

EVALUATION OF LABORATORY BATCH TESTS FOR ENHANCED BIOLOGICAL PHOSPHORUS REMOVAL

Utvärdering av laboratoriebatchförsök för biologisk fosforavskiljning

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Abstract

Phosphorus release batch tests can be used for evaluation of an activated sludge process with enhanced biological phosphorus removal (EBPR or bio-P). The test method is described and different aspects of the result evaluation are discussed. The concentration of dissolved phosphorus in a sample from the activated sludge is followed during an anaerobic period within a batch reactor or a beaker. The release of phosphorus is used to estimate the activity of phosphate accumulating organisms (PAOs). In addition to the phosphorus analyses, VFA (volatile fatty acids) can be analysed in order to estimate the fraction of glycogen accumulating organisms (GAOs) competing with PAOs for available VFA. Potassium and magnesium can be analysed to evaluate if chemical precipitation affect the test results. Chemical precipitation, temperature, pH and presence of nitrate or oxygen are example of factors that can influence results from the test.

Key words – Batch tests, EBPR, phosphorus release.

Sammanfattning

Fosforläppförsök kan användas för att utvärdera en aktivt slam-process med bio-P (biologisk fosforavskiljning, EBPR). Testmetoden beskrivs och olika aspekter på utvärderingen av resultaten diskuteras. Koncentrationen av löst fosfor i ett aktivt slam-prov i bägare eller reaktor följs under en anaerob period. Fosforläppet används för att uppskatta aktiviteten av fosfatackumulerande organismer (PAO). Förutom fosforanalyserna kan VFA (flyktiga fettsyror) analyseras för att uppskatta hur stor andel glykogenackumulerande organismer (GAO) som konkurrerar med PAO om tillgänglig VFA. Med hjälp av analyser av kalium och magnesium kan effekten av kemisk fällning under försöket uppskattas. Kemisk fällning, temperatur, pH och närvaro av nitrat eller syre är exempel på faktorer som kan påverka resultaten av försöken.

Introduction

Enhanced biological phosphorus removal (EBPR or bio-P) relies on alternating anaerobic and aerobic/anoxic conditions. When the sludge is kept anaerobic with an easily degradable carbon source available, the polyphosphate-accumulating organisms (PAOs) take up the carbon source for storage within the cells with a simultaneous release of polyphosphate. The anaerobic release and the subsequent aerobic or anoxic uptake of phosphate are well known phenomena, even if the bio-P process is not yet fully understood. Phosphorus release in laboratory batch tests can be used in order to characterise the phosphorus-removal potential of an activated sludge. The capacity of the sludge to release phosphorus under well-defined laboratory conditions is a good indicator of the potential for the bio-P function at the plant.

The test can be used to determine whether there is a bio-P process working at a plant. During introduction of

the bio-P process, that can take months, the P-release test is an easy way to see the first and increasing sign of the process, which was utilised in Tykesson *et al.* (2004). If the test is performed more regularly at one plant the results can be used for optimisation of the operation. The test results can be used for modelling or as a simple tool for identifying periods with problems. The effect of variations in P-removal capacity can be evaluated and measures can be taken to prevent negative effects. For example the consequence of decreased load during the industrial holiday can be evaluated and minimised the next time by dosing external carbon source during this period.

The laboratory test can also be used to characterise the potential for bio-P of a wastewater or an external carbon source. It is then important to use an activated sludge with known capacity, so that different carbon sources can be compared against one well-functioning bio-P sludge.

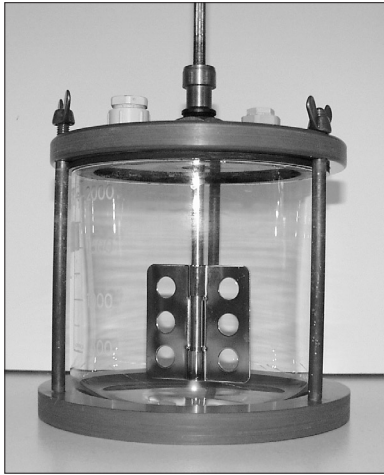


Figure 1. Example of equipment used for the laboratory test.

