Challenge
Simple and standard-compliant determination of phosphorus in sewage sludge after microwave-assisted acid digestion.

Solution
Spectrophotometric determination of the phosphorus concentration in sewage sludge using the SPECORD PLUS.

Intended audience
This AppNote is aimed to users who work in environmental analysis, such as sewage treatment plants.

Determination of Phosphorus by UV/Vis Spectroscopy According to DIN EN ISO 6878:2004 [1] in Sewage Sludge

Introduction

Phosphorus is an essential nutrient for all organisms and is found in all living cells. The macronutrient is significantly involved in processes of energy production and generation as well as in the regulation of the acid-base balance. Phosphorus is also an important nutrient for plant growth and is therefore used in agriculture as a fertilizer. One source of phosphorus is sewage sludge, which is a waste product of wastewater treatment in municipal sewage treatment plants. The phosphorus content in sewage sludge can vary widely depending on its origin.
Phosphorus was added to the „List of Critical Raw Materials for the European Union“ [2] by the European Commission. In 2012, the German government also emphasized the need for sustainable phosphorus management for resource conservation in relevant material flows. Furthermore, the German Sewage Sludge Ordinance (GSSO, AbfKlärV) [3] contains the obligation to recover phosphorus as of 2029. According to the requirements of the GSSO, phosphorus must be recovered if sewage sludge contains more than 20 grams of phosphorus per kilogram of dry matter. Sewage sludge is considered waste, but offers a high resource potential due to its high content of valuable nutrients such as calcium and potassium. Depending on its pollutant content, sewage sludge can be used as fertilizer on agricultural soils or in landscaping.
The European Union has passed several directives that have to be adopted by the member states to regulate the application of sewage sludge to soils (e.g., the „Sewage Sludge Directive 86/278/EEC“ [4]). These permit the use of sludge from sewage treatment plants for fertilizer purposes only if the requirements specified by the GSSO are met. The GSSO regulates the requirements for the use of sewage sludge for fertilizer purposes on agricultural soils. To ensure the unrestricted suitability of soils intended for sewage sludge application, the ordinance provides for regular pollutant tests of these soils and sets limit values in this

respect that may not be exceeded. As a result, the content of nutrients (e.g., P) and pollutants (e.g., Cd, Hg, Pb) in sludge from municipal and industrial wastewater treatment must be checked regularly.
After sample preparation according to DIN EN 16174:2012 (method B) - a microwave-assisted acid digestion of the sewage sludge - UV/Vis spectrophotometry is a fast and simple analytical method to detect the concentration of phosphorus in sewage sludge. The standard procedure is described in DIN EN ISO 6878.
The accuracy of the method was investigated using the reference material NIST 2781 and a sewage sludge sample that was part of a national round robin test.

Materials and Methods

Instrumentation and software settings

All measurements were carried out by a SPECORD 200 PLUS spectrophotometer equipped with a holder for standard cuvettes for cuvettes with a path length of 10 - 50 mm. For the measurements according to DIN EN ISO 6878, 10 mm glass cuvettes (OS) were used. Using the software ASpect UV, the absorbance was measured for the parameter phosphorus in the photometry module. The software settings are listed in Table 1.

Table 1: Software settings for measurement according to DIN EN ISO 6878

	Phosphor
Measurement mode	Absorption
Wavelength [nm]	880
Integration time [s]	0.1

Sample preparation

The sample preparation of the sewage sludge was performed according to DIN EN 16174:2012 (method B), which requires a microwave-assisted acid digestion.Prior to the analysis, the contents of the sample bottles were homogenized by shaking them upside down. Afterwards, approximately 0.5 g of homogenized sample were accurately weighed (0.0001 g) and added to a digestion vessel (DAP60). Successively, 0.5 mL of DI water, 6 (± 0.1) mL HCl, and 2 (± 0.1) mL HNO_3 were added. The mixture was swivelled carefully and left standing for at least 15 minutes before the vessel was closed. Subsequent heating (175 °C, 10 min) was performed in a speedwave XPERT microwave digestion system. Afterwards the vessels were allowed to cool down to room temperature (RT) to avoid foaming and splashing. The solutions were transferred to a graduated polypropylene tube, diluted to 50 mL with DI water, and centrifuged (1125xg, 10 min).

