua regia* from photometric P determination with molybdenum blue. Even the high nitrate concentrations in winter in eutrophic water bodies are considerably lower (e.g. Selig et al. 2006).

Concentration range

TP in seston or in unfiltered water samples is mainly measured as phosphate by the molybdenum blue method. The molybdenum blue method is the dominant method in water analytics, which measurement range is between 0.05 and 10 µmol l^{-1}. The limit of quantification of 0.05 µmol l^{-1} may be reduced considerably (Gimbert et al. 2007), if Continuous Flow Analysers with very long cuvettes are used. Currently, the limit of quantification for the whole procedure (digestion and determination) is 0.22 µmol l^{-1} in a 5 cm cuvette.

Protocol

Preparation

- Freeze sample at -20 °C.
- Thaw sample in hot water before further preparation.

Digestion

- Fill Teflon vessel with 10 ml well shaken water sample (total sample),
- Add 1.0 ml alkaline persulfate solution.
- Per run, at least 2 standards (once 10 µmol l^{-1} organically bound phosphorus, e.g. diphenyl phosphate, triphosphate or glucose-6-phosphate, and once phosphate) and at least 2 blanks have to be digested.
- Close digestion vessels.
- Digest in a common microwave at 450 W for 50 s or in a laboratory microwave LAVIS 1000 two times with program 7 (tab. 4.1.5-1).
- Wait at least 5 min after digestion before opening and removal of sample solution (Attention: high pressure!), wait longer, if vessels are warm.

Neutralisation

- Transfer each sample into a graduate test tube, rinse with 1 ml ultra-pure water (add to sample).
- Neutralisation of sample with pH-indicator 3-nitrophenol:
  - Add 3 drops of indicator solution,
  - Add some drops of ammonia solution until solution turns yellow,
  - Back titration to colourless solution with 1N HCl (Fig. 4.1.5-1 and 2).
- Fill neutralised samples to 15 or 20 ml with ultra-pure water.
- For samples without considerable intrinsic colouration a sample turbidity value does not need to be determined.

Figure 4.1.5-1 Yellow solution (nitrophenol) after addition of ammonia solution

Table 4.1.5-1 Digestion program for microwave Lavis 1000 for seston

Level	Max. Power (W)	Ramp (min)	Temperature (°C)	Holding (min)
1	1000	2		15:00
2	1000	2		15:00

Measurement

- photometrically as molybdenum blue (chapter 5.2.3)
- correction of dilution by neutralisation (equation 4.2.2-1)

Equation 4.1.5-1 Calculation of the TP concentration in seston and correction of dilution by neutralisation

$$TP = F_{PO_4} \times (E_{sample} - E_{RBW}) \times FD$$

TP Total Phosphorus (µmol l^{-1})

F_{PO4} Factor of calibration line for phosphate, with extinction on the x-axis and P concentration on the y-axis.

E_{sample} extinction at 885 nm, Cuvette length for calibration and measurement have to be the same

E_{RBW} Reagent blank value

$$FD = \frac{\text{total volume}}{\text{digestion volume}}$$

FD factor of dilution of Neutralisation:
Total volume after Neutralisation (ml)
Digestion volume (ml)

Reagents

- stir alkaline persulfate: 25 g potassium peroxide disulfate ($K_2S_2O_8$ nitrogen-poor), 15 g boric acid and 7,5 g sodium hydroxide in a 500 ml volumetric flask with around 400 ml ultra-pure water until complete dissolution. Fill to 500 ml.
- 3-nitrophenol: dissolve 0.3 g nitrophenol in ethanol or 0.08 g in 100 ml ultra-pure water.
- Ammonia solution: dilute concentrated ammonia solution 1:4 with ultra-pure water
- 1 N HCl: fill a 1 litre volumetric flask with around 750 ml of ultra-pure water; add 85,5 ml concentrated HCl slowly (Attention: heats up!), fill to 1 litre after cooling down to room temperature.

References

Berthold M, Zimmer D Schumann R (2015) A simplified method for total phosphorus digestion with potassium persulphate at sub-boiling temperatures in different environmental samples. Rostocker Meeresbiol Beitr 25: 7–25

DIN EN ISO 6878: 2004 Water quality - Determination of phosphorus - Ammonium molybdate spectrometric method, DOI: 10.31030/9552789

Gimbert LJ, Haygarth PM, Worsfold PJ (2007) Determination of nanomolar concentrations of phosphate in natural waters using flow injection with a long path length liquid waveguide capillary cell and solid-state spectrophotometric detection. Talanta 21: 1624-1628, DOI: 10.1016/j.talanta.2006.07.044

Hansen H P, Koroleff F (1999) Determination of nutrients. In: Grasshoff K, Kremling K, Ehrhardt M (Eds.) Methods of seawater analysis. Wiley-VCH, Weinheim 3. ed. 159-251, DOI: 10.1002/9783527613984.ch10

Selig U, Baudler H, Krech M & Nausch G (2006) Nutrient accumulation and nutrient retention in coastal waters – 30 years investigation in the Darß-Zingst Bodden chain. Acta Hydrochim Hydrobiol 34: 9-19, DOI: 10.1002/aheh.200500616

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