There is no standardised or generally accepted method for phosphorus release- and uptake tests. The basic principle is easy and always similar. Details in the test procedure might vary and be of great importance for the results. Further the evaluation is not always carried out in the same way and may depend both on details in the test procedure and on the evaluator's personal opinion. Batch tests are often used in research laboratories with the purpose of describing the EBPR mechanism, which normally requires more advanced equipment. Literature where the method is used for process evaluation in full-scale applications is not very frequent. In Tykesson *et al.* (2002) and Brdjanovic and van Loosdrecht (2000) the test was used for modelling purposes. Another description of the batch-test procedure is found in Janssen *et al.* (2002).

The basic idea of the selected method evaluated in this paper was its simplicity. It should be possible to carry out the test at a laboratory without any advanced equipment. In spite of the simplicity there are a lot of details influencing the experimental conditions chosen and the evaluation of the results. Principles and problems with the evaluation of the test and how different factors affect the results are discussed. Depending on the purpose of the batch test, the method can be adjusted for each case, which might reduce the simplicity.

Method

The principle of the method is to expose activated sludge to anaerobic conditions in a simple laboratory reactor, with a carbon source added in the beginning of the anaerobic period. The concentration of dissolved phosphorus is monitored during the test period. The acti-

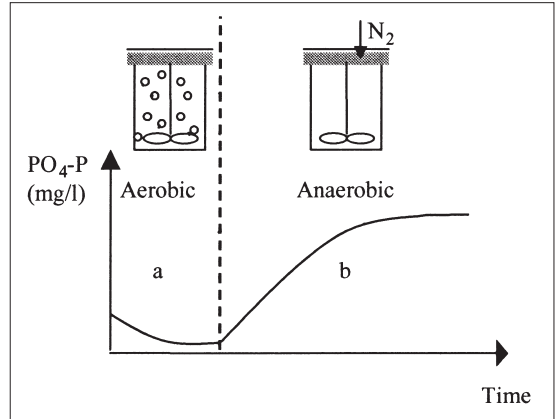


Figure 2. The experimental period and expected variation of dissolved phosphorus concentration during a phosphorus release batch test.

vated sludge used in the test is collected from the end of the aerated part (or period) of the plant, where as much phosphorus as possible is stored within the biomass.

Beakers with continuous mixing and possibilities to aerate and to add nitrogen gas are used for the tests. An example of equipment that can be used is shown in Figure 1. A normal beaker and a magnetic stirrer can be as good as this equipment. During the test, samples are taken at regular intervals. The samples are directly filtered in order to stop the biological processes and then analysed to determine the dissolved phosphate concentration (and other substances if relevant).

The test procedure is normally divided into two phases (see Figure 2):

- pre-aeration period
- anaerobic phosphate-release period

The purpose of the *pre-aeration period* (Figure 2a) is to maximise the storage of phosphorus but also to oxidise present VFAs to ensure a known carbon source and concentration when the anaerobic release starts. Phosphorus might have been released during the transportation from the plant to the laboratory or the phosphorus uptake might not have been optimal at the plant. Air is supplied through a diffuser. During this period the concentration of dissolved phosphorus is expected to decrease.

The second period is the *anaerobic release period* (Figure 2b). The aeration is stopped and anaerobic conditions are induced by supplying the reactor with nitrogen gas. The nitrogen gas is either bubbled through the activated sludge or supplied at the top of the reactor to ensure the absence of oxygen. At the beginning of the period the carbon source, usually acetate, is added. Acetate can be replaced by another carbon source (or wastewater) that is chosen to be tested. If the bio-P

process is working well, the concentration of dissolved phosphorus increases and reaches the maximum concentration before the end of this release period. If the carbon source is not in excess it will limit the release.

With the desired simplicity of the method the experiments can be done without pH-control. However, the pH is important for the process and should be measured regularly during the experiment. Since a pH-meter then has to be available, manual control of pH is suggested even if an automatic pH-controller is lacking.

To analyse the dissolved substances such as phosphate the samples have to be filtrated directly in order to remove the active biomass and stop the reaction.

Suspended solids (SS) and volatile suspended solids (VSS) of the sludge is important for comparison as the sludge concentration is different at different plants and changes over time as well. VSS is the organic part of the sludge – a rough measure of the amount of bacteria. The concentration of SS changes during the experiment as the poly-P that is released is included in the SS but not in the VSS. The variation of the other storage products, PHA and glycogen seems to be less important and the VSS concentration is more constant during the experiment.