Samples and reagents

For the photometric measurement, the standards were prepared according to DIN EN ISO 6878:2004. First, an orthophosphate stock solution with 50 mg/L phosphorus was prepared. For this purpose, 0.2197 (± 0.0001) g potassium dihydrogen phosphate were dissolved in approx. 800 mL distilled water in a 1000 mL graduated flask, mixed with 10 mL sulphuric acid and filled up to the mark with distilled water. The orthophosphate standard solution was prepared from the stock solution, 20 mL of the stock solution was pipetted into a 500 mL graduated flask and made up to the mark with distilled water. The standard solution has a phosphorus concentration of 2 mg/l. For the preparation of the calibration standards, different volumes of the ortophosphate standard solution were transferred into 50 mL graduated flasks and first filled up with approx. 40 ml distilled water (see Table 2).
For the formation of the dye 1 mL ascorbic acid, as well as 2 ml acid molybdate solution were added to each volumetric flask shortly before the measurement while shaking. Subsequently, the flask was filled up to the mark with distilled water and mixed well.

Table 2: Phosphorus concentration of the calibration standards

Calibration standard	Volume standard solution [ml]	Phosphorus concentration [mg/l]
Standard 1	1	0.04
Standard 2	2	0.08
Standard 3	3	0.12
Standard 4	4	0.16
Standard 5	5	0.20
Standard 6	8	0.32
Standard 7	10	0.40
Standard 8	15	0.60
Standard 9	20	0.80

Calibration

To determine the phosphorus concentration, the first step was to create a calibration curve. The calibration standards were measured with the parameters shown in Table 1.

Linear regression was chosen as the regression model. The calibration curve is shown in Figure 1.

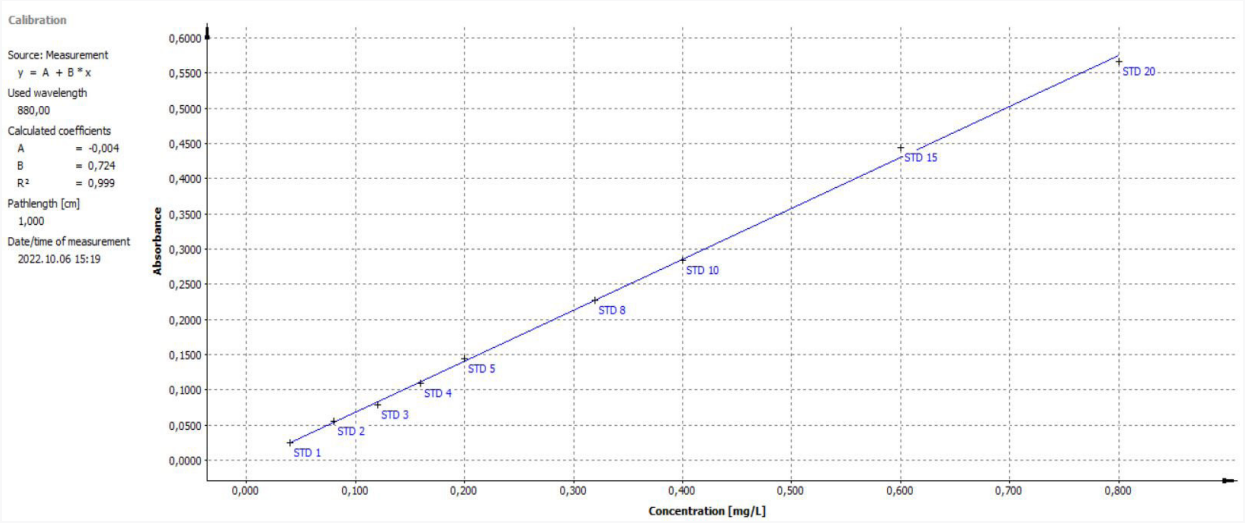

Figure 1: Calibration curve

The absorbance of the blank value measurement with distilled water is automatically subtracted from the absorbances of the measured calibration standards in the ASpect UV software. The calculated absorbances of the calibration standard solutions are given as a function (y-axis) of the concentrations of the standard solutions (x-axis). The last is given in milligrams per litre (mg/l). The relationship between absorbance and concentration shows a linear dependence in the indicated concentration range. The ASpect UV software automatically calculates the slope and the R^2 value of the calibration curve. Also, the concentration of the unknown samples is automatically calculated as a function of measured absorbance. In addition, the automatic calculation of the concentration is carried out, if information about dilution and initial sample weight is available. This eliminates calculation errors and saves the user valuable laboratory time.

Measurements

For the measurements, the reference material NIST 2781 and a sewage sludge sample were prepared fivefold according to the standards. Table 3 shows weight and volume of the samples used.

Table 3: Sample weight and volume

Sample	Weight [g]	Volume digestion [mL]	Volume sample [mL]	Volume flask [mL]
Reference material 1	0.5126	50	0.05	50
Reference material 2	0.4992	50	0.05	50
Reference material 3	0.5010	50	0.05	50
Reference material 4	0.5208	50	0.05	50
Reference material 5	0.5329	50	0.05	50
Round robin test sample 1	0.5390	50	0.05	50
Round robin test sample 2	0.5216	50	0.05	50
Round robin test sample 3	0.5431	50	0.05	50
Round robin test sample 4	0.5324	50	0.05	50
Round robin test sample 5	0.5438	50	0.05	50

The prepared samples were measured within 10 to 30 minutes. It should be noted, that the analytical results are time-dependent and the absorption increases with time. Therefore, the samples should be measured quickly and after the same reaction time. Reference, blank, and samples were analyzed one after the other. The reference was measured against distilled water. For the blank sample, distilled water was used and treated identically to the sewage sludge samples. The absorbance of the blank sample was automatically subtracted from the subsequent samples.