Results

Phosphorus release

The bio-P process can be evaluated from the phosphorus measurements. Two different measures are suggested:

- maximum release
- initial/maximum release rate

As an example one test is evaluated and used to show how the different values can be calculated. From Figure 3 and forward all figures are results from real batch tests, which better illustrates the need for variable evaluation methods. P-values calculated per gram volatile suspended solids are recommended when the purpose is to

compare the capacity of the biomass. In experiments where the same sludge is compared at the same time, the choice of unit, mg P/l or mg P/g VSS, is less important. Further, in experiments where chemical precipitation is important and studied it might be better to express the results in mg P/l, as the phosphorus concentration as such is important.

The *maximum release*, measured as the difference between the maximum and minimum level of phosphates in phase b (Figure 2), can be a good measure of the bio-P potential of the biomass. The calculation is illustrated in Figure 3. It is important to ensure that the supply of carbon source and the time allowed are enough to avoid limitations of the maximum release. Problems with precipitation as a result of high concentrations of phosphorus can also influence the result and if it is suspected initial P-release rate might be a better measurement.

The *release rate* is often calculated and used as a measure of the size of the phosphorus-accumulating biomass. It is expected that the initial release will be the fastest and used for the calculation. However, sludge from different plants behaves differently and in the example in Figure 4 the initial release rate is slow and it is better to use the maximum release rate found later during the anaerobic period. This slow start of the release can be a result of oxygen or nitrate remaining in the beginning of the anaerobic period. In Figure 4 the period between 1.75 and 3 hours is chosen for calculation of the release rate. By using just the total phosphate difference during this period, the release rate is $4.7 \text{ mg P}/(\text{gVSS}\cdot\text{h})$ and by using the linear regression during the same period the release rate is calculated to $4.5 \text{ mg P}/(\text{gVSS}\cdot\text{h})$.

It is difficult to standardise the method for calculation of the release rate, but it is nevertheless important to choose a method that gives comparable values. A certain time can be used either with a fixed or a subjective starting point. It might be a problem to find a suitable and fair method when sludge from different plants, behaving differently, are compared.

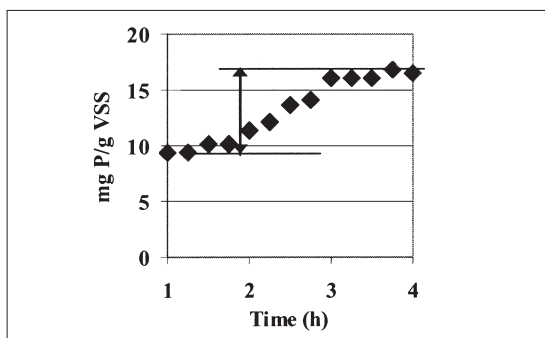


Figure 3. Calculation of the maximum release.

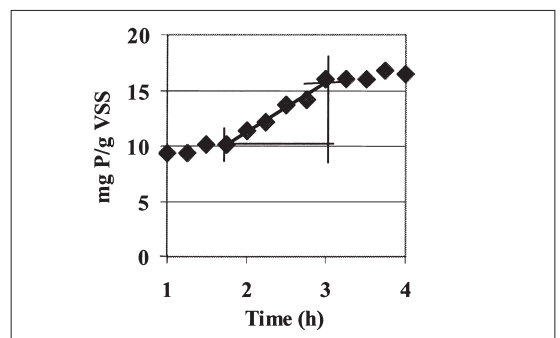


Figure 4. Calculation of the release rate.

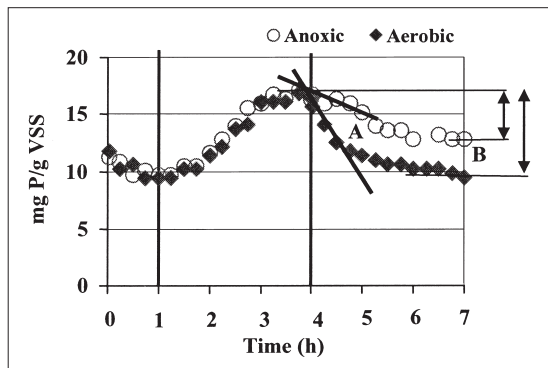


Figure 5. Calculation of potential for denitrifying dephosphatation by phosphate-uptake rates (A) and maximum phosphate uptake (B).

Extended measurements

With modifications from the suggested simple methods, a lot more information about the process can be obtained. Examples are:

- Phosphate uptake
- Capacity for anoxic uptake
- P/VFA-ratio for evaluating the presence of GAO

An additional third period can be added to the test. The *phosphate uptake* is then measured as a decrease in dissolved phosphorus. To measure the aerobic uptake the activated sludge is aerated as in the pre-aeration period. There are two main reasons for not including the third period. The first reason is the time. With only phase 1 and 2, the time period suggested is already 4 hours and with additionally 3 hours the test period is very long. Another reason can be that if carbon source is remaining when this period starts it can decrease the P-uptake.

To find the anoxic uptake, nitrate can be added to the reactor while the nitrogen gas is still being supplied. If two different batches are used in parallel, one batch can be aerated while the other one is given an addition of

Table 1. Conversion factors for the ratio P_{rel}/VFA_{up} .

| P/HAc mg P/mg COD | P/HAc mg P/mg HAc | P/C mole P/mole C |
|----------------------|----------------------|----------------------|
| 1 | 1.07 | 1.04 |
| 0.93 | 1 | 0.97 |
| 0.96 | 1.03 | 1 |

nitrate. By comparing the aerobic- and anoxic uptake rates (Figure 5A) or maximum uptakes (Figure 5B) it is possible to get an estimation of the potential for *anoxic uptake* (denitrifying dephosphatation).

Glycogen-accumulating organisms, GAOs, also use VFA anaerobically, without releasing any phosphorus. Measurements of the VFA uptake can be used to evaluate the *presence of GAOs*. A lower P/VFA ratio means a higher abundance of GAOs. For this evaluation the VFA has to be measured frequently in addition to the phosphorus.

Filipe *et al.* (2001) and Smolders *et al.* (1994) have proposed theoretical models with P/VFA-ratios 0.57 and 0.48 P-mole/C-mole at pH 7. The models were investigated from laboratory reactors with acetate as the sole carbon source. According to Barnard and Scruggs (2003) the ratio for a well-established PAO population is 0.5 mg P/g HAc and if a ratio much lower than that is found it can be assumed that the acetic acid was taken up by GAOs. Table 1 shows the conversion factors between P/VFA in mg/mg (COD and HAc) and P/C in mole/mole for acetate. As seen in the table the values are close to each other. For rough estimations of the ratios conversion is not necessary. However, if other carbon sources are used the differences are considerable.

Example of a P-release test is shown in Figure 6 a. The P and VFA concentrations are analysed for the same samples. In Figure 6 b the P/VFA ratio is calculated by linear regression. The ratio was found to be 0.42 mg $P_{rel}/mg VFA_{up}$ (=0.41 P-mole/C-mole).

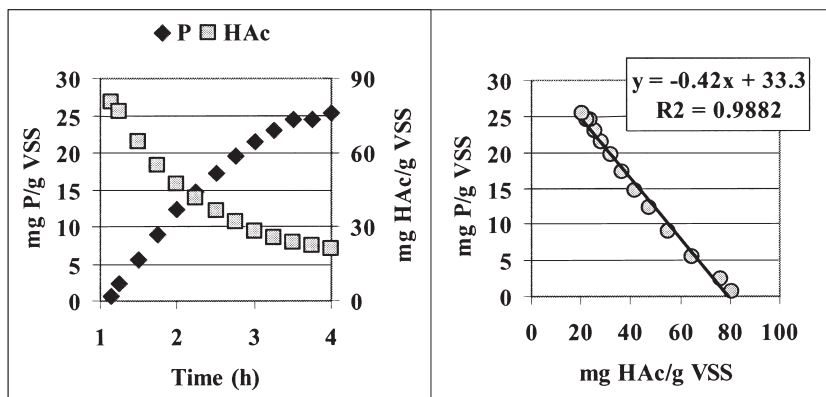


Figure 6. a. P-release and VFA(HAc)-uptake during the anaerobic period of the batch test. b. Linear regression of for finding the relation between P and HAc.