Results and Discussion

It is important to note, that the influence of interfering factors on the absorption must be checked before the measurement. If the sample contains arsenate, it must be reduced to arsenite with thiosulphate according to DIN EN ISO 6878:2004 and, only then, further sample preparation can be carried out as described above. For turbid and colored samples, DIN EN ISO 6878:2004 provides for treatment with a compensation solution consisting of sulphuric and ascorbic acid. If the presence of such interfering factors can be excluded, the samples are treated with the detection reagents as described in the standard. The accuracy of the method was investigated by analysing the standard reference material NIST 2781. Table 4 shows the values and recoveries for the tested reference material as well as for the proficiency test sample. According to the certificate of analysis, the standard reference material contains 24300 mg/kg of phosphorus. The phosphorus concentration was calculated according to formula 1.

$$c_P = \frac{V_D}{m_W} * \frac{V_F}{V_S} * c_{P\ measured} \qquad (1)$$

c_p = Phosphorus concentration [mg/g]
V_D = Volumen digestion [L]
M_W = Weight [g]
V_F = Volume flask [L]
V_S = Volume sample [L]
$c_{P\ measured}$ = Measured concentration value of the software ASpect UV

Table 4: Recovery of phosphorus concentration in reference material NIST 2781 and round robin test sample

Sample	Concentration value ASpect UV [mg/L]	Phosphorus concentration [mg/kg]	Recovery [%]
Reference material 1	0.2418	23586	97.1
Reference material 2	0.2371	23748	97.7
Reference material 3	0.2418	24132	99.3
Reference material 4	0.2618	25134	103.0
Reference material 5	0.2642	24789	102.0
Average value	**0.2493**	**24278**	**99.9**
Round robin test sample 1	0.2684	24898	103.0
Round robin test sample 2	0.2530	24252	100.3
Round robin test sample 3	0.2651	24406	100.9
Round robin test sample 4	0.2643	24822	102.7
Round robin test sample 5	0.2626	24145	99.9
Average value	**0.2627**	**24505**	**101.3**

Table 4 summarizes the measured values for the recovery of the phosphorus concentration in the investigated sample and in the reference material. The average recovery of 99.9 % for the reference material and 101.3 % for the round robin test sample, demonstrates the excellent suitability of the SPECORD 200 PLUS as a precise measuring instrument for the determination of phosphorus in sewage sludge.

Conclusion

The SPECORD 200 PLUS double-beam spectrophotometer enables the fast, precise and simple determination of the concentration of phosphorus in sewage sludge according to DIN EN ISO 6878. The automatic calculation of the concentration as a function of dilution and weight in the ASpect UV software supports the error-free determination of concentration values. A sophisticated range of accessories allows the use of cuvettes with different shapes and path length as well as automation for up to 116 samples.

Figure 2: SPECORD 200 PLUS

Recommended device configuration

Table 5: Overview of used devices and possible accessories

Article	Article number	Description
SPECORD 200 PLUS	823-0200P-2-R	Powerful double beam spectrophotometer, providing excellent measurement results. Analysis of liquid, gaseous, powderous and solid samples possible.
Cassette Sipper System	820-60141-P	For the measurement of liquid samples in continuous flow without change of the measuring cuvette. Many samples can be measured one after the other in short time via suction tube and flow cell. Can be combined with the APG Basic xyz sampler.
APG basis xyz-sampler	820-60300-0	For fully automatic quantitative sampling and analysis of up to 116 samples consecutively within shortest time and with low effort. Can be combined with various sample racks (to be ordered separately).

References

[1] DIN EN ISO 6878:2004; Bestimmung von Phosphor – Photometrisches Verfahren mittels Ammoniummolybdat

[2] Mitteilung der Kommission an das Europäische Parlament, den Rat, den Europäischen Wirtschafts- und Sozialausschuss und den Ausschuss der Regionen, Brüssel, 2020

[3] Verordnung über die Verwertung von Klärschlamm, Klärschlammgemisch und Klärschlammkompost (Klärschlammverordnung - AbfKlärV), Ausfertigung 2017

[4] 86/278/EWG Richtlinie des Rates über den Schutz der Umwelt und insbesondere der Böden bei der Verwendung von Klärschlamm in der Landwirtschaft, 1986

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