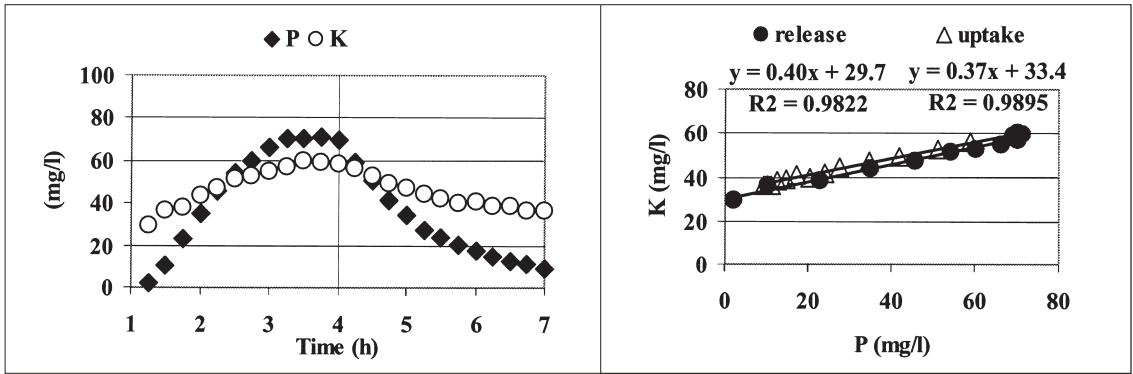


Figure 7. a. Phosphorus(P) and potassium (K) release and uptake during a batch test. b. Linear regression for the proportion between K and P.

Other measurements can also be used in a P-release batch test. Analyses of different dissolved or solid metals, such as coagulants for chemical precipitation and the counter-ions for polyphosphates (Mg and K) can be useful, especially at plants with combined biological phosphorus removal and chemical precipitation.

In research experiments, more complicated analyses such as PHA and glycogen content are often used in order to investigate the mechanism of the bio-P process. Example where these analyses are used on full-scale sludge can be found in Saunders *et al.* (2003) and suggested methods in Bond *et al.* (1999).

Precipitation

Precipitation of phosphate compounds is improved by high concentrations of phosphorus and the precipitation agent. The optimal conditions for precipitation are different for different compounds. If part of the sludge sample is chemical sludge or if the natural concentration of, for example, calcium in the wastewater is high, precipitation might occur during the release test.

In the case of chemical precipitation at the plant, metal hydroxides as well as metal phosphates are formed. If simultaneous precipitation or chemical sludge recirculation is applied these compounds are also present in the sludge during the test and the metal hydroxides can take part in the phosphorus-removal process. They precipitate dissolved phosphorus. The amount of dissolved phosphorus measured during the test will then be less than the phosphorus that is actually released. The measured differences in concentration are much higher in a laboratory test than what can be found in a full-scale plant with continuous flow.

Potassium and magnesium work as counter ions during phosphorus release and uptake. If the ratios K/P and Mg/P are constant for the EBPR process, the release (or

uptake) of the counter-ions can be a better measurement of the activity, as they tend to precipitate less (especially K). But literature shows that these ratios are not as constant as could be expected (Tykesson and Jansen, 2005). However, measurements of the counter-ions can identify cases where chemical precipitation has a great effect on the result of the test. As an example the P- and K-release and uptake during a test is shown in figure 7 a. Figure 7 b shows the same results, where the proportion between K and P was found. During the release period the K/P ratio was 0.37 mg K/mg P and during the uptake period the ratio was 0.40. Converted into molar ratios these values correspond to 0.32 and 0.29 mole K/mole P. This is similar to results found in literature. If the ratios had been significantly higher, chemical precipitation would have been assumed to have influenced the P-release and uptake.

Precipitation with Ca is enhanced by a high pH and high concentrations of Ca and P. If the Ca-concentration in the wastewater is high and the P-release is good it is important to keep the pH low. The suggested value is pH 7.

Effects of experimental conditions on the results

Carbon addition

The easily degradable carbon source is added in the test at the same time as the anaerobic period starts. Acetate, in the form of sodium acetate, is often used.

For measuring the maximum release there has to be an excess of carbon, ensuring that it is not limiting the release. For experiments with full-scale sludge acetate addition to a concentration of 300 mg COD/l is normally sufficient for the whole anaerobic period, depending on sludge composition and concentration.

If the uptake period is included in the test it has to be taken into account that acetate left in the aerobic period affects the phosphorus uptake. It is suggested that the PAOs will use this acetate instead of using the internal storage products, and thus the P-uptake rate will be reduced (Janssen *et al.*, 2002). Washing the sludge and subsequently adding phosphate is thus recommended (Brdjanovic *et al.*, 1999). Alternatively the anaerobic release can be done twice, with the second time adding an optimised amount of acetate according to the results in the first release test. No carbon source will then be present in the aerobic period (Janssen *et al.*, 2002). This will be time-consuming and complicates the test.

Pre-aeration

The sludge sample is preferably taken out from the aerated tank at the treatment plant, when the phosphate concentration inside the biomass is high. If the level of phosphorus stored in the biomass is not at its maximum or if phosphorus has been released during the transportation or storage, the pre-aeration period is very important. The excess of easily degradable carbon source, that can have been produced by hydrolyse during the storage, will also be oxidised during the aeration. As a consequence added carbon source will be the only carbon source taken up anaerobically.

During the aeration, especially in small reactors where the surface is comparatively large, the aeration will cause loss of CO₂, which will increase pH (Battistoni *et al.*, 1997). pH is important for the biological phosphorus release and also for the effect of precipitation.

If the sludge is used directly after the sampling from the treatment plant the pre-aeration can sometimes with advantage be omitted or shortened.

pH

The P-release rate increases with increased pH (Smolders *et al.*, 1994) and a constant and very similar pH is necessary to make good comparisons between experiments. Batch tests performed at pH 7 is therefore recommended. Also the effect of precipitation in the tests is dependent on pH. Calcium precipitation is for example much more likely to occur at pH 8 than at pH 7.

Automatic pH-control gives a constant pH at desired level, but it also makes the method more complicated and there is need for more instruments, which is inconsistent with the basic idea of a simple method. Anyhow if a pH-meter is available it is not too complicated to control the pH manually. HCl and NaOH-solutions can be used. Suitable molarity is different for different sludge but 0.5M–1M is suggested. During the aeration the pH will increase rapidly due to loss of CO₂, but during the anaerobic period the pH-changes will normally be minor.

To ensure anaerobic conditions no electron acceptor such as oxygen or nitrate should be present in the reactor. After addition of the carbon source, the oxygen level should be reduced to zero as soon as possible. How soon all oxygen has disappeared depends on the oxygen-uptake rate of the sludge. The presence of oxygen reduces the phosphorus-release rate. This effect can be seen in the test shown in Figure 8. The initial phosphorus release is slow and it can be seen that as soon as no oxygen is present, the release rate increases.

There are different practical alternatives for the elimination of oxygen after the pre-aeration period. If the carbon source is added immediately after the aeration has stopped, the oxygen will delay the phosphorus release and some of the carbon source will be used aerobically. This consumption of the carbon source is important if a wastewater with limited amounts of available carbon source for the bio-P bacteria is tested, but is less important if surplus of for example acetate is added.

Another solution is to wait for the oxygen to be consumed before the carbon addition. The oxygen is then only consumed by the endogenous respiration and it can in some cases take a very long time. If nitrogen gas is bubbled through the activated sludge the elimination of oxygen is much faster.

Nitrogen gas is normally added to the reactor during the whole anaerobic period, either bubbled through the sludge or on the top, in order to reduce the chances for oxygen to reach the sludge. Experiments without nitrogen gas can also be acceptable for less accurate estimations of the P-release.

If nitrate is present in the sludge, or is produced by nitrification during the pre-aeration period, it might also

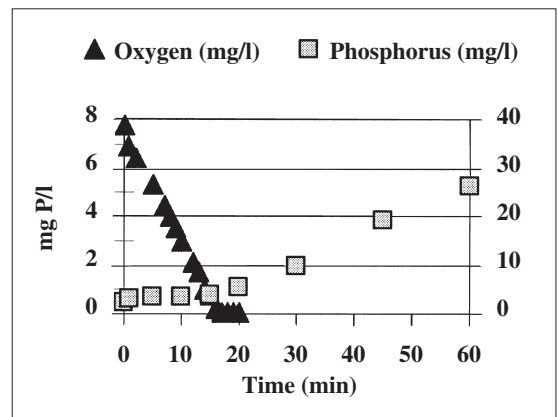


Figure 8. Phosphorus release and oxygen concentration during the start of a batch test.

influence the result of the phosphate-release test. Nitrate can act as the electron acceptor needed for the phosphorus uptake. Denitrification of all the nitrate has to occur before anaerobic condition is fully induced, which also might slow down the initial release of phosphorus. The denitrification requires carbon source and the nitrate will not be consumed at any significant rate before the addition, and hence it will definitely influence the phosphorus release and the carbon consumption.

If the experiments are done in order to evaluate a specific carbon source or if the ratio of P released/C taken up is calculated, nitrate and oxygen has to be considered. It is always a good idea to measure both the oxygen and nitrate concentration in the beginning of the anaerobic period to document the possible effect on the results. If the initial release is slow due to oxygen or nitrate, the initial period can be excluded when the P-release rate is calculated.

Temperature

The temperature chosen for the phosphorus-release tests can either be the temperature at the plant at the time of sampling or a constant temperature, for example 20°C. Taking into account the adaptation of bacteria to different temperatures, the prevailing plant temperature would be most suitable. Using the same temperature each time can perhaps facilitate the comparison from time to time or from plant to plant. In Figure 9 batch tests performed at two different temperatures are shown. The initial release is slow because of oxygen remaining in the anaerobic period. The oxygen-uptake rate is also dependent on the temperature and the oxygen is present for a longer time in the reactor at 15°C than in the reactor at 22°C. The linear regression is made from 20 and 15 minutes respectively and up to 90 minutes. A higher release rate is seen with the higher temperature, but the maximum release is more similar at the temperatures tested.

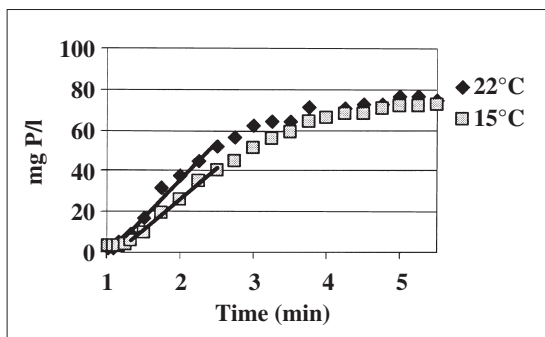


Figure 9. Temperature effect on the phosphorus release rate.

Discussion

The phosphorus-release and uptake test is assumed to be valuable for the practitioners in the field. The detailed evaluation of the test above suggests that it is difficult to standardise the method in order to make it suitable for all cases and for sludge from different plants. Before the test is carried out it is necessary to decide some details about the practical performance:

Duration – The needed time period for release is different for different types of sludge. If the phosphorus release has stopped and reached a constant level the time period is enough. A decision has also to be taken for the length of the test regarding phosphorus release only or phosphorus uptake as well.

Carbon source addition – If a reference carbon source, for example acetate, is used it is requested to be sufficient for the maximum release, in order to ensure that the carbon source is not limiting the release. The concentration of bio-P bacteria and volatile suspended solids are for example important for the amount carbon source needed. If an uptake test also is performed, the excess carbon is critical for the uptake rate. If a wastewater is tested, it is necessary to choose the quantity of the added wastewater and the dilution aspects has to be considered as well.

pH – If pH regulation is possible pH 7 is suggested, but depending on the purpose of the test other values can be selected.

Temperature – The temperature has an effect on the phosphorus release. For long time series it has to be decided whether a standardised temperature of for example 20°C or the actual temperature at the plant is used. For single tests and for comparisons between different plants a temperature of 20°C is suggested.

Use of nitrogen gas – Nitrogen can be used for ensuring anaerobic conditions either by addition at the top or bubbled through the activated sludge. It is effective to bubble nitrogen gas through the reactor in order to speed up the removal of the oxygen after the pre-aeration period.

Conclusions

Even if the phosphorus release batch test is simple and does not require any advanced equipment, there are many different factors influencing the result that makes it difficult to standardise the procedure and make it suitable for all purposes and different plants.

Phosphorus-release rates and maximum phosphorus release are the most common ways of measuring the bio-P activity with the test. Phosphorus uptake can also be measured, which requires a longer test period.

Estimation of the fraction of GAOs compared to PAOs can easily be done by analysing the uptake of carbon source.

pH is important for the result of a batch test and a controlled pH is suggested if possible.

Other factors that can affect the test and have to be considered during the test and the evaluation of the results are oxygen and nitrate during the anaerobic phase and temperature.

Phosphorus batch tests are easy to perform but more complicated to evaluate.